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Predicting microbial growth: growth responses of salmonellae in a laboratory medium as affected by pH, sodium chloride and storage temperature

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The growth responses of salmonellae (mixed inoculum of *Salmonella thompson, S. stanley* and S. *infantis*) as affected by NaCl concentration, pH level and storage temperature were studied in laboratory medium. Growth curves were obtained at 5 concentrations of NaCl (0.5–4.5%, w/v), 5 pH levels (5.6–6.8) and 5 storage temperatures (10–30 °C). Sigmoid curves (Gompertz form) were fitted to the data and the curve parameters used to produce a polynomial model from which predicted growth curves could be generated for any combination of NaCl, pH and storage temperature within the limits studied. From those growth curves values for growth rate, generation time, lag time and other values such as time to a 1000-fold increase in numbers were derived. Such a model offers a cost-effective approach to understanding the microbial growth response in foods, and forms a data-base against which other controlling factors could be evaluated. Some problems of fitting curves to microbial growth data and of modelling such data are discussed.

Key words: Salmonellae; Predictive modelling; Growth curves; Temperature; Sodium chloride; pH

Introduction

There are no known means to prevent the occasional contamination of agricultural products by microbes of concern, such as *Salmonella, Clostridium botulinum, Cl. perfringens, Campylobacter.* Food processors must therefore assume they may be present and consciously take measures to kill them or prevent their multiplication. Food products that are repackaged (e.g. after slicing) will be exposed to the risk of post process contamination with organisms of human origin such as *Staphylococcus aureus* and *Salmonella*. It has become apparent in recent years that a better understanding of the effect of the food components and storage conditions on the growth responses of the microbes normally present in food products would obviate the need for much bacteriological testing of those products.

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Assuming sufficient nutrients are available, microbial growth in foods is controlled primarily by pH, water activity (a_w) and storage temperature, with additional factors such as preservatives, modified atmosphere packaging or heat treatment also contributing. In practice several of those factors act in combination, usually at levels which singly would not be sufficient to control microbial growth.

In food microbiology much effort has been directed at defining limiting values for growth of single factors, such as a_w or temperature, for a wide range of microbes. With the exception of *Cl. botulinum*, there have been few studies of factors acting in combination to prevent microbial growth, due in part to experimental difficulties and inadequately automated microbiological methods. The consequence is a poor appreciation of the relative contributions of physical and chemical factors controlling microbial growth in many food systems. Given an adequate data base the response of many microbes in foods could largely be predicted from knowledge of the formulation, the process and the storage conditions.

The growth responses of a range of organisms have been studied in laboratory medium in the presence of different combinations of preservatives, at several pH levels and storage temperatures including *Cl. botulinum* (Baird-Parker and Freame, 1967; Roberts and Ingram, 1973) *Vibrio parahaemolyticus* (Beuchat, 1973) *Staph. aureus* (Bean and Roberts, 1974) *Pseudomonas fragi, Enterobacter liquefaciens, Lactobacillus* sp., *Brochothrix thermosphacta* (Roberts et al., 1979) and *Escherichia coli, Salmonella* sp., *Cl. perfringens,* faecal streptococci (Gibson and Roberts, 1986a, b).

Such information has improved our understanding of how combinations of factors affect the growth of some organisms of concern to the food industry. It also provides a database against which additional factors, such as prior sub-lethal injury, history of inoculum or other food preservatives, could be tested. The deficiency of those data was that results are largely expressed as 'growth/no growth' after a given incubation period, with no indication of rate or amount of growth.

The desire to minimise the levels of nitrite added to cured meats prompted extensive studies on factors controlling the growth of *Cl. botulinum.* The extensive literature was reviewed critically by Tompkin (1983) and by Roberts and Gibson (1986). The most systematic investigations used a model cured meat system to establish the relative contributions of various factors, acting alone and in combination, to control the growth of *Cl. botulinum* in pasteurized cured meats (Roberts et al., 1981a,b,c). The resultant mathematical model predicted the probability of toxin production (i.e. growth) of *Cl. botulinum* types A and B in the model system as a function of NaCl, nitrite, heat treatment, the presence or absence of other preservatives such as iso-ascorbate, polyphosphate or nitrate, and incubation (storage) temperature (Robinson et al., 1982). A similar logistic regression analysis has been used to calculate the probability of toxin production from one spore of *Cl. botulinum* in fish homogenate, as a function of temperature and inoculum size (Lindroth and Genigeorgis, 1986). Regression analysis was also used by Jensen et al. (1987) to model growth of *Cl. botulinum* in laboratory medium and by Ikawa and Genigeorgis (1987) for fish fillets stored under modified atmospheres.

Predicting the probability of growth is an improvement on earlier data which

merely showed whether or not growth would occur because the consequences of manipulating several factors at once can be predicted. Models which predict the probability of toxin production are adequate for organisms such as *Cl. botulinum* where any toxin production is unacceptable. When a certain amount of microbial growth may be tolerated, the ability to estimate the extent of growth of key microbes with respect to time and temperature of storage in conditions representative of foods would be of even greater benefit to the food industry. Models which relate generation times of a wide range of organisms to temperature have been proposed (Ratkowsky et al., 1982, 1983) and are relevant to foods (Gill, 1984; Smith, 1985). The square-root model has recently been extended to take account of the effect of water activity/sodium chloride concentration on growth rate of a strain of *Staphylococcus xylosus* (McMeekin et al., 1987).

Given knowledge of the growth responses of microbes of concern with respect to physical and chemical properties of foods i.e. pH, a_w, level of preservatives, storage temperature, models can be constructed which predict the likelihood and extent of growth. An example is available for one strain of *Staph. aureus* and one strain of *S. typhimurium* grown in UHT milk at a range of temperatures and pH levels, with a_w adjusted with glucose (Broughall et al., 1983; Broughall and Brown, 1984).

Ideally, modelling should contain no element of subjective judgement e.g. of rates of growth. Sigmoid curves, such as the logistic and Gompertz functions, fit microbiological growth data well, but a relatively large number of data points are required. With the intention of gaining experience in applying such objective means of analysis, and of the problems of acquiring sufficient data to warrant such curve fitting routines, growth responses of *Cl. botulinum* were determined in the model pork slurry system containing 1.5–4.5% sodium chloride at a range of storage temperatures (15–27 °C) (Gibson et al., 1987). The growth curves were fitted by both Gompertz and logistic functions and estimates of lag time, growth rate and generation time calculated for each fit. Generally, better fits were obtained with the Gompertz than the logistic function. Even using a selective botulinum medium (Gibson, 1986) counting was laborious, and absence of counts at particular parts of the growth curve sometimes caused the curve-fitting programme to fail. As a consequence insufficient treatment combinations were evaluated for a reliable model of growth of *Cl. botulinum* to be developed.

Consequently a simpler experimental system was chosen in which the growth responses of salmonellae in a laboratory medium, adjusted to a range of pH and NaCl levels and stored at temperatures between 10 and 30 °C, were studied. A more comprehensive data base was generated with the aim of producing a model to describe the growth responses of salmonellae under the conditions studied. That model was then tested against the growth responses of salmonellae inoculated into a simulated cured pork product at seven combinations of storage temperature and NaCl level, and against data published by others.

This paper reports our experience modelling parameters derived by Gompertz curve-fitting, the curve for each treatment combination containing more determinations of viable numbers than usually performed.

Materials and Methods

Strains used

Spontaneous antibiotic-resistant mutants of *Salmonella thompson, S. stanley* and *S. infantis*, isolated in two steps using Tryptone Soya Agar (TSA) plates containing (1) rifampicin (0.05 mg/ml) and (2) nalidixic acid (0.05 mg/ml), were provided by B M Mackey (IFR-BL).

Media

Tryptone Soya Broth (TSB, Oxoid CM129), adjusted to pH 7.2 (with 1 M HCl) prior to autoclaving, was used to culture the inocula.

For growth curve studies NaCl was added to TSB during preparation to give final concentrations of 0.5–4.5% (w/v). Broths at each NaCl level were made in bulk and adjusted to the appropriate pH level using 1 M HCl (approx. pH 5.6, 5.9, 6.2, 6.5 or 6.8) prior to dispensing in 300 ml volumes and autoclaving at 121° C for 15 min.

Tryptone Soya Agar (TSA) was prepared using TSB plus 1% agar (Lab M No. 2, London Analytical & Bacteriological Media Ltd., 50 Mark Lane, London EC3R 7QJ) and adjusted to pH 7.2 prior to autoclaving. Rifampicin and nalidixic acid (Sigma) were added aseptically to the molten tempered agar (50° C) each to a final concentration of 0.05 mg/ml in the antibiotic agar, designated TSAA. The antibiotic solutions were prepared immediately prior to adding to the agar, rifampicin was dissolved in ethanol and nalidixic acid in sterile distilled water to give 5 mg/ml.

Pork slurries

Pork slurries (minced defatted pork : water in the ratio 1 : 1.5) were prepared as described by Gibson et al. (1987). Target NaCl levels of 1.5, 2.5, 3.9 and 4.5% (w/v) were added. The slurries were inoculated and dispensed into 1 oz screw-capped bottles (approx. 28 g/bottle).

Inoculum

Each strain was grown to the stationary phase in TSB for 24 h at 35° C. 1 ml of each culture (approx. 1×10^9/ml) was mixed and diluted to approx. 3×10^5/ml, 1 ml of which was inoculated into each 300 ml volume of TSB to give a final inoculum of approx. 1×10^3/ml. For pork slurries the inoculum was diluted to give a final concentration of approx. 1×10^3/g slurry.

Experimental procedure

TSB in 300 ml volumes was equilibrated overnight to the intended incubation temperature. Each 300 ml was inoculated with 1 ml of the mixed strain inoculum

using a 1 ml repeating syringe (Eppendorf Multipette 4780, Baird and Tatlock Ltd, London). Immediately after inoculation the broths were dispensed aseptically in 10 ml volumes into sterile 1 oz bottles with screw caps using a peristaltic pump (Accuramatic Mk 5, Jencons). A count was made from one sample on TSAA immediately after dispensing and the time noted. The remaining 29 bottles were placed at the appropriate incubation temperature. The procedure of inoculating and dispensing the broths was monitored using thermocouples and carried out as quickly as possible to ensure no significant change in temperature occurred prior to storage.

Pork slurries were prepared immediately prior to inoculation from minced pork thawed overnight at 4°C. During preparation and dispensing, the slurry temperature did not rise above 15°C. Slurries were sampled as described by Gibson et al. (1987) and one count per slurry made immediately after dispensing. The remaining bottles were placed at the temperature of storage.

At intervals during storage decimal dilutions were made from separate bottles of TSB or 'stomached' slurry and 20 μl of each plated onto TSAA (slurry sampling is fully described by Gibson et al., 1987). The plates were incubated at 35°C for approx. 20 h and colonies counted. Counts are calculated according to the method of Farmiloe et al. (1954) and expressed at $\log_{10}$/ml of TSB or g of slurry and plotted against time (h).

From each count made on TSAA from stored slurries inoculated with salmonellae two to five colonies were picked and streaked on Brilliant Green Agar (BGA, Oxoid CM329) for confirmation of *Salmonella*.

Determination of pH, NaCl, fat and moisture

NaCl concentrations of the TSB were determined using a PTI-54 salt analyser and pH measurements of TSB and slurry were made using a Kent EIL 7045/46 pH meter. Chloride determinations on slurry samples were made according to the Official Method of the Society for Analytical Chemistry (Hansen, 1973). Fat and moisture determinations on slurry samples were carried out according to the method of Bostain et al. (1985). The combinations of pH, NaCl and storage temperatures studied in TSB and pork slurries are listed in Tables I and II.

Experimental design

Initially the experiment was planned in three stages each based on a second-order central composite design (John and Quenouille, 1977). In each stage, it was intended that one of the independent variables (temperature, salt or pH) was held constant while the remaining two took the standardized levels -1, $-1/\sqrt{2}$, 0, $1/\sqrt{2}$, $+1$ where -1 and $+1$ corresponded to the limits of the independent variables in the experiment (viz. 0.5, 1.08, 2.5, 3.41, 4.5% NaCl; 10, 12.9, 20, 27.1, 30°C and pH level 5.60, 5.78, 6.2, 6.62, 6.8). The constant variable was set at zero.

This approach was taken because it was impossible to complete the whole experiment (i.e. 125 treatment combinations) at once. It also allowed the first stage

TABLE I

Growth rate, lag time and generation time derived from calculated curve parameters for growth of salmonellae in media

Code	NaCl (% w/v)	pH	Temp. (°C)	Derived values			Residual sums of squares	Residual degrees freedom
				Growth rate [a]	Lag time [b]	Gen. time [c]		
1	0.77	6.48	20	0.30	3.8	1.0	1.3	18
2	1.33	6.13	15	0.12	14.2	2.6	0.3	16
3	1.33	6.13	25	0.51	4.9	0.6	1.4	17
4	2.66	6.12	10	0.02	74.9	16.2	0.9	22
5	2.67	6.12	20	0.25	7.4	1.2	0.4	17
6	2.69	6.12	20	0.25	7.3	1.2	0.6	17
7	2.73	6.12	20	0.26	7.6	1.2	0.2	17
8	2.65	6.12	20	0.26	7.4	1.2	0.2	17
9	2.70	6.12	20	0.25	7.0	1.2	0.3	17
10	2.75	6.12	20	0.25	7.2	1.2	0.3	17
11	2.65	6.12	20	0.25	7.8	1.2	0.3	17
12	2.75	6.12	20	0.25	6.8	1.2	0.3	17
13	2.75	6.12	30	0.63	2.8	0.5	0.5	12
14	4.06	6.02	15	0.07	38.4	4.6	3.7	23
15	4.06	6.02	25	0.32	7.0	0.9	1.4	16
16	4.50	5.90	20	0.16	16.8	1.9	0.6	21
17	0.82	6.22	10	0.03	83.5	8.9	3.0	23
18	0.82	6.22	15	0.11	8.7	2.7	2.2	23
19	0.82	6.22	25	0.51	2.5	0.6	0.6	13
20	0.82	6.22	27	0.42	2.0	0.7	2.8	15
21	2.72	6.07	15	0.09	5.7	3.4	0.6	17
22	4.50	5.99	25	0.30	10.3	1.0	3.0	13
23	4.50	5.99	27	0.30	13.2	1.0	5.4	14
24	4.50	5.99	30	0.36	6.3	0.8	6.5	16
25	1.30	5.63	10	0.02	44.6	18.5	0.8	16
26	1.30	5.63	15	0.10	24.3	3.1	6.3	15
27	1.30	5.63	20	0.25	7.2	1.2	0.6	13
28	1.30	5.63	25	0.51	5.0	0.6	1.6	13
29	1.30	5.63	30	0.99	5.6	0.3	10.0	13
30	1.30	5.89	15	0.12	26.0	2.6	4.5	15
31	1.30	5.89	25	0.44	5.1	0.7	1.2	14
32	1.32	6.20	10	0.02	42.9	17.7	2.4	19
33	1.32	6.20	20	0.29	8.9	1.0	1.7	12
34	1.32	6.20	30	1.06	5.9	0.3	8.2	11
35	1.31	6.40	15	0.12	23.4	2.6	4.8	18
36	1.31	6.40	25	0.52	5.7	0.6	0.4	12
37	1.28	6.77	10	0.05	70.7	6.3	5.9	11
38	1.28	6.77	15	0.11	21.7	2.7	2.6	15
39	1.28	6.77	20	0.32	8.8	0.9	2.2	15
40	1.28	6.77	25	0.50	4.7	0.6	1.2	12

TABLE I (continued)

Code	NaCl (% w/v)	pH	Temp. (°C)	Derived values			Residual sums of squares	Residual degrees freedom
				Growth rate [a]	Lag time [b]	Gen. time [c]		
41	1.28	6.77	30	0.94	4.2	0.3	9.5	11
42	4.56	6.02	10	0.02	209.8	18.4	3.2	9
43	4.56	6.02	15	0.07	53.6	4.2	0.7	10
46	2.64	5.95	15	0.09	14.6	3.2	1.8	16
49	3.75	5.95	10	0.02	115.7	18.7	7.8	17
50	4.52	6.60	10	0.03	222.5	11.0	6.7	11
51	4.50	6.37	10	0.03	209.8	11.4	2.0	10
52	3.76	6.64	10	0.02	152.7	13.8	6.8	15
53	3.70	6.37	10	0.02	102.4	17.8	0.5	17
54	2.64	6.60	10	0.03	26.8	12.0	0.3	16
55	2.66	6.34	10	0.03	47.3	11.6	0.7	16
56	4.52	6.60	15	0.06	36.1	5.3	0.6	12
57	4.50	6.37	15	0.06	45.1	5.0	0.7	12
58	3.76	6.64	15	0.05	10.4	6.0	0.6	15
59	3.70	6.37	15	0.06	24.4	5.2	0.4	14
60	3.75	5.95	15	0.05	13.1	6.6	0.3	12
61	2.64	6.60	15	0.11	16.0	2.7	0.6	18
62	2.66	6.34	15	0.11	14.4	2.7	0.6	18
63	2.62	5.64	30	0.52	3.9	0.6	0.8	13
64	2.60	6.68	30	0.50	2.6	0.6	0.2	13
65	1.10	5.94	30	0.64	2.5	0.5	0.1	12
66	1.08	6.49	30	0.67	2.4	0.4	0.4	12
67	0.72	5.63	25	0.35	2.1	0.9	0.3	13
68	0.70	6.77	25	0.36	2.7	0.8	0.3	13
69	4.46	6.65	25	0.26	11.1	1.2	0.9	14
70	4.46	5.66	25	0.24	11.6	1.2	0.8	15

[a] Growth rate ($\log_{10}$/h).
[b] Lag time (h).
[c] Generation time (h).

to act as a pilot study to assess the feasibility of modelling the response surface and to test the belief gained during previous work with *Cl. botulinum* (Gibson et al., 1987) and that of other workers (Broughall and Brown, 1984) in a curved quadratic-type response surface. Practical limitations made it difficult to achieve precisely the calculated conditions listed above, so the following target levels were chosen:

storage temperature (°C)	10, 15, 20, 25, 30
NaCl (% w/v)	0.5, 1.0, 2.5, 3.9, 4.5
pH	5.6, 5.9, 6.2, 6.5, 6.8.

TABLE II

Growth rate, lag time and generation time derived from calculated curve parameters for salmonellae in pork slurries

Code	NaCl (% w/v)	NaCl (% on water)	pH	Temp. (°C)	Derived values			Residual sums of squares	Residual degrees freedom
					Growth rate [a]	Lag time [b]	Gen. time [c]		
71	4.41	5.06	5.48	20	0.11	16.03	2.69	0.49	15
72	4.41	5.06	5.48	25	0.23	14.13	1.31	0.36	9
73	4.41	5.06	5.48	30	0.25	3.74	1.20	0.52	9
74	2.71	3.11	5.53	15	0.10	14.48	2.94	0.29	10
75	1.61	1.83	5.52	15	0.11	20.30	2.70	0.22	10
76	3.71	4.29	5.49	15	0.07	21.65	4.04	0.39	14
77	4.41	5.06	5.48	15	0.05	39.05	5.61	1.81	16

[a] Growth rate ($\log_{10}$/h).
[b] Lag time (h).
[c] Generation time (h).

Stage 1 (Codes 1–16)

Growth of salmonellae was monitored in 16 combinations of salt level and storage temperature at constant pH (approx. 6.1–6.2 after autoclaving). Eight combinations were replicates (2.5% NaCl stored at 20 °C; codes 5–12), to estimate the reproducibility of the growth curves.

A more flexible approach was taken in subsequent stages to include a wider range of combinations of factors than required by the central composite design.

Stage 2 (Codes 17–24)

A further 8 combinations of NaCl level and storage temperature at pH approx. 6.2 were studied to test the model for both interpolation and extrapolation, and the observations included in subsequent model building.

Stage 3 (Codes 25–41)

The third stage was based on the initial design but with NaCl held constant at 1.0% and pH level varied, plus additional combinations of salt and temperature at the highest and lowest pH levels.

Stages 4 and 5 (Codes 42–52 and 63–70, respectively)

The inclusion of pH in the model revealed a number of gaps in the sampling strategy which led to uncertainty in the predicted behaviour. Some of the gaps in the design arose because of the uncertainty of the growth response of the organism. For example, treatment combinations at high salt, low pH and low storage temperatures were initially omitted because growth was likely to be prohibitively slow. Treatment combinations at low NaCl and high storage temperatures were initially omitted because frequent counting was required. The fourth and fifth stage of the experiment included some of those combinations.

Stage 6 pork slurries (Codes 71–77)

Four slurries containing NaCl (% w/v) approx. 1.5, 2.5, 3.5 or 4.5 were incubated at 15 °C. A further three slurries containing NaCl (% w/v) approx. 4.5 were incubated at 20, 25 and 30 °C.

Statistical modelling

Modelling was carried out in two stages following Broughall et al. (1983), Broughall and Brown (1984) and Jefferies and Brain (1984). The first stage involved modelling the bacterial growth curve by a Gompertz function (Jefferies and Brain, 1984; Gibson et al., 1987). The second stage of modelling concentrated on describing the variation of the parameters of the growth curve as a function of growth conditions.

At each combination of salt, temperature and pH the bacterial count was modelled as a function of time using the Gompertz growth curve given by

$$L(t) = A + C \exp\{-\exp[-B(t-M)]\}$$

where $L(t)$ is the $\log_{10}$ count at time t. This allows the growth curve to be summarised in four parameters B, M, C and A where A is the asymptotic log-count as t decreases indefinitely, C is the asymptotic amount of growth that occurs as t increases indefinitely, and B is the relative growth rate at M, where M is the time at which the absolute growth rate is a maximum. These parameters were used to derive growth rate, generation time and lag time. Other quantities, such as time to reach a given increase in numbers may also be derived

$$\text{growth rate } (\log_{10} \text{ count/h}) = \frac{BC}{e}$$

$$\text{lag (h)} = \frac{M-1}{B}$$

$$\text{generation time (h)} = \frac{\log_{10}(2)e}{B \times C}$$

All curves were fitted and parameters derived using the Maximum Likelihood Program (MLP) package (Ross et al., 1980).

The function chosen to model the parameters was a polynomial of the form

$$y = a + b_1 s + b_2 t + b_3 p + b_4 s^2 + b_5 t^2 + b_6 p^2 + b_7 st + b_8 sp + b_9 tp + e$$

In this expression y is the response variable, i.e. the parameter to be modelled, s, t and p represent NaCl (% w/v), temperature (°C) and pH, and e represents a random error. Such a model has no theoretical basis, but can be viewed as a Taylor's series approximation to a true underlying theoretical function (Box and Draper, 1987), and was chosen for simplicity and to reflect our belief in a quadratic

response surface. Separate models were specified for each of the parameters B, M, C and A in which the value of any given parameter was predicted independently of the other parameters. In reality these parameters are not independent and the values of one parameter depend in part on the value of the others. This approach nevertheless provided a reasonable approximation to the underlying function. In modelling the parameters it was necessary to impose the constraint that the predicted values could not be negative. Fitting this polynomial model directly to the parameter values could lead to negative predicted values, so all modelling was performed after taking the natural logarithm of B, M, C and A which had the further advantage of stabilizing the variance, thus strengthening any tests of the model. Such a step was necessary because the variances of the parameters increased in proportion to their magnitude.

Results

Curve fitting

Between 15 and 20 observations were taken during the growth period for each treatment combination and good curve fits were obtained. From those fitted curves the parameters B, M, C and A were derived (see Materials and Methods, Statistical modelling) and lag (h), growth rate ($\log_{10}$ increase/h) and generation time (h) calculated for growth in TSB (see Table I). An indication of the goodness of fit of the Gompertz function to the data is given by the Residual Sum of Squares: the smaller the tabulated value, the closer the fit.

Modelling the parameters

Plots of B, M, C and A and their natural logarithm against temperature, salt and pH confirmed the need for a variance-stabilizing transformation prior to modelling and that the response surface was curved. While some systematic variation might have been expected from having conducted the experiment in several stages, there was little evidence of this.

In all cases the initial bacterial count per ml (g) was approx. 1×10^3, thus A should be independent of conditions. The final count, represented by $A + C$ may be expected to vary systematically with conditions. In fact no systematic variation was found for C. As an initial approach, the mean values for A (3.25) and C (5.97) were substituted to keep the model as simple as possible. Subsequent modelling involved only B and M.

Following the first stage of the experiment the above model excluding terms involving pH, was fitted and used to predict responses for conditions in the second stage. The models were then updated to include those second stage data. Predicted values for B and M agreed well with observed values and data from the second stage of the experiment did not significantly alter the model.

With the third stage of the experiment which included pH terms, the full model was fitted. This produced two models (including and excluding cross-product terms) giving markedly different predicted behaviour. Initially the models involving cross-product terms (salt × temperature, salt × pH and temperature × pH) were fitted:

$$\ln B = -29.4 + 5.49s + 0.722t + 4.40p - 0.161s^2 - 0.00636t^2 - 0.166p^2 - 0.00472st - 0.857sp - 0.0473tp$$

$$\ln M = 40.64 - 7.33s - 0.555t - 8.24p + 0.191s^2 + 0.00761t^2 + 0.510p^2 - 0.00231st + 1.092sp - 0.0161tp$$

TABLE III

Comparison of observed values for B and M in tryptone soya broth with those predicted by the third stage model

Code	B [a]	B_1 [c]	B_2 [d]	M [b]	M_1 [c]	M_2 [d]
42	0.007	0.006	0.005	344.5	458.1	328.7
43	0.039	0.022	0.020	79.5	114.2	84.9
46	0.049	0.041	0.049	35.1	34.6	43.3
49	0.077	0.008	0.010	246.4	204.9	231.2
50	0.014	0.007	0.002	295.5	2975.6	306.0
51	0.014	0.007	0.003	279.6	1304.3	297.9
52	0.011	0.010	0.004	244.3	1115.4	216.7
53	0.008	0.009	0.006	227.8	511.1	206.6
54	0.008	0.013	0.010	152.3	298.6	155.2
55	0.010	0.012	0.011	149.9	209.8	153.4
56	0.027	0.026	0.005	73.5	777.9	79.0
57	0.030	0.025	0.010	78.1	334.8	76.9
58	0.019	0.037	0.012	61.8	295.1	56.0
59	0.029	0.036	0.020	59.5	132.4	53.4
60	0.017	0.031	0.037	72.5	51.3	59.7
61	0.057	0.049	0.032	33.5	79.8	40.1
62	0.056	0.046	0.038	32.4	54.9	39.6
63	0.256	0.274	0.599	7.8	4.0	9.2
64	0.246	0.376	0.156	6.7	16.8	7.3
65	0.320	0.317	0.356	5.6	7.4	7.3
66	0.331	0.370	0.352	5.4	6.6	6.7
67	0.170	0.190	0.158	8.0	16.2	10.9
68	0.175	0.263	0.295	8.5	7.0	8.9
69	0.129	0.146	0.020	18.8	183.6	16.7
70	0.124	0.108	0.270	19.7	6.9	20.9

[a] B, relative growth rate (observed value).

[b] M, time (h) to maximum growth rate (observed value).

[c] B_1, M_1, values for B and M predicted by the third stage model including cross product terms (interactions).

[d] B_2, M_2, values for B and M predicted by the third stage model excluding cross product terms (NaCl × temperature, NaCl × pH and temperature × pH interactions).

While the fitted values for the observed data were reasonable, predicted values for some untested conditions were not. The problem was most severe for M, where at 0.5% salt M decreased with increasing pH, but at 1.5% salt M reached a minimum at about 5.9. At all other salt levels M increased with increasing pH. The simpler models, which did not involve the cross-product terms,

$$\ln B = -14.6 + 0.323s + 0.435t + 1.74p - 0.0920s^2 - 0.00670t^2 - 0.117p^2$$

$$\ln M = 27.5 - 0.300s - 0.466t - 5.77p + 0.0923s^2 + 0.00783t^2 + 0.451p^2$$

TABLE IV

Generation times of salmonellae grown in tryptone soya broth, pH 6.0–6.2

Temp. (°C)	NaCl (% w/v) [a]	pH	Generation time (h)		Standard error	Code [c]
			Observed	Predicted [b]		
10	0.82	6.22	8.9	11.62	2.24	17
	1.32	6.20	17.7	10.70	1.65	32
	2.66	6.12	16.2	9.84	1.30	4
	3.70	6.37	17.8	10.85	1.33	53
	4.56	6.02	18.4	12.04	2.29	42
15	0.82	6.22	2.7	3.20	0.46	18
	1.33	6.13	2.6	3.09	0.33	2
	2.64	5.95	3.2	3.08	0.33	46
	3.75	5.95	6.6	3.44	0.40	60
	4.56	6.02	4.2	4.14	0.59	43
20	0.77	6.50	1.0	1.12	0.15	1
	1.32	6.20	1.0	1.14	0.12	33
	2.67	6.12	1.2	1.23	0.13	5
	3.50	6.20	–	1.45	0.15	NT
	4.50	5.90	1.9	1.80	0.26	16
25	0.82	6.22	0.6	0.56	0.08	19
	1.32	6.20	0.6	0.58	0.06	3
	2.50	6.20	–	0.65	0.07	NT
	4.06	6.02	0.9	0.95	0.11	15
	4.50	5.99	1.0	1.11	0.16	22
30	0.50	6.20	–	0.36	0.07	NT
	1.32	6.20	0.3	0.38	0.06	34
	2.50	6.20	–	0.46	0.07	NT
	3.50	6.20	–	0.63	0.11	NT
	4.50	5.99	0.8	0.88	0.18	24

[a] Analysed NaCl (% w/v).

[b] Predicted generation times were calculated using analysed NaCl concentrations and measured pH values.

[c] Code: see Table I for full list of combinations studied and calculated values for growth rate, lag and generation time. NT, not tested.

predicted behaviour which reflected our experience. In particular they gave much better predictions of subsequent observations in the fourth and fifth stages (Table III).

The fourth and fifth experimental stages were devised to test the behaviour predicted by the models and to fill gaps in the sampling strategy which led to the problems observed above. Hence, they concentrate on high and low temperatures. Following the fourth stage the models were fitted and tested against the observed values of the fifth stage. Again the model was updated to include all the observations.

TABLE V

Time for a 1000-fold increase in numbers of salmonellae in tryptone soya broth

Temp. (°C)	NaCl (% w/v)	pH	Time [a]		Code [c]
			Observed (h)	Predicted (h) [b]	
10	0.82	6.22	176	180	17
	1.32	6.20	237	171	32
	2.66	6.12	249	188	4
	3.70	6.37	288	236	53
	4.56	6.02	394	372	42
15	0.82	6.22	37	47	18
	1.33	6.13	41	45	2
	2.64	5.95	47	52	46
	3.75	5.95	85	70	60
	4.56	6.02	98	99	43
20	0.77	6.50	14	17	1
	1.32	6.20	19	17	33
	2.67	6.12	20	19	5
	3.50	6.20	–	23	NT
	4.50	5.90	36	38	16
25	0.82	6.22	8.5	9.4	19
	1.32	6.20	10.7	9.2	3
	2.50	6.20	–	10	NT
	4.06	6.02	16	17	15
	4.50	5.99	20	21	22
30	0.50	6.20	–	7.7	NT
	1.32	6.20	9.6	7.2	34
	2.50	6.20	–	8.0	NT
	3.50	6.20	–	10.7	NT
	4.50	5.99	14.2	17	24

[a] Time to nearest hour, initial inoculum approx. 1×10^3 (mean $\log_{10} 3.25$).

[b] Tabulated values calculated from predicted lag and predicted growth rate.

[c] Code: see Table I for full list of codes and NaCl and pH levels studied. NT, not tested.

The final models for B and M took the form:

$$\ln B = -23.5 + 1.496s + 0.487t + 4.29p - 0.0608s^2 - 0.00563t^2 - 0.293p^2 - 0.01261st - 0.171sp - 0.0124tp$$

$$\ln M = 29.19 - 0.409s - 0.5518t - 6.02p + 0.0924s^2 + 0.007710t^2 + 0.448p^2 + 0.00118st + 0.0166sp + 0.01367tp$$

Those models were used to generate Gompertz curves for all combinations of NaCl, pH level and storage temperatures within the range of levels studied i.e. NaCl, 0.5–4.5% (w/v) at intervals of 0.5%; pH level 5.6–6.8 at intervals of 0.1 unit; storage temperature 10–30 °C at 5 °C intervals. From the predicted parameters B, M, C and A values for growth rate, lag time and generation time were derived. A comparison of a range of predicted and observed generation times obtained in TSB is given in Table IV.

In a comparison similar to Table IV between observed and predicted lag times in TSB, agreement was better at 15–30 °C than at 10 °C, at which temperature

TABLE VI

Comparison of generation times (h) of salmonellae grown in pork slurry with those predicted from growth in tryptone soya broth (TSB)

Temp. (°C)	NaCl (% w/v) [a]	pH	Observed (pork slurry)	Predicted (TSB) [b]	Standard error
15	1.61	5.52	2.70	3.89	0.85
	2.71	5.53	2.94	3.47	0.82
	3.71	5.49	4.04	3.60	1.05
	4.41	5.48	5.61	3.93	1.31
20	4.41	5.48	2.69	1.72	0.52
25	4.41	5.48	1.31	1.00	0.29
30	4.41	5.48	1.20	0.76	0.25
	(% on water)				
15	1.83	5.52	2.70	3.76	0.83
	3.11	5.53	2.94	3.45	0.86
	4.29	5.49	4.04	3.85	1.23
	5.06	5.48	5.61	4.50	1.75
20	5.06	5.48	2.69	2.05	0.73
25	5.06	5.48	1.31	1.24	0.43
30	5.06	5.48	1.20	0.99	0.38

[a] Analysed NaCl expressed as % w/v and % on water.

[b] Predicted generation times were calculated using the analysed NaCl concentrations and measured pH values.

predicted values were, with one exception, greater than observed values by approx. 2 standard errors. Broughall et al. (1983) also noted a better fit of generation time than lag time to their model.

In an attempt to assess the overall utility of the model, the predicted time for a 1000-fold increase in number was compared with the observed time (Table V).

Pork slurries

Moisture and fat levels of four pork slurries ranged from 86.49–87.95% moisture (mean 87.20%) and 0.95–1.9% (w/v) fat (mean 1.06%). Storage temperatures and analytical values for NaCl and pH level are included in Table II. At least two colonies were picked from each presumptive salmonella count made. Of 105 colonies picked from slurry counts on TSAA, all gave typical *Salmonella* reactions on BGA. Gompertz curves were fitted to the data and the derived lag time, growth rate and generation time listed in Table II. A comparison of observed generation times with those predicted using the above model is given in Table VI.

Discussion

The specific approach taken here, namely characterising growth by a Gompertz function and describing the parameters in terms of the growth conditions, was motivated by consideration of objectivity, simplicity and utility. One advantage of using the Gompertz curve is that it does not assume a constant growth rate. That the specific growth rate of an organism is not constant over the growth period, but increases to a maximum then decreases, is well established (Jason, 1983; Broughall and Brown, 1984; Gibson et al., 1987). Providing sufficient data are available, fitting a sigmoid curve of the Gompertz type provides a more objective characterization of the growth curve than calculating generation and lag times from a slope 'judged by eye' to be in the exponential phase of growth (Broughall et al., 1983). One drawback to using the Gompertz curve is that a relatively large number of data points (at least 10 and preferably 15) are required. Similar arguments apply to the logistic curve, but it assumes that acceleration and deceleration of growth rate are identical i.e. the curve is 'symmetrical'. Experience has led us to prefer the Gompertz curve.

Although the 'growth curves' produced by Broughall et al. (1983) contained an element of subjective curve fitting, it was the first serious attempt to build a model to predict the growth responses of microbes of concern to the food industry as affected by a_w and storage temperature. That work was expanded to include pH level as a variable, and surfaces describing the model in 3 dimensions were published (Broughall and Brown, 1984). Such data, including the calculation of predicted time to a particular increase in numbers (e.g. 1000 fold) of either salmonellae or *Staph. aureus* provides useful information to manufacturers of milk-based products.

Our model is simpler than the Arrhenius model used by Broughall et al. (1983) in

which the parameters of the non-linear Arrhenius equation were replaced by empirically derived functions of water activity, and subsequently of pH (Broughall and Brown, 1984). The strategy used by Broughall et al. (1983) involved modelling bacterial count, after the initial lag phase, as a function of generation time and lag time. Lag and generation times were then related to temperature using the non-linear Arrhenius equation, and the parameters of this equation were related to a_w and pH (Broughall and Brown, 1984). In fact the parameters were replaced by functions of a_w and pH. An alternative strategy was proposed by Einarsson and Eriksson (1986) who modelled bacterial count as a polynomial function of time, inoculum level and storage temperatures. Such a model does not contain parameters that can be interpreted directly in terms of growth behaviour as is the case with the sigmoid curves, and more importantly such parameters cannot easily be derived.

The polynomial model used here makes no assumptions about underlying relationships, simply providing an approximation to them. Being a linear model parameter estimation is readily accomplished and does not require lengthy optimisation routines. For this reason no attempt was made to replace the Gompertz

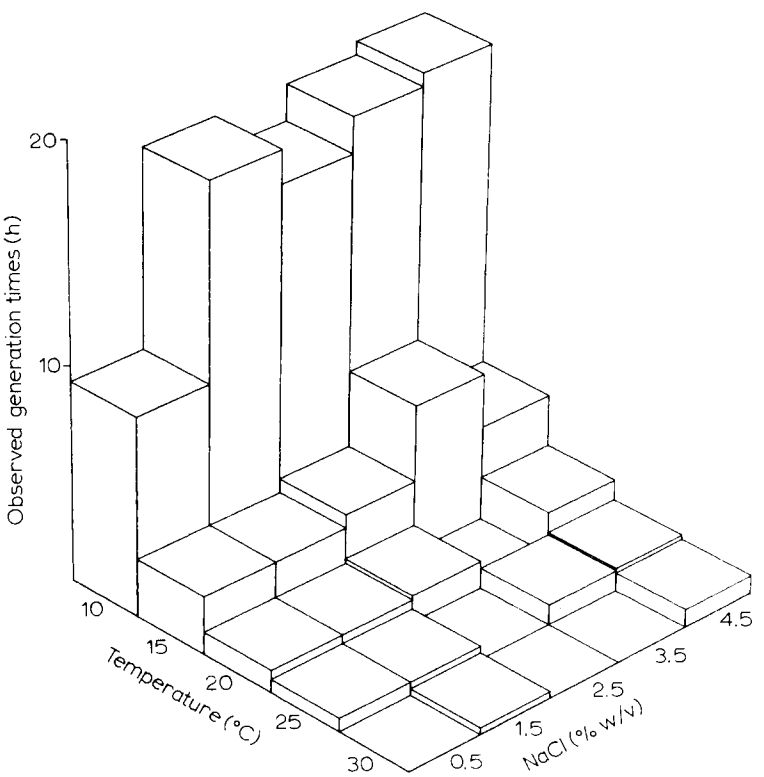

Fig. 1. The effect of NaCl level and temperature on growth of salmonellae in tryptone soya broth at pH approx. 6.2 – observed generation times (h).

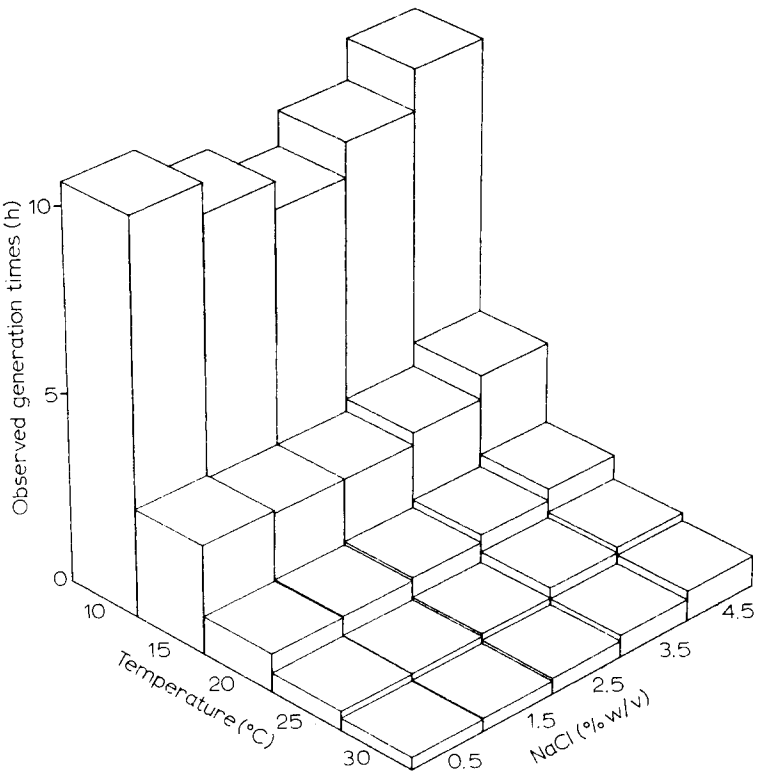

Fig. 2. The effect of NaCl level and temperature on growth of salmonellae in tryptone soya broth at pH approx. 6.2 – predicted generation times (h).

parameters by functions of growth conditions and fitting the resulting model. Polynomial models in general have several disadvantages. They are not constrained and so can give unexpected predictions, such as negative parameter values. In part this was circumvented by taking logarithms, but this does not prevent the model giving infinitely large parameter values. With any model, the further one predicts away from studied conditions the greater likelihood of unrealistic predictions.

Interest of food microbiologists is generally focussed on the more directly interpretable growth rate, lag and generation times, but modelling was carried out on the natural parameters of the Gompertz curve. This allows the whole growth curve to be predicted, and lag, growth rates, or any other quantities of interest such as the time to attain a given increase in numbers, to be readily derived from the predicted curve.

The curved response surface of our model is illustrated in Figs. 1–4 which express in three dimensions the effects of NaCl and storage temperature on the observed and predicted values for generation time, and time to a 1000-fold increase in numbers, at pH approx. 6.2.

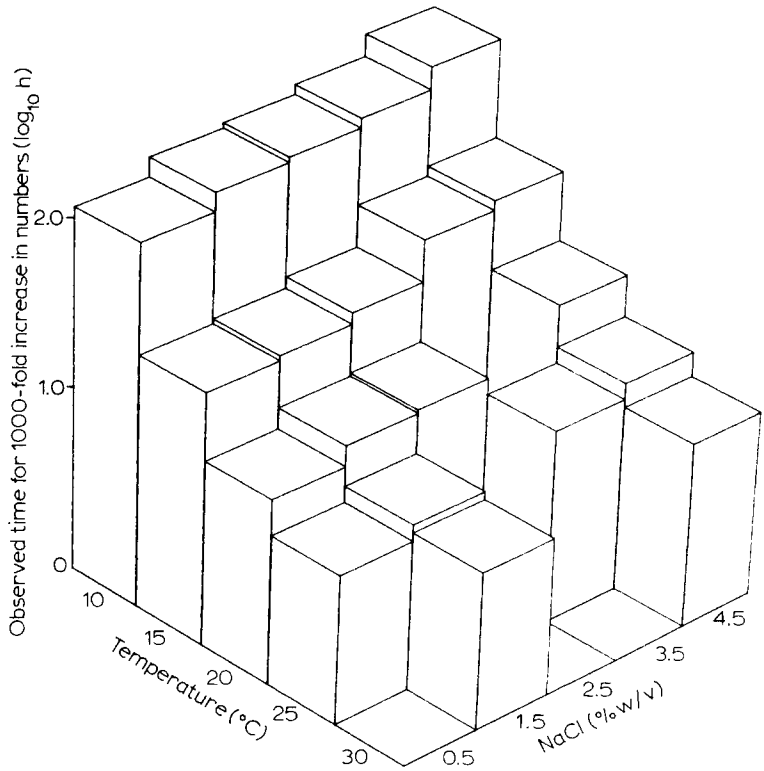

Fig. 3. The effect of NaCl level and temperature on growth of salmonellae in tryptone soya broth at pH approx. 6.2 – observed time for 1000-fold increase in numbers (log_{10} h).

Table IV summarizes observed and predicted generation times for some combinations of NaCl, pH and storage temperature studied. For each 'observed' generation time the corresponding 'predicted' generation time, together with the standard error, has been calculated by substituting those NaCl, pH and storage temperatures into the derived model. In most cases there is good agreement between the observed and the predicted values, which have standard errors of less than ± 0.5 h. The standard errors of predicted generation times at 10 °C were much larger than those at 15–30 °C probably due to greater variability in counts.

Table VI compares generation times predicted from the model in broth with those observed in pork slurries. In broths the NaCl added is expressed as % w/v, but when added to meat the NaCl is usually expressed as % salt on water, which takes into account the fat and protein content, rather than % w/v. Predicted values using NaCl expressed as salt on water were, with one exception, within ± 1 standard error of the observed values; despite the pH of the slurries being below those studied and some of the % salt on water values being above those studied. Predicted values should not ideally, be calculated outside the limits of the database used to develop the model.

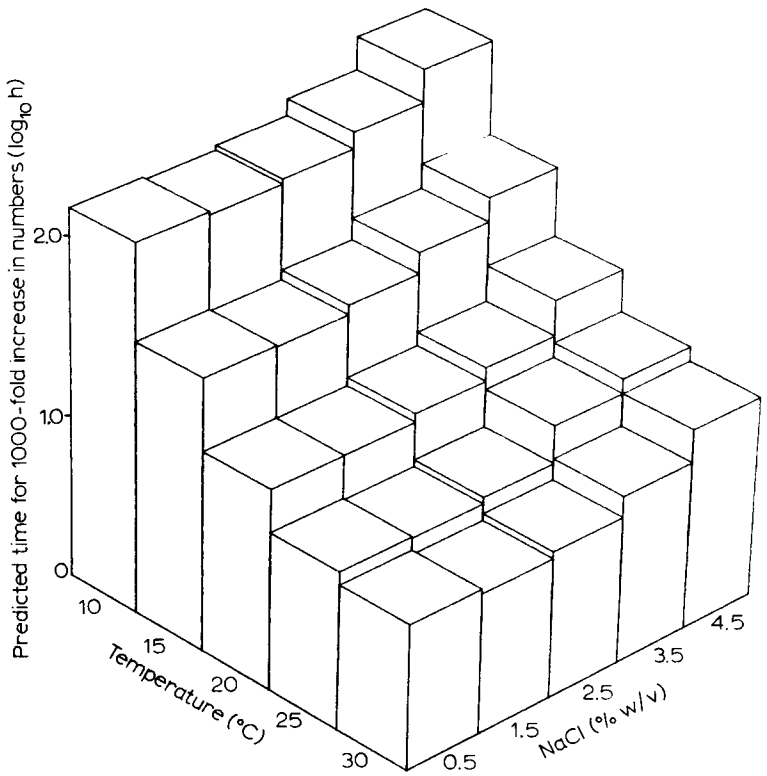

Fig. 4. The effect of NaCl level and temperature on growth of salmonellae in tryptone soya broth at pH approx. 6.2 – predicted time for 1000-fold increase in numbers (log_{10} h).

Much of the published data relating to growth of salmonellae in foods has been restricted to minimum conditions for growth e.g. minimum temperature for growth (summarized by Mackey et al., 1980) and minimum a_w for growth (see reviews by Troller, 1980; Sperber, 1983; Troller, 1986). Relatively little information is available on the growth responses of salmonellae in conditions relevant to formulated foods. Some readily available data are summarised in Tables VII and VIII. Where plots of counts against time were published, we derived co-ordinates from the data points, fitted a Gompertz function to those points, and calculated lag and generation time (see Table VIII).

Generation times published by other workers of salmonellae growing on or in meat are compared with our predictions in Table VII. Where the pH level of each individual study was not given, we have predicted the generation time of salmonellae at the temperatures and maximum and minimum pH levels stated. Since 0% NaCl was not studied in our experiments we assumed a level of 0.5% NaCl for all of our predictions. There was good agreement between predicted generation times and those published, with the exception of *S. typhimurium* inoculated into blended

TABLE VII

Published generation times for salmonellae compared with predicted values

Temp. (°C)	Substrate	pH [a]	Strain	Generation time (h)		pH	Standard error	Ref.
				Published	Predicted			
8	beef slices	5.6–6.3	d	35	33.96 22.06	5.6 6.5	11.97 5.56	1
10	blended mutton	5.7–6.3	e	9.65	16.95 12.10	5.7 6.3	4.79 2.77	2
10	beef surface	5.6–6.5	f	8–26	18.30 11.33	5.6 6.5	5.70 2.66	3
12.5	beef surface	5.6–6.5	f	5.2–10.2	9.00 5.73	5.6 6.5	2.45 1.16	3
15	beef surface	5.6–6.5	f	2.9–3.5	4.75 3.11	5.6 6.5	1.17 0.57	3
15	blended mutton	5.7–6.3	e	2.65	4.43 3.28	5.7 6.3	0.97 0.59	2
15	beef slices	5.6–6.3	d	5.2	4.75 3.11	5.6 6.3	1.17 0.57	1
20	blended mutton	5.7–6.3	e	1.6	1.53 1.18	5.7 6.3	0.30 0.20	2
20	beef slices	5.6–6.3	d	2.2	1.64 1.13	5.6 6.3	0.36 0.19	1
25	blended mutton	5.7–6.3	e	0.75	0.70 0.56	5.7 6.3	0.14 0.09	2
25	beef slices	5.6–6.3	d	1.0	0.75 0.55	5.6 6.3	0.16 0.09	1
30	blended mutton	5.7–6.3	e	0.49	0.43 0.35	5.7 6.3	0.10 0.07	2
30	beef slices	5.6–6.3	d	0.9	0.45 0.35	5.6 6.3	0.11 0.07	1
35	blended mutton	5.7–6.3	e	0.39	0.35 0.30	5.7 6.3	0.11 0.09	2
40	blended mutton	5.7–6.3	e	0.30	0.37 0.33	5.7 6.3	0.19 0.16	2
10	milk, a_w 0.98	NS [b]	e	20 [g]	10.65	7.0	3.87	4
12	milk, a_w 0.98	NS	e	5.0 [g]	6.22	7.0	2.11	4
16	milk, a_w 0.98	NS	e	1.9 [g]	2.42	7.0	0.75	4
20	milk, a_w 0.98	NS	e	1.2 [g]	1.13	7.0	0.34	4
20	milk, a_w 0.98	6.5	e	1.2–2.4 [g]	1.13	6.5	0.19	5

TABLE VII (continued)

Temp. (°C)	Substrate	pH [a]	Strain	Generation time (h)		pH	Standard error	Ref.
				Published	Predicted			
26	milk, a_w 0.98	NS	e	0.7 [g]	0.51	7.0	0.15	4
30	milk, a_w 0.98	NS	e	0.5 [g]	0.37	7.0	0.13	4
30	milk, a_w 0.98	6.5	e	< 1.2 [g]	0.35	6.5	0.07	5

[a] pH range of meat measured, but pH level not specified for each generation time.
[b] NS, pH level of milk not stated, predicted value given for pH 7.0.
[c] 0.5% NaCl (% w/v) values used unless NaCl level specified in reference.
[d] *Salmonella oranienburg*.
[e] *Salmonella typhimurium*.
[f] Various strains tested.
[g] Generation times predicted by other workers.

References:
1. Shaw and Nicol, 1969.
2. Smith, 1985.
3. Mackey et al., 1980.
4. Broughall et al., 1983.
5. Broughall and Brown, 1984.

mutton (pH range 5.7–6.3) and incubated at 10 °C. The published generation time was 9.65 h (Smith, 1985) while the predicted generation time for 3 mixed strains was 12.10–16.95 h depending on pH (range 5.7–6.3).

TABLE VIII

Generation times for salmonellae calculated from published growth data

Temp (°C)	Substrate	pH	Strain	Generation time (h)		Standard error	Ref.
				Calculated [a]	Predicted [b]		
6.7	trypticase broth	7.3	c	29.32	29.18	17.68	1
7.5	trypticase broth	7.3	c	16.67	22.78	13.48	1
8.3	trypticase broth	7.3	c	14.55	17.91	10.38	1
8	nutrient broth 1% NaCl	7.4	c	24.44	19.60	12.45	2
10	ground pork no NaCl added	5.75	d	7.82	16.35	4.44	3
10	ground pork 2% NaCl added	5.75	d	10.73	11.49	2.17	3
10	ground pork 3.5% NaCl added	5.75	d	20.88	10.61	2.28	3

[a] Generation times calculated by fitting Gompertz curves through data points plotted by original authors.
[b] Predicted values calculated from model at appropriate pH value and NaCl concentration (0.5% w/v NaCl assumed if no level stated).
[c] S. *heidelburg*.
[d] S. *derby*, S. *enteriditis*, S. *thompson*.

References:
1. Matches and Liston, 1968.
2. Matches and Liston, 1972.
3. Alford and Palumbo, 1969.

Nevertheless, our observed generation time at 10 °C in broth containing 0.82% (w/v) NaCl and pH 6.22 was 8.9 h, which is similar to the 9.65 h quoted by Smith (1985). A wide range of generation times (8–26 h) were obtained when a mixture of strains of salmonellae were grown on beef surfaces at 10 °C (Mackey et al., 1980). At higher storage temperatures (20–40 °C) there was excellent agreement between the published generation times and those predicted by our model.

Some predicted generation times for salmonellae in milk are included in Table VI but comparison with our predicted values must be viewed with care since those of Broughall et al. (1983) and Broughall and Brown (1984) were obtained for one strain of *S. typhimurium* in milk with a_w adjusted with glucose, while our predictions are from growth of three salmonellae in laboratory medium with the a_w adjusted with NaCl. With the exception of 10 °C storage, there was close agreement between those generation times and our predicted values.

Retrospective microbiological examination of foods has failed to provide information relevant to other formulations or storage conditions. Since the main factors determining the nature and extent of microbial growth have often been identified, it should be possible, in principle, to model growth response of microbes of concern with respect to those factors.

In this paper we have obtained a relatively large amount of data in terms of both numbers of observations on each growth curve, and variety of growth conditions. This has enabled us to construct a stable model of the variation in growth response to simultaneous variation in temperature, salt and pH. Stability of the model is desirable in two respects. The primary aim was to provide predictions of the growth response (e.g. lag, generation time) in untested media and foods. Also, models of this nature provide a new basis for understanding growth responses: they permit straightforward comparison between different growth conditions.

Having developed a model, its robustness and relevance to a wide range of circumstances should be evaluated. Models of bacterial growth response with respect to temperatures are already being used in food process control (Smith, 1985; Gill, 1986). The similarity of predicted growth parameters for salmonellae across a range of concentrations of sodium chloride, pH values and incubation temperatures suggests strongly that modelling offers the most cost-effective approach to understanding and controlling microbial growth in foods.

Acknowledgement

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References

Alford, J.A. and Palumbo, S.A. (1969) Interaction of salt, pH, and temperature on the growth and survival of salmonellae in ground pork. Appl. Microbiol., 17, 528–532.

Baird-Parker, A.C. and Freame, B. (1967) Combined effect of water activity, pH and temperature on the growth of *Clostridium botulinum* from spore and vegetative cell inocula. J. Appl. Bacteriol. 30, 420–429.

Bean, P. and Roberts, T.A. (1974) The effect of pH, sodium chloride and sodium nitrite on heat resistance of *Staphylococcus aureus* and growth of damaged cells in laboratory media. Proc. 4th Int. Congress of Food Science and Technology. III 93–102 (IUFOST), Madrid, Sept. 1974.

Beuchat, L.R. (1973) Interacting effects of pH, temperature and salt concentration on growth and survival of *Vibrio parahaemolyticus*. Appl. Microbiol. 25, 844–846.

Bostain, M.L., Fish, D.L., Webb, N.B. and Arey, J.J. (1985) Automated methods for determination of fat and moisture in meat and poultry products: collaborative study. J. Assoc. Anal. Chem. 68, 876–880.

Box, G.E.P. and Draper, N.R. (1987) The use of graduating function. In: Empirical Model-Building and Response Surfaces, Chapt. 2, John Wiley and Sons, New York, pp. 20–33.

Broughall, J.M. and Brown, C. (1984) Hazard analysis applied to microbial growth in foods: development and application of three-dimensional models to predict bacterial growth. Food Microbiol. 1, 13–22.

Broughall, J.M., Anslow, P.A. and Kilsby, D.C. (1983) Hazard analysis applied to microbial growth in foods: Development of mathematical models describing the effect of water activity. J. Appl. Bacteriol. 55, 101–110.

Einarsson, H. and Eriksson, S.G. (1986) Microbial growth models for prediction of shelflife of chilled meat. In: Meat Chilling 1986. Symp. Int. Institute of Refrigeration Commission C2 (Food Science and Technology), Bristol, 10–12 September 1986, pp. 397–402. IIR, Paris.

Farmiloe, F.J., Cornford, S.J., Coppock, J.B.M. and Ingram, M. (1954) The survival of *Bacillus subtilis* spores in the baking of bread. J. Sci. Food Agric. 5, 292–304.

Gibson, A.M. (1986) Selective recovery of proteolytic strains of *Clostridium botulinum* types A and B from model cured meat systems. Food Microbiol. 3, 213–222.

Gibson, A.M. and Roberts, T.A. (1986a) The effect of pH, water activity, sodium nitrite and storage temperature on the growth of *Escherichia coli* and salmonellae in a laboratory medium. Int. J. Food Microbiol. 3, 183–194.

Gibson, A.M. and Roberts, T.A. (1986b) The effect of pH, sodium chloride, sodium nitrite and storage temperature on the growth of *Clostridium perfringens* and faecal streptococci in laboratory media. Int. J. Food Microbiol. 3, 195–210.

Gibson, A.M., Bratchell, N. and Roberts, T.A. (1987) The effect of sodium chloride and temperature on the rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. J. Appl. Bacteriol. 62, 479–490.

Gill, C.O. (1984) Prevention of early spoilage in livers. Proc. 30th Eur. Meet. Meat Research Workers, Bristol, 1984, pp. 240–241.

Gill, C.O. (1986) Temperature function integration for hygiene evaluation of food processing procedures. Food Technol. Aust. 38, 203–204.

Hansen, N.W. (1973) Determination of chloride. In: Official Standardized and Recommended Methods of Analysis: Meat Products and Meat Extracts, 2nd edn., Society for Analytical Chemistry, London, p. 155.

Ikawa, J.Y. and Genigeorgis, C. (1987). Probability of growth and toxin production by nonproteolytic *Clostridium botulinum* in rockfish fillets stored under modified atmospheres. Int. J. Food Microbiol. 4, 167–181.

Jason, A.C. (1983) A deterministic model for monophasic growth of batch cultures of bacteria. Antonie van Leeuwenhoek, 49, 513–536.

Jeffries, C.J. and Brain, P. (1984) A mathematical model of pollen tube penetration in apple styles. Planta 160, 52–58.

Jensen, M.J., Genigeorgis, C. and Lindroth, S. (1987) Probability of growth of *Clostridium botulinum* as affected by strain, cell and serological type, inoculum size and temperature and time of incubation in a model broth system. J. Food Safety 8, 109–126.

John, J.A. and Quenouille, M.H. (1977). Response surface methods. In: Experiments: Design and Analysis, 2nd edn., Charles Griffin and Co. Ltd., London, pp. 162–181.

Lindroth, S.E. and Genigeorgis, C.A. (1986) Probability of growth and toxin production by nonproteolytic *Clostridium botulinum* in rockfish stored under modified atmospheres. Int. J. Food Microbiol. 3, 167–181.

Mackey, B.M., Roberts, T.A., Mansfield, J. and Farkas, G. (1980) Growth of *Salmonella* on chilled meat. J. Hyg. (Cambridge) 85, 115–124.

Matches, J.R. and Liston, J. (1968). Low temperature growth of *Salmonella* J. Food Sci. 33, 641–645.
Matches, J.R. and Liston, J. (1972) Effects of incubation temperature on the salt tolerance of *Salmonella*. J. Milk Food Technol. 35, 39–44.
McMeekin, T.A., Chandler, R.E., Doe, P.E., Garland, C.D., Olley, J., Putro, S. and Ratkowsky, D.A. (1987) Model for combined effect of temperature and salt concentration/water activity on the growth rate of *Staphylococcus xylosus*. J. Appl. Bacteriol. 62, 543–550.
Ratkowsky, D.A., Olley, J., McMeekin, T.A. and Ball, A. (1982) Relationship between temperature and growth rate of bacterial cultures. J. Bacteriol. 149, 1–5.
Ratkowsky, D.A., Lowry, R.K., McMeekin, T.A., Stokes, A.N. and Chandler, R.E. (1983) Model for bacterial culture growth rate throughout the entire biokinetic temperature range. J. Bacteriol. 154, 1222–1226.
Roberts, T.A. and Gibson, A.M. (1986) Chemical methods for controlling *Clostridium botulinum* in processed meats. Food Technol. (Champaign) 40, 163–171 and 176.
Roberts, T.A., Gibson, A.M. and Robinson, A. (1981a) Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurised cured meats. I. In pork slurry in the pH range 5.54–6.36. J. Food Technol. 16, 239–266.
Roberts, T.A., Gibson, A.M. and Robinson, A. (1981b) Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurised cured meats. II. In pork slurry in the pH range 6.27–6.72. J. Food Technol. 16, 267–281.
Roberts, T.A., Gibson, A.M. and Robinson, A. (1981c) Prediction of toxin production by *Clostridium botulinum* in pasteurised pork slurry. J. Food Technol. 16, 337–355.
Roberts, T.A. and Ingram, M. (1973) Inhibition of growth of *Clostridium botulinum* at different pH values by sodium chloride and sodium nitrite. J. Food Technol. 8, 467–475.
Roberts, T.A., Britton, C.R. and Shroff, N.N. (1979) The effect of pH, water activity, sodium nitrite and incubation temperature on growth of bacteria isolated from meats. In: B. Jarvis, J.H.B. Christian and H.D. Michener, (Eds.), Food Microbiology and Technology, Medecina Viva, Parma, Italy. pp. 57–71.
Robinson, A., Gibson, A.M. and Roberts, T.A. (1982) Factors controlling the growth of *Clostridium botulinum* types A and B in pasteurized, cured meats. V. Prediction of toxin production: Non-linear effects of storage temperature and salt concentration. J. Food Technol. 17, 727–744.
Ross, G.J.S., Hawkins, D., Jones, R.D., Kempton, R.A., Fauckner, F.B., Payne, R.W. and White, R.P. (1980) Maximum Likelihood Program. Rothamstead Experimental Station, Rothampstead, U.K.
Shaw, M.K. and Nicol, D.J. (1969) Effect of the gaseous environment on the growth on meat of some food poisoning and food spoilage organisms. Proc. 15th Eur. Meet. Meat Research Workers, Helsinki, 1969. pp. 226–232.
Smith, M.G. (1985) The generation time, lag time, and minimum temperature of growth of coliform organisms on meat, and the implications for codes of practice in abattoirs. J. Hyg. Cambridge 94, 289–300.
Sperber, W.H. (1983) Influence of water activity on foodborne bacteria – A review. J. Food Protect. 46, pp. 142–150.
Tompkin, R.B. (1983) Nitrite. In: A.L. Branen and P.M. Davidson (Eds.), Antimicrobials in Foods, Marcel Dekker, Inc., New York, NY, pp. 205–256.
Troller, J.A. (1980) Influence of water activity on microorganisms in foods. Food Technol. (Champaign) 34, 76–80 and 82–83.
Troller, J.A. (1986) Water relations of foodborne bacterial pathogens – An updated review. J. Food Protect. 49, 656–670.