Model for the non-thermal inactivation of *Listeria monocytogenes* in a reduced oxygen environment

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The effect and interactions of temperature (4–42°C), lactic acid concentration (0–2%) (pH 3·3–7·3), sodium chloride content (0·5–19·0%), and sodium nitrite concentration (0–200 μg ml^{-1}) on the survival of a three strain mixture of Listeria monocytogenes *in an oxygen-limited environment was studied using sealed, nitrogen-flushed flasks containing brain heart infusion broth. A total of 206 inactivation curves were generated, representing 157 different combinations of the four variables. The inactivation curves were fitted to both a linear and a non-linear primary model which were used to calculate the time to achieve a 4-D (99·99%) inactivation (t_{4D}). Natural logarithmic transformed t_{4D} values were subsequently used to generate secondary models of the four independent variables using response surface analysis. Qualitatively, t_{4D} was inversely related to lactic acid concentration, temperature, and sodium chloride concentration, with the effect of sodium nitrite being pH dependent. Quantitatively, the impact of the four variables was similar to that observed previously for aerobic conditions (Buchanan et al. 1994). While sodium nitrite clearly accelerated the inactivation of* L. monocytogenes *in an acidic environment, comparison of the anaerobic model with an earlier aerobic model indicated that restricting oxygen levels did not enhance the activity of the curing agent. Once validated, the models should provide a rapid means for estimating the survival of* L. monocytogenes *in acidified foods.*

Introduction

One of the primary means of preserving foods and controlling foodborne pathogens is through acidification. When placed in an adverse acidic environment, pathogenic bacteria are inactivated at a rate that is characteristic of that particular species. That rate is dependent on a number of factors such as pH, acidulant identity, acidulant concentration, water activity, and temperature (Ahamad and Marth 1989, El-Shenawy and Marth 1989, Parish and Higgins 1989, Sorrells et al. 1989, Cole et al. 1990, Buchanan et al. 1993, 1994, Buchanan and Golden 1994). Recently, we explored the feasibility of modelling the non-thermal inactivation of *Listeria monocytogenes* (Buchanan et al. 1994). A large body of experimental data was acquired and used to develop response surface models of the effects of temperature, lactic acid concentration (or pH), sodium chloride content (water activity), and sodium nitrite concentration on the 'time to achieve a 4-D (99·99%) inactivation' (t_{4D}). Oxygen availability

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is one of the factors that can influence the growth and survival of *L. monocytogenes* and other foodborne pathogens, particularly in conjunction with the use of sodium nitrite as an inhibitor (Buchanan et al. 1989). The increased interest in being able to predict the inactivation of pathogens in ready-to-eat, uncooked, fermented and non-fermented dried foods prompted us to develop a second dataset for *L. monocytogenes* where the amount of oxygen was limited. These data were then used to develop additional response surface models for describing the effects and interactions of temperature, lactic acid concentration (or pH), sodium chloride content, and sodium nitrite concentration on the survival of *L. monocytogenes* under conditions of reduced oxygen availability.

Materials and Methods

With the exception of the use of nitrogen-flushed, sealed flasks, the techniques employed are the same as those of Buchanan et al. (1994). These techniques are described briefly below.

Micro-organisms

A three strain mixture of *Listeria monocytogenes* (Scott A, HO-V-S and V-7) was used. The strains were cultured individually for 24 h at 37°C and then combined to produce an inoculum of approximately 10^9 cfu ml^{-1}.

Test system

Double strength brain heart infusion broth (BHI) (Difco, Detroit, MI) was supplemented with crystalline sodium chloride and 85% lactic acid to achieve the desired concentrations between 0·5–19·0% and 0·0–2·0%, respectively. After recording the pH, the medium was dispensed in 50 ml portions to 250 ml trypsinizing flasks. The side-arm was sealed with a rubber septum, the flask mouth was sealed, and the flask was autoclaved. Immediately prior to inoculation, filter-sterilized sodium nitrite was added to achieve concentrations of 0–200 μg ml^{-1}. The volume of the sodium nitrite solution and the inoculum (see below) was taken into account in the preparation of the medium.

Inactivation studies

Each flask was inoculated with 1·5 ml of the three-strain inoculum to achieve an initial population density of approximately 10^8 cfu ml^{-1}. The flasks were then flushed with sterile nitrogen for 10 min and sealed. Past studies have indicated that this reduces the level of oxygen to 100–150 ppm. The flasks were incubated on rotary shakers (150 r min^{-1}, 2 cm radius) in incubators at 4, 12, 19, 28, 37, or 42°C. Periodically, 0·1 ml samples were removed through the rubber septum using a sterile syringe + hypodermic needle. After diluting appropriately in 0·1% peptone water, viable counts on tryptose agar (Difco) were performed using a Spiral Plater (Spiral Systems, Inc., Cincinnati, OH) or spread plates (at lower population densities). All plates were incubated for 24 h at 37°C. Sampling continued for 6 months or until the counts fell below the lower limit of detection ($Log_{10} < 1·03$ cfu ml^{-1}).

Survivor curves

Survivor curves were generated by fitting the data to both a linear (Eqn 1) (Buchanan et al. 1993) and a non-linear model (Eqn 2) (Whiting and Buchanan 1993).

Linear primary model

$$\begin{aligned} Y &= Y_0 && [t < t_L] \\ Y &= Y_0 + M(t - t_L) && [t > t_L] \end{aligned} \quad (1)$$

Where:

$Y = Log_{10}$ count at time t	Log_{10} (cfu ml^{-1})
$Y_o = Log_{10}$ count at time t = 0	Log_{10} (cfu ml^{-1})
M = Slope of the survivor curve	[Log_{10}(cfu ml^{-1}]/h
t = Time	h
t_L = Duration of lag period	h

Non-linear primary model

$$Y = Y_0 + \log_{10}\left[\frac{F_1(1 + e^{-b_1 t_L})}{(1 + e^{b_1(t-t_L)})} + \frac{(1 - F_1)(1 + e^{-b_2 t_L})}{(1 + e^{b_2(t-t_L)})}\right] \quad (2)$$

Where:

$b_1 = 2·3/D_1$ = Inactivation rate of major population group

$b_2 = 2·3/D_2$ = Inactivation rate of minor population group

F_1 = Fraction of population in major group

$(1 - F_1)$ = Fraction of population in minor group

D-values for the linear model were calculated by taking the negative reciprocal of the M value, and those for the non-linear models according to the relationship described above. The t_{4D} were calculated

Table 1. Effect of temperature, sodium chloride, lactic acid and sodium nitrite on the kinetics of non-thermal inactivation of *Listeria monocytogenes* based on survivor curves generated using linear and non-linear primary models

Independent variables						Linear model					Non–linear model					
T	S	L	N	pH	n	Y_o	D	M	t_L	t_{4D}	N_o	F_1	D_1	D_2	t_L	t_{4D}
42	0·5	0·50	0	4·3	1	7·8	6·7	–0·149	0	26·9	7·8	–7·0	6·4	0·0	0	27·6
42	0·5	0·50	100	4·3	1	7·7	1·3	–0·788	0	5·1	7·7	–7·0	1·2	0·0	0	5·0
42	0·5	1·00	0	3·8	1	7·8	6·8	–0·147	0	27·3	7·8	–7·0	6·5	0·0	0	27·9
42	4·5	0·40	50	4·7	1	7·7	6·7	–0·148	3	30·3	7·7	–7·0	6·5	0·0	4	30·8
42	7·8	0·40	50	4·7	1	7·7	6·5	–0·154	4	30·0	7·7	–7·0	6·4	0·0	4	30·2
42	10·3	0·00	10	7·3	3	7·9	19·2	–0·052	0	76·9	7·9	–7·0	17·9	0·0	0	77·0
42	10·3	0·00	200	7·2	1	8·0	35·4	–0·028	24	165·0	8·0	–7·0	33·8	0·0	30	166·9
42	10·3	0·25	0	4·8	1	7·7	14·5	–0·069	0	58·2	7·7	–7·0	13·7	0·0	0	58·9
42	10·3	0·50	50	4·3	1	8·0	6·2	–0·161	0	24·8	8·0	–7·0	5·9	0·0	0	25·6
42	10·3	0·50	150	4·3	1	7·9	1·1	–0·897	0	4·5	7·9	–5·4	1·0	13·3	0	4·4
42	10·3	1·00	0	3·9	1	7·7	6·8	–0·147	0	27·3	7·7	–7·0	6·5	0·0	0	27·8
42	10·3	1·00	10	3·9	3	8·0	1·1	–0·876	0	4·6	8·0	–7·0	1·1	0·0	0	4·6
42	10·3	1·00	200	3·9	1	7·7	0·4	–2·363	0	1·7	7·7	–7·0	0·4	0·0	0	1·7
42	10·3	2·00	50	3·4	1	7·5	0·6	–1·699	0	2·4	7·5	–7·0	0·6	0·0	0	2·4
42	13·1	1·00	50	3·8	1	7·8	1·1	–0·880	0	4·6	7·8	–7·0	1·1	0·0	0	4·6
42	19·0	0·00	0	7·3	1	8·1	34·4	–0·029	24	161·6	8·1	–7·0	33·9	0·0	24	162·5
42	19·0	0·25	100	4·7	1	8·2	2·4	–0·420	0	9·5	8·2	–5·1	2·1	19·8	0	9·0
42	19·0	1·00	150	3·8	1	8·2	0·7	–1·426	0	2·8	8·2	–4·2	0·2	1·9	0	0·9
42	19·0	2·00	0	3·3	1	8·0	0·5	–2·156	0	1·8	8·0	–7·0	0·4	0·0	0	1·9
42	19·0	2·00	200	3·3	1	7·9	0·3	–3·490	0	1·2	7·9	–7·0	0·3	0·0	0	1·2
37	0·5	1·00	0	3·9	1	8·2	8·4	–0·119	2	35·6	8·2	–7·0	8·1	0·0	2	36·2
37	0·5	2·00	0	3·3	1	8·2	8·1	–0·124	2	34·3	8·2	–7·0	7·8	0·0	2	34·9
37	6·3	0·50	200	4·4	1	8·5	13·9	–0·072	2	57·6	8·5	–7·0	13·4	0·0	2	58·6
37	6·3	2·00	0	3·3	1	8·5	7·6	–0·132	2	32·3	8·5	–7·0	7·4	0·0	2	32·8
37	7·8	0·50	100	4·4	1	7·8	6·9	–0·144	2	29·8	7·8	–7·0	6·4	0·0	2	28·9
37	10·3	0·00	0	7·3	1	8·0	57·6	–0·017	72	302·4	8·0	–7·0	57·3	0·0	72	303·0
37	10·3	0·00	10	7·3	3	7·8	119·5	–0·008	0	477·9	7·9	–2·5	56·5	286·7	24	258·3
37	10·3	0·00	50	7·3	1	8·1	44·0	–0·023	134	309·7	8·1	–7·0	43·2	0·0	138	311·2
37	10·3	0·00	200	7·3	1	8·0	45·1	–0·022	81	261·3	8·0	–7·0	49·6	0·0	55	255·6
37	10·3	1·00	0	3·9	3	7·9	10·3	–0·096	0	41·1	7·9	–7·0	9·8	0·0	0	42·1
37	10·3	1·00	10	3·9	5	7·8	4·7	–0·447	0	18·7	7·8	–7·0	4·5	0·0	0	19·3
37	10·3	1·00	50	3·8	2	8·0	1·1	–0·885	0	4·5	8·0	–7·0	1·1	0·0	0	4·5
37	10·3	1·00	100	3·8	2	8·0	0·9	–1·075	0	3·7	8·0	–7·0	0·9	0·0	0	3·8
37	10·3	1·00	200	3·8	2	7·9	0·2	–4·560	0	0·9	7·9	–7·0	0·2	0·0	0	0·9
37	13·1	0·50	0	4·4	1	8·2	15·0	–0·067	2	62·1	8·2	–7·0	14·4	0·0	2	63·2
37	13·1	1·50	50	3·7	1	7·9	0·7	–1·404	0	2·8	7·9	–7·0	0·7	0·0	0	2·9
37	19·0	0·00	0	7·3	1	8·1	53·7	–0·019	24	238·8	8·1	–7·0	52·5	0·0	24	241·0
37	19·0	0·00	200	7·3	1	7·9	77·9	–0·013	37	348·8	7·9	–7·0	76·9	0·0	32	350·6
37	19·0	0·50	200	4·4	1	8·5	8·8	–0·114	0	35·1	8·5	–7·0	10·9	0·0	0	46·8
37	19·0	2·00	200	3·3	1	8·2	0·3	–2·902	0	1·4	8·2	–7·0	0·3	0·0	0	1·4
28	0·5	0·50	0	4·3	1	8·0	99·6	–0·010	6	404·3	8·0	–7·0	94·7	0·0	6	410·7
28	0·5	2·00	100	3·3	1	7·8	0·3	–3·507	0	1·2	7·8	–7·0	0·3	0·0	0	1·2
28	4·5	0·40	50	4·6	1	8·0	16·2	–0·062	6	70·6	8·0	–7·0	15·7	0·0	6	71·2
28	6·3	0·37	0	4·7	1	7·9	106·1	–0·009	54	478·8	7·9	–7·0	104·1	0·0	51	480·4
28	6·3	0·37	150	4·7	1	8·1	3·9	–0·259	0	15·4	8·1	–4·8	1·3	36·9	0	5·7
28	6·3	0·50	5	4·4	3	8·2	41·9	–0·024	0	167·5	8·2	–7·0	33·6	0·0	0	144·7
28	6·3	0·50	15	4·4	3	7·9	40·7	–0·025	0	162·6	7·9	–7·0	32·7	0·0	0	140·9
28	6·3	1·50	15	3·7	3	6·5	16·6	–0·061	0	66·48	7·3	–2·3	2·4	44·2	0	10·3
28	6·3	1·50	150	3·6	1	7·7	5·8	–0·173	0	23·1	7·7	–7·0	5·6	0·0	0	23·9
28	6·3	2·00	200	3·3	1	8·0	0·8	–1·221	0	3·3	8·0	–7·0	0·8	0·0	0	3·3
28	7·8	0·40	50	4·6	1	8·0	128·6	–0·008	2	516·3	8·0	–7·0	121·4	0·0	2	523·5

Table 1. — continued

Independent variables						Linear model					Non–linear model					
T	S	L	N	pH	n	Y_o	D	M	t_L	t_{4D}	N_o	F_1	D_1	D_2	t_L	t_{4D}
28	7·8	1·00	50	3·9	1	8·0	9·4	–0·107	0	37·4	8·0	–7·0	8·9	0·0	0	38·5
28	7·8	1·50	50	3·6	1	7·9	2·7	–0·373	0	10·7	7·9	–7·0	2·6	0·0	0	11·1
28	10·3	0·50	150	4·4	1	8·1	27·2	–0·037	0	108·7	8·1	–7·0	25·9	0·0	0	111·7
28	10·3	1·00	0	3·9	1	6·9	14·1	–0·071	2	58·4	6·9	–7·0	13·5	0·0	2	59·0
28	13·1	0·00	5	7·3	3	8·0	113·5	–0·009	588	1042·1	8·0	–7·0	119·7	0·0	560	1039·4
28	13·1	0·00	15	7·3	3	8·1	120·7	–0·008	506	989·0	8·1	–7·0	133·0	0·0	451	983·3
28	13·1	0·00	150	7·3	1	8·0	109·7	–0·009	74	512·8	8·0	–7·0	104·1	0·0	51	480·4
28	13·1	0·37	50	4·8	1	8·1	64·1	–0·016	6	262·5	8·1	–7·0	65·9	0·0	6	286·8
28	13·1	0·37	200	4·8	1	5·4	62·2	–0·016	4	252·6	5·4	–7·0	57·6	0·0	4	250·2
28	13·1	0·50	50	4·4	3	8·1	39·4	–0·025	0	157·6	7·8	–7·0	34·0	0·0	0	146·3
28	13·1	1·00	50	3·9	1	7·9	13·0	–0·770	0	51·8	7·9	–7·0	12·3	0·0	0	53·0
28	13·1	1·00	200	3·9	1	8·0	0·5	–2·111	0	1·9	8·0	–7·0	0·5	0·0	0	1·9
28	13·1	1·50	5	3·6	3	8·2	1·4	–0·702	2	7·7	8·2	–4·0	1·4	39·6	2	7·66
28	13·1	1·50	100	3·6	1	8·3	0·7	–1·358	0	3·0	8·3	–7·0	0·7	0·0	0	3·0
28	13·1	2·00	0	3·3	1	8·1	0·9	–1·118	0	3·6	8·1	–7·0	0·8	0·0	0	3·6
28	13·1	2·00	150	3·3	1	8·0	0·5	–2·163	0	1·8	8·0	–7·0	0·4	0·0	0	1·9
28	19·0	0·00	200	7·3	1	8·1	199·8	–0·005	0	799·4	8·1	–7·0	186·1	0·0	0	801·5
28	19·0	0·50	0	4·3	1	8·2	57·6	–0·017	0	230·5	8·2	–7·0	54·8	0·0	0	236·0
28	19·0	0·50	150	4·3	1	8·2	17·2	–0·058	0	68·6	8·2	–5·3	0·8	67·4	0	3·2
28	19·0	0·50	200	4·3	1	8·0	3·1	–0·322	0	12·4	8·0	–7·0	5·3	0·0	0	22·8
28	19·0	1·00	0	3·9	1	8·1	19·8	–0·050	0	79·4	8·1	–7·0	19·0	0·0	0	81·9
28	19·0	1·00	100	3·9	1	8·2	0·8	–1·289	0	3·1	8·2	–7·0	0·7	0·0	0	3·2
28	19·0	1·50	50	3·6	1	8·1	0·8	–1·312	0	3·0	8·1	–7·0	0·7	0·0	0	3·1
28	19·0	1·50	200	3·6	1	7·8	0·7	–1·390	0	2·9	7·8	–7·0	0·7	0·0	0	3·0
28	19·0	2·00	50	3·4	1	8·0	0·8	–1·125	0	3·3	8·0	–7·0	0·8	0·0	0	3·4
19	0·5	1·00	10	4·1	3	8·0	66·8	–0·015	96	363·1	8·0	–4·0	52·1	3255·2	126	334·6
19	0·5	1·50	200	3·7	1	7·9	0·7	–1·460	0	2·7	7·9	–7·0	0·7	0·0	0	2·8
19	0·5	2·00	0	3·3	1	7·9	28·3	–0·035	0	113·0	7·9	–7·0	26·7	0·0	0	115·1
19	0·5	2·00	200	3·3	1	7·7	0·5	–2·060	0	1·9	7·7	–7·0	0·5	0·0	0	2·0
19	4·5	0·40	50	4·6	1	8·1	125·1	–0·008	0	500·5	8·1	–7·0	117·4	0·0	0	505·4
19	6·3	0·50	150	4·4	1	8·0	33·3	–0·030	0	133·2	8·0	–7·0	31·7	0·0	0	136·6
19	6·3	1·00	200	3·9	1	8·3	1·0	–1·040	0	3·8	8·3	–7·0	0·9	0·0	0	3·9
19	6·3	1·50	0	3·6	1	7·9	20·8	–0·048	0	83·2	7·9	–7·0	19·9	0·0	0	85·8
19	7·8	0·40	50	4·6	1	8·0	121·4	–0·008	2	487·5	8·0	–7·0	114·3	0·0	2	493·2
19	10·3	0·00	150	7·3	1	8·0	952·5	–0·001	24	3833·9	8·0	–7·0	8·2	0·0	24	56·9
19	10·3	0·50	100	4·4	1	6·7	33·5	–0·030	4	138·1	6·7	–7·0	31·6	0·0	4	138·1
19	10·3	1·00	50	3·9	1	8·0	1·5	–0·690	0	5·8	8·0	–7·0	1·3	0·0	0	5·7
19	10·3	1·50	200	3·6	1	7·9	0·5	–2·085	0	1·9	7·9	–7·0	0·5	0·0	0	2·0
19	10·3	2·00	0	3·3	1	7·8	3·9	–0·254	0	15·8	7·8	–5·2	1·4	82·3	0	6·0
19	10·3	2·00	200	3·3	1	8·0	0·7	–1·456	0	2·8	8·0	–7·0	0·5	0·0	0	2·1
19	13·2	0·00	15	7·3	3	7·5	692·9	–0·001	200	2971·5	7·5	–7·0	514·5	0·0	360	2461·4
19	13·2	0·25	100	4·8	1	8·0	272·2	–0·004	24	1112·8	8·0	–7·0	255·5	0·0	24	1112·6
19	13·2	0·50	200	4·5	1	8·0	66·6	–0·015	0	266·5	8·0	–7·0	63·0	0·0	0	271·2
19	19·0	0·00	0	7·3	1	8·1	291·9	–0·003	110	1278·2	8·1	–7·0	290·7	0·0	56	1282·6
19	19·0	0·00	200	7·3	1	7·7	337·0	–0·003	136	1484·8	7·7	–7·0	332·6	0·0	97	1488·4
19	19·0	0·50	150	4·5	1	7·9	94·3	–0·011	0	377·0	7·9	–7·0	88·4	0·0	0	380·6
19	19·0	1·00	10	4·1	3	7·0	57·0	–0·018	0	227·9	7·6	–3·0	13·3	146·8	0	57·4
19	19·0	1·50	200	3·7	1	7·8	8·4	–0·119	0	33·7	7·8	–7·0	8·1	0·0	0	35·0
19	19·0	2·00	150	3·3	1	7·8	0·8	–1·207	0	3·3	7·8	–7·0	0·8	0·0	0	3·4
12	0·5	0·50	100	4·5	1	7·9	61·5	–0·016	24	269·9	7·9	–7·0	59·7	0·0	24	271·7
12	0·5	0·50	200	4·5	1	8·0	68·2	–0·015	2	274·7	8·0	–7·0	63·0	0·0	2	272·2
12	0·5	1·00	100	3·9	1	8·0	12·3	–0·081	0	49·3	8·0	–7·0	11·8	0·0	0	50·6
12	0·5	1·00	200	3·9	1	7·8	12·4	–0·080	0	49·7	7·8	–7·0	11·8	0·0	0	50·9
12	0·5	1·50	50	3·7	1	7·8	12·8	0·078	0	51·4	7·8	–7·0	12·2	0·0	0	52·5
12	0·5	1·50	200	3·7	1	7·9	5·7	–0·177	0	22·6	7·9	–4·2	5·5	0·0	0	23·5
12	4·5	0·40	50	4·6	1	8·2	66·2	–0·015	0	264·8	8·2	–7·0	62·8	0·0	0	270·5

Table 1.—continued

Independent variables						Linear model					Non–linear model					
T	S	L	N	pH	n	Y_o	D	M	t_L	t_{4D}	N_o	F_1	D_1	D_2	t_L	t_{4D}
12	4·5	1·50	150	3·7	1	8·0	0·8	–1·226	0	3·3	8·0	–7·0	0·8	0·0	0	3·4
12	6·3	0·50	5	4·4	3	7·6	155·8	–0·006	576	1199·0	7·6	–7·0	134·0	0·0	576	1112·6
12	6·3	1·00	150	3·7	1	7·6	0·7	–1·458	0	2·8	7·9	–7·0		0·0	0	2·8
12	6·3	1·50	5	3·7	3	7·7	99·5	–0·010	0	398·0	7·7	–7·0	79·2	0·0	0	341·09
12	7·8	0·40	50	4·6	1	8·0	61·8	–0·016	6	253·2	8·0	–7·0	58·8	0·0	6	256·4
12	7·8	2·00	50	3·3	1	7·9	0·8	–1·199	0	3·3	7·9	–7·0	0·8	0·0	6	9·2
12	10·3	0·25	150	4·8	1	8·0	60·6	–0·017	0	242·4	8·0	–7·0	57·3	0·0	0	246·7
12	10·3	0·50	100	4·5	1	7·9	56·8	–0·018	0	227·1	7·9	–7·0	53·9	0·0	0	232·0
12	10·3	1·00	0	4·0	1	7·7	61·6	–0·016	79	325·1	7·7	–7·0	58·6	0·0	93	328·1
12	10·3	2·00	50	3·4	1	7·8	5·7	–0·175	0	22·9	7·8	–3·6	0·5	12·4	0	2·0
12	13·2	0·00	0	7·3	1	8·0	1252·6	–0·001	550	5560·3	8·0	–7·0	945·9	0·0	879	4712·5
12	13·2	0·00	200	7·3	1	7·9	697·1	–0·001	705	3493·4	7·9	–7·0	628·3	0·0	825	3353·7
12	13·2	0·50	5	4·4	3	8·0	119·1	–0·008	576	1052·3	8·0	–7·0	103·6	0·0	576	990·9
12	13·2	1·00	50	4·0	1	8·0	34·8	–0·029	2	141·2	8·0	–7·0	33·1	0·0	2	143·7
12	13·2	1·50	5	3·7	3	8·2	46·1	–0·022	0	184·5	7·7	–7·0	42·7	0·0	0	183·9
12	13·2	2·00	200	3·3	1	7·9	0·8	–1·189	0	3·41	7·9	–7·0	0·8	0·0	0	3·4
12	19·0	0·00	0	7·3	1	7·9	931·9	–0·001	623	4350·5	7·9	–7·0	804·4	0·0	788	4044·2
12	19·0	0·00	15	7·3	3	7·5	628·5	–0·002	600	3114·1	7·5	–7·0	538·2	0·0	600	2772·6
12	19·0	0·25	0	4·8	1	8·0	579·6	–0·002	404	2722·2	8·0	–7·0	562·5	0·0	404	2699·2
12	19·0	0·50	200	4·3	1	8·1	27·3	–0·037	0	109·1	8·1	–7·0	25·9	0·0	0	111·3
12	19·0	1·00	50	4·0	1	8·0	33·6	–0·030	1	135·5	8·0	–7·0	32·0	0·0	1	138·2
12	19·0	1·50	0	3·7	1	8·0	32·4	–0·031	2	131·8	8·0	–7·0	31·0	0·0	2	134·5
12	19·0	2·00	150	3·4	1	7·8	0·8	–1·203	0	3·3	7·8	–7·0	0·8	0·0	0	3·4
4	0·5	0·50	0	4·3	1	8·2	402·8	–0·002	314	1924·9	8·2	–7·0	397·7	0·0	308	1927·0
4	0·5	0·50	200	4·4	1	8·1	37·8	–0·026	0	151·3	8·1	–7·0	35·9	0·0	0	154·4
4	0·5	1·50	100	3·7	1	7·9	8·8	–0·113	0	35·4	7·9	–7·0	8·5	0·0	0	36·6
4	0·5	2·00	0	3·3	1	7·9	54·8	–0·018	0	219·2	7·9	–7·0	51·8	0·0	0	223·1
4	0·5	2·00	100	3·3	1	8·2	1·2	–0·816	0	4·9	8·2	–3·8	0·6	11·5	0	2·7
4	4·5	0·40	50	4·6	1	8·4	81·4	–0·012	0	325·5	8·4	–7·0	77·0	0·0	0	331·3
4	4·5	1·00	100	4·0	1	8·0	12·9	–0·077	0	51·8	8·0	–7·0	12·4	0·0	0	53·2
4	6·3	0·50	150	4·4	1	8·4	70·8	–0·014	0	283·2	8·4	–7·0	67·3	0·0	0	289·8
4	6·3	2·00	0	3·3	1	8·3	12·5	–0·080	0	50·1	8·3	–7·0	11·9	0·0	0	51·3
4	7·8	0·40	50	4·6	1	8·5	77·1	–0·013	0	308·4	8·5	–7·0	74·7	0·0	0	321·7
4	7·8	1·00	100	3·9	1	8·0	12·5	–0·080	0	50·0	8·0	–7·0	11·9	0·0	0	51·2
4	10·3	0·00	10	7·3	3	8·0	951·8	–0·001	240	4047·1	8·0	–1·1	359·1	2338·3	449	1900·4
4	10·3	0·25	100	4·8	1	7·5	58·8	–0·017	229	463·8	7·6	–7·0	60·0	0·0	217	457·7
4	10·3	0·50	50	4·5	1	7·9	86·1	–0·012	99	443·2	8·0	–7·0	83·9	0·0	106	443·7
4	10·3	1·00	10	3·9	3	8·0	107·9	–0·009	0	431·7	7·3	–7·0	77·0	0·0	132	441·4
4	10·3	1·00	200	3·9	1	8·2	3·4	–0·299	0	13·4	8·2	–7·0	3·2	0·0	0	13·7
4	10·3	2·00	50	3·3	1	7·9	3·4	–0·296	0	13·5	7·9	–7·0	3·2	0·0	0	13·9
4	13·2	0·50	100	4·5	1	8·4	117·2	–0·009	0	468·6	8·4	–7·0	111·0	0·0	0	477·9
4	13·2	1·50	50	3·7	1	8·4	6·1	–0·164	0	24·3	8·4	–7·0	5·9	0·0	0	25·2
4	13·2	2·00	200	3·3	1	7·9	1·4	–0·735	0	5·4	7·9	–7·0	1·3	0·0	0	5·4
4	19·0	0·50	50	4·4	1	7·8	78·5	–0·013	97	410·8	7·8	–7·0	75·8	0·0	111	415·0
4	19·0	1·00	100	3·9	1	8·3	2·8	–0·358	0	11·2	8·3	–7·0	2·7	0·0	0	11·5
4	19·0	1·00	200	3·9	1	7·9	1·2	–0·871	0	4·6	7·9	–7·0	1·1	0·0	0	4·6
4	19·0	1·50	0	3·7	1	8·2	6·5	–0·154	0	26·1	8·2	–7·0	6·2	0·0	0	26·8
4	19·0	1·50	200	3·7	1	7·9	1·2	–0·871	0	4·6	7·9	–7·0	0·8	0·0	0	3·3
4	19·0	2·00	0	3·3	1	8·1	5·4	–0·186	0	21·5	8·1	–7·0	5·2	0·0	0	22·5
4	19·0	2·00	200	3·3	1	8·2	2·8	–0·356	0	11·2	8·2	–7·0	2·7	0·0	0	11·6

T, temperature (°C); S, sodium chloride (%); L, lactic acid (%); N, sodium nitrite (μg ml^{-1}); n, number of replicates; t_L, lag period prior to inactivation (h); D, D value (90% decline) (h); t_{4D}, time to 4–D (99·99%) inactivation (h); F, fraction of initial population in major group; Y_o, initial population density [$\log_{10}$(cfu ml^{-1})]; M, slope of inactivation curve.

using Eqns 3 and 4 for the linear and non-linear models, respectively.

$$t_{4D} = t_L + (4 \times D) \quad (3)$$

$$t_{4D} = \frac{LN[((1 + e^{-b_1 t_L})/0{\cdot}0001) - 1] + b_1 t_L}{b_1} \quad (4)$$

As per the rationale provided with the aerobic model (Buchanan et al. 1994), only the D_1 value was considered in calculating t_{4D} values for the non-linear primary model.

Secondary models

Response surface analysis was used to generate secondary models of t_{4D} values on the independent variables (SAS Institute Inc. 1989) after a natural logarithmic transformation of the t_{4D} data was used to stabilize the variance. Four-variable models on temperature/sodium chloride/lactic acid/sodium nitrite and temperature/sodium chloride/pH/sodium nitrite were generated using both quadratic and cubic polynomials. Separate models were generated for the datasets based on linear and non-linear primary models. The analyses were also submitted to backward stepwise regression analysis. A probability of $< 0{\cdot}1$ was used as the retention criterion for that analysis.

Results and Discussion

A total of 206 inactivation curves were generated, representing 157 different combinations of the four independent variables (Table 1). The types of inactivation curves encountered were similar to those previously described for aerobic cultures (Buchanan et al. 1994). This included some of the milder variable combinations supporting an initial growth of *L. monocytogenes* as indicated by a two- to 10-fold increase in population density prior to

Table 2. Cubic response surface equations for the effects and interactions of temperature (T) × sodium chloride (S) × sodium nitrite (N) × lactic acid (L) and temperature × sodium chloride × sodium nitrite × pH (P) on the natural logarithm of the t_{4D} for *Listeria monocytogenes*

(2a) T × S × N × L, Linear primary model

$LN(T_{4D}) =$ 8·0065 + 0.0661T − 0·0157S − 0·4165L − 0·02N + 0·0103TS − 0·0902TL + 0·000235TN − 0·1722SL + 0·000705SN − 0·0341LN − 0·00702T^2 − 0·00323S^2 + 0·1939L^2 + 0·0000596N^2 − 0·00215TSL − 0·00000205TSN + 0·0000411TLN + 0·000509SLN − 0·0000555TS^2 − 0·000000488TN^2 + 0·0243TL^2 − 0·000157T^2S + 0·00000227T^2N + 0·00171T^2L + 0·0258SL^2 − 0·00000451SN^2 + 0·00417S^2L − 0·00000101S^2N + 0·0000404LN^2 + 0·00772L^2N + 0·000071T^3 + 0·0000184S^3 − 0·2595L^3 + 0·000000145N^3

(2b) T × S × N × P, Linear primary model

$LN(T_{4D}) =$ 12·4609 + 0·1821T − 0·6281S − 8·7146P − 0·0805N + 0·000315TS − 0·0756TP + 0·000413TN + 0·1951SP + 0·00188SN + 0·00578PN − 0·00207T^2 + 0·00336S^2 + 2·7168P^2 + 0·000276N^2 + 0·00139TSP + 0·00000468TSN − 0·0000668TPN − 0·000237SPN − 0·0000519TS^2 − 0·00000105TN^2 + 0·00779TP^2 − 0·000115T^2S + 0·00000599T^2N − 0·000603T^2P − 0·0174SP^2 − 0·00000332SN^2 − 0·000666S^2P + 0·00000106S^2N − 0·0000213PN^2 + 0·000499P^2N + 0·0000541T^3 + 0·0000302S^3 − 0·2168P^3 − 0·00000014N^3

(2c) T × S × N × L, Non–linear primary model

$LN(T_{4D}) =$ 7·2905 + 0·0277T − 0·0711S + 4·1022L − 0·0233N + 0·0121TS − 0·1275TL + 0·000443TN − 0·3833SL + 0·000655SN − 0·0265LN − 0·00606T^2 + 0·0162S^2 − 3·2198L^2 − 0·0000331N^2 − 0·00225TSL − 0·0000102TSN + 0·0000366TLN + 0·000464SLN − 0·0000904TS^2 − 0·000000436TN^2 + 0·0447TL^2 − 0·000176T^2S + 0·000000965T^2N + 0·00163T^2L + 0·1004SL^2 − 0·00000342SN^2 + 0·00629S^2L + 0·00000209S^2N + 0·0000226LN^2 + 0·00656L^2N + 0·0000689T^3 − 0·00072S^3 + 0·4133L^3 + 0·000000545N^3

(2d) T × S × N × P, Non linear primary model:

$LN(T_{4D}) =$ 13·0056 + 0·2783T − 0·7439S − 10·5912P − 0·0577N + 0·00362TS − 0·1414TP + 0·000634TN + 0·0354SP + 0·00157SN + 0·000241PN − 0·0013T^2 + 0.0536S^2 + 3·6283P^2 + 0·000115N^2 + 0·00039TSP − 0·00000104TSN − 0·0000155TPN − 0·000135SPN − 0·0000464TS^2 − 0·00000173TN^2 + 0·01489TP^2 − 0·000101T^2S + 0·00000179T^2N − 0·000482T^2P + 0·0202SP^2 − 0·00000302SN^2 − 0·00779S^2P + 0·00000094S^2N + 0·00000411PN^2 + 0·000229P^2N + 0·0000463T^3 − 0·00076S^3 − 0·3246P^3 + 0·00000016N^3

T = 4–42°C; S = 0·5–19·0% (w/v, g L^{-1} ÷ 10); N = 0–200 μg ml^{-1}; L = 0·0–2·0%; P = 3·3–7·3.

inactivation. As before, in these instances the Y_o value was fixed and the period of growth was included as part of the t_L value. The D values and t_{4D} values based on the linear and non-linear primary models were similar.

Qualitatively, the results were similar to those previously observed with aerobic cultures (Buchanan et al. 1994). The t_{4D} values were inversely related to the lactic acid concentration, incubation temperature, and sodium chloride content. The effect of sodium nitrite was pH (lactic acid) dependent.

Quadratic (not shown) and cubic (Table 2) response surface models were generated for the natural logarithm (LN) transformed t_{4D} data for both linear and non-linear primary model datasets. Separate sets of models were generated on lactic acid/temperature/sodium chloride/sodium nitrite and pH/temperature/sodium chloride/sodium nitrite. Based on a comparison of R^2 values, the cubic model enhanced slightly the fit between the models and the observed data (Table 3); however, the extent of the improvement was not as large as previously observed with aerobic cultures (Buchanan et al. 1994). The R^2 values also indicated a slightly better fit using the t_{4D} values from the linear primary model. Evaluation of scatter diagrams comparing predicted vs observed values for the four cubic models, indicated that models provided reasonable fits with the experimental data, particularly considering the rather variable nature of non-thermal inactivation and the broad range of the t_{4D} values. The scatter diagram for the P × T × S × N model (model 2b, Table 2) based on t_{4D} values derived from the linear primary model is provided as an example (Fig. 1). Confidence intervals for the predictions (not shown) were estimated by solving a matrix of the variances of the individual terms. This matrix is available upon request.

The cubic models were submitted to backward stepwise regression as a means of simplifying the equations (Table 4). This procedure eliminated approximately half of the equations' terms, while having a minimal impact on the observed R^2 values (Table 3). Again, the scatter diagram for the P × T × S × N model based on t_{4D} values from the linear primary (model 4b, Table 4) is provided as an example

Table 3. R^2 values for the four-variable response surface models generated from the non-thermal inactivation datasets

Full regression	Quadratic model	Cubic model
Linear dataset		
T/S/N/L	0·879	0·907
T/S/N/P	0·889	0·909
Non-linear dataset		
T/S/N/L	0·863	0·899
T/S/N/P	0·867	0·895
Backward regression	**Quadratic model**	**Cubic model**
Linear dataset		
T/S/N/L	0·878	0·901
T/S/N/P	0·886	0·901
Non-linear dataset		
T/S/N/L	0·859	0·894
T/S/N/P	0·866	0·889

T, temperature (°C); S, sodium chloride (%); L, lactic acid (%); N, sodium nitrite (μg ml^{-1}); P, pH.

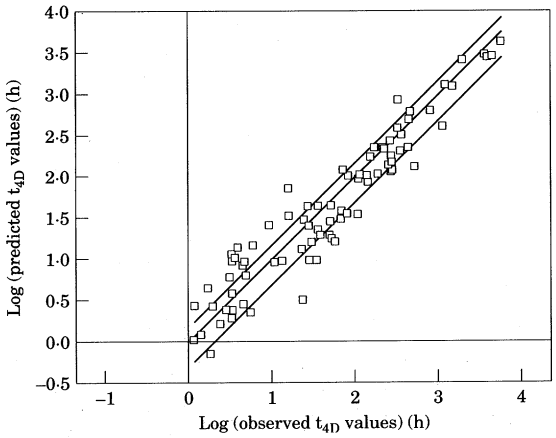

Figure 1. Comparison of predicted vs observed t_{4D} values for the full cubic P × T × S × N model of the non-thermal inactivation of *Listeria monocytogenes* under oxygen restricted conditions. This model (model 2b) is based on the t_{4D} values derived from the linear primary model. The centre line is the 'line of identity' and the others represent ± 50% of the observed value.

(Fig. 2). Comparison of the t_{4D} values predicted by the full and backward regression models for the variable combinations examined experimentally indicated strong agreement between the

Table 4. Response surface models generated by submitting cubic models (Table 2) of the natural logarithm of the t_{4D} for *Listeria monocytogenes* to backward regression analysis

(4a) T × S × N × L, Linear primary model

$LN(T_{4D}) = 7{\cdot}8753 - 0{\cdot}0138N + 0{\cdot}00553TS - 0{\cdot}1135TL + 0{\cdot}000246TN - 0{\cdot}0984SL - 0{\cdot}0392LN - 0{\cdot}00191T^2 + 0{\cdot}000073N^2 + 0{\cdot}000728SLN + 0{\cdot}0314TL^2 - 0{\cdot}000128T^2S + 0{\cdot}00138T^2L - 0{\cdot}00000275SN^2 + 0{\cdot}0000453LN^2 + 0{\cdot}00897L^2N - 0{\cdot}1938L^3$

(4b) T × S × N × P, Linear primary model

$LN(T_{4D}) = -0{\cdot}2139 - 0{\cdot}6544S - 0{\cdot}0456N - 0{\cdot}0162TP + 0{\cdot}000231TN + 0{\cdot}2285SP + 0{\cdot}00145SN + 0{\cdot}7822P^2 + 0{\cdot}0000905N^2 - 0{\cdot}000264SPN + 0{\cdot}00519TP^2 - 0{\cdot}000946T^2P - 0{\cdot}0197SP^2 + 0{\cdot}000516P^2N + 0{\cdot}0000335T^3 - 0{\cdot}0856P^3$

(4c) T × S × N × L, Non–linear primary model

$LN(T_{4D}) = 7{\cdot}1345 + 2{\cdot}7473L - 0{\cdot}0202N + 0{\cdot}00406TS - 0{\cdot}1316TL + 0{\cdot}000289TN - 0{\cdot}3497SL - 0{\cdot}0245LN - 0{\cdot}00183T^2 + 0{\cdot}0169S^2 - 1{\cdot}6725L^2 + 0{\cdot}00061SLN + 0{\cdot}0408TL^2 - 0{\cdot}000105T^2S + 0{\cdot}00137T^2L + 0{\cdot}0769SL^2 - 0{\cdot}0000019SN^2 + 0{\cdot}00476S^2L + 0{\cdot}00716L^2N - 0{\cdot}000743S^3 + 0{\cdot}00000042N^3$

(4d) T × S × N × P, Non–linear primary model

$LN(T_{4D}) = -3{\cdot}8146 + 0{\cdot}2401T - 0{\cdot}6625S - 0{\cdot}0447N - 0{\cdot}1236TP + 0{\cdot}000289TN + 0{\cdot}000398SN + 0{\cdot}0606S^2 + 1{\cdot}4449P^2 + 0{\cdot}0126TP^2 - 0{\cdot}00029T^2P + 0{\cdot}0247SP^2 - 0{\cdot}00828S^2P + 0{\cdot}000124P^2N - 0{\cdot}000955S^3 - 0{\cdot}1818P^3$

T = 4–42°C; S = 0·5–19·0% (w/v, g $l^{-1} \div 10$); N = 0–200 μg ml^{-1}; L = 0·0–2·0%; P = 3·3–7·3.

results from the two models ($R^2 = 0{\cdot}95$) (Fig. 3). While a number of the cross-product terms were eliminated by the backward regression, retention of a number of these terms indicated significant two and three way interactions among the variables.

While all of the eight equations adequately described the experimental data, it appears that either the full cubic (model 2b, Table 2) or backward regressed (model 4b, Table 4) equations for T × S × P × N gave the overall best fits and would be the most useful for working with

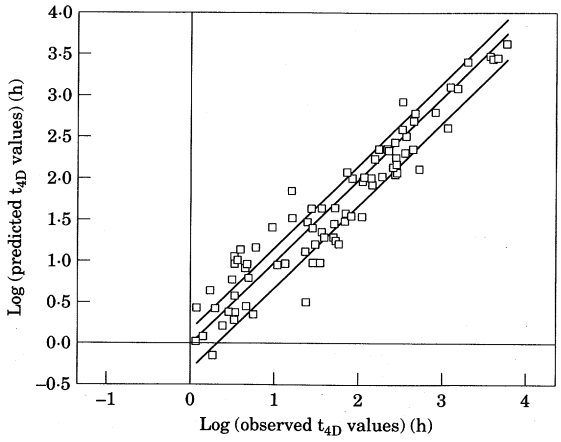

Figure 2. Comparison of predicted vs observed t_{4D} values for the cubic backward stepwise regression P × T × S × N model of the non-thermal inactivation of *Listeria monocytogenes* under oxygen restricted conditions. This model (model 4b) is based on the t_{4D} values derived from the linear primary model. The centre line is the 'line of identity' and the others represent ± 50% of the observed value.

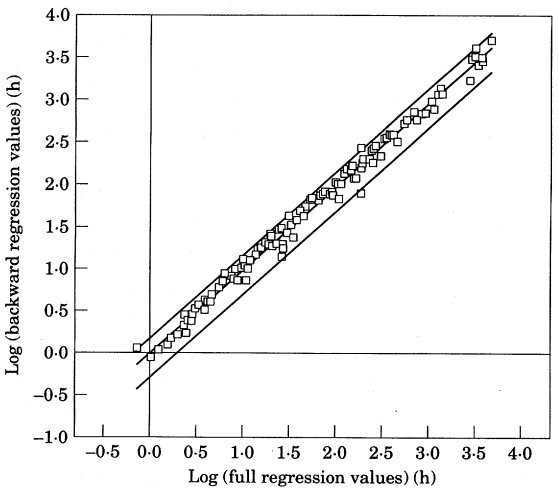

Figure 3. Comparison ($R^2 = 0{\cdot}95$) of the t_{4D} values predicted by the cubic P × T × S × N models based on a full regression (model 2b) and a subsequent stepwise backward regression. The centre line is the 'line of identity' and the others represent ± 50% of the value predicted by full regression model.

food systems. Intuitively, the use of the backwards regressed model is attractive because it is simpler and has been reduced to only statistically significant terms. However, it is important to note that, regardless of statistical significance, both equations are empirical, and computer-assisted applications are not hampered by the number of terms in the equations. While the concentration of the organic acid has distinct impact on inactivation rates due to anion effects, pH seems to be the more important determinant at concentrations that are likely to be used in foods (Buchanan et al. 1993, Buchanan and Golden 1994). Further, the often substantial buffering capacity of food systems and the relative ease of measuring pH suggest that the pH-based models would have greater applicability in relation to predicting the behaviour of *L. monocytogenes* in foods. However, the usefulness and potential accuracy of models for predicting non-thermal inactivation in foods would be enhanced by considering both variables, and experimentation is underway to differentiate the effects of pH and lactate concentration for both the aerobic and anaerobic datasets.

Sodium nitrite can have a significant impact on the growth and survival of a number of Gram-positive foodborne pathogens including *L. monocytogenes* (Shahamat et al. 1980, Buchanan et al. 1989, 1994). The curing agent accelerated the inactivation of *L. monocytogenes* under conditions of limited oxygen availability (Table 1), with the activity being dependent on the pH of the system. It had substantial activity under acidic conditions, but had little or no activity at pH values $\geq$ 6·5. Restricting oxygen levels has been reported to increase the bacteriostatic activity of sodium nitrite for *L. monocytogenes* (Buchanan et al. 1989) and *Staphylococcus aureus* (Buchanan and Solberg 1972, Smith and Palumbo 1980, Fang et al. 1985). However, comparison of t_{4D} values predicted by the current anaerobic model (model 2b) with values calculated using the equivalent aerobic model (Buchanan et al. 1994) indicated that the enhanced non-thermal inactivation of *L. monocytogenes* achieved by the introduction of sodium nitrite was not affected by oxygen availability (Fig. 4). It is possible that the nitrogen-flushed, sealed-flask system used to generate

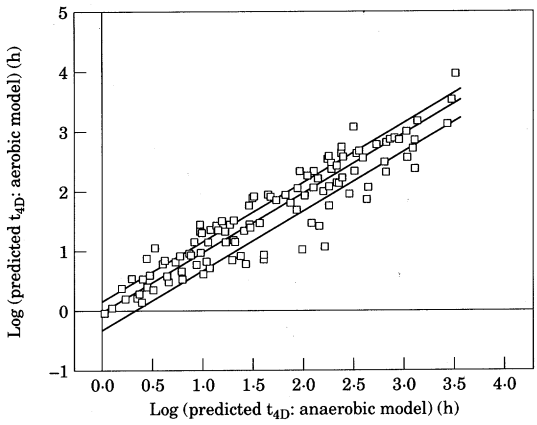

Figure 4. Comparison of the t_{4D} values predicted for sodium nitrite-containing cultures by anaerobic (current study) and aerobic (Buchanan et al. 1994). Both models were the full cubic response surface equations derived from the experimental data using the linear primary model. The centre line is the 'line of identity' and the others represent ± 50% of the anaerobic value.

an oxygen-limited environment was not sufficiently anaerobic to demonstrate an effect. However, this is unlikely since a similar system was used to demonstrate that anaerobiosis did enhance the inhibition of *L. monocytogenes* growth (Buchanan et al. 1989, Buchanan and Phillips 1990).

Comparison of the predicted inactivation times against the limited quantitative data available on the non-thermal inactivation of *L. monocytogenes* in foods (Buchanan et al. 1994) suggest that the models should provide a rapid means of acquiring initial estimates of the pathogen's survival. However, as with any model generated in microbiological medium, effective validation of both the aerobic and anaerobic models will require additional quantitative data on the non-thermal inactivation of *L. monocytogenes* in a variety of acidified or fermented foods.

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