ELSEVIER

International Journal of
Food Microbiology 32 (1996) 73–90

International Journal of Food Microbiology

Predictive models of the effect of temperature, pH and acetic and lactic acids on the growth of *Listeria monocytogenes*

Susan M. George*, Laura C.C. Richardson, Michael W. Peck

Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney Lane, Norwich, NR4 7UA, UK

Received 31 October 1995; revised 20 February 1996; accepted 22 March 1996

Abstract

The combined effect of temperature (1–20°C), pH (4.5–7.2) and acetic acid (0–10 000 mg/l; model 1) or lactic acid (0–20 000 mg/l; model 2) on growth of *Listeria monocytogenes* in laboratory media was studied. Growth curves at various combinations of temperature, pH and acid concentration were fitted by the model of Baranyi and Roberts (1994), and specific growth rates derived from the curve fit were modelled. Predictions of growth from the models were compared with data in the literature, and this showed the models to be suitable for use in predicting growth of *L. monocytogenes* in a range of foods including meat, poultry, fish, egg and milk and dairy products. The two models are compatible, i.e. they give similar predictions for cases when no acid is present.

Keywords: Listeria monocytogenes; Predictive modelling; Food safety; Acetic acid; Lactic acid; pH; Temperature

* Corresponding author. Tel: +44 1603 255000; fax: +44 1603 507723.

0168-1605/96/$15.00 © 1996 Elsevier Science B.V. All rights reserved
PII S0168-1605(96)01108-7

1. Introduction

Listeria monocytogenes is well established as a foodborne pathogen that causes listeriosis, a disease with a high mortality rate. It is tolerant of acidic conditions and low temperature, and is of particular concern in minimally processed foods that rely on refrigeration for preservation. Foods implicated in listeriosis outbreaks include coleslaw (Schlech et al., 1983), milk (Fleming et al., 1985) soft cheeses (James et al., 1985; Bille and Glauser, 1988) and most recently pork tongues in aspic (Jacquet et al., 1995), pork rillettes (Anonymous, 1993) and brie cheese (Goulet et al., 1995).

Much has been published on the limiting temperature and pH for growth of *L. monocytogenes* (Conner et al., 1986; George et al., 1988; Parish and Higgins, 1989; Farber et al., 1989; Sorrells et al., 1989), and some researchers have studied the effect of either lactic and acetic acids in combination with temperature and pH (Ahamad and Marth, 1989; Farber et al., 1989; Sorrells et al., 1989; Sorrells and Enigl, 1990; Conner et al., 1990; Young and Foegeding, 1993). In these studies, either acetic or lactic acid was used to adjust the pH but the quantity was not measured, or a measured amount of acid was added but the resultant pH was not recorded. Other researchers have used lactic and acetic acids or their salts combined with other preservatives to enhance their inhibitory effect (El-Shenawy and Marth, 1991, 1992; Chen and Shelef, 1992; Schlyter et al., 1993; Oh and Marshall, 1994; Pelroy et al., 1994).

Mathematical modelling has been used to describe growth responses of microorganisms to combinations of preservative factors (McMeekin et al., 1993; McClure et al., 1994). Modelling provides information on interactions between two or more factors and allows interpolation in the region of observations. These models can be used to predict growth of microorganisms in foods, and this may reduce the amount of challenge testing needed to ensure food safety. Models have been published for *L. monocytogenes* describing the effects of temperature alone (Duh and Schaffner, 1993), temperature in combination with pH and NaCl or a_w (Cole et al., 1990; Wijtzes et al., 1993), and pH, NaCl, nitrite, and gaseous atmosphere (Buchanan et al., 1989; Buchanan and Phillips, 1990). All models were developed using laboratory media. Duffy et al. (1994) modelled the combined effects of nitrite with ascorbate on growth in meats, and Guerzoni et al. (1994) modelled growth of *L. monocytogenes* in dairy products and model food systems. The models of Duh and Schaffner (1993), Wijtzes et al. (1993) and Buchanan and Phillips (1990) were shown to provide a valid prediction of growth in foods and other laboratory media. However, predictive models have not been constructed describing the effect of acetic or lactic acids on growth of *L. monocytogenes*, although Houtsma et al. (1994) modelled the effect of sodium lactate and sodium chloride on growth of the non-pathogenic *Listeria innocua*.

The aim of this study was to determine the effect of temperature and pH combined with either lactic acid or acetic acid on the growth of *L. monocytogenes*, and to construct two kinetic growth models. Growth was in laboratory media so that conditions other than those under test were optimal. Acids were added independently of pH, and the concentration of undissociated acid was used for

modelling purposes. It was also determined whether predictions from the models provided a good description of the growth of *L. monocytogenes* in foods.

2. Materials and methods

2.1. Strains

In preliminary experiments a total of 60 strains of *L. monocytogenes* were screened for tolerance of lactic and acetic acids. These included clinical isolates and isolates from foods implicated in listeriosis outbreaks and covering a range of serotypes (1a, 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4c, 4d, 4e and 7). A single strain, *L. monocytogenes* F6861, serotype 4b was selected because of its tolerance of both acetic and lactic acids and was used for all growth experiments. This strain was obtained from Dr. R. Weaver, CDC, Atlanta, USA, and had been isolated from Mexican-style cheese involved in a listeriosis outbreak in the USA in 1985 (James et al., 1985).

2.2. Media

Tryptone soya broth (Oxoid CM129) with 3 g/l yeast extract (Oxoid) and 10 g/l additional glucose (TSYGB) was used for optimal growth (George and Lund, 1992). Batches were made up to 80% volume, and 10 or 20% organic acid stock solution was added to give up to 10 000 mg/l final concentration of acetic acid and up to 20 000 mg/l final concentration of lactic acid. The pH was adjusted using HCl or KOH before making up to final volume. The pH was adjusted independently of the amount of organic acid added and the media were sterilized by filtration to prevent pH changes. Sterile media (100 ml volume) were transferred to sterile 250-ml conical flasks. This gave a large surface area and small depth of medium with a large headspace. Flasks of media were equilibrated at the intended incubation temperature prior to inoculation.

2.3. Inocula

Cultures for inocula were preconditioned at the temperature at which experiments were carried out. Overnight cultures (20 h at 30°C) in tryptose phosphate broth (TPB; Difco Laboratories Ltd.) were used to inoculate TPB preincubated at the appropriate temperature. Inocula for experiments at 20°C were incubated at 30°C for 20 h, for 12°C experiments at 12°C for 4 days, and for 8°C, 4°C and 1°C experiments, at 8°C for 6 days. Inoculum cultures were diluted in peptone salt dilution fluid (PSDF) (ICMSF, 1978) to give an initial count of 10^2–10^3 cells/ml after inoculation.

2.4. Experimental procedure

Test broths equilibrated to temperature were inoculated, mixed, and samples removed to determine the initial inoculum concentration and pH. Test flasks were incubated in shaking waterbaths (80–100 strokes/min) (thermoregulator, TE-8J, in shaking waterbath, SB-16, Techne, Cambridge, Ltd.) to maintain temperature and increase oxygenation. The temperature was monitored throughout the experiment and the pH checked frequently to ensure that no change occurred before growth commenced. Samples were taken at appropriate intervals to determine viable counts by dilution in PSDF and plating 100-μl volumes onto triplicate plates of tryptose agar (TA; Difco Laboratories Ltd.). Plates were incubated at 35°C for 48 h. The mean of three plate counts was used to calculate the number of colony forming units/ml. Up to 35 samples were taken per growth curve, although for most curves between 10 and 20 samples were taken.

2.5. Experimental design

Combinations of conditions appropriate to a wide range of foods were selected to define boundaries for growth experiments. A total of 96 and 142 combinations of conditions were tested for models 1 (acetic) and 2 (lactic), respectively. The following range of conditions was tested:

acetic acid experiments; temperature 4–20°C, pH 4.5–7.0, total acetic acid 0–10 000 mg/l.

lactic acid experiments; temperature 1–20°C, pH 4.3–7.2, total lactic acid 0–20 000 mg/l.

2.6. Construction of the models

Figs. 1 and 2 show the combinations of pH and acid at each temperature. At some conditions more than one growth curve was made. In order to generate models covering as wide a range of temperature and pH as possible, conditions were selected that were limiting for growth. Model 1 (acetic) was constructed from 119 curves, and model 2 (lactic) from 161 curves.

2.7. Modelling

The first stage was to fit a sigmoid function to the growth data. The method used was that described by Baranyi and Roberts (1994) and derives μ, maximum specific growth rate, and h_0, the product of μ and lag time. In the second stage, a quadratic polynomial of the controlling factors was fitted to the derived parameter μ. This lead to a multivariate linear regression of the form:

$$\ln\mu = c_1 + c_2T + c_3P + c_4U + c_5T \times P + c_6T \times U + c_7P \times U + c_8T^2 + c_9P^2 + c_{10}U^2$$

where $\ln\mu$ is the natural logarithm (ln) of the specific growth rate (μ) derived from the first stage, T, P and U denote temperature (°C), pH and undissociated acid (mg/l) and $c_1 - c_{10}$ are the coefficients to be estimated. The pK_a of acetic and lactic acid were calculated from Robinson and Stokes (1959). The natural logarithm, as a link function, was used to damp the variance of μ (McClure et al., 1993). This modelled maximum specific growth rate, μ, was used to predict the doubling time: $t_D = \ln 2/\mu$, and the time to 10^3 increase: $t_{1000} = (\ln 10^3 + h_0)/\mu$. For the latter quantity, h_0 was taken as constant (Baranyi and Roberts, 1994).

In order to determine whether predictions from the models provided a valid description of the growth of *L. monocytogenes* in foods, predictions were compared with doubling times and time to a 10^3 increase reported in the literature. Where times were not quoted, the doubling time was calculated from the maximum slope of published growth curves and time to 10^3 increase was measured from the plots. These comparisons showed for which food products predictions compare well with observations of growth in the food.

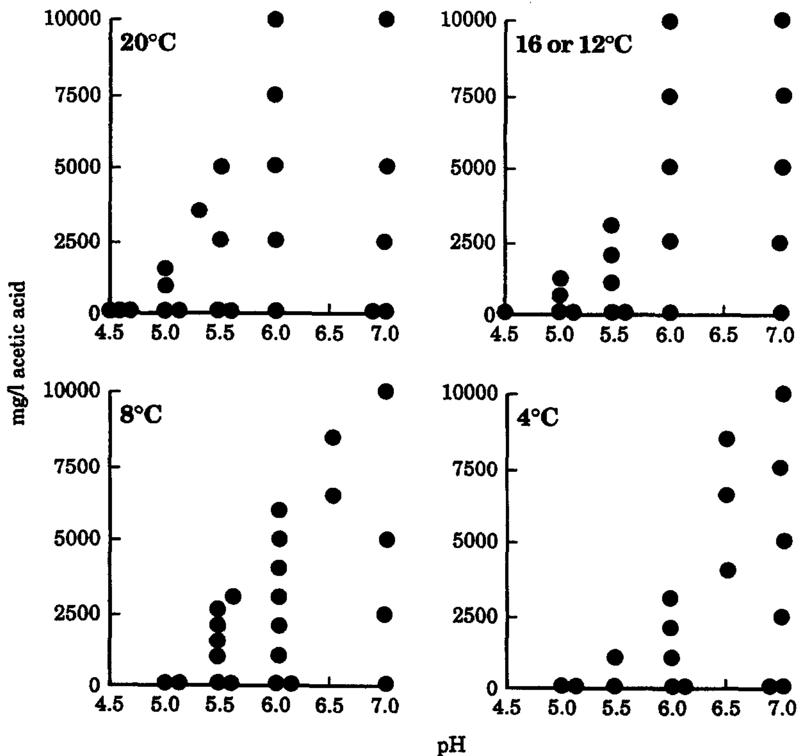


Fig. 1. Model 1 (acetic) experimental plan showing combinations of temperature, pH and acetic acid concentration tested.

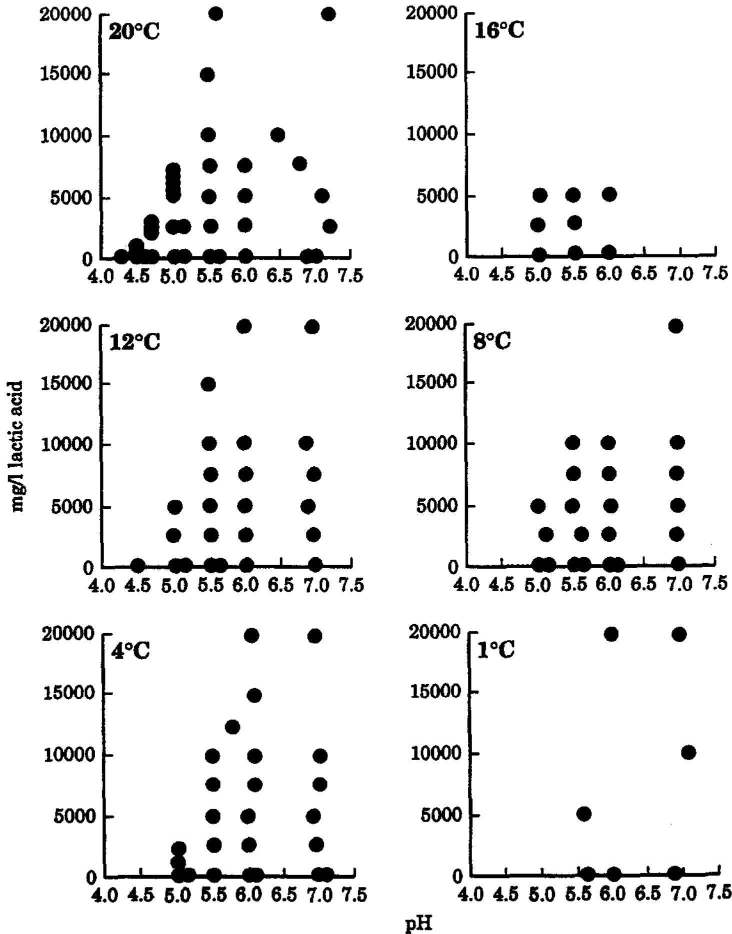


Fig. 2. Model 2 (lactic) experimental plan showing combinations of temperature, pH and lactic acid concentration tested.

3. Results

The minimum pH for detected growth of *L. monocytogenes* in 50 days at 20°C was 4.3 when the pH was adjusted with HCl. Growth was not observed in 50 days at pH 4.0. At 12°, 8° and 4°C growth was observed at pH 5.0 but not at pH 4.5. The minimum pH for growth at 1°C was 5.4, with growth not observed at pH 5.0 in 50 days. Acetic acid had a greater effect on growth than lactic acid at the same pH, when total weight of acid was compared. In the presence of 2500 mg/l lactic acid at pH 5.0 at 20°C the doubling time was 2.3 h, but at the same temperature and pH with 2500 mg/l acetic acid no growth was detected in 50 days. However, growth was observed at pH 5.5, 20°C, in the presence of 2500 mg/l acetic acid with a doubling time of 5.4 h.

The first stage of modelling was to fit growth curves to bacterial counts using the method of Baranyi and Roberts (1994). The curves fitted the data well for both the acetic acid and the lactic acid data sets; examples are shown in Fig. 3. The second stage of modelling, describing the variation of the specific growth rate derived in stage 1, showed a good fit of both models; the R^2 statistics were 97.0% for model 1 and 94.6% for model 2. The residual mean square error (RMSE) for $\ln\mu$, for model 1 was 0.20 and for model 2 was 0.24. The smaller the figure for the RMSE the closer the fit to the actual data (Box and Draper, 1987). There was good agreement between experimental (derived in stage 1) and the fitted (derived from multivariate linear regression) specific growth rates for both models (Table 1).

To compare the two models, predictions of doubling times were made for the same conditions of pH and temperature from each model (Fig. 4). The points all fall on or very close to the line of unity, demonstrating that the models are compatible and either can be used for making predictions for conditions where there is no acid present.

4. Discussion

Petran and Zottola (1989) reported the minimum pH for growth of the Scott A strain of *L. monocytogenes* at 30°C as pH 4.7 and did not observe growth at pH 4.5, and Sorrells et al. (1989) reported a minimum pH of 4.4 for growth at 10°C of four

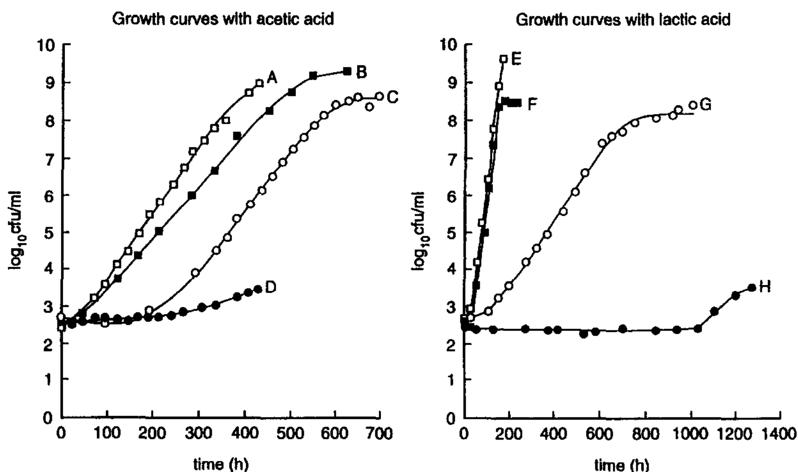


Fig. 3. Some typical fitted growth curves with actual counts of *Listeria monocytogenes*. Growth conditions were as follows: (A) 8°C, pH 6.0, 2000 mg/l acetic acid (calculated 110 mg/l undissociated); (B) 4°C, pH 7.0, 2500 mg/l acetic acid (14.7 mg/l undiss.); (C) 20°C, pH 5.3, 3500 mg/l acetic acid (778 mg/l undiss.); (D) 12°C, pH 5.5, 3000 mg/l acetic acid (463 mg/l undiss.); (E) 12°C, pH 5.5, 10 000 mg/l lactic acid (226 mg/l undiss.); (F) 20°C, pH 4.7, 2000 mg/l lactic acid (252 mg/l undiss.); (G) 4°C, pH 6.1, 20 000 mg/l lactic acid (118 mg/l unidss.); (H) 8°C, pH 5.0, 5000 mg/l lactic acid (345 mg/l undiss.). Points are actual counts and growth curves were fitted using the model of Baranyi and Roberts (1994).

Table 1
Comparison of measured specific growth rates (μ) with specific growth rates fitted by models for *Listeria monocytogenes*

Model 1						Model 2					
Temp(°C)	pH	Acetic acid		Specific growth rate (h^{-1})		Temp(°C)	pH	Lactic acid		Specific growth rate (h^{-1})	
		Total (mg/l)	Undiss.[c] (mg/l)	Measured[a]	Fitted[b]			Total (mg/l)	Undiss. (mg/l)	Measured	Fitted
20	4.5	0	0	0.140	0.145	20	4.7	2000	252	0.127	0.126
20	7.0	10000	57	0.316	0.329	20	7.2	20000	9.2	0.451	0.364
16	5.0	0	0	0.169	0.170	16	5.5	2500	5.6	0.234	0.239
12	6.0	5000	272	0.040	0.043	12	5.0	5000	341	0.066	0.066
8	5.5	2000	311	0.011	0.013	8	7.0	7500	5.55	0.090	0.082
4	6.5	6500	120	0.015	0.016	4	6.1	20000	118	0.021	0.030
						1	5.7	10000	150	0.009	0.011

[a]Specific growth rate (μ) generated by curve fitting in first stage of modelling.
[b]Specific growth rate (μ) fitted by model.
[c]Undissociated acid (calculated).

strains when pH was adjusted with HCl. Other studies in this laboratory have demonstrated growth of the Scott A strain at pH 4.3 (Lund, 1993) and weak growth of strain 88/1, a cheese isolate, at pH 4.2 (George and Lund, unpublished data). In both cases the pH was adjusted with HCl and the incubation temperature was 20°C. Acetic acid appeared more inhibitory to growth of *L. monocytogenes* than lactic acid in terms of total acid added as weight per volume. This observation agrees with previous findings that acetic acid increased the minimum pH for growth more than lactic acid (Farber et al., 1989; Sorrells et al., 1989). A similar conclusion was reached in studies of the relative effect of equimolar concentrations of acetic and lactic acids (Young and Foegeding, 1993). The greater effectiveness of acetic acid is partly a reflection of its lower pK_a, giving a greater proportion of acid in the undissociated form. The pK_a of acetic acid at 20°C was calculated as 4.7558, the pK_a of lactic acid as 3.8594 (Robinson and Stokes, 1959). In contrast, at an equivalent concentration of undissociated acid (in terms of weight), lactic acid appeared more inhibitory to growth than acetic acid. For example, at 12°C and pH 5.5, growth was observed in the presence of a calculated concentration of 463 mg/l undissociated (3000 mg/l total) acetic acid, but not in the presence of 452 mg/l undissociated (20 000 mg/l total) lactic acid. This is consistent with observations

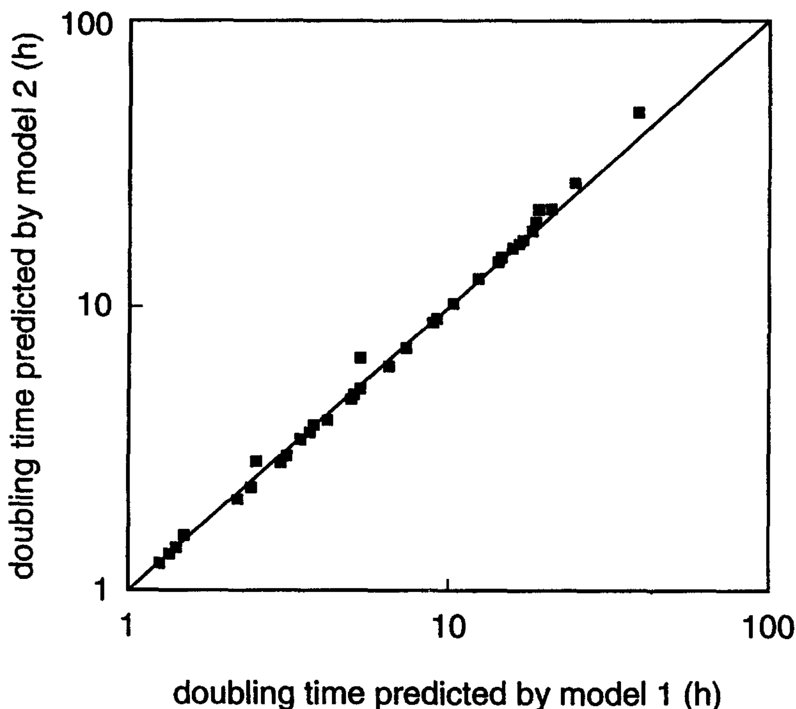


Fig. 4. Comparison of predicted doubling times at the same conditions of temperature (4–20°C) and pH (4.5–7.0) from models describing the effect of acetic acid (model 1) and lactic acid (model 2) on growth of *Listeria monocytogenes*.

Table 2
Comparison of published and predicted growth data for *Listeria monocytogenes*

Ref[a]	Substrate	Temp (°C)	pH	Acetic acid		Doubling time (h)		Time to 10^3 increase (days)		Estimated[b] variables	Strain
				Total (mg/l)	Undiss (mg/l)	Published	Predicted	Published	Predicted		
1	Difco TB	7	5.9	500	38.2	20.8	13	11.7	7.1	pH	V7
1	Difco TB	13	5.9	500	37.6	4.7	4.0	3.1	2.2	pH	CA
2	Difco TB	13	5.6	640	81	7.2	5.8	5	3.2	Acetic acid	V7
3	Chicken slurry	15	5.6	3660	332	27.8	12.8	22	7.0	Acetic acid	Unknown
4	Turkey	4	6.2	850	30.6	72	23.2	30	12.5	Acetic acid	Mixed
5	Frankfurter	5	6.0	10 000	556	198	346	—	—	Acetic acid	Mixed

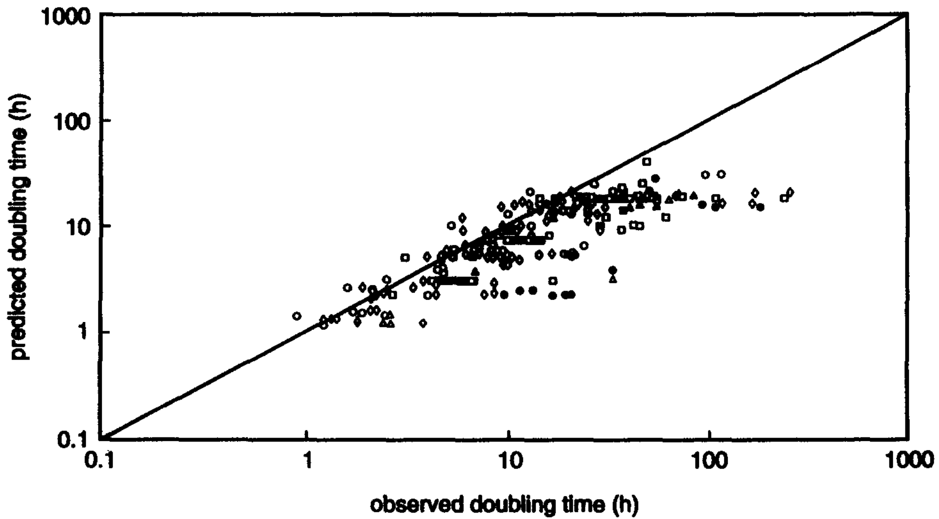
Ref	Substrate	Temp (°C)	pH	Lactic acid		Doubling time (h)		Time to 10^3 increase (days)		Estimated variables	Strain
				Total (mg/l)	Undiss (mg/l)	Published	Predicted	Published	Predicted		
1	Difco TB	7	6.4	500	1.47	16.8	9.0	—	—	pH	CA
1	Difco TB	13	6.4	500	1.45	4.2	2.8	—	—	pH	CA
2	Difco TB	13	5.6	870	15.7	9	3.9	4	2.4	Lactic acid	V7
2	DifcoTB	13	5.0	1220	83	12	7.5	7.5	4.6	Lactic acid	V7
6	Strained beef	20	6.3	16 000	57.8	4.8	1.5	2.2	0.9	Lactic acid	Scott A
7	Frankfurter	4.4	6.0	16 000	120	50	21.6	—	—	Lactic acid	Unknown
8	Crawfish	4	6.0	5000	37.5	18.4	21.7	7	13	pH	Scott A
8	Crawfish	4	6.0	10 000	75	28.1	22.6	12	14	pH	Scott A
9	Beef	10	6.1	12 000	70	32	5.76	—	—	Lactic acid	Scott A

[a]Reference.
[b]Estimated variables: Ref. 1, pH was estimated from the concentration of acid added. Ref. 2, acid concentration was estimated from the measured pH. Refs. 3, 4, 7 and 9, acid concentration was calculated from the concentration of sodium salt added. References: 1, Ahamad and Marth, 1989; 2, El-Shenawy and Marth, 1989, 1991, 1992; 3, Lachica, 1992; 4, Schlyter et al., 1993; 5, Palumbo and Williams, 1994; 6, Hudson and Mott, 1993c; 7, de Koos, 1992; 8, Oh and Marshall, 1994; 9, Stillmunkes et al., 1993.

made by Young and Foegeding (1993) that lactic acid had a greater inhibitory effect on growth rate, when equimolar amounts of undissociated acids were compared at an equal pH.

In all cases the Baranyi model fitted the growth data well; examples are shown in Fig. 3. A comparison of predicted specific growth rates from each model with the specific growth rates generated in the first stage of the model showed good

(a)



(b)

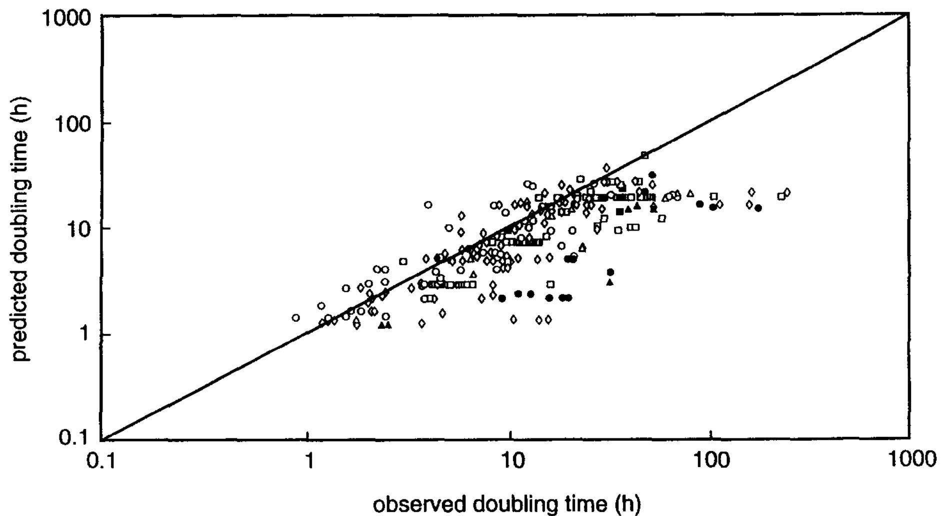


Fig. 5(a,b)

agreement, particularly at lower acid concentrations and a pH of 5.0–6.5 (Table 1). For example, the predicted growth rate at 12°C, pH 5.0 and 5000 mg/l lactic acid was identical to the experimental growth rate for the same conditions. However, the lactic acid model tended to predict slower growth than that observed at pH 7.2. This may have been due to the inclusion in the model of only a few curves at this high pH. The models should not be used to make predictions outside of the range of conditions used in their development.

The validity of these models for predicting growth was investigated by comparing predictions with growth data in foods and laboratory media from published literature. Table 2 gives examples of predicted doubling time and time to 10^3 increase in cell numbers compared with published values. In most cases the models predicted faster growth than reported. Figs. 5 and 6 show double log plots of observed doubling time and time to 10^3 increase, against predicted values from published data. The plots contain 290 acetic and 318 lactic data points from 62 references. The observed and predicted doubling times and times to 10^3 increase appear similar (close to the line of unity). Many predicted doubling times and times to 10^3 increase are shorter than observed which may be due to the presence of controlling factors not included in our models. For example Manu-Tawiah et al. (1993) observed slower than expected growth of *L. monocytogenes* on pork chops and attributed this to competing microflora. Slower growth than expected was also recorded in pork liver sausage (Weaver and Shelef, 1993) and may have been due to the presence of other preservatives such as salt and nitrite. All points of comparison with growth on vegetables fall below the line of unity indicating that growth is considerably slower than predicted by our models, thus making these models of less use for predicting growth in vegetables. However, the models may be used to predict growth of *L. monocytogenes* in meat, poultry, milk, dairy products, fish and eggs.

Fig. 5. Comparison of doubling times from published data with predictions from the models for *Listeria monocytogenes*. (a) Model 1, acetic acid. (b) Model 2, lactic acid. Data are taken from experiments in: meat and poultry (◇); milk and dairy products (□) egg (▲); fish (△); media (○); vegetables (●); other (■). References include those used in Table 2 and the following; Berrang et al., 1989; Beuchat and Brackett, 1990; Buchanan et al., 1989; Buchanan and Klawitter, 1990; Buchanan and Phillips, 1990; Carlin and Nguyen-the, 1994; Chen and Hotchkiss, 1993; Chen and Shelef, 1992; Conner et al., 1990; Denis and Ramet, 1989; Dickson, 1990; Donnelly and Briggs, 1986; Dorsa et al., 1993; El-Gazzar et al., 1991; El-Shenawy and Marth, 1988; Erickson and Jenkins, 1992; Farrag and Marth, 1989; Farrag et al., 1990; Ferguson and Shelef, 1990; Ferreira and Lund, 1992; Gill and Reichel, 1989; Glass and Doyle, 1989; Grant et al., 1993; Grau and Vanderlinde, 1990, 1992, 1993; Huang et al., 1992; Hudson, 1992; Hudson and Mott, 1993a,b,c; Ingham et al., 1990; Jemmi and Keusch, 1992; Leung et al., 1992; Manu-Tawiah et al., 1993; Marshall and Schmidt, 1988; Marshall et al., 1991, 1992; Oh and Marshall, 1993; Papageorgiou and Marth, 1989; Pearson and Marth, 1990; Petran and Zottola, 1989; Qvist, pers comm.; Rosenow and Marth, 1987; Ryser and Marth, 1987; Schillinger et al., 1991; Shelef and Addala, 1994; Sionkowski and Shelef, 1990; Steinbruegge et al., 1988; Walker et al., 1990; Wang and Shelef, 1992; Weaver and Shelef, 1993; Wimpfheimer et al., 1990; Zeitoun and Debevere, 1991.

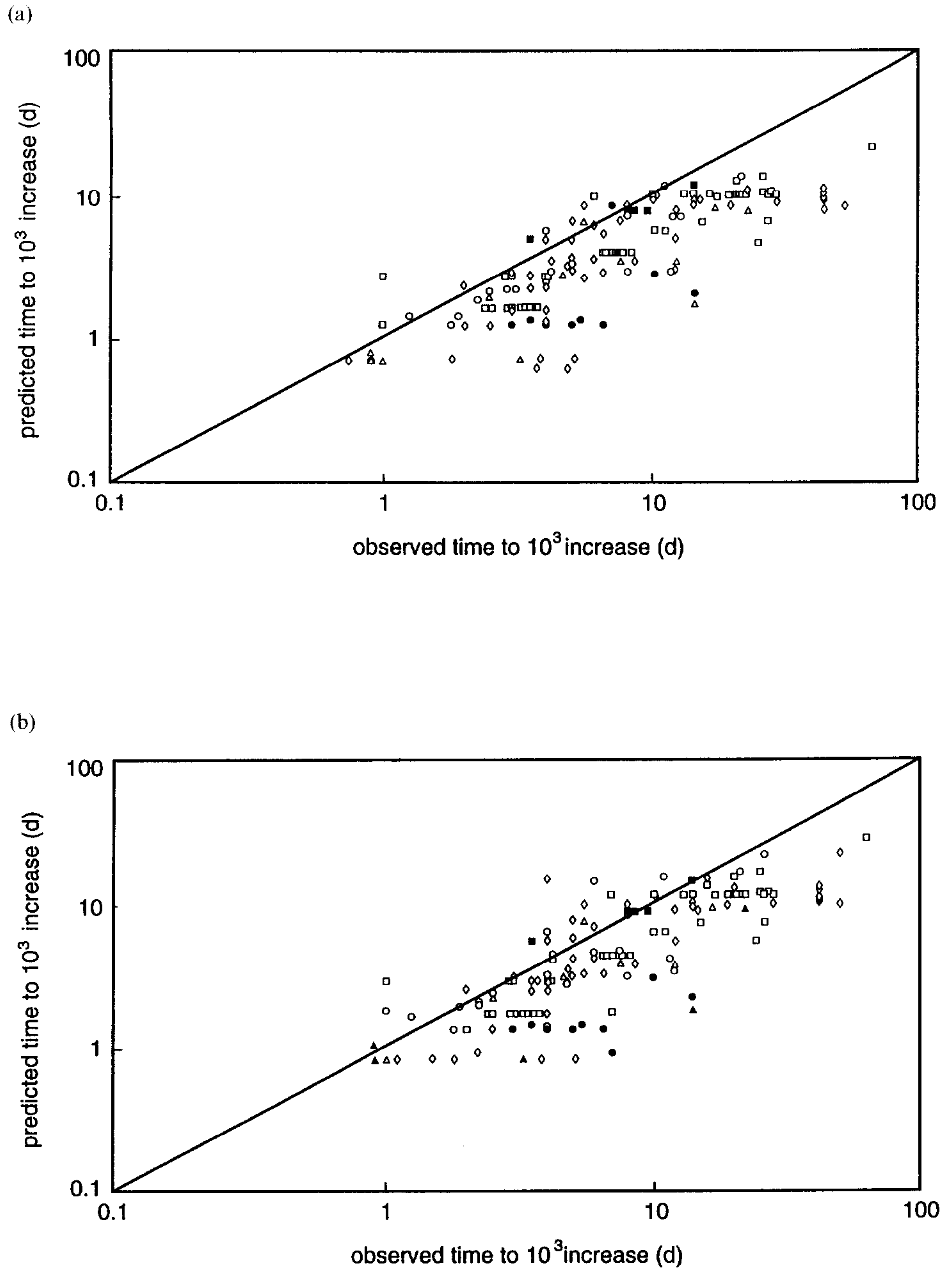


Fig. 6. Comparison of time to 10^3 increase from published data with predictions from the models for *Listeria monocytogenes*. (a) Model 1, acetic acid. (b) Model 2, lactic acid. Symbols and references used as in Fig. 5.

In this article two models have been described that provide good predictions of growth of *L. monocytogenes* in foods containing acetic acid or lactic acid. These two models can be used to predict growth of *L. monocytogenes* in foods in which these acids are present.

Acknowledgements

The authors wish to thank Dr. J. Baranyi for assistance with the modelling and many helpful discussions, and Dr. B.M. Lund for her contribution to the development of this project. This work was funded by the Ministry of Agriculture, Fisheries and Food as part of their programme on the predictive modelling of foodborne pathogens. The aim of this programme was to develop predictive models for inclusion in Food MicroModel. Food MicroModel software for the personal computer was developed by, and is available from, Food MicroModel Ltd., Leatherhead, Surrey, UK.

References

Ahamad, N. and Marth, E.H. (1989) Behaviour of *Listeria monocytogenes* at 7, 13, 21 and 35°C in tryptose broth acidified with acetic, citric, or lactic acid. J. Food Prot. 52, 688–695.

Anonymous (1993) Epidémie de listériose à lysovar 2671-108-312 en France. Bull. Epidémiol. Hebd. 34, 157–158.

Baranyi, J. and Roberts, T.A. (1994) A dynamic approach to predicting bacterial growth in food. Int. J. Food Microbiol. 23, 277–294.

Berrang, M.E., Brackett, R.E. and Beuchat, L.R. (1989) Growth of *Listeria monocytogenes* on fresh vegetables stored under controlled atmosphere. J. Food Prot. 52, 702–705.

Beuchat, L.R. and Brackett, R.E. (1990) Survival and growth of *Listeria monocytogenes* on lettuce as influenced by shredding, chlorine treatment, modified atmosphere packaging and temperature. J. Food Sci. 55, 755–758, 870.

Bille, J. and Glauser, M.-P. (1988) Listerioses en Suisse. Bull. Bundesamtes Gesundheitswesen. 3, 28.

Box, G.E.P. and Draper, N.R. (1987) Least squares for response surface work. In: Empirical Model-building and Response Surfaces, John Wiley, New York, pp. 34–103.

Buchanan, R.L. and Klawitter, L.A. (1990) Effects of temperature and oxygen on the growth of *Listeria monocytogenes* at pH 4.5. J. Food Sci. 55, 1754–1756.

Buchanan, R.L. and Phillips, J.G. (1990) Response surface model for predicting the effects of temperature, pH, sodium chloride content, sodium nitrite concentration and atmosphere on the growth of *Listeria monocytogenes*. J. Food Prot. 53, 370–376.

Buchanan, R.L., Stahl, H.G. and Whiting, R.C. (1989) Effects and interactions of temperature, pH, atmosphere, sodium chloride, and sodium nitrite on the growth of *Listeria monocytogenes*. J. Food Prot. 52, 844–851.

Carlin, F. and Nguyen-the, C. (1994) Fate of *Listeria monocytogenes* on four types of minimally processed green salads. Lett. Appl. Microbiol. 18, 222–226.

Chen, J.H. and Hotchkiss, J.H. (1993) Growth of *Listeria monocytogenes* and *Clostridium sporogenes* in cottage cheese in modified atmosphere packaging. J. Dairy Sci. 76, 972–977.

Chen, N. and Shelef, L.A. (1992) Relationship between water activity, salts of lactic acid and growth of *Listeria monocytogenes* in a meat model system. J. Food Prot. 55, 574–578.

Cole, M.B., Jones, M.V. and Holyoak, C. (1990) The effect of pH, salt concentration and temperature on the survival and growth of *Listeria monocytogenes*. J. Appl. Bacteriol. 69, 63–72.

Conner, D.E., Brackett, R.E. and Beauchat, L.R. (1986) Effects of temperature, sodium chloride, and pH on growth of *Listeria monocytogenes* in cabbage juice. Appl. Environ. Microbiol. 52, 59–63.

Conner, D.E., Scott, V.N. and Bernard, D.T. (1990) Growth, inhibition and survival of *Listeria monocytogenes* as affected by acidic conditions. J. Food Prot. 53, 652–655.

Denis, F. and Ramet, J.P. (1989) Antibacterial activity of the lactoperoxidase system on *Listeria monocytogenes* in trypticase soy broth, UHT milk and French soft cheese. J. Food Prot. 52, 706–711.

Dickson, J.S. (1990) Survival and growth of *Listeria monocytogenes* on beef tissue surfaces as affected by simulated processing conditions. J. Food Safety 10, 165–174.

Donnelly, C.W. and Briggs, E.H. (1986) Psychotrophic growth and thermal inactivation of *Listeria monocytogenes* as a function of milk composition. J. Food Prot. 49, 994–998.

Dorsa, W.J., Marshall, D.L., Moody, M.W. and Hackney, C.R. (1993) Low temperature growth and thermal inactivation of *Listeria monocytogenes* in precooked crawfish tail meat. J. Food Prot. 56, 106–109.

Duffy, L.L., Vanderlinde, P.B. and Grau, F.H. (1994) Growth of *Listeria monocytogenes* on vacuum-packed cooked meats: effects of pH, a_w, nitrite and ascorbate. Int. J. Food Microbiol. 23, 377–390.

Duh, Y.-H. and Schaffner, D.W. (1993) Modelling the effect of temperature on the growth rate and lag time of *Listeria innocua* and *Listeria monocytogenes*. J. Food Prot. 56, 205–210.

El-Gazzar, F.E., Bohner, H.F. and Marth, E.H. (1991) Growth of *Listeria monocytogenes* at 4, 32, and 40°C in skim milk and in retentate and permeate from ultrafiltered skim milk. J. Food Prot. 54, 338–342.

El-Shenawy, M.A. and Marth, E.H. (1988) Inhibition and inactivation of *Listeria monocytogenes* by sorbic acid. J. Food Prot. 51, 842–847.

El-Shenawy, M.A. and Marth, E.H. (1989) Inhibition or inactivation of *Listeria monocytogenes* by sodium benzoate together with some organic acids. J. Food Prot. 52, 771–776.

El-Shenawy, M.A. and Marth, E.H. (1991) Organic acids enhance the antilisterial activity of potassium sorbate. J. Food Prot. 54, 593–597.

El-Shenawy, M.A. and Marth, E.H. (1992) Behaviour of *Listeria monocytogenes* in the presence of sodium propionate together with food acids. J. Food Prot. 55, 241–245.

Erickson, J.P. and Jenkins, P. (1992) Behaviour of psychrotrophic pathogens *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Aeromonas hydrophila* in commercially pasteurized eggs held at 2, 6.7 and 12.8°C. J. Food Prot. 55, 8–12.

Farber, J.M., Sanders, G.W., Dunfield, S. and Prescott, R. (1989) The effect of various acidulants on the growth of *Listeria monocytogenes*. Lett. Appl. Microbiol. 9, 181–183.

Farrag, S.A. and Marth, E.H. (1989) Growth of *Listeria monocytogenes* in the presence of *Pseudomonas fluorescens* at 7 or 13°C in skim milk. J. Food Prot. 52, 852–855.

Farrag, S.A., El-Gazzar, F.E. and Marth, E.H. (1990) Fate of *Listeria monocytogenes* in sweetened condensed and evaporated milk during storage at 7 or 21°C. J. Food Prot. 53, 747–750.

Ferguson, R.D. and Shelef, L.A. (1990) Growth of *Listeria monocytogenes* in soymilk. Food Microbiol. 7, 49–52.

Ferreira, M.A.S.S. and Lund, B.M. (1992) The effect of citric acid on growth of *Listeria monocytogenes*. Abstract 148. Listeria 1992. 11th International Symposium on Problems of Listeriosis, Copenhagen, Denmark.

Fleming, D.W., Cochi, S.C., MacDonald, K.L., Brondum, J., Hayes, P.S., Plikaytis, B.D., Holmes, M.B., Audurier, A., Broome, C.V. and Reingold, A.L. (1985) Pasteurized milk as a vehicle for infection in an outbreak of listeriosis. N. Engl. J. Med. 312, 404–407.

George, S.M. and Lund, B.M. (1992) The effect of culture medium and aeration on growth of *Listeria monocytogenes* at pH 4.5. Lett. Appl. Microbiol. 15, 49–52.

George, S.M., Lund, B.M. and Brocklehurst, T.F. (1988) The effect of pH and temperature on initiation of growth of *Listeria monocytogenes*. Lett. Appl. Microbiol. 6, 153–156.

Gill, C.O. and Reichel, M.P. (1989) Growth of the cold-tolerant pathogens *Yersinia enterocolitica*, *Aeromonas hydrophila* and *Listeria monocytogenes* on high-pH beef packaged under vacuum or carbon dioxide. Food Microbiol. 6, 223–230.

Glass, K.A. and Doyle, M.P. (1989) Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. Appl. Environ. Microbiol. 55, 1565–1569.

Goulet, V., Jacquet, C., Vaillant, V., Rebiere, J., Mouret, E., Lorente, C., Maillot, E., Staïner, F., Haury, B., Pierre, O. and Rocourt, J. (1995) Bouffée épidémique de listériose. Bull. Epidémiol. Hebd. 23, 106-107.

Grant, I.R., Nixon, C.R. and Patterson, M.F. (1993) Comparison of the growth of *Listeria monocytogenes* in unirradiated and irradiated cook-chill roast beef and gravy at refrigeration temperatures. Lett. Appl. Microbiol. 17, 55-57.

Grau, F.H. and Vanderlinde, P.B. (1990) Growth of *Listeria monocytogenes* on vacuum-packaged beef. J. Food Prot. 53, 739-741.

Grau, F.H. and Vanderlinde, P.B. (1992) Occurrence, numbers and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. 55, 4-7.

Grau, F.H. and Vanderlinde, P.B. (1993) Aerobic growth of *Listeria monocytogenes* on beef lean and fatty tissue: equations describing the effects of temperature and pH. J. Food Prot. 56, 96-101.

Guerzoni, M.E., Lanciotti, R., Torriani, S. and Dellaglio, F. (1994) Growth modelling of *Listeria monocytogenes* and *Yersinia enterocolitica* in food model systems and dairy products. Int. J. Food Microbiol. 24, 83-92.

Huang, I.P., Yousef, A.E., Marth, E.H. and Matthews, M.E. (1992) Thermal inactivation of *Listeria monocytogenes* in chicken gravy. J. Food Prot. 55, 492-496.

Hudson, J.A. (1992) Efficacy of high sodium chloride concentrations for the destruction of *Listeria monocytogenes*. Lett. Appl. Microbiol. 14, 178-180.

Hudson, J.A. and Mott, S.J. (1993a) Growth of *Listeria monocytogenes*, *Aeromonas hydrophila* and *Yersinia enterocolitica* in pâté and a comparison with predictive models. Int. J. Food Microbiol. 20, 1-11.

Hudson, J.A. and Mott, S.J. (1993b) Growth of *Listeria monocytogenes*, *Aeromonas hydrophila* and *Yersinia enterocolitica* on cold-smoked salmon under refrigeration and mild temperature abuse. Food Microbiol. 10, 61-68.

Hudson, J.A. and Mott, S.J. (1993c) Growth of *Listeria monocytogenes*, *Aeromonas hydrophila* and *Yersinia enterocolitica* on cooked beef under refrigeration and mild temperature abuse. Food Microbiol. 10, 429-437.

Houtsma, P.C., Kusters, B.J.M., de Wit, J.C., Rombouts, F.M. and Zwietering, M.H. (1994) Modelling growth rates of *Listeria innocua* as a function of lactate concentration. Int. J. Food Microbiol. 24, 113-123.

ICMSF (1978) Microorganisms in Foods. Vol 1. Their Significance and Methods of Enumeration. The International Commission on Microbiological Specifications for Foods. 2nd ed. University of Toronto Press, Toronto, pp. 320

Ingham, S.C., Escude, J.M. and McCown, P. (1990) Comparative growth rates of *Listeria monocytogenes* and *Pseudomonas fragi* on cooked chicken loaf stored under air and two modified atmospheres. J. Food Prot. 53, 289-291.

Jacquet, C., Catimel, B., Brosch, R., Buchrieser, C., Dehaumont, P., Goulet, V., Lepoutre, A., Veit, P. and Rocourt, J. (1995) Investigations related to the epidemic strain involved in the French listeriosis outbreak in 1992. Appl. Environ. Microbiol. 61, 2242-2246.

James, S.M., Fannin, S.L., Agee, B.A., Hall, B., Parker, E., Vogt, J., Run, G., Williams, J., Lieb, L., Salmingen, C., Prendergast, T., Werner, S.B. and Chin, J. (1985) Listeriosis outbreak associated with Mexican-type cheese. Morb. Mortal. Wkly. Rep. 34, 357-359.

Jemmi, T. and Keusch, A. (1992) Behaviour of *Listeria monocytogenes* during processing and storage of experimentally contaminated hot-smoked trout. Int. J. Food Microbiol. 15, 339-346.

de Koos, J.T. (1992) The use of sodium lactate in meat and poultry preservation. Eur. Food Drink Rev. Summer, 37-41.

Lachica, R.V. (1992) Monitoring and control of cold tolerant pathogens in chilled foods. Act. Rep. Res. Dev. Assoc. 44, 64-76.

Leung, C.-K., Huang, Y.W. and Harrison, M.A. (1992) Fate of *Listeria monocytogenes* and *Aeromonas hydrophila* on packaged channel catfish fillets stored at 4°C. J. Food Prot. 55, 728-730.

Lund, B.M. (1993) Quantification of factors affecting the probability of development of pathogenic bacteria, in particular *Clostridium botulinum*, in foods. J. Ind. Microbiol. 12, 144-155.

Manu-Tawiah, W., Myers, D.J., Olson, D.G. and Molins, R.A. (1993) Survival and growth of *Listeria monocytogenes* and *Yersinia enterocolitica* in pork chops packaged under modified gas atmospheres. J. Food Sci. 58, 475–479.

Marshall, D.L. and Schmidt, R.H. (1988) Growth of *Listeria monocytogenes* at 10°C in milk preincubated with selected pseudomonads. J. Food Prot. 51, 277–282.

Marshall, D.L., Wiese-Lehigh, P.L., Wells, J.H. and Farr, A.J. (1991) Comparative growth of *Listeria monocytogenes* and *Pseudomonas fluorescens* on precooked chicken nuggets stored under modified atmospheres. J. Food Prot. 54, 841–843, 851.

Marshall, D.L., Andrews, L.S., Wells, J.H. and Farr, A.J. (1992) Influence of modified atmosphere packaging on the competitive growth of *Listeria monocytogenes* and *Pseudomonas fluorescens* on precooked chicken. Food Microbiol. 9, 303–309.

McClure, P.J., Baranyi, J., Boogard, E., Kelly, T.M. and Roberts, T.A. (1993) A predictive model for the combined effect of pH, sodium chloride and storage temperature on the growth of *Brochothrix thermosphacta*. Int. J. Food Microbiol. 19, 161–178.

McClure, P.J., Blackburn, C. de W., Cole, M.B., Curtis, P.S., Jones, J.E., Legan, J.D., Ogden, I.D., Peck, M.W., Roberts, T.A., Sutherland, J.P. and Walker, S.J. (1994) Modeling the growth, survival and death of microorganisms in foods: the UK Food Micromodel approach. Int. J. Food Microbiol. 23, 265–275.

McMeekin, T., Olley, J.N., Ross, T. and Ratkowsky, D.A. (1993) In: Predictive Microbiology: Theory and Application, Research Studies Press Ltd., Taunton, Somerset, UK.

Oh, D-H. and Marshall, D.L. (1993) Influence of temperature, pH, and glycerol monolaurate on growth and survival of *Listeria monocytogenes*. J. Food Prot. 56, 744–749.

Oh, D-H. and Marshall, D.L. (1994) Enhanced inhibition of *Listeria monocytogenes* by glycerol monolaurate with organic acids. J. Food Sci. 59, 1258–1261.

Palumbo, S.A. and Williams, A.C. (1994) Control of *Listeria monocytogenes* on the surface of frankfurters by acid treatments. Food Microbiol. 11, 293–300.

Papageorgiou, D.K. and Marth, E.H. (1989) Behaviour of *Listeria monocytogenes* at 4 and 22°C in whey and skim milk containing 6 or 12% sodium chloride. J. Food Prot. 52, 625–630.

Parish, M.E. and Higgins, D.P. (1989) Survival of *Listeria monocytogenes* in low pH model broth systems. J Food Prot. 52, 144–147.

Pearson, L.J. and Marth, E.H. (1990) Behaviour of *Listeria monocytogenes* in the presence of cocoa, carrageenin, and sugar in a milk medium incubated with and without agitation. J. Food Prot. 53, 30–37.

Pelroy, G.A., Peterson, M.E., Holland, P.J. and Eklund, M.W. (1994) Inhibition of *Listeria monocytogenes* in cold-process (smoked) salmon by sodium lactate. J. Food Prot. 57, 108–113.

Petran, R.L. and Zottola, E.A. (1989) A study of factors affecting growth and recovery of *Listeria monocytogenes* Scott A. J. Food Sci. 54, 458–460.

Robinson, R.A. and Stokes, R.H. (1959) Electrolyte Solutions, Butterworths, London, pp. 517–521

Rosenow, E.M. and Marth, E.H. (1987) Growth of *Listeria monocytogenes* in skim, whole and chocolate milk, and in whipping cream during incubation at 4, 8, 13, 21 and 35°C. J. Food Prot. 50, 452–459.

Ryser, E.T. and Marth, E.H. (1987) Fate of *Listeria monocytogenes* during the manufacture and ripening of camembert cheese. J. Food Prot. 50, 372–378.

Schillinger, U., Kaya, M. and Lucke, F.-K. (1991) Behaviour of *Listeria monocytogenes* in meat and its control by a bacteriocin-producing strain of *Lactobacillus sake*. J. Appl. Bacteriol. 70, 473–478.

Schlech, W.F., Lavigne, R., Bortolussi, R., Allen, A., Haldane, E., Hightower, A., Johnson, S., King, S., Nicholls, E. and Broome, C. (1983) Epidemic listeriosis — evidence for transmission by food. N. Engl. J. Med. 308, 203–206.

Schlyter, J.H., Glass, K.L., Loeffelholz, J., Degnan, A.J. and Luchansky, J.B. (1993) The effects of diacetate with nitrite, lactate or pediocin on the viability of *Listeria monocytogenes* in turkey slurries. Int. J. Food Microbiol. 19, 271–281.

Shelef, L.A. and Addala, L. (1994) Inhibition of *Listeria monocytogenes* and other bacteria by sodium diacetate. J. Food Safety 14, 103–115.

Sionkowski, P.J. and Shelef, L.A. (1990) Viability of *Listeria monocytogenes* strain Brie-1 in the avian egg. J. Food Prot. 53, 15–17.

Sorrells, K.M. and Enigl, D.C. (1990) Effect of pH, acidulant, sodium chloride and temperature on the growth of *Listeria monocytogenes*. J. Food Safety 11, 31–37.
Sorrells, K.M., Enigl, D.C. and Hatfield, J.R. (1989) Effect of pH, acidulant, time and temperature on the growth and survival of *Listeria monocytogenes*. J. Food Prot. 52, 571–573.
Steinbruegge, E.G., Maxcy, R.B. and Liewen, M.B. (1988) Fate of *Listeria monocytogenes* on ready to serve lettuce. J. Food Prot. 51, 596–599.
Stillmunkes, A.A., Prabhu, G.A., Sebranek, J.G. and Molins, R.A. (1993) Microbiological safety of cooked beef roasts treated with lactate, monolaurin or gluconate. J. Food Sci. 58, 953–958.
Walker, S.J., Archer, P. and Banks, J.G. (1990) Growth of *Listeria monocytogenes* at refrigeration temperatures. J. Appl. Bacteriol. 68, 157–162.
Wang, C. and Shelef, L.A. (1992) Behaviour of *Listeria monocytogenes* and the spoilage microflora in fresh cod fish treated with lysozyme and EDTA. Food Microbiol. 9, 207–213.
Weaver, R.A. and Shelef, L.A. (1993) Antilisterial activity of sodium, potassium or calcium lactate in pork liver sausage. J. Food Safety 13, 133–146.
Wijtzes, T., McClure, P.J., Zwietering, M.H. and Roberts, T.A. (1993) Modelling bacterial growth of *Listeria monocytogenes* as a function of water activity, pH and temperature. Int. J. Food Microbiol. 18, 139–149.
Wimpfheimer, L., Altman, N.S. and Hotchkiss, J.H. (1990) Growth of *Listeria monocytogenes* Scott A, serotype 4 and competitive spoilage organisms in raw chicken packaged under modified atmospheres and in air. Int. J. Food Microbiol. 11, 205–214.
Young, K.M. and Foegeding, P.M. (1993) Acetic, lactic and citric acids and pH inhibition of *Listeria monocytogenes* Scott A and the effects on intracellular pH. J. Appl. Bacteriol. 74, 515–520.
Zeitoun, A.A.M. and Debevere, J.M. (1991) Inhibition, survival and growth of *Listeria monocytogenes* on poultry as influenced by buffered lactic acid treatment and modified atmosphere packaging. Int. J. Food Microbiol. 14, 161–170.