ELSEVIER

International Journal of
Food Microbiology 32 (1996) 133–144

International Journal of Food Microbiology

Predictive modeling of the growth of *Listeria monocytogenes* in CO_2 environments

J.M. Farber[a,*], Y. Cai[a], W.H. Ross[b]

[a]*Bureau of Microbial Hazards, Food Directorate, Health Protection Branch, Postal Locator 2204A2, Health Canada, Ottawa, Ontario K1A 0L2, Canada*

[b]*Bureau of Biostatistics and Computer Applications, Health Protection Branch, Health Canada, Ottawa, Ontario K1A 0L2, Canada*

Received 14 October 1995; revised 19 April 1996; accepted 29 April 1996

Abstract

The effects of pH (5.5, 6.5), temperature (4, 7 and 10°C) and carbon dioxide (10, 30, 50, 70 and 90%) on the growth and/or survival of a five strain mixture of *Listeria monocytogenes* were examined in brain heart infusion broth. All three variables had a major influence on the growth characteristics of the organism. As expected, both the lag time and generation time increased as the CO_2 level increased, and as pH and temperature decreased. Growth over a 30-day period was observed at all parameter combinations tested, except at pH 5.5, 4°C in the presence of either 50, 70 or 90% carbon dioxide. Two primary models, the Gompertz and Baranyi equations, were compared for their ability to describe the growth of *L. monocytogenes*. In general, the Gompertz model predicted both longer lag and shorter generation times, compared to the Baranyi model. The Baranyi model appeared to fit the overall data better than the Gompertz model. However, these differences were often small. Response surface models were developed for predicting the effects and interactions of the three independent variables on the growth and/or survival of *L. monocytogenes* in the different modified atmospheres. Results demonstrate the importance of strict temperature control for maintaining the advantages of food shelf life extension in enriched carbon dioxide environments. The information obtained in this study could be used as a guide to manufacturers of modified-atmosphere packaged foods, especially when designing products in which this organism may be a concern.

* Corresponding author. Tel.: +1 613 9570895; fax: +1 613 9410280; e-mail: jfarber@hpb.hwc.ca

PII S0168-1605(96)01117-8

Keywords: *Listeria monocytogenes*; Predictive modeling; Modified-atmospheres; Carbon dioxide; Baranyi and Gompertz model's

1. Introduction

Modified-atmosphere packaging (MAP) can generally be described as a type of packaging where the food product is stored in something other than air. MAP foods have become increasingly popular in North America, as food manufacturers have become aware of consumers needs for fresh refrigerated foods with extended shelf life. In one of the more common types of MAP, a single gas flushing is used, usually preceded by the application of a vacuum. The gases normally used for this latter type of MAP include carbon dioxide (CO_2), oxygen (O_2) and/or nitrogen (N_2). The most important gas from a microbiological standpoint is CO_2, which can inhibit the growth of many microorganisms (Daniels et al., 1985; Dixon and Kell, 1989; Farber, 1991). High levels of CO_2 can inhibit spoilage organisms such as the pseudomonads, thereby extending the shelf life of a product. Although a lot of information exists in the area of MAP technology with regard to packaging films, machinery, etc., research into the microbiological safety of MAP foods has lagged behind.

There has been an increased interest of late in developing mathematical models that can describe the growth characteristics of microorganisms. These models can be used to predict how microorganisms will behave or change with time in a certain food product. In addition, intrinsic and extrinsic parameters affecting the rate of change can also be modeled. *Listeria monocytogenes* is a psychrotrophic foodborne pathogen which can cause serious human illness (Farber and Peterkin, 1991). There have been a number of studies whereby empirical mathematical models that describe the effects and interactions of temperature, sodium chloride, sodium nitrite, pH and atmosphere (aerobic or anaerobic) on the growth kinetics of *L. monocytogenes*, have been developed (Buchanan and Phillips, 1990; Wijtzes et al., 1993). In addition, non-thermal inactivation models for the organism have also recently been described (Buchanan et al., 1994). However, to our knowledge, there is no published data describing the behaviour of *L. monocytogenes* in different modified atmospheres containing various levels of CO_2.

Since the growth in sales of MAP foods in North America is likely to increase even more in the future, the present study was done in an initial attempt to model the growth of *L. monocytogenes* in different CO_2 environments, with the ultimate goal of developing models that can accurately predict the behaviour of the organism in MAP ready-to-eat foods.

2. Materials and methods

2.1. Organisms

L. monocytogenes strains Scott A (serotype 4b), F6861 (serotype 4b), and HPB strain numbers 2 (serotype 4b), 410 (serotype 1/2a), and 395 (serotype 1/2b) were used as a pool for inoculation experiments. Cultures were stored at 4°C in a semisolid medium consisting of meat extract (5.0 g), peptone (10.0 g), NaCl (3.0 g), $Na_2HPO_4 \cdot 12H_2O$ (2.0 g), and agar (10.0 g) dissolved in 1 l of distilled water, final medium pH 7.4 (Institut Pasteur, Paris). Organisms were inoculated onto tryptose agar (Difco Laboratories, Detroit, MI), incubated at 30°C for 48 h and then grown at 30°C for 24 h in 5 ml of tryptic soy broth (Difco) containing 0.6% yeast extract. One millilitre from each overnight culture was pooled, centrifuged and the pellet resuspended in 5 ml peptone water. For inoculation studies, appropriate dilutions of the cultures were made in 0.1% (w/v) peptone water.

2.2. Sample set-up

Experiments were done using Mason jars containing inoculated brain heart infusion broth (Difco Labs). The initial pH values of the sterile broth were adjusted to either 5.5 or 6.5 using 0.1 N HCl. In addition, final pH values were done on select samples. Two small holes were made in the lids of the jars in order to facilitate two rubber septa. After squeezing the rubber septa into the holes, a silicone seal was placed around the edges to avoid air leakage. After sealing, a vacuum was pulled (500 mmHg vacuum; 1 min) and then each jar was flushed 2–3 times with the appropriate gas mixture by inserting inlet and outlet needles (18G-1.5 inch) through the septa. A Multivac proportional gas mixer (Model KM100-3M) was used to give the desired initial proportions of CO_2 and N_2 in the package headspace as follows: MA_1 (10% CO_2, 90% N_2), MA_2 (30% CO_2, 70% N_2), MA_3 (50% CO_2, 50% N_2), MA_4 (70% CO_2, 30% N_2), and MA_5 (90% CO_2, 10% N_2). Samples were then stored at 4, 7 and 10°C for time periods of up to 1 month.

For most experiments, to obtain an even inoculum, 1 l of sterile BHI broth contained in a 3 l flask was inoculated with the five strain pool of *L. monocytogenes* to give a final concentration of approximately 10^3 cells/ml. Then, 100 ml portions were aseptically transferred into sterilized 500 ml capacity glass Mason jars. At various time intervals, samples were placed in 0.1% (w/v) peptone water and appropriate dilutions spread plated in duplicate onto plates of tryptic soy agar containing 0.6% yeast extract (TSA-YE), which were incubated at 30°C for 3 days. During the enumeration phase, two colonies per plate were picked and confirmed as *L. monocytogenes*, following methodology previously described (Farber et al., 1994).

2.3. Gas analysis

Gas atmospheres within the jars were analyzed with a Varian gas chromatograph (Model 3300, Varian Canada, Inc., Toronto, Ont.) fitted with a thermal conductivity detector, and using poropak Q (80–100 mesh) and molecular sieve 5A (80–100 mesh) columns in series. Helium was used as carrier gas at a flow rate of 30 ml/min. The column oven was initially set at 50°C for 3 min, and then was temperature programmed to increase by 50°C/min to 150°C. The injector and detector were set at 160 and 200°C, respectively. Gas samples were withdrawn using a 1.0 ml gas-tight Pressure-Lok syringe (Precision Sampling Corp., Boston Range, LA), through the septa present on the lids of the jars. Peaks were recorded and analyzed with a Varian integrator (Model 4270, Varian Canada, Inc.). Gas chromatographic analysis was done on days 0, 7, 14, 21 and 30 for the 4 and 7°C storage experiments, and on days 0, 7 and 15 for the 10°C experiment.

2.4. Statistical analysis of MAP growth data

The primary objective of the statistical analysis of the growth data was to estimate a response surface for various growth parameters over the region of the experimental design. The method of estimation is a standard two-stage procedure. In the first stage, $\log_{10}$(maximum growth) (γ), generation time (τ), and lag time (λ) were estimated for each combination of the experimental variables. Growth parameter estimates were obtained by fitting both the Gompertz function (model G), as proposed by Gibson et al. (1988), and the model (model B) proposed by Baranyi et al. (1993). This allowed for an assessment of the potential impact of the choice of growth curve model on the resulting response surface. For model G, the lag time was defined according to McMeekin et al. (1993).

For the second stage of the estimation procedure, a quadratic response surface was fit to the natural logarithm of each growth parameter. The parameters were transformed by logarithms so that the approximate variances of their estimators was more homogeneous over the design space (Atkinson, 1987). As a result the models were of the form

$$\theta = a_1 + a_2P + a_3T + a_4C + a_5PT + a_6PC + a_7TC + a_8T^2 + a_9C^2$$

where θ denotes the natural logarithm (not $\log_{10}$) of the growth parameter to be modeled, P = pH level, T = temperature, C = CO_2, and a_i (i = 1,...,9) are the coefficients of the response surface. Note that there is no quadratic term for pH since it occurs at only two levels. At both stages of the estimation procedure, ordinary least squares was used to produce parameter estimates. All experiments were done with duplicate samples and were repeated twice.

3. Results

3.1. Estimation of growth parameters

The results for headspace gases are not presented. However, the gas levels varied only slightly (± 5%) during storage, regardless of the temperature or pH. Growth curve parameter estimates obtained from both models can be seen in Table 1. In

Table 1
Fitted generation time (τ), lag time (λ), and difference in MSE (δ) between models

pH	Temperature (°C)	CO_2 (%)	N[a]	τ_G[b] (days)	τ_B[c] (days)	λ_G (days)	λ_B (days)	δ[d]
5.5	4	0	17	0.653	0.766	2.669	1.612	0.045
5.5	4	10	10	0.942	0.932	7.168	6.954	0.015
5.5	4	30	10	1.867	1.793	9.828	9.634	−0.002
5.5	7	0	13	0.584	0.571	3.260	3.236	−0.026
5.5	7	10	10	0.472	0.585	1.007	0.145	0.027
5.5	7	30	10	0.583	0.696	1.453	0.366	0.036
5.5	7	50	12	0.870	0.970	3.107	2.357	0.015
5.5	7	70	16	1.446	1.602	5.191	4.098	−0.004
5.5	7	90	16	2.056	2.254	3.171	2.566	−0.003
5.5	10	0	12	0.223	0.289	1.465	0.888	0.057
5.5	10	10	13	0.266	0.318	0.852	0.470	0.008
5.5	10	30	13	0.347	0.434	1.543	0.863	−0.003
5.5	10	50	13	0.381	0.463	1.786	1.160	−0.007
5.5	10	70	13	0.625	0.770	2.303	1.419	−0.043
5.5	10	90	13	1.202	1.264	4.217	3.789	−0.002
6.5	4	0	15	0.565	0.654	1.748	0.891	0.059
6.5	4	10	10	0.757	0.857	5.914	4.855	0.010
6.5	4	30	10	1.008	1.077	7.284	6.424	−0.005
6.5	4	50	10	1.825	1.907	3.111	3.164	0.003
6.5	4	70	10	1.503	1.432	8.413	8.305	0.003
6.5	4	90	10	1.999	1.534	11.319	12.793	0.000
6.5	7	0	12	0.241	0.300	2.060	1.520	0.054
6.5	7	10	7	0.318	0.395	0.293	0.000	−0.037
6.5	7	30	10	0.450	0.550	0.510	0.000	0.025
6.5	7	50	10	0.557	0.671	1.035	0.000	0.062
6.5	7	70	13	0.832	0.989	2.740	1.059	0.017
6.5	7	90	13	0.821	0.978	2.320	0.877	0.013
6.5	10	0	12	0.210	0.269	1.295	0.713	0.068
6.5	10	10	10	0.231	0.281	0.697	0.253	0.028
6.5	10	30	11	0.317	0.379	0.686	0.213	0.007
6.5	10	50	13	0.321	0.401	0.991	0.350	0.005
6.5	10	70	13	0.382	0.473	1.248	0.480	0.031
6.5	10	90	13	0.548	0.620	1.430	0.617	0.023

[a] N, number of data points for each growth curve.
[b] G, Gompertz model.
[c] B, Baranyi model.
[d] δ, difference in model mean squared error ($MSE_G - MSE_B$).

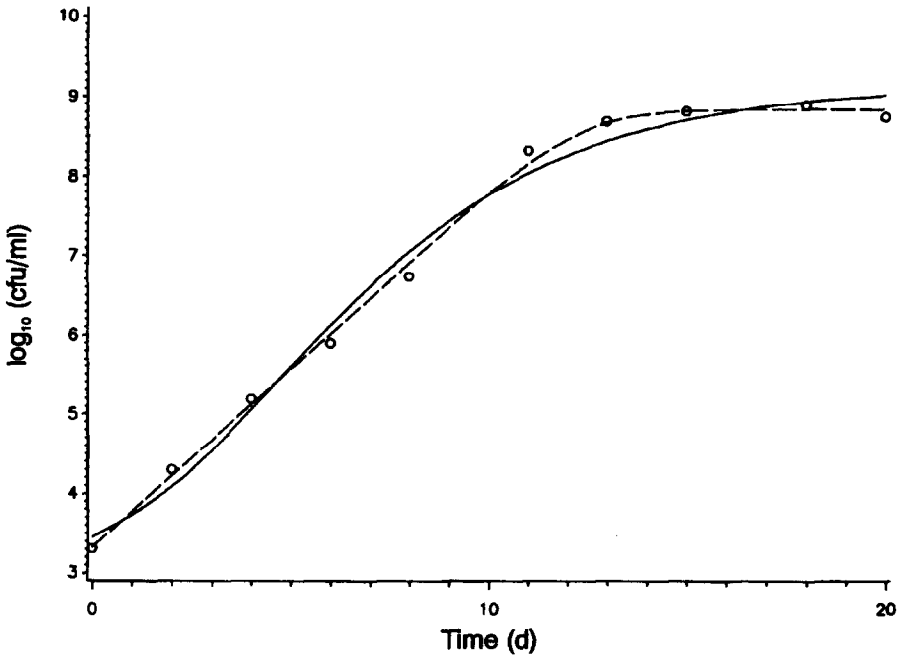

Fig. 1. Observed and fitted values for *L. monocytogenes* growing in BHI broth under the following conditions: pH = 6.5; temperature = 7°C; CO_2 = 50%. ——, Gompertz growth curve; – – –, Baranyi growth curve.

general, both the lag and generation time increased as the CO_2 concentration increased, and as pH and temperature decreased (Table 1). At both pH 5.5 and 6.5, large differences were observed in both the lag and generation time at different incubation temperatures. For example (pH 6.5) at 4, 7 and 10°C under an atmosphere of 90% CO_2, fitted Baranyi values for lag time were 12.8, 0.877 and 0.617 days, respectively, while corresponding generation time values were 1.534, 0.978 and 0.620 days, respectively (Table 1). In addition, much larger lag and generation times were observed for experiments run at pH 5.5 as compared to pH 6.5 (Table 1). In fact, there was an actual decline in numbers of *L. monocytogenes* of about 0.3–0.6 logs when present in BHI broth stored at 4°C at pH 5.5, in the presence of 50, 70 or 90% CO_2 (results not shown).

In general, estimates of generation time based on model G were consistently larger than those based on model B. In addition, with only two exceptions, lag time estimates for model G were larger than those derived from model B. Fig. 1 displays the data and both fitted models for the growth of *L. monocytogenes* in BHI in the following environment: pH = 6.5, temperature = 7°C, and CO_2 = 50%.

From the mean squared error (MSE) analysis (MSE is defined as the residual sum of squares divided by the degrees of freedom for the model), it can be seen that model B improves the overall fit compared to model G in 70% (23/33) of the cases,

i.e. if $\delta > 0$, then model B fits more closely than model G (see Table 1). Note, however, that for a pH of 5.5, model G improves the overall fit compared with model B in 53% (8/15) of the cases. The data indicate that estimates of λ were the most variable.

3.2. Response surface estimation

Transformation of the parameter estimates was considered, in order to make the data consistent with the assumptions of least squares. In particular, a common transformation was chosen so that the approximate variance of the parameter estimates was homogeneous across the design of the experiment.

Parameter estimates for the quadratic response surfaces (QRS) for each growth parameter and model combination were obtained (Table 2). All the quadratic terms are included in the fitted model for each parameter. MSE and r^2 values are also included in Table 2. Plots of the quadratic response surface for $\ln(\tau)$ and $\ln(\lambda)$ using the Baranyi model can be seen in Figs. 2 and 3, respectively.

A linear response surface (LRS) model was compared with the QRS model (results not shown) for goodness of fit, using the usual F test (Seber, 1977). The LRS was only adequate for generation time, with lack of fit for lag time evident in both the B and G models.

3.3. Replicated design points

Estimates of maximum specific growth rates and lag time derived from seven additional replicated experiments can be seen in Table 3. In addition, the corre-sponding predicted values obtained from the response surface were also recorded.

Table 2
Coefficients for response surface equations of the natural logarithm of the growth parameters[a]

Growth parameters	γ_G^b	γ_B^c	τ_G	τ_B	λ_G	λ_B
Constant	0.4583	0.2513	3.9651	2.9465	8.6656	4.8719
pH	0.1863	0.2099	−0.4823	−0.3604	−0.5995	0.1280
Temperature	0.3101	0.3335	−0.6517	−0.4742	−1.2944	−1.19333
CO_2^d	−1.6688	−1.672	5.8669	5.6668	3.8859	10.1099
pH*Temp	−0.0177	−0.0208	0.0395	0.03049	0.0152	−0.04453
pH*CO_2^d	0.0852	0.0867	−0.6203	−0.6344	−0.4399	−1.41277
Temp*CO_2^d	0.1031	0.1007	−0.0802	−0.03586	−0.1544	−0.24792
$Temp^2$	−0.0143	−0.0143	0.0153	0.0076	0.0703	0.08419
CO_2^d	0.1996	0.1663	−0.0349	−0.2589	1.2957	1.83963
Root MSE	0.066	0.062	0.180	0.162	0.523	0.776
R^2	0.846	0.882	0.948	0.946	0.740	0.700

[a] $\ln\gamma$ = ln(log$_{10}$maximum population density); $\ln\tau$ = ln(generation time) in days; $\ln\lambda$ = ln(lag time) in days.
[b] G, Gompertz model.
[c] B, Baranyi model.
[d] CO_2 levels measured as a proportion, rather than a percentage, i.e. 0.1 rather than 10%.

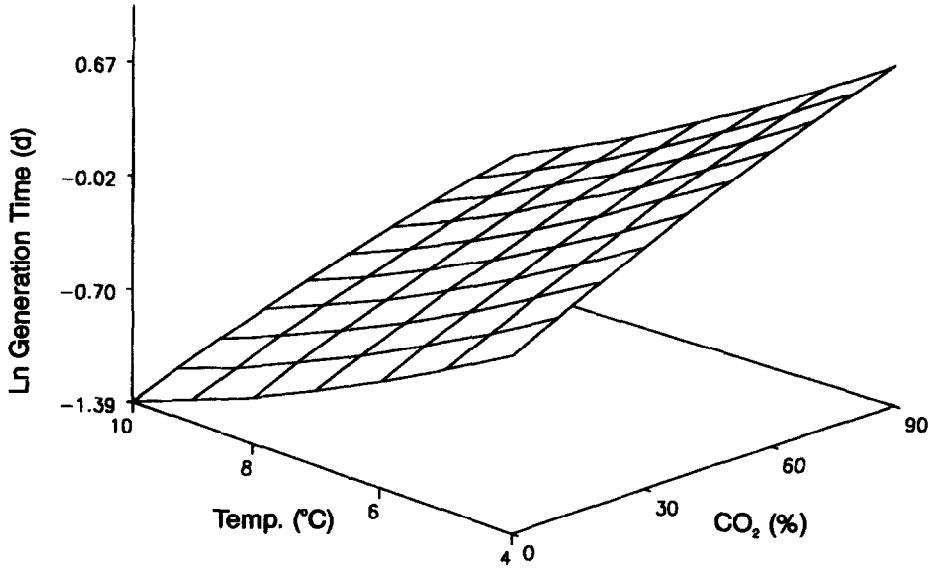

Fig. 2. Quadratic response surface for $\ln(\tau)$, Baranyi model, pH 6.5.

Generally, the agreement was quite good. The largest deviations between observed and predicted growth parameters occurred for pH = 6.5, temperature = 4°C, and CO_2 = 70% (Table 3). In this case, the estimate of the maximum population density was very imprecise, i.e. a large standard deviation was observed (results not shown).

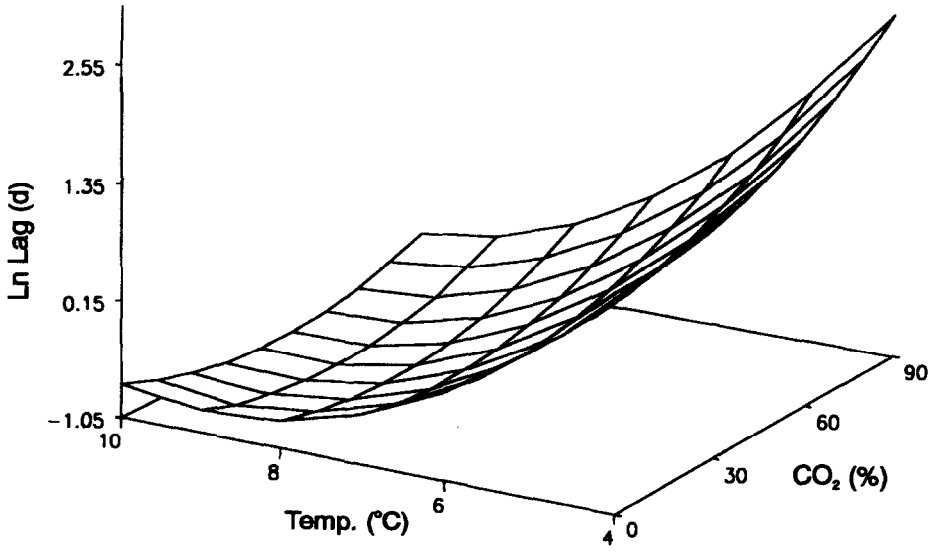

Fig. 3. Quadratic response surface for $\ln(\lambda)$, Baranyi model, pH 6.5.

Table 3
Fitted and predicted (Pred.) generation (τ) and lag times (λ) in days for replicated runs

pH	Temperature(°C)	CO_2 (%)	τ_G[a]		λ_G		τ_B[b]		λ_B	
			Fitted	Pred.	Fitted	Pred.	Fitted	Pred.	Fitted	Pred.
5.5	4	10	0.825[c]	1.032	3.347	5.744	0.432	0.462	2.051	3.755
5.5	7	30	0.466	0.659	1.659	1.769	0.248	0.338	1.036	0.980
5.5	10	70	0.549	0.693	3.111	2.391	0.270	0.359	2.442	1.516
6.5	4	70	1.861	1.711	10.54	7.635	0.888	0.699	8.861	7.212
6.5	7	30	0.340	0.446	1.353	0.947	0.195	0.241	0.597	0.534
6.5	7	50	0.465	0.572	1.967	1.152	0.241	0.299	1.358	0.610
6.5	10	90	0.428	0.500	1.890	1.533	0.225	0.254	1.244	0.542

[a]G, Gompertz model.
[b]B, Baranyi model.
[c]Number represents days.

4. Discussion

There has been considerable interest of late in developing mathematical models that describe the effects of different parameters on the growth of foodborne pathogens. The ultimate aim of these models is to enable one to accurately predict the shelf life and/or safety of foods. *L. monocytogenes* is one of the foodborne pathogens to which much attention has been paid recently, and there have been a number of studies looking at predictive models that can describe the behaviour of this organism both in broth and in foods (Buchanan and Phillips, 1990; Hudson and Mott, 1993; Wijtzes et al., 1993; Guerzoni et al., 1994). However, little if any published work is available to describe the interactive effects of various parameters on the growth of this pathogen in modified atmospheres containing various levels of CO_2.

This study has shown the extreme importance of both pH and especially temperature in controlling the growth of *L. monocytogenes* in modified atmospheres. In particular, over a period of 30 days with an initial pH of 5.5, the organism was unable to grow at 4°C in the presence of $\geq 50\%$ CO_2. However, at the same initial pH, a slight increase in storage temperature to 7°C allowed for fairly good growth of the organism, with an observed generation time of either 2.056 (Gompertz) or 0.979 (Baranyi) days. Razavilar and Genigeorgis (1992) also found that the inhibitory effect of CO_2 on the growth of *Listeria* spp. was enhanced with decreasing storage temperature.

This study has confirmed that the major effects of CO_2 on *L. monocytogenes* are bacteriostatic (i.e., decreases growth rate and increases lag time), although at low pH (5.5) and temperature (4°C), decreases in numbers of *L. monocytogenes* of up to 0.5 logs compared to the initial counts, were observed in the presence of high levels of CO_2 ($\geq 50\%$). Hart et al. (1991) examined the growth of *L. monocytogenes* on skinless chicken breast meat stored at 1, 6 and 15°C under various gas atmospheres. At 1°C, the organism failed to grow under any of the gas atmospheres, including air, while at 6°C, the organism grew slowly in an atmosphere of 30% CO_2 remainder N_2, but did not increase in number under an atmosphere of 30% CO_2, remainder air or 100% CO_2, over the 15 days storage period.

Wimpfheimer et al. (1990) found that under a MA of 75% CO_2, 25% N_2, both *L. monocytogenes* as well as other aerobic bacteria failed to grow on raw chicken stored at either 4, 10 or 27°C. However, in a MA containing some O_2 (72.5:22.5:5; $CO_2/N_2/O_2$), *L. monocytogenes* increased in number by almost 6 logs at 4°C over the 21 days storage period, while at the same time the aerobic colony count was greater than 4 logs lower compared to growth in air at the same temperature.

Marshall et al. (1991) and Marshall et al. (1992) did a series of experiments examining the influence of different MAs on the competitive growth of both *L. monocytogenes* and *Pseudomonas fluorescens* on precooked chicken nuggets stored at 3, 7 or 11°C. The atmospheres used were either air, 76% CO_2:13.3% N_2:10.7% O_2 or 80% CO_2:20% N_2. From the results of initial experiments, it was apparent that although *L. monocytogenes* was capable of growing on the surface of the chicken

nuggets, both MAs appeared effective in either increasing the lag phase or reducing the rate of growth of the organism. Similar to the results of Wimpfheimer et al. (1990), MA containing no O_2 was more restrictive to the growth of the organism.

Farber and Daley (1994) found that *L. monocytogenes* was capable of growing on turkey roll in MA containing CO_2 levels as high as 50%, i.e. *L. monocytogenes* strain Scott A was unable to grow on turkey roll stored under an atmosphere of 70% CO_2:30% N_2, either at 4 or 10°C. In all instances, *Listeria* did not grow as well in any of the MA, compared to growth in air. It is possible that background microorganisms and/or inhibitory chemicals combined with high CO_2 environments may act synergistically or additively to inhibit the growth of *L. monocytogenes.*

In general, there was considerable agreement between predictions using the Gompertz model and those obtained from the Baranyi model. There were systematic differences in the lag time estimates of the two models, with the lag times being consistently larger in the Gompertz model. These differences reflect the differences in shape of the two models, with the Gompertz model being more highly curved throughout the exponential phase than the Baranyi model.

Comparison of the mean square errors for each model showed that, overall, the Baranyi model produced a closer fit to the growth data. However, these differences were often small. The range of estimated standard errors of the regressions was 0.03 to 0.38 for the Gompertz model and 0.03 to 0.42 for the Baranyi model. Fig. 1 illustrates the closeness of predicted growth values from each model. Both the Gompertz and Baranyi models were 'fail-safe' in terms of the predicted lag phase, i.e. both models predicted lag phases that were shorter than the fitted values. For generation time, however, model predictions were not always 'fail-safe', but were, nevertheless, very close to the fitted values. In conclusion, the mathematical models generated by this study provide 'first estimates' of the potential for growth of *L. monocytogenes* in MAP foods. Future research will concentrate on modeling the growth of *L. monocytogenes* 'in situ' in MAP foods.

References

Atkinson, A.C. (1987) Plots, Transformations and Regression: An Introduction to Graphical Methods of Diagnostic Regression Analysis, pp. 80–105. Clarendon Press, Oxford.

Baranyi, J., Roberts, T.A. and McClure, P.H. (1993) A non-autonomous differential equation to model bacterial growth. Food Microbiol. 10, 43–59.

Buchanan, R.L., Golden, M.H., Whiting, R.C., Phillips, J.G. and Smith, J.L. (1994) Non-thermal inactivation models for *Listeria monocytogenes*. J. Food Sci. 59, 179–188.

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.

Daniels, J.A., Krishnamurth, R. and Rizvi, S.S.H. (1985) A review of effects of CO_2 on microbial growth and food quality. J. Food Prot. 48, 532–537.

Dixon, N.M. and Kell, D.B. (1989) The inhibition by CO_2 of the growth and metabolism of microorganisms. J. Appl. Bacteriol. 67, 109–136.

Farber, J.M. (1991) Microbiological aspects of modified-atmosphere packaging technology – a review. J. Food Prot. 54, 58–70.

Farber, J.M. and Daley, E. (1994) Fate of *Listeria monocytogenes* on modified-atmosphere packaged turkey roll slices. J. Food Prot. 57, 1098–1100.

Farber, J.M. and Peterkin, P.I. (1991) *Listeria monocytogenes*, a foodborne pathogen. Microbiol. Rev. 55, 476–511.

Farber, J.M., Warburton, D.W. and Babiuk, T. (1994) MF-HPB 30. Isolation of *Listeria monocytogenes* from all foods and envrionmental samples. In: Compendium of Analytical Methods, Vol. 2. Polyscience Publications, Morin Heights, Quebec.

Gibson, A.M., Bratchell, N. and Roberts, T.A. (1988) Predicting microbial growth: growth responses of salmonellae in a laboratory medium as affected by pH, sodium chloride and storage temperature. Int. J. Food Microbiol. 6, 155–178.

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.

Hart, C.D., Mead, G.C. and Norris A.P. (1991) Effects of gaseous environment and temperature on the storage behaviour of *Listeria monocytogenes* on chicken breast meat. J. Appl. Bacteriol. 70, 40–46.

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

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.

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.

McMeekin, J.A., Olley, J.N., Ross, T. and Ratkowsky, D.A. (1993) Predictive Microbiology: Theory and Application. Wiley, New York.

Razavilar, V. and Genigeorgis, C. (1992) Interactive effect of temperature, atmosphere and storage time on the probability of colony formation on blood agar by four *Listeria* species. J. Food Prot. 55, 88–92.

Seber, G.A.F. (1977) Linear Regression Analysis, pp. 96–125. Wiley, New York.

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* and competitive spoilage organisms in raw chicken packaged under modified atmospheres and in air. Int. J. Food Microbiol. 11, 205–214.