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Modeling lactic acid bacteria growth in vacuum-packaged cooked meat emulsions stored at three temperatures

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Abstract

The effect of three storage temperatures on the growth of lactic acid bacteria (LAB) in cooked meat emulsions packaged in low oxygen permeability film was investigated. Bacterial counts at 0°C, 8°C and 15°C were fitted to the Gompertz equation and the maximum specific growth rate (μ) was obtained as derived parameter, this value being maximal at 15°C (1.16 days^{-1}). Arrhenius and root square models were used to describe the effect of different storage temperatures on maximum specific growth rate. The models were statistically validated and the mean square error (MSE), coefficient of determination (R^2), bias factor and accuracy factor were used to evaluate and compare the performance of predictive models. The effect of temperature was better interpreted by root square model than by Arrhenius type model, showing the least deviations from the observed value, which would lead to "fail-safe" prediction of shelf-life. Since the final number of LAB did not show significant changes with storage temperature after 25 days, a temperature abuse during storage will not result in an increase of spoilage by LAB.

Keywords: Lactic acid bacteria; Vacuum-packaging; Growth

1. Introduction

In recent years, interest has increased in using mathematical models to describe the growth of micro-organisms as a function of specific environmental conditions (e.g. temperature, water activity, pH, oxygen availability, product composition). Predictive modeling provides a fast and yet relatively inexpensive way to get reliable estimates on microbial growth and survival (Mc Meekin et al., 1993). Mathematical models can be used to describe the fate of micro-organisms in the manufacturing process design and optimization for production and distribution of food products (Alavi et al., 1999).

Predictive microbiology has focused on understanding the effect of intrinsic and extrinsic parameters on the growth/inactivation of food pathogens. Growth models have been developed in laboratory media for *Staphylococcus aureus* (Sutherland et al., 1994), *Bacillus cereus* (Sutherland et al., 1996), *Yersinia enterocolitica* (Sutherland and Bayliss, 1994), *Clostridium botulinum* (Graham et al., 1996), *Escherichia coli* O157:H7 (Sutherland et al., 1997; Pond et al., 2001) and *Listeria monocytogenes* (Dalgaard and Jørgensen, 1998; te Giffel and Zwietering 1999). Modeling can also be applied to predict the microbial shelf-life of foods (Witjzes et al., 1995; Baranyi et al., 1995; Devlieghere et al., 1998; Malakar et al., 1999).

Lactic acid bacteria (LAB) were identified as major spoilage populations of vacuum-packaged emulsion-type sausages and other processed meats stored at refrigeration temperatures (Borch et al., 1996; Korkeala and Björkroth, 1997; Samelis et al., 2000). As a result of LAB activities, sour off-flavors and off-odors, milky exudates, swelling of the pack, and/or greenish color can be observed. The rate of spoilage is affected by a combination of intrinsic and extrinsic factors such as the composition, water activity, packaging method, and storage temperature. The dominance of LAB is unaltered by the chill temperature used during extended storage periods (Korkeala et al., 1989). Controlling the progress of spoilage in processed meats is difficult as these bacteria are psychotropic, microaerophilic, and

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resistant to nitrite, salt, and smoke (Franz and von Holy, 1996).

Temperature is a major factor responsible for microbial spoilage. Both the growth rate and lag phase are highly temperature dependent. Thus, the effect of temperature on microbial stability has been widely studied (Ratkowsky et al., 1982; Zwietering et al., 1991; McMeekin et al., 1992; Buchanan, 1993; Almonacid-Merino and Torres, 1993).

Both primary and secondary models have been used in predictive microbiology. Primary models describe the changes in microbial population as a function of time, and secondary models describe the responses by parameters of primary models to changes in environmental conditions (Whiting, 1995). Arrhenius- and Bélehrádek-type (square-root model) models are two major classes of secondary models that have been proposed to describe the effect of temperature on microbial growth.

The objective of this work was to develop growth models and determine growth rates of LAB in cured and cooked meat emulsions at three different temperatures during 50 days of storage to compare the most suitable secondary models.

2. Materials and methods

2.1. Cooked sausage processing

Cooked sausages were manufactured according to a procedure used in a local meat processing plant. Their composition were: 46% beef; 35% pork; 15% pork fat; 2% NaCl; powder milk 0.8%; curing salts 0.2%; binding and flavoring additives 1%. Two separate batches were prepared by thoroughly mixing the ingredients. The mix was then emulsified, filled into natural casings, and cooked to a core temperature of 75°C. Samples were immediately transported to the laboratory under refrigerating conditions. Sausages (80 g portions, 3 sausage units) were vacuum-packaged using a film (ENVARIL SAICIF, Argentine). The diffusion coefficient of the film was $19\,cm^3\,m^{-2}\,24\,h^{-1}\,atm^{-1}$ to oxygen at 25°C and 75% RH. The package was sealed at a final vacuum of 0.95 mm Hg using an RAPI-VAC S-750 vacuum packaging machine (SERVIVAC SRL, Argentine). Some samples were immediately analysed (day 0) while the remaining were stored at 0°C, 8°C and 15°C for 54 days. A new package was opened at each sampling interval (3 days).

2.2. Microbiological analysis

At each sampling time, after removing casing, 10 g of sample were aseptically transferred to a stomacher bag containing 90 ml of sterile 0.1% (w/v) peptone water and homogenized in a stomacher (Lab-Blender 400, Seward, London, UK) for 2 min at room temperature. The stomached samples were serially diluted with sterile 0.1% peptone water and surface plated (0.1 ml) in duplicate onto MRS (Merck) agar plates. Bacterial colonies were counted after incubating aerobically at 30°C for 3 days. Two separate determinations were performed and results were expressed as log colony forming units per gram ($cfu\,g^{-1}$).

2.3. Growth model

For each storage temperature the experimental data were fitted to the modified-Gompertz equation (Gibson and Roberts, 1989)

$$\log N = A + C\exp(-\exp(-B(t-M))), \quad (1)$$

where $\log N$ is the logarithm of microbial counts [$\log_{10}(cfu\,g^{-1})$] at time t, A is the asymptotic log count as time decreases indefinitely [$\log_{10}(cfu\,g^{-1})$], C is the log count increment as time increases indefinitely [$\log_{10}(cfu\,g^{-1})$], B is the relative maximum growth rate at time M [day^{-1}], and M is the time required to reach the maximum rate growth [day]. Each curve was generated using three sets of experimental data.

2.4. Effect of temperature on specific growth rate

The maximum or specific growth rate of each growth temperature was estimated from the modified Gompertz model (Eq. (1)) [$\mu = BC/e$; ($\log_{10}(cfu\,g^{-1})day^{-1}$]. The effect of storage temperature on specific growth rate was described by the Arrhenius and square root models.

(a) *Arrhenius model*: The Arrhenius equation is expressed as

$$\ln\mu = \ln A - \left(\frac{E_a}{RT}\right), \quad (2)$$

where μ is the specific growth rate (day^{-1}), A is a parameter to be fitted (day^{-1}), R is the gas constant ($8.314\,J\,K^{-1}\,mol^{-1}$), T the is absolute temperature (K), and E_a is interpreted as the activation energy for bacterial growth ($kJ\,mol^{-1}$).

(b) *Square root model*: The square root model is an empirical model for describing the dependence of bacterial growth rate on temperature. The model was first suggested by Ratkowsky et al. (1982) and can be expressed as

$$\sqrt{\mu} = b(T - T_{min}), \quad (3)$$

where b is a regression coefficient [$(day^{-1})^{-1/2}\,°C^{-1}$] and T_{min} is the theoretical minimum temperature for growth.

2.5. Statistical analysis

All models were obtained by fitting the data to Eqs. (1)–(3) with the least squares and Marquardt algorithm for linear and nonlinear models, respectively. Confidence intervals were calculated by using the Student's t-value. Residual analysis and lack of fit test were used to assess the statistical acceptability of the models. Kolomogorov–Smirnov test was performed to verify the normality of the residuals, Cochran test for variance homogeneity, and Durbin–Watson test for autocorrelation. All the tests were performed by using STATGRAPHICS® *Plus* version 4.0 (Statistical Graphics Corp).

The lack of fit test determines if the regression model adequately fits the data. For each model, the residual sum of squares (RSS) of all μ data was calculated and compared with the measurement error. This comparison between the lack of fit and the measurement error can be quantified statistically by the F-test (Zwietering et al., 1994). Significant lack of fit indicates that the model does not adequately fit the response.

2.6. Model comparison

The following indices were used: Mean square error (MSE) and regression coefficient (R^2) were used to compare models. The lower the MSE, the better the adequacy of the model to describe the data (Sutherland et al., 1994).

$$MSE = \frac{RSS}{n} = \frac{\sum(\mu_{observed} - \mu_{predicted})^2}{n}, \quad (4)$$

where RSS is the residual sum of squares, n the number of degrees of freedom, $\mu_{observed}$ the observed specific growth rate, and $\mu_{predicted}$ the predicted specific growth rate.

The bias factor is an estimate for the mean difference between the observed and predicted values. It can be calculated from

$$bias\,factor = 10^{\left(\sum \log(\mu_{observed}/\mu_{predicted})/n\right)}. \quad (5)$$

If there is no structural deviation and the bias factor = 1, the model is exact (Ross, 1996).

The accuracy factor is the measure for the mean absolute difference between the predicted and observed values.

$$accuracy\,factor = 10^{\left(\sum|\log \mu_{predicted}/\mu_{observed}|/n\right)}. \quad (6)$$

The larger the value, the less accurate the average estimate (Ross, 1996).

3. Results and discussion

Fig. 1 illustrates the growth of LAB in cooked and cured meat packaged in a low permeability film. All the curves can be fitted with the Gompertz model. The final population in meat was between between 7.50 and 8.00 log cfu g^{-1}. The time necessary to reach the stationary phase were 8, 14 and 25 days at 15°C, 8°C and 0°C, respectively. These results are partially in agreement with those reported by Giannuzzi et al. (1998) who found that LAB growing in vacuum-packaged beef cuts stored at 10°C, 9°C and 7°C took approximately 7, 10 and 16 days to reach the stationary phase, respectively. When the storage at 0°C was compared, the cooked meat emulsions required 25 days to reach the stationary phase, in contrast to 35 days reported for beef cuts in similar conditions.

The derived parameter μ (specific growth rate) for LAB in cured and cooked meat emulsions packaged in low permeability film at the studied temperatures are listed in Table 1. The estimated values of coefficients for the three models together with the plotted values of μ as a function of temperature are shown in Fig. 2. When comparing these data with those obtained by Giannuzzi et al. (1998) for Arrhenius and root square models ($\ln A = 35.764$ [days^{-1}] and $E_a = 84.66$ [kJ mol^{-1}] and $P = 0.429$ [(log(cfu g^{-1})days^{-1})$^{1/2}$] and $q = 0.043$ [(log(cfu g^{-1})days^{-1})$^{1/2}$°C^{-1}], respectively) μ values for LAB growing in cooked meat emulsions were observed to be less affected by temperature than LAB growing in beef cuts in the same packaging conditions. These differences can be attributed to interactions between the factors, which influence the growth of LAB (nutrients status, water availability).

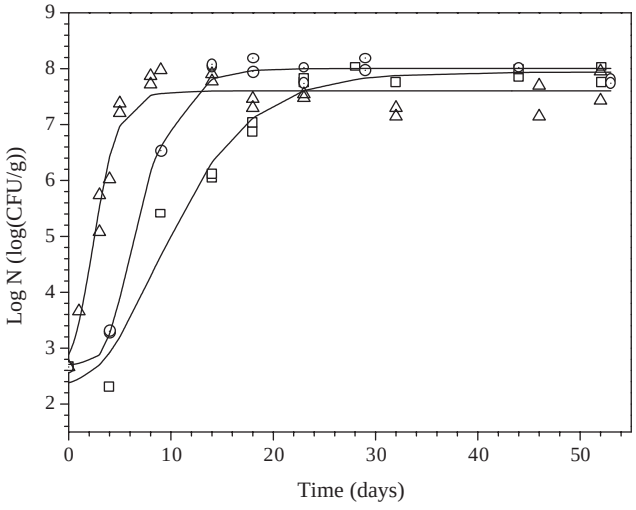

Fig. 1. Fitting of the Gompertz model to microbial counts of LAB growing in cooked meat emulsions packaged in low permeability film. (□) 0°C, $R^2 = 0.96$; (○) 8°C, $R^2 = 0.98$ and (△) 15°C, $R^2 = 0.99$.

The models were statistically validated using the residual analyses and lack of fit test. The Kolmogorov Smirnov and Cochran test showed that, in all cases, residuals were normally distributed with constant variance. The Durbin–Watson values were greater than 1.4, indicating that there is no autocorrelation in the residuals. The mathematical and statistical comparisons of the general and specific models for prediction of growth of LAB in vacuum-packaged meat emulsions are presented in Table 2. Comparison of the MSE for the two models showed that square root model produced the closest prediction of the growth data with a value of 0.0027. The R^2 values observed for the specific models, Arrhenius and root square, were greater than the values obtained by te Giffel and Zwietering (1999) for meat products. Moreover, R^2 values for the two analysed models were higher than 0.90, indicating that they explained higher fractions of the total variation.

Table 1
Estimated μ (specific growth rate) obtained from Gompertz parameters

Temperature (°C)	Estimated μ (day^{-1})	95% confidence interval
0	0.412	0.345–0.476
8	0.640	0.615–0.663
15	1.158	1.059–1.259

The indices bias and accuracy provide an objective indication of model performance. It has been shown that these factors were also valuable tools for evaluation of the performance of predictive models (Ross, 1996; Neumeyer et al., 1997; Dalgaard and Jørgensen, 1998; te Giffel and Zwietering 1999). A bias factor less than 1.0 indicates that the model is, in general, fail-safe. Results in Table 2 show values of 0.99 for square root model, this predicted value being higher than the observed value. Eq. (2) (Arrhenius model) showed a bias factor of 1.11, indicating that the prediction resulted in a lower level of confidence.

Since the bias factor provides no indication of the average accuracy of estimates because under- and

Table 2
Values of mathematical and statistical indices for Arrhenius and square root models

Indices	Equation type	
	Arrhenius model	Square root model
MSE[a]	0.0122	0.0027
R^2[b]	0.94	0.93
Bias factor	1.11	0.99
Accuracy factor	1.38	1.05

[a] MSE: mean square error.
[b] R^2: regression coefficient.

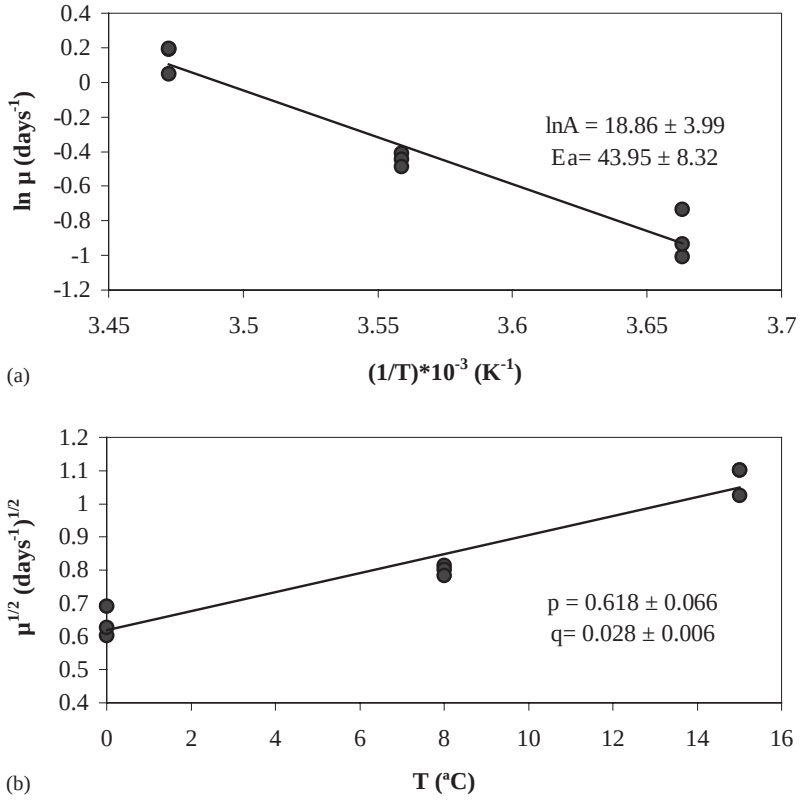

Fig. 2. Effect of temperature on specific growth rate of LAB in meat emulsions packaged in low permeability film: (a) Arrhenius model and (b) square root model.

over-prediction tend to cancel out, the accuracy factor can be calculated. As shown in Table 2 for the studied models, the values of accuracy factor indicate that on average the predictions differ from observations by 5% and 38% for square root and Arrhenius models, respectively. These results corroborate that Arrhenius equation failed to accurately predict LAB growth rate in the studied meat emulsions. Although the differences observed between the models, the obtained values may be considered satisfactory on the bases that these were derived from data obtained using non-sterile and inhomogeneous food. Dalgaard and Jørgensen (1998) calculated accuracy factors ranging from 1.4 to 4.0 for growth rates of *Listeria monocytogenes* in various types of seafood.

The two assayed models were statistically acceptable and fitted the experimental data of specific growth rate temperatures while, for the prediction performance, the root square model was better than Arrhenius model. These results agree with those of Stannard et al. (1985), who reported that the square root equation better described microbial growth of psychotropic food spoilage as a function of temperature when compared with simple Arrhenius equation, this being attributed to the fact that Arrhenius equation was originally proposed to describe the temperature dependence of the specific reaction rate constant in chemical reactions and does not adequately describe the effect of temperature on bacterial growth. On the other hand, Phillips and Griffiths (1987) also preferred square root equation to predict the effects of temperature on psychotropic growth in dairy products. The Arrhenius equation did not fit the data as well, presumably because the activation energy was dependent of the specific bacterial strains.

On the basis that microbial testing in foods is expensive and time consuming, mathematical models become a useful tool to provide a matrix of microbial growth responses to a broad range of storage conditions. The present work allowed to predict LAB growth in vacuum-packaged meat emulsions at different storage temperatures providing a sound and logical approach for addressing food safety hazards.

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