EVALUATION OF PREDICTIVE MODELS DESCRIBING THE GROWTH OF *LISTERIA MONOCYTOGENES*

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Abstract
Various mathematical models have been developed to predict the growth of micro-organisms in food and to estimate shelf-life and safety of food products. Before they can be used in practice, predictive models must be shown to predict accurately the behaviour of micro-organisms in foods. Validation of models should involve comparison of predicted responses to observations in product, independent of those used to generate the model. Typically, growth rates or generation times predicted by the model are compared to those observed for the same organism in foods.
In this study, we used general applicable models and specific growth models for *Listeria* to evaluate the ability to predict the growth of this pathogen on meat products. Literature values, obtained from a large number of publications, for growth rates were compared with predictions given by different models by graphical and mathematical analysis.
Overall, the gamma model performed best. However, only small differences between the various models were observed. Model predictions were accurate within a factor of about two. These predictions should therefore not be considered as absolute, but in many cases the accuracy will be sufficient to use these models as a tool in management decisions.

Introduction
In recent years, the interest in developing mathematical models to describe growth of micro-organisms as a function of controlling factors (e.g. water activity (a_w) , pH, temperature and oxygen availability) has increased. Predictive growth models have been developed in model media for a range of pathogens, for example *Staphylococcus aureus* (Sutherland *et al.*, 1994), *Listeria monocytogenes* (Farber *et al.*, 1996; McClure *et al.*, 1997) , *Bacillus cereus* (Sutherland *et al.*, 1996), *Yersinia enterocolitica* (Adams *et al.*, 1991; Sutherland and Bayliss, 1994) and *Escherichia coli* O157:H7 (Sutherland *et al.*, 1997).
L. monocytogenes has been recognized as an important foodborne pathogen that causes listeriosis. Outbreaks of listeriosis have been associated with milk, cheese, vegetables and salads, and meat products. The organism is particularly problematic for the food industry because it is widespread in the enviroment. *L. monocytogenes* is able to grow over a wide range of temperatures (-1.5 to 45 °C), pH values (4.39 to 9.4), and osmotic pressures (NaCl concentrations up to 10 %). It is also facultatively anaerobic (ICMSF, 1996).
Several mathematical models to describe the combined effect of temperature, pH, a_w, $NaNO_2$, CO_2 concentrations and irradiation on growth of *L. monocytogenes* have been published (Grau and Vanderlinde, 1993; Patterson *et al.*, 1993; Duffy *et al.*, 1994 and Farber *et al.*, 1996).
To determine whether predictions provide good description of growth in foods, models should be validated to evaluate the predictive ability of a model.The accuracy of models can be assessed graphically by plotting the observed values against the corresponding predictions of a model. Furthermore, mean-square-error (MSE), r^2 values and the recently described indices, bias factor and accuracy factor (Ross, 1996), can also be used

as an indication of the reliability of models when applied to foods.
The aim of this study was to determine the accuracy of general models (the Gamma concept (Zwietering *et al.*, 1996) and Pathogen Modeling Program (Buchanan, 1993)) and specific models (applied to estimate the growth rate of *Listeria* on meat(products) Grau and Vanderlinde, 1993; Patterson *et al.*, 1993; Duffy *et al.*, 1994 and Farber *et al.*, 1996) to predict the growth of *L. monocytogenes* on meat products. Therefore, growth rates on meat products reported in literature were compared with predictions given by the various growth models, by means of graphical and mathematical analysis.

Materials and methods

Models

General models

Gamma-concept (Zwietering *et al.*, 1996):

This model is based on the fact that the effect of various factors affecting the growth rate of micro-organisms can be combined by multiplying the separate effects. The effect of water activity is assumed to be linear, the effect of pH parabolic and the effect of temperature is supposed to follow the quadratic Ratkowsky equation:

$$\mu = c(a_w - a_{w,\min})(pH - pH_{\min})(pH_{\max} - pH)(T - T_{\min})^2 \quad (1)$$

The equation can be extended to include models describing the influence of additional effects, e.g preservatives, packaging conditions. The various hurdles can be quantified by separating the effects:

$$\gamma = \frac{\mu}{\mu_{opt}} = \gamma(T) \cdot \gamma(pH) \cdot \gamma(a_w) \quad (2)$$

The relative effect of one variable can be described by the gamma-factor of that variable:

$$\gamma(T) = \left(\frac{T - T_{\min}}{T_{opt} - T_{\min}}\right)^2 \quad (3a)$$

$$\gamma(pH) = \frac{(pH - pH_{\min})(pH_{\max} - pH)}{(pH_{opt} - pH_{\min})(pH_{\max} - pH_{opt})} \quad (3b)$$

$$\gamma(a_w) = \frac{(a_w - a_{w\min})}{(1 - a_{w\min})} \quad (3c)$$

Symbols:
T_{min} , pH_{min} , a_{wmin}= organism parameters; minimal growth temperature, pH and water activity of a micro-organism;
T_{opt} , pH_{opt}= organism parameters; optimal growth temperature and pH;
pH_{max}= organism parameter; maximal pH at which growth just stops;
T, pH and a_w = product- and process parameters; storage temperature, pH and water activity of the product under study.

Pathogen Modeling Program (PMP, version 5.0) (Buchanan, 1993):

Pathogen Modeling Program (PMP) was developed at the US Department of Agriculture. PMP is based on large experimental data sets. Polynomial models (like the example given in equation 4) are used to predict the growth of various pathogenic micro-organisms as function of controlling growth factors (e.g. temperature, pH, a_w, micro-organisms as function of controlling growth factors (e.g. temperature, pH, a_w, availability of oxygen)

$$\ln(\mu) = a + bT + cT^2 + dpH + epH^2 + fa_w + ga_w^2 + hTpH + iTa_w + jpHa_w \quad (4)$$

Specific models

Modified Arrhenius equation (Grau and Vanderlinde, 1993); to describe the effect of temperature and pH on growth of *L. monocytogenes* on beef

$$\ln(gen/h) = A_0 + A_1/T + A_2/T^2 + A_3/pH + A_4/pH^2 \quad (5)$$

T = temperature (K); A_0 - A_4 = coefficients for the equation: A_0 = -232.64; A_1 x10^{-5} =1.4041; A^2x10^{-7} = -2.1908; A^3x10^{-2} = 1.1586; A^4(10^{-2} = -4.0952

Quadratic equation (Patterson *et al.*, 1993); to estimate the effect of irradiation and temperature on the growth rate of *L. monocytogenes* on poultry:

$$(gen/dag) = A_0 + A_1T + A_2D + A_3T^2 + A_4D^2 + A_5TD + A_6TD^2 + A_7DT^2 \quad (6)$$

In this evaluation, irradiation was not taken into account (D =0); this leads to equation 7 in which only the effect of temperature on growth of *L. monocytogenes* is described:

$$(gen/dag) = A_0 + A_1T + A_3T^2 \quad (7)$$

T = temperature (^oC); D = irradiation dose (kGy); A_1 - A_7 = coefficients for the quadratic equation; A_0 = -0.899; A_1 = 0.252; A_3 = -0.045

Quadratic equation (Duffy *et al.*, 1994) to describe the effect of pH and a_w on growth of *L. monocytogenes* on meat at 5 ^{0}C:

$$(gen/h) = A_0 + A_1pH + A_2a_w + A_3pHa_w + A_4pH^2 + A_5a_w^2 \quad (8)$$

A_0= -19.684; A_1 = 0.5085; A_2 = 36.254; A_3 = -0.4970; A_4 = 0.0046939 (A_4 was left out since this term was shown to be not significant); A_5 = -16.581.

Quadratic equation (Farber *et al.*, 1996) to predict the influence of temperature, pH and CO_2 concentration on growth of *L. monocytogenes:*

$$\ln(gen/dag) = A_0 + A_1 pH + A_2 T + A_3 CO_2 + A_4 pH\,T + A_5 pH\,CO_2 + A_6 T\,CO_2 + A_7 T^2 + A_8 CO_2^2 \quad (9)$$

In this case CO_2 not taken into account (CO_2= 0). This leads to the following quadratic equation:

$$\ln(gen/dag) = A_0 + A_1 pH + A_2 T + A_4 pH\,T + A_7 T^2 \quad (10)$$

T = temperature (°C); CO_2 = percentage CO_2 in the package; $A1$-A_8 = coefficients for the quadratic equation; A_0 = 3.9651; A_1 = -0.4823; A_2 = -0.6517; A_4 = 0.0395; A_7 = 0.0153.

Validation
Validation can be carried out on the basis of the same data as the model was set up with, i.e. internal validation. External validation uses new data, e.g. literature data, to assess the quality of the predictions of the model. The adequacy of a model to predict data can be assessed graphically or on the basis of mathematical and statistical parameters.

Graphical comparison

Literature values for growth rate in foods can be plotted against the corresponding predictions of a model. From this plot, predictions which would be unsafe in practice can be visualized readily, and the overall reliability of the model assessed.

Mathematical/statistical comparison

Several mathematical and statistical indices can be used to evaluate the performance of predictive growth models. These are described below.

1) percentage goodness of fit (Grau and Vanderlinde, 1993)

$$100 \times \frac{predicted\ \mu - observed\ \mu}{observed\ \mu} \quad (11)$$

The difference between the calculated and experimental growth rate (μ (h^{-1})) expressed as a percentage of the experimental value.

2) Mean square error (MSE) (Adair *et al.*, 1989; Sutherland *et al.*, 1994).

$$MSE = \frac{RSS}{n} = \frac{\sum(\mu\ observed - \mu\ predicted)^2}{n} \quad (12)$$

The MSE, the residual sum of squares divided by the number of datum points, is a measure of variability remaining after fitting a model, that is not accounted for by

deliberate changes in factors such as temperature, pH and a_w. This error may come from several sources including natural variability, systematic errors and bias. The lower the MSE; the better the adequacy of the model to fit the data.

3) "multiple regression coefficient" or "coefficient of determination" (r^2) (Grau en Vanderlinde, 1993; Duffy *et al.*, 1994; Sutherland *et al.*, 1994).

The r^2 statistic is often used as an overall measure of the fit attained. It measures the fraction of the variation about the mean that is explained by the fitted model.The higher the value ($0 < r^2 < 1$), the better is the prediction by the model.

4) bias factor (Ross, 1996). The bias factor answers the question whether, on average, the observed values lie above or below the line of equivalence and, if so, by how much. It gives the structural deviations of a model.

$$bias\ factor = 10^{(\sum \log(GTpredicted/GTobserved)/n)} \tag{13}$$

A bias factor < 1 indicates a 'fail safe' model, i.e. observed generation times were larger than predicted values, so that predicted values give a margin of safety.

5) accuracy or precision factor (Ross, 1996). The accuracy factor averages the distance between each point and the line of equivalence as a measure of how close, on average, predictions are to observations.

$$accuracy\ factor = 10^{(\sum |\log(GTpredicted\ /\ GTobserved)|/n)} \tag{14}$$

The larger the value, the less accurate is the average estimate. An accuracy factor of 2 indicates that the prediction is, on average, a factor of 2 different from the observed value, i.e. either half as large or twice as large.

Results and discussion

The reliability of the models predicting growth of *Listeria* in meat products was tested by comparing predictions with observations for growth in meat(products) reported in literature. The literature validation excercise revealed marked deficiencies in the literature itself. In many publications information about the foods, experimental design and/or methods was incomplete or data were not suitable for curve-fitting and deriving kinetic parameters. A total of 83 data sets from 20 references were used.
In Figure 1 an example of the comparison of observed and predicted growth rates is given for the Gamma model.

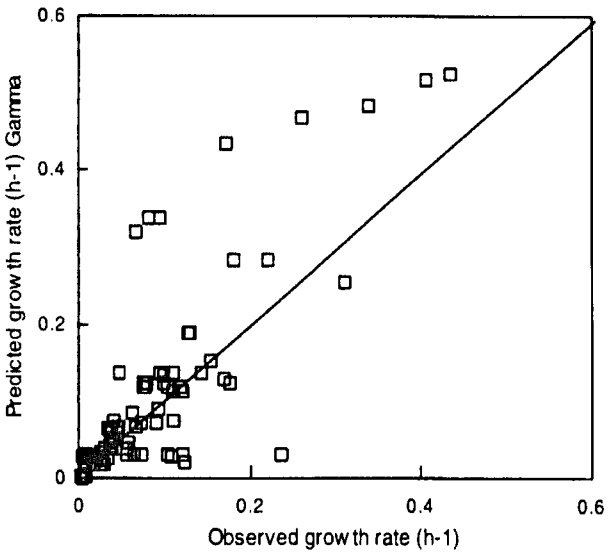

Figure 1 Comparison of published growth rates (h-1) and those predicted using the Gamma model for Listeria in meat(products).

In general, good agreement across the range of growth conditions was shown between observed and predicted values. The trend over a large range of decades (0.002 -0.5) is predicted well. Most points fall close to the line of equivalence, indicating that the model predicts growth rates similar to those reported in published studies. Sometimes, there was poor agreement, this may be due experimental error, natural variability, model inaccuracy, additional relevant factors influencing growth (e.g. preservatives, modified atmosphere packaging) not (yet) implemented in the models or near-limiting growth conditions.
For the other models similar trends were observed, except for the model of Duffy et al., 1994. This model was developed to describe growth of *Listeria* on meat at 5 °C, therefore, it can not be extrapolated for use at other temperatures. Only the effect of water activity and pH were taken into account in this model, while temperature is generally the most important controlling factor in these type of products.
The mathematical and statistical comparison of 6 models (both general and specific models) for prediction of growth of *Listeria* on meat(products) is given in Table 1.

Table 1 Evaluation of 6 models predicting growth of *Listeria* on meat(products) according to various mathematical/statistical characteristics (83 data sets)

Model	Gamma	PMP	Gvdl	Patterson	Duffy	Farber
> 70 %[a]	25	33	30	27	29	33
MSE	0.0058	0.0089	0.0148	0.0083	0.0083	0.0062
R^2	0.655	0.627	0.477	0.294	0.053	0.179
bias	0.918	0.778	0.719	0.705	1.080	0.828
accuracy	1.733	1.780	1.768	1.885	2.398	1.861

[a] = the number of observations for which the predicted growth rate differs more than 70% from the reported growth rate.

Comparing the various mathematical and statistical characteristics in Table 1, it can be observed that overall the Gamma model performs best. However, differences between the models were small. All models are 'fail safe' models (bias factor < 1), except the model of Duffy *et al.*, 1994. As mentioned before this model should not be extrapolated, as it has only been developed to describe growth at 5 °C.
The agreement between the various models and the literature data shows that the concept of predictive microbiology can give appropriate predictions. Predictive models offer guidelines to the likelihood of bacterial growth under given conditions, assist in product development, eliminating some bacterial testing during product formulation studies. However, models can only be used to support decisions. The accuracy factor of about 2 implies that while the models can be used as tool for management decisions they should not be relied upon as the sole determinant of the products' safety.

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