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Validation of predictive models describing the growth of *Listeria monocytogenes*

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Abstract

In this study, predictions for growth rate of *Listeria* on food products were evaluated by both general applicable models and specific growth models. Literature values, obtained from a large number of publications, for growth rates in/on a variety of foods were compared by graphical and mathematical analysis with predictions given by various models. Apart for the great advantage of being generally applicable, the general models performed best. However, only small differences between the various models were observed. Model predictions were accurate within a factor of about two to four, depending on the type of product. The predictions should therefore not be considered as absolute; it is important to understand the limitations of the performance of models. All results and all assumptions should be criticised, but in many cases the accuracy will be sufficient to use these types of models as a tool in management decisions.

Keywords: Predictive microbiology; Growth models; Validation; *Listeria monocytogenes*; Food

1. Introduction

In recent years, the interest in developing mathematical models to describe the growth of microorganisms 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), *Bacillus cereus* (Sutherland et al., 1996), *Yersinia enterocolitica* (Adams et al., 1991; Sutherland and Bayliss, 1994), *Clostridium botulinum* (Graham et al., 1996) and *Escherichia coli* O157:H7 (Sutherland et al., 1997).

Listeria 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 environment (Farber and Peterkin, 1991). *L. monocyto-*

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genes 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).

For *L. monocytogenes*, specific mathematical models to describe the combined effect of temperature, pH, a_w, organic acids, $NaNO_2$, CO_2 concentrations and irradiation on growth have been published (Buchanan and Phillips, 1990; Duh and Schaffner, 1993; Grau and Vanderlinde, 1993; Patterson et al., 1993; Duffy et al., 1994, Farber et al., 1996; George et al., 1996; Murphy et al., 1996, Fernández et al., 1997; McClure et al., 1997).

Predictive microbiology aims at the quantitative estimation of microbial growth in foods using mathematical modelling. To determine whether predictions provide good description of growth in foods, models should be validated to evaluate their predictive ability. 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.

Recently, the performance of various models in predicting the behaviour of *L. monocytogenes* on seafood was compared by Dalgaard and Jørgensen (1998). To obtain data for this validation, challenge studies and storage tests were performed.

The aim of this study was to determine the accuracy of general models and specific models to predict the growth of *L. monocytogenes* in/on food products. The general models that were evaluated are: the Gamma concept (Zwietering et al., 1996), Pathogen Modeling Program (PMP) (Buchanan, 1993) and food micromodel (FMM) (Food MicroModel Ltd., Randalls Road, Leatherhead, Surrey, KT22 7RY, UK). Specific models to estimate the growth of *Listeria* include the models developed by Grau and Vanderlinde, 1993; Patterson et al., 1993; Duffy et al., 1994; Farber et al., 1996; Murphy et al., 1996.

Growth rates in/on foods reported in literature were compared with predictions given by the various growth models, by means of graphical and mathematical analysis.

2. Models

In Table 1 the various models that were evaluated in order to determine their accuracy in predicting growth of *L. monocytogenes* in/on foods are shown. To be able to compare the models, only the controlling factors temperature, pH and a_w were considered. For all considered models the reported parameters were used, so no fitting occurred.

2.1. General models

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

The Gamma-concept is based on the assumption that the effect of various factors affecting the growth

Table 1
Models describing the growth rate of *Listeria monocytogenes*

Model	Controlling factors	Type	No. of parameters
General			
Gamma	*T*, pH, a_w	Square root	6
PMP[a]	*T*, pH, a_w	Polynomial (2nd order)	10
FMM[b]	*T*, pH, a_w, nitrite, lactate	Polynomial (2nd order)	10–21
Specific			
Grau and Vanderlinde, 1993	*T*, pH	Modified Arrhenius	5
Duffy et al., 1994	pH, a_w	Polynomial (2nd order)	5
Farber et al., 1996	*T*, pH, CO_2	Polynomial (2nd order)	9
Patterson et al., 1993	*T*, irradiation	Polynomial (3th order)	8
Murphy et al., 1996	*T*, pH, NaCl	Polynomial (3th order)	12

[a] PMP = Pathogen Modeling Program.
[b] FMM = Food micromodel.

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, for example preservatives or packaging conditions. The advantage of this approach is that for every variable determining growth rate, the relative effect 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:

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

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

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

For the calculations the following characteristics of *L. monocytogenes* were used: T_{min}: −1.5°C; T_{opt}: 37°C; pH_{min}: 4.39; pH_{opt}: 7; pH_{max}: 9.6; $a_{w,min}$: 0.92; μ_{opt}: 2 h^{-1}.

*2.1.2. Pathogen Modeling Program (PMP, version 5.0) (*Buchanan, 1993*) and Food MicroModel (FMM, version 2.5)*

In PMP and FMM, polynomial models are used to predict the growth of various pathogenic micro-organisms as function of controlling growth factors (e.g. temperature, pH, a_w, availability of oxygen). FMM pathogen models are principally based upon research sponsored by the Ministry of Agriculture, Fisheries and Food (MAFF) although some other models have been made available from other sources. The data used to generate the models were obtained from extensive experiments performed in microbiological culture media. Then, the models were validated for a variety of food products (e.g. McClure et al., 1993; Sutherland and Bayliss, 1994; Sutherland et al., 1994, 1996, 1997).

2.2. Specific models

*2.2.1. Modified Arrhenius equation (*Grau and Vanderlinde, 1993*)*

The combined effect of temperature and pH on growth of *L. monocytogenes* on lean beef can be described by a modified and additive Arrhenius equation of the form:

$$\ln(\mu) = A_0 + A_1/T + A_2/T^2 + A_3/pH + A_4/pH^2 \quad (4)$$

μ = specific growth rate (h^{-1}); T = temperature (K); A_0–A_4 = coefficients for the equation: $A_0 = -232.64$; $A_1 = 1.4041 \cdot 10^5$; $A_2 = -2.1908 \cdot 10^7$; $A_3 = 1.1586 \cdot 10^2$; $A_4 = -4.0952 \cdot 10^2$

*2.2.2. Third order polynomial model (*Patterson et al., 1993*)*

To estimate the effect of temperature and irradiation on the growth rate of *L. monocytogenes* on poultry meat a polynomial function was derived:

$$\mu = A_0 + A_1T + A_2D + A_3T^2 + A_4D^2 + A_5TD + A_6TD^2 + A_7DT^2 \quad (5)$$

For the purpose of the present evaluation, irradiation was not taken into account ($D = 0$); this leads to Eq. (6) in which only the effect of temperature on growth of *L. monocytogenes* is described:

$$\mu = A_0 + A_1T + A_3T^2 \quad (6)$$

μ = specific growth rate (in log cfu g^{-1} day^{-1}; according to the strict definition of specific growth rate this should be given as $time^{-1}$); T = temperature (°C); D = irradiation dose (kGy); A_1–A_7 = coefficients for the quadratic equation; $A_0 = 0.42$; $A_1 = -0.036$; $A_3 = 0.01$.

*2.2.3. Quadratic equation (*Duffy et al., 1994*)*

To describe the effect of pH and a_w on the growth rate of *L. monocytogenes* on cooked meats at 5°C a quadratic equation was developed:

$$\mu = A_0 + A_1pH + A_2a_w + A_3pHa_w + A_4pH^2 + A_5a_w^2 \quad (7)$$

μ = specific growth rate (h^{-1}); $A_0 = -19.684$; $A_1 = 0.5085$; $A_2 = 36.254$; $A_3 = -0.4970$; $A_4 = 0.0046939$ (A_4 was left out since this term was shown to be not significant); $A_5 = -16.581$.

*2.2.4. Quadratic equation (*Farber et al., 1996*)*

A response surface model was developed to predict the effect and interaction of temperature, pH and CO_2 concentration on growth of *L. monocytogenes*:

$$\ln(GT) = A_0 + A_1 pH + A_2 T + A_3 CO_2 + A_4 pHT + A_5 pHCO_2 + A_6 TCO_2 + A_7 T^2 + A_8 CO_2{}^2 \quad (8)$$

In the present comparison CO_2 is not taken into account ($CO_2 = 0$). This leads to the following quadratic equation:

$$\ln(GT) = A_0 + A_1 pH + A_2 T + A_4 pHT + A_7 T^2 \quad (9)$$

GT = generation time (days); T = temperature (°C); CO_2 = level of CO_2 measured as a proportion in the package; A_1–A_8 = coefficients for the quadratic equation; $A_0 = 2.9465$; $A_1 = -0.3604$; $A_2 = -0.4742$; $A_4 = 0.03049$; $A_7 = 0.0076$.

*2.2.5. Third order polynomial model (*Murphy et al., 1996*)*

Growth curves of *L. monocytogenes* in reconstituted skim milk powder were fitted using the Gompertz function:

$$L(t) = A + Ce^{-e(-B[t-M])} \quad (10)$$

$L(t)$ = log count of bacteria at time t (h) (log cfu ml^{-1}); A = initial level of bacteria (log cfu ml^{-1}); C = number of log cycles of growth (log cfu ml^{-1}); M = time at which the absolute growth rate is maximal (h); B = relative growth rate at M (log cfu ml^{-1} h^{-1})

The specific growth rate can be calculated by: $\mu = (BC/e)$.

For growth of *L. monocytogenes* in skim milk the following Gompertz parameters B and C were determined:

$$\ln B = -48.0193 + 0.5612T + 0.1934NaCl + 18.0587pH - 0.0098T^2 - 0.0375NaCl^2 - 2.6085pH^2 - 0.0214TNaCl - 0.0442TpH + 0.1272pH^3 + 0.0030TNaClpH + 0.0008T^2pH \quad (11)$$

$$\ln C = -29.0563 + 0.0754T - 0.0674NaCl + 13.4553pH - 0.0025T^2 + 0.0165NaCl^2 - 1.9810pH^2 - 0.0032TpH + 0.00003T^3 - 0.014NaCl^3 + 0.0969pH^3 \quad (12)$$

T = temperature (°C); NaCl = sodium chloride concentration (%). It should be noted that the amount of significant numbers differs considerably, e.g. 48.0193 and 0.00003, this may result in large prediction errors. The last number is multiplied with a large number (T^3), resulting in a relevant addition.

3. Validation

Validation can be carried out on the basis of the same data as the model was set up with to determine if the model can describe the experimental data sufficiently, i.e. internal validation. External validation uses new data, obtained from storage and challenge tests or growth rate data reported in literature, 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 indices.

3.1. 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. For this, examination and analysis of residual plots can also be useful.

3.2. Mathematical/statistical comparison

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

3.2.1. Mean square error

$$\mathrm{MSE} = \frac{\mathrm{RSS}}{n} = \frac{\sum(\mu_{\mathrm{observed}} - \mu_{\mathrm{predicted}})^2}{n} \qquad (13)$$

The MSE, the residual sum of squares divided by the number of degrees of freedom (DF), is a measure of variability remaining, that is not accounted for by deliberate changes in factors such as temperature, pH and a_w. Since no parameters are estimated the number of degrees of freedom equals the number of datum points. This remaining variability may come from several sources including natural variability and systematic errors. The lower the MSE the better the adequacy of the model to describe the data (Adair et al., 1989; Sutherland et al., 1994).

The models were also validated statistically by an *F*-ratio test. The MSE of the models was compared with the measurement error. For this comparison, an average measurement error, 0.00638 with 45 DF, was used, estimated from replicate experiments performed by Zwietering et al. (1994); Houtsma et al. (1996).

3.2.2. Regression coefficient or coefficient of determination

The regression coefficient (r^2) is often used as an overall measure of the prediction attained. It measures the fraction of the variation about the mean that is explained by a model. The higher the value ($0 < r^2 < 1$), the better is the prediction by the model (Grau and Vanderlinde, 1993; Duffy et al., 1994; Sutherland et al., 1994).

3.2.3. Bias factor

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.

$$\text{bias factor} = 10^{\left(\sum \log\left(\frac{\mathrm{GT}_{\mathrm{predicted}}}{\mathrm{GT}_{\mathrm{observed}}}\right)/n\right)} = 10^{\left(\sum \log\left(\frac{\mu_{observed}}{\mu_{predicted}}\right)/n\right)} \qquad (14)$$

$\mathrm{GT}_{\mathrm{predicted}}$ = the predicted generation time; $\mathrm{GT}_{\mathrm{observed}}$ = the observed generation time; n = the number of observations; $\mu_{\mathrm{predicted}}$ = the predicted specific growth rate; μ_{observed} = the observed specific growth rate.

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 (Ross, 1996).

3.2.4. Accuracy or precision factor

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.

$$\text{Accuracy factor} = 10^{\left(\sum |\log \mu_{\mathrm{predicted}}/\mu_{\mathrm{observed}}|/n\right)} \qquad (15)$$

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. If there is no structural deviation (bias = 1, both positive and negative deviations, on average the model is exact), inaccuracies can still be shown by the accuracy factor (Ross, 1996).

4. Materials and methods

A literature search (1990–1997) was conducted to obtain data on the growth of different *L. monocytogenes* strains in/on a variety of foods. The foods were divided into seven groups: meat (products), milk, dairy products, cheese, vegetables, fish and egg products. The experimental set-up and results reported in the publications were analyzed. Product characteristics (T, pH and a_w) of the foods were obtained from the papers or estimated if they were not specifically mentioned. Growth rates were taken directly from the publications or they were estimated from reported growth data.

Predictions of growth rates were then made using the general and specific models described earlier, followed by the validation and comparison of the models by the criteria mentioned under graphical (Section 3.1) and mathematical comparison (Section 3.2).

After comparing the models, the ability of the models to determine important process steps in a food chain was assessed in an example. Growth was predicted by three models; the Gamma concept, the model described by Grau and Vanderlinde (1993)

and the model developed by Patterson et al. (1993). As a first, rough indication it is supposed that no inactivation of *Listeria* occurs in the process except when the temperature is higher than the maximum temperature for growth. It is supposed in using the models for determination of important processing steps that no multiplication takes place ($\mu = 0$).

5. Results and discussion

The literature validation exercise 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. It was necessary to make assumptions about some conditions of pH and a_w. This has also been noted by others (Buchanan and Phillips, 1990; Sutherland et al., 1994; Ross, 1996; Neumeyer et al., 1997). Using literature data results in larger variability caused by differences in applied methods, experimental set-up, products, strains etc. but it reflects reality. Moreover, it should be noted that in order to validate predictive models, collecting and interpreting literature data and predicting growth rates by mathematical models takes less time than performing experiments e.g. challenge tests. In total, about 300 data sets from 50 references were used.

5.1. Predictions using the Gamma concept

For *L. monocytogenes* (T_{min}: −1.5°C; T_{opt}: 37°C; pH_{min}: 4.39; pH_{opt}: 7; pH_{max}: 9.6; $a_{w,min}$: 0.92; μ_{opt}: 2 h^{-1}) in crawfish (T: 4°C; pH: 6 and a_w: 0.99), the Gamma factors can be calculated as: $\gamma(T) = 0.020$; $\gamma(pH) = 0.853$ and $\gamma(a_w) = 0.875$. The total γ value is 0.015, so the growth rate can be estimated as 0.030. This gives a quantification of each of the hurdles.

The reduction factor, $1/\gamma$, of a variable is the factor with which the optimum growth rate is reduced. The main reduction of growth rate of *L. monocytogenes* on crawfish is achieved with temperature i.e. a factor 50 reduction. In Table 2 more examples of reduction factors for growth of *L. monocytogenes* in various food products are shown.

Table 2
Reduction factors ($1/\gamma$) of temperature, pH and a_w for *Listeria monocytogenes* in foods calculated with the Gamma model

Product	$1/\gamma$ (T)	$1/\gamma$ (pH)	$1/\gamma$ (a_w)
Pork (4, 5.8, 0.98)[a]	*50*	1.3	1.3
Salami (7, 4.6, 0.93)	*20*	*6.6*	*8*
Crab (5, 7, 0.99)	*35*	1.0	1.1
Crawfish (4, 6.0, 0.99)	*50*	1.1	1.1
Milk (4, 6.6, 0.993)	*50*	1.0	1.1
Cottage cheese (7, 5.1, 0.988)	*20*	2.1	1.2
Broccoli (15, 6.5, 0.98)	*5.4*	1.0	1.3

[a] Product parameters: temperature, pH and a_w.

The factors quantitatively influencing the growth rate are presented in italics.

These data show that for most food products temperature is the only quantitatively important factor in controlling growth of *L. monocytogenes*. In products, such as salami, in which both the pH and a_w are low, these factors also contribute considerably to a decreased growth rate as compared with the optimal growth rate.

5.2. Comparison of predictions

5.2.1. Graphical comparison

From plots, in which measured growth rates are shown as a function of predictions by a model and from residual plots, predictions which would be unsafe in practice can be visualized easily and the overall reliability of models can be assessed.

In Fig. 1 an example of the comparison of observed and predicted growth rates in milk is given for the Gamma model. Fig. 1a shows the results on a linear scale, in Fig. 1b the square root of the observed growth rate is plotted as a function of the (square root of the) predicted growth rate and Fig. 1c presents the values on a logarithmic scale. Plots of residuals against predictions were examined for growth rate, log transformation and square root transformation (data not shown). These plots suggest that log transformation is most suitable as this results in a homogeneous error distribution. The advantage

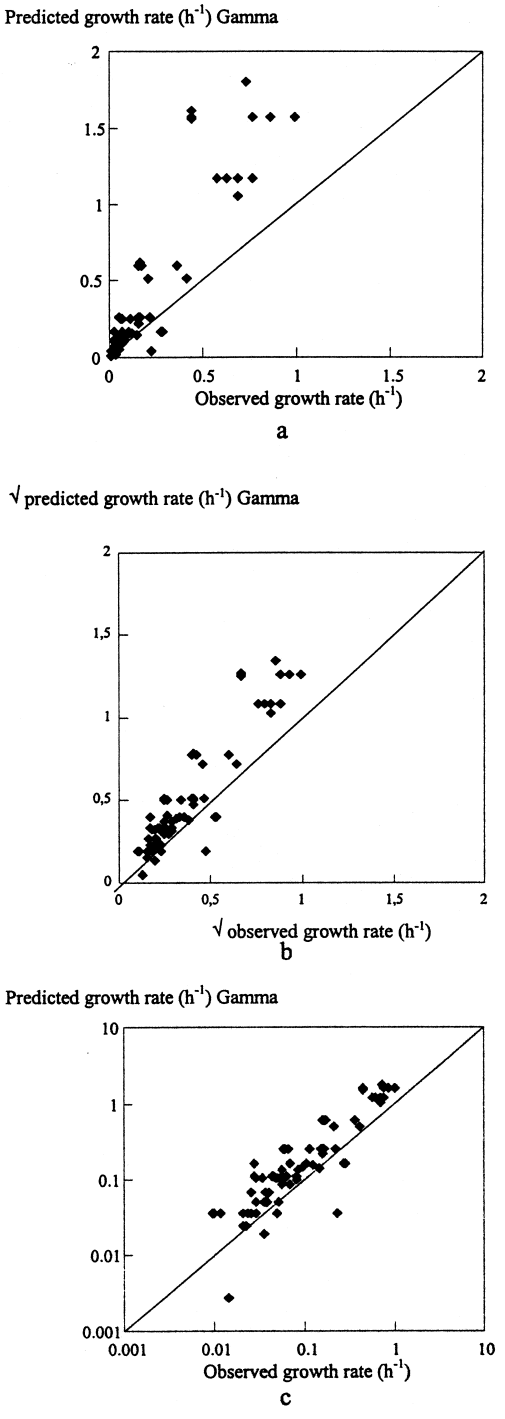

Fig. 1. Comparison of published growth rates (h^{-1}) and those predicted using the Gamma concept for *Listeria* in milk. (a) Linear scale; (b) square root; (c) logarithmic scale.

of showing the growth rate on a log scale is that the points are more evenly spread over a larger range. Furthermore, it is not unusual that predictions deviate by a factor 10 from observations. A disadvantage is that structural deviations, as observed in Fig. 1a, may remain unnoticed in a log plot, Fig. 1c. Therefore, it is useful to examine untransformed data as well as log or square root transformed data.

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 (0.001–1.0) is predicted well. Most points fall close to the line of equivalence, i.e. the predicted value is equal to the observed value, 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. Growth predictions under sub-optimal conditions, e.g. low temperatures, are however relevant for the food industry as this situation most likely occurs in the industry. In a recent study, PMP and FMM were reported not to accurately predict the growth of *L. monocytogenes* in various types of seafood. It has been suggested that expanding the models with additional factors such as lactate and phenol may provide more accurate predictions by Dalgaard and Jørgensen (1998).

In Fig. 2, the predictive ability of the various models is compared for growth of *Listeria* on meat. No difference between raw and cooked meat products nor between different types of meat (pork, beef, chicken) could be noted (data not shown). The three general models tested predict development of the organism equally well, the orders of magnitude estimated for growth rate were comparable. The Gamma concept can be used over a wider range of conditions than the others since it is based on the limits of growth. As can be observed in the graph, at very low temperatures or if a combination of conditions becomes unfavourable for growth, both PMP and FMM cannot be used to predict growth whereas small growth rates could be predicted applying the Gamma concept. However, the model predicts in most of these cases slower growth than actually occurs. For practical application these growth limit-

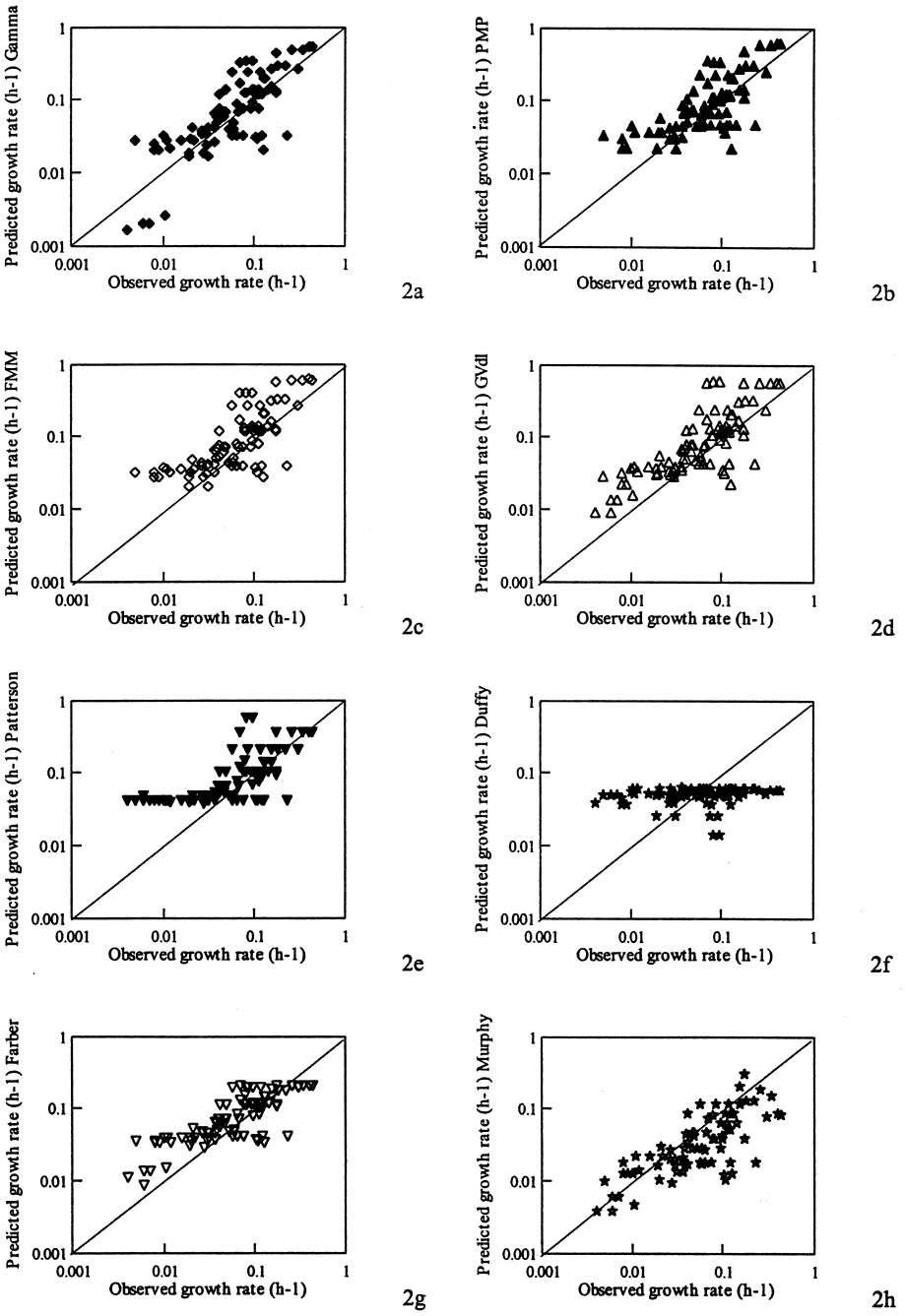

Fig. 2. Evaluation of eight models for prediction of growth of *Listeria monocytogenes* on meat (products).

ing conditions often are especially important. PMP and FMM do not predict growth of *L. monocytogenes* below 4 and 1°C, respectively. A third order polynomial model as applied in the model of Murphy et al. (1996) does not give better predictions for this organism.

For the specific models similar trends were observed, except for the model of Duffy et al., 1994.

This model was developed to describe the growth of *Listeria* on meat at 5°C. Therefore, it cannot be extrapolated for use at other temperatures. Only the effects of a_w and pH were taken into account in this model, while temperature is generally the most important controlling factor in most food products as can be observed in Table 2. Fig. 2 shows that if only temperature is taken into account as in a model published by Patterson et al. (1993), the model can still provide a reasonable description of experimental data.

Although the specific models were devised to predict behaviour of *L. monocytogenes* on meat, the results for other foods indicate that reasonable estimations for growth could be made.

The results of our study are comparable with those reported by others. Reasonable agreement between observed growth rates available in literature and predicted growth rates has been shown for several spoilage bacteria and pathogens, e.g. *Brochothrix thermosphacta* (McClure et al., 1993), *St. aureus* (Sutherland et al., 1994; Walls et al., 1996), *B. cereus* (Sutherland et al., 1996), *Cl. botulinum* (Graham et al., 1996), *L. monocytogenes* (Wijtzes et al., 1993; Dalgaard and Jørgensen, 1998) and *Y. enterocolitica* (Sutherland and Bayliss, 1994). In general, published growth rates were slower than predicted values, i.e. in most cases fail-safe predictions are given. This is not unexpected, since in most cases rich, liquid, broth media were used to develop models. This provides optimal growth conditions and models based on data generated in this way tend to give fail-safe predictions.

Table 3
Evaluation of general models predicting the growth of *Listeria monocytogenes* on foods according to various mathematical/statistical characteristics

Product (no.)[a]		Models		
		Gamma	PMP[b]	FMM[c]
Meat (92)	MSE	0.0066	0.0090	0.0105
	r^2	0.63	0.64	0.62
	Bias	0.84	0.75	0.74
	Accuracy	1.78	1.74	1.73
Fish (22)	MSE	0.0034	0.0056	0.0058
	r^2	0.85	0.86	0.87
	Bias	0.91	0.64	0.77
	Accuracy	1.78	1.82	1.65
Egg (15)	MSE	0.1105	0.1856	0.1512
	r^2	0.15	0.13	0.15
	Bias	0.35	0.30	0.30
	Accuracy	3.01	3.31	3.28
Milk (98)	MSE	0.1549	0.3549	0.1409
	r^2	0.90	0.87	0.85
	Bias	0.57	0.45	0.56
	Accuracy	1.98	2.31	1.91
Dairy products (18)	MSE	0.0864	0.1237	0.0741
	r^2	0.68	0.60	0.66
	Bias	0.54	0.42	0.49
	Accuracy	2.41	3.00	2.39
Cheese (32)	MSE	0.1047	0.0812	0.1013
	r^2	0.58	0.80	0.53
	Bias	0.98	1.15	0.90
	Accuracy	1.84	1.92	2.01
Vegetables (21)	MSE	0.2504	0.6437	0.2852
	r^2	0.80	0.74	0.83
	Bias	0.35	0.29	0.32
	Accuracy	2.87	3.48	3.10

[a] No. = number of data.
[b] PMP = pathogen modeling program.
[c] FMM = Food MicroModel.

5.2.2. Mathematical comparison

The mathematical and statistical comparisons of the general and specific models for prediction of growth of *L. monocytogenes* on food (products) are presented in Tables 3 and 4, respectively. Comparison of the MSE for the general models showed that, overall, the Gamma concept produced the closest prediction of the growth data. However, the differences were often small. The range of estimated standard errors was 0.003 to 0.25 for the Gamma concept, 0.006 to 0.64 for PMP and 0.006 to 0.29 for FMM. The results presented in Table 4 for the specific models reveal similar trends. Table 5 shows the statistical validation of the general models by the *F*-ratio test. The results indicate that values were not significantly different from the measurement error for meat (Gamma model and PMP) and fish (all three models shown in italics in Table 5). If the *F*-ratio test is accepted, this indicates that the models describe the observed growth rates quite well, since the test is quite rigorous. The measurement error used is based on replicate experiments performed under equal controlled conditions, this cannot be expected when growth rates are taken from the literature. For the other types of food, the variance was significantly larger than a typical growth rate

Table 4
Evaluation of specific models predicting the growth of *Listeria monocytogenes* on foods according to various mathematical/statistical characteristics

Product (no.)[a]		Models				
		GVdl[b]	Patterson	Duffy	Farber	Murphy
Meat (92)	MSE	0.0146	0.0081	0.0077	0.0036	0.0063
	r^2	0.48	0.35	0.04	0.47	0.31
	Bias	0.68	0.68	1.06	0.76	1.56
	Accuracy	1.83	1.91	2.36	1.74	1.84
Fish (22)	MSE	0.0054	0.0007	0.0032	0.0014	0.0013
	r^2	0.86	0.84	0.01	0.57	0.81
	Bias	0.72	0.76	0.70	0.78	1.83
	Accuracy	1.70	1.61	2.20	1.64	1.94
Egg (15)	MSE	0.2920	0.1177	0.0056	0.0069	0.1005
	r^2	0.15	0.24		0.14	0.21
	Bias	0.24	0.30	1.14	0.55	0.28
	Accuracy	4.25	3.28	2.01	2.15	3.56
Milk (98)	MSE	0.1508	0.0289	0.1460	0.1461	0.1409
	r^2	0.90	0.90	0.21	0.12	0.85
	Bias	0.54	0.74	1.88	1.83	1.76
	Accuracy	1.97	1.54	3.24	3.38	1.97
Dairy products (18)	MSE	0.1285	0.0428	0.0154	0.0161	0.1480
	r^2	0.57	0.62	0.05	0.02	0.59
	Bias	0.26	0.34	1.17	0.47	1.18
	Accuracy	4.04	3.25	1.37	3.32	1.58
Cheese (32)	MSE	0.0880	0.1050	0.2632	0.2381	0.2043
	r^2	0.59	0.65	0.10	0.003	0.09
	Bias	0.63	0.86	2.28	1.14	0.71
	Accuracy	2.11	1.80	3.85	2.33	2.05
Vegetables (21)	MSE	0.2543	0.0925	0.0374	0.0334	0.0608
	r^2	0.85	0.74	0.29	0.01	0.43
	Bias	0.32	0.42	1.73	1.03	0.74
	Accuracy	3.11	2.43	2.98	2.82	1.76

[a] No. = number of data.
[b] GVdl = Grau and Vanderlinde.

variance. For the other general and specific models similar results were obtained (data not shown).

The r^2 statistic is often used as an overall measure of fit attained. It represents the fraction of the variation that is explained by a model. The higher the r^2, the better the data are predicted by a model. For the general models, the r^2 values were comparable and ranged from 0.13– 0.15 for egg products to 0.85–0.90 for milk products (Table 3). The r^2 values observed for the specific models are, in general, lower than for the general models for all types of food (Table 4).

The indices bias and accuracy provide an objective indication of model performance. In contrast to the traditional statistical methods, these indices are not based on the deviation between observed and mean response. This causes a problem in evaluating performance of models by novel data because the main response is not known. The bias and accuracy factor test the hypothesis that the model under evaluation predicts the true mean or represents it better than another model (Ross, 1996). It has been shown that these factors were valuable tools for evaluation of the performance of predictive models (Neumeyer et al., 1997; Dalgaard and Jørgensen, 1998).

In calculating the bias factor, over- and underprediction are given equal weight in determining the average deviation. A bias factor less than one

Table 5
Statistical analysis of the Gamma concept, pathogen modeling program (PMP) and food micromodel (FMM) used for predicting the growth of *Listeria monocytogenes* on foods

Model	f^{a}_{Gamma}	f_{PMP}	f_{FMM}	F^{b}
Meat	*1.03*	*1.41*	1.65	1.56
Fish	*0.53*	*0.88*	*0.91*	1.78
Egg	17.3	29.1	23.7	1.89
Milk	24.3	55.6	22.1	1.56
Dairy products	13.5	19.4	11.6	1.84
Cheese	16.4	12.7	15.9	1.70
Vegetables	39.2	100.9	44.7	1.80

[a] $f = MS_{model}/MS_{meas.error}$ [$MS_{meas.error} = 0.00638$ (Zwietering et al., 1994; Houtsma et al., 1996)]; MS_{model} = mean square error = RSS/DF; DF = degrees of freedom (DF equals the number of data, since no parameters were estimated); RSS = residual sum of squares.
[b] $F = F$-table value (95% confidence).

indicates that a model is, in general, fail-safe. The general models predict for most food products faster growth than in fact occurs, except for estimations by the PMP for multiplication of the organism in cheese. The values were about 0.3 for growth in egg products and vegetables, indicating that the models are very conservative because they predict generation times, on average, one third of that actually observed. Values of about 0.8–0.9 were reached for growth predictions in/on meat, fish and cheese. The model of Grau and Vanderlinde (1993) and the model described by Patterson et al. (1993) also give fail-safe predictions, while for the models published by Duffy et al. (1994); Farber et al. (1996) bias factors of more than 1 were calculated for most foods. In previous reports bias factors close to 1 have been noted for *St. aureus*, while values ranging from 0.52 to 1.15 for psychrotrophic pseudomonads were published (Neumeyer et al., 1997). For behaviour of *L. monocytogenes* on seafood bias factors ranging from 1.0 to 3.9 have been reported, depending on the type of product (Dalgaard and Jørgensen, 1998).

The bias factor provides no indication of the average accuracy of estimates because under- and over-prediction tend to cancel out. Therefore, the accuracy factor can be calculated. As shown in Tables 3 and 4 the values for the different models depended on the type of product and ranged from 1.7 to 3.5 for the general models and from 1.4 to 4.3 for the specific models. For example, an accuracy factor of 1.7 indicates that on average the predictions differ from observations by 70%. In other publications accuracy factors calculated based on experiments varied from 1.26 for *St. aureus* (Ross, 1996) and from 1.1 to 1.4 for growth of psychrotrophic pseudomonads (Neumeyer et al., 1997). These values were derived from data obtained in experiments performed in simple, homogenous systems and thus represent a high degree of control. The model accuracy decreased as the degree of experimental control was reduced. Using non-sterile, inhomogenous foods or literature data resulted in lower levels of confidence. Dalgaard and Jørgensen (1998) calculated accuracy factors ranging from 1.4 to 4.0 for growth rates of *L. monocytogenes* in various types of seafood. For *St. aureus* an accuracy factor of 1.53 (Ross, 1996) and for pseudomonads values of about 1.3 (Neumeyer et al., 1997) were reported based on literature data. Orders of magnitude can be predicted and in many cases the accuracy of the estimates will be sufficient to take management decisions. This is illustrated by the example presented in Section 5.3.

The low agreement between the predicted growth rates and measured values for eggs is most likely due to the presence of natural antimicrobial components in these products, e.g. lysozyme, conalbumin and avidin (Jay, 1996). The protective cover of many fruits and vegetables and the low pH values, below which many micro-organisms cannot grow are important factors in inhibiting growth of bacteria. In addition, naturally occurring substances are present in these type of products e.g. essential oils in herbs and spices, hydroxycinnamic acid derivatives in fruits and vegetables and glucosinolates in cruciferous plants (cabbage, broccoli etc.) yielding isothiocyanate upon mechanical disruption, which possesses antibacterial activity (Jay, 1996).

For the products for which the F test was not accepted and/or the bias factor was lower than 0.9, the growth rates were corrected by a certain factor to obtain a bias factor of 1, i.e. on average predicted values are equal those observed. In this way, an extra γ-factor is introduced in Eq. (2). This γ is dependent on the type of product (for egg and vegetables 0.35, for milk 0.57 and for dairy products 0.54). To have a safety margin resulting in more fail-safe predictions it is also possible to correct growth rates to a bias factor of 0.9. The growth rates and the mathematical and statistical indices were in egg, milk, dairy products and vegetables were calculated again by the Gamma concept (Table 6). In Fig. 3 the corrected

Table 6
Use of corrected growth rates calculated by the Gamma concept, to obtain a bias factor of 1, for predicting the growth of *Listeria monocytogenes* on foods

Model	γ Product	MSE	Accuracy	f^{a}_{Gamma}	F^{b}
Egg	0.35	0.0100	2.58	1.57	1.89
Milk	0.57	0.0117	1.43	1.83	1.56
Dairy products	0.54	0.0114	2.14	1.79	1.84
Vegetables	0.35	0.0059	1.43	0.92	1.80

[a] $f = MS_{model}/MS_{meas.error}$ [$MS_{meas.error} = 0.00638$ (Zwietering et al., 1994; Houtsma et al., 1996)]; MS_{model} = mean square error = RSS/DF; DF = degrees of freedom (DF equals the number of data, since no parameters were estimated); RSS = residual sum of squares.
[b] $F = F$-table value (95% confidence).

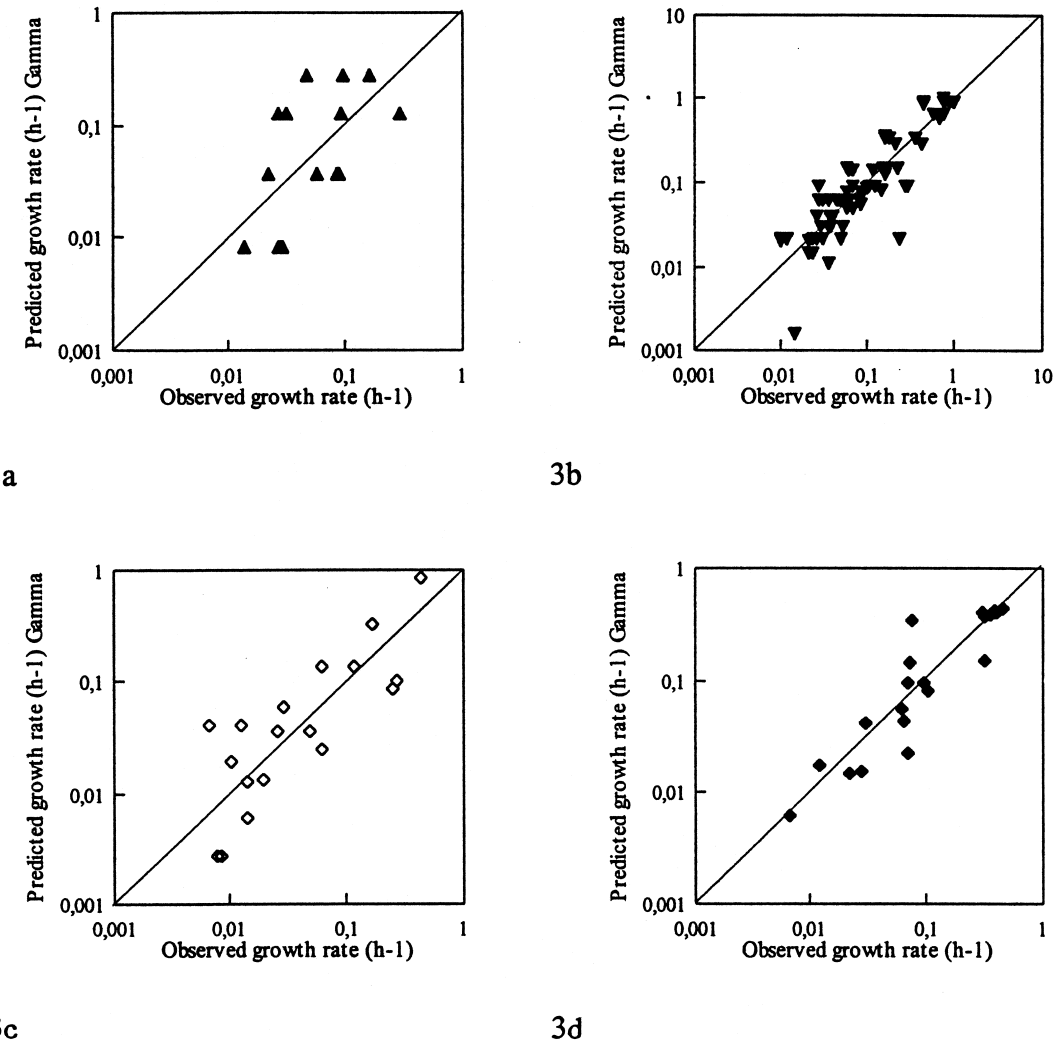

Fig. 3. Comparison of published growth rates (h^{-1}) and corrected growth rates calculated using the Gamma concept for eggs (a), milk (b), dairy products (c) and vegetables (d).

growth rates are plotted to the observed values for the different product groups. As observed in Table 6 and Fig. 3, the correction of growth rates led to better predictions: a decrease in the MSE, lower values for the accuracy factor and acceptance of the F ratio test for three of the four product groups. Only for milk the F ratio test was not accepted, however, the difference between the test value and the F-table value was very small.

5.3. Application of predictive models for determination of important processing steps

In Fig. 4 a prediction of the development of *L. monocytogenes* in a process for the production of sliced, cooked ham is presented. It can be observed that the models give quantitatively different results. However, the conclusion reached is the similar, i.e. in all three cases the risk determining steps were the same. These were mainly cooling and storage steps. The example shows that a model containing only the effect of temperature can already make reasonable estimations compared with other models. This is due to the fact that temperature is, as shown by calculating the γ-factors, quantitatively the only important factor inhibiting growth of *Listeria* in most food products. Prediction with various models can increase confidence in the prediction, without large time investment.

6. Conclusions

No standard method or set of criteria has been published by which a model can be validated. In this paper, the accuracy of general and specific models describing growth of *L. monocytogenes* was evaluated by various criteria. The use of one criterium to evaluate predictive ability of models may fail to reveal some forms of systematic deviation between observed and predicted behaviour. Therefore, the application of a set of criteria is recommended for assessment of performance of models. The bias and accuracy factor can be used to provide an indication of the performance of models. However, it is important to examine measured and predicted growth rates graphically, with both untransformed and log or square root transformed data, to be able to observe trends and structural deviations.

With the use of the Gamma concept the growth rate can be determined for various organisms as function of various variables. Furthermore, the effect

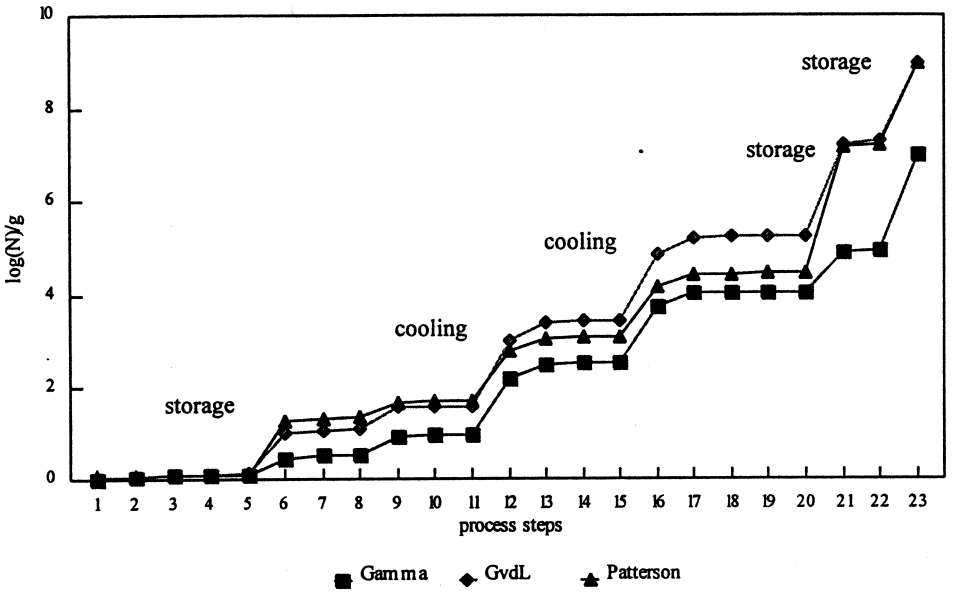

Fig. 4. Growth (log N g^{-1}; $N_0 = 1$ g^{-1}) of *Listeria monocytogenes* on cooked sliced ham during the production process predicted by three models.

of the various hurdles can be quantified by separating the respective effects. The Gamma model is shown to be at least as good as other models. It was demonstrated that there was reasonable agreement between the various sets of data though marked differences exist in terms of experimental conditions, strains and growth media used in the experiments.

Predictive microbiology enables quantitative estimation of growth of micro-organisms. However, it is important that users of models do understand the limitations of performance of models. Critical use of models is necessary, all results and all assumptions should be criticised. Only the order of magnitude of growth can be predicted by models but in most cases this is sufficient. Models can be used to support decisions, prevent experiments, design experiments, and perform the relevant experiments. A model is a useful 'discussion partner' giving you good ideas, pointing you in the right direction, but like other discussion partners is not always right.

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