Development of a new mathematical modelling approach for prediction of growth kinetics of *Listeria monocytogenes* in milk

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Summary

The main objective of the present study was to develop a new modelling method, inverse dynamic modelling approach, as an alternative to two-step modelling approach, which is traditionally used *n* predictive food microbiology. For this purpose, the growth data of *Listeria monocytogenes* in milk subjected to isothermal and non-isothermal storage conditions were gathered from previously published growth curves. The bacterial growth data were described as a function of time and temperature using the direct two-step, direct one-step and inverse dynamic modelling approaches based on the Baranyi and Huang models. Maximum specific growth rate (μ_{max}) and lag phase duration (λ) estimated by different modelling approaches and primary models were statistically compared. Results revealed that there was no significant difference ($p > 0.05$) between the growth kinetic parameters obtained from direct and inverse modelling approaches. The prediction capability of inverse dynamic modelling approach was validated by externally gathering growth curves. The inverse dynamic modelling approach provided satisfactory statistical indices (0.99 > Bias factor > 1.10 and 1.16 > Accuracy factor > 1.19), meaning that it can be reliably used as an alternative way of describing the growth behaviour of *Listeria monocytogenes* in milk in a fast way with a minimal labour requirement.

Keywords

inverse dynamic modelling; milk; growth kinetic; food safety; predictive microbiology

Milk is a nutrient-rich and health-promoting beverage, but it is easily perishable within a short time. Furthermore, milk may be potentially risky due to being a favourable environment for growth of pathogenic bacteria if it is not properly processed, packaged, distributed and stored [1]. Among food-borne pathogenic bacteria, *Listeria monocytogenes* is one of the most abundant one that can be isolated from milk and milk products [2]. Actually, *L. monocytogenes* is expected to be efficiently inhibited by pasteurization process in case that cross contamination occurs in any time before consumption, and milk may become a potentially unsafe beverage [2]. In such case, although temperature control is crucial, the conditions to which milk subjected during its transportation and marketing are beyond the manufacturer's direct control, and it frequently deviate from the specifications set. Additionally, there is no temperature control from the time the products leave the retail store to the time of domestic storage and consumption. Uncontrolled temperature conditions can enable even a few cells of *L. monocytogenes* to reach high numbers (> 2 log CFU·ml^{-1}) that may lead to serious health risks [3].

Predictive food microbiology is a relatively new field of theoretical research in food microbiology that combines traditional food microbiology knowledge with the disciplines of mathematics and statistics to describe the behaviour of bacteria in food under variable environmental conditions [4]. Through modelling studies in predictive food microbiology, undesirable microbial effects that cause food-borne diseases can be stopped or reduced. Mathematical models used in predictive microbiology are basically primary or secondary models [5]. Primary models describe behaviour of microorganism as a function of time under a static environmental condition. Secondary models are used to determine the effects of environmental factors and/or food matrixes on model parameters.

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Temperature is one of the most important environmental factors that directly affect the growth behaviour of microorganisms in foods.

The most widely used modelling approach in predictive microbiology is a two-step modelling approach in which a sequential fitting process of primary and secondary models is applied. In this process, the primary model is fitted to the growth data points. Then, the growth kinetic parameters derived from the primary model are defined as a function of environmental conditions such as temperature, pH and water activity in the secondary model [6]. Even though this modelling approach is generally satisfactory, there are some pitfalls. The major disadvantage lies in the potential accumulation and propagation of errors due to the ordered non-linear regression process performed twice [7]. The one-step modelling approach is an alternative way of describing growth behaviour by overcoming the weaknesses of a two-step process. In this approach, primary and secondary models are fitted simultaneously to all growth data points from different conditions [8, 9]. The one-step modelling approach can also be applied to fit the growth data points gathered under dynamic conditions, which enables to determine the kinetic parameters from dynamic experiments without performing isothermal experiments that are usually time-consuming and labour-intensive [10, 11].

Both two-step and one-step modelling approaches used so far in predictive food microbiology are direct modelling processes. In these modelling approaches, primary and secondary models are used for stable environmental conditions, and then the models obtained from two-step or one-step modelling approaches are evaluated considering their predictive capabilities at changing environmental conditions. As an alternative to direct modelling techniques, inverse dynamic modelling approach is a new modelling method. Rather than using isothermal experiments, which are often time-consuming and labour-intensive, this method directly uses the behaviour of microorganisms subjected to dynamically varying temperature conditions, which minimizes experimental effort in a single step, being a fast and inexpensive way of modelling [10]. Therefore, the inverse dynamic modelling approach has a considerable potential as a simulation tool to predict growth behaviour of microorganisms and can pave a new alternative way for doing experimental plan in predictive food microbiology [11]. In this regard, the advantages of the inverse dynamic modelling approach have increasingly aroused interests in predictive food microbiology field and some inspirational works were published recently [11–13].

The main objective of this study was to comprehensively evaluate and validate the prediction capability of the inverse dynamic modelling approach based on the Baranyi and Huang models by considering growth kinetic parameters of *L. monocytogenes* in milk at isothermal and non-isothermal storage conditions.

MATERIALS AND METHODS

Study structure

The work was carried out in five main separate phases: i) the growth data points of *L. monocytogenes* in milk for isothermal and non-isothermal storage conditions were gathered, ii) the two-step and one-step modelling approaches including different primary models (the Baranyi and Huang models) and secondary model of Ratkowsky were applied to obtain growth kinetics for the direct modelling approach, iii) the dynamic versions of the same models were used to obtain growth kinetics for the inverse modelling approach, iv) the growth kinetics obtained from direct and inverse modelling methods were statistically compared and v) externally collected maximum growth rate values were used for model validation. The flow chart outlining the main steps followed in the present study is shown in Fig. 1. Details of the study phases are explained in the following subsections.

Data collection

The bacterial growth data of *L. monocytogenes* in milk at isothermal and non-isothermal storage conditions were extracted from the previously published study [14]. The experimental set-up to monitor *L. monocytogenes* in milk was explained in detail in the work of Xanthiakos et al. [14]. In brief, fresh commercial pasteurized whole milk (less than 6 h after packaging) was used for inoculation to obtain 3–4 log CFU·ml^{-1}. Milk cartons were then either stored under controlled isothermal conditions (4 °C, 8 °C, 12 °C and 16 °C) or under programmed changing temperature conditions in high-precision (± 0.2 °C) low-temperature incubators (model MIR 153; Sanyo Electric, Ora-Gun, Gunma, Japan). The temperature of samples during storage was monitored using electronic temperature-monitoring devices (Cox Tracer; Cox Technologies, Belmont, North Carolina, USA). Duplicate samples from each storage temperature were taken at appropriate time intervals to allow for efficient kinetic analysis of micro-

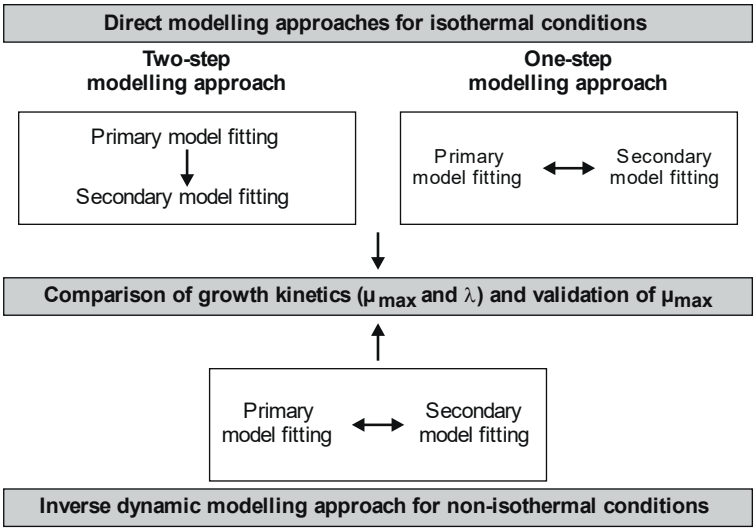

Fig. 1. The flow chart outlining the main steps followed in the present study.

μ_{max} – maximum specific growth rate of microorganisms; λ – lag phase duration.

Tab. 1. Primary models used in this work for isothermal conditions.

Model	Equation	Number
Baranyi	$y(t) = y_0 + \mu_{max}F(t) - \ln\left(1 + \frac{e^{\mu_{max}F(t)} - 1}{e^{(y_{max} - y_0)}}\right)$	(1)
	$F(t) = t + \frac{1}{v}\ln\left(e^{-vt} + e^{-\mu_{max}\lambda} - e^{(-vt-\mu_{max}\lambda)}\right)$	(2)
Huang	$y(t) = y_0 + y_{max} - \ln\left(e^{y_0} + [e^{y_{max}} - e^{y_0}].e^{-\mu_{max}B(t)}\right)$	(3)
	$B(t) = t + \frac{1}{4}\ln\left(\frac{1 + e^{-4(t-\lambda)}}{1 + e^{4\lambda}}\right)$	(4)

t – time (in hours); $y(t)$ – count of microorganisms at time t (expressed as natural logarithm of colony forming units per gram); y_0 – initial count of microorganisms (expressed as natural logarithm of colony forming units per gram); y_{max} – maximum count of microorganisms (expressed as natural logarithm of colony forming units per gram); μ_{max} – maximum specific growth rate of microorganisms (expressed as natural logarithm of colony forming units per hour); λ – lag phase duration (in hours); ν – rate of increase in the limiting substrate, assumed to be equal to μ_{max}.

Tab. 2. Primary models used in this work for non-isothermal conditions.

Model	Equation	Number
Baranyi	$\frac{dy(t)}{dt} = \mu_{max}\left[\frac{1}{1 + \exp(-Q(t))}\right]\{1 - \exp(y(t) - y_{max})\}$	(5)
	$\frac{dQ(t)}{dt} = \mu_{max}$	(6)
	$y(0) = y_0$ and $Q(0) = \ln(q_0)$	(7)
Huang	$\frac{dy(t)}{dt} = \mu_{max}\left[\frac{1}{1 + \exp[-4(t-\lambda)]}\right]\{1 - \exp(y(t) - y_{max})\}$	(8)
	$y(0) = y_0$	(9)

t – time (in hours); $y(t)$ – count of microorganisms at time t (expressed as natural logarithm of colony forming units per gram); y_{max} – maximum count of microorganisms (expressed as natural logarithm of colony forming units per gram); μ_{max} – maximum specific growth rate of microorganisms (expressed as natural logarithm of colony forming units per hour); λ – lag phase duration (in hours); y_0 – initial count of microorganisms (expressed as natural logarithm of colony forming units per gram); q_0 – initial dimensionless variable related to physiological state of the cells; $Q(t)$ – a dimensionless variable related to physiological state of the cells at time t.

bial growth. For enumeration of *L. monocytogenes*, 0.1 ml volumes of serial dilutions of milk were spread on the surface of PALCAM agar plates (Merck, Darmstadt, Germany) and incubated at 30 °C for 48 h. In the current study, the data collection process for the growth curves was performed using GetData Graph Digitizer 2.26 software (Digital River, Cologne, Germany) by which the growth data points could be extracted precisely with one decimal accuracy.

Primary models

Two different primary models, namely, Baranyi [15, 16] and Huang [16] models were employed for fitting of the growth data points obtained at isothermal and non-isothermal storage conditions using Eqs. 1–9 shown in Tab. 1 and Tab. 2, respectively.

Secondary models

The Ratkowsky model [17] was employed for the determination of relationship between storage temperature and μ_{max} using the Eq. 10:

$$\sqrt{\mu_{\max}} = b_1(T - T_0) \tag{10}$$

where T is storage temperature (in degrees Celsius), T_0 is theoretical lowest temperature at which microbial growth is observable (in degrees Celsius), $\mu_{\max}$ is maximum specific bacterial growth rate (expressed as unit per hour), b_1 is regression coefficient.

Additionally, lag phase duration (λ) was defined as a function of $\mu_{\max}$ with respect to temperature using the Eq. 11 [18]:

$$\lambda = \frac{b_2}{\mu_{\max}(T)} \tag{11}$$

where b_2 is regression coefficient, $\mu_{\max}(T)$ is a function of storage temperature (T).

Parameter estimation

For the direct two-step and one-step modelling approaches, the parameters were calculated by means of NonLinearModel command, which uses Levenberg Marquardt algorithm, in the Matlab 8.3.0.532 (R2014a) software (MathWorks, Natick, Massachusetts, USA). Determination of suitable starting values in non-linear regression procedure is essential step to estimate the accurate parameters.

For the inverse dynamic modelling approach, each of the parameters (b_1, b_2 and T_0) was estimated with ga command, which uses genetic algorithm in Global Optimization Toolbox, in the Matlab software to minimize difference between observed and fitted growth data using the objective function given by Eq. 12:

$$obj = \min \sum_{i=1}^{n} [f_{\text{fit}}(b_1, b_2, T_0) - f_{\text{obs}}]^2 \tag{12}$$

where *obj* is the objective function, minimization of difference between f_{fit} and f_{obs}, f_{fit} are the fitted values that satisfy the objective function, f_{obs} is the observed growth data and n is the number of experiments.

Since the ordinary differential equations in the dynamic models of Baranyi and Huang have no analytical solution [10], numerical solution was obtained by using the function ode23 in Matlab, which is based on the Runge–Kutta method.

The 95% confidence intervals (*CI*) of the model parameters were calculated using Eq. 13 [16]:

$$CI = \text{par} \pm t_n\sqrt{S} \tag{13}$$

where par is the parameter estimated by fitting, t_n is calculated using the inverse of Student's t cumulative distribution function, S is a vector of the diagonal elements from the estimated covariance matrix of the coefficient estimates, $S = (X^{\mathrm{T}}X)^{-1}s^2$, X is the Jacobian of the fitted values with respect to the parameters, X^{T} is the transpose of X and s^2 is the mean squared error.

Comparison of the goodness of fit

The comparison of the performance of the models was carried out by using the root mean square error (*RMSE*), adjusted coefficient of determination (R^2_{adj}), corrected Akaike information criterion (AIC_C) and Bayesian information criterion (*BIC*) using Eqs. 14–17, respectively:

$$RMSE = \sqrt{\sum_{i=1}^{n} \frac{(x_{\text{obs}} - x_{\text{fit}})^2}{n - s}} \tag{14}$$

$$R^2_{\text{adj}} = 1 - \left(\frac{n-1}{n-s}\right)\left(\frac{SSE}{SST}\right) \tag{15}$$

$$AIC_{\text{C}} = (n)\ln\left(\frac{SSE}{n}\right) + 2(s+1) + \\ + \frac{2(s+1)(s+2)}{n-s-2} \tag{16}$$

$$BIC = n\ln\left(\frac{SSE}{n}\right) + s\ln(n) \tag{17}$$

where x_{obs} is experimental bacterial growth, x_{fit} is the fitted value, n is the number of experiments, s is the number of parameters of the model, *SSE* is the sum of squares of errors and *SST* is the total sum of squares.

Both Baranyi and Huang models use ln scale to fit the counts of microorganisms, but reporting

microbial outcomes is frequently done in log scale. Therefore, conversion from ln scale to log scale was done throughout the study to report all goodness-of-fit results.

Statistical analysis

Growth kinetic parameters obtained from all modelling approaches and primary models were subjected to one-way analysis of variance (ANOVA) using the Matlab software. Statistical differences between the means of growth kinetic parameters were determined by post hoc analysis using Tukey's multiple range test. The differences between the means were regarded as statistically significant when $p \leq 0.05$.

Validation of the models

The prediction capability of individual modelling approaches and primary models was evaluated through independent maximum specific growth rate data of *L. monocytogenes* in milk collected from ComBase database (University of Tasmania, Hobart, Australia). Arbitrary selected eight *L. monocytogenes* growth curves for milk products stored at temperatures ranging from 4 °C to 15 °C were used to test the prediction performance of the models. The comparison was done considering bias (B_f) and accuracy (A_f) factors [19] are given in Eqs. 18 and 19, respectively:

$$B_f = 10^{\frac{\sum_{i=1}^{n} \log(x_{pred}/x_{obs})}{n}} \quad (18)$$

$$A_f = 10^{\frac{\sum_{i=1}^{n} \log(x_{pred}/x_{obs})}{n}} \quad (19)$$

where x_{pred} refers to predicted μ_{max} (expressed as natural logarithm of colony forming units per hour), x_{obs} refers to experimental μ_{max}, n refers to the number of experimental growth data.

RESULTS AND DISCUSSION

The experimental *L. monocytogenes* counts collected from previously published curves for milk at the storage temperatures of 4 °C, 8 °C, 12 °C and 16 °C [14] were used to implement the two-step and one-step modelling approaches based on the Baranyi and Huang models (Fig. 2, Fig. 3). The initial bacterial counts of *L. monocytogenes* were on average 3.6 ± 0.1 log CFU·g^{-1} (mean ± 95% *CI*) for all temperatures. Storage duration was directly related to storage temperature and ranged from 792 h to 144 h (33 days to 6 days) with an increase in storage temperature from 4 °C to 16 °C. The *L. monocytogenes* counts could reach the level ranging from 7.5 ± 0.1 log CFU·g^{-1} to 8.6 ± 0.1 log CFU·g^{-1} (mean ± 95% *CI*) at the end of storage depending on the storage temperature. This demonstrated that the growth potential of *L. monocytogenes* in milk was enhanced with the increasing storage temperature.

The goodness-of-fit of both primary models involved in the traditionally used two-step modelling approach was evaluated by calculating their statistical indices (*RMSE*, R^2_{adj}, AIC_C and *BIC*). The *RMSE* values obtained from the two-step modelling approach were 0.699 and 0.909 for the

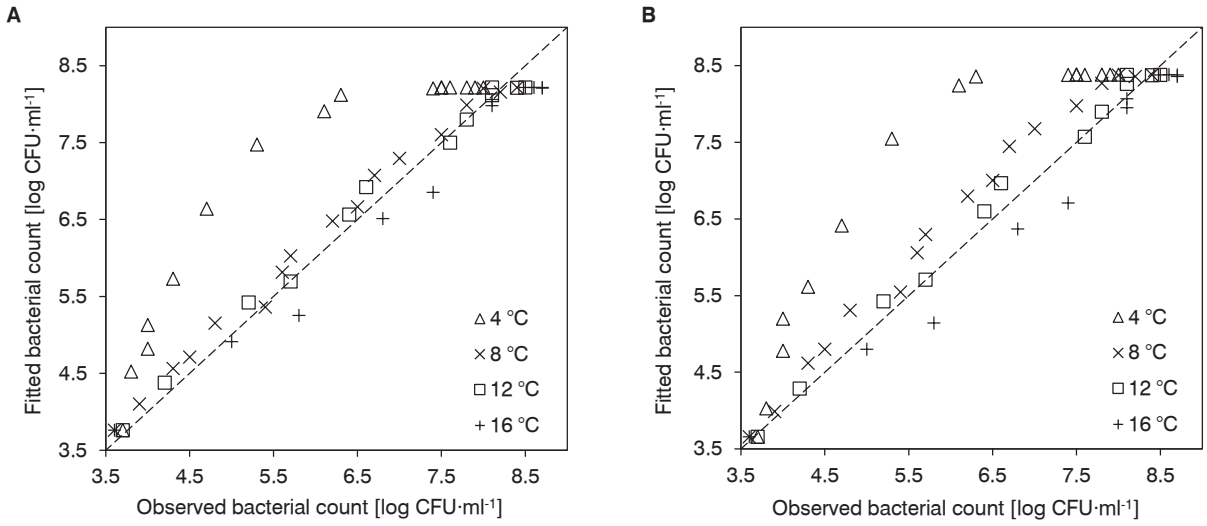

Fig. 2. The observed and fitted *Listeria monocytogenes* counts in milk using direct two-step modelling approach.

A – Baranyi model, B – Huang model.

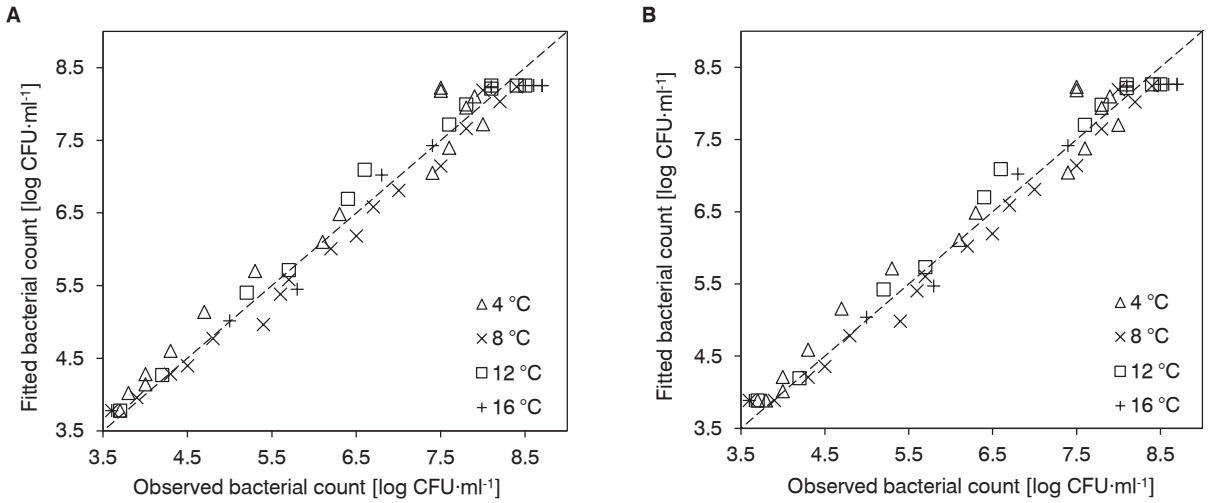

Fig. 3. The observed and fitted *Listeria monocytogenes* counts in milk using direct one-step modelling approach.

A – Baranyi model, B – Huang model.

Baranyi and Huang models, respectively, and the R^2_{adj} values were 0.825 and 0.705 for the Baranyi and Huang models, respectively (Tab. 3). The Baranyi model yielded lower *RMSE* and higher R^2_{adj} values. This means that the fitting capability of the Baranyi model was better than that of the Huang model when direct two-step modelling was used to describe the growth behaviour of *L. monocytogenes* in milk. In addition, the AIC_C and *BIC* indices of Baranyi model were –32.3 and –25.8, respectively, while the AIC_C and *BIC* indices of Huang model were –2.4 and 4.1, respectively. These results indicated that the Baranyi model was more successfully to estimate growth behaviour of *L. monocytogenes* in milk in the direct two-step modelling approach (Tab. 3).

All the statistical indices (*RMSE*, R^2_{adj}, AIC_C and *BIC*) obtained from the one-step modelling approach indicated that the fitting capability of the Baranyi model for the one-step modelling approach was better than that of the Huang model, analogically as it appeared in the two-step modelling approach (Tab. 3). In addition, the statistical evaluation regarding the fitting capability of the primary models based on the one-step modelling approach showed that the fitting capability each of the primary models was better than that of the traditionally used two-step modelling approach (Tab. 3). These results showed that the one-step modelling approach enhanced the fitting performance of the models and could be more reliably used for estimation of *L. monocytogenes* in milk regardless of the primary models. Additionally, the lack of fitting capability of the direct two-step modelling approach for 4 °C was eliminated by implementation of the direct one-step modelling approach (Fig. 3).

The degrees of freedom of the one-step modelling approach proposed in this study was 52 (the number of observations – the number of parameters in the global model), while the degrees of freedom of the traditional two-step modelling approach used by Xanthiakos et al. [14] was only 3 for the Ratkowsky model and was a maximum of 12 for the primary models at various temperatures ranging from 4 °C to 16 °C. It is important to underline that in particular the Ratkowsky model with a low number of degrees of freedom may be regarded as providing results that are suspicious and uncertain. From this point of view, the one-step modelling approach has a higher number of degrees of freedom, which decreases confidence intervals and uncertainty of the parameters compared to the traditionally used two-step modelling approach. Therefore, regardless of the primary model, the one-step modelling approach significantly ($p < 0.05$) improved the prediction capability of the models for quantitative description of *L. monocytogenes* in milk.

When the one-step modelling approach was used, the minimum counts of *L. monocytogenes* in milk were predicted to be 3.8 ± 0.2 log CFU·g^{-1} and 3.9 ± 0.1 log CFU·g^{-1} (mean ± 95% *CI*) for the Baranyi and Huang models, respectively. The experimental minimum counts were between 3.6 log CFU·g^{-1} and 3.7 log CFU·g^{-1} corresponding to an average of 3.7 ± 0.1 log CFU·g^{-1} (mean ± 95% *CI*), which showed that the Baranyi

Tab. 3. Growth kinetic parameters (with 95% confidence interval) estimated using combinations of modelling approaches and primary models.

Primary models	Modelling approach	Model parameters		*RMSE*	R^2_{adj}	*AICc*	*BIC*
		T_0 [°C]	b_1 [°C^{-1}, h$^{0.5}$]	b_2 [–]				
Baranyi	Direct two-step	–4.9 (–5.4, –4.3) ^{a}	0.019 (0.019, 0.020) ^{ab}	0.98 (0.73, 1.23) ^{a}	0.6994	0.8254	–32,3	–25,8
	Direct one-step	–1.2 (–1.5, –0.8) ^{a}	0.025 (0.024, 0.026) ^{c}	1.72 (0.51, 2.94) ^{a}	0.3129	0.9651	–124,0	–117,5
	Inverse dynamic	–2.0 (–2.0, –1.9) ^{a}	0.023 (0.023, 0.024) ^{bc}	1.03 (0.96, 1.10) ^{a}	0.1826	0.9888	–232,5	–192,5
Huang	Direct two-step	–2.8 (–4.9, –0.7) ^{a}	0.018 (0.015, 0.021) ^{a}	0.83 (0.62, 1.04) ^{a}	0.9091	0.7051	–2,4	4,1
	Direct one-step	–1.2 (–1.5, –0.8) ^{a}	0.026 (0.024, 0.028) ^{c}	1.78 (0.90, 2.67) ^{a}	0.3160	0.9648	–120,6	–114,1
	Inverse dynamic	–4.7 (–5.0, –4.5) ^{a}	0.020 (0.020, 0.021) ^{ab}	1.54 (1.50, 1.58) ^{a}	0.2663	0.9762	–179,7	–175,5

Superscripts with different letters show that values within the same model parameter are significantly different ($p \leq 0.05$).
RMSE – root mean square error, R^2_{adj} – adjusted coefficient of determination, *AIC* – Akaike information criterion, *BIC* – Bayesian information criterion.

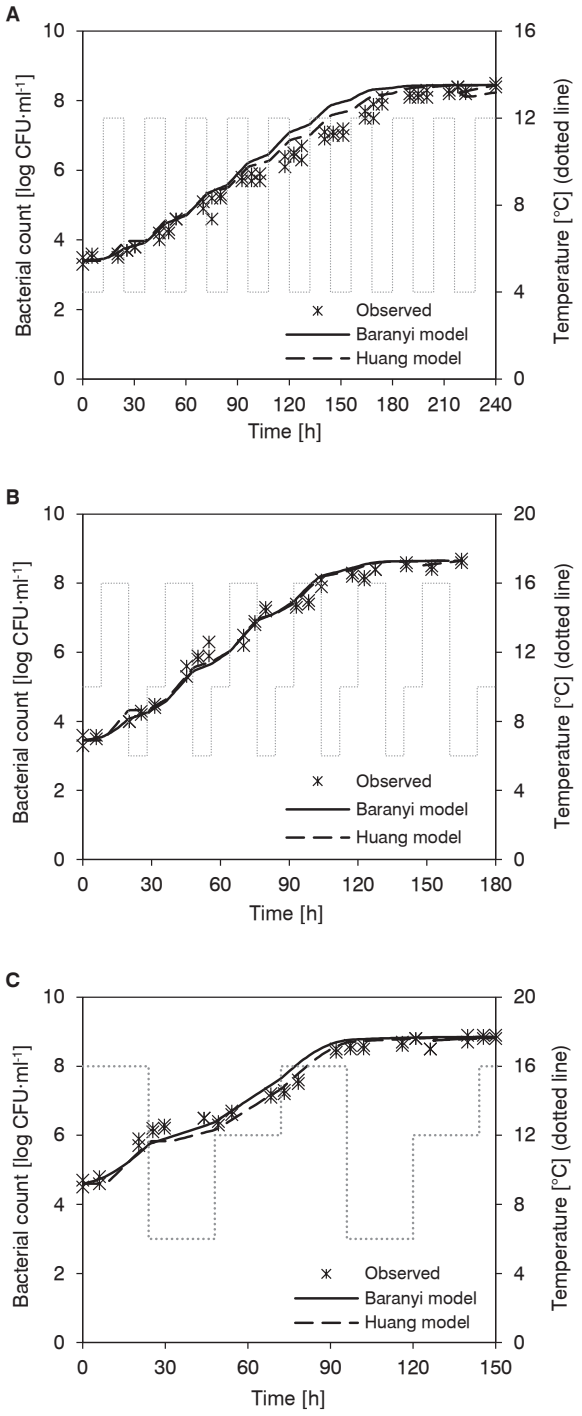

Fig. 4. The observed and fitted *Listeria monocytogenes* counts in milk using inverse dynamic modelling approach.

A – temperature profile 1, B – temperature profile 2, C – temperature profile 3.
Temperature profile 1 refers to cycling that is 12 h at 4 °C and 12 h at 12 °C.
Temperature profile 2 refers to cycling that is 8 h at 10 °C, 12 h at 16 °C and 8 h at 6 °C.
Temperature profile 3 refers to cycling that is 24 h at 16 °C, 24 h at 6 °C and 24 h at 12 °C.
The dashed line shows the changing temperature during storage.

model provided better prediction performance for maximum counts in comparison with the Huang model.

The one-step modelling approach predicted the maximum counts of *L. monocytogenes* to be 8.3 ± 0.3 log CFU·g^{-1} and 8.1 ± 0.3 log CFU·g^{-1} (mean ± 95% *CI*) for the Baranyi, and Huang models, respectively. The maximum counts were experimentally found to be within the range of 8.4–8.7 log CFU·g^{-1}. This indicated that each primary model successfully estimated maximum counts of *L. monocytogenes* in milk.

The experimental *L. monocytogenes* counts in milk at dynamic storage temperatures ranging from 4 °C to 16 °C were used for dynamic inverse modelling approach based on Baranyi and Huang models (Fig. 4). *RMSE* and R^2_{adj} values of both primary models involved in inverse dynamic modelling approach were calculated. The *RMSE* values were 0.183 and 0.267 for the Baranyi and Huang models, respectively, and R^2_{adj} values were 0.989 and 0.976 for the Baranyi and Huang models, respectively. The Baranyi model yielded lower *RMSE* and higher R^2_{adj} values. This means that the fitting capability of the Baranyi model was better than that of the Huang model when inverse dynamic modelling was used to describe the growth behaviour of *L. monocytogenes* in milk.

T_0 and b_1 are the Ratkowsky parameters showing the relationship between temperature and maximum growth rate of the microorganism. T_0 was equal to –2.0 ± 0.1 °C and –4.7 ± 0.3 °C (mean ± 95% *CI*) for the Baranyi and Huang models, respectively, in inverse dynamic modelling

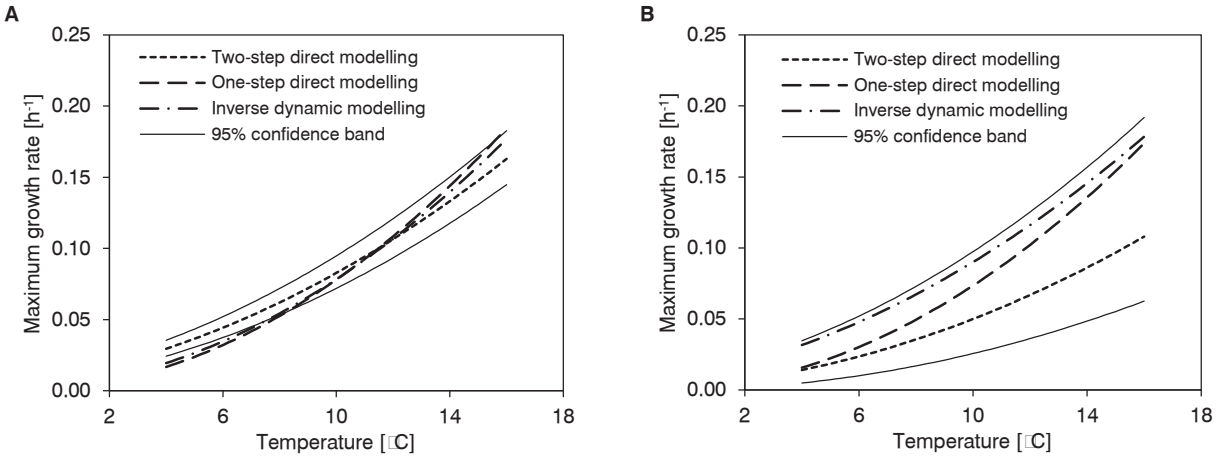

Fig. 5. The effect of storage temperature on the maximum growth rate calculated with individual models.
A – Baranyi model, B – Huang model.

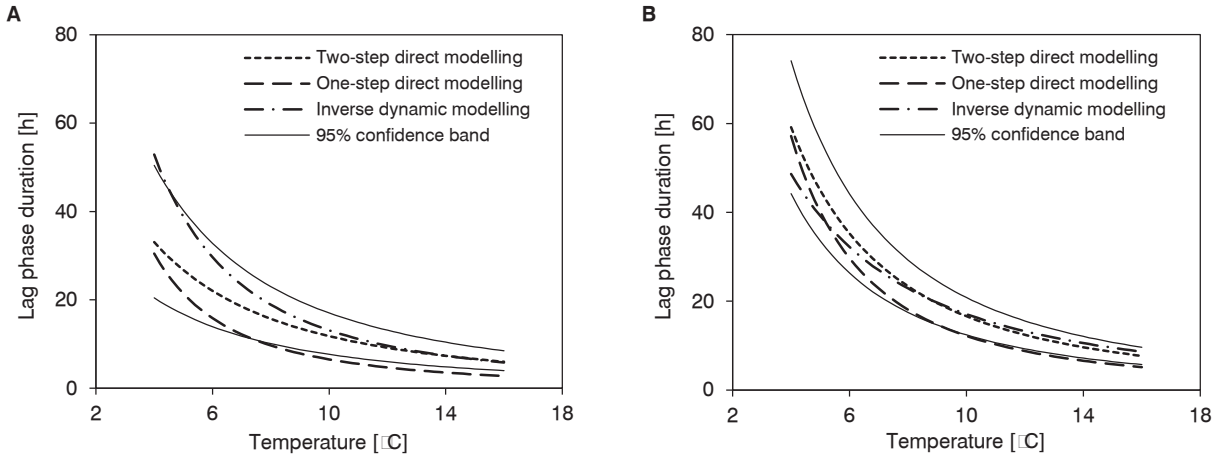

Fig. 6. The effect of storage temperature on the lag phase duration calculated with individual models.
A – Baranyi model, B – Huang model.

approach. Meanwhile, b_1 was found to range from 0.020 to 0.024 for both Baranyi and Huang models in the inverse dynamic modelling approach. These results are in a good agreement with the work conducted by XANTHIAKOS et al. [14] who reported that the values of T_0 and b_1 ranged from –1.6 °C to –3.0 °C and 0.023 to 0.25 ($°C^{-1}. h^{0.5}$), respectively, with 95% *CI*. Additionally, in this work T_0 and b_1 were calculated ranging from –0.7 °C to –4.9 °C and from 0.019 to 0.028 ($°C^{-1}. h^{0.5}$), respectively, when the direct modelling approach was employed. All these results showed that the inverse dynamic modelling approach could estimate the minimum temperature for growth of *L. monocytogenes* in milk.

Another Ratkowsky parameter, b_2 directly related to the lag phase duration of the microorganism, was calculated as 1.03 ± 0.07 and 1.54 ± 0.04 (mean ± 95% *CI*) for the Baranyi and Huang models in the inverse dynamic modelling approach, respectively. These results are within the range of findings reported by XANTHIAKOS et al. [14] who calculated b_2 with average values ranging from 1.34 to 4.37 for temperatures from 4 °C to 16 °C, suggesting that the inverse dynamic modelling approach can predict b_2 parameter of *L. monocytogenes* in milk.

While simulating the growth behaviour of microorganisms, accurately determining the exponential phase, in which the growth rate reaches a maximum value and variations in organoleptic properties of foods also reach maximum, and the lag phase, in which organoleptic properties almost do not change, are very important. μ_{max} and λ are the most important critical parameters to describe the growth behaviour of microorganisms in food, and temperature is a key determinant for both growth parameters [20, 21]. The kinetic parameters including μ_{max} and λ belonging to *L. monocytogenes* in milk for each modelling approach including the Baranyi and Huang models are shown in Fig. 5 and Fig. 6, respectively. As expected, the figures demonstrate that μ_{max} increased and λ decreased with an increase in storage temperature. Additionally, μ_{max} and λ values of *L. monocytogenes* in milk obtained from the inverse dynamic modelling approach are within the range of findings obtained by two-step direct modelling approach considering 95% *CI*. These results indicated that the inverse dynamic modelling approach can be used for predicting μ_{max} and λ parameters of *L. monocytogenes* in milk and is an alternative to traditionally used two-step modelling approach for estimating growth parameters of microorganisms.

Validation of the models is a fundamental

Tab. 4. Observed and predicted maximum growth rate values of *Listeria monocytogenes* in milk using combinations of modelling approaches and primary models.

Source ID	Product	Temperature [°C]	pH	Observed μ_{max} [h^{-1}]	Predicted μ_{max} [h^{-1}]					
					Baranyi model			Huang model		
					Direct two-step	Direct one-step	Inverse dynamic	Direct two-step	Direct one-step	Inverse dynamic
BJ00_15	Whole milk	4	6.8	0.026	0.032	0.017	0.019	0.014	0.018	0.032
L168_4	Milk	7	7	0.066	0.057	0.042	0.042	0.029	0.046	0.057
BJ00_05	Whole milk	8	6.8	0.040	0.066	0.053	0.052	0.036	0.057	0.067
L14_LM	Whole milk	10	6.6	0.069	0.089	0.079	0.076	0.050	0.085	0.090
L867_LM	UHT milk	12	6.7	0.103	0.114	0.109	0.103	0.067	0.118	0.116
L30_LM	Whole milk	13	6.6	0.116	0.128	0.126	0.118	0.076	0.137	0.130
BJ00_10	Whole milk	14	6.8	0.118	0.143	0.145	0.135	0.086	0.157	0.146
L204_LM	UHT milk	15	6.6	0.183	0.158	0.164	0.152	0.097	0.179	0.162
Bias factor B_f					1.12	1.03	0.99	0.68	1.10	1.10
Accuracy factor A_f					1.20	1.18	1.16	1.46	1.21	1.19

Observed maximum growth rate values were collected from ComBase database (University of Tasmania, Hobart, Australia).

process by which the prediction ability of the developed models can be tested using previously published or newly generated data. In this regard, externally and arbitrarily collected μ_{max} values of *L. monocytogenes* in milk products from ComBase database were used for assessing the prediction power of the modelling approaches and primary models considering B_f and A_f (Tab. 4). The statistical indices of B_f were 1.12 and 0.68 for the traditionally used direct two-step modelling approach based on the Baranyi and Huang models, respectively. B_f factor of 1 indicates no structural deviation of the model. B_f factor of 1.12 indicated that the Baranyi model overestimated by 12 % whereas the B_f factor of 0.68 indicated that the Huang model underestimated by 32 % when the direct two-step modelling approach was employed. Additionally, the direct two-step modelling approach provided statistical indicates of A_f were 1.20 and 1.46 for the Baranyi and Huang models, respectively, meaning that on average the predicted value was 20 % and 46 % different (either less or greater) from the observed values when the Baranyi and Huang models were used, respectively. These results simply mean that the Huang model failed to accurately predict μ_{max} values of *L. monocytogenes* in milk products when the direct two-step modelling approach was employed. However, the lack of prediction capability of the Huang model was eliminated by using the direct one-step modelling approach providing the statistical indices were $B_f = 1.10$ and $A_f = 1.21$. Additionally, the inverse dynamic modelling approach based on both primary models yielded satisfactory statistical indices ($0.99 > B_f > 1.10$ and $1.16 > A_f > 1.19$), confirming that the inverse dynamic modelling approach considerably improved models' prediction capability regardless of the primary model used and can be reliably used as an alternative way of describing the growth behaviour of *L. monocytogenes* in milk.

CONCLUSION

In this work, the Baranyi and Huang models were used to evaluate the fitting capabilities of direct two-step, direct one-step and inverse dynamic modelling approaches. The direct one-step modelling approach improved the fitting capabilities of the Baranyi and Huang models when compared with the direct two-step modelling approach. The inverse dynamic modelling approach based on both models not only produced more accurate predictions, but also decreased the requirement of experimental effort to describe the growth behaviour of *L. monocytogenes* in milk, clearly meaning that the inverse dynamic modelling approach is more efficient, accurate and cost-effective way of developing kinetic models. In other words, the inverse dynamic modelling approach has a considerable potential to be used as an alternative simulation method to the direct two-step modelling approach, which is traditionally used in predictive microbiology, because of providing an opportunity to make new types of experimental designs that are more informative and less time-consuming.

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