Application of a machine learning-based regression method to describe *Listeria monocytogenes* behaviour in milk

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Summary
The main aim of the present study was to develop a prediction tool to describe *Listeria monocytogenes* behaviour in milk by employing traditionally used models (the re-parametrized Gompertz, Baranyi and Huang models) and an alternatively proposed machine learning-based regression model. The fitting capability of both groups of models was evaluated and compared considering their statistical indices (coefficient of determination R^2, root mean square error *RMSE*). The machine learning-based regression model provided better predictions (with R^2 of 0.958 and *RMSE* of 0.407) than the traditionally used models. The prediction capability of both methodologies was tested considering externally collected data from the literature. The machine learning-based regression model in the validation process gave satisfactory statistical indices (bias factor of 1.016 and accuracy factor of 1.056), which is better prediction power than the traditionally used models. These results indicated that the machine learning-based regression method can be reliably employed as an alternative way of describing the growth behaviour of *L. monocytogenes* in milk. Therefore, the software developed in this work has a significant potential to be used as an alternative simulation method to traditionally used approach in the field of predictive microbiology.

Keywords
data mining; prediction tool; gaussian process regression; predictive microbiology

Listeria monocytogenes, belonging to the most important pathogenic microorganisms, is transmitted due to the consumption of contaminated food. *L. monocytogenes* can grow and remain in raw milk in a broad range of temperatures and pH as in a favourable environment for growth of pathogenic bacteria. From a public health view, milk can be considered as a potentially risky food product especially if it is not properly processed, packaged, distributed and stored [1, 2].

Predictive food microbiology is a theoretical branch of food microbiology. It assesses and models microbial quantity as a function of environmental changes using mathematics and statistics [3]. Mathematical models applied in predictive microbiology are generally classified into three categories as primary, secondary and tertiary models [4]. Behaviour of microorganisms under static environmental conditions is described by primary models as a function of time, while secondary models are used to determine the effects of environmental factors and/or food matrices on model parameters. Then, primary and secondary models may be combined in a computer software to tertiary models. Although this conventional modelling approach is generally satisfactory, it may have some disadvantages. The main drawback is the potential accumulation and propagation of errors due to the twice-ordered non-linear regression process [5, 6].

In recent years, interest in the use of machine learning algorithms has increased in many research areas. This has been triggered by the collective possibilities of three advancing technologies: first, devices to rapidly capture large amounts of digital data; second, an exponential increase in affordable computing power and data storage; and third, a global system of interconnected com-

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puter networks for rapid data transfer. There are several published works using machine learning applications in food safety and modelling [7–9]. As various approaches allow identification of underlying relationships between explanatory variables and response variables from a dataset, machine learning-based regression methods can predict the behaviour of populations and have the potential to improve the predictive accuracy of bacterial population behaviour. However, the use of machine learning algorithms to predict the behaviour of microorganisms in food is not yet widespread. Only one paper on this topic was published by HIURA et al. [10] who used the eXtreme gradient boosting tree as a machine learning algorithm to directly predict the bacterial population behaviour of *L. monocytogenes*. In fact, numerous machine learning-based regression methods are available such as support vector machine regression and random forest regression. These are applied in food safety [11] and agriculture [12].

In the current study, external data were employed to predict the behaviour of bacterial populations of *L. monocytogenes* in milk using a machine learning-based regression method. Its prediction capability was compared with models traditionally used in predictive food microbiology to predict the counts of microorganisms, namely, the re-parametrized Gompertz, Baranyi and Huang models.

MATERIAL AND METHODS

The study was performed in five parts: i) population data points of *L. monocytogenes* in milk were gathered from the ComBase database (Tasmania Institute of Agriculture, Tasmania, Australia) as Excel (Microsoft, Redmond, Washington, USA) files, ii) data pre-processing including feature selection was performed, iii) various traditional primary models (re-parametrized Gompertz, Baranyi and Huang models) and a machine learning-based regression method based on gaussian process regression (GPR) were applied to predict the microorganism population, iv) prediction capability of the methods was assessed considering their corresponding coefficient of determination, root mean square error, Akaike information criterion, Bayesian information criterion and acceptable prediction zone criteria and v) the prediction capability of both methodologies was tested by considering the externally collected data from the literature, which were not included in ComBase database, using the software developed in this work. All these processes were done in Matlab 9.10.0.1710957 (R2021a) software (MathWorks, Natick, Massachusetts, USA). The flow chart showing the steps followed in the study is presented in Fig. 1. Details of the parts of the current work are explained in the following subsections.

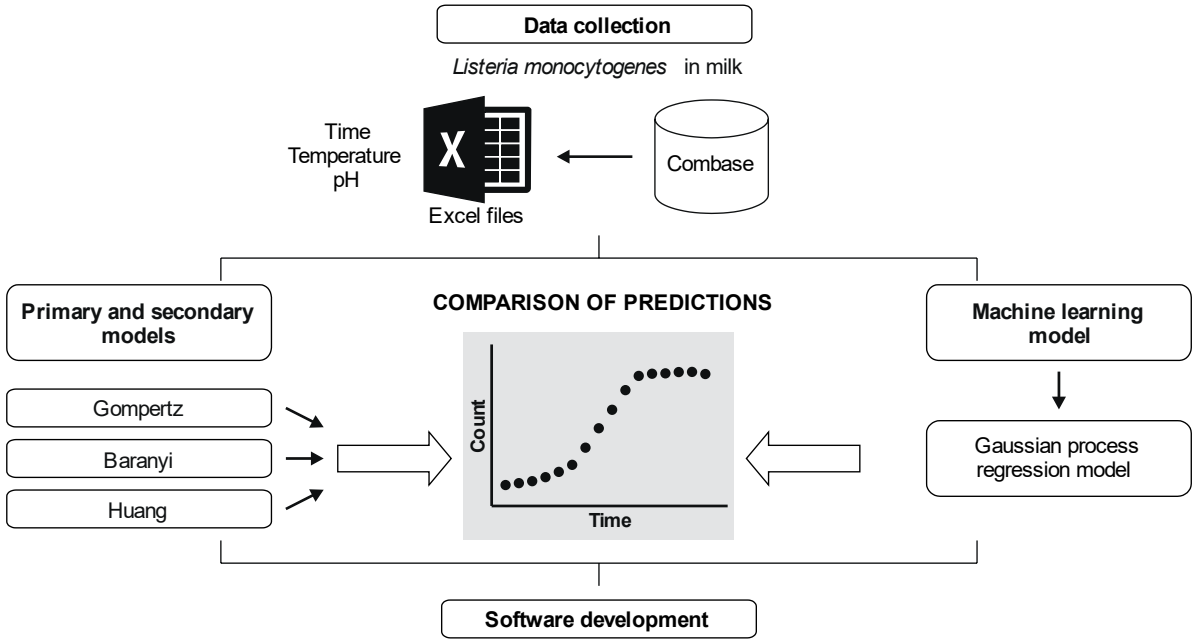

Fig. 1. Flow chart outlining the main steps followed in the present study.

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

Model	Equation	Number
Gompertz	$y(t) = y_0 + (y_{\max} - y_0) \cdot \exp\left\{-\exp\left[\frac{\mu_{\max} \cdot e}{(y_{\max} - y_0)} \cdot (\lambda - t) + 1\right]\right\}$	(1)
Baranyi	$y(t) = y_0 + \mu_{\max} F(t) - \ln\left(1 + \frac{e^{\mu_{\max} F(t)} - 1}{e^{(y_{\max} - y_0)}}\right)$	(2)
	$F(t) = t + \frac{1}{v}\ln\left(e^{-vt} + e^{-\mu_{\max}\lambda} - e^{(-vt-\mu_{\max}\lambda)}\right)$	(3)
Huang	$y(t) = y_0 + y_{\max} - \ln\left(e^{y_0} + [e^{y_{\max}} - e^{y_0}].e^{-\mu_{\max} B(t)}\right)$	(4)
	$B(t) = t + \frac{1}{4}\ln\left(\frac{1 + e^{-4(t-\lambda)}}{1 + e^{4\lambda}}\right)$	(5)

Gompertz – re-parametrized Gompertz model, t – time (in hours), $y(t)$ – counts of microorganisms at time t (expressed as natural logarithm of counts in colony forming units per millilitre), y_0 – initial counts of microorganisms (expressed as natural logarithm of counts in colony forming units per millilitre), $y_{\max}$ – maximum counts of microorganisms (expressed as natural logarithm of counts in colony forming units per millilitre), $\mu_{\max}$ – maximum specific growth rate of the microbial culture (expressed as natural logarithm of counts in colony forming units per hour), λ – lag phase duration (in hours), v – the rate of increase of the limiting substrate, assumed to be equal to $\mu_{\max}$.

Data collection

The ComBase database provides approximately 60 000 bacterial data obtained from research organisations and research papers. In this database, bacterial data are available with their specific features and conditions including food category, food name, temperature, pH, water activity, conditions and time, which enables to classify microbial factors and responses. In order to describe the growth behaviour of *L. monocytogenes* in milk, 317 bacterial data points were collected from the ComBase database with their specific and individual information (time, temperature and pH) as Excel files.

Data pre-processing

The microbial responses collected from the ComBase database were stored with their record ID. For each record ID belonging to a certain data set, the objective variable (response variable) was described as the microbial population in milk. Other variables including time (in hour), temperature (in degrees Celsius) and pH were defined as predictor variables. The microbial population (expressed as natural logarithm of counts in colony forming units per millilitre) at 0 h were defined as the initial microbial population for each record ID. Data with a time of 0 h were coded as 0 and other data were coded as 1 to separate initial counts from others. Pre-processing was done in Matlab 9.10.0.1710957 (R2021a) software.

Modelling

Primary models

Three different primary models, namely, the re-parametrized Gompertz [13], Baranyi [14] and Huang [15] models were employed using one-step modelling approach [16, 17] for fitting of the growth data points obtained from ComBase database using Eqs. 1–5 given in Tab. 1.

Secondary models

Ratkowsky model [18] was employed to determine the effects of storage temperature and pH on maximum specific growth rate of microorganisms ($\mu_{\max}$) using Eq. 6 which is only valid in the sub-optimal temperature and pH range:

$$\mu_{\max} = b_1(T - T_0)^2 \times (pH - pH_{\min}) \quad (6)$$

where T is storage temperature (in degrees Celsius), T_0 is theoretical lowest bacterial growth temperature (in degrees Celsius), pH is acidity of the food product, $pH_{\min}$ is theoretical lowest bacterial growth pH, $\mu_{\max}$ 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 Eq. 7 [19]:

$$\lambda = \frac{b_2}{\mu_{max}(T, pH)} \quad (7)$$

where b_2 is regression coefficient, $\mu_{\max}(T,pH)$ is a function of temperature and pH, which leads λ to be defined as a function of storage temperature and pH.

Gaussian process regression

Gaussian process regression (GPR) is a non-linear, non-parametric Bayesian approach and

flexible, fully probabilistic model [20]. Gaussian distribution is based on the concept of infinite-dimensional generation of normal distributions with multivariate algorithm. Gaussian processes are used for statistical modelling, regression to multiple target values and higher dimensions of mapping. In the most basic setting, Gaussian process models a hidden function based on a limited set of observations. Squared exponential kernel was used for GPR. Gaussian process gives the best linear unbiased prediction at unsampled locations.

GPR is trained with 10-fold cross validation, which is the best way to deal with the overfitting problem. In 10-fold cross validation, the dataset is divided into 10 equally sized partitions. In each iteration, one-fold was used for testing and others were used for training. Then, all tests in each iteration were combined to acquire predictions for the dataset. Cross-validation provides unbiased evaluation. Training without validation leads to overfitting and provides unsatisfactory validation results.

Goodness-of-fit

Comparison of the performance of the models was carried out by using the root mean square error (*RMSE*), coefficient of determination (R^2), corrected Akaike information criterion (*AICc*) and Bayesian information criterion (*BIC*) using Eqs. 8–11, respectively:

$$RMSE = \sqrt{\sum_{i=1}^{n} \frac{(x_{obs} - x_{fit})^2}{n - s}} \tag{8}$$

$$R^2 = 1 - \left(\frac{SSE}{SST}\right) \tag{9}$$

$$AICc = (n)\ln\left(\frac{SSE}{n}\right) + 2(s+1) + \cdot + \frac{2(s+1)(s+2)}{n-s-2} \tag{10}$$

$$BIC = n\ln\left(\frac{SSE}{n}\right) + s\ln(n) \tag{11}$$

where x_{obs} is the experimental quantity of bacteria, 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.

The acceptable prediction zone (APZ) procedure may be used for validation of the overall performance of all kinds of predictive models [21]. A prediction is taken into consideration as desirable by the APZ technique when the residual (observed – predicted) is in the range of from –1 log CFU·ml^{-1} to 0.5 log CFU·ml^{-1} (fail-safe) and fail dangerous if the residuals are outside this range. *pAPZ* is the proportion of residuals within APZ range.

Validation and software development

The prediction capability of traditional modelling approaches and of the machine learning approach was evaluated with the bacterial growth data of *L. monocytogenes* in milk extracted from the published study [1]. The comparison was done considering bias (B_f) and accuracy (A_f) factors given in Eq. 12 and Eq. 13, respectively:

$$B_f = 10^{\frac{\sum_{i=1}^{n}\log(x_{pred}/x_{obs})}{n}} \tag{12}$$

$$A_f = 10^{\frac{\sum_{i=1}^{n}\log(x_{pred}/x_{obs})}{n}} \tag{13}$$

where x_{pred} refers to the predicted counts of the microorganism (expressed as logarithm of colony forming units per millilitre), x_{obs} refers to experimental counts of microorganisms (expressed as logarithm of colony forming units per millilitre) and n refers to the number of experimental data points.

In order to show validation results visually, a prediction software was developed in this study employing traditionally used models (re-parametrized Gompertz, Baranyi and Huang) and the proposed alternative machine learning-based regression method (GPR). All these processes were done in Matlab 9.10.0.1710957 (R2021a) software.

RESULTS AND DISCUSSION

Record ID, temperature (in degrees Celsius), pH and time (in hours) for *L. monocytogenes* in milk were obtained from the ComBase database, the histograms of gathered data are given in Fig. 2. Totally, 317 data points were employed. Maximum specific growth rate (μ_{max}), which is one of the most important growth kinetic parameters, can be modelled with respect to environmental factors such as temperature and pH. Temperature plays a key role in affecting microbial growth behaviour in food [22]. Temperature ranged from 5 °C to 35 °C, which are the possible temperatures to which food products are subjected. Other important factor that is directly affecting the growth behaviour of microorganisms is pH. In this study, pH ranged from 4.32 to 7.00 (Fig. 2).

Goodness-of-fit of the traditional models (re-parametrized Gompertz, Baranyi and Huang models) and of the machine learning-based re-

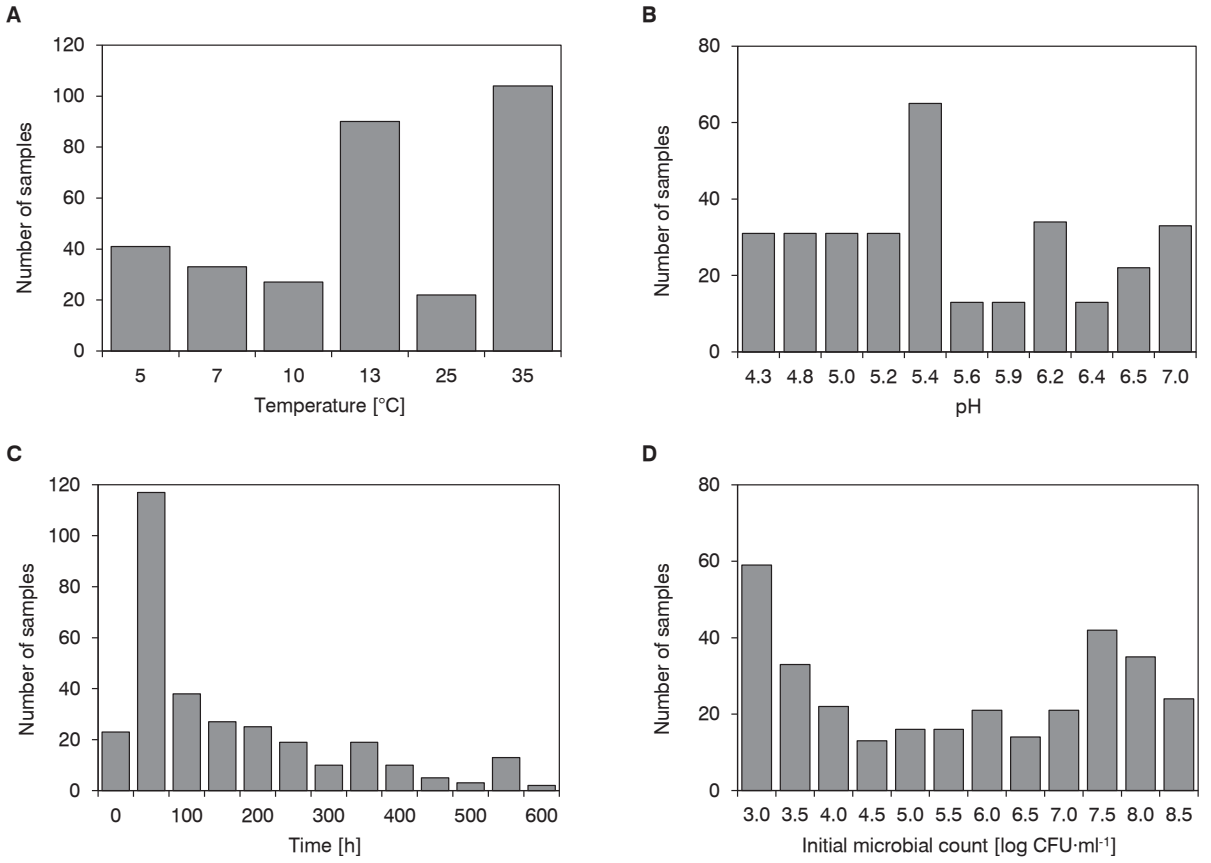

Fig. 2. Histograms of the variables.

A – temperature, B – pH, C – time, D – initial microbial count.

gression method (Gaussian process regression) for estimation of the bacterial data points of *L. monocytogenes* in milk was assessed by analysing their statistical indices (R^2, *RMSE*, *AICc*, *BIC* and *pAPZ*) (Tab. 2). The R^2 values obtained from the conventional models were in the range of 0.832–0.861 and the *RMSE* values ranged from 0.739 to 0.813. On the other hand, Gaussian process regression provided R^2 and *RMSE* of 0.958 and 0.407, respectively. This result showed that the machine learning-based regression method provided better fitting performance than any of the traditional models (re-parametrized Gompertz, Baranyi and Huang models). HIURA et al. [10] also used a machine-learning algorithm to predict the behaviour of *L. monocytogenes* in various food products such as beef, culture medium or pork and reported that R^2 and *RMSE* values were a maximum of 0.80 and a minimum of 0.96, respectively. The results indicated that the machine-learning approach used in this study provided satisfactory fitting performance.

Values $-119.1 < AICc < -179.6$ and $-163.1 < BIC < -102.6$ were obtained for the conventional models, while *AICc* of –557.0 and *BIC* of –540.4 were for obtained for the machine learning-based regression method. These results indicated that Gaussian process regression used in this study yielded excellent prediction capability although the traditional secondary modelling step, in which the effects of environmental factors and/or food

Tab. 2. Comparison of the performance of the models for training data.

Models	R^2	*RMSE*	*AICc*	*BIC*	*pAPZ*
Gompertz	0.861	0.739	–179.6	–163.1	0.970
Baranyi	0.855	0.754	–166.5	–149.9	0.965
Huang	0.832	0.813	–119.1	–102.6	0.963
GPR	0.958	0.407	–557.0	–540.4	0.989

Gompertz – re-parametrized Gompertz model, GPR – Gaussian process regression, R^2 – coefficient of determination, *RMSE* – root mean square error, *AICc* – corrected Akaike information criterion, *BIC* – Bayesian information criterion, *pAPZ* – proportion of the number of higher residuals (observed–predicted) ranging from –1 log CFU·ml^{-1} to +0.5 log CFU·ml^{-1} within the number of all predictions.

matrixes on model parameters are determined, was skipped.

No standard is available in predictive microbiology for classification of model performance. However, in the US education system, an established performance criterion is that a test score of 70 % correct answers is the minimum for classification of acceptable performance [23]. This established criterion is used in the APZ method. Thus, when the proportion of residuals in APZ (*pAPZ*) is 0.7, the model is classified as providing acceptable predictions. *pAPZ* for the re-parametrized Gompertz, Baranyi and Huang models was 0.970, 0.965 and 0.963, respectively, while *pAPZ* for the Gaussian process regression 0.989. This would mean that the traditional models (re-parametrized Gompertz, Baranyi and Huang models) and the machine learning-based regression method yielded acceptable prediction performance and Gaussian process regression had the best performance (Fig. 3).

External validation based on independent experiments is necessary to reliably use the models developed. Although Gaussian process regression gave the best fitting performance for the bacterial data points of *L. monocytogenes* in milk, the prediction capability of re-parametrized Gompertz, Baranyi, Huang and Gaussian process regression were analysed also with external data sets that were not used for training of the models. The comparison for predicted microbial counts was done by considering the statistical indices (R^2, *RMSE,* B_f, A_f and *pAPZ*; Tab. 3). The R^2 values obtained from each of the conventional models were in the range of 0.823–0.930 and the *RMSE* values ranged from 0.442 to 0.701. On the other hand, Gaussian process regression provided R^2 and *RMSE* of 0.938 and 0.415, respectively. This result showed that the machine learning-based regression method provided better prediction capability than any of the traditional models (re-parametrized Gompertz, Baranyi and Huang models).

Values $1.019 < B_f < 1.071$ and $1.059 < A_f < 1.103$ were obtained for the conventional models, while B_f of 1.016 and A_f of 1.056 were obtained for the machine learning-based regression method. B_f

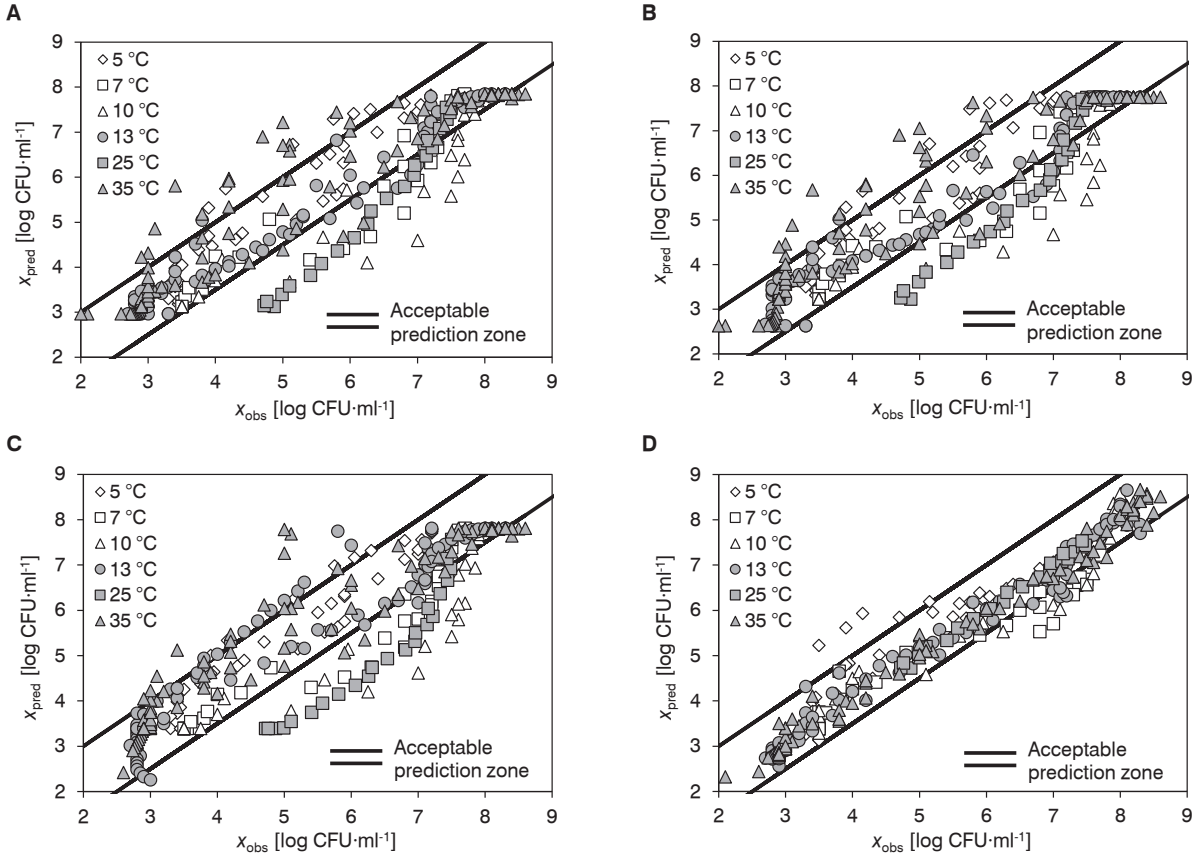

Fig. 3. Observed and model-fitted counts of *Listeria monocytogenes* in milk in the training phase.

A – re-parametrized Gompertz model, B – Baranyi model, C – Huang model, D – Gaussian process regression model. x_{pred} – predicted counts of the microorganism, x_{obs} – observed counts of microorganisms.

factor of 1 indicates no structural deviation of the model. B_f factor of 1.016 indicated that the model overestimated the maximum by 1.6 % whereas A_f of 1.056 showed that, on average, the predicted value was by a maximum of 5.6 % different (either smaller or larger) from the observed value. These results suggest that Gaussian process regression can be safely used because the error rates are relatively small. Additionally, *pAPZ* was 0.989 for Gaussian process regression, which means that 98.9 % of all data were in the range of the acceptable prediction zone (Fig. 4).

The comparison for the predicted microbial counts done by considering the statistical indices (R^2, *RMSE*, B_f, A_f and *pAPZ*) confirmed that Gaussian process regression could be reliably used as an alternative way of describing the growth behaviour of *L. monocytogenes* in milk. Therefore, the software developed in this work has a significant potential to be used as an alternative simulation method by which the predictions can be done similarly to the primary and secondary model steps in the traditionally used approach in the field of predictive microbiology. The developed software is provided in GitHub platform with "*Listeria-monocytogenes*-behaviour-in-milk" repository (GitHub, San Francisco, California, USA).

Tab. 3. Comparison of the performance of the models for external validation data.

Models	R^2	*RMSE*	B_f	A_f	*pAPZ*
Gompertz	0.881	0.574	1.047	1.084	0.970
Baranyi	0.930	0.442	1.019	1.059	0.965
Huang	0.823	0.701	1.071	1.103	0.963
GPR	0.938	0.415	1.016	1.056	0.989

Gompertz – reparametrized Gompertz model, GPR – gaussian process regression, R^2 – adjusted coefficient of determination, *RMSE* – root mean square error, B_f – bias factor, A_f – accuracy factor calculated based on microbial counts (expressed as logarithm of counts in colony forming units per millilitre), *pAPZ* – proportion of the number of higher residuals (observed–predicted) ranging from –1 log CFU·ml^{-1} to +0.5 log CFU·ml^{-1} within the number of all predictions.

Maximum specific growth rate (μ_{max}) and lag phase duration (λ) are the critical parameters

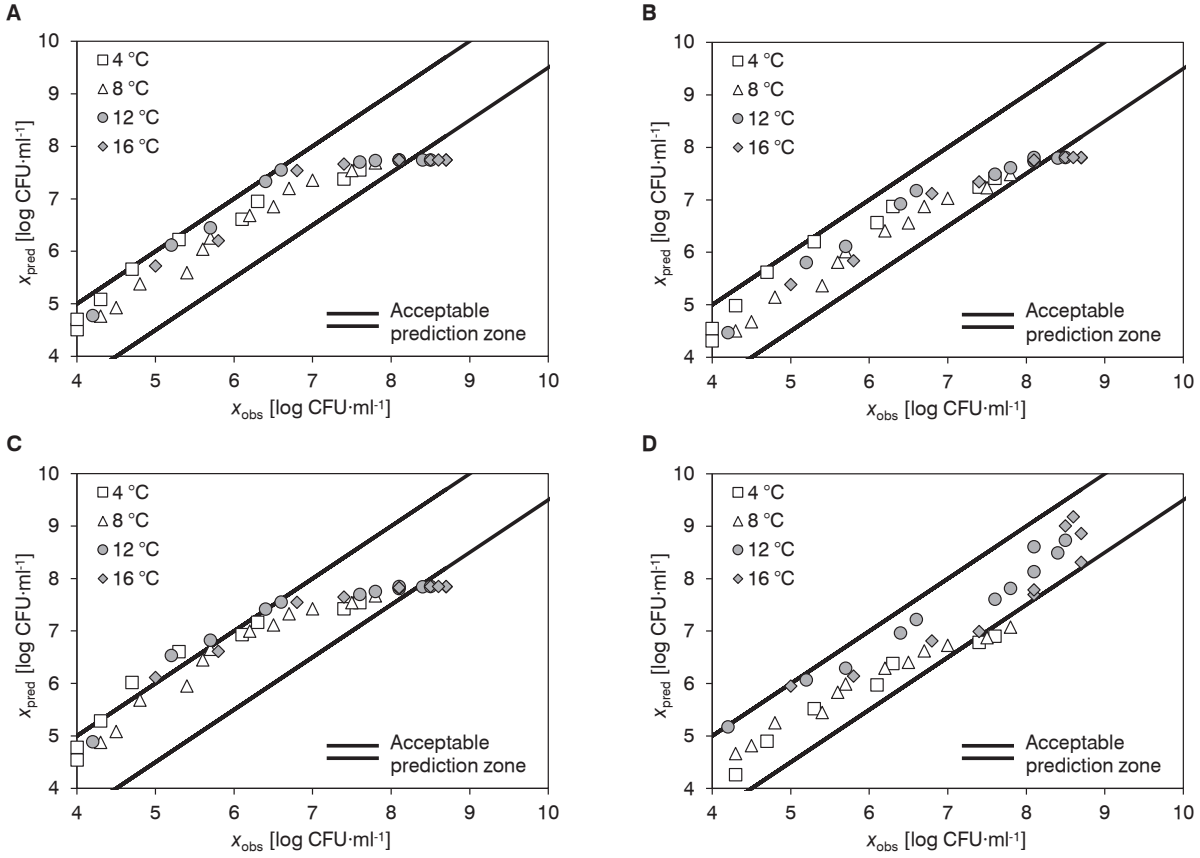

Fig. 4. Observed and predicted counts of *Listeria monocytogenes* in milk in the validation phase.

A – re-parametrized Gompertz model, B – Baranyi model, C – Huang model and D – Gaussian process regression model. x_{pred} – predicted counts of the microorganism, x_{obs} – observed counts of microorganisms.

Tab. 4. Parameters derived from the primary and secondary models.

Primary model	T_0 [°C]		b_1		b_2		pH_{min}	
	Mean	95% *CI*	Mean	95% *CI*	Mean	95% *CI*	Mean	95% *CI*
Gompertz	–0.80	(–0.82, –0.78)	0.016	(0.014, 0.018)	0.40	(0.38, 0.42)	4.7	(4.6, 4.8)
Baranyi	–0.84	(–0.85, –0.83)	0.021	(0.020, 0.022)	0.29	(0.28, 0.30)	4.7	(4.6, 4.8)
Huang	–1.3	(–1.4, –1.2)	0.013	(0.012, 0.014)	0.47	(0.46, 0.48)	4.5	(4.5, 4.6)

Gompertz – re-parametrized Gompertzmodel, T_0 – theoretical lowest bacterial growth temperature, b_1, b_2 – regression coefficients, pH_{min} – theoretical lowest bacterial growth pH, 95% *CI* – 95% confidence interval of the parameters.

to describe the growth behaviour of microorganisms in food. The parameters to describe μ_{max} and λ derived from the re-parametrized Gompertz, Baranyi and Huang models are given in the Tab. 4. Unfortunately, both of these parameters (μ_{max} and λ) could not be determined using the machine learning approach. Therefore, this may be considered as the first important limitation of this methodology compared to traditional modelling methods in predictive microbiology. A second limitation can be the fact that the prediction power of the machine learning regression method directly depends on the dataset size. If the number of data is not enough, the machine learning method may not be used for prediction of microorganism behaviour, meaning it requires a big dataset to be employed for modelling. Additionally, this modelling work can only be used for prediction of *L. monocytogenes* in milk at certain conditions. However, this is also valid for all the modelling works in predictive microbiology carried out by traditional modelling. On the other hand, the machine learning approach enables simultaneous modelling of microbial survival and growth behaviour, meaning that the machine learning methodology can be practically applied to microbial growth data and inactivation data at the same time.

CONCLUSION

In this work, the prediction capabilities of traditionally used models (re-parametrized Gompertz, Baranyi and Huang models) and the alternatively proposed machine learning-based regression method (Gaussian process regression) were evaluated and compared for description of L. monocytogenes behaviour in milk. All models provided satisfactory prediction capability but Gaussian process regression was superior over the traditionally used models for fitting and prediction. The results indicated that Gaussian process regression can be reliably employed as an alternative way to describe simultaneously growth and survival of microorganisms in food products and has a significant potential to be used as an alternative simulation method by skipping the secondary model step in the two-step modelling approach traditionally used in predictive microbiology.

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