Development and validation of one-step modelling approach for prediction of mushroom spoilage

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Summary
The primary aims of this work were to improve the prediction capability of the traditionally used two-step modelling approach with the most popular primary growth models in the predictive food microbiology field, and to validate the prediction capability of the one-step modelling approach, a proposed alternative way to traditional modelling approach. For this purpose, the growth behaviour of *Pseudomonas* spp. existing in the natural microflora of button mushrooms (*Agaricus bisporus*) was simulated with two-step and one-step modelling approaches. The Baranyi model yielded the best fitting performance when it was employed in the two-step modelling approach. The fitting capability of all the primary models was also compared using the one-step modelling approach. No matter which primary model was used, the one-step modelling approach significantly improved the prediction capability of the models, and all the primary models gave root mean squared error lower than 0.299 and adjusted coefficient of determination higher than 0.948. Successfully validated Baranyi model in one-step modelling approach provided the highest prediction capability and exhibited considerable potential to be used as a prediction tool. This indicated that the one-step modelling approach could be reliably employed to assess and predict mushroom spoilage as a function of time and storage temperature.

Keywords
one-step modelling approach; simulation; mushroom spoilage; prediction

The button mushroom (*Agaricus bisporus*) is the most widely known and consumed mushroom species in the world because of high levels of nutrients [1, 2] and plays an important role in mushroom industry [3]. After being harvested, the button mushrooms can be easily perishable within a short time due to lack of cuticle that could defend them against physical deterioration or microbial contamination. Hence, the button mushrooms are very vulnerable to contamination by microorganisms during being grown and processed. In this regard, *Pseudomonas* spp. are known as the most ubiquitous microorganisms causing the mushroom spoilage [4, 5].

Predictive microbiology is the integration of traditional microbiology knowledge with the disciplines of mathematics and statistics to describe microbial behaviour under various environmental conditions. This mathematical knowledge enables us to estimate the behaviour of pathogens and spoilage microorganisms on or in foods subjected to the conditions at which microbiological data are unavailable [6]. In this regards, the main purpose of predictive microbiology is to predict microbial behaviour that can prevent food spoilage, as well as food-borne illnesses, by employing mathematical models. Primary and secondary models are commonly used in predictive food microbiology [7, 8]. For the first class, the modified Gompertz, logistic, Baranyi and Huang models are the most popular ones describing microbial growth data as a function of time at constant environmental conditions. Secondary models provide information on how the obtained parameters from primary models change with respect to one or more environmental or cultural factors (e.g. composition of atmosphere, pH, temperature or salt level). Temperature is one of the most important environmental factors directly affecting the growth behaviour of microorganisms in foods, and its effect is widely simulated using the Ratkowsky model [9].

The two-step modelling approach, in which the primary and secondary models are separately fitted to the growth data and kinetic parameters respectively, is the most popular modelling procedure followed in the predictive food microbiology. However, there are some drawbacks concerning the use of the two-step modelling approach. A ma-

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jor drawback is accumulation and propagation of errors due to the two sequentially performed non-linear regression procedures [10]. In other words, the sequentially performed primary and secondary model fittings generally result in a decrease in the overall prediction capability of the model and an increase in uncertainty of the estimated parameter. This occurs in particular when the number of data referring to various environmental conditions is not big enough, which causes low degree of freedom. Furthermore, this approach often fails to estimate the lag time duration although it yields relatively reliable information on the value of maximum specific growth rate [11, 12]. To avoid these disadvantages of two-step modelling approach, alternatively, a one-step modelling can be applied to simulate microbiological data and kinetic parameters. In this approach, primary and secondary modelling of the growth and temperature (as a changing environmental factor) data is performed simultaneously. The use of this approach frequently provides better prediction, lower uncertainty, more precise coefficients and robust confidence interval than the traditionally used two-step modelling approach [13, 14]. These advantages are more pronounced at high biological variation in microbiological data and when not enough microbiological data for the secondary model are available [15, 16].

Predictive models developed with one-step modelling approach are a relatively new way of simulating the growth behaviour of microorganisms. The one-step modelling approach has been employed up to now for a limited number of food products, including liquid eggs, potato salad and oyster mushroom [16–18]. In this point, the availability and predictive ability of the model, which was developed by MANTHOU et al. [16], could be further improved by considering the wider temperature range to which mushrooms are usually subjected during storage, delivery and retail marketing. Therefore, it is important to investigate and evaluate the prediction capability of one-step modelling approach considering the microbial growth data of *Pseudomonas* spp. on button mushroom, which is the most extensively consumed edible mushroom all over the world, at possible temperatures that mushrooms are generally subjected to. Additionally, there is no published study that compared one-step and two-step modelling approaches using the microbial counts, which directly indicate the microbiological quality of food products.

The main objective of this work was to develop and validate one-step modelling approach, a proposed alternative way to traditional modelling approach to assess and predict mushroom spoilage considering the *Pseudomonas* spp. counts existing in the natural microflora of button mushrooms (*Agaricus bisporus*) stored at various temperatures ranging from 4 °C to 28 °C. For this purpose, the experimental growth data of *Pseudomonas* spp., collected from the previously published curves for button mushrooms, were simulated with two-step and one-step modelling approaches. Four different primary models (the modified Gompertz, logistic Baranyi and Huang models) and the most-known secondary model (Ratkowsky) were employed to predict the *Pseudomonas* spp. counts on button mushrooms as a combined function of time and storage temperature. Two-step and one-step modelling approaches and the primary models used in these modelling approaches were compared considering their goodness-of-fit indices. The modelling approach with the best goodness-of-fit index was determined. Validation was performed with the externally collected growth data previously published *Pseudomonas* spp. on button mushrooms.

MATERIALS AND METHODS

Data collection

Growth data of *Pseudomonas* spp. were collected from the previously published curves for button mushrooms under various isothermal temperatures (4, 12, 20 and 28 °C) [19]. The followed experimental procedure of obtaining these microbiological data was explained in detail previously [19]. In brief, button mushrooms were collected at the closed cap stage (cap diameter 3.5–4.5 cm) and directly transported to the research laboratory at 4 °C, without any treatments. The mushrooms were put in polystyrene trays, which were not overwrapped with any packaging material. The microbiological analyses were done for each temperature with an appropriate sampling frequency. The *Pseudomonas* spp. counts during the storage were determined in three different trays at each storage temperature for each sampling point for a maximum duration of 240 h (10 days). Twenty-one growth data were used for each temperature of 4 °C and 12 °C, while twenty-four data points were employed for each temperature of 20 °C and 28 °C. This means that ninety growth data in total were used for one-step modelling approach.

Modelling

For the two-step and one-step modelling approaches, the modified Gompertz, logistic Baranyi and Huang models were used due to being

Tab. 1. Primary models.

Model	Equation	Number
Modified Gompertz	$x(t) = x_0 + (x_{\max} - x_0) \cdot \exp\left\{-\exp\left[\frac{r_{\max} \cdot e}{(x_{\max} - x_0)} \cdot (\lambda - t) + 1\right]\right\}$	1
Logistic	$x(t) = x_0 + \frac{(x_{\max} - x_0)}{\left\{1 + \exp\left[\frac{4 \cdot r_{\max}}{(x_{\max} - x_0)} \cdot (\lambda - t) + 2\right]\right\}}$	2
Baranyi	$y(t) = y_0 + \mu_{\max} F(t) - \ln\left(1 + \frac{e^{\mu_{\max} F(t)} - 1}{e^{(y_{\max} - y_0)}}\right)$	3
Huang	$y(t) = y_0 + \mu_{\max} - \ln\left(e^{y_0} + [e^{y_{\max}} - e^{y_0}] \cdot e^{-\mu_{\max} B(t)}\right)$	4

t – time (in hours); $x(t)$ – count of microorganisms at time t (expressed as logarithm of colony forming units per gram); x_0 – initial count of microorganisms (expressed as logarithm of colony forming units per gram); x_{max} – maximum count of microorganisms (expressed as logarithm of colony forming units per gram); r_{max} – growth rate (expressed as logarithm of colony forming units per hour); λ – lag phase duration (in hours); $y(t)$ – count of microorganisms (expressed as natural logarithm of colony forming units per gram) at time t; 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); $F(t)$ – adjustment function described by Baranyi and Roberts [21]; $B(t)$ – adjustment functionsdescribed by Huang [10].

the most popular sigmoid functions that describe the growth behaviour of microorganisms as a function of time. The modified Gompertz and logistic models at constant environmental conditions are defined by Eq. 1 and Eq. 2, respectively [20].

The Baranyi and Huang models are other extensively used primary models that are described by Eq. 3 and Eq. 4, respectively [10, 21].

Model equations are given in Tab. 1.

Ratkowsky model was used to determine the relationship between storage temperature and the maximum specific growth rate (μ_{max}) using Eq. 5:

$$\sqrt{\mu_{\max}} = b_1(T - T_0) \tag{5}$$

where T is the storage temperature (in degrees Celsius), T_0 is the theoretical lowest temperature at which microbial growth is observable (in degrees Celsius), μ_{max} is the maximum specific growth rate of microorganisms (expressed as natural logarithm of colony forming units per hour), b_1 is the regression coefficient.

The modified Gompertz and logistic models use decadic logarithmic scale, but the Baranyi and Huang models use natural logarithmic scale. Therefore, the growth rate values (r_{max}) obtained from the modified Gompertz and logistic models were multiplied by ln(10) to get the maximum specific growth rate values (μ_{max}).

The lag phase duration (λ) was correlated with the μ_{max} using Eq. (6):

$$\lambda = \frac{b_2}{\mu_{max}(T)} \tag{6}$$

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

For the traditionally used two-step modelling approach, the primary and secondary models were separately fitted to the growth data and kinetic parameters, respectively. For one-step modelling approach, the primary and secondary models were simultaneously fitted to *Pseudomonas* spp. growth data and mushroom storage temperature. All parameters were calculated using NonLinearModel command, which uses Levenberg-Marquardt algorithm in the Matlab 8.3.0.532 (R2014a) software (MathWorks, Natick, Massachusetts, USA). Determination of starting values in the non-linear regression procedure is a critical step to estimate the accurate parameters. Starting values for the parameters, x_0/y_0 and x_{max}/y_{max} were selected as the minimum and maximum counts of microorganisms at the entire temperature range, respectively. The starting values of b_1 and T_0 for the maximum growth rate and b_2 for the lag phase duration were estimated by using ga command, which uses genetic algorithm in Global Optimization Toolbox of the Matlab software so that the estimated parameters could not get stuck in possible local optimal points.

Comparison of the primary models' estimation capacity

Comparison of models regarding how well they can describe the observed growth data was done with statistical indices such as root mean square

error (*RMSE*) and adjusted coefficient of determination (R^2_{adj}) values given by Eq. 7 and Eq. 8, respectively:

$$RMSE = \sqrt{\sum_{i=1}^{n} \frac{(x_{obs} - x_{fit})^2}{n - s}} \quad (7)$$

$$R^2_{adj} = 1 - \left(\frac{n-1}{n-s}\right)\left(\frac{SSE}{SST}\right) \quad (8)$$

where x_{obs} is the value obtained in experiments, x_{fit} is the fitted value, n is the number of observations, s is the number of parameters of the model, *SSE* is the sum of squared residuals and *SST* is the total sum of squares.

Because the primary models use different scale for the counts of microorganisms, *RMSE* values of the modified Gompertz and logistic models cannot be directly compared with *RMSE* value of the Baranyi and Huang models. Therefore, the conversion from the natural logarithm scale to decadic logarithm scale was done to compare *RMSE* values of all the primary models.

Statistical analysis

RMSE and R^2_{adj} values obtained from two-step and one-step modelling approaches were subjected to one-way analysis of variance (ANOVA) using the Matlab 8.3.0.532 (R2014a) software. Statistical differences between the modelling approaches were determined by post hoc analysis using Tukey's test. The differences between the means were regarded as statistically significant if $p \leq 0.05$.

Validation of the global model

Validation is a necessary step in predictive food microbiology needed for reliable use of the models considering the independent experimental data. Therefore, literature search was done for validation of the global model, which is developed in order to predict *Pseudomonas* spp. counts on the button mushrooms, and independent external growth data were collected from a previously published work on button mushrooms [22]. In this study, data collection for validation was performed using image processing toolbox by which the growth data points could be extracted accurately to Matlab 8.3.0.532 (R2014a). Twenty-three growth data were used to perform model validation. Comparison of experimental growth data with the predicted data was done with the bias (B_f) and accuracy (A_f) factors [23–25] given in Eq. 9 and Eq. 10, respectively:

$$B_f = 10^{\frac{\sum_{i=1}^{n} \log(x_{pred}/x_{obs})}{n}} \quad (9)$$

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

where x_{pred} refers to *Pseudomonas* spp. counts (expressed as logarithm of colony forming units per gram), x_{obs} refers to experimental *Pseudomonas* spp. counts (expressed as logarithm of colony forming units per gram), n refers to the number of experimental growth data.

B_f and A_f show how close are simulated data to experimental data, value of 1 for both B_f and A_f meaning that there is a perfect agreement between experimental and predicted *Pseudomonas* spp. count data. Additionally, two validation criteria known as mean deviation (*MD*) and mean absolute deviation (*MAD*) were calculated to assess the prediction capability of the models, as stated by Le Marc et al. [26]. A value of *MD* and *MAD* close to 0 shows that the prediction capability of the model is perfect.

RESULTS AND DISCUSSION

The experimental *Pseudomonas* spp. counts collected from the previously published curves for button mushrooms at the storage temperatures of 4, 12, 20 and 28 °C were modelled using two-step and one-step modelling approaches (Tab. 2 and Tab. 3). The initial bacterial counts of *Pseudomonas* spp. were on average 7.05 ± 0.14 log CFU·g^{-1} for all temperatures. Storage duration was directly related to storage temperature and ranged from 240 h to 84 h (10 days to 3.5 days) with an increase in storage temperature from 4 °C to 28 °C. The *Pseudomonas* spp. counts could reach the level ranging from 8.64 ± 0.13 log CFU·g^{-1} to 10.76 ± 0.05 log CFU·g^{-1} at the end of storage depending on the storage temperature (Tab. 1 and Tab. 2). This demonstrated that the growth potential of *Pseudomonas* spp. on button mushrooms was enhanced with the increasing storage temperature.

The goodness-of-fit of all primary models involved in the traditionally used two-step modelling approach was evaluated by calculating their *RMSE* and R^2_{adj} values (Tab. 4). The *RMSE* values obtained from the primary models based on the two-step modelling approach were between 0.549 and 0.490, and R^2_{adj} values were between 0.826 and 0.861. Among four different primary models, the Baranyi model yielded the lowest *RMSE* and the highest R^2_{adj} values. This means that the fitting capability of the Baranyi model was superior

over other primary models when two-step modelling was used to describe the growth behaviour of *Pseudomonas* spp. on button mushrooms.

The *RMSE* and R^2_{adj} values of all the primary models based on one-step modelling approach ranged from 0.294 to 0.299 and from 0.948 to 0.950, respectively. The Baranyi model had the best goodness-of-fit parameters, similar as in the two-step modelling approach, but there was no significant differences ($p > 0.05$) between primary models when they were employed in one-step modelling approach. The statistical evaluation regarding the fitting capability of the primary models based on one one-step modelling approach showed that the fitting capability of the primary models was better than that of the traditionally used two-step modelling approach. These results showed that one-step modelling approach could be reliably used for estimation of *Pseudomonas* spp. counts on button mushrooms.

The degrees of freedom of the one-step modelling approach proposed in this study was 85 (the number of observations – the number of parameters in the global model), but the degrees of freedom of the traditional two-step modelling approach used by TARLAK et al. [19] was only 2 for the Ratkowsky model and was maximum 20 for the Baranyi model at various temperatures ranging from 4 °C to 28 °C. It is important to underline that especially Ratkowsky model with a low degree of freedom might be regarded as giving the results which are suspicious and uncertain. From this point of view, the one-step modelling approach has higher degree of freedom, which decreases confidence intervals and uncertainty of the parameters compared to the traditionally used two-step modelling approach [13, 14]. Therefore, no matter which primary model was used, the one-step modelling approach significantly ($p < 0.05$) improved the prediction capability of the models for quantitative description of *Pseudomonas* spp. counts on button mushrooms.

When the one-step modelling approach was used, the minimum counts of *Pseudomonas* spp. were found to be 7.02 ± 0.11 log CFU·g^{-1}, 6.70 ± 0.19 log CFU·g^{-1}, 7.06 ± 0.07 log CFU·g^{-1} and 7.10 ± 0.07 log CFU·g^{-1} for the modified Gompertz, logistic, Baranyi and Huang models,

Tab. 2. Observed and fitted growth data of *Pseudomonas* spp. populations on button mushrooms stored at 4 °C and 12 °C.

Storage temperature [°C]	Storage time [h]	Observed data [log CFU·g^{-1}]	Fitted data [log CFU·g^{-1}]							
			One-step modelling approach				Two-step modelling approach			
			Modified Gompertz model	Logistic model	Baranyi model	Huang model	Modified Gompertz model	Logistic model	Baranyi model	Huang model
4	0	7.05 ± 0.14	7.07	7.04	7.06	7.10	7.12	7.00	7.07	7.14
	24	7.25 ± 0.05	7.13	7.14	7.13	7.10	7.20	7.10	7.15	7.14
	60	7.45 ± 0.09	7.31	7.35	7.33	7.24	7.52	7.45	7.47	7.41
	96	8.04 ± 0.13	7.61	7.63	7.63	7.66	8.07	7.99	8.03	8.09
	144	8.43 ± 0.06	8.15	8.13	8.15	8.21	8.85	8.74	8.90	8.97
	192	8.65 ± 0.05	8.76	8.71	8.73	8.76	9.44	9.31	9.64	9.67
	240	8.64 ± 0.13	9.33	9.31	9.31	9.30	9.79	9.66	9.96	9.97
12	0	7.05 ± 0.14	7.07	7.04	7.06	7.10	7.41	7.36	7.31	7.14
	24	7.27 ± 0.10	7.26	7.29	7.26	7.13	7.12	7.00	7.07	7.14
	48	7.72 ± 0.12	7.67	7.68	7.68	7.72	8.16	8.14	7.93	7.97
	72	8.62 ± 0.22	8.25	8.23	8.25	8.30	8.98	8.93	8.75	8.80
	100	9.60 ±0.18	9.00	8.97	8.96	8.97	9.61	9.53	9.60	9.61
	144	10.05 ± 0.21	9.94	9.98	10.00	9.96	10.03	9.93	10.01	10.02
	192	10.20 ± 0.18	10.54	10.54	10.56	10.56	10.14	10.05	10.03	10.03

Observed data in the table are given as average values ± standard deviations.

Tab. 3. Observed and fitted growth data of *Pseudomonas* spp. populations on button mushrooms stored at 20 °C and 28 °C.

Storage temperature [°C]	Storage time [h]	Observed data [log CFU·g^{-1}]	Fitted data [log CFU·g^{-1}]							
			One-step modelling approach				Two-step modelling approach			
			Modified Gompertz model	Logistic model	Baranyi model	Huang model	Modified Gompertz model	Logistic model	Baranyi model	Huang model
20	0	7.05 ± 0.14	7.07	7.04	7.06	7.10	7.12	7.00	7.07	7.14
	12	7.15 ± 0.08	7.22	7.25	7.22	7.10	7.34	7.29	7.23	7.14
	24	7.38 ± 0.12	7.53	7.56	7.55	7.55	7.93	7.93	7.65	7.63
	36	7.66 ± 0.06	8.00	7.99	8.00	8.05	8.66	8.66	8.27	8.31
	48	8.71 ± 0.13	8.54	8.51	8.51	8.55	9.27	9.24	8.94	8.97
	72	9.79 ± 0.02	9.56	9.58	9.55	9.53	9.90	9.83	9.88	9.87
	96	10.51 ± 0.03	10.26	10.30	10.35	10.31	10.10	10.01	10.02	10.03
	120	10.76 ± 0.05	10.65	10.63	10.60	10.62	10.15	10.06	10.03	10.04
28	0	7.05 ± 0.14	7.07	7.04	7.06	7.10	7.12	7.00	7.07	7.14
	12	7.17 ± 0.16	7.36	7.40	7.37	7.32	7.62	7.61	7.40	7.27
	24	7.78 ± 0.22	8.03	8.02	8.03	8.08	8.71	8.73	8.24	8.27
	36	8.57 ± 0.28	8.87	8.84	8.82	8.85	9.53	9.50	9.21	9.21
	48	9.43 ± 0.16	9.62	9.64	9.61	9.58	9.92	9.86	9.85	9.84
	60	10.27 ± 0.15	10.16	10.21	10.25	10.21	10.08	10.00	10.01	10.01
	72	10.55 ± 0.05	10.52	10.53	10.55	10.55	10.14	10.05	10.03	10.03
	84	10.69 ± 0.07	10.74	10.69	10.62	10.65	10.16	10.07	10.03	10.04

Observed data in the table are given as average values ± standard deviations.

Tab. 4. Kinetic parameters of *Pseudomonas* spp. growth on button mushrooms.

Modelling approach	Primary models	Model parameters					*RMSE*	R^2_{adj}
		x_0 [log CFU·g^{-1}]	x_{max} [log CFU·g^{-1}]	T_0 [°C]	b_1	b_2		
Two-step modelling	Modified Gompertz	7.10 ± 0.06	10.18 ± 0.09	−13.54 ± 2.70	(7.44 ± 0.65) × 10^{-3}	0.67 ± 0.22	0.549	0.826
	Logistic	6.96 ± 0.10	10.08 ± 0.07	−12.60 ± 2.36	(7.69 ± 0.61) × 10^{-3}	0.53 ± 0.23	0.541	0.831
	Baranyi	7.07 ± 0.06	10.03 ± 0.01	−17.42 ± 1.83	(9.91 ± 0.53) × 10^{-3}	0.96 ± 0.15	0.490	0.861
	Huang	7.14 ± 0.06	10.04 ± 0.01	−17.87 ± 2.38	(9.57 ± 0.65) × 10^{-3}	0.88 ± 0.38	0.494	0.859
One-step modelling	Modified Gompertz	7.02 ± 0.11	11.03 ± 0.21	−13.59 ± 0.83	(6.41 ± 0.28) × 10^{-3}	0.70 ± 0.24	0.299	0.948
	Logistic	6.70 ± 0.19	10.82 ± 0.16	−13.54 ± 0.81	(6.43 ± 0.28) × 10^{-3}	0.43 ± 0.35	0.298	0.948
	Baranyi	7.06 ± 0.07	10.63 ± 0.10	−13.70 ± 0.80	(6.31 ± 0.25) × 10^{-3}	0.73 ± 0.19	0.294	0.950
	Huang	7.10 ± 0.07	10.67 ± 0.11	−13.73 ± 0.83	(9.20 ± 0.33) × 10^{-3}	0.55 ± 0.12	0.297	0.949

Values in the table represent estimated average values ± standard errors.
x_0 – initial count of microorganisms; x_{max} – maximum count of microorganisms; T_0 – theoretical lowest temperature at which microbial growth is observable; b_1, b_2 – regression coefficients; *RMSE* – root mean square error; R^2_{adj} – adjusted coefficient of determination.

respectively (Tab. 4). The experimental minimum counts were between 6.91 log CFU·g^{-1} and 7.19 log CFU·g^{-1} corresponding to an average of 7.05 ± 0.14 log CFU·g^{-1}, which showed that all primary models except for the logistic model successfully estimated the minimum *Pseudomonas* spp. populations on button mushrooms.

The one-step modelling approach showed that maximum counts of *Pseudomonas* spp. were 11.03 ± 0.21 log CFU·g^{-1}, 10.82 ± 0.16 log CFU·g^{-1}, 10.63 ± 0.10 log CFU·g^{-1} and 10.67 ± 0.11 log CFU·g^{-1} for the modified Gompertz, logistic, Baranyi and Huang models, respectively (Tab. 4). The maximum counts were experimentally found to be changing within the range of 8.64 ± 0.13 log CFU·g^{-1} to 10.76 ± 0.05 log CFU·g^{-1}. This indicated that the Baranyi and Huang models provided better prediction performance for maximum counts in comparison with modified Gompertz and logistic models.

The μ_{max} and λ values play a critical role in the description of microbial growth behaviour, and temperature is one of the most important environmental factors directly affecting both of these growth kinetic parameters [23]. In this work, μ_{max} and λ values were not directly given in Tab. 4 when the one-step modelling approach was employed. Instead, b_1, b_2 and T_0, the other parameters deriving from the secondary Ratkowsky model and being directly related to μ_{max} and λ, were presented in Tab. 4. Concerning the secondary model's parameter T_0, its estimated valued were –13.59 ± 0.83 °C, –13.54 ± 0.81 °C, –13.70 ± 0.80 °C and –13.73 ± 0.83 for the modified Gompertz, logistic, Baranyi and Huang models, respectively (Tab. 4). At this point, it needs to be highlighted that the estimated T_0, which is the temperature-intercept of the Ratkowsky model, refers only to the theoretical lowest temperature, which can be much lower than that actually observed [25]. Although this value is not quite logical, nevertheless it gives the idea that *Pseudomonas* spp. can proliferate extensively on button mushrooms as they are a nutrient-rich substrate for their growth. Taking into account the secondary model's parameters (b_1, b_2 and T_0) given in Tab. 4, μ_{max} increased from 0.029 h^{-1} to 0.164 h^{-1}, from 0.029 h^{-1} to 0.164 h^{-1}, from 0.029 h^{-1} to 0.160 h^{-1} and from 0.027 h^{-1} to 0.149 h^{-1} with the increasing storage temperature for the modified Gompertz, logistic, Baranyi and Haung models, respectively (Fig. 1). An exact opposite tendency was observed for λ decreasing from 55.0 h to 9.8 h, from 33.9 h to 6.1 h, from 58.1 h to 10.5 h and 52.1 h to 9.4 h for the modified Gompertz, logistic, Baranyi and Haung models, respectively, as the temperature was increased from 4 °C to 28 °C (Fig. 2). These results reflect the facts that μ_{max} and λ values are inversely correlated and mushrooms should be kept at low temperatures in order to reduce their microbial contamination.

All of the primary models involved in the one-step modelling approach yielded high goodness-of-fit values. Therefore, the prediction capability of each primary model was evaluated. For this purpose, the data predicted by all primary models based on one-step modelling approach and the previously published growth data by WANG et al. [22] for *Pseudomonas* spp. on mushrooms were compared, statistical values for validation of the models are given in Tab. 5. B_f and A_f were respectively calculated as 0.99 and 1.03 by the Baranyi model, both being the closest to 1 among all primary models. These results indicated that the Baranyi model involved in one-step modelling

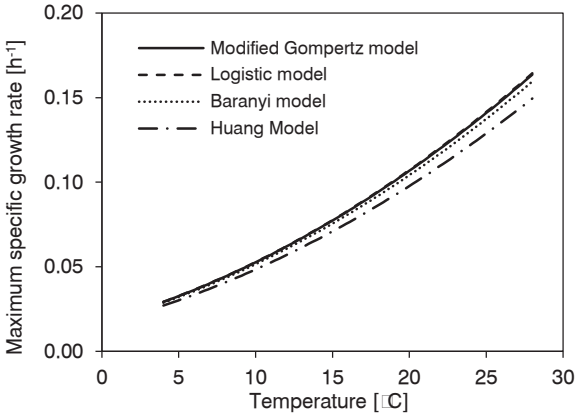

Fig. 1. The effect of storage temperature on maximum specific growth rate.

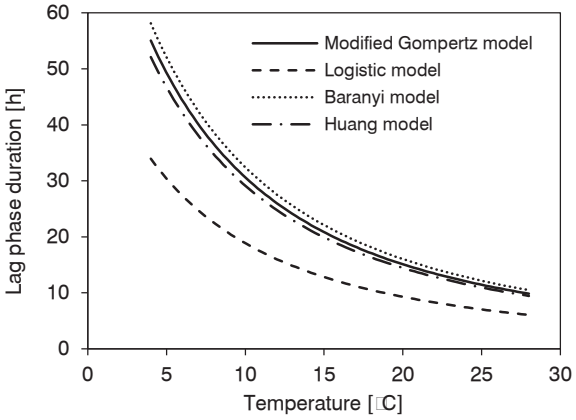

Fig. 2. The effect of storage temperature on lag phase duration.

Tab. 5. Validation criteria of the primary models involved in the one-step modelling approach.

Primary Models	Validation criteria			
	B_f	A_f	*MD*	*MAD*
Modified Gompertz	1.01	1.04	0.04	0.25
Logistic	0.93	1.08	0.41	0.44
Baranyi	0.99	1.03	–0.09	0.21
Huang	0.97	1.04	–0.21	0.25

B_f – bias factor; A_f – accuracy factor; *MD* – mean deviation; *MAD* – mean absolute deviation.

approach had the best capability to predict *Pseudomonas* spp. counts on mushrooms stored at temperatures ranging from 4 °C to 28 °C. *MD* and *MAD* values obtained for Baranyi model based on the one-step modelling approach were found to be –0.09 log CFU·g^{-1} and 0.21 log CFU·g^{-1}, respectively. The *MD* value of –0.09 log CFU·g^{-1} indicated that on average the global model overestimated 0.09 log CFU·g^{-1}, while the *MAD* value of 0.21 log CFU·g^{-1} showed that on average the predicted values were 0.21 log CFU·g^{-1} different (either higher or lower) from the observed ones. All these prediction performance indices revealed that the Baranyi model developed in this work considering the one-step modelling approach can be reliably used to predict the *Pseudomonas* spp. counts on the button mushrooms stored for any time and at any temperature ranging from 4 °C to 28 °C (Fig. 3). The modelling approach described in this work with accurate and robust prediction performance provided valuable information for performing quantitative prediction of *Pseudomonas* spp. counts on button mushrooms.

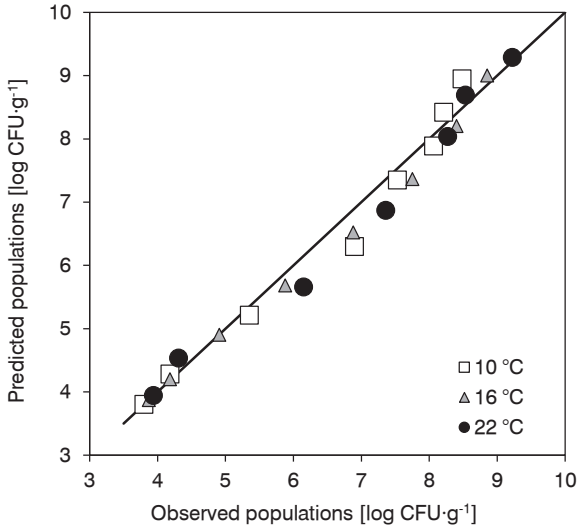

Fig. 3. Observed and predicted *Pseudomonas* spp. populations on button mushrooms.

The observed data were collected from WANG et al. [22].

CONCLUSIONS

The fitting capability of the modified Gompertz, logistic, Baranyi and Huang models, which are the most popular primary models describing the microbial growth as a function of time at constant environmental conditions, were firstly compared employing the two-step modelling approach. The Baranyi model yielded the best fitting performance when it was employed in the traditionally used two-step modelling approach. The fitting capability of all the primary models was also compared using the one-step modelling approach proposed in this study. No matter which primary model was used, the one-step modelling approach significantly ($p < 0.05$) improved the prediction capability of the models for the quantitative description of *Pseudomonas* spp. counts on button mushrooms. The successfully validated Baranyi model involved in one-step modelling approach exhibited considerable potential to be used for prediction of *Pseudomonas* spp. counts as a function of time and storage temperature, if the initial counts are known. Hence, this global model could be employed as a more accurate and robust alternative to the traditionally used two-step modelling approach to determine the microbial spoilage of mushrooms, as *Pseudomonas* spp. counts are a reliable indicator of spoilage.

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