Development and validation of growth models using one-step modelling approach for determination of chicken meat shelf-life under isothermal and non-isothermal storage conditions

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Summary

The main objective of the present study was to investigate and simulate the effect of storage temperature on spoilage of aerobically stored chicken meat using two-step and one-step modelling approaches. For this purpose, the fitting capability of various primary models was evaluated for total bacterial counts in aerobically stored chicken meat. The one-step modelling approach considerably improved the fitting capability whichever primary model was used. The statistical indices (bias factor = 0.999 and accuracy factor = 1.194) showed that the best prediction performance for prediction of maximum specific bacterial growth rate values was obtained by the one-step modelling approach involving the Huang model. The prediction performance of the Huang model was also assessed for various non-isothermal storage conditions, and this model provided satisfactory statistical indices (1.027 > bias factor > 1.064 and 1.069 > accuracy factor > 1.085). The validated one-step modelling approach exhibited good performance as a prediction tool for the determination of chicken meat spoilage; therefore the chicken meat shelf-life was predicted as a function of storage temperature. The shelf-life decreased from 58 h (2.4 days) to 16 h (0.7 days) when the storage temperature was increased from 4 °C to 15 °C.

Keywords

dynamic model; microbiological quality; growth kinetics; predictive microbiology; shelf-life

Meat is among nutrient-dense foods and is a source of protein. Meat can be roughly classified into red and white meat based on the rational content of myoglobin in muscle fibre which is high and low, respectively [1]. Because white meat is relatively low cost and has low fat content, it is widely consumed all over the world. Chicken meat consumption plays an important role in income of white meat industry and has recently increased widely all over the world [2–4]. Unfortunately, chicken meat is a highly perishable food product even when kept under refrigeration, which may result in an important economic loss for the white meat industry [5]. Total bacterial counts are directly related to the spoilage of the chicken meat, and *Pseudomonas* spp. is one of the most abundant bacterial genera, naturally existing in chicken microbiota [6].

The main purpose of predictive microbiology is to predict microbial behaviour by means of mathematical models, which can help to prevent food spoilage and food-borne illnesses [7]. Primary and secondary models are commonly used in predictive food microbiology [8]. For the primary models, 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. Alternatively, recently Robazza et al. [9] have proposed a new growth model based on central limit theorem in order to describe the growth behaviour of *Pseudomonas* spp. in fish meat and have reported that this new model gave the better fitting performance than the models which are widely used as primary models in predictive food microbiology. The secondary models indicate how obtained para-

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meters from primary models change with respect to one or more environmental or cultural factors (e.g. gas composition, pH, temperature and salt level). Temperature is one of the most important environmental factors directly affecting the growth behaviour of microorganisms in foods, and its effect has been widely described by the Ratkowsky model [10].

Under real life conditions, environmental factors are not always constant during the pass time for the food product reaches consumers [11]. Therefore, dynamic models are essential to model by taking into account the changing environmental conditions a food product really subjects to [7]. The most likely variable environmental condition is the temperature that considerably affects the growth of microorganisms in foods. Dynamic models considering the effect of the changing temperature are important to model the effect of the temperature on microbial growth under non-isothermal conditions. In this regard, various temperature cycling scenarios that food products can be subjected to can be considered, and the effects of these changes on microbial growth behaviour can be predicted with dynamic models.

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. The major drawback is accumulation and propagation of errors due to the performance of two sequential non-linear regressions [12]. Besides, this approach generally results in high uncertainty for estimation of secondary model parameters because of the low number of degrees of freedom. To avoid these disadvantages of the two-step modelling approach, alternatively, a one-step modelling approach can be applied to simulate microbial data and kinetic parameters. In this approach, primary and secondary modelling for the growth and temperature (as a changing environmental factor) data is performed simultaneously. The use of this approach frequently provides better prediction performance, lower uncertainty, more precise coefficients and a more robust confidence interval than the two-step modelling approach [13, 14]. These advantages are much more noticeable when high biological variation in microbial data is observed and not enough microbiological data for the secondary model are available [15, 16].

In this work, the microbial growth in aerobically stored chicken meat was simulated with the two-step and one-step modelling approaches. The fitting capability of both approaches was compared using various primary models. Each of the models was assessed in a validation step for specific maximum growth rate data. The primary model that gave the best prediction performance was employed to predict total bacterial counts (*TBC*) in aerobically stored chicken meat and to calculate the shelf-life of chicken meat.

MATERIALS AND METHODS

Data collection

The bacterial growth data points based on *TBC* were collected from a previously published study conducted with aerobically stored chicken meat [6]. In that study, the microbiological analysis used chicken breast fillets, homogenized by a stomacher homogenizer in quarter-strength Ringer‘s solution, decimally diluted solutions being plated on tryptic glucose yeast agar (Biolife Italiana, Milan, Italy). This is a medium for enumeration of mesophilic aerobic and facultatively anaerobic microorganisms in water, food and animal feed. The plates were incubated at 30 °C for 48 h. The growth data points were extracted either from the growth curves or obtained directly from tabulated data. Data from growth curves were extracted using GetData Graph Digitizer 2.26 software (Digital River, Cologne, Germany) with nominal uncertainty of the measurement of ± 0.1 log CFU·g^{-1}. For simulation work in this study, 14 growth data points were collected separately for the storage temperatures of 4 °C, 10 °C and 15 °C corresponding to 42 growth data points from the source study [6].

For assessing prediction performance of this simulation work under non-isothermal storage conditions, two different temperature scenarios which can simulate changes in temperatures during processing, transportation and storage of chicken meat were considered:

- temperature profile 1 – 16 h at 6 °C and 8 h at 10 °C,
- temperature profile 2 – 16 h at 6 °C, 4 h at 9 °C and 4 h at 13 °C.

Modelling

Various popular primary models, namely, the modified Gompertz model, logistic, Baranyi and Huang models, and the central limit theorem-based growth model, which is alternative to traditionally used growth models, were involved in the two-step and one-step modelling approaches [9, 11, 17, 18]. Primary models used in this study are presented in Tab. 1.

Tab. 1. Primary models.

Model	Equation	Equation number
Modified Gompertz	$x(t) = x_0 + A \cdot \exp\left\{-\exp\left[\frac{r_{\max} \cdot e}{A} \cdot (\lambda - t) + 1\right]\right\}$	1
Logistic	$x(t) = x_0 + \frac{A}{\left\{1 + \exp\left[\frac{4 \cdot r_{\max}}{A} \cdot (\lambda - t) + 2\right]\right\}}$	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
Central limit theorem	$y(t) = y_0 + \mu_{max} \sqrt{\frac{\pi}{2\sqrt{m}}} (\lambda - t^*).erf(t)$ $erf(t) = erf\left[\sqrt{2}\left(\frac{t^* - t}{t^* - \lambda}\right)\right] - erf\left[\sqrt{2}\left(\frac{t^*}{t^* - \lambda}\right)\right]$	5

t – time (in hours); *x*(*t*) – counts of microorganisms at time *t* (expressed as logarithm of colony forming units per gram); x_0 – initial counts of microorganisms (expressed as logarithm of colony forming units per gram); A – ($x_{max} - x_0$); x_{max} – maximum counts 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*) – counts of microorganisms (expressed as natural logarithm of colony forming units per gram) at time *t*; y_0 – initial counts of microorganisms (expressed as natural logarithm of colony forming units per gram); y_{max} – maximum counts of microorganisms (expressed as natural logarithm of colony forming units per gram); μ_{max} – maximum specific growth rate (expressed as natural logarithm of colony forming units per hour); *F*(*t*) – adjustment function described by Baranyi and Roberts [17]; *B*(*t*) – adjustment functions described by Huang [18]; *m* – characterizes the physiological state of the cells.; t^* – time of inflection point (in hours); *erf* – the Gaussian error function.

Because the primary models use different scale for the counts of microbial populations, the growth rates values (r_{max}) obtained from the modified Gompertz and logistic models after fitting were converted to the maximum specific growth rate values (μ_{max}).

The Ratkowsky model described by Ratkowsky et al. [10] was employed to relate storage temperature with μ_{max} using Eq. (6):

$$\sqrt{\mu_{\max}} = b_1(T - T_0) \quad (6)$$

where T is 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 (expressed as natural logarithm of colony forming units per hour), and b_1 is the regression coefficient.

Lag phase (λ) was defined as a function of μ_{max} with respect to temperature using Eq. (7) [19]:

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

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

For the two-step and one-step modelling approaches, each of the parameters was 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 the non-linear regression procedure is an essential step to estimate accurate parameters. Randomly choosing starting points for the parameters might lead the estimated parameters to possible local optimal points around the global one in particular in case of the one-step modelling approach. Therefore, the starting points of the parameters were selected by using ga command, which uses genetic algorithm in Global Optimization Toolbox of Matlab software for the two-step and one-step modelling approaches. Following successful iteration process for the non-linear regression procedure, the global optimum values of the parameters were estimated.

Comparison of the goodness of fit of the global models

The comparison of the global models' estimation capabilities was performed by taking into consideration the root mean square error (*RMSE*) and the adjusted coefficient of determi-

nation (R^2_{adj}), Akaike information criteria (*AIC*) and Bayesian information criteria (*BIC*) using Eqs. 8–11, respectively [20]:

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

$$R^2_{adj} = 1 - \left(\frac{n-1}{n-s}\right)\left(\frac{SSE}{SST}\right) \quad (9)$$

$$AIC = n \ln\left(\frac{SSE}{n}\right) + 2s \quad (10)$$

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

where x_{obs} is the experimental counts of microorganisms, 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.

As the primary models use different scale for microbial counts, *RMSE*, *AIC* and *BIC* values of the modified Gompertz and logistic models cannot be directly compared with *RMSE*, *AIC* and *BIC* values of the Baranyi, Huang and central limit theorem-based growth models. Therefore, conversion from the ln scale to log scale was done to compare *RMSE*, *AIC* and *BIC* values of all the primary models.

Statistical analysis

The statistical indices (*RMSE*, R^2_{adj}, *AIC* and *BIC*) obtained from the 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

Verification of the developed models in the predictive food microbiology is crucial to be reliably employed as a simulation tool. The prediction performance of the global models was assessed via independent maximum bacterial growth rate data of *TBC* collected from the previously published work [5]. Two maximum growth rate data were reported separately for the different nine storage temperatures within the range of 0–25 °C corresponding to data of Dominguez and Schaffner [5]. Averages of these data were used for the comparison with the maximum bacterial growth rate data predicted by each of the global models. The comparison was done considering the bias (B_f) and accuracy (A_f) factors [21] given in Eq. 12 and Eq. 13, respectively:

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

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

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

B_f and A_f are the indicators of prediction performance of the models, and a value of 1 for B_f and A_f means that there is a perfect agreement between experimental and maximum growth rate data. Additionally, two validation criteria known as mean deviation (*MD*) and mean absolute deviation (*MAD*) were calculated to evaluate the prediction capability of the models for non-isothermal storage conditions, as stated by Le Marc et al. [22]. A value of *MD* and *MAD* close to 0 shows that the prediction capability of the model is perfect.

Shelf-life prediction

TBC in aerobically-stored chicken meat stored at the temperature range of 4–15 °C can be predicted as a function of time and storage temperature using Eq. 14 proposed by Buchanan et al. [23]:

$$\begin{aligned} x(t) &= x_0 && \text{for } t \leq \lambda \\ x(t) &= x_0 + r_{max}(t - \lambda) && \text{for } \lambda < t < t_{max} \\ x(t) &= x_{max} && \text{for } t \geq t_{max} \end{aligned} \quad (14)$$

where t is time (in hours), $x(t)$ is the counts of microorganisms (expressed as logarithm of colony forming units per gram) at time t, x_0 is the initial counts of microorganisms (expressed as logarithm of colony forming units per gram), t_{max} is the time (in hours) when *TBC* reaches maximum, r_{max} is the fitted maximum bacterial growth rate (expressed as logarithm of colony forming units per hour) at the storage temperature (in degrees Celsius) and λ is the lag phase duration (in hours).

These equations can also be used to assess the microbial spoilage and predict the product shelf-life. In this study, the shelf-life of chicken meat was defined as the time which *TBC* in chicken meat reach a threshold value of 7 log CFU·g^{-1} [5].

Tab. 2. Observed and fitted data of total bacterial counts in aerobically stored chicken meat at different temperature.

Storage time (days)	Total bacterial counts [log CFU·g^{-1}]										
	Observed data	Fitted data – two-step modelling approach					Fitted data – one-step modelling approach				
		Modified Gompertz model	Logistic model	Baranyi model	Huang model	Central limit theorem	Modified Gompertz model	Logistic model	Baranyi model	Huang model	Central limit theorem
Storage at 4 °C											
0	5.1 ± 0.3	5.4	5.5	5.3	5.3	5.1	5.3	5.4	5.2	5.2	5.1
0.4	5.2 ± 0.2	5.6	5.7	5.6	5.6	5.4	5.5	5.6	5.5	5.5	5.4
0.8	5.7 ± 0.2	5.8	5.9	5.9	5.9	5.7	5.8	5.8	5.9	5.9	5.8
1	6.2 ± 0.3	6.0	6.0	6.1	6.1	5.9	5.9	5.9	6.0	6.0	6.0
1.4	6.3 ± 0.4	6.3	6.3	6.4	6.4	6.2	6.3	6.2	6.3	6.3	6.3
2	6.8 ± 0.3	6.9	6.8	6.8	6.8	6.7	6.9	6.7	6.8	6.8	6.9
3	7.3 ± 0.3	7.7	7.7	7.6	7.6	7.4	7.8	7.6	7.6	7.6	7.7
4	8.6 ± 0.4	8.5	8.5	8.3	8.3	8.1	8.5	8.5	8.3	8.3	8.4
5	9.2 ± 0.2	9.0	9.1	9.0	9.0	8.6	9.0	9.1	9.1	9.1	9.0
6	9.5 ± 0.1	9.3	9.5	9.5	9.5	8.9	9.3	9.4	9.5	9.5	9.4
7	9.7 ± 0.2	9.6	9.7	9.7	9.7	9.2	9.5	9.6	9.7	9.7	9.7
8	10.0 ± 0.4	9.7	9.8	9.8	9.8	9.3	9.7	9.7	9.8	9.8	9.8
9	9.9 ± 0.2	9.8	9.8	9.8	9.8	9.4	9.8	9.8	9.8	9.8	9.9
10	9.8 ± 0.1	9.8	9.8	9.8	9.8	9.5	9.8	9.8	9.8	9.8	10.0
Storage at 10 °C											
0	5.1 ± 0.3	5.4	5.5	5.3	5.3	5.1	5.3	5.4	5.2	5.2	5.1
0.4	5.9 ± 0.5	5.9	5.9	6.0	6.0	5.9	5.8	5.8	5.9	5.9	5.9
0.8	6.8 ± 0.4	6.6	6.5	6.6	6.6	6.6	6.6	6.5	6.6	6.6	6.6
1	7.0 ± 0.3	7.0	6.8	6.9	6.9	7.0	7.0	6.9	7.0	6.9	7.0
1.4	7.5 ± 0.3	7.7	7.6	7.6	7.6	7.7	7.8	7.7	7.6	7.6	7.7
2	8.4 ± 0.4	8.6	8.6	8.5	8.5	8.6	8.6	8.7	8.6	8.6	8.6
3	9.1 ± 0.1	9.4	9.5	9.6	9.6	9.5	9.4	9.6	9.7	9.7	9.5
4	9.6 ± 0.2	9.7	9.8	9.8	9.8	9.9	9.7	9.8	9.8	9.8	9.8
5	9.9 ± 0.1	9.9	9.8	9.8	9.8	10.0	9.8	9.8	9.8	9.8	9.8
6	10.1 ± 0.3	9.9	9.8	9.8	9.8	10.0	9.9	9.8	9.8	9.8	9.9
7	9.8 ± 0.1	9.9	9.8	9.8	9.8	10.0	9.9	9.8	9.8	9.8	9.9
8	9.9 ± 0.4	9.9	9.8	9.8	9.8	10.0	9.9	9.8	9.8	9.8	9.9
9	9.7 ± 0.2	9.9	9.8	9.8	9.8	10.0	9.9	9.8	9.8	9.8	9.9
10	10.0 ± 0.1	9.9	9.8	9.8	9.8	10.0	9.9	9.8	9.8	9.8	9.9

Tab. 2. continued

Storage time (days)	Total bacterial counts [log CFU·g^{-1}]										
	Observed data	Fitted data – two-step modelling approach					Fitted data – one-step modelling approach				
		Modified Gompertz model	Logistic model	Baranyi model	Huang model	Central limit theorem	Modified Gompertz model	Logistic model	Baranyi model	Huang model	Central limit theorem
Storage at 15 °C											
0	5.1 ± 0.3	5.4	5.5	5.3	5.3	5.1	5.3	5.4	5.2	5.2	5.1
0.4	7.2 ± 0.2	6.3	6.2	6.3	6.3	6.4	6.3	6.2	6.3	6.3	6.3
0.8	7.5 ± 0.4	7.5	7.3	7.4	7.4	7.7	7.5	7.4	7.5	7.4	7.5
1	8.1 ± 0.2	8.0	7.9	7.9	7.9	8.2	8.1	8.1	8.0	8.0	8.1
1.4	9.0 ± 0.2	8.8	8.9	8.8	8.8	9.1	8.9	9.0	9.0	9.0	8.9
2	9.2 ± 0.1	9.5	9.6	9.7	9.7	9.8	9.5	9.6	9.7	9.7	9.5
3	9.6 ± 0.3	9.8	9.8	9.8	9.8	10.2	9.8	9.8	9.8	9.8	9.8
4	9.5 ± 0.1	9.9	9.8	9.8	9.8	10.2	9.9	9.8	9.8	9.8	9.8
5	10.3 ± 0.4	9.9	9.8	9.8	9.8	10.2	9.9	9.8	9.8	9.8	9.8
6	10.4 ± 0.2	9.9	9.8	9.8	9.8	10.2	9.9	9.8	9.8	9.8	9.8
7	9.8 ± 0.3	9.9	9.8	9.8	9.8	10.2	9.9	9.8	9.8	9.8	9.8
8	9.6 ± 0.1	9.9	9.8	9.8	9.8	10.2	9.9	9.8	9.8	9.8	9.8
9	9.7 ± 0.0	9.9	9.8	9.8	9.8	10.2	9.9	9.8	9.8	9.8	9.8
10	9.6 ± 0.1	9.9	9.8	9.8	9.8	10.2	9.9	9.8	9.8	9.8	9.8

Observed data in the table are given as mean ± standard deviation obtained from Lytou et al. [6].

RESULTS AND DISCUSSION

The growth data points extracted from the previously published study for aerobically-stored chicken meat [6] were separately considered and fitted using two-step and one-step modelling approaches involving the modified Gompertz, logistic, Baranyi, Huang and central limit theorem-based growth models. While the initial *TBC* values were on average of 5.1 ± 0.3 log CFU·g^{-1} for each temperature of 4 °C, 10 °C and 15 °C, the maximum *TBC* reached 10.0 ± 0.4 log CFU·g^{-1} for the storage at 4 °C, 10.1 ± 0.3 log CFU·g^{-1} for the storage at 10 °C and 10.4 ± 0.2 log CFU·g^{-1} for the storage at 15 °C (Tab. 2). However, there was no significant difference ($p > 0.05$) between storage temperatures in terms of maximum *TBC*. This result shows that microbial growth in aerobically-stored chicken meat was not significantly affected by the storage temperature.

The fitting capability of the traditional two-step modelling approach based on various primary models (the modified Gompertz, logistic, Branyi, Huang and central limit theorem-based growth models) was compared by taking into account *RMSE*, R^2_{adj}, *AIC* and *BIC* values (Tab. 3). All statistical indices belonging to two-step modelling approach, *RMSE*, R^2_{adj}, *AIC* and *BIC* values, showed that the modified Gompertz model gave significantly ($p < 0.05$) better fitting performance for the simulation of the microbial growth data. This simply means that the fitting ability of the modified Gompertz model was superior over other primary models when two-step modelling was employed to describe the microbial growth in aerobically-stored chicken meat.

When the two-step and one-step modelling approaches were compared, the *RMSE* and R^2_{adj} values of each of the primary models based on one-step modelling approach were maximum 0.287 and minimum 0.972, respectively. Additionally, *AIC* and *BIC* values were smaller than –95.564

Tab. 3. Comparison of statistical indices for two-step and one-step modelling approaches.

Modelling approach	Statistical index	Modified Gompertz model	Logistic model	Baranyi model	Huang model	Central limit theorem
Two-step modelling	RMSE	0.287	0.309	0.292	0.293	0.393
	R^2_{adj}	0.972	0.968	0.971	0.971	0.945
	AIC	–100.323	–94.059	–98.866	–98.458	–73.802
	BIC	–102.847	–96.584	–101.391	–100.983	–76.327
One-step modelling	*RMSE*	0.278	0.287	0.282	0.287	0.260
	R^2_{adj}	0.974	0.972	0.973	0.973	0.976
	AIC	–101.656	–95.564	–100.653	–100.263	–108.317
	BIC	–104.180	–98.089	–103.177	–102.787	–110.841

Statistical indices are calculated using overall total bacterial counts for storage temperature 4 °C, 10 °C and 15 °C.
RMSE – root mean square error, R^2_{adj} – adjusted coefficient of determination, *AIC* – Akaike information criterion, *BIC* – Bayesian information criterion.

and –98.089, respectively, for each primary model (Tab. 3). These results indicated that one-step modelling approach significantly ($p < 0.05$) improved the goodness of fit results whichever primary model was employed. In other words, the statistical evaluation regarding the fitting capability of the primary models based on one-step modelling approach indicated that the fitting capability of the primary models was better than that of the traditionally used two-step modelling approach. These results simply mean that one-step modelling approach could be reliably used for the estimation of *TBC* in chicken meat. Furthermore, even the worst goodness of fit results obtained from one-step modelling approach were better than the best goodness of fit result obtained from the two-step modelling approach. Therefore, one-step modelling approach was considered for further simulation works on microbial growth and to describe the shelf-life of aerobically stored chicken meat.

The degree of freedom in the traditional two-step modelling approach used by Lytou et al. [6] was only 1 for the Ratkowsky model. On the other hand, the number of degrees of freedom belonging to the one-step modelling approach proposed in this study was 38 for each global model. It is known that the high number of degrees of freedom while employing non-linear regression procedure results in a decrease in uncertainty and in an increase in reliability of the model parameters [12]. From this point of view, the two-step modelling approach with only one degree of freedom for the Ratkowsky model may be regarded as giving the results that are doubtful and ambiguous. Additionally, the one-step modelling approach with a higher number of degrees of freedom than that in the two-step modelling approach yielded higher confidence of the estimated parameters.

When simulating the growth behaviour of microorganisms, accurate determination of the ex-

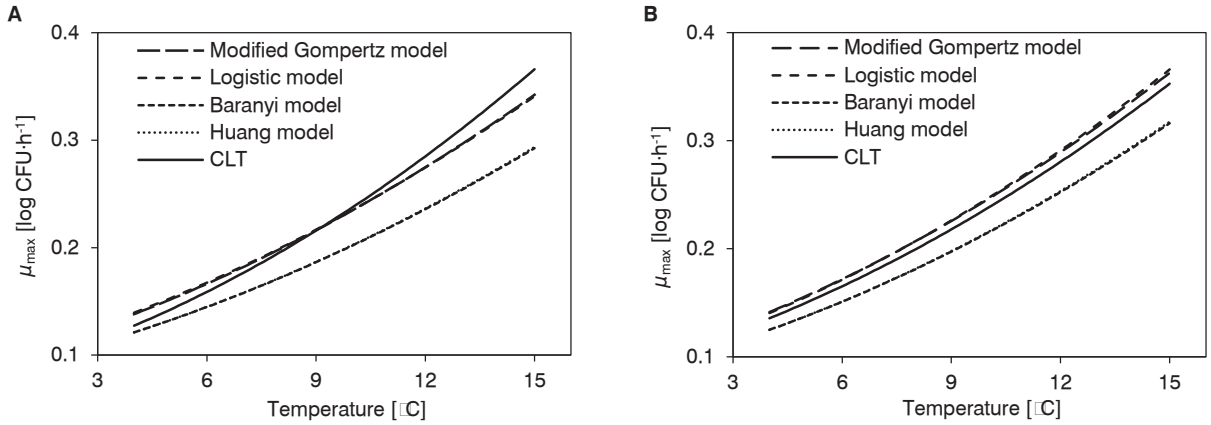

Fig. 1. The effect of storage temperature on the maximum growth rate.

A – two-step modelling approach, B – one-step modelling approach.
CLT – central limit theorem-based growth model.

ponential phase in which the growth rate reaches a maximum value and the variations in organoleptic properties of foods also reach maximum, and accurate determination of the lag phase in which organoleptic properties almost do not change, is very important. μ_{max} values obtained from the primary models for both two-step and one-step modelling approaches are shown in Fig. 1. As expected, μ_{max} values increased with the increasing storage temperature from 4 °C to 15 °C for all primary models and for both modelling approaches. The other finding related to μ_{max} was that the one-step modelling approach estimated μ_{max} values significantly ($p < 0.05$) higher than the two-step modelling approach. A similar trend was previously obtained for μ_{max} values of *Pseudomonas* spp. on mushrooms [24]. λ value was not detected for any storage temperature and primary models used in two-step and one-step modelling approaches. This result is in good agreement with the findings of LYTOU et al. [6], who did not report any λ values for the storage temperature in the range of 4 °C to 15 °C. This result obviously revealed that bacteria did not need any time to adapt to new conditions and could immediately start to grow in aerobically stored chicken meat.

Validation is an important step to check how well the developed models are working. Therefore, the prediction power of the global models obtained from the one-step modelling approach was evaluated with the indices of B_f and A_f. There was no significant difference ($p > 0.05$) among the fitting capabilities of any primary models used for one-step modelling and all models gave high goodness of fit results. Therefore, each of the primary models was processed in a validation step. For validation, independent maximum microbial growth rate data were collected from the work of DOMINGUEZ and SCHAFFNER [5] conducted for chicken meat aerobically stored at temperatures between 0 °C and 25 °C. The maximum growth rate values were predicted via all global models involving various primary models, and they were compared with the observed maximum growth data published [5] (Tab. 4). The statistical indices of B_f and A_f showed that, although their fitting capabilities were high to describe the microbial growth in aerobically stored chicken meat, modified Gompertz, logistic and central limit theorem-based growth models failed to accurately predict μ_{max} values of *TBC*. On the other hand, B_f and A_f values were calculated as 0.999 and 1.194, respectively, for the Huang model, which showed that it yielded the prediction power by far superior over the other primary models (Tab. 4). A B_f factor of 1 indicates no structural deviation of the model. The B_f factor of 0.999 indicated an overestimation of 0.1 % whereas the A_f factor of 1.194 showed that, on average, the predicted value was by 19.4 % different (either smaller or larger) from the observed values. The B_f and A_f values were calculated as 0.917 and 1.207, respectively, using the Ratkowsky model parameters reported by LYTOU et al. [6]. These results confirmed that the prediction power

Tab. 4. Observed and predicted maximum growth rate values of bacteria in aerobically stored chicken meat using one-step modelling approach.

Storage temperature [°C]	Maximum growth rate [log CFU·h^{-1}]						
	Observed values		Predicted values				
	Minimum	Maximum	Modified Gompertz model	Logistic model	Baranyi model	Huang model	Central limit theorem
0	0.0128	0.0162	0.0190	0.0184	0.0150	0.0149	0.0172
5	0.0251	0.0267	0.0461	0.0458	0.0380	0.0380	0.0434
7	0.0394	0.0549	0.0602	0.0602	0.0502	0.0503	0.0572
10	0.0435	0.0522	0.0850	0.0854	0.0715	0.0718	0.0815
15	0.1268	0.1365	0.1358	0.1372	0.1156	0.1162	0.1315
18	0.1612	0.1820	0.1719	0.1742	0.1471	0.1479	0.1672
20	0.1718	0.1747	0.1984	0.2013	0.1702	0.1712	0.1934
22	0.2365	0.2404	0.2267	0.2304	0.1949	0.1962	0.2215
25	0.2229	0.4903	0.2728	0.2776	0.2352	0.2368	0.2672
Bias factor B_f			1.164	1.168	0.996	0.999	1.120
Accuracy factor A_f			1.240	1.237	1.197	1.194	1.211

Observed maximum growth rate values were collected from the work of DOMINGUEZ and SCHAFFNER [5] conducted for chicken meat aerobically stored at temperatures between 0 °C and 25 °C. Predicted maximum growth rate values were estimated using one-step modelling approach.

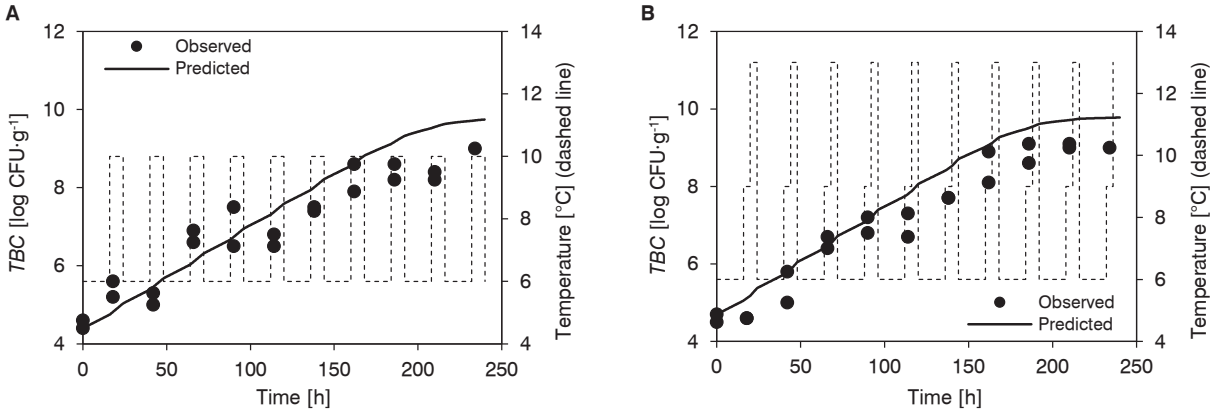

Fig. 2. Observed and predicted total bacterial counts in chicken meat subjected to non-isothermal storage conditions.

A – Temperature profile 1, B – Temperature profile 2.
Temperature profile 1 refers to cycling that is 16 h at 6 °C and 8 h at 10 °C. Temperature profile 2 refers to cycling that is 16 h at 6 °C, 4 h at 9 °C and 4 h at 13 °C.
TBC – total bacterial counts.The dashed line shows the changing temperature during storage.

Tab. 5. Evaluation of prediction power of Huang model for non-isothermal storage conditions.

Dynamic profiles	Bias factor B_f	Accuracy factor A_f	Mean deviation *MD*	Mean absolute deviation MAD
Temperature profile 1	1.027	1.085	0.27	0.59
Temperature profile 2	1.064	1.069	0.46	0.49

Temperature profile 1 refers to cycling that is 16 h at 6 °C and 8 h at 10 °C. Temperature profile 2 refers to cycling that is 16 h at 6 °C, 4 h at 9 °C and 4 h at 13 °C.

of the one-step modelling approach involving the Huang model in this work was higher than the prediction power of the two-step modelling approach used by LYTOU et al. [6].

In order to assess the prediction performance of the one-step modelling approach involving the Huang model, which gave the best prediction performance for the estimation of μ_{max} values of *TBC*, by considering the microbial growth in chicken meat under aerobical non-isothermal storage conditions, the differential form of the Huang model described by MILKIEVICZ [20] was used, and the predictions were compared with the observed growth data points from the study of LYTOU et al. [6] (Fig. 2). Here, data collection process for growth curves was performed using GetData Graph Digitizer 2.26 software. The statistical indices (B_f, A_f, *MD* and *MAD*) for the comparison of predicted and observed values are given in Tab. 5. B_f and A_f close to one for both of the non-isothermal profiles showed that the dynamic model used in this work had a high capability to predict *TBC* in the chicken meat under aerobical non-isothermal storage conditions. The *MD* and *MAD* values ranging from 0.27 log CFU·g^{-1} to 0.59 log CFU·g^{-1} for both of the non-isothermal profiles also confirmed that the Huang model had a good prediction performance for quantitative prediction of *TBC* in chicken meat in both isothermal and non-isothermal storage conditions.

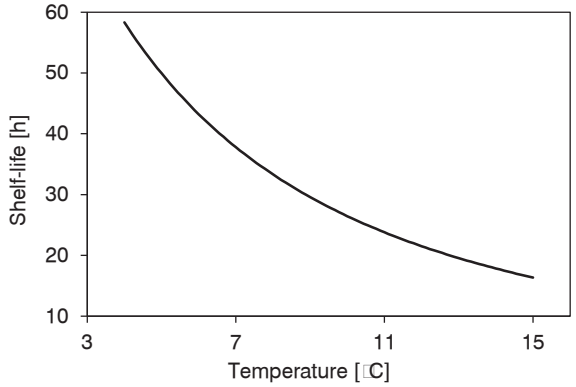

Fig. 3. Shelf-life prediction as a function of temperature using one-step modelling approach.

Shelf-life was predicted using Huang model, initial bacterial counts x_0 = 5.1 log CFU·g^{-1} and limiting bacterial counts $x(t)$ = 7 log CFU·g^{-1}.

As the spoilage of chicken meat is directly linked to *TBC*, the Huang model was employed

to predict the shelf-life of chicken meat as a function temperature. For this purpose, *TBC* of 7 log CFU·g^{-1} was considered as the limiting microbiological criterion for chicken meat. The effect of temperature on microbial spoilage was correlated with the shelf-life of chicken meat (Fig. 3). The temperature was found to be a critical factor affecting the shelf-life of chicken meat. As the temperature increased, the shelf-life of chicken meat decreased considerably. For instance, the shelf-life was calculated as 58 h (2.4 days) and 16 h (0.7 days) at 4 °C and 15 °C, respectively. In other words, the shelf-life was almost four-fold lower at 15 °C than at 4 °C. This is in accordance with practical knowledge that chicken meat should not be subjected to higher temperatures and always be stored refrigerated, preferably at less than 4 °C throughout the shelf-life.

CONCLUSION

No matter which primary model was used, the one-step modelling approach noticeably improved the prediction capability of the models for quantitative prediction of *TBC* in aerobically stored chicken meat. Successfully validated Huang model has a potential to be used as a simulation tool to predict *TBC* as a function of time and storage temperature. Additionally, use of the link between *TBC* and the shelf-life of the chicken meat could enable the chicken meat producers to assess microbial spoilage and reliably predict product shelf-life subjected to both isothermal and non-isothermal storage conditions.

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