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Prediction of growth kinetics of *Pseudomonas* spp. in meat products under isothermal and non-isothermal storage conditions

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ABSTRACT

The main objective of the present study was to develop and validate a new alternative modelling method to predict the shelf-life of food products under non-isothermal storage conditions. The bacterial growth data of the *Pseudomonas* spp. was extracted from published studies conducted for aerobically-stored fish, pork and chicken meat and described with two-step and one-step modelling approaches employing different primary models (the modified Gompertz, logistic, Baranyi and Huang models) under isothermal storage temperatures. Temperature dependent kinetic parameters (maximum specific growth rate 'μ_{max}' and lag phase duration 'λ') were described as a function of storage temperature via the Ratkowsky model integrated with each primary model. The Huang model based on the one-step modelling approach yielded the best goodness of fit results (RMSE = 0.451 and adjusted-R^2 = 0.942) for all food products at isothermal storage conditions, therefore, was also used to check it's the prediction capability under non-isothermal storage conditions. The differential form of the Huang model provided satisfactorily statistical indexes ($1.075 > B_f > 1.014$ and $1.080 > A_f > 1.047$) indicating reliably being able to use to describe the growth behaviour of *Pseudomonas* spp. in fish, pork and chicken meat subjected to non-isothermal storage conditions.

Keywords: Dynamic condition, Microbiological quality, *Pseudomonas* spp., growth kinetic, Predictive microbiology

Introduction

Meat is among nutrient-dense foods and is a source of protein. Fish, pork and chicken meat play an important role in meat industry; however, are highly perishable food products even when kept under refrigeration, which may result in an important economic loss (Bruckner et al., 2013; Dominguez and Schaffner, 2007; Koutsoumanis, 2001). Initial microbial quality and storage conditions have a direct effect on product shelf-life, and *Pseudomonas* spp. is one of the most abundant bacterial genera, naturally existing in fish, pork and chicken microbiota (Bruckner et al., 2013; Ghollasi-Mood et al., 2017; Lytou et al., 2016; Koutsoumanis, 2001).

Microbial load in food can be determined with traditional microbiological enumeration techniques. Even more, the results of these techniques give us only information about specific time and condition. But the growth behaviour of microorganisms depends on changing environmental factors. Therefore, the traditional enumeration techniques are not adequately practical. Predictive microbiology is a tool used to describe microbial behaviour in food. Although traditional microbiological methods have high costs and time-consuming results, these methods are still used simultaneously with predictive microbiology to describe microbial behaviour in the development of products and processes (Bovill et al., 2001).

The main objective of predictive microbiology is to predict microbial behaviour, which 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 (Whiting, 1995). 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. The secondary models indicate how obtained the growth parameters 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 is widely described using the Ratkowsky model (Ratkowsky et al., 1982).

Under real life conditions, environmental factors are not always constant during the pass time for the food product reaches consumers (Zwietering et al., 1994). Therefore, dynamic models are essential to model by taking into account the changing environmental conditions which a food product really subjects to (Pérez-Rodríguez and Valero, 2013). Dynamic models considering the effect of changing temperature are important to model the effect of the temperature on microbial growth under non-isothermal conditions.

Generally, the primary and secondary models are separately fitted to the growth data and kinetic parameters, respectively and this is the most popular modelling procedure followed in the predictive food microbiology. But there are some drawbacks concerning about this modelling approach. The major drawback is to lead to be accumulation and propagation of errors due to being sequentially performed nonlinear regression two times (Huang, 2017). To avoid these disadvantages of two-step modelling approach, alternatively, a one-step modelling approach can be applied while simulating 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. Therefore, the use of this approach frequently provides better prediction performance, lower uncertainty, more precise coefficients and robust confidence interval than the two-step modelling approach (Jewell, 2012; Martino and Marks, 2007).

In the present study, the growth behaviour of *Pseudomonas* spp. naturally existing in fish, pork and chicken microbiota were described with both two-step and one-step modelling approaches for isothermal storage conditions. The fitting capabilities of both approaches were compared and the approach which gave better fitting performance was tested under non-isothermal storage conditions.

Materials and Methods

Experimental Data

The bacterial growth data of *Pseudomonas* spp. were extracted from the published works performed for fish, pork and chicken meat (Bruckner, 2010; Bruckner et al., 2013; Koutsoumanis, 2001). While there were six isothermal storage conditions (0, 2, 5, 8, 10 and 15 °C) to simulate the bacterial growth behaviour for fish (Koutsoumanis, 2001), there were five isothermal storage conditions (2, 4, 7, 10 and 15 °C) for pork and chicken meat (Bruckner, 2010; Bruckner et al., 2013). The experimental set-ups to monitor *Pseudomonas* spp. in the targeted food products (fish, pork and chicken meat) were explained in detail in the respective studies (Bruckner, 2010; Bruckner et al., 2013; Koutsoumanis, 2001). In brief, food products were transported to the laboratory under temperature-controlled refrigeration conditions. As soon as they arrived and the initial microbiological analyses of them were performed, and they were started to keep at aerobically storage conditions. For microbiological analyses, food samples (25 g) were added aseptically to 225 mL

of 0.1% peptone water with salt (NaCl, 0.85%), and the mixture was homogenized for 60 s with a stomacher. A 10-fold dilution series of the homogenate was prepared using saline peptone diluents. Appropriate dilutions were transferred to *Pseudomonas* Agar Base with CFC supplement (Oxoid) incubating at 20-25 °C for 48 h. In the current study, data collection process for the growth curves was performed using GetData Graph Digitizer 2.26 software (www.getdata-graph-digitizer.com) by which the growth data points could be extracted accurately with one decimal precision.

Modelling

Four different primary models namely the modified Gompertz (Zwietering et al., 1990), logistic (Zwietering et al., 1990), Baranyi (Baranyi and Roberts, 1994) and Huang (Huang 2017) models were fitted with the two-step and one-step modelling approaches as they are the most used sigmoid functions that describe the bacterial growth behaviour and are defined by Eqs (1), (2), (3) and (4), respectively at constant environmental conditions:

$$y(t) = y_0 + (y_{max} - y_0).\exp\left\{-\exp\left[\frac{\mu_{max}.e}{(y_{max}-y_0)}.(\lambda - t) + 1\right]\right\} \quad (1)$$

$$y(t) = y_0 + \frac{(y_{max}-y_0)}{\left\{1+\exp\left[\frac{4.\mu_{max}}{(y_{max}-y_0)}.(\lambda-t)+2\right]\right\}} \quad (2)$$

$$y(t) = y_0 + \mu_{max}F(t) - \ln\left(1 + \frac{e^{\mu_{max}F(t)}-1}{e^{(y_{max}-y_0)}}\right) \quad (3)$$

$$y(t) = y_0 + y_{max} - \ln(e^{y_0} + [e^{y_{max}} - e^{y_0}].e^{-\mu_{max}B(t)}) \quad (4)$$

F(t) and *B*(t) are the adjustment functions that are respectively described by Baranyi and Roberts (1994) and Huang (2017):

$$F(t) = t + \frac{1}{v}\ln\left(e^{-vt} + e^{-\mu_{max}\lambda} - e^{(-vt-\mu_{max}\lambda)}\right) \quad (5)$$

$$B(t) = t + \frac{1}{4}\ln\left(\frac{1+e^{-4(t-\lambda)}}{1+e^{4\lambda}}\right) \quad (6)$$

where t is the time (h), $y(t)$ is the concentration of bacterial populations (ln CFU/g) at time t, y_0 is the initial concentration of bacterial populations (ln CFU/g), y_{max} is the maximum concentration of bacterial populations (ln CFU/g), μ_{max} is the maximum specific bacterial growth rate (1/h), λ is the duration of lag phase (h) and v is the rate of increase of limiting substrate, assumed to be equal to μ_{max}.

The Ratkowsky model (Ratkowsky et al., 1982) was employed for the determination of relationship between storage temperature and μ_{max} using the Eq. (7):

$$\sqrt{\mu_{max}} = b_1(T - T_0) \quad (7)$$

where T is the storage temperature (°C), T_0 is the notional temperature (°C), μ_{max} is the maximum specific bacterial growth rate (1/h), b_1 is the regression coefficient.

Additionally, λ was defined as a function of μ_{max} with respect to temperature using the Eq (8) (Robinson et al., 1998):

$$\lambda = \frac{b_2}{\mu_{max}(T)} \quad (8)$$

where b_2 is the regression coefficient, $\mu_{max}(T)$ is the 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 Inc., Natick, MA, USA). Determination of suitable starting values in nonlinear regression procedure is necessary step to estimate the accurate parameters. The starting values for the parameters, y_0 and y_{max} were selected as the minimum and maximum concentration of bacterial populations considering the entire temperature range, respectively. Randomly choosing starting points for the parameters, b_1, b_2 and T_0 might lead the estimated parameters to possible local optimal points around global one for especially the one-step modelling approach. Therefore, the starting points of these 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 nonlinear regression procedure, the global optimum values of the parameters were obtained.

Comparison of the Goodness of Fit of the 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

determination (adjusted-R^2) using Eqs. (9) and (10) respectively (Milkievicz et al. 2020):

$$\mathrm{RMSE} = \sqrt{\sum_{i=1}^{n} \frac{(\mathrm{observed_i} - \mathrm{fitted_i})^2}{n - s}} \qquad (9)$$

$$\text{adjusted-}R^2 = 1 - \left(\frac{n-1}{n-s}\right)\left(\frac{\mathrm{SSE}}{\mathrm{SST}}\right) \qquad (10)$$

where $\mathrm{observed_i}$ is the experimental bacterial growth, *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. RMSE and adjusted-R^2 were calculated for entire data sets, which correspond to 5 for fish and 6 for pork and chicken meat considering observed and fitted values as log CFU/g.

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 model that gave the best fitting capability to model the growth behaviour of *Pseudomonas* spp. existing in fish, pork and chicken microbiota were assessed by considering the growth data obtained from non-isothermal storage conditions. The comparison was done considering each of the global models' corresponding the bias (B_f) and accuracy (A_f) factors (Ross, 1996) given in Eqs. (11) and (12), respectively:

$$B_f = 10^{\frac{\sum_{i=1}^{n} \log(y_{\mathrm{predicted}}/y_{\mathrm{observed}})}{n}} \qquad (11)$$

$$A_f = 10^{\frac{\sum_{i=1}^{n} |\log(y_{\mathrm{predicted}}/y_{\mathrm{observed}})|}{n}} \qquad (12)$$

where $y_{predicted}$ refers to predicted maximum growth rate (log CFU/h), $y_{observed}$ refers to experimental maximum growth rate (log CFU/h), *n* refers to the number of data.

The B_f is a measure of average variation between the predictions and observations. The model yielding B_f greater than 1 is considered as 'fail dangerous', while the model providing B_f less than 1 is considered as 'fail safe'. A value of 1 for B_f indicates that there is a perfect agreement between the predictions and observations. The A_f measures the average difference between the predictions and observations by disregarding whether the difference is positive or negative. The larger A_f value, the less accurate is the average estimate (Ross, 1996). 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. (2008). A value of MD and MAD closing to 0 shows that the prediction capability of the model is perfect.

Results and Discussion

The growth data of the *Pseudomonas* spp. existing in fish, pork and chicken meat microbiota were fitted using two-step and one-step modelling approaches, and the statistical indicates were given in Table 1. RMSE and adjusted-R^2 values presented in Table 1 indicate the overall fitting capabilities for two-step modelling approach, which means that RMSE and adjusted-R^2 values were calculated after consecutively done primary and secondary model fitting for entire data sets for each food product. The statistical indices showed that Huang model gave the best fitting performance for each food product. The fitting capability of the Baranyi model was the second. The Modified Gompertz and logistic models yielded almost the same fitting capabilities, which means that both of the primary models could not estimate the growth behaviour of *Pseudomonas* spp. as good as the Huang and Baranyi models estimated when the-wo step modelling approach was employed.

It is known that the degree of freedom while employing nonlinear regression procedure is important to decrease in uncertainty and increase in reliability of the model parameters (Huang, 2017). While doing simulation with one-step modelling approach, primary and secondary modelling is performed simultaneously considering whole experimental data set, which means that the simulation with one-step modelling approach has always higher degrees of freedom than the simulation with two-step modelling approach. Therefore, the improvement obtained from one-step modelling approach can be attributed to higher degrees of freedom in one-step modelling approach.

One-step modelling approach, an alternative way to traditionally used two-step modelling approach, was employed to quantitatively detect *Pseudomonas* spp. count. The statistical indices, RMSE and adjusted-R^2 values, showing the fitting capability of one-step modelling approach were presented for each food product in Table 1. The RMSE and adjusted-R^2 values of each of the primary models and each food product based on one-step modelling approach were calculated maximum 0.466 and minimum 0.938, respectively. These results showed that no matter which primary model was used, the one-step modelling approach gave considerably better prediction performance when the one-step modelling approach was employed. Therefore, the growth kinetics obtained from

the one-step modelling approach for each food product (fish, pork and chicken meat) and each primary model (the modified Gompertz, logistic, Baranyi and Huang models) were given in Table 2.

The Huang model based on the one-step modelling approach showed that maximum counts of *Pseudomonas* spp. were 8.1 ± 0.1, 9.5 ± 0.1 and 9.4 ± 0.1 for the fish, pork and chicken meat, respectively (Table 2), while the maximum counts were experimentally found to be of 8.30 ± 0.30, 9.8 ± 0.2 and 9.6 ± 0.2, for the fish, pork and chicken meat, respectively. This indicated that the Huang model provided suitable prediction performance for maximum counts of *Pseudomonas* spp. in each food product.

Table 1. Comparison of fitting capability of different primary models based on two-step and one-step modelling approaches

Food products	Primary models	Modified Gompertz		Logistic		Baranyi		Huang	
	Modelling approach	2-step*	1-step	2-step*	1-step	2-step*	1-step	2-step*	1-step
Fish	RMSE	0.572	0.466	0.586	0.460	0.567	0.452	0.543	0.451
	Adjusted-R^2	0.907	0.938	0.903	0.940	0.909	0.941	0.916	0.942
Pork	RMSE	0.609	0.383	0.506	0.406	0.607	0.440	0.573	0.430
	Adjusted-R^2	0.941	0.977	0.959	0.974	0.941	0.969	0.948	0.971
Chicken	RMSE	0.540	0.260	0.423	0.263	0.389	0.259	0.397	0.256
	Adjusted-R^2	0.933	0.984	0.959	0.984	0.965	0.984	0.964	0.985

RMSE: root mean square error and Adjusted-R^2: adjusted coefficient of determination, calculated overall data sets for each food product considering observed and fitted values as log CFU/g.

* RMSE and adjusted-R^2 values calculated after consecutively done primary and secondary model fitting for entire data sets for each food product.

Table 2. Kinetic parameters of *Pseudomonas* spp. in different food products using one-step modelling approach.

Food product	Primary models	y_0 (log CFU/g)	y_{max} (log CFU/g)	T_0 (°C)	b_1	b_2
Fish	Modified Gompertz	3.4 ± 0.2	8.3 ± 0.1	-8.52 ± 0.50	0.0260 ± 0.0014	2.35 ± 0.88
	Logistic	2.9 ± 0.3	8.2 ± 0.1	-8.55 ± 0.49	0.0255 ± 0.0014	1.25 ± 1.28
	Baranyi	3.3 ± 0.2	8.1 ± 0.1	-8.58 ± 0.46	0.0238 ± 0.0011	1.41 ± 0.69
	Huang	3.4 ± 0.1	8.1 ± 0.1	-8.58 ± 0.46	0.0236 ± 0.0010	1.45 ± 0.51
Pork	Modified Gompertz	3.2 ± 0.2	9.8 ± 0.2	-14.30 ± 1.25	0.0179 ± 0.0012	2.65 ± 1.04
	Logistic	2.3 ± 0.1	9.7 ± 0.2	-14.28 ± 1.30	0.0173 ± 0.0011	0.00 ± 0.00
	Baranyi	3.3 ± 0.2	9.5 ± 0.1	-14.01 ± 1.27	0.0165 ± 0.0012	1.61 ± 0.82
	Huang	3.4 ± 0.1	9.5 ± 0.1	-14.03 ± 1.24	0.0165 ± 0.0011	1.78 ± 0.64
Chicken	Modified Gompertz	3.9 ± 0.1	9.8 ± 0.2	-7.77 ± 0.37	0.0289 ± 0.0011	2.55 ± 0.65
	Logistic	3.3 ± 0.2	9.6 ± 0.1	-7.76 ± 0.37	0.0284 ± 0.0010	1.14 ± 0.96
	Baranyi	3.9 ± 0.1	9.4 ± 0.1	-7.65 ± 0.35	0.0272 ± 0.0009	1.77 ± 0.46
	Huang	4.0 ± 0.1	9.4 ± 0.1	-7.62 ± 0.35	0.0270 ± 0.0008	1.74 ± 0.36

While simulating the growth behaviour of microorganisms, accurately determining the exponential phase in which the growth rate reaches maximum value and the variations in organoleptic properties of foods also reach maxima 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 behavior of microorganisms on food, and temperature has a key role in affecting directly both of these growth parameters (Huang, 2008). The kinetic parameters including μ_{max} and λ belonging to *Pseudomonas* spp. for each food product (fish, pork and chicken meat) and each primary model (the modified Gompertz, logistic, Baranyi and Huang models) were shown in Figure 1 and Figure 2, respectively. As it is expected, the figures demonstrate that μ_{max} increased and λ decreased because of rising storage temperature. At this point, it needs to be highlighted that the logistic model tented to yield λ smaller than other primary models (modified Gompertz, Baranyi and Huang models) no matter for which food product was. Additionally, logistic model's statistical indices about b_2, which are used to calculate λ, were higher than other models for chicken and fish, which means a weakness of the logistic model about describing λ. These results are in a good agreement with the findings reported by Tarlak, (2020) for mushroom.

Validation is an important step to check how well the developed models are working. The Huang model is the best primary model simulating the growth behaviour of *Pseudomonas* spp. in fish, pork and chicken meat, therefore, Huang model was used to test the prediction capability for the *Pseudomonas* spp. concentration under non-isothermal storage conditions (Figure 3). The statistical values for validation of the Huang model are given in Table 3. B_f and A_f were calculated maximum 1.075 and 1.080, respectively for all food products (fish, pork and chicken meat). A B_f and A_f of 1 indicates no structural deviation of the model. The B_f factor of 1.075 indicated that the model overestimates less than 7.5% whereas the A_f factor of 1.080 showed that on average the predicted value was less than 8.0% different (either smaller or larger) from the observed value for each of the food products. In addition, MD and MAD values were less than 0.39 and 0.41, respectively considering all food products (fish, pork and chicken meat). All these statistical indexes show that the Huang model can be reliably used to predict the growth behaviour of *Pseudomonas* spp. in fish, pork and chicken meat at not only isothermal but also non-isothermal storage conditions. Because the spoilage of fish, pork and chicken meat is directly linked with *Pseudomonas* spp. concentration, the one-step modelling approach could be also used for the prediction of product shelf life.

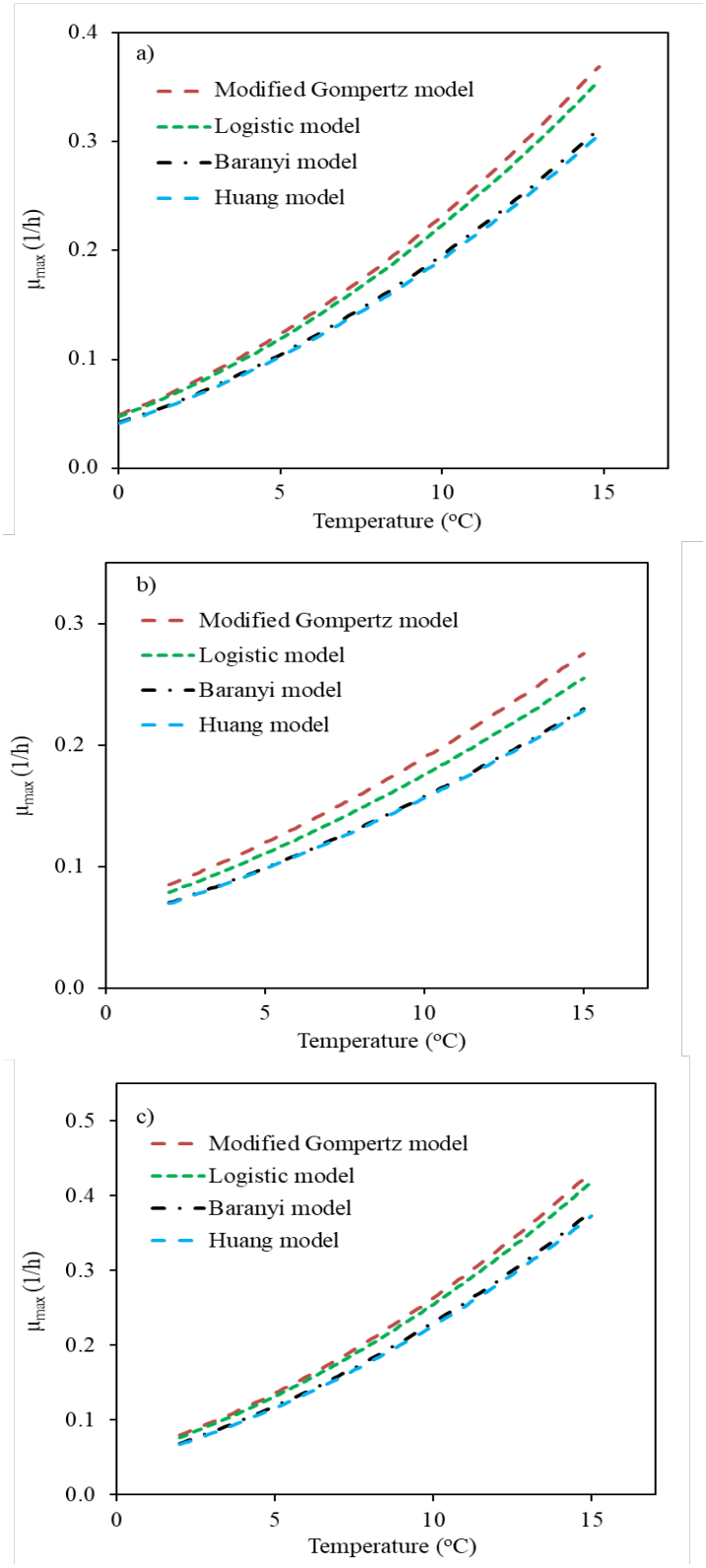

Figure 1. The effect of storage temperature on the maximum specific growth rate (μ_{max}) values obtained from one-step modelling approach for (a) fish, (b) pork and (c) chicken meat.

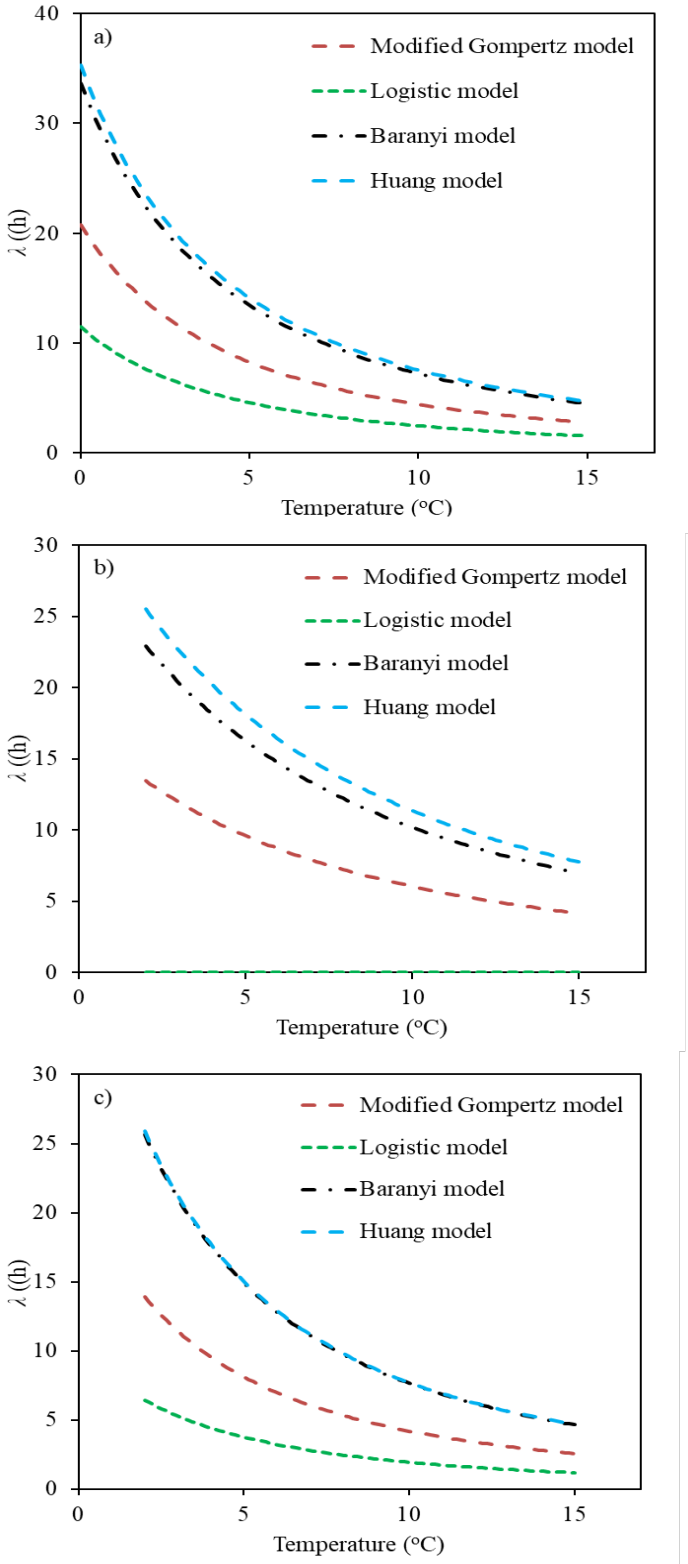

Figure 2. The effect of storage temperature on the lag phase duration (λ) values obtained from one-step modelling approach for (a) fish, (b) pork and (c) chicken meat.

Table 3. Validation criteria of one-step modelling approach based on the Huang model.

Food products	B_f	A_f	MD	MAD
Fish	1.014	1.059	0.02	0.33
Pork	1.075	1.080	0.39	0.41
Chicken	1.016	1.047	0.18	0.31

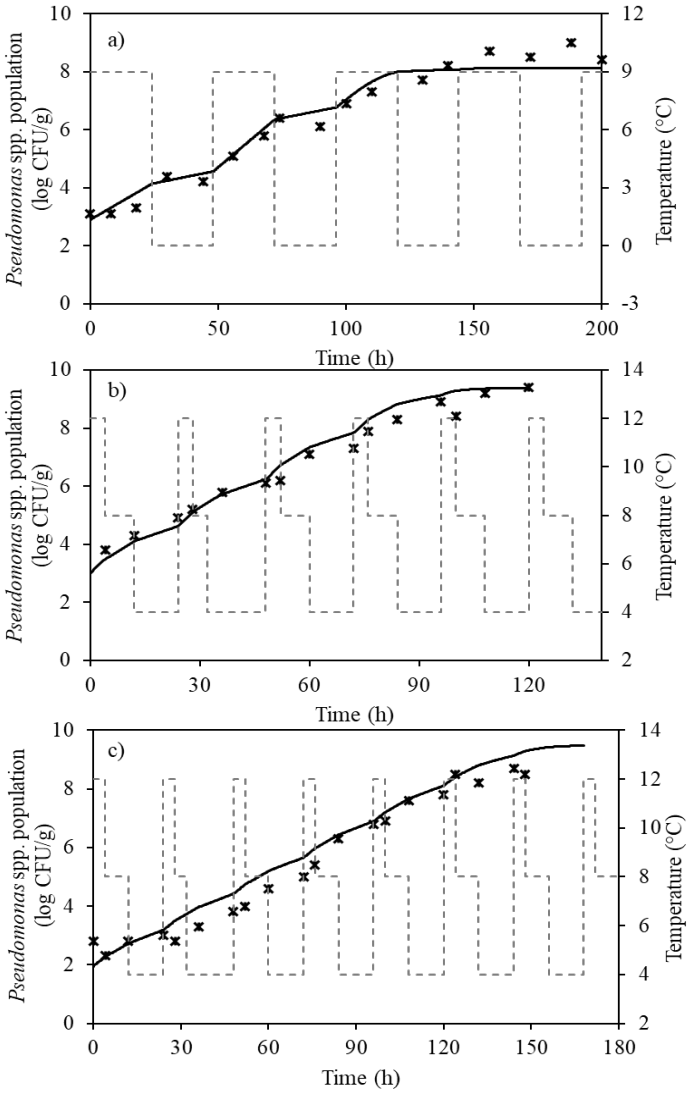

Figure 3. The prediction of *Pseudomonas* spp. concentration in (a) fish, (b) pork and (c) chicken meat subjected to non-isothermal storage conditions. Observed (*) and predicted (−) *Pseudomonas* spp. concentration. The dashed lines (--) show the changing temperature during storage.

Conclusion

No matter which primary model was used, the one-step modelling approach considerably improved the prediction capability of the models, which were published for the quantitative prediction of *Pseudomonas* spp. concentration in aerobically stored fish, pork and chicken meat. The successfully validated differential form of the Huang model merged with the Ratkowsky model provided valuable information to evaluate and simulate the growth behaviour of the *Pseudomonas* spp. in aerobically stored fish, pork and chicken meat under non-isothermal conditions in which the food products are usually subjected to during storage, delivery and retail marketing. The predictive models used in this work have a high potential to be used as a simulation tool for the meat processors to follow the microbiological quality of the food products before they reach to the consumers.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Ethics committee approval: Author declare that this study does not include any experiments with human or animal subjects.

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Disclosure: -

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