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Mathematical modelling for predicting the growth of *Pseudomonas* spp. in poultry under variable temperature conditions

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ABSTRACT

A dynamic growth model under variable temperature conditions was implemented and calibrated using raw data for microbial growth of *Pseudomonas* spp. in poultry under aerobic conditions. The primary model was implemented using measurement data under a set of fixed temperatures. The two primary models used for predicting the growth under constant temperature conditions were: Baranyi and modified Gompertz. For the Baranyi model the maximum specific growth rate and the lag phase at constant environmental conditions are expressed in exact form and it has been shown that in limit case when maximal cells concentration is much higher than the initial concentration the maximum specific growth rate is approximately equal to the specific growth rate. The model parameters are determined in a temperature range of 2–20 °C. As a secondary model the square root model was used for maximum specific growth rate in both models.
In both models the main assumption, that the initial physiological state of the inoculum is constant and independent of the environmental parameters, is used, and a free parameter was implemented which was determined by minimizing the mean square error (MSE) relative to the measurement data. Two temperature profiles were used for calibration of the models on the initial conditions of the cells.

1. Introduction

A mathematical model for microbial growth is a necessary component in the efficient assessment of food contamination, shelf life and risk assessment in supply chains (Lindqvist and Westöö, 2000; Poshet et al., 2003; Olafsdottir et al., 2006).

One of the most important components in quantitative microbial risk assessment (QMRA) is the development of predictive microbial growth models which are able to take into account the influence of the variation of environmental parameters on the microbial growth along the supply chain (Nauta, 2002; Nauta et al., 2005; Orriss and Whitehead, 1999).

In recent times a considerable effort has been invested in the development of mathematical models of microbial dynamics in food products and a number of mathematical models and expressions for predictive microbial growth in food have been developed (Baranyi and Roberts, 1995; Van Impe et al., 2005; Buchanan et al., 1997; Zwietering et al., 1990).

In all cases the microbial growth under variable environmental conditions are described by first order kinetics i.e. by a single or by a system of ordinary differential equations of first order (Poschet et al., 2005; Swinnen et al., 2004; Van Impe et al., 1995).

One of the most important environmental parameters, from the food safety and quality point of view, is temperature. Considering the temperature changes along the supply chain, the use of dynamic models which are able to take into account the influence of temperature variation on microbial growth is essential for prediction of products' shelf life when considering spoilage microorganisms and/or for risk assessment when considering food borne pathogens (Baranyi et al., 1995; Van Impe et al., 1992; Kreyenschmidt, 2003; Bobelyn et al., 2006; Giannakourou et al., 2005).

To obtain a dynamic mathematical model for predictive microbial growth under variable temperature conditions, in the static experimental approach two steps are employed. In the first step the microbial growth data under constant environmental conditions is generated and this procedure is repeated for different sets of environmental parameters (Koutsoumanis, 2001; Baert et al., 2007; Koseki and Isobe, 2005).

The obtained raw data is fitted with a so-called primary model i.e. with model curves which describe the growth of the microbial population with time. Some of the most common primary models are modified Gompertz, Baranyi and Roberts and Logistic models (Ratkowsky et al., 1983; Juneja et al., 2006; Fujikawa et al., 2004; Corradini et al., 2005).

Once the model parameters under stationary environmental conditions are determined the second step is performed and the parameters' dependence on temperature is evaluated.

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In this paper the microbial growth of *Pseudomonas* spp. in poultry under variable temperature conditions is considered. The two alternative models previously tested and used, Baranyi and Roberts and modified Gompertz are employed to develop the mathematical model for microbial growth of *Pseudomonas* in poultry under temperature shift to low temperature (Baranyi et al., 1995; Van Impe et al., 1995). This is of practical relevance for predicting the shelf life since the similar temperature shifts often occur within chilled food supply chains (Koutsoumanis, 2001).

The static measurements are used to obtain the temperature dependence of the model parameters needed in the secondary model. Data from dynamic experiments are used for model calibration to the initial conditions of the cells.

For the dynamic temperature conditions in both models a free parameter was used to optimize the model for the specific dynamic conditions and initial state of the cells (Baranyi et al., 1995). This will be explained in the following sections.

2. Materials and methods

2.1. Experimental design

Chickens were slaughtered, cooled by air chilling for 3 h and 25 min and afterwards dissected into single filets at a poultry slaughtering and processing company in Germany. Filets were wrapped in foil, packed in a cardboard box (5–10 kg) and delivered to a German wholesaler close to the laboratory. Fillets were then transported in a cooling box from the wholesaler to the laboratory. The time between the slaughtering of the chicken and the first investigation was between 18 and 20 h.

In the laboratory filets were sliced into 100 g pieces under aseptic conditions, put in individual pouches and wrapped with low density polyethylene films. Initial bacterial count of *Pseudomonas* spp. was log 3.78 ± 0.77 cfu/g. The pieces were stored under controlled temperature conditions in high precision low temperature incubators (Sanyo MIR 153) at 2, 4, 10, 15 and 20 °C. Two different dynamic storage experiments were conducted. In the first non-isothermal experiment the following cycle was used: 24 h at 2 °C, 12 h at 10 °C. In the second cycle the temperature was varied in the following fashion: 10 h at 4 °C, 5 h 10 °C, 4 h 15 °C. During the experiments temperature was controlled every 5 min by data loggers (Testo 151). Samples from the filets were taken at appropriate time intervals and the number of *Pseudomonas* spp. was investigated. Every measurement was repeated at least 6 times.

2.2. Sample preparation and microbiological analysis

For the analysis of *Pseudomonas* spp. a representative product sample of 25 g was transferred to a Stomacher-bag and homogenized for 60 s in a Stomacher 400 (Kleinfeld Labortechnik) with 225 g chilled saline peptone diluent (0.85% NaCl with 0.1% petone). Further appropriate 10-fold dilution of the homogenate were made with saline peptone diluent. For each dilution blank, two replicas were prepared. 0.1 ml from each appropriate dilution step were spread on the surface of dried media into petri dishes. *Pseudomonas* spp. were determined by using Pseudomonas agar base (Oxoid) plus CFC supplement (Oxoid). Petri dishes were aerobically incubated at 25 °C for 48 h.

2.3. The primary model and mathematical modelling under dynamic temperature conditions

In this work the Baranyi and Roberts and modified Gompertz models are used as primary models to fit the raw data for microbial growth under a set of constant temperatures in order to obtain the primary model curves.

These models are general and could be used for different types of micro organisms and food (Baranyi et al., 1995; Van Impe et al., 2005). All the model parameters which are related to the *Pseudomonas* dynamics are obtained by fitting the growth curve on the measurement data for *Pseudomonas* in poultry at different temperatures. In this way the model parameters for each selected temperature are obtained. In the next step the secondary model is developed where the functional dependence of the model parameters on temperature is achieved.

The microbial growth under dynamical temperature conditions according to the Baranyi and Roberts model is described by the following set of differential equations with appropriate initial conditions (Baranyi et al., 1995; Baranyi and Roberts, 1995; Baranyi et al., 1993; Baranyi and Roberts, 1994)

$$\frac{dq(t)}{dt} = \nu \cdot q(t); \quad q(0) = q_0 \tag{1}$$

$$\frac{dN(t)}{dt} = \mu_0 \cdot \alpha(t) \cdot \left(1 - \left(\frac{N(t)}{N_{\max}}\right)^m\right) \cdot N(t); \ \alpha(t) = \frac{q(t)}{1+q(t)}; \ N(0) = N_0, \tag{2}$$

where q_0 and $q(t)$ are the quantities which are related to the critical substance necessary for growth and characterize the physiological state of the culture in the moment of inoculation and later time, respectively. The temperature-dependent specific growth rate, expressed in [1/h], is denoted by μ_0. N_0, $N_{\max}$ and $N(t)$ are initial, maximal and actual cell concentration, respectively, expressed in [cfu/g], m is a shape parameter for which $m=1$ was assumed. The adjustment function, which takes into account the lag phase during which the population adapts to the new environment is denoted by $\alpha(t)$. The relative growth rate ν relates to the critical substance and determines the quickness of the transition from the lag phase to the exponential phase. The growth of the bacterial culture is a result of production of the critical substances by certain enzymatic reactions and it has been assumed that after inoculation, the critical substance increases at the same specific rate as the cells in the exponential phase (Baranyi et al., 1995). This suggests that specific growth rate for quantity q is equal to the relative growth rate of the colonies (i.e. $\nu=\mu_0$) (Baranyi and Roberts, 1994). The "logistic" part in Eq. (2) limits the population growth to the value $N_{\max}$.

The specific growth rate is determined by the environmental conditions, e.g., temperature, pH, NaCl%, water activity. In this work only temperature dependence is considered and the other environmental parameters are considered to be constant (Buchanan et al., 1993; Buchanan et al., 1989).

The main assumptions for the dynamical temperature conditions are that the specific growth rate is changing instantaneously with temperature and parameters $N_{\max}$ and q_0 are temperature independent (Baranyi et al., 1995).

If the temperature variation in time is described by the temperature profile $T(t)$, the above system of equations is solved by integration in the following way (Baranyi and Roberts, 1994):

$$q(t) = q_0 \cdot \exp\left(\int_0^t \mu_0(T(t_1))dt_1\right) \tag{3}$$

$$y(t) = y_0 + A(t) - \ln\left(1 + \frac{\exp(A(t)) - 1}{\exp(y_{\max} - y_0)}\right); \ A(t) = \int_0^t \mu_0(T(t_1)) \cdot \alpha(t_1) \cdot dt_1; \tag{4}$$

$$y(t) = \ln(N(t)); \quad y_0 = \ln(N_0); \quad y_{\max} = \ln(N_{\max}); \quad h = \ln(1 + 1/q_0).$$

The quantity $y(t)$ in the equations mentioned above is the natural logarithm of the cell concentration $N(t)$.

Function $A(t)$ expresses a time delay in growth during the transitions from lag phase to exponential growth phase, and these transitions are determined by the rise of the critical substance given by the quantity $q(t)$ in Eq. (2).

Instead of using parameter q_0, the quantity $h=\ln(1+1/q_0)$ is more stable in practical calculations. For constant environmental conditions the growth curve for the Baranyi and Roberts model has been expressed by the following equation (Baranyi et al., 1995):

$$y(t) = y_{\max} + \ln\left(\frac{1-\exp(-h)+\exp(\mu_0 t-h)}{\exp(y_{\max}-y_0)+\exp(\mu_0\cdot t-h)-\exp(-h)}\right). \quad (5)$$

The maximum specific growth rate could be defined as the actual specific growth rate at the inflection point of the growth curve as shown in Fig. 1. The inflection point and maximum specific growth rate for the Baranyi and Roberts model are evaluated in Eqs. (A1)–(A4). The instant and maximum specific growth rates are expressed by the following relations respectively:

$$\mu(t) = \frac{1}{N(t)}\frac{dN(t)}{dt} = \frac{dy(t)}{dt}; \quad (6)$$

$$\mu_{\max} = \mu(t_{\text{infl}}) = k\cdot\mu_0; \quad k = \frac{(1+q_0)\cdot\left(\frac{N_{\max}}{N_0}-1\right)}{\left(1+\sqrt{(1+q_0)\cdot\left(\frac{N_{\max}}{N_0}-1\right)+1}\right)^2}; \quad 0<k<1;$$
$$N_{\max} >> N_0 \Rightarrow k\approx 1; \quad \mu_{\max}\approx\mu_0. \quad (7)$$

where $\mu(t)$ is the actual specific growth rate at instant t and $\mu_{\max}$ is the maximum specific growth rate. From the above equation it could be seen that the correction factor k is in range between zero and one and in the limiting case of $N_{\max}\gg N_0$, k is approximately equal to one and the maximum specific growth rate is approximately equal to the specific growth rate μ_0, which follows from Eq. (7). If the model parameters N_0 and $N_{\max}$ are temperature independent, the correction parameter k is also temperature independent.

By using Eq. (6) for the inflection point the natural logarithm of the cell concentration at the inflection point-y_{infl} is given by the following equation:

$$y_{\text{infl}} = y(t_{\text{infl}}) = \ln(N_{\max}) - \frac{1}{2}\ln\left(\frac{N_{\max}}{N_0}+q_0\cdot\left(\frac{N_{\max}}{N_0}-1\right)\right). \quad (8)$$

Here it is important to emphasize that μ_0 is a model parameter in the Baranyi and Roberts model with the dimension of the specific growth rate and $\mu_{\max}$ is the maximum specific growth rate i.e. actual specific growth rate at the inflection point of the growth curve.

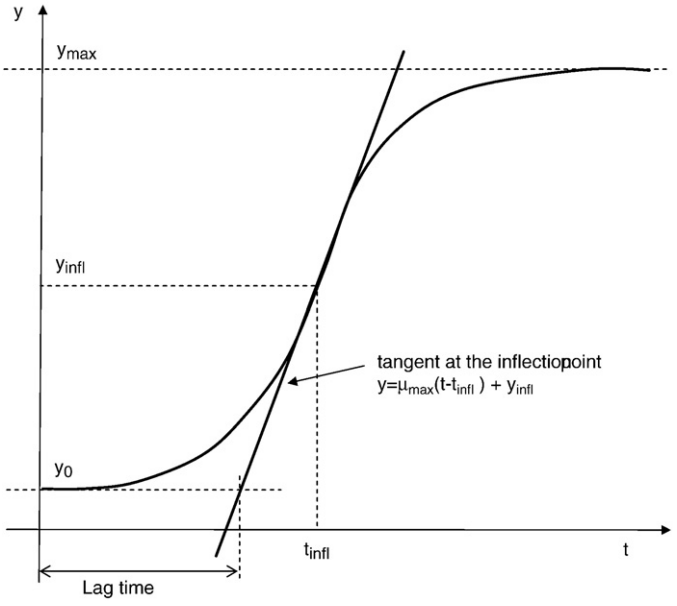

Fig. 1. The schematic view of the growth curve with corresponding lag phase and inflection point in the case of the Baranyi and Roberts model.

According to Fig. 1 the lag phase at constant environmental conditions could be exactly expressed using the correction factor k from Eq. (7) and the model parameters h and μ_0 with the following exact equations:

$$\lambda = t_{\text{infl}} - \frac{y_{\text{infl}}-y_0}{\mu_{\max}} = \frac{h}{k\cdot\mu_0} = \frac{h}{\mu_{\max}},$$
$$\lambda_{\text{aprox}} = \frac{h}{\mu_0} \Rightarrow \lambda = \frac{\lambda_{\text{aprox}}}{k} \quad (9)$$

where λ and λ_{approx} are exact and approximate lag phase respectively.

Under the same conditions as given in Eq. (7) the following limiting relations hold (Baranyi et al., 1993; Swinnen et al., 2004):

$$N_{\max} >> N_0 \Rightarrow \lambda\cdot\mu_0 \approx h \Rightarrow \lambda = \lim_{t\to+\infty}(t-A(t)). \quad (10)$$

As the quantity h only depends on the initial physiological state of the colony, it could be concluded from the equations mentioned above that the product of the specific growth rate and the lag phase is independent of the environmental parameters after inoculation (Baranyi et al., 1995).

According to Eq. (9) the lag phase for the Baranyi and Roberts model should be corrected with the factor k given by Eq. (7). As the correction factor is always less than one, the estimated lag time by the exact equation will always be higher than the lag time estimated by the approximate equation ($\lambda>\lambda_{\text{approx}}$). Also from (7) follows that the maximum specific growth rate for the Baranyi and Roberts model $\mu_{\max}$ will always be smaller than μ_0.

The modified Gompertz primary model for microbial growth has been expressed by the following equation (Zwietering et al., 1990; Juneja et al., 2007; Gibson et al., 1988; Buchanan et al., 1993):

$$y(t) = A + C\cdot\exp(-\exp(-B\cdot(t-M)))$$
$$= A + C\cdot\exp\left(-\exp\left(-\frac{e\cdot\mu_{\max}}{C}\cdot(t-\lambda)+1\right)\right) \quad (11)$$

where A, C, B and M are model parameters and y, λ, $\mu_{\max}$ have the same meaning as in (4–7), i.e. the natural logarithm of the cell concentration, lag phase and maximum specific growth rate respectively, e is base of the natural logarithm.

The parameter M is the inflection point of the sigmodial curve given by (11), $A+C$ is the natural logarithm of the maximal concentration $y_{\max}$, and parameter A is the asymptotic value for $y(t)$ if the time is taken as minus infinity.

The maximum specific growth rate and the approximate expression for lag phase for the modified Gompertz model has been expressed by (Zwietering et al., 1990; Gibson et al., 1988):

$$\mu_{\max} = \frac{B\cdot C}{e}; \quad \exp(B\cdot M) >> 1 \Rightarrow \lambda \approx M - \frac{1}{B}. \quad (12)$$

The exact expression for lag phase, evaluated in A5 and A6 has been expressed by the following relation (McMeekin et al., 1993):

$$\lambda = M - \frac{1}{B}(1-\exp(1-\exp(B\cdot M))). \quad (13)$$

In the case of dynamical temperature conditions, first order kinetics should be used and growth of the bacterial populations has been described by the following first order differential equations with corresponding initial conditions (Van Impe et al., 1995):

$$\frac{dy(t)}{dt} = \frac{e\cdot\mu_{\max}}{C}\cdot(y-A)\cdot\ln\left(\frac{C}{y-A}\right); \quad y(0) = A + C\exp(-\exp(B\cdot M)). \quad (14)$$

The model parameter M is included only in the initial conditions. For constant environmental conditions the above relation is equivalent to the one given by Eq. (11).

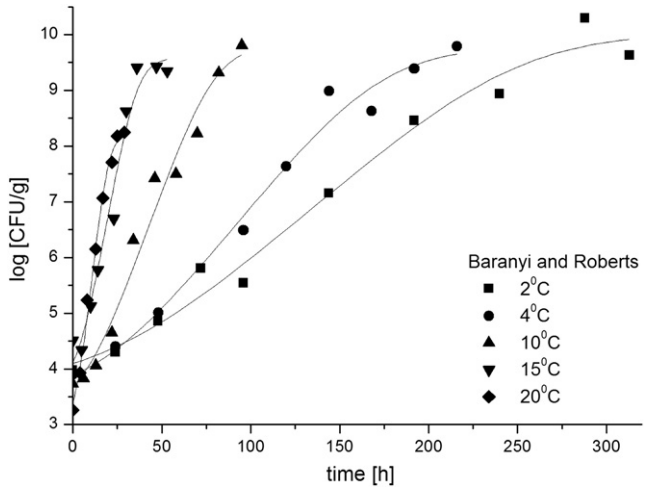

Fig. 2. Raw data and fitted growth curves for Baranyi and Roberts model for five different temperatures (<*h*> = 1.0442).

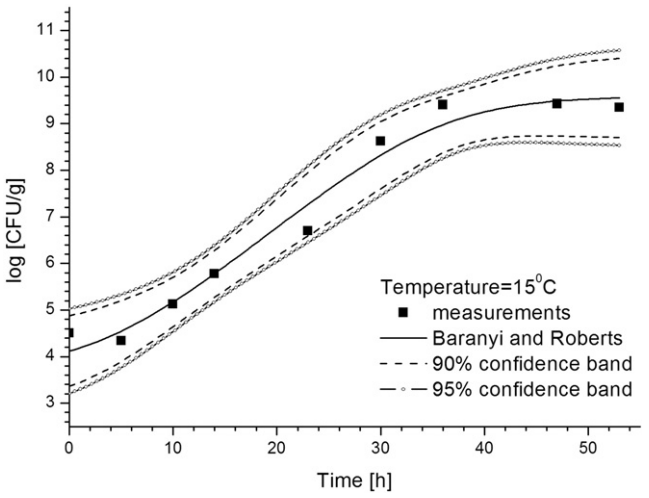

Fig. 4. Fitted growth curves for Baranyi and Roberts model (5) at 15 °C with 90% and 95% confidence bands.

If we assume that parameters *A* and *C* are temperature independent and that only parameter *B* is a function of the temperature the solutions has been expressed by the following integral equation (Van Impe et al., 1995):

$$y(t) = A + C \cdot \exp\left(-\exp\left(-\int_0^t \frac{e \cdot \mu_{\max}(T(t_1))}{C} \cdot dt_1 + C_0\right)\right), \quad (15)$$

$$C_0 = B(T(0)) \cdot M \approx \frac{e}{C}h + 1; \quad h = \mu_{\max} \cdot \lambda.$$

By doing so, in both Eqs. (15) and (4) there is the same free parameter *h*, which could be used to optimize the model during the calibration process by comparison with data from measurements obtained under dynamical temperature conditions. It is important to emphasize that in the above equation we used connection between parameter h and the maximum specific growth rate and the lag phase from the Baranyi model given by Eq. (9).

2.4. Secondary model

To obtain the temperature dependence of the model parameters, the growth curves for constant temperature given by Eqs. (5) and (11) for Baranyi and Roberts and modified Gompertz primary models, respectively, were fitted on the measurement data for five different temperatures: 2, 4, 10, 15 and 20 °C. For this temperature range (and also the temperature range for dynamic conditions of 2 °C–20 °C) the Ratkowsky square root model was used as a secondary model for specific growth rate for the both models (Ratkowsky et al., 1983, 1982; Zwietering et al., 1991; Amézquita et al., 2005):

$$\sqrt{\mu_{\max}} = b \cdot (T - T_{\min}), \quad (16)$$

where *b* is a model parameter expressed in $[(h^{0.5}\,°C)^{-1}]$ and $T_{\min}$ is the conceptual minimal temperature for microbial growth. Both parameters are obtained by fitting the experimental data for specific growth under different constant temperatures. The above model is valid only for the temperature range $[T_{\min}, T_{\text{opt}}]$, where T_{opt} is the optimal temperature for microbial growth.

The maximal bacterial concentration (Baranyi et al., 1995; Koutsoumanis, 2001) as well as the initial physiological state of the cells (Baranyi et al., 1995; Amézquita et al., 2005) are assumed to be temperature independent in the considered temperature range.

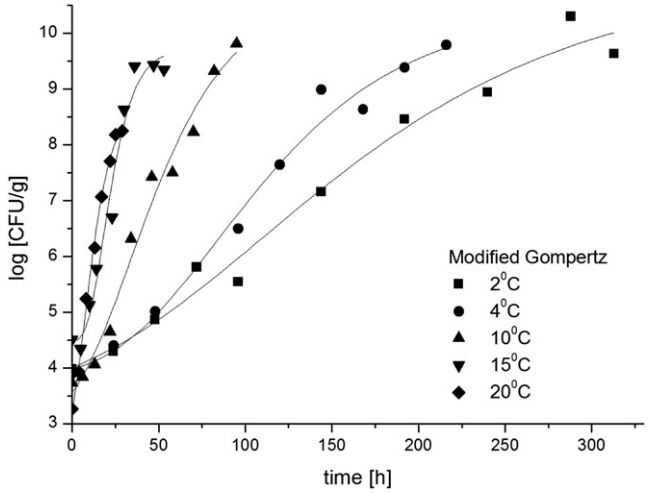

Fig. 3. Raw data and fitted growth curves for modified Gompertz model for five different temperatures.

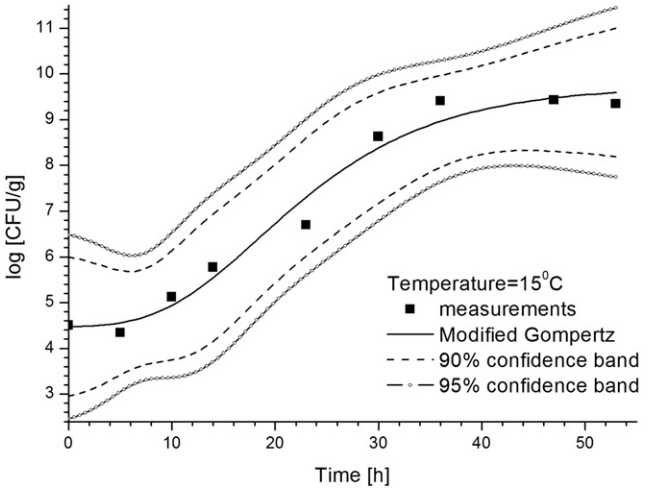

Fig. 5. Fitted growth curves for modified Gompertz model (11) at 15 °C with 90% and 95% confidence bands.

Table 1
The estimated values and standard errors for parameters in the Baranyi and Roberts primary model in the first step, where all model parameters are variable, with pseudo R^2 and MSE (mean square error)

Tem [°C]	y_0 log [cfu/g]	y_{max} log [cfu/g]	μ_{max} [1/h]	h	MSE	Pseudo R^2
2	3.97±0.32	10.15±0.46	0.0264±0.0045	0.601±0.845	0.1373	0.9822
4	3.95±0.30	9.73±0.35	0.043±0.0075	1.3607±1.0124	0.1145	0.9860
10	3.53±0.33	10.14±0.78	0.08102±0.0146	0.3897±0.8864	0.1559	0.9804
15	4.45±0.26	9.51±0.23	0.2355±0.0423	2.659±1.1504	0.1083	0.9858
20	3.22±0.15	8.40±0.18	0.2545±0.0279	0.2098±0.4237	0.0235	0.9963

Table 2
The estimated vales and standard errors for recalculated parameters in the Baranyi and Roberts primary model for fixed parameter h, with pseudo R^2 and MSE (mean square error)

	$\langle h \rangle = 1.0442$				
Tem [°C]	y_0 log[cfu/g]	y_{max} log[cfu/g]	μ_{max} [1/h]	MSE	Pseudo R^2
2	4.10±0.20	10.05±0.34	0.0284±0.0022	0.1228	0.98215
4	3.88±0.208	9.80±0.31	0.043±0.0027	0.09707	0.98577
10	3.69±0.22	9.84±0.46	0.091±0.007	0.14771	0.97842
15	4.12±0.24	9.59±0.29	0.185±0.0165	0.1406	0.9779
20	3.39±0.14	8.24±0.15	0.304±0.0173	0.03656	0.99293

2.5. Model application

Obtained measurement data were converted in ln(cfu/g) and were fitted to the growth curves given by Eqs. (5) and (11) for the primary Baranyi and modified Gompertz model respectively. In the case of Baranyi and Roberts in the first step the four model parameters: y_0, y_{max}, μ_{max}, and h in Eq. (5), were obtained by non-linear regression using the software ORIGIN 7.5. As suggested by various authors (Baranyi et al., 1995; Amézquita et al., 2005), the parameter h obtained for different temperatures is averaged and mean values $\langle h \rangle$ were used in the next step utilizing the same equation. This follows from the assumption that if the measurement procedures are standardised then initial physiological state of the colony, i.e. quantity h should be the same for different temperatures during the static measurements (Baranyi and Roberts, 1994).

In the second step the three remaining model parameters: y_0, y_{max} and μ_{max} are recalculated using Eq. (5) with fixed value of the quantity $\langle h \rangle$ from the first step. The specific growth rate obtained in this way was used in the secondary model.

3. Results and discussion

In Figs. 2 and 3 the fitted growth curves at constant temperatures for Baranyi and Roberts (Eq. (5)) and modified Gompertz primary models (Eq. (11)) are shown respectively.

In Figs. 4 and 5 the fitted growth curves at 15 °C with 90% and 95% confident bands for the Baranyi and Roberts and modified Gompertz model, respectively, are presented.

In Tables 1 and 2 the numerical values calculated for the model parameters with standard deviation, mean square error (MSE) and pseudo R^2 (Bates and Watts, 1988; Bickel and Doksum, 2001; Wackerly et al., 2002) in Baranyi and Roberts primary model before and after fixing parameter h are presented, respectively.

From the above tables it can be seen that after fixing the parameter h on the average value the recalculated specific growth rate in the second step has a smaller standard error in all cases. Also it could be noticed that in the first step parameter h has the biggest uncertainty. From the standard errors for each parameter the marginal confidence intervals could be calculated using the student distributions (Bates and Watts, 1988).

In Table 3 the numerical values of the model parameters with standard errors, MSE and pseudo R^2 for the modified Gompertz model are presented.

In Table 4 the estimated values using the exact and approximated Eqs. (7), (9), (12), (13) and (A3) are summarized. The inflection point, the maximum specific growth rate, exact lag time, and approximated lag time in the Baranyi and Roberts model are calculated using the Eqs. (A3), (7) and (9). On the other hand, the lag time and maximum specific growth rate in the modified Gompertz model are calculated using the relations 12 and 13, respectively. The inflection point for the modified Gompertz growth curve is parameter M.

From the numerical results in Table 4 it could be seen that a good agreement between the two models at constant temperature is obtained for the maximum specific growth rates, and a less good agreement for the results of lag phase. As the confidence intervals for parameters in the Gompertz model at constant temperature (Table 3) are larger than the ones for the Baranyi and Roberts model (Table 2) the lag times in Table 4 obtained by the Baranyi and Roberts model for this specific case are obtained with a higher confidence then the ones obtained by the Gompertz model.

The results in Table 4 for lag time and maximum specific growth rate suggest that the exact Eqs. (7) and (9) instead of approximations should be used for the Baranyi and Roberts model in the cases when the correction factor k in Eq. (7) is considerably smaller than one. From the results in Table 4 one can conclude that one of the most important parameters for the shelf life, the lag time, decreases with temperature from 39.83 h to 3.95 h as temperature increases from 2 to 20 °C. In the same temperature range the specific growth rate increases from 0.026 1/h to 0.263 1/h according to the Baranyi and Roberts model. Similar conclusions follow from results obtained by the modified Gompertz model. In Figs. 6 and 7 the temperature dependence of the specific

Table 4
The calculated values for inflection point, maximum specific growth rate exact and approximated lag time and correction factor k in the Baranyi and Roberts model and lag time and specific growth rate in the modified Gompertz model

	Baranyi and Roberts					Gompertz	
Temperature [°C]	t_{infl} [h]	μ_{max} [1/h]	λ-exact [h]	λ-approx [h]	k	λ [h]	μ_{max} [1/h]
2	133.794	0.0262	39.835	36.702	0.923	20.364	0.0259
4	92.644	0.0375	27.811	25.574	0.921	26.653	0.0402
10	42.656	0.0849	12.291	11.410	0.9297	6.102	0.0858
15	19.1837	0.1672	6.242	5.623	0.903	8.883	0.2027
20	10.6891	0.2637	3.959	3.432	0.871	0.951	0.2593

Table 3
The estimated vales and standard errors for parameters in the modified Gompertz primary model with pseudo R^2 and MSE (mean square error)

T [°C]	A log[cfu/g]	B [1/h]	C log[cfu/g]	M [h]	MSE	Pseudo R^2
2	3.548±1.076	0.00945±0.004	7.468±2.098	108.466±24.197	0.1471	0.9816
4	3.866±0.498	0.0166±0.005	6.554±1.094	83.714±10.879	0.1133	0.9861
10	3.064±1.0935	0.0303±0.012	7.697±2.2	33.341±7.245	0.1354	0.983
15	4.469±0.3391	0.1047±0.022	5.262±0.591	18.403±2.095	0.1651	0.9783
20	2.619±0.6245	0.1135±0.022	6.209±0.904	7.398±1.521	0.022	0.9965

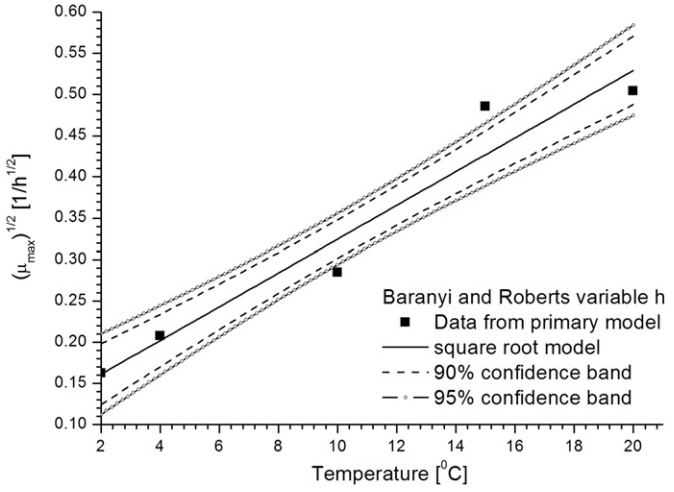

Fig. 6. Square root model (16) for temperature dependence of the specific growth rate in the Baranyi and Roberts model for variable parameter h with 90% and 95% confidence bands.

growth rate in the Baranyi and Roberts model for variable and fixed parameter *h* are presented, respectively. The 90% and 95% confidence bands for the square root model are given as well.

In Fig. 8 the joint 90% and 95% confidence intervals for the parameters in the square root model for fixed and variable parameter *h* in the Baranyi and Roberts model are presented. The highest posterior density (HPD) regions approach is used (Bates and Watts, 1988; Bickel and Doksum, 2001). From the above figure it could be noticed that the surface of the joint interval for the parameters in the square root model is much smaller for the fixed than for the variable parameter *h*. This implicates that the results obtained using the square root model for *Pseudomonas* in the Baranyi and Roberts model obtained in the second step using fixed value for parameter *h*, as explained above, have higher confidence than the ones obtained using variable parameter *h* in the first step.

In Table 5 the estimated values for the parameter in the square root model for the fixed and variable parameter *h* in the Baranyi and Roberts model are presented.

From all presented results in Figs. 6–8 and Table 5 it can be concluded that uncertainty of the parameters in the square root model is much smaller if the parameter *h* is fixed.

The square root model is used for modelling the temperature dependence of the maximum specific growth rate in the modified Gompertz model.

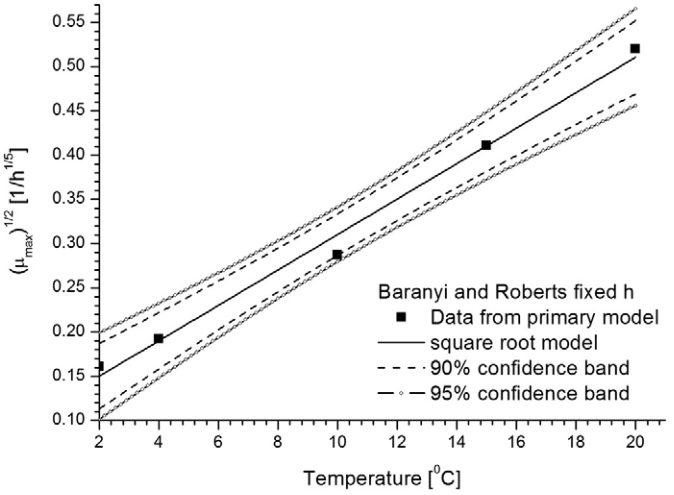

Fig. 7. Square root model (16) for temperature dependence of the specific growth rate in the Baranyi and Roberts model for the fixed parameter *h* with 90% and 95% confidence bands.

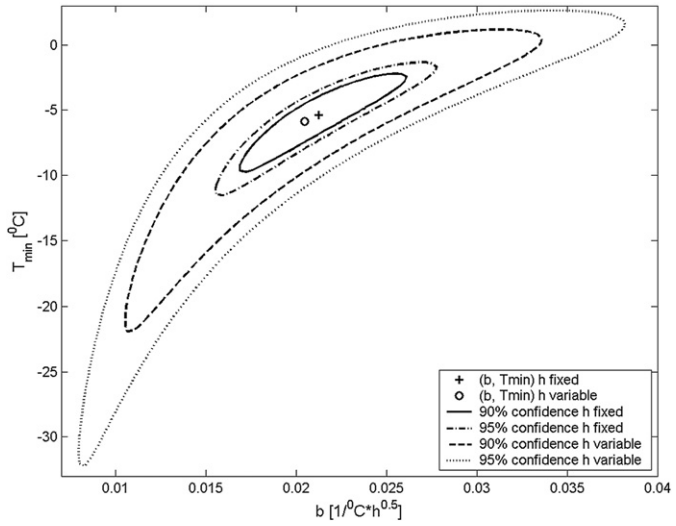

Fig. 8. Joint 90% and 95% confidence intervals for the parameters b and T_{min} in the square root model (16) for the temperature dependence of the specific growth rate in the Baranyi and Roberts model.

In Fig. 9 the maximum growth rate as a function of temperature in the case of the modified Gompertz model is shown together with the 90% and 95% confidence bands for the square root model. In Fig. 10 the joint 90% and 95% confidence intervals for the parameters in the square root approach for the Gompertz model are presented. From the Fig. 9 it can be seen that the joint confidence intervals for parameters in the square root approach for the Gompertz model is bigger than the same interval for fixed h in the Baranyi and Roberts model and smaller for variable parameter h.

In Table 5 the estimated values for the parameters b and T_{min} as well as MSE and pseudo R^2 for the modified Gompertz model are presented.

The detailed experimental validation and statistical comparison of the presented model is beyond the scope of this paper. For the used models there has been extensive discussion in the literature with their characteristics under variable conditions (Baranyi and Roberts, 1994; Baranyi, et al., 1995; Van Impe et al., 1995; Zwietering et al., 1991).

The presented models are calibrated using the initial conditions (initial physiological state of the culture) for the two temperature profiles for *Pseudomonas* spp. in poultry. The growth curves are shown in Figs. 11 and 12.

In both cases the six replications of measurements under same temperature conditions are averaged and used for calibration of the models.

To be able to calibrate the model under dynamical temperature conditions a free parameter is used in both models, as suggested in Section 2.5 and Eqs. (4) and (15).

For both the models the free parameter used in the calibration was the initial physiological state of the culture, namely q_0, or h. In both

Table 5
Estimated parameters in the square root approach in the Baranyi and Roberts and in the Gompertz model

Baranyi and Roberts			
b [1/°C h$^{1/2}$]	T_{min} [°C]	MSE	Pseudo R^2
Variable h			
0.02045 ± 0.0029	−5.8812 ± 2.466	0.00188	0.9432
Fixed h			
0.0212 ± 0.00115	−5.382 ± 0.9186	0.0003	0.9913
Gompertz			
0.02015 ± 0.00161	−5.816 ± 1.3886	0.00058	0.98117

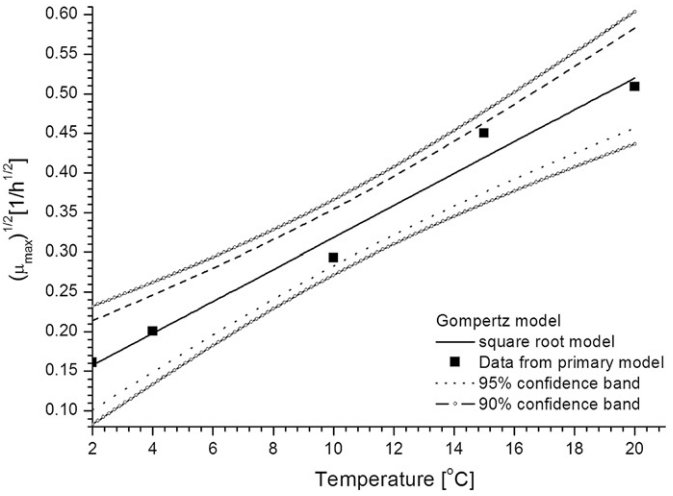

Fig. 9. Square root model for temperature dependence of the specific growth rate in the Gompertz model with 90% and 95% confidence bands.

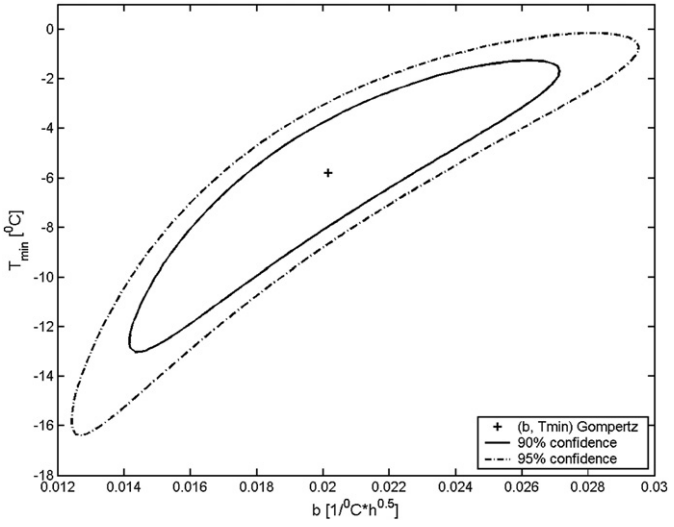

Fig. 10. Joint 90% and 95% confidence intervals for the parameters b and T_{min} in the square root model for the temperature dependence of the specific growth rate in the Gompertz model.

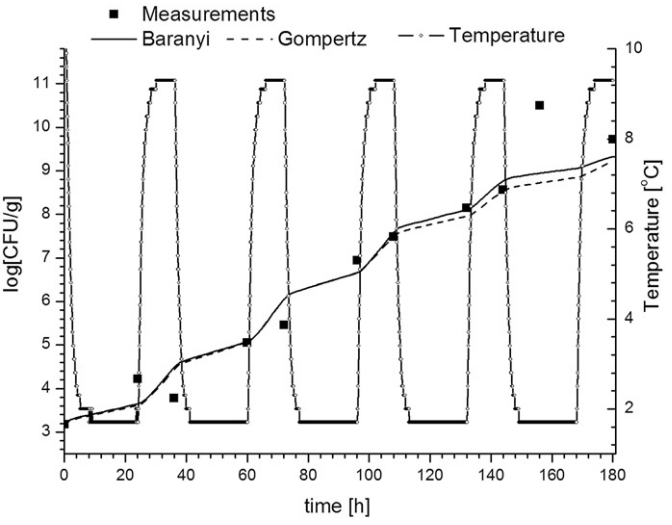

Fig. 11. The growth curves for the *Pseudomonas* spp. in poultry under dynamical temperature conditions (temperature profile 1) obtained using the Baranyi and Roberts in Eq. (4) and the modified Gompertz model in Eq. (15) compared with measurement results.

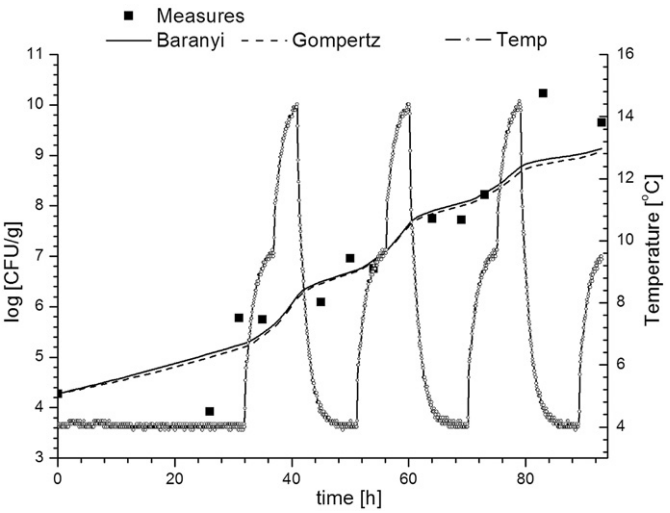

Fig. 12. The growth curves for the *Pseudomonas* spp. in poultry under dynamical temperature conditions (temperature profile 2) obtained using the Baranyi and Roberts in Eq. (4) and the modified Gompertz model in Eq. (15) compared with measurement results.

cases the free parameter was obtained by minimizing the MSE. If the initial conditions differ, perhaps because different supply chain is analyzed, the model should be readjusted by recalculating the free parameter to the new initial conditions.

The obtained results for variable parameters h for Baranyi and Roberts and modified Gompertz model, respectively, and the relevant statistics for the calibration of the mathematical models used for the growth of *Pseudomonas* spp. in poultry for two temperature profiles 1 and 2 are presented in Table 6.

4. Conclusions

Baranyi and Roberts and modified Gompertz models have been applied for the prediction of growth of *Pseudomonas* spp. in poultry under variable temperature conditions. The secondary models used are the Ratkowsky model (square root model) in both cases. The exact expressions for the maximum specific growth rate, lag time and inflection point in the case of the Baranyi and Roberts model are presented. The modified Gompertz model under dynamical temperature conditions is changed introducing the same free parameter h as in Baranyi and Roberts model. The dynamical measurements are used for calibration of the models on the initial conditions of the cells and free parameters are obtained by minimising the MSE.

A good agreement between models and data is obtained and similar values for parameter h are obtained in both cases in the dynamic regime.

The presented results in this study implied that both alternative approaches are suitable for modelling the microbial growth of *Pseudomonas* spp. in poultry under dynamic temperature conditions for predicting the shelf life.

Table 6
The estimated optimal values for the model parameter h in the Baranyi and Roberts and modified Gompertz models, respectively, for two temperature profiles and relevant statistics for the calibration of the models through comparison with measured data

Baranyi and Roberts			Modified Gompertz		
h	MSE	pseudo R^2	h	MSE	Pseudo R^2
Temperature profile 1					
0.5573	0.62547	0.936	0.461	0.6542	0.9229
Temperature profile 2					
0.4106	0.60911	0.8999	0.361	0.5964	0.8953

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Appendix A

The instant specific growth rate is given by the following relation:

$$\mu(t) = \frac{dy(t)}{dt} = \frac{1}{N(t)}\frac{dN(t)}{dt} = -\frac{\exp(t\cdot\mu_0)\cdot q_0\cdot(1+q_0)\cdot(N_0-N_{\max})\cdot\mu_0}{(1+q_0\cdot\exp(t\cdot\mu_0))\cdot(N_{\max}+q_0\cdot(N_0\cdot(\exp(t\cdot\mu_0)-1)+N_{\max}))}. \quad (A1)$$

The specific growth rate has maximum at inflection point and it is obtained using the following equations:

$$\frac{d\mu(t)}{dt} = \frac{d^2y(t)}{dt^2} = \frac{\exp(t\cdot\mu_0)\cdot q_0\cdot(1+q_0)\cdot(N_0-N_{\max})\cdot(\exp(2\cdot t\cdot\mu_0)\cdot q_0^2\cdot N_0+q_0\cdot(N_0-N_{\max})-N_{\max})\cdot\mu_0^2}{(1+q_0\exp(t\cdot\mu_0))^2\cdot(N_{\max}+q_0\cdot((\exp(t\cdot\mu_0)-1)\cdot N_0+N_{\max}))^2} = 0 \Rightarrow \quad (A2)$$

$$t_{\mathrm{infl}} = \frac{1}{2\cdot\mu_0}\cdot\mathrm{Ln}\left(\frac{N_{\max}+q_0\cdot(N_{\max}-N_0)}{N_0\cdot q_0^2}\right) \quad (A3)$$

$$\mu_{\max} = \mu(t_{\mathrm{infl}}) = k\cdot\mu_0;\quad k = \frac{(1+q_0)\cdot\left(\frac{N_{\max}}{N_0}-1\right)}{\left(1+\sqrt{(1+q_0)\cdot\left(\frac{N_{\max}}{N_0}-1\right)+1}\right)^2};\quad 0<k<1; \quad (A4)$$

$N_{\max} >> N_0 \Rightarrow k\approx 1;\quad \mu_{\max}\approx\mu_0.$

The natural logarithm of cell concentration at the inflection point and at the initial time ($t=0$) are given by the following equations (McMeekin et al., 1993):

$$y(t_{\mathrm{infl}}) = A + \frac{C}{e};\quad y_0 = y(t=0) = A + C\cdot\exp(-\exp(B\cdot M)) \quad (A5)$$

The lag time is defined as interception between tangent line at inflection point and horizontal line at y_0 (McMeekin et al., 1993):

$$y_0 = (\lambda - M)\frac{B\cdot C}{e} + y(t_{\mathrm{infl}}) \Rightarrow \lambda = M - \frac{1}{B}(1-\exp(1-\exp(B\cdot M))) \quad (A6)$$

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