African Journal of Biotechnology Vol. 10(38), pp. 7468-7479, 25 July, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.823
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Identifiability of Baranyi model and comparison with empirical models in predicting effect of essential oils on growth of *Salmonella typhimurium* in rainbow trout stored under aerobic, modified atmosphere and vacuum packed conditions

Mustafa Tahsin Yılmaz*

Food Technology Department, Safiye Cikrikcioglu Vocational College, Erciyes University, 38039, Kayseri-Turkey. E-mail: mtyilmaz@erciyes.edu.tr. Tel: +90 352 4374901/41523. Fax: +90 352 4371383.

Accepted 3 June, 2011

The structural identifiability properties of the Baranyi model were analyzed in fitting the effect of oregano and thyme essential oils on growth of *Salmonella typhimurium* in rainbow trout stored under aerobic (AP), modified atmosphere (MAP) and vacuum packed (VP) conditions. Although, formally proven to be structurally identifiable using the Taylor-series approach, the Baranyi model was not practically identifiable in the presence of experimental data. In addition, performance of the Baranyi model was compared with those of the empirical modified Gompertz and logistic models and Huang models. Higher values of R^2, modeling efficiency and lower absolute values of mean bias error, root mean square error, mean percentage error and chi-square were obtained with modified Gompertz and logistic models than those obtained with the Huang and Baranyi models. The essential oil and packing treatments had remarkable delaying effects on the growth of *S. typhimurium*. Considering the obtained results in this study, the empirical modified Gompertz and logistic models can be used more effectively than the mechanistic Huang and Baranyi models to predict the effect of plant essential oils on growth potential of *Salmonella* in fish products stored under aerobic, MAP/VP conditions.

Key words: Identifiability of Baranyi model, predictive microbiology, *Salmonella typhimurium*, essential oil, packing treatments.

INTRODUCTION

A great number of mathematical models have been developed so far to predict microbial growth (Grijspeerdt and Vanrolleghem, 1999). These models are generally classified as empirical and mechanical models (France and Thormley, 1984; McMeekin and Ross, 2002). In practice; however, the majority of the models, either purely mechanistic or empirical are defined be-tween the two extremes. On the other hand, Whiting and Buchanan (1993) proposed the more common accep-table classification scheme: primary, secondary and tertiary models. One of the early empirical mathematical models is the first order kinetic growth model that was used to describe microbial growth of *Listeria monocytogenes* (Grau and Vanderlinde, 1992; Li et al., 2007). A linear relationship between changes in cell con-centration could be well described by this model at each consecutive time period. However, the main drawback of this model is the fact that lag time has to be determined from experimental data and cannot be determined by the model regression (Schmidt, 1992). In this respect, the Gompertz equation was first used by Gibson et al. (1987) to fit the microbial growth curve although it was originally developed to describe human mortality as a function of age (Causton, 1977). However, the same pro-blem was the case for this model; namely, it was not effective for the description of lag phase in some cases (Li et al., 2007).

In order to overcome this problem, Gibson et al. (1987) proposed the modified Gompertz model, which was

considered to be the best sigmoidal model for growth curves (Zwietering et al., 1991; McMeekin et al., 1993). However, mechanistic approaches are not used to derive the modified Gompertz model. As known, the mechanistic considerations include all intrinsic and extrinsic factors regulating cellular metabolism and interpreting the modeled response with regard to known phenomena and processes. Therefore, providing a biological basis for interpretation of its parameters is difficult. Some attempts have been made to develop more mechanistic growth models (Li et al., 2007). In this respect, Baranyi and Roberts (1994, 1995) developed the Baranyi model. The model is well known to be capable of showing a good fit for bacterial growth curves, that is, *Bacillus* spp., *Brochothrix thermospacta*, *Clostridium* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Staphylococcus* spp., *Yersinia enterocolitica* and *Salmonella typhimurium* (Baranyi and Roberts, 1994, 1995; Pin et al., 2002; Fujikawa et al., 2004; Lopez et al., 2004). So far, the Baranyi model has become one of the most commonly preferred growth models due to the fact that it has a good fitting capability, that it can be applied for dynamic environmental conditions and that most of the model parameters are biologically interpretable (Pin et al., 2002; Lopez et al., 2004; van Impe et al., 2005). Moreover, although the application of the modified Gompertz model for the description of microbial survival/inactivation and growth is well documented and described in the literature (Zwietering et al., 1990; Buchanan, 1993; Erkmen, 2008, 2009), the Baranyi model is being adopted more and more over the modified Gompertz model (Sutherland et al., 1997). One of the most striking properties of the Baranyi model is the fact that it is a truly dynamic model dealing with time varying environmental conditions, which is an indispensible property with respect to the increasing attention paid to shelf life prediction of foods and quantitative risk analysis of food production cycles (Grijspeerdt and Vanrolleghem, 1999).

The success of the model-based predictions greatly depends on the reliability of the model parameters, which makes parameter estimate process important to obtain very sensitive results. In this respect, determining identifiability properties of the parameters obtained by the model-based predictions comes into prominence to evaluate the statistical significance of the parameter estimates. The identifiability of the parameter estimates gains importance when parameters cannot be significantly predicted or estimates are strongly correlated with each other (Grijspeerdt and Vanrolleghem, 1999). Many problems related with parameter identifiability can be overcome by a good experimental design (Baranyi et al., 1996). However, the identifiability properties of the predictive microbiological properties have been reported not to always get the attention they surely deserve (Grijspeerdt and Vanrolleghem, 1999). Therefore, this study was undertaken to determine the identifiability properties of the Baranyi model in fitting the effect of oregano and thyme essential oils on growth of *S. typhimurium* in rainbow trout stored under aerobic (AP), modified atmosphere (MAP) and vacuum packed (VP) conditions. It was also aimed to compare the performance of Baranyi model with the most common used empirical and mechanistic models under these conditions.

MATERIALS AND METHODS

Product

Fresh rainbow trout *Oncorhynchus mykiss* fillets were obtained from a local fish company in Kayseri, Turkey and immediately transferred in ice boxes to the laboratory. The skin was removed aseptically. The trout fillets were cut in pieces (average weight 30 g), which were immediately sealed in polyethylene bags. Aseptic conditions were kept during preparation and handling. Experiments were carried out on three different batches of fish fillets.

Extraction of essential oils

Dried oregano (500 g) (*Origanum vulgare* L.) and thyme (*Thymus vulgaris* L.) that were identified by the scientists of botany in Erciyes University in Kayseri, Turkey were purchased from a local retail spice market. The essential oils of the species were extracted by the Clevenger hydro distillation method. Plant materials (100 g) were cut into small pieces, placed in a distillation apparatus with 2 L of double distilled water and hydro distilled for 3 h. After the oils were dried over anhydrous sodium sulphate, they were stored at 4°C until analyses.

Culture preparation and inoculation experiments

Trout samples were inoculated with an 18 h culture of *S. typhimurium* ATCC 14028. The culture was activated (10^4-10^5 cfu/ml) in Nutrient Broth (Merck, Darmstadt, Germany), after incubation at 37°C for 24 h and finally inoculated on the trout up to a final population of 5 $\log_{10}$ cfu/g.

Essential oil application and packing treatments

Prior to essential oil applications, samples were tested for the presence of *S. typhimurium* and the pathogen was not detected. The number of *S. typhimurium* was evaluated in three samples, that is, control (fillets without essential oil application), oregano (fillets added with oregano essential oil) and thyme (fillets added with thyme essential oil). For this purpose, appropriate volumes of oregano and thyme distillates were applied by surface spraying, yielding 0.2 % (v/w) (organoneptically accepted level determined in a preliminary study) essential oil level per sample.

The number of *S. typhimurium* was also evaluated in the samples packed under three gas atmospheres, that is, air, modified atmosphere (30% CO_2/70%N_2/4 ppm O_2 gas mixture at 1.2 ppm moisture) and vacuum. For the aerobic storage (AP), the samples were placed in sterile Petri dishes. For modified atmosphere packing (MAP), the samples were packed with polyethylene films with low O_2 permeability (O_2 transmission rate of 5 ml/m^2/24 h at 23°C and 75% R.H.), and for vacuum packed samples, the samples were packed with Cryovac BB405 bags (Cryovac A/S, Oslo, Norway), using a vacuum machine (Ünal Machine Equipments, KVG 010 model, Adapazarı, Turkey). After packing, samples were stored at 4±1°C prior to enumeration of the pathogen cells.

Enumeration of *S. typhimurium* cells

S. typhimurium was plated on Brilliant Green Phenol Red Agar (Merck) and enumerated after incubation at 37°C for 24 h.

Fitting of the empirical and mechanistic models

Modified Gompertz model (Equation 1) and logistic (Equation 2) were used as empirical models to fit the microbial growth curves of the bacteria (Gibson et al., 1987).

$$\log N = \log N_0 + a\exp\left\{-\exp\left[-B(t-m)\right]\right\} \quad (1)$$

Where, log *N* (log cfu/g) is the logarithm of the cell number *S. typhimurium* at time *t*; log N_0 (log cfu_0) the logarithm of the cell number *S. typhimurium* counts at time 0; *a* the count increment as time increases indefinitely, that is number of log cycles of growth (log cfu/g); *B* is the specific growth rate at time *m* (1/h) and *m* the time at which the absolute growth rate is at a maximum (h).

$$\log N = \log N_0 + a/\left\{1+\exp(d-ct)\right\} \quad (2)$$

Where, log *N*, log N_0 and *a* have the same meaning as Equation 2; *d* a dimensionless parameter and *c* the specific growth rate (1/h). Because modified Gompertz and logistic models are sigmoidal models, both of them are more appropriate to fitting growth curves complete with all three phases, involving lag, exponential and stationary phases. When incomplete growth was the case, especially once the stationary phase data are missing, these models may not be suitable for fitting the growth curves (Juneja et al., 2009). For the modified Gompertz model, the maximum specific growth rate (log cfu/g/h) can be calculated from

$$\mu_{\max} = Ba/e \text{ (where e = 2.7182)} \quad (3)$$

and for the logistic model, the maximum specific growth rate (log cfu/g/h) can be derived from

$$\mu_{\max} = ac/4 \quad (4)$$

The Huang model (Huang, 2008) that has been recently developed for fitting the bacterial growth curves was also used in this study. This model is especially appropriate for fitting growth curves without stationary phase when λ is defined as the duration of the lag phase.

$$\ln N = \ln N_0 + \mu_{\max}\left\{t + \frac{1}{25}\ln\frac{1+\exp\left[-25(t-\lambda)\right]}{1+\exp(25\lambda)}\right\} \quad (5)$$

Where, ln *N* (ln cfu/g) is the logarithm of the cell number *S. typhimurium* at time *t*; ln N_0 (ln cfu/g) the logarithm of the cell number *S. typhimurium* counts at time 0; $\mu_{\max}$ the maximum specific growth rate (ln cfu/g/h) and λ is the duration of the lag phase (h).

The Baranyi model (Baranyi and Roberts, 1994) was also used to fit the sigmoidal bacterial growth curves under constant temperature conditions.

$$y(t) = y_0 + \mu_{\max}F(t) - \frac{1}{m}\ln\left(1+\frac{\exp(m\mu_{\max}F(t)) - 1}{\exp\left[m(y_{\max}-y_0)\right]}\right) \quad (6)$$

$$F(t) = t + \frac{1}{\mu_{\max}}\ln\left[\exp(-vt) + \exp(-h_0) - \exp(-vt-h_0)\right];$$

and where, $y(t) = \ln(x(t))$ (cfu/g) is the logarithm of the cell number *S. typhimurium* with $x(t)$; y_0 (ln (x_0); $y_{\max} = \ln(x_{\max})$, x_0 being the initial and $x_{\max}$ the asymptotic cell concentration, respectively; $\mu_{\max}$ the maximum specific growth rate (ln cfu/g/h); *m* a curvature parameter to characterize the transition from the exponential phase; *v* a curvature parameter to characterize the transition to the exponential phase, h_0 a dimensionless parameter quantifying the initial physiological state of the cells. From that λ (h) can be calculated as $h_0/\mu_{\max}$.

For the curvature parameters, Baranyi (1997) suggested $v = \mu_{\max}$ and $m = 1$, the values that were also adopted in this study. Baranyi and Roberts (1994) also reported that the parameter h_0 is approximately constant in a situation in which the pre-inoculation history of the cells is identical. However, this parameter varies in reality (Juneja et al., 2009); therefore, the growth data obtained were first fitted with the Baranyi model to obtain all four parameters y_0, $y_{\max}$, h_0 and $\mu_{\max}$. After a mean value of the parameter h_0 for all growth curves was determined and the value of h_0 was fixed with the average value, $\overline{h_0}$, the growth data were fitted again with the Baranyi model to obtain the values of y_0, $y_{\max}$ and $\mu_{\max}$ for each growth curve (Juneja et al., 2009).

Structural identifiability of the Baranyi model

The statistical significance of the parameter estimates can be evaluated by the identifiability of the parameters. In this respect, it is possible to give a unique value to a parameter of a mathematical model, being related to theoretical identifiability (Vanrolleghem and Dochain, 1998). With structural identifiability analysis, it can be revealed that only certain combinations of parameters are identifiable. The method developed by Pohjanpalo (1978) and based on the Taylor series expansion of the model was used to analyze the structural identifiability. In this method, the successive derivatives are examined to check if they include information on the parameters to be identified. There are four parameters in the Baranyi model; therefore, if the model parameters can be written as a combination of any four derivatives of the Taylor series expansion, the structural identifiability is proven (Grijspeerdt and Vanrolleghem, 1999).

In this respect, let the model be indicated by $f(t)$ and $(d^i f/dt^i)$ (0) by z_i, then it can be proven that the four parameters can be written as a combination of the z_i's:

$$\mu_{\max} = \frac{\sqrt{z_2^4 + 3z_3^2 - 2z_2z_4}}{z_2}$$

$$h_0 = \frac{\ln 2}{\frac{z_2^2 - z_3}{\sqrt{z_2^4 + 3z_3^2 - 2z_2z_4}} + 1} \quad (7)$$

$y_0 = z_1$

$$y_{\max} = z_1 - \frac{\ln 2}{\frac{z_2^2 - z_3}{\sqrt{z_2^4 + 3z_3^2 - 2z_2z_4}} + 1} - \ln\left(\frac{1}{2} - \frac{z_2^2 + z_3}{2\sqrt{z_2^4 + 3z_3^2 - 2z_2z_4}}\right)$$

This analysis indicates that the Baranyi model is structurally identifiable, which demonstrates that this model structure makes the attainment of identifiable parameters possible with the condition that an ideal set of data is obtained (Grijspeerdt and Vanrolleghem, 1999).

Fitting of data and non-linear regression of the derived models

A non-linear regression procedure in Statistica software (Release 5.0, Statsoft Inc., Tulsa, OK, USA) was used to fit each individual set of growth data to the four primary models, minimizing the sum of squares of the difference between experimental data and the fitted model, that is, loss function (observed−predicted). The Quasi-Newton algorithm option of the non-linear regression procedure was used during numerical iteration to search for the calculated parameters of each model. After several iterations in the non-linear procedure, the starting values were converged to estimated values of the parameters.

Performance of the derived models using statistical analysis

The performance of the derived models (Equations 1, 2 and 5, 6) was compared using various statistical parameters such as the mean percentage error (*MPE*), the mean bias error (*MBE*), the root mean square error (*RMSE*), the modeling efficiency (*EF*) and chi-square (χ^2) in addition to R^2. These statistics allow for the detection of the differences between experimental data and the model estimates. These parameters can be estimated as following (Toğrul and Arslan, 2004):

$$MPE = \frac{1}{n}\sum_{i=1}^{n}\left[\log N_{exp,i} - \log N_{pre,i} / \log N_{exp,i}\right] \times 100 \quad (8)$$

$$MBE = \frac{1}{n}\sum_{i=1}^{n} \log N_{pre,i} - \log N_{exp,i} \quad (9)$$

$$RMSE = \left[\frac{1}{n}\sum_{i=1}^{n} \log N_{exp,i} - \log N_{pre,i}^{\ 2}\right]^{1/2} \quad (10)$$

$$EF = \frac{\sum_{i=1}^{n} \log N_{exp,i} - \log N_{exp,ave}^{\ 2} - \sum_{i=1}^{n} \log N_{pre,i} - \log N_{exp,i}^{\ 2}}{\sum_{i=1}^{n} \log N_{exp,i} - \log N_{exp,ave}^{\ 2}} \quad (11)$$

$$\chi^2 = \frac{\sum_{i=1}^{n} \log N_{exp,i} - \log N_{pre,i}^{\ 2}}{n - n_u} \quad (12)$$

Where, log $N_{exp,i}$ (log cfu/g) is the experimental logarithm of the cell number *S. typhimurium* at time t; log $N_{pre,i}$ is the predicted logarithm of the cell number *S. typhimurium* at time *t* (log cfu/ g); log $N_{exp,ave}$ is the average of logarithm of the cell number *S. typhimurium* at time *t* (log cfu/g); n is the number of data points and n_u is the number of model parameters.

RESULTS AND DISCUSSION

Fitting of the mathematical models to microbial growth curves

Figures 1a to c, d to f and 2a to c, d to f show the fitting of modified Gompertz, logistic, Huang and Baranyi models, respectively to the experimental data for the effect of essential oil addition on *S. typhimurium* growth in rainbow trout stored under aerobic, MAP and VP conditions. The empirical models, modified Gompertz and logistic models were fitted to each individual growth curve satisfactorily with R^2 values ranging from 0.93 to 0.99 (Table 1). On the other hand, the mechanistic models, Huang and Baranyi models yielded in relatively poor fit as compared to the empirical models, resulting in R^2 values ranging from 0.76 to 0.99 (Table 2).

The effect of essential oil and packing treatments

The derived parameters for the modified Gompertz model, namely *a*, *B*, *m*, μ_{max} and R^2 at different essential oil treatments and packing applications as a function of time are presented in Table 1. μ_{max} values were observed to range between 0.03 to 0.08 log cfu/g/h. When the essential oil applications were compared with each other within packing treatments, thyme oil addition was observed to reduce the μ_{max} values of *S. typhimurium* in the fillets stored under all the packing types. However, the effect of oregano oil to decrease μ_{max} value could be observed when the fillets were vacuum-packed (Table 1). These results indicated that essential oil application delayed the growth of *S. typhimurium* and thyme oil could delay the growth of the pathogen even under aerobic conditions. It was reported that in the presence of various essential oils (oregano, pimiento, horseradish, mint), *Salmonella* species was inhibited under aerobic conditions in precooked roast beef (Ward et al., 1998) and beef (Cutter, 2000). It is also well known that many spices and herbs have an effect to delay the onset of spoilage or prevent the growth of foodborne pathogens because their essential oils possess antimicrobial activity (Nychas and Tassou, 2000).

In this study, packing treatments were also observed to have considerable delaying effects on the growth of *S. typhimurium*, which can be seen by the lower μ_{max} values determined for the fillets that were packed under MAP and VP conditions (Table 1). When the packing types were compared with each other within the essential oil applications, MAP was observed to have a remarkable delaying effect on the growth of *S. typhimurium* under all atmospheric conditions, even in the fillets without any essential oil application (control samples) (Table 1). On the other hand, VP could delay the growth of *S. typhimurium* in the fillets only treated with essential oils. Comparable results were also obtained with the logistic model. The μ_{max} values obtained from this model

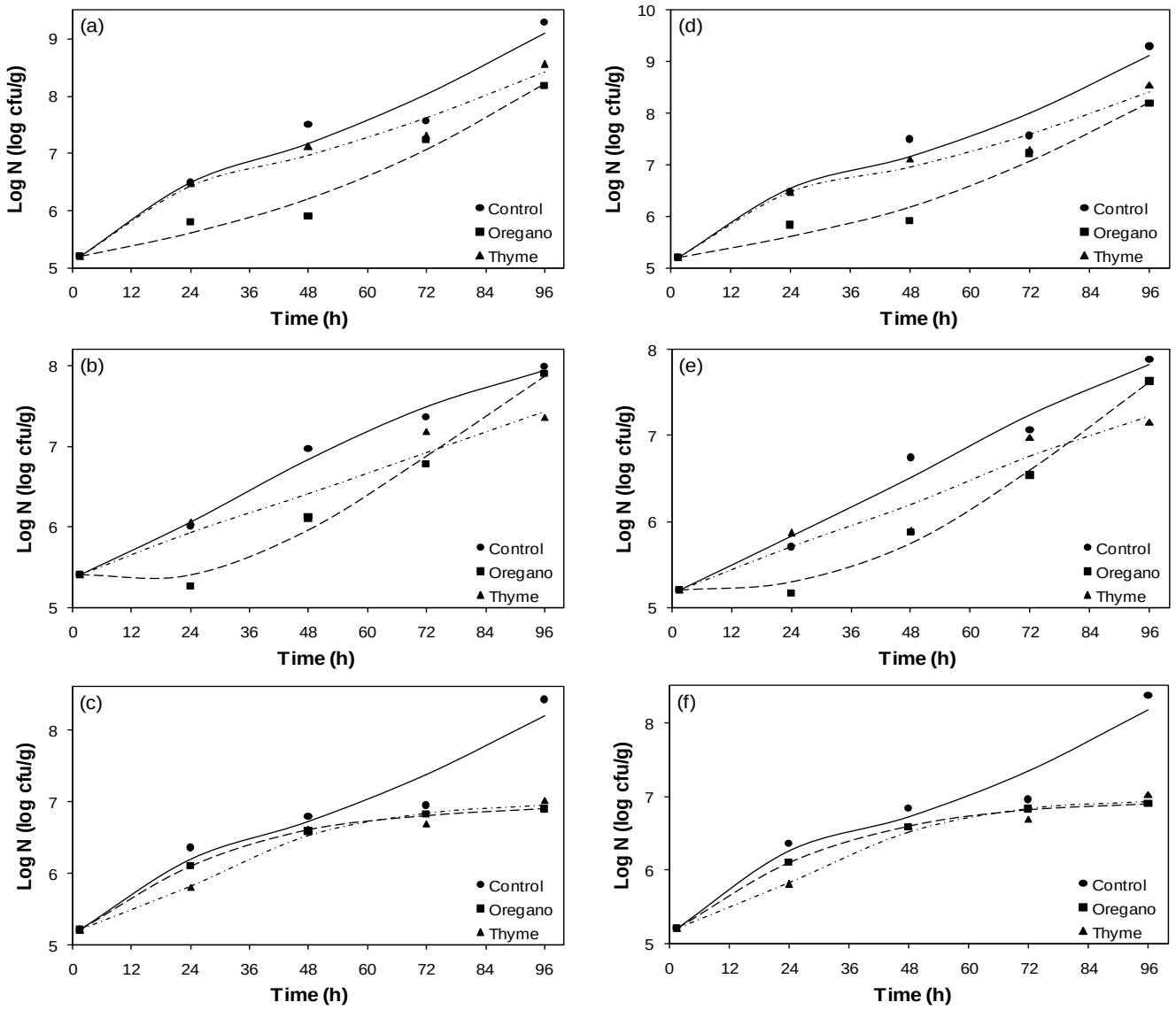

Figure 1. Fitting of modified Gompertz (a, b, c) and logistic models (d, e, f) to experimental data (data point) for the effect of essential oils on *Salmonella typhimurium* growth in rainbow trout stored under (a-d) aerobic, (b-e) MAP and (c-f) VP conditions.

generally showed a similar trend with the μ_{max} values obtained by the modified Gompertz model. In the case of mechanistic models, the similar trend was observed in the μ_{max} values calculated by the Huang model and these values were generally higher in the control and air-packed samples (Table 2). On the other hand, the similar trends obtained by the modified Gompertz, logistic and Huang models were not provided by the Baranyi model, as seen by the μ_{max} values obtained by this model.

Comparison of the performance of empirical and mechanistic models

Tables 1 and 2 show the performance of the Equations 1, 2 and 5, 6. The higher the values of EF and the lower the values of *MPE*, *MBE*, *RMSE* and $\chi 2$ are, the better the goodness of fit will be (Toğrul and Arslan, 2004). In this respect, it can be seen that the modified Gompertz and logistic models, described the relationship with a very good fit values; however, statistical analysis indicated that this was not the case for the mechanistic models, Huang and Baranyi models. Briefly, the higher values of *EF* and the lower absolute values of *MBE*, *RMSE*, *MPE* and χ^2 could be obtained with the statistically fitted empirical models, for example, the modified Gompertz and logistic models.

The correlations between observed and predicted data for the behavior of *S. typhimurium* growth in rainbow trout calculated with the modified Gompertz, logistic, Huang

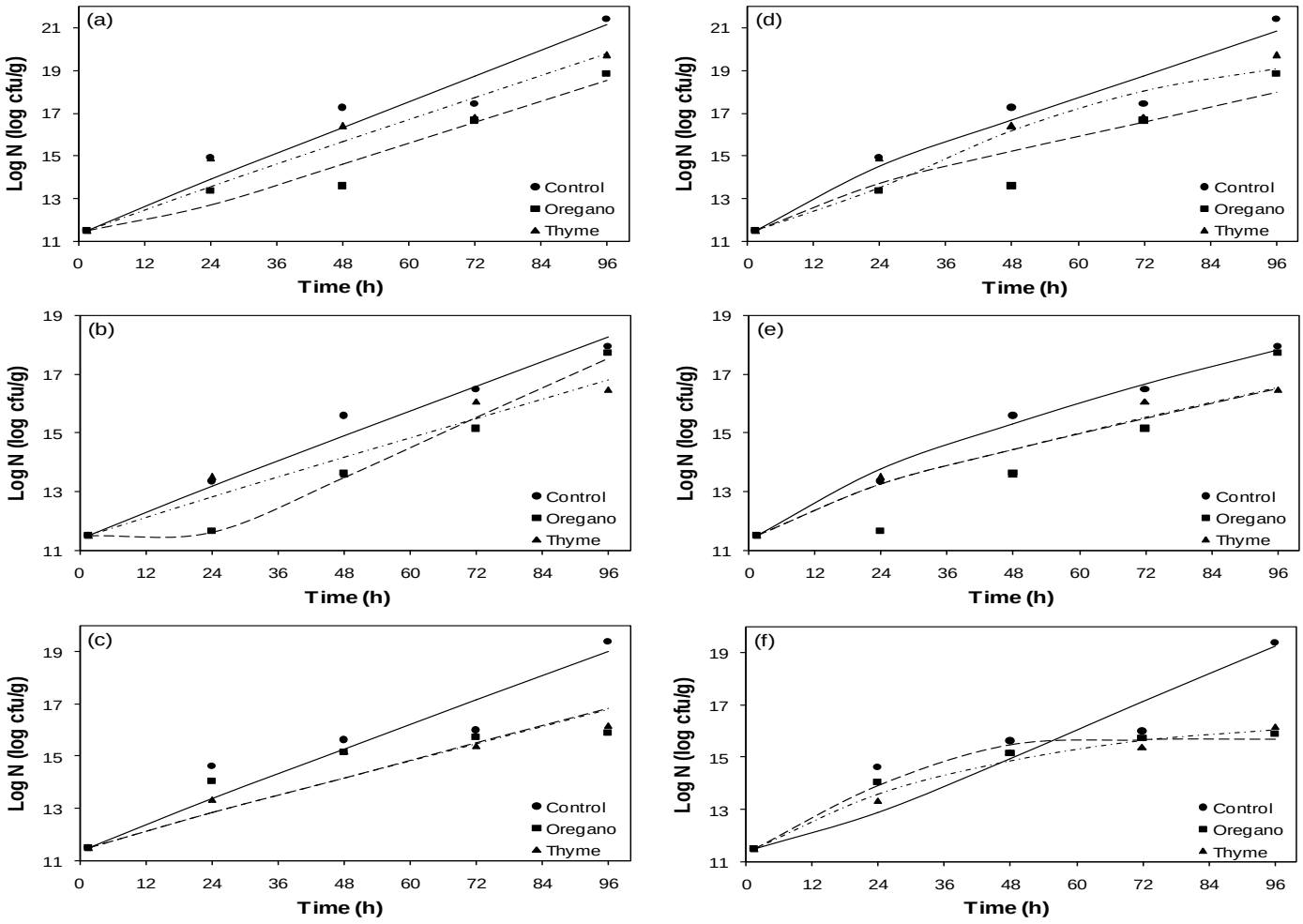

Figure 2. Fitting of Huang (a, b, c) and Baranyi models (d, e, f) to experimental data (data point) for the effect of essential oils on *Salmonella typhimurium* growth in rainbow trout stored under (a-d) aerobic, (b-e) MAP and (c-f) VP conditions.

and Baranyi models for AP, MAP and VP conditions are illustrated in Figures 3 and 4, respectively. The modified Gompertz and logistic models showed a good fit to all growth curves as assessed using the correlation coefficients between experimental and predicted values; however, Huang and Baranyi models yielded in relatively lower fit values. Therefore, it can be suggested that modified Gompertz and logistic models allowed a better prediction of the effects of oregano and thyme essential oils on growth of *S. typhimurium* in rainbow trout stored under aerobic, MAP/VP conditions.

Practical identifiability of the Baranyi model and functional analysis to test sensitivity

After it was previously shown that the Baranyi model is structurally identifiable, it was necessary to determine what the identifiability properties were when using experimental data. The experimental data always includes a certain level of noise and this has an effect on the parameter estimation process. Therefore, it should be aimed to determine the possibility to give the parameters unique values with experimental data (Grijspeerdt and Vanrolleghem, 1999).

To determine identifiability of the Baranyi model, sensitivity functions an indicator for the identifiability of the selected model should be developed. It can be shown by the sensitivity functions at what times the parameters are most sensitive to the experimental data, implying that the information content of an experiment can be increased by sampling at time when the sensitivity functions have the highest values (Vialas et al., 1985). If the sensitivity equations are proportional, the covariance matrix is singular, indicating that the model is not practically identifiable (Robinson, 1985). If they are nearly proportional, the parameter estimation is highly correlated. Therefore, it is possible to have a quick indication for the identifiability of the model by plotting the sensitivity functions. The sensitivity functions developed for the Baranyi model can be calculated by taking the partial derivatives of the model with respect to the four parameters, μ_{max}, h_0, y_0

Table 1. Parameters of modified Gompertz and logistic models for the effect of essential oils on *Salmonella typhimurium* growth in rainbow trout stored under aerobic, VP (vacuum packed) and MAP (modified atmosphere packed) conditions.

Parameter	AP			MAP			VP		
	Control	Oregano	Thyme	Control	Oregano	Thyme	Control	Oregano	Thyme
Modified Gompertz									
a (log cfu/g)	47.7	27.3	36.9	3.6	5.2	4.5	47.1	2.0	2.0
B (1/h)	0.005	0.008	0.004	0.028	0.022	0.013	0.004	0.046	0.050
m (h)	284.7	189.0	295.6	39.1	77.4	68.3	342.6	9.0	21.2
μ_{max} (log cfu/g/h)	0.08	0.08	0.06	0.04	0.04	0.02	0.08	0.03	0.04
R^2	0.99	0.99	0.99	0.99	0.93	0.99	0.99	0.96	0.99
Regression parameters									
MPE	-0.031	0.022	0.008	-0.068	-0.328	-0.069	0.019	-0.001	-0.033
MBE	-0.007	-0.006	-0.004	0.002	0.014	-0.002	-0.008	0.000	0.001
RMSE	0.310	0.200	0.197	0.124	0.121	0.214	0.270	0.020	0.110
EF	0.91	0.96	0.93	0.97	0.98	0.87	0.88	0.99	0.96
χ^2	0.13	0.05	0.05	0.02	0.02	0.06	0.10	0.001	0.01
Logistic									
a (log cfu/g)	35.7	6.6	29.0	3.7	4.1	2.9	35.1	2.0	2.0
d (dimensionless)	3.4	3.3	3.2	2.2	3.6	1.9	3.6	1.1	1.9
c (1/h)	0.015	0.030	0.013	0.039	0.045	0.032	0.014	0.056	0.066
μ_{max} (log cfu/g/h)	0.13	0.05	0.09	0.03	0.05	0.02	0.12	0.03	0.03
R^2	0.98	0.96	0.99	0.99	0.99	0.98	0.99	0.96	0.99
Regression parameters									
MPE	-0.124	0.127	-0.002	-0.162	-0.284	-0.006	-0.046	0.001	-0.005
MBE	-0.002	-0.011	-0.004	0.005	0.012	-0.006	-0.003	-0.001	0.001
RMSE	0.297	0.193	0.184	0.169	0.118	0.207	0.237	0.017	0.104
EF	0.91	0.962	0.94	0.95	0.98	0.88	0.90	0.99	0.95
χ^2	0.12	0.05	0.05	0.04	0.02	0.06	0.08	0.00	0.01

and y_{max}, respectively (Grijspeerdt and Vanrolleghem, 1999):

$$\frac{\partial y}{\partial \mu_{max}} = -\frac{\exp\ h_0 + t\mu_{max}\left[\exp\ y_0 - \exp\ y_{max}\right] t}{\left[-1+\exp\ h_0 + \exp\ t\mu_{max}\right]\left[-\exp\ y_0 + \exp\ h_0 + y_{max} + \exp\ y_0 + t\mu_{max}\right]}$$

$$\frac{\partial y}{\partial h_0} = \frac{\exp\ h_0\left[\exp\ y_0 - \exp\ y_{max}\right]\left[-1+\exp\ t\mu_{max}\right]}{\left[-1+\exp\ h_0 + \exp\ t\mu_{max}\right]\left[-\exp\ y_0 + \exp\ h_0 + y_{max} + \exp\ y_0 + t\mu_{max}\right]} \quad (13)$$

$$\frac{\partial y}{\partial y_0} = \frac{\exp(h_0 + y_{max})}{\left[-\exp\ y_0 + \exp\ h_0 + y_{max} + \exp\ y_0 + t\mu_{max}\right]}$$

$$\frac{\partial y}{\partial y_{max}} = \frac{\exp(y_0)\left[-1+\exp\ t\mu_{max}\right]}{\left[-\exp\ y_0 + \exp\ h_0 + y_{max} + \exp\ y_0 + t\mu_{max}\right]}$$

The development of the different sensitivity functions with time using the estimated parameter values is illustrated in Figure 5. In this figure, it could be seen that more than two sensitivity functions are proportional to each other, indicating that the model is not identifiable. In addition, ($\delta y/\delta y_o$) and ($\delta y/\delta y_{max}$) are linearly correlated, but they are not proportional (Figure 5) as an intercept is seen to exist when a linear regression is performed between them. However, visual inspection may be illusive since linear combinations of the sensitivity functions are inspec- linear analysis may be helpful in this respect (Grijspeerdt and Vanrolleghem, 1999).

In addition, the correlation matrixes of the parameter estimates, constructed from the estimated parameter values of AP, MAP and VP samples, showed that the parameter estimates are highly correlated between one another, also indicating the model is not identifiable in the case of the experimental conditions in this study.

Table 2. Parameters of modified Huang and Baranyi models for the effect of essential oils on *Salmonella typhimurium growth* in rainbow trout stored under aerobic, VP and MAP conditions.

Parameter	AP			MAP			VP		
	Control	Oregano	Thyme	Control	Oregano	Thyme	Control	Oregano	Thyme
Huang									
λ (h)	-0.9	9.6	-1.0	-0.9	24.4	-1.0	-1.0	-1.0	-1.0
μ_{max} (ln cfu/g/h)	0.10	0.08	0.09	0.07	0.08	0.06	0.08	0.06	0.06
R^2	0.82	0.92	0.73	0.91	0.99	0.84	0.84	0.90	0.79
Regression parameters									
MPE	1.460	-0.166	1.989	0.801	-0.009	0.616	1.360	2.586	1.649
MBE	-0.224	0.000	-0.286	-0.111	0.000	-0.092	-0.205	-0.362	-0.225
RMSE	0.976	0.652	0.889	0.480	0.226	0.558	0.900	0.942	0.682
EF	0.82	0.92	0.742	0.92	0.99	0.84	0.75	-0.566	0.58
χ^2	1.27	0.57	1.054	0.31	0.07	0.42	1.08	1.18	0.62
Baranyi									
y_{max} (ln cfu/g)	18.0	117.0	1.2	7.7	122.1	127.5	11.6	-26.5	2.5
y_0 (ln cfu/g)	-239.8	-116.9	-6.6	-7.5	-121.9	-127.6	-12.3	-30.7	-2.3
μ_{max} (ln cfu/g/h)	0.09	0.06	0.12	0.06	0.04	0.04	0.09	0.15	0.04
R^2	0.87	0.83	0.89	0.97	0.76	0.85	0.90	0.87	0.93
Regression parameters									
MPE	0.340	-2.327	1.712	-0.487	-3.777	-0.309	2.472	0.104	-0.169
MBE	-0.069	0.248	-0.264	0.053	0.384	0.023	-0.359	-0.016	0.015
RMSE	0.830	0.944	1.083	0.340	1.101	0.530	1.105	0.240	0.281
EF	0.87	0.83	0.617	0.96	0.75	0.85	0.62	0.90	0.93
χ^2	0.92	1.19	1.56	0.15	1.62	0.38	1.63	0.10	0.11

$$\begin{pmatrix} & \mu_{\max} & h_0 & y_{\max} & y_0 \\ \mu_{\max} & 1 & 0.04 & -0.997 & 0.997 \\ h_0 & 0.04 & 1 & -0.117 & 0.119 \\ y_{\max} & -0.997 & -0.117 & 1 & -1.000 \\ y_0 & 0.997 & 0.119 & -1.000 & 1 \end{pmatrix}_{\text{(AP samples)}}$$

$$\begin{pmatrix} & \mu_{\max} & h_0 & y_{\max} & y_0 \\ \mu_{\max} & 1 & -0.41 & -0.808 & 0.827 \\ h_0 & -0.41 & 1 & 0.869 & -0.852 \\ y_{\max} & -0.808 & 0.869 & 1 & -0.999 \\ y_0 & 0.827 & -0.852 & -0.999 & 1 \end{pmatrix}_{\text{(MAP samples)}}$$

$$\begin{pmatrix} & \mu_{\max} & h_0 & y_{\max} & y_0 \\ \mu_{\max} & 1 & 0.843 & -0.899 & 0.898 \\ h_0 & 0.843 & 1 & -0.993 & 0.994 \\ y_{\max} & -0.899 & -0.993 & 1 & -1.000 \\ y_0 & 0.898 & 0.994 & -1.000 & 1 \end{pmatrix}_{\text{(VP samples)}}$$

One of the most remarkable models is the Baranyi model (Baranyi and Roberts, 1994) that was developed to describe the process of adjustment of microbial cells by hypothetical adjustment functions $\alpha_n(t)$ and $\beta_n(t)$. In the model, the adjustment function $\alpha_n(t)$ denotes that the product of the lag parameter and the maximum specific growth are regarded to be a simple transition of the initial physiological state of cells and the inhibition function $\beta_n(t)$ describes the transition from exponential phase to

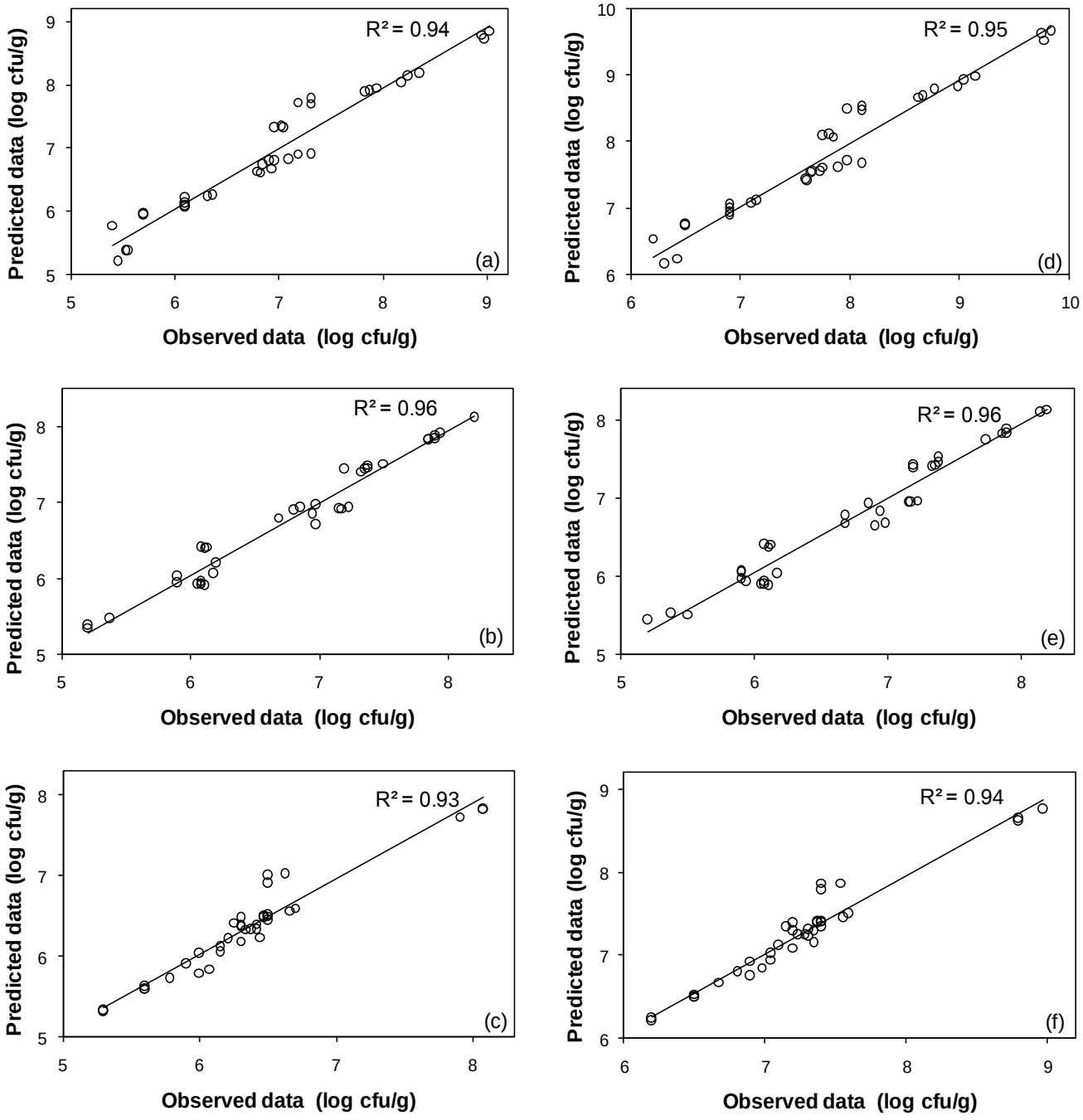

Figure 3. Correlations between observed and predicted data for the behavior of *Salmonella typhimurium* growth in rainbow trout calculated with modified Gompertz (a, b, c) and logistic models (d,e,f) for (a-d) aerobic, (b-e) MAP and (c-f) VP conditions.

stationary phase (Baranyi and Roberts, 1994, 1995). In the model, the following differential equations are used to describe the microbial populations in a batch culture (Baranyi and Roberts, 1994; Li et al., 2007):

$$\begin{cases} \frac{\delta N}{\delta t} = \alpha_n(t)\,\mu_{max} N \beta_n(t) & 0 \le t < +\infty;\ N > 0 \\ N(0) = N_0 & t = 0;\ N_0 > 0 \end{cases} \quad (14)$$

$$\alpha_n(t) = q(t) / \left[1 + q(t)\right] \quad (15)$$

$$\frac{\delta q(t)}{\delta t} = \mu_{max} q(t),\ q(t=0) = q_0 \quad (16)$$

$$\beta_n(t) = 1 - \left(N / N_{max}\right)^m = 1 - N^m / N_{max}^m \quad (17)$$

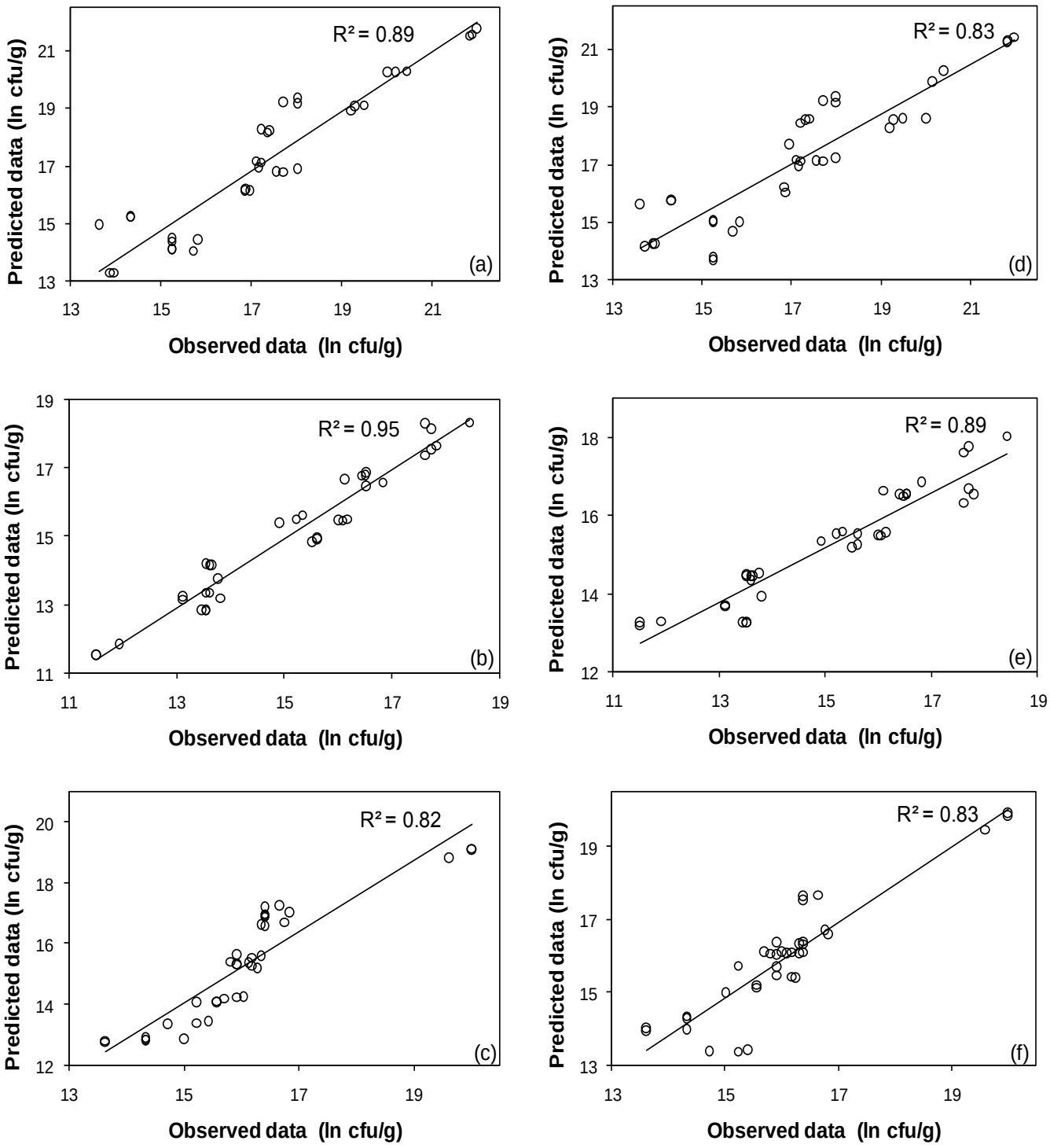

Figure 4. Correlations between observed and predicted data for the behavior of *Salmonella typhimurium* growth in rainbow trout calculated with Huang (a, b, c) and Baranyi models (d, e, f) for (a-d) aerobic, (b - e) MAP and (c - f) VP conditions.

concentration of microbes at time t (cfu/g); N_0 is the initial concentration of microbes at time t = 0 (cfu/g); $q(t)$ is the physiological state of the cells at time t (h); μ_{max} is the maximum growth rate; q_0 is the initial physiological state of the cells at time t = 0; N_{max} is the maximum growth population of cells (cfu/g) and m is the positive number characterizing the curvature of the growth curve at the transition between the exponential phase and stationary phase.

In the case of this study; however, the Baranyi model

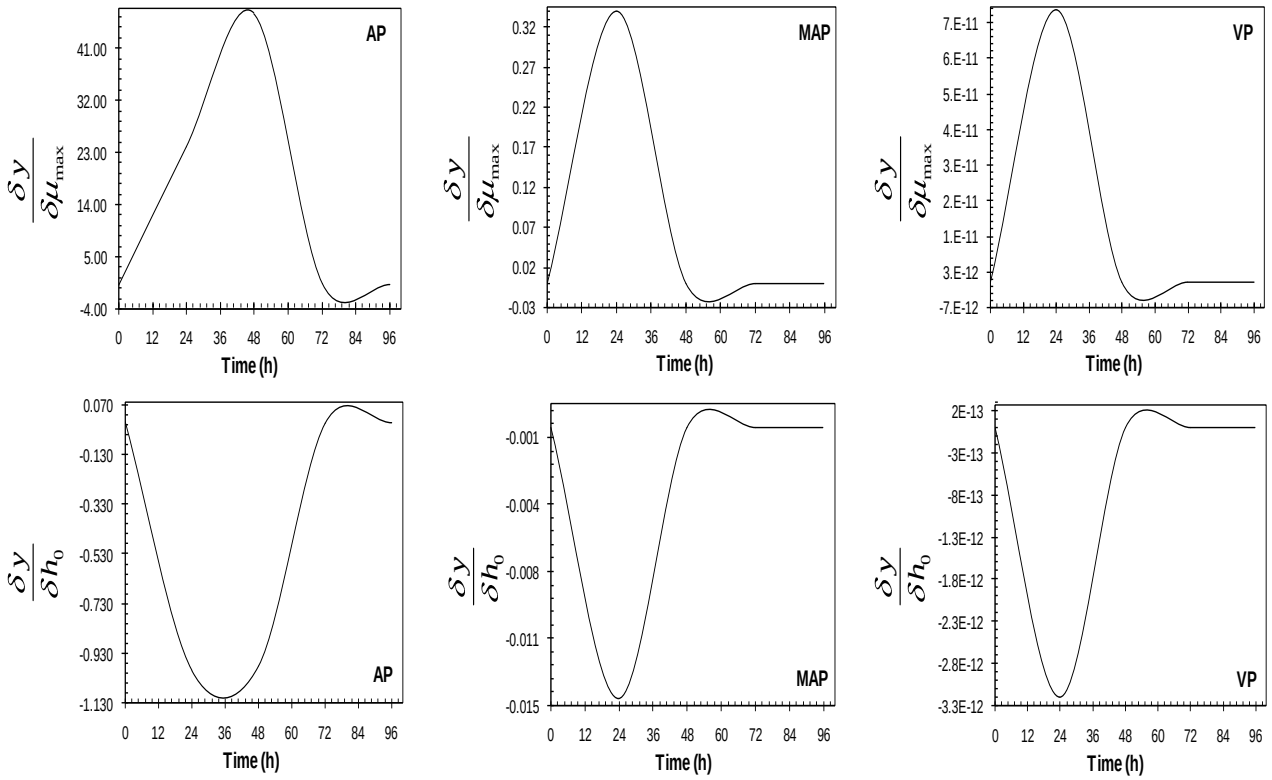

Figure 5. Sensitivity functions derived from the partial derivatives of the estimated parameters calculated by the Baranyi model using Equation 13 for AP (air packed), MAP (modified atmosphere packed) and VP (vacuum packed) samples.

was determined not to be identifiable and yielded in a relatively poor fit as compared to the modified Gompertz and logistic models. This could be explained by the fact that the physiological state of the cells at incubation in the model is presumed to be constant for the change of incubation temperature (Baranyi and Roberts, 1994), probably causing the model not to yield a very good fit to the experimental data in this study. Accordingly, some deficits are still present in the model (Li et al., 2007). $q(t)$ is defined as the physiological state of the cells at incubation, which is assumed to be constant (Baranyi and Roberts, 1994). It could be inferred from Equation 16 that the $q(t)$ of the cells shows an exponential and unbound growth once the growth is initiated. However, this is biologically impossible (Li et al., 2007). The assumption of the parameter q_0 remaining constant was indicated to be correct only for positive temperature changes, for example, changing the incubation temperature from 4 to 35°C (Alavi et al., 1999; Swinnen et al., 2004). Furthermore, it was determined that the q_0 parameter values decreased with lower incubation temperatures (Alavi et al., 1999). The reason could also be explained by the fact that usual nonlinear regression programs cannot fit the model thoroughly due to the large number of parameters in the model and its sensitivity to the number of data points and their distribution (Buchanan et al., 1997; Baranyi, 1997).

Conclusions

There is a repeating debate in which the growth models are better to describe the bacterial growth. It has been discussed that the mechanistic models such as the Baranyi model were more preferred than the empirical models such as modified Gompertz and logistic models. However, in this study, the two empirical models were directly compared with the more mechanistic Huang and Baranyi models, revealing that the modified Gompertz and logistic models were better than the Huang and Baranyi models for describing the effect of oregano and thyme essential oils on the growth of *S. typhimurium* in rainbow trout stored under aerobic, MAP/VP conditions. The Baranyi model was performed in two-steps. In the first step, the h_0 of each individual curves was determined and in the second step, an average of all h_0 values was taken, indicating that the analysis with Baranyi model was more-time consuming than that with the other models. As to Huang model, the maximum specific growth rate (μ_{max}) and the duration of the lag phase (λ) of each growth curve could be obtained in one step process. The identifiability properties of the Baranyi model were also analyzed and it was concluded that the Baranyi model was not practically identifiable in the presence of the experimental data in spite of being structurally identifiable.

Considering the obtained results in this study, the empirical modified Gompertz and logistic models can be used more effectively than the mechanistic Huang and Baranyi models to predict the effect of plant essential oils on growth potential of *Salmonella* in fish products stored under aerobic, MAP/VP conditions. The modified Gompertz and logistic models can be used to set critical limits in HACCP procedure for the storage of fish products in order to delay or reduce potential growth of *S. typhimurium.*

REFERENCES

Alavi SH, Puri VM, Knabel SJ, Mohtqar RH, Whiting RC (1999). Development and validation of a dynamic growth model for *Listeria monocytogenes* in fluid whole milk. J. Food Prot. 62: 170-178.

Baranyi J (1997). Simple is good as long as it is enough. Food Microbiol. 14: 391-394.

Baranyi J, Roberts TA (1994). A dynamic approach to predicting bacterial growth in food. Int. J. Food Microbiol. 23: 277-294.

Baranyi J, Roberts TA (1995). Mathematics of predictive food microbiology. Int. J. Food Microbiol. 26: 199-218.

Baranyi J, Ross T, McMeekin TA, Roberts TA (1996). Effects of parameterization on the performance of empirical models in 'predictive microbiology'. Food Microbiol. 13: 83-91.

Buchanan RL (1993). Predictive food microbiology. Trends Food Sci. Technol. 4: 6-11.

Buchanan RL, Whiting RC, Damert WC (1997). When is simple good enough: a comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves. Food Microbiol. 14: 313-326.

Causton DR (1977). A Biologist's Mathematics, Arnold, London.

Cutter CN (2000). Antimicrobial effect of herb extract against *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella typhimurium* associated with beef. J. Food Prot. 63: 601-607.

Erkmen O (2008). Modeling the effects of sucuk production technique on *Listeria monocytogenes*, aerobic bacteria and lactic acid bacteria during ripening and storage. Food Bioprod. Process. 86: 220-226.

Erkmen O (2009). Mathematical modeling of *Salmonella typhimurium* inactivation under high hydrostatic pressure at different temperatures. Food Bioprod. Process. 87: 68-73.

France J, Thormley JHM (1984). Mathematical Models in Agriculture, Butterworths, London.

Fujikawa H, Kai A, Morozumi S (2004). A new logistic model for *Escherichia coli* growth at constant and dynamic temperatures. Food Microbiol. 21: 501-509.

Gibson AM, Bratchell N, Roberts TA (1987). The effect of sodium chloride and temperature on the rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. J. Appl. Bacteriol. 62: 479-490.

Grau FH, Vanderlinde PB (1992). Occurrence, numbers and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Protect. 55: 4-7.

Grijspeerdt K, Vanrolleghem P (1999). Estimating the parameters of the Baranyi model for bacterial growth. Food Microbiol. 16: 5936-605.

Huang L (2008). Growth kinetics of *Listeria monocytogenes* in broth and beef frankfurters-determination of lag phase duration and exponential growth rate under isothermal conditions. J. Food Sci. 73: E235-E242.

Juneja VK, Valenzuela Melendres M, Huang L, Subbiah J, Thippareddi, H (2009). Mathematical modeling of growth of *Salmonella* in raw ground beef under isothermal conditions from 10 to 45 ºC. Int. J. Food Microbiol. 131: 106-111.

Li H, Xie G, Edmonson, A (2007). Evolution and limitations of primary mathematical models in predictive microbiology. Int. J. Food Microbiol. 109(8): 608-626.

Lopez S, Prieto M, Dijkstra J, Dhanoa MS, France J (2004). Statistical evaluation of mathematical models for microbial growth. Int. J. Food Microbiol. 96: 289-300.

McMeekin TA, Olley J, Ross T, Ratkowsky DA (1993). Predictive Microbiology: Theory and Application, John Wiley, New York.

McMeekin TA, Ross T (2002). Predictive microbiology: providing a knowledge-based framework for change management. Int. J. Food Microbiol. 78: 133-153.

Nychas GJE, Tassou CC (2000). Preservatives: Traditional Preservatives-oils and spices. In: Encyclopedia of Food Microbiology (Eds Robinson R, Batt C, Patel P) London. Academic Press. pp. 1717-1722.

Pin C, Gonzalo FGDG, Ordonez JA, Baranyi J (2002). Analysing the lag-growth rate relationship of *Yersinia enterocolitica*. Int. J. Food Microbiol. 73: 197-201.

Pohjanpalo H (1978). System identifiability based on the power series expansion of the solution. Math. Biosci. 41: 21-33.

Robinson JA (1985). Determining microbial parameters using nonlinear regression analysis: advantages and limitations in microbial ecology. Adv. Microb. Ecol. 8: 61-114.

Schmidt SK (1992). Models for studying the population ecology of microorganisms in natural systems, Modelling the Metabolic and Physiologic Activities of Micro organisms, Wiley, New York, pp. 31-59.

Sutherland JP, Bayliss AJ, Braxton DS, Beaumont AL (1997). Predictive modelling of *Escherichia coli* O157:H7: Inclusion of carbondioxide as a fourth factor in a pre-existing model. Int. J. Food Microbiol. 37: 113-120.

Swinnen IAM, Bernaerts K, Dens EJJ, Geeraerd AH, Van Impe JF (2004). Predictive modelling of the microbial lag phase: a review. Int. J. Food Microbiol. 94: 137-159.

Toğrul H, Arslan N (2004). Mathematical model for prediction of apparent viscosity of molasses. J. Food Eng. 62: 281-289.

van Impe JF, Poschet F, Geeraerd AH, Vereecken KM (2005). Towards a novel class of predictive microbial growth models. Int. J. Food Microbiol. 100: 97-105.

Vanrolleghem PA, Dochain D (1998). Bioprocess model identification. In Advanced instrumentation, data interpretation and control of biotechnol.l processes. (Eds J. Van Impe, P. Vanrolleghem, D. Iserentant) pp. 251-318. Dordrecht, The Netherlands, Kluwer Academic Pub.

Vialas C, Cheru A, Gentil S (1985). An experimental approach to improve the monod model identification. In IFAC Modelling and Control of Biotechnol. Processes. pp. 175-179, Noordwijkerhout, Holland.

Ward SM, Delaquis PJ, Holley RA, Mazza G (1998). Inhibition of spoilage and pathogenic bacteria on agar and pre-cooked roast beef by volatile horseradish distillates. Food Res. Int. 31: 19-26.

Whiting RC, Buchanan RL (1993). A classification of models for predictive microbiology. Food Microbiol. 10: 175-177.

Zwietering MH, Jongenburger I, Rombouts FM, Van't Riet K (1990). Modeling of the bacterial growth curve. Appl. Environ. Microb. 56: 1875-1881.

Zwietering MH, de Koos JT, Hasenack BE, de Wit JC, Van't Riet K (1991). Modelling of bacterial growth as a function of temperature. Appl. Environ. Microb. 57: 1094-1101.