Mathematical Models for Prediction of Temperature Effects on Kinetic Parameters of Microorganisms' Inactivation: Tools for Model Comparison and Adequacy in Data Fitting

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Maria M. Gil, [1]

Fátima A. Miller, [2]

Teresa R. S. Brandão, [2]

Cristina L. M. Silva, [2]✉

Email clsilva@porto.ucp.pt

[1] MARE—Marine and Environmental Sciences Centre, ESTM, Instituto Politécnico de Leiria Campus 4|Santuário Nossa Senhora dos Remédios, Apartado 12, 2520-641 Peniche, Portugal **AQ2**

[2] CBQF—Centro de Biotecnologia e Química Fina, Escola Superior de Biotecnologia, Centro Regional do Porto da Universidade Católica Portuguesa, Rua Arquiteto Lobão Vital, 172, 4200-374 Porto, Portugal

Abstract

Microbial inactivation often follows a sigmoidal kinetic behaviour, with an initial lag phase, followed by a maximum inactivation rate period and tending to a final asymptotic value. Mathematically, such tendencies may be described by using primary kinetic models (Gompertz based model is one example) that describe microbial survival throughout processing time when stressing conditions are applied. The parameters of kinetic models are directly affected by temperature. Despite the number of mathematical equations used to describe the dependence of the kinetic parameters on temperature (so-called secondary models), there is a lack of studies regarding model comparison and adequacy in data fitting. This work provides a review of mathematical models that describe the temperature dependence of kinetic parameters related to microbial thermal inactivation. Regression analysis schemes and tests seeking model comparison are presented. A case study is included to provide guidance for the assessment of secondary model adequacy and regression analyses procedures. When modelling temperature effects on sigmoidal inactivation kinetics of microorganisms, one should be aware about the regression methodology applied. The most adequate models according to the two-step regression methodology may not be the best selection if a global fit is applied.

Keywords

Microbial inactivation
Maximum inactivation rate
Shoulder parameter
Temperature effects

Abbreviations

BF	Bias factor
CFU	Colony-forming unit
SHW	Standardised half width
SSE	Sum of squares of residuals
SST	Total sum of squares

Nomenclature

A_{Arr_i}	Parameter i of the Arrhenius model that relates L with T ($i = 1, 2$)
A_{Dav_i}	Parameter i of the Davey model that relates L with T ($i = 1, 2, 3$)
A_{Ratk_i}	Parameter i of the Ratkowsky modified model that relates L with T ($i = 1, 2$)
A_{WLF_i}	Parameter i of the Williams-Landel-Ferry model that relates L with T ($i = 1, 2, 3$)

A_{Hip_i}	Parameter *i* of the hyperbole model that relates *L* with T ($i = 1, 2, 3$)
C_{pel_i}	Parameter *i* of the Peleg model that relates $k_{\max}$ with *T* ($i = 1, 2, 3$)
C_{Ratk_i}	Parameter *i* of the Ratkowsky model that relates $k_{\max}$ with *T* ($i = 1, 2$)
C_{RatkM_i}	Parameter *i* of the Ratkowsky modified model that relates $k_{\max}$ with *T* ($i = 1, 2$)
C_{WLF_i}	Parameter *i* of the Williams-Landel-Ferry model that relates $k_{\max}$ with *T* ($i = 1, 2, 3$)
df	Degrees of freedom
D	Decimal reduction time (s; min)
e	Residual
E_a	Activation energy (J mol^{-1})
$f(x_i,\theta)$	Mathematical function with θ parameters, evaluated at x_i
F	*F* distribution
k	Inactivation rate (s^{-1}; min^{-1})
L	Lag or shoulder parameter (s; min)
n	Number of experimental points
n_r	Number of replicates
N	Microbial load (CFU mL^{-1})
p	Number of model parameters
R	Gas constant (8.314 J mol^{-1} K^{-1})
R^2	Coefficient of determination
t	Time (s; min)
T	Temperature (K; °C)
x	Independent variable
y	Dependent response variable; logarithm of microbial loads normalised in relation to initial values
z	Temperature change required to change *D* value by a factor of 10 (°C)
z'	Temperature change required to change *L* parameter by a factor of 10 (°C)

Greek Symbols

훼α	Significance level (%)
휀ε	Experimental error
ρ	Correlation coefficient between parameters estimates
$\boldsymbol{\theta}$	Vector of model parameters

Subscripts

adj	Adjusted value
aver	Average value
i	at observation i
inact	Inactivation
max	Maximum value
min	Minimum value
pred	Predicted by the model
pure	Related to pure error
r	at replicate r
ref	Reference value
res	Residual value
0	Initial value

Introduction

The bacterial spoilage of foods and the growth of pathogens are major industry concerns, because they directly affect consumers' health and safety. To provide an adequate margin of safety against food-borne pathogens, effective thermal treatments must be conveniently designed.

Predictive microbiology (i.e. the use of mathematical models to describe microbial kinetic behaviour) can be valuable at quantifying pathogen's survival within specific environmental conditions. It may also help to determine the extent to which existing thermal processes could be modified in order to attain major food quality retention. These predictions may be important in planning Hazard Analysis and Critical Control Points (HACCP) programs, since they can provide expected changes of microbial populations, when exposed to specific conditions.

According to Whiting and Buchanan (1994), mathematical models that describe the number of surviving microorganisms with time, under particular environmental and culture conditions (such as temperature, pH and water activity), are classified as primary models. The predictions of those models may be expressed in colony-forming units per millilitre, toxin formation, substrate levels, metabolic products (which are direct measures of the response) or absorbance or impedance (which are indirect measures of the response).

High temperature is the major factor that affects microorganisms' survival. The heat inactivation of microbial spores and the mortality of vegetative cells exposed to heat have been assumed to vary log-linearly with time. However, depending on microbial thermal resistance/microbial supporting media, and on the extent and severity of the thermal process, deviations from this linear behaviour may be observed. Thus, the survival curves are reflections of heat resistance distributions. An overestimation of the heat resistance may have a negative impact on foods' quality attributes (since an extended thermal process may degrade nutrients and organoleptic characteristics) and an underestimation increases the probability of microbial contamination (Peleg and Cole 1998).

Microbial inactivation behaviour can be generally characterised by the following three features (i.e. sigmoidal tendencies): an initial *shoulder* or *lag* phase (L), followed by a *maximum inactivation rate* ($k_{\max}$) period, tending to an asymptotic value of the microbial load (often referred to as *tail* or residual population, N_{res}). These features can be considered parameters of the kinetic primary models. Gompertz and Baranyi-based models are examples of expressions that are able to describe such tendencies (Gil et al. 2011; Char et al. 2009; Huang 2009; Miller et al. 2009a; Mataragas et al. 2008; Perni et al. 2006).

Gompertz equation can be considered a three-parameter model ($k_{\max}$, L and $\log(N_{\mathrm{res}}/N_0)$), which is able to describe a complete or incomplete sigmoidal behaviour (with/without shoulder or tailing effects):

$$y_{\mathrm{inact}}(t) = \log\left(\frac{N}{N_0}\right) = \log\left(\frac{N_{\mathrm{res}}}{N_0}\right)\ \exp\left(-\exp\left(-\frac{k_{\max}\ \exp(1)}{\log\left(\frac{N_{\mathrm{res}}}{N_0}\right)}(L-t)+1\right)\right)$$

where y_{inact} is the logarithm of microbial loads normalised in relation to initial value (N_0).

This work provides a revision of mathematical models that describe the temperature dependence of kinetic parameters related to microbial thermal inactivation. Regression analysis schemes and tests seeking model comparison are presented. A case study is included to provide guidance for the assessment of secondary model adequacy and regression analyses procedures.

Dependence of Kinetic Parameters on Temperature

The parameters of the kinetic primary models are directly affected by the temperature and by other environmental factors, such as pH and water activity. Mathematically, this dependence can be described by using the so-called secondary models.

Concerning microbial growth, this dependence has been extensively studied, particularly for maximum growth rate (Zwietering et al. 1991; McMeekin et al. 1993; Ross and McMeekin 1994; McDonald and Sun 1999; Ross and Dalgaard 2004). In relation to microbial inactivation, the works of Juneja et al. (2001), Peleg et al. (2002), Valdramidis et al. (2005a), Gil et al. (2006) and Van Asselt and Zwietering (2006) are examples in which secondary models were used. However, scarce information related to the initial shoulder (for inactivation) or lag (for growth) is available (Oscar 2002; Xiong et al. 1999; Zaika and Phillips 2005).

Despite the considerable number of mathematical equations used as secondary models, there is a lack of studies regarding model comparison and their adequacy in data fitting.

The Models for Maximum Inactivation Rate

The models commonly used to describe maximum inactivation rate dependence on temperature, in different contexts of study, are presented and discussed below. Those models were originally applied to provide an interpretation of microbial growth rates in response to temperature, but they can also be valuable functions for inactivation behaviour.

Arrhenius Equation

The dependence of $k_{\max}$ on temperature (T) can be expressed by an Arrhenius type equation or by its linearized forms (Laidler 1969; Schoolfield et al. 1981; Ratkowsky et al. 1982; Jay 1996; Zanoni et al. 1997; Peleg et al. 2002; Rudra et al. 2010; Zhang et al. 2010):

$$k_{\max}(T) = k_{\mathrm{ref}} \exp\left(-\frac{E_{\mathrm{a}}}{R}\left(\frac{1}{T} - \frac{1}{T_{\mathrm{ref}}}\right)\right) \qquad 2$$

where k_{ref} is the inactivation rate at a finite reference temperature (T_{ref}), E_{a} the activation energy and R the universal gas constant.

Several authors proclaimed that Arrhenius equation is clearly inadequate for microbiological systems (Mohr and Krawiec 1980; Ratkowsky et al. 1982). Furthermore, Peleg et al. (2002) highlighted that the equation gives equal weight to deviations at the low and high temperature regions, which could be limitative.

Ratkowsky Model and Modified Forms

Ratkowsky et al. (1982) proposed the following model:

$$k_{\max}(T) = \left[C_{\mathrm{Ratk}_1}\,(T - T_{\min})\right]^2 \qquad 3$$

where C_{Ratk_1} and $T_{\min}$ are model parameters; $T_{\min}$ is the theoretical minimum temperature for growth or inactivation.

However, according to Ratkowsky et al. (1983), this equation is not the most appropriate if high temperatures are considered.

Zwietering et al. (1991) proposed modifications in the Ratkowsky model aiming at describing bacterial growth rate throughout an entire temperature range (i.e. considering high temperatures):

$$k_{\max}(T) = \left[C_{\mathrm{RatkM}_1}\,(T - T_{\min})\right]^2 \left\{1 - \exp\left[C_{\mathrm{RatkM}_2}\,(T - T_{\max})\right]\right\} \qquad 4$$

where C_{RatkM_1} , C_{RatkM_2} , $T_{\min}$ and $T_{\max}$ are model parameters; $T_{\max}$ marks the end of the temperature range where growth is still possible. Any further rise of temperature will result in heat inactivation (Koseki et al. 2011; Velugoti et al. 2011).

Oscar (2002) confirmed that Eq. 3 describes satisfactorily *Salmonella typhimurium* growth rate and *Escherichia coli* growth rate in mechanically tenderised beef meat (Huang 2010). To avoid observed data heteroscedasticity, Alber and Schaffner (1992) and Gibson et al. (1988) suggested transformations of Eq. 3, by natural logarithm and square root.

Peleg Model

Peleg et al. (2002) proposed an alternative logarithmic-logistic model for the dependence of microbial inactivation rate on temperature:

$$k_{\max}(T) = \ln\left\{1 + \exp\left[C_{\mathrm{Pel}_1}\left(T - C_{\mathrm{Pel}_2}\right)\right]\right\}^{C_{\mathrm{Pel}_3}} \qquad 5$$

where C_{Pel_1} , C_{Pel_2} and C_{Pel_3} are model parameters (C_{Pel_2} is the marker of the temperature range where the changes accelerate (Corradini and Peleg 2003)).

This equation is purely empirical and circumvents the excessive weight given to the range of low temperatures. However, there is a lack of sensitivity when dealing with relatively small temperature changes.

Bigelow Model

Microbiologists often prefer to quantify the inactivation behaviour by definition of decimal reduction time, D value (Mafart 2000; Van Asselt and Zwietering 2006; Leguérinel et al. 2007; Miller et al. 2009a). The Bigelow model expresses de dependence of D value with temperature:

$$D = D_{\mathrm{ref}}\ 10^{\frac{T_{\mathrm{ref}} - T}{z}} \qquad 6$$

where D_{ref} is the decimal reduction time at reference temperature and z is the thermal resistance constant (i.e. the number of temperature degrees raise which leads to a tenfold reduction of D value).

In sigmoidal inactivation tendencies, $k_{\max}$ is proportional to the reciprocal of D (i.e. $k_{\max} = 1/D$):

$$k_{\max}(T) = k_{\mathrm{ref}} 10^{\frac{T - T_{\mathrm{ref}}}{z}} \qquad 7$$

If D_{ref} and z are integrated into the Bigelow model, predictions for the specific inactivation rate at a given temperature can be obtained. Valdramidis et al. (2005a) reported that the Bigelow model adequately described the inactivation rate of *E. coli*.

Williams-Landel-Ferry Model

Williams-Landel-Ferry (WLF) equation, firstly applied to the description of polymer viscosity as a function of temperature (William et al. 1955), can also be used to describe $k_{\max}$ temperature dependence:

$$\log(k_{\max}(T)) = -\frac{C_{\mathrm{WLF}_1}\left(T - C_{\mathrm{WLF}_3}\right)}{C_{\mathrm{WLF}_2} + (T - T_{\min})} \quad 8$$

Herein, C_{WLF_1}, C_{WLF_2}, $T_{\min}$ and C_{WLF_3} are model parameters.

Peleg et al. (2002) found that the formulation of WLF model as logarithmic relationships makes it particularly sensitive to rates at the lower limits of the temperatures tested.

The Models for Shoulder

Regarding the dependence of initial lag (for growth) or shoulder (for inactivation) on temperature, *L* (*T*), scarce information is available (concerning lag: Oscar 2002; concerning shoulder: Xiong et al. 1999; Zaika and Phillips 2005). The recognised high variability of this parameter is the main difficulty associated to modelling the temperature effect. This can be explained by diverse factors, such as the growth phase (i.e. exponential or stationary) of the selected microorganisms for the thermal inactivation studies or by the recover media used, which have been demonstrated to influence the initial delay of the microbial response (Miller et al. 2009a; Miller et al. 2009b).

Despite these limitations, secondary models for predicting lag time as a function of temperature have been proposed by some authors. The models commonly used to describe lag time/shoulder as a function of temperature, in different contexts of study, are presented and discussed below.

Arrhenius Type Equation

As a consequence of the exponential dependence of the shoulder parameter with temperature, an Arrhenius type equation can be considered (Gil et al. 2006):

$$L(T) = A_{\mathrm{Arr}_1} \exp\left(A_{\mathrm{Arr}_2} \left(\frac{1}{T} - \frac{1}{T_{\mathrm{ref}}} \right) \right) \quad 9$$

where A_{Arr_1} and A_{Arr_2} are model parameters.

However, Schaffner (1995) used the Arrhenius model to predict the lag time as a function of temperature for three psychrotrophic bacteria and found a high degree of parameter correlation.

Davey Model

Oscar (2002), based on a transformation of the Arrhenius model proposed by Davey (1991), used the following equation for predicting the potential lag time of *S. typhimurium* on cooked chicken:

$$L(T) = A_{\mathrm{Dav}_1} + \frac{A_{\mathrm{Dav}_2}}{T} + \frac{A_{\mathrm{Dav}_3}}{T^2} \quad 10$$

where A_{Dav_1} , A_{Dav_2} and A_{Dav_3} are model parameters.

The author concluded that the Davey model predicted well the lag time, when high temperatures were used.

Ratkowsky Based Models

The Ratkowsky model was originally applied to provide an interpretation of microbial growth rates in response to temperatures, but it can also be used for shoulder dependence on temperature (Miller et al. 2010, 2011):

$$L(T) = \left[A_{\mathrm{Ratk}_1} \left(T - A_{\mathrm{Ratk}_2} \right) \right]^2 \quad 11$$

Duh and Schaffner (1993) and Zwietering et al. (1991), inspired on Ratkowsky model, used the inverse of the previous function, as follows:

$$L(T) = 1/\left[A_{\mathrm{Ratk}_1} \left(T - A_{\mathrm{Ratk}_2} \right) \right]^2 \quad 12$$

where A_{Ratk_1} and A_{Ratk_2} are model parameters.

Bigelow Type Models

Miller et al. (2009a) applied a Bigelow type relation to predict shoulder as a function of temperature, for the sigmoidal thermal inactivation kinetics of *Listeria innocua* in broth:

$$L(T) = L_{\mathrm{ref}} \; 10^{\frac{T_{\mathrm{ref}} - T}{z'}} \tag{13}$$

where L_{ref} is the shoulder at a reference temperature and z' is the temperature required for a tenfold reduction of L.

Williams-Landel-Ferry Model

Schaffner (1995) applied this model to predict lag time as a function of temperature, for the growth behaviour of three psychotropic bacteria:

$$\log(L(T)) = \frac{A_{\mathrm{WLF}_1} \; \left(T - A_{\mathrm{WLF}_3}\right)}{A_{\mathrm{WLF}_2} + T - T_{\min}} \tag{14}$$

herein, A_{WLF_1} , A_{WLF_2} , $T_{\min}$ and A_{WLF_3} are parameters.

Hyperbole Models

A hyperbole model was suggested by Adair et al. (1989) and Gill et al. (1988):

$$L(T) = \frac{A_{\mathrm{Hip}_1}}{T - A_{\mathrm{Hip}_2}} \tag{15}$$

Zwietering et al. (1994) and Duh and Schaffner (1993) proposed alternative secondary models for bacterial growth, derived from the previous model:

$$L(T) = \exp\left[A_{\mathrm{Hip}_1} / \left(T - A_{\mathrm{Hip}_2}\right)\right] \tag{16}$$

$$L(T) = \left[A_{\mathrm{Hip}_1} / \left(T - A_{\mathrm{Hip}_2}\right)\right]^{A_{\mathrm{Hip}_3}} \tag{17}$$

where A_{Hip_1} , A_{Hip_2} and A_{Hip_3} are model parameters.

Oscar (2002) elected Eq. 15 as the best model to describe lag time dependence on temperature.

Data Analysis Methodology

Data analysis is an important stage of the research, involving multiple approaches and statistical procedures that contribute to descriptive and inductive (or inference) targets. While descriptive statistics are used to describe the basic features of the data in a study, inferential statistical analysis infers properties about a population: this includes testing hypotheses and deriving estimates. So, after experimental data have been gathered, the next step focuses on analyses procedures. Regression analysis schemes and tests seeking model comparison are crucial tools, as well as the assessment of the parameter estimation. These topics will be following reviewed.

Regression Analysis Procedures

Once a model is selected, data fitting is the subsequent procedure seeking parameter estimation. The fitting procedures do not depend on the complexity of the model assumed (Van Boekel 1996). As discussed by Bard (1974), model fitting requires (i) definition of a function which is a suitable measure of the deviation the experimental data and the model, usually referred as the *objective function*, and (ii) seeking the value (s) of the parameter (s) at which the objective function is minimum or maximum, as appropriate (this step is usually referred to as *optimisation* procedure).

Least-squares estimation is the most widely used regression procedure. This scheme consists on finding parameters’ values that minimise the sum of squares of the residuals (SSE):

$$\text{SSE} = \sum_{i=1}^{n} e_i^2 = \sum_{i=1}^{n} \left(y_i - f(x_i, \theta)\right)^2 \qquad 18$$

where e_i is the residual (or error), i.e. the deviation between the experimental point and the predicted value of the response variable at observation i; y_i is the experimental measured response at observation i (e.g. log of microbial load); $f(x_i, \boldsymbol{\theta})$ is the model prediction for y_i; and x_i is the independent variable at observation i and $\boldsymbol{\theta}$ is the parameter vector (e.g. shoulder and maximum inactivation rate). The total number of points is n.

Linear regression is a well-known procedure. A linear model in regression analysis designation is the one that is linear in the parameters (and not necessarily in the variables). This means that the first derivative (s) of the model with respect to the parameter (s) is (are) independent of the parameter (s) and, as a result, higher order derivative (s) are equal to zero.

The least-squares fitting is only appropriate when several assumptions are fulfilled (Walpole and Myers 1993): (i) the model function is the correct one, and it includes important variables and not irrelevant ones; (ii) the experimental error of the data is attributed only to the response variable y_i, which implies that the values observed for x_i do not have any associated error; and (iii) the experimental errors (ε_i) in the dependent variable are normally distributed, their average is zero and their variance is constant (homoscedastic errors), which are uncorrelated and there is no systematic error in data. If the analysis of the residuals e_i reproduces these assumptions, it may be concluded that the model is appropriate to fit experimental data. To evaluate the ability of each model to describe the data without violating these assumptions, the examination of the residuals' distribution and the plotting of the residuals, in time sequence or against the fitted values of the response variable, are valuable tools. This aspect will be later discussed.

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In a large number of situations, microbial kinetics requires non-linear models in data fitting. In such situations, no exact solutions are available for parameter estimation, because the derivatives depend on the parameters themselves. A solution must be found by linear approximation. Hence, the non-linear least-squares procedure, aiming at minimising SSE, cannot be solved straightforward. Instead, the problem must be solved iteratively, requiring initial estimations. Numerical procedures commonly used for function minimisation can be found in Johnson and Frasier (1985): Nelder-Mead (simplex concept), Newton-

Raphson, Gauss-Newton, Steepest Descendent and Marquardt-Levenberg.

Often, the kinetic parameter dependence on temperature (or any other environmental factor) is obtained in two steps. In a first step, kinetic data is gathered at fixed temperatures within a range of interest and kinetic parameters are estimated by fitting the convenient (primary) model to data. In a second step, the kinetic parameter dependence on temperature is obtained. This procedure often leads to large confidence intervals of the model parameters, particularly if a small number of temperatures are considered (Lund 1983). To overcome this drawback, Arabshahi and Lund (1985) proposed a one-step method, or global fit, for analysing kinetic data. In such approach, one single fit of the isothermal kinetic data is performed by using a mathematical model that encompasses the temperature effect (this meaning that primary and secondary models are merged). This leads to unbiased and more precise estimations, resulting in narrow confidence intervals of the parameter estimates. There is a more effective utilisation of raw data, circumventing the estimation of unnecessary parameters (Haralampu et al. 1985; Cohen and Saguy 1985; Membré et al. 1997; Skandamis et al. 2002; Brandão 2004; Valdramidis et al. 2005b).

Although the application of one-step method is recommended, mainly when the confidence intervals of the kinetic parameters are statistically meaningless, the one-step and the two-step methods may lead to different results concerning the selection of the secondary models involved. This means that, if a two-step methodology is chosen, the primary and secondary models assumed (based on statistical ability to fit the experimental data) may differ from the ones selected if the one-step approach is applied (one single global model obtained by primary and secondary models conjoint). In the case of sigmoidal inactivation kinetics, since both the shoulder and maximum inactivation rate parameters are temperature dependent, two secondary models should be considered. If a two-step regression approach is chosen, the more convenient secondary models of each kinetic parameter are selected according to the separate regression analysis performed (i.e. analysis of the residuals, goodness of fit and precision of the parameter estimates). However, if a one-step approach is chosen, the interaction of both secondary models in the single global one directly affects the fitting ability. In such situation, since different regression approaches (one step and two steps) could lead to different secondary model selection, both approaches should be carefully studied. These considerations are not conveniently studied by the modellers and the global regression analysis is often applied without any particular devotion to these unexpected situations.

Assessment of Model Adequacy

An accurate regression analysis, as well as an appropriate choice of experimental conditions, may contribute to improved parameter estimation. However, statistical analyses to estimate the unknown parameters of a model are not completed if the parameters are not evaluated in terms of reliability and precision and if goodness-of-fit is not assessed.

Although a considerable effort has been given to the development of alternative models to describe microbial inactivation, there is a lack of dedication to the assessment of the estimation. Measures of the performance and reliability of mathematical models will be following presented.

Analysis of the Residuals

The quality of the regression can be assessed by a convenient analysis of the residuals (i.e. difference between the observed response variable and the value predicted by the model). Residuals should be independent, random and normally distributed (with means equal to zero and constant variance). If these assumptions are not violated, the fit of the model to data is adequate. The diagnostic plotting of the residuals may be helpful in the detection of a proper model form.

Randomness can be evaluated by the runs test, which allows analysing the way experimental points are distributed along the model curve (Walpole and Myers 1993; Van Boekel 1996; Brandão 2004). The probability value (*P* value) of the runs test indicates the chance of obtaining fewer runs than observed in the experiment. *P* values lower than 0.5 indicate lack of fit (with 5% significance level) as the runs observed are fewer than expected, indicating clustering of residuals with the same sign or systematic bias (Motulsky and Ransnas 1987).

Residual normality can be assessed by applying Kolmogorov-Smirnov test (Neter et al. 1996; Guimarães and Cabral 1997).

In some cases, the residuals have a tendency in the pattern distribution (heteroscedasticity), which reveals inconstant variance of the errors. To evaluate the ability of each model to describe the data without showing this behaviour, the bias factor (BF) should also be calculated.

The BF of the model predictions can be evaluated by calculating the antilogarithm of the average of the logarithm of the ratio between the predicted values by the model ($y_{i\text{pred}}$) and the observed ones (y_i) (Ross 1996):

$$\text{BF} = 10^{\sum_{i=1}^{n} \log\left(y_{i\text{pred}}/y_i\right)/n} \qquad 19$$

If the BF is equal to 1, a perfect agreement between predictions and observed values occurs (perfect model fit). If lower or higher values than 1 are obtained, this is suggestive of systematic under or over model estimation, respectively.

Goodness-of-Fit

Goodness-of-fit can be evaluated using the adjusted coefficient of determination (R^2_{adj}), lack of fit and F test (for comparison of two fits to one data set).

The quality of a fit is commonly confined to the calculation of the coefficient of determination, R^2. However, if the comparison between models with different number of parameters is the final goal, the coefficient of determination adjusted, R^2_{adj} (adjusted R^2 by dividing both the numerator and denominator by their respective degrees of freedom; Neter et al. 1996), should be used:

$$R^2_{\text{adj}} = 1 - \left(\frac{n-1}{n-p}\right)\frac{\text{SSE}}{\text{SST}} \qquad 20$$

In this, n is the number of experimental observations, p the number of model parameters, SSE the sum of squares of the residuals and SST the total sum of squares (of regression and residuals; $\mathrm{SST} = \sum_{i=1}^{n} \left(y_i - \overline{y}\right)^2$, being y_{aver} the average value of the response). The values of R^2_{adj} vary between 0 and 1, meaning the proportion of the total variation is explained by the model.

The concept of lack of fit can also be used to compare models, being this extremely important in regression analysis procedures. If the model is appropriate to describe the data, the residuals represent only experimental error. If the model is not adequate, a lack of fit is observed and an additional variation, besides a pure error from repeated observations, occurs (Walpole and Myers 1993; Neter et al. 1996):

$$\mathrm{SSE}_{\mathrm{lack\ \ of\ \ fit}} = \mathrm{SSE} - \mathrm{SSE}_{\mathrm{pure}} = \sum_{i=1}^{n} \left(y_i - y_{i_{\mathrm{pred}}}\right)^2 - \sum_{r=1}^{n_r} \left(y_{ri} - y_{r_{\mathrm{aver}}}\right)^2 \tag{21}$$

where y_{raver} is the average value of the replicates for a given x_i and n_r the number of replicates.

If $\mathrm{SSE}_{\mathrm{lack\ of\ fit}}$ is much smaller than $\mathrm{SSE}_{\mathrm{pure}}$, this means that the sample variation around the regression line is a pure error resulting from the variation that occurs among repeated observations; thus, the model is adequate. Otherwise, the model is not adequate.

This can be quantified at an α level of significance, comparing the critical F value ($F_{\alpha, df_{SSE} - df_{SSEpure}, df_{SSEpure}}$) with a calculated F value (Zwietering et al. 1991; Walpole and Myers 1993; Neter et al. 1996):

$$F_{\mathrm{calculated}} = \frac{\left(\mathrm{SSE} - \mathrm{SSE}_{\mathrm{pure}}\right) / \left(df_{\mathrm{SSE}} - df_{\mathrm{SSE}_{\mathrm{pure}}}\right)}{\mathrm{SSE}_{\mathrm{pure}} / df_{\mathrm{SSE}_{\mathrm{pure}}}} \tag{22}$$

where df_{SSE} are the degrees of freedom of the residuals ($df_{\mathrm{SSE}} = n - p$) and df_{SSEpure} the degrees of freedom of pure error (i.e. total number of points minus the number of different x_i values). The model is considered adequate, if $F_{\mathrm{calculated}}$ is lower than $F_{\alpha, df_{SSE} - df_{SSEpure}, df_{SSEpure}}$.

The F statistics can also be used with the purpose of comparing the quality of the fit of two different models (with different number of parameters) to the same data set (Motulsky and Ransnas 1987):

$$F_{\text{calculated}} = \frac{(\text{SSE}_1 - \text{SSE}_2)/\left(df_1 - df_2\right)}{\text{SSE}_2/df_2} \tag{23}$$

The subscripts 1 and 2 refer to model fits with fewer and higher number of parameters, respectively. The value of $F_{\text{calculated}}$ should be compared to a critical F value for a given ~~훠~~α level of significance ($\boxed{F_{\alpha,df_1-df_2,df_2}}$).

An $F_{\text{calculated}}$ superior to the critical percentile of the F distribution indicates that the model with more parameters fits the data significantly better than the model with fewer. Otherwise, the model with fewer parameters should be elected.

When the models have equal number of parameters, the following F statistics can be used instead of the previous equation (Motulsky and Ransnas 1987):

$$F = \frac{\text{SSE}_1}{\text{SSE}_2} \tag{24}$$

The model with lower SSE is most likely to be correct.

Precision of the Parameter Estimates

Precision of the parameter estimates can be evaluated by the standardised half width at 95%, SHW (i.e. halved confidence interval at 95%, divided by the estimate $\boxed{\equiv \frac{\text{confidence interval}_{95\%}}{2} \times \frac{1}{\mathit{estimate}} \times 100}\Big)$.

However, when a model has more than one parameter, the confidence interval of each parameter does not provide full statistical information due to correlations between parameter estimates. In such cases, a better interpretation of precision can be based on the contours of the parameters' joint confidence regions at (100–2~~훠~~α)%. The joint confidence contour can be calculated by (Box et al. 1978; Bates and Watts 1988; Seber and Wild 1989):

$$\text{SSE}_{(1-2\alpha)\%} = \text{SSE}_{\min}\left(1 + \frac{p}{n-p} F_{2\alpha\%(p,n\ \ -p)}\right) \tag{25}$$

where SSE (100–2~~훼~~α)% is the sum of squares of residuals of a contour region of (100–2~~훼~~α)% confidence level and F is the (2~~훼~~α)% significance point of the F distribution with p and $n - p$ degrees of freedom.

A significance level of ~~훼~~ α = 5% is commonly used, thus giving joint confidence regions at 90%.

Case Study Using Experimental Data from *L. innocua* Inactivation in Broth

A case study is presented to provide guidance for the assessment of secondary model adequacy and regression analyses procedures. Experimental data of thermal inactivation of *L. innocua* in broth was considered within the temperature range of 52.5 and 65.0 °C (Miller et al. 2009a). Three replicates at each temperature were considered.

The Gompertz based model was used to describe microbial inactivation (Eq. 1) with complete or incomplete sigmoidal behaviour (with/without shoulder or tailing effects). Depending on the environmental stressing conditions (i.e. temperature was the one studied in this work), the three mentioned tendencies may (or may not) occur. In relation to the *tail* (residual survival level), researchers still do not agree whether the phenomenon is simply an experimental drawback in cell enumeration (i.e. the tails occur at low cell concentration, often around the detection level threshold) or is actually a resistant residual population in relation to the stressing condition applied. In the temperature range of this study, the tail was not evident, which is bared by the considerable low values of log (N_{res}/N_0) estimates (no horizontal asymptote).

Some of the secondary models previously discussed were used to describe maximum inactivation rate and shoulder dependence on temperature. In this approach, log (N_{res}/N_0) was assumed to be independent of temperature, which was proven by ANOVA procedures.

The reference temperature (Eqs. 2, 7 and 9) was assumed to be 331.9 K (i.e. middle value of the range 52.5–65.0 °C), as parameter estimation is improved (Cohen et al. 1994).

Regression analyses were performed using STATISTICA 6.0 (StatSoft, Inc. 2001, Tulsa, OK, USA) software, applying the Levenberg-Marquardt method to minimise the sum of squares of residuals.

The selection of the proposed secondary models was statistically evaluated taking into consideration analysis of the residuals, goodness-of-fit and precision of the parameter estimates.

Two-Step Methodology (Non-Global Fit)

~~nactivation~~Inactivation data were analysed according to a *two-step* procedure. In the first step, the Gompertz model (Eq. 1) was fitted to experimental data and kinetic parameters, k_{max} and L, were estimated (Table 1). Model adequacy in data fitting was proven for all temperatures tested (randomness and normality of the residuals were verified; R^2_{adj} varied between 0.88 and 0.99). Examples of model fitting can be seen in Fig. 1.

Table 1

Estimated kinetic parameters of the Gompertz model and R^2_{adj} values (Miller et al. 2

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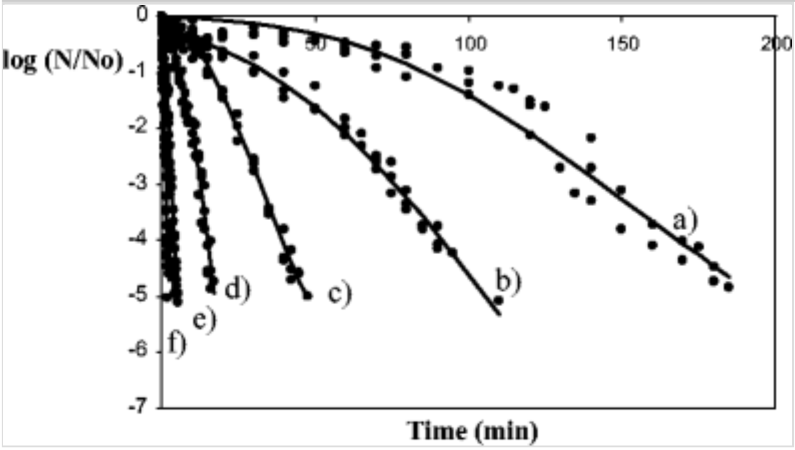
T (°C)	Replicate	$\log\left(\frac{N_{res}}{N_0}\right)$	SHW$_{95\%}$ (%)	k_{max} (min^{-1})	SHW$_{95\%}$ (%)	*L* (min)	SHW$_{95}$ (%)
52.5	1	−16.6	92.3	0.05	47.0	82.7	53.1
	2	−13.6	285.0	0.05	164.4	87.6	132.5
	3	−11.3	80.9	0.04	31.7	80.3	35.1
55.0	1	−16.1	99.4	0.08	58.9	40.0	77.9
	2	−8.4	72.5	0.05	29.0	11.4	161.2
	3	−9.2	20.3	0.06	7.8	21.5	23.5
57.5	1	−8.9	36.1	0.14	9.6	9.2	31.3
	2	−9.5	22.3	0.14	6.5	12.3	15.5
	3	−9.3	28.2	0.16	8.6	13.0	16.3
60.0	1	−10.1	42.4	0.42	13.0	4.50	26.1
	2	−14.6	4.3	0.39	3.7	5.74	9.3
	3	−16.6	97.1	0.50	46.5	7.09	61.3
62.5	1	−5.6	20.5	1.22	13.0	0.7	34.4

T (°C)	Replicate	$\log\left(\frac{N_{res}}{N_0}\right)$	SHW$_{95\%}$ (%)	k_{max} (min^{-1})	SHW$_{95\%}$ (%)	*L* (min)	SHW$_{95}$ (%)
	2	−9.4	38.8	1.19	9.0	0.9	39.2
	3	−8.4	59.6	1.10	13.3	0.6	78.0
65.0	1	−5.7	18.7	2.30	8.7	0.01	834.4
	2	−7.5	44.8	2.17	10.7	0.09	181.4
	3	−10.1	89.0	2.57	24.6	0.27	143.2

Fig. 1

Results from fitting the Gompertz model to *L. innocua* experimental data obtained by Miller et al. (2009a) (black circle). Two-step method. **a** 52.5 °C. **b** 55.0 °C. **c** 57.5 °C. **d** 60.0 °C. **e** 62.5 °C. **f** 65.0 °C

AQ5



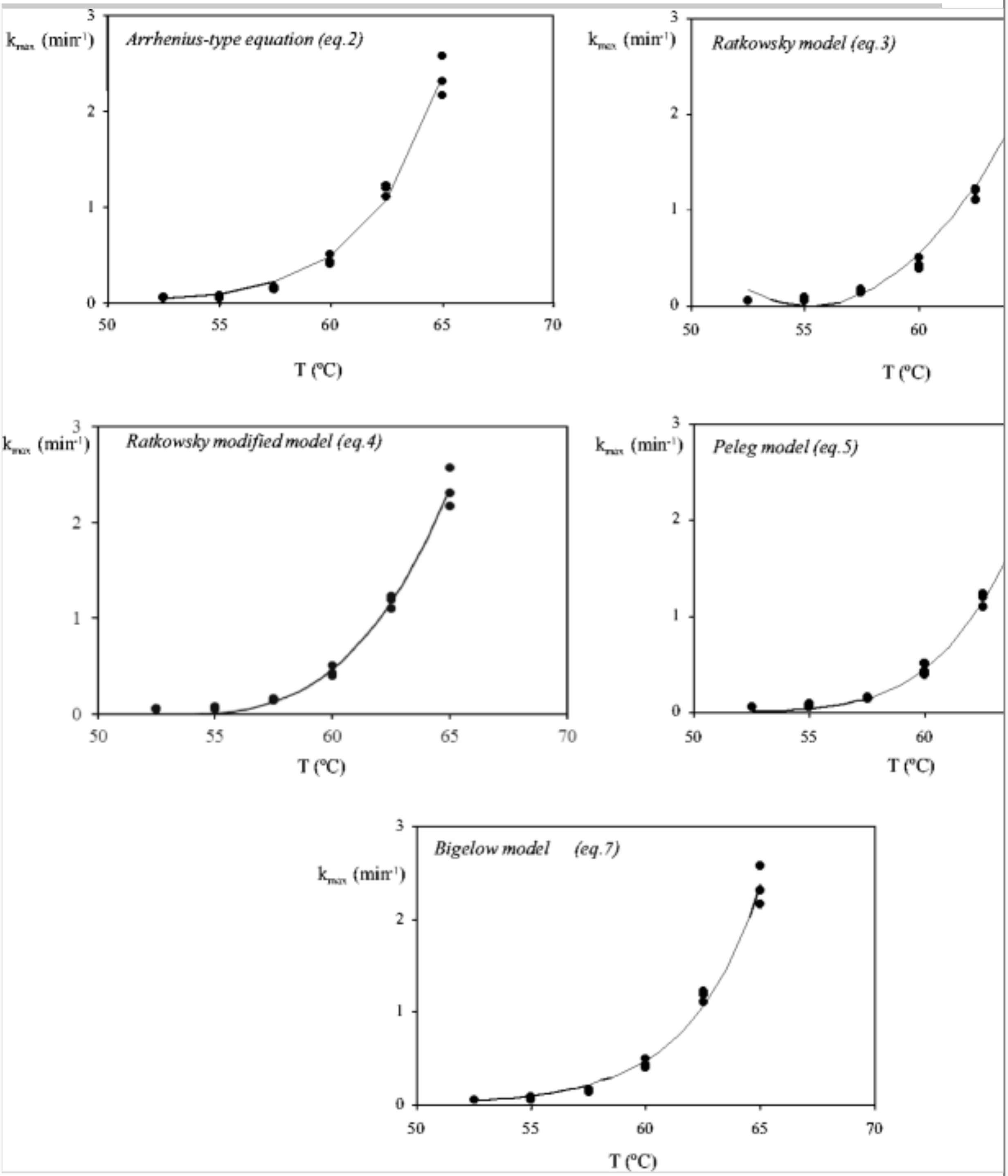
In the second step, the models following presented were used to describe the dependence of k_{max} and *L* on temperature.

Assessment of Models for k_{max} Dependence on Temperature

Five secondary models were used to describe the influence of temperature on k_{max} (Eqs. 2, 3, 4, 5 and 7). Data fits are in Fig. 2. One can observe that all fits are quite similar; however, Ratkowsky and Ratkowsky modified models compromise predictions at low temperatures. At temperatures below 55 °C (corresponding to T_{min} parameter in the equations), an overestimation is obtained for k_{max} when Ratkowsky model (Eq. 3) was selected. Concerning the Ratkowsky modified model (Eq. 4), an underestimation occurs and meaningless negative values of $k_{\max}$ are predicted.

Fig. 2

Dependence of the maximum inactivation rate on temperature (the lines represent models fits to *L. innocua* experimental data obtained by Miller et al. 2009a, black circle)



Estimates of the parameters and related precision, evaluated by the ratio of the standard error at 95% to the estimate ($\mathrm{SHW}_{95\%}$), are shown in Table 2. Concerning parameters’ precision, worse results were obtained with Ratkowsky modified model (considerable high $\mathrm{SHW}_{95\%}$ obtained). For the remaining tested models, precision of the estimates varied between 1.2% (for Ratkowsky model) and 86.2% (for Peleg model).

Table 2

Parameter estimates of the secondary models for k_{max} and L, precision assessed by the standardised half width at 95%

Kinetic parameter	**Secondary model**	**Parameter of the secondary model**	**Estimate**	**$\mathrm{SHW}_{95\%}$ (%)**
k_{max} (min^{-1})	Arrhenius (Eq. 2)	k_{ref} (min^{-1}) E_a ($\mathrm{J\ mol}^{-1}$)	3.23×10^{-1} 2.98×10^{5}	18.6 10.0
	Ratkowsky model (Eq. 3)	C_{Ratk_1} ($°\mathrm{C}^{-1}\ \mathrm{min}^{-0.5}$) T_{min} (°C)	1.53×10^{-1} 5.52×10^{1}	7.9 1.2
	Ratkowsky modified model (Eq. 4)	C_{RatkM_1} ($°\mathrm{C}^{-1}\ \mathrm{min}^{-0.5}$) T_{min} (°C) C_{RatkM_2} ($°\mathrm{C}^{-1}$) T_{max} (°C)	5.20×10^{-1} 5.31×10^{1} -5.27×10^{-3} 5.31×10^{1}	4.0×10^{3} 2.6×10^{3} 7.8×10^{3} 5.3×10^{3}
	Peleg model (Eq. 5)	C_{Pel_1} ($°\mathrm{C}^{-1}$) C_{Pel_2} (°C) C_{Pel_3}	4.41×10^{-1} 6.08×10^{1} 1.24×10^{0}	86.2 1.6 76.2
	Bigelow model (Eq. 7)	k_{ref} (min) z (°C)	7.08×10^{0} 7.24×10^{0}	18.7 10.2
L (min)	Arrhenius (Eq. 9)	A_{Arr_1} (min) A_{Arr_2} (K^{-1})	5.11×10^{0} 4.82×10^{4}	52.4 19.5
	Davey model (Eq. 10)	A_{Dav_1} (min) A_{Dav_2} (min °C) A_{Dav_3} (min $°\mathrm{C}^2$)	2.68×10^{3} -3.30×10^{5} 1.02×10^{7}	36.6 2.2×10^{3} 32.7
	Ratkowsky model (Eq. 11)	A_{Ratk_1} ($°\mathrm{C}^{-1}\ \mathrm{min}^{0.5}$) A_{Ratk_2} (°C)	9.17×10^{-1} 6.19×10^{1}	18.2 2.3
	Inverse of Ratkowsky model (Eq. 12)	A_{Ratk_1} ($°\mathrm{C}^{-1}\ \mathrm{min}^{-0.5}$) A_{Ratk_2} (°C)	3.93×10^{-2} 4.97×10^{1}	24.7 1.4
	WLF (Eq. 14)	A_{WLF_1} (min) A_{WLF_3} (°C)	2.14×10^{5} 6.23×10^{1}	7.9×10^{6} 11.4

Kinetic parameter	Secondary model	Parameter of the secondary model	Estimate	SHW$_{95\%}$ (%)
		A_{WLF_2} (°C)	-1.09×10^6	7.9×10^6
	Hyperbola (Eq. 15)	A_{Hip_1} (min) A_{Hip_2} (°C)	6.55×10^1 5.17×10^1	38.6 0.6
	Exponential of hyperbola (Eq. 16)	A_{Hip_1} (min) A_{Hip_2} (°C)	2.60×10^1 4.66×10^1	28.7 3.7
	Modified hyperbola (Eq. 17)	A_{Hip_1} (min) A_{Hip_2} (°C) A_{Hip_3}	2.15×10^1 4.55×10^1 3.95×10^0	31.8 39.2 205.1

Statistical indicators for secondary model selection are shown in Table 3. The analysis of the residuals showed that randomness was verified in all studied models (proved by runs test results; significance level of 3%). The BF values reflected that best fits were obtained with Arrhenius and Bigelow models (BF values closest to 1). Ratkowsky model was the worst case, because no BF was calculated to Ratkowsky modified model (since meaningless $k_{\mathrm{max}} < 0$ values were predicted by the model). Residual normality was verified in all situations (results from Kolmogorov-Smirnov test).

Table 3

Statistical indicators for secondary models selection (*two-step* methodology): analysis of the residuals (BF, randomness and normality) and goodness-of-fit (R^2_{adj} and lack-of-fit)

Kinetic parameter	Secondary model	Residuals			Goodness-of-fit	
		BF	Randomness	Normality	R^2_{adj}	Lack-of-fit
k_{max} (min^{-1})	Arrhenius	1.12	Yes	Yes	0.99	No
	Ratkowsky model	0.60	Yes	Yes	0.98	Yes

[a] Not calculated, since meaningless $k_{\mathrm{max}} < 0$ values were predicted by the model

[b] Not calculated, since meaningless $L < 0$ values were predicted by the model

Kinetic parameter	Secondary model	Residuals			Goodness-of-fit	
		BF	Randomness	Normality	R^2_{adj}	Lack-of-fit
	Ratkowsky modified model	–[a]	Yes	Yes	0.99	No
	Peleg model	0.72	Yes	Yes	0.99	No
	Bigelow model	1.15	Yes	Yes	0.99	No
	Arrhenius	1.26	Yes	Yes	0.96	No
	Davey model	–[b]	No	Yes	0.93	Yes
	Ratkowsky model	2.05	No	Yes	0.88	Yes
L (min)	Inverse of Ratkowsky model	2.54	Yes	Yes	0.97	No
	WLF	1.20	Yes	Yes	0.96	No
	Hyperbola	3.08	Yes	Yes	0.95	No
	Exponential of hyperbola	2.89	Yes	Yes	0.96	No
	Modified hyperbola	2.03	Yes	Yes	0.97	No

[a] Not calculated, since meaningless $k_{max} < 0$ values were predicted by the model

[b] Not calculated, since meaningless $L < 0$ values were predicted by the model

R^2_{adj} values were high (≥ 0.98) in all cases, which means that, in the worst cases, the model explained 98% of the total variation.

The concept of lack of fit was also used for model assessment. Only the Ratkowsky model revealed a significant amount of variation due to lack of fit, which means that there is a large portion of the variation around the regression line in addition to the pure error.

Based on the criteria mentioned above, the Arrhenius equation (Eq. 2) and the Bigelow (Eq. 7) and Peleg (Eq. 5) models were the best secondary models regarding the description of the temperature dependence of k_{max} of *L. innocua*: highest R^2_{adj}, the lowest prediction bias and no lack of fit (residuals represent only experimental error).

Pair-wise comparisons of these three models (in terms of fitting the same data set) were done by applying an *F* test. Pair-wise difference in the *SSE* between Arrhenius equation and Bigelow model was not significant to elect the best one in terms of goodness-of-fit. Therefore, when comparing the latest ones with Peleg model, an *F* value superior to the critical percentile of the *F* distribution was obtained in both cases, indicating that the model with more parameters (Peleg model) fits the data significantly better than the simpler model. Thus, the selected model (between the Arrhenius equation, the Bigelow and Peleg model) based on the goodness-of-fit and residual analysis was the Peleg model.

Besides, Peleg model has more parameters (three parameters) than the other models (two parameters), the number of experimental data points considered (18 points) did not compromise the regression procedure and the precision of the estimates already discussed. Better fitting is expected when models with more parameters are used. However, it is better to use less complex models with less number of parameters.

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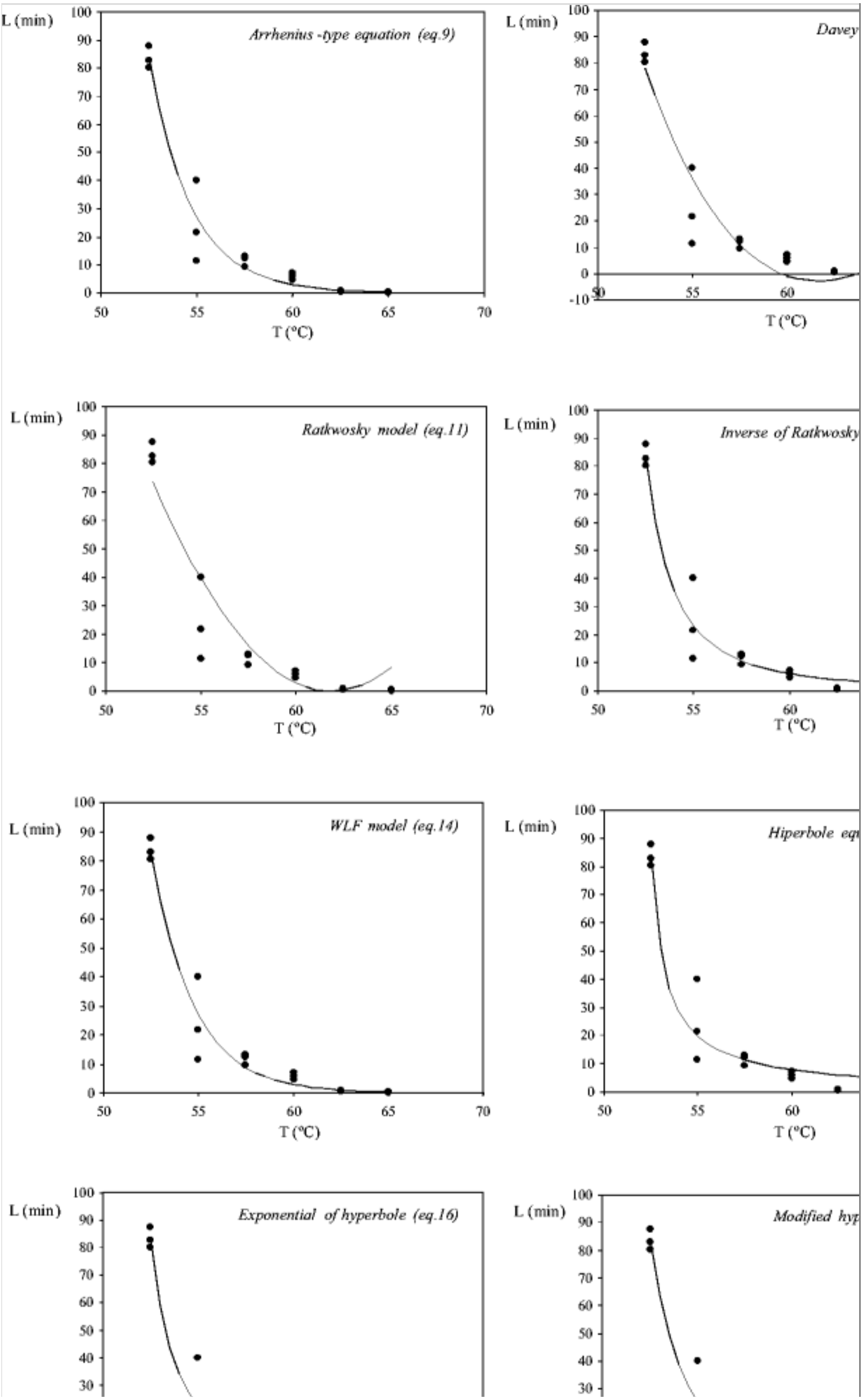
Assessment of Models for *L* Dependence on Temperature

Eight secondary models were used to describe the influence of temperature on shoulder (Eqs. 9, 10, 11, 12, 14, 15, 16 and 17). Data fits are in Fig. 3.

Fig. 3

Dependence of shoulder on temperature (the lines represent models fits to *L. innocua* experimental data obtained by Miller et al. 2009a, black circle)


L (min)
Arrhenius -type equation (eq.9)
T (°C)
Davey
Ratkwosky model (eq.11)
Inverse of Ratkwosky
WLF model (eq.14)
Hiperbole eq
Exponential of hyperbole (eq.16)
Modified hyp
0
10
20
30
40
50
60
70
80
90
100
-10
50
55
60
65
70

One can easily observed that an under estimation is obtained when Davey model (Eq. 10) was fitted to experimental data, leading to negative prediction of shoulder parameter (which is biologically improbable). Consequently, this model was not considered.

At the highest temperature values, an overestimation of L occurred when Ratkowsky model (Eq. 11) was considered. The hyperbola equation (Eq. 15) and the exponential of the hyperbola equation (Eq. 16) did not properly describe the shoulder parameter at high temperatures. This fact can be explained by the non-hyperbolic behaviour of shoulder with temperature, contrarily to the results reported by Adair et al. (1989) and Gill et al. (1988).

Parameter estimates and related precision are in Table 2. Results of the assessment of model adequacy are in Table 3. The analysis of the residuals showed that randomness was not verified when the Ratkowsky model (Eq. 11) was considered, which was proved by runs test results (significance level of 3%). Consequently, this model was excluded.

The bias factor values reflected that best fits were obtained with Arrhenius and WLF models (BF values closest to 1). All the other studied models showed systematic bias. Therefore, hyperbola (Eq. 15), exponential of hyperbola (Eq. 16), modified hyperbola (Eq. 17) and inverse of Ratkowsky (Eq. 12) models were excluded, besides the proven residual randomness and normality and considerable high R^2_{adj} values. In relation to modified hyperbole model, Oscar (2002) arrived at the conclusion that the exponent parameter (A_{Hip_3}) should vary between 1 and 2, in order to decrease prediction bias. In our case, this parameter was 4.

Also for Arrhenius and WLF models, residual randomness and normality were verified. The R^2_{adj} values were quite similar and satisfactorily high (0.96). Consequently, it can be concluded that both models are statistically adequate. However, results from the F test pair-wise comparisons allowed concluding that Arrhenius was the most adequate.

Concerning parameters' precision, evaluated by SHW of the estimates at 95%, satisfactory results were obtained for all models. Exception was observed for the WLF, which showed the largest confidence intervals.

In conclusion, the best model for describing the shoulder dependence on temperature was the Arrhenius-type equation, which satisfied all statistical criteria assumed.

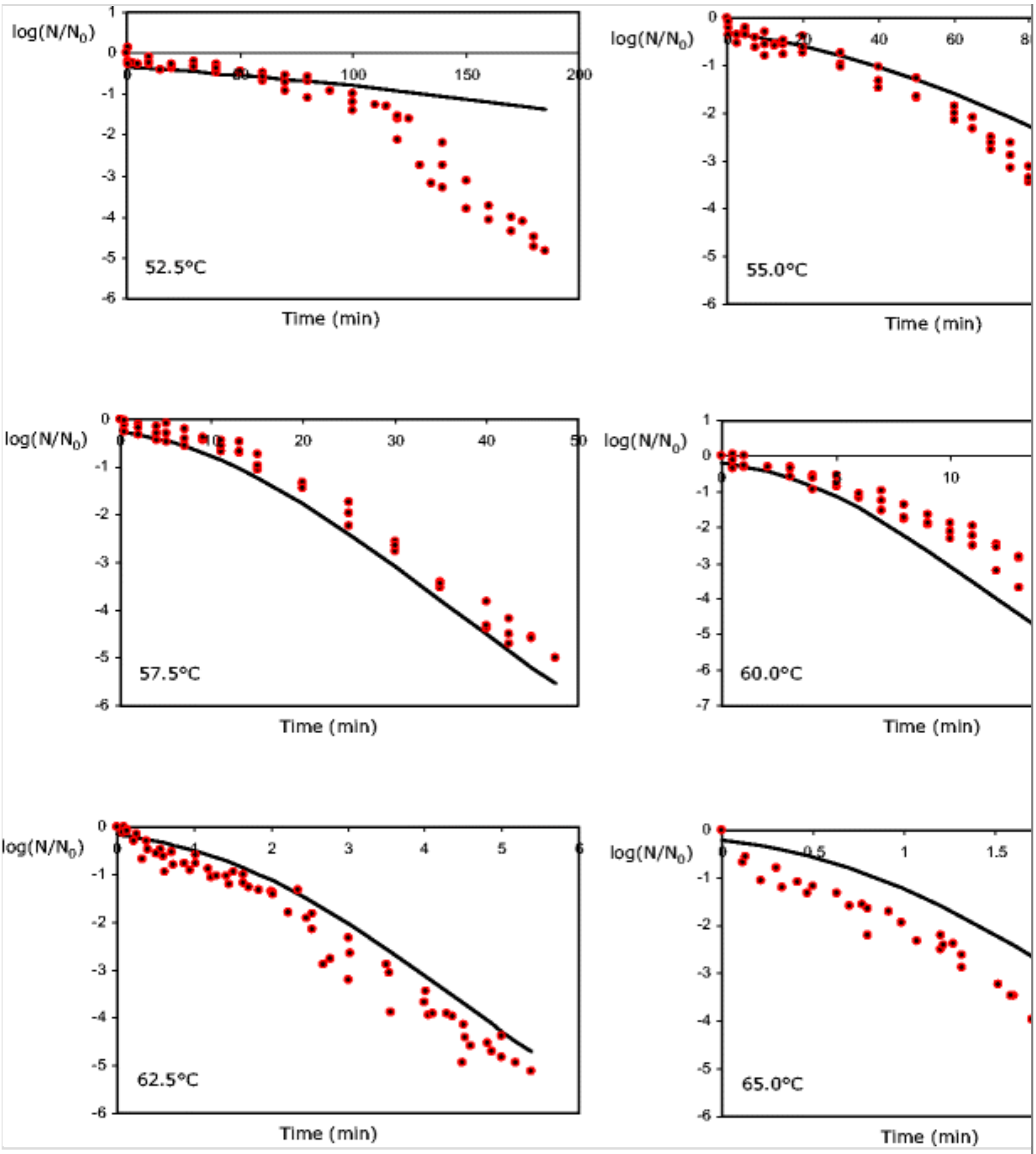
Predictions of the Inactivation of *L. innocua*

Based on the criteria for model selection, the most appropriate models to describe temperature dependence of k_{max} and L were Peleg model (Eq. 5) and Arrhenius equation (Eq. 9), respectively. In order to validate this selection, inactivation of *L. innocua* was predicted by including these models into the Gompertz equation (Eq. 1). The values of the parameters used in the prediction were $\log(N_{res}/N_0) = -10$, $C_{\mathrm{Pe1}_1} = 0.441\ ^\circ\mathrm{C}^{-1}$, $C_{\mathrm{Pe1}_2} = 60.82\ ^\circ\mathrm{C}$, $C_{\mathrm{Pe1}_3} = 1.24$, $A_{\mathrm{Arr}_1} = 5.11$ min and $A_{\mathrm{Arr}_2} = 48{,}200\ \mathrm{K}^{-1}$ (parameters estimated in a *two-step* methodology). Results are in Fig. 4. It can be observed that model predictions deviated considerably from the experimental behaviour. This can be explained by the poor estimation capacity at low temperatures of the Peleg model. This model is clearly inadequate to account for changes that occur at relatively low temperatures, due to lack of sensitivity. Peleg et al. (2002) also arrived at a similar conclusion. Confronting the Arrhenius predictions for the shoulder time, a smooth underestimation is observed for some temperatures (i.e. 57.5 and 60.0 °C). Despite precision of parameter had been attained, accuracy was sacrificed, which directly affected the predictive ability of the model.

Fig. 4

Predictions of *L. innocua* inactivation, including the secondary equations (Peleg model, Eq. 5, and Arrhenius equation, Eq. 9) into the Gompertz equation (the dots represent *L. innocua* experimental data obtained by Miller et al. 2009a)

AQ7



Results showed that one should be aware if a combined model is used to predict microbial inactivation, on the basis of commonly applied secondary model parameters. Although the temperature effects on $k_{\max}$ and L were well described by a Peleg model and Arrhenius equation, respectively, the prediction of *L. innocua* inactivation may not be adequate if a *two-step* methodology is applied.

One-Step Methodology (Global Fit)

Combinations of the five models considered for $k_{\max}$ and the eight models considered for shoulder were merged with the Gompertz model. A total of

40 regressions were therefore performed, fitting the global model to experimental data obtained at all temperatures (also all replicates together). Thirty-six possible combinations of the secondary models were straight away excluded on the basis of residual analysis and goodness-of-fit (results not showed). Thus, only four possible combinations were studied: (1) $k_{\text{max-Arrhenius}} - L_{\text{Ratkowsky}}$, (2) $k_{\text{max-Bigelow}} - L_{\text{Ratkowsky}}$, (3) $k_{\text{max-Peleg}} - L_{\text{Arrhenius}}$ and (4) $k_{\text{max-Peleg}} - L_{\text{Ratkowsky}}$. Global fits of Gompertz model including these selected combinations are in Fig. 5. The statistical assessments of global model adequacy are in Table 4. Although the global regressions were performed in one-step procedure, the statistical assessment was carried out for each temperature aiming at a better understanding of model predictions. If assessment had been considered on the basis of the global regressions, large underestimations would balance with large overestimations, which would compromise the evaluation.

Fig. 5

One-step regression of experimental data of *L. innocua* at 52.5, 55.0 and 57.5 °C (top plot) and 60.0, 62.5 and 65.0 °C (bottom plot) using (1) $k_{\text{max-Arrhenius}} - L_{\text{Ratkowsky}}$ (short dotted line), (2) $k_{\text{max-Bigelow}} - L_{\text{Ratkowsky}}$ (long dotted line), (3) $k_{\text{max-Peleg}} - L_{\text{Arrhenius}}$ (black line) and (4) $k_{\text{max-Peleg}} - L_{\text{Ratkowsky}}$ (grey line). *Black circle*: *L. innocua* experimental data obtained by Miller et al. (2009a)

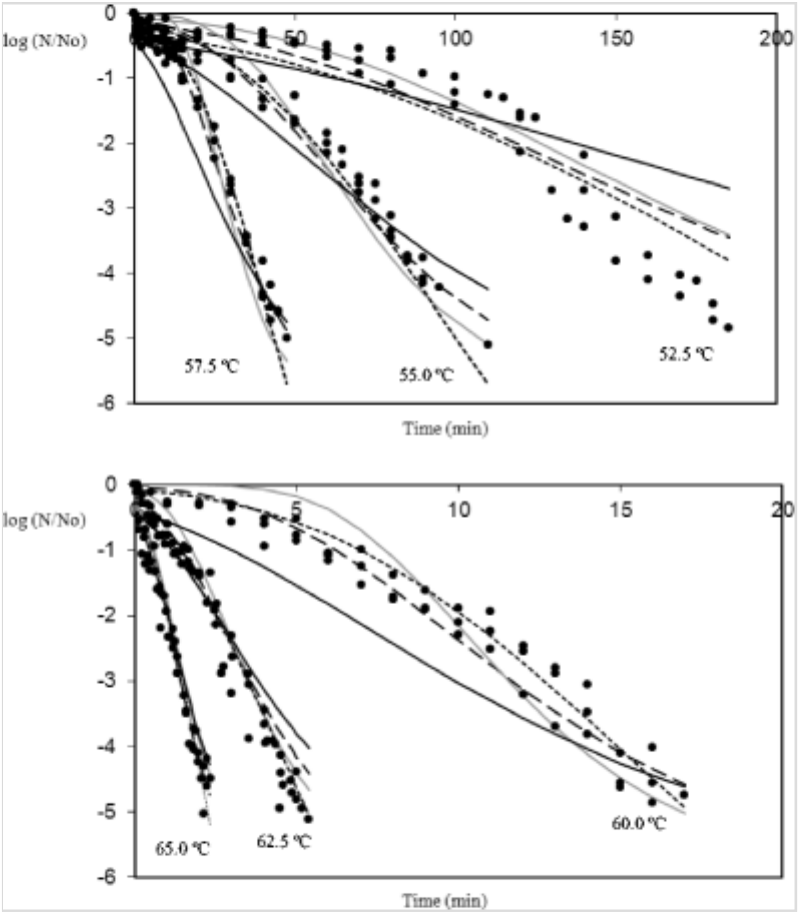


Table 4

Statistical indicators of the global regressions analyses: analysis of the residuals (BF and normality) and goodness-of-fit (R^2_{adj})

Secondary models			T (°C)	Residuals		
Combination	$k_{max} = f(T)$	$L = f(T)$		**BF**	**Randomness**	**Normal**
1	Arrhenius	Ratkowsky	52.5	0.78	No	No
			55.0	1.19	No	Yes
			57.5	1.23	No	No
			60.0	1.20	No	Yes
			62.5	0.87	No	Yes
			65.0	1.11	No	Yes
2	Bigelow	Ratkowsky	52.5	0.95	No	No
			55.0	1.39	No	Yes
			57.5	1.31	No	Yes
			60.0	1.15	No	Yes

Secondary models			**T (°C)**	**Residuals**		
Combination	**$k_{max} = f(T)$**	**$L = f(T)$**		**BF**	**Randomness**	**Normal**
3	Peleg	Arrhenius	62.5	1.07	No	Yes
			65.0	1.18	No	Yes
			52.5	0.78	No	No
			55.0	0.80	No	Yes
			57.5	0.54	No	Yes
			60.0	0.58	No	Yes
			62.5	0.91	No	No
			65.0	1.11	Yes	Yes
4	Peleg	Ratkowsky	52.5	1.30	No	No
			55.0	3.07	No	Yes
			57.5	7.70	No	Yes
			60.0	4.30	No	Yes
			62.5	1.45	No	Yes
			65.0	1.24	Yes	Yes

It was observed that residual normality was not verified in 25% of the cases, and randomness was strongly sacrificed. Nevertheless, the bias factor and R^2_{adj} values reflected that combinations 1 and 2 were the best cases. The bias factor averaged 1.06 and 1.17 for $k_{max\text{-}Arrhenius} - L_{Ratkowsky}$ and $k_{max\text{-}Bigelow} - L_{Ratkowsky}$, respectively. The values of R^2_{adj} were 0.95 and 0.93. In all tested combinations, the lowest R^2_{adj} values were obtained for the lower temperature extreme, corresponding to the worse predictive ability of the global fit.

When a global fit is performed, besides the well-documented and discussed parameter estimation improvements (Valdramidis et al. 2005b; Membré et al. 1997), considerations about the performance of each individual fit are often neglected. In our case study, when the global fit was performed, the assumptions assumed for the residuals (randomness and normality) were broken and, consequently, the regression was compromised as well as the predictive ability of the global model.

Curiously, if the secondary models selected according to the results of the *two-step* methodology (Peleg model to describe $k_{\max}$ (T) and Arrhenius to describe L (T)) are assumed in the global fit (Table 2; combination 3), the quality of the regression decreased.

Seeking parameter estimation comparison (of *two steps* and global fits), combination 1 was used. Estimates of the parameters and their precision are shown in Table 5. Results showed that parameter estimation was improved when a global fit was performed: smaller $\mathrm{SHW}_{95\%}$ (see Tables 2 and 5) and low correlation coefficients between parameters ($\rho_{k_{\mathrm{ref}}-E_{\mathrm{a}}} = -0.44$ and $\rho_{A_{\mathrm{Ratk1}}-A_{\mathrm{Ratk2}}} = -0.30$). Joint confident regions of parameters estimates at 90% were also constructed (Eq. 25; Fig. 6). The *two-step* procedure led to higher confidence regions and to an increased collinearity of the estimates: narrow regions were observed and higher correlation coefficients between parameters were obtained when compared to one-step scheme ($\rho_{k_{\mathrm{ref}}-E_{\mathrm{a}}} = -0.97$ and $\rho_{A_{\mathrm{Ratk1}}-A_{\mathrm{Ratk2}}} = -0.92$). This was expected to occur, since several works reported that global fit procedure allows a more complete and effective utilisation of raw data, avoiding of unnecessary parameters, thus leading to unbiased and more precise estimations (Arabshahi and Lund 1985; Valdramidis et al. 2005a). These regions are a much better representation of the estimates than individual confidence intervals since it makes evident the correlation between parameters. The extremes of the regions correspond approximately to the 95% confidence intervals for individual parameters (Haralampu et al. 1985), which can also be seen in Fig. 6.

Table 5

Parameter estimates from the one-step regression analysis, precision assessed by the

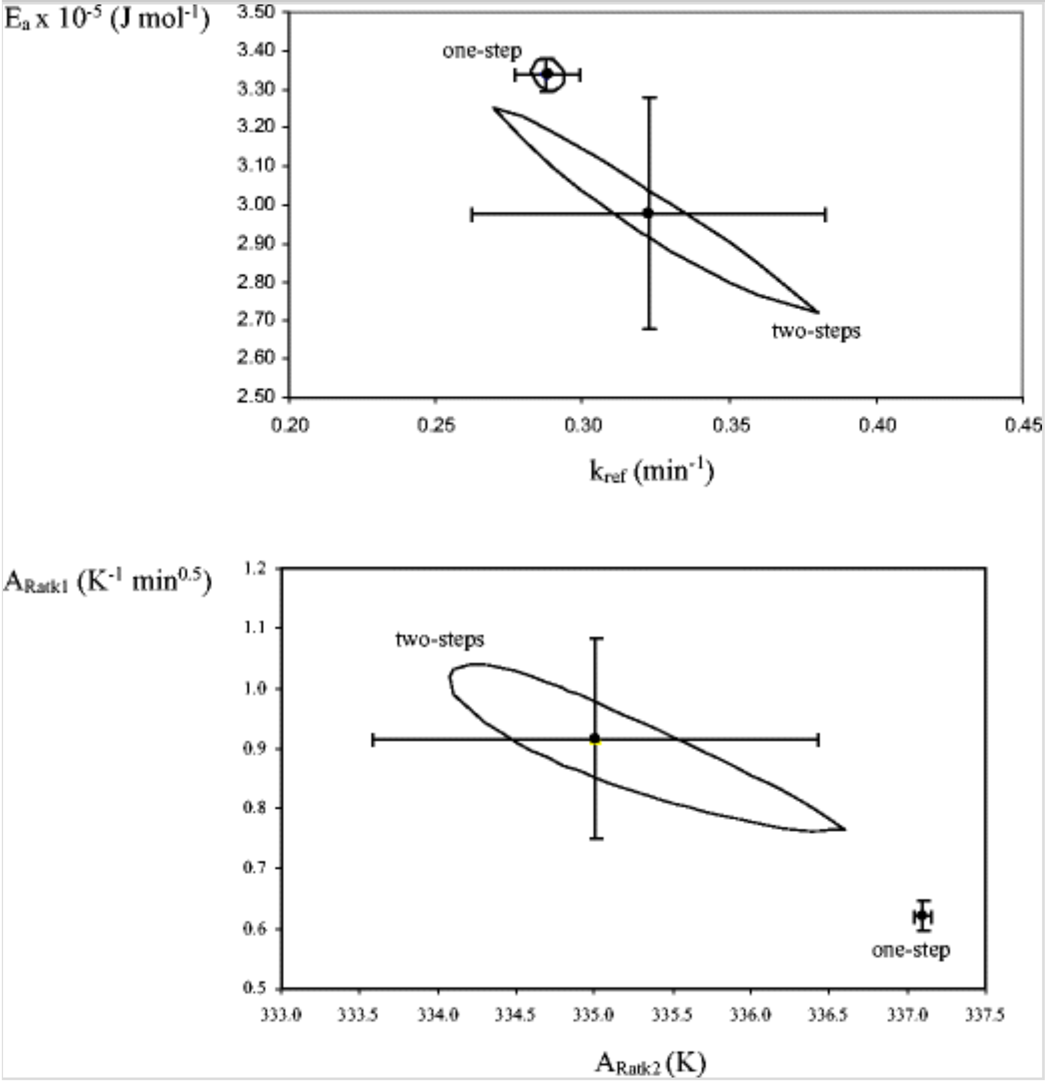
Secondary models	**log (N_{res}/N_0)**		**$k_{\max}$ (min^{-1})**		
Arrhenius ($k_{\max} = f(T)$)/Ratkowsky model ($L = f(T)$)	log (N_{res}/N_0)	$\mathrm{SHW}_{95\%}$ of log (N_{res}/N_0) (%)	k_{ref} (min^{-1})	E_{a} (J mol^{-1})	$\mathrm{SHW}_{95\%}$ of k_{ref} (%)
$T_{\mathrm{ref}} = 331.9$ K					

Secondary models	**log (N_{res}/N_0)**			**k_{max} (min^{-1})**	
	−12.03	10.84	0.29	3.34×10^5	4.01

$T_{ref} = 331.9$ K

Fig. 6

Joint confidence regions of the estimates at 90% of the Arrhenius model that relates k_{max} with T (Eq. 2) (upper plot) and of the estimates of the Ratkowsky model that relates L with T (Eq. 11) (bottom plot); Comparison of one-step and two-step regressions (the lines correspond to the individual confidence intervals at 95%)



Besides this, the two different regression schemes yield different parameter estimates, as there is no interception of the regions and the solutions of the *one-step* procedure are not enclosed into the *two-step* contour. A significant difference was obtained for L value, which can be explained by the collinearity between parameters. Nevertheless, differences in the individual values of $k_{\max}$ at given temperature were not observed.

Accuracy of log (N_{res}/N_0) predicted by both *two-step* and *one-step* procedure was analysed (accuracy stands for deviation to the experimental value). Curiously, log (N_{res}/N_0) predicted by *one-step* procedure deviated considerably from the experimental values, for the six temperatures. This means that, besides precision of parameter is attained, accuracy is sacrificed, which directly affects the predictive ability of the model to the residual population.

Conclusions

This review highlights important topics for testing the adequacy of mathematical models used for predictive purposes.

When modelling temperature affects sigmoidal inactivation kinetics of microorganisms, one should be aware about the regression methodology applied. Models that relate kinetic parameters with temperature and that are selected according to a *two-step* regression procedure may not be the adequate ones if a global fit is applied. These two regression procedures may also lead to different parameter estimates and to different model predictions.

One should be aware that if a global regression of sigmoidal microbial inactivation isothermal data is performed on the basis of commonly applied secondary models, a careful assessment of models combination is required. This was proven with the case study. Although the temperature effects on $k_{\max}$ and L were well described by a Peleg model and Arrhenius equation, respectively, the prediction of *L. innocua* inactivation may not be adequate if a *two-step* methodology is applied. If a *one-step* approach is chosen, the interaction of the secondary models directly affects the fitting ability of the global model assumed.

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