Available online at www.sciencedirect.com
ScienceDirect
Journal of Food Engineering 80 (2007) 581–593
JOURNAL OF FOOD ENGINEERING
www.elsevier.com/locate/jfoodeng

Nonlinear regression technique to estimate kinetic parameters and confidence intervals in unsteady-state conduction-heated foods

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Received 1 February 2006; received in revised form 17 May 2006; accepted 22 June 2006
Available online 7 September 2006

Abstract

Due to difficulty in computing, confidence intervals (CIs) for kinetic parameters and the predicted dependent variable (Y) in nonlinear models are often not reported. The purpose of this work was to present a straightforward method to calculate asymptotic CIs for kinetic parameters and the associated Y variable for nonisothermal survivor or retention curves. The novelty of this work was that (1) confidence bands (CBs) and prediction bands (PBs) for predicted Y (microbial survival ratio or nutrient retention) were computed along with CIs for the parameters (using Matlab®), and (2) confidence regions for the parameters were computed by an iterative method. Both the k–E and the D–z model were used. Three case studies were used. Kinetic parameters for microbial death (Cases 1 and 2) in an unsteady-state conduction-heated canned food and for thiamin concentration (Case 3) were estimated using a nonlinear regression technique. Upper 95% prediction bands gave a more conservative (safer) limit than the Y value predicted by the model, up to a 0.84 log difference. Given the availability and ease of use of nonlinear regression software, researchers can consider using the proposed method as a template for kinetic parameter estimation, confidence interval, and confidence region computation. These data are essential for accurate estimates of food safety.

Keywords: Confidence interval; Prediction band; Confidence region; Conduction heating; Nonisothermal; Unsteady-state heating; Nonlinear regression; Kinetic parameters

1. Introduction

Common methods to estimate kinetic parameters in high-moisture foods include heating liquid samples in capillary tubes, and heating small samples of moist foods—such as ground beef—for different times at different constant temperature. The kinetics could apply to nutrient destruction or microbial death, for example. These methods usually work well, because during heating of small samples of high-moisture foods, there are short lag times and negligible temperature gradients within the sample. However, in the literature surveyed, there is no standard method to estimate kinetic parameters in low-moisture, conduction-heated foods subject to temperatures above 100 °C, such as vegetable pastes, candies, confectionaries, breads, and extruded grains. Isothermal experiments at temperatures above 100 °C require more sophisticated design, because samples must be heated in a pressure vessel or oil bath, and some temperature-measuring device (e.g., a thermocouple) must penetrate the sample container while still maintaining a perfect seal to prevent moisture loss and maintain pressure. As temperatures and pressure increase, measuring sample temperature may become impractical, so the experimenter may choose to predict sample temperature using thermal properties. Even with

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doi:10.1016/j.jfoodeng.2006.06.023

Nomenclature

Greek

α	thermal diffusivity, m^2/s, or probability
β_E, β_z	time–temperature history, min
γ	R/l
Δt	time interval of integration for β, min
η	any parameter
μ	average of distribution
ρ	r/R dimensionless radial position
ρ_{ij}	correlation coefficient between the ith and jth parameters
λ	eigenvalue, dimensionless; for cylinder, λ_m satisfies $\lambda_m J_1(\lambda_m) - (\frac{hR}{k})J_0(\lambda_m) = 0$; for slab, λ_n satisfies $\lambda_n \tan \lambda_n = (\frac{hl}{k})$
ξ	z/l dimensionless axial position
π	3.14156
σ	standard deviation
τ	heating Fourier number $= \alpha t/R^2$
τ_w	cooling Fourier number $= \alpha t_{cool}/R^2$
ω_j	roots of $J_0(\omega) = 0$
C_a	measured mass-average survival ratio or mass-average retention, dimensionless
$\widehat{C}_a$	predicted mass-average survival ratio or mass-average retention, dimensionless
D_r	time to reduce microorganism population by 90% at reference temperature T_r, min
E_a	activation energy, J/g mol
F_α	critical value of the F distribution for a given $(1-\alpha)$ confidence level and degrees of freedom. For example, in Excel, finv (95% confidence, p, $n-p$) = finv(0.05, 2, 24) = 3.40. In matlab, the identical expression is finv(0.95, 2, 24) = 3.4
h	heat-transfer coefficient, $W/(m^2\,K)$
i,j,k,n	integer indices
J_0	zero order Bessel function
J_1	first-order Bessel function
k	thermal conductivity in Eq. (1), W/m K
k_r	rate constant at reference temperature T_r, min^{-1}
l	half the container length, normalized = 1.0
ln	natural logarithm
log	base 10 logarithm
MSE	mean square error $= SS_{best\text{-}fit}/(n-p)$
N	number of microorganisms
N_0	initial number of microorganisms
n	number of data points
p	number of parameters = 2 in the present work
r	position in radial direction, m
R	container radius, normalized = 1.0
RMSE	root mean square error= $\sqrt{\sum(Y_i - \widehat{Y}_i)^2/(n-p)}$
R_g	gas law constant
$SS_{best\text{-}fit}$	minimum sum-of-squares of errors, i.e., sum-of-squares when nonlinear regression fits the curve
$SS_{all\text{-}fixed}$	target value for sum-of-squares to compute parameter joint confidence regions
t	time, min
t_{cool}	cooling time, min
t_n	end time of process, min
T	temperature, K
T_r	reference temperature, K
T_s	steam or retort temperature, °C
T_w	cooling water temperature, °C
T_i	initial temperature, °C
Y_a	measured dependent variable
$\widehat{Y}_a$	predicted dependent variable
z	can axial coordinate, m
z	temperature change causing a 10-fold change in D, °C

small samples, often very long lag times (time to reach nearly a temperature plateau, or time to reach 99% of the temperature increase) and large temperature gradients make the isothermal method challenging for many conduction-heated foods.

Some researchers have proposed alternate methods for conduction-heated solids, such as heating thin samples (~2 mm of soy flour, van den Hout, Meerdink, & Van't Riet, 1999; 13.5-mm diameter test tubes with gelatinized starch solutions, Dolan, Steffe, & Morgan, 1989) and using transient heat-transfer theory to predict the temperature within the sample.

Other researchers have presented calculation methods to estimate thermal and kinetic parameters in conduction heating of foods in larger sealed containers, such as cans. Lenz and Lund (1977a) used nonlinear regression to estimate the thermal diffusivity of pureed peas, pureed lima beans, baked beans and applesauce. Lenz and Lund (1977b) used a distribution of activation energies and thermal diffusivities in a Monte Carlo technique to compute 95% confidence intervals for thermal death time (F_0) and for mass-average retention of thiamin, chlorophyll, and betanin in various foods. Lenz and Lund (1980) used nonlinear regression to estimate activation energy and rate constant for thiamin destruction in conduction-heated canned pea puree. They also computed the asymptotic 95% confidence intervals for the reaction rate constant and the thermal death time.

Other researchers have presented calculation methods to estimate kinetic parameters in conduction heating of foods in larger sealed containers, such as cans. Similar to Lenz and Lund (1977a, 1980), Welt et al. (1997) used sealed cans of pea puree inoculated with *Bacillus stearothermophilus* spores. Using a finite-difference procedure, they calculated

puree temperatures throughout the cans at any time. Once the temperatures were known, they used the paired equivalent isothermal exposures (PEIE) method to converge to the rate constant and activation energy for microbial destruction that minimized the sum-of-squares of errors. The advantages of Lenz and Lund's (1980) and Welt et al's. (1997) experimental and calculation methods were that lag-time and temperature gradients were not problems, and that moisture loss at temperatures greater than 100 °C was prevented because the cans were sealed. However, there were some disadvantages with the estimation procedures: (1) both methods are multi-step and are therefore labor-intensive; (2) the PEIE method requires writing extensive computer code for the iterative estimation procedure; (3) in the PEIE method, negative activation energies result when two pairs of data move opposite to that expected (i.e., increased microbial count with time); and (4) the PEIE method may be deficient when two dynamic thermal exposures yield similar equivalent temperatures. The one-step regression method proposed in the present work avoided all four of these difficulties, as described later.

Nasri, Simpson, Bouzas, and Torres (1993) used nonlinear regression to estimate kinetic parameters ($D_{121.1\,°C}$ and z) of thiamin degradation in pea puree. They used the jackknife method to estimate the standard error of the parameters. However, they did not estimate confidence intervals for the parameters, and did not include standard error or confidence intervals for the predicted Y (mass-average thiamin concentration in the can). Because Y is a complicated exponential function of the parameters, the relationship of Y-variability to parameter-variability is not straightforward. Especially in the study of microbial death, the confidence interval for Y is more important in assessing food safety than the confidence intervals for the parameters.

The correlation between the two kinetic parameters means that the confidence interval of one parameter depends on the value of the other parameter. Therefore, joint confidence regions can be used to see the correlation. Fernández, Ocio, Fernández, Rodrigo, and Martinez (1999) and Fernández, Ocio, Fernández, Rodrigo, and Martinez (2001) plotted the 90% joint confidence regions for $D_{90\,°C}$ and z for thermal resistance of two *Bacillus cereus* strains heated under isothermal conditions, and under nonisothermal conditions at different heating rates, respectively. Claeys, Ludikhuyze, van Loey, and Hendrickx (2001) and Claeys, Ludikhuyze, and Hendrickx (2001) plotted the 90% joint confidence regions for D and z for inactivation of alkaline phosphatase, lactoperoxidase, and β-lactoblobulin, and for hydroxymethlfurfural for isothermal and nonisothermal conditions. It appears that in these four papers, the elliptical approximation was used for the confidence contour. In the present work, we show the true confidence contour so that it can be compared to the elliptical approximation.

In summary, the literature reviewed shows no standardized method to report confidence intervals for multiple kinetic parameters and for predicted dependent variable for nonisothermal conduction-heated foods. Therefore, the purpose of this work is to show, for unsteady-state conduction-heated foods, a convenient method to estimate (1) kinetic parameters, their asymptotic confidence intervals, and their joint confidence regions, and (2) asymptotic confidence intervals for the predicted dependent variable Y.

2. Overview of method

2.1. Published data used in this study

For comparison purposes, data from two studies were used. For Cases #1–2 in the present work, the work of Welt et al. (1997) and the data of Welt (1996) were used. In their study, cans of pea puree, inoculated with spores of *Bacillus stearothermophilus*, were heated at 12 different time–temperature combinations using three different retort temperatures (Table 1). The number of spores surviving for each can was measured and recorded as a survival ratio $= N_i/N_0$, where $N_0 = 9(\pm 1) \times 10^5$ CFU/mL. For case 3, data from Nasri et al. (1993) were used.

2.2. Equations

2.2.1. Analytical heating solution for finite cylindrical geometry

2.2.1.1. From time = 0 until steam off. Temperatures were predicted using conduction heat transfer for heating with

Table 1
Retort temperature, heating time, and measured survival ratios for 24 heated cans (Welt, 1996, pp. 190–191)

Can number	Retort temperature (°C)	Heating time (min)	Measured survival ratios, N/N_0
1	n/a	0	0.891
2	n/a	0	1.109
3	104.4	50	0.689
4	104.4	50	0.769
5	104.4	90	0.656
6	104.4	90	0.744
7	104.4	180	0.387
8	104.4	180	0.476
9	104.4	180	0.279
10	104.4	180	0.266
11	104.4	240	0.0755
12	104.4	240	0.0697
13	104.4	240	0.0703
14	104.4	240	0.0667
15	104.4	275	0.0418
16	104.4	275	0.0401
17	104.4	305	0.00725
18	104.4	305	0.00855
19	112.8	27.5	0.642
20	112.8	32.5	0.433
21	112.8	44	0.159
22	120.6	19	0.500
23	120.6	24	0.266
24	120.6	27.5	0.093

Can size was 0.0602 diameter by 0.0348 m height. Survival ratio for can #2 was >1.0 because the denominator of survival ratio for all cans was the mean of survivor numbers for cans 1 and 2 (mean $= 9 \times 10^5$ CFU/mL).

uniform initial temperature. The slab part of this solution is from Carslaw and Jaeger (1959, p. 122, Eq. (12)); the radial solution part is from Myers (1971, p. 122, Eq. (3.3.20)). This solution assumes a constant finite heat-transfer coefficient along all boundaries of the finite cylinder:

$$\frac{T(\rho,\xi,t)-T_s}{T_i-T_s}=\sum_{m=1}^{\infty}\frac{2\left(\frac{hl}{k}\right)\cos(\lambda_m\xi)\sec(\lambda_m)\exp\left[-\lambda_m^2\left(\frac{\alpha t}{l^2}\right)\right]}{\left(\frac{hl}{k}\right)\left(\frac{hl}{k}+1\right)+\lambda_m^2}\times\sum_{n=1}^{\infty}\frac{2\left(\frac{hR}{k}\right)J_0(\lambda_n\rho)\exp\left[-\lambda_n^2\left(\frac{\alpha t}{R^2}\right)\right]}{\left[\left(\frac{hR}{k}\right)^2+\lambda_n^2\right]J_0(\lambda_n)} \quad (1)$$

2.2.1.2. From steam off until all temperatures in can were below significant microbial kill temperature. When cooling begins at the end of heating, there is a non-uniform initial temperature distribution throughout the can. The analytical conduction heat-transfer solution for this case is from Lenz and Lund (1977a, Eq. (7)), and assumes an infinite heat-transfer coefficient along the boundary:

$$\frac{T-T_w}{T_s-T_i}=4\sum_{j=1}^{\infty}\sum_{i=0}^{\infty}\times\frac{(-1)^i\exp\left[-\left(\gamma^2\left(i+\frac{1}{2}\right)^2\pi^2+\omega_j^2\right)\tau_w\right]J_0(\omega_j\rho)}{\left(i+\frac{1}{2}\right)\pi\omega_jJ_1(\omega_j)}\times\cos\left[\left(i+\frac{1}{2}\right)\pi\xi\right]\left\{\left(\frac{T_s-T_w}{T_s-T_i}\right)-\exp\left[-\left(\omega_j^2+\left(i+\frac{1}{2}\right)^2\pi^2\gamma^2\right)\tau\right]\right\} \quad (2)$$

Cooling temperatures were calculated using Eq. (2) until all Gauss node temperatures in the can were below 80 °C.

Tolerance criteria: Additional terms in Eqs. (1) and (2) were added until the relative difference ($sum_{new} - sum_{old}/sum_{old}$) in the summation was $\leqslant 10^{-6}$.

Kinetic parameters were estimated based on a first-order kinetic model and Arrhenius relationship ($k = f(T)$), using nlinfit in Matlab®, described later.

Note: If one has access to finite-element (such as FEMLAB®) or finite difference software, that software can be used to replace Eqs. (1) and (2).

2.2.2. Models for microbial survival ratio (or nutrient retention) ($0 < N/N_0 < 1.0$) at any point within the can

$$k\text{–}E\text{ model}: \quad Y(k_r,\rho,\xi,t)=N(k_r,\rho,\xi,t)/N_0=\exp[-k_r\beta_E(\rho,\xi,t)] \quad (3)$$

A model is linear in a parameter if the first derivative of the dependent variable with respect to that parameter is not a function of that parameter. (Another way of describing this condition is that the second derivative with respect to that parameter equals zero.) If a model is nonlinear in a parameter, then the parameter cannot be solved for directly, but must be solved for by nonlinear regression ("directed" trial-and-error). If a model is linear in a parameter, then an initial guess is not needed for that parameter. Fewer nonlinear parameters usually will indicate better convergence. Therefore, we first determine which parameters in the model are nonlinear. For the two models in the present work, all parameters are nonlinear, as shown by the following:

The k–E model is nonlinear in both k_r and E_a because

$$\frac{\partial Y}{\partial k_r}=-\beta_E\exp(-k_r\beta_E)=f(k_r) \quad (4)$$

and

$$\frac{\partial Y}{\partial E_a}=(k_r/R)\beta'_E\exp(-k_r\beta_E)=f(E_a) \quad (5)$$

$$D\text{–}z\text{ model}: \quad Y(D_r,\rho,\xi,t)=\log(N/N_0)=-(1/D_r)\beta_z(\rho,\xi,t) \quad (6)$$

The D–z model is nonlinear in both D_r and z because

$$\frac{\partial Y}{\partial D_r}=\frac{\beta_z}{D_r^2}=f(D_r) \quad (7)$$

and

$$\frac{\partial Y}{\partial z}=\frac{\beta'_z}{D_rz^2}=f(z) \quad (8)$$

Definition of beta and beta prime:

$$k\text{–}E\text{ model}: \quad \beta_E(\rho,\xi,t)=\int_{\tau=0}^{t}\exp\left[\frac{-E_a}{R_g}\left(\frac{1}{T(\rho,\xi,\tau)}-\frac{1}{T_r}\right)\right]d\tau \quad (9)$$

$$\beta'_E(\rho,\xi,t)=\int_{\tau=0}^{t}\left(\frac{1}{T(\rho,\xi,\tau)}-\frac{1}{T_r}\right)\times\exp\left[\frac{-E_a}{R_g}\left(\frac{1}{T(\rho,\xi,\tau)}-\frac{1}{T_r}\right)\right]d\tau \quad (10)$$

$$D\text{–}z\text{ model}: \quad \beta_z(\rho,\xi,t)=\int_{\tau=0}^{t}10^{\left(\frac{T(\rho,\xi,\tau)-T_r}{z}\right)}d\tau \quad (11)$$

$$\beta'_z(\rho,\xi,t)=\int_{\tau=0}^{t}[T(\rho,\xi,\tau)-T_r]10^{\left(\frac{T(\rho,\xi,\tau)-T_r}{z}\right)}d\tau \quad (12)$$

Beta calculated by N-point trapezoidal rule:

k–E model:

$$\beta_E(\rho,\xi,t)\cong\sum_{n=0}^{N-1}\frac{\Delta t}{2}\left\{\exp\left[\frac{-E_a}{R_g}\left(\frac{1}{T(\rho,\xi,t_{n+1})}-\frac{1}{T_r}\right)\right]+\exp\left[\frac{-E_a}{R_g}\left(\frac{1}{T(\rho,\xi,t_n)}-\frac{1}{T_r}\right)\right]\right\} \quad (13)$$

D–z model:

$$\beta_z(\rho,\xi,t)\cong\sum_{n=0}^{N-1}\frac{\Delta t}{2}\left\{10^{\left(\frac{T(\rho,\xi,t_{n+1})-T_r}{z}\right)}+10^{\left(\frac{T(\rho,\xi,t_n)-T_r}{z}\right)}\right\} \quad (14)$$

To minimize computer time spent on Eqs. (13) and (14), one should use smaller time intervals when temperature is changing rapidly, and longer time intervals when temperature is moving slowly. Therefore, in the present work, from time = 0 until the time ("plateau time") the temperatures at all 9 Gauss points in the can reached within 2 °C of T_s, $\Delta t = 60$ s. To speed calculations when the temperature was not changing rapidly, from "plateau time" until cooling time, $\Delta t = 1200$ s. During cooling, $\Delta t = 30$ s.

Arrhenius relationship of rate to temperature:

$$k\text{–}E \text{ model:} \quad k(T) = k_r \exp\left[-\left(\frac{E_a}{R}\right)\left(\frac{1}{T}-\frac{1}{T_r}\right)\right] \tag{15}$$

$$D\text{–}z \text{ model:} \quad D(T) = D_r 10^{\frac{-(T-T_r)}{z}} \tag{16}$$

2.2.3. Predicted mass-average survival ratio (or nutrient retention)

$$k\text{–}E \text{ model:} \quad \widehat{C}_a(k_r,t) = \widehat{Y}_a(k_r,t) = \frac{2\int_{\xi=0}^{l}\int_{\rho=0}^{R}\exp[-k_r\beta_E(\rho,\xi,t)]\rho\,d\rho\,d\xi}{R^2 l} \tag{17}$$

$$D\text{–}z \text{ model:} \quad \widehat{C}_a(k_r,t) = \frac{2\int_{\xi=0}^{l}\int_{\rho=0}^{R}10^{\left[-\frac{1}{D_r}\beta_z(\rho,\xi,t)\right]}\rho\,d\rho\,d\xi}{R^2 l} \tag{18}$$

Mass-average survival ratio (or nutrient retention) calculated using 3-point Gauss integration.

$$k\text{–}E \text{ model:} \quad \widehat{C}_a(k_r,t_n) = \widehat{Y}_a(k_r,t_n) \cong \frac{2}{R}\sum_{j=1}^{3}\sum_{i=1}^{3}\exp[-k_r\beta_E(\rho_{ij},\xi_{ij},t_n)]\rho_{ij}w_i w_j \tag{19}$$

$$D\text{–}z \text{ model:} \quad \widehat{C}_a(D_r,t_n) \cong \frac{2}{R}\sum_{j=1}^{3}\sum_{i=1}^{3}10^{\left[-\frac{1}{D_r}\beta_z(\rho_{ij},\xi_{ij},t_n)\right]}\rho_{ij}w_i w_j \tag{20}$$

The predicted dependent variable for the D–z model is the logarithm of the mass-average concentration:

$$D\text{–}z \text{ model:} \quad \widehat{Y}_a = \log_{10}(\widehat{C}_a) \tag{21}$$

The choice of numerical integration technique is not trivial, because the computational time increases rapidly with the number of nodes. Gauss is more accurate than trapezoidal integration, but is not convenient when limits change, such as a time-series in Eq. (9). When limits are fixed, such as Eqs. (17) and (18), Gauss integration should be used (Eqs. (19) and (20)), because a minimum number of nodes can give extremely high accuracy (exact for a $2n-1$ polynomial, where n is number of nodes). For example, numerical integration on 2D functions similar to Eq. (17) showed that 3-node Gauss gave an error of 0.000788%. 9-Node and 50-node trapezoidal gave an error of −0.7087% and −0.01888%, respectively. The fact that 50-nodes trapezoidal could not match 3-nodes Gauss shows that Gauss is always preferred.

2.2.4. Sum-of-squares of errors to minimize by nonlinear regression

The parameters $k_{121.1\,°C}$ and E_a, or $D_{121.1\,°C}$ and z were estimated as the pair that minimized sum-of-squares of errors:

$$k\text{–}E \text{ model:} \quad \mathrm{SS}_{\text{best-fit}} = \sum_i[(C_a)_i - (\widehat{C}_a)_i]^2 \tag{22}$$

$$D\text{–}z \text{ model:} \quad \mathrm{SS}_{\text{best-fit}} = \sum_i[(\log C_a)_i - (\log \widehat{C}_a)_i]^2 \tag{23}$$

2.2.5. Asymptotic confidence intervals and confidence/prediction bands

For nonlinear models, the best method for computing the asymmetric confidence intervals is the Monte Carlo method (Van Boekel, 1996). The Monte Carlo method is beyond the scope of this work. A common approximation of nonlinear confidence intervals is the asymptotic confidence interval, which is symmetric (Van Boekel, 1996). This approximation may underestimate the true confidence interval (Johnson & Faunt, 1992). Although asymptotic CIs lack in theoretical reliability, they are computationally expedient and conceptually appealing. Matlab® has two commands for asymptotic confidence intervals:

for parameters: nlparci(beta, residuals, Jacobian),
for predicted Y value: nlpredci(model, x, parameters, residuals, Jacobian, alpha, simultaneousoption, predictionoption).

These two functions use the equations (Seber & Wild, 1989) based on the t distribution, which give a symmetric confidence interval at every point. The confidence bands are the boundaries that have a 95% chance of containing the true regression line. The prediction band is the area where 95% of all the data are expected to lie (Motulsky & Christopoulos, 2004). The prediction band will be wider than the confidence band. In the present work, the 95% confidence intervals for each of the two parameters were calculated using nlparci, and the 95% simultaneous (simultaneousoption = "on") confidence band (predictionoption = "curve") and 95% prediction band (predictionoption = "observation") was calculated for the predicted Y using nlpredci. For the predicted Y, we say "band" because we used the simultaneous option, as opposed to "interval" if we had used the non-simultaneous option.

2.2.6. Standard error and correlation coefficient

Standard error σ_i of each parameter was estimated per Van Boekel (1996), where σ_i is the square root of the corresponding diagonal of the symmetric parameter variance–covariance matrix

$$\mathbf{cov}(\mathbf{a}) = (\mathbf{X}^{\mathbf{T}}\mathbf{X})^{-1}(\mathrm{MSE}) = \begin{pmatrix} \sigma^2_{k_r} & \sigma_{k_r E_a} \\ \sigma_{k_r E_a} & \sigma^2_{E_a} \end{pmatrix} \tag{24}$$

and $\mathbf{X}$ is the Jacobian

$$\mathbf{X} = \begin{pmatrix} \left(\frac{\partial Y_1}{\partial k_r}\right) & & \left(\frac{\partial Y_1}{\partial E_a}\right) \\ \vdots & \ddots & \vdots \\ \left(\frac{\partial Y_n}{\partial k_r}\right) & & \left(\frac{\partial Y_n}{\partial E_a}\right) \end{pmatrix} \tag{25}$$

(The variables k_r and E_a in Eqs. (24) and (25) were replaced with D_r and z, respectively, when the D–z model was used.)

The correlation coefficient between the two parameters was $\rho_{k_r E_a} = \sigma_{k_r E_a}/(\sigma_{k_r}\sigma_{E_a})$, and $-1.0 \leqslant \rho \leqslant 1.0$, where higher values of $|\rho|$ indicate more difficulty in the estimation process.

2.2.7. Parameter joint confidence regions

The joint confidence region for two parameters, η_1 and η_2, can be defined as the set of points (η_1, η_2) where the sum-of-squares of errors is less than or equal to a constant value determined by the level of confidence required. Specifically, the constant value is (Motulsky & Christopoulos, 2004)

$$\mathrm{SS}_{\text{all-fixed}} = \mathrm{SS}_{\text{best-fit}}\left(\frac{p}{n-p}F_\alpha(p, n-p) + 1\right) \tag{26}$$

The confidence contour bounding the confidence region was computed per the iterative method of Motulsky and Christopoulos (2004). Their method is as follows: (1) fix parameter 1 (along the y-axis) equal to best-fit value already determined. (2) Allow parameter 2 (along x-axis) to vary using a root-finder program (function fmincon in Matlab) until $\mathrm{SS} \cong \mathrm{SS}_{\text{all-fixed}}$. Because the contour is an oval shape, there will be two values (roots) of parameter 2 (E or z) that will satisfy this criterion. By using two different starting values of parameter 2—one on each side of parameter $2_{\text{best-fit}}$ in the root-finder—the two roots can be found. (3) Fix parameter 1 at a slightly lower value, ~95% of the best-fit value. Repeat #2. (4) Repeat #3 until the two roots of parameter 2 are almost equal, such as within 1–5% of each other. This completes the lower half of the contour. (5) Repeat #3–4, but use *increasing* values of parameter 1, using starting value = 1.05 × parameter $2_{\text{best-fit}}$. This procedure completes the upper half of the contour.

For all cases below, the temperatures at the Gauss nodes at the specified times were calculated first via Eqs. (1) and (2), and then stored to be accessed during parameter estimation (Eqs. (22) and (23)). Alternatively, one could compute and supply temperatures via a separate finite-difference or finite-element program, such as FEMLAB®.

3. Case studies

The following three cases studies were used as examples to illustrate how to estimate the parameters and how to compute predicted $\widehat{Y}_a$, confidence intervals and prediction intervals for parameters and Y, and the 95% and 99% confidence regions for the two parameters.

3.1. Case 1. k–E model, data of Welt (1996)

Y_a, heating times, and retort temperatures were those listed in Table 1. $T_r = 380$ K, because we found by trial-and-error that the convergence was good and the correlation between k_r and D was low. $k_{121.1\,^\circ\mathrm{C}}$ and E were estimated by minimizing Eq. (22). Predicted survival ratios $\widehat{Y}_a$ were computed per Eq. (17). Internal can temperatures in Eq. (13) were generated by Eqs. (1) and (2), which used the heating times and boundary conditions $h = 5500$ W/m^2 K for heating, and $h = \infty$ for cooling (because we did not find an analytical solution for finite h). Retort temperatures are listed in Table 1. Initial uniform can temperature = 0 °C. Cooling water temperature = 25 °C.

3.2. Case 2. D–z model, data of Welt (1996)

Same as Case 1, except using the corresponding D–z model equations. $T_r = 381$ K, because we found by trial-and-error that the convergence was good and the correlation was low. $D_{121.1\,^\circ\mathrm{C}}$ and z were estimated by minimizing Eq. (23). Predicted survival ratios $\widehat{C}_a$ were computed using Eq. (20). The predicted dependent variable $\widehat{Y}_a$ was calculated via Eq. (21). Identical with Case 1, internal can temperatures in Eq. (14) were generated by Eq. (1) and (2), which used the heating times and boundary conditions $h = 5500$ W/m^2 K for heating, $h = \infty$ for cooling. Retort temperatures are listed in Table 1.

3.3. Case 3. D–z model, data of Nasri (1993)

Same as Case 2, except Nasri et al.'s (1993) data (Table 2) were used. $T_r = 376$ K. Initial uniform can temperature = 37.5 °C. Cooling water temperature = 13 °C.

4. Results and discussion

All k_r and D were estimated at T_r, rather than at 121.1 °C, to avoid high correlation with E and z, respectively. All correlations between k and E and between D and z were not high (Table 3, $|\rho|$ ranged 0.247–0.766, where $|\rho| \geqslant 0.99$ indicates excessive correlation). To allow comparison at one temperature, all k and D were reported at 121.1 °C per Eqs. (15) and (16). As expected, the nonlinear regression fits for all three cases were good (Table 3, root-mean squared error ranged 0.099–0.260 for all three cases).

4.1. Case 1

4.1.1. Parameter values

The rate constant and activation energy estimated in the present work were 47% and 14% higher ($k_{121.1\,^\circ\mathrm{C}} = 0.382$ vs. 0.26 min^{-1}, $E = 284$ vs. 250 kJ/g mol, Table 3), than Welt et al. estimated using the PEIE method. The 95% confidence interval for rate constant in the present work (0.322, 0.443, Table 3) did not even include the mean value = 0.26 min^{-1} from Welt et al. (1997). Likewise, Welt

Table 2
Retort temperature, heating time, and measured retention ratios for thiamin for 18 heated cans (Nasri et al., 1993, Table 1 in their paper)

Can number	Retort temperature (°C)	Heating time (min)	Measured retention ratios, C_a	Log (retention ratio)
1	116	92	0.806	−0.094
2	116	92	0.755	−0.122
3	116	92	0.738	−0.132
4	115	100	0.653	−0.185
5	115	100	0.721	−0.142
6	115	100	0.694	−0.159
7	112	137	0.657	−0.182
8	112	137	0.637	−0.196
9	112	137	0.679	−0.168
10	109	211	0.51	−0.292
11	109	211	0.561	−0.251
12	109	211	0.624	−0.205
13	106	367	0.475	−0.323
14	106	367	0.408	−0.389
15	106	367	0.483	−0.316
16	103	675	0.3	−0.523
17	103	675	0.261	−0.583
18	103	675	0.313	−0.504

We generated the individual measured retention ratios using a normal distribution with the reported standard deviation and reported mean values of the triplicate runs from Nasri et al. (1993). Can size was 0.081 diameter by 0.1111 m height.

et al.'s (1997) 95% confidence intervals for both parameters did not include the values in the present work (0.382 min^{-1} and 284 kJ/g mol). The RMSE based on Welt et al.'s (1997) reported parameter estimates was slightly lower than ours (0.0913 vs. 0.099), implying that they may have used means, rather than individual survival ratios for the cans (Table 1). In that case, the fact that our parameter estimates are different from theirs indicates that researchers should use individual data, and not means, when estimating parameters.

4.1.2. Parameter confidence intervals (CIs)

The width of the confidence intervals we estimated for $k_{121.1\,°C}$ and E were 73% and 243% *larger*, respectively, than those estimated by Welt et al. (1997) (0.121 vs. 0.07 min^{-1}, and 102.9 vs. 30 kJ/g mol). These larger CIs will lead to larger confidence intervals for the dependent variable, but *how much* larger was beyond the scope of this work. The practical result is that for the same data, Welt et al.'s (1997) CI results imply that the product is safer than it actually is. Therefore, standard methods for computing CIs are needed to make more accurate food safety conclusions.

4.1.3. Parameter confidence regions (CRs)

The nearly elliptical form of the 95% and 99% confidence regions (Fig. 1) shows the low correlation = 0.247 for $T_r = 380$ K. Higher correlation coefficient would show a more slanted, more "stretched" ellipse. The correlation coefficient was highly dependent on the reference temperature chosen. For example, the correlation coefficient became more negative at $T_r < 380$ K (confidence regions more elliptical and slanted to the left), and became more positive as $T_r > 380$ K (confidence regions more elliptical and slanted to the right). The same pattern was found in Cases 2 and 3 below. Therefore, when reporting confidence regions for parameters using the Arrhenius model, one should also report both the reference temperature and the correlation coefficient.

Table 3
Statistical results of parameter estimates

	Root mean square error	Number of data	Parameter estimate	Standard error	Correlation coefficient $\rho_{k_r E_a}$ or $\rho_{D_r z}$ (reference temperature)	95% asymptotic confidence interval	Results from other researchers
Case 1 Welt (1996) data *k–E* model	0.0990	24	$k_{121.1\,°C} = 0.382\ min^{-1}$ $E = 284.0$ kJ/g mol	0.0291 min^{-1} 24.8 °C	0.247 ($T_r = 380$ K)	(0.322, 0.443) (232.5, 335.4)	$k_{121.1\,°C} = 0.26\ min^{-1}$ 95% confidence interval (0.23, 0.30) $E = 250$ kJ/g mol 95% confidence interval (235, 265) RMSE[a] = 0.0913
Case 2 Welt (1996) data *D–z* model	0.260log	24	$D_{121.1\,°C} = 6.31$ min $z = 11.36$ °C	0.586 min 1.31 °C	0.766 ($T_r = 381$ K)	(5.09, 7.53) (8.64, 14.1)	$D_{121.1\,°C} = 8.9$ min $z = 11.4$ °C CIs not reported RMSE[a] = 0.363
Case 3 Nasri et al. (1993) data *D–z* model	0.0314log	18	$D_{121.1\,°C} = 287.9$ min $z = 29.1$ °C	8.47 min 3.08 °C	−0.545 ($T_r = 376$ K)	(269.9, 305.8) (22.54, 35.6)	$D_{121.1\,°C} = 304 \pm 32$min $z = 30 \pm 3$ °C RMSE[b] = 0.0168

[a] RMSE computed in the present work based on Welt et al.'s (1997) parameter estimates.
[b] RMSE computed in the present work based on Nasri et al.'s (1993) parameter estimates on 6 means.

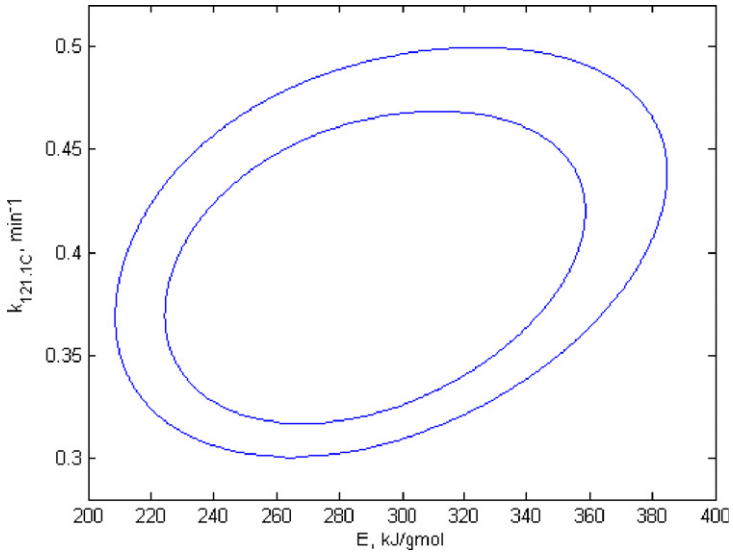

Fig. 1. Case 1. Welt (1996) data. 95% (inner) and 99% (outer) joint confidence region for parameters $k_{121.1\,^{\circ}C}$ and E ($T_r = 380$ K). All points represent constant sum-of-squares of errors per Eq. (26). Computed iteratively per Motulsky and Christopoulos (2004).

4.1.4. Dependent variable

The 95% confidence bands and 95% prediction bands for the survival ratio $\widehat{Y}_a$ are shown separately for each retort temperature (Figs. 2–4). In general, both the confidence and prediction bands were smaller at short and long times, and reached a maximum at some time in the middle (Fig. 2 shows the trend best). This non-constant size of CBs is common for nonlinear models (Bates & Watts, 1988, pp. 59–60). The width of the CBs ranged from 0 to 0.235, while prediction bands ranged from 0.519 to 0.570. As expected, more than half the points lie outside the CB, while all the points lie within the PB (Fig. 2). These plots reveal the power of using a nonisothermal method over an isothermal method, namely, that the rate constant and activation energy over a large temperature range can be found with fewer experiments. In this case, the temperature range of the material in the cans was 0 °C to ~120 °C. Covering a range that large with isothermal experiments would be prohibitively expensive and time-consuming.

4.2. Case 2

4.2.1. Parameter values

Analogous to Case 1, the $D_{121.1\,^{\circ}C}$ value (inversely proportional to rate constant) we estimated was 29% lower (6.31 vs. 8.9 min) than Welt et al. (1997) estimated, while the z value was the same (11.36 vs. 11.4 °C).

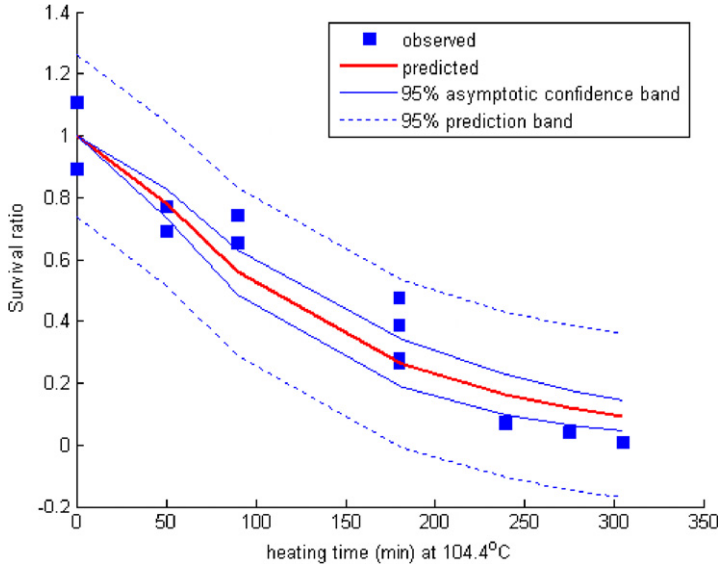

Fig. 2. Case 1. Welt (1996) data. 95% asymptotic confidence band and 95% prediction band for survival ratio for cans heated at retort temperature of 104.4 °C. Prediction curve, confidence band, and prediction bands were constructed by minimizing total sum-of-squares for the data in Figs. 2–4.

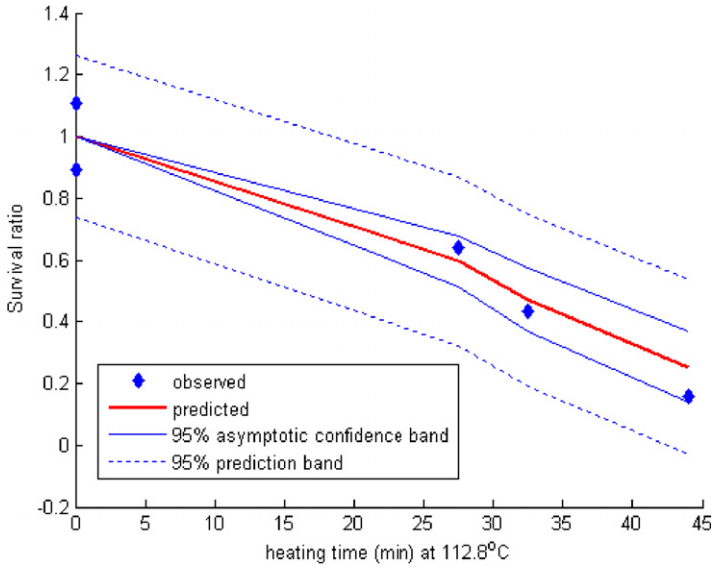

Fig. 3. Case 1. Welt (1996) data. 95% asymptotic confidence band and 95% prediction band for survival ratio for cans heated at retort temperature of 112.8 °C.

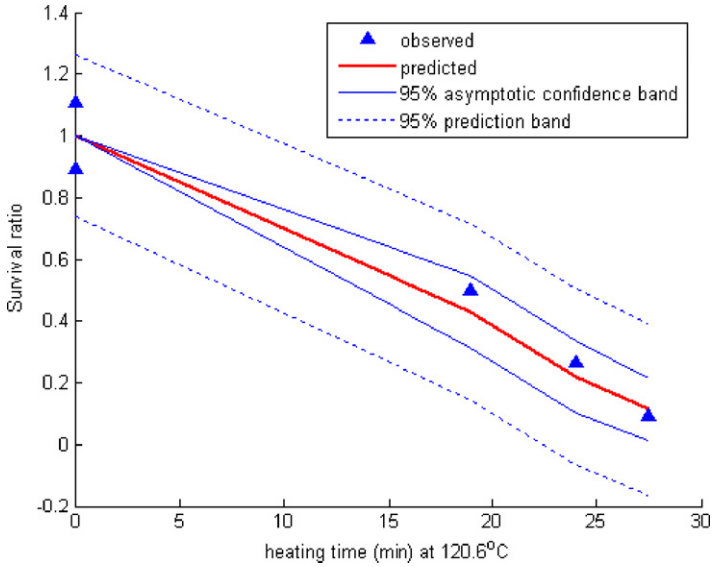

Fig. 4. Case 1. Welt (1996) data. 95% asymptotic confidence band and 95% prediction band for cans heated at retort temperature of 120.6 °C.

4.2.2. Parameter CRs

The 95% and 99% CRs slant to the right at 381 K (Fig. 5, positive correlation = 0.766, Table 3). Note that the range of the CI strongly depended on the estimates. For example, the 95% CI for z at the estimated value of $D_{121.1\,^{\circ}C} = 6.31$ min was (8.64, 14.1) (Table 3), while at $D_{121.1\,^{\circ}C} = 7.5$ min, the 95% CI values for z was much higher, approximately (11.4, 16.9) (Fig. 5). Note that these true contours are very different from the commonly used ellipse approximations.

4.2.3. Dependent variable

The confidence and prediction bands for $\log(N/N_0)$ at the 3 retort temperatures are shown in Figs. 6–8. The bands consistently increase with heating time in all plots. The confidence and prediction band widths ranged from 0 to 0.992 log, and 1.37 to 1.69 log, respectively. Both the small RMSE = 0.260 log (Table 3) and the relatively small size of the CBs (Figs. 6–8) show that the nonlinear fit was reasonably good. As with Case 1, more than half of the data at 104.4 °C fell outside the confidence band, but all data fell within the prediction band (Fig. 6). Note that although the model predicted a 1.54 log reduction after 305 min heating at retort temperature = 104.4 °C, the 95% prediction band showed only a 0.81 log reduction on the conservative (upper) side (Fig. 6). That is, one would expect 95% of the data to fall between −0.81 and −2.3 log, which is a 1.5 log difference. A processor should consider using the

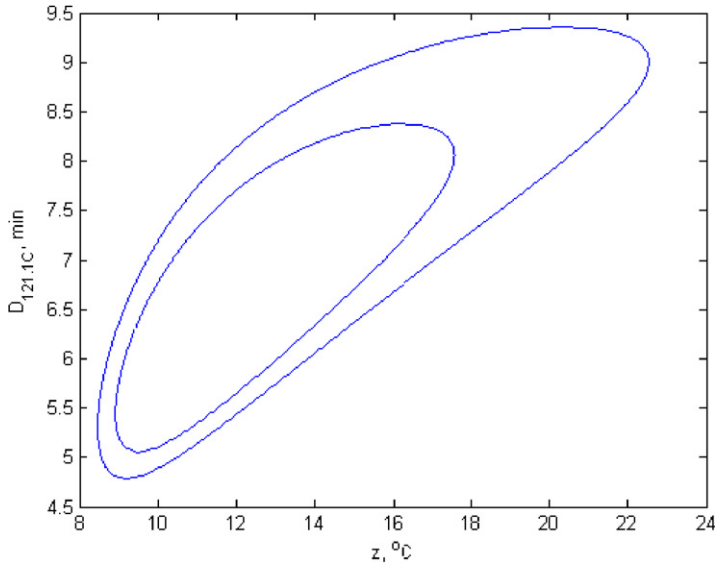

Fig. 5. Case 2. Welt (1996) data. 95% (inner) and 99% (outer) joint confidence region for parameters $D_{121.1\,^{\circ}C}$ and z ($T_r = 381$ K). All points represent constant sum-of-squares of errors per Eq. (26). Computed iteratively per Motulsky and Christopoulos (2004).

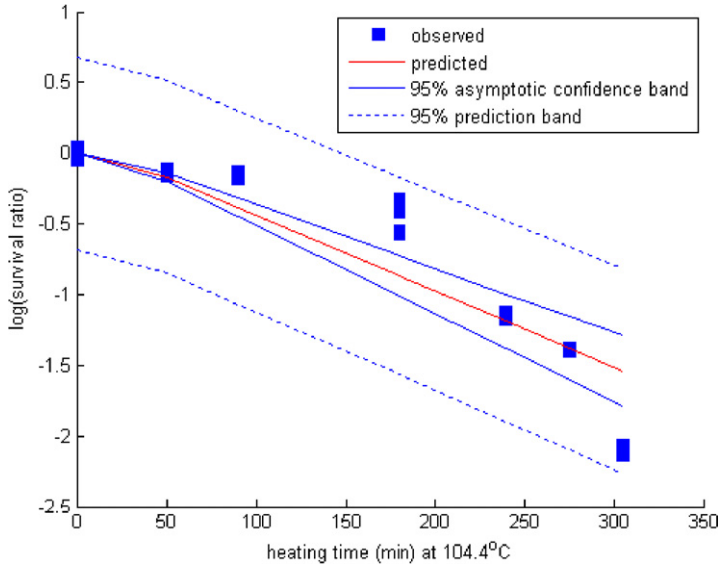

Fig. 6. Case 2. Welt (1996) data. 95% asymptotic confidence band and 95% prediction band for log(survival ratio) for cans heated at retort temperature of 104.4 °C. Prediction curve, confidence band, and prediction bands were constructed by minimizing total sum-of-squares for the data in Figs. 6–8.

upper 95% PB, rather than the predicted Y, as a conservative estimate. These types of error estimates can be helpful when predicting food safety.

4.3. Case 3

4.3.1. Parameter values

Our estimate of $D_{121.1\,^{\circ}C} = 287.9$ min was 5% lower than Nasri et al.'s (1993) estimate of 304 min, but our 95% confidence interval (269.9, 305.8) included their value (Table 3). Likewise, our z estimate was statistically the same as Nasri et al.'s (1993) (29.1 vs. 30 °C).

4.3.2. Parameter CIs

Nasri et al. (1993) did not report CIs, but reported standard error. The standard error of z was identical in our work and in Nasri et al.'s (3 °C). However, unlike in Case 1, our standard error of $D_{121.1\,^{\circ}C}$ was about 1/4 the size of that computed by Nasri et al. (8.47 vs. 32 min). There are two possible causes for this large difference: (1) Nasri et al. (1993) used only the 6 mean survival ratios in their nonlinear regression, while we used all 18 individual survival ratios; (2) The jackknife method Nasri et al. (1993) used is different from the asymptotic method we used. The jackknife method and other Monte Carlo methods

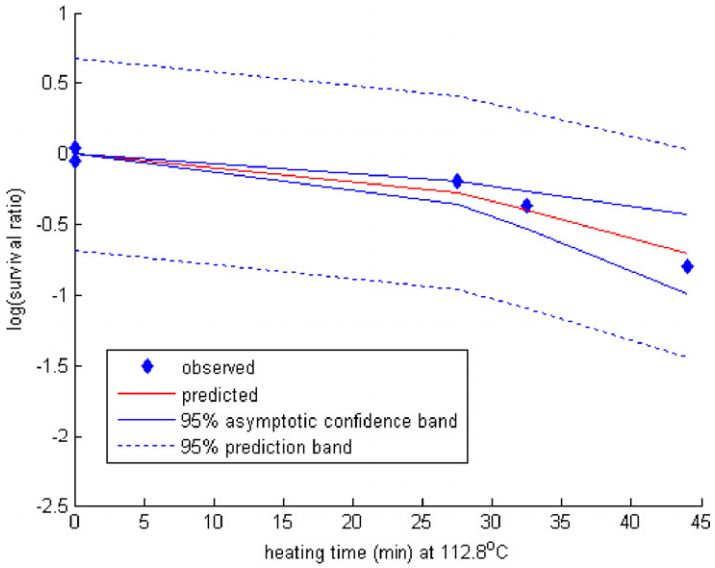

Fig. 7. Case 2. Welt (1996) data. 95% asymptotic confidence band and 95% prediction band for log(survival ratio) for cans heated at retort temperature of 112.8 °C.

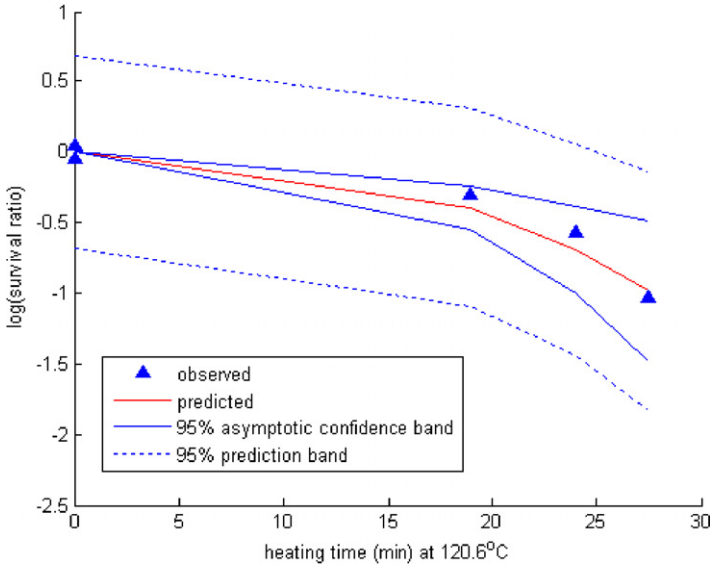

Fig. 8. Case 2. Welt (1996) data. 95% asymptotic confidence band and 95% prediction band for log(survival ratio) for cans heated at retort temperature of 120.6 °C.

are usually considered to give more realistic estimates of the error than asymptotic methods, because the Monte Carlo methods are based on repeated iterations of the subsets of the data. These results show that the method used to obtain the standard error and CIs should be reported, because the difference can be large.

4.3.3. Parameter confidence regions (CRs)

The 95% and especially the 99% CR were both heavily skewed (Fig. 9) from the elliptical shapes often used as approximations. On the 95% confidence contour, the wide variation of z (23–42 °C) in the region of ca. $270 \leqslant D_{121\,^{\circ}C} \leqslant 306$ min may have been because of the limited data set covering a small range (less than one log) of survival ratios (Table 2).

4.3.4. Dependent variable

Graphical confidence and prediction bands for the dependent variable over time could not be shown, because there was only one heating time for each of the six retort temperatures (Table 2). The individual 95% confidence bounds and prediction bounds for each retort condition (Table 4) show a minimum at a middle time (211 min at 109 °C retort temperature). This behavior was opposite from that in Case 1.

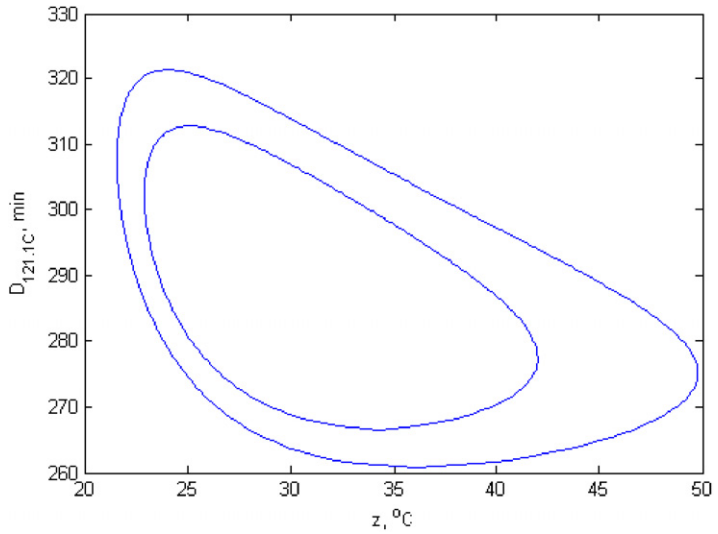

Fig. 9. Case 3. Nasri et al. (1993) data. 95% (inner) and 99% (outer) joint confidence region for parameters $D_{121\,^{\circ}C}$ and z ($T_r = 376$ K). All points represent constant sum-of-squares of errors per Eq. (26). Computed iteratively per Motulsky and Christopoulos (2004).

Table 4
Case 3. Nasri et al. (1993) data

Retort temperature (°C)	Heating time (min)	Predicted log(retention)	95% confidence band width (log)	95% prediction band with (log)
116	92	−0.139	0.0494	0.1762
115	100	−0.145	0.0481	0.1758
112	137	−0.177	0.0457	0.1752
109	211	−0.239	0.0440	0.1748
106	367	−0.354	0.0475	0.1757
103	675	−0.535	0.0897	0.1914

95% Confidence intervals and 95% prediction intervals for log(retention) for each of six retort time–temperature conditions.

5. Conclusions

Most literature that reports confidence intervals for kinetic parameters has not reported the confidence interval for predicted Y, even though the predicted Y is more important than the estimated parameters for food safety purposes. In this article, three case studies were examined. The confidence intervals for parameters and the confidence and prediction intervals for predicted Y were computed. Both the k–E and the D–z model were used. Upper 95% prediction bands gave a more conservative (safer) limit than the Y value predicted by the model, up to a 0.84 log difference. This result indicates that for food safety considerations, the upper prediction band is more important than the model prediction (regression line). Confidence regions for the parameters were sometimes highly skewed from the typical elliptical approximations used in most of the literature. The intent of this work was to propose a framework for other researchers to use when reporting error in kinetic studies. Because this nonlinear regression method is easy to use, and can be implemented conveniently using Matlab® or Visual Basic for Applications on Excel®, we hope that researchers will use the method on other data to disprove or confirm our results.

Acknowledgements

We thank Dr. Bradley Marks for helpful comments in preparing this manuscript. This research has been supported in part by NSF grant DMS0405330.

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