Food Research International 89 (2016) 614–619

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

One-step global parameter estimation of kinetic inactivation parameters for *Bacillus sporothermodurans* spores under static and dynamic thermal processes

F. Cattani [a], K.D. Dolan [b,c], S.D. Oliveira [a], D.K. Mishra [c,d], C.A.S. Ferreira [a], P.M. Periago [e], A. Aznar [e], P.S. Fernandez [e], V.P. Valdramidis [f,*]

[a] *Laboratório de Imunologia e Microbiologia, Faculdade de Biociências, PUCRS, Brazil*
[b] *Department of Food Science & Human Nutrition, Michigan State University, East Lansing, MI 48824, USA*
[c] *Department of Biosystems & Agricultural Engineering, Michigan State University, East Lansing, MI 48824, USA*
[d] *Department of Food Science, Purdue University, West Lafayette, IN, USA*
[e] *Department of Food Engineering and Agricultural Machinery, Institute of Vegetable Biotechnology, Regional Campus of International Excellence "Campus Mare Nostrum", Technical University of Cartagena (UPCT), P. Alfonso XIII, No. 48, 30203 Cartagena, Spain*
[f] *Department of Food Studies and Environmental Health, Faculty of Health Sciences, University of Malta, Msida, Malta*

ARTICLE INFO

Article history:
Received 15 May 2016
Received in revised form 8 August 2016
Accepted 22 August 2016
Available online 26 August 2016

Keywords:
Thermal technologies
High heat resistance
Bacillus sporothermodurans
Spore forming bacteria, one-step regression

ABSTRACT

Bacillus sporothermodurans produces highly heat-resistant endospores, that can survive under ultra-high temperature. High heat-resistant sporeforming bacteria are one of the main causes for spoilage and safety of low-acid foods. They can be used as indicators or surrogates to establish the minimum requirements for heat processes, but it is necessary to understand their thermal inactivation kinetics. The aim of the present work was to study the inactivation kinetics under both static and dynamic conditions in a vegetable soup. Ordinary least squares one-step regression and sequential procedures were applied for estimating these parameters. Results showed that multiple dynamic heating profiles, when analyzed simultaneously, can be used to accurately estimate the kinetic parameters while significantly reducing estimation errors and data collection.

1. Introduction

The safety of shelf-stable food products, such as vegetable soups, depends on the processes applied to inactivate heat resistant spores. The application of heat is both an important method of preserving foods and a means of developing texture, flavour and colour of products. However, thermal treatments are not always sufficient to inactivate all bacterial spores, especially those that are highly heat resistant and non-pathogenic (Hornstra, Ter Beek, Smelt, Kallemeijn, & Brul, 2009). Thermal inactivation of bacterial spores has been the topic of many studies due to their high heat resistance. *Bacillus sporothermodurans* can produce highly heat-resistant spores (HRS) that may survive UHT treatment (135 to 142 °C for a few seconds) (Hammer, Lembke, Suhren, & Heeschen, 1995; Pettersson, Lembke, Hammer, Stackebrandt, & Priest, 1996). *B. sporothermodurans* spores have been found to resist temperatures above 130 °C with D_{140} ranging from 3.4–7.9 s and *z*-values ranging from 13.1–14.2 °C (Huemer, Klijn, Vogelsang, & Langeveld, 1998).

An essential issue for food manufacturers is the effective application of thermal treatments without damaging the desirable sensory and nutritional qualities in a food product (Richardson, 2004). Therefore, the need for ensuring the microbiological quality and safety of food products has stimulated interest in using mathematical models for quantifying and predicting microbial survival during thermal processing. It is a traditional approach to estimate microbial model parameters under isothermal conditions and they are then validated under dynamic realistic conditions. For thermal processing, graphical integration is used to estimate the survival of bacteria under dynamic conditions (Conesa, Andreu, Fernández, Esnoz, & Palop, 2009). Recent reports have highlighted that (inactivation) model equations and their associated parameter values obtained under static conditions (e.g., acid, thermal, etc) cannot be used directly for predicting dynamic conditions (Janssen et al., 2008; Valdramidis, Geeraerd, Bernaerts, & Van Impe, 2006). This result can be attributed either to the model structure properties (Janssen et al., 2008), the induced stress resistance phenomena (Valdramidis, Geeraerd, & Van Impe, 2007; Velliou et al., 2011), or the effect on the accuracy and precision of the parameter estimates (Jewell, 2012; Valdramidis, Geeraerd, Bernaerts, & Van Impe, 2008). A way to tackle

* Corresponding author.
E-mail addresses: fernandacattani@yahoo.com.br (F. Cattani), pablo.fernandez@upct.es (P.S. Fernandez), vasilis.valdramidis@um.edu.mt (V.P. Valdramidis).

http://dx.doi.org/10.1016/j.foodres.2016.08.027
0963-9969/

these phenomena is by implementing approaches in which parameter estimates are obtained under realistic dynamic environments, closer to real processing conditions and, therefore, to the inactivation treatment that the spores are exposed to (Chen, 2013; Dogan, Weidendorfer, Mueller-Merbach, Lembke, & Hinrichs, 2009; Dolan, Valdramidis, & Mishra, 2013; Dolan, Yang, & Trampel, 2007; Huang, 2013; Peleg, Normand, & Campanella, 2003; Valdramidis et al., 2008). These nonlinear parameter studies, also known as inverse problems, have been based on dynamic (simulated) data of specific temperature profiles.

It is also important to highlight that inverse problems in quantitative food microbiology may have a number of potential pitfalls, such as lack of convergence, convergence to local minima, over-parameterization, high correlation, leading to large parameter confidence intervals, and poor sequential estimation as data are successively added (Dolan & Mishra, 2012). Therefore, the objective of this study was to characterize the microbial resistance of *B. sporothermodurans* spores in vegetable soup under static and dynamic temperature conditions and apply parameter estimation techniques.

2. Materials and methods

2.1. Microorganism and spore crop preparation

Bacillus sporothermodurans IC4 (Unilever Netherlands Sourcing Unit Oss) was isolated from an Indian curry soup and was able to survive high heat treatments. In order to sporulate the microorganism, a freeze-dried sample was rehydrated in Nutrient Broth (NBScharlau, Barcelona, Spain) and incubated at 37 °C for 12-14 h under continuous agitation. Once turbidity was evident, the culture was streaked to check purity on Nutrient Agar (NA Scharlau). Plates containing Fortified Nutrient Agar (FNA) (Mazas, Gonzalez, Lopez, Gonzalez, & Sarmiento, 1995) were used as sporulation media. A bacterial suspension was prepared by flooding the NA plates, after incubated at 37 °C for 24 h, with buffered peptone water. This suspension was collected with sterile pipettes and used as inoculum. Plates containing the sporulation medium were inoculated with 0.2 mL of the suspension and allowed to dry under aseptic conditions in a laminar-flow cabinet. The plates containing sporulation media were then incubated at 37 °C for at least 4 days, until a sporulation rate reaching at least 90% was accomplished. Spores were harvested and then centrifuged three times (5000 g for 10 min, at 4 °C). Spores of *B. sporothermodurans* were suspended in distilled water and stored at −20 °C until further use.

2.2. Vegetable soup

A vegetable soup treated by ultra high temperature (UHT) processing was purchased from a local supermarket. Ingredients were water, onions, carrots, leek, celery, olive oil and salt. Nutritional information (100 mL): energy, 9 kCal/40 kJ; protein content 0.5 g; carbohydrates 1.4 g; fat 0.2 g; NaCl 0.4 g. The final pH was 6.2.

2.3. Isothermal and non-isothermal heat treatment

All heat treatments were carried out in a thermoresistometer Mastia that can be programmed to perform isothermal and non-isothermal experiments. In brief, the instrument consists on a stainless steel vessel of 400 mL volume which can be pressurized through a manometer. Temperature control is performed with a PLC, by means of a PID (Proportional Integral Derivative) controller. The vessel was filled with 350 mL of the substrate, pressurized and then set to the treatment temperature. Preliminary tests were performed in distilled water to verify that the heat resistance of the strain was similar to previously published studies (data not shown). For isothermal treatments, the vessel was filled with the vegetable soup as heating medium. The instrument was sterilized with distilled water, cooled, emptied, and immediately filled with vegetable soup and heated to the temperature of treatment. This temperature was kept constant through all the experiment and, once the heating temperature was stabilized at the set point, the *B. sporothermodurans* spore suspension was injected (0.2 mL). The samples were collected into sterile test tubes at preset time intervals, appropriately diluted, immediately plated, and incubated. Before sampling, the contents of the sampling tube of the thermoresistometer were discarded. The temperatures for isothermal treatments were 118, 121, 124, and 127 °C. Experiments were performed in triplicate for each temperature.

For non-isothermal treatments the thermoresistometer was programmed to change in temperature according to selected temperature profiles. The non-isothermal treatments for the vegetable soup were run in a temperature range from 80 to 121 °C at a rate of 1.5 °C/min and from 75 to 121 °C at a rate of 2.6 °C/min. These temperature profiles were selected to simulate typical processing conditions used in the food industry to process low acid soups. The number of survivors in each sample was established by decimal serial dilutions in sterile peptone water, and plated on Nutrient Agar. Plates were incubated at 37 °C for 48 h to determine the number of bacterial spores expressed as CFU/mL. All experiments were performed in triplicate for each profile tested.

2.4. Model structure selection

A preliminary assessment of the isothermal data was performed using GInaFiT (Geeraerd, Valdramidis, & Van Impe, 2005). The most suitable primary model structure appeared to be the classical log-linear first order inactivation model (refer to the results section).

$$\frac{d\log_{10}N(t)}{dt} = -\frac{1}{D_{ref}}10^{\left(\frac{T-T_{ref}}{z}\right)} \quad (1)$$

Herein, $\log_{10}N(t)$ represents the microbial cell density [log (CFU/mL)], D_{ref} is the decimal reduction time (min) and z the thermal resistance constant (°C). In the case of the dynamic temperature profiles, temperature T, which was recorded every 5 s, was plugged into Eq. (1). Linear interpolation was performed for estimating temperatures between the recorded values.

2.5. Scaled sensitivity coefficients

The plot of the scaled sensitivity coefficients X' versus the independent variable of time can be used to assess the degree of linear dependence and to compare the maximum absolute value of each X' to the total change of the dependent variable. Therefore, the scaled sensitivity coefficient for each parameter was computed as follows for the dynamic temperature profiles:

$$X\prime_{\beta_\iota} = \beta_\iota \frac{\partial log N(t)}{\partial \beta_\iota} \quad (2)$$

where β_i was the ith parameter. The units of X' are the same as those for log$N$, and can be compared directly. The parameters with the largest and most uncorrelated X' will be easiest to estimate, and will have the smallest relative errors (Beck & Arnold, 2007; Dolan & Mishra, 2013).

2.6. Parameter estimation

2.6.1. Ordinary least squares and estimation of reference temperature

Two different types of parameter identification approaches were applied during the regression analysis of Eq. (1): (i) all isothermal data were analyzed simultaneously, (ii) dynamic profiles were analyzed individually and (ii) all data of the two dynamic profiles were analyzed simultaneously. For these approaches, the ordinary Least Squares (OLS) minimization in MATLAB (Version 2011a), lsqnonlin or nlinfit (nonlinear regression routine in the statistical toolbox), and ode45 (Runge-

Kutta adaptive 4th- and 5th-order method) were used to estimate the model parameters. Statistical analysis included the estimation of sum of squared error (SSE), mean squared error (MSE), root mean squared error (RMSE), estimation of the parameters standard error, relative error (%), and the 95% confidence interval of the estimated parameters.

The best-fit parameter estimates (D_{ref}, z, and all log$N(0)$ parameters) were those resulting from minimizing the sum of squares of residuals, where residual = $\log N_{observed} - \log N_{predicted}$, where $\log N_{observed}$ are the data, and $\log N_{predicted}$ is computed from Eq. (1). Parameter asymptotic errors were computed as the square root of the corresponding diagonal of the var.-covariance matrix (cov(a), below) (Dolan, 2003; van Boekel, 1996)

$$\mathbf{cov}(\mathbf{a}) = \left(\mathbf{X}^{\mathrm{T}}\mathbf{X}\right)^{-1}(MSE) = \begin{pmatrix} \sigma^2_{D_r} & \sigma_{D_r z} \\ \sigma_{D_r z} & \sigma^2_{z} \end{pmatrix} \quad (3)$$

where $\mathbf{X}$ is the sensitivity matrix, an n x p matrix (n is the number of data; p is the number of parameters) of sensitivity coefficients, where each column corresponds to one parameter, and the jth sensitivity coefficient for the ith parameter = $\partial X_j/\partial \beta_i$, and j ranges from 1 to n, while i ranges from 1 to p. MSE is the mean square error = (sum of squares)/$(n - p)$. The cov(a) matrix used in the present work had more columns than the two shown in Eq. (3), because there were initial value parameters $\log N(0)_1$, $\log N(0)_2$, etc.

The structure of the Bigelow model requires the assignment of a finite reference temperature. Therefore, an optimum T_{ref} was determined by analysing the correlation coefficient between D_{ref} and z versus T_{ref} for a number of arbitrary chosen values and interpolating where the correlation coefficient ~0 (Dolan et al., 2013; Schwaab & Pinto, 2007). There was a different optimum T_{ref} for each data set because each set had different temperature profiles, i.e. one T_{ref} for results for all static data combined, another T_{ref} for ramp 1 only, another T_{ref} for ramp 2 only, and one T_{ref} for both ramp 1 and 2 combined. For comparison purposes, the D_{ref} values were converted to one common $T_{ref,}$ discussed in Results.

2.6.2. Sequential estimation

For non-isothermal data, a sequential estimation was applied in which the model for a given data set was assessed by how well each parameter approaches a constant before the end of the experiment. Similar to Dolan et al. (2013), this sequential procedure was developed using the matrix inversion lemma (Beck & Arnold, 1977) based on the Gauss minimization method. To compare parameters to each other, the value of the sequential parameter was normalized as follows: Normalized sequential parameter = parameter value at time t/last value of parameter at end time.

3. Results and discussion

Heat resistance of *B. sporothermodurans* was characterized over a wide range of temperatures (both for isothermal and non-isothermal treatments) in the vegetable soup. The survival curves of *B. sporothermodurans* IC4 were generated under isothermal and dynamic conditions. The optimum reference temperature for the combined ramp set was found to be 121 °C. The correlation coefficient between D_{ref} and z was −0.25. Although not equal to zero, this correlation coefficient gave the lowest relative error (2.82%) for D_{ref}. This optimum temperature = 121 °C was near the high end of the temperature range, consistent with theory: "When D- and z-values are used and food temperature is varying, the optimum value of reference temperature…is close to the maximum temperature achieved by the food material. Thus, the reference temperature selected should be very close to the maximum process temperature" (Datta, 1993). The optimum T_{ref} for the other experiments is shown in Table 1, and is also near the high end of their respective temperature ranges.

The isothermal results (Fig. 1) showed that due to the high resistance of the spores, temperature as high as 127 °C was required with a treatment time of 3 min in order to achieve a 2.5-log microbial inactivation. At 121 °C, a treatment time of 15 min was required to reduce microbial populations by 3-log cycles. Table 1 and Fig. 1 show the results of the regression analysis for the isothermal data. The application of a one-step regression analysis of all the isothermal data prevents accumulation of fitting errors (Valdramidis et al., 2005). The estimated parameter of the present work resulted in higher D_{121} values than those obtained from distilled water in which *B. sporothermodurans* IC4 was sporulated in mushroom soup agar at 121 °C (van Zuijlen et al., 2010).

Table 1
Parameter estimates, standard errors, 95% confidence intervals and statistical indices of the performed parameter identification techniques.

All static data					
Parameters	Estimates	SE	Rel. error	95% CI	95% CI
$D_{122.6\ °C}$ (min)	3.63	0.147	4.05%	3.34	3.92
z (°C)	9.20	0.465	5.05%	8.28	10.13
$\log_{10N(0)1}$ (log10(cfu/mL))	4.59	0.093	2.03%	4.41	4.77
$\log_{10N(0)2}$ (log10(cfu/mL))	4.82	0.081	1.67%	4.66	4.98
$\log_{10N(0)3}$ (log10(cfu/mL))	4.63	0.074	1.60%	4.48	4.78
$\log_{10N(0)4}$ (log10(cfu/mL))	4.50	0.094	2.08%	4.32	4.69
$D_{121\ °C}$ (min, converted from Dr)	5.42			4.98	5.86
SSE, MSE, RMSE = 12.62, 0.11, 0.333					
Ramp 1					
$D_{120.2\ °C}$ (min)	6.03	0.2154	3.57%	5.55	6.41
z (°C)	16.1	2.9822	18.48%	10.13	22.16
$\log_{10N(0)1}$ (log10(cfu/mL))	4.38	0.0547	1.25%	4.27	4.49
$D_{121\ °C}$ (min, converted from Dr)	5.38			5.00	5.76
SSE, MSE, RMSE = 1.68, 0.04, 0.20					
Ramp 2					
$D_{116\ °C}$ (min)	15.40	1.00	6.48%	13.38	17.43
z (°C)	21.6	6.77	31.34%	7.87	35.34
$\log_{10N(0)2}$ (log10(cfu/mL))	4.46	0.09	1.94%	4.29	4.64
D_{121C} (min, converted from Dr)	7.55			6.57	8.53
SSE, MSE, RMSE = 1.23, 0.034, 0.182					
Ramp 1 and Ramp 2					
$D_{121\ °C}$ (min)	5.68	0.1604	2.82%	5.36	6.00
z (°C)	11.2	0.7552	6.77%	9.65	12.65
$\log_{10N(0)1}$ (log10(cfu/mL))	4.29	0.0363	0.85%	4.21	4.36
$\log_{10N(0)2}$ (log10(cfu/mL))	4.39	0.0545	1.24%	4.28	4.50
SSE, MSE, RMSE = 3.04, 0.038, 0.195					

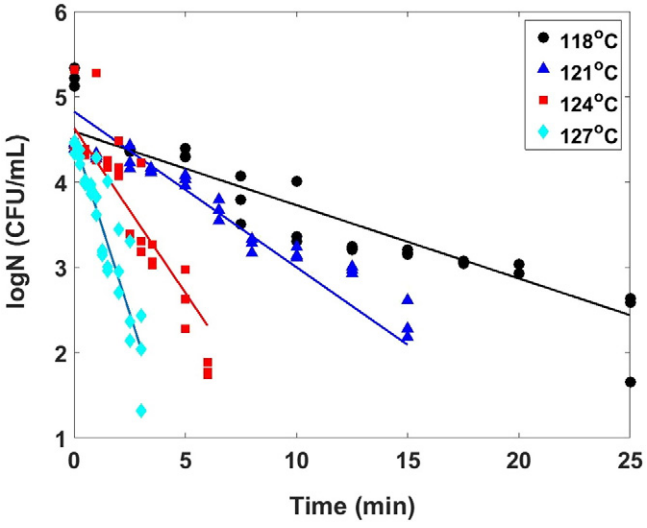

Fig. 1. Regression analysis of all the static data by the use of Eq. (1) at the following temperatures: 118, 121, 124 and 127 °C.

This is an expected result considering the protective effect of nutrients that are present in the soup.

Based on the model structure selection of the isothermal data, regression analysis was also performed for the non-isothermal data by using the same model (Fig. 2, Fig. 3, Table 1). For comparison purposes, the D values were converted to a common $T_{ref} = 121$ °C (Table 1). Only $D_{121°C}$ for Ramp 2 (7.55 min) was significantly different ($P < 0.05$) from $D_{121\,°C}$ for the combined ramps (5.68 min), as can be seen by inspection of the 95% confidence intervals for the $D_{121\,°C}$. Specifically, the 95% CI for Ramp 2 (6.56, 8.53) does not contain the $D_{121\,°C}$ for the combined ramp (5.68 min). The z-values obtained were slightly higher compared to those published in the literature for this microorganism. They ranged from 7 to 8.3 °C when suspended in distilled water (van Zuijlen et al., 2010) and from 6.6 to 7.3 °C when suspended in McIlvaine buffer at pH values of 7, 5 and 3 or courgette puree (Esteban, Huertas, Fernández, & Palop, 2013).

Although the $D_{121\,°C}$ value for the static data (5.42 min) was not significantly different from that for the combined ramps (5.68 min), the accuracy of the latter was greater. The relative error for the D value was 30% smaller for the combined ramp (2.8 vs. 4%). The reverse was the case for the z values: The z value for the isothermal test (9.2 °C) was 18% smaller than that for the combined ramp dynamic set (11.2 °C). The relative errors for the z values were 25% smaller for the static test (5.05%) than for the dynamic test (6.77%). Regarding the fit of the model to the data, RMSE value for the dynamic data (0.195 logCFU/mL) was 40% smaller than that for the static data (0.333 logCFU/mL) In summary, the dynamic parameter results were more accurate for the D value and RMSE; and not as accurate for the z value. Even though the z value was less accurate for the dynamic results, the dynamic results were obtained using fewer data (84 dynamic data versus 120 static data), and running only 2 dynamic tests versus 4 static tests. Overall, the dynamic data results were more accurate than those for the static data.

Initially, regression analysis of the dynamic data was performed for each individual dynamic profile, i.e., ramp1, ramp2. Despite the high fitting capacity of the model (ramp1 with RMSE = 0.20, ramp2 with RMSE = 0.18), the parameter estimates had high relative errors and the D_{121} and z values were significantly different between the two ramps (5.38 vs. 7.55 min). The estimates of z for ramp1 and ramp2 were 16.1 and 21.6 °C, respectively. The estimate of z for the combined ramps was 11.2 °C. The reason for this wide variation was that the error for z for each ramp individually was too large (z1 and z2 errors were 18.5 and 31.3%, respectively). The errors were so large that the confidence intervals contained the combined-ramp estimate of 11.2 °C (Table 1).

The cause of the large errors in z is the difficulty in estimating the temperature-sensitivity parameter (z) with only one dynamic experiment A minimum of two dynamic experiments are required for accurate estimation of z. The reason for needing at least two dynamic experiments is as follows: For only one dynamic experiment, the optimum reference temperature T_{ref} must be found to uncorrelate D_{ref} and z. However, after finding optimum T_{ref}, the sensitivity coefficient (SSC) for z is then typically too small for accurate estimation. Therefore, the better solution is to combine two dynamic temperature experiments and find the optimum T_{ref}. For this combined case, at least one SSC for z will be uncorrelated with the SSC for D_{ref} (desirable) while the other SSC for z will be large. The result will be a much more accurate estimate for z.

In the present case, the SSC for z for ramp1 is small, but highly uncorrelated with SSC for D_{ref} (Fig. 4). The SSC for z for ramp2 is also uncorrelated and is sufficiently large to allow z to be estimated more accurately. Fig. 4 also shows that slower experiments allow more accurate estimation of z, because the SSC for z is larger during the times most of the data were being collected (time = 20–50 min). Referring to a previous study, slower heating rates were also shown to give more accurate estimates for inactivation of *E. coli.* K12 (Dolan et al., 2013). Regarding the accuracy of fit, the RMSE values for Ramp 1 only (0.20 log(cfu/mL)), Ramp 2 only (0.182), and combined Ramps (0.195) are nearly the same. However, a low value of RMSE is valuable only when parameter estimates are accurate. For Ramp 1 only and Ramp 2 only, the z values are highly inaccurate.

Previous studies have also shown that the more the microbial system is excited, i.e., application of stressed environments (in this case by the application of temperature variations) the more the obtained information related to the microbiological responses (Valdramidis et al., 2008). In addition, these types of microbiological results can be generated more easily saving time and resources compared to the traditional methodology of the isothermal data approach. For example isothermal data points were collected from isothermal experiments, while only 22 non-isothermal data points were collected in dynamic studies. It should also be highlighted that the estimated parameters (from the experimental sets of ramp1, ramp2) are derived from a set of dynamic experiments that cover the complete temperature range of a realistic industrial process environment (i.e., heating up, holding and cooling profile, as it happens typically in retort processing).

In addition, when the scaled sensitivity coefficients were analyzed (Fig. 4) it was evident that both *Dref* and z could be easily estimated because a) the absolute values of X' for *Dref* were large and uncorrelated (different shapes) for both ramps, and b) the absolute value of X' for z was large and uncorrelated for ramp 2. The X' for z for ramp1 was highly uncorrelated (different shape and crossed the zero line), but contributed less to the estimation of z because its absolute value was smaller than X' z for ramp2 (Fig. 4). These results are also evident in Table 1. The large values of X'log(N)1 at ramp 1 and X'log(N)2 at ramp2 were not shown in Fig. 4 to avoid clutter, but they indicate that these parameters can

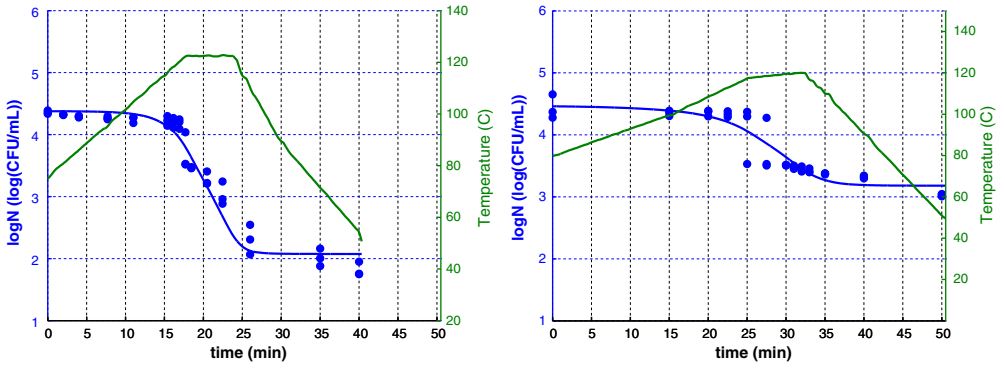

Fig. 2. Regression analysis of data collected in ramp 1 (A) for a temperature profile from 80 to 121 °C and ramp 2 (B) for a temperature profile from 75 to 121 °C; by the use of Eq. (1) in both cases. Temperature profile appears with an overlaid line.

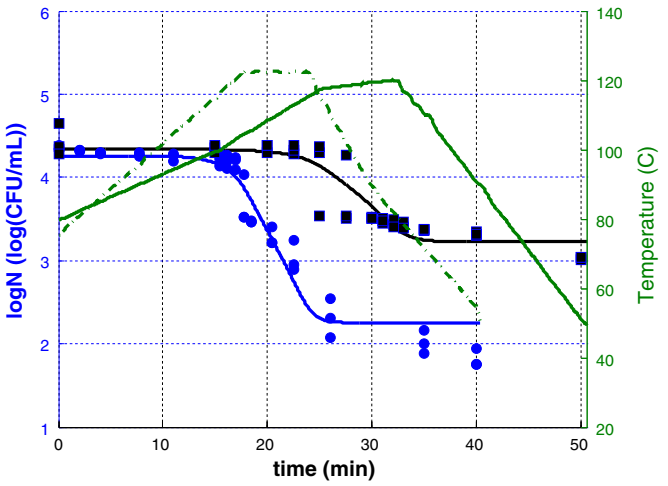

Fig. 3. Regression analysis of both data collected during ramps 1 (-) and 2 (–) by the use of Eq. (1). Temperature profile appears with an overlaid line.

be easily estimated with small variance of the parameter estimates. The initial values in many models often are the most accurately estimated parameters, as in this case.

Sequential estimation of parameters in a model provides good information into building the model and determining the uncertainty in parameters. If parameters come to a constant value after certain time then the experiment can be stopped, as further data will not improve the parameter estimates. Fig. 5 illustrates the progression with each datum for parameters *Dref* and *z*, which are the most important for characterizing microbial inactivation kinetics. Parameters approached a constant after 22 and 40 min for *D*ref and *z* value, respectively. The data collection could have been reduced by completing the dynamic experiments when time reached 40 min (as evident from the experimental data).

4. Conclusions

The implementation of regression analysis in which parameter estimates are obtained under dynamic environments is imperative in order to assess microbial inactivation kinetics in realistic conditions. Although this type of studies has been previously discussed on some dynamic (simulated) data of specific temperature profiles, further characterization of target microorganisms (spores for sterilization, or vegetative organisms for pasteurization) will be required in future research activities. This conclusion is drawn considering that inactivation parameters estimated under non-isothermal conditions give accurate and precise estimates with fewer total data than that for isothermal conditions.

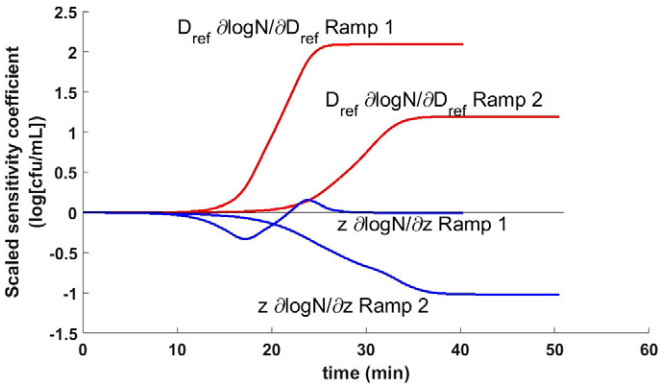

Fig. 4. Scaled sensitivity coefficients for Eq. (1) for both ramp1 and ramp2.

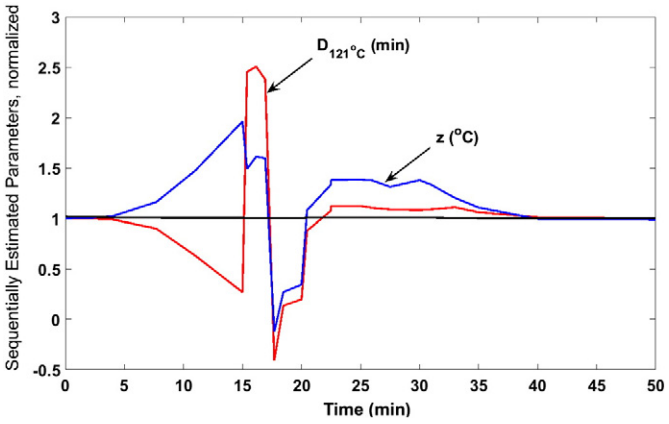

Fig. 5. Sequentially estimated D_{ref} and z for Eq. (1).

Acknowledgements

Fernanda Cattani acknowledges Programa Capes/Fundação Carolina (Brazil) for financial support. This project was partly funded by the Ministry of Economy and Competitiveness (MINECO) of the Spanish Government and European Regional Development Fund (ERDF) through project AGL2013- 48993-C2-1-R. This research was also partly supported by a Marie Curie FP7-Reintegration-Grant (303939) within the 7th European Community Framework Programme under the project Development of novel Disinfection Technologies for Fresh Produce (DiTec), and by the COST ACTION CA15118 Mathematical and Computer Science Methods for Food Science and Industry (FoodMC).

References

Beck, J., & Arnold, K. (1977). *Parameter estimation in engineering and science.* New York: Wiley.

Beck, J., & Arnold, K. (2007). *Parameter estimation in engineering and science.* New York: Wiley.

Chen, G. (2013). A comparison of two methods for estimating microbial survival parameters from dynamic survival data. *International Journal of Food Science & Technology*, *48*(5), 1109–1113. http://dx.doi.org/10.1111/ijfs.12058.

Conesa, R., Andreu, S., Fernández, P. S., Esnoz, A., & Palop, A. (2009). Nonisothermal heat resistance determinations with the thermoresistometer Mastia. *Journal of Applied Microbiology*, *107*(2), 506–513. http://dx.doi.org/10.1111/j.1365-2672.2009.04236.x.

Datta, A. K. (1993). Error-estimates for approximate kinetic-parameters used in food literature. *Journal of Food Engineering*, *18*(2), 181–199. http://dx.doi.org/10.1016/0260-8774(93)90035-I.

Dogan, Z., Weidendorfer, K., Mueller-Merbach, M., Lembke, F., & Hinrichs, J. (2009). Inactivation kinetics of *Bacillus* spores in batch- and continuous-heating systems. *Lwt-Food Science and Technology*, *42*(1), 81–86. http://dx.doi.org/10.1016/j.lwt.2008.05.005.

Dolan, K. D. (2003). Estimation of kinetic parameters for nonisothermal food processes. *Journal of Food Science*, *68*(3), 728–741. http://dx.doi.org/10.1111/j.1365-2621.2003.tb08234.x.

Dolan, K. D., & Mishra, D. K. (2012). *Parameter identification under dynamic temperature conditions in inactivation kinetics: Nova science publishers.*

Dolan, K. D., & Mishra, D. K. (2013). Parameter estimation in food science. *Annual Review of Food Science and Technology*, *4*(4), 401–422. http://dx.doi.org/10.1146/annurev-food-022811-101247.

Dolan, K. D., Yang, L., & Trampel, C. P. (2007). Nonlinear regression technique to estimate kinetic parameters and confidence intervals in unsteady-state conduction-heated foods. *Journal of Food Engineering*, *80*(2), 581–593. http://dx.doi.org/10.1016/j.jfoodeng.2006.06.023.

Dolan, K. D., Valdramidis, V. P., & Mishra, D. K. (2013). Parameter estimation for dynamic microbial inactivation: Which model, which precision? *Food Control*, *29*(2), 401–408. http://dx.doi.org/10.1016/j.foodcont.2012.05.042.

Esteban, M. D., Huertas, J. P., Fernández, P. S., & Palop, A. (2013). Effect of the medium characteristics and the heating and cooling rates on the nonisothermal heat resistance of *Bacillus sporothermodurans* IC4 spores. *Food Microbiology*, *34*(1), 158–163. http://dx.doi.org/10.1016/j.fm.2012.11.020.

Geeraerd, A. H., Valdramidis, V. P., & Van Impe, J. F. (2005). GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. *International Journal of Food Microbiology*, *102*(1), 95–105. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.11.038.

Hammer, P., Lembke, F., Suhren, G., & Heeschen, W. (1995). Characterization of a heat resistant mesophilic *Bacillus* species affecting quality of UHT-milk — A preliminary report. *Kieler Milchwirtschaftliche Forschungsberichte*, *47*(4), 297–305.

Hornstra, L. M., Ter Beek, A., Smelt, J. P., Kallemeijn, W. W., & Brul, S. (2009). On the origin of heterogeneity in (preservation) resistance of *Bacillus* spores: Input for a 'systems' analysis approach of bacterial spore outgrowth. *International Journal of Food Microbiology*, *134*(1-2), 9–15. http://dx.doi.org/10.1016/j.ijfoodmicro.2009.02.011.

Huang, L. (2013). Determination of thermal inactivation kinetics of *Listeria monocytogenes* in chicken meats by isothermal and dynamic methods. *Food Control*, *33*(2), 484–488. http://dx.doi.org/10.2016/j.foodcont.2013.03.049.

Huemer, I. A., Klijn, N., Vogelsang, H. W. J., & Langeveld, L. P. M. (1998). Thermal death kinetics of spores of *Bacillus sporothermodurans* isolated from UHT milk. *International Dairy Journal*, *8*(10−11), 851–855. http://dx.doi.org/10.1016/s0958-6946(98)00129-0.

Janssen, M., Verhulst, A., Valdramidis, V., Devlieghere, F., Van Impe, J. F., & Geeraerd, A. H. (2008). Inactivation model equations and their associated parameter values obtained under static acid stress conditions cannot be used directly for predicting inactivation under dynamic conditions. *International Journal of Food Microbiology*, *128*(1), 136–145. http://dx.doi.org/10.1016/j.ijfoodmicro.2008.06.009.

Jewell, K. (2012). Comparison of 1-step and 2-step methods of fitting microbiological models. *International Journal of Food Microbiology*, *160*(2), 145–161. http://dx.doi.org/10.1016/j.ijfoodmicro.2012.09.017.

Mazas, M., Gonzalez, I., Lopez, M., Gonzalez, J., & Sarmiento, R. M. (1995). Effects of sporulation media and strain on thermal-resistance of *Bacillus cereus* spores. *International Journal of Food Science and Technology*, *30*(1), 71–78.

Peleg, M., Normand, M. D., & Campanella, O. H. (2003). Estimating microbial inactivation parameters from survival curves obtained under varying conditions — The linear case. *Bulletin of Mathematical Biology*, *65*(2), 219–234. http://dx.doi.org/10.1016/s0092-8240(02)00097-6.

Pettersson, B., Lembke, F., Hammer, P., Stackebrandt, E., & Priest, F. G. (1996). *Bacillus sporothermodurans*, a new species producing highly heat-resistant endospores. *International Journal of Systematic Bacteriology*, *46*(3), 759–764.

Richardson, P. (2004). Improving the thermal processing of foods. *Improving the thermal processing of foods*. Woodhead Publishing Limited (xvii +507 pp.-xvii +507 pp.).

Schwaab, M., & Pinto, J. C. (2007). Optimum reference temperature for reparameterization of the Arrhenius equation. Part 1: Problems involving one kinetic constant. *Chemical Engineering Science*, *62*(10), 2750–2764. http://dx.doi.org/10.1016/j.ces.2007.02.020.

Valdramidis, V. P., Belaubre, N., Zuniga, R., Foster, A. M., Havet, M., Geeraerd, A. H., & Kondjoyan, A. (2005). Development of predictive modelling approaches for surface temperature and associated microbiological inactivation during hot dry air decontamination. *International Journal of Food Microbiology*, *100*(1-3), 261–274. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.10.025.

Valdramidis, V. P., Geeraerd, A. H., Bernaerts, K., & Van Impe, J. F. (2006). Microbial dynamics versus mathematical model dynamics: The case of microbial heat resistance induction. *Innovative Food Science & Emerging Technologies*, *7*(1-2), 80–87. http://dx.doi.org/10.1016/j.ifset.2005.09.005.

Valdramidis, V. P., Geeraerd, A. H., & Van Impe, J. F. (2007). Stress-adaptive responses by heat under the microscope of predictive microbiology. *Journal of Applied Microbiology*, *103*(5), 1922–1930. http://dx.doi.org/10.1111/j.1365-2672.2007.03426.x.

Valdramidis, V. P., Geeraerd, A. H., Bernaerts, K., & Van Impe, J. F. M. (2008). Identification of non-linear microbial inactivation kinetics under dynamic conditions. *International Journal of Food Microbiology*, *128*(1), 146–152. http://dx.doi.org/10.1016/j.ijfoodmicro.2008.06.036.

van Boekel, M. A. J. S. (1996). Statistical aspects of kinetic modeling for food science problems. *Journal of Food Science*, *61*(3), 477-+.

van Zuijlen, A., Periago, P. M., Amezquita, A., Palop, A., Brul, S., & Fernandez, P. S. (2010). Characterization of *Bacillus sporothermodurans* IC4 spores; putative indicator microorganism for optimisation of thermal processes in food sterilisation. *Food Research International*, *43*(7), 1895–1901. http://dx.doi.org/10.1016/j.foodres.2009.11.011.

Velliou, E. G., Derlinden, E. v., Cappuyns, A. M., Nikolaidou, E., Geeraerd, A. H., Devlieghere, F., & Impe, J. F. v. (2011). Towards the quantification of the effect of acid treatment on the heat tolerance of *Escherichia coli* K12 at lethal temperatures. *Food Microbiology*, *28*(4), 702–711. http://dx.doi.org/10.1016/j.fm.2010.06.007.