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Mathematical quantification of the induced stress resistance of microbial populations during non-isothermal stresses

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ABSTRACT

This contribution presents a mathematical model to describe non-isothermal microbial inactivation processes taking into account the acclimation of the microbial cell to thermal stress. The model extends the log-linear inactivation model including a variable and model parameters quantifying the induced thermal resistance.

The model has been tested on cells of *Escherichia coli* against two families of non-isothermal profiles with different constant heating rates. One of the families was composed of monophasic profiles, consisting of a non-isothermal heating stage from 35 to 70 °C; the other family was composed of biphasic profiles, consisting of a non-isothermal heating stage followed by a holding period at constant temperature of 57.5 °C. Lower heating rates resulted in a higher thermal resistance of the bacterial population. This was reflected in a higher D-value. The parameter estimation was performed in two steps. Firstly, the D and z-values were estimated from the isothermal experiments. Next, the parameters describing the acclimation were estimated using one of the biphasic profiles. This set of parameters was able to describe the remaining experimental data.

Finally, a methodology for the construction of diagrams illustrating the magnitude of the induced thermal resistance is presented. The methodology has been illustrated by building it for a biphasic temperature profile with a linear heating phase and a holding phase. This diagram provides a visualization of how the shape of the temperature profile (heating rate and holding temperature) affects the acclimation of the cell to the thermal stress. This diagram can be used for the design of inactivation treatments by industry taking into account the acclimation of the cell to the thermal stress.

1. Introduction

Mathematical modelling simplifies processes occurring in the physical world through a series of hypotheses, so that they can be described using systems of (differential) equations. These equations usually have a different number of constant coefficients (model parameters) which are unknown and commonly have to be estimated using experimental data. Once the model parameters have been estimated, the model has to be validated against an independent data set and different conditions before further use. If the independent experiment cannot be accurately simulated using the fitted model, then new hypotheses should be incorporated thus modifying the model structure by adding new equations, new parameters or new relationships among them (Vilas et al. 2016).

Mathematical modelling is a key tool to assist food microbiology (Baranyi and Roberts 1995; Ferrer et al. 2009). They mainly serve two purposes: prediction and inference. Prediction tries to simulate the evolution of the microbial density during the different stages of the food chain (e.g. storage or processing). It can be used, for instance, to evaluate the health risk associated to the consumption of the food product. Inference refers to the interpretation of the model parameters. In those models where they have a clear biological meaning, conclusions can be drawn based on the values estimated. For instance, one can conclude that a food matrix promotes bacterial growth if the growth rate estimated in this medium is higher than the one obtained in a reference laboratory medium.

The first models used in predictive microbiology assumed that the bacterial population reduction followed a log-linear relationship with time during an isothermal treatment. These types of models allowed the prediction of the microbial load at times different to the ones recorded

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in the experiment, leading to the definition of the D-value: the time required to cause a ten-fold reduction in the microbial population. Bigelow (1921) showed that this parameter follows a negative log-linear relationship with temperature and Esty and Meyer (1922) defined the concept of *ideal destruction curve*, mathematically describing this relationship. This result led other researchers to extend this model defining the z-value: the temperature increase required to reduce the D-value a 90% (Stumbo 1973). This model allowed predicting the response of the microbial population to isothermal heat treatments at temperatures different from the experimental ones.

Heat treatments applied in industry typically have complex profiles far from isothermal conditions (Vicente and Machado 2011). Mathematical models to describe dynamic inactivation profiles have been developed by extending the ones used for isothermal treatments. The most popular examples are those by Geeraerd et al. (2000) - who extended the log-linear model- and Peleg and Cole (1998) or Mafart et al. (2002) - who extended a Weibullian isothermal model. Peleg and Normand (2004) described a mathematical model for the parameter estimation directly from non-isothermal experiments. However, experimental observations have shown that predictions using model parameters estimated from isothermal experiments usually fail to predict dynamic inactivation (Janssen et al. 2008). The models usually overpredict the microbial inactivation, especially for slow heating rates, as observed for *Escherichia coli* by Valdramidis et al. (2006), Hassani et al. (2007) and Mackey et al. (1993), for *Enterococus faecium*, *Listeria monocytogenes* and *Staphylococcus aureus* by Hassani et al. (2006a, 2006b, 2007), for *Salmonella* by Hansen and Knøchel (1996), Hassani et al. (2007), Mackey and Derrick (1987) and Quintavalla and Campanini (1991) and for vegetative cells of *Bacillus stearothermophilus* by Mackey et al. (1993). Authors have justified this deviation arguing that the slow heating rate allows the microbial population to develop an acclimation to the thermal stresses, increasing its resistance. This is reflected as an increase of the D-value (or equivalent model parameter) estimated from the experimental data.

Hassani et al. (2007) studied the stress acclimation of different bacterial populations by fitting the Weibull primary inactivation model to a set of non-isothermal experiments with constant heating rate. This model (Eq. (1)) extends the model by Mafart et al. (2002), which considers that the time required to inactivate the microbial population follows a Weibull distribution with scale factor δ and shape factor p. The shape factor defines the concavity of the survivor curves. In this model, δ is equal to the time required for the first ten-fold reduction of the initial microbial population. Hassani et al. (2007) proposed a log-linear secondary model for it with respect to the heating rate (Hr) with coefficients a and b in order to account for the stress acclimation.

$$\log\frac{N}{N_0} = -\left(\frac{t}{\delta(Hr)}\right)^p$$
$$\log\delta = a + b{\cdot}Hr \tag{1}$$

This model allowed them to successfully predict the inactivation curve for the different temperature profiles, with constant heating rates, tested. Nevertheless, this model can only reproduce survivor curves with a unique curvature, thus, limiting its use for complex profiles including several heating stages or cooling phases.

Dolan et al. (2013) reviewed and compared several models for the description of dynamic experiments. Among them, the model by Valdramidis et al. (2007) extends the model defined by Geeraerd et al. (2000) to describe biphasic temperature profiles including a heating phase at constant rate and a holding phase. The resulting model is provided in Eq. (2), where C_c is related to the physiological state of cells, k_{max}, k_1, k_2 and $AsymD_{ref}$ are model parameters, T is the temperature and T_{ref} is a reference temperature. Following the notation the original authors used in their paper, the term dT/dt stands for the constant heating rate during the heating phase.

$$\frac{dN}{dt} = -k_{max}(T)\left(\frac{1}{1+C_c}\right)\left(k_1\frac{dT/dt}{k_2+dT/dt}\right)N$$
$$\frac{dC_c}{dt} = -k_{max}(T)C_c$$
$$k_{max}(T) = \frac{\ln 10}{AsymD_{ref}}\exp\left(\frac{\ln 10}{z}(T-T_{ref})\right) \tag{2}$$

With this model, Valdramidis et al. (2007) successfully described the biphasic inactivation profiles tested. However, this model has similar issues to the one by Hassani et al. (2007). The inclusion of the heating rate explicitly limits the range of temperature profiles that can be simulated.

Corradini and Peleg (2009) implemented an additional term in the model described by Peleg and Cole (1998) in order to describe the thermal acclimation of the microbial population. The resulting model is depicted in Eq. (3), where n, k, k_{adapt}, T_c, T_{limit} and a are model parameters to be estimated. The first derivative of temperature with respect to time at time t is represented by $\nu(t)$, according to the authors notation.

$$\frac{d\log_{10}S}{dt} = -b(T,\nu(t))\cdot n\cdot\left(-\frac{\log_{10}S}{b(T,\nu(t))}\right)^{\frac{n-1}{n}}$$
$$b(T,\nu) = \frac{\ln\{1+\exp[k(T-T_c)]\}}{1+\exp\{k_{adapt}[T_{c,adapt}(\nu(t))-T]\}}$$
$$T_{c,adapt} = T_{limit} - a{\cdot}\nu(t) \tag{3}$$

With this model, they were able to describe previously published dynamic inactivation curves of *E. coli* and L. *monocytogenes*. On the other hand, this model introduces the induced thermal resistance with an equation that only considers the instantaneous heating rate. Therefore, it does not consider the acclimation that may have been developed during earlier stages of the treatment.

Stasiewicz et al. (2008) applied a different modification to the model by Peleg and Cole 1998 to include the induced thermal resistance. The model they proposed is shown in Eq. (4), where n, β_1 and β_2 are unknown model parameters. The induced thermal resistance is represented by the variable τ, which grows when the temperature is within the limits defined by (HS_{lower},HS_{upper}).

$$\frac{d\log_{10}S}{dt} = -b(T,\tau)\cdot n\cdot\left(-\frac{\log_{10}S}{b(T,\tau)}\right)^{\frac{n-1}{n}}$$
$$\ln b(T,\tau) = \ln b(T_{ref}) - \beta_1\left(\frac{1}{T(t)}-\frac{1}{T_{ref}}\right) - \beta_2\tau$$
$$\tau = \int_{t_{T=HS_{lower}}}^{t_{T=HS_{upper}}}[T(t)-HS_{lower}]dt \tag{4}$$

Using this model they were able to describe the thermal inactivation of *Salmonella* in ground turkey. Nevertheless, the variable τ is unbounded and $b(T,\tau)$ may become negative if the cells are kept within the (HS_{lower},HS_{upper}) temperature range for a long period. This would imply that the cells grow, even if the temperature is within the lethal range for the bacteria.

In this contribution, a novel dynamic inactivation model, which takes into account the resistance of the microbial population induced by the thermal stress, is presented. The model has been built as an extension of the log-linear inactivation model, including an additional variable that accounts for the cell acclimation. The model has been tested against two different families of dynamic inactivation profiles measured for *E. coli*. The experimental data generated are presented in Section 2.1. The hypotheses followed for the development of the model, as well as the differential equations describing it, are outlined in Section 2.2. The mathematical methods used for the solution of the differential equations, for the model fitting and for the evaluation of the goodness of fit are described in Sections 2.3, 2.4 and 2.5, respectively. Section 3 discusses the performance of the model. Finally, Section 4 presents the