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Predictions of microbial thermal inactivation in solid foods: isothermal and non-isothermal conditions

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Abstract

This work focuses on the use of the Gompertz-inspired model to predict the thermal inactivation behaviour of microorganisms obtained in solid food products, validated for isothermal and non-isothermal conditions. Experiments were carried out in parsley, artificially inoculated with *Listeria innocua*. For the isothermal conditions tested, the predictive ability of the model was confined. The higher the temperature, the higher deviations observed (i.e. the model underestimates the inactivation behaviour). However, for the non-isothermal condition tested, the model predicted the microbial response accurately.

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Keywords: Microbial inactivation; solid foods; isothermal and non-isothermal; experimental validation; Gompertz-inspired model.

1. Introduction

The selection of an appropriate mathematical model, as well as the identification and quantification of the relevant factors that affect microorganism inactivation, are two difficult tasks in predictive modelling. The majority of bacterial data relating heat treatments to thermal death kinetics are usually obtained in medium. The broths are inoculated with known concentrations of the target organism and placed in a controlled thermostatic environment. Samples are removed at given times and viable microbial cells are enumerated. A model that describes the kinetic behaviour is then selected and kinetic parameters are estimated on the basis of regression analysis procedures.

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However, when the microorganism is on a solid food surface, its kinetic response may be considerable different. Assumption that broth-data-based models are conservative, because of the ideal conditions, might not be valid. For inactivation, it is widely known that bacterial pathogens tend to be more resistant to heat in real food products than in broth-based media [1].

To assess the predictive ability of a model in pathogen inactivation is an important topic, since dangerous underestimations should be avoided. Mathematical models developed from data obtained in broth should be validated in "real" food systems, where there is wider influencing factors rather than the stressing conditions such as temperature, pH or water activity. The main objective of this work was to assess the use of Gompertz-inspired model expressed in terms of relevant factors (temperature, pH and water activity), developed on the basis of experiments carried out in broth, in predictions of *Listeria innocua* inactivation in parsley surface. Both isothermal and time-varying temperature conditions were considered.

2. Model description

2.1. Isothermal conditions

A Gompertz-inspired model that included the temperature, pH and water activity effects on shoulder, maximum inactivation rate and tail parameters (merging eqs. 2, 3 and 4 into the Gompertz-inspired model expressed by eq. 1) was used [1]:

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

herein, y_{inact} represents the microbial cell density: logarithm of the microbial load (N) at a certain process time (t), normalized to the initial content (N_0); L is the time parameter (or shoulder) and k_{max} the maximum inactivation rate; N_{res} is the residual microbial load.

$$\log(k_{\max}) = \sum_{i=0}^{n}\sum_{j=0}^{n}\sum_{k=0}^{n} G_{k_{\max}\, ijk}\; a_w{}^{k}\; pH^{j}\; T^{i} \quad (2)$$

$$\log(L) = \sum_{i=0}^{n}\sum_{j=0}^{n}\sum_{k=0}^{n} G_{L\, ijk}\; a_w{}^{k}\; pH^{j}\; T^{i} \quad (3)$$

where n is the order of the polynomial to be assumed and G are polynomials coefficients (i=1,…, n; j=1,…, n; k=1,…, n).

$$\log\left(\frac{N_{res}}{N_0}\right) = \sum_{i=0}^{n}\sum_{j=0}^{n} G_{Tail\, ij}\; a_w{}^{k}\; pH^{j} \quad (4)$$

where n is the order of the polynomial to be assumed and G are polynomials coefficients (i=1,…, n; j=1,…, n).

The values of the parameters (coefficients of the polynomial equation) used in the simulation were the ones previously estimated considering inactivation experimental data of *L. innocua* obtained at different conditions in broth[2].

2.2. Non-Isothermal conditions

To predict the inactivation behaviour of *L. innocua* in parsley under non-isothermal conditions, a combined model including the temperature effect (merging eqs. 5 and 6) into the integrated expression of the Gompertz-inspired model was used [1].

$$k_{\max}(T) = k_{ref}\ \exp\left(-\frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right) \tag{5}$$

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

$$L(T) = \left[A_{Ratk_1}\left(T - A_{Ratk_2}\right)\right]^2 \tag{6}$$

where A_{Ratk1} and A_{Ratk2} are Ratkowsky models parameters.
The values of the parameters used in the simulation were the ones previously estimated [2].

The following equation expresses the variation of $\log(N/N_0)$ with time, for time-varying temperature conditions:

$$\log\left(\frac{N}{N_0}\right) = \int_0^t \left[\begin{array}{l} -k_{\max}(T(t), a_w, pH)\exp(1)\exp\left(-\dfrac{k_{\max}(T(t), a_w, pH)\,e}{\log\left(\dfrac{N_{res}}{N_0}\right)(a_w, pH)}\left(L(T(t), a_w, pH) - t'\right) + 1\right) \\ \times \exp\left(-\exp\left(-\dfrac{k_{\max}(T(t), a_w, pH)\,e}{\log\left(\dfrac{N_{res}}{N_0}\right)(a_w, pH)}\left(L(T(t), a_w, pH) - t'\right) + 1\right)\right) \end{array}\right] dt' \tag{7}$$

3. Methodology

All microbial inactivation data was experimentally obtained by Miller *et al.*[2]. Since the model was developed for a specific range of pH (from 4.5 to 7.5) and water activity (from 0.95 to 0.99) values, a solid food with characteristics within these ranges was chosen. Parsley was the selected one ($pH_{parsley}$=6.2 and $a_{w\text{-}parsley}$=0.98). Moreover, parsley is an aromatic herb with a great potential of contamination. This re-enforces the interest on selecting parsley as a case to study. For non-isothermal conditions, the model developed was dependent on media temperature history.

4. Results and discussion

4.1. Isothermal conditions

Using the experimental temperature conditions, pH and water activity values of the parsley, the microbial load was predicted. The experimental results and model predictions are included in Figure 1. It is obvious the inadequacy of model predictions, particular for the higher temperatures tested. For 52.5 °C, the model slightly overestimates the microbial inactivation. However, and as temperature increases from 60.0 to 65.0 °C, dangerous underestimations were attained. This reveals that when *Listeria innocua* was at parsley’s surface, additional thermal resistance was observed.

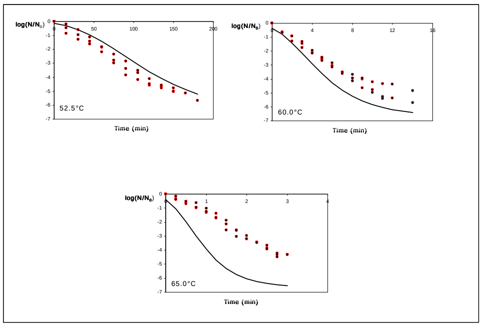

Fig. 1. Inactivation of *L.innocua* in parsley at 52.5, 60.0 and 65.0 ºC: experimental data (dots) and model predictions (continuous black line).

4.2. Non-Isothermal conditions

Using the experimental time-varying temperature conditions, the microbial load was predicted. The experimental results and model predictions are including in Figure 2. Accurate predictions were attained (contrarily to isothermal situations) and results showed the ability of the Gompertz-inspired model (developed with experimental data obtained in broth) in estimating microbial response in food, under non-isothermal conditions.

It was found that no microbial growth was verified during the come-up-time and there was a limit of temperature below which no inactivation was observed: the minimum temperature for inactivation was approximately 60.0 ºC for a heating rate of 1.9 ºC/min. Microbial hypothesis concerning initialization of thermal inactivation in broth was validated in parsley. Microorganisms, after exposed to a slow temperature evolution, would have time to adapt their plasma membranes and might increase their resistance to subsequent lethal heat treatments. Moreover, the heat resistance greatly depends on the physiological state (and therefore the previous environment). This fact can explain the predictive ability of the model, only under non-isothermal conditions.

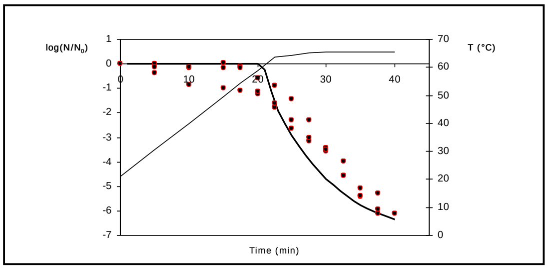

Fig. 2. Inactivation of *L. innocua* in parsley under non-isothermal conditions: experimental data (dots) and model predictions (continuous black line). Thinner black line indicates the temperature history.

5. Conclusions

The model developed and expressed in terms of the most relevant variables studied (i.e. temperature, pH and water activity) allowed predictions that will certainly contribute to improvements of the preliminary designs of adequate thermal treatments.

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