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Combined effects of temperature, pH and water activity on predictive ability of microbial kinetic inactivation model

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Abstract

It is well known that temperature is the key factor controlling the microbial survival/inactivation. However, the interactive effects of further stressing environmental conditions may influence microbial behaviour. The objective of this work was to include, in the inactivation model, temperature, pH and a_w effects using a black box polynomial model, aiming at accurate prediction. Data of *Listeria innocua* obtained within the temperature range of 52.5 and 65.0 °C, pH of 4.5, 6.0 and 7.5, and a_w of 0.95 and 0.99 were used for model assessment. The relations of such parameters with temperature, a_w and pH were assumed to be polynomials.

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Keywords: Maximum inactivation rate; shoulder parameter; tail; temperature, pH and water activity effects; polynomial functions.

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1. Introduction

Temperature is the key factor controlling the survival/inactivation of bacteria. Nevertheless, other adverse factors such as low pH values and reduced water activity influence the microbial response. The study of main and combined effects of temperature, water activity, and pH on kinetic parameters is important for a complete process assessment and control. Several authors studied the influence of those effects per si on microbial behaviour. However, significant interactions between environmental factors are not commonly assessed. Besides the considerable attempt in modelling kinetic parameters as function of environmental influences, the predictive ability of the inactivation

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behaviour is scarcely assessed (and often compromised). One can ask “what is the sense of using so diverse secondary models (such as Arrhenius, Peleg, Ratkowsky type models) if deplorable predictions of microbial survival are observed when those models are merged?”

Polynomial models still receive considerable attention in describing such relations. The flexible behaviour, making possible the purely empirical but underlying relation between microbial kinetic parameters and environmental influences, makes polynomial modelling approaches a promising field, for an accurate prediction of the microbial survival.

The objective of this work was to include, in the inactivation model, temperature, pH and water activity effects using a black box polynomial model, aiming at accurate prediction. The relations of maximum inactivation rate (k_{max}) and shoulder (L) on environmental factors were purely empirical. The log variations of k_{max} and L on temperature were assumed to be polynomials. The pH and water activity effects were then included in those models. Tail [$\log(N_{res}/N_0)$] was assumed to be independent of temperature, but dependent on pH and water activity (this relation was also assumed to be polynomial). The predictive ability of the inactivation model, expressed in terms of all environmental factors, was assessed.

2. The models

Gompertz inspired model can be used to describe sigmoidal microbial inactivation, under isothermal conditions:

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

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.

The log variations of k_{max} and L on temperature, pH and water activity (a_w) can be described by polynomials:

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

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

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).

Regarding the tail parameter ($\log(N_{res}/N_0)$), the dependence on pH and water activity can also be described by polynomials:

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

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

3. Materials and methods

3.1. Experimental design

Preliminary analyses were performed in order to evaluate if temperature, water activity and pH had a significant effect on *L. innocua* inactivation. Experiments were designed according to a 2^3 factorial design at two temperature levels (52.5°C, 65.0°C), two pH levels (4.5, 7.5) and two water activity levels (0.95, 0.99). Results were analysed using Experimental Design package of STATISTICATM v 6.0 (Statsoft_, Tulsa, OK, USA).

3.2. Microbial inactivation data

All microbial inactivation data was experimentally obtained by Miller *et al.* [1].

3.3. Modelling procedures

Inactivation experimental data of *Listeria innocua* obtained at different conditions of temperature, pH and water activity[1] (according to the experimental design defined in §3.1) were fitted separately with the Gompertz-inspired model (eq. 1). The model parameters (k_{max}, L and $\log(N_{res}/N_0)$) were estimated at each one of the combined factors. Secondary models, that express the relations of kinetic parameters on environmental conditions, were purely empirical (eqs. 2-4). Model parameters were estimated by non-linear regression analysis, using a flexible black box modelling approach. The log variations of k_{max} and L on temperature were assumed to be tertiary-order polynomials (or lower). The tail ($\log(N_{res}/N_0)$) was assumed to be independent of temperature (this was assessed but not shown), but dependent on pH and water activity. The variation of tail parameters on pH was also assumed to be secondary-order polynomial.

3.4. Statistical analysis

Model fitting procedures were performed using STATISTICA 6.0 (StatSoft, Inc. 2001, Tulsa, OK, USA) software, by least squares non-linear regression analysis. The adequacy of the models was tested by the coefficient of determination, and residuals analyses (randomness and normality).

4. Results and discussion

The Gompertz inspired model was fitted to inactivation experimental data of *L. innocua,* leading to a set of 28 k_{max} (T, pH, a_w) and L (T, pH, a_w) values. To test the effect of the mentioned environmental factors on *L. innocua*, a factorial analysis was previously performed. It was concluded that kinetic parameters were significantly affected by all factors (at a significance level of 6.5%), being the major effect due to the temperature (for k_{max} and L) and pH (for tail). In face of that, a study with the objective of modelling the influence of those environmental factors on the inactivation behaviour was conducted.

To predict the inactivation behaviour, a combined model including the main and combined effects of environmental factors (merging eqs. 2, 3 and 4 into the Gompertz-inspired model) was used. Assuming given parameters, simulated values of microbial load were calculated and are presented in Figure 1. The values of the parameters used in the simulation were previously estimated (results not shown).

From the results presented in Figure 1, it can be concluded that, in the range of environmental factors studied, the model predicts successfully the inactivation kinetics (accurate predictions were observed). Besides the model developed is confined to *L. innocua* kinetics in the media studied, this is certainly a contribution to design more efficient thermal inactivation processes with controlled influence of the stressing environmental factors. However, and for an insightful control of the water activity influence, further levels of this factor should be studied.

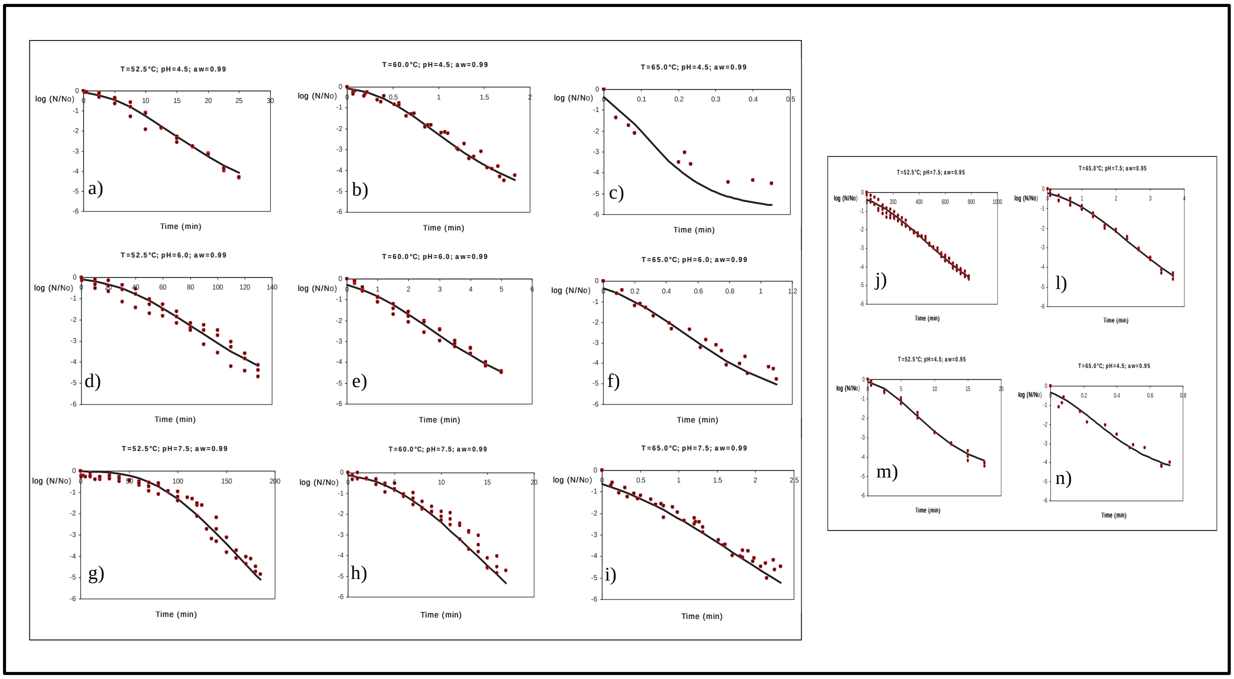

Fig. 1. Inactivation model predictions (continuous black line) of experimental inactivation data of *Listeria innocua* (black dots); a_w = 0.99 (left plot) and a_w=0.95 (right plot): a) T=52. 5 °C and pH=4.5; b) T=60.0 °C and pH=4.5; c) T=65.0 °C and pH=4.5; d) T=52. 5 °C and pH=6.0; e) T=60.0 °C and pH=6.0; f) T=65.0 °C and pH=6.0; g) T=52. 5 °C and pH=7.5; h) T=60.0 °C and pH=7.5; i) T=65.0 °C and pH=7.5; j) T=52. 5 °C and pH=7.5; l) T=65.0 °C and pH=7.5; m) T=52. 5 °C and pH=4.5; n) T=65.0 °C and pH=4.5.

The combined decrease of pH and increase of temperature and water activity is responsible for the decrease of microbial resistance. Therefore, it can be concluded that low water activity is protective within the considered temperature range and that a pH decrease results in a decreased microbial tolerance to heat treatments. This type of microbial responses is commonly referred in literature. The pH impact is less evident at higher temperatures.

5. Conclusions

Models that describe the effect of temperature, pH and water activity on sigmoidal behaviour (assessed by the shoulder period, maximum inactivation rate and tail) were successfully developed on the basis of polynomial functions. When these mathematical relationships were included in the primary kinetic model, accurate predictions of the inactivation data were attained, thus validating the predictive ability of the model expressed in terms of the stressing environmental factors studied. Nevertheless, one should be aware that the model was developed for a particular microorganism and for a particular media, and caution should be taken if the model predictions are extended to other bacteria behaviour. Extrapolations using environmental conditions out of the studied range may conduct to erroneous results, as well.

Besides these limitations, the model developed may be used as an initial step for predicting the inactivation pattern of *Listeria* under particular stressing conditions. This can be a valuable tool for designing efficient inactivation processes involving these bacteria.

References

[1] Miller, FA, Ramos, B, Gil, MM, Brandão, TRS, Teixeira, P, Silva, CLM. Influence of pH, type of acid and recovery media on the thermal inactivation of *Listeria innocua*. *Int J Food Microbiol* 2009: **133**: 121-128.