Designing experiments for microbial inactivation kinetics studies

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Abstract

The Gompertz model with an initial shoulder followed by a maximum inactivation rate (k_{max}) was assumed to describe microbial inactivation. Model kinetic parameters (i.e. shoulder and k_{max}) were considered to be temperature dependent and a Ratkowsky equation and an Arrhenius–type relationship were used to describe such dependence. For predictive purposes it is of major importance to estimate model parameters with high precision. Precision increases with the number of experimental points. However, and in many situations, when replicates of a number of experimental conditions equal to the number of model parameters is considered, maximum precision is attained. Optimal experimental conditions can be obtained on the basis of *D*-optimal design concept (i.e. minimization of the generalized variance of the parameter estimates).

The main objective of this work was to design experiments using *D*-optimal criterion for processes described by a Gompertz inactivation model under isothermal conditions, aiming at parameter estimation with maximum precision.

For a single given temperature, Gompertz model is a two-parameter model (shoulder and k_{max} are the kinetic parameters) and results showed that the two optimal sampling times corresponds to the times required for 89.09 % and 99.97 % of microbial inactivation.

If a temperature range is considered, the temperature effect can be included in the Gompertz model using Ratkowsky and Arrhenius equations. In such situation the final expression becomes a four-parameter model. Optimal experimental conditions corresponds to four experiments: two conducted at each extreme temperature (T_{min}=52.5 ºC and T_{max}=65 ºC), one at the average temperature of the range tested (T_{ave}=58.8 ºC), and the remained one at a temperature 3% lower than the maximum extreme ($T_{3\%<Tmax}$=65.0 ºC). At each temperature, the sampling times corresponds to 99.95% (T_{min}), 97.49% (T_{ave}), 99.92% ($T_{3\%<Tmax}$) and 99.21% (T_{max}) of inactivation.

Keywords

D-optimal design, predictive microbiology, thermal inactivation.

Introduction

Predictive microbiology is gaining considerable importance in the food processing domain, particularly in the design of efficient and safe inactivation treatments. This terminology designates the use of mathematical models in the description of microbial responses to environmental stressing factors, such as temperature, pH or water activity. Microbial inactivation can be mathematically described by a modified Gompertz model, which includes an initial shoulder (L) followed by a maximum inactivation rate (k_{max}) period. The kinetic model parameters are temperature dependent and a Ratkowsky (square-root) equation or an Arrhenius-type relationship can be used to express such relationship.

Kinetic models should predict the microbial behaviour accurately and precisely, which depends mutually on the adequacy of the model and on parameters` quality. If the mathematical model is properly chosen and the prime objective is to improve parameter estimation, underlying statistical theories can be applied. The criterion aiming at minimisation of parameters` variance, nominated as *D*-optimal design, is an appropriate and commonly used approach seeking parameters' precision (Brandão *et al.* 2001). Precision increases with the number of experimental points. However, in many situations, maximum precision is

attained if replicates of a number of experimental conditions equal to the number of model parameters are considered.
The main objective of this work was to define optimal experimental conditions for isothermal inactivation processes described by a Gompertz model, aiming at kinetic parameters estimation with improved precision.

Mathematical considerations

The model

If one single temperature is chosen from a range, the microbial inactivation model assumed was the one based on the Gompertz equation:

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

where N is the microbial content at time t; 0 and res are indexes denoting initial and residual, respectively. This is a two-parameter model, being k_{max} and L the kinetic parameters (the maximum inactivation rate and the shoulder parameter, respectively).
If a range of temperatures is considered, the temperature dependence of k_{max} and L may be included in the previous equation. Assuming an Arrhenius-type relationship for k_{max} and a Ratkowsky equation for L, the Gompertz model becomes a four-parameter model as follows:

$$y_{inact}(t) = \log\left(\frac{N_{res}}{N_0}\right)\exp\left(-\exp\left(\frac{\left(-k_{ref}\exp\left(-\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right)\right)e}{\log\left(\frac{N_{res}}{N_0}\right)}\left(\left(C_{Ratk}(T-T_{min})\right)^2-t\right)+1\right)\right) \quad (2)$$

where k_{ref} is the inactivation rate at a finite reference temperature T_{ref} , E_a is the process activation energy, and C_{Ratk} and T_{min} are Ratkowsky equation parameters; R is the universal gas constant.

Design criterion

D-optimal experiments were planed by minimisation of parameters` variance, which corresponds mathematically to the minimisation of the determinant of the variance-covariance matrix $[\mathbf{F}^T\mathbf{F}]^{-1}$ (pxp) (or maximisation of $|\mathbf{F}^T\mathbf{F}|$) (Atkinson, 1982). The elements of the matrix **F** are the partial derivatives of the mathematical model used to describe the process (eqns 1 or 2) with respect to each parameter, evaluated at all experimental conditions. If the number of model parameters (p) equals the number of experimental conditions (n), **F** is a square matrix (pxp) and, consequently, the determinant becomes $\Delta \equiv \left|F^T F\right| = |F|^2$.

Methods

Single temperature

Considering a single isothermal experiment, microbial inactivation is described by eqn 1 and two parameters should be estimated: k_{max} and L. If two sampling times are planned (n=2), the expression of the determinant becomes:

$$\Delta = \left|F^T F\right| = \left(\sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial k_{max}}\right)_{t_i}^2 \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial L}\right)_{t_i}^2 - \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial k_{max}} \times \frac{\partial y_{inact}}{\partial L}\right)_{t_i}^2\right) \quad (3)$$

The two sampling times (t_1 and t_2) that maximise $|\Delta|$ were calculated numerically, using the analysis tool packages available in Microsoft® Office Excel. The corresponding microbial content was then calculated using eqn. 1.

Preliminary estimates of k_{max} and L required for calculation were the ones presented in Table 1 (and were representative of *Listeria innocua* inactivation). Six temperatures, in the range 52.5 ºC to 65.0 ºC, were considered and $\log(N_{res}/N_0)$ was assumed to be -5 in all cases.

Range of temperatures

If a temperature range is considered, inactivation behaviour is described by eqn 2 and four parameters should be estimated: E_a, k_{ref} from Arrhenius equation and C_{Ratk} and T_{min} from Ratkowsky model. If four sampling conditions (i.e. temperature/sampling time) are planned (n=4), the determinant becomes:

$$\Delta=\left|F^TF\right|=\begin{vmatrix} \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial k_{ref}}\right)_i^2 & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial k_{ref}}\times\frac{\partial y_{inact}}{\partial E_a}\right)_i & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial k_{ref}}\times\frac{\partial y_{inact}}{\partial C_{Ratk}}\right)_i & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial k_{ref}}\times\frac{\partial y_{inact}}{\partial T_{min}}\right)_i \\ \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial E_a}\times\frac{\partial y_{inact}}{\partial k_{ref}}\right)_i & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial E_a}\right)_i^2 & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial E_a}\times\frac{\partial y_{inact}}{\partial C_{Ratk}}\right)_i & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial E_a}\times\frac{\partial y_{inact}}{\partial T_{min}}\right)_i \\ \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial C_{Ratk}}\times\frac{\partial y_{inact}}{\partial k_{ref}}\right)_i & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial C_{Ratk}}\times\frac{\partial y_{inact}}{\partial E_a}\right)_i & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial C_{Ratk}}\right)_i^2 & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial C_{Ratk}}\times\frac{\partial y_{inact}}{\partial T_{min}}\right)_i \\ \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial T_{min}}\times\frac{\partial y_{inact}}{\partial k_{ref}}\right)_i & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial T_{min}}\times\frac{\partial y_{inact}}{\partial E_a}\right)_i & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial T_{min}}\times\frac{\partial y_{inact}}{\partial C_{Ratk}}\right)_i & \sum_{i=1}^{n}\left(\frac{\partial y_{inact}}{\partial T_{min}}\right)_i^2 \end{vmatrix} \qquad (4)$$

where the sampling conditions corresponds to a sampling time t_i for one experiment at a temperature T_i. The sampling conditions that maximize $|\Delta|$ were calculated as described before.

Preliminary estimates of k_{ref}, E_a, C_{Ratk} and T_{min} required for calculation were assumed to be 0.29 min^{-1}, 3.34×10^5 J mol^{-1}, 0.38 K^{-1} $min^{-0.5}$ and 337.1 K, respectively (and were representative of *Listeria innocua* inactivation). The temperature range was 52.5 ºC to 65.0 ºC and $\log(N_{res}/N_0)$ was considered -5.

Results and discussion

Single temperature

If one single isothermal condition is chosen, the Gompertz model is a two-parameter model. Consequently, optimal experimental design implies replicates of two sampling times. In this case, results showed that the two sampling times that minimize the parameters` variance were temperature dependent (see Table 1, Figure 1). Curiously, optimal experiments consist always on a number of replicates taken at the times corresponding to 89.09 % (i.e. $\log(N/N_0)$=-0.96) and to 99.97 % (i.e. $\log(N/N_0)$=-3.52) of inactivation.

Table 1: Variables used in *D*-optimal experimental design definition, and corresponding sampling conditions

Variables			Optimal sampling			
T (ºC)	k_{max} (min^{-1})	L (min)	t_{1-opt} (min)	$\log(N/N_0)_{t1-opt}$	t_{2-opt} (min)	$\log(N/N_0)_{t2-opt}$
52.5	0.0404	69.06	91.85	-0.962	162.1	-3.516
55.0	0.0756	39.58	51.76	-0.962	89.31	-3.516
57.5	0.141	10.82	17.36	-0.962	37.52	-3.516
60.0	0.452	6.06	8.10	-0.962	14.38	-3.516
62.5	1.14	0.682	1.49	-0.962	3.98	-3.516
65.0	2.18	0.030	0.45	-0.962	1.75	-3.516

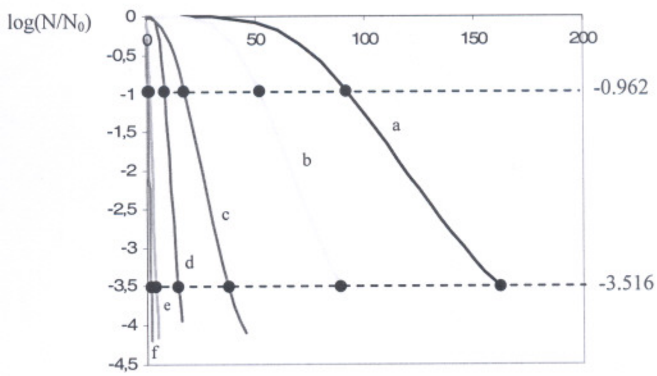

Figure 1: Predicted inactivation data. The dots represent the optimal sampling.
a) 52.5 °C; b) 55.0 °C; c) 57.5 °C; d) 60.0 °C; e) 62.5 °C; f) 65 °C

Heuristic designs, with experimental points equally spaced in the time scale, are the most common experimental sampling used. To assess the efficiency of such designs, it was calculated the ratio between $|\Delta|$ for 10 sampling points equally spaced in the time scale and the one for 5 replicates of each optimal times (thus being a measurement of design efficiency). The efficiency of the heuristic design was 26, 18, 33, 31, 43 and 76% for the experiments conducted at 52.5, 55.0, 57.5, 60.0, 62.5 and 65.0 °C, respectively. If *D*-optimal design was chosen, the confidence intervals of k_{max} and L decrease 40% and 59%, respectively (at 52.5 °C) and 12% for both parameters (at 65.0 °C), and parameters would be estimated with improved precision.

If a temperature range is considered, the Gompertz model is a four-parameter model. Optimal experimental design consists of four experiments conducted within the experimental range: one at each extreme temperature (T_{min}=52.5 °C and T_{max}=65 °C), one at the average temperature of the range tested (T_{ave}=58.8 °C), and the remained one at a temperature 3% lower than the maximum extreme ($T_{3\%<Tmax}$=65.0 °C). At each temperature, the sampling times corresponds to 99.95% (T_{min}), 97.49% (T_{ave}), 99.92% ($T_{3\%<Tmax}$) and 99.21% (T_{max}) of inactivation.

The ratio between the determinant calculated with 18 sampling points equally spaced in time (at each one of the six temperatures), and 27 replicates of each one of the four optimal sampling conditions, was also assessed. For the sake of comparison, it was assumed a number of replicates, so that the two designs would have the same number of data points. The efficiency of the heuristic design was only 1.6%. If *D*-optimal design was chosen, the confidence intervals of k_{ref}, E_a, C_{Rakt} and T_{min} would decrease 64%, 88%, 80% and 72%, respectively. Once again, this shows that the application of *D*-optimal conditions would improve estimation.

Conclusions

Application of *D*-optimal design concept to microbial inactivation processes described by a Gompertz model, may considerably improve parameters´ precision, when compared to commonly used heuristic designs.

References

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