Optimal Experimental Design for Estimating the Kinetic Parameters of the Bigelow Model

Luís M. Cunha, Fernanda A. R. Oliveira,* Teresa R. S. Brandão & Jorge C. Oliveira

Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200 Porto, Portugal

ABSTRACT

The optimum experimental design for systems following the Bigelow model was studied by determining the sampling conditions that lead to a minimum confidence region for a number of observations equal to the number of parameters. For isothermal conditions, it was found that this corresponds to the sampling times when the fractional concentration of the decaying factor (η_i) is equal to e^{-1} *and that the experiments should be performed in the limit range of temperatures chosen. These results are identical to those described in the literature for a first-order Arrhenius model. For non-isothermal experiments with linearly increasing temperature, the optimal experimental design is obtained with a maximum heating rate, a minimum initial temperature and sampling times when the product of the fractional concentrations is* e^{-2} *(with* $\eta_1 \cong 0.70$ *and* $\eta_2 \cong 0.19$*). The influence of the heating rate on the precision of the estimates is more significant for high* z *values and the influence of the initial temperature is more significant for low values of the heating rate.* © *1997 Elsevier Science Limited.*

NOMENCLATURE

a	Hunter colour scale value
b	Hunter colour scale value
D_{ref}	Decimal reduction time at a reference temperature T_{ref} (min)
D_T	Decimal reduction time at a given temperature T (min)

*To whom correspondence should be addressed. Fax: (351) 2 590351, E-mail: fernanda@esb.ucp.pt.

E_a Activation energy
$\mathbf{F}$ Matrix of the derivatives of the response function in order to the model parameters
k_T Reaction rate constant at a given temperature (min^{-1})
L Hunter colour scale value
m Heating rate (°C/min)
N Number or concentration of a micro-organism or quality factor
n Number of experimental points
N_0 Initial concentration of a component or quality factor
p Number of parameters
R Universal gas constant
T Temperature (°C)
T_0 Initial temperature (°C)
t_i Time for the ith experiment (min)
T_{ref} Reference temperature (°C)
v^*_i Normalised reaction rate evaluated at t_i (min^{-1})
v^*_{max} Maximum normalised reaction rate (min^{-1})
z Thermal death time parameter (°C)

Greek symbols
Δ Determinant of $\mathbf{F}$ (min^{-1} in eqn (2), $min^{-1}°C^{-1}$ in eqns (6) and (14))
Δ_{max} Modulus of the determinant of $\mathbf{F}$ for a fixed temperature profile ($min^{-1}°C^{-1}$)
Δ_{opt} Modulus of the determinant of $\mathbf{F}$ for optimal conditions ($min^{-1}°C^{-1}$)
η_i Fractional concentration for the ith experiment
θ Vector of p parameters
ζ Efficiency factor (%)

INTRODUCTION

Preservation of foods by thermal processing is based on reducing the number of vegetative organisms and bacterial spores. Frequently, the lethality desired or achieved in a given process is estimated using experimental kinetic data for the pathogens thermal death rate. Although the concepts and mathematics of process design and evaluation are not complex, the suitability of different mathematical models to describe the reality is a subject of concern and discussion. Even for the simplest options, the so-called TDT (or Bigelow) model and the first order (or Arrhenius) model, discussion still exists on the best alternative (e.g. Jonsson *et al.*, 1977; Manji and van de Voort, 1985; Pflug, 1987; Ramaswamy *et al.*, 1989).

For more than 70 years, the model resulting from the empirical observations made by Bigelow (1921) has been the basis for the design of thermal processes used by the canning industry for low acid foods (Nunes *et al.*, 1991). It is commonly accepted that at high temperatures and for relatively short times the logarithm of the number of viable cells decreases linearly with time, the slope being the reciprocal of the decimal reduction time (D), and that the logarithm of D decreases linearly with temperature, the reciprocal of the slope being named the z value. The D value at a reference temperature (D_{ref}) and the z value are the basis of the thermal death time method (TDT), which is the current standard in industrial practice for most

sterilisation processes (Nunes *et al.*, 1991). Assessment of quality loss in thermally processed foods has involved in many cases the application of a similar kinetic model for quality indicators (e.g. Shin and Bhowmik (1995) have used this model to describe kinetics of colour change in pea puree).

The kinetic parameters of the mathematical models are estimated by regression of experimental data obtained in most cases with isothermal experiments. The use of non-isothermal methods was first introduced by Rogers (1963) and was reported for the study of reaction kinetics (Rhim *et al.*, 1989a; Nunes *et al.*, 1991) and for the kinetics of colour change (Rhim *et al.*, 1989b). These methods have significant advantages: minimisation of experimental requirements, overcoming thermal lag problems and providing a dynamic situation closer to the reality of most thermal processes. In all cases cited above a first-order rate with an Arrhenius type dependency on temperature was considered. Linear temperature profiles are the most common because they are very easy to obtain experimentally. The Arrhenius equation considers an exponential variation of the rate constant with the reciprocal of temperature and this eventually leads to an integral that does not have an exact analytical solution (Rhim *et al.*, 1989a). This is a clear drawback of the method, as the error introduced by either approximate analytical solutions or by numerical resolution of the integral impairs the accuracy of the regression. Curiously, the Bigelow model would not have this problem, as the integration of the process equation when the rate constant varies exponentially with temperature (instead of its reciprocal) is straightforward (Miles and Mackey, 1994). Moreira *et al.* (1993) have compared isothermal and non-isothermal methods for estimation of mass diffusion kinetics with an Arrhenius type temperature dependency, using numerical integration.

The estimation of kinetic parameters from experimental data involves the application of statistical methods in two phases: experimental design and data analysis. Although much more emphasis is often put on the latter, one should realise that the general value of the information contained in the data is actually established when the experiment is designed and even a very careful data analysis is unable to recover information that is not present in the data (Bates and Watts, 1988). As Lenz and Lund (1980) stressed, much of the data currently found in the literature could have been obtained with considerable less effort by proper choice of experimental conditions. Furthermore, the quality of the experimental data greatly determines the quality of the parameters obtained in terms of precision and accuracy. This is of utmost importance if microbiological or chemical model parameters are then used in process design or assessment (Van Boekel, 1996).

Box and Lucas (1959) proposed an optimum design criterion for nonlinear models, based on establishing the sampling conditions that lead to a minimum confidence region, for a standard situation of a number of observations (n) equal to the number of parameters (p), which is also known as the D-optimal design (Bates and Watts, 1988). This criterion was applied to a first order model with Arrhenius temperature dependency at isothermal conditions (Box and Lucas, 1959) and to diffusional processes with an Arrhenius temperature dependency at non-isothermal conditions (Oliveira *et al.*, 1995).

The main objective of this work was to establish the experimental conditions corresponding to the D-optimal design for systems described by the Bigelow model for both isothermal and non-isothermal experimental plans.

MATHEMATICAL METHODS

For any choice of the design variable (i.e., the independent variable, t) the size of the parameters joint confidence region is proportional to the Jacobian $|(\mathbf{F}^T\mathbf{F})|^{-1/2}$ of the derivative matrix $\mathbf{F}$ (where $\mathbf{F} \equiv [fi,j]$, with $fi,j = \partial\eta i/\partial\theta j$ evaluated at $t = t_i$, with i ranging from 1 to n. η represents the system response and θ a kinetic parameter). Thus a logical choice of the design criterion is to choose sampling points so that the size of this joint confidence region is minimised, that is, the determinant $D \equiv |\mathbf{F}^T\mathbf{F}|$ should be maximised. According to Box and Lucas (1959), if a sequence of n observations is to be designed for a p-parameter model, the D-optimal design can be simplified from the maximisation of $D \equiv |\mathbf{F}^T\mathbf{F}|$ to the maximisation of $\Delta \equiv \text{mod}(|\mathbf{F}|)$, in the case where $n = p$ (Δ denotes the modulus of the determinant of the matrix $\mathbf{F}$). Atkinson and Hunter (1968) showed that for a number of observations higher than the number of parameters, the optimal design often corresponds to r replications of the optimal p sampling times ($r = n/p$).

In our work the initial concentration was not considered to be a model parameter. By logical reasoning, if one desires to estimate this value, the extra optimal sampling time would be zero.

Isothermal conditions

Single temperature

The variation of the system response with time at a constant temperature T, for the Bigelow model, is given by:

$$\eta_i = 10^{\left(-\frac{t_i}{D_T}\right)} \tag{1}$$

where η is the fractional concentration of colony forming microbial units, or of a quality factor, at time t_i, and D_T is the decimal reduction time at the experimental temperature, T. According to the definition of Δ:

$$\Delta = \text{mod}\left(\left|\frac{\partial\eta_i}{\partial D_T}\right|\right) = \text{mod}\left(\frac{t_i \ln(10) 10^{-t_i/D_T}}{D_T^2}\right) = \text{mod}\left(-\frac{\eta_i \ln(\eta_i)}{D_T}\right) \tag{2}$$

The optimum sampling time for estimating D_T, and corresponding η value, was calculated analytically from the zero of the derivative of eqn (2) in relation to time.

Range of temperatures

Applying the temperature dependency relationship to eqn (1) for an isothermal experiment at temperature T_i:

$$\eta_i = 10^{\left(-\frac{t_i}{D_{\text{ref}} 10^{\frac{T_{\text{ref}} - T_i}{z}}}\right)} \tag{3}$$

Deriving eqn (3) in relation to the model parameters, D_{ref} and z, yields:

$$\frac{\partial \eta_i}{\partial D_{ref}} = \frac{t_i \ln(10)\ 10^{\left[-\frac{t_i}{D_{ref} 10^{\frac{T_{ref}-T_i}{z}}} - \frac{T_{ref}-T_i}{z} \right]}}{D_{ref}^2} = -\frac{\eta_i \ln(\eta_i)}{D_{ref}} \tag{4}$$

$$\frac{\partial \eta_i}{\partial z} = \frac{t_i \ln(10)^2\ 10^{\left[-\frac{t_i}{D_{ref} 10^{\frac{T_{ref}-T_i}{z}}} - \frac{T_{ref}-T_i}{z} \right]}}{D_{ref} z^2} (T_{ref}-T_i)$$

$$= -\frac{\eta_i \ln(\eta_i) \ln(10)}{z^2} (T_{ref}-T_i) \tag{5}$$

From eqns (4) and (5) the determinant Δ was built for two sampling times, t_1 and t_2, the former being the sampling time for one experiment at T_1 and the latter for one experiment at T_2. For the sake of simplification, Δ is indicated in terms of η_1 and η_2:

$$\Delta = \mathrm{mod}\left(\begin{vmatrix} \frac{\partial \eta_1}{\partial D_{ref}} & \frac{\partial \eta_1}{\partial z} \\ \frac{\partial \eta_2}{\partial D_{ref}} & \frac{\partial \eta_2}{\partial z} \end{vmatrix} \right) = \mathrm{mod}\left(\frac{\eta_1 \ln(\eta_1) \eta_2 \ln(\eta_2) \ln(10)}{D_{ref} z^2} (T_2 - T_1) \right) \tag{6}$$

The maximum value of Δ can be obtained by calculating analytically the zero values of the derivatives of eqn (6) in order to η_1 and η_2, or by finding the conditions that maximise $\mathrm{mod}(T_2 - T_1)$, $\mathrm{mod}(\eta_1 \ln(\eta_1))$ and $\mathrm{mod}(\eta_2 \ln(\eta_2))$ simultaneously.

Non-isothermal conditions

Non-isothermal kinetic models are based on three equations: (i) the rate of reaction, (ii) the temperature dependency of the kinetic parameters, and (iii) the time–temperature relationship (Rhim *et al.*, 1989a). For a constant heating rate, m, the latter is:

$$T(t) = T_0 + mt \tag{7}$$

where T_0 is the initial temperature. The process equation becomes:

$$\ln(\eta_i) = \frac{\ln(10)}{D_{ref}} \int_0^{t_i} 10^{\left(\frac{T_0 + mt - T_{ref}}{z} \right)} \mathrm{d}t \tag{8}$$

Solving the integral analytically and rearranging:

$$\eta_i = 10^{\left\{-\frac{z}{D_{\text{ref}} m \ln(10)} 10^{\left(\frac{T_0 - T_{\text{ref}}}{z}\right)} \left[10^{\left(\frac{mt_i}{z}\right)} - 1\right]\right\}} \tag{9}$$

Deriving this equation in relation to D_{ref} and z:

$$\frac{\partial \eta_i}{\partial D_{\text{ref}}} = \frac{z\left[10^{\left(\frac{mt_i}{z}\right)} - 1\right] 10^{\left\{\frac{T_0 - T_{\text{ref}}}{z} - \frac{z}{D_{\text{ref}} m \ln(10)} 10^{\left(\frac{T_0 - T_{\text{ref}}}{z}\right)} \left(10^{\left(\frac{mt_i}{z}\right)} - 1\right)\right\}}}{D_{\text{ref}}^2 m}$$

$$\frac{\partial \eta_i}{\partial z} = \frac{10^{\left\{\frac{T_0 - T_{\text{ref}}}{z} - \frac{z}{D_{\text{ref}} m \ln(10)} 10^{\left(\frac{T_0 - T_{\text{ref}}}{z}\right)} \left(10^{\left(\frac{mt_i}{z}\right)} - 1\right)\right\}}}{D_{\text{ref}} m z} * \tag{10}$$

$$* \left\{\left(10^{\left(\frac{mt_i}{z}\right)} - 1\right)[(T_0 - T_{\text{ref}}) \ln(10) - z] + 10^{\left(\frac{mt_i}{z}\right)} mt_i \ln(10)\right\} \tag{11}$$

These equations may be simplified if written in terms of η_i:

$$\frac{\partial \eta_i}{\partial D_{\text{ref}}} = -\frac{\eta_i \ln(\eta_i)}{D_{\text{ref}}} \tag{12}$$

$$\frac{\partial \eta_i}{\partial z} = -\frac{\eta_i \ln(\eta_i)}{z^2} \left\{[T_0 - T_{\text{ref}}]\ln(10) - z + \frac{X_i \ln(X_i) z}{X_i - 1}\right\}$$

with

$$X_i = 1 - \frac{\ln(\eta_i) D_{\text{ref}} m}{z 10^{\frac{T_0 - T_{\text{ref}}}{z}}} \tag{13}$$

X_i is a dummy variable used for condensed notation.

The determinant Δ is therefore:

$$\Delta = \text{mod}\left(\frac{\eta_1 \ln(\eta_1) \eta_2 \ln(\eta_2)}{D_{\text{ref}} z}\left[\frac{X_2 \ln(X_2)}{(X_2 - 1)} - \frac{X_1 \ln(X_1)}{(X_1 - 1)}\right]\right) \tag{14}$$

The two values of the response function that maximise Δ were determined numerically from eqn (14) using *Mathematica*® (for Windows 2.2, enhanced version, Wolfram, 1993).

RESULTS AND DISCUSSION

Optimal design for isothermal conditions

Single temperature
The optimal sampling conditions obtained from eqn (2) are:

$$t = \frac{D_T}{\ln(10)}\ ;\ \eta = e^{-1} \tag{15}$$

Thus, the best design will be to take a sample at the time when the concentration is 36.8% of the initial value. These results are identical to those obtained by Box and Lucas (1959) for a first order decay model, considering the relationship between D_T and the reaction rate, k_T, of the first-order model (Ramaswamy *et al.*, 1989):

$$D_T = \frac{\ln(10)}{k_T} \tag{16}$$

Temperature range
From eqn (6), for a range of temperatures, Δ is maximised when $\text{mod}(T_2 - T_1)$ is maximum (T_1 and T_2 are the limits of the temperature range considered) and η_1 and η_2 are both e^{-1} at the respective temperature (that is, the sampling times at temperatures T_1 and T_2 are such that the concentration has decreased to 36.8% in both cases). These results are evident from eqn (6) and the previous result for a single temperature.

Considering the relationship between the z-value and the activation energy, E_a, of the Arrhenius model ($z = RTT_{ref}\ln(10)/E_a$, Ramaswamy *et al.*, 1989) and the relation between D_T and k_T expressed by eqn (16), it can be seen that the sampling times for a system following the Bigelow model over a range of temperatures are identical to the sampling times obtained by Box and Lucas (1959) for the optimal design of a system following the first-order decay model with an Arrhenius relationship.

Optimal design for non-isothermal conditions

As the complexity of eqn (14) prevented a direct analytical solution, the optimal design for the non-isothermal Bigelow model was numerically computed for different sets of the parameters D_{ref} and z. The η_1 and η_2 values that maximised Δ were determined for different values of the heating rate, m and of the initial temperature, T_0. It was found that, for all conditions, the value of Δ increased with the heating rate and decreased with the initial temperature, up to a given limit. The values of m and T_0 beyond which there was no significant increase in the value of Δ were considered to be the mathematically optimum conditions, regardless of their physical realisability, and the corresponding value of Δ will be named Δ_{opt}.

For these optimal conditions we have then found that for all the situations tested the sampling times t_1 and t_2 were such that the fractional concentrations had exactly the same values. This solution of the optimisation problem was a pair of irrational numbers: $\eta_1 = 0.70322\ \ldots$ and $\eta_2 = 0.19245\ \ldots$. It was necessary to establish mathe-

matically if these would be the solutions for all possible combinations of the system parameters. When the solution of an optimisational problem (zero of a derivative) is irrational, it is sometimes possible to find simple implicit equations that characterise the solution. By mathematical manipulations, we found that all pairs of η_1 and η_2 values that verify the system:

$$\eta_1 \times \eta_2 = \frac{1}{e^2} \tag{17}$$

$$\eta_2 = \eta_1^{e^{[1/(1+\ln(\eta_1))]}} \tag{18}$$

are solutions of the equation obtained by finding the zero of the derivative of eqn (14) in relation to time (this can be verified by replacing eqns (17) and (18) in eqn (14)). It is noteworthy that eqn (17) expresses that the two sampling points are symmetrical in a logarithmic scale in relation to e^{-1} (the optimum sampling time for a constant temperature).The feasibility of the optimal experimental design thus obtained needed careful consideration, as the values of m and T_0 may be outside reasonable limits. Table 1 shows the results obtained for $T_0 = 20°C$ and it can clearly be seen that the optimal experimental design may lead to sampling times that are too small and heating rates that are too high to be physically feasible, although the choice of T_0 is much more favourable than what could be used in microbial death kinetics.

It is therefore necessary to analyse the sensitivity of the precision of the estimated parameters to the experimental conditions, m and T_0, so that suboptimal designs can

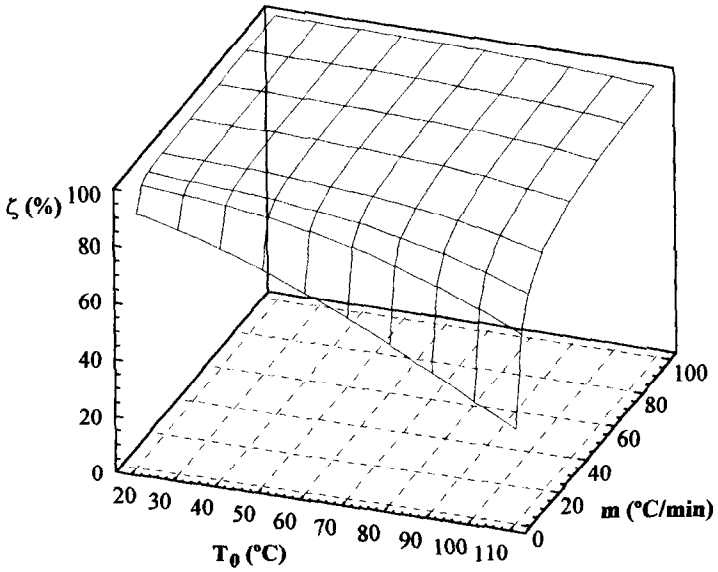

Fig. 1. Influence of the initial temperature (T_0) and of the heating rate (m) on the efficiency (ζ) of the optimal design for the Bigelow model under non-isothermal conditions (D_{ref} = 48 min, z = 59°C and T_{ref} = 121°C).

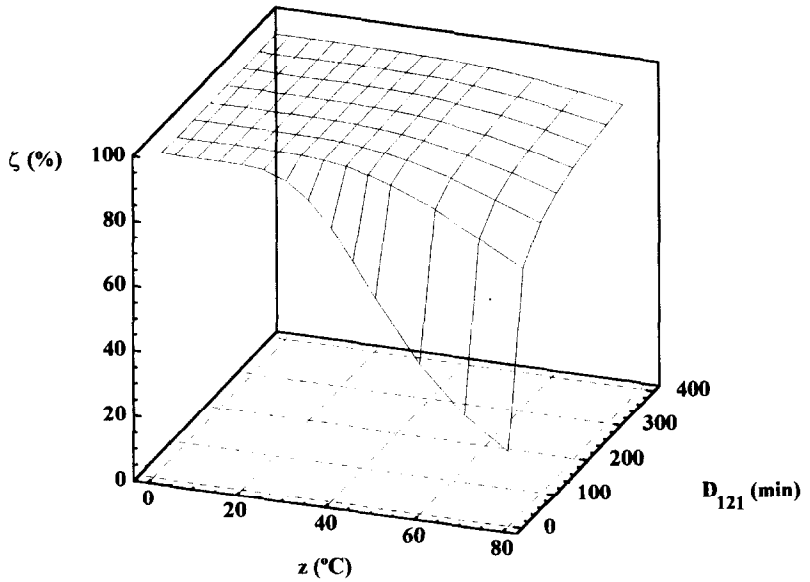

Fig. 2. Influence of D_{ref} and of the z-values on the efficiency (ζ) of the optimal design for the Bigelow model under non-isothermal conditions (T_{ref} = 121°C).

be defined. As the precision of the estimates is measured by the size of the confidence regions, it can be said that the ratio of the determinant Δ for a given situation to the optimum value, Δ_{opt}, is a fractional measurement of the precision of that situation compared to the maximum possible precision. For this purpose we have defined an efficiency factor, $\zeta = \Delta_{max}/\Delta_{opt} * 100$, where Δ_{max} is the maximum value of the determinant that verifies any given physical restriction (maximum m or minimum T_0). A suboptimal design will be obtained by specifying the physical limits of m and T_0 and then determining the sampling times for those conditions.

The sensitivity of the regression precision to m and T_0 for a given set of kinetic parameters (D_{ref} = 48 min; z = 59°C, T_{ref} = 121°C – see Table 2) is shown in Fig. 1. It can be seen that a very good precision can be obtained when the initial temperature is low. Increasing the heating rate improves the precision of suboptimal designs

TABLE 1

Optimal Sampling Times and Heating Rate for the Non-isothermal Bigelow Model, with T_{ref} = 121°C and T_0 = 20°C

D_{ref} (min)	z (°C)	m (°C/min)	t_1 (min)	t_2 (min)
4	10	20	2.7	3.1
4	40	100000	0.019	0.0022
80	10	100	0.82	0.90
80	40	100000	0.0024	0.0027

TABLE 2
Kinetic Data for the Variation of Some Foodstuff Properties, with $T_{ref} = 121°C$ (Adapted from Hallström *et al.*, 1988[a])

	z (°C)	*D_{ref} (min)*
Chemical changes		
Non-enzymatic browning	17–39	0.4–40
Denaturation of proteins	5–10	5
Lysine	21	750
Vitamin destruction		
In general	20–30	100–1 000
Thiamine	20–30	38–380
Ascorbic acid (vit. C)	51	245
Pantothenic acid	31	250–6 400
Riboflavin (vit. B_2)	28	2 800
Folic acid	37	2 800
Enzyme inactivation		
In general	7.55	1–10
Peroxidase	26–37	2–3
Lipases (from *Pseudomonas*)	25–37	1.2–1.7[b]
Proteases (from *P. frourosceus*)	20–35	4–27[c]
Proteases (from *Pseudomonas*)	32	0.5–1.7[b]
Micro-organisms		
B. stearothermophilus	7–13	3.5–6.8
B. subtilis	6.8–13	0.4–0.76
B. cereus	9.7	0.038–0.065
B. megaterium	8.8	0.04
C. botulinum	8–12	0.1–0.3
C. perfringens	10	
C. sporogenes	9–13	0.15–2.6
C. sporogenes (PA 3679)	10.6	0.48–1.4
C. thermosaccharolyticum	1.7–10	3–22
C. nigrificans	8–10	2–3
Cooking value: overall quality estimation		
Peas	17–28	12.5
Sugar beets	29	2.0
Whole corn	36	2.4
Broccoli	44	4.4
Squash	23	1.5
Carrots	15	1.4
Green beans	14–29	1.4
Potatoes	21	1.2
Colour		
Chlorophyll (spinach, pea puree)	38–80	14–350
Carotenoids (paprika)	19	0.038
Betamin (beetroots)	59	48

[a]Table 1.4, from page 25, with permission of Chapman & Hall. International Thomson Publishing Services Ltd
[b]At D_{150}; [c]at D_{120}–D_{150}.

significantly, but for high initial temperatures very high heating rates would be required, which can only be achieved in special equipment (such as a thermo-resistometer). The effect of the kinetic parameters on the sensitivity is shown in Fig. 2, for a given set of experimental conditions ($m = 1$°C/min, $T_0 = 20$°C). It can be seen that for a low initial temperature high precisions are obtained in virtually every range of D_{ref} and z of interest, as the efficiency of the suboptimal design only decreases in the area where z is very high and D_{ref} is low, but this does not occur in food processing: quality factors have high z but also high D_{ref} while micro-organisms show low D_{ref}, but low z as well. The magnitude of kinetic parameters found in literature is shown in Table 2, from the values collected by Hallström *et al.* (1988). It can be concluded that the efficiency of the suboptimal design needs to be considered only if there is a restriction on the initial temperature, either because the model does not apply at low temperatures (which is the obvious case of microbial thermal death), or because the heating medium cannot be initiated at a low temperature (this is the case of thermostatic oil baths).

It should be stressed that the choice of the reference temperature does not show any effect on the optimal design.

Reaction rate concept

The thermal degradation rate is influenced by the kinetic parameters (D_{ref} and z) and by the experimental conditions (T_0 and m). Figure 3(a) shows the kinetic patterns for generic values of these four parameters and indicates the influence of each parameter on the curve. Evidently the shape of this curve is directly influenced by the changes on the rate of thermal degradation. To study if optimal and sub-optimal designs implied any given type of shape, the reaction rate was calculated from the model equations:

$$v_i^* = \left| \frac{1}{N_0} \frac{dN}{dt} \right| = \left| -10^{\left\{ \frac{mt_i + T_0 - T_{ref}}{z} - \frac{z}{D_{ref} m \ln(10)} 10^{\left(\frac{T_0 - T_{ref}}{z} \right)} \left[10^{\left(\frac{mt_i}{z} \right)} - 1 \right] \right\}} \frac{\ln(10)}{D_{ref}} \right| \quad (19)$$

where v^*_i is the reaction rate at the sampling time t_i. Figure 3(b) represents the reaction rates corresponding to the curves of Fig. 3(a), showing that there is a maximum rate at the time where the decay curve has an inflection point.

Figure 4 shows the initial reaction rate and the reaction rates of the two sub-optimal sampling times for several designs, normalised with the maximum reaction rate in each case, so that all designs fall in the same curves. It can be seen that sampling designs of high efficiency have low initial reaction rates and that the reaction rate of the first sampling point is lower than that of the second sampling point. In these cases ($\zeta > 80\%$), the maximum reaction rate occurs between the two sampling points. Situations where the initial reaction rate is high have very low design efficiencies and the first sampling point has a higher reaction rate than the

second. Designs of high efficiency in terms of precision therefore lead to a curve of concentration versus time showing a clear shoulder, with the inflection point in between the two sampling times.

CASE STUDY

To clarify the application of these concepts in food research, a case study is provided, using literature data. Shin and Bhowmik (1995) studied the kinetics of total colour retention ($-La/b$, from the Hunter colour scale) and the kinetics of *C. botulinum* spores thermal death in the thermal processing of pea puree. These quality and safety indicators show very different degradation (or death) rates and therefore provide a good basis for a case study. The system parameters are shown in Table 3.

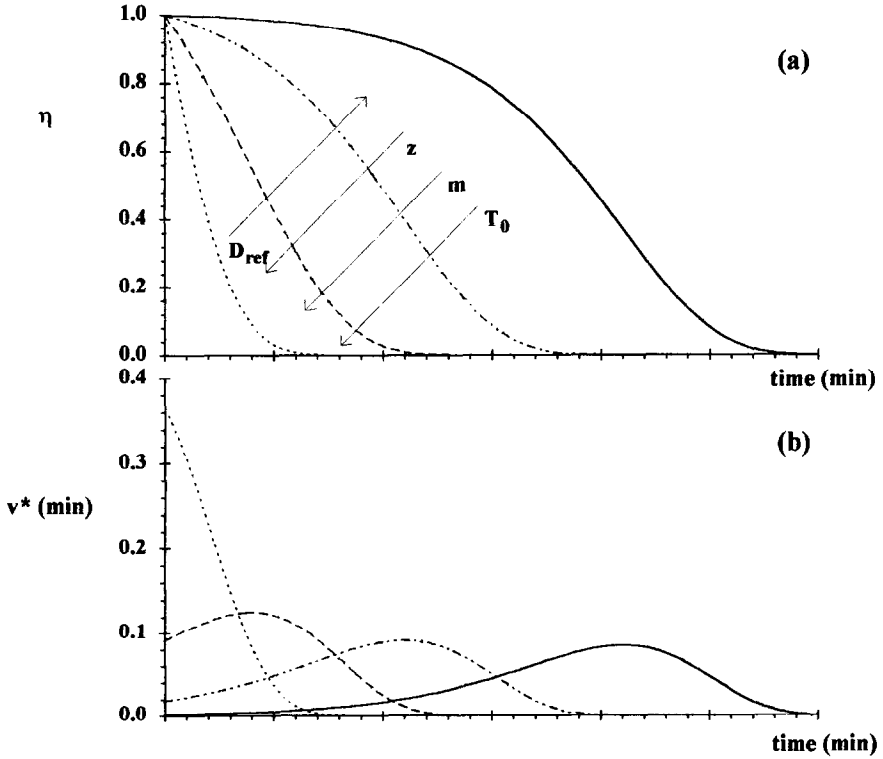

Fig. 3. (a) Some typical thermal degradation profiles for the Bigelow model under non-isothermal conditions as influenced by the kinetic parameters (D_{ref} and z) and by the experimental conditions (expressed in terms of T_0 and m). (b) Corresponding normalized reaction rate profiles. The arrows indicate the effect of increasing the different parameters.

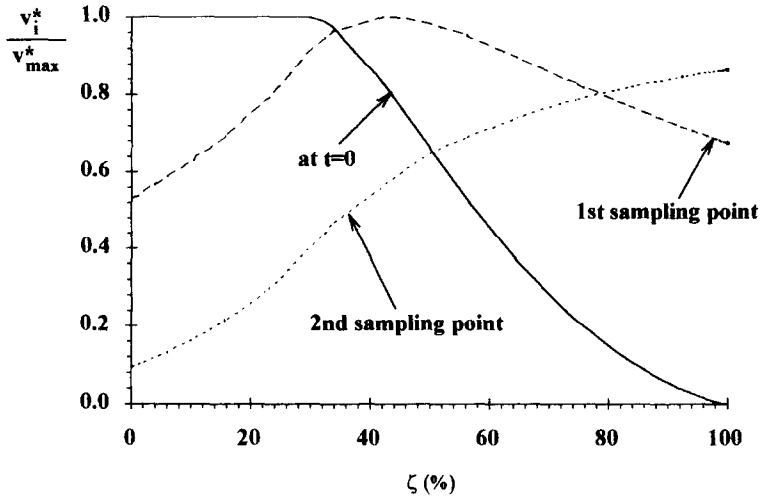

Fig. 4. Influence of the normalized reaction rates taken at each of the optimal sampling times and for $t = 0$ on the efficiency (ζ) of the optimal design for the Bigelow model under non-isothermal conditions.

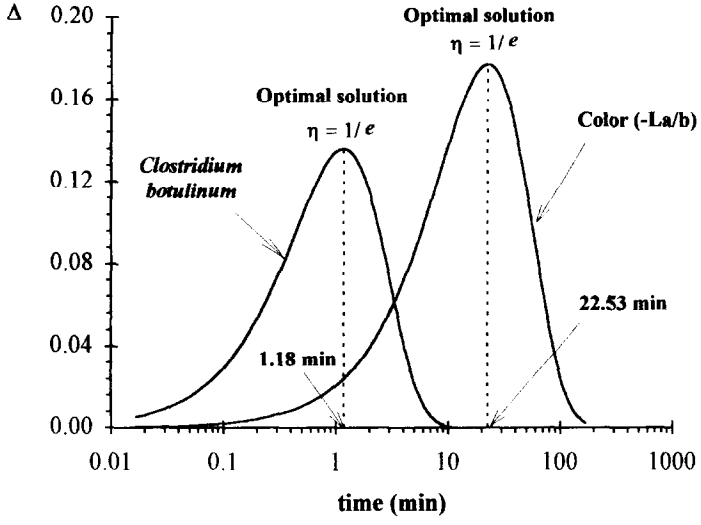

Fig. 5. The design *locus* for the microbial and colour systems (Shin and Bhowmik, 1995) assuming the Bigelow model for a single temperature ($T = 110°C$). (The value of Δ for the colour system was multiplied by 25 for the sake of clearness).

Isothermal conditions

For the optimal sampling time for a single experiment, 110°C was considered. D_{110} was calculated ($D_{110(\text{microbial})} = 2.7$ min; $D_{110(\text{colour})} = 51.9$ min) and then by application of eqn (15) each of the optimal sampling times was obtained. Figure 5 shows the value of Δ as a function of the time at which the sample is taken, to visualise the maximum precision that is obtained with a sampling time where the conversion is $1/e$ (1.2 and 22.5 min for microbial and quality factors, respectively).

Considering that one would wish to verify the system parameters D_{ref} and z, in the temperature range 100 to 120°C, eqn (6) is used to calculate Δ. The optimal design implies performing one experiment at 100°C and another at 120°C and for each obtaining a sample at the time when the fractional concentration at that temperature is $1/e$. The optimum sampling times are therefore different for the two factors.

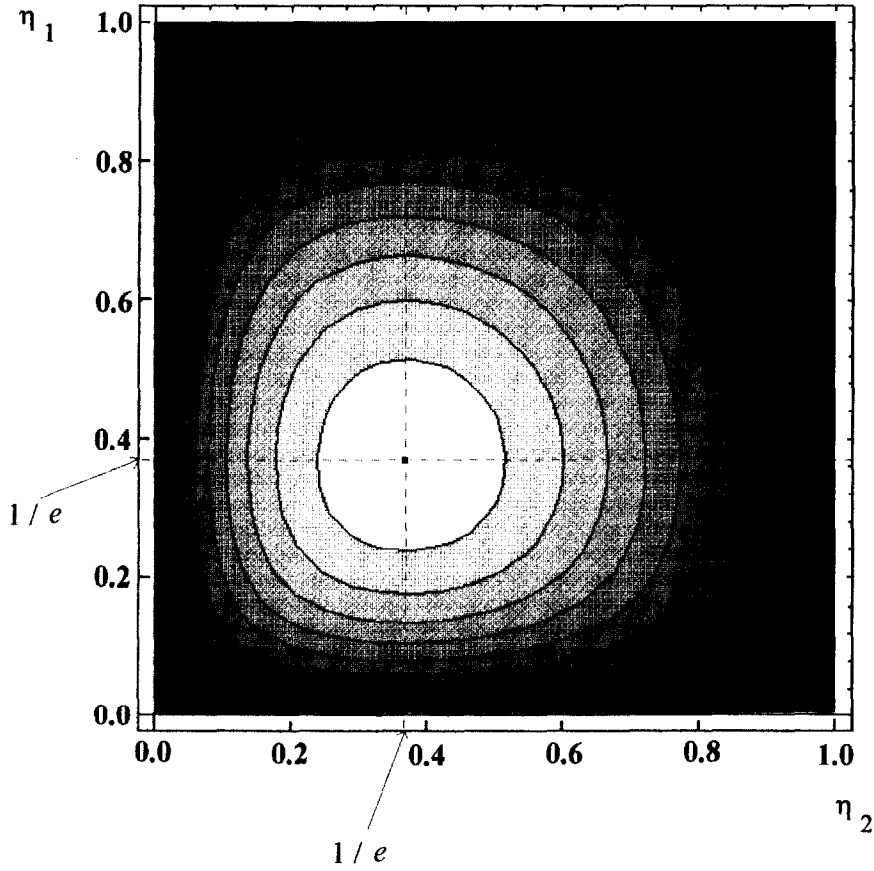

Fig. 6. Contour plot of Δ in the space of η_1 and η_2 for the isothermal conditions in a range of temperatures – eqn 6 ($D_{\text{ref}} = 28.58$ min, $z = 42.87$°C, $T_1 = 100$°C and $T_2 = 120$°C) (darker areas correspond to lower values of Δ).

TABLE 3
Optimal Sampling Times for Kinetic Studies of Microbial Death and Colour Degradation in Pea Puree (Shin and Bhowmik, 1995) with the Bigelow Model at Isothermal Conditions (for a Range of Temperatures $100°C \leq T \leq 120°C$)

System	$D_{121.1}$ *(min)*	z *(°C)*	T *(°C)*	t_i^a *(min)*
			100	11.75
Clostridium botulinum spores	0.21	10.00		
			120	0.12
			100	38.55
Total colour ($-La/b$)	28.58	42.87		
			120	13.17

[a] $\eta_i = 1/e$ in all cases.

The results are shown in Table 3. Figure 6 visualises the precision of the sampling design. Contour plots of iso-Δ values are shown for the fractional concentration at the sampling time used in the experiment at 100°C versus that of the experiment at 120°C, showing the maximum Δ when both are equal to $1/e$. It is important to note

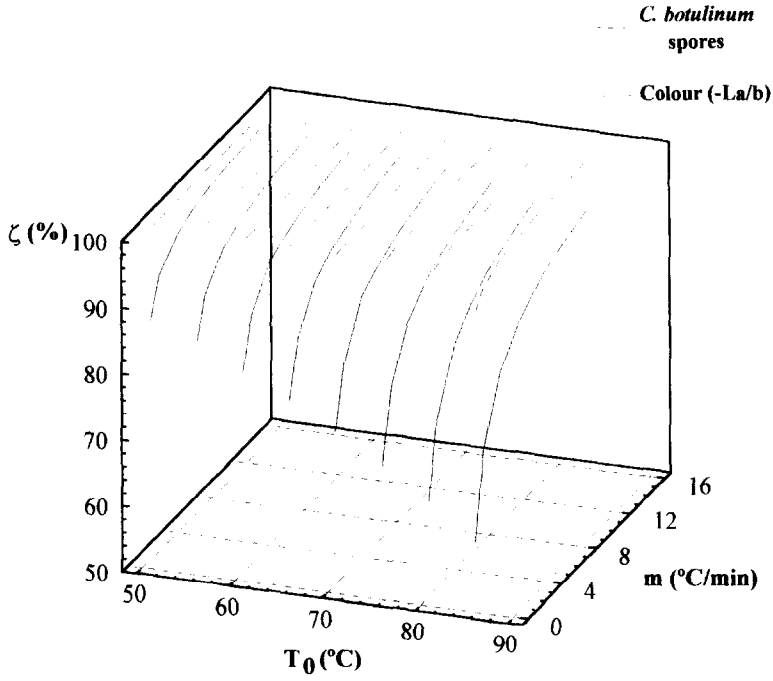

Fig. 7. Influence of the heating rate (m) and of the initial temperature (T_0) on the efficiency (ζ) of the optimal design for the Bigelow model under non-isothermal conditions, for two biological systems from pea puree (Shin and Bhowmik, 1995).

that for the microbial death kinetics, the sampling time at 120°C is too low. Non-isothermal methods might therefore be preferable.

Non-isothermal conditions

To establish the optimal design for a non-isothermal experiment, the physical limits of a Julabo heating oil (Julabo thermal H, JULABO Labortechnik GMBH, Seelbach, Germany) were considered. The minimum recommended temperature is 50°C and the maximum heating rate is 14°C/min.

Figure 7 shows the efficiency of suboptimal designs for several combinations of heating rate and initial temperature. For the microbial death kinetics the initial temperature does not affect the efficiency too much. For an initial temperature of 85°C very high efficiencies are obtained using heating rates above 4°C/min. Low initial temperatures should not be considered, as the microbial thermal death model does not obviously apply at 50°C. For the quality factor (colour change), however, high initial temperatures cause a significant loss of efficiency in the precision, which only the highest heating rates could compensate somewhat. However, this would imply a very short initial sampling time, specially for the microbial death rate, which is not physically reasonable. Therefore, this figure shows that the experiments for determining the kinetic parameters for the microbial system and for the quality system should not be the same. For the former, high initial temperature should be used, and for the latter the lowest initial temperature is recommended. In these conditions, heating rates around 4 to 8°C/min are good enough to have optimum precision.

For instance, for the loss of colour, the initial temperature of 50°C and the heating rate of 8°C/min would have as optimum sampling times 10.4 and 14.0 minutes, with design efficiency close to 100%. For the microbial death kinetics, the initial temperature of 85°C and heating rate of 4°C/min would give a design efficiency close to 100%, but the sampling times would be 5.2 and 6.9 minutes. These values are very close and the error in the measurement of time, which is not accounted in this statistical analysis, is an additional source of error. It would be best to use a heating rate of 1°C/min, with sampling times of 15.1 and 21.6 minutes, although the efficiency in the precision would then be around 93%. If the microbial death kinetics could be considered from 80°C onwards, then the initial temperature of 80°C with a heating rate of 1°C/min would have close to 100% efficiency, with sampling times of 19.9 and 26.5 minutes. The final temperature (temperature at the second sampling time) would be around 107°C, which is relatively low. A more reasonable design in relation to process temperatures would include higher initial temperatures and would have decreased precision. For instance, for a high initial temperature of 100°C and a heating rate of 1°C/min, the sampling times are 3.6 and 7.9 minutes and the efficiency is only 48%.

CONCLUSIONS

The *D*-optimal experimental designs for the Bigelow model and the first-order/Arrhenius decay model under isothermal conditions are identical: this implies that experimental data obtained by the application of optimal design may be used to estimate the parameters of each model with similar precision.

Non-isothermal experiments using linearly increasing temperature histories should be started at the lowest possible temperature and use the highest possible heating rate. This is particularly important if the reaction under study has a low sensitivity to temperature (high z-values). When high heating rates may be applied, the effect of the initial temperature becomes less important.

High efficiencies of the precision (above 80% of the maximum possible precision, quantified in terms of the size of the confidence region) imply a low initial reaction rate and that the maximum reaction rate occurs between the two sampling points.

These conclusions should not be generalised for other kinetics. It is curious to note, for instance, that in a similar study conducted for diffusional processes where the influence of the temperature in the mass transfer rate is much lower, optimal results were obtained at intermediate heating rates, with both high and low heating rates decreasing the design efficiency (Oliveira *et al.*, 1995).

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