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ORIGINAL ARTICLE

Stress-adaptive responses by heat under the microscope of predictive microbiology

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Abstract

Aims: In previous studies the microbial kinetics of *Escherichia coli* K12 have been evaluated under static and dynamic conditions (Valdramidis *et al.* 2005, 2006). An acquired microbial thermotolerance following heating rates lower than 0·82°C min^{-1} for the studied micro-organism was observed. Quantification of this induced physiological phenomenon and incorporation, as a model building block, in a general microbial inactivation model is the main outcome of this work.

Methods and Results: The microbial inactivation rate observed (k_{obs}) under time-varying temperature conditions is studied and expressed as a function of the heating rate (dT/dt). Hereto, a model building block related to the microbial physiology (k_{phys}) under stress conditions is developed. Evaluation of the performance of the developed mathematical approach depicts that physiological adaptation is an essential issue to be considered when modelling microbial inactivation.

Conclusions: Consideration, at a mathematical level, of microbial responses resulting in physiological adaptations contribute to the reliable quantification of the safety risks during food processing.

Significance and Impact of the Study: By taking into account the physiological adaptation, the microbiological evolution during heat processing can be accurately assessed, and overly conservative or fail dangerous food processing designs can be avoided.

Introduction

Bacteria once exposed to a mild stress exhibit an ability called stress-adaptive response (SAR), which enables them to resist further stresses (homologous or heterologous). If this stress is because of a heat treatment the resulting physiological phenomenon is named 'acquired microbial thermotolerance' (Yousef and Courtney 2003). The same phenomenon is also reported in the literature as adaptive response, induced thermotolerance, habituation, acclimatization or stress hardening (Yousef and Courtney 2003).

Several literature studies focused on the effect of the increasing heating rates (similar to those employed for minimally processed food products) on the acquired microbial thermotolerance as it influences clearly the observed microbial inactivation kinetics. These studies (as summarized next) have been performed for evaluating the kinetics of different bacteria, i.e. *Escherichia coli*, Salmonellae, *Listeria monocytogenes*, *Staphylococcus aureus* and also the yeast *Saccharomyces cerevisiae*.

Tsuchido *et al.* (1974, 1982) studied the effect of a temperature elevating process (for linearly increasing temperatures from 0 to 50°C and heating rates of 1, 2·1, 5, 27, 750°C min^{-1} (Tsuchido *et al.* 1974) and of 0·3, 0·6, 1, 10, 17·5°C min^{-1} (Tsuchido *et al.* 1982), on the subsequent isothermal death of *E. coli* K12 inoculated in a

nutrient broth. The thermal resistance of the cells of *E. coli* K12 decreased with increase of the heating rates. Similar results were obtained by Valdramidis *et al.* (2006) for the same strain and when the temperature increased from 30 to 55°C and the heating rates varied between 0·15 and 1·64°C min^{-1}.

Thompson *et al.* (1979) and Juneja and Marks (2003) also encountered this phenomenon by studying the inactivation kinetics for *Salmonella* Typhimurium (linearly increasing temperature from 47 to 60°C) and *Salmonella* spp. (linearly increasing temperature from 10 to 58°C), respectively, in beef products. The heating rates under investigation were 0·1–0·2°C min^{-1} for the former and 0·27–0·8°C min^{-1} for the latter case study. These studies indicate that cells during slow come-up times are able to adjust to environmental changes and thus become more resistant. The observed change in the heat resistance of *Salmonellae* was in accordance with the studies of Mackey and Derrick (1987). In that study the survival of *S.* Typhimurium when heated at a rate of 0·6–10°C min^{-1} in a broth system and at a temperature increasing linearly from 20 to 55°C was under investigation.

An inverse relation between induced thermotolerance and heating rate was also reported in case studies of *L. monocytogenes*. On the one hand, Quintavalla and Campanini (1991) observed that heating cells of *L. monocytogenes* in meat emulsions at rising temperatures of 0·5°C min^{-1} from 40°C to different final constant temperatures, i.e. 60, 63, 66°C, increased the heat resistance of the organism. On the other hand, Stephens *et al.* (1994), found that at heating rates between 5·0 and 0·7°C min^{-1} (up to a subsequent holding temperature of 60°C), there were cells that developed an increased thermotolerance. Kim *et al.* (1994) proved that the heat resistance of *L. monocytogenes* (serotype 1) in ground pork increased with the decrease of the heating rate from 8 to 1·3°C min^{-1}. Hansen and Knochel (1996) evaluated the effects on the heat resistance in sous-vide cooked beef. The product was inoculated with the same microorganism and at heating rates of 0·3, 0·6 and 10°C min^{-1}, and a significant increase on the heat resistance especially for pH values higher than 5·8, was observed. Finally, Hassani *et al.* (2005) also observed for the same microorganism that the slower the heating rate the greater the induced microbial heat resistance at a linearly increasing temperature profile from 30 to 70°C (heating rates 0·5 to 9°C min^{-1} and for different levels of pH, i.e. 4, 5·5, 7·4). Similar results were obtained at pH levels of 5·5 and 7·4, when the same experimental protocol was followed for studying the inactivation kinetics of *Staph. aureus* (Hassani *et al.* 2006).

Observations like those encountered for vegetative micro-organisms can be found for the yeast *S. cerevisiae* (Marechal *et al.* 1999; Martinez de Marañón *et al.* 1999; Guyot *et al.* 2005).

In most of these studies the microbial heat resistant at the final constant temperatures is quantified. However, to the best of the author's knowledge, a sound mathematical approach making use of differential equations and incorporating the effect of the heating rate on the microbial inactivation has not been developed yet.

The following general expression for the inactivation of a microbial population N in a homogeneous food (model) product as a function of time can be proposed and consists of the following set of n differential equations (Vereecken *et al.* 2000; Bernaerts *et al.* 2004; Van Impe *et al.* 2005).

$$\frac{dN}{dt} = -k(\cdot) = -k(N, <env>, <phys>) \cdot N \qquad (1)$$

where N is the cell density of the microbial species (CFU ml^{-1}), <*env*> is the actual (micro)environmental conditions (not or only slightly influenced by the microbial evolution) such as temperature, high pressure, salt concentration, water activity and so on and <*phys*> is the physiological state of the species, for instance, as influenced by the temperature history.

During previous research within our team, the microbial inactivation of *E. coli* K12 under dynamic conditions was predicted based on static model developments and by taking into account N, <*env*> and <*phys*> (only reflecting the physiological state of the cells at static thermal experimental conditions) in $k(\cdot)$ [see eqn (1)]. A consistent underestimation of the microbial population level, or, in other words, a consistent overestimation of the inactivation achieved, was observed (Valdramidis *et al.* 2006). The aim of this study is to develop a sound differential equation that describes accurately the microbial inactivation kinetics by incorporating physiological adjustments during the experiments, in this case, for changing temperature conditions. Therefore, another factor also related to the physiological state (<*phys*>) of *E. coli* K12 MG1655, namely, as influenced by the exposure to a mild thermal stress, is developed.

Materials and methods

Microbial data

In our work, *E. coli* K12 MG1655 strain was chosen as a surrogate for the food-borne pathogen *E. coli* O157:H7. Survival data at static and dynamic temperature conditions of early stationary phase cultures as described in previous research (Valdramidis *et al.* 2005, 2006) were used. For these experiments sealed sterile glass capillary tubes (Hirschmann) filled with 100 μl of cell suspension

of early stationary phase cultures of *E. coli* K12 MG1655 were immersed in a circulating water bath (GR150-S12, Grant). On the one hand the isothermal microbial inactivation data were obtained at the constant temperatures of 52, 54, 54·6 and 55°C (Valdramidis *et al.* 2005). On the other hand the dynamic experimental data (partially duplicated) were generated at six different heating rates of 0·15, 0·20, 0·40, 0·55, 0·82, 1·64°C min^{-1} with an initial and a final temperature of 30 and 55°C, respectively (for more details see Valdramidis *et al.* 2006).

Available model based on static data

A reduced version (namely, not incorporating the so-called tailing, as it was not apparent in the experimental data) of the dynamic model of Geeraerd *et al.* (2000), has been used for estimating the inactivation parameters following a global identification procedure in which all the static data are used.

$$\frac{dN}{dt} = -k_{max}(T) \cdot \left(\frac{1}{1+C_c}\right) \cdot N \quad (2)$$

$$\frac{dC_c}{dt} = -k_{max}(T) \cdot C_c \quad (3)$$

Herein, $1/(1+C_c)$ is a factor influenced by the physiological state of the cells at static experimental thermal conditions and it describes the so-called shoulder effect (Geeraerd *et al.* 2000, 2005). This factor can be denoted as $k(<phys1>)$. C_c is called the critical component of the cells [-], and it follows a first-order decrease [eqn (3)], while k_{max} is the specific inactivation rate [1/min]. Under static conditions this model reads as follows, with S_l denoting the shoulder length [min]:

$$N(t) = N(0) \cdot \exp(-k_{max} \cdot t) \cdot \frac{\exp(k_{max} \cdot S_l)}{1 + (\exp(k_{max} \cdot S_l) - 1) \cdot \exp(-k_{max} \cdot t)} \quad (4)$$

Observe that this static inactivation model equation is a simple analytical expression suitable for the description of such survivor curve shapes: all parameters have a clear biological/graphical meaning and the shoulder effect is easily recognizable, including the occasions where S_l is zero or very large (approaching infinity). In these limiting cases, the third factor reduces to one (eqn (4) reduces to classical log-linear inactivation) or to $\exp(k_{max}\, t)$ [eqn (4) reduces to $N(t)=N(0)$], respectively. The effect of the temperature on parameter k_{max} was described by the use of the Bigelow (1921) model [eqn (5)]

$$k_{max}(T) = \frac{\ln 10}{\mathrm{Asym}D_{ref}} \cdot \exp\left(\frac{\ln 10}{z} \cdot (T - T_{ref})\right) \quad (5)$$

Herein, $\mathrm{Asym}D_{ref}$ (min) denotes the asymptotic decimal reduction time at a reference temperature T_{ref} (°C) and z (°C) is the thermal resistance constant (Juneja *et al.* 2001). The microbial parameters were identified by the use of eqns (2), (3) and (5). The estimated parameters for $T_{ref} =$ 53·5°C were as follows: $\mathrm{Asym}D_{ref} = 26{\cdot}46$ (min) ± 1·86, $z = 3{\cdot}34$ (°C) ± 0·31, $\log Cc(0) = 0{\cdot}85$ (-) ± 0·21.

When studying the microbial evolution under the dynamic temperature profiles the temperature evolution was given by the modified Dabes kinetics. The Dabes-type model (written with the parameters of interest of this study) is given by the equation $t = A \cdot T + (B \cdot T)/(T_{diff} - T)$(Roels 1983). If $A = (t_{crit} - B)/T_{diff}$ then the modified Dabes kinetics as described by Van Impe *et al.* (1994) is given by eqn (6). Observe that T_o is an intercept added to the modified Dabes model.

$$T = T_o + T_{diff} \cdot \frac{(t + t_{crit}) - \sqrt{(t + t_{crit})^2 - 4 \cdot (t_{crit} - B) \cdot t}}{2 \cdot (t_{crit} - B)} \quad (6)$$

Parameter T_o (°C) represents the initial temperature (equals 30°C in all cases), T_{diff} (°C) is the difference between the initial and the final temperatures (equals 25°C in all cases), B (min) refers to a shape parameter (fixed at $5 \cdot 10^{-4}$min as it takes very low values in all studied cases) and the t_{crit} (min) is the critical time, i.e. the time to reach the holding temperature $T_o + T_{diff}$ (see also Fig. 1). Estimates for the t_{crit} (min) were 15·27, 30·61, 45·41, 61·98, 128·89, 168·27 min for the heating rates of 1·64, 0·82, 0·55, 0·40, 0·20, 0·15°C min^{-1}, respectively (Valdramidis *et al.* 2006).

For performing predictions of the microbial population during the different heating profiles as based on the isothermal inactivation kinetics, eqn (6) is plugged into (5) and the latter to eqns (2) and (3). $N(0)$ equals the experimental microbial population at time zero and parameters $\mathrm{Asym}D_{ref}$, z and C_c are the ones estimated before. Additionally, the following microbiological hypotheses are considered [similar to the approach discussed in Valdramidis *et al.* (2006)]: (i) thermal inactivation starts at 49·5°C, consequently till that time k_{max} of eqn (5) is set equal to zero, (ii) potential microbial growth during the start of the come-up phase is not considered and would, in any case, be very limited.

Characterization of the observed inactivation rate

The microbial inactivation rate observed during the dynamic experimental conditions is studied. This is cperformed by evaluating a so-called observed inactivation rate, k_{obs} (min^{-1}), i.e. the actual slope of the micro-

bial inactivation curve. For estimating these slopes a reparameterized model of eqn (4) is proposed, in which k_{max} is replaced by k_{obs} and S_l by M_d (as explained next):

$$N(t) = N(0) \cdot \exp(-k_{obs} \cdot t) \cdot \frac{\exp(k_{obs} \cdot M_d)}{1 + (\exp(k_{obs} \cdot M_d) - 1) \cdot \exp(-k_{obs} \cdot t)} \quad (7)$$

or in log-transformation:

$$\log(N(t)) = \log(N(0)) - \frac{k_{obs} \cdot t}{In(10)} + \frac{k_{obs} \cdot M_d}{In(10)} - \log(1 + (\exp(k_{obs} \cdot M_d) - 1) \cdot \exp(-k_{obs} \cdot t)) \quad (8)$$

k_{obs} (min^{-1}) denotes the observed inactivation rate under dynamic conditions, $\log(N(0))$ (CFU ml^{-1}) is the initial microbial load and parameter M_d (min) (M_d stands for the microbial delay) denotes the observed delay on the microbial inactivation during dynamic conditions which can be attributed either to temperatures too low to yield microbial inactivation or to an (induced) microbial heat resistance (see example of Fig. 1). Obviously, M_d has a clearly different meaning in comparison with the so-called shoulder length S_l described for the original version of the model in which this shoulder length has significance only in static conditions (Geeraerd *et al.* 2000). The focus of using this model is to estimate the observed inactivation rate of the dynamic experimental studies. In case M_d is larger than the time to reach the holding temperature, t_{crit}, then the region where k_{obs} is identified, represents a static environment (no change of temperature) as in the example represented in Fig. 1.

Identification of a new model building block

For studying the observed inactivation rate, k_{obs}, with respect to the heating rate (dT/dt), a Monod-type equation is chosen. In order to create an unscaled mathematical expression the k_{obs} is divided by the maximum specific inactivation rate, k_{max}, at which the come-up time is equal to zero (i.e. $k_{max\,55}$ of the static experiment at 55°C which equals 0·26 min^{-1}) (Valdramidis *et al.* 2005). Consequently, the following expression is derived having two parameters, i.e. k_1, k_2 (see also Fig. 2).

$$k(<phys2>) = \frac{k_{obs}}{k_{max\,55}} = k_1 \cdot \frac{(dT/dt)}{k_2 + (dT/dt)} \quad (9)$$

$k(<phys2>)$ is then the physiological state of the cells as influenced by the dynamic temperature environment they experience. This equation implies that the lower the heating rate, dT/dt, the lower the physiological state $k(<phys2>)$. Mathematically speaking. eqn (9) tends to a point of (0, 0) with infinite slope. Although experimentally this point cannot be part of an experimental study because at $dT/dt = 0$ the final temperature of 55°C is not reached, the way the dependent variable of the function evolves with respect to dT/dt is the desirable one (i.e. the lower the heating rate the more the induced microbial heat resistance to be expected). The necessity of having a second factor [next to the $k(<phys1>)$ indicated previously in this text] describing the physiological state of the cells arises because of the need of incorporating the dynamic temperature influences on the microbial kinetics. Additionally, clear separation of the 'shoulder' effect and the 'induced heat resistance' effects is desirable for interpreting separately these phenomena.

Simulations of the microbial inactivation kinetics when the effect of the microbial adaptation is considered are performed by the use of the following equation [based on the general expression of eqn (1) and the model of Geeraerd *et al.* (2000)].

$$\frac{dN}{dt} = -k_{max}(T) \cdot \left(\frac{1}{1 + C_c}\right) \cdot k(<phys2>) \cdot N \quad (10)$$

$$\frac{dC_c}{dt} = -k_{max}(T) \cdot C_c \quad (11)$$

The third factor is represented by eqn (9) and is the additional model building block that aims at describing the microbial stress-adaptive responses as discussed before. The effect of the temperature on parameter k_{max} is described by the use of the Bigelow (1921) model [eqn (5)] and the temperature evolution is given by the modified Dabes kinetics [eqn (6)]. Simulations with the new developed modelling approach [eqns (10) and (11)] are performed by using the isothermal inactivation kinetic parameters $AsymD_{ref}$, z and $C_c(0)$, the experimental $N(0)$, the identifed parameters of eqn (9) and the already discussed microbiological hypotheses.

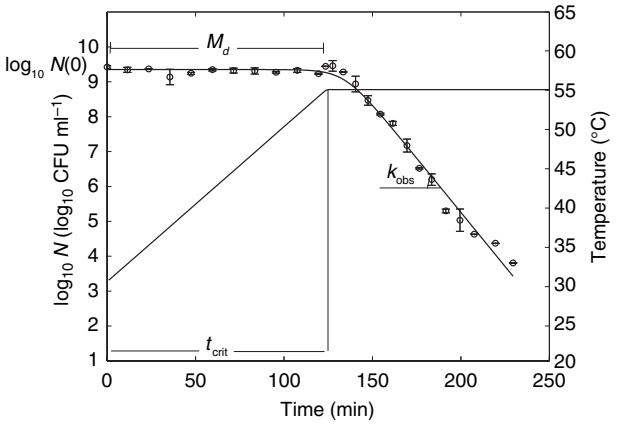

Figure 1 Graphical example visualizing the significance of parameters k_{obs}, M_d and $\log(N(0))$ based on eqn (8). The heating rate of the chosen profile is 0·20°C min^{-1}.

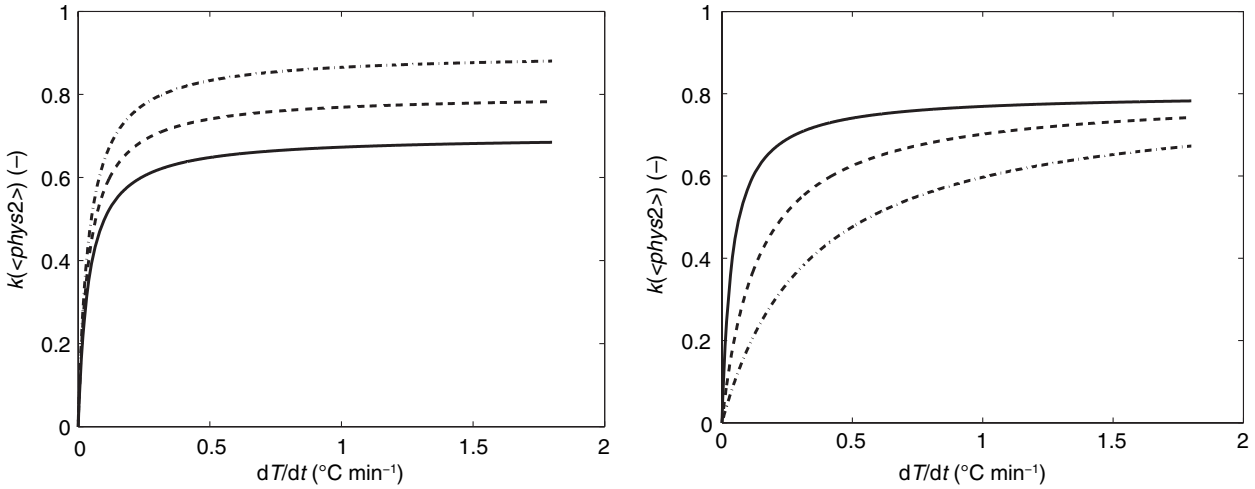

Figure 2 Simulations of eqn (9) for different values of k_1 and k_2. Left plot: k_1 = 0·7 (—), 0·8 (- -), 0·9 (-·) and k_2 = 0·04 (°C min^{-1}). Right plot: k_1 = 0·8 and k_2 = 0·04 (—), 0·14 (- -) (°C min^{-1}), 0·34 (-·).

Performance capability

In our case study, the accuracy and bias factors (which are consistent with the least squares algorithm of fitting models) presented by Baranyi *et al.* (1999) are considered in order to evaluate the performance capability of the developed model.

$$A_f = 10^{\sqrt{\frac{\sum_{i=1}^{n}(\log_{10}\hat{N}_i - \log_{10}N_i)^2}{n}}} \quad (12)$$

$$B_f = 10^{\frac{\sum_{i=1}^{n}(\log_{10}\hat{N}_i - \log_{10}N_i)}{n}} \quad (13)$$

where $\log_{10}\hat{N}_i$ denotes the predicted microbial load and n is the number of the experimental measurements. The indices of eqns (12) and (13) are adapted from their original form in order to be used for inactivation kinetics and to compare residuals of observed and simulated microbial load [in a similar way that Geysen *et al.* (2006) used them for growth kinetics]. On the one hand, the accuracy factor indicates the spread of results around the simulation or, in other words, how close, on average, simulations are to observations (values close to 1 are indicative of small deviations). On the other hand, the bias factor evaluates whether the observed values lie above the simulation line (i.e. $B_f < 1$, underprediction of microbial load or fail-dangerous) or below the simulation line (i.e. $B_f > 1$, overprediction of microbial load or fail-safe) (Ross 1996).

Results

As discussed in Materials and methods, if $M_d > t_{crit}$ then the region in which k_{obs} is identified is a region in which temperature does not change (as is the case for the example in Fig. 1). For this study and for all the examined data sets, $M_d > t_{crit}$ except for the case where the heating rate is 0·15°C min^{-1}. Therefore, the parameter k_{obs} at a heating rate of 0·15°C min^{-1} is estimated with M_d fixed to the value of the corresponding t_{crit}. Consequently, k_{obs} will represent the observed inactivation rate during the final static temperature, 55°C, of the dynamic experiments, similarly to the other heating profiles at hand. The estimated parameters k_{obs} and M_d of eqn (8) are tabulated in Table 1.

At a second step the evolution of the $k(<phys2>)$ with respect to the heating rate (dT/dt) is evaluated by eqn (9). Parameters k_1 and k_2 are estimated following a nonlinear fitting procedure (Fig. 3) of this Monod-type equation and result in k_1 = 0·696 (-) (±0·009), k_2 = 0·042 (°C min^{-1}) ± 0·005 (SE).

Finally, microbial simulations of the population level taking into account the induced microbial heat resistance are performed with eqns (11) and (14) [as based on eqns (9) and (10)].

$$(dN/dt) = -k_{max}(T)\cdot\left(\frac{1}{1+C_c}\right)\cdot\left(k_1\cdot\frac{(dT/dt)}{k_2+(dT/dt)}\right)\cdot N \quad (14)$$

A posteriori, it can be observed that the newly introduced factor can be re-arranged in a similar way [having the general format $1/(1+v)$] like the factor describing the shoulder effect, i.e. as $k(<phys1>)$. If it is assumed that $T_k = k_2/(dT/dt)$ then the third factor of the right hand side of eqn (14) can be reformulated as $k_1 \cdot 1/(1+T_k)$.

In Fig. 4, the performance of the developed mathematical approach incorporating the acquired induced microbial heat resistance is illustrated and confronted with the

Table 1 Estimated parameters k_{obs}, M_d and their standard errors by using eqn (7), for the studied heating rates

(d*T*/d*t*) (°C min^{-1})	k_{obs} (min^{-1}) ± SE	M_d (min) ± SE
1·64	0·1677 ± 0·0076	23·77 ± 2·94
0·82	0·1652 ± 0·0074	38·31 ± 2·68
0·55	0·1657 ± 0·0100	55·59 ± 3·66
0·40	0·1608 ± 0·0052	72·44 ± 2·46
0·20	0·1431 ± 0·0049	134·13 ± 2·30
0·15	0·1241 ± 0·0074	157·72 ± 5·47
0·15	0·1356 ± 0·0092	Fixed to 168·72

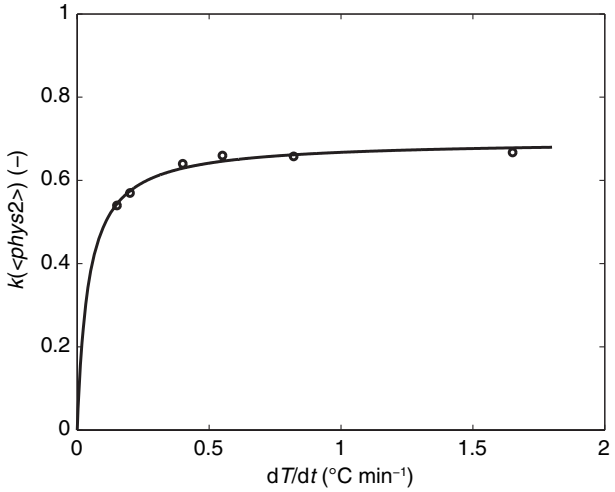

Figure 3 Fitting of eqn (9) for estimating parameters k_1 and k_2. $R^2_{adj} = 1 - \frac{(n-1)}{(n-p)} \cdot \frac{(SSE)}{(SSTO)} = 0{\cdot}960$, where n and p, are number of data points and number of parameters, respectively, whereas SSE, SSTO, are the sum of squared errors and the total sum of errors.

microbial inactivation data at hand. Additionally, these simulations are plotted next to the microbial predictions performed without incorporating the factor describing the induced microbial heat resistance [i.e. the third factor of the right hand side of eqn (14) is not incorporated] as discussed before in the section 'Available model based on static data'. The deviation of the models from the experimental data is tabulated in Table 2.

Discussion

A model building block related with the microbial physiology (k_{phys}) under stress conditions is developed. Evaluation of the performance of the developed mathematical approach depicts that physiological adaptation is an essential issue to be considered when modelling microbial inactivation. Despite the empiricism of the developed equation, it can be observed that the equation suitably describes a qualitative observation originating from the physiological responses of the cells, i.e. the lower the heating rate, the larger the induced microbial heat resistance to be expected. In a previous study, an empirical temperature shift term (i.e. not the actual temperature, but the actual temperature lowered by some °C is being used in the model), correcting for the effect of increased resistance, was incorporated in a microbial modelling approach (Baranyi *et al.* 1996). The current alternative approach aims at introducing an independent factor that is estimated in relation to the heating rate and respects the observed phenomenon of induced heat resistance. The development of a more mechanistic approach is an important alley towards the future, and may require thorough studies that focus on the quantification of the molecular adaptations (e.g. production of heat shock proteins) or on the exploitation of microscopical experiments.

It should be stated that for this study the developed model [eqn (14)] is used only for simulation purposes. These simulations cannot be called 'predictions' as they are based on information from dynamic data (namely, k_{obs}). The model based on the static data, on the other hand, is used for predicting the microbial inactivation kinetics under unexplored dynamic temperature conditions. The developed approach could describe more accurately the microbial load at all the examined heating rates than the classical approach in which the add-in model building block $k(<phys2>)$ is not used. The low values of A_f indicate a small deviation of the microbial population simulations from the experimental data (see Table 2). Particularly, the approach based on eqn (14) could describe (in all cases) with higher accuracy the microbial population than the other approach. When the model quality is studied for all the experimental sets, the microbial simulations of eqn (14) gave B_f values close to one. On the contrary, predictions based on the modelling approach in which the microbial-induced heat resistance is not incorporated (Valdramidis *et al.* 2006) resulted in very low B_f values, or pronounced fail-dangerous situations. This means that the developed modelling approach eventuated in simulations that describe reliably the microbial load and can be considered for ensuring the microbial safety of the examined product under the studied microbial kinetics.

As discussed in the Materials and methods, for this study the accuracy factor, A_f, and the bias factor, B_f, as described by Baranyi *et al.* (1999) are used in order to evaluate the preformance capability of the model. Various statistical indices are suggested in the literature in order to compare competing models. Among these indices Jeyamkondan *et al.* (2001) referred to graphical plots, mean relative percentage residual (MRPR), mean absolute relative residual (MARR) and root mean squared residual (RMSR). Similarly, Ross (1996) suggested the use of the

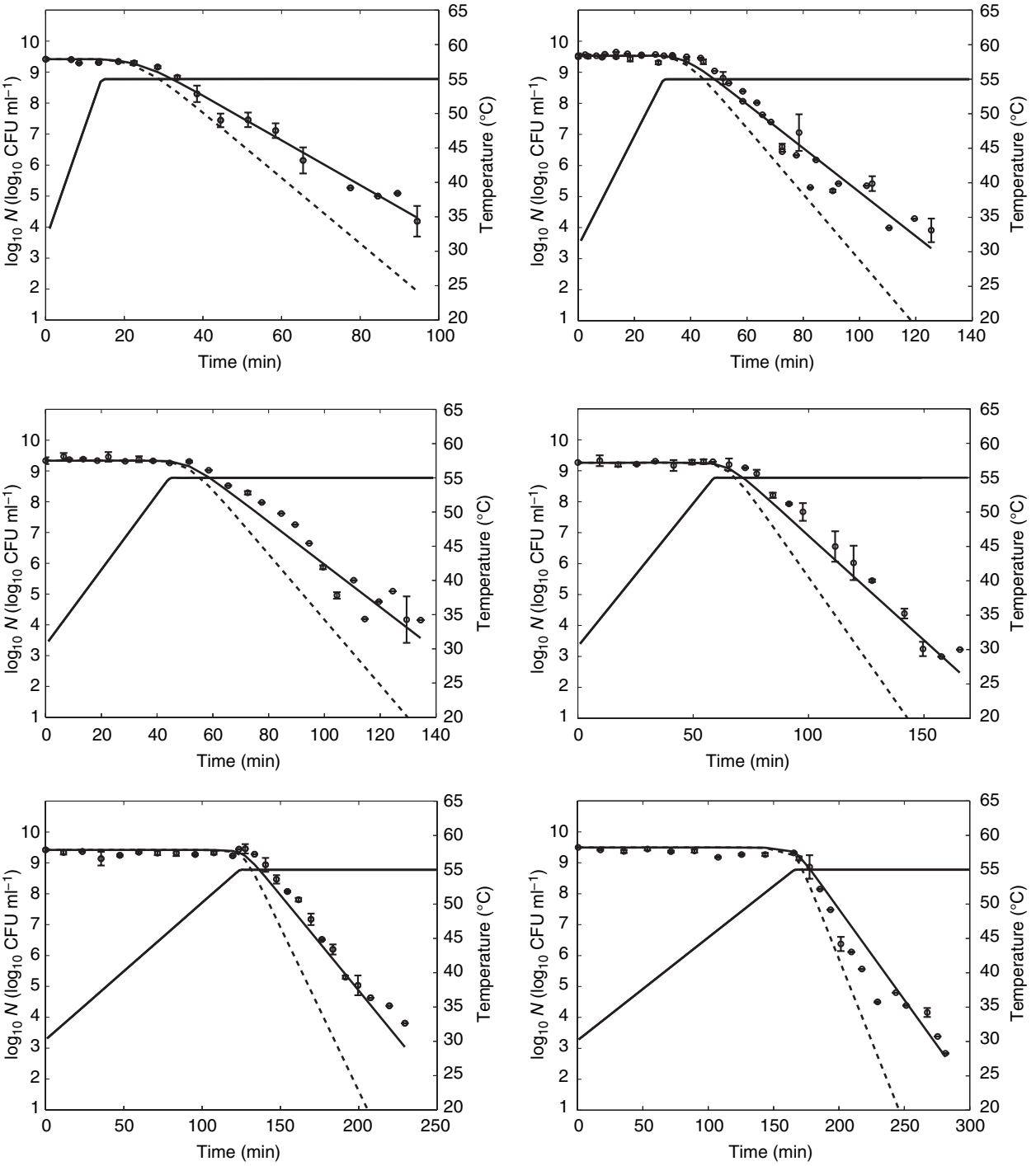

Figure 4 Microbial inactivation experiments of *Escherichia coli* K12 and corresponding temperature profile at all studied heating rates. From left to right, first row: 1·64, 0·82°C min^{-1}, second row: 0·55, 0·40°C min^{-1}, third row: 0·20, 0·15°C min^{-1}. Thick line: simulations by incorporating the factor $k(<phys2>)$ [eqn (14)], dashed line predictions without taking into account the $k(<phys2>)$ factor (Valdramidis *et al.* 2006).

bias (B_f) and accuracy factors (A_f) for assessing the predictive performance of secondary models (e.g. growth rate or generation time). Additionally, Campos *et al.* (2005) discussed the use of the robustness index (RI) and the mean relative error (RE) for comparing experimental and predicted microbial counts. Among these statistics the MRPR, MARR and RE should be considered with caution as normalization takes place (i.e. residuals are divided by

Table 2 A_f and B_f comparison for the modelling approaches presented in Valdramidis *et al.* (2006) and the new approach [eqn (14)]

(dT/dt) (°C min^{-1})	Approach presented in Valdramidis *et al.* (2006)	Approach based on eqn (14)
	A_f, B_f	A_f , B_f
1·64	14·61, 0·17	1·58, 1·06
0·82	16·97, 0·17	2·12, 0·96
0·55	43·81, 0·07	2·33, 0·73
0·40	81·49, 0·05	2·12, 0·64
0·20	127·47, 0·05	2·08, 0·71
0·15	231·40, 0·05	2·99, 1·68

the observed or predicted data). This normalization is built on the assumption that the variability is larger for larger values of the observed variable. If this would be the case [which can be proven experimentally, see, e.g. Alber and Schaffner (1992) and Zwietering *et al.* (1994)] then the use of these statistics entails the necessity of using also a relevant cost function [e.g. weighted sum of squared errors (WSSE)] during parameter identification. In other words, if such variability increase would be a reality, then this phenomenon should also be taken into account during parameter estimation, and not only during the evaluation of the model.

Previous studies in which microbial predictions were developed based on isothermal inactivation kinetics have shown a lacking on the prediction capability of the implemented microbial inactivation models under dynamic conditions (Hassani *et al.* 2005, 2006; Valdramidis *et al.* 2006). Cheroutre-Vialette and Lebert (2000) argue about the use of differential equations describing variable conditions when the inactivation parameters are transposed from static conditions to evaluate variable conditions. The representation of these physiological changes because of a (slowly) increasing temperature by the use of an add-in building block in a differential equation can work as a correcting factor for improving the modelling accuracy. This factor represents the acquired (or induced) microbial thermotolerance. It can be attributed to different causes among which is the heat shock protein production. For example, the stress-adaptive responses of *E. coli* O157:H7 in a model beef gravy system were associated to an increase in the levels of hsps proteins, e.g. GroEL, DnaK [see Juneja and Novak 2003, for a general review on synthesis of heat shock proteins, or for some representative examples see Juneja *et al.* (1998) and Tsuchido *et al.* (1992)]. Nevertheless, some studies argue that other factors such as plasma membrane phospholipid denaturation could be involved in this phenomenon (Morozov *et al.* 1997; Guyot *et al.* 2005).

From these, it can be concluded that any microbial response resulting in physiological adaptations should be considered mathematically. Consequently, reliable quantification of the safety risks and of the processing impacts avoiding any fail-dangerous or overly conservative (fail-safe) heat-processing designs can be achieved. In further research, the presented methodology will be validated (concerning the performance of microbial predictions) on several experimental data generated in dynamic conditions.

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