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A model describing the relationship between regrowth lag time and mild temperature increase for *Listeria monocytogenes*

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Abstract

In order to comply with the consumer demand for ready-to-eat and look 'fresh' products, mild heat treatment will be used more and more in the agrofood industry. Nonetheless there is no tool to define the most appropriate mild heat treatment. In order to build this tool, it is necessary to study and describe the response of a bacterial population to a mild increase in temperature, from the dynamic point of view. The response to a mild increase in temperature, defined by stress duration and temperature, consisted in a mortality phase followed by the lag time of the survivors and their exponential growth. The effect of the mild increase in temperature on the mortality phase was described in a previous paper (Bréand et al., Int. J. Food Microbiol., in press). The effect of the stress duration on the lag was presented in a previous paper (Bréand et al., Int. J. Food Microbiol. 38 (1997) 157–167). In particular, the biphasic relationship between the lag and the stress duration was observed and modelled with a four parameter nonlinear model: the primary model (Bréand et al., Int. J. Food Microbiol. 38 (1997) 157–167). The study presented in this paper deals with the effect of the stress temperature on the biphasic relationship between the lag time and the stress duration. The secondary models describing the effect of the stress temperature on this biphasic relationship, were empirically built from our experimental data concerning *Listeria monocytogenes*. This work pointed out that the higher the stress temperature, the narrower the range of stress duration for which the lag time increased. Since the primary and the secondary models of the lag time were available, the global model describing the effect of the mild increase duration and temperature directly on the lag was fitted. This model allowed an improvement of the parameter estimator precision. The potential contribution in mild heat treatment optimization of this global model and the one built for the mortality phase (Bréand et al., Int. J. Food Microbiol., in press) is discussed. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: *Listeria monocytogenes*; Mild heat treatment; Model

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1. Introduction

The ability of bacteria surviving a mild temporary increase in temperature to initiate exponential growth after a lag time was pointed out at the beginning of the century by Oerskov (1925). The exponential growth rate did not depend on the mild increase in temperature (Jackson and Woodbine, 1963; Kim et al., 1994; Bréand et al., 1997). On the contrary, the lag time depended on the duration (stress duration: d_s; Bréand et al., 1997) during which the population was placed under a non-viable temperature (stress temperature: T_S; Bréand et al., 1997) and on the value of the stress temperature.

Up to now, the studies of the response of a bacterial population to a mild increase in temperature dealt principally with the injured cellular sites (Ray, 1986).

When a dynamic approach was presented, both characteristics of a temporary mild increase in temperature were rarely considered. For example Jackson and Woodbine (1963) observed the increase of the lag time with the stress duration but they studied only one stress temperature (60°C). A non-monotonic relationship between the lag time and the stress duration was observed for *Salmonella typhimurium* but the authors (Mackey and Derrick, 1984) only studied one stress temperature (52°C). An increase of the lag time with the stress temperature was observed for *Staphylococcus aureus* (Batish et al., 1990) but only one stress duration was studied. When the effect of the stress duration and temperature on the lag time was studied (Kaufman et al., 1959), the experimental design was such that little information could be extracted.

Yet the relationship between lag time and stress temperature and duration is important from the point of view of food microbiology.

Nowadays the consumer demand for ready-to-eat products is greater and greater. The safety of these products rely partly on a mild increase in temperature which would allow to warrant the microbial safety and the functional properties of the product. In fact, there is no objective tool to define such mild heat treatment. The method which consists in considering only the number of survivors does not seem to be the most efficient. In fact this method assumes that the longer the stress duration, the safer the treatment. In a previous paper, we have shown that the extension of the treatment duration might not be the best way to warrant the safety of a product. Indeed, for a fixed stress temperature, the lag time first increased with the stress duration and then decreased as the stress duration increased (Bréand et al., 1997). So far, there is no information about the effect of stress temperature on lag time.

Moreover in the case of the contamination of a product, if the pathogens have been injured for example by a mild increase in temperature, there is no tool to determine the recovery time (Mackey and Derrick, 1984). Yet this recovery time depends on environmental fluctuations (Mackey and Derrick, 1984). If the effect of the stress temperature and duration on the lag time or recovery time was known, it would allow us to determine the required recovery time.

Consequently from the agrofood viewpoint, the knowledge of the relationship between the lag time and the stress duration and temperature seems of particular interest. In a previous paper we presented the study of the effect of the stress duration on the lag time. This study deals with the effect of the stress temperature on the lag time.

The aim of the study presented in this paper is to build a mathematical model describing the relationship between the parameters of the *lag* vs. d_s primary model (Bréand et al., 1997) and the stress temperature. Once these secondary models were built, the global model describing the effect of the stress duration and temperature was fitted.

As the biological explanations about the studied relationship are still unavailable and should be complex, our mathematical models were empirically built from our experimental data concerning *Listeria monocytogenes*.

2. Materials and methods

2.1. Bacterial strains and medium

The reference strain *Listeria monocytogenes* CIP 7831 ATCC 35152 was used in this study. The media used for this study were the same as the ones presented in our previous paper (Bréand et al., 1997).

2.2. Temperature change

The temperature increase studied herein was defined as follows: an abrupt increase from a pre-stress temperature (T_{bs}) fixed between T_{min} and T_{max} to a stress temperature (T_s) higher than T_{max} for a given stress duration d_s. Temperature is then quickly decreased to an after-stress temperature (T_{as}) fixed between T_{min} and T_{max}. Pre-stress and after-stress temperatures were equal to 35°C.

As we were interested in the response of a bacterial population to a mild temperature increase the stress temperatures chosen were only a little greater than T_{max}. As the maximal growth temperature of the studied *L. monocytogenes* was roughly 48–49°C (Charles-Bajard, 1996), the studied stress temperatures varied between 50° and 60°C; 13 experiments were performed at 50, 52, 53, 54, 55, 56, 56.5, 57, 57.5, 58, 58.5, 59 and 60°C. The data concerning the stress temperatures of 53, 54 and 55°C were not available for our previous paper (Bréand et al., 1997).

The stress durations varied, simply for experimental convenience, between 0 and 120 min except for 53, 54 and 55°C for which the stress duration was extended up to 240 min.

For the control experiments, done for each studied stress temperature, T_{bs}, T_s and T_{as} were equal to 35°C. Therefore the 'control lag time' was induced by the medium change only.

The experiments were realized according to the protocol presented in our previous paper (Bréand et al., 1997).

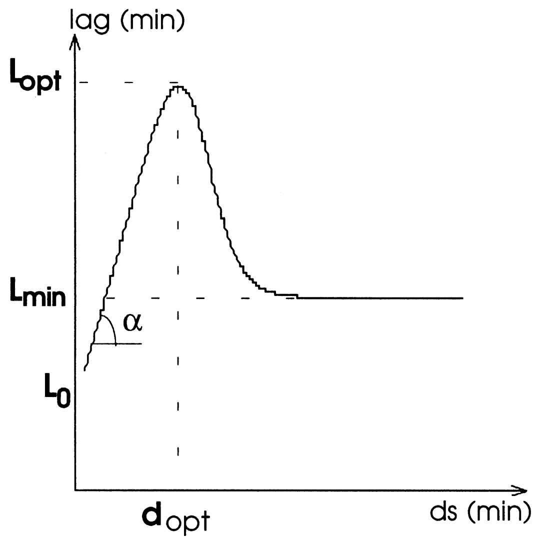

Fig. 1. The four parameters of the primary model describing the relationship between the lag time and the stress duration (d_s) (Bréand et al., 1997) and the slope of the linear part. L_0, the lag time for a null stress duration, i.e. the lag time induced by the medium change; L_{opt}, the greatest lag time; d_{opt}, the abscissa for the greatest lag time; L_{min}, the lag time for the large stress durations; α, the slope of the linear part of the relationship.

2.3. Data analysis

2.3.1. Estimation of the lag time for each stress duration

A specific process to estimate the lag time was set and presented in our previous paper (Bréand et al., 1997).

2.3.2. Relations studied

The nonlinear model with four parameters presented in the paper of Bréand et al. (1997), was used to describe the biphasic relationship between the lag time (*lag*) and the stress duration (d_s). The significance of these four parameters is presented in Fig. 1.

Furthermore, the slope, α, of the first linear phase (Fig. 1) was introduced to describe the effect of the stress temperature (T_s) on the *lag* vs. d_s relationship. The parameter α considered, L_{opt} is a function of the four other parameters α, d_{opt}, L_0, and L_{min}. Moreover, the parameter L_0, corresponding to the lag time when there is no increase in temperature, can actually be considered as an initial condition.

Consequently, to describe the effect of T_s on *lag* vs. d_s, in order to build a global model describing the effect of d_s and T_s on *lag*, only three relations have to be modelled:

d_{opt} vs. T_s,

α vs. T_s,

L_{min} vs. T_s.

2.3.3. Model building

2.3.3.1. Secondary models

For the data considered in this paper, three models were built. These are expressed as Eqs. (1)–(3):

$$d_{opt} = c_1 \times 10^{c_2 T_s} + \varepsilon, \quad (1)$$

$$\alpha = c_3 \times 10^{c_4 T_s} + \varepsilon \quad (2)$$

$$L_{min} = c_5 + \varepsilon \quad (3)$$

ε is the error term considered as additive, gaussian and homoscedastic.

2.3.3.2. Global model

The global model describing the effect of d_s and T_s on *lag* was built by replacing the parameters of the primary model by their corresponding secondary models (Eqs. (1)–(3)). Nevertheless, since:

- a great variability has been observed for L_0 estimated by fitting the *lag* vs. d_s model, the data were centred on this parameter and L_0 was fixed to 0 in the global model,
- no significant difference ($P = 5\%$) was detected between $|c_2|$ and $|c_4|$, c_4 was replaced by $-c_2$ in the global model.

Finally, the non linear global model describing the effect of d_s and T_s on *lag* with four parameters (c_1, c_2, c_3, c_5), is expressed as Eq. (4):

$$lag(d_s, T_s) = c_5 + \frac{A(d_s, T_s)}{[1 + \exp(B(d_s, T_s) - 1.12]} + \varepsilon$$

with

$$A(d_s, T_s) = [c_1 \times 10^{c_2 T_s} \times d_s - c_5]$$

and

$$B(d_s, T_s) = \left[5.06 \times \frac{c_1 \times 10^{c_2 T_s} \times (d_s - c_3 \times 10^{-c_2 T_s})}{c_1 \times 10^{c_2 T_s} \times c_3 \times 10^{-c_2 T_s} - c_5} \right] \quad (4)$$

ε is the error term considered as additive, gaussian and homoscedastic; constants were not arbitrarily fixed but resulted from the building of the primary model (Bréand et al., 1997).

2.3.4. Prediction of the threshold time

To predict the threshold time of a heat stressed bacterial population of initial density $\log_{10}(N_0)$, the death phase, the growth lag time of the survivors and the exponential growth phase have to be considered. For a threshold equal to 100 times the inoculum $\log_{10}(N_0)$, the threshold time (i.e. the required time for the population to reach a fixed threshold of density) was predicted with Eq. (5):

$$t_{threshold} = lag(d_s, T_s) + \frac{(100 \times \log_{10}(N_0) - \log_{10}(N(d_s\, T_s))}{\mu_{max}} \times \ln(10). \quad (5)$$

The predictions of the threshold time were calculated for all the studied increases in temperature with Eq. (5).

The growth lag time ($lag(d_s, T_s)$) was predicted with the global model defined by Eq. (4), the number of survivors ($\log_{10}(N(d_s, T_s))$) was predicted with the global model presented in a previous paper (Bréand et al., in press), μ_{max} was predicted with a growth rate model specific to *Listeria monocytogenes* (Charles-Bajard, 1996).

2.3.5. Parameter estimator

Even though the models expressed by Eq. (1) and Eq. (2) could be linearized, they were kept in their nonlinear form. Consequently, except for Eq. (3), all fits were performed by nonlinear regression using the ordinary least squares criterion. For the fit of Eq. (4) to the data, that is to say the lag times observed for the studied (d_s, T_s) couples, the initial values of its four parameters were taken to be the estimators obtained by fitting the secondary models (Eqs. (1)–(3)).

2.3.6. Evaluation of fit

For the secondary models, the autocorrelation and the heteroscedasticity in the residual distribution were examined with the graph of the residuals versus the stress temperature since the regression function of these models was monotonic.

For the global model, the autocorrelation and the heteroscedasticity were examined with the graph of the residuals versus the predicted lag time since the predicted lag time took into account the stress duration and the stress temperature.

For the global and the secondary models, the normality of the residual distribution was examined by plotting the residual percentiles versus the standardized normal percentiles: the residual probability plot.

2.3.7. Parameter correlation

The correlation between model (1) to (4) parameters was examined with the graphical representation of the confidence regions (Beale, 1960; Lobry et al., 1991).

As a matter of fact, too strong a correlation between parameters decreased the precision of their estimator and indicated an overparameterization (Ratkowsky, 1990). The curvature of the model could also be assessed with the graphical representation of the confidence region.

The strong nonlinearity of the model was assessed according to the projection of the parameter confidence region. If the shape of the projection, in a two parameter plan, of the confidence region was not elliptical, the strong nonlinearity of the model was detected.

2.3.8. Confidence intervals

In this study since the data set size was small, the Jackknife method (Tukey, 1958) was used to estimate the 95% confidence intervals of the parameters. This iterative method allows the parameters and the confidence intervals to be estimated.

3. Results

3.1. Relation d_{opt} vs. T_s

Fig. 2a shows a decreasing relationship between d_{opt} (Fig. 1) and the stress temperature. Moreover the secondary model defined by Eq. (1) allowed a good description of the data (Fig. 2a). The value obtained for the root mean square error was 17.32.

Autocorrelation in the residual distribution was not detected by the examination of the scattergram displaying the residuals against the stress temperature. Two strong residuals were obtained for $T_{\mathrm{s}} =$ 53°C and 54°C, respectively (Fig. 2a). Nevertheless the size of the available data set was too small to conclude a heteroscedasticity in the residual distribution. The hypothesis of normality of the residual distribution was not questioned after the examination of the residual probability plot since it presented a linear trend.

As usual with the exponential function, a strong correlation was observed between the model Eq. (1) parameters (c_1 and c_2). This strong correlation explains in part the poor precision of the estimators of c_1 and c_2 (Table 1).

3.2. Relation α vs. T_s

Fig. 2b shows an increasing relationship between α and T_{s}. The secondary model defined by Eq. (2) was used to describe the data (Fig. 2b). The value obtained for the root mean square error was 1.81.

Neither autocorrelation nor heteroscedasticity was detected by the examination of the residuals vs. T_{s} plot. Since the residual probability plot presented a linear trend, the hypothesis of the normality of the residuals was not rejected.

As for the relationship between d_{opt} and T_{s}, a strong correlation was observed between the two parameters of Eq. (2). Once again this can explain the large magnitude of the 95% confidence intervals on the estimators (Table 1).

Given this information and the size of the available data set, the model defined by Eq. (2) was considered as a satisfying descriptor of the relationship between α and T_{s}.

3.3. L_{min} vs. T_s

For the available data set and given the poor precision of the estimators (Fig. 2c), the relationship between L_{min} and the stress temperature was described by Eq. (3). That is to say the relationship between L_{min} and T_{s} was described by the mean of the values estimated for L_{min} (Table 1).

3.4. Global model

Since a primary model describing the effect of stress duration on lag time (Bréand et al., 1997), and the secondary models describing the effect of stress temperature on the parameters of this primary model (Fig. 2) were defined, a global model describing the effect of stress duration and temperature on the lag

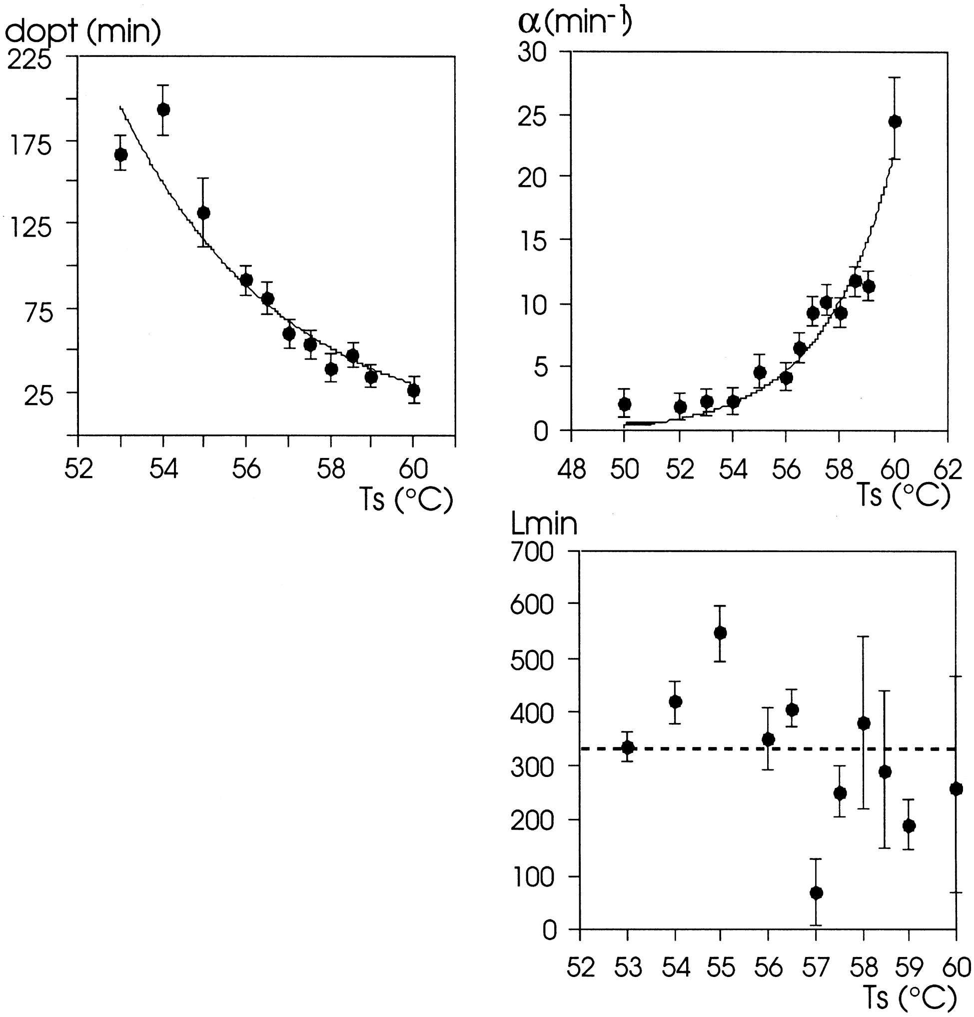

Fig. 2. The effect of the stress temperature on three of the parameters of the primary model. (a) The relationship between d_{opt} and T_s. The fit with the model Eq. (1) is presented. (b) The relationship between α and T_s. The fit with the model Eq. (2) is presented. (c) The relationship between L_{min} and T_s. The fit by the model Eq. (3) is presented.

Table 1
The estimators of the parameters of the secondary and global models defined by Eqs. (1)–(4). The 95% confidence intervals estimated with the Jackknife are indicated in brackets

	c_1	c_2	c_3	c_4	c_5
Eq. (1)	195 [161; 228]	−0.14 [−0.17; −0.12]			
Eq. (2)			1.49 [0.43; 2.55]	0.16 [0.04; 0.28]	
Eq. (3)					350 [260; 440]
Eq. (4)	201 [179; 223]	−0.15 [−0.16; 0.14]	1.58 [1.49; 1.68]		1.33 [102; 165]

was built. A root mean square error of 57.58 was obtained by fitting Eq. (4) to the data, that is to say the observed lag times for all the studied couples (d_s, T_s).

Neither autocorrelation nor heteroscedasticity was detected through the examination of the graph of the residuals versus the estimated lag time. The normality of the residual distribution was not questioned after the examination of the residual probability plot. The greatest observed lag times seem underestimated by the global model, according to the scattergram of the predicted lag time versus the observed lag time (Fig. 3).

No too strong nonlinearity of the global model was detected by the examination of the projection of the confidence region.

According to the examination of the projections of the confidence region, no correlation was detected between c_1 and c_5, c_2 and c_5, c_3 and c_5. However, a correlation was observed between c_1 and c_2, c_1 and c_3, c_2 and c_3.

The estimators of c_1, c_2, c_3, obtained by fitting the global model were not significantly different from those obtained by fitting the secondary models (Table 1). Concerning c_5, its estimator obtained by fitting Eq. (3) to the centred data was a little higher than the one obtained by fitting the global model.

Moreover, the parameters were estimated with better precision by fitting the global model (Table 1). Actually, the magnitude of the 95% confidence intervals of the estimators was smaller with the global model than with the secondary models (Table 1).

The filled contour plot of the theoretical surface compiled after the fit of the global model Eq. (4) to the lag times is presented in Fig. 4a.

The same procedure was followed for the global model describing the survivor number (Bréand et al., in press). The filled contour plot of the theoretical surface describing the survivor number is presented in Fig. 4b.

The lack of overlap between the increases in temperature which maximized the lag time and those which minimized the survivor number, is deduced from the comparison of Fig. 4a and b.

The filled contour plot corresponding to the pre-

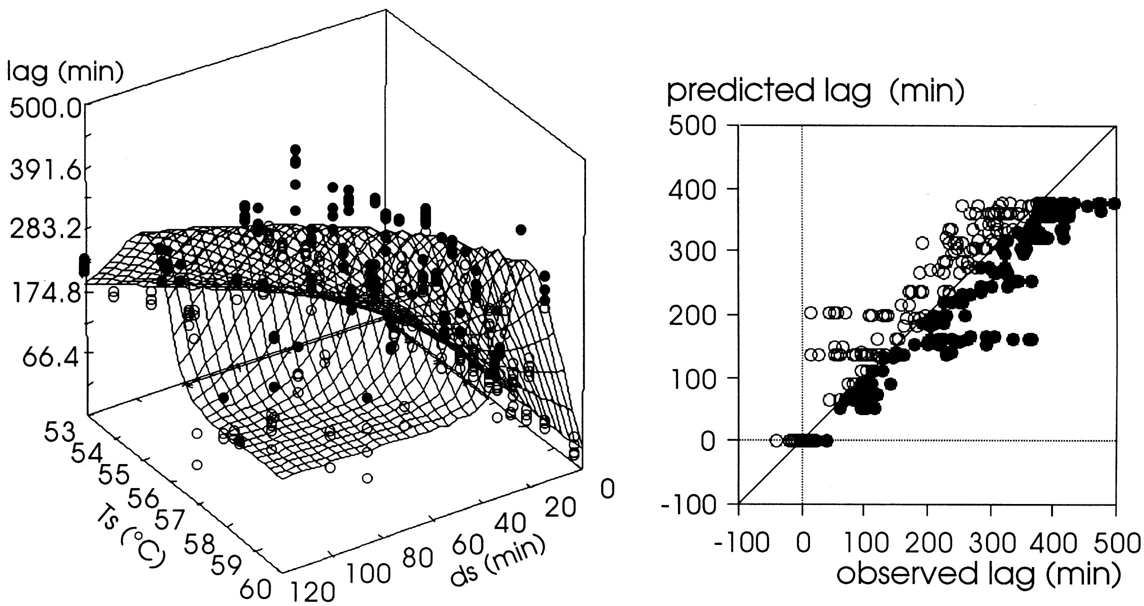

Fig. 3. The fit by the global model Eq. (4) of the lag times observed for all the couples (d_s, T_s) studied. The theoretical surface was superimposed on the data. The points in white were such that the model predicted a value greater than the observation. The points in black were such that the model predicted a value smaller than the observation.

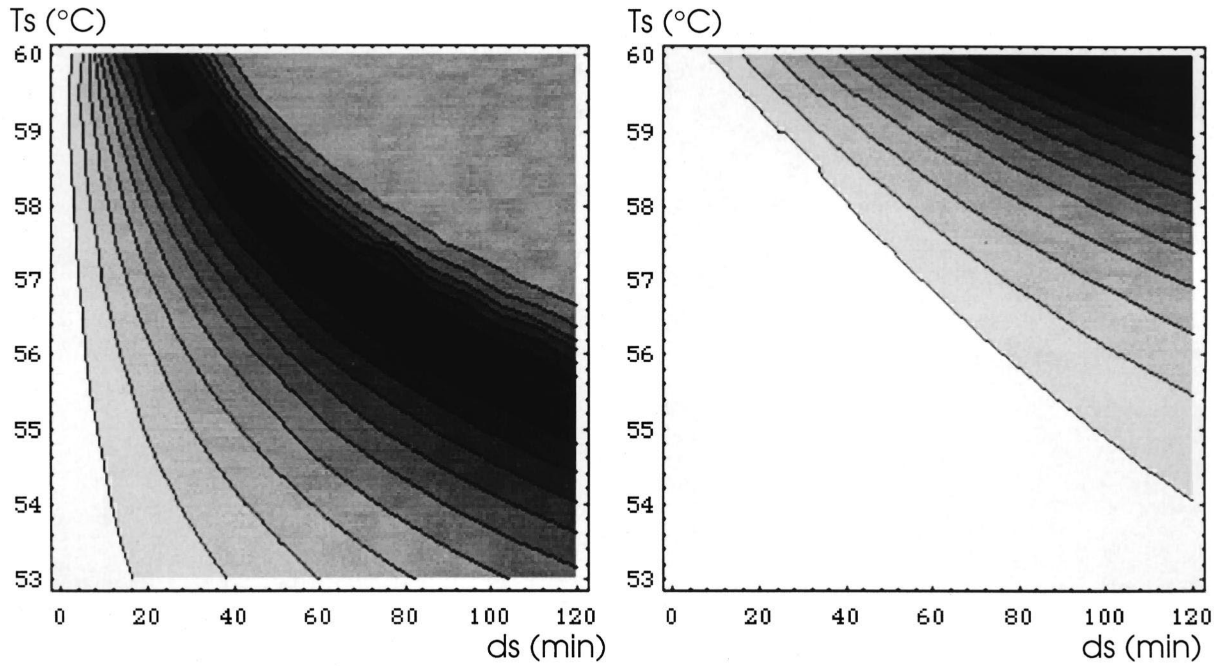

Fig. 4. (a) The filled contour plot of the global model describing the effect of (d_s, T_s) on the lag time, the darker the gray, the higher the lag time. (b) The filled contour plot of the global model (Bréand et al., in press) describing the effect of (d_s, T_s) on the survivor number, the darker the gray, the lesser the survivor number.

dicted threshold time is presented in Fig. 5. According to Fig. 5, two sets of increase in temperature could maximize the threshold time:

- the one corresponding to the minimization of the survivor number,
- the one corresponding to the maximization of the survivor growth lag time.

4. Discussion

The study presented in this paper dealt with the effect of stress temperature on the biphasic relationship between lag time and stress duration. For the description of this biphasic relationship, a primary model with four parameters was built (Bréand et al., 1997). The effect of stress temperature on the *lag* vs. d_s relationship was thus studied by mathematically describing the relationship between these parameters and the stress temperature.

For the studied *Listeria monocytogenes*, the stress duration inducing the greatest lag time, d_{opt}, decreased with the stress temperature. Moreover, given the size of the sample and the information indicated in Section 3, an exponential model allowed a satisfactory description of the data. This result indicated that the stress duration after which the lag time did not increase, was shorter and shorter as the stress temperature increased. That is to say, the lengthening of the stress duration was not the best strategy to warrant the safety of a foodstuff even for high stress temperature.

The slope of the linear part of the relationship between *lag* and d_s increased with the stress temperature. Once again an exponential function allowed a satisfactory description of the data, given the sample size and the information cited in Section 3. Nonetheless, if the underestimation of the data for the low values of the stress temperatures (Fig. 2b) is observed for other samples, the exponential model defined by Eq. (2) will be modified. The scattergram presented on Fig. 2b indicates that the increase of the lag time up to peak, is all the faster as the stress temperature is high.

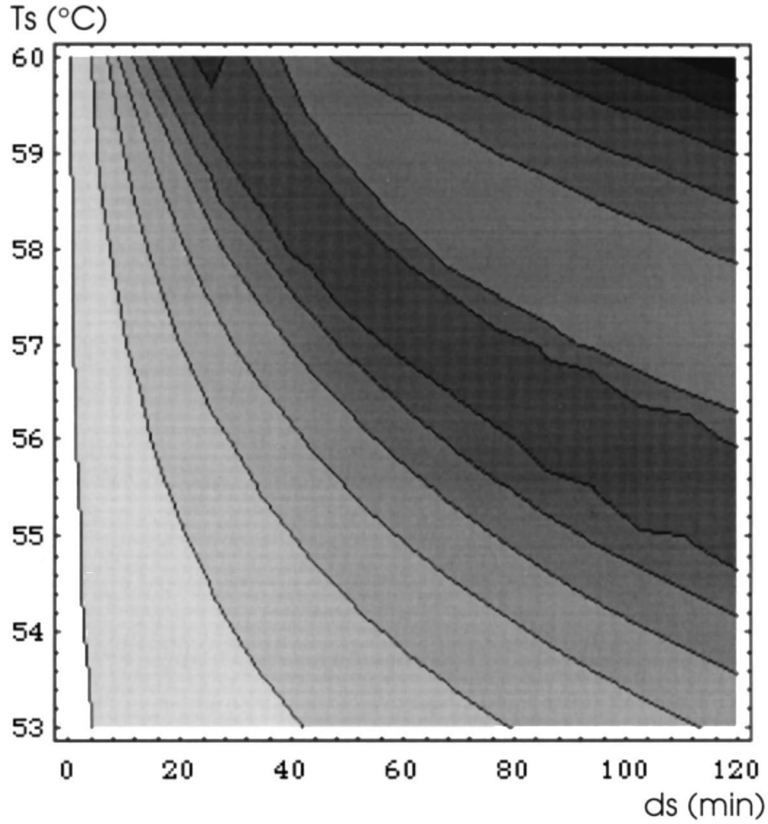

Fig. 5. The filled contour plot of the threshold time predicted for each studied increase in temperature. The darker the gray, the higher the predicted threshold time. The lines represented the points (d_s, T_s) for which an equal threshold time was predicted.

These two previous results which have never been cited in the literature, linked with the two previous papers on the mortality phase and on the *lag* vs. d_s relationship (Bréand et al., 1997; Bréand et al., in press), led to a better understanding of the response of *Listeria monocytogenes* to a mild increase in temperature. As expected, the higher the stress temperature and the longer the stress duration, the smaller was the survivor number (Bréand et al., in press). Concerning the lag time of the survivors, the result was more surprising. A biphasic relationship between lag time and stress duration was described (Bréand et al., 1997). Moreover, the decreasing relationship between the abscissa of the optimal lag time (d_{opt}) and the stress temperature (Fig. 2) indicated that the higher the stress temperature, the narrower the range of the stress duration for which the lag time increased.

By replacing the parameters of the *lag* vs. d_s primary model (Bréand et al., 1997) by their corresponding secondary models, a global model describing the effect of d_s and T_s on the lag was built. This model considered four parameters among the existing parameters of the secondary models Eqs. (1)–(3). An underestimation was observed for the greatest lag times. If such a bias is observed for other data sets, the global model will have to be modified. The precision of the estimators of the parameters of the secondary models was improved when the global model was fitted to the lag times observed for all the studied couples (d_s, T_s). The ability of the global model to improve the precision of the estimators of the secondary model parameters was already stressed in the case of the description of the effect of d_s and T_s on the number of survivors of a mild increase in temperature (Bréand et al., in press). Consequently, from the point of view of predictive microbiology, it seems interesting to fit the global model in order to improve the precision of the parameter estimators.

With the global model, sets of increases in temperature inducing the same lag time have been defined (Fig. 4b). This model can become an interesting tool to estimate the required recovery time, or lag time, before the identification of pathogens. Indeed, injury due to an increase in temperature induces the modification of the abilities of the survivors to grow. The main difference between non-injured and injured bacteria was the lack of growth of injured bacteria on the media classically used in the identification process (Hurst, 1977; Mackey and Derrick, 1984; Ray, 1986; Salamah, 1990). In the case of pathogens or microorganisms used as indicators of the quality of a product such as water, this difference was particularly dramatic. Thus, in order to improve the identification process, the injured bacteria are placed in a nonselective media during recovery time (Mackey and Derrick, 1982, 1984). The aim of this step is the recovery of the bacteria since the recovered bacteria should have the same growth ability as the non-injured ones. The difficulty of this process is the determination of the recovery time. In fact, this time depends on the environmental conditions which induced the injury (Mackey and Derrick, 1982, 1984). The global model built herein could become an objective tool to estimate the required recovery time in the case of mild heat-induced injury. The required information for this estimator of the recovery time is the characteristics d_s and T_s of the increase in temperature, and the lag time of the population in the case of lack of increase in temperature, L_0. In the case of contamination during the manufacturing of a foodstuff, this information seems easily available.

A mortality phase followed by the lag time and the exponential growth of the survivors is the response to a mild increase in temperature such as those studied herein (Bréand et al., 1997). Yet, so far, in order to define the mild heat treatment to apply to warrant the safety of a foodstuff, only the mortality phase was considered. This criterion probably correct in the case of pasteurization, is no longer sufficient for mild increases in temperature. In fact, mild increase in temperature does not allow eradication of the entire bacterial population. Thus, by considering only the mortality phase to define mild heat treatment, the evolution of the survivors was not taken into account. The fact that the mild heat increase which minimized the number of survivors did not maximize the lag time of the survivors stressed the necessity to consider both the mortality and the lag to define a mild heat treatment. This fact was deduced from the global modelling of mortality and lag time. The comparison of the two figures Fig. 4a and b reveals that the sets of increase in temperature for which the lag is maximum, does not overlap the sets of increase in temperature for which the survivor number is minimum. This observation concerning *Listeria monocytogenes* indicates that the heat treatment which minimizes the number of survivors is not the safest since the lag time of the survivors can be relatively short. By combining these two figures, a new strategy to define mild heat treatment comes into view. The idea is to choose the heat treatment according to a criterion which allows a compromise between the minimization of the number of survivors and the maximization of the lag time. Such a criterion can be the threshold time. In fact, in the case of the heat stressed population, the threshold time depended on the death, the regrowth lag and the exponential growth of the survivors.

Since the mathematical tools for the description of the lag time (Eq. (4)) and the mortality (Bréand et al., in press) were available, and the mathematical tool describing *Listeria monocytogenes* growth phase published in the literature (Charles-Bajard, 1996), the threshold time could be predicted.

So far the way to maximize the threshold time was to minimize the survivor number. The filled contour plot of the predicted threshold time shows that the minimization of the survivors effectively induced the maximization of the threshold time (Fig. 5). The filled contour plot of the predicted threshold time also shows that the maximization of the lag time could induce the maximization of the threshold time (Fig. 5). Our future work could be to implement a method to estimate the confidence bands for the sets of points (d_s, T_S) for which a same threshold time was predicted, this could improve the reliability of the contour plot.

On the other hand, in order to improve the mathematical models presented in this paper and to validate the result presented by Fig. 5, coming from a prediction, it is necessary to pursue the gathering of data.

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