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Modelling pH evolution and lactic acid production in the growth medium of a lactic acid bacterium: Application to set a biological TTI

M. Ellouze [a,b,*], M. Pichaud [a], C. Bonaiti [a], L. Coroller [c], O. Couvert [d], D. Thuault [d], R. Vaillant [a]

[a] CRYOLOG S.A. Département R&D, 58, Boulevard Gustave Roch, 44261 Nantes, France
[b] Unité MASQ, Ecole Nationale Vétérinaire de Maisons Alfort, 7 Avenue du Général de Gaulle 94700 Maisons Alfort, France
[c] Laboratoire Universitaire de Biodiversité et Ecologie Microbienne, Université Européenne de Bretagne, 6 Rue de l'Université, 29000 Quimper, France
[d] Unité IDEA, ADRIA, A. Créac'h Gwen, 29196 Quimper, France

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ABSTRACT

Time temperature integrators or indicators (TTIs) are effective tools making the continuous monitoring of the time temperature history of chilled products possible throughout the cold chain. Their correct setting is of critical importance to ensure food quality.
The objective of this study was to develop a model to facilitate accurate settings of the CRYOLOG biological TTI, TRACEO®. Experimental designs were used to investigate and model the effects of the temperature, the TTI inoculum size, pH, and water activity on its response time.
The modelling process went through several steps addressing growth, acidification and inhibition phenomena in dynamic conditions. The model showed satisfactory results and validations in industrial conditions gave clear evidence that such a model is a valuable tool, not only to predict accurate response times of TRACEO®, but also to propose precise settings to manufacture the appropriate TTI to trace a particular food according to a given time temperature scenario.

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1. Introduction

Foodborne diseases have become a major concern in the food industry and have inflicted significant burdens on society. According to studies made by the World Health Organisation, 30% of the population in industrialized countries suffer from food borne diseases every year (WHO, 2007). For example, in Europe, 6860 outbreaks were reported in 2004, affecting 42,447 people, thirteen of whom died (EFSA, 2006).

Refrigerated goods are often involved in food poisoning episodes. Hence, to prevent economic losses and to reduce the impact of foodborne diseases, the cold chain must be fully respected. Moreover, with recent regulatory changes, food business processors are now urged to develop traceability tools to monitor the goods' temperature from the factory all the way through to the consumer. Time Temperature Indicators or Integrators (TTIs) can bring about a solution to this new need.

TTIs can be defined as simple and inexpensive devices used to show measurable time temperature dependent changes related to the food to which they are attached (Taoukis and Labuza, 1989).

These changes are usually expressed by a visible response such as a mechanical deformation, a colour change or movement (Taoukis and Labuza, 1989). Depending on the involved working principle, they can be classified as biological, chemical or physical systems (Yan et al., 2008). Several TTIs have been developed and patented, the principles and applications of which have been largely reviewed (Taoukis and Labuza, 2003).

Commercially available TTIs include a number of diffusion (Monitor Mark® and Freshness Check® by the 3M Company, USA) enzymatic (CheckPoint® by the VITSAB Company, Sweden) polymer based (Lifelines Freshness Monitor® and Fresh Check® by Lifelines Technology, USA) and microbiological systems (TRACEO® and (eO)® by CRYOLOG) (Kerry et al., 2006).

Microbial TTIs advance all others because their response is closely related to microbial food spoilage. In fact, bacterial growth and metabolism in such TTIs directly reflect bacterial growth and metabolism in the traced food (Vaikousi et al., 2008).

CRYOLOG is a young French company which develops and markets a line of biological TTIs to trace sensitive goods. These TTIs are set according to the food category to trace and provide clear information about the product quality loss for both consumers and food retailers, thus allowing appropriate monitoring of the cold chain.

In the current research, a particular biological TTI was studied. TRACEO® is a small adhesive blue tag in which selected strains of lactic acid bacteria (LAB) are trapped. Once put on the bar code of the package of the traced chilled food and depending on the time temperature profile the system goes through, TRACEO® delivers a clear twofold response: an irreversible colour change from blue to

* Corresponding author. CRYOLOG S.A. Département R&D, 58, Boulevard Gustave Roch, 44261 Nantes, France. Tel.: +33 1 43 96 71 78; fax: +33 1 43 96 70 90.
E-mail address: mellouze@cryolog.com (M. Ellouze).

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pink and a simultaneous opacification reaction once the product has experienced critical temperature abuses or once it has reached its use by date. The opacification reaction prevents correct reading of the bar code. The product is therefore rejected by the scanner at check out, thus enabling automated and systematic detection of altered food in markets. The visible colour change from blue to pink warns the consumer that the food has become spoiled.

These two simultaneous changes depend on the acidification of the TTI medium resulting from the LAB growth brought about by the temperature storage conditions. Our study aims at using a new modelling approach to predict the response time of TRACEO® taking into account LAB growth, lactic acid production and pH decrease in the TRACEO® medium. As a second step, the model provides the correct settings (pH, water activity, inoculum size) to manufacture specific TTIs so that their responses closely match the quality loss of the particular product to be traced.

2. Materials and methods

2.1. TTI composition

The TRACEO® medium is based on a nutrient medium supplemented with a specific chromatic indicator and a precipitating molecule. Further details can be found in the TRACEO® patent (R, Vaillant, 17.09.01, French Patent Office No. FR 2 829 854 B1).

A preliminary study (data not shown) made it possible to identify the best micro organism to use in the TTIs. It was selected among several strains of lactic acid bacteria for its acidification capacities and its ability to grow in the TTI medium without being inhibited by the chromatic indicator. *Carnobacterium piscicola* isolated from dairy products showed the best profile and was therefore selected.

2.2. Experimental design

We investigated the effects of four factors of interest. The tested levels are those usually used by CRYOLOG to produce industrial TTIs. The effects of the temperature (3 °C and 20 °C), the pH (7.5 and 9), the water activity (0.955 and 0.994) and the inoculum size (4 log CFU/ml and 8 log CFU/ml) on the time of response of the TTI, were thus studied with a full factorial design 2^4. Twelve central points were added and eight extra runs were performed, as suggested by the Statgraphics (5.1) software, to take into account the central level value of each factor. The final experimental design was thus made up of 36 trials which were randomized and repeated.

According to the experimental design, sausage-like packagings were prepared, inoculated and stored at different constant temperatures. Inoculums were obtained from fresh cultures which were diluted to the target values. These "laboratory TTIs" offered a larger medium quantity than classical TTIs thus facilitating microbiological and pH analysis. At pre-established times, aliquots were taken and used for both pH measurements (Hanna Instruments HI-8418 pH meter) and LAB countings (modified version of the normalized method NF V04-503 using the TRACEO® Agar medium as *C. piscicola* was inhibited by some of the MRS components). The colour change and the opacification reaction of the TTIs were monitored by scoring their colour evolution on a scale from 1 to 6 before putting them on bar codes and scanning them to check scan rejection.

2.3. Global modelling approach

The modelling approach involved three major steps, including simulations of (1) growth, (2) lactic acid production and (3) pH decrease. Steps (2) and (3) both change the growth conditions. Step by step simulation was therefore required to simulate the growth, the lactic acid production and the pH decrease at the end of each step i to be considered as initial conditions in the following step $i+1$.

2.4. Modelling approach

2.4.1. Modelling growth

Growth of *C. piscicola* was modelled by the primary model of Rosso (1995).

$$\ln(N) = \begin{cases} \ln(N_0) & , t \le \text{lag} \\ \ln(N_{\max}) - \ln\left(1 + \left(\frac{N_{\max}}{N_0} - 1\right).\exp(-\mu_{\max}(t - lag))\right) & , t > \text{lag} \end{cases} \quad (1)$$

where N(CFU/ml) is the bacterial concentration at the instant t, N_0 (CFU/ml) the initial bacterial concentration, $N_{\max}$(CFU/ml) the maximum bacterial concentration, $\mu_{\max}(h^{-1})$ the maximum specific growth rate and lag(h) the lag time.

The model parameters were estimated for each run by the least square fitting method (LSQCURVEFIT, MATLAB 6.1, Optimization Toolbox, The Math-works). The parameters lag and $\mu_{\max}$ were further investigated by secondary models.

Eq. (2) made it possible to take into account the effects of the LAB physiological state k on the lag time.

$$k = \mu_{\max} \cdot \text{lag} \quad (2)$$

The effects of the environmental factors on $\mu_{\max}$ were evaluated using the gamma concept which was first proposed by Zwietering et al. (1992) and further developed by several studies (Wijtzes et al., 1998, 2001; Zwietering et al., 1996).

$$\mu_{\max} = \mu_{\text{opt}} \cdot \gamma_T(T) \cdot \gamma_{\text{pH}}(\text{pH}) \cdot \gamma_{\text{AH}}(\text{AH}) \cdot \gamma_{\text{aw}}(\text{aw}) \quad (3)$$

where T (°C) is the temperature, aw is the water activity, AH (mM) the concentration of undissociated lactic acid, $\mu_{\max}$ (h^{-1}) the maximum specific growth rate for a given condition of temperature, pH, water activity and undissociated organic acid, and $\mu_{\text{opt}}(h^{-1})$ the optimal growth rate observed in optimal growth conditions. μ_{opt} is a medium and microbe dependent parameter.

Each γ_i term reflects the effect of the correspondent environmental factor i on the maximum growth rate. The effects of temperature, pH and water activity were modelled by the Cardinal Parameter Model (CPM) (Rosso, 1995).

$$\gamma(X) = \begin{cases} 0 & , X \le X_{\min} \\ \frac{(X - X_{\max}) \cdot (X - X_{\min})^n}{(X_{\text{opt}} - X_{\min})^{n-1} \cdot [(X_{\text{opt}} - X_{\min}) \cdot (X - X_{\text{opt}}) - (X_{\text{opt}} - X_{\max}) \cdot (X_{\text{opt}} + X_{\min} - nX)]} & , X_{\min} < X < X_{\max} \\ 0 & , X \ge X_{\max} \end{cases} \quad (4)$$

where X is the studied environmental factor (temperature, pH or aw), $X_{\min}$ the level of the factor below which no growth occurs, $X_{\max}$ the level of the factor above which no growth occurs, X_{opt} the level of the factor at which $\mu_{\max}$ is equal to its optimum value μ_{opt} and n a shape parameter ($n=2$ for Temperature, $n=1$ for pH and $n=1$ for aw).

The lactic acid effect on the bacterial growth rate was modelled by Eq. (5) (Presser et al., 1997; Breidt and Fleming, 1998; Le Marc et al., 2002).

$$\gamma(\text{AH}) = 1 - \left(\frac{[AH]}{MIC}\right)^s \quad (5)$$

where MIC(mM) is the minimal theoretical concentration of undissociated lactic acid and s a shape parameter.

$X_{\min}$, $X_{\max}$, X_{opt} and MIC are the cardinal values of the strain. These microbe dependent parameters, are independent from the growth medium (Pinon et al., 2004; Neysens and De Vuyst, 2005) and were consequently estimated in a BHI medium supplemented with 0.2% glucose and 0.3% yeast extract according to the works of Cuppers and Smelt (1993) Experiments were carried out in a Bioscreen C (Labsystems, Helsinki, Finland). Four monofactorial designs were used to evaluate the effect of each factor as shown in Table 1. The non

Table 1
Levels of temperature, pH, water activity and lactate concentration tested on the four monofactorial designs to characterize the cardinal values of *C. piscicola*

Studied factors	Factors range	Other factors levels
Temperature (°C)	1.75–35.00	pH=7; aw=0.997; [AH]=0 mM
pH	5.10–10.40	T=30 °C; aw=0.997; [AH]=0 mM
aw	0.931–0.999	T=30 °C; pH=7; [AH]=0 mM
Lactic acid (mM)	0–120	T=30 °C; aw=0.999; pH=5.2

studied factors were kept at optimal levels. To estimate the lactic acid effect, a pH of 5.2 was used so as to identify the effects of the undissociated form of the lactic acid. Further details on the methodology of the assessment of cardinal values can be found in Membré et al. (2002).

Once the cardinal values assessed, the secondary model (Eq. (3)) was used to simulate the maximum growth rate $\widehat{\mu}_{max}$ for each run of the experimental design. These simulated $\widehat{\mu}_{max}$ values were compared to the observed μ_{max} values and the best μ_{opt} estimate was assessed as the value which minimizes the sum of squared errors between $\widehat{\mu}_{max}$ and μ_{max}.

2.4.2. Modelling the buffer effect model

The buffer effect model was used to simulate the medium pH knowing the total lactic acid concentration in the medium. Specific trials were made to characterize the buffering properties of the TRACEO® medium. Increasing quantities (100 µl) of lactic acid (1 M) were added to the medium and the pH decrease was monitored (Hanna Instruments HI-8418 pH meter).

The experimental curves were best described by a modified logistic model (Whiting, 1993).

$$\mathrm{pH(Lac)} = \mathrm{pH_0}\left[f\frac{1+e^{-k_1\cdot\lambda}}{1+e^{-k_1\cdot(Lac-\lambda)}} + (1-f)\frac{1+e^{-k_2\cdot\lambda}}{1+e^{-k_2\cdot(Lac-\lambda)}}\right] \quad (6)$$

where Lac (mM) is the total lactic acid concentration produced by *C. piscicola*, $\mathrm{pH_0}$ the initial pH of the medium, λ (mM) the constant minimal lactic acid concentration required to observe an acidification reaction, k_1 the constant acidification rate for the first part of the kinetic, k_2 the constant acidification rate for the second part of the kinetic, and f the pH observed at the end of the first acidification curve.

Parameter f is difficult to interpret. For the sake of simplification, it is replaced by its logit transformation shown in Eq. (7) where the parameter F estimates the relative importance of the two acidification processes

$$F = \log_{10}\left(\frac{f}{1-f}\right). \quad (7)$$

2.4.3. Modelling the lactic acid production model

Eq. (8) is commonly used in the literature, to predict lactic acid concentrations (Vereecken and Van Impe, 2002; Poschet et al., 2005).

$$\frac{\mathrm{dLac}}{\mathrm{d}t} = Y_p\frac{\mathrm{d}N}{\mathrm{d}t} \quad (8)$$

where Y_p (mM/cell.h) is the lactic acid quantity produced by cellular division and time unit.

The bacterial strain characteristics and the TRACEO® medium composition are in line with the hypothesis required to use such a model. Indeed, *C. piscicola* has a mainly homofermentative metabolism with the lactic acid being the only end product. Moreover, the medium rich composition prevents from substrate limitations. The use of the model of Vereecken and Van Impe (2002) is therefore fully justified.

Step by step evaluations of the bacterial population, the produced lactic acid and the consequent pH drop were performed. By minimizing the sum of the squared errors between observed and simulated pH values for the 36 first trials of the experimental design, Y_p was assessed. The remaining set of 36 runs was left aside for validation.

Only undissociated lactic acid inhibits the growth. The total lactate concentration was therefore transformed into inhibitory lactate concentration with the Anderson Hasselbach relation shown in Eq. (9) before integrating it in the secondary model.

$$\mathrm{pH} = \mathrm{p}Ka + \log\left(\frac{\mathrm{Lac}}{[\mathrm{AH}]}\right) \quad (9)$$

where the lactic acid pKa is 3.86.

2.5. Evaluating the response time of the TTI

To evaluate the accuracy of the response times of the TTIs, experimentally observed response times were compared to those simulated by the model.

First, observed response times were obtained for each run of the experimental design according to the following procedure. In a former study (data not shown) the previously described colour scale (1–6) was used by operators to score the colour change of the TTIs in several temperature scenarios. The corresponding pH changes and the final response times were recorded for each experiment. Results have shown that the final colour change is observed at a score of 5 corresponding to a TTI pH of 6.35 at which the opacification reaction was completely achieved, as the scan rejected the bar code. A logistic model was then developed to predict the response time of the TTIs by using pH monitoring as shown in Eq. (10)

$$t = \frac{1}{\alpha}\left[\ln\left(\frac{\mathrm{pH}-pH_{\min}}{\mathrm{pH}_{\max}-pH}\right)-\beta\right] \quad (10)$$

where $\mathrm{pH_{max}}$ is the asymptotic maximum pH (initial pH value), $\mathrm{pH_{min}}$ the asymptotic minimum pH as t increases, t the time, pH the pH of response of the TTI (pH=6.35), α the relative pH decrease rate and β the time at which the absolute pH decrease rate is maximum.

This logistic model was thus applied to each run of the experimental design to assess the parameters $\mathrm{pH_{max}}$, $\mathrm{pH_{min}}$, α and β of each trial to be used to predict the correspondent observed response time.

Second, simulated response times were obtained directly from the global model as explained in Fig. 1 using the remaining set of 36 runs that were not used to generate the model. Given the initial conditions, namely the inoculum size, the water activity, the temperature and the initial pH, and assuming that the initial undissociated lactic acid concentration is zero, it was possible to simulate the instantaneous growth rate $\mu_{max,t}$ for a δt time, and to deduce the cell density N_t using

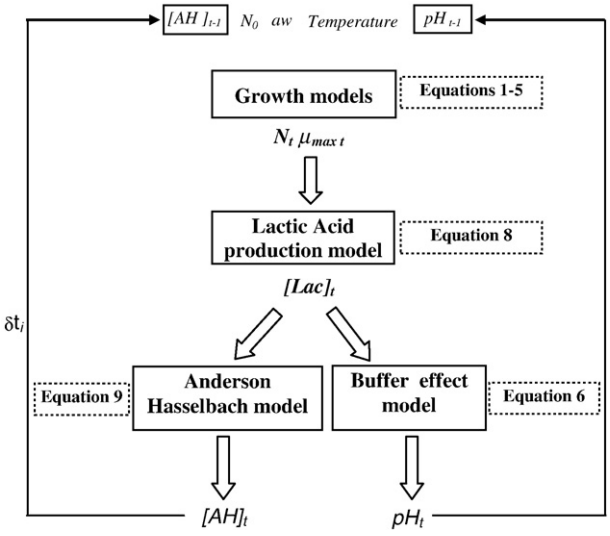

Fig. 1. The global model design.

the growth models. The lactic acid production model was then used to evaluate the lactic acid produced during this period. Finally, the Anderson Hasselbach equation converted the total lactic acid concentration Lac into undissociated lactic acid concentration [AH] while the buffer effect model transformed the total lactate concentration Lac to the correspondent pH value. As long as the pH was higher than 6.35, (the particular pH value at which the colour of the TTI changed from blue to pink), another simulation cycle was performed, taking into account the new values of pH_t and the new concentration of undissociated lactic acid $[AH]_t$. The initial values of the temperature and the water activity remained unchanged. The simulated response time was thus calculated when the simulated pH value reaches 6.35 as the sum of all the cycles' durations.

As a final step, simulated and observed response times were compared for each run and the Bias and the Accuracy Factors (respectively Bf and Af) were used to evaluate the model's goodness of fit. These factors provide an indication of the deviation between the predictions and the observations (Ross, 1996)

$$\mathrm{Bf} = 10^{\frac{\sum \log(t_{\mathrm{simulated}}/t_{\mathrm{observed}})}{n}} \tag{11}$$

$$\mathrm{Af} = 10^{\frac{\sum |\log(t_{\mathrm{simulated}}/t_{\mathrm{observed}})|}{n}}. \tag{12}$$

2.6. Model validation

We evaluated the model's ability to provide correct TRACEO® settings suitable to a specific product (shelf life of 10.5 days) with a specific product time temperature profile (one third of the shelf life at 4 °C and the rest of the shelf life at 8 °C). New experiments were carried out.

The time temperature scenario was given as an input to the model which provided several combinations of the possible settings to produce the adequate TTI for such a food under the specified time temperature profile. Twenty industrial prototypes of TTIs were then manufactured with respect to the model settings, put on bar codes and stored according to the time temperature scenario.

At different time intervals, the TTI colour was monitored with regard to the 1–6 colour scale reference. The TTI ability to prevent correct reading of the bar code was also checked. Once the colour score reached the value of 5 and the scan rejected the bar code, the experimental response time was assessed. All observed response times were then compared to the theoretical response time (shelf life of the food = 10.5days).

3. Results and discussion

The global model involves several parameters to be estimated. As for the growth models, the parameters are the optimal growth rate μ_{opt}, the *C. piscicola* physiological parameter k, the maximum population density N_{max} and the *cardinal values* (X_{min}, X_{opt}, X_{max}, MIC and the shape parameter s). The four parameters of the buffer effect model are F, k_1, k_2 and λ. Finally, the only parameter involved in the lactic acid production model is Y_p.

Table 2
Cardinal values of *Carnobacterium piscicola*

Factors (X)	T (°C) [CL 95%]	pH [CL 95%]	aw [CL 95%]	[AH] (mM) [CL 95%]
X_{min}	−5.42 [−8.07; −2.78]	5.12 [4.90; 5.33]	0.924 [0.916; 0.933]	–
X_{opt}	31.61 [29.01; 34.22]	7.27 [6.42; 8.13]	0.997	–
X_{max}	36.47 [33.13; 39.82]	10.24 [9.74; 10.73]	0.997	–
MIC	–	–	–	7.77 [7.24; 8.30]
s	–	–	–	0.48 [0.42; 0.54]

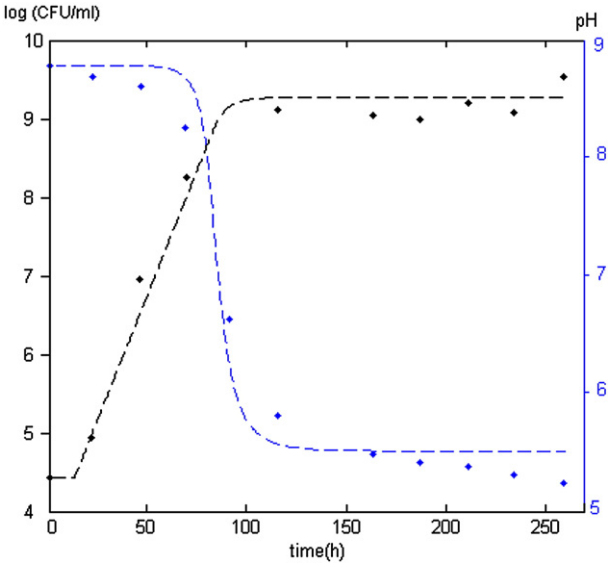

Fig. 2. Example of a fitting of experimental viable counts (log CFU/ml) and pH (.) on the growth and pH evolution models (dashed lines) for a 4 log inoculum TTI stored at 20 °C.

3.1. The growth parameters

The Cardinal values were obtained by fitting the experimental data of the four monofactorial designs to the secondary growth model. Results are shown in Table 2. These results are in line with cardinal values of LAB reported in the literature (Desmazeaud and de Roissart, 1994).

μ_{opt} is a medium and microbe specific parameter. In our work, its accurate estimation is very important since an overestimated μ_{opt} will make the model simulate faster growth leading to a premature pH decrease and an underestimation of the TTI response time. On the contrary, if the μ_{opt} is underestimated, the model will be a fail safe model as the predicted response times time will be overestimated.

Correct μ_{opt} estimation requires environmental conditions, namely pH, water activity and temperature to remain as constant as possible throughout the runs used to generate the estimate. However, in most trials, dynamic conditions were observed: the lactic acid, which is a primary growth metabolite, is steadily released as *C. piscicola* cells grow, causing a continuous pH decrease. Such a situation leads to poor μ_{opt} estimation. To overcome this issue, only selected runs were used to estimate this parameter. In fact, we observed that trials with an inoculum size of 10^4 CFU/ml did not sustain a significant acidification in the early exponential phase as shown in Fig. 2, the pH change became significant (decrease of 0.5 pH unit) only in the late exponential phase. As a result, we neglected the lactic acid production in the runs with an inoculum size of 10^4 CFU/ml and used them to estimate the μ_{opt}.

We validated this approximation as the fitting results presented in Table 3 show that the best estimate is obtained with 4 log inoculum runs. In fact, the root mean squared error values (RMSE) dropped from 0.1133 when using all the experiments to 0.0266 when using the 4 log inoculum trials only. We therefore used the μ_{opt} value of 0.898 h^{-1} for the rest of the work.

Table 3
Estimates of the optimal growth rate μ_{opt} and the associated RMSE for several subsets of the experimental design

Runs	μ_{opt} (h^{-1})	RMSE
Inoculum 4 log +6 log +8 log	1.085	0.1133
Inoculum 4 log +6 log	1.020	0.0425
Inoculum 4 log	0.898	0.0266

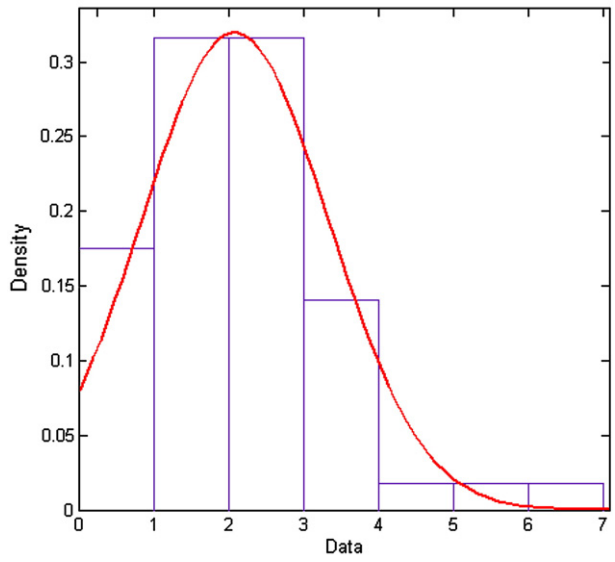

Fig. 3. Distribution of the physiological parameter *k*. The bars represent the observations and the line represent the fitting of the normal distribution *N*(2.28, 1.3).

An accurate evaluation of the lag phase is also important to accurately estimate the acidification reaction start and to get correct response times for the TTIs. The physiological parameter *k* which relates the maximum growth rate to the lag (Eq. (2)), was thus evaluated for the runs of the experimental design. Only one run out of the 12 repetitions of the central point was taken into account, so as to give the same weight to each trial conditions. As the parameter *k* is always positive, the values were adjusted to a truncated normal distribution (mu=2.28, sigma=1.3) depicted in Fig. 3. The normal distribution was used because several authors have demonstrated in different works that the physiological parameter *k* is normally distributed (Powell et al., 2006; Tamplin et al., 2005). Hence, as our global model is designed for a deterministic approach, we chose to use the median value as an estimate of the parameter *k*=2.13.

The decimal logarithm of the maximum population density log (N_{max}) is also normally distributed (mu=9.24, sigma=0.19). It seems that it is independent from the environmental factors at least at the studied levels, so we used the median value log(N_{max})=9.27 as an estimate for this parameter.

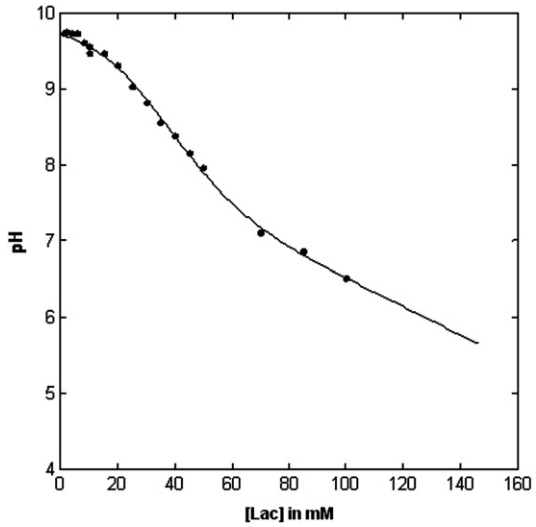

Fig. 4. pH evolution as a function of total lactate concentration. The line represents the fittings according to the buffer effect model on experimental data (■).

Table 4
Parameters estimates of the buffer effect model

Parameter	Estimation
λ (mM)	13.120
k_1	0.027
k_2	0.085
a	−0.213
b	1.554

3.2. The buffer effect model parameters

The evolution of the pH of the medium with increasing lactic acid concentrations (Fig. 4) is a sigmoid which can be described by a logistic model.

Once we had fitted the data to the adapted logistic model (Eq. (6)) we observed constant acidification rates k_1 and k_2 for all the experiments. Furthermore, the parameter λ which reflects the required lactic acid concentration to initiate the acidification reaction, seemed to remain unchanged. Hence, the fitting was performed again keeping the parameters λ, k_1 and k_2 at fixed values. Only pH_0 and *F* varied with regards to the experimental pH.

Fitted pH_0 was very close to the target initial pH value. We therefore assumed that pH_0 is the initial experimental pH. The data also showed that the parameter *F* depends on pH_0. A linear regression was proposed as shown in Eq. (13)

$$F = a.pH_0 + b. \quad (13)$$

Table 4 presents the estimates of the parameters of the buffer effect model. The goodness of fit showed in Fig. 4 offers clear evidence that the adapted logistic model is suitable to describe the pH evolution of the TTI medium with regards to lactic acid production.

3.3. The lactic acid production parameter

The accurate estimation of the lactic acid production parameter Y_p is of paramount importance for the global model. In fact, a precise estimation will provide correct information for the secondary growth model, which will properly simulate the corresponding inhibitory

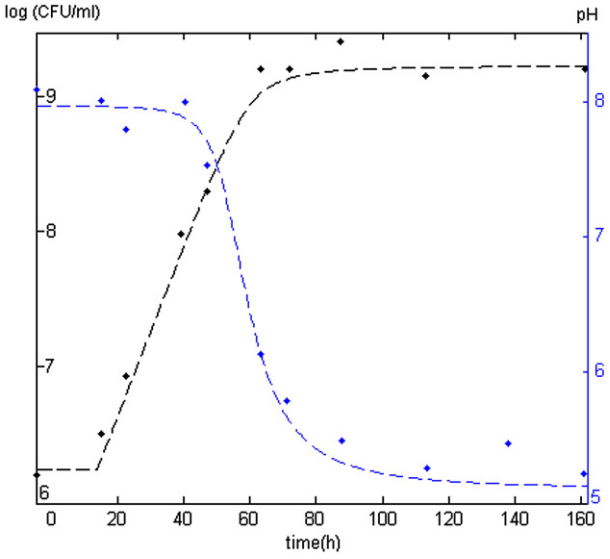

Fig. 5. Fitting of experimental viable counts (log CFU/ml) and pH (.) on the growth and pH evolution models (dashed lines) for a TTI (6 log inoculum, aw=0.975, pH=8.25) stored at 11.5 °C.

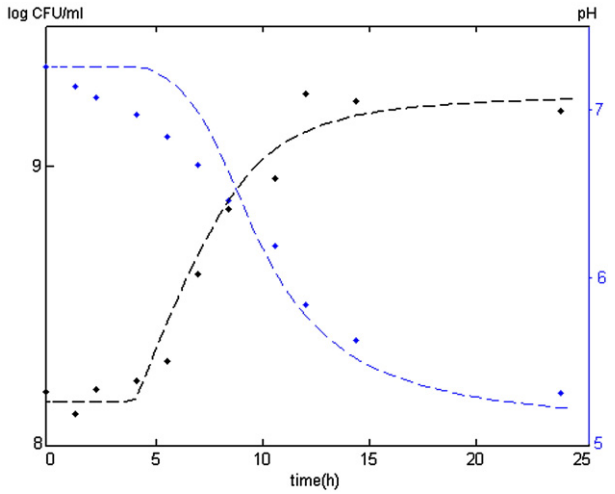

Fig. 6. Fitting of experimental viable counts (log CFU/ml) and pH (.) on the growth and pH evolution models (dashed lines) for an 8 log inoculum TTI stored at 20 °C.

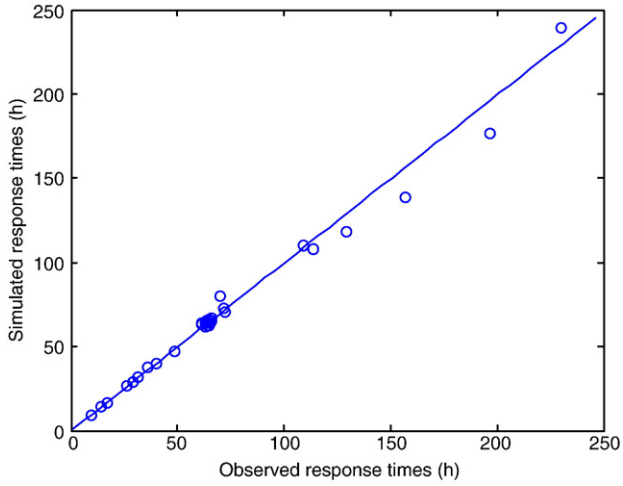

Fig. 7. Observed and simulated response times for runs 36 to 72.

effect, thus leading to realistic estimates of growth and lactic acid evolution. On the contrary, inadequate Y_p estimation will make the model simulate unrealistic lactic acid concentrations leading to inaccurate growth predictions and inadequate response times of the TTIs.

We estimated the yield of lactic acid production Y_p to $4.95.10^{-8}$ mM $h^{-1}.CFU^{-1}$. Our results are in the same range as other authors' findings of $7.23.10^{-8}$ mmol $h^{-1}.CFU^{-1}$ (Vereecken and Van Impe, 2002). We believe that the slight difference is most probably due to the use of different growth media and different lactic acid bacteria strains in the two studies.

3.4. Model evaluation

The goodness of fit of our model is quite satisfactory, with a RMSE value of 0.266 for pH simulations and 0.119 for growth simulations. Fig. 5 illustrates the goodness of fit for one particular trial of the experimental design. The bias factor (1.17) and the accuracy factor (1.04) indicate that there is a good agreement between predicted and observed response times. Indeed, these two indicators show that the predictions differ from the observations by 17% and that there is only a 2% deviation of predictions with reference to observations.

However, as shown in Fig. 6, when the initial inoculum is high, (10^8 CFU/ml), the observed acidification reaction is faster than the model's predictions at the beginning of the experiment. Indeed, even if the model succeeds in accurately predicting the growth, it fails in simulating the initial pH evolution. In this case, observed pH values seem to fall off immediately whereas the model predicts an initial shoulder phase , which leads to inaccurate fittings.

We believe that this initial quick pH drop is due to the inoculum preparation procedure. In fact, inoculum 4 and 6 TTIs are inoculated using the appropriate dilution of the preculture whereas inoculum 8 TTIs are directly inoculated from the preculture where the concentration of lactic acid is important. As a result, when transferred to the TTI medium, the cells will passively release the lactic acid , which they accumulated when they were in the preculture medium, thus causing an artificial and immediate pH decrease in the TTI medium. These findings lead us to conclude that a washing procedure should be included when using high inoculums. The buffering capacities of the medium should also be improved to avoid such an artificial pH drop.

Even if we acknowledge that our model sometimes fails in accurately predicting the initial pH evolution in some trials, it always succeeds in predicting the pH decrease in the 6.35 area, where the response time of the TTI is observed. Our model is therefore able to simulate correct response times. In fact, when comparing the observed and simulated response times (Fig. 7) a good correlation is obtained (R^2=0.985) and proves the accuracy of the model.

3.5. Model validations

Finally, the model's ability to provide correct settings for TTIs given the food shelf life and the storage conditions was checked. The experimentally observed response times of the prototypes were compared to the theoretical shelf life of the food (10.5 days). Results

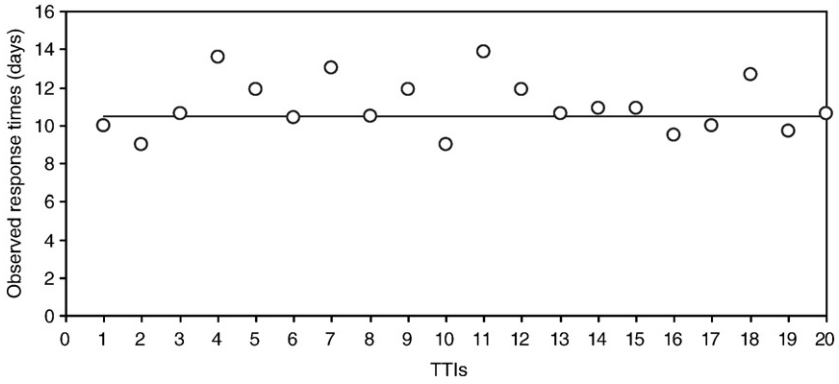

Fig. 8. Observed response times (o) for twenty industrial TTIs set according to the model. The line represents the target response time (10.5 days).

presented in Fig. 8 are, as expected, very satisfactory. The values ranged from 8.9 days to 14 days. The average observed time is 10.83 days and the median value is only 1% higher than the product shelf life. Such results clearly prove that the observed responses of industrial TRACEO®, set according to our model, are in agreement with the predicted responses.

4. Conclusion

The aim of this paper was to provide an accurate tool to set TRACEO® biological TTIs, given the food shelf life and a time temperature scenario. To reach this goal, several models were used in order to simulate the LAB growth, the acidification reaction and the pH decrease in the TRACEO® medium. The Rosso primary model (Rosso, 1995) and the secondary cardinal model (Rosso, 1995) were used as growth models to take into account the effects of the environmental conditions, while the Vereecken and Van Impe (2002) model was used to assess lactic acid production. As for the buffer effect model, the modified logistic model was well adapted to our data but further research will focus on the improvement of the TRACEO® buffering capacities to avoid artificial pH drops at the beginning of some trials.

Further research will also be carried out to ensure optimal evaluation of the global model parameters. A sensitivity analysis is worth conducting so as to identify the most important parameters and improve their estimation, trying to take into account the uncertainty related to each parameter estimation as well. Moreover, our global strategy could be changed to estimate the whole set of parameters at once, thus avoiding biased estimates of the key parameter Y_p , which is the last parameter to be estimated in the current strategy, after all the other parameters have been assessed with their possible associated errors.

Despite these minor adjustments, the global model showed great fitting capacities and it can now be used for accurate simulations of TRACEO® response times for specific settings. Moreover, it proposes precise settings to be used for TRACEO® to trace selected food.

Our research strategy will now focus on larger scale validations of TRACEO® responses in conditions of drastic temperature abuses for several settings and different shelf lives. Parallel studies on food will also be conducted. As LAB are the major alteration micro organisms, our lactic acid bacteria based TTI is likely to successfully mimic the behaviour of the natural food flora of many goods. Our validated device could fundamentally change the approach of food business processors to setting the shelf lives of their products. They could then rely on a “dynamic food shelf life” , which is specific to the traced food under the very specific storage conditions it goes through, thus improving the quality of their products.

Such a concept can also be used in other fields such as the pharmaceutical field where the respect of the storage temperature of medicines and vaccines is crucial.

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References

Breidt, F., Fleming, H.P., 1998. Modeling of the competitive growth of *Listeria monocytogenes* and *Lactococcus lactis* in vegetable broth. Applied and Environmental Microbiology 64 (9), 3159–3165.
Cuppers, H.G.A.M., Smelt, J.P.P.M., 1993. Time to turbidity measurements as a tool for modelling spoilage by *Lactobacillus*. Journal of Industrial Microbiology 12, 168–171.
Desmazeaud, M.J., de Roissart, H., 1994. Métabolisme général des bactéries lactiques. In: De Roissart, H., Luquet, F.M. (Eds.), Bactéries Lactiques: Aspects Fondamentaux et Technologiques. Lorica, Paris, pp. 169–179.
EFSA, 2006. Trends and sources of zoonoses, zoonotic agents and antimicrobial resistance in the European Union in 2004. The EFSA Journal 310–2005, 18–19.
Kerry, J.P., O'Grady, M.N., Hogan, S.A., 2006. Past, Current and potential utilisation of active and intelligent packaging systems for meat and muscle-based products: a review. Meat Science 74, 113–130.
Le Marc, Y., Huchet, V., Bourgeois, C.M., Guyonnet, J.P., Mafart, P., Thuault, D., 2002. Modelling the growth runs of *Listeria* as a function of temperature, pH and organic acid concentration. International Journal of Food Microbiology 73, 219–237.
Membré, J.-M., Leporq, B., Vialette, M., Mettler, E., Perrier, L., Thuault, D., Zwietering, M., 2002. Temperature effect on bacterial growth rate: quantitative microbiology approach including cardinal values and variability estimates to perform growth simulations on/in food. International Journal of Food Microbiology 100, 179–186.
Neysens, P., De Vuyst, L., 2005. Kinetics and modelling of sourdough lactic acid bacteria. Trends in Food Science and Technology 16, 95–103.
Pinon, A., Zwietering, M., Perrier, L., Membre, J.M., Leporq, B., Mettler, E., Thuault, D., Coroller, L., Stahl, V., Vialette, M., 2004. Development and validation of experimental protocols for use of cardinal models for prediction of microorganism growth in food products. Applied and Environmental Microbiology 70 (2), 1081–1087.
Poschet, F., Vereecken, K.M., Geeraerd, A.H., Nicolaï, B.M., Van Impe, J.F., 2005. Analysis of a novel class of predictive microbial growth models and application to coculture growth. International Journal of Food Microbiology 100, 107–124.
Powell, M.R., Tamplin, M., Marks, B., Campos, D.T., 2006. Bayesian synthesis of a pathogen growth model: *Listeria monocytogenes* under competition. International Journal of Food Microbiology 109, 34–46.
Presser, K.A., Ross, T., Ratkowsky, D.A., 1997. Modelling the growth rate of *Escherichia coli* as a function of temperature, pH and lactic acid concentration, and water activity. Applied and Environmental Microbiology 63 (6), 1773–1779.
Ross, T., 1996. Indices for performance evaluation of predictive models in food microbiology. Journal of Applied Microbiology 81 (5), 501–508.
Rosso, L. 1995. Modélisation et microbiologie prévisionnelle: Elaboration d'un nouvel outil pour l'agro - alimentaire, PhD thesis. Université Claude BERNARD, Lyon, France.
Tamplin, M.L., Paoli, G., Marmer, B.S., Phillips, J., 2005. Models of the behaviour of *Escherichia coli 0157:H7* in raw sterile ground beef stored at 5 to 46 °C. International Journal of Food Microbiology 100, 335–344.
Taoukis, P.S., Labuza, T.P., 1989. Applicability of time-temperature indicators as shelf life monitors of food products. Journal of Food Science 54, 783–788.
Taoukis, P.S., Labuza, T.P., 2003. Time-temperature indicators (TTI). In: Ahvenainen, R. (Ed.), Novel Food Packaging Techniques. Woodhead Publishing Limited, Cambridge, pp. 103–126.
Vaikousi, H., Biliaderis, C.G., Koutsoumanis, K.P., 2008. Development of a Microbial Time Temperature Indicator (TTI) Prototype for Monitoring Microbiological Quality of Chilled Foods. Applied and Environmental Microbiology 74 (10), 3242–3250.
Vereecken, K.M., Van Impe, J.F., 2002. Analysis and practical implementation of a model for combined growth and metabolite production of lactic acid bacteria. International Journal of Food Microbiology 73, 239–250.
Whiting, R.C., 1993. Modeling bacterial survival in unfavorable environments. Journal of Industrial Microbiology 12, 240–246.
WHO, 2007. Food Safety and Food Borne Illness. Fact Sheet No. 237. http://www.who.int/mediacentre/factsheets/fs237/en/.
Wijtzes, T., Rombouts, F.M., Kant-Muermans, M.L., Van't Riet, K., Zwietering, M.H., 2001. Development and validation of a combined temperature, water activity, pH model for bacterial growth rate of *Lactobacillus curvatus*. International Journal of Food Microbiology 63, 57–64.
Wijtzes, T., Van't Riet, K., Huis in't Veld, J.H., Zwietering, M.H., 1998. A decision support system for the prediction of microbial food safety and food quality. International Journal of Food Microbiology 42, 79–90.
Yan, S., Huawei, C., Limin, Z., Fazheng, R., Luda, Z., Hengtao, Z., 2008. Development and characterization of a new amylase type time-temperature indicator. Journal of Food Control 3, 315–319.
Zwietering, M.H., Wijtzes, T., De Wit, J.C., Van't Riet, K., 1992. A decision support system for the prediction of the microbial spoilage in foods. Journal of Food Protection 55, 973–979.
Zwitering, M.H., De Wit, J.C., Notermans, S., 1996. Application of predictive microbiology to estimate the number of *Bacillus cereus* in pasteurized milk at the time of consumption. Interntional Journal of Food Microbiology 30, 55–70.