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Modeling the growth of *Lactobacillus viridescens* under non-isothermal conditions in vacuum-packed sliced ham

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ABSTRACT

Lactic acid bacteria (LAB) are responsible for spoiling vacuum-packed meat products, such as ham. Since the temperature is the main factor affecting the microbial dynamic, the use of mathematical models describing the microbial behavior into a non-isothermal environment can be very useful for predicting food shelf life. In this study, the growth of *Lactobacillus viridescens* was measured in vacuum-packed sliced ham under non-isothermal conditions, and the predictive ability of primary (Baranyi and Roberts, 1994) and secondary (Square Root) models were assessed using parameters estimated in MRS culture medium under isothermal conditions (between 4 and 30 °C). Fresh ham piece was sterilized, sliced, inoculated, vacuum-packed, and stored in a temperature-controlled incubator at five different non-isothermal conditions (between 4 and 25 °C) and one isothermal condition (8 °C). The mathematical models obtained in MRS medium were assessed by comparing predicted values with *L. viridescens* growth data in vacuum-packed ham. Its predictive ability was assessed through statistical indexes, with good results (bias factor between 0.95 and 1.03; accuracy factor between 1.04 and 1.07, and RMSE between 0.76 and 1.33), especially in increasing temperature, which predictions were safe. The model parameters obtained from isothermal growth data in MRS medium enabled to estimate the shelf life of a commercial ham under non-isothermal conditions in the temperature range analyzed.

1. Introduction

Meat products are susceptible to microbial spoilage, for having pH close to neutral and being rich in nutrients. The acceptable spoilage point can be set as a tolerable level of bacteria, occurrence of undesirable odors or unacceptable appearance. The shelf life of such products depends on the number and type of microorganisms, mostly bacteria, initially present and its subsequent growth (Borch et al., 1996).

The color of cured meats is one of the most important factors affecting consumer acceptability. Green discoloration in cured meats is a recurrent problem for the meat industry and is usually caused by specific microorganisms, which are able to produce oxidizing substances that act on the cured meat pigments (Grant and McCurdy, 1986). *Lactobacillus viridescens* has been described as the organism frequently responsible for microbial greening in cured sausage and ham products (Niven et al., 1949, Sharpe, 1962, Tittsler et al., 1952). The major difference between this specie and the other *lactobacilli* is a much lower minimum growth temperature (Niven and Evans, 1956), what characterizes it as a potential spoilage agent of chilled meat products.

The temperature has great influence on the kinetics of microbial growth, especially for chilled foods, because it usually varies greatly during transport, retail and at home (Kilcast and Subramaniam, 2000). Hence the mathematical modeling of microbiological growth is aimed at developing models that can be applied to describe non-isothermal conditions to mimic real time-temperature situations (Gougouli and Koutsoumanis, 2010, Ross and McMeekin, 1999).

Many traditional methods used for determining the shelf life of foods do not consider temperature fluctuations along the distribution and storage food chain, simulating a constant environment, which rarely happens. Short periods of high temperatures can spoil the meat product, and then it is clear the importance of studying growth of lactic acid bacteria under non-isothermal conditions.

The complexity of non-isothermal modeling is that many isothermal experiments at different temperatures within a determined range are necessary to obtain reliable parameters estimation. These experiments can by carried out in culture medium. Then, the construction of

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Table 1
Non-isothermal temperature profiles designed to assess the growth of L. *viridescens* in ham with the plateaus of temperature (T, in °C) and time to temperature shift (t_{shift}, in hours).

Profile	T_1 [t_{shift1}]	T_2 [t_{shift2}]	T_3 [t_{shift3}]	T_4 [t_{shift4}]	T_5 [t_{shift5}]	T_6 [t_{shift6}]
NI-1	4 [63.0]	8 [91.6]	12 [105.0]	16 [168.0]		
NI-2	12 [20.1]	16 [32.0]	20 [39.8]	25 [60.0]		
NI-3	25 [4.3]	20 [10.8]	16 [20.7]	12 [37.5]	8 [71.6]	4 [168.0]
NI-4	16 [11.9]	12 [32.0]	8 [72.9]	4 [192.0]		
NI-5	12 [16.7]	8 [50.9]	4 [155.5]	8 [189.7]	12 [248.0]	
I-8	8 [384]					

secondary models capable of describing the dependence of the primary parameters on the temperature is necessary to be possible to simulate the microorganism growth at dynamic temperatures. The most common method for validating models developed in culture medium using new data is to carry out experiments directly in the food product of concern (Baert et al., 2007).

The aim of the present study was to assess the predictive power of a mathematical model, using parameters obtained from isothermal experiments in culture medium, to describe the growth of *L. viridescens* in vacuum-packed sliced ham under non-isothermal conditions.

2. Materials and methods

2.1. Microorganism

L. viridescens (CCT 5843 ATCC 12706, Lote 22.07) used in this study was purchased in lyophilized form from the collection of cultures of the André Tosello Foundation of Tropical Cultures (Fundação Tropical de Culturas André Tosello, Campinas, Brazil). The strains were rehydrated, grown in MRS (de Man, Rogosa and Sharpe, 1960) broth medium (Acumedia Manufactures, Inc. Lansing, Michigan, USA), and stored in Eppendorf tubes with MRS medium containing 20% glycerol at −24 °C until its use.

2.2. Inocula

The reactivation of the culture for preparing the inocula was carried out in MRS medium at 30 °C for 18 h, reaching bacterial concentration of 10^9 CFU/g. Then, successive dilutions were performed in test tubes containing MRS until the concentration of, approximately, 6×10^4 CFU/g.

2.3. Sample preparation

In order to eliminate the natural bacterial flora, a whole piece of ham was superficially sterilized with alcohol 70% (v/v) and sliced in laminar flow chamber. The slices (about 20 g) were inoculated with 1 mL of inocula, put into a sterile mixer bag, and packaged in a vacuum plastic bag. The samples were stored in a temperature-controlled incubator (Dist, Florianópolis, Brasil).

2.4. Growth conditions

The growth of *L. viridescens* in vacuum-packed sliced ham was evaluated in six different temperature profiles, covering the temperature range in domestic refrigerators (since 4 °C) and the ambient temperature (until 25 °C). The selected temperature profiles are shown in Table 1. The temperature into the incubator was recorded by data logger (Testo174, Lenzkirch, Germany) every five minutes. Temperature variations were registered looking for refrigeration failures along the preservation of ham in order to consider the influence of temperature increase and decrease during the product shelf life. All experiments were conducted until the stationary growth phase.

2.5. Sampling

In pre-determined time intervals, two samples (duplicate) were taken to determinate the *L. viridescens* cells concentration in ham. As the homogenization packages were packed together with the ham, peptone water (1%, v/v) was added to the whole sample in the ratio 9:1 [volume peptone water (mL): ham mass (g)] and the solution was mixed for 60 s in stomacher (ITR model 1204) to carry out the first dilution. The following tenfold dilutions were performed in test tubes containing peptone water (1%, w/v). Then, 1 mL of each dilution was transferred to sterile Petri dishes and double layer of agar MRS (Difco Laboratories, Detroit, USA) was placed. All the procedures were carried out in laminar flow chamber. After the solidification, the inverted plates were incubated at 30 °C for 48 h. The results were expressed as log (N), where N is the LAB concentration at time t [CFU/g].

2.6. Mathematical modeling

The predictions of the microbial growth under non-isothermal conditions were carried out using the Baranyi and Roberts (1994) model in a differential form, according to Eqs. (1) and (2), and the initial condition in Eq. (3). In these equations, Q is related to the physiological state of the cells [dimensionless]; μ is the maximum specific growth rate [1/h]; N_{max} is the maximum population [CFU/g]; and N is the population [CFU/g] along the time.

$$\frac{d(\ln\ N)}{dt} = \mu\left[\frac{1}{1+\ \exp(-Q(t))}\right]\left[1-\exp\left(\ln\left(\frac{N}{N_{max}}\right)\right)\right] \quad (1)$$

$$\frac{d(Q(t))}{dt} = \mu \quad (2)$$

$$\ln(N(t=0)) = \ \ln(N_0) \quad (3)$$

The secondary models used in this study to describe the influence of temperature on the primary growth model parameters were obtained through isothermal *L. viridescens* growth data in MRS medium. The square root model (Ratkowsky et al., 1982), shown in Eq. (4), was used to describe the influence of temperature on the maximum specific growth rate (μ). The natural logarithm of the maximum population (y_{max}) was described by an arithmetic average of the values obtained isothermally in MRS (Eq. (5)) (standard deviation = 1.0). The h_0 is the initial physiological state of the cells [dimensionless] and it was set equal to zero, since there was no lag phase in culture medium. In this equation, T is the temperature [°C], T_{min} [°C] is the theoretical

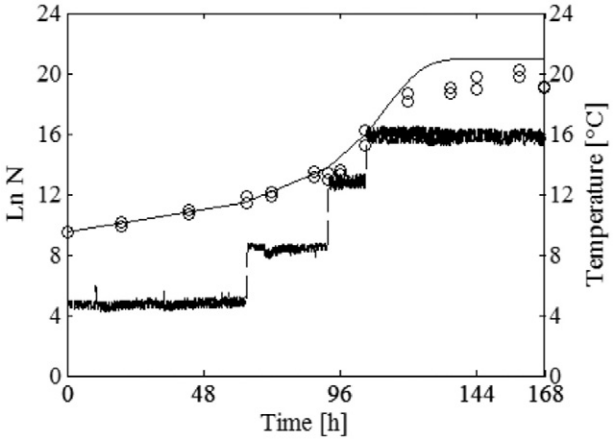

Fig. 1. *L. viridescens* growth in ham under non-isothermal condition NI-1 (symbols), prediction by Baranyi and Roberts model (continuous line) and experimental temperature (dashed line).

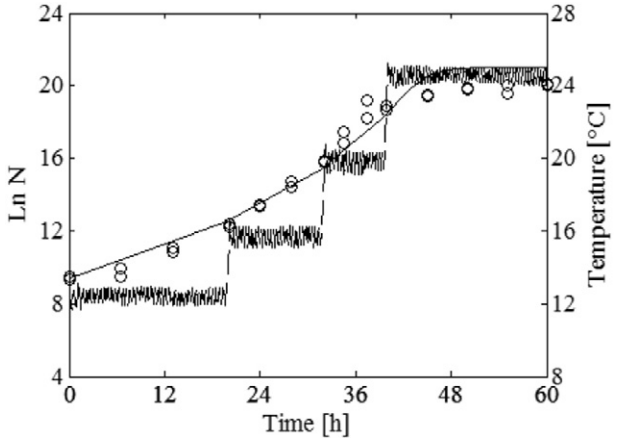

Fig. 2. *L. viridescens* growth in ham under non-isothermal condition NI-2 (symbols), prediction by Baranyi and Roberts model (continuous line) and experimental temperature (dashed line).

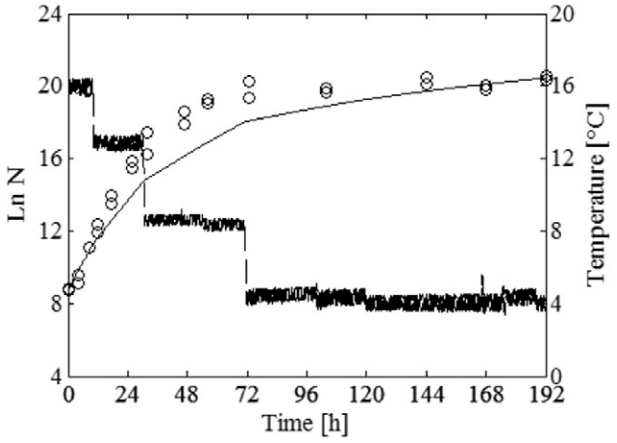

Fig. 4. *L. viridescens* growth in ham under non-isothermal condition NI-4 (symbols), prediction by Baranyi and Roberts model (continuous line) and experimental temperature (dashed line).

temperature for the minimal microbial growth, and b [1/(h$^{0.5}$ °C)] is an empirical parameter. The parameters of the primary and secondary models were obtained by Camargo (2015), in which b was equal to 0.029 and T_{min} was equal to −1.3, with R^2 equal to 0.993.

$$\sqrt{\mu} = b(T - T_{min}) \tag{4}$$

$$y_{max} = 21.0 \tag{5}$$

The resolution of the differential equations were carried out using the Matlab software (version 7, MathWorks, Natick, USA) using the Runge-Kutta method (ode23 function). The temperature profile used in the predictions was acquired by data recorded in data logger.

2.7. Statistical analysis

For comparing the statistical indices, the responses obtained experimentally and the responses predicted by the models were transformed into natural logarithmic base. Then, the comparison between the values was carried out by using RMSE, and bias and accuracy factors (Ross, 1996), shown in Eqs. (6), (7), and (8), respectively, in which $value_{pred}$ are the values predicted by the model, $value_{exp}$ are the experimental growth data and n is the number of experimental data.

$$RMSE = \sqrt{\frac{\sum (value_{exp} - value_{pred})^2}{n}} \tag{6}$$

$$bias\ \ factor = 10^{\left(\sum \frac{\log\left(value_{pred}/value_{exp}\right)}{n}\right)} \tag{7}$$

$$accuracy\ \ factor = 10^{\left(\sum \frac{\left|\log\left(value_{pred}/value_{exp}\right)\right|}{n}\right)} \tag{8}$$

3. Results and discussion

Curves of the observed values and the predicted curve by the mathematical models for *L. viridescens* growth in ham under non-isothermal conditions are shown in Figs. 1 to 6.

The values of RMSE, bias and accuracy factors from comparing predicted and experimental, for the five different non-isothermal conditions and one isothermal condition, are shown in Table 2. The bias factor ranged from 0.95 to 1.035 and the accuracy factor was less than 1.07 indicating that the predictions had a small deviation when compared to the observed values.

In increasing temperature profiles (NI-1 and NI-2), the bias factor is greater than one, characterizing safe predictions. The predictions are in

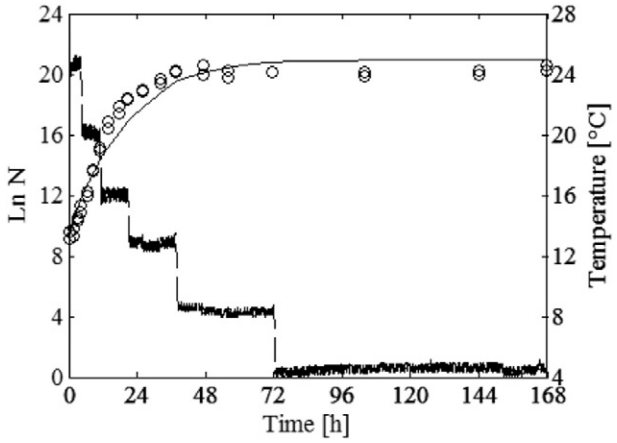

Fig. 3. *L. viridescens* growth in ham under non-isothermal condition NI-3 (symbols), prediction by Baranyi and Roberts model (continuous line) and experimental temperature (dashed line).

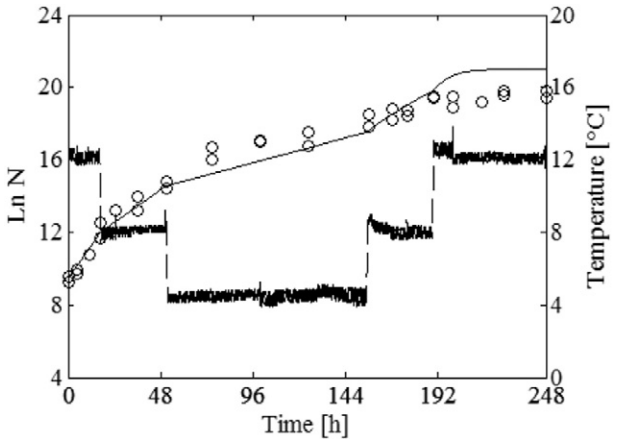

Fig. 5. *L. viridescens* growth in ham under non-isothermal condition NI-5 (symbols), prediction by Baranyi and Roberts model (continuous line) and experimental temperature (dashed line).

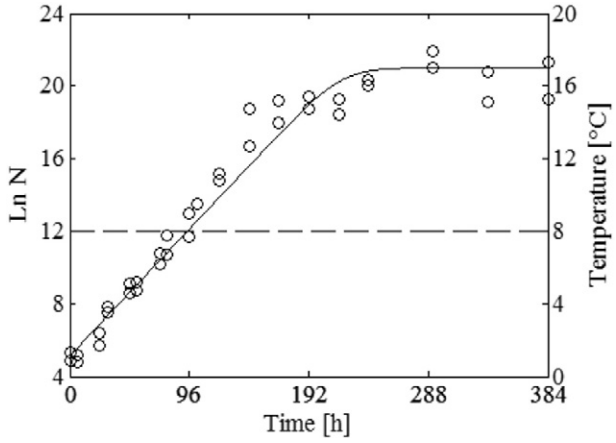

Fig. 6. *L. viridescens* growth in ham under isothermal condition I-8 (symbols), prediction by Baranyi and Roberts model (continuous line) and experimental temperature (dashed line).

agreement with the observed bacterial concentrations until 100 h at NI-1 (Fig. 1) and until 40 h at NI-2 (Fig. 2), and there is an overestimation of the stationary phase in both temperature conditions.

In decreasing temperature profiles (NI-3, NI-4, and NI-5 (from 0 to 156 h)), the model underestimated the experimental growth (bias factor less than one), featuring a dangerous prediction, and there is no significant overestimation of the stationary phase. These safe and dangerous predictions could had occurred because it is necessary a certain time for the heat transfer from the incubator atmosphere to the inoculated ham samples, retarding microbial response to that temperature shift; and/or the BAL needed to adapt themselves (metabolic changes) to the new environmental condition (intermediate lag phases) (Longhi et al., 2013, Swinnen et al., 2005). In addition, bacterial growth in the food matrix, instead of the MRS medium used to obtain the model, may affect the microbial growth. Ham is more complex than the culture medium (Antwi et al., 2007) and this can be another factor resulting in the deviations between the experimental growth data in ham and the predictions.

For the isothermal condition I-8 [8 °C], it was observed similar behavior to that observed in decreasing non-isothermal conditions, since the statistical indexes for this condition were of the same order of magnitude and the bias factor was slightly less than one. It shows that this modeling approach can be applied to any temperature conditions within the range studied (between 4 °C and 25 °C), whether it is decreasing, increasing or constant.

It is important to highlight that most deviations were observed near the stationary phase, which was maintained as a constant value for any case. Many authors (Augustin and Carlier, 2000, Baranyi and Roberts, 1994, Dalgaard and Koutsoumanis, 2001) consider that lag and exponential are the microbial growth phases of greatest interest because, in most foods, the spoilage occurs before the stationary phase, as happened in all conditions assessed in this study. For ham and meat products in general, the point for the spoilage caused by lactic acid bacteria is defined as 10^7 CFU/g (Slongo et al., 2009).

Table 2
Results of the statistical indices obtained in the assessment of the model predictive ability for each different temperature profile.

Condition	Bias factor	Accuracy factor	RMSE
NI-1	1.03	1.04	0.92
NI-2	1.01	1.04	0.76
NI-3	0.99	1.05	0.90
NI-4	0.95	1.07	1.33
NI-5	1.00	1.04	0.84
I-8	0.99	1.06	0.99

It is possible to observe a discreet lag phase in experimental data at NI-2 non-isothermal condition that was neglected by the model description, once it has been developed in culture medium, in which no lag phase was observed.

According to Longhi et al. (2013), growth predictions of *Lactobacillus plantarum* in culture medium tend to be better when the experimental temperatures are close to the optimum growth temperature. For growth of *L. viridescens* in ham, the optimum growth temperature is 30 °C and the best prediction, according to this author, would be for NI-3, which was not observed. Such behavior suggests that this microorganism has greater development in chilling temperatures than near optimal temperature, characterizing it as a spoilage agent of chilled meat products, as reported by Borch et al. (1996).

The ability of secondary models to represent the dependence of the parameters with the temperature and the quality of the fitting of primary models to experimental data influences the prediction of microbial growth in non-isothermal conditions. Then, it is possible to say that the performance of a non-isothermal model depends on the performance of the primary and secondary models (Masana, 1999, Corradini and Peleg, 2005). In this study, even with primary and secondary models developed in culture medium, it was possible to predict the lactic acid bacteria growth in the food of interest (ham) with small deviations, what makes this modeling viable for industrial application in order to assess the product shelf life.

4. Conclusion

The model proposed, using parameters estimated from isothermal growth curves in culture medium, is suitable to describe *L. viridescens* growth under dynamic conditions in refrigerated vacuum packed meat products and characterize its shelf life due to spoilage. However, as the model has been developed in culture medium, the ham composition and/or structure could induce to deviations in the predictions, more remarkably for decreasing temperature conditions where the fail was dangerous. Therefore, it is extremely important to evaluate predictions in all possible conditions in the real scenario. Predictions from primary and secondary models are a useful tool to predict the behavior of bacteria of interest in real conditions of the refrigeration chain.

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