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

Nathália Buss da Silva [a], Daniel Angelo Longhi [b], Wiaslan Figueiredo Martins [a], João Borges Laurindo [a], Gláucia Maria Falcão de Aragão [a], Bruno Augusto Mattar Carciofi [a,*]

[a] *Department of Chemical and Food Engineering, Federal University of Santa Catarina, Florianópolis, SC, Brazil*
[b] *Food Engineering, Federal University of Paraná, Jandaia do Sul, PR, Brazil*

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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.

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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

* Corresponding author at: Laboratório de Propriedades Físicas de Alimentos, Departamento de Engenharia Química e Engenharia de Alimentos, Campus Universitário, Trindade, Florianópolis, SC 88040-900, Brazil.
E-mail addresses: nathaliabuss@gmail.com (N.B. Silva), ealdaniel@ufpr.br (D.A. Longhi), wiaslanmartins@gmail.com (W.F. Martins), jb.laurindo@ufsc.br (J.B. Laurindo), glaucia.aragao@ufsc.br (G.M.F. Aragão), bruno.carciofi@ufsc.br (B.A.M. Carciofi).

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
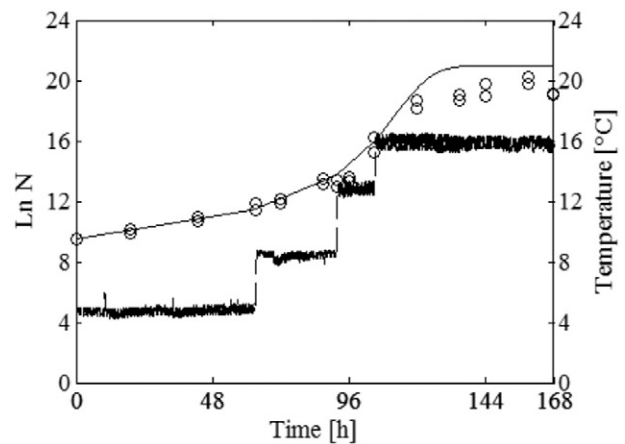

Fig. 1. L. *viridescens* growth in ham under non-isothermal condition NI-1 (symbols), prediction by Baranyi and Roberts model (continuous line) and experimental temperature (dashed line).