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Mathematical modeling of *Lactobacillus viridescens* growth in vacuum packed sliced ham under non isothermal conditions

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Abstract

Lactic acid bacteria (LAB) are responsible for the spoilage of vacuum packed meat products, as ham. Temperature is the main factor affecting the microbial dynamics and its variation during the production, distribution and storage of foods is considerable. Thus, the use of mathematical models to describe the microbial behavior under variable temperatures can be very useful in predicting the food shelf life. This study evaluated the growth of *Lactobacillus viridescens* in sliced ham under non isothermal conditions, and assessed the predictive ability of the Baranyi and Roberts model using parameters obtained isothermally in culture medium (MRS). To obtain the BAL growth, the fresh ham piece was sterilized, sliced, inoculated with bacteria and stored in a temperature-controlled incubator. For the establishment of the secondary models, the primary model parameters were obtained isothermally in the culture medium at 4, 8, 12, 16, 20 and 30 ° C, in which there was no lag phase observed; the square root model was selected to describe the dependence of the μ_{max} parameter (maximum specific growth rate) with the temperature, and the y_{max} parameter (maximum population) was represented by an average because there was no significant influence of the temperature. The mathematical models were validated with *L. viridescens* growth data in ham under five variable temperature conditions (NI-1 (4-8-12-16 °C), NI-2 (12-16-20-25 °C), NI-3 (25-20-16-12-8-4 °C), NI-4 (16-12-8-4 °C) and NI-5 (12-8-4-8-12 °C)), and its predictive ability were assessed through statistical indexes (bias factor, accuracy factor and RMSE), with good results (bias factor between 0.9450 and 1.0326; accuracy factor between 1.0382 and 1.0682, and RMSE between 0.7641 and 1.3317), especially in increasing temperature, where the prediction was safe. The validated model can be used to estimate the shelf life of a commercial ham under different temperature conditions.

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Keywords: lactic acid bacteria; dynamic modeling; meat products

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1. Introduction

The color of cured meats is one of the most important factors affecting consumer acceptability. Green discoloration in cured meats is a periodic problem for the meat industry and is usually caused by particular microorganisms which are able to produce oxidizing substances that act on the cured meat pigments[1]. *Lactobacillus viridescens* has been described as the organism frequently responsible for microbial greening in cured sausage and ham products [2,3,4].

The temperature has great influence on the kinetics of microbial growth, especially for chilled foods, when it usually varies greatly during transport, retail and at home[5]. Due to this, the mathematical modeling of the microbial growth is directed to obtaining models describing non-isothermal environment and make it possible to predict the shelf -life of foods in real conditions[6,7].

The most common method for validating models using new data is to carry out experiments directly on the food product of concern[8]. The objective of this study was to assess the predictive ability of a mathematical model to describe the growth of *L. viridescens* under non isothermal conditions, in order to simulate a real environment this products are subjected, using parameters obtained in culture medium at constant temperatures.

2. Materials and methods

2.1. Microorganism, inoculum, sample preparation, growth conditions and sampling

The *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 (Campinas, Brazil). The strains were rehydrated, grown in Man, Rogosa and Sharpe (MRS) - *Lactobacillus* medium (Acumedia Manufactures, Michigan, USA), and stored in Eppendorf tubes with MRS containing 20 % glycerol at −24 ºC until its use.

The reactivation of the culture for preparing the inoculum was carried out in MRS at 30 ºC for 18 h, when the concentration of 10^9 CFU/g is obtained. Then, successive dilutions were performed in test tubes containing MRS until the concentration of 6 x 10^4 CFU/g.

In order to eliminate the natural bacterial flora, the 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 inoculum and packed into a sterile mixer bag and then in a vacuum plastic bag. The samples were stored in a temperature-controlled incubator (Dist, Florianópolis, Brasil).

The growth of *L. viridescens* in vacuum-packed sliced ham was evaluated in five non isothermal conditions. The programmed temperature profiles are shown in Table 1. The temperature of the incubator was recorded in data logger (Testo174, Lenzkirch, Germany) in every five minutes. In pre-determined time intervals, two samples (duplicate) were taken to determine the *L. viridescens* cells concentration in ham by plate count method. The results were expressed as log (*N*), where *N* is the LAB concentration [CFU/g] at time *t*[h].

Table 1 – Non isothermal temperature profiles designed to assess the growth of *L. viridescens* in ham with the plateaus of temperature (T, in ºC) 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]	

2.3 Mathematical modeling and statistical analysis

The predictions of the microbial growth under non-isothermal conditions were carried out using the Baranyi and Roberts[9] model in a differential form, Eq. (1) and (2), with the initial conditions $\ln(N(0)) = \ln(N_0)$ and $\ln(Q(0)) =$