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Optimal experimental design to model spoilage bacteria growth in vacuum-packaged ham

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ABSTRACT

Proper mathematical models with trustworthy estimated parameters are essential to accurately predict the shelf life of foodstuffs. Traditionally, many isothermal experiments are performed to estimate these parameters and their temperature dependence. Alternatively, Optimal Experimental Design (OED) of non-isothermal experiments reduces the experimental workload and costs. The objective of this study was to apply OED to estimate the growth parameters of *Weissella viridescens* in vacuum-packaged ham under non-isothermal conditions. Results of non-isothermal experiments (temperature ranging from 4 to 25 °C) were used for parameters estimation and independent data for model validation. Baranyi and Roberts primary model and the Square Root secondary model fitted well to the data, as verified by R^2, greater than 0.986, RMSE, lower than 0.470, and by narrow confidence interval of the estimated parameters. In the model validation, a good agreement between predicted values and experimental data was observed (RMSE = 0.344, bias = 0.997 and accuracy = 1.017), indicating that the growth parameters of *W. viridescens* in vacuum-packaged ham were successfully estimated with OED.

1. Introduction

Microbial growth is the most common cause of food spoilage, which can be noticeable by visible growth (slime, colonies), textural changes (degradation of polymers) or undesirable odors and flavors (Gram et al., 2002). The temperature of chilled foods, unlike the pH and water activity, may vary extensively throughout the complete production and distribution chain (Van Impe et al., 1992), in which the increase of the refrigeration temperature tends to reduce the shelf life of non-shelf stable meat products (McDonald and Sun, 1999). *Weissella viridescens* has been identified as one of the main specific spoilage organisms of meat products which cause green discoloration (Borch et al., 1996; Nychas et al., 2008; Dušková et al., 2013; Dalcanton et al., 2013). *W. viridescens* is a ubiquitous organism in meat plants, which some strains have high heat resistance (D-value of 71 min in ham slices at 65 °C) (Milbourne, 1983) and can survive to high pressure processing at 600 MPa for 10 min at 22 °C (Han et al., 2011).

The traditional approach for estimating the microbial growth parameters in foods is the sequential two steps modelling (TSM): a primary model is fitted to the microbial growth data (measurements of temporal response of the microorganism to a single set of conditions) and, then, a secondary model is fitted to the primary model parameters (establish the dependence of model parameter to changes in environmental conditions) (Whiting and Buchanan, 1993). The estimation of model parameters with satisfactory accuracy from the TSM approach needs a lot of experimental data and long time, resulting in high workload and costs. Despites these disadvantages, the TSM approach has been the most reported in the literature (e.g. Juneja et al., 2007; Gospavic et al., 2008; Zhou et al., 2008; Dalcanton et al., 2013; Longhi et al., 2014).

The Optimal Experimental Design (OED) approach has been used successfully to estimate the microbial growth parameters, specially of pathogenic bacteria, with great accuracy and less workload and costs in comparison with TSM approach (Bernaerts et al., 2002; Grijspeerdt and De Reu, 2005; Van Derlinden et al., 2013; Longhi et al., 2017). In OED approach, optimal changes

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between different levels of a factor (e.g. temperature) are designed taking into account the model sensitivity to the parameters variations, to the experimental noise and to the constraints of the system (Versyck et al., 1999; Franceschini and Macchietto, 2008). Then, the parameters are estimated with the simultaneous fitting of the primary and secondary models to the experimental data. Even OED tool presenting advantages, it has not been widely used in predictive microbiology, probably due to the complexity of the calculations in the optimization step. There are few studies in the literature for the estimation of growth parameters of spoilage microorganisms in foods with OED approach (García et al., 2015; Vilas et al., 2017). Thus, the objective of this study was to use OED approach to estimate and validate the growth parameters of *W. viridescens* in vacuum-packaged ham slices under optimal predetermined non-isothermal storage conditions.

2. Material and methods

2.1. Microorganism

Lyophilized *W. viridescens* strain (CCT 5843 ATCC 12706, Lot 22.07) was purchased from André Tosello Foundation of Tropical Cultures (Campinas, Brazil). The strains were rehydrated, grown into Man, Rogosa and Sharpe (MRS) broth (Acumedia Manufactures, Michigan, USA), and stored at −24 °C in Eppendorf tubes containing MRS:glycerol medium (4:1 in volume).

2.2. Experimental procedures

Samples of two slices of ham (1.5 mm thick each, approximate total mass of 20 g) were cut aseptically with a slicer (Metvisa, model CFIE 250, Brusque, Brazil) from fresh pieces of ham (Seara, Itajaí, Brazil). All the samples used in the present study were from the same lot, acquired in a local market (Florianópolis, Brazil). The strains of *W. viridescens* were reactivated in MRS medium at 30 °C for 18 h, and 1 mL of inoculum (6.10^4 CFU g^{-1}, approximately) was spread between the two ham slices. The samples were vacuum packaged in sterile bags and placed inside an incubator with temperature control (Dist, Florianópolis, Brazil) subjected to five different time-temperature profiles. The temperature around the samples was recorded by data loggers (Testo 174, Lenzkirch, Germany) every 5 min. Measurements of pH (TESTO, model 205, Sparta, USA), water activity (Aqualab, model SERIES 3TE, Pullman, USA) and sodium chloride (method proposed by Aliño et al., 2001) were also carried out to verify the physicochemical composition. The microbial growth was determined in duplicate by plate count method (concentration expressed in CFU g^{-1}) until the stationary growth phase.

2.3. Growth modelling

The Baranyi and Roberts primary model (Baranyi and Roberts, 1994), given by Equations (1) and (2), was used to describe the growth of *W. viridescens* in ham over time. The Square Root secondary model (Ratkowsky et al., 1982), given by Equation (3), was used to describe the dependence of μ_{max} parameter with the temperature. In Equations (1)–(3), y [ln CFU g^{-1}] is the natural logarithm of the cell concentration N [CFU g^{-1}] over time t [h], Q [dimensionless] is related to the physiological state of the cells over time t, μ_{max} [h^{-1}] is the maximum specific growth rate, y_{max} [ln CFU g^{-1}] is the natural logarithm of the maximum cell concentration, T [°C] is the temperature, T_{min} [°C] is the theoretical temperature for minimal microbial growth and b [$h^{-0.5}$ °C^{-1}] is an empirical parameter. The initial conditions to solve Equations (1) and (2) are $y(0) = y_0$ and $Q(0) = Q_0$, respectively, in which y_0 [ln CFU g^{-1}] is the value of the natural logarithm of initial cell concentration and Q_0 [dimensionless] is the value related to the initial physiological state of cells. Equation (4) gives the mathematical relationship between the parameters Q_0 and h_0.

$$\frac{dy}{dt} = \mu_{max}\left[\frac{1}{1+\exp(-Q)}\right][1-\exp(y-y_{max})] \quad (1)$$

$$\frac{dQ}{dt} = \mu_{max} \quad (2)$$

$$\sqrt{\mu_{max}} = b(T - T_{min}) \quad (3)$$

$$Q_0 = \ln\left[\frac{1}{\exp(h_0)-1}\right] \quad (4)$$

2.4. Optimal non-isothermal experiments

Experimental data of the growth of *W. viridescens* in ham were obtained for five different optimal non-isothermal conditions, from which two conditions assessed the microbial growth with increasing temperatures (IT), two assessed the microbial growth with decreasing temperatures (DT), and one assessed the microbial growth with decreasing and increasing temperatures (DIT). Each temperature profile was designed shifting the temperature between the following predetermined plateaus: 4, 8, 12, and 16 °C (profile $IT_{4\text{-}8\text{-}12\text{-}16}$), 12, 16, 20, and 25 °C (profile $IT_{12\text{-}16\text{-}20\text{-}25}$), 16, 12, 8, and 4 °C (profile $DT_{16\text{-}12\text{-}8\text{-}4}$), 25, 20, 16, 12, 8, and 4 °C (profile $DT_{25\text{-}20\text{-}16\text{-}12\text{-}8\text{-}4}$), and 12, 8, 4, 8, and 12 °C (profile $DIT_{12\text{-}8\text{-}4\text{-}8\text{-}12}$). The temperature profiles were designed to extend the temperature range in each experiment (at least 8 °C) and, together with other temperature profile (two IT or two DT profiles), lead to further extension of the temperature range (to 21 °C). The temperature plateaus were chosen based in preliminary tests, previous studies in culture medium performed in our laboratory (Longhi et al., 2017; Silva et al., 2017) and the literature (Bernaerts et al., 2002; Grijspeerdt and De Reu, 2005; Balsa-Canto et al., 2008). The following restrictions were taken into account: (i) each temperature profile was composed by, at least, four temperature plateaus, because the use of multiple temperature steps would improve the information content of the experimental data (Bernaerts et al., 2002); (ii) the maximum difference between temperature plateaus was 5 °C, aiming to avoid intermediate lag phases (Grijspeerdt and De Reu, 2005); (iii) one experimental data was collected at each optimal time to shift between temperature plateaus ($t_{shift(n)}$) and, at least, three experimental data (about equidistant in the time interval) at each temperature plateau, aiming the improvement of confidence intervals of estimated parameters.

Each optimal time to shift between temperature plateaus ($t_{shift(n)}$) and each final experimental time (t_f) were obtained by minimizing the E-modified criterion (ratio of the largest to the smallest eigenvalue) of the Fisher Information Matrix (*FIM*), given by Equation (5), in which $\left(\frac{\partial y}{\partial p}\right)$ is the sensitivity matrix (i,j) of the model response (y_i) to the model parameters (p_j) variation, $\left(\frac{\partial y}{\partial p}\right)^T$ is the transpose of $\left(\frac{\partial y}{\partial p}\right)$, and W is a weighting matrix. The E-modified criterion is not sensitive to the number and location of sampling times and the increase of experimental data would improve the size and shape of the confidence hyperellipsoids (Balsa-Canto et al., 2008).