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Predictive modeling of *Pseudomonas fluorescens* growth under different temperature and pH values

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ABSTRACT

Meat is one of the most perishable foods owing to its nutrient availability, high water activity, and pH around 5.6. These properties are highly conducive for microbial growth. Fresh meat, when exposed to oxygen, is subjected to the action of aerobic psychrotrophic, proteolytic, and lipolytic spoilage microorganisms, such as *Pseudomonas* spp. The spoilage results in the appearance of slime and off-flavor in food. In order to predict the growth of *Pseudomonas fluorescens* in fresh meat at different pH values, stored under refrigeration, and temperature abuse, microbial mathematical modeling was applied. The primary Baranyi and Roberts and the modified Gompertz models were fitted to the experimental data to obtain the growth parameters. The Ratkowsky extended model was used to determine the effect of pH and temperature on the growth parameter μ_{max}. The program DMFit 3.0 was used for model adjustment and fitting. The experimental data showed good fit for both the models tested, and the primary and secondary models based on the Baranyi and Roberts models showed better validation. Thus, these models can be applied to predict the growth of *P. fluorescens* under the conditions tested.

Introduction

Fresh meat is a highly perishable product owing to its high nutritious content. Many interrelated factors influence the shelf life and freshness of meat such as storage temperature, atmospheric oxygen (O_2), endogenous enzymes, moisture, light and most importantly, micro-organisms.[1] Therefore, meat is a complex niche with particular physicochemical characteristics that allow colonization and development of a wide variety of organisms,[2] making it highly susceptible to be contaminated by spoilage bacteria.[3] Microbial spoilage can lead to food disposal and consequent economic losses to the industry and a decrease in consumer trust.[4]

Various conservation methods are available to increase the shelf life of fresh meat including refrigeration.[5] The use of cold temperatures, such as refrigeration and freezing, is considered the most effective method of retarding or inhibiting microbial growth in meat products during transportation or storage for maintaining product quality and extending shelf life.[6] The use

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of refrigeration to preserve food is based on the reduced rate of metabolism in microorganisms at low temperatures.[7] The rate of enzyme-catalyzed reactions is temperature dependent, and considerably slows down at low temperatures. Therefore, it is extremely important to control and maintain the refrigeration temperature within the acceptable limits to ensure the security and integrity, and to extend the shelf life of meat products.[8]

However, even in cold stored meat, the organoleptic spoilage by microbial consumption of meat nutrients, such as sugars and free amino acids, and the release of undesired volatile metabolites cannot be eliminated completely. These activities may be performed at low temperatures by psychrotrophic bacteria compromising preserving effect of low temperature.[5] These microorganism can thrive well at low temperatures via modifying their cytoplasmic membrane and increasing the unsaturated fatty acids levels, which keep the membrane in semifluid state, thereby facilitating the transport of nutrients and enzymes.[9] Their ability to grow at low temperatures is one of the challenges to the meat industry while considering the meat quality and public health.[10]

Pseudomonas spp. stand out among the psychotropic microorganisms, particularly, those involved in the spoilage of meat stored at low temperatures.[11] They are Gram-negative, aerobic, proteolytic and lipolytic microorganisms mostly associated with the deterioration of fresh meat. The most commonly found pseudomonas in meat is *Pseudomonas fluorescens*, which is characterized by producing a soluble pigment called pyoverdine.

Predictive microbiology can be used to assess the quality of food susceptible to deterioration by these spoiling microorganisms considering the fact that the behavior of the microbial population and its effect on environment can be predicted, as its status is known at any given time.[12] Thus, predictive models can be used to predict product quality and microbiological safety considering the product characteristics and processing and storage conditions.[13]

This study aimed to construct and validate the mathematical models for predicting *P. fluorescens* growth at different temperatures and pH conditions.

Material and methods

Inoculum standardization and maintenance

Freeze-dried *P. fluorescens* strain ATCC 13525 was stored at −18 °C in a freezing medium containing 15 mL glycerol, 0.5 g bacteriological peptone, 0.3 g yeast extract, 0.5 g NaCl, and 100 mL distilled water. Prior to use, the strain was activated in brain heart infusion (BHI) broth (HiMedia, Mumbai, India) and incubated at 28 °C for 24 h to obtain the number of cells necessary for standardization.

Effect of storage temperature and pH of the medium on the growth of P. fluorescens in meat broth

Aliquots of the standardized inoculum were transferred to 100 mL meat broth (10 g meat extract, 10 g meat peptone, 5 g tryptone, and 5 g glucose in 1 L water) at a final concentration of 10^4 CFU mL^{-1}, and incubated at 4 °C, 7 °C, and 12 °C.

The pH of the medium was adjusted to 5.5, 6.0, and 6.3 with 2 M NaOH or 2 M HCl using a digital pH meter (Digimed DM20, Campo Grande, Brazil).

The growth of *P. fluorescens* at each pH and temperature condition (4 °C and pH 5.5; 4 °C and pH 6.0; 4 °C and pH 6.3; 7 °C and pH 5.5; 7 °C and pH 6.0; 7 °C and pH 6.3; 12 °C and pH 5.0; 12 °C and pH 6.0; and 12 °C and pH 6.3) was monitored at 3, 6, 9, 12, 24, 30, 36, 48, 54, 60, 72, 84, 96, 108, and 120 h of incubation. At each interval, an aliquot of 1 mL was transferred to a tube containing 9 mL of 0.1% (w/v) peptone water, and was serially diluted. Subsequently, a 0.01-mL aliquot from appropriate dilutions was taken and plated on Trypticase Soy Agar (TSA) plates (HiMedia, Mumbai, India) by microdrop technique. The plates were incubated at 28 °C for 24 h, and the colonies were counted.

Analysis of growth data for obtaining models

The model for *P. fluorescens* growth in meat broth was developed using the two-stage methodology. In the first stage, the maximum specific growth rate (μ_{max}), and the lag phase (λ) were calculated for each experimental combination. The growth parameters were obtained by the modified Gompertz and Baranyi and Roberts equation to the experimental data by using DMFit 3.0 program. In the second stage, the estimates obtained for μ_{max} were adjusted for the extended Ratkowsky model to determine the effect of temperature and pH on the maximum specific growth rate of *P. fluorescens*, according to the following equation:

$$\mu_{max} = a(\mathrm{pH} - \mathrm{pH}_{mim})(T - T_{mim})^2 \quad (1)$$

where a is the regression constant, and pH_{mim}, T_{mim} are the minimum pH and minimum temperature, respectively, estimated theoretically for the microbial growth.

Validation of the results by statistical analysis of models

The following statistical parameters were calculated for the validation of the models: correlation coefficient (R^2), mean square error (RMSE), bias factor, and accuracy factor.[14] The correlation coefficient (R^2) describes the model fit throughout the length of the curve; the closer the value of R^2 is to one, the better the model fit is.

The mean square error (RMSE) is given by Eq. (2), and presents the model error relative to the data, that is, how close are the predicted values to the observed values; where the closer to zero indicates a better fit.

$$\mathrm{RMSE} = \frac{\mathrm{SQR}}{n} = \Sigma(\mathrm{valueobs} - \mathrm{valuepred})^2 \quad (2)$$

where valueobs is the experimental value, valuepred is the value predicted by the model, SQR is the sum of square residuals, and n is the number of degrees of freedom (number of data points – number of model parameters).

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