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Mathematical modelling of temperature effect on growth kinetics of *Pseudomonas* spp. on sliced mushroom (*Agaricus bisporus*)

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ABSTRACT

The growth data of *Pseudomonas* spp. on sliced mushrooms (*Agaricus bisporus*) stored between 4 and 28 °C were obtained and fitted to three different primary models, known as the modified Gompertz, logistic and Baranyi models. The goodness of fit of these models was compared by considering the mean squared error (MSE) and the coefficient of determination for nonlinear regression (pseudo-R^2). The Baranyi model yielded the lowest MSE and highest pseudo-R^2 values. Therefore, the Baranyi model was selected as the best primary model. Maximum specific growth rate (r_{max}) and lag phase duration (λ) obtained from the Baranyi model were fitted to secondary models namely, the Ratkowsky and Arrhenius models. High pseudo-R^2 and low MSE values indicated that the Arrhenius model has a high goodness of fit to determine the effect of temperature on r_{max}. Observed number of *Pseudomonas* spp. on sliced mushrooms from independent experiments was compared with the predicted number of *Pseudomonas* spp. with the models used by considering the B_f and A_f values. The B_f and A_f values were found to be 0.974 and 1.036, respectively. The correlation between the observed and predicted number of *Pseudomonas* spp. was high. Mushroom spoilage was simulated as a function of temperature with the models used. The models used for *Pseudomonas* spp. growth can provide a fast and cost-effective alternative to traditional microbiological techniques to determine the effect of storage temperature on product shelf-life. The models can be used to evaluate the growth behaviour of *Pseudomonas* spp. on sliced mushroom, set limits for the quantitative detection of the microbial spoilage and assess product shelf-life.

1. Introduction

Mushrooms have been consumed as a source of food and medicine for centuries, because of their high amounts of proteins, minerals and bioactive compounds (Wani et al., 2010). The cultivated button mushroom (*Agaricus bisporus*) is the most common edible mushroom in the world. *Agaricus bisporus* has a very short shelf-life because it has no cuticle to protect it from physical deterioration or microbial attack (Brennan et al., 2000). Although no outbreak of pathogenic microorganisms such as *E. coli* O157:H7 and *L. monocytogenes* has been reported (Guan et al., 2012) for *Agaricus bisporus*, it is very susceptible to contamination with *Pseudomonas* spp. which are abundant in nature (González-Fandos et al., 2006; Simón et al., 2005). The *Pseudomonas* spp. are responsible for causing spoilage, and the initial count of *Pseudomonas* spp. on cultivated mushrooms is quite high, ranging from 6.9 to 8.1 $\log_{10}$ CFU/g (Simón et al., 2005; Venturini et al., 2011).

Predictive food microbiology aims to estimate the microbial growth using mathematical models under different conditions. These mathematical models are generally classified into three main categories known as primary, secondary and tertiary models (Whiting, 1995). Primary models describe the growth data as a function of time under a constant environmental condition. Sigmoidal type models such as the modified Gompertz, logistic and Baranyi models are widely used as primary models for fitting microbial growth data. Secondary models describe the effects of environmental factors, such as temperature, pH and water activity (a_w) on the parameters of the primary models, including maximum specific growth rate and lag phase duration. One of the most important environmental factors from the food safety point of view is temperature. The most widely used secondary model to determine the relationship between temperature and maximum specific growth rate is known as the Ratkowsky or square root model (Ratkowsky et al., 1982). Tertiary models combine both the primary and secondary models with user-friendly application software or expert systems to assess microbial behaviour under specific conditions (Wang et al., 2013; Whiting, 1995).

Predictive models are considered as important tools to assess product shelf-life and food safety, to perform hazard analysis and set critical control points, and to develop risk assessment plans. Predictive

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models are a very quick, efficient and cost effective way of assessing the potential for growth or predicting the growth of microorganisms under specific conditions without needing long, expensive and time consuming analysis techniques and specialized people. Traditional microbial enumeration techniques give information only for conditions that the analysis was conducted, and they are not practical to determine the growth of microorganisms under variable environmental conditions. Predictive models, on the other hand, are useful in predicting the growth of microorganisms in foods during processing and storage in real time situations (Bovill et al., 2001). Predictive models were used to describe the growth behaviour of spoilage microorganisms in which the time to reach a specified target level under variable temperature conditions is the most important parameter. The growth behaviour of *Pseudomonas* spp. being one of the most abundant microorganisms widely isolated from various foods has been studied and modelled using some predictive models. Koutsoumanis (2001) used the logistic model to describe the growth behaviour of *Pseudomonas* spp. in fish stored at temperatures ranging from 0 to 15 °C. Gospavic et al. (2008) used the modified Gompertz and the Baranyi models to implement a growth model for estimating the growth of *Pseudomonas* spp. in poultry under variable temperature conditions. Zhang et al. (2011) used the Baranyi model to fit the number of *Pseudomonas* spp. in beef stored between 0 and 20 °C. Bruckner et al. (2013) used the modified Gompertz model to define growth behaviour of *Pseudomonas* spp. in pork and poultry meat stored between 2 and 15 °C. Dabadé et al. (2015) used the Baranyi and the modified Gompertz models to describe the growth behaviour of *Pseudomonas* spp. in tropical fresh shrimp stored at temperatures ranging from 0 to 28 °C. Lytou et al. (2016) used the Baranyi model to describe the growth of total viable bacteria including *Pseudomonas* spp. in marinated and unmarinated chicken breast fillets stored at 4, 10 and 15 °C, and correlated these data with shelf-life. Psychrotropic bacteria, mainly *Pseudomonas* spp., have been isolated as the most ubiquitous microorganisms responsible for the spoilage of mushrooms (Simón et al., 2005). Temperature is the most important factor affecting the growth rate. The relationship between the growth rate of *Pseudomonas* spp. and the storage temperature of sliced mushrooms was not investigated. Therefore, it is important to investigate and model the microbial growth of *Pseudomonas* spp. on sliced mushrooms.

The main objective of this work was to develop primary and secondary models to describe the growth of *Pseudomonas* spp. on sliced mushrooms. First, several representative primary models such as the modified Gompertz, logistic and Baranyi models were used to describe the microbial growth data of *Pseudomonas* spp. on sliced mushrooms at different storage temperatures (4, 12, 20 and 28 °C). The goodness of fit of these three models were compared by considering the mean squared error (MSE) and the coefficient of determination for nonlinear regression (pseudo-R^2). Then, the maximum specific growth rate and lag phase duration derived from the primary model which has the best goodness of fit were correlated with the temperature using the Ratkowsky and Arrhenius models. Observed growth data from the independent experiments were used to validate the models. The bias factor (B_f) and accuracy factor (A_f) were employed to validate the models used. Finally, mushroom spoilage and mushroom shelf-life were correlated with the temperature using the models.

2. Materials and methods

2.1. Sample preparation and microbiological analysis

Cultivated button mushrooms (*Agaricus bisporus*) were obtained from MUPA Agriculture and Industry Inc. (Izmit, Kocaeli, Turkey). The mushrooms were harvested at the closed cap stage with the cap diameter of 3.5–4.5 cm. After harvesting, mushrooms were immediately shipped to the laboratory under chilled conditions. Stipes were trimmed at 1 cm, and mushrooms were sliced carefully (5 mm wide) using a presterilized stainless steel knife. The slices were placed in polystyrene trays (200 g/tray) with the dimension of 22.5 × 13.5 × 3 cm. The trays were not overwrapped with any packaging material and placed in an incubator at 4, 12, 20 and 28 °C. The temperature in the incubator did not vary more than ± 0.2 °C.

Twenty-five grams of mushrooms were aseptically weighed and homogenized using a stomacher (Interscience, Bag Mixer 400VW, USA) at high speed for 2 min with 225 ml of sterile peptone (0.1%, w/v) water (Oxoid, Basingstoke, UK) for each temperature with the corresponding sampling frequency. Dilutions (10^{-2} – 10^{-10}) were made in serial dilution tubes by taking 1 ml of sample with 9 ml of 0.1% (w/v) sterile peptone water. *Pseudomonas* spp. were determined in King's B medium (King et al., 1954), following incubation at 25 °C for 48 h. Three different trays were used at each temperature for each sampling point. Results were expressed as the average of three measurements in terms of $\log_{10}$ CFU/g. Gormley (1975) reported that the mushrooms with L^* value lower than 69 were regarded as unacceptable by the consumers. Therefore, prior to microbiological analyses, the L^* value of the sliced mushrooms was measured using a Chroma Meter (CR-400, Konica Minolta Inc., Tokyo, Japan) equipped with a D_{65} illuminant source, and microbiological analyses were carried out until the L^* value of the sliced mushrooms was lower than 69. Sampling frequency was based on the storage temperature to determine the growth behaviour of *Pseudomonas* spp. on sliced mushrooms.

2.2. Modelling

2.2.1. Primary models

The modified Gompertz, logistic and Baranyi models were commonly used in literature as primary models which are sigmoidal functions that describe growth behaviour of microorganisms as a function of time at constant environmental conditions. The modified Gompertz and logistic models are defined by Eqs. (1) and (2), respectively (Zwietering et al., 1990):

$$x(t) = x_0 + (x_{max} - x_0)\exp\left\{-\exp\left[\frac{r_{max}\,.\,e}{(x_{max} - x_0)}(\lambda - t) + 1\right]\right\} \quad (1)$$

$$x(t) = x_0 + \frac{(x_{max} - x_0)}{\left\{1 + \exp\left[\frac{4r_{max}}{(x_{max} - x_0)}(\lambda - t) + 2\right]\right\}} \quad (2)$$

where t is the time (h), x(t) is the number of microorganisms ($\log_{10}$ CFU/g) at time t, x_0 is the initial number of microorganisms ($\log_{10}$ CFU/g), x_{max} is the maximum number of microorganisms ($\log_{10}$ CFU/g), r_{max} is the maximum specific growth rate ($\log_{10}$ CFU/h) and λ is the lag phase duration (h).

The Baranyi model at constant environmental conditions is given by:

$$x(t) = x_0 + r_{max}\,.\,A(t) - \frac{1}{\ln(10)}.\ln\left(1 + \frac{e^{r_{max}\,.\,\ln(10).A(t)} - 1}{10^{(x_{max} - x_0)}}\right) \quad (3)$$

where A(t) is the adjustment function described by Baranyi and Roberts (1994), t is the time (h), x(t) is the number of microorganisms ($\log_{10}$ CFU/g) at time t, x_0 is the initial number of microorganisms ($\log_{10}$ CFU/g), x_{max} is the maximum number of microorganisms ($\log_{10}$ CFU/g), r_{max} is the maximum specific growth rate ($\log_{10}$ CFU/h).

The growth data of *Pseudomonas* spp. obtained at isothermal conditions were fitted to three different primary models (the modified Gompertz, logistic and Baranyi models) and all parameters were calculated using ORIGIN 9.0 software (OriginLab Corporation, Northampton, MA, USA) which uses Levenberg-Marquardt algorithm.

2.2.2. Secondary models

Secondary models are used to describe the effects of different environmental factors such as temperature, pH, a_w, oxygen availability, additives etc. on the parameters of the primary models (Juneja et al., 2007). After the growth data were fitted to each primary model,