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Estimation of *Aspergillus flavus* growth under the influence of different formulation factors by means of kinetic, probabilistic, and survival models

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Abstract

A Box-Behnken design was conducted to determine the effect of casein concentration (0, 5, or 10%), corn oil (0, 3, or 6%), a_w (0.900, 0.945, or 0.990), pH (3.5, 5.0, or 6.5), concentration of cinnamon essential oil (CEO: 0, 200, or 400 ppm), and incubation temperature (15, 25, or 35°C) on the growth of *A. flavus* during 50 days of incubation. Potato dextrose agars were adjusted to the different levels of tested factors and poured into Petri dishes, once solidified were inoculated with mold spores and incubated at studied temperatures. Mold response was modeled using Gompertz and quadratic polynomial equations. The obtained polynomial regression model (allowed the significant ($p<0.05$) for linear, quadratic, and interaction effects for the Gompertz equation coefficients' parameters to be identified) adequately described ($R^2>0.97$) mold growth. Additionally, in order to describe growth/not-growth boundary, collected data after 50 days of incubation were classified according to the observed response as 1 (growth) or 0 (not growth), then a binary logistic regression was used to model growth interface. Mold growth probability strongly depend on casein, oil, temperature, and a_w, as well as variations of pH and CEO concentration, being lower for those systems with higher content of CEO (>180 ppm). Furthermore, survival analysis using failure time was utilized to estimate the time at which mold growth began. The time to fail was directly related to the temperature and CEO concentration; for systems formulated with more than 200 ppm of CEO, time to fail was >30 days for low protein and fat contents. The three tested approaches to describe *A. flavus* response, adequately predicted growth rate and lag time, or growth probability, or the time in which growth will occur. The use and selection of any of these approaches will depend on the intended application.

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Keywords: Aspergillus flavus; survival estimation; kinetic models; probabilistic models

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1. Introduction

Mathematical modeling tools, together with experimental data, are utilized for estimating microbial responses in order to define processing and storage conditions for processed foods. Today, predictive microbiology considers kinetic models that allow predicting microbial growth in a wide range of conditions. These models, however, are not able to predict information under conditions that result in no growth[1]. With the aim of developing predictive models that describe the growth/no-growth boundary, some probabilistic models have been evaluated as useful tools for defining the combination of factors to prevent the growth of microorganisms[2, 3]. Furthermore, time-to-fail models have been used to estimate the time at which microbial growth occurs[4]. Molds are toxicological and spoilage microorganisms that may produce mycotoxins; particularly, *Aspergillus* species have the ability to grow in a wide range of environmental conditions and foods. Mold growth in food products depends on several factors such as product composition, pH, a_w, temperature, composition of the atmosphere, presence and concentration of preservatives, and storage time. Since a_w and temperature are the most important factors for *Aspergillus* responses, several approaches take into account such factors during the estimation of spoilage (failure) time[5, 6]. However, most available models ignore factors such as food composition and structure, as well as potential microbial interactions and the presence of antifungal agents[7]. In this work, the response of *A. flavus* in a food model system under different conditions was obtained. Then, a probabilistic model that considers the combinations of studied factors (a_w, pH, fat, protein, cinnamon essential oil (CEO), and incubation temperature) was developed to predict the growth boundary for *A. flavus*. The performance of the growth/no-growth and time-to-fail models are also presented, comparing the obtained predictions with those obtained through traditional growth kinetics using the Gompertz equation.

2 Materials and Methods

2.1 Experimental design and inoculation procedure

A Box-Behnken design was used to evaluate the effect of different factors on *A. flavus* lag time and radial growth. The studied variables were incubation temperature (15, 25, 35°C), casein concentration (0, 5, 10%), corn oil concentration (0, 3, 6%), a_w (0.900, 0.945, 0.990), CEO (0, 200, 400 ppm), and pH (3.5, 5.0, 6.5). Every combination was evaluated by triplicate. For each experiment, model systems were prepared with a sucrose solution to adjust a_w, casein (Sigma Chemical Co., Steinheim, Germany), corn oil (Mazola, Monterrey, Mexico) and potato dextrose agar (3.9 g /100 g solution). The pH was adjusted with 0.1 N HCl or NaOH solutions (Merck, Darmstadt, Germany) as appropriate. In systems containing corn oil, Tween 20 at 2% (w/w) (Chemical Meyer, Tláhuac, Mex) was added as emulsifier. Systems were poured into Petri dishes and depending on the experimental design, tested CEO (Aromatic Chemicals Potosinos SA de CV, San Luis Potosi, Mex) was added. *A. flavus* (ATCC 18672), obtained from the Food Microbiology Laboratory at the University of the Américas Puebla, was grown in PDA (Becton Dickinson de Mexico SA de CV, Cuautitlan, Edo. Mex) at 25°C during 7 days. The culture surface was washed and spores were recovered to obtain a suspension of $\approx 10^6$ spores/mL. Petri dishes were inoculated with 5 µL of the spore suspension and incubated at selected temperatures in sealed containers (avoiding anoxic conditions). Mold growth was daily monitored and the diameter of the colonies was measured for 50 days.

2.2 Modeling of growth curves.

The mold growth curves were modeled by the Gompertz equation, Eq. (1), by fitting the model parameters using non-linear regression.

$$Log\left(\frac{D}{D_0}\right) = Aexp(-\exp(b-ct)) \quad \text{where} \quad \lambda = (b-1)/c \quad \text{and} \quad \mu = A(b-1)/\lambda \qquad (1)$$

where: µ is the maximum growth rate (1/h), A is the maximum growth, λ is the phase of adaptation (h), D is the colony diameter (mm) at time t (h), D_0 is the initial diameter of the colony (mm), and *a*, *b*, *c* are Gompertz's eq. parameters. In order to determine the parameters dependence on the evaluated factors (temperature, a_w, % protein, % fat, pH, CEO concentration (ppm)), a response surface design was utilized for obtaining the coefficients of a polynomial model for the significant ($p<0.05$) variables and interactions.