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Modeling *Penicillium expansum* growth response to thyme essential oil at selected water activities and pH values using surface response methodology

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Abstract

Our objective was to evaluate, using a full factorial design, the effects of selected water activities (0.990, 0.945, or 0.900), pHs (5, 4, or 3), and thyme essential concentration (TEO, 0, 25, 50, or 100 ppm) oil on *Penicillium expansum* lag time (λ) and radial growth rate (μ_m) obtained by modeling mold response using Gompertz equation, and corresponding polynomial quadratic models. Potato-dextrose agar formulated with every studied factor combination was inoculated with 10^3 spores/ml, and incubated at 25ºC up to 30 days. Mold colony diameter was periodically measured during incubation and adjusted with Gompertz equation to determine λ and μ_m. Decreasing a_w and pH, and increasing TEO concentration decreased μ_m and increased λ. At low a_w and pH, the increase in TEO concentration had a dramatic effect on *P. expansum* response since 25 ppm of TEO inhibited its growth for 30 days at 25ºC. Gompertz parameters exhibited that *P. expansum* was sensitive to the evaluated combined factors, allowing us to construct a secondary predictive growth model. TEO in combination with a_w and pH reduction effectively inhibited *P. expansum* growth.

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Keywords: Penicillium expansum; kinetic model; thyme essential oil; predictive models

1. Introduction

Antimicrobial agents are chemical additives or naturally-present compounds that delay or inactivate microbial growth in food products, and therefore slow down quality deterioration and help maintain food safety. Many researchers have concluded that the evolution of food additives should be based on a balance of risks and benefits,

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so that in the future the most beneficial will be those that fulfill several functions, depending on the food to which they are added[1]. Antimicrobials are currently produced synthetically, but these are also present naturally in various food components[2]. Thus, they can be classified as antimicrobial compounds naturally present in the food or added intentionally. Among antimicrobials approved for direct use are organic acids and their derivatives, some esters, nitrites, nitrates, and several others[3]. Thyme is used as a flavoring and as a medicinal plant; the main components of its essential oil are thymol, anethole, and borneol (in its leaves), as well as carvacrol and cineole (in the whole plant). Thyme is produced in great amounts in some states of Mexico; so far, it is primarily used to enhance the flavor of dishes and for culinary preparations. However, few investigations have been made on the effect of its essential oil to inhibit fungal growth[4]. Today's consumers associate safe foods with fresh or least-processed and are demanding foods with characteristics similar to those of their fresh counterparts: without the addition of chemical additives, including those that serve as antimicrobial agents. This has led to a search for new forms of conservation, and to study the effect that different natural products in combination with some traditional forms may have on food preservation. The use of natural products to achieve an extended shelf-life while maintaining microbiological stability and safety is a current trend in alternative in the search for antimicrobial agents[2]. Therefore, it is important to study the essential oil of thyme used as a natural antimicrobial agent to inhibit mold growth, and also evaluate the effect of changing pH and/or a_w on the antimicrobial activity of this essential oil. The antimicrobial activity of essential oils from several plants and spices has been recognized for many years. However, data on the effect of essential oils in combination with other factors on mold growth is still scarce. Additionally, there are few models to predict performance when natural preservatives are used in combination with other factors. Our objective was to evaluate, using a full factorial design, the effects of selected a_w (0.990, 0.945, or 0.900), pHs (5, 4, or 3), and concentration of TEO (0, 25, 50, or 100 ppm) on *Penicillium expansum* lag time (λ) and radial growth rate (μ_m) obtained by modeling mold response using Gompertz's equation, and corresponding polynomial quadratic models.

2 Materials and Methods

Thyme was acquired from a local market in San Francisco Tepeyecac (Puebla, Mexico). The essential oil was obtained by steam-distillation in a Clevenger-type apparatus. The oil was analyzed using a gas chromatograph (Agilent Technologies 6850N) coupled to a mass spectrometer detector (MSD, Agilent 5975C). A capillary column (Agilent, 5% phenyl methyl polysiloxane, 30 m length, 250 mm diameter and 0.25 µm thick) was used. 1 µl of oil was injected using a 10:1 split at a temperature of 300°C. The ramp column temperature started at 60°C for 2 min, increasing to 250°C at 10°C/min and was maintained for 10 min. The carrier gas was helium at a constant flow rate of 1.1 mL/min. The mass spectrometer was operated at 70 eV and maintained at 200°C[5, 6]. *P. expansum* was obtained from the Food Microbiology collection – Universidad de las Américas Puebla and maintained in PDA slants at 25°C for 10 days[7]. Spores were harvested washing the culture surface with 10 ml of sterile Tween (0.1%) aqueous solution and the spore suspension was used as inocula the same day[5, 7]. Solid model systems were prepared using a PDA base; following a full factorial design including studied factors and levels. Agar was adjusted to different a_w values with sodium chloride, the amount needed was calculated by Chirife and Favetto's equation [8]:

$$a_w = 1 - K * m$$

where: K is a constant characteristic of the solute (0.03710 for NaCl) and m is solute molality in the aqueous phase. Model systems with the adjusted a_w were sterilized at 121°C for 15 min, cooled and the pH (5.0, 4.0 or 3.0) was adjusted with citric acid (10%) previously sterilized. The amounts of acid needed to achieve the desired pH were previously determined by titration curves. TEO (0, 25, 50, or 100 ppm) was thoroughly mixed with agar and poured into Petri dishes and allowed to solidify. Each of the prepared systems was inoculated (10^6 spores/plate). Three replicates of each combination were performed. Inoculated systems were kept at 25°C in airtight plastic chambers. The diameter of the mold colony was measured with a digital caliper every 24 h starting from the day when growth became visible. Mold growth response was adjusted to Gompertz's equation[9, 10]:

$$ln\left(\frac{D_t}{D_0}\right) = Aexp\left[-exp\left[\frac{\mu_m e}{A}\right](\lambda - t) + 1\right]$$

where: D_t (cm) is the average colony diameter at time t (h), D_0 (mm) is the average initial diameter, A is the maximum growth, μ_m is the maximum growth rate (1/h), and λ is the lag phase (h). With the parameters obtained from Gompertz model, an analysis of the experimental surface-response design was carried out: