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Modeling growth for predicting the contamination level of guava nectar by *Candida pelliculosa* under different conditions of pH and storage temperature

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The combined effects of temperature (2–46°C) and pH (1.55–6.25) on the growth of *Candida pelliculosa* isolated from guava nectar produced in Cameroon were studied using a turbidity method, ie measurement of optical density at 630 nm. A quadratic polynomial model was constructed to predict the effects and interactions of these two environmental conditions on the maximal optical density obtained ($r^2 = 0.97$). The relation between optical density and population density of *C. pelliculosa* (CFU ml^{-1}) was also established using an exponential regression ($r^2 = 0.99$). According to the model, maximal growth conditions were 37°C and pH 6.25 for obtaining the maximal optical density of 1.25 corresponding to about 60×10^6 CFU ml^{-1}. A good agreement of the model was found between the predicted values and the observed values of maximal optical density. The model was validated by the experimental values of maximal optical density obtained in the growth of *C. pelliculosa* in commercial guava nectar (pH 3.15).

Keywords: *Candida pelliculosa*; pH; temperature; quadratic polynomial model; guava nectar

Introduction

The shelf-life of many foods and the growth of microorganisms depend on the effects of multiple variables such as temperature, pH and type of acidulant, water activity and character of humectant, absorption and desorption isotherms, oxygen availability, carbon dioxide levels, redox potential, nutrient content and availability, and the presence of antimicrobials [3,6,14]. In a wide range of foods and beverages with high sugar content and low pH, yeasts are often the most common spoilage microorganisms [5,13,21].

In Cameroon, guava nectar is the most important of the fruit juices and nectars produced (about 70% of the production), followed by pineapple juice, passion-fruit and grapefruit nectars. The majority of small-scale industries involved in the production of these beverages do not use chemical preservatives, but only pasteurization and/or deep-freezing as preservation methods [10,20].

Yeasts most frequently isolated from fruit juices and nectars produced at the pilot plant unit of Njombe Agronomic Research Station in Cameroon included *Candida* spp and *Saccharomyces* spp [19]. In Nigeria in orange juice, *Pichia* spp, *Candida* spp and *Saccharomyces* spp were the most prevalent [8]. These spoilage microorganisms were generally responsible for the poor quality and frank spoilage of products during storage, which could become very significant in developing countries because of the lack of an adequate quality control policy and infrastructure in their industries.

During recent years, research in predictive microbiology has increased [3,7,14,18]. Development of mathematical models would allow manufacturers to optimize quality and safety in food production and distribution chains and aid day-to-day decision making.

Few data are available on response surface models for predicting the effects of environmental conditions on the growth of yeasts in tropical fruit juices and nectars. *Candida pelliculosa* is one of the prevalent yeasts isolated from fruit juices and nectars produced at the industrial level in Cameroon. This study was conducted to establish a mathematical model for predicting the growth of this yeast in guava nectar as a function of pH and storage temperatures.

Materials and methods

Micro-organism and media

The same strain of *Candida pelliculosa* isolated from fermented pineapple juice, guava and passion-fruit nectars produced between September 1994 and February 1995 by the 'Société Agro-Industrielle des Fruits de l'Ouest' (SAIFO) of Cameroon was used for the investigation. The strain was identified with 99.9% probability by the Diagnostic Service of Biomerieux (France) using ID 32 C strips.

Pure cultures of yeasts were stored at 2°C in Potato Dextrose Broth (PDB, Sigma, France). Enumeration of CFU's (poured plates) was performed on Potato Dextrose Agar (PDA, Biokar Diagnostics, France) acidified to pH 3.5 with 10% tartaric acid, after incubation at 32°C for 48 h.

Commercial guava nectar (pH 3.15; 10.5° Brix) was centrifuged and adjusted to different pH values with 20% citric acid or 2 N NaOH. Then it was filter-sterilized using a Min-

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isart sterile disposable syringe filter holder (SM 16555, pore size 0.45 μm, Sartorius GmbH, Germany). For the preparation of inocula (pre-culture), isolated colonies from PDA were picked and grown at 32°C for 72 h in commercial guava nectar (pH 3.15) which had been centrifuged and sterilized at 110°C for 30 min.

Experimental procedures

A factorial design was used to determine the effects and interactions of temperature and pH. The growth kinetics were established in duplicate for different temperatures (2, 5, 10, 15, 25, 32, 35, 37, 39 and 46°C), and pH values (1.55, 1.85, 2.00, 2.50, 3.15, 4.00, 5.00 and 6.25).

Growth studies were done directly in spectrophotometric cuvettes. Cuvettes were sterilized by soaking them overnight in 95% ethanol, followed by drying them under a flow of sterile air and subsequently in an oven at 50°C for 2–3 days. Duplicate sterilized cuvettes were then filled with 1 ml filter-sterilized guava nectar at different pH values. They were inoculated with 20 μl of the pre-culture containing 3.6×10^6 to 3.9×10^6 CFU ml^{-1} of *C. pelliculosa*, corresponding to an initial cell count in the cuvette after inoculation of about 7.2×10^4 to 7.8×10^4 CFU ml^{-1}. They were then closed hermetically with sterile stoppers and incubated at different temperatures. The optical density (OD_{630}) of each cuvette was read every 3 h at 630 nm. The cuvettes were stirred manually before each reading.

Statistical analyses and model development

Taking into consideration the maximal ambient temperature in Cameroon and in the major part of tropical countries (about 37°C), and the pH of fruit juices and nectars, only temperatures from 2°C to 37°C and pH from 2.00 to 6.25 were included in the model. The statistical analyses were performed using the version 5.0 of STAT-ITCF ('Institut Technique des Céreales et des Fourrages', Paris, France) computer program. The quadratic polynomial model was constructed with the experimental values to define the mathematical relationship between maximal optical density resulting from the growth of *C. pelliculosa* in guava nectar, initial pH values of the culture media (P variable) and incubation temperatures (T variable in degrees centigrade). An exponential regression was used to define the relationship between CFU ml^{-1} of *C. pelliculosa* and OD_{630}.

Results and discussion

An exponential regression was used to define the relationship between the number of viable cells on PDA and the OD resulting from the growth of *C. pelliculosa* in commercial guava nectar. That relationship, with the corresponding calibration curve shown in Figure 1, was defined as:

$$\text{CFU ml}^{-1} \text{ of } \textit{Candida pelliculosa} = (3190794.27) \cdot e^{2.29(\text{OD})},$$

with $r^2 = 0.99$, and d.f. = 8 .

The growth kinetics were measured on duplicate cultures. Some examples of growth curves of *C. pelliculosa* in guava nectar at different pH values at 32°C are shown in Figure 2. The maximal optical densities occurred after about 90 h incubation. The storage period generally adopted by fruit juice industries to detect fermented product before distribution was about 1 week, so we did not take into account the incubation time for the construction of our mathematical model.

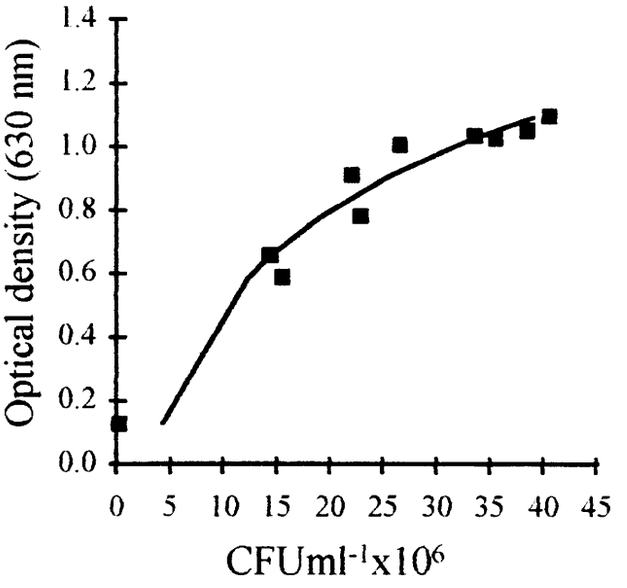

Figure 1 Calibration curve of the growth of *Candida pelliculosa* in commercial guava nectar (pH 3.15; 10.5° Brix) at 32°C.

Figure 3 shows a three-dimensional plot of the measured maximal optical density (mean of two replicates) in relation to the growth parameters pH and temperature. The highest value of maximal OD (1.095 for about 39.2×10^6 CFU ml^{-1}) was obtained at 37°C and pH 6.25. The maximal OD values obtained at pH 1.55–2.00 were very low, especially at 2°C and 46°C.

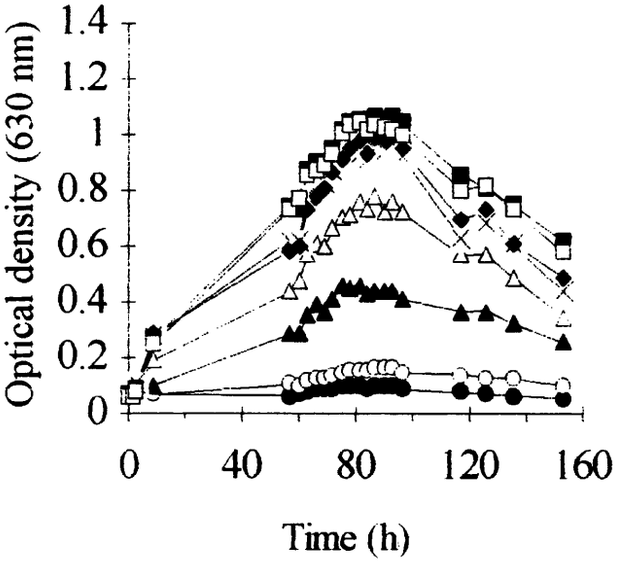

Figure 2 Growth curves of *Candida pelliculosa* in guava nectar at different pH values: 1.55 (●), 1.85 (○), 2.00 (▲), 2.50 (△), 3.15 (×), 4.00 (◆), 5.00 (■) and 6.25 (□) at 32°C.

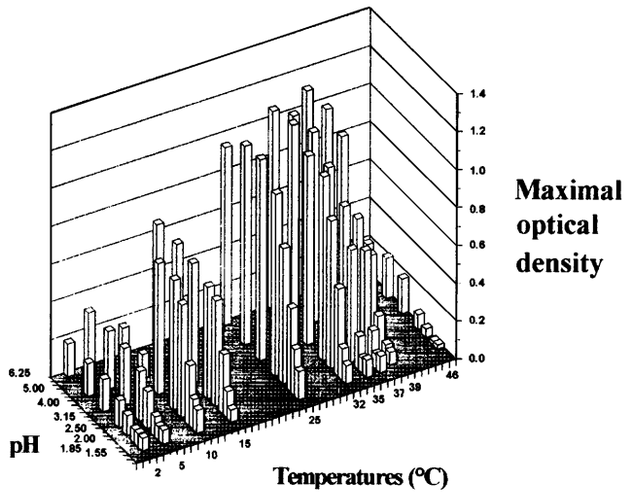

Figure 3 The three-dimensional plot of the maximal optical density values at 630 nm (mean of two replicates) resulting from the growth of *Candida pelliculosa* in guava nectar at different pH values and storage temperatures.

The mathematical equation for the quadratic polynomial model constructed, with 84 degrees of freedom (d.f.) was:

$$\text{Max OD} = 0.0288T + 0.0726P - 0.0008T^2 - 0.012P^2 + 0.0056T \cdot P$$

Transformation of the parameters included in the model, for example max OD to ln(max OD), in order to improve the model as is usually done [2,4,11,16,22] was not necessary to construct our model.

The maximal optical density values predicted by the model were plotted at different temperatures and pH values (Figure 4). A good agreement ($r^2 = 0.97$) was found between the predicted values and the observed values of maximal optical densities as shown in Figure 5.

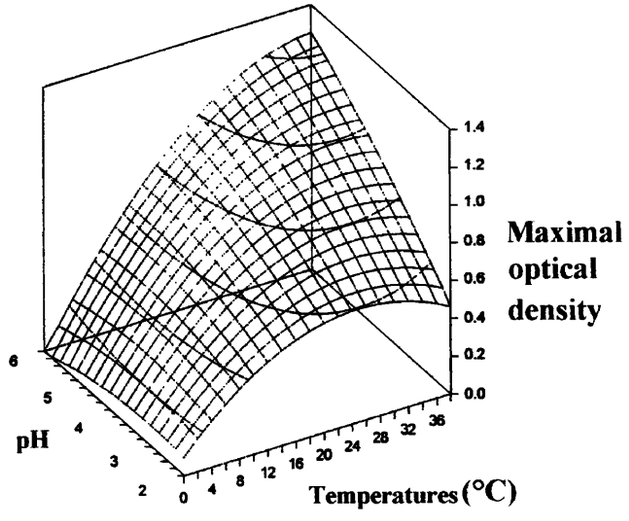

Figure 4 The predicted effects of storage temperature and pH on the growth of *Candida pelliculosa* in guava nectar, derived from the response surface equation.

According to the model, the maximal population density of *C. pelliculosa* (at maximal OD value of 1.25) was about 5.6×10^7 CFU ml^{-1} at pH 6.25 and 37°C. In commercial guava nectar (pH 3.15) stored at 10°C (refrigeration temperature), 25, 32 and 37°C (ambient temperatures in different Cameroonian and tropical regions), the predicted maximal population densities of *C. pelliculosa* were respectively: 9.9×10^6 CFU ml^{-1} (max OD value of 0.49), 1.9×10^7 CFU ml^{-1} (max OD value of 0.77), 1.9×10^7 CFU ml^{-1} (max OD value of 0.78), and 1.7×10^7 CFU ml^{-1} (max OD value of 0.73). On the other hand, at pH 2.50 (guava pulp) and temperature values of 10, 25, 32 and 37°C, the predicted maximal population densities of the yeast were respectively: 8.7×10^6 CFU ml^{-1} (max OD value of 0.44), 1.4×10^7 CFU ml^{-1} (max OD value of 0.64), 1.3×10^7 CFU ml^{-1} (max OD value of 0.61), and 1.1×10^7 CFU ml^{-1} (max OD value of 0.54).

This model, as well as all the multifactorial models, presents three essential advantages: allowing a study of many factors and their interactions, and good correlation with experimental values. But this type of model could become very complex and fragile especially when too many factors are included [2,17]. This is why authors of multifactorial models validate their mathematical model with other experimental values obtained under the same conditions [15].

The present model was validated by another experiment carried out on growth of the same yeast in commercial guava nectar (pH 3.15). The values of the maximal optical density obtained were 0.83 (predicted value of 0.73) at 37°C, 0.72 (predicted value of 0.75) at 35°C, 0.82 (predicted value of 0.78) at 32°C, 0.79 (predicted value of 0.77) at 25°C, 0.70 (predicted value of 0.63) at 15°C, 0.70 (predicted value of 0.49) at 10°C, 0.42 (predicted value of 0.43) at 8°C, 0.28 (predicted value of 0.32) at 5°C and 0.16 (predicted value of 0.20) at 2°C.

The model was not validated with commercial pineapple

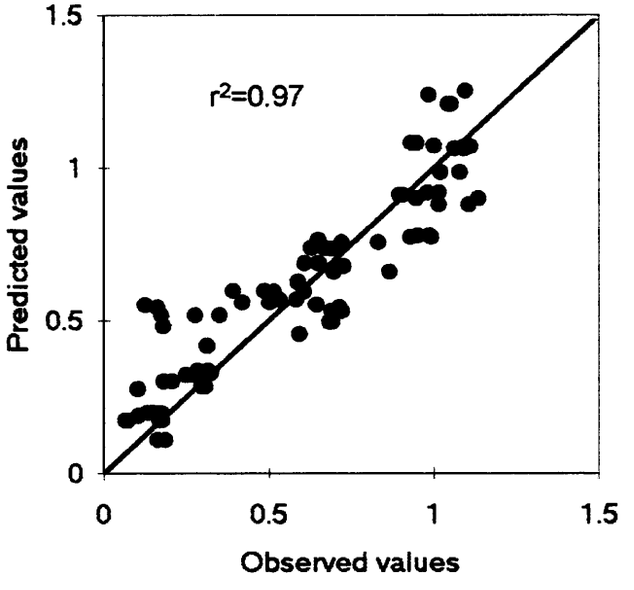

Figure 5 Relationship between predicted values and experimental values of the maximal optical density.

juice (pH 3.95) and passion fruit nectar (pH 3.03). Yeast growth was faster and more abundant in these beverages than in guava nectar, possibly because pineapple juice and passion fruit pulp contain more available sugar (13.5 g per 100 g and 13.0 g per 100 g respectively) than guava pulp with only 5.5 g per 100 g of edible parts [9]. The average available sugar contents of pineapple, passion fruit and guava, nutrients needed for the growth of the yeast [1,12], were reported to be: fructose (3.7 g, 4.0 g and 2.6 g per 100 g, respectively), glucose (3.7 g, 4.8 g and 2.1 g per 100 g, respectively), and sucrose (6.1 g, 4.2 g and 0.7 g per 100 g, respectively) [9]. These fruits contain an acceptable amount of proteins, minerals and micronutrients such as vitamins [9] also needed by the yeast [1,12]. This confirms that the culture medium composition and the environmental conditions both affect the growth of microorganisms [3,6,14].

To predict the growth of microorganisms in beverages, it would be necessary for producers of fruit juices and nectars to develop in some cases a mathematical model for each product and for each spoilage microorganisms found in the beverages.

The choice of maximal optical density as a parameter in the model, among others such as growth rate, arose from the fact that it gives a general idea of the maximal population density of the yeast in the beverage and could easily be compared with standards for fruit juices and nectars, in relation to pH and the preservation temperatures of the product contaminated by *C. pelliculosa*.

According to the present model, fruit juices and nectars could be fermented by *C. pelliculosa* at different storage temperatures (2–37°C) and pH values (2.00–6.25). The growth of the yeast could be limited by acidifying the beverages (with citric acid for example) and storing the pasteurized products at lower temperatures. In all instances, efficient pasteurization associated with aseptic packaging, eliminating the risk of recontamination, would preserve good commercial and microbiological qualities of fruit juices and nectars during storage and distribution.

Although our model appears to slightly underestimate the maximal optical density values compared to the experimental values at some temperatures, the use of turbidity as well as other rapid microbiology methods such as indirect conductimetry [6] seem to be convenient for monitoring yeast activity and generating a large amount of data that can be used to develop predictive models for growth of food spoilage yeasts.

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