International Journal of Food Microbiology 193 (2015) 23–28

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Modeling the growth of *Byssochlamys fulva* and *Neosartorya fischeri* on solidified apple juice by measuring colony diameter and ergosterol content

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ARTICLE INFO

Article history:
Received 22 August 2013
Received in revised form 18 June 2014
Accepted 3 October 2014
Available online 13 October 2014

Keywords:
Fungal growth
Predictive microbiology
Food mycology
Mathematical modeling

ABSTRACT

Byssochlamys fulva and *Neosartorya fischeri* are heat-resistant fungi which are a concern to food industries (e.g. apple juice industry) since their growth represents significant economic liabilities. Although the most common method used to assess fungal growth in solid substrates is by measuring the colony's diameter, it is difficult to apply this method to food substrates. Alternatively, ergosterol contents have been used to quantify fungal contamination in some types of food. The current study aimed at modeling the growth of the heat-resistant fungi *B. fulva* and *N. fischeri* by measuring the colony diameter and ergosterol content, fitting the Baranyi and Roberts model to the results, and finally establishing a correlation between the parameters of the two analytical methods. Whereas the colony diameter was measured daily, the quantification of ergosterol was performed when the colonies reached diameters of 30, 60, 90, 120 and 150 mm. Results showed that *B. fulva* and *N. fischeri* were able to grow successfully on solidified apple juice at 10, 15, 20, 25 and 30 °C, and the Baranyi and Roberts model showed good ability to describe growth data. The correlation curves between the parameters of colony diameter and ergosterol content were obtained with satisfactory statistical indexes.

1. Introduction

Apples are fruits that can offer many possibilities for industrialization. Apple juice is one of the most popular products obtained from apples with great commercial importance for some countries' regions (e.g. the south of Brazil). Apple juice is also a source of many beneficial components for health, such as antioxidant substances (Lu and Foo, 2000; Ibrahim et al., 2011).

A major concern of the apple juice industry is mainly related to the toxigenic and spoilage microorganisms that can survive the pasteurization conditions (Bahçeci and Acar, 2007). *Byssochlamys fulva* and *Neosartorya fischeri* are fungi species conspicuous for the production of resistant ascospores that enable them to survive at temperatures usually used for the pasteurization of fruit juices. Additionally, these fungi are commonly found in fruit juices where they are important spoilage agents (Tournas, 1994; Piecková et al., 1994; Salomão et al., 2007).

Fungal spoilage is often visible in the form of colonies on food surface and thus fungal growth has been studied by measuring the colonies' diameter over a time period. The measurement of colony diameter is the most common method to evaluate fungal growth on solid substrates (Taniwaki et al., 2010; Panagou et al., 2010; Zimmermann et al., 2011). A growth rate function may be derived by plotting the colonies' diameter measurements over a time period. Consequently, their radial growth rate and detection time may be calculated under several conditions (Gibson and Hocking, 1997).

The measurement of hyphal extension rate, usually reported as radial growth rate, is probably the simplest and the most direct method to measure fungal growth. Growth estimates for filamentous fungi, however, is more complicated due to the formation of surface colonies and hyphae all over the food (Marín et al., 2005).

Fungal growth rate, or rather, the increase in colony diameter over a time period, can be empirically described by the Baranyi and Roberts (1994) model, which was originally developed for bacterial growth. The model has been successfully used to describe the growth of bacteria, yeast and filamentous fungi (Gibson et al., 1994; Valik et al., 1999; Marín et al., 2008). From this primary model, the detection time (λ) and the maximum growth rate (μ_{max}) parameters are estimated, which subsequently may be used for a secondary modeling if growth curves for different constant conditions are available. Modeling of colony diameter may be useful for research purposes, albeit not a routine

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http://dx.doi.org/10.1016/j.ijfoodmicro.2014.10.006
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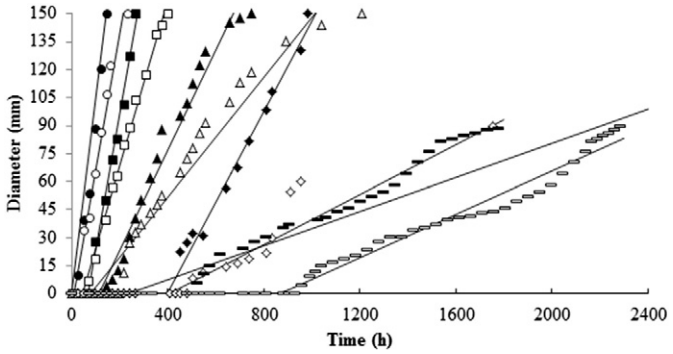

Fig. 1. *B. fulva* (filled symbol) and *N. fischeri* (unfilled symbol) growth curves assessed by colony diameter measurements on solidified apple juice. The continuous lines (–) represent the fitting of the Baranyi and Roberts model to the experimental data at 30 °C (●○), 25 °C (■□), 20 °C (▲△), 15 °C (◆◇) and 10 °C (▬▭).

measurable parameter of food analysis. Ergosterol analysis, which accounts for the total fungal population in food samples, may be an alternative (Marín et al., 2006, 2008; Taniwaki et al., 2006).

Ergosterol, an important component in fungal membranes, is involved in numerous biological functions, such as membrane fluidity regulation, activity and distribution of integral proteins and control of the cellular cycle. These facts make ergosterol and its biosynthetic pathway essential for fungal growth (Bard et al., 1993; Alcazar-Fuoli et al., 2008). Seitz et al. (1977) pioneered a High Performance/Pressure Liquid Chromatography (HPLC) method for the quantification of ergosterol that has become widely used to estimate the degree of fungal infection in grain and other plant materials. This method was used by some authors to calculate the degree of fungal infection in solid medium, grain and other foods (Kadakal et al., 2005; Marín et al., 2006, 2008; Taniwaki et al., 2006).

Ergosterol content has been used to determine fungal contamination in cereals, juices and other food products (Gourama and Bullerman, 1995; Saxena et al., 2001; Taniwaki et al., 2001, 2006; Kadakal et al., 2005; Marín et al., 2005, 2008). The analysis is related to total fungal biomass and it has been correlated to colony diameter growth (Marín et al., 2008; Taniwaki et al., 2009).

The current study aims at modeling the growth of the heat-resistant fungi *B. fulva* and *N. fischeri* from data of colony diameter and ergosterol content so that temperature influence on the growth parameters of these fungi on solidified apple juice and the correlation between the parameters of the two analytical methods could be verified.

2. Material and methods

2.1. Microorganisms and preparation of spore suspension

B. fulva IOC 4518 strain was isolated from apple juice concentrate by Salomão et al. (2008) and *N. fischeri* was isolated and identified in samples taken from an apple nectar processing line by Salomão (2002). The preparation of *B. fulva* and *N. fischeri* spores started by the pre-sporulation in Petri dishes containing Potato Dextrose Agar (PDA) media (pH 3.5) for 7 days at 30 °C. The collected spores were added to the sporulation plates containing Malt Extract Agar (MEA) media and incubated for 30 days at 30 °C. After this period, 1 mL of sterile distilled water was added to each plate, which was scraped with a rubber spatula. The entire plate content was filtered through 4 layers of sterile gauze and centrifuged at 3500 rpm (2000 times the force of gravity (g)) for 15 min. This procedure was repeated until no hyphae were seen under the microscope. The final suspension was prepared with the precipitate in a minimum volume of water, sufficient to obtain a highly concentrated suspension (10^5 spores/mL) (Salomão et al., 2007). *B. fulva* and *N. fischeri* suspensions were transferred to a flask and kept at 4 °C until use. A microscopic observation was carried out before use to insure the absence of germinated spores and hyphae.

2.2. Spore suspension quantification

Dilutions of *B. fulva* and *N. fischeri* spore suspensions were carried out to verify the number of spores in the suspension. For this procedure, 16 × 100 mm (diameter × length) screw-cap tubes with 4.5 mL sterile distilled water and 0.5 mL of each suspension were used. The above prepared solutions (1/10 dilutions) were then activated by inserting the tubes into a thermostatic bath (Tecnal-TE-184, Brazil) at 80 °C for 10 min and the tubes were then immediately placed in an ice bath (previous study in our laboratory). The procedure halted the dormancy of the spores.

Further, appropriate decimal dilutions were performed and plated on PDA media plus tartaric acid solution (1/10, mass/volume) until pH 3.5 was reached and 0.1% (in mass) of a Rose Bengal solution (5/100 mass/volume) to inhibit the spread of other undesirable microbial organisms (Baglioni et al., 1999). The plates were wrapped in a plastic film to prevent drying. The colonies were enumerated after an incubation period of 3–5 days at 30 °C. Counts were expressed as CFU/mL and each test was individually analyzed in triplicate trials.

2.3. Growth medium

Diluted apple juice was prepared from clarified and concentrated apple juice (70 °Brix) supplied by Fischer S/A (Videira, Santa Catarina, Brazil). The juice's pH was adjusted to 3.8 by a sodium hydroxide (1 mol/L) or hydrochloric acid 1 (1 mol/L) solution. Soluble solid content was adjusted to 12 °Brix (refractometer AR200 Reichert, USA) by dilution with distilled water. A hygrometer (Aqua Lab Model Series Models 3TE, Decagon Devices, USA) was used to measure the juice's water activity (a_w), equal to 0.99 for diluted apple juice. Growth medium was prepared with 100 mL of the formulated juice plus 1.5 g of agar. The mixture was heated, maintained at 115 °C for 1 min (previous study in our laboratory) and the growth medium was then placed in Petri dishes (150 mm diameter).

2.4. Growth kinetics of *B. fulva* and *N. fischeri*

The growth kinetics of *B. fulva* and *N. fischeri* was analyzed by measuring the colony diameter on the surface of solidified apple juice and the ergosterol content in the plates over a time period at five different

Table 1
R^2 and *RMSE* (mm) values for fitting the Baranyi and Roberts model to the experimental data of colony diameter for *B. fulva* and *N. fischeri* on solidified apple juice at 10, 15, 20, 25 and 30 °C.

Microorganism	Statistical index	Temperature				
		10 °C	15 °C	20 °C	25 °C	30 °C
B. fulva	R^2	0.987	0.993	0.979	0.992	0.955
	RMSE	5.51	4.12	8.28	5.12	12.19
N. fischeri	R^2	0.983	0.934	0.956	0.994	0.974
	RMSE	3.96	10.21	10.88	4.05	9.15

Table 2
μ_{max} (mm/h) and λ (h) parameters obtained by fitting the Baranyi and Roberts model to the experimental data of colony diameter for *B. fulva* and *N. fischeri* on solidified apple juice at 10, 15, 20, 25 and 30 °C.

Microorganism	Parameter	Temperature				
		10 °C	15 °C	20 °C	25 °C	30 °C
B. fulva	μ_{max}	0.0754	0.2427	0.2683	0.7278	1.0410
	λ	472.0	398.3	113.9	68.1	23.3
N. fischeri	μ_{max}	0.0583	0.0457	0.1606	0.4433	0.7319
	λ	868.3	235.9	78.7	45.1	10.3