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Modeling of *Byssochamys nivea* and *Neosartorya fischeri* inactivation in papaya and pineapple juices as a function of temperature and soluble solids content

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ABSTRACT

This study aimed to model the inactivation of *B. nivea* and *N. fischeri* ascospores in pineapple and papaya juices as influenced by temperature (78, 80, 85, 90 and 92 °C) and soluble solids concentration (10, 13, 20, 27 and 30 °Brix). First, a primary model was used to fit the Weibull model to inactivation data obtained from a combination of temperature and soluble solids concentration and to calculate δ (time for the first decimal reduction) and p (shape parameter). Then, a secondary model was used to describe how the inactivation kinetic parameters of these fungi in pineapple and papaya juices varied with the changes in temperature and soluble solids concentration. The shape parameter (p) was fixed for each strain and at temperature and soluble solids concentration studied. The results indicated that both linear and quadratic effects of temperature as well as the interaction between temperature and total soluble solids were deemed significant on δ value for both *B. nivea* and *N. fischeri* (except for *B. nivea* in papaya juice). This study contributes to the field by bringing new predictive models describing the influence and interactions of mild temperature conditions and soluble solids contents of fruit juices on the inactivation kinetics of heat-resistant fungi.

1. Introduction

Fungi such as *Penicillium*, *Aspergillus*, *Alternaria* are the main microorganisms associated with spoilage of a wide variety of foods (Pitt & Hocking, 1999). Regardless of this, these genera mainly include species that are not able to tolerate harsh food processing conditions. On the other hand, some species of *Byssochlamys*, *Neosartorya*, *Talaromyces* and *Eupenicillium*, comprise fungi presenting high chemical and heat resistances (Suresh, Ethiaraj, & Jayaram, 1996; Tournas, 1994; Valik and Pieckova, 2001). Therefore, these fungi are of major relevance for the stability of thermally processed foods, such as fruit juices and purees.

The thermal and chemical resistances of *B. nivea, B. fulva, N. fischeri, T. flavus* are related to the presence of structures known as ascospores, which confers the ability to survive after heating at least at 80 °C per 30 min (Kotzekidou, 1997; Pitt & Hocking, 1999; Tournas, 1994). As ascospores are exposed to sub lethal temperature conditions, they are activated, germinate and can multiply on the product during storage. This can further lead to spoilage and extensive economic losses for the industry (Slongo and Aragao, 2006). Several D values (the time at a specific temperature needed to cause one log cycle reduction in the population of a target microorganism) or inactivation kinetic parameters for heat-resistant fungi have been reported in the literature (Engel & Teuber, 1991; Delgado, Sant'Ana, Granato, & Massaguer, 2012a,b; Rajashekhara, Suresh, & Ethiraj, 1996; Sant'Ana, Rosenthal, & Massaguer, 2008, Tournas & Traxler, 1994). D-values at 90 °C ranging from <2 min to 6 min, for example, have been found for different species of heat-resistant fungi. It is known that D-values and other inactivation kinetic parameters may vary with substrata, pH, soluble solids contents, water activity, presence of

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preservatives, among other factors (Delgado et al., 2012a, b; Engel & Teuber, 1991; Rajashekhara et al., 1996; Tournas & Traxler, 1994).

Because of their high heat-resistance, ascospores of the species *B. nivea* and *N. fischeri* were used as targets of fruit juices thermal processes for several years (Eicher and Ludwig, 2002; Sant'Ana et al., 2008). However, the importance of heat-resistant fungi as targets of thermal processing of acidic foods declined in the last decades with the emergence of *Alicyclobacillus*, an acid-othermophilic sporeforming bacterium presenting *D*-values higher than those reported for heat-resistant fungi (McKnight, Eiroa, Sant'Ana, & Massaguer, 2010; Spinelli, Sant'Ana, Rodrigues, & Massaguer, 2009, 2010). Because of the high heat-resistance of *Alicyclobacillus* spores, several industries have applied thermal processes that can reach up to 115°C/15–30 s in order to produce shelf-stable fruit juices. Although thermal processing at these time and temperature conditions will ensure the inactivation of heat-resistant fungi in fruit juices (Tribst, Sant'Ana, & Massaguer, 2009), the over concern with *Alicyclobacillus* has led to a lack of interest on heat-resistant fungi. Nonetheless, the importance of heat-resistant fungi as fruit juice spoilers should not be underestimated because these microorganisms can pose shelf-stability problems in mild thermally processed fruit juices and acidic foods. This gains importance in an era of consumer concerns about ultraprocessed foods (Moubarac, Martins, Claro, Levy, Cannon, & Monteiro, 2013) and willingness to purchase foods not subjected to intense processing (Ragaert, Verbeke, Devlieghere, & Debevere, 2004). Additionally to spoilage problems, heat-resistant fungi such as *B. nivea* and *B. fulva* can produce mycotoxins, such as patulin, potentially posing a threat for food safety (Sant'Ana et al., 2008; Sant'Ana et al., 2012). In the scenario described above, the importance of heat-resistant fungi for the microbiological quality and safety of fruit juices is then revisited.

Several studies regarding the inactivation kinetics of heat-resistant fungi can be found in the literature (Engel & Teuber, 1991; Delgado et al., 2012a; Rajashekhara et al., 1996; Sant'Ana et al., 2008, Tournas & Traxler, 1994). Regardless of this, studies dealing with the modeling of combined factors and their interactions on thermal resistance of heat-resistant fungi are scarce. It is known that temperature and soluble solids contents are two major factors influencing on inactivation kinetics of heat-resistant fungi (Tournas, 1994; Tribst et al., 2009). Therefore, the objective of this study were to determine the inactivation kinetic parameters of *B. nivea* and *N. fischeri* ascospores in pineapple and papaya juices and to assess the impact of temperature and soluble solids concentration on these parameters through a secondary modeling approach.

2. Material and methods

2.1. Microorganisms and preparation of suspensions of spores

B. nivea LB01 and *N. fischeri* LB11 isolated from fruit juices and belonging to the culture collection of the Laboratory of Quantitative Food Microbiology at the University of Campinas, SP, Brazil, were used in this study. The fungi were grown on Potato Dextrose Agar (PDA, Himedia Laboratories, Mumbai, India) at 30 °C for seven days. Then, the colonies were washed with sterile distilled water. Roux bottles containing 180 mL of Malt Extract Agar (MEA, Difco Laboratories, Detroit, MI, USA) were inoculated with 0.5 mL following incubation at 30 °C for 30 days. The suspension of ascospores were obtained as previously described by Sant'Ana, Rosenthal, and Massaguer (2009) and further stored at 4 °C until used. The concentration of ascospores was determined after activation at 80 °C for 10 min, followed by serial dilutions in sterile 0.1% peptone water and plating in MEA. The concentration of the ascospores suspensions was adjusted at 10^7 ascospores/mL (Delgado et al., 2012a,b).

2.2. Preparation of fruit juices

Commercial pineapple (pH 3.7 and 16 °Brix) and papaya (pH 3.9 and 13 °Brix) juices free of preservatives were used in the experiments. Soluble solids concentration values (10, 13, 20, 27 and 30 °Brix) were adjusted using sucrose or sterile distilled water. The soluble solids concentration was measured using a refractometer (model Abbe, Atago, Tokyo, Japan). The juices were subjected to thermal treatment at 105 °C for 10 min to inactivate any potential contaminants (Sant'Ana et al., 2009).

2.3. Determination of B. nivea and N. fischeri ascospores heat resistance in pineapple and papaya juices at different temperatures and soluble solid concentrations

Estimation of thermal inactivation kinetics of *B. nivea* and *N. fischeri* ascospores in pineapple and papaya juices was performed in thermal death tubes (TDT, 8 mm external diameter, 6 mm internal diameter and 1 mm wall thickness). The TDT tubes were filled with 1 mL of pineapple and papaya juices at different soluble solid concentrations and 1 mL of the ascospore suspension, resulting in a final concentration of 10^6 ascospores/mL. The procedures for determining heat resistance were those previously described by Sant'Ana et al. (2009).

In order to investigate the influence of interactions between temperature and soluble solids on thermal inactivation kinetics of *B. nivea* and *N. fischeri* ascospores, a central composite design for two factors was used. The ranges of the factors were 78, 80, 85, 90 and 92 °C, for temperature, and 10, 13, 20, 27 and 30 °Brix, for soluble solids concentration. The conditions studied comprise the range of temperature and soluble solids which mild-processed fruit juices are subjected during processing and commercialization (Tables 1 and 2 contain the experimental design).

2.4. Modeling of B. nivea and N. fischeri ascospores inactivation in pineapple and papaya juices as a function of temperature and soluble solid concentration

2.4.1. Primary modeling

As the inactivation kinetics data was found to mainly follow a nonlinear trend, the Weibull model (Equation (1)) described by Mafart, Couver, Gaillard, and Leguerinel (2002) was chosen to fit the data of survival of heat-resistant molds studied herein (primary modeling). The GinaFiT software (Geeraerd, Valdramidis, & Van Impe, 2005) was used to fit the model to the data and to estimate the main parameters of inactivation of the Weibull model, *i.e.*, δ and p.

$$\log\frac{N}{N_0} = -\left(\frac{t}{\delta}\right)p \quad (1)$$

Where: N (population at time t), N_0 (initial population), t (time), δ (time for the first decimal reduction) and p (shape parameter). The δ (unity is time) represents the probability distribution describing the time interval for failure to occur (microbial death). The p value (which is dimensionless) describes the curvature of the microbial survival along the time. If $p = 1$ the susceptibility of microbial cells does not change throughout the time, while for $p > 1$ and $p < 1$, the microbial cells become more and less susceptible throughout the time (van Boekel, 2002). The standard deviation and R^2 values were obtained for models dealing with inactivation of *B. nivea* and *N. fischeri* ascospores in the different conditions assessed.