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Proposal of a generic model to predict the time to reject low acid fruit pulps contaminated by *Byssochlamys fulva*

Proposta de um modelo genérico para predizer o tempo de rejeição de polpas de frutas de baixa acidez contaminadas por *Byssochlamys fulva*

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Resumo
Uma ampla variedade de microrganismos pode contaminar produtos alimentícios. Devido ao seu baixo pH, frutas são frequentemente degradadas por fungos. O crescimento de fungos em frutas depende de características do alimento, assim como das condições armazenamento. Neste contexto, a microbiologia preditiva é uma opção para melhor compreender esses fenômenos e estabelecer estratégias visando evitar o crescimento microbiano em alimentos. Deste modo, o objetivo deste estudo foi desenvolver um modelo capaz predizer o tempo para a deterioração de frutas de baixa acidez contaminadas por esporos de *Byssochlamys fulva*, em função da temperatura, do pH e do teor de açúcares da fruta. Para tanto, o comportamento de crescimento desse microrganismo foi inicialmente estudado em meio de cultura ágar-batata-dextrose em função da temperatura (20, 28, 36ºC), pH (4, 5, 6) e teor de açúcares (0, 5, 10, 15% m/v). Um equacionamento matemático foi construído para quantificar o efeito desses fatores sobre a cinética de crescimento do fungo e sobre o tempo de rejeição do produto, constituído pelo modelo linear e um modelo do tipo Gibson. Curvas de crescimento de *B. fulva* em polpas de manga, melão, mamão e tomate foram obtidas experimentalmente. A partir delas foi determinado o tempo de rejeição dessas polpas e esse valor foi comparado com aquele predito pelo modelo matemático construído, no intervalo de 20ºC a 36ºC. O equacionamento desenvolvido descreveu satisfatoriamente o tempo de rejeição para esses alimentos, mostrando melhores resultados para a polpa de melão, para a qual a exatidão da predição foi superior a 90%. Em síntese, o modelo proposto neste trabalho mostra-se viável para predizer o tempo de vida de prateleira de alimentos contaminados por esporos desse fungo, principalmente quando armazenados em condições de baixa temperatura.
Palavras-chave: Microbiologia Preditiva. Crescimento. Fungo. Vida de Prateleira.

Abstract
A wide variety of microorganisms can contaminate food products. Due to low pH, fruits are mostly spoiled by fungi. The growth of fungi on fruits depends on the characteristics of the food and on environmental factors. In this context, the predictive microbiology is one option for studying and designing strategies to control microbial growth on foods. Thus, the aim of this work was to develop a model to predict the growth and the spoilage of low acid fruits contaminated by *Byssochlamys fulva* spores based on the storage temperature, pH and sugar content of the fruit. The growth behavior of this microorganism in modified potato-dextrose culture medium was studied as a function of temperature (20, 28, 36ºC), pH (4, 5, 6) and sugar content (0, 5, 10, 15% m/v). A mathematical model was constructed to quantify the effect of these factors on the growth kinetics of this fungus and on the time to spoil the product, using linear model and Gibson-type model. The growth curves of *B. fulva* in pulps of mango, melon, papaya and tomato were obtained experimentally from which the time to spoil was determined and compared with the results predict by the mathematical model in the range of 20ºC to 36ºC. The developed model described satisfactorily the time to spoil, showing better results for melon pulp, for which the accuracy of the model was greater than 90%. The proposed model has a great potential to predict the shelf life of those foods contaminated by this fungus, mainly under lower storage temperature conditions.
Keywords: Predictive Microbiology. Growth. Fungi. Shelf Life.

Nomenclature

S	sugar content [%]
T	temperature [ºC]
t_r	time to reject [h]
y	colony radius [mm]
y_0	initial colony radius [mm]
y_{obs}	colony radius observed experimentally [mm]
y_{pred}	colony radius predicted by the mathematical model [mm]
λ	lag phase time [h]
μ	radial growth rate [$mm.h^{-1}$]

1. Introduction

Fruits and vegetables are important foods in the human diet because they are source of vitamins, phytochemicals and antioxidants. Food and Agriculture Organization of the United Nations (FAO) recommends the consumption of at least 400 grams per day of these foods. Therefore, the world production of fruit and vegetables has been increasing in recent years. On the other hand, a significant portion of the total fruits and vegetables produced in the world is wasted by microbial spoilage of food. Pre-harvest and post-harvest conditions, transportation, processing and environmental conditions (pH, temperature, water activity/moisture, oxygen level) are some factors that can increase the susceptibility of fruit to invasion and growth of microorganisms (Alegbeleye *et al.*, 2022).

Foods can be contaminated by a wide variety of bacteria and fungi during any of the stages of harvest, processing and storage. The types of microorganism that will colonize each medium and the extent to which they will grow depend on the characteristics of the food product and on environmental factors (Huis In't Veld, 1996).

Fungi are capable of metabolizing a wider variety of substrates than many other microorganisms. They are more tolerant to low pH levels, to low water activity and to temperature fluctuations (Alegbeleye *et al.*, 2022). When they grow on a food product, fungi lead to changes that can be detected by the human senses, such as the appearance of superficial pigmentation, formation of slime, and production of secondary metabolites (acids, gases or alcohols) that create

unpleasant smells and flavors, leading to the rejection of the product. The wastage due to fungal growth on foods is responsible for significant economic losses and environmental impacts (Alegbeleye *et al.*, 2022; Dantigny *et al.*, 2005; Huis In't Veld, 1996). Furthermore, fungi are also capable of synthesizing toxins that are harmful to human health, such as aflatoxins, ochratoxins and byssochlamic acid (Garcia *et al.*, 2009; Panagou *et al.*, 2007; Welke *et al.*, 2009). Controlling the growth of these microorganisms on food is therefore not just a matter of avoiding economic losses and environmental impacts; it is also a question of public health.

Many different species of filamentous fungi are of relevance to food processing. *Byssochlamys fulva* is a species that merits particular interest because of the following characteristics: it produces heat resistant spores (Kotzekidou, 1997); it can grow in environments with low oxygen concentrations or with modified atmospheres (Chapman *et al.*, 2007; Taniwaki *et al.*, 2010); it can produce mycotoxins above the threshold concentration at which they pose a health risk to the consumer (Sant'Ana *et al.*, 2010a); it can spoil clarified juice even at very low initial contamination level (Sant'Ana *et al.* 2010b), it has a fast colony growth rate that can reach 20 mm per day (Valík and Piecková, 2001) and its spores are capable of resisting the action of the typical sanitizers used in food industry (Salomão *et al.*, 2011) . Also, this microorganism can grow in refrigerated foods, depending on the storage conditions and the initial temperature of the product (Silva *et al.*, 2013, Silva *et al.*, 2014).

In the context of food production, predictive microbiology is one option for designing strategies to control microbial growth. In this field of study it has become customary to structure the mathematical models used on two principal levels: primary models describe variations in microbial population sizes over time; and secondary models evaluate how the parameters in the primary models (growth rate, lag phase duration) are affected by environmental factors such as temperature, pH, the water activity and the presence of inhibitors (Whiting and Buchanan, 1993). In the literature, there are many primary models such as linear, logistic, modified Gompertz and Baranyi models. Many studies have demonstrated that the growth behavior of fungi is successfully described by linear model (Baert *et al.*, 2007; Cuppers *et al.*, 1997; Dantigny, 2021; Gougouli *et al.*, 2011; Lahlali *et al.*, 2007; Lee, Magan, 2010; Parra, Magan, 2004)

The effects of environmental factors on the growth kinetics of fungi have been modeled mathematically in several published studies. Cuppers *et al.* (1997) studied the effects of temperature and sodium chloride concentration on the growth of *Penicillium roqueforti*, *Trichoderma harzianum*, *Paecilomyces variotti*, *Aspergillus niger* and *Emericella nidulans*. Parra and Magan (2004) investigated the effects of temperature and water activity on growth of *Aspergillus niger*. Mousa *et al.* (2011) also modeled the effect of these environmental factors, but on the growth of *Aspergillus flavus*. Silva *et al.* (2010) studied the effects of pH and temperature on growth of *Aspergillus* section *Nigri* IOC 4573. Gougouli *et al.* (2011) studied the effects of temperature and inoculum size on the radial growth rate of a series of fungi isolated in yoghurt production facilities. Specifically with relation to *B. fulva*, Panagou *et al.* (2010) analyzed the influence of temperature and of water activity on the development of this microorganism and of *B. nivea*.

In the literature there are many secondary models to evaluate the effects of environmental factors on the growth kinetic of microorganisms, such as Belehrádek model, Arrhenius model, Arrhenius-Davey model and polynomial models (Panagou *et al.*, 2003; Ross, McMeekin, 1994; Samapundo *et al.*, 2005). However, according to Garcia *et al.* (2009), the model proposed by Gibson *et al.* (1994) deserves special attention because it was the first secondary model developed specifically for fungi, while the other models cited were developed to study the growth behavior of bacteria.

Some models were also developed to predict the growth behavior of fungi in specific fruit products. Sant'Ana *et al.* (2010a) have studied the production of patulin by *B. nivea* and *B. fulva* in apple juice. The lag time and growth rate of *Aspergillus* section *Nigri* IOC 4573 in mango nectar as a function of temperature and pH were studied by Silva *et al.* (2010). The growth parameters of *B. fulva* in papaya pulp were evaluated by Silva *et al.* (2013). Wang *et al.* (2017) investigated the growth of *Penicillium expansum* and production of patulin in kiwi juice. Tremarin *et al.* (2017) have

studied the influence of soluble solid content and storage temperature on the growth of *B. fulva* in agar-added apple juices, developing a model to predict the growth of this microorganism in apple juice as a function of these factors. Santos *et al.* (2020) have investigated the effect of sugar concentration and storage temperature on the time for visible growth (t_v) of ascospores of heat-resistant moulds, including *Byssochlamys spp*. The authors have observed that reduction in temperature (14ºC to 7ºC) have a more pronounced effect on the distribution of t_v than the effect of sugar content (44 to 59ºBrix). For *B. fulva*, the authors have reported visible colony growth after 8-23 days at 14ºC. According to Dantigny (2021), t_v parameter is more relevant in the study of fungal growth behavior than the radial growth rate. A food product is considered to be spoiled when the mould colony is visible (2-3 mm in diameter). Thus, the time for visible growth is direct related to the mould-free shelf life of food products which makes it a parameter of practical application in the industrial environment. Therefore, the construction of a model relating directly the characteristics of the product and the storage conditions with the t_v parameter becomes a distinctive approach in the predictive microbiology. This approach allows estimating in a more objective way how the changes in the composition of the food and processing conditions affect the shelf life of food products. Also, this approach is appropriate to integrate the model with optimization routines in the field of product development.

To the better of our knowledge, there are no studies related to the development of a general model to predict the time for visible growth of *B. fulva* in fruit pulps, based on their main properties, such as sugar content and pH, from room temperature to excessive temperature. In view of this gap in the current literature, the objectives of this study were to: (i) evaluate the effects of the substrate pH, sugar content (glucose) and temperature on the growth kinetics of *B. fulva*; (ii) develop a predictive model using linear model and Gibson-type model to estimate the time to reject fruits contaminated with *B. fulva* as a function of these intrinsic and extrinsic factors; and (iii) apply the proposed model thus developed to predict the time for visible growth of *B. fulva* in low acid fruit pulp (mango, melon, papaya and tomato) comparing to experimental data, in order to verify if a simplified model can estimate satisfactorily the time to spoil real food products caused by this microorganism.

2. Material and Methods

2.1 Preparation of the inoculum

Lyophilized spores of *B. fulva* isolated from fruit were obtained from the Fundação André Tosello's tropical cultures collection (Campinas, SP, Brazil), identified as strain NRRL 1125. The rehydration of the spores was done in sterile water in the laboratory, according to a specific protocol provided by the supplier. They were then added to a Petri dish containing a potato dextrose agar (PDA) culture medium and incubated in a bacterial growth chamber (Biopar, Brazil) at 28°C for 12 days to allow the colony matrix to form, followed by refrigerated storage until their use in the experiments. Fragments of the colony matrix were transferred into test tubes containing malt-extract-agar culture medium (MEA) and returned to the bacterial growth chamber at 28°C for 21 days. Microscopic observations revealed the presence of many spores in the colony at this time.

The surface of the fungus colony grown in MEA was washed in two 1 mL aliquots of a previously sterilized aqueous solution of Tween-20 at 5% (v/v) in a horizontal laminar flow chamber and then stored in a falcon tube. The spore suspension was mixed and standardized to a concentration of $3x10^6$ spores/mL. This suspension was used to inoculate plates immediately after preparation and no thermal spore activation treatment was therefore used, following a similar procedure to that described by Panagou *et al.* (2010).

2.2 Preparation of the culture mediums

The basic culture medium used for experiments was prepared by dissolving 2% (m/v) of potato dextrose in distilled water. Varying quantities of anhydrous glucose were then added to adjust

the sugar content depending on which experiment was being prepared. Likewise, the pH was adjusted to the appropriate level using drops of 1 mol/L hydrochloric acid or 1 mol/L aqueous solution of sodium hydroxide. The pH of each solution was measured using a digital pH meter (Ion Ph B500, Brazil). Finally, bacteriological agar was added to obtain a solid medium. Agar concentrations varied from 1.4% to 1.8% (m/v) because the pH of the medium affects its capacity to gel.

The resulting solutions were sterilized in an autoclave (Phoenix AV 50, Brazil) at 121°C for 15 minutes, and then transferred to 90 mm diameter Petri dishes in a laminar flow chamber. Plates were kept in a cabinet at 28°C for 24 hours to rule out contamination and then put in refrigerated storage until used in the experiments.

2.3 Inoculation and monitoring of radial growth

An automatic pipette was used to inoculate the center of each plate with 2 μL of the spore suspension. The plates were divided into three groups and stored in cabinets at the conditions set out in the experiment plan. The edges of the plates were sealed with plastic film to prevent water loss from the culture medium and to avoid contamination during the experiment.

Plates were observed daily and the diameter reached by each colony in four distinct directions was marked until the entire surface of the culture medium was covered by the colony. At the end of the experiments the diameters marked were measured, thereby providing the radius vs. time data used for mathematical modeling. Calculations were based on the mean radius of the four diameter measurements made on any given day.

2.4 Experimental design

The experimental design used for this study employs a complete factorial treatment for three factors: temperature (20, 28, 36°C), pH (4, 5, 6) and sugar content (0, 5, 10, 15 % m/v), with triplicates of each treatment.

2.5 Fitting of predictive models

The software RStudio version 1.0.136 was used to conduct linear regression on the experimental data for colony radius (y) vs. time (t) in order to obtain parameters for the linear model according to Equation 1. Radial growth rate (μ) was evaluated from the slope of the linear regression. The point at which the straight line intersects with the x-axis provides an estimate of the lag phase time (λ). The initial colony radius (y_0) was set to zero.

$$y = y_0 + \mu.(t - \lambda) \tag{1}$$

where y is the colony radius, [mm], y_0 is the initial colony radius, [mm], μ is the radial growth rate [mm.h^{-1}], t is time, [h], and λ is the lag phase time, [h].

Also the time to reject (t_r) the spoilage product was estimated according to Equation 2. For the purposes of this study, t_r was defined as the moment in which the colony radius reached 1.5 mm that was assumed as a critical radius (y_{crit}). According to Gibson *et al.* (1994) and Dantigny (2021) fungal colonies of this size are visible to the naked eye and therefore lead consumers to reject the product.

$$t_r = \frac{y_{crit}}{\mu} + \lambda \tag{2}$$

where t_r is the time to reject the product, [h], y_{crit} is critical colony radius, [mm], μ is the radial growth rate [mm.h^{-1}], and λ is the lag phase time, [h].

Analysis of variance (ANOVA) was used to determine which environmental factors had a significant effect on the parameter t_r, before proceeding to construction of the time to reject model. These tests were conducted using the software Bioestat 5.0 and the significance level (α) adopted

was 0.05. The statistical analysis of the effect of pH and sugar content on the parameter t_r was stratified by temperature in order to evaluate in which condition these intrinsic factor are more important in the product shelf life.

After the statistical analyses, for each treatment the mean values of the time to reject (t_r) were adjusted using the Gibson-type model capable of relating them to temperature (T), pH and sugar content (S). Gibson *et al.* (1997) found that the natural logarithm of the radial growth rate showed a relationship with the square root of water activity. The Gibson-type model used in this study is based on the works of Cuppers *et al.* (1997) and Tassou *et al.* (2007), as shown by Equation 3. Once more the software RStudio was used to determine the values of the coefficients for fitting the predictive model.

$$\ln(t_r) = a_0 + a_1.T + a_2.pH + a_3.S + \frac{a_4}{T} + a_5.\sqrt{S} + a_6.pH.\sqrt{S} \quad (3)$$

where t_r is the time to reject the product, [h], T is the temperature, [ºC], S is percentage of sugar [%], and a_0, a_1, a_2, a_3, a_4, a_5, a_6 are the model coefficients.

2.6 Validation of mathematical models

The predictive model (Eq. 3) was validated by comparing the time to reject predicted by the mathematical equations with the results obtained in independent experiments. Table 1 lists the values used for the environmental factors in the validation experiments. The performance of the proposed model was quantified using the accuracy factor (AF) indicator, as shown in Equation 4.

$$AF = 10^{\sum_{i=1}^{n} \frac{\left|\log\left(t_{r,pred,i}\right) - \log\left(t_{r,obs,i}\right)\right|}{n}} \quad (4)$$

where $t_{r,pred}$ is the time to reject the product predicted by the model, [h], $t_{r,obs}$ is the time to reject the product obtained by experimental data, [h], and n is the number of experimental data.

Table 1 – Values of environmental factors used in the validation experiments performed for the proposed model

Validation scenario	T / ºC	pH	Sugar content / % m/v	Number of curves used in validation stage
(I)	24	4.5	8	5
(II)	24	5.5	8	5
(III)	20	6	0	3
(IV)	28	4	0	2
(V)	28	6	10	2

2.7 Application of the proposed model

The proposed model developed in this work was applied to predict the time to reject pulps of low acid fruits (mango, melon, papaya and tomato) contaminated by *B. fulva*. The goal of these experiments was to investigate the potentialities and the limitations of this simplified model to predict the growth of this fungus in real food, just based on the temperature, pH and sugar content of this substrate. For this purpose, independent experiments were carried out according to the procedure described in section 2.2 and 2.3. However, in this case the PDA medium was replaced by pulps of mango (*Mangifera indica* L., variety Tommy Atkins), melon (*Cucumis melo* L., variety Cantaloupe), papaya (*Carica papaya* L., variety Formosa and Golden) and tomato (*Solanum lycopersicum*, variety Caqui). The literature was searched to obtain the values for typical sugar content and pH of those fruit pulps, as shown in Table 2, in order to predict the time to reject the product, considering the typical range of variation of the properties of those fruits. The performance of the proposed model to predict the t_r by *B. fulva* in real foods was quantified using the AF indicator, as shown previously in Equation 4. Also, the bias factor was evaluated according to Equation 5. The bias factor was used to graduate the performance of the model following the interpretation proposed

by Ross (1999) apud Mejiholm *et al.* (2010): (i) 0.95-1.11 prediction is in a good zone; (ii) 0.87-0.95 or 1.11-1.43 prediction is in an acceptable zone and (iii) < 0.87 or > 1.43 prediction is in an unacceptable zone.

$$Bias = 10^{\sum_{i=1}^{n} \frac{\log(t_{r,pred,i}) - \log(t_{r,obs,i})}{n}} \quad (5)$$

where $t_{r,pred}$ is the time to reject the product predicted by the model, [h], $t_{r,obs}$ is the time to reject the product obtained by experimental data, [h], and n is the number of experimental data.

Table 2 – Typical values (mean ± standard deviation) for pH and sugar content in pulp of mango, melon, papaya and tomato, according to the literature.

Fruit pulp	pH	Sugar content / % m/v	References
Mango	4.10 ± 0.11	4.02 ± 1.08	Benevides *et al.* (2008)
Melon	5.45 ± 0.20	10.09 ± 2.08	Pizarro *et al.* (2006)
Papaya	5.25 ± 0.16	9.06 ± 2.28	Shinagawa (2009) and Santana *et al.* (2003)
Tomato	4.35 ± 0.10	2.13 ± 0.33	Ferreira *et al.* (2010) and Ferreira *et al.* (2012)

3. Results and Discussion

3.1 Growth curves and parameters of linear model

Performing the experimental procedure described in the previous section, one hundred and eight growth curves were obtained by *B. fulva*. The coefficient of determination (R^2) of the linear model varied from 0.938 to 0.999. Therefore, the linear model was fitted adequately to the experimental data and this model was able to describe accurately the growth behavior of this particular strain of fungus.

Figure 1 shows the growth curves for *B. fulva* in artificial substrate with a pH of 6.0 and 10% of sugar content for temperatures of 20ºC (♦), 28ºC (x) and 36ºC (•). It can be observed that, within the temperature range investigated in this study, higher temperatures increase the microorganism's growth rate. All growth curves obtained in this study for the fungus are similar to those shown in Figure 1, with varying conditions in terms of incubation temperature and the pH and sugar content of the culture medium.

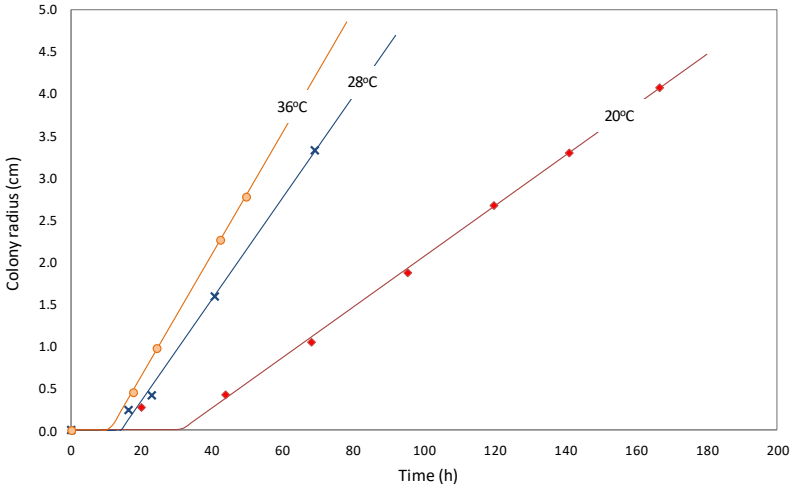

Figure 1 - Growth curves for *B. fulva* in artificial culture medium with pH = 6.0 and 10% of sugar content, plotting experimental data (dot) and the fitted mathematical model (--), for temperatures of 20ºC (♦), 28ºC (x) and 36ºC (•).

Table 3 lists the means and the standard deviations for radial growth rate, lag phase time and time to reject the product in each treatment, as obtained by the linear model. The statistical analysis of these results showed that there was lower variation for parameter μ than for parameter λ, within each treatment.

Table 3 – Means and standard deviations for radial growth rate (μ, in mm.day^{-1}), estimated lag phase time (λ, in h) of *B. fulva*, time to reject the product (t_r, in h) and the variation coefficient (VC) for the different combinations of temperature, pH and sugar content, predicted by linear model.

Temperature (ºC)	Sugar content (%)	pH	μ / mm.day^{-1}	λ / h	t_r / h	VC / %
20	0	4	6.67±0.34	21.3±3.1	26.7±2.9	10.8
		5	6.27±0.07	16.5±0.7	22.2±0.7	3.2
		6	6.54±0.04	22.6±0.7	28.1±0.7	2.4
	5	4	7.55±0.35	28.7±2.6	33.5±2.5	7.4
		5	7.89±0.28	30.3±4.0	37.1±1.1	3.0
		6	4.83±0.35	14.4±0.2	26.5±0.0	0.1
	10	4	7.41±0.33	31.2±6.8	39.6±3.5	8.9
		5	6.81±0.52	22.4±6.5	30.8±4.2	13.6
		6	6.45±0.15	21.2±1.5	26.8±1.7	6.2
	15	4	6.75±0.27	27.6±3.9	33.0±3.7	11.3
		5	6.63	37.3	42.7	-
		6	6.16	35.2	41.0	-
28	0	4	13.56±0.28	13.8±0.3	16.4±0.4	2.2
		5	14.57±0.92	12.4±0.9	14.8±0.8	5.3
		6	10.56±0.01	7.8±0.6	11.3±0.6	5.5
	5	4	13.81±0.57	7.1±2.9	8.1±0.4	5.0
		5	13.23±0.82	21.9±0.8	24.6±0.6	2.6
		6	14.16±0.25	11.3±0.9	13.9±0.9	6.5
	10	4	13.16±0.48	14.6±2.1	16.2±0.4	2.6
		5	13.51±0.24	12.4±1.3	15.0±1.3	8.7
		6	14.52±0.11	14.3±0.3	16.8±0.3	1.8
	15	4	7.96±0.10	9.1±0.9	16.6±0.9	5.1
		5	10.05±0.32	21.9±1.3	25.5±1.2	4.7
		6	9.90±0.15	23.7±1.1	27.3±1.1	4.1
36	0	4	17.24±1.61	8.1±3.0	10.2±2.8	27.5
		5	18.64±0.97	10.0±1.3	11.9±1.2	10.5
		6	19.33±1.26	10.7±1.7	12.5±1.5	12.2
	5	4	19.47±0.51	11.4±1.1	13.2±1.2	8.7
		5	19.42±0.16	12.0±0.7	13.8±0.7	4.8
		6	19.21±0.64	10.2±0.2	12.1±0.2	1.6
	10	4	16.00±0.27	10.9±0.5	13.1±0.5	3.9
		5	16.15±1.67	11.4±2.8	13.6±2.6	19.0
		6	16.61±0.67	9.6±1.2	11.8±1.1	9.5
	15	4	14.21±0.54	10.6±1.1	13.1±1.0	7.8
		5	15.71±0.37	11.9±1.1	14.2±1.1	8.0
		6	12.87±2.12	9.5±1.5	12.3±1.1	8.8

These findings are in agreement with the literature. According to Swinnen *et al.* (2004) accurate predictions of the lag phase time are very difficult to obtain because there are many factors having influence on this growth parameter. For the time to reject the product, the variation coefficient (VC) was also evaluated. The values of VC ranged from 0.1 to 27.5%. Also, most values of VC were below 10%. Statistically, it means low dispersion of t_r data, therefore dataset can be considered homogeneous.

The results obtained in this study are in agreement with the literature. Valík and Piecková (2001) studied the growth of *B. fulva* in Sabouraud agar, adopting the Baranyi model to assess growth parameters and reporting figures for the colony growth rate (in terms of the diameter) of the order of 20 mm.day^{-1}, at a pH of 6.0 and a temperature of 25ºC. In the present study, the radial growth rate was estimated from 9.9 to 14.5 mm.day^{-1}, for temperature of 28ºC and pH of 6.0. It is important to point out that there are differences between the strains and culture mediums used in each study.

In turn, Panagou *et al.* (2010) studied the growth of the same fungus in a malt extract agar medium. Using the Baranyi model, they estimated a maximum growth rate close to 26 mm.day^{-1} at a temperature of 35ºC, and 10 mm.day^{-1} at 20ºC. In the present work, using another primary model, 60% of the growth rates fell within the range of 16 and 19.5 mm.day^{-1} at 36ºC, whereas at 20ºC, the growth rates varied from 4.8 to 7.9 mm.day^{-1}. It can therefore be concluded that the growth rate calculated in the present study are similar to results reported in the literature. Related to lag phase time Panagou *et al.* (2010) observed values from 35 to 42 h at 20ºC, which is a little larger than the lag times observed in the present study (14 to 37 h at 20ºC). For 35ºC, the same authors reported values of 15 to 24 h that are larger than those found in this work, since λ oscillated between 8 and 12 hours at 36ºC. In addition to the differences between the strains used in each work, it is possible to conclude that malt extract agar and Sabouraud agar are culture mediums more favorable to the growth of *B. fulva* than potato dextrose agar used in the present work.

3.2 Investigation of the effects of environmental factors

Before constructing the time to reject predictive model, the analysis of variance (ANOVA) was used to test whether environmental factors (T, pH and sugar content-S) had a significant effect on the parameter t_r. The results of this analysis revealed that temperature (p-value = 0.0002), pH, sugar content and the interaction pH x sugar content have significant effect on time to reject the product due to unacceptable levels of *B. fulva* contamination as shown in Table 4, since the colony radius has reached the critical size, becoming visible to the naked eye.

Table 4 – P-values for the effects of pH, sugar content (S) and the interaction pH x S on the parameter t_r at 20ºC, 28ºC and 36ºC.

Intrinsic factors	20ºC	28ºC	36ºC
pH	0.0175	0.0002	0.089
S	< 0.0001	0.0001	0.052
pH x S	< 0.0001	0.0001	0.279

Statistical analysis showed that, within the range of values analyzed here, variations in the pH have a significant effect on the time to reject a product contaminated by *B. fulva* in optimal (28ºC) and suboptimal growing temperatures (20ºC). In turn, the sugar content had effect on this quality parameter, within the ranges investigated here, except 36ºC. This result is in agreement with the literature. Panagou *et al.* (2007) have demonstrated the effect of the pH on the growth rate of *Monascus ruber*, a heat-resistant fungus. The authors have observed this effect only in optimum or suboptimum conditions for temperature and water activity.

Thus, all these factors were included in the predictive model. Since the correlation test performed in software-R has indicated a strong correlation (0.955) between sugar content and pH x sugar content, the last one was removed from the final model.

Table 5 lists the coefficients of fit for Gibson-type model (Equation 3), which describes the effects of environmental factors (T, pH, S) on the parameter t_r. This table also contains the results for the standard deviation error and p-value for each parameter. The adjusted r^2 of this model was 0.786.

Table 5 – Values of the estimated coefficients for Gibson-type model, the standard deviation e p-value for each coefficient

Parameter	Estimated value	Standard deviation error	p-value
a_0	-0.5437	1.811	0.7661
a_1	0.0306	0.033	0.3591
a_2	0.0119	0.043	0.7832
a_3	0.0269	0.023	0.2512
a_4	63.5703	23.348	0.0107
a_5	-0.0219	0.088	0.8045

3.3 Validation of the kinetic model

The predictive model developed during the preceding stages were applied to estimate the time to reject a product contaminated by *Byssochlamys fulva* spores for each of the validation scenarios listed in Table 1. The AF indices were calculated for all the scenarios used in this stage, in order to provide a better assessment of the model's performance in the validation procedure. The results of these calculations are shown in Table 6.

Table 6 – Values of the time to reject a product contaminated by *B. fulva* spores obtained experimentally and by the predictive model and the accuracy factor of the prediction.

Validation scenario	T / ºC	pH	Sugar content / % m/v	Range of t_r obtained experimentally / h	Estimated t_r by the predictive model / h	Accuracy factor (AF)
(I)	24	4.5	8	22.4 to 24.7	21.0	1.14
(II)	24	5.5	8	17.4 to 19.7	21.3	1.18
(III)	20	6	0	26.6 to 28 5	27.6	1.03
(IV)	28	4	0	21.9 to 22.0	13.9	1.58
(V)	28	6	10	9.9 to 12.7	17.4	1.55
				Over all medium accuracy		1.21

The results listed in Table 6 show that the model's accuracy varied from 42% (scenario IV) to 97% (scenario III), with a typical accuracy of close to 79%. The validation procedure of the proposed model in different scenarios also made it possible to identify conditions under which its predictions are reliable and in which conditions its performance is unsatisfactory, as shown in Figure 2.

Therefore, the model constructed in this study accurately predicted the time to product rejection across all scenarios, since the data were distributed close to the diagonal line as can be seen in Figure 2. Only scenarios IV and V had significant deviation from the diagonal line. However, the scenario IV has shown a safe failure. On the other hand, in the scenario V, the prediction is in an unsafe failure zone, because the time predicted by the model is greater than the experimental observation. Summing up, at high temperature, intermediate sugar content and high pH values, the model's prediction is in the unsafe failure zone. Thus, the model's predictions should be treated with caution under these conditions. However, at low temperature, low sugar content and high pH values, the model's prediction is very accurate.

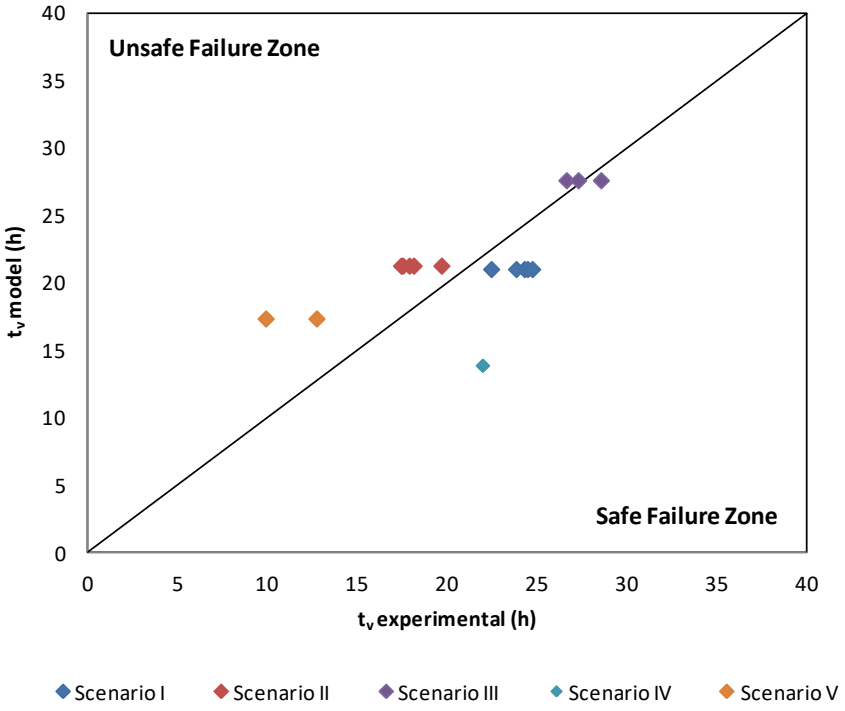

Figure 2 – Comparison between the time to reject a product predicted by the model and obtained experimentally in different validation scenarios, showing in which condition the model prediction is in the unsafe and safe failure zone.

3.5 Application of the proposed model for real foods

Pulps of mango, melon, papaya and tomato fruit were inoculated with spores of *B. fulva*. The growth of this fungus in such substrate was monitored. These experimental data were used to compare the fungal growth in real food and the fungal growth predicted by the proposed model. The time to reject these fruit pulps predicted by the mathematical model was obtained just using the pH and sugar content values that are typical for these fruits and in a range of storage temperature conditions for which the experimental data were obtained. The authors chose to use data from the literature for pH and sugar content instead of measuring them directly in order to evaluate the robustness of the proposed model. The results are listed in Table 7.

Table 7 – Values of the time to reject real pulp fruits contaminated by *B. fulva* spores obtained experimentally and by the predictive model, the bias factor and the accuracy factor of the prediction.

Pulp fruit	pH	Sugar content / % m/v	T / ºC	Value of t_r obtained experimentally / h	Estimated t_r by the predictive model / h	Bias factor	Accuracy factor (AF)
Mango	4.1	4.02	20	41.6	28.8	0.69	1.31
			28	15.2	14.8	0.97	
			36	7.5	11.4	2.13	
Melon	5.42	10.09	20	31.6	33.5	1.06	1.07
			28	15.2	17.3	1.13	
			36	13.2	13.3	1.03	
Papaya	5.25	9.06	20	27.7	32.7	1.19	1.44
			28	13.1	16.8	1.30	
			36	6.5	12.9	2.03	

			20	40.7	27.8	0.69	
Tomato	4.35	2.13	28	7.5	14.3	1.93	1.49
			36	9.4	11.0	1.18	

The results listed in Table 7 have shown that model's accuracy varied from 1.07 (melon pulp) to 1.49 (tomato pulp). Thus, the proposed model was able to predict the growth behavior of this fungus on fruit pulps with accuracy varying from 51% to 93%. It is a good achievement because a simplified model was able to predict the time to reject real pulp fruits, just considering the pH, the sugar content and the storage temperature of the food. The best predictions of the model were observed for *B. fulva* contaminating the melon pulp. On the other hand, the worst predictions were found for this fungus growing in the tomato pulp. Silva *et al.* (2013) studied the influence of the initial product temperature and the air stream velocity on the shelf life of papaya pulp contaminated by this fungus during refrigeration process. The authors have found time to rejection about 20 h at 28ºC. In the present study, the time to reject for the papaya pulp was estimated at 14 h at the same temperature, just considering the typical pH, sugar content and the storage temperature of this product.

Giffel and Zwietering (1999) also discussed the use of statistical indices to validate mathematical models in predictive microbiology. According to these authors the accuracy is reduced when the growth medium is a real food rather than an artificial culture medium. The authors reported that AF varied from 1.7 to 3.5 when they have studied the growth of *Listeria monocytogenes* in a variety of food substrates. Related to the growth of fungi in food, Baert *et al.* (2007) studied the growth of *Penicillium expansum* on an artificial substrate and a real food. The validation analysis performed in that work showed better performance of the model on artificial medium than for real food for which AF varied from 1.26 to 3.00. Thus, the performance of the simplified proposed model in the present study can be considered satisfactory, since the AF varied from 1.07 to 1.49 to predict the growth behavior of *B. fulva* in real foods.

Figure 3 shows the bias factor for each pulp fruit in the range of temperature studied in the present work. The bias factor obtained in the present study indicated that most predictions are in the range of acceptable zone, i.e., from 0.87 to 1.43 according to the graduate performance proposed by Ross (1999). Confirming the previous results, for melon pulp the predictions are in the good zone, since bias factor are in the range of 0.95 to 1.11.

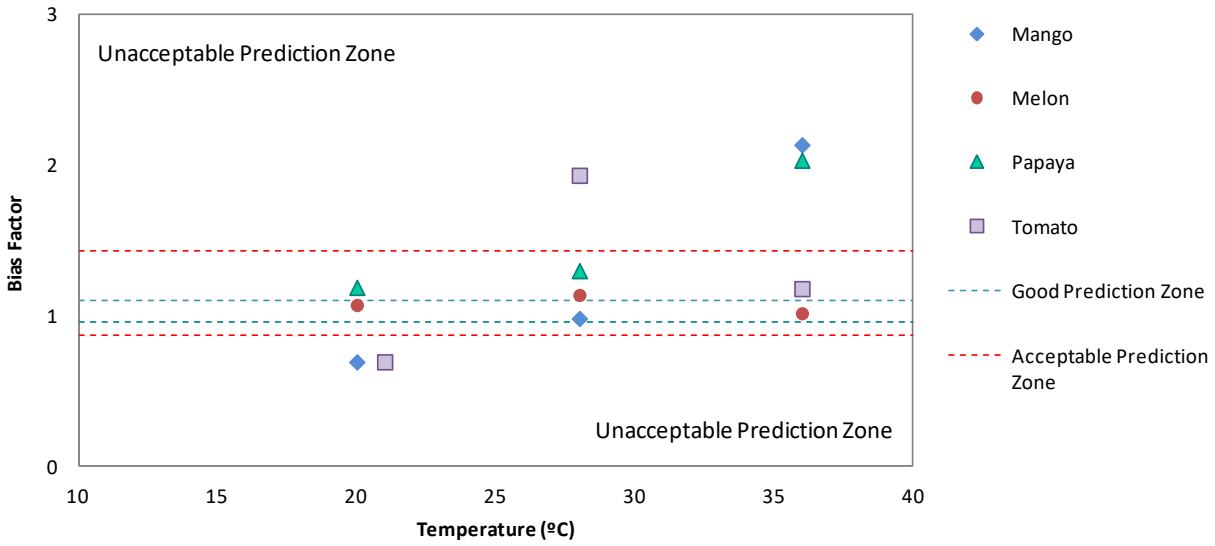

Figure 3 – Bias factor evaluated for each fruit pulp at 20ºC, 28ºC and 36ºC, showing a good prediction zone (between green lines), acceptable prediction zone (between red lines) and unacceptable prediction zone.

Considering the effect of the temperature, the results in Figure 3 have also shown that at lower temperature the model's predictions are in the safe failure zone (bias factor < 1), because the time to reject the product is shorter than that observed experimentally. On the other hand, at higher temperature the time to reject the product predicted by the mathematical model is greater than that observed experimentally. Thus, in this case the model's prediction is in the unsafe failure zone (bias factor >> 1), mainly for pulp of papaya and mango fruits.

4. Conclusion

This study has explored the mathematical model's applicability for predicting *B. fulva* growth in real foodstuffs. Specifically, the time to reject pulp of fruits contaminated by *B. fulva* spores was investigated from the perspective of mathematical modeling. The study has shown that the linear model was able to satisfactory describe the growth behavior of this fungus. The pH and the sugar content (glucose) of the substrate and the temperature have effect on the time to reject the food product contaminated by the spores of this fungus. At temperatures between 20ºC and 28ºC, which are within the typical range for microbial growth, variations in pH can significantly affect the growth rate of *B. fulva* in the product. This is because small changes in pH can impact the metabolism and reproduction rates of microorganisms. As a result, the time to reject the product was also affected by the pH in this range of temperature, as demonstrated in this study. At higher temperature, such as 36ºC, the impact of pH on the growth and development of *B. fulva* may be negligible because the temperature itself may have a greater effect on microbial growth, surpassing the impact of pH variation. So, at such temperature, the time to reject the product was not affected by the pH.

The performance of the Gibson-type model was satisfactory describing the effects of these variables in the time to reject the product, since the accuracy factor varied from 1.03 to 1.58 in the validation stage. At low temperature, low sugar content and high pH values, the model's prediction was very accurate.

The application of the proposed model to predict the time for visible growth of *B. fulva* in low acid fruit pulp revealed a good agreement with experimental data for melon pulp. For others fruit pulps (mango, papaya and tomato) the model's prediction should be treated with caution. The results have also shown that at lower temperature the model's predictions are in the safe failure zone, while at high temperature the model's predictions are in the unsafe failure zone. Thus, the authors understand that the proposed model has limitations when applied to predict the time to reject products at high temperatures. Therefore, further studies are suggested to improve prediction of the proposed model in such condition, including other intrinsic factors not evaluated in this work, such as water activity, acidity level and redox potential of fruit pulp.

Finally, the model proposed in this article can be applied in the process of development of products based on fruit pulp, in order to identify the best strategies to extend their shelf life, when the contamination by *B. fulva* spores is a relevant situation. In addition, this model can be integrated with optimization routines to identify optimal conditions to maximize the shelf life of products based on fruit pulp in terms of pH adjustment, sugar content and storage temperature.

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References

Alegbeleye, O., Odeyemi, O. A., Strateva, M., & Stratev, D. (2022) Microbial spoilage of vegetables, fruits and cereals. *Applied Food Research*, 2(1), 100122. https://doi.org/10.1016/j.afres.2022.100122.

Baert, K., Valero, A., De Meulenaer, B., Samapundo, S., Ahmed, M. M., Bo, L., Debevere, J., Devlieghere, F. (2007). Modeling the effect of temperature on the growth rate and lag phase of *Penicillium expansum* in apples. *International Journal of Food Microbiology*, 118(2), 139-150. https://doi: 10.1016/j.ijfoodmicro.2007.07.006.

Benevides, S. D., Ramos, A. M., Stringheta, P. C., Castro, V. C. (2008). Qualidade da manga e polpa da manga Ubá. *Ciência e Tecnologia de Alimentos*, 28(3), 571-578. https://doi.org/10.1590/S0101-20612008000300011.

Chapman, B., Winley, E., Fong, A. S. W., Hocking, A. D., Stewart, C. M., Buckle, K. A. (2007). Ascospore inactivation and germination by high pressure processing is affected by ascospore age. *Innovative Food Science and Emerging Technology*, 8(4), 531-534. https://doi.org/10.1016/j.ifset.2007.04.011.

Cuppers, H. G. A. M., Oomes, S., Brul, S. (1997). A model for combined effects of temperature and salt concentration on growth rate of food spoilage molds. *Applied Environmental Microbiology*, 63(10), 3764-3769. https://doi: 10.1128/aem.63.10.3764-3769.1997.

Dantigny, P. (2021). Applications of predictive modeling technique to fungal growth in foods. *Current Opinion in Food Science*, 38, 86-90. https://doi.org/10.1016/j.cofs.2020.10.028.

Dantigny, P., Guilmart, A., Bensoussan, M. (2005). Basis of predictive mycology. International *Journal of Food Microbiology*, 100(1-3), 187-196. https:// doi: 10.1016/j.ijfoodmicro.2004.10.013.

Ferreira, R. M. A., Lopes, W. A. R., Aroucha, E. M. M., Mano, N. C. S., Sousa, C. M. G. (2012). Caracterização física e química de híbridos de tomate em diferentes estádios de maturação produzidos em Baraúna, Rio Grande do Norte. *Revista Ceres*, 59(4), 506-511. https://doi.org/10.1590/S0034-737X2012000400011.

Ferreira, S. M. R., Quadros, D. A., Karkle, E. N. L., Lima, J. J., Tullio, L. T., Freitas, R. J. S. (2010). Qualidade pós-colheita do tomate de mesa convencional e orgânico. *Food Science and Technology*, 30(4), 858-864. https://doi.org/10.1590/S0101-20612010000400004.

Garcia, D., Ramos, A. J., Sanchis, V., Marín, S. (2009). Predicting mycotoxins in foods: a review. *Food Microbiology*, 26(8), 757-769. https://doi.org/10.1016/j.fm.2009.05.014

Gibson, A. M., Baranyi, J. I., Pitt, J. I., Eyles, M. J., Roberts, T. A. (1994). Predicting fungal growth: the effect of water activity on *Aspergillus flavus* and related species. *International Journal of Food Microbiology*, 23(3-4), 419-431. http:// doi:10.1016/0168-1605(94)90167-8.

Gibson, A. M.; Hockin, A. D. (1997). Advances in the predictive modeling of fungal growth in food. *Trends in Food Science & Technology*, 8(11), 353-358. https://doi.org/10.1016/S0924-2244(97)01065-0.

Giffel, M. C., Zwietering, M. H. (1999). Validation of predictive models describing the growth of *Listeria monocytogenes*. *International Journal of Food Microbiology*, 46(2), 135-149. http://doi: 10.1016/s0168-1605(98)00189-5.

Gougouli, M., Kalantzi, K., Beletsiotis, E., Koutsoumanis, K. P. (2011). Development and application of predictive models for fungal growth as tools to improve quality control in yogurt production. *Food Microbiology*, 28(8), 1453-1462. http:// doi: 10.1016/j.fm.2011.07.006

Huis In't Veld, J. H. J. (1996). Microbial and biochemical spoilage of foods: an overview. *International Journal of Food Microbiology*, 33(1), 1-18. https://doi.org/10.1016/0168-1605(96)01139-7

Kotzekidou, P. (1997). Heat resistance of *Byssochlamys nivea*, *Byssochlamys fulva* and *Neosartorya fischeri* isolated from canned tomato paste. *Journal of Food Science*, 62(2), 410-437. https://doi.org/10.1111/j.1365-2621.1997.tb04014.x

Lahlali, R., Serrhini, M. N., Friel, D., Jijakli, M. H. (2007). Predictive modelling of temperature and water activity (solutes) on the in vitro radial growth of *Botrytis cinerea* Pers. *International Journal of Food Microbiology*, 114(1), 1-9. https:// doi: 10.1016/j.ijfoodmicro.2006.11.004.

Lee, H. B., Magan, N. (2010). The influence of environmental factors on growth and interactions between *Embellisia allii* and *Fusarium oxysporum* f. sp. cepae isolated from garlic. *International Journal of Food Microbiology*, 138(3), 238-242. https://doi.org/10.1016/j.ijfoodmicro.2010.01.019

Mejholm, O., Gunvig, A., Borggaard, C., Blom-Hanssen, J., Mellefont, L., Ross, T., Leroi, F., Else, T., Visser, D., Dalgaard, P. (2010). Predicting growth rates and growth boundary of Listeria monocytogenes – An international validation study with focus on processed and ready-to-eat meat and seafood. *International Journal of Food Microbiology*, 141(3), 137-150. http://dx.doi.org/10.1016/j.ijfoodmicro.2010.04.026

Mousa, W., Ghazali, F. M., Jinap, S., Ghazali, H. M., Radu, S. (2011). Modelling the effect of water activity and temperature on growth rate and aflatoxin production by two isolates of *Aspergillus flavus* on paddy. *Journal of Applied Microbiology*, 111(5), 1262-1274. https:// doi: 10.1111/j.1365-2672.2011.05134.x.

Panagou, E. Z., Chelonas, S., Chatzipavlidis, I., Nychas, G. E. (2010). Modelling the effect of temperature and water activity on the growth rate and growth/no growth interface of *Byssochlamys fulva* and *Byssochlamys nivea*. *Food Microbiology*, 27(5), 618-627. https://doi: 10.1016/j.fm.2010.02.005.

Panagou, E. Z., Kodogiannis, V., Nychans, G. J. E. (2007). Modelling fungal growth using radial basis function neural networks: the case of the ascomycetous fungus *Monascus ruber* van Tieghem. *International Journal of Food Microbiology*, 117(3), 276-286. https://doi.org/10.1016/j.ijfoodmicro.2007.03.010

Panagou, E. Z., Skandamis, P. N., Nychans, G. J. E. (2003). Modelling the combined effect of temperature, pH and Aw on the growth rate of *Monascus ruber*, a heat-resistant fungus isolated from green table olives. *Journal of Applied Microbiology*, 94(1), 146-156. https://doi: 10.1046/j.1365-2672.2003.01818.x.

Parra, R., Magan, N. (2004). Modelling the effect of temperature and water activity on growth of *Aspergillus niger* strains and applications for food spoilage moulds. *Journal of Applied Microbiology*, 97(2), 429-438. https://doi: 10.1111/j.1365-2672.2004.02320.x.

Pizarro, C. A. C., Benedetti, B. C., Haj-Isa, N. M. A. (2006). Avaliação de melão minimamente processado armazenado em diferentes temperaturas e embalagens. *Food Science and Technology*, 26(2), 246-252. https://doi.org/10.1590/S0101-20612006000200003

Ross, T., McMeekin, T. A. (1994). Predictive microbiology. *International Journal of Food Microbiology*, 23(3-4), 241-264. https:// doi: 10.1016/0168-1605(94)90155-4.

Salomão, B. C. M., Muller, C., Massaguer, P. R., Aragão, G. M. F. (2011) Aplicação do dicloroisocianurato de sódio e ácido peracético para redução de esporos de Penicillium expansum, Byssochlamys fulva e Alicyclobacillus acidoterrestris na superfície de maçãs e em soluções aquosas. *Alimentos e Nutrição*, 22(2), 219-230. https://repositorio.ufrn.br/handle/123456789/32327.

Samapundo, S., Devlieghere, F., De Meulenaer, B., Geeraerd, A. H., Van Impe, J. F., Debevere, J. M. (2005). Predictive modelling of the individual and combined effect of water activity and temperature on the radial growth of *Fusarium verticilliodes* and *F. proliferatum* on corn. *International Journal of Food Microbiology*, 105(1), 35-52. https:// doi:10.1016/j.ijfoodmicro.2005.06.007.

Sant'Ana, A. S., Simas, R. C., Almeida, C. A. A., Cabral, E. C., Rauber, R. H., Mallmann, C. A., Eberlin, M. N., Rosenthal, A., Massaguer, P. R. (2010a). Influence of package, type of apple juice and temperature on the production of patulin by *Byssochlamys nivea* and *Byssochlamys fulva*. *International Journal of Food Microbiology*, 142(1-2), 156-163. https:// doi:10.1016/j.ijfoodmicro.2010.06.017.

Sant'Ana, A. S., Dantigny, P., Tahara, A. C., Rosenthal, A., Massaguer, P. R. 2010b. Use of a logistic model to assess spoilage by *Byssochlamys fulva* in clarified apple juice. *International Journal of Food Microbiology*, 137(2-3), 299-302. https:// doi:10.1016/j.ijfoodmicro.2009.11.029.

Santana, L. R. R., Matsuura, F. C. A. U., Cardoso, R. L. (2003). Genótipos melhorados de mamão (*Carica papaya* L.): avaliação tecnológica dos frutos na forma de sorvete. *Food Science and Technology*, 23(suppl), 151-155. https://doi.org/10.1590/S0101-20612003000400028.

Santos, J. L. P., Samapundo, S., Van Impe, J., Sant'Ana, A. S., Devlieghere, F. (2020). Effect of sugar concentration (ºBrix) and storage temperature on the time to visible growth of individual ascospores of six heat-resistant moulds isolated from fruit products. *Food Control*, 108, 106880. https://doi.org/10.1016/j.foodcont.2019.106880.

Shinagawa, F. B. (2009). *Avaliação das características bioquímicas da polpa de mamão (Carica papaya L.) processada por alta pressão hidrostática*. Dissertação de Mestrado, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brasil.

Silva, A. R., Sant'Ana, A. S., Massaguer, P. R. (2010). Modelling the lag time and growth rate of *Aspergillus* section Nigri IOC 4573 in mango nectar as a function of temperature and pH. *Journal of Applied Microbiology*, 109(3), 1105-1116. https://doi:10.1111/j.1365-2672.2010.04803.x.

Silva, P. R. S., Tessaro, I. C., Marczak, L. D. F. (2013). Integrating a kinetic microbial model with a heat transfer model to predict *Byssochlamys fulva* growth in refrigerated papaya pulp. *Journal of Food Engineering*, 118(3), 279-288. https://doi.org/10.1016/j.jfoodeng.2013.04.014

Silva, P. R. S., Tessaro, I. C., Marczak, L. D. F. (2014). Modeling and simulation of *Byssochlamys fulva* growth on papaya pulp subjected to evaporative cooling. *Chemical Engineering Science*, 114, 134-143. https://doi.org/10.1016/j.ces.2014.04.010

Swinnen, I. A. M., Bernaerts, K., Dens, E. J. J., Geeraerd, A. H., Van Impe, J. F. (2004). Predictive modeling of the microbial lag phase: a review. *International Journal of Food Microbiology*, 94(2), 137-159. https:// doi: 10.1016/j.ijfoodmicro.2004.01.006.

Taniwaki, M. H., Hocking, A. D., Pitt, J. I., Fleet, G. H. (2010). Growth and mycotoxin production by fungi in atmospheres containing 80% carbon dioxide and 20% oxygen. *International Journal of Food Microbiology*, 143(3), 218-225. https://doi: 10.1016/j.ijfoodmicro.2010.08.030.

Tassou, C. C., Panagou, E. Z., Natskoulis, P., Magan, N. (2007). Modelling the effect of temperature and water activity on the growth of two ochratoxigenic strains of *Aspergillus carbonarius* from Greek wine grapes. *Journal of Applied Microbiology*, 103(6), 2267-2276. https://doi: 10.1111/j.1365-2672.2007.03480.x.

Tremarin, A.; Aragão, G. M. F.; Salomão, B. C. M.; Brandão, T. R.; Silva, C. L. M. (2017). Modeling the soluble solids and storage temperature effects on *Byssochlamys fulva* growth in apple juice. *Food Bioprocess Technology*, 10, 720-729. https://doi 10.1007/s11947-016-1854-3.

Valík, L., Piecková, E. (2001). Growth modelling of heat-resistant fungi: the effect of water activity. *International Journal of Food Microbiology*, 63(1-2), 11-17. https://doi: 10.1016/s0168-1605(00)00386-x.

Wang, Y., Shan, T., Yuan, Y., Zhang, Z., Guo, C. Yue, T. (2017). Evaluation of *Penicillium expansum* growth, patulin accumulation, nonvolatile compounds and volatile profile in kiwi juices of different cultivars. *Food Chemistry*, 228, 211-218. https://doi: 10.1016/j.foodchem.2017.01.086.

Welke, J. E., Hoeltz, M., Dottori, H. A., Noll, I. B. (2009). Ocorrência de fungos termorresistentes em suco de maçã. *Brazillian Journal of Food Technology*. II SSA, 78-83. http://bjft.ital.sp.gov.br/especiais/especial_2009/v11_edesp_14.pdf

Whiting, R. C., Buchanan, R. L. (1993). A classification of models in predictive microbiology – a reply to K. R. Davey. *Food Microbiology*, 10(2), 175-177.