Thermal inactivation of *Byssochlamys nivea* in pineapple juice combined with preliminary high pressure treatments

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ABSTRACT

Byssochlamys nivea is a thermal resistant filamentous fungi and potential micotoxin producer. Recent studies have verified the presence of ascospores of such microorganism in samples of pineapple nectars and juices. Although the majority of filamentous fungi have limited heat resistance and are easily destroyed by heat, *Byssochlamys nivea* ascospores have shown high thermal resistance. The aim of this work was to evaluate the application of linear and Weibull models on thermal inactivation (70, 80 and 90ºC) of *Byssochlamys nivea* ascospores in pineapple juice after pretreatment with high pressure (550MPa or 650MPa during 15min). Following the treatments, survival curves were built up for each processing temperature and adjusted for both models.

It was observed that the survival curves of *B. nivea* ascospores at 70°C after both previous treatment and at 80°C after 550MPa for 15 minutes fitted well in linear and Weibull models. In the others treatments, the Weibull model showed better fit. On 90ºC ascospores inactivation (control), without previously high pressure treatment, the Weibull model also showed a better adjustment, presenting a larger R^2 and a smaller RMSE. In the others controls treatments (70 and 80ºC without previously high pressure treatment), it was verified the activation of *B. nivea* ascospores, then it was not possible to adjust the models. Thus, a 5-log reduction (t_5) treatment, as recommended by the Food and Drug Administration, on *Byssochlamys nivea* ascospores in pineapple juice was observed in this work at 90ºC/15.38min with previously high pressure treatment of 650MPa for 15min.

Keywords: Byssochlamys nivea; thermal inactivation; pineapple juice.

INTRODUCTION

Previous study focusing on fruit juices and nectars regarding microbiology safety along industrial production lines showed contamination by thermal resistance moulds even in products after pasteurization and packaging. *Byssshochlamys nivea* has proved to be the most thermal resistance mould isolated from pineapple products in that study. Such a mould has the capacity of growing in the product during storage, leading to its deterioration and risk for the consumers.

The few *Byssshochlamys* species that have thermal resistance as a characteristic produce resistant spores, named ascospores. Most food deterioration associated to such species is due to the survival of ascopores to pasteurization process [1]. Thermal resistance is attributed to the presence of sexual spores (ascospores) that have great resistance to pH variation, presence of sugar, fat acids, etc [2].

Another worrying factor is the toxin potential production by those thermal resistance moulds, mainly in fruit derivate products. The generous *Byssochlamys* can produce patulin, byssochlamic acid, bissotoxin A, assymetrin and variotin. Those composts can act in the central neural system leading to trembling, convulsions and dead in animals [2]. Thus, thermal resistant moulds of such specimens can be considered a potential serious hazard for food safety.

Studies have shown that thermal resistant moulds are able to survive either to heat or to pressure processes. Furthermore, under certain conditions both heat and pressure individually can activate the ascospores for further germination [3,4]. Activation is related to damage to cell wall without cell inactivation [3]. Based on such aspects, this studied has aimed at evaluating thermal resistance of *B. nivea* inoculated in pineapple juice following pressure treatment. It has also aimed at evaluate linear and Weibull models to describe the thermal

inactivation patterns under different operational conditions of pressure (550MPa or 650MPa during 15min) and temperature (70, 80 and 90ºC) in subsequent treatments.
Weibull model has described non-linear inactivation of several microorganisms under different experimental conditions [5,6,7,8,9,10,11,12,13,14] and linear model has been used in some studies in comparative terms [10, 11, 13, 15] in thermal resistan moulds survival curves.

MATERIALS & METHODS

***Byssochlamys nivea*:** *B. nivea* strain originated from microorganisms collection of the University of Santa Catarina (UFSC). The strain was isolated from strawberry pulp by Aragão [16]. The mould was inoculated directly in the juice samples aiming at resulting in a concentration of 10^5-10^6 ascospores/mL.

Pineapple Juice: Pineapple juice commercialized in local market (Portugal) was used in the study and soluble solids analyses resulted in 12ºBrix.

Thermal Inactivation Combined with Preliminary High Pressure Treatments: For the preliminary high pressure treatment applied to the inoculated pineapple juice previously to thermal treatment the following conditions were used: 550 e 650MPa for 15 minutes. Initial temperature of high pressure treatment was set at 20 ºC. Samples inoculated with the mould (15mL) were inserted in sterilized polyethylene bags and pressurized. After on, 10mL of the sample were transferred to sterile eppendorf tubes and immersed in thermostatic baths, adjusted to the following temperatures: 70, 80 e 90ºC for 0, 5, 10, 15, 20 e 25 minutes. Following the thermal treatment, tubes containing samples were immediately cooled down in ice bath and aseptically opened. Serial dilution and pour plating were then carried out, using double concentrated Malt Extract Agar (20mL) added with rose bengal (0,25%), followed by homogenization. After mixture solidification, the plates were inoculated at 30ºC for 7 days. Analyses were done in duplicate.
Survival thermal curves were built up using the microbial counting resulting for each temperature and treatment time. Weibull and linear models were used to describe mould inactivation in pineapple juice using Statistica 8.0 program.

Linear Model: For the application of such model it was assumed that microbial inactivation at constant temperature follows 1st order kinetics, based on the following equation:

$$\log_{10} S(t) = -\frac{t}{D} \qquad (t \geq 0) \tag{1}$$

In which: $S(t) = \frac{N}{N_0}$; t = time (min); D = time for decimal reduction (min).

According to such a model, all population cells have the same probability of death [8,10]. The plot $\log_{10} S(t)$ *versus* time (min) will be linear allowing determination of D-value.
D-vaule is the time of decimal reduction being defined as the time required to decrease in 90% microbial population at a fixed temperature. When microbial population is represented in semi-log coordinates, D-value is the time required for reducing one logarithmic order the number of microorganisms. D does not depend on the initial population, considering it only relates to the linear inclination. Exposition of microbial population to higher temperatures lead to a decrease in D-value [17].

Modelo de Weibull: Weibull model assume that cell or spore population have different resistance and survival cell is a cumulative form of letal distribution. In terms of survival, a cumulative distribution form of Weibull can be written by Equation 2, as follows:

$$S(t) = \exp\left(-\left(\frac{t}{\alpha}\right)^{\beta}\right) \tag{2}$$

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$$\log_{10} S(t) = -\frac{1}{2.303}.\left(\frac{t}{\alpha}\right)^{\beta} \tag{3}$$

In which: $S(t) = \frac{N}{N_0}$; t = time (min); α and β are distribution parameters: α is named scale parameter, whose unit is min or sec; and β is named form parameter, used as a behavior index.

However, many authors [7, 8, 10, 18] may rather write down Equation 2 as follows (Equation 4):

$$\log_{10} S(t) = -b.t^n \quad (4)$$

In which: $n = \beta$ and $b = \frac{1}{2.303}.\alpha^{-n}$ (min^{-1} ou sec^{-1})

The model represented in Equation 4 shows as the main advantage the simplicity and is sufficiently robust to describe survival curves that present shoulders (concave), where n > 1, and curves with tails (convexes), where < 1. Concave curves (n > 1) indicate that cumulative damage result in the increase of cell sensitiveness, and the convexes (n < 1) show higher resistance or ability of microorganism for adapting to a stressing treatment. When n = 1, the model is linear [6, 9, 10, 15].

In Weibull model, t_d is the time required to decrease 1 logarithmic cycle $\log_{10}$ of microbial population. The value t_1, related to the primary reduction, is analogous to D-value in the linear model. t_d can be determined by Equation 5.

$$t_d = \left(\frac{d}{b}\right)^{1/n} \quad (5)$$

In which: d = number of reductions in initial population.

D-values higher than 2 determines cumulative time of the process.

The time of thermal treatment will be the one related to the model that better fit to the survival curve of target microorganism, in the case of the present study the *B. nivea* ascospores.

Parameter models were adjusted by minimum square method usinh Statidtica 8.0 program.

RESULTS & DISCUSSION

Figure 1 shows survival curves for *B. nivea* ascospores after treatment at 550 e 650 MPa for 15 min. adjusted using Linear and Weibull. Table 1 presents determinant coefficients (R^2) and mean square of residues (MQ_E) for each model and for each treatment. Models presented residues with normal distribution.

As it can be seen from Table 1, survival curves of *B. nivea* ascospores at 70°C after both previous treatment and at 80°C after 550MPa for 15 minutes fitted well in linear and Weibull models. In the others treatments, the Weibull model showed better fit. On 90ºC ascospores inactivation (control), without previously high pressure treatment, the Weibull model also showed a better adjustment, presenting a larger R^2 and a smaller RMSE. In the others controls treatments (70 and 80ºC without previously high pressure treatment), it was verified the activation of *B. nivea* ascospores, then it was not possible to adjust the models. Table 2 shows resulting parameters for Linear model (D-value) and for Weibull (b e n) for each treatment applied to the mould.

The parameter of form (n) varied proportionally with the temperature in both previous high pressure treatment (Table 2). D-value from Linear model was significant for all survival curves, while the parameters of Weibull model were significant for some of the treatments (in bold). All parameters presented low standard-deviation, assuring in that way the repeatability. .

According to Figure 1 by carryng out previous pressure treatment at 650 MPa for 15 min. thermal resistance higher resistance to inactivation was verified for the *B. nivea* ascospores inoculated in pineapple juice at 70 and 80ºC, or possibly a higher capacity of the spores to adapt to the treatment (tail formation n < 1). On the other hand, in both treatment at 90ºC cumulative damage resulted in a higher ascospores sensitiveness (shoulder formation n > 1). In the survival curves after high pressure treatment at 550 MPa/ 15 min. tail formation at 70 and 80ºC was observed, and both almost a linear behavior was observed (Figure 1 and Table 2).

By utilizing b and n values (Table 2) t_1 and t_5 values were determined using Equation 5. In Table 3, t_1 and t_5 values related to Weibull model are presented, as well as D and 5D associated to linear model.

In this sense, after pressure application at 550MPa/15min. it will be necessary thermal treatment for 16.50min. on the juice to obtain 5 logarithmic reductions in *B. nivea* ascospores population, while with preliminary treatment at 650MPa for 15min 15.38min of heat treatment would be required. Preliminary high pressure treatment contributes to ascospores inactivation at 90ºC and avoided activation in treatments at 70 and 80ºC (data not show).

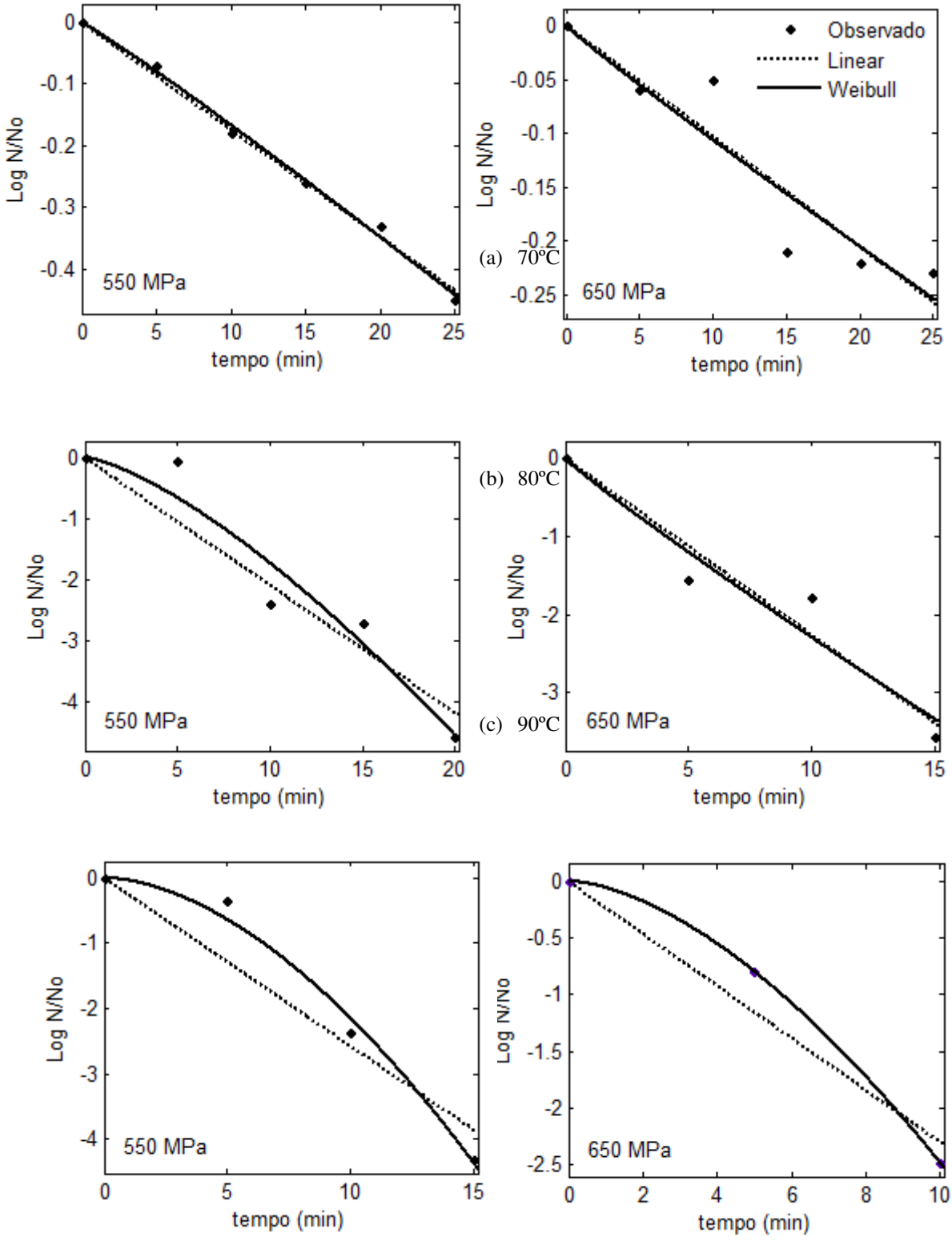

Figure 1: Survival curves for *B. nivea* ascospores after treatment at 550 MPa for 15 min and 650 MPa for 15 min estimated by linear and Weibull models in pineapple juice at 70ºC (a), 80ºC (b) and 90ºC (c).

Table 1 Determinant coefficients (R^2) and residues square mean (MQ_E) from survival curve of *B. nivea* ascospores inoculated in pineapple juice fitted using linear and Weibull models, after treatment at 550 MPa for 15 minutes and 650 MPa for 15 minutes

Ascospores of *B. nivea* in pineapple juice								
	After treatment at 550 MPa / 15 min				After treatment at 650 MPa / 15 min			
	Weibull Model		Linear Model		Weibull Model		Linear Model	
	R^2	MQ_E	R^2	MQ_E	R^2	MQ_E	R^2	MQ_E
Temperatures								
70ºC	0,99	$6,4.10^{-4}$	0,99	$7,9.10^{-4}$	0,87	0,0068	0,87	0,0069
80ºC	0,94	0,90	0,91	1,39	0,93	0,43	0,93	0,44
90ºC	0,99	0,12	0,91	1,11	0,99	$8,3.10^{-6}$	0,95	0,16

Table 2: Parameters for linear and Weibull model and survival curve of *B. nivea* ascosporos in pineapple juice after treatment at 550 MPa for 15 minutes and 650 MPa for 15 minutes

Ascospores of *B. nivea* in pineapple juice						
	After treatment at 550 MPa / 15 min			After treatment at 650 MPa / 15 min		
	Weibull Model		Linear Model	Weibull Model		Linear Model
	n ± DP	b ± DP	D ± DP	n ± DP	b ± DP	D ± DP
Temperatures						
70ºC	1,06±0,063	0,014±0,0027	57,47±1,12	0,94±0,32	0,012±0,011	98,38±9,68
p-level	***0,0001***	***0,0060***	***0,0000***	***0,0443***	*0,3604*	***0,0001***
80ºC	1,07±0,28	0,16±0,13	5,08±0,39	0,93±0,049	0,27±0,03	4,43±0,49
p-level	***0,0179***	*0,2958*	***0,0000***	*0,3072*	*0,5649*	***0,0122***
90ºC	1,76±0,25	0,037±0,02	3,89±0,49	1,65±0,0055	$0,055±6,9.10^{-4}$	4,34±0,48
p-level	***0,0190***	*0,2621*	***0,0042***	***0,0021***	***0,0079***	***0,0120***

Table 3: t_1 e t_5 values (Weibull modelo) and D e 5D (Linear modelo) determined for *B. nivea* ascóspores in pieapple juice at 70, 80 and 90ºC after treatment at 550 MPa for 15 minutes and 650 MPa for 15 minutes

Ascospores of *B. nivea* in pineapple juice								
	Afetr treatment at 550 MPa / 15 min				After treatament at 650 MPa / 15 min			
	Weibull Model		Linear Model		Weibull Model		Linear Model	
	t_1(min)	t_5(min)	D(min)	5D(min)	t_1(min)	t_5(min)	D(min)	5D(min)
Temperatures								
70ºC	56,06	256,06	57,47	287,35	110,51	612,36	98,38	491,90
80ºC	5,54	24,95	5,08	24,40	4,09	23,07	4,43	22,15
90ºC	6,61	**16,50**	3,89	19,45	5,80	**15,38**	4,34	21,7

CONCLUSION

Preliminary pressure treatment contributed to *B. nivea* asospores inactivation in pineapple juice at 90ºC and avoid inactivation and 70 and 80ºC. Weibull model fitted better for the most applied treatments. It was required 16.50min at 90ºC after treatment at 550MPa/15min and 15.38min after 650MPa/15 min., in order to obtain 5 log-reduction, as recommended by FDA [19]. However, considering the long time required for inactivation, implying low process efficiency, high energy demand an prejudice to quality aspects such as sensory attributes, further studies are necessary to improve the process aiming at having a industrial application.

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