THERMAL INACTIVATION OF *BYSSOCHLAMYS NIVEA* IN PINEAPPLE NECTAR 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. 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 nectar 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 survival curves at 90°C after high pressure pretreatment at 550 MPa/15 min did not fit well to linear and Weibull models. For all the other treatments, the Weibull model presented a better fit. At 90ºC without pressure treatment, the Weibull model also showed a better adjustment, having a larger R^2 and a smaller RMSE. Regarding the process effectiveness, a 5-log reduction (t_5), as recommended for pasteurization, was only achieved for *Byssochlamys nivea* ascospores presented in pineapple nectar at 90ºC/10.7 min with previous high pressure treatment of 650 MPa for 15 min. Considering the high intensity and energy demanding process with possibly product damage, other preventive and alternative treatments are being investigated.

Keywords: *Byssochlamys nivea*. thermal inactivation. pineapple nectar.

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. *Bysshochlamys 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, depending on storage and packaging characteristics, leading to its deterioration and risk for the consumers.

The few *Bysshochlamys* species that have thermal resistance as a characteristic produce resistant spores, named ascospores. Most food deterioration associated to such species is because of the survival of ascopores to pasteurization process (SPLITTSTOESSER, 1991). Thermal resistance is attributed to the presence of sexual spores (ascospores) that have great resistance to pH variation, presence of sugar, fat acids, etc. (TOURNAS, 1994).

Another worrisome factor is the potential production of toxin by those thermal resistance moulds, mainly in fruit derivate products. The generous *Byssochlamys* can produce patulin, byssochlamic acid, bissotoxin A, assymetrin and variotin. Those compounds can act in the central neural system leading to trembling, convulsions and death in animals (TOURNAS, 1994). 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 (DIJKSTREHUIS and TEUNISSE, 2004; EICHER and LUDWIG, 2002). Activation is related to damage to cell wall without cell inactivation (DIJKSTREHUIS and TEUNISSE, 2004). Based on such aspects, this study has aimed at evaluating thermal resistance of *B. nivea* inoculated in pineapple juice following pressure treatment. It has also aimed at evaluating 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 (MAFART *et al.,* 2002; MARTINUS and VAN BOEKEL, 2002; BUZRUL and ALPAS, 2004; BUZRUL *et al.*, 2005; ALBERT and MAFART, 2004; BUZRUL and ALPAS, 2007; CHEN, 2007; ARAGÃO *et al.*, 2007; HUANG, 2009; SANT'ANA *et al.*, 2009) and linear model has been used in some studies in comparative terms (HUANG, 2009; CHEN, 2007; BUZRUL and ALPAS, 2007; CHEN and HOOVER, 2004) in the adjustment of thermal resistant moulds survival curves.

MATERIAL AND 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 (1989). The mould was inoculated directly in juice samples aiming at resulting in a concentration of 10^5-10^6 ascospores/mL.

Pineapple Nectar Pineapple nectar 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 nectar previously submitted to thermal treatment, the following conditions were used: 550 e 650MPa for 15 minutes. Initial temperature of high pressure treatment was set at 20ºC. Samples inoculated with mould (15 mL) were inserted in sterilized polyethylene bags and

pressurized. After on, 10 mL 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 results for each temperature and treatment time. Weibull and linear models were used to describe mould inactivation in pineapple nectar by using *Statistica* 8.0 program.

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

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

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

According to such a model, all population cells have the same probability of death (BUZRUL *et al*, 2005; BUZRUL e ALPAS, 2007). The plot $\log_{10}$ S(t) *versus* time (min) will be linear allowing determination of D-value.

D-value 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 leads to a decrease in D-value (SINGH & HELDMAN, 1995).

Weibull Model Weibull model assumes 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:

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

and

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

In which: $S(t) = \dfrac{N}{N_0}$; 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 (BUZRUL and ALPAS, 2007; BUZRUL and ALPAS, 2004; BUZRUL *et al.*, 2005; PELEG, 1999) may rather write down Equation 2 as follows (Equation 4):

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

in which: $n = \beta$ and $b = \dfrac{1}{2.303}.\alpha^{-n} \,(\text{min}^{-1} \text{ or } \text{sec}^{-1})$

The model is very simple and sufficiently robust to describe survival curves that present shoulders (concave), where n > 1, and curves with tails (convex), where < 1. Concave curves (n > 1) indicate that cumulative damage results in the increase of cell sensitiveness, and the convex curves (n < 1) show higher resistance or ability of microorganism for adapting to a stressing treatment. When n = 1, the model is linear (BUZRUL and ALPAS, 2007; ALBERT and MAFART, 2005; CHEN and HOOVER, 2004; MARTINUS and VAN BOEKEL, 2002)

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.

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

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

D-values higher than 2 determine 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, i.e., *B. nivea* ascospores.

Parameter models were adjusted by the least squares method using *Statistica* 8.0 program.

RESULTS AND DISCUSSION Figure 1 shows survival curves, adjusted by Linear and Weibull models, for *B. nivea* ascospores after treatment at 550 e 650 MPa for 15 min. Table 1 presents R-squared values and error mean square (MS_E) for each model and for each treatment. Models presented residues with normal distribution.

As it can be seen from Table 1, for all treatments after treatment at 550MPa/15 min, Weibull model showed better fit than linear model, and the same was verified for survival curves of *B. nivea* ascospores at 70 and 80ºC, after 650 MPa/ 15 min (graph b of Figure

1). Table 2 shows resulting parameters for Linear model (D-value) and for Weibull (b and n) for each treatment applied to the mould.

The parameter of form (n) did not vary proportionally with the temperature when previous high pressure treatment at 650 MPa for 15 min (Table 2) was used, as observed by Sant'Ana *et al.* (2009). D-value from Linear model was statistically significant for all survival curves, while the parameters of Weibull model were statistically significant for some of the treatments (in bold). All parameters presented low standard deviation, showing then repeatability.

According to Figure 1 by carrying out previous pressure treatment at 550 MPa for 15 min, thermal resistance higher resistance to inactivation was verified for the *B. nivea* ascospores inoculated in pineapple nectar at 70ºC, or possibly a higher capacity of the spores to adapt to the treatment (tail formation n < 1). On the other hand, at 80 and 90ºC, cumulative damage resulted in a higher ascospores sensitiveness (shoulder formation n > 1). In the survival curves after high pressure treatment at 650 MPa/15 min, it was observed a tail formation at 70 and 90ºC; at 80ºC, almost a linear behavior was observed (n = 1.024 ± 0.15) (Figure 1).

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 a pressure application at 550 MPa/15 min, it will be necessary a thermal treatment for 18.75 min on the nectar in order to obtain 5 logarithmic reductions in *B. nivea* ascospores population, while with preliminary treatment at 650 MPa for 15 min, 18.69 min 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 shown).
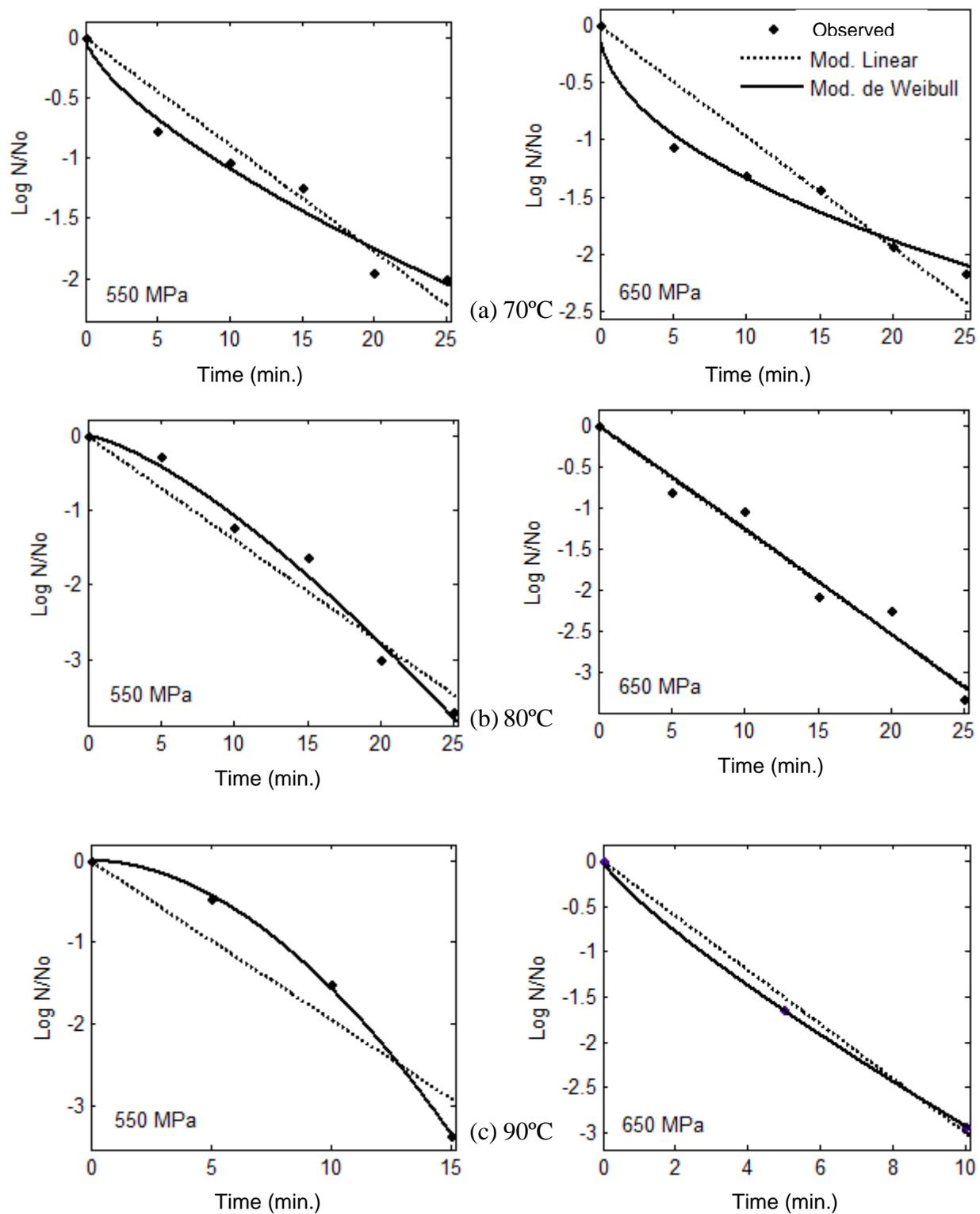
Figure 1: Survival curves for *B. nivea* ascospores, after treatment at 550 MPa for 15 min and 650 MPa for 15 min, estimated by linear and Weibull models in pineapple nectar at 70ºC (a) 80ºC (b) and 90ºC (c).

Table 1 R^2 and error mean square (MS_E) from survival curve of *B. nivea* ascospores inoculated in pineapple nectar fitted by 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 nectar							
	After treatment at 550 MPa/15 min				After treatment at 650 MPa/15 min			
	Weibull Model		Linear Model		Weibull Model		Linear Model	
Temperatures	R^2	MS_E	R^2	MS_E	R^2	MS_E	R^2	MS_E
70ºC	0.97	0.088	0.92	0.21	0.98	0.055	0.83	0.50
80ºC	0.99	0.15	0.95	0.50	0.97	0.21	0.97	0.21
90ºC	0.99	0.0039	0.90	0.64	0.99	3.4×10^{-7}	0.99	0.028

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

	Ascospores of *B. nivea* in pineapple nectar					
	After treatment at 550 MPa/15 min			After treatment at 650 MPa/15 min		
	Weibull Model		Linear Model	Weibull Model		Linear Model
Temperatures	n ± SD	b ± SD	D ± SD	n ± SD	b ± SD	D ± SD
70ºC	0.69±0.073	0.22±0.073	11.27±0.71	0.50±0.073	0.42±0.0088	10.34±0.91
p-level	*0.0037*	*0.0395*	*0.0000*	*0.0024*	*0.0085*	*0.0000*
80ºC	1.39±0.14	0.043±0.0019	7.20±0.44	1.024±0.15	0.12±0.054	7.88±0.34
p-level	*0.0006*	*0.0878*	*0.0000*	*0.0026*	*0.0942*	*0.0000*
90ºC	1.90±0.062	0.019±0.0032	5.14±0.65	0.83±5.8×10^{-4}	0.44±5.5×10^{-4}	3.33±0.12
p-level	*0.0011*	*0.0258*	*0.0000*	*0.0004*	*0.0008*	*0.0013*

Table 3. t_1 e t_5 values (Weibull model) and D e 5D (Linear model) determined for *B. nivea* ascospores in pineapple nectar and integral 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 nectar							
	Afetr treatment at 550 MPa/15 min				After treatment at 650 MPa/15 min			
	Weibull Model		Linear Model		Weibull Model		Linear Model	
Temperatures	t_1(min)	t_5(min)	D(min)	5D(min)	t_1(min)	t_5(min)	D(min)	5D(min)
70ºC	8.97	92.47	11.27	56.35	5.67	141.72	10.34	51.70
80ºC	9.62	30.61	7.20	36.00	7.92	38.18	7.88	39.40
90ºC	8.05	18.78	5.14	25.70	2.69	18.69	3.33	16.65

CONCLUSION Preliminary pressure treatment contributed to *B. nivea* ascospores inactivation in pineapple nectar at 90ºC and avoided inactivation at 70 and 80ºC. Weibull model fitted better all applied treatments. It was required 18.75 min at 90ºC, after treatment at 550MPa/15min and 18.69 min after 650MPa/15 min, to obtain 5 log-reduction, as recommended by FDA (2001). However, the long time required for inactivation implies in low process efficiency and high energy demands, and may also compromise quality aspects such as nutritional value and sensory attributes. Further studies are necessary to improve the process with the goal of industrial applications.

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