BEHAVIOUR OF *Neosartorya fischeri* ASCOSPORES IN PINEAPPLE JUICE

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For heat-resistance determinations, a *Neosartorya fischeri* ascospore suspension, obtained at three different conditions of temperature (20, 30 and 35°C) and age (1, 2 and 3 months), was heat treated in pineapple juice with three different ratio conditions. The 1/k (min) values obtained were statistically analysed through the software Statistica 6.0. At 80°C, when there was a level change in the ratio factor from a lower (10) to a higher one (38), the thermal resistance (1/k) increased in approximately 41%. The increase of the ascospore production age and temperature caused an increase on the thermal resistance approximately equal to 28% and 14%, respectively. Through the results, it could be concluded that *N.fischeri* presented lower thermal resistance for heating mediums with lower ratio values, or rather, the increase of the soluble solids concentration caused an increase of its thermal resistance. It was also observed that the ascospore production age and temperature increased significantly the thermal resistance of this mould in pineapple juice.

KEY-WORDS: RATIO; HEAT-RESISTANCE; Neosartorya fischeri; PINEAPPLE JUICE.

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1 INTRODUCTION

The variety and quantity of fruits juices available to the consumer continue to increase. New process and preservation methods have resulted in an improvement of the quality of these products and increased the consumer demand. Deterioration of these juices may be due to microbiological spoilage as well as chemical or physical spoilage. Fruits and their products, such as juices, concentrates and canned fruits, are highly susceptible to yeast and fungal spoilage (PENÃ, FARIA and MASSAGUER, 2004). The study of thermal resistant mould is of extreme importance since it has been frequently implied in the deterioration of these juices, mainly due to their ability to survive at normal heat treatment applied in the juice industry (RAJASHEKLARA, SURESH and ETHIRAJ, 2000).

According to MISLIVEC, BEUCHAT and COUSIN (2001), due to their ability of adaptation to the different conditions, moulds and yeasts are frequently found as contaminants in foods, in equipments that were not adequately sanitized, as well as sub processed foods.

Ascospores of moulds belonging to the genus *Neosartorya, Byssochlamys, Talaromyces* and *Eupenicillium* can often survive to commercial thermal treatment, grow and cause spoilage (KOTZEKIDOU, 1997; RAJASHEKLARA, SURESH and ETHIRAJ, 2000; SALOMÃO et al., 2004). These heat resistant moulds are also known to produce various mycotoxins during their growth in fruit products and therefore are a major concern to public health (BEUCHAT, 1988; RAJASHEKLARA, SURESH and ETHIRAJ, 2000). Several heat resistant fungi has been reported as capable to survive to temperatures of 90°C and/or greater for several minutes, depending on the species and/or product (PIECKOVÁ and SAMSON, 2000).

There are many factors that contribute to the survival and to the increasement of the thermal resistance of these moulds, as the presence of organic acids, soluble solids content, types of heating mediums, addition of preservatives and the time and the temperature of ascospore production. RAJASHEKLARA, SURESH and ETHIRAJ (1996), in research carried out with *N.fischeri* ascospores using mango and grape juices as heating mediums at different contents of soluble solids (10°Brix for dilluted juice and 45°Brix for concentrated juice), verified that the thermal resistance of this mould became higher in concentrated juices. This mould is able to cause fruit disintegration by its ability to produce various pectinolytic and disintegrative enzymes (UGWUANYI and OBETA, 1999).

Even though heat processing with a varying temperature-time schedule is a critical unit operation, chemical measures such as use of acidulants and preservatives are also utilized to achieve commercial sterility in food products. The thermal tolerance extent of ascospores varies from strain to strain and with the heating medium composition (RAJASHEKLARA, SURESH and ETHIRAJ, 1996; KOTZEKIDOU, 1997).

The influence of different factors on the *N. fischeri* ascospores thermal resistance using experimental planning was not found in the literature. The present research main objective was to study the influence of the factors related to *N.fischeri* heat resistance in pineapple juice. Through the fractionary experimental planning, it was possible to associate the different factors that contribute to the survival and to the thermal resistance of *N. fischeri* ascospores. It was obtained, in this manner a better analysis of these factors and of its interactions.

2 MATERIAL AND METHODS

2.1 EXPERIMENTS

The assays were carried out in accordance with the experimental planning distribution (Table 1). The temperatures and ages of ascospores production were 25°C for 1 and 3 months, 30°C for 2 months and 35°C for 1 and 3 months. The heating medium ratio was 10, 24 and 38 in accordance with the range used in the industry (personal communication) (RODRIGUES and IEMMA, 2005).

TABLE 1 - LEVEL AND FACTORS USED IN THE EXPERIMENTAL PLANNING FOR PINEAPPLE JUICE

Factors	Level		
	- 1	0	+ 1
Temperature of ascospore production (ºC)	25	30	35
Heating medium Ratio (ºBrix/acidity)	10	24	38
Age of ascospore production (months)	1	2	3

2.2 PREPARATION OF ASCOSPORES

The mould *N. fischeri* used in the present investigation was isolated from productive processes of apple nectar in previous studies and identified according to the standard procedures (PITT and HOCKING, 1985). The isolated mould culture was maintained in the laboratory on ascospore suspension. Ascospores were prepared after 1 and 3 months of growth at 25 and 35°C and 2 months of growth at 30°C on malt extract agar (MEA, pH 5.4), according to PITT and HOCKING (1985). Ascospores were harvested by flooding the medium surface with 25 mL of sterile water and gently rubbing the mold surface with a sterile glass rod and glass pearl. The ascospore suspensions were diluted to 10^8 ascospore/mL.

2.3 CHEMICAL ANALYSIS

The acidity of each fruit juice was determined by titulation. The results were expressed as percentage of acidity, calculated according to predominant acidy (citric acid (m/m)) (PREGNOLATTO and PREGNOLATTO, 1985). The determination of the content of total soluble solids (°Brix) was carried out with a refractometer (ABBÉ-refractometer-Instrument). The results were expressed as grams of total soluble solids contained in 100 g of sample. Once the °Brix and the acidity had been determined, the ratio could be calculated as the relation between them.

To set the low ratio values, first of all the °Brix of the juice was fixed (12°Brix) and, then, a 20% citric acid solution was added to the medium until the acidity of the medium reached 1.2. For the ratio determination, the relation between the juice °Brix and acidity found after the addition of the citric acid was done.

To set the high ratio values, firstly, the natural acidity of the juices was fixed (0.5 for pineapple juice). Then, a 60% sugar solution was added to the medium until 19°Brix. For the ratio determination, the relation between °Brix adjusted by sugar addition and the juice acidity was done. The ratio values found was 38.

2.4 HEAT RESISTANCE

Thermal resistance studies were conducted in commercial pineapple juice (pH 3.4 and different conditions of °Brix and acidity). For the heating, 0.2 mL of ascospores suspension was added in 1.8 mL of juice in TDT tubes stamped with blowpipe O_2/GLP. The tubes were heated in a thermostatically controlled water bath (TE-184 ± 0.1°C) at 80, 85 and 90°C. The come-up time was previously measured and the procedure was started when the required temperature was reached. At each time interval, duplicate tubes were removed and immediately cooled on ice water (SALOMÃO, SLONGO and ARAGÃO, 2007).

For all tested temperatures, there was a sample relating to time 0, which was submitted only to a previously defined activation treatment (85°C/10 min). To determine the initial number of ascospores, the suspensions were diluted as needed in duplicate plates and pour plated using malt extract agar (Biolife, Milano, Italy) containing 50 mg/L of rose Bengal. Plates were incubated at 30°C and counted after 3 to 5 days.

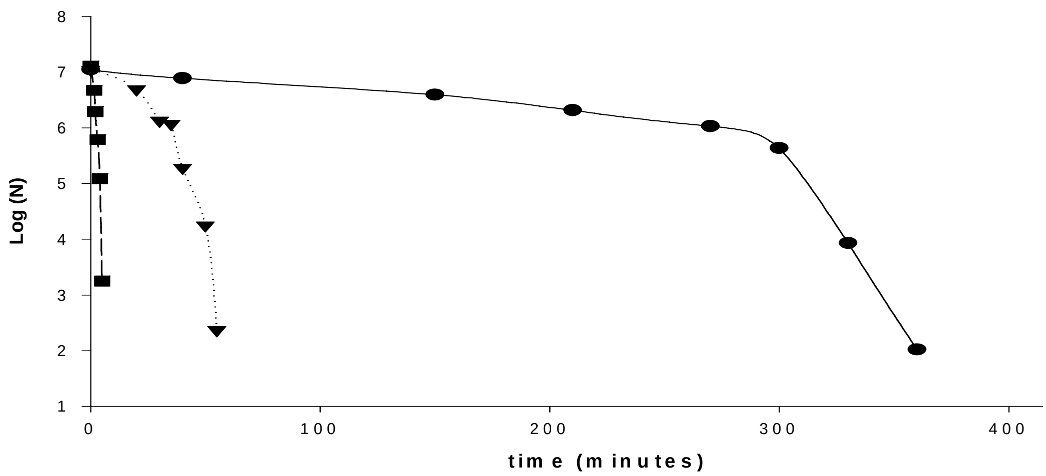
2.5 CALCULATION OF ASCOSPORES THERMAL DEATH RATE

The calculation of *D* and *Z* values is valid only for a logarithmic death rate. But heat resistant molds survivor curves often exhibit a shoulder and an acceleration death rate. As these curves were no linear, it was inappropriate to calculate the traditional *D* values. To overcome this problem, ALDERTON and SNELL (1970) used an empirical formula $(\log No - \log N_1)^a = kt + C$, where *No* is the initial number of ascospores, and N_1 is the surviving number of ascospores per milliliter of heating media at a given time; *a* is the reciprocal of the slope of the curve plotting log $(\log No - \log N_1)$ against log *t*; *k* is a death rate constant and the slope of the linearized curve; *C* is the intercept and *t* is the heating time in minutes. If the death rate followed the expression exactly and there were no experimental errors, *C* would be zero. The 1/*k* value is derived from the formula $1/k = t/(\log No - \log N_1)^a$, assuming $C = 0$, and is similar to $D = t/(\log No - \log N_1)$ in function. The 1/*k* value is defined as the nonlogarithmic death rate and was calculated from thermal inactivation curves that decreased at least 3 log cycles.

3 RESULTS AND DISCUSSION

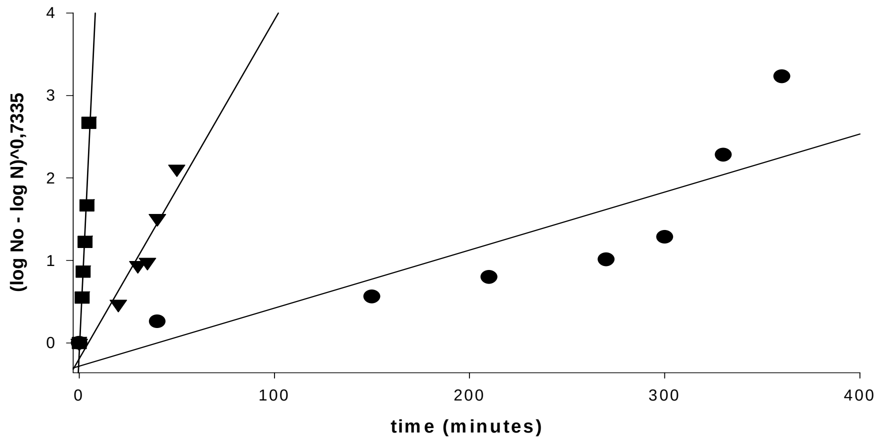
The thermal resistances of the *N.fischeri* ascospores were carried out in all 11 assays. An example of thermal inactivation from one assay of the experimental planning is shown in Figure 1. This figure presents all the curves at different temperatures with heating medium ratio equal to 38 and ascospore suspensions produced after 3 month at 35°C. The non-linearity of the curves can be observed at milder temperatures (80°C and 85°C). As the temperature increases (90°C) the curve becomes almost linear. KING, BAYNE and ALDERTON (1979) also observed this phenomenon and verified that the high temperatures masked the curves non-linearity, being necessary the utilization of lower temperatures to observe this phenomenon. The same result was observed by ENGEL and TEUBER (1991); SURESH, ETHIRAJ and JAYARAM (1996); BAGLIONI, GUMERATO and MASSAGUER (1999); SALOMÃO et al. (2004); SLONGO, MIORELLI and ARAGÃO (2005); SALOMÃO, SLONGO and ARAGÃO (2007).

FIGURE 1 - THERMAL INACTIVATION CURVE OF *N. fischeri* ASCOSPORES AT (-●-) 80°C, (-▼-) 85°C AND (- ■-) 90°C IN PINEAPPLE JUICE (RATIO 38, TEMPERATURE OF ASCOSPORES PRODUCTION 35°C AND AGE 3 MONTHS)



In Figure 2, the linearized curves from Figure 1, to obtain 1/k values are presented.

FIGURE 2 - LINEARIZED SURVIVAL CURVES OF *N. fischeri* ASCOSPORES AT (-•-) 80°C, (-▼-) 85°C AND (- ■-) 90°C IN PINEAPPLE JUICE (RATIO 38, TEMPERATURE OF ASCOSPORES INCUBATION 35°C AND AGE 3 MONTHS)



The equations of each line are presented below:

$$(\log No - \log N)^{0,4859} = 0,007t - 0,0585$$ ($r^2 = 0,9801$) for 80°C eq. 01

$$(\log No - \log N)^{0,4859} = 0,0459t - 0,0636$$ ($r^2 = 0,9009$) for 85°C eq. 02

$$(\log No - \log N)^{0,4859} = 0,4704t - 0,1258$$ ($r^2 = 0,9350$) for 90°C eq. 03

Table 2 contains the results obtained for all assays. The exponent "a" values, calculated at 80°C and the 1/k values (min) for the different assays carried out at 80, 85 and 90°C are presented. This table also shows the Z* values for each condition. The r^2 values, for all assays, varied from 0.83 to 0.99.

TABLE 2 – MATRIX OF THE 2^3 FACTORIAL EXPERIMENTAL PLANNING AND THE PARAMETERS OF HEAT RESISTANCE "a", 1/k (min) AND Z* (ºC)

Assays	Factors R	A	T	"a"	1/k 80ºC (min)	1/k 85ºC (min)	1/k 90ºC (min)	Z*(ºC)
1 - (25-1-10)	-1	-1	-1	0.156	56.2	12.5	1.5	6.4
2 - (25-3-10)	-1	-1	+1	0.163	62.1	14.8	1.5	6.1
3 - (25-1-38)	-1	+1	-1	0.172	71,4	17.9	1.7	5.8
4 - (25-3-38)	-1	+1	+1	0.170	87.7	20.4	2.2	5.9
5 - (35-1-10)	+1	-1	-1	0.170	88.5	14.3	1.4	5.9
6 - (35-3-10)	+1	-1	+1	0.186	88.4	15.0	1.8	5.4
7 - (35-1-38)	+1	+1	-1	0.166	113.6	21.9	1.7	6.0
8 - (35-3-38)	+1	+1	+1	0.726	142.8	19.3	2.0	5.9
9 - (30-2-24)	0	0	0	0.486	142.8	21.8	2.1	5.5
10 - (30-2-24)	0	0	0	0.575	135.1	21.9	2.2	5.6
11 - (30-2-24)	0	0	0	0.538	135.1	20.2	2.1	5.5

T = Temperature (°C). R = Ratio (ºBrix/acidity). A = Age (months).

The results showed in the Table 2 were subjected to the analyses of variance and effect estimate though the software Statistica 6.0. The factors are considered statistically significant when $p < 0.05$.

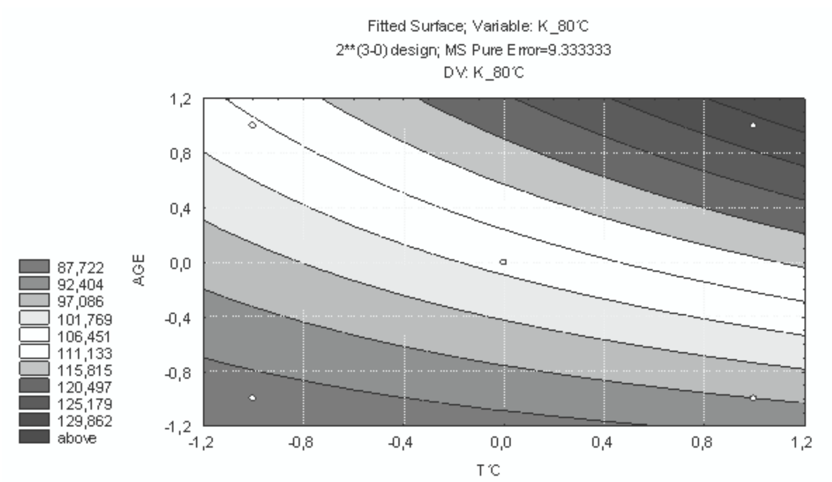
Table 3 presents the statistical analysis results. It is observed that for the response variable 1/k at 80°C all factors showed significant effect ($p< 0.05$). Analysing the 1/k values at 85°C it is verified that all factors presented significant effect. At 90°C, the factors temperature and age of ascospores presented significant effect. For the response variable Z*, the significant factors were the ratio and the age of ascospore production.

TABLE 3 – ANALYSES OF VARIANCE OF ESTIMATE EFFECT FOR THE RESPONSE VARIABLE – 1/k AND Z*

Factors	Response variable 1 / k						Response variable Z*	
	1/k - 80°C		1/k - 85°C		1/k - 90°C			
	Effect	Value- p	Effect	Value- p	Effect	Value- p	Effect	Value- p
(3) – TºC	14.71	0.0208	4.57	0.02	0.259	0.007	0.125	0.084
(1)- *Ratio*	40.85	0.0027	3.33	0.037	0.006	0.787	-0.25	0.023
(2) – Age	28.20	0.0058	4.39	0.021	0.385	0.003	-0.36	0.011
(1) for (2)	3.63	0.234	2.18	0.08	0.003	0.890	0.216	0.030
(1) for (3)	7.99	0.658	-0.39	0.61	0.126	0.028	0.180	0.043
(2) for (3)	7.75	0.069	-1.10	0.23	-0.074	0.072	0.067	0.226

Analyzing the response variable 1/k value at 80°C it is verified that all factors analysed were significant and presented an estimate increase of *N.fischeri* thermal resistance equal to 41%, 28% and 14%, for ratio, age and temperature, respectively (from a lower factors level to a higher factor level). Figures 3 and 4 present the level curves for the 1/k values at 80°C considering temperature *versus* age of ascospore and ratio *versus* age of ascospore, respectively. Through these observations, it was seen that *N.fischeri* presents higher thermal resistance for heating mediums with higher ratio and with older ascospores. This fact is influenced by the increase of the temperature of ascospores production.

FIGURE 3 – RESPONSE SURFACE AND LEVEL CURVE FOR 1/K VALUES AT 80°C FOR THE FACTORS TEMPERATURE *VERSUS* AGE OF ASCOSPORES

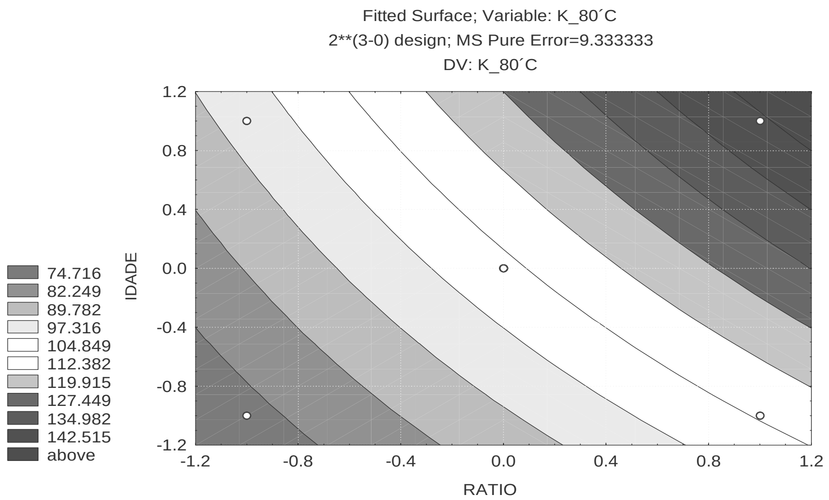


The equation of the level curve in Figure 3 correlating the factors temperature *versus* age of ascospores is presented below:

$$1/k = 103.3 + 7.35 * T°C + 14.10 * Age \qquad \text{eq. } 04$$

In this research, the increasement of the ratio value from lower to higher levels was obtained by addition of sucrose solution to the heating medium. It was verified that this change caused greater thermal resistance than the one caused by the other factors.

FIGURE 4 – RESPONSE SURFACE AND LEVEL CURVE FOR THE RESPONSE VARIABLE 1/K VALUES AT 80°C FOR THE FACTORS RATIO *VERSUS* AGE OF ASCOSPORES



The equation of the level curve in Figure 4 correlating the factors ratio *versus* age of ascospores is presented below:

$$1/k = 103.3 + 20.43 * Ratio + 14.10 * Age \qquad \text{eq. 05}$$

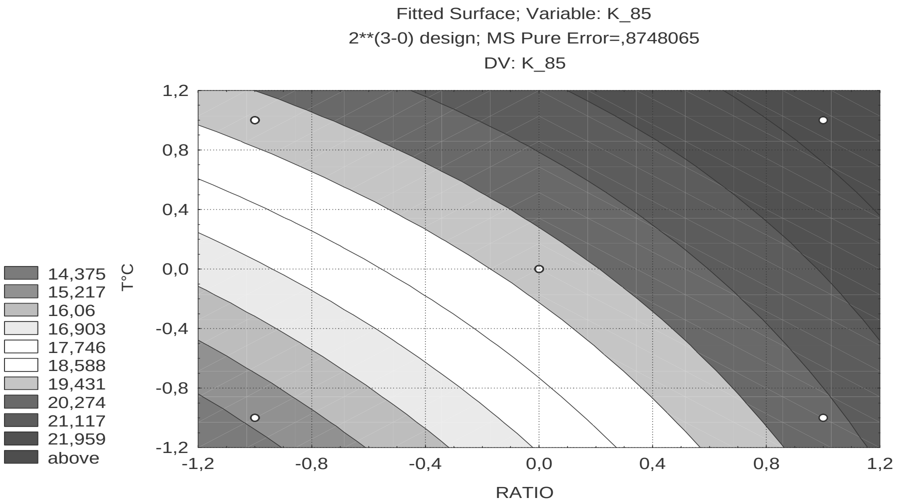
SALOMÃO (2002) studied *N. fischeri* ascospores produced at 30°C for 1 month, using apple juice as heating medium (pH 3.5 and ratio 48) and obtained a $1/k_{80°C}$ value of 153 minutes, that is higher than those found in this research. However, considering the difference between the ratio in that study and in this study (which higher ratio value is 38), it can be said that the values of 1/k parameters found are next to the value found by SALOMÃO (2002) and RAJASHEKLARA, SURESH and ETHIRAJ (2000) (*N. fischeri* ascospores, in mango and grape juices containing different concentrations of soluble solids; 10 and 45°Brix).

The results obtained also show that the age of ascospore increases, considerably, the mold thermal resistance. TOURNAS and TRAXLER (1994) isolated *N. fischeri* from pineapple juice and procedured a study with its ascospores with 1, 2, 3 and 6 months of age, heated in dionised water. Those authors verified that the ascospores with higher age presented higher thermal resistance than those in lower age. CONNER and BEUCHAT (1987) observed that *N. fischeri* ascospores with 21 days of production showed more heat resistance than ascospores with 8 days of production.

It was observed that the ascospores presented lower thermal resistance in heating medium with low ratio values. To these medium was added 20% citric acid solution until to reach the desired ratio. Due to the fact that these heating mediums are more acid, there was a sensibilization of these ascospores and, consequently, the reduction of its thermal resistance. The citric acid action in the thermal resistance reduction of *N. fischeri* ascospores was also verified by RAJASHEKLARA, SURESH and ETHIRAJ (1996), in study carried out with mango juice and different organic acids (malic, citric and tartaric acids). The authors verified that the citric acid caused the highest thermal inactivation and consequent sensibilization of these ascospores. SALOMÃO, SLONGO and ARAGÃO (2007) verified that the thermal resistance of *N. fischeri* ascospores decreases in more acid heat medium.

Evaluating the response variable 1/k at 85°C it was observed that the change in the analised factors from the lower levels to the higher ones caused an increment in the *N. fischeri* thermal resistance. However, this increase is just equal to 3.3, 4 and 4%, respectively, for ratio, temperature and age of ascospore. It is noticed through Figure 5 that the ratio has an important effect on the thermal resistance, where its increase lead to a considerable increment of the thermal resistance. It is also noticed that the temperature of ascospore production has lower effect on the thermal resistance increase. When these factors went from lower level, to higher ones, they presented an increase of 3 and 4%, respectively, in the *N. fischeri* thermal resistance. Analysing the level curve of Figure 5 it is possible to verify the influence of these factors.

FIGURE 5 – RESPONSE SURFACE AND LEVEL CURVE FOR 1/K VALUES AT 85°C FOR THE FACTORS TEMPERATURE *VERSUS* RATIO



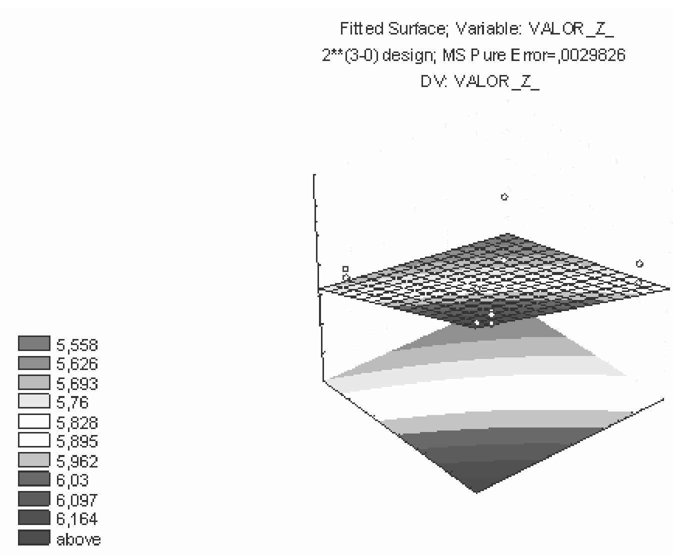
The equation of the level curve in Figure 5 correlating the factors ratio versus temperature of ascospores production is showed below:

$$1/K = 18.965 + 1.667*RATIO + 2.195*T^{o}C \qquad \text{eq. 06}$$

Concerning to the response variable 1/k at 90°C it was observed that the temperature and the age of ascospore presented a slight significant effect in the heat resistance. SALOMÃO et al. (2004), in study carried out with *N. fischeri* ascospores in apple juice at different pH values, verified that at 90°C the 1/k values were almost the same.

Figure 6 shows the response surface for the response variable Z*, when the factors ratio and the age of ascospore presented significant effect on the reduction of the Z* values. It is observed that, as the levels of the two factors increases, the Z* value reduces approximately 0.25% and 0.36%, respectively.

FIGURE 6 – RESPONSE SURFACE OBTAINED FOR THE FACTORS RATIO *VERSUS* AGES OF ASCOSPORES FOR THE RESPONSE VARIABLE Z*



The equation of the level curve in Figure 6 correlating the factors ratio *versus* age of ascospores production is showed below:

$$Z^* = 5.813 - 0.1255^*Ratio - 0.1830^* age \qquad eq. \quad 07$$

SALOMÃO (2002) in study carried out with *N. fischeri* ascospores, produced at 30°C during 1 month, and using apple juice (11.3°Brix, pH 3.5) as heating medium founded a Z* value equal to 4.6°C. ARAGÃO (1989), in study carried out with *N. fischeri* in strawberry juice as heating medium (15°Brix, pH 3.0), found an Z* value of 6.2°C. BEUCHAT (1986) investigated the heat resistance of *N. fischeri* and also reported the same behavior.

4 CONCLUSION

In the higher temperature, the effects caused by the variation of the factors were not very expressive if compared with the effects obtained at 80°C. The results showed that high thermal treatment temperature mask the real effects caused by the variation of the factors. Thus, it was observed that all the analysed factors increased the thermal resistance of the mould *N. fischeri*. It was also verified that as higher as the heating mediums ratios as higher as the thermal resistance. This fact can be explained due to the protective effect that the presence of soluble solids causes to the *N. fischeri* ascospore. The opposite effect can be observed in the lower ratio values, where the ascospores sensibilization happened and the ascospores become less tolerant to heat.

It is important to observe that during pineapple juice processing the thermal treatment is not enough to reduce the presence of *N. fischeri* in the final product and its presence in the raw material must be minimized.

RESUMO

COMPORTAMENTO DE ASCÓSPOROS DE *Neosartorya fischeri* EM SUCO DE ABACAXI

A determinação da resistência térmica de ascósporos de *Neosartorya fischeri* em suco de abacaxi foi realizada em três diferentes condições de *ratio* do suco, temperatura e idade de produção dos ascósporos. Os valores de 1/k obtidos foram submetidos à análise estatística, usando-se o *software Statistica 6.0*. O fator *ratio* para a variável resposta 1/k a 80°C ao passar do nível inferior (10) para nível superior (38) apresentou estimativa de aumento da termorresistência de aproximadamente 41%. O aumento da idade do fungo e da temperatura de incubação elevaram a resistência térmica em aproximadamente 28% e 14%, respectivamente. Os resultados evidenciaram que o fungo *N. fischeri* apresentou menor resistência térmica para meios de aquecimento com baixos valores de *ratio*, ou seja, a elevação da concentração de sólidos solúveis provocou aumento na sua resistência térmica. Constatou-se ainda que a idade, bem como a temperatura de incubação aumentam significativamente a resistência térmica desse fungo.

PALAVRAS-CHAVE: ASCÓSPOROS; RATIO; RESISTÊNCIA TÉRMICA; N. fischeri, SUCO DE ABACAXI.

REFERENCES

1 1 ALDERTON, G.; SNELL, N. Chemical states of bacterial spores. Heat resistance and its kinetics at intermediate water activity. **Applied Microbiology**, v. 19, p.565-572, 1970.

2 ARAGÃO, G. M. F. **Identificação e determinação da resistência térmica de fungos filamentosos termorresistentes isolados da polpa de morango**. Campinas, 1989. 165 p. Dissertação (Mestrado em Ciência dos Alimentos), Universidade de Campinas.

3 BAGLIONI, F.; GUMERATO, H.F.; MASSAGUER, P.R. Ocorrência de fungos filamentosos termo-resistentes em polpa de tomate envasada assepticamente. **Ciência e Tecnologia de Alimentos**, v. 19 n.2, p. 258-263, 1999.

4 BEUCHAT, L. R. Spoilage of acid products by heat resistant moulds. **Dairy Food Environmental Sanitation**, v.18, p.588-593, 1988.

5 CONNER, D. E.; BEUCHAT, L.R. Heat resistance of ascospores of *Neosartorya fischeri* as affected by sporulation and heating medium. **International Journal of Food Microbiology**, v.4, p.303-312, 1987.

6 ENGEL, G.; TEUBER, M. Heat resistance of *Byssochlamys nivea* in milk and cream. **International Journal of Food Microbiology**, v.12, p.225-234, 1991.

7 KING, A.D.; BAYNE, H.G.; ALDERTON, G. Nonlogaritimic death rate calculations for *Byssochlamys fulva* and other microorganisms. **Applied Environmental Microbiology**, v.37, n. 3, p.596-600, 1979.

8 KOTZEKIDOU, P. Heat resistance of *Byssochlamys nivea*, *Byssochlamys fulva* and *Neosartorya fischeri* isolated from canned tomato paste. **Journal of Food Science**, v.62, n. 2, p.410-437, 1997.

9 MISLIVEC, P.B.; BEUCHAT, L.R.; COUSIN, M. A. Detection and enumeration of heat resistant molds. In: VANDERZANT, C.; SPLISTOESSER, D.F. (Eds.). **Compendium of methods for the microbiological examination of foods.** 3rd ed. Washington: APHA, 2001. p 17, 251-253.

10 PENÃ, L.W.; FARIA, A.J.; MASSAGUER, P.R. Development of a predictive model on the growth of the spoilage mould, *Paecilomyces variotii*, in pineapple juice. **Fruit Processing**, v 6, p.420-426, 2004.

11 PIECKOVÁ, E.; SAMSON, R.A. Heat resistance of *Paecilomyces variotii* in sauce and juice. **Journal of Industrial Microbiology and Biotechnology**, v.24, n.4, p. 227-230, 2000.

12 PITT, J.I.; HOCKING, A.D. **Fungi and food spoilage**. Sydney: Academic Press, 1985. 560 p.

13 PREGNOLATTO, W.; PREGNOLATTO, N.P. (ed.). **Normas analíticas do Instituto Adolfo Lutz**: métodos químicos e físicos para análise de alimentos. São Paulo: Instituto Adolfo lutz, 1985. 365 p.

14 RAJASHEKLARA, E.; SURESH. E.R.; ETHIRAJ, S. Influence of different heating media on thermal resistance of *Neosartorya fischeri* isolated from papaya fruit. **Journal of Applied Bacteriology**, v.81, p.337-340, 1996.

15 RAJASHEKHARA, E.; SURESH, E.R.; ETHIRAJ, S. Modulation of thermal resistance of ascospores of *Neosartorya fischeri* by acidulants and preservatives in mango and grape fruit. **Food Microbiology**, v.17, p.269-275, 2000.

16 RODRIGUES, M. I.; IEMMA, A F. Planejamento de experimentos e otimização de processos. Campinas: Casa do Pão Editora, 2005.

17 SALOMÃO, B.C.M. **Isolamento, identificação e estudo da resistência térmica de fungos filamentosos termorresistentes em produtos de frutas**. Florianópolis, 2002, 92 p. Dissertação (Mestrado em Engenharia de Alimentos), Universidade Federal de Santa Catarina.

18 SALOMÃO, B.C.M.; COSTA, C.A.; MASSAGUER, P.R.; ARAGÃO, G.M.F. Influência de diferentes pH do meio de aquecimento na resistência térmica de *Neosartorya fischeri* isolado do processo produtivo de néctar de maçã. **Alimentos e Nutrição**, v.15, n.1, p.7-10, 2004.

19 SALOMÃO, B.C.M.; SLONGO, A.P.; ARAGÃO, G.M.F. Heat resistance of *Neosartorya fischeri* in various juices. **Lebensmittel-Wissenschaft und-Technologie**, v.40, p. 676-680, 2007.

20 SLONGO, A.P.; MIORELLII, S.; ARAGÃO, G.M.F. Influência de diferentes fatores na termorresistência de *Neosartorya fischeri* em suco de mamão. **Alimentos e Nutrição**, v.16, n.4, 377-387, 2005

21 SURESH, E.R.; ETHIRAJ, S.; JAYARAM, H.L. Heat resistance of *Neosartorya fischeri* isolated from grapes. **Journal of Food Science and Technology**, v. 33, p.76-78, 1996.

22 TOURNAS, V.; TRAXLER, R. W. Heat resistance of a *Neosartorya fischeri* strain isolated from pineapple juice frozen concentrate. **Journal of Food Protection**, v.57, n.9, p.814-816, 1994.

23 UGWUANYI, J. O.; OBETA, J. A. N. Pectinolytic and cellulolytic activities of heat resistant fungi and their macerating effects on mango and African mango. **Journal of the Science of Food and Agriculture**, v.79, p.1054-1059, 1999.