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Effect of sweet solutes and potassium sorbate on the thermal inactivation of *Z. bailii* in model aqueous systems

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Abstract

The effect of solute type (glucose and polyols) and potassium sorbate (KS) on *Zygosaccharomyces bailii* thermal inactivation was evaluated in acidified aqueous model systems. Thermal inactivation curves obtained were fitted with Baranyi equation and parameters of this model were estimated and used to establish the effect of water activity (a_w), solute added and KS on *Z. Bailii* survival. Results obtained showed that addition of KS (0.025%, w/w) in general promoted an increase in the rate of heat inactivation. The use of polyols to depress a_w to 0.985 in the absence of KS produced no effect on rate of heat inactivation. On the contrary, the use of glucose (10%, w/w) enhanced it. This trend was also observed when a_w was depressed to 0.971 by glucose or xylitol or glucose-polyols. Moreover, a_w depression in systems containing KS produced different effects on thermal inactivation rate depending on system composition. A synergic effect on the rate of inactivation of *Z. bailii* was observed by the combined use of KS and sorbitol, xylitol or glucose to depress a_W to 0.985–0.988. This behavior might allow to decrease the severity of the thermal treatment with no detrimental effect on sterility.

Keywords: Sweet solutes; Potassium sorbate; Thermal inactivation; *Z. bailii*

1. Introduction

High water activity of juices, and other fruit products allows microbial growth. These products are preserved by the combined use of hurdles such as low pH, depression of water activity by solute addition, chemical preservatives and thermal treatment (Stiles, Duffy, & Schaffner, 2002). Spoilage yeasts, such as *Saccharomyces cerevisiae*, *Candida lipolytica* and *Zygosaccharomyces bailii* are able to overcome these hurdles (Stiles et al., 2002). In particular, the latter, is an osmophilic, acid tolerant and preservative resistant yeast (Jenkins, Poulus, Cole, Vandeven, & Legan, 2000; Warth, 1977).

Z. bailii is able to grow at a pH as low as 2.2 and in the presence of 600–750 mg/L of sorbic (Praphailong & Fleet, 1997; Thomas & Davenport, 1985). However, this abilities can be affected by the acidification agent, the solute used to reduce the a_w as well as by other solutes present in the food (Lenovich, Buchanan, Worley, & Restaino, 1988; Thomas & Davenport, 1985).

Consumer demand for healthier products such as low calorie ones lead to the replacement of sucrose by alternative sweeteners and by bulking agents. Polyols can fulfill both requirements reducing the energetic input from 4 kcal/g of sucrose to 2.6, 2.4 or 1.6 Kcal in the case of adding sorbitol, xylitol or mannitol, respectively (O'Brien, 2002). Moreover, use of xylitol reduces caries and plaque white (O'Brien, 2002) and may help to reduce the chance of acute otitis media in children (Pszczola, 1999). Additionally, polyols can depress the water activity, enhance texture and mouthfeel (O'Brien, 2002).

Incorporation of a preservative such as sorbic acid or its potassium salt into a low pH food provides an additional hurdle to control spoilage (Gliemmo, Campos, &

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Gerschenson, 2004). However, it must be taken into account that environmental factors and processing steps such as pH, solute added, thermal treatment applied, can enhance or reduce the action of the preservative. The combined use of KS and heat exerts a synergistic lethal effect on yeasts (Makdesi & Beuchat, 1996). In relation to the solute used as humectant, it was reported that polyols and sugars increase the heat resistance of bacteria, yeast and fungal spores (Corry, 1976a, 1976b; Lenovich et al., 1988; Smith, Benedict, Haas, & Palumbo, 1983). But this behavior depends on solute used as humectant and on the nature of the microorganism (Almagro et al., 2000).

There is no information about the effect of polyols on survival of *Z. bailii* and on the antimicrobial action of sorbates in their presence. Therefore, this study examines the effect of some polyols, glucose and potassium sorbate on the survival during thermal treatment of *Z. bailii* in acidified aqueous systems.

2. Materials and methods

2.1. Inoculum preparation

Z. bailii NRRL 7256 inoculum was prepared in Sabouraud broth (Biokar Diagnostics, Beauvais, France) at 25 °C until stationary phase was achieved (31 h).

2.2. Model system formulation

Model systems were formulated based on Sabouraud broth and the different composition in humectants is given in Table 1. Polyol concentrations were selected to give a final a_w of 0.985 (systems A–C) and level added was smaller than the maximum admitted by Argentine Food Code. A Glucose concentration of 10% (w/w) which depressed a_w to 0.988 was chosen according to the maximum level admitted by Argentine Food Code for glucidic content modified products. In some cases, enough glucose was added to systems containing a polyol or glucose to get a final a_w of 0.971 (systems E–H). For comparison purposes, a system containing enough xylitol to depress a_w to 0.971 (system I) and a system containing enough glucose to attain a a_w of 0.900 (system J) were studied.

Water activity was measured with an Aqualab dewpoint electronic humidity meter (Decagon Devices Inc., Pullman, Washington, USA). The experimental error in a_w determination is $\pm 0.005 a_w$ units when using humidity meter according to Roa and Tapia de Daza (1991) and, as a consequence, there are no significant differences between a_w values of 0.985 and 0.988.

The level of KS used was 0.025% w/w and it was chosen taking into account that the minimum inhibitory concentration (MIC) for studied systems was within the range of 0.020–0.025% (w/w) as it was previously established (Gliemmo et al., 2004). Control system free of preservative and/or humectants were prepared for comparison purposes. The pH was adjusted to 3.0 by addition of citric acid before autoclaving.

A volume of 99.00 ml of each system, with and without KS, was dispensed by triplicate into flasks (250 ml, Erlenmeyer) and autoclaved. After autoclaving, the flasks were tempered at 50.0 (±0.5) °C and 1 mL of Sabouraud broth containing the inocula was aseptically dispensed into the flasks to obtain a population of 1×10^5 CFU/mL. Flasks were constantly agitated on an orbital shaker and maintained at 50.0 (±0.5) °C in a forced convection constant-temperature chamber.

2.3. Sampling and analysis

Different aliquots were removed from each flask at selected times over a maximum time of 60 min for systems without preservative, and 30 min for the rest of the systems. Aliquots were used for determining the viable population of *Z. bailii* by surface plating or by pour plating on Sabouraud agar. (Biokar Diagnostics, Beauvais, France). The plates were incubated at 25 (±0.5) °C. After 7–10 days of incubation, colonies were counted and thermal inactivation curves were constructed.

2.4. Data analysis

The thermal inactivation curves were determined in triplicate and modeled using Baranyi equation (Xiong, Xie, Edmondson, Linton, & Sheard, 1999). It expresses the logarithmic relation between N and N_0 (number of microorganisms present at time t and zero, respectively) as a function of time (t)

$$\log\frac{N}{N_0} = \log\left[q_B + (1 - q_B)e^{-k(t-B(t))}\right],$$

where $q_B = (N_{min}/N)$; N_{min} is the minimum cell concentration remaining in the tailing phase; k is the maximum rela-

Table 1
Model system composition

Composition[a] (%, w/w)	Systems										
	A	B	C	D	E	F	G	H	I	J	K
Sorbitol	12.90	–	–	–	12.90	–	–	–	–	–	–
Xylitol	–	11.00	–	–	–	11.00	–	–	19.60	–	–
Mannitol	–	–	13.00	–	–	–	13.00	–	–	–	–
Glucose	–	–	–	10.00	10.00	10.00	10.00	22.00	–	48.21	–
a_w	0.985	0.985	0.985	0.988	0.971	0.971	0.971	0.971	0.971	0.900	1.000

[a] Sabouraud broth: quantity enough for 100 g.

tive death rate while $B(t)$, is the lag time function, and is defined as:

$$B(t) = \frac{r}{3}\left(\frac{1}{2}\ln\frac{(r+2)^2}{r^2 - rt + t^2} + \sqrt{3}\ \arctan\frac{2t-r}{r\sqrt{3}} + \sqrt{3}\ \arctan\frac{1}{\sqrt{3}}\right),$$

where the lag parameter r is the time required for the relative death rate to reach half of the maximum relative death rate k.

The parameters of survival curves were estimated for each system from the respective models by nonlinear regression analysis of data. An analysis of variance (ANOVA) and the least significant difference (LSD) test were applied to establish significant differences between parameters calculated for all systems. Besides, an ANOVA of two factors (humectant and KS level or a_w and KS level) was performed to analyze possible interactions (Sokal & Rohlf, 1969). In all cases, statistical significance was considered at a 5% level ($\alpha = 0.05$).

The statistical analysis was performed using the Statgraphics computer program (Statgraphics Plus for Windows, version 3.0, 1997, Manugistics, Inc., Rockville, MD, USA).

3. Results and discussion

Thermal inactivation curves were satisfactory fitted using Baranyi model. Fig. 1 shows experimental data and predicted curve for the system containing 13% (w/w) of sorbitol and 0.025% w/w of KS (panel a). For the rest of the systems analyzed, thermal inactivation curves modeled showed a similar pattern. To check the performance of applied equation, the correlation coefficient (R^2) and the root mean square error (RMSE) between experimental data and those predicted by the model were estimated. Results showed R^2 values within the range of 0.97–0.99 and RMSE values between 0.1 and 0.3 demonstrating a good fit of data to Baranyi model.

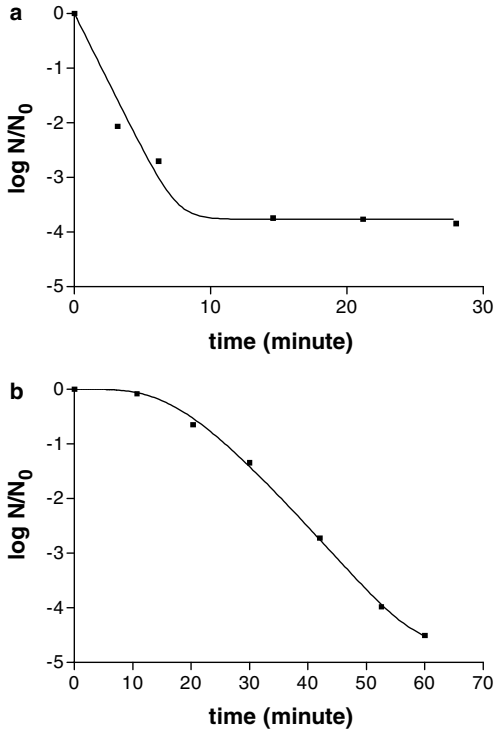

Fig. 1. Thermal inactivation curve fittings for *Z. bailii*. Panel a: system containing sorbitol and 0.025% (w/w) of potassium sorbate (KS). Panel b: system free of humectants and KS (■) experimental data, (-) full model fitting.

Among the three parameters of Baranyi model, r, k and q_B, k was the most sensitive followed by r and q_B as it was previously stated by Xiong et al. (1999). In studied systems, curves obtained did not exhibited shoulder (Fig. 1, panel a) with the exception of that one of the system free of humectants and KS (Fig. 1, panel b). Moreover, no tail was detected in this system throughout the time assayed. However, for the rest of the systems, a tail was generally observed suggesting the presence of a heat resistant subpopulation, probably due to the formation of a small number of ascospores which were more resistant than the vegetative cells. The same behavior was reported by Corry (1976a) for osmophilic yeast heated in solutions of sugars and polyols.

3.1. Effect of potassium sorbate on yeast thermal inactivation

The presence of 0.025% w/w of KS increased significantly the rate of heat inactivation for all studied systems (Fig. 2) with the exception of the system containing enough quantity of xylitol to depress a_w to 0.971, in this case no significant effect of KS addition was observed. The increase in the rate of heat inactivation generally observed is a trend previously reported by Beuchat (1982) who found that 0.010% (w/w) of KS enhanced the rate of yeasts inactivation in several juices. Moreover, addition of 0.060–0.120% (w/w) of KS to fresh apple cider, peach slices and fruit salad acted synergistically with heat to extend storage life; even low levels of the preservative such as 0.005% (w/w) can significantly decrease D values (Beuchat, 1982; Golden & Beuchat, 1992).

3.2. Effect of a_w depression on yeast thermal inactivation

Depression of a_w from 1 to 0.985 produced no significant effect on the rate of thermal inactivation when a polyol is used to depress water activity (Fig. 3, panel a, systems K vs A, B, C). But, when a 10% of glucose was added, an increase in the rate of thermal inactivation was observed (Fig. 3, panel a, system D). This trend was enhanced when enough glucose to get an a_w of 0.971 was used (systems D vs H). Also, a_w depression from 0.985 to 0.971 by the addition of glucose to systems containing a polyol or by the addition of more xylitol to the system containing this humectant, increased the rate of thermal inactivation (Fig. 3, panel a, systems A, B, C vs E, F, G,

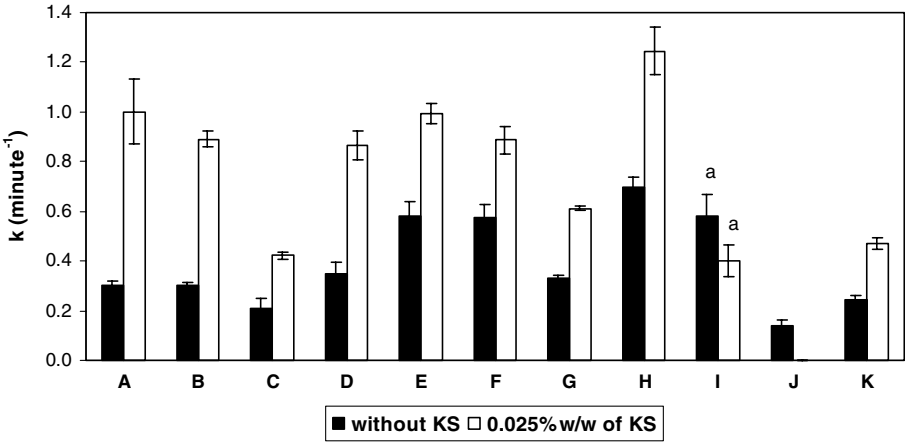

Fig. 2. Thermal inactivation rate constants of *Z. bailii* in model systems as a function of potassium sorbate (KS) content. Vertical bars represent standard deviation of the mean. Columns followed by the same letter are not significantly different ($p \leqslant 0.05$). Capital letters below each column make reference to systems described in Table 1.

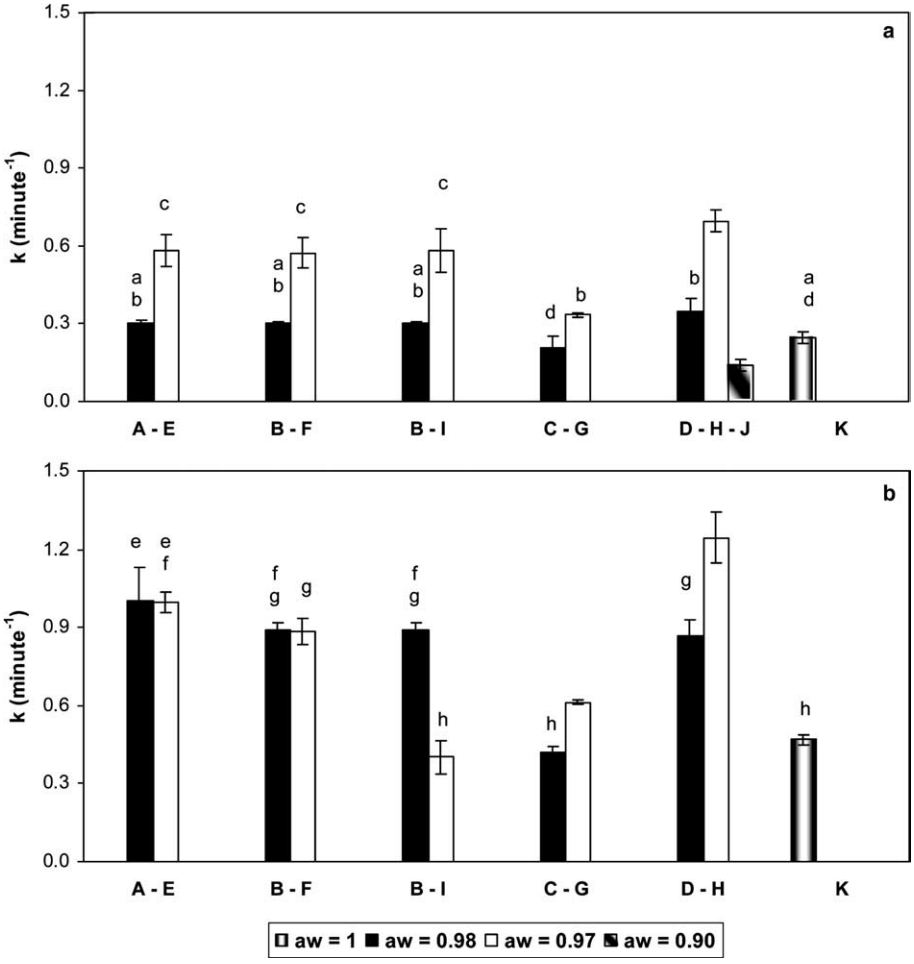

Fig. 3. Thermal inactivation rate constants of *Z. bailii* in model systems as a function of water activity depression and humectant added. Panel a: systems without KS added. Panel b: systems containing 0.025% (w/w) of KS. Vertical bars represent standard deviation of the mean. Columns followed by the same letter are not significantly different ($p \leqslant 0.05$). Capital letters below each column make reference to systems described in Table 1.

respectively, and system B vs I). The order of increase was: glucose/mannitol < glucose/sorbitol = glucose/xylitol = xylitol < glucose 22%.

In summary, results obtained showed that the effect of a_w depression to 0.985 on thermal inactivation depended on solute used. On the contrary, in general, a depression

in a_w to 0.971 promoted an increase in the rate of thermal inactivation being glucose the solute that exerted the strongest increment. However, the effect of a_w depression on thermal inactivation commonly reported is the increase in heat resistance of microorganisms. Corry (1976a, 1976b) found that the heat resistance of *Zygosaccharomyces rouxii* and *Zygosaccharomyces pombe* was enhanced in solutions of sugars and polyols containing 0.1 M phosphate buffer, pH 6.5 at a water activity of 0.95. Golden and Beuchat (1992) reported that the use of glucose or sucrose to depress a_w to 0.93 in an acid broth (pH 4.5) enhanced heat resistance of cells of *Z. rouxii*. It was postulated that the increase in heat resistance exhibited by cells at reduced a_w values is in relation with the dehydration of the cell that took place and with the concomitant increase in stability of cell protein in the dry state (Gibson, 1973; Smith et al., 1983). From another point of view, it must be stressed that sensitivity to heat at reduced water activity may depend on the type of microorganism, the solute used and its concentration. As an example, the effect of heat on rate of inactivation at 49 °C of *Penicillium puberulum* was not influenced by sucrose level within the range of 0–60% (w/w). Moreover, no change in *Z. rouxii* and *Torulopsis globosa* D_{55} value was observed when a_w was depressed from 0.995 to 0.980 by the addition of sucrose, but the use of enough quantity of this sugar to get an a_w of 0.90 promoted an increase in D_{55} value (Gibson, 1973).

As it was previously mentioned, in the systems studied in this research, a_w depression to 0.985–0.971 produced no change or increased rate of thermal inactivation depending on a_w value and solute used. When glucose was used as humectant it was observed an increase in the rate of thermal inactivation in the water activity range of 0.988–0.971 but depression of a_w to 0.900 promoted a decrease in the rate of thermal inactivation (Fig. 3, panel a, systems K, D and H vs J) suggesting that a larger a_w depression promoted the protective effect commonly reported in bibliography. As a consequence, it can be concluded that the effect of a_w depression depends on its magnitude and that much work must be done to elucidate the particular effect exerted by a_w depression on thermal inactivation.

3.3. Effect of a_w depression on yeast thermal inactivation in the presence of KS

Depression of a_w to 0.985 induced an increase in the rate of thermal inactivation for systems containing KS, and sorbitol, xylitol or glucose as humectants (Fig. 3, panel b, systems K vs A, B and D). However, the use of mannitol (system C) produced no change in this parameter. An additional depression of a_w to 0.971 produced different trends. The incorporation of glucose when sorbitol or xylitol were used as humectants did not produced an extra increase in the rate of thermal inactivation. However, addition of glucose to systems containing mannitol or glucose promoted a significant increase in that rate (Fig. 3, panel b, systems G and H). Finally, the use of additional xylitol to get a water activity of 0.971 (system I) produced a decrease in the rate of thermal inactivation (Fig. 3, panel b, system B vs I). This latter trend is in agreement with the increase in heat resistance commonly reported in media of depressed water activity (Corry, 1976a, 1976b).

When a_w was depressed to 0.900 by addition of glucose, yeast death took place in approximately 5 min of thermal treatment since *Z. bailii* viable count was reduced from 1×10^4 to 1 CFU/ml. Mentioned trend did not allow to estimate accurately the death rate. Anyhow, it was demonstrated that depression of a_w to 0.900 by addition of glu-

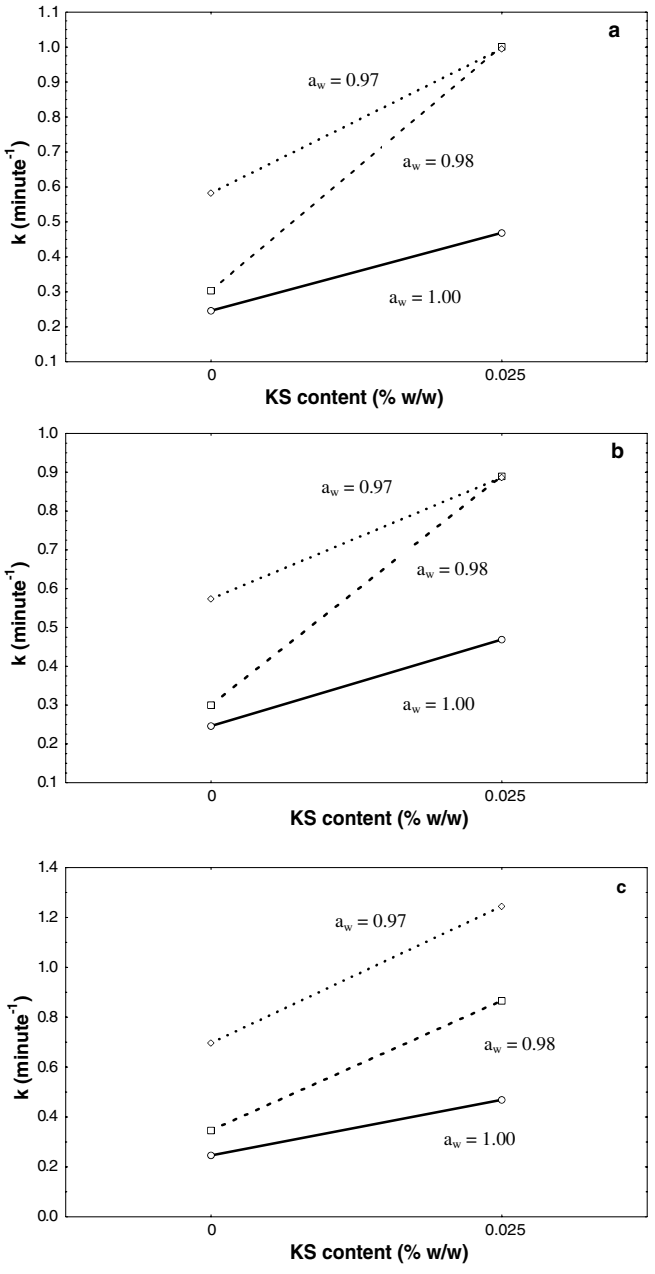

Fig. 4. Thermal inactivation rate constants of *Z. bailii* in model systems as a function of potassium sorbate (KS) content and water activity depression. Panel a: systems containing sorbitol. Panel b: systems containing xylitol. Panel c: systems containing glucose.

cose increased the rate of thermal inactivation with reference to systems D and H (Fig. 3, panel b). This trend is opposite to the one observed in the absence of KS and would suggest the existence of an interaction between a_w depression by glucose addition and KS as it will be discussed later.

It must be highlighted that from the point of view of minimizing addition of solutes and, at the same time, improving thermal inactivation, it is more convenient an a_w depression to 0.985–0.988 through the use of sorbitol, xylitol or glucose in the presence of KS (Fig. 3, panel b, systems A, B, D vs E, F, H).

In relation to the evaluation of the interaction between the effect of a_w depression, solute added and KS presence on the rate of thermal inactivation, it was observed through ANOVA, a significant interaction ($p \leqslant 0.05$) among all variables analyzed. Moreover, a synergic action was observed on the rate of inactivation by the combined use of KS and enough quantity of sorbitol, xylitol or glucose to get an a_w of 0.985–0.988 (Fig. 4, panel a, b and c). This trend is in agreement with the findings of Beuchat (1981) who reported that the inhibitory action of KS against molds is enhanced by high sucrose concentration.

Results obtained in this study showed that thermal inactivation depends on water activity, solute used as humectant and presence of KS. Depression of a_w produced different effects on inactivation depending on solute used as humectant and on the presence of KS. Addition of the latter together with the use of sorbitol, xylitol or glucose to get an a_w of 0.985–0.988 produced a synergic action on the rate of inactivation. This behavior could allow to decrease the severity of thermal treatments with no detrimental effect on sterility of the product.

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