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Effect of aspartame and other additives on the growth and thermal inactivation of *Zygosaccharomyces bailii* in acidified aqueous systems

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ABSTRACT

Growth and thermal inactivation curves were obtained for *Zygosaccharomyces bailii* in acidified aqueous systems resembling low sugar products. Growth curves were modeled using Gompertz equation while thermal inactivation curves were fitted with the Baranyi equation. The parameters of the models were estimated and used to establish the effect of aspartame and other additives (sorbate, xylitol and glucose) on the growth and survival of *Z. bailii*. Aspartame addition produced different effects on growth rate depending on the solute added and the potassium sorbate concentration. The joint use of xylitol and aspartame showed the lowest growth rates in the absence or in the presence of 0.005% w/w sorbate. Regardless of subinhibitory levels of KS, the addition of aspartame increased the population of the stationary phase of the systems containing glucose or xylitol, suggesting that *Z. bailii* metabolizes aspartame. The use of aspartame increased the thermal inactivation rates of all systems. Glucose or xylitol addition to the system containing aspartame and sorbate increased the heat sensitivity of *Z. bailii*. In the absence of sorbate, only glucose decreased the heat resistance of the yeast, whereas xylitol did not affect it. The results obtained highlight the importance of considering the effect of system composition when evaluating microbial stability of food systems.

1. Introduction

Over the past decades, obesity has reached epidemic rates worldwide becoming a health problem. Obese people are at an increased risk for diabetes, cardiovascular disease and hypertension, among other chronic diseases (World Health Organization, 2012). These facts led to the development of foods with low sugar content, such as juices, jellies and jams.

Decrease in pH, water activity depression by solute addition, thermal treatment and the use of preservatives are some of the hurdles applied to preserve low sugar content foods. These hurdles prevent the growth of pathogens but are overcome by spoilage yeasts, such as *Saccharomyces cerevisiae*, *Candida lipolytica* and *Zygosaccharomyces bailii* (Deák, 2007; Gabriel, 2012; Stiles, Duffy, & Schaffner, 2002; Stratford, 2006). In particular, the latter is an osmophilic, acid tolerant and preservative resistant yeast (Jenkins, Poulus, Cole, Vandeven, & Legan, 2000; Warth, 1977). It is able to grow under anaerobic conditions and has minimum nutritional needs. It's resistant to pasteurization and cleaning agents. For example, it can grow in the presence of 600–750 mg/l of sorbic acid and at pH levels less than the pKa of the preservative (Praphailong & Fleet, 1997; Thomas & Davenport, 1985). Besides causing food spoilage, its growth results in significant economic losses (Cheng, Moghraby, & Piper, 1999; Thomas & Davenport, 1985). These facts show that the knowledge of the effects of additives on growth and thermal inactivation of *Z. bailii* is of fundamental interest to manufacturers of acidic products.

The decrease in sucrose levels for the formulation of low sugar content foods may affect sensory and microbiological properties. These negative effects may be compensated by the addition of alternative sweeteners, bulking agents and preservatives. The methyl ester of aspartylphenylalanine, commonly known as aspartame (APM), can fulfill the first requirement. It tastes sweet like sugar and has a potency 200 times higher than that of sucrose (Chattopadhyay, Raychaudhuri, & Chakraborty, 2011; Homler, 1984). Few reports have studied the effect of APM on the growth of oral anaerobes and lactic acid bacteria (Keating & White, 1990; Keller, Nash, Newberg, & Shazer, 1991; Keller, Newberg, Krieger, & Shazer, 1991; Wyss, 1993). Some oral bacteria are able to growth at APM levels 10-fold lower than those used in sweeteners (Wyss, 1993). Keller, Newberg, et al. (1991) related the degradation of APM with the metabolism of yogurt cultures. However, the effect of APM on the growth and thermal inactivation of yeasts has not been studied yet.

Xylitol is a bulking agent that possesses a sweet taste, depresses water activity (a_w) and improves texture and mouthfeel. It has no after taste and is safe for diabetics. According to the US Food and Drug Administration, its use allows reducing the nutritional input from 4.0 kcal/g for sucrose to 2.4 kcal/g for xylitol. It shows antimicrobial properties since it reduces caries and white plaque and may help to decrease the chance of acute otitis media in children (Chen,

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Jiang, Chen, & Qin, 2010; Pszczola, 1999). It also has the ability to act as a quorum sensing antagonist in a gram-negative marker strain (Mukherji, Joshi-Navare, & Prabhune, 2013). Moreover, it reduces the growth of *Z. bailii* (Gliemmo, Campos, & Gerschenson, 2006a) and acts synergistically with potassium sorbate (KS) increasing the thermal inactivation rate of this yeast (Gliemmo, Campos, & Gerschenson, 2006b). The latter preservative is the salt of sorbic acid, a lipophilic weak acid extensively used as a preservative in the food industry. Sorbic acid and potassium sorbate are collectively known as sorbates. Their effectiveness to inhibit *Z. bailii* growth is strain dependent. The minimum inhibitory concentrations are in the range of 7.3 to 9 mM for different *Z. bailii* strains at pH 4.0 and 25 °C. These values are higher than the ones observed for other food spoilage yeasts. They show the high resistance of *Z. bailii* to sorbic acid which may be attributed to the presence of a small number of phenotypically resistant cells in the population (Martorell, Stratford, Steels, Fernández-Espinar, & Querol, 2007; Steels, James, Roberts, & Stratford, 2000).

Current consumers demand products that are preservative-free or with reduced levels of them highlighting the importance of studying the effect of changes in formulation on antimicrobial action. In this connection, synergistic interactions between humectants (glucose, xylitol or sorbitol) and KS were reported for the inhibition of growth and the increase of thermal inactivation of *Z. bailii* (Gliemmo et al., 2006a,b). These facts may allow to maintain the biological activity of the preservative diminishing the amount that is used, or to decrease the severity of the thermal treatment with no detrimental effect on sterility.

There is no information about the effect of APM on the growth and thermal inactivation of *Z. bailii*, therefore this study examines the effect of this sweetener and the existence of interactions between xylitol, glucose and KS on growth and thermal inactivation of *Z. bailii* in aqueous systems that model low sugar foods.

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 early stationary phase was achieved (24 h).

2.2. Model system formulation for growth and thermal inactivation studies

Model systems were prepared using Sabouraud broth. Their composition is given in Table 1. Xylitol, glucose and APM contents were chosen according to the maximum level admitted by Argentine Food Code for glucidic content modified foods.

Concentrations of 0.005 and 0.010% w/w of KS were added to each system for studying yeast growth. For inactivation studies, 0.025% w/w of KS was added. The latter level was chosen taking into account that the minimum inhibitory concentration against *Z. bailii* growth in studied systems was within the range of 0.020–0.035% w/w as it was found in preliminary studies (data not shown).

Table 1
Model system composition.

Composition (% w/w)[a]	Systems					
	A	B	C	D	E	F
Aspartame	0.500	–	0.500	–	0.500	–
Xylitol	–	–	11.00	11.00	–	–
Glucose	–	–	–	–	10.00	10.00
a_w	1.000	1.000	0.985	0.985	0.988	0.988

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

Control systems free of preservative and/or humectants were prepared for comparison purposes.

The water activity was measured with an Aqualab dew point electronic humidity meter (Decagon Devices Inc., Pullman, Washington, USA). The experimental error in a_w determination is ± 0.005 a_w units when using electronic humidity meters according to Roa and Tapia de Daza (1991). The pH was adjusted to 3.00 by addition of citric acid before autoclaving.

Previous studies have shown that pH and KS content do not change significantly by autoclaving but APM does (Gliemmo, Campos, & Gerschenson, 2004; Homler, 1984). Therefore, systems free of APM were elaborated, dispensed into flasks and autoclaved. Then, an aliquot of APM solution sterilized by membrane filtration (cellulosic, white plain, 0.22 μm, Micron Separations Inc., USA) was added aseptically to each autoclaved flask reaching the composition indicated in Table 1.

An aliquot of 14.25 ml of the systems used for the growth assay was placed in 60 ml amber glass flasks. Then, 0.75 ml of Sabouraud broth containing the inoculum was aseptically added to each one of these systems in duplicate obtaining a population of 1.10^6 CFU/ml. Next, they were incubated at 25 (± 0.5) °C.

An aliquot of 99.90 ml of the systems used for the evaluation of thermal inactivation was placed in 250 ml amber glass flasks. After autoclaving, they were tempered at 50.0 (± 0.5) °C and the inoculum (1 ml) was aseptically dispensed in triplicate into the flasks to obtain a population of 1.10^5 CFU/ml. The flasks were constantly agitated at 60 rpm on an orbital shaker (Shaker Pro, Vicking, Buenos Aires, Argentina) and maintained at 50.0 (± 0.5) °C in a forced convection constant temperature chamber.

2.3. Sampling

2.3.1. Growth studies

The yeast growth was measured by turbidimetry following the previously described procedure (Gliemmo et al., 2006a). Briefly, 1.00 ml aliquots were removed from each flask at selected times and the absorbance at 540 nm was measured (Spectrophotometer Shimadzu UV-1203, Japan). The relationship between concentration and absorbance/turbidity is only linear over a limited range corresponding approximately to a tenfold increase in cell numbers. For this reason, the samples with an absorbance greater than 0.3 were diluted with the supernatant obtained after centrifugation of each sample (3000 rpm for 10 min) as it was suggested by Dalgaard, Ross, Kamperman, Neumeyer, and McMeekin (1994). Besides, this supernatant was used as a blank for measurements. Growth curves were constructed with the measurements of the absorbance of the samples.

2.3.2. Thermal inactivation studies

Different aliquots were removed from each flask at selected times over a maximum time of 60 min for preservative-free systems, and 30 min for the rest of the systems. These aliquots were used for determining the viable population of *Z. bailii* by surface plating on Sabouraud agar (Biokar Diagnostics, Beauvais, France). The plates were incubated at 25 (± 0.5) °C. After 7 days of incubation, colonies were counted and thermal inactivation curves were constructed.

2.4. Cell surface hydrophobicity

In order to check the possible effect of xylitol and APM on cell surface, cell surface hydrophobicity was determined using the Microbial Adhesion to Hydrocarbon Test (Li & McLandsborough, 1999; Rosenberg & Gutnick, 1980). Briefly, aliquots of systems A, B and D were inoculated with *Z. bailii* and incubated at 25 (± 0.5) °C for 26 h in order to obtain log phase cells. Then, they were centrifugated at 10,000 rpm for 10 min to separate the growth medium. Pellets were washed twice and resuspended in 10 ml Ringer's solution at

pH 3.0, reaching a turbidity greater than the 0.5 Mc Farland standard in each tube. Four milliliter aliquots of each suspension was dispensed into two tubes. One milliliter of xylene was added to one of them (*Am*), while the other, which remained without xylene, was used as the control (*Ac*). After 10 min of incubation, tubes were vortexed for 1 min, and kept at room temperature for 30 min for phase separation. Then, 2.00 ml of the aqueous phase was removed and transferred to an empty tube. This cycle was repeated twice. Once the three separations were done, the optical density at 600 nm was determined using a spectrophotometer (Shimadzu UV-1203, Japan), which was zeroed using Ringer's solution at pH 3.0. The absorbance of the microbial assay tubes (*Am*) and the absorbance of the control (*Ac*) were used to calculate the adhesion to hydrocarbon as:

$$\% = \frac{Ac - Am}{Ac} \cdot 100. \quad (1)$$

2.5. Data analysis

2.5.1. Analysis of growth curves

The yeast growth curves were determined in duplicate and modeled using the modified Gompertz equation (McKellar & Lu, 2004; McMeekin, Olley, Ross, & Ratkowsky, 1993):

$$y = A \cdot \exp\left\{-\exp\left[1 + \frac{\mu}{A}(\lambda - t)\right]\right\} \quad (2)$$

which expresses the change of the turbidity (y) produced by the yeast population vs. the time (t). The growth parameters are the specific growth rate (μ), the lag phase time (λ) and the asymptotic value (A). The specific growth rate evaluated does not correspond to the maximum growth rate, as the optical density method is less sensitive than the viable method (Biesta-Peters, Reij, Joosten, Gorris, & Zwietering, 2010; Dalgaard & Koutsoumanis, 2001; Skara et al., 2012; Valero, Perez-Rodriguez, Carrasco, Garcia-Gimeno, & Zurera, 2006).

The growth parameters were estimated for each system by nonlinear regression analysis of data and using the modified Gompertz equation. In the cases of the joint presence of xylitol and 0.010% w/w of KS, yeast growth curves could not be satisfactorily modeled by the modified Gompertz equation and specific growth rate was determined by linear regression of data. No information about the asymptotic value could be obtained.

2.5.2. Analysis of thermal inactivation curves

The thermal inactivation curves were determined in triplicate and modeled using the Baranyi model (Baranyi, Roberts, & McClure, 1993) with the modification proposed by Xiong, Xie, Edmondson, Linton, and Sheard (1999) in order to model inactivation curves. The applied model 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):

$$\log\frac{N}{N_0} = \log\left[q_B + (1 - q_B)e^{-k(t - B(t))}\right] \quad (3)$$

where $q_B = (N_{\min} / N)$; $N_{\min}$ is the minimum cell concentration remaining in the tailing phase; k is the maximum relative death rate while $B(t)$, is the lag time function, and is defined as:

$$B(t) = \frac{r}{3}\left(\frac{1}{2}\ln\frac{(r+2)^2}{r^2 - rt + t^2} + \sqrt{3}\cdot\arctan\frac{2t - r}{r\sqrt{3}} + \sqrt{3}\cdot\arctan\frac{1}{\sqrt{3}}\right) \quad (4)$$

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 (k, r and q_B) were estimated for each system from the respective models by nonlinear regression analysis of data.

2.5.3. Model adequacy check-up

To check the adequacy of applied equations, an analysis of variance (ANOVA) was performed. The root mean square error (RMSE) between experimental data and those predicted by the models were estimated and taken as the measure of goodness of fit, as suggested by Ratkowsky (2004).

2.5.4. Analysis of growth and survival parameters and cell surface hydrophobicity values

The analysis of variance and the Least Significant Difference (LSD) test were applied to establish differences between parameters of growth and survival curves and cell surface hydrophobicity values. Also analyses of variance of two factors (APM/a_w, APM/KS level or a_w/KS level) were performed to establish possible interactions (Sokal & Rohlf, 1969). In all cases, the statistical significance was evaluated at a 5% level ($\alpha = 0.05$). The statistical analyses were performed using the Statgraphics computer program (Statgraphics Plus for Windows, version 5.1, 2001, Manugistics, Inc., Rockville, Maryland, USA).

3. Results and discussion

Growth and thermal inactivation curves were satisfactorily fitted using Eqs. (2) and (3), respectively. The RMSE values were within the range of 0.09 and 0.3 indicating a good correlation between the observed and the predicted values of responses.

Experimental data and modeled curves of growth and thermal inactivation of *Z. bailii* are shown in Figs. 1 and 2. The modeled curves showed similar patterns. The presence of studied additives did not modify the pattern of growth and thermal inactivation curves. The latter presented a nonlinear shape in agreement with those observed by van Boekel (2002). Moreover, they exhibited in general a tail (Fig. 2).

Since turbidimetry does not allow the detection of populations lower than 10^6–10^7 CFU/ml (Dalgaard et al., 1994), only the specific growth rate and the asymptotic value were used to evaluate the influence of additives on *Z. bailii* growth.

The maximum relative death rate (k) was the most sensitive of the three parameters of the Baranyi model and it was followed by r and q_B as previously stated by Xiong et al. (1999). For this reason, k was the only parameter reported for the studied systems.

3.1. Effect of APM on Z. bailii

3.1.1. Growth studies

In the absence of KS, APM addition to the humectant-free system or to the one containing xylitol decreased growth rate (Fig. 3, panel I, systems B, D vs. A, C, respectively). In systems containing glucose no significant differences were observed by APM addition (Fig. 3, panel I, system F vs. E).

In the presence of KS different effects on growth rate were observed (Fig. 3, panel I). In the absence of KS or in the presence of 0.005% w/w, the growth rate decreased due to addition of APM and increased in the presence of 0.010% w/w of KS showing a remarkable antagonic effect ($p \leq 0.05$) of APM on KS antimicrobial action observed by the joint presence of 0.010% w/w of KS and APM in the system containing xylitol (Fig. 4, panel I).

For the systems containing APM: the lowest growth rate was observed for the one containing xylitol with or without 0.005% w/w of KS (Fig. 3, panel I, system C).

For the control system, APM addition promoted different effects on the asymptotic value: the former slightly decreased in the absence or in the presence of 0.010% w/w of KS and it did not vary in the

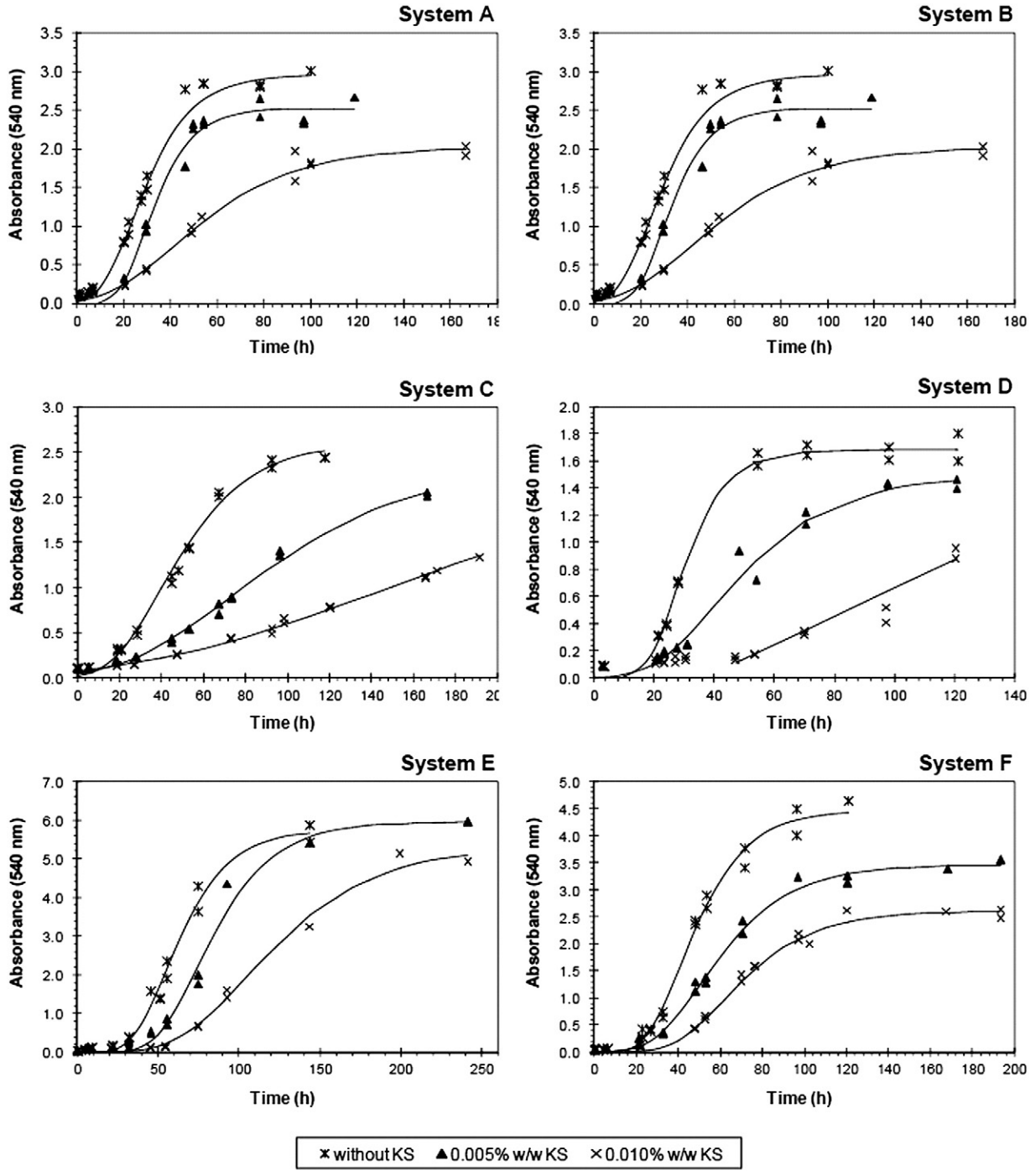

Fig. 1. Growth curve fittings of *Z. bailii* for model systems containing different levels of sorbate. (Points) experimental data, (—) full model fitting.

system containing 0.005% w/w of KS (Fig. 3, panel II, system B vs. A). In systems of depressed a_w, the presence of APM increased the asymptotic value. According to growth curves, the time necessary to reach the stationary phase was larger for these systems (data not shown) explaining in this way the increase in the asymptotic value with no increase in growth rate (Fig. 3, panel I).

Keller, Newberg, et al. (1991) related APM degradation with the metabolism of *Lactobacillus bulgaricus*, *Streptococcus termophilus* and commercial yogurt cultures. Aspartame degradation increased when microorganisms entered in the exponential growth phase and decreased in the stationary phase. A similar trend was reported in chocolate milk (Keller, Nash, et al., 1991). These authors suggested that the sweetener may be metabolized by microorganisms in a similar way to other peptides. Other authors reported that APM levels normally used in fermented dairy products (0.02–0.03%) did not affect the growth of lactic acid starters and probiotic bacteria (Keating & White, 1990; Vinderola, Costa, Regenhardt, & Reinheimer, 2002). On the other hand, some oral anaerobes that require phenylalanine for growth used APM in the absence of this amino acid (Wyss, 1993). Mollapour and Piper (2001) isolated a gene from *Z. bailii* that showed that when it was heterologously expressed in *S. cerevisiae* it could enable the growth of the latter on phenylalanine.

So far, the effect of the joint use of APM and weak acid preservatives against microbial growth has not been studied.

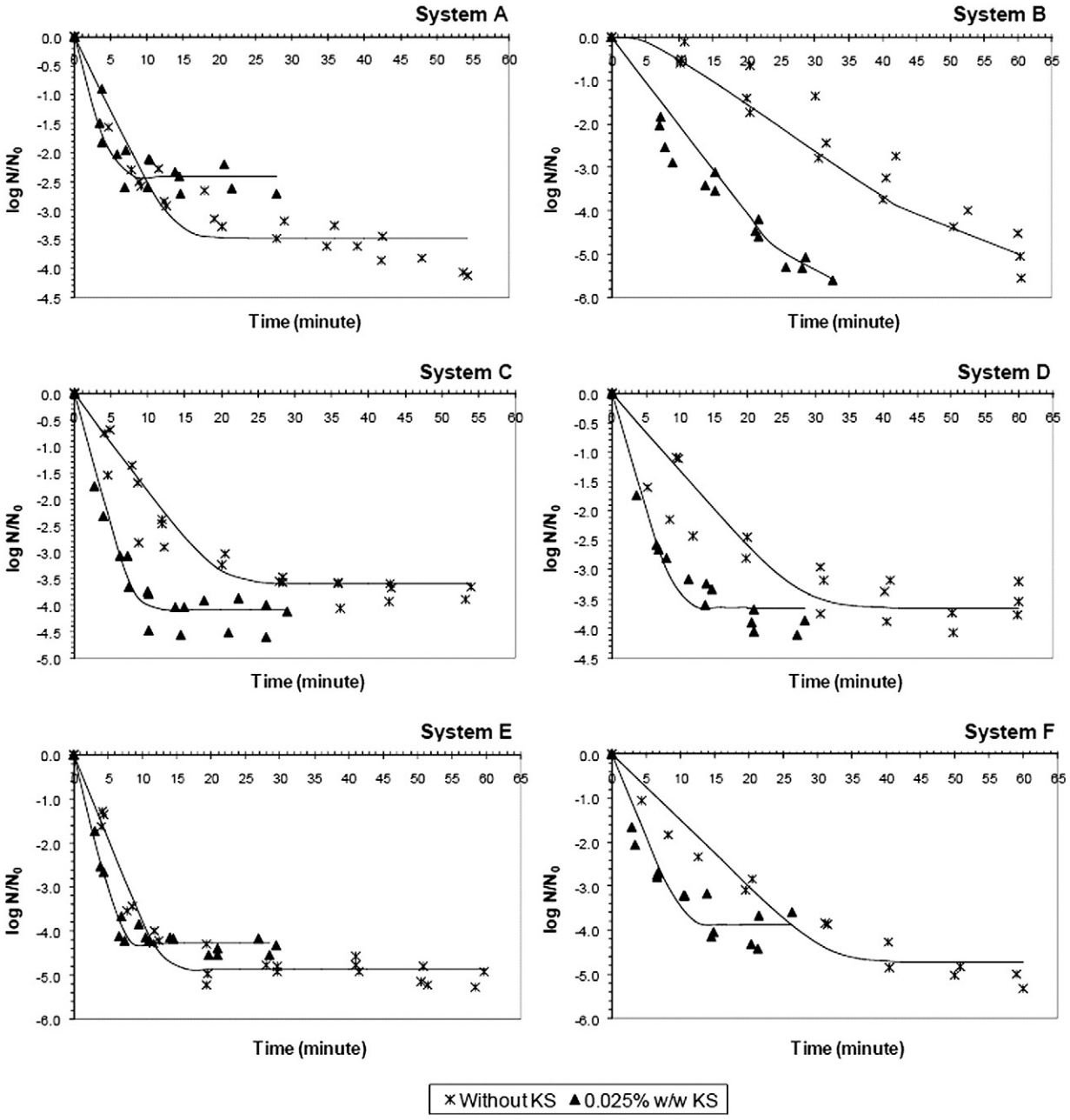

Fig. 2. Thermal inactivation curve fittings of *Z. bailii* for model systems containing different levels of sorbate (KS). (Points) experimental data, (—) full model fitting.

Z. bailii is resistant to weak acid preservatives since it has the ability to expel the protons – formed by the dissociation of the acid – out of the cell. This action occurs through a mechanism that implies the use of plasma membrane H+-ATPase. Simultaneously, a net K+ influx together with the efflux of dissociated preservative may allow the cell to maintain charge balance (Fleet, 1992; Macpherson, Shabala, Rooney, Jarman, & Davies, 2005; Praphailong & Fleet, 1997). The resistance of *Z. bailii* to weak acid preservatives increases when the yeast has enough energy to pump the protons out of the cell (Thomas & Davenport, 1985). This requirement may be obtained through glucose addition. In the present study, addition of APM to systems containing glucose increased the population that reached the stationary phase, suggesting that *Z. bailii* might use the sweetener as energy source.

In summary, APM addition showed different effects on *Z. bailii* growth according to system composition. In general, it increased the population of the stationary phase and this trend might be related with the ability of *Z. bailii* to metabolize APM.

3.1.2. Thermal inactivation studies

Regardless of the presence of KS, the addition of APM increased the death rate in all the systems that were studied (Fig. 3, panel III).

Aspartame and KS acted synergistically increasing the death rate of *Z. bailii* in the control systems (systems A and B) and in the presence of xylitol (systems C and D) as it was tested by an ANOVA ($p \leq 0.05$). As an example, the case of the control system is shown in Fig. 4 (panel III).

At pH 3.00, APM has a predominantly positive charge (pKa = 2.40) and may interact by electrostatic forces with cell membrane components compromising its structural integrity. In this way, APM might be more susceptible to the action of heat treatment. Otherwise, KS and heat treatment act synergistically on thermal inactivation of yeasts,

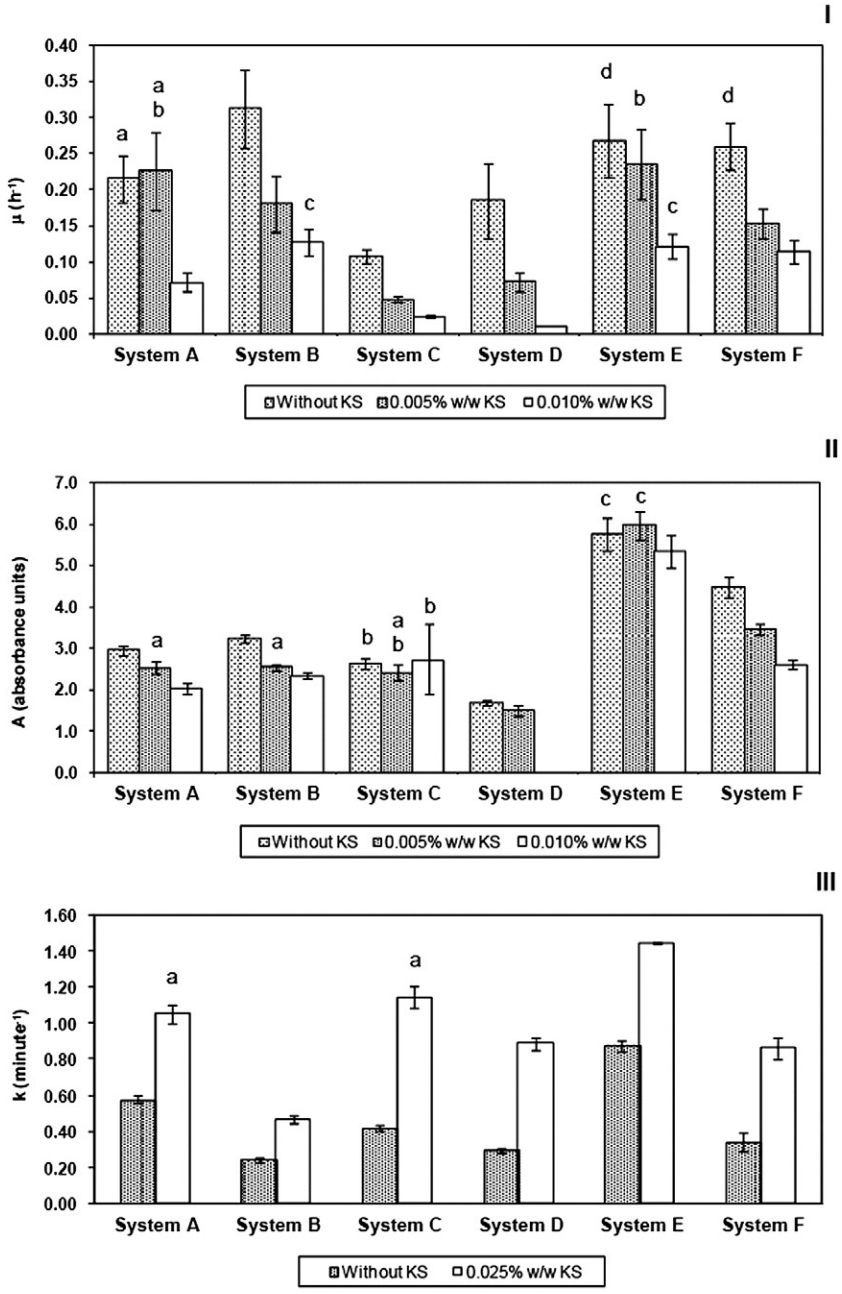

Fig. 3. Growth and thermal inactivation parameters of *Z. bailii* in model systems as a function of sorbate (KS) level. Panel I: growth rate (μ), panel II: asymptotic value (A), and panel III: thermal inactivation rate (k). Bars followed by the same letter inside each panel are not significantly different ($p \leq 0.05$). The absence of letters means that a significant difference ($p \leq 0.05$) was found. Vertical lines over the bars represent standard deviation of the mean.

since KS decreases the ability of microorganisms to recover from the injury caused by heat treatment (Beuchat, 1981; Gliemmo et al., 2006b; Tsuchido & Shibasaki, 1980). Therefore, the occurrence of these two facts may explain the synergistic effect between KS and APM on thermal inactivation observed in this study.

No information has been previously reported about the effect of APM on thermal inactivation of microorganisms. It is noteworthy that the increase in thermal inactivation promoted by the presence of APM may reduce the severity of the heat treatment needed for yeast inactivation.

3.2. Effect of KS on Z. bailii in the presence of APM

3.2.1. Growth studies

In general, the growth rate and the asymptotic value decreased with the increase in KS content (Fig. 3, panels I and II).

The cell may adapt to stress caused by the presence of weak acid through different mechanisms (Kubo & Lee, 1998). Lambert and Stratford (1999) suggested that the microorganism must pump outside the excess of protons in order to achieve the optimal pH to enter in the exponential phase. According to this, the decrease in growth rate promoted by KS presence may have occurred because the cells used the energy of the environment for maintenance lowering the yield of biomass (Quintas, Leyva, Sotoca, Loureiro-Dias, & Peinado, 2005).

3.2.2. Thermal inactivation studies

The addition of KS significantly increased the rate of thermal inactivation in all studied systems (Fig. 3, panel III). This trend was independent of the presence of APM and is due to the ability of KS to retard or inhibit the reparation of thermally damaged cells, as it was

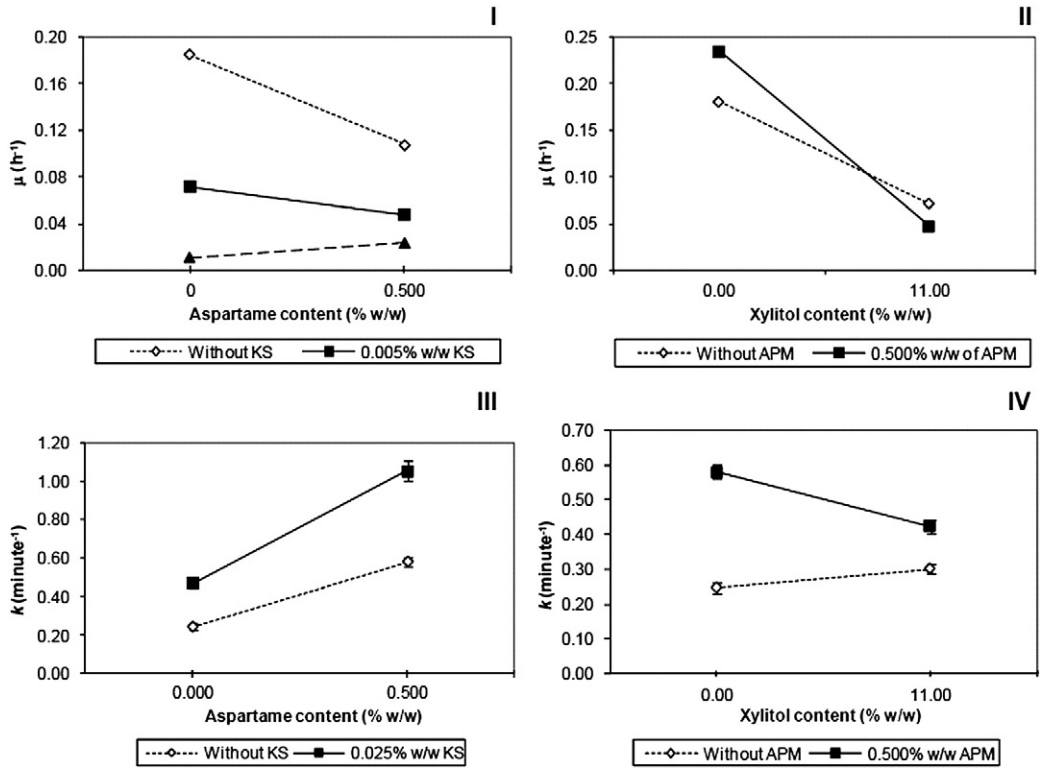

Fig. 4. Interaction graphs of growth rate (μ) and thermal inactivation rate (k) of *Z. bailii*. Panel I: interaction between aspartame (APM) and potassium sorbate (KS) in the presence of xylitol. Panel II: interaction between xylitol and APM in the presence of 0.005% w/w of KS. Panel III: interaction between APM and KS in free-humectant systems. Panel IV: interaction between xylitol and APM in the absence of KS. Vertical bars represent standard deviation of the mean.

mentioned above. The increase in thermal inactivation of yeasts by KS was previously reported for several juices, fresh apple cider, peach slices and fruit salad (Beuchat, 1982; Golden & Beuchat, 1992; Veiga & Madeira-Lopes, 2000).

3.3. Effect of xylitol and glucose on Z. bailii in the presence of APM

3.3.1. Growth studies

Independently of KS presence, the addition of xylitol decreased growth rate (Fig. 2, panel I, system A vs. C) suggesting an inhibitory action of this humectant on *Z. bailii* growth. This action did not depend on APM presence (Fig. 3, panel I, system B vs. D). However, a significant interaction between APM and xylitol was observed in systems containing 0.005% w/w of KS since the decrease in growth rate produced by xylitol addition was higher in the presence of APM (Fig. 4, panel II). This interaction was not observed in the presence of 0.010% w/w of KS due to the antagonic effect between KS and APM, as it was reported above.

The effect of xylitol addition on the asymptotic value did not show a clear trend.

The use of glucose increased growth rate except for the system containing 0.005% w/w of KS where no significant differences were found (Fig. 3, panel I, system A vs. E). Independently of KS presence, asymptotic value increased by the addition of glucose (Fig. 3, panel II, system A vs. E). For the systems containing KS, this trend can be linked with the fact that glucose increases yeasts' resistance to weak acids as it was previously reported (Rodrigues & Pais, 2000; Warth, 1977).

Also, it was suggested that cells might use APM as an energy source. This fact can be related with the evidence that in the absence of APM, glucose promoted a decrease in yeast growth (Fig. 3, panel I, system B vs. F).

It is interesting to remark that the effect of glucose on *Z. bailii* growth was dependant on APM presence because the addition of glucose to APM-free systems decreased growth rate (Fig. 3, panel I, system B vs. F). Conversely the addition of glucose to APM containing systems generally increased growth rate (Fig. 3, panel I, system A vs. E). These results suggest an interaction between glucose and APM.

3.3.2. Thermal inactivation studies

In the absence of KS, xylitol addition decreased death rate of *Z. bailii* whereas in the presence of the preservative it did not vary (Fig. 3, panel III, system A vs. C). An ANOVA ($p \leq 0.05$) showed the existence of an antagonism between xylitol and APM in systems free of KS since in the absence of APM, xylitol increased and in the presence of APM, it decreased death rate (Fig. 4, panel IV).

Irrespective of KS and APM presence, glucose addition increased the rate of thermal inactivation (Fig. 3, panel III, systems A, B vs. E, F, respectively). Moreover, the highest rates of thermal inactivation were observed for the system containing APM, glucose and KS (Fig. 3, panel III, system E).

The effect of a_w depression on thermal inactivation which is commonly reported, is the increase in the heat resistance of microorganisms (Deák, 2007). It may be related to the dehydration of the cell that took place and the concomitant increase in the stability of cell protein in the dry state (Gibson, 1973; Smith, Benedict, Haas, & Palumbo, 1983). However, sensitivity to heat at reduced a_w may depend on the solute used, its concentration, the type of microorganism and its growth phase. In a previous work, we observed that a_w depression to 0.985 by polyols addition produced no effect on heat inactivation rate of *Z. bailii* in broth at pH 3.00 (Gliemmo et al., 2006b). Martorell et al. (2007) reported that the thermal resistance of *Z. bailii* and *Zygosaccharomyces rouxii* increased with the increase of the glucose level. After 4 h of thermal treatment, the resistance decreased.

It may be concluded that to increase the sensitivity of yeast against heat treatment, 10.00% w/w of glucose or 11.00% w/w of xylitol should be used in systems containing KS and APM. However, in the absence of KS, glucose may be selected rather than xylitol since the latter increased yeast heat resistance.

3.4. Effect of APM and xylitol on cell surface hydrophobicity

The addition of APM or xylitol promoted an increase in the percentage of cell surface hydrophobicity from 90.0 ± 1.1 to 96.3 ± 0.5 for the system containing APM and to 96.6 ± 0.3 for the one containing xylitol. It was proposed that a change in the amount of cells that can adhere to a solvent is an expression of a change in surface structure. In the case of an increase, it was suggested that lipids are released to the medium making the cells more susceptible to other stress factors (Tsuchido, Katsui, Takeuchi, Takano, & Shibasaki, 1985). This fact helps to explain the decrease in growth rate and in thermal resistance observed for APM or xylitol addition in the absence of KS (Fig. 3, panels I and III, system B vs. A and D, respectively).

4. Conclusions

Aspartame addition produced different effects on growth rate depending on system composition. It is noteworthy that: i) the joint use of xylitol and APM showed the lowest growth rates in the absence or in the presence of 0.005% w/w of KS, and ii) regardless of KS presence within the range of 0.005–0.010% w/w, the addition of APM increased the population at the stationary phase of the systems of depressed a_w suggesting that *Z. bailii* metabolizes APM.

Regarding thermal inactivation, the presence of APM increased the rates of all systems. Moreover, a synergistic effect was observed between APM and KS or between APM and KS/xylitol. Glucose addition to the system containing APM increased the heat sensitivity of *Z. bailii* independently of KS presence.

As KS concentration increased, growth rate and the maximum population of the stationary phase gradually diminished whereas thermal inactivation rates increased.

Addition of APM or xylitol promoted changes in cell surface structure making the cells more susceptible to other stress factors. This fact is probably linked to the decrease in growth rate and thermal resistance observed for APM or xylitol addition in the absence of KS.

It must be stressed that the use of 0.500% w/w of APM could allow decreasing the severity of thermal treatment. This trend is enhanced when 0.025% w/w of KS and 10.00% w/w of glucose are also present in the acid aqueous systems resembling low sugar foods. However, if a post-process contamination with *Z. bailii* occurs and the right conditions are given for the yeast to grow the presence of APM would enhance this growth since the sweetener can be metabolized by the yeast. The abovementioned results were obtained by researching on model systems. Similar trends are expected to be observed in food products.

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