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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