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# Effect of steviosides and system composition on stability and antimicrobial action of sorbates in acidified model aqueous systems

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ABSTRACT

The effect of steviosides on sorbate stability and on its antimicrobial action was studied in aqueous systems (pH 3.0). The use of steviosides decreased sorbate destruction in all the systems. Its effect on nonenzymatic browning (NEB) depended on the system composition. From the point of view of microbial stability, the steviosides promoted a slight increase in the minimum inhibitory concentration (MIC) of sorbates against *Zygosaccharomyces bailii* and *Zygosaccharomyces rouxii*. However, the main effect of steviosides was the protected action on sorbate destruction. This action was essential to ensure that the preservative residual level was higher than the MIC of the preservative to prevent the growth *Z. bailii* or *Z. rouxii* during storage. The results reported highlight that the use of steviosides in aqueous model systems resembling low-calorie sweet products can be useful to protect potassium sorbate (KS) from destruction and depending on the system composition also to decrease browning development.

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## 1. Introduction

Humans are predisposed to like sweet foods and this trend together with a sedentary life style has led to an alarming increase in diabetes and obesity. As a consequence, the development of healthier light foods with fewer calories is a must. Sugar performs many functions in formulations apart from the development of the sweet taste; it contributes to the desired viscosity and texture, controls hygroscopicity, moisture migration and also depresses water activity (Sandrou & Arvanitoyannis, 2000). For these mentioned reasons, the development of reduced sugar products requires the inclusion of many additives to provide all the functions of sugar, among them; an intense sweetener, a bulk agent and an antimicrobial, all of which are currently included in formulations.

According to Haliday (2009), the top high intensity sweeteners used for food and beverages are saccharin and aspartame. The latter is a dipeptide that can react with carbonyllic compounds taking part in nonenzymatic browning reactions and inducing undesirable changes in color (Gliemmo, Campos, & Gerschenson, 2001). From another point of view, products containing aspartame require a specific label since they contain phenylalanine.

Today, there is a trend to replace synthetic additives with natural ones; in this sense stevia is gaining interest. This sweetener is extracted from the leaves of *Stevia rebaudiana* Bertoni, a plant native to Paraguay; it is 300 times sweeter than sucrose and also has a low glycaemic index making it attractive for diabetic people (Geuns, 2003). The use of stevia as a food additive has been permitted in USA since 2008 and is expected to obtain its approval in Europe by mid-2010 (Parischa, 2010). According to Kroyer (1999), stevia is stable in aqueous solutions in a pH range of 3–10 and under thermal treatment up to 80 °C, the effect on the stability of some vitamins was also studied however, there is no information about its effect on other additives.

Polyols can provide the bulk and texture given by sucrose with the advantage of having fewer calories per gram and do not promote browning development (Gliemmo, Campos, & Gerschenson, 2004). They possess some health benefits such as, reducing the risk of tooth decay and keeping down blood glucose and insulin levels (O'Brien Nabors, 2002). Xylitol is the sweetest polyol, being as sweet as sucrose (Sandrou & Arvanitoyannis, 2000) and in addition, it can exert a slight antimicrobial action on *Z. bailii* (Gliemmo, Campos, & Gerschenson, 2006; Gliemmo et al., 2004).

Low calorie foods are often sweetened by a mixture of sweeteners. In this way, the possibility of exceeding the acceptable daily intake is decreased and also, a smaller amount of each sweetener is needed to ensure a specific sweet level since a synergistic action on the sweetness intensity is verified by the joint addition of two sweeteners (Kroger, Meister, & Kava, 2006).

The partial or total elimination of sugars from a product produces an increase in water activity decreasing preservation factors, therefore to solve this problem; an antimicrobial agent was usually added. Sorbic acid and its potassium salt (KS), commonly named as sorbates, are frequently used in acidic foods. Its activity is strongly

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influenced by the type of food, the conditions of processing and storage, and the level of the preservative (Sofos, 2000). It is known that KS is prone to oxidation in aqueous solutions. This reaction is accompanied by an increase in the concentration of carbonylic compounds which polymerizes rapidly to brown pigments (Arya & Thakur, 1988). Oxidative degradation and browning development depends on pH, the water activity, the presence of other additives, and the conditions of storage and processing (Campos & Gerschenson, 1996; Gerschenson & Campos, 1995). Moreover, if sorbate degradation took place to an important extent being the residual level lower than the amount needed to control microbial growth, the stability of the food might be affected.

The spoilage flora in low sugar foods due to their low pH, high water activity values and the presence of preservatives is mainly composed by some lactic acid bacteria and fungal flora, usually dominated by acid-tolerant yeasts (Tapia, Argaiz, Lopez Malo, & Diaz, 1995), such as *Zygosaccharomyces bailii* and *rouxii*. Particularly, *Z. bailii* can grow in the presence of high levels of sorbic acid and at pH values lower than the pKa of the preservatives (Warth, 1977). The yeast growth could be affected by additives present in the food (Lenovich, Buchanan, Worley, & Restaino, 1988).

As previously mentioned, there is no information about the effect of stevia on the stability and antimicrobial action of sorbates. Therefore, this study examines the effect of stevia and other sweeteners such as glucose, aspartame and xylitol on (1) sorbate stability and browning development in acidified aqueous systems during storage at 35 °C and (2) the MIC of the antimicrobial concerning *Z. bailii* and *Z. rouxii* growth in aqueous systems.

## 2. Materials and methods

### 2.1. Sorbate stability

#### 2.1.1. Model system formulation

The composition of the different model systems is given in Table 1. The concentrations of all the components were within the level admitted by the Argentine Food Code for modified jams, jellies, and fruit stews. In particular, the steviosides and aspartame concentration used was selected after an informal sensory evaluation showed that the mixture was moderately sweet. Control systems free of the different components were formulated for comparison purposes.

Water activity ($a_w$) was measured with an Aqualab dewpoint electronic humidity meter (Decagon Devices Inc., Pullman, Wa., U.S.A.).

In all of the cases, the pH was adjusted to 3.0 by addition of citric acid. Potassium sorbate (Sigma, St. Louis, Mo., U.S.A.), glucose, and citric acid (Merck Química Argentina, Buenos Aires, Argentina) used were of reagent grade. Steviosides (90% w/w of a mixture of steviosides and 10% w/w of maltodextrin) (Inmobal Nutrer, Argentina), xylitol and aspartame (Gelfix, Argentina) were of food grade.

A volume of 15 mL of each model system was dispensed in duplicate into 60 mL dark glass flasks and stored at 35 °C ± 1 °C for 60 days in forced convection constant temperature chambers. The flasks were hermetically sealed to prevent evaporation. Each system was stored in duplicate and sampled at 10 prefixed time intervals. After storage, residual KS, nonenzymatic browning, pH and $a_w$ were measured.

#### 2.1.2. Analysis

The sorbates were dosed according to the AOAC oxidation method (AOAC, 1990), which includes a steam distillation followed by oxidation to malonaldehyde and measurement at 532 nm of the pigment formed between malonaldehyde and thiobarbituric acid. The precision of the technique, evaluated by means of the variance coefficient was 3.4%, as established previously by Campos, Gerschenson, Alzamora, and Chirife (1991).

Nonenzymatic browning was evaluated by means of color measurement in a colorimeter (Minolta Co. Ltd., Osaka, Japan). The CIE tristimulus values were calculated for illuminant C, 2°. From these data, the color chromatic coordinate was calculated ($x$) and the browning index (BI) (Buera, Petriella, & Lozano, 1985) was estimated as:

$$BI = \frac{100x(x-0.31)}{0.172}$$

where 0.31: illuminant C chromatic coordinate, and 0.172: spectral pure color chromatic coordinate minus illuminant C chromatic coordinate.

The water activity was measured at 25 °C with a Decagon CX-1 hygrometer (Decagon, Pullman, Wa., U.S.A.). The equipment was calibrated using NaCl solutions of 1.00, 2.00, 3.00 and 4.00 % w/w, as it was recommended by Chirife and Resnik (1984) for the prediction of high water activity values. The experimental error in determination is ±0.005 units when using this humidity meter according to Roa and Tapia de Daza (1991).

The pH was determined with a pH meter (Cole-Parmer, Chicago, Il., U.S.A.) provided with a glass electrode.

All the determinations were conducted in duplicate.

#### 2.1.3. Sorbate antimicrobial action

*2.1.3.1. Test microorganisms and inocula preparation.* Yeasts used for testing the efficiency of sorbates in the systems studied were *Z. bailii* NRRL 7.256 and *Z. rouxii* ATCC 28.253. The inocula were prepared in Sabouraud broth (Biokar Diagnostics, Beauvais, France) at 25 °C until the stationary phase was achieved (24 h).

*2.1.3.2. Model system formulation and minimum inhibitory concentration determination.* The composition of the different model systems is given in Table 2. The pH was adjusted to 3.0 by adding citric acid before autoclaving. Preliminary data showed that the pH values and KS content do not change significantly by autoclaving. After autoclaving, a series of twofold dilutions of each system ranging from 0.00 to 0·04% (v/v), was prepared in Sabouraud broth and portions of 50 µl of the serial dilutions were pipetted into the wells of microtiter plates, together with 50 µl of a $10^5$ CFU/ml culture of each yeast. The microtiter

**Table 1**
Model system composition.

| Composition (% w/w) | System | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J | K | L |
| Potassium sorbate | 0.134 | – | 0.134 | 0.134 | – | 0.134 | 0.134 | – | 0.134 | 0.134 | – | 0.134 |
| Stevioside | – | 0.350 | 0.350 | – | 0.350 | 0350 | – | 0.350 | 0.350 | – | 0.350 | 0.350 |
| Aspartame | – | – | – | – | – | – | – | – | – | 0.050 | 0.050 | 0.050 |
| Glucose | – | – | – | 10.000 | 10.000 | 10.000 | – | – | – | – | – | – |
| Xilitol | – | – | – | – | – | – | 11.000 | 11.000 | 11.000 | – | – | – |
| water | 99.80 | 99.60 | 99.50 | 89.86 | 89.65 | 89.52 | 88.87 | 88.52 | 88.65 | 99.20 | 99.70 | 99.60 |
| $a_w$ | 1.00 | 1.00 | 1.00 | 0.985 | 0.985 | 0.985 | 0.985 | 0.985 | 0.985 | 1.00 | 1.00 | 1.00 |

$a_w$: water activity.

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