Food Research International 43 (2010) 2171–2175

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

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

V.M. Hracek [a,1], M.F. Gliemmo [a,b,1], C.A. Campos [a,b,*]

[a] Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria (1428), Argentina
[b] Member of Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina

ARTICLE INFO

*Article history:*
Received 11 April 2010
Accepted 21 July 2010

*Keywords:*
Steviosides
Sorbate stability
Nonenzymatic browning
*Zygosaccharomyces bailii*
*Zygosaccharomyces rouxii*
MIC

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.

© 2010 Elsevier Ltd. All rights reserved.

## 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

* Corresponding author. Departamento de Industrias, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria (1428), Argentina. Tel./fax: +54 11 45763366.
*E-mail address:* carmen@di.fcen.uba.ar (C.A. Campos).
[1] Tel./fax: +54 11 45763366.

0963-9969/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodres.2010.07.031

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.

**Table 2**
Model system composition used for the study of sorbate antimicrobial action.

| Composition (% w/w) | System | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | M | N | O | P | Q | R | S | T |
| Sabouraud broth | 100 | 99.65 | 90.00 | 89.65 | 89.00 | 88.65 | 99.95 | 99.60 |
| Potassium sorbate | 0–0.040 | 0–0.040 | 0–0.040 | 0–0.040 | 0–0.040 | 0–0.040 | 0–0.040 | 0–0.040 |
| Stevioside | – | 0.350 | – | 0350 | – | 0.350 | – | 0.350 |
| Aspartame | – | – | – | – | – | – | 0.050 | 0.050 |
| Glucose | – | – | 10.000 | 10.000 | – | – | – | – |
| Xilitol | – | – | – | – | 11.000 | 11.000 | – | – |
| $a_w$ | 1.000 | 1.000 | 0.988 | 0.988 | 0.985 | 0.985 | 1.000 | 1.000 |

$a_w$: water activity.

plates were incubated at 25 °C for 48 h. A negative and a positive control, was also performed. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of KS inhibiting the visible growth of each yeast on the wells (Alderman & Smith, 2001). The experiment was done in duplicate and replicated one.

*2.1.3.3. Data analysis.* The rate constants for sorbic acid degradation and nonenzymatic browning were estimated by linear regression analysis of data. In all of the cases, the statistical significance of the regressions was evaluated by means of an analysis of variance. Significant differences among kinetic rate constants were evaluated by means of a covariance analysis for the equality of the slopes at a 95% confidence level (Sokal & Rohlf, 1980).

## 3. Results and discussion

### 3.1. Model systems stability

In all systems studied, the sorbate degradation exhibited 1st-order rate constants, whereas nonenzymatic browning showed zero-order kinetics. These trends agree with previously published information (Gerschenson & Campos, 1995; Gliemmo et al., 2001, 2004). Figs. 1 and 2 show rate constants for the potassium sorbate and browning development, respectively.

### 3.2. Sorbic acid degradation

As can be observed in Fig. 1, the use of steviosides decreased sorbates destruction in all the systems independently of water activity (systems C vs. A, F vs. D, I vs. G, L vs. J). It is known that sorbates can form complexes with starch and as a consequence, some properties of the preservative could be modified (Duckova & Mandak, 1981; Kurup, Wan, & Chan, 1995). Probably, steviosides due to their glycoside structure could also form complexes with sorbates through hydrogen bonding and in this way protect the preservative from degradation.

The addition of aspartame in the absence of steviosides did not modify preservative destruction (system J vs. A). However, in the presence of steviosides, the aspartame addition protected sorbates from destruction (system L vs. C). The diverse effect of aspartame on preservative stability was reported in previous studies (Gliemmo, Calviño, Tamasi, Gerschenson, & Campos, 2008) and it was related to the different trends that this polypeptide could exert on KS degradation depending on the system composition. According to Thakur, Singh, and Arya (1994), in aqueous solutions containing sugars, some amino acids and proteins protected KS from degradation. This behavior was explained by the formation of melanoidins which could retard rancidity development in foods. Taking into account that aspartame is a polypeptide, that sorbic acid is structurally similar to fatty acids, and that steviosides are structurally similar to sugars, the same trend might be expected in relation to sorbic acid degradation.

The addition of enough glucose or xylitol to depress water activity to 0.985, in the absence of steviosides increased preservative destruction (system D, G vs. A). This trend had been previously reported (Gliemmo et al., 2008, 2004) and was related to the effect of $a_w$ depression on sorbates stability.

The incorporation of steviosides in systems with depressed water activity produced a protective effect (systems F, I vs. D, G, respectively).

It must be stressed that the systems containing the joint presence of steviosides, with glucose or with aspartame exhibited the smallest preservative degradation rates. As a consequence of this behavior, the half life of the preservative, estimated from destruction rate constants, increased from 12 days to 293 for glucose (system D vs. F) and from 20 days to 204 for aspartame (system J vs. L) when steviosides were present.

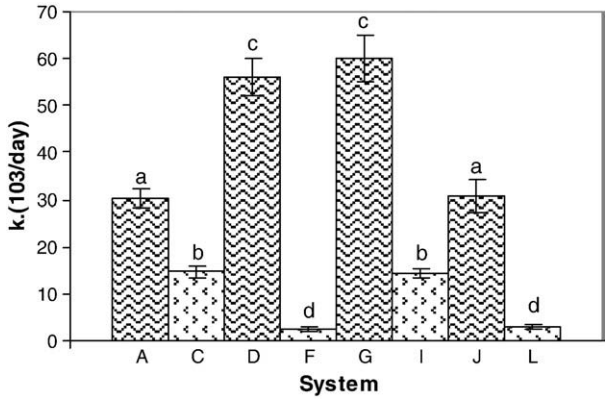


**Fig. 1.** Rate constants for sorbic acid degradation. The bars followed by the same letter are not significantly different (P>0.05) according to Least Significant Differences (LSD) Test. Error bars represent standard error of rate constants. Systems without steviosides; systems with steviosides.

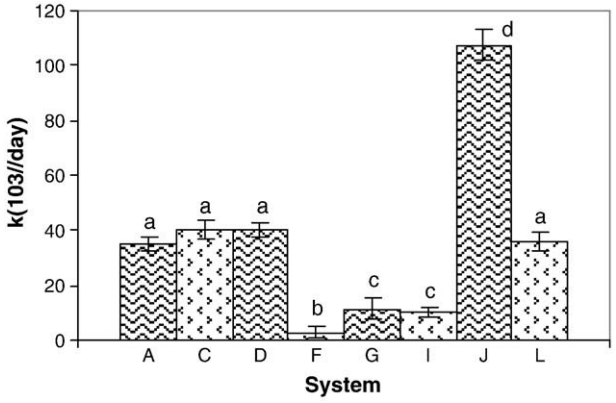


**Fig. 2.** Rate constants for nonenzymatic browning. The bars followed by the same letter are not significantly different (P>0.05) according to Least Significant Differences (LSD) Test. Error bars represent standard error of rate constants. Systems without steviosides; systems with steviosides.

### 3.3. Browning development

In the absence of KS, the systems did not exhibit browning during the storage time (systems B, E, H and K). On the contrary, in the presence of the preservative (systems A, C, D, F, G, I), nonenzymatic browning took place due to the polymerization of carbonylic compounds produced by KS oxidation or present in sugars. When xylitol was present (system G), browning was decreased since xylitol did not induce pigment formation because of the lack of carbonylic groups in its structure.

The addition of aspartame in the absence of EE increased browning development (system J vs. A) since pigment formation occurs as a result of the Maillard reaction between aspartame and carbonylic compounds. This trend had been reported in previous studies (Gliemmo et al., 2001, 2004, 2008).

The addition of steviosides decreased browning in systems containing glucose or aspartame (system F vs. D and L vs. J). Probably, this trend would be related to the smallest preservative destruction observed in these systems and as a consequence, the scarce number of carbonylic compounds formed.

It must be mentioned that the addition of steviosides, in the system free of humectants and in the one containing xylitol (system C, I vs. A, G, respectively), produced no effect on browning suggesting that the effect of steviosides on browning depends on the system composition.

### 3.4. Sorbate antimicrobial action

The preservative MIC value in the systems studied is shown in Table 3. As can be seen, the MIC values for *Z. bailii* were higher than the ones for *Z. rouxii* with the exception of system O, in which they were equal. This trend is related to the fact that *Z. bailii* exhibited a higher tolerance to sorbates than *Z. rouxii* (Praphailong & Fleet, 1997). The MIC values for *Z. bailii* were within the range of 0.0250–0.0400 % (w/w) and for *Z. rouxii,* in the range of 0.0150–0.0200 % (w/w). These levels were in accordance with previously reported MIC values for the yeasts studied in liquid media (Praphailong & Fleet, 1997; Gliemmo et al., 2004; Rivera Carriles, Argaiz, Palou, & Lopez Malo, 2005). For both yeasts, the MIC of the preservative depended on the system composition.

The addition of steviosides promoted an increase in the MIC for the systems containing glucose or xylitol inoculated with *Z. bailii*. In the case of systems inoculated with *Z. rouxii*, the MIC showed a slight increase when the systems contained xylitol or aspartame. Probably, depending on the composition of the system, the yeast could be able to metabolize steviosides leading to a higher population level. As a consequence, a higher concentration of the preservative is necessary to inhibit yeast growth.

To elucidate the significance of preservative destruction on the microbial stability, the residual sorbate concentration, after 2 months of storage at 35 °C, was calculated from destruction-rate constants (Fig. 1). The results obtained showed that preservative residual levels for all the systems were significantly lower than the *Z. bailii* MIC (Fig. 3) unless steviosides were present. The same trend was observed for the systems containing glucose or xylitol (Systems D and G) for the *Z. rouxii* MIC. The results reported showed that the residual KS concentration would not ensure microbial stability for products with a shelf-life equal to or longer than 2 months.

The fact that an osmophilic yeast as *Z. bailii* can grow in acidic media containing preservatives is of economic importance for the processing of beverages and other sweet products (Tapia et al., 1995) and the use of additives that can prevent yeast growth can be a useful tool to improve beverage preservation.

**Table 3**
Minimum inhibitory concentration (MIC) of KS against *Z bailii* and *Z rouxii.*

| MIC KS (% w/w) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| System | M | N | O | P | Q | R | S | T |
| *Z. bailii* | 0.0300 | 0.0400 | 0.0250 | 0.0275 | 0.0300 | 0.0350 | 0.0400 | 0.0350 |
| *Z rouxii* | 0.0175 | 0.0175 | 0.0250 | 0.0250 | 0.0175 | 0.0200 | 0.0175 | 0.0200 |

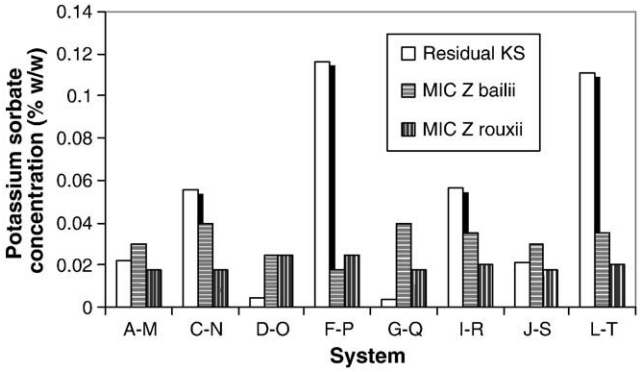


**Fig. 3.** Minimum inhibitory concentration (MIC) and potassion sorbate (KS) residual concentration after 2 months of storage.

## 4. Conclusions

Steviosides and other components of the systems influenced sorbate stability and its antimicrobial activity, as a result of this influence the main trends observed were:

a) The use of steviosides decreased sorbate destruction in all the systems independently of water activity. The effect on browning depended on the system composition: in systems containing glucose or aspartame, browning decreased. However, in the system free of humectants and in the one containing xylitol, no effect on browning development was detected.
b) The addition of aspartame in the absence of steviosides did not modify the preservative destruction and increased browning since pigment formation occurs as a result of the Maillard reaction between aspartame and carbonylic compounds. Nevertheless, in the presence of steviosides, aspartame addition protected sorbates from destruction.
c) The addition of enough glucose or xylitol to depress water activity to 0.985, in the absence of steviosides increased the preservative destruction but when steviosides were present, produced a protected effect.
d) The joint presence of steviosides, with glucose or with aspartame exhibited the smallest preservative degradation rates.
e) From the point of view of microbial stabiliy, steviosides promoted an increase in the MIC for the systems containing glucose or xylitol and inoculated with *Z. bailii.* In the case of the systems inoculated with *Z. rouxii*, the MIC increased when the systems contained xylitol or aspartame.
f) After 2 months of storage, the addition of steviosides is generally necessary to keep the KS level higher than the MIC of the preservative to prevent the growth *Z. bailii* or *Z. rouxii.*

The results reported here emphasize the importance of the correct sweeteners and humectants selection in order to minimize the KS destruction and to ensure microbial stability of aqueous model systems resembling low-calorie sweet products. In particular, the use of steviosides seems to exhibit several advantages, it protected the KS from destruction and depending on the system composition also decreased browning development.

## Acknowledgments

We acknowledge the financial support from the Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y

Tecnológicas de la República Argentina and Agencia Nacional de Investigaciones Científicas y Tecnológicas de la República Argentina.

## References


Alderman, D. J., & Smith, P. (2001). Development of draft protocols of standard reference methods for antimicrobial agent susceptibility testing of bacteria associated with fish diseases. *Aquaculture*, *196*, 211—243.

AOAC. (1990). Sections 20.098–20.101. Official Methods of Analysis. Ed. Association of Official Analytical Chemists, Washington, DC.

Arya, S. S., & Thakur, B. R. (1988). Degradation products of sorbic acid in aqueous solutions. *Food Chemistry*, *29*, 41—49.

Buera, M. P., Petriella, C., & Lozano, R. D. (1985). Definition of color in the non-enzymatic browning. *Die Farbe*, *33*, 316—326.

Campos, C. A., & Gerschenson, L. N. (1996). Effect of certain additives on sorbates stability. *Food Research International*, *29*(2), 147—154.

Campos, C. A., Gerschenson, L. N., Alzamora, S. M., & Chirife, J. (1991). Determination of sorbic acid in r beef: An improved procedure. *Journal of Food Science*, *56*(3), 863—866.

Chirife, J., & Resnik, S. L. (1984). Unsaturated solutions of sodium chloride as reference sources of water activity at various temperatures. *Journal of Food Science*, *49*(6), 1486—1488.

Duckova, K., & Mandak, M. (1981). Interaktion modifizierter staken mit sorbinsaure. *Pharmazie*, *36*(H9), 634—635.

Gerschenson, L. N., & Campos, C. A. (1995). Sorbic acid stability during processing and storage of high moisture foods. In G. Barbosa Cánovas, & J. Welti Chanes (Eds.), *Food preservation by moisture control. Fundamentals and applications* (pp. 761—790). Lancaster, Pa: Technomic Publishing Co.

Geuns, J. M. C. (2003). Molecules of interest stevioside. *Phytochemistry*, *64*, 913—921.

Gliemmo, M. F., Calviño, A. M., Tamasi, O., Gerschenson, L. N., & Campos, C. A. (2008). Interactions between aspartame, glucose and xylitol in aqueous systems containing potassium sorbate. *LWT – Food Science and Technology*, *41*, 611—619.

Gliemmo, M. F., Campos, C. A., & Gerschenson, L. N. (2001). Interaction between potassium sorbate and aspartame in aqueous model sugar systems. *Journal of Food Science*, *66*(3), 428—431.

Gliemmo, M. F., Campos, C. A., & Gerschenson, L. N. (2004). Effect of sweet humectants on stability and antimicrobial activity of sorbates. *Journal of Food Science*, *69*(2), 39—44.

Gliemmo, M. F., Campos, C. A., & Gerschenson, L. N. (2006). Effect of several humectants and potassium sorbate on the growth of *Zygosaccharomyces bailii* in model aqueous systems resembling low sugar products. *Journal of Food Engineering*, *77*, 761—770.

Haliday, J. (2009). Global use of bulk and high intensity sweeteners. *Food Navigator.com* (http://www.foodnavigator.com/Product-Categories/Sweeteners-intense-bulk-polyols/Global-food-use-of-bulk-and-high-intensity-sweeteners).

Kroger, M., Meister, K., & Kava, R. (2006). Low-calorie sweeteners and other sugar substitutes: A review of the safety issues. *Comprehensive Reviews in Food Science and Food Safety*, *5*, 35—47.

Kroyer, G. (1999). The low calorie sweetener stevioside: Stability and interactions with food ingredients. *LWT – Food Science and Technology*, *32*, 509—512.

Kurup, T. R. R., Wan, L. S. C., & Chan, L. W. (1995). Interaction of preservatives with macromolecules. Part II. Cellulose derivatives. *Pharmaceutics Acta Helvetiae*, *70*, 187—193.

Lenovich, L. M., Buchanan, R. L., Worley, N. J., & Restaino, L. (1988). Effect of solute type on sorbate resistance in *Zygosaccharomyces rouxii*. *Journal of Food Science*, *53*(3), 914—916.

O'Brien Nabors, L. (2002). Sweet choices: sugar replacements for foods and beverages. *Food Technology, 56* (7), 28–34, 45.

Parischa, S. (2010). Stevia: beverages and replacing aspartame. *Food Navigator.com*. (http://www.foodnavigator.com/Product-Categories/Sweeteners-intense-bulk-polyols/Stevia-beverages-and-replacing-aspartame).

Praphailong, W., & Fleet, G. H. (1997). The effect of pH, sodium chloride, sucrose, sorbate and benzoate on the growth of food spoilage yeasts. *Food Microbiology*, *14*, 459—468.

Rivera Carriles, K., Argaiz, A., Palou, E., & Lopez Malo, A. (2005). Synergistic inhibitory effect of citral with selected phenolics against *Zygosccharomyces bailii*. *Journal of Food Protection*, *68*, 602—606.

Roa, V., & Tapia de Daza, M. S. (1991). Evaluation of water activity measurement with a dew point electronic humidity meter. *LWT — Food Science and Technology*, *24*(3), 208—213.

Sandrou, D. K., & Arvanitoyannis, S. (2000). Low-fat calorie foods: Current state and perspectives. *Critical Reviews in Food Science and Nutrition*, *40*(5), 427—447.

Sofos, J. N. (2000). Sorbic acid. In A. S. Naidú (Ed.), *Natural Food Antimicrobial Systems, Chap. 23*. Boca Raton, Florida: CRC Press.

Sokal, R., & Rohlf, F. (1980). *Introducción a la bioestadística* (pp. 130—219). Barcelona, Spain: Editorial Reverté.

Tapia, M. S., Argaiz, A., Lopez Malo, A., & Diaz, R. V. (1995). Microbial stability assessment in high and intermediate moisture foods: Special emphasis on fruits products. In G. Barbosa Cánovas, & J. Welti Chanes (Eds.), *Food preservation by moisture control. Fundamentals and applications* (pp. 575—602). Lancaster, Pa.: Technomic Publishing Co.

Thakur, B. R., Singh, R. K., & Arya, S. S. (1994). Chemistry of sorbates — A basic perspective. *Food Reviews International*, *10*(1), 71—91.

Warth, A. D. (1977). Mechanism of resistance of *Saccharomyces bailii* to benzoic, sorbic and other weak acids used as food preservatives. *The Journal of Applied Bacteriology*, *43*, 15—30.