The Low Calorie Sweetener Stevioside: Stability and Interaction with Food Ingredients

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The stability of the low calorie sweetener stevioside during different processing and storage conditions, as well as the effects of its interaction with the water-soluble vitamins ascorbic acid, thiamin, riboflavin, pyridoxine and nicotinic acid, the organic acids acetic acid, citric acid, tartaric acid and phosphoric acid, the other common low calorie sweeteners saccharin, cyclamate, aspartame, acesulfame, neohesperidin dihydrochalcone, and caffeine in coffee and tea, were evaluated. Incubation of solid stevioside at elevated temperatures for 1 h showed good stability up to 120°C, whilst forced decomposition was noticed at temperatures exceeding 140°C. In aqueous solution stevioside was remarkably stable in a pH range of 2-3; however, under strong acidic conditions (pH 1), a significant decrease in the stevioside concentration was detected. Up to 4 h of incubation with individual water-soluble vitamins in aqueous solution at 80°C showed no significant changes with regard to stevioside and the B-vitamins, whereas a protective effect of stevioside on the degradation of ascorbic acid was observed, resulting in a significant delayed degradation rate. In the presence of other individual low calorie sweeteners, practically no interaction was found at room temperature after 4 months of incubation in aqueous media. Stability studies of stevioside in solutions of organic acids showed a tendency towards enhanced decomposition of the sweetener at lower pH values, depending on the acidic medium. In stevioside-sweetened coffee and tea, very few significant changes in caffeine content or in stevioside content were found.

Introduction

Stevioside, a high intensity non-nutritive sweetener, is extracted from the leaves of Stevia rebaudiana Bertoni, a sweet plant native to north-eastern Paraguay. It is a white, crystalline, odourless powder which is approximately 300 times sweeter than sucrose (1). Structurally, stevioside (13-2-O-β-D-glucopyranosyl-2-glucopyranosyloxyl)kaur-16-en-19-oic-acid β-D-glucopyranosyl ester) is a glycoside with a glucosyl and a sophorosyl residue attached to the aglycone steviol, which has a cyclopentanohydrophenanthrene skeleton. Stevioside and extracts of S. rebaudiana leaves are commercially available and used in many countries including Japan and several South American countries as sweetener for a variety of food and beverages (2). At present, applications for Stevia sweeteners do not exist in the European Community because of specific European Union (EU) requirements with regard to safety evaluations. In the last few years, biomedical research, mainly in Asian countries, has demonstrated no significant toxic activities of stevioside in a wide variety of biological systems and has confirmed its lack of mutagenic (3), subchronic toxic (4), carcinogenic (5), teratogenic (6), or contraceptive (6) effects. The metabolism of stevioside in humans was investigated by detection of its metabolites in urine and faeces after single oral dose application (7). To date, few data are available on the practical applications of stevioside in foods and beverages and there is a lack of detailed knowledge regarding its stability during different processing and storage conditions and of its interaction with other food ingredients or food additives, with special regard to its application in appropriate food categories. Good stability for stevioside has been reported for storage in solution with water, soy sauce and vegetable protein hydrolysates at 30°C for 30 days. Some loss, however, was found for storage in vinegar and after heating to 80°C (8). No significant changes were observed during long-term storage in carbonated phosphoric and citric acidified beverages at room temperature, whilst some degradation occurred at 37°C (9). In this study, the stability of stevioside under different processing and storage conditions, as well as the effects of its interaction with water-soluble vitamins, organic acids, caffeine and other low calorie sweeteners, were evaluated. This knowledge is essential for the effective application of stevioside in foods and for the future formulation of appropriate, functional foods.
Materials and Methods

To evaluate the stability and interaction properties of stevioside (Sigma Chemical Company, St. Louis, MO, U.S.A.), model experiments consisting of incubation with the individual food ingredients at relevant temperatures for a proposed time period were performed. In all tests, stevioside was applied in concentrations of 0.5 g/L in consideration of its sweetening power and practical applications.

Analysis methods

Stevioside was determined by high-performance liquid chromatography (HPLC) analysis, as described elsewhere (10), with slight modifications. A Perkin Elmer (PE, Norwalk, CT, USA) high-pressure liquid chromatography, Model 200 Series, equipped with a 20 µL injection system (Rheodyne, Cotati, CA, USA) and a UV-spectrometer detection system (LCD Spectro Monitor 1204 A; Laboratory Data Control, Riviera Beach, FL, USA) was used for analysis. Separation was performed with a Waters (Milford, MA, USA) µBondapak C18-column (3.9 x 300 mm) and methanol-water (65:35 v/v) as the elution solvent at a flow rate of 2 mL/min. The detection wavelength was 210 nm. Under these analytical conditions, the typical retention time (tR) of stevioside was 7.7 min and the detection limit was 0.1 µg. Additionally, steviolbioside, a potential degradation product of stevioside, was identified in the HPLC chromatogram of stevioside analysis (tR = 13.5 min). The potential formation of glucose was determined by enzymatic analysis (11). B-group vitamins were analysed using Association of Official Analytical Chemists methods (12) and ascorbic acid by enzymatic assay (13). The low calorie sweeteners saccharin, cyclamate, aspartame and acesulfame were analysed by thin-layer chromatography (TLC) (14, 15) and neohesperidin dihydrochalcone by HPLC (16). The caffeine content of the coffee and tea was analysed by HPLC (17). All analytical data represent the mean values of triplicate measurements with relative standard deviations s<sub>r</sub> < 3%.

Stability studies of stevioside at elevated temperatures

Fifty milligrams of solid stevioside were incubated in a sealed glass vial at different temperatures from 40 up to 200 °C for 1 h. For quantitative analysis, HPLC was used to evaluate stevioside degradation at the specific temperatures.

Stability studies of stevioside at different pH at elevated temperatures

Aqueous solutions of stevioside (0.5 g/L) were heated in sealed glass vials at temperatures of 60 and 80 °C for time periods of 1 and 2 h at different pH values from pH 1 to pH 10, which were individually adjusted by appropriate buffer systems (Table 1). Losses in stevioside content and formation of steviolbioside and glucose were determined.

Table 1 Buffer systems

<table>
<thead>
<tr>
<th>pH</th>
<th>Buffer solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Potassium chloride/hydrochloric acid</td>
</tr>
<tr>
<td>2, 3, 4</td>
<td>Sodium citrate/hydrochloric acid</td>
</tr>
<tr>
<td>5, 6</td>
<td>Sodium citrate/sodium hydroxide</td>
</tr>
<tr>
<td>7</td>
<td>Potassium dihydrogen phosphate/disodium phosphate</td>
</tr>
<tr>
<td>8</td>
<td>Boric acid/hydrochloric acid</td>
</tr>
<tr>
<td>9, 10</td>
<td>Boric acid/potassium chloride/sodium hydroxide</td>
</tr>
</tbody>
</table>

Stability studies of stevioside in organic acids

Stevioside was dissolved in aqueous solutions (0.5 g/L) of the organic acids acetic acid, citric acid, tartaric acid, and phosphoric acid. Concentrations of 1 and 10 g/L were used, respectively, for the different organic acid systems. The samples were stored in sealed glass vials at room temperature in the dark for different time periods of up to 4 months. Quantitative HPLC analysis was used to follow the progress of chemical degradation.

Interaction of stevioside with water-soluble vitamins

In an aqueous system, stevioside (0.5 g/L) was incubated in binary mixtures of the water-soluble vitamins ascorbic acid, thiamin, riboflavin, pyridoxine, and nicotinic acid at 80 °C for up to 4 h in sealed glass vials. The vitamins were applied in concentrations according to their recommended daily allowances (RDA) (18), which are given in Table 2. The interaction effects were monitored periodically by quantitative analysis by comparison to pure standard substances which had been submitted to the same procedure.

Interaction of stevioside with other low calorie sweeteners

Binary aqueous solutions of stevioside (0.5 g/L) were prepared with the individual low calorie sweeteners saccharin, cyclamate, aspartame, acesulfame, and neohesperidin dihydrochalcone and incubated in sealed glass vials at room temperature in the dark for up to 4 months, as well as at a temperature of 80 °C for up to 4 h. Each of the low-calorie sweeteners tested was applied in concentrations according to their sweetening power comparable to approximately 15 g sucrose/100 mL. Degradation of the individual sweeteners was analysed periodically by HPLC and TLC, respectively.

Stability and interaction of stevioside in a coffee and tea beverage

In consideration of practical applications, stevioside was used to sweeten hot coffee and tea (0.5 g/L) and kept at 80 °C up to 4 h. Samples taken at 1-h intervals were analysed by HPLC for changes in stevioside and caffeine content.
### Results and Discussion

**Stability studies of the pure sweetener**

Incubation of the solid sweetener stevioside at elevated temperatures for 1 h showed good stability up to 120 °C, whilst at temperatures exceeding 140 °C forced decomposition was seen which resulted in total decomposition by heating to 200 °C as shown in Fig. 1. As a consequence, the application of stevioside as a sweetening agent might not be suitable or recommended in baking or other processes requiring high temperatures.

In aqueous solution stevioside is remarkably stable over a wide range of pH values and temperature. Under thermal treatment in a pH range of 2–10 over 2 h practically no degradation of stevioside could be observed at 60 °C and only slight losses up to 5% (pH 2 and 10) occurred on heating to a temperature of 80 °C. Under strong acidic conditions (pH 1) forced decomposition of stevioside was observed which resulted in total decomposition after incubation at a temperature of 80 °C for 2 h (Fig. 2). Only traces of steviolbioside and glucose, degradation products of sterioside, were detected which could be attributed to the rupture of the C19 ester bond in the Sweetener.

**Stability studies in organic acids**

Stability studies of stevioside at room temperature in dilute solutions of the organic acids (1 and 10 g/L) acetic acid, citric acid, tartaric acid and phosphoric acid over time periods of up to 4 months showed a tendency towards enhanced decomposition of the sweetener at lower pH values, depending on the acidic medium. While no evidence was found of degradation after 4 months of storage at room temperature in 1 g/L solutions of acetic acid (pH 3.1), citric acid (pH 2.6) and tartaric acid (pH 2.6), losses of 30% occurred in equivalent solutions of phosphoric acid (pH 2.2). In 10 g/L solutions of acetic acid (pH 2.6), citric acid (pH 2.1), tartaric acid (pH 2.1) and phosphoric acid (pH 1.6) losses in stevioside concentration of 2, 22, 33 and 75% were observed after 4 months of storage, respectively. The time-dependent degradation rates are shown in Fig. 3.

**Interaction studies with vitamins**

Incubation of stevioside for up to 4 h with individual water-soluble B-group vitamins and vitamin C in aqueous solution at 80 °C showed no significant change in sweetener or in vitamin concentration for the B-vitamins under thermal treatment and in untreated samples. In the case of vitamin C, however, incubation at 80 °C resulted in a time-dependent degradation of ascorbic acid, where-as a protective effect of stevioside on the degradation of ascorbic acid was observed, resulting in a delayed degradation rate of vitamin C in the presence of stevioside (27% after 4 h incubation) compared to the analogous treated standard substance (13% after 4 h incubation). The time-dependent degradation rates of ascorbic acid during the storage experiments are shown in Fig. 4.
solutions of stevioside with other individual low-calorie sweeteners, saccharin, cyclamate, aspartame, acesulfame and neohesperidin dihydrochalcone, were investigated. Excellent stability and no interaction between the individual sweeteners were found in the course of thermal treatment at 80°C for up to 4 h as well as over 4 months of incubation at room temperature, indicating that there are no chemical objections to the simultaneous use of stevioside with other low-calorie sweeteners.

**Stability and interaction studies in hot beverages**

Thermal treatment of stevioside-sweetened coffee and tea at 80°C over a time period of 4 h resulted in no significant influence on either the caffeine or the stevioside content. Only minimal losses of stevioside, up to 5%, were seen after 4 h incubation of tea or coffee, indicating that no interaction effects should be expected under practical conditions of the preparation and consumption of the hot beverages.

**Conclusion**

Stability and interaction studies show that the low-calorie sweetener stevioside shows good stability under normal conditions of use. However, under extreme temperature and pH conditions, chemical degradation of the sweetener occurs. Furthermore, specific aspects of a possible interaction with other food ingredients should be taken into consideration, especially in its application in different categories of food.

**References**


