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Stevioside and *Stevia*-sweetener in food: application, stability and interaction with food ingredients

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Abstract The stability of the natural sweetener stevioside during different processing and storage conditions as well as the effects of its interaction with water-soluble vitamins, food relevant organic acids and other common low calorie sweeteners and its application in coffee and tea beverages were evaluated. 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 noticed. In aqueous solutions stevioside is remarkable stable in a pH range 2–10 under thermal treatment up to 80°C, however, under strong acidic conditions (pH 1) a significant decrease in the stevioside concentration was detected. Up to 4 h incubation of stevioside with individual water-soluble vitamins in aqueous solution at 80°C showed no significant changes in 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 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 a stevioside-sweetened coffee and tea beverage, practically, no significant chances neither in caffeine content nor in stevioside content could be noticed. Furthermore an overview of already performed studies in literature about the Stevia-sweetener stevioside and rebaudioside A is given.

Keywords Low calorie sweetener · Stevioside · Stability · Interaction · Organic acids · Water-soluble vitamins · Caffeine

1 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 and is a white, crystalline and odorless powder and approximately 300 times sweeter than sucrose (Soejarto et al. 1983). Structurally, stevioside (13-[2-*O*-β-D-glucopyranosyl-α-glucopyranosyl)oxy]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 cyclopentanonhydrophenanthrene skeleton. Stevioside and extracts of *Stevia 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 (Kinghorn and Soejarto 1985). At present, general regulations for *Stevia*-sweeteners for applications in food do not exist in the European Community.

So far, little data have been available on the practical applications in foods and beverages and there is

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a lack of detailed knowledge about the stability during different processing and storage conditions and about the interaction of stevioside with other food ingredients or food additives with special regard to its application in appropriate food categories.

Good stability of stevioside has been reported in solutions in water, soy sauce and vegetable protein hydrolyzates at storage at 30°C for 30 days; however, some losses were found in vinegar and after heating at 80°C (Shirakawa and Onishi 1979).

Chang and Cook (1983) found that during long-term storage tests of stevioside and rebaudioside A in carbonated phosphoric and citric acidified beverages no significant changes were observed at storage at 4°C and at room temperature for at least 3 months, whilst some degradation occurred at 37°C with 17% loss after 4 months of storage in the citric acid beverage and 36% loss in the phosphoric beverage. As degradation products steviolbioside, glucose, and some non identified substances were detected. Rebaudioside A showed no significant changes during 4 months of storage at 4°C, 3 months at room temperature, or 1 month at 37°C in either citric acid or phosphoric acid beverages. After 4 months of storage at 37°C a degradation of 13% (citric acid system), respectively, 25% (phosphoric acid system) was observed. No degradation products of rebaudioside A were detected. Heating at 60°C for up to 137 h in the citric and the phosphoric acid systems did not cause any appreciable degradation of stevioside or rebaudioside A. Stevioside concentration did not decrease in citric acid beverages, and only 4% decrease was noted in the phosphoric acid beverages. The corresponding figures for rebaudioside A were 3% in citric and 6% in phosphoric acidified beverages. However, heating the citric acid systems at 100°C for 13 h resulted in degradation of stevioside up to 68% and of rebaudioside A up to 76%. In the phosphoric acid system losses up to 86%, respectively, 87% were detected after 13 h. Fewer degradation products were observed for rebaudioside A than for stevioside and in the phosphoric acid system the degradation reactions were more pronounced. When exposing to sunlight (3000 Langleys) Chang and Cook (1983) detected no significant changes in stevioside concentration in either the phosphoric or citric acid beverages. Surprisingly, considerable degradation of rebaudioside A was observed with losses of 22% in the phosphoric and 18% losses and the citric acid beverages. In contrary, by repeating the study under similar conditions Clos et al. (2008) found that both stevioside and rebaudioside A are stable to sunlight exposure and no significant differences in the photostability between stevioside and rebaudioside A was observed.

In our study, the stability of stevioside during different processing and storage conditions as well as the effects of its interaction with water-soluble vitamins, organic acids, other low calorie sweeteners and caffeine at applications in coffee and tea beverages was evaluated. This knowledge seems to be essential for its effective application in food and for formulating appropriate natural sweetened foods.

2 Materials and methods

2.1 Study design

For the evaluation of the stability and interaction properties model experiments were performed by incubation of pure stevioside with the individual food ingredients at relevant temperatures for a proposed time period. In all tests, stevioside was applied in concentrations of 0.5 g/L in consideration of its sweetening power and practical applications.

2.2 Methods of analysis

Stevioside were determined by HPLC analysis. Separation was performed with a C18-column and methanol–water (65:35 v/v) as elution solvent and detection at 210 nm wavelength. The potential formation of glucose was determined by enzymatic analysis (Bergmeyer et al. 1974). Vitamins of the B-group were analyzed by AOAC methods (AOAC 1984) and ascorbic acid by enzymatic assay (Beutler 1984). The low calorie sweeteners saccharin, cyclamate, aspartame, acesulfame and were analyzed by thin layer chromatography (Kroyer and Washüttl 1979; Kroyer et al. 1993) and neohesperidin dihydrochalcone by HPLC (Fischer 1977). The caffeine content of the coffee and tea beverages was analyzed by HPLC (Terada and Sakabe 1984). All analytical data represent the mean values of triplicate measurements with relative standard deviations $<3\%$.

2.3 Stability studies of stevioside at elevated temperatures

50 mg of the solid sweetener 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.

2.4 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 in different pH ranges from pH 1 to 10 which were individually adjusted by appropriate buffer systems. Losses in stevioside content and formation of steviolbioside and glucose were determined.

2.5 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. For the different organic acid systems concentrations of 1 and 10 g/L were used, respectively. The samples were stored in sealed glass vials at room temperature in the dark for different time periods up to 4 months. Quantitative HPLC analysis was used to follow the progress of chemical degradation.

2.6 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 up to 4 h in sealed glass vials. The vitamins were applied in concentrations according to their recommended daily allowances (RDA). The effects of interaction were monitored periodically by quantitative analysis in comparison to pure standard substances which are submitted to the same procedure.

2.7 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 up to 4 months as well as at a temperature of 80°C up to 4 h. Each of the tested low calorie sweeteners were applied in concentrations according to their sweetening power comparable to approximately 15 g sucrose/100 mL. Degradation of the individual sweeteners was analyzed periodically by HPLC and TLC, respectively.

2.8 Stability and interaction of stevioside in a coffee and tea beverage

In consideration of practical applications stevioside was used to sweeten a hot coffee and tea beverage (0.5 g/L) and kept at 80°C up to 4 h. Samples taken at 1 h interval were analyzed by HPLC for changes in stevioside and caffeine content.

3 Results and discussion

3.1 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 noticed resulting in total decomposition by heating up to 200°C as documented in Fig. 1. As a consequence, the application of stevioside as sweetening agent might not be suitable and recommended for baking processes or processes requiring high temperatures.

In aqueous solution stevioside is remarkable stable over a wide range of pH and temperature. Under thermal treatment in a pH range 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 by heating at a temperature of 80°C. Under strong acidic conditions (pH 1) forced decomposition of stevioside was noticed resulting in total decomposition after incubation at a temperature of 80°C for 2 h (Fig. 2). As degradation product of stevioside only traces of steviolbioside and glucose were detected which could be attributed to the rapture of the C19 ester bond in stevioside.

3.2 Stability studies in organic acids

Stability studies of stevioside at room temperature in diluted solutions of the organic acids (1 and 10 g/L)

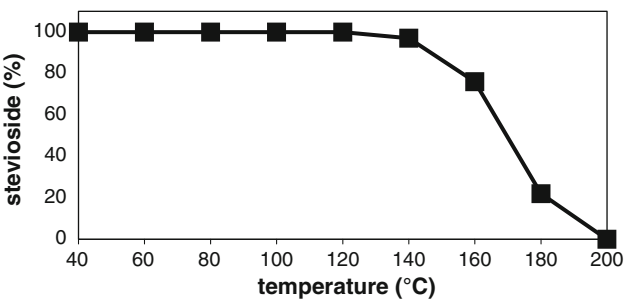

Fig. 1 Stability and degradation rate of stevioside as solid substance at elevated temperatures

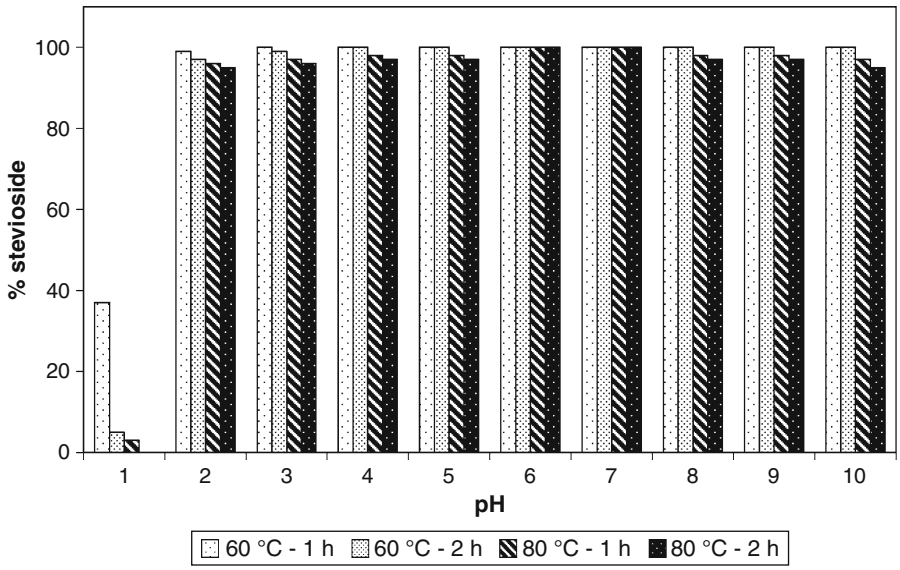

Fig. 2 pH-stability and degradation rate of stevioside in a pH range of 1–10 under thermal treatment

acetic acid, citric acid, tartaric acid and phosphoric acid during time periods up to 4 months showed a tendency towards enhanced decomposition of the sweetener at lower pH values depending on the acidic medium. While there could be found no evidence of degradation after 4 months 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 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 storage, respectively. The time dependent degradation rates are documented in Fig. 3.

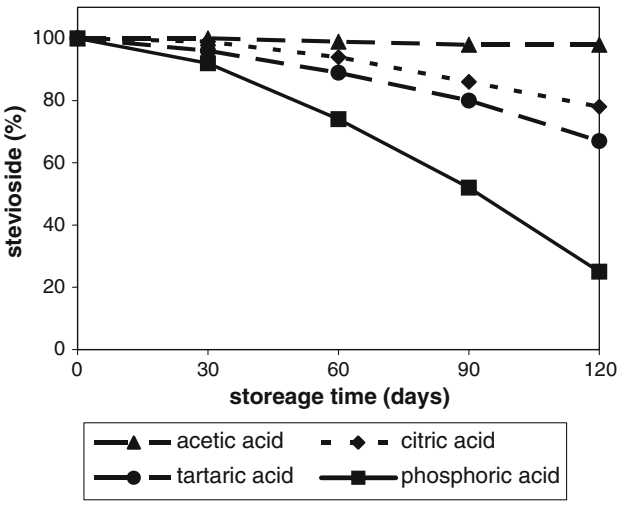

Fig. 3 Degradation rate of stevioside in 1% organic acids at room temperature

3.3 Interaction studies with vitamins

Up to 4 h incubation of stevioside with individual water-soluble vitamins of the B-group and vitamin C in aqueous solution at 80°C showed no significant changes neither in sweetener concentration nor in vitamin concentration in regard to the B-vitamins under thermal treatment as well as in untreated samples. In the case of vitamin C, however, incubation at 80°C resulted in a time dependent degradation of ascorbic acid, whereas a protective effect of stevioside on the degradation of ascorbic acid was observed resulting in a delayed degradation rate of vitamin C in 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 documented in Fig. 4.

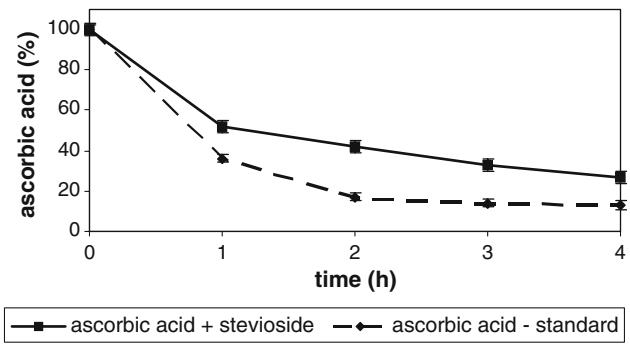

Fig. 4 Degradation rate of ascorbic acid as standard substance and in presence of stevioside, *filled square on solid line* ascorbic acid + stevioside, *filled diamond on broken line* ascorbic acid – standard

3.4 Interaction studies with other low calorie sweeteners

With regard to the practical application of low calorie sweeteners in synergistic mixtures thereof binary aqueous solutions of stevioside with the other individual low calorie sweeteners saccharin, cyclamate, aspartame, acesulfame and neohesperidin dihydrochalcone were investigated. Thereby, excellent stability and no interaction between the individual sweeteners were found in the course of thermal treatment at 80°C up to 4 h as well as of 4 months incubation at room temperature indicating that there are chemically no objections to the simultaneous application of stevioside with other low calorie sweeteners.

3.5 Stability and interaction studies in hot beverages

By thermal treatment at 80°C of stevioside-sweetened coffee and tea beverages over a time period of 4 h no significant influence neither on the caffeine nor on the stevioside content could be observed. Only minimal losses of stevioside up to 5% could be noticed after 4 h warm-keeping of tea or coffee indicating that under practical conditions of preparing and consumption of the hot beverages no effects of interaction should be expected.

4 Conclusion

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

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