AJHS.
Asian Journal of Home Science
Volume **8** | Issue 2 | December, 2013 | 739-742

Research Paper

Physiochemical characterization of stevioside and its compatilibility in selected food products

■ S. JANKI ALIAS PRIYA **AND** MAYA PRAKASH

Received: 28.05.2013; **Revised:** 04.11.2013; **Accepted:** 22.11.2013

See end of the paper for authors' affiliations

Correspondence to :
S. JANKI ALIAS PRIYA
Department of Foods and Nutrition, Acharya N.G. Ranga Agricultural University, HYDERABAD (A.P.) INDIA
Email: janagipriya@yahoo.com

■ **ABSTRACT :** Diabetes and obesity are the two threatening problems of the decade that demand the restricted use of sugar by the patients. Both the disorders are dependant on the glucose concentrations of the blood. It is the time for the researcher to introduce new compounds that can satisfy the individuals by replacing the taste of the sugar that can relieve them from the stress of sugar (Sucrose) restriction in their diet. In view of the above demand the current work aims to study one of the natural and an alternative sweetener, stevioside. The work involves the physico chemical characterization of the stevioside and analyzing its compatibility with the selected foods in comparison to the regular sugar. Techniques like sensory analysis, instrumental analysis and statistical analysis were employed. Sensory analysis includes the Threshold Test, Dose Response Test and time intensity Test. HPLC was used as a part of instrumental analysis. The study proved that the sweetness perception of stevioside increased with its concentration up to a limit after which the perception of bitter started. This indicates that the stevioside can be used as an alternative sweetener within the specified concentrations. Moreover, the study also revealed that the sweetness equivalence values for the high potency sweetness are highly system dependent and may vary in different food products. Using water as a medium it has been shown that sweetness of steviosides at 0.0197 and 0.013g had sweetness level equivalent to the sucrose concentrations of 3 per cent and 10 per cent, respectively.

■ **KEY WORDS :** Stevioside, Diabetes, Obesity, Sweetener, Dose response test, HPLC

■ **HOW TO CITE THIS PAPER :** Priya, S. Janaki Alias and Prakash, Maya (2013). Physiochemical characterization of stevioside and its compatilibility in selected food products. *Asian J. Home Sci.*, **8** (2): 739-742.

Sweet compounds always induce a positive hedonic response in humans and this response, which is found in the neonate, is often thought to be inborn. Sweet taste is said to have been experienced even by a five- month-old fetus. Since the beginning of the century sugar consumption has been on the rise.

Sugar has always been the most important sweetening ingredient of the human diet. Sugar (sucrose) is a natural sweetener that provides 4 calories per gram. It is acknowledged that excess sugar ingestion amounts to increase energy intake, which, in turn can lead to weight, gain and chronic disease associated with obesity and dental caries (Glinsmann *et al.*, 1998). Obesity is a risk factor for heart disease, cancer, diabetes and some other diseases.

Disadvantages of regular sugar:

There are many health hazards due to sugar consumption. Sugar suppresses the immune system. It lowers the amount of vitamin E in the blood (Veromann *et al.*, 2003). It increases the amount of liver fat (Veromann *et al.*, 2002). Sugar can produce a significant rise in triglyceride (Scanto and Yudkin, 1969) and it reduces low-density lipoprotein (Albrink and Ullrich, 1993). Government guidelines recommend eating much smaller amounts of sugar and sugar foods. As mentioned, high intake of glucose may lead to diabetes and obesity. These two conditions intern demands the restricted use of the regular sugar in the diet. Therefore, there is a need for sugar substitutes, which can help to reduce calorie intake. The food industry has responded to this trend by diverting its resources in to the development of alternative

sweeteners (Sardesai and Waldshans, 1991). Any food that adds sweet flavors to foods is called sweeteners. Sweeteners can be divided in to two broad classes; Bulk sweeteners, which provide energy (calories) and intense sweeteners, which have a sweet taste but are effectively non-caloric (Grenby, 1991). The sweeteners from natural sources with potential for commercial use include stevioside, rebaudioside, stevioside is a natural sweetener extracted from leaves of *Stevia rebaudiana* (Bertoni). Bertoni [A] is a white, crystalline, odorless powder, which is approximately 100-300 times sweeter than sucrose (Kroyer, 1999).

Stevioside sweetener is used in a wide variety of products, including soft drinks, tabletop sweeteners, confectionaries, fruit products, processed seafood products, processed vegetable products (*http://www.legco.gov.hk*). Stevioside is also used in ice cream, peach juice, dry desert mix, chocolate raspberry bars, and coconut protein bars. It is a high intensity non-nutritive sweetener which prevents the risk of high calorie accumulation on consumption. Hence, it is recommended to be used by the diabetic and obese patients.

■ RESEARCH METHODS

Keeping the above discussed points in mind the present study was under taken with an objective to analyze the physiochemical properties of the stevioside and study its compatibility with the selected food samples as a sweetener.

Preparation of stevioside:

Stevioside was extracted from the plant *Stevia rebaudiana* bertoni. The processing of the sample was done at CFTRI (Central Food Technological Research Institute), Mysore. The steps followed for the extraction procedure are detailed in the following Fig. A.

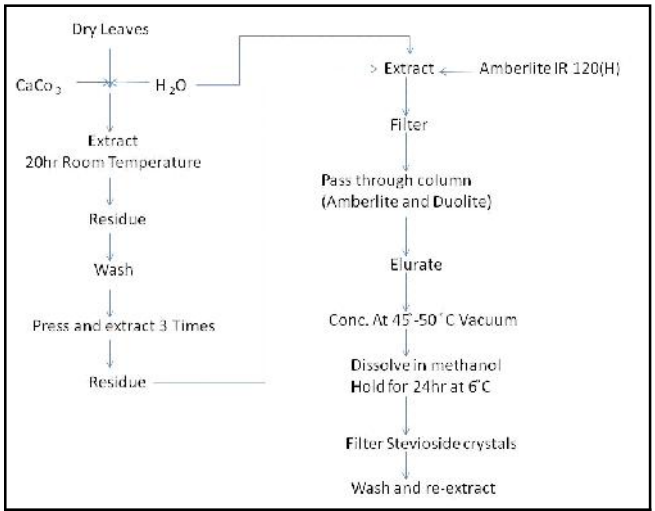

Fig. A: The protocol for the processing of the stevioside extraction form the plant material

Analysis of sreviosid:

Stevioside sweetener's equi-sweetness concentration was analyzed by sensory and instrumental methods.

Sensory analysis:

This was done using three different approaches Threshold Test, Dose Response Test and Time intensity test.

Panel training:

A group of 10 panelists was trained in three different sessions for psychometric (Threshold, Dose - response and Time intensity studies) and quantitative descriptive analysis tests.

The members of the panel were drawn from the scientific staff familiar with sensory analysis techniques and who had experience in the sensory evaluation of artificial or intense sweeteners. Samples were evaluated in the booth room maintained at temperature of 22±2°C and relative humidity of 50±5 % under fluorescent lighting equivalent to daylight.

Threshold test:

Threshold tests are conducted as per the method given in IS: 5126 (1969) and by chamber IV and Wolf (1996). Sweetness thresholds were determined for stevioside sample processed at CFTRI, Mysore along with sucrose, in pure aqueous media. Filtered and UV-treated water (Aqua guard, India, pH 6.0) was used for all the sensory experiments. The concentration series required for setting up the limits were determined by dilution technique.

Preparation of stevioside sample:

The sample was prepared in two series, the arithmetic and the geometric series which includes the concentrations of blank 0 per cent, 3, 6,9,12,15 per cent stock solutions and 0 per cent Blank with 2, 4, 8, 16 and 32 per cent stock solutions, respectively. The concentration of stock was maintained to be 0.025g / 100ml

Dose response study:

In dose-response study prior to the evaluation of test sample the panelists were given 2 and 32 per cent, sucrose solutions which served an anchoring points for 'low' and 'high' levels of sweetener intensities, respectively. The samples were served in 25ml 3-digit coded beakers. Solutions were served in increasing concentrations of sweetener. Panelist rated the samples for taste attributes using 15 cm scale where 1.25 meant detection threshold of the attribute and 13.75 indicate saturation threshold. The panelists were asked to mark the intensity of sweetness. Based on the data collected from the panelists the equi-sweetness level of the sweetener was detected.

Time intensity / temporal response study:

In the time intensity study panelists were trained to mark the scores on a structured quantitative descriptive analysis scale of 15cm with anchoring at low (1.25cm) and high (13.75cm) thresholds of 0.002 per cent and 0.1 per cent stevioside solution, respectively. The panelists were asked to mark the perceived intensity of sweetness on the scale at regular intervals of 10s, starting from the onset of sweetness perception up to a total period of 60s. Ten milliliter's of the sample (0.01%) was given in a 25ml beaker to the panelist. The panelist was asked to mark the intensity of the perceived sensation on the score card as soon as he/she takes the sample into the mouth. This marks the onset of sweetness. Then the panelist was asked to hold the sample in the mouth for 10s, after which he/she was asked to swallow and mark the intensity. Further markings of sweetness intensity were done after every 10s up to 60s. Time intensity profile was obtained by plotting mean scores of stevioside sweetness versus time in seconds. This study was taken up to measure the lingering effect of stevioside sweetness with time.

High performance liquid chromatography:

0.5 mg of stevioside was taken in 1 ml of deionoised water. It was degassed before the use to avoid the air bubble in the column. From that 10 µl of the sample is injected on the column an HPLC was run.

■ RESEARCH FINDINGS AND DISCUSSION

The psychophysical characterization of sweetener (stevioside) was done through experiments like threshold determination, dose response studies and time intensity. Acceptability in *Kheer*, black coffee, use level coffee and lime beverage was analyzed with a comparison to normal sugar. The results of the study are summarized below:

Organoleptic evaluation of stevioside:

As a part of this analysis the threshold values of steviocide in water and milk medium was analyzed and the results are shown as under.

From the Table 1 it is clear that the stevioside solution has sweetness level equivalent to that 0.4 per cent sugar solution in water medium

Table 1: Threshold values in milk and water from stevioside

		Arithmetic Mean (g %)	Geometric mean (g %)
Water	Stevioside	0.0025	0.002
	Sugar	0.4	0.42
Milk	Stevioside	Mean score (g%) 0.004	

Dose response studies are helpful in finding out the equisweetness levels of stevioside. Stevioside solutions of 0.003, 0.009, 0.027 and 0.081 per cent have sweetness levels that are almost equal to sweetness of sugar solution 2, 4, 8 and 16 gram per cent, respectively. It was also noticed that higher concentration (more than 0.6 gram %) of stevioside had distinct bitterness, which had adverse effect on sweetness perception (Table 2 and Fig. 1).

Table 2: Dose response pattern of stevioside and sucrose

Sr. No.	Sucrose (g %)		Stevioside (g %)	
	Conc	Mean ± S.D.	Conc	Mean + S.D.
1.	2	1.5± 0.51	0.003	2± 0.21
2.	4	2.8± 0.68	0.009	3± 0.06
3.	8	4.6± 1.2	0.027	6± 0.71
4.	16	7± 0.96	0.081	7.2± 0.42
5.	32	13± 1.22	0.243	5.5 ± 0.93
6.	-	-	0.719	3.2± 1.13

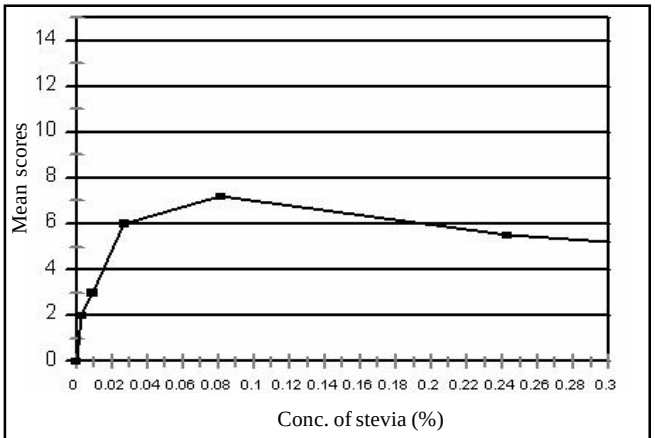

Fig. 1: Dose response graph for stevioside

The time intensity study showed that the Lingering sweetness perception in stevioside was more at 0.01 gram per cent when compared to sucrose; this was influenced by the concentration gradient.

Table 3: Time intensity profile of stevioside

Sr. No.	Time (sec.)	Score	Mean score			
			Plain water		Lime juice	
			Sucrose	Stevioside	Sucrose	Stevioside
1.	10	13.75	11.19	10.67	11.00	10.24
2.	20	13.75	9.88	9.17	9.86	8.99
3.	30	13.75	7.81	7.92	7.93	7.64
4.	40	13.75	6.19	6.83	6.29	6.14
5.	50	13.75	5.06	5.92	4.89	4.81
6.	60	13.75	3.50	5.00	3.39	4.07
7.	70	13.75	1.94	3.95	2.21	2.96

HPLC analysis showed the retention time of stevioside to be 2156.59 mv (milli volts) to check the quality of sample.

Table 4 :

Sr. No.	Particulars	Stevioside
1.	Area mV(milli volts)-sec	2156.593
2.	Retention time (Minutes)	5.33

From the above Table it was found that area (Milli Volts/ sec) of stevioside sample was found to be 2156.59 where as retention time was 5.33 min. Thus, it reveals that the stevioside sample contains no impurities

Compatability of stevioside in various foods like coffee, black coffee, lime beverages which showed the following common observations. Organoleptic evaluation and statistical t – test showed differences between stevioside coffee, sucrose coffee, stevioside black coffee, sucrose black coffee, stevioside Lime juice and Sucrose lime juice. Much difference was not found between the color, body, fresh aroma, sweetness etc. But the difference was found in the lingering sweetness and bitterness.

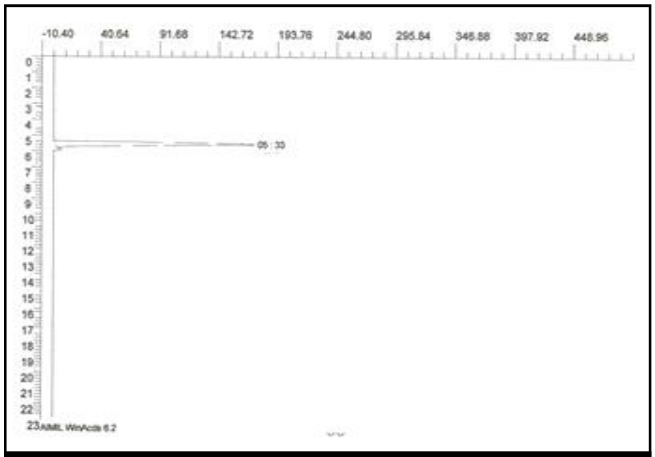

Fig. 3: HPLC results:

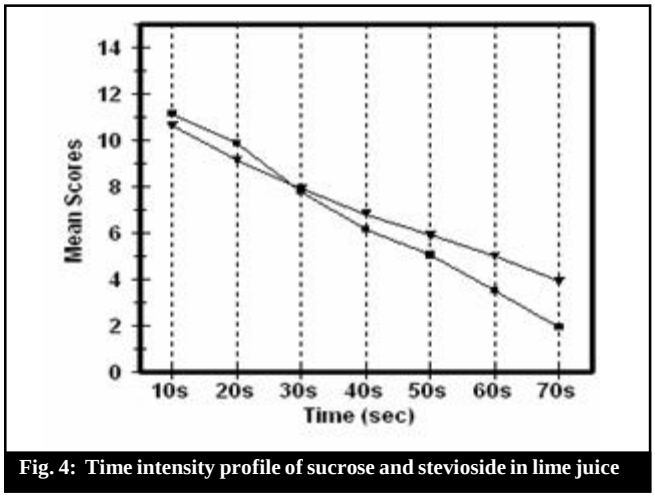

Fig. 4: Time intensity profile of sucrose and stevioside in lime juice

Conclusion:

From the above study on stevioside evaluation with a comparison to sucrose and its compatibility in various food products revealed that the stevioside have near equivalence of sweetness with Sucrose and can be used as an alternative to it in various food products like milk, coffee, *Kheer* etc. However, the sweetness equivalence was maintained only for a limited concentration after which the bitterness was perceived by the users. This indicates that the stevioside substitution for sugar must be within the limits concentration. Hence, the work concludes that steviocide can be used as an effective sweetener as an alternative to sucrose. This use can ultimately solve the problem of feed restriction to the diabetic and obese patients.

Authors' affiliations:
MAYA PRAKASH, Department of Sensory Science, Central Food Technological Research Institute, MYSORE (KARNATAKA) INDIA

■ REFERENCES

Albrink, M. and Ullrich, I.H. (1986). Interaction of dietary sucrose and fibre on serum lipids in healthy young men fed high carbohydrate diets. *American J. Clin. Nutr.,***43**:419-428.

Bell, F. (1954). Stevioside: A unique sweetening agent. *Chem. Indian*, pp 897-898.

Birch, G.G., Green, L.F. and Coulson, C.B. (1971). In: Sweetness and Sweeteners. *Intense Sweetness Natural Origin*, **17**: 35.

Chambers IV, E. and Wole, M.B. (1996). Sensory testing methods. American Society for Testing and Materials, W. Conshohocken, PA.

Glinsmann, W., Irausquin, H. and Youngmee, K. (1986). Evaluation of health aspects of sugar contained in carbohydrate sweeteners. F.D.A. Report of Sugars Task Force, 39 pp.

Kroyer, G.T. (1999). The low calorie sweetener stevioside: stability and interaction with food ingredients. Lebensm -*Wiss.u –Technol*, **32**: 509-512.

Pamplona, R., *et al.* (1993) Mechanisms of glycation in atherogenesis. *Med. Hypotheses*, **40**(3):174-181.

Scanto, S. and Yudkin, J. (1969). The effect of dietary sucrose on blood lipids, serum insulin, platelet adhesiveness and body weight in human volunteers, *Postgraduate Med. J.*, **45**:602-607.

Trevor, H. Grenby (1997). Dental aspects of the use of sweeteners. *Pure & Appl. Chem.*, **69** (4): 709-714.

Veromann, S. *et al.* (2003). Dietary sugar and salt represent real risk factors for cataract development. *Ophthalmologica*, **217**(4): 302-307.

Viswanath, M. Sardesai and Tammi, H. Waldshans (1991). Natural and synthetic intense sweeteners. *J. Nutr. Biochem.,***2**: 36-244.