Physico-chemical and sensory characteristics of steviolbioside synthesized from stevioside and its application in fruit drinks and food

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Abstract Steviolbioside (Sb) was synthesized from stevioside and characterized by infrared, nuclear magnetic resonance (¹H NMR and ¹³C NMR) spectroscopy. The purity melting point, solubility, acute toxicity, heat stability and sensory properties of Sb were evaluated. Physico-chemical and sensory properties of low calorie fruit drinks and shortened cake prepared by replacing sugar with Sb were evaluated. Sb was stable in neutral or acidic aqueous solutions maintained at 100 °C for 2 h. The sweetness intensity rate of Sb was found to be about 44 and 18.51 times sweeter than 0.5% and 10% sucrose solution, respectively. Sb solutions had sweet taste without bitterness compared to stevioside. No significant differences between the organoleptic properties of cakes prepared using sugar and those prepared replacing sugar with 50% Sb were observed. All drinks replacing sugar with Sb at

66% level had the highest overall acceptability scores comparable to those prepared using sugar alone.

Keywords Steviolbioside · Stevioside · Low calorie · Sensory properties · Sweetness

Introduction

Nowadays, global strategies are focused on using sugar substitute in food industries to satisfy consumer demand and dietary modifications to slow obesity development, and to close the gap between the sugar production and consumption. Since then, stevioside has been known with growing interest as a natural non-nutritive sweetener which is isolated from the plant *Stevia rebaudiana* Bertoni.

Stevia rebaudiana Bertoni is a perennial herb of significant economic value due to its high content of natural, dietetically valuable sweeteners in its leaves and has many medical effects (Abou-Arab et al. 2010; Gasmalla et al. 2014). The leaves of stevia contain ent-kaurene-type diterpene glycosides (Fig. 1), namely, stevioside 1, rebaudiosides A-F, steviolbioside 2, and dulcoside A, which are responsible for the typical sweet taste (Kinghorn et al. 1999). Stevioside 1 is the prevalent sweetener, accounting for 3–12% (w/w) of dried leaves and 90% in total glycosides with a sweetening potential 300 times that of sucrose and is known to be noncaloric (Moussa et al. 2003; Chat-sudthipong and Muanprasat 2009). Minor components are present in this plant, including rebaudiosides (B, C, F), steviolbioside 2 and dulcoside A (1–2% in total) (Geuns 2003). Some evidence confirms that steviolbioside and rebaudioside B are not genuine constituents of *S. rebaudiana* but are rather formed by partial hydrolysis during the extraction process (Kim and Dubois 1991).

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Fig. 1 Structure of stevioside 1 derivatives

In conjunction with sweetness, stevioside 1 contains a bitterness astringency, and detrimental aftertaste (Moussa et al. 2003; Cardello et al. 1999). The bitter aftertaste associated with stevioside 1 has been a problem for using it as a sweetener. The flavour companies have been trying to find ways to cover it without detracting from the distinguished benefits of its natural status (Massoud and Amin 2005).

Several studies have reported the antihyperglycaemic, insulinotropic, glucagonostatic and antihypertensive effects of stevia glycosides (Cardello et al. 1999).

Lately, we studied the production and physico-chemical evaluation of new stevia amino acid sweeteners from the natural stevioside (Khattab et al. 2015). In our continuing research to discover a natural non-caloric sweetener, we have focused on a novel minor steviolglycoside, steviolbioside that is synthesized from stevioside and studied its physicochemical and sensory characteristics. In addition, we investigated the effect of sucrose replacement by steviolbioside in low calorie fruit (mango and apple) drinks and cake on the quality of the final products.

The aim of the work is the improvement of stevioside in terms of overcoming its bitterness and astringency by synthesizing derivatives such as steviolbioside, which will improve the scope of utilizing stevioside as non-caloric sweetener which should be favorable for health conscious individuals.

Materials and methods

Materials

Stevioside was purchased from AWA for food additives (Alexandria, Egypt). Sucrose and sodium benzoate were

obtained from El Nasr Pharmaceutical Chemical Co. Cairo, Egypt. Ascorbic acid was obtained from S. LLChuangfeng Industrial Park, Zichuang Zibo city-Shandong (China); β-carotene 10% CWS (E 160 a) manufactured by ROHA Dyechem, Egypt; anhydrous citric acid was obtained from Weifang Ensign Industry Co.LTD China; Caramel colour (E150d) from Delta Aromatic International Co. 439, Pyramids, Giza, Egypt. Givaudan apple flavour was obtained from Schweiz. AG, Switzerland. Carboxy methyl cellulose (Yulog) was obtained from Shangdong yulong Cellulose Technology Co. LTD, China. Inulin (Frutafit® TEX), of long average chain length ≥ 23 monomers (Sensus, Brenntag Química, Spain) was used in this study. Wheat flour (72% extraction), sunflower oil, egg yolk, milk, sugar, baking powder, vanilla flavour, and salt were purchased from the commercial markets in Alexandria, Egypt. All of the aforementioned materials are food grade.

Fruit drinks were obtained as follows: natural mango pulp, 14° Brix from Cairo Agro. Processing Company, Egypt, pasteurized. Natural concentrated apple juice 70° Brix, from Delta Aromatic International Co., Giza, Egypt.

The solvents used were of HPLC reagent grade. The chemicals were obtained from Sigma Aldrich, Germany.

The stevioside purity was checked by reverse-phase high performance liquid chromatography (RP-HPLC), using an Agilent HPLC attached to a UV-visible Agilent 1200 PDA detector. A solution of stevioside was prepared and filtered through a 0.45 μm filter prior to use in HPLC. The injection volume was 70 μl. A Zorbax NH₂ column (5 μm 4.6 × 250 mm) was used. A linear gradient over 20 min (84–50% CH₃CN in H₂O, (pH = 5, H₃PO₄)) at a flow rate 2.0 mL/min was used to elute stevioside which was detected at 210 nm (Vanek et al. 2001). Pure stevioside (Sigma-Aldrich, USA) was used as reference standard.

Chemical analysis

Melting points were determined using a Mel-Temp apparatus and the obtained values are uncorrected. IR spectrum was performed using Perkin-Elmer system 1600 FTIR spectrophotometer in the region 400–4000 cm⁻¹ as KBr pellets. Nuclear Magnetic resonance spectra (¹H NMR and ¹³C NMR spectra) were recorded on a JOEL 500 MHz spectrometer with chemical shifts reported in parts per million (ppm) and are referenced relative to the used solvent (e.g. MeOH at δ H 3.35, 4.78 ppm for CD₃-OD). Elemental analyses were accomplished on Perkin-Elmer 2400 elemental analyzer, and the obtained values were found within $\pm 0.3\%$ of the theoretical values. TLC on silica gel-protected aluminum sheets (Type 60 GF254, Merck) was used to follow-up the reaction and to check the compounds purity, using a UV-lamp at λ254 nm.

Hydrolysis of stevioside 1

Stevioside **1** (5 g, 6.21 mmol) and NaOH (4 g, 100 mmol) were dissolved in 50 mL MeOH at room temperature. The reaction mixture was heated under reflux for 7 h under continuous stirring. The reaction mixture was cooled to room temperature and then neutralized with 1 N HCl. The desired product was obtained by the following two methods (Khatab et al. 2015);

Method A: The solvent was evaporated under vacuum to give a white solid, which was recrystallized with methanol-acetone (1:1) mixture to yield pure steviolbioside **2** in yield (2.99 g) 75% with mp of 198–199 °C.

Method B: The reaction mixture was extracted with *n*-BuOH. The organic layer was washed with water and evaporated under vacuum to give a crude solid which was recrystallized with methanol-acetone (1:1) mixture to yield pure steviolbioside **2** in yield (3.48 g) 87.5% with mp of 198–199 °C.

The obtained product had the following characteristics; IR (KBr): 3500–3300 (br., O–H), 2918 (sp³ C–H), 1720 (C=O acid), 1643 (C=C) cm^{−1}. ¹H NMR (CDCl₃): δ 0.83–0.86 (1H, m, CH), 0.94 (3H, s, CH₃), 0.97–1.09 (2H, m, CH), 1.16 (3H, s, CH₃), 1.39–1.43 (2H, m, CH), 1.48–1.53 (3H, m, CH), 1.57–1.69 (1H, m, CH), 1.77–1.93 (6H, m, CH), 2.01–2.14 (3H, m, CH), 2.17–2.20 (2H, m, CH), 3.29–3.62 (16H, m, 13 CH–O, 3 OH, D₂O exchangeable), 4.58–4.66 (7H, m, O–CH–O, =CH₂, 4 OH), 5.22–5.27 (1H, m, O–CH–O). ¹³C NMR: δ 15.23, 15.42, 18.93, 19.89, 20.00, 21.86, 21.93, 28.04, 28.11, 29.43, 37.31, 37.74, 37.89, 39.35, 40.64, 41.28, 41.41, 41.71, 43.37, 44.13, 44.25, 53.67, 53.80, 56.64, 56.73, 61.17, 61.36, 61.89, 62.37, 68.75, 70.07, 70.19, 70.60, 71.09, 74.04, 74.91, 76.44, 76.54, 76.84, 76.90, 76.94, 76.99, 77.16, 77.32, 78.59, 80.71, 86.48, 87.02, 87.74, 95.78, 96.03, 102.37, 102.94, 104.62, 152.32, 180.55. *Anal. Calcd* for C₃₂H₅₀O₁₃: C, 59.80; H, 7.84; found: C, 60.08; H, 8.11.

The purity of steviolbioside **2** was determined by HPLC using the following conditions; detection at wavelength of 220 nm and Agilent 1200 PDA detector. A Zorbax NH₂ column (5 μm 4.6x250 mm) was used for the separation, with a linear gradient of 84 to 55% CH₃CN in H₂O/ (pH = 5, H₃PO₄), over 15 min at a flow rate of 2.0 mL/min. The retention time *t_R* of steviolbioside **2** was 5.519 min (100%).

Physical characteristics of steviolbioside 2

The melting points of pure sucrose, stevioside (St, **1**), and steviolbioside (Sb, **2**) were determined. The solubility of steviolbioside **2** in water, ethanol, methanol, and chloroform was determined according to Soejarto et al. (1983). Heat stability of synthesized steviolbioside **2** sweetener in

acidic, neutral and alkaline solutions (at pH range of 2.5 to 9) at 60 °C and 100 °C for 2 h was examined by TLC and confirmed by HPLC as described by Chang and Cook (1983).

Acute toxicity

The oral acute toxicity of synthesized steviolbioside **2** was evaluated using male mice (20 g each, Medical Research Institute, Alexandria University) according to Verma et al. (1994). The animals were divided into groups of six mice each. Steviolbioside **2** was suspended in 1% gum acacia and given orally in doses of 50, 150, 300 mg/kg. The mortality percentage in each group was recorded after 24 h. Additionally, the tested product was evaluated for its par-enteral acute toxicity in groups of mice of six animals each. Steviolbioside **2**, or its vehicle, or propylene glycol (control) were given by intraperitoneal injection.

Sweetness intensity and taste quality evaluation

Sensory tests were carried out in a standardized room. The participants for sensory studies consisted of staff members from the Food Science and Technology Department, Faculty of Agriculture, Alexandria University. All panellists (*n* = 32, both sexes aged 48 ± 12 years) were exposed to a variety of sweeteners (Fructose, glucose, sucrose) prior to the test and trained to understand the hedoning scale of the sensory properties. Moreover, Panellists were asked to carry out magnitude estimation (vs. 0.5, 2, 5, 7, 10 and 15% sucrose) according to the formulae developed by DuBois et al. (1991). Sweetness intensity and taste quality (sweet, sour, bitter, sweet aftertaste, and other taste attributes for e.g. astringency and bitterness aftertaste) were evaluated (Hanger et al. 1996).

Technological methods

Cake preparation

Shortened cakes were prepared using the following ingredients: 100% flour, 60% sugar, 7.5% skimmed milk, 30% fresh whole eggs, 50% butter, 5% baking powder, 1.2% salt, vanilla and 50% water. All ingredients were mixed for 10 min using a Kitchen-Aid Professional mixer. All ingredients were placed into metallic and lard coated pans, and baked in an electric oven for 30 min at 200 °C. The sucrose was substituted by the synthesized steviolbioside **2** at ratio 50, 75 and 100%. The amount of each sweetener was calculated according to its sweetness equivalence. The relative sweetness of stevioside **1** and steviolbioside **2** are 125 and 20 time of sucrose. Inulin was used as bulking agent. Replacement of sucrose in cakes was applied

according to Zahn et al. (2010), where the appropriate amounts of water were added to inulin (2:1 V/W ratio) in order to achieve the proper dispersion of inulin to act as a filling replacer. Sensory evaluation of all the cake preparations was conducted by a trained panellists based on their appearance, hardness, moistness, cohesiveness and overall acceptability. The panellists were asked to judge the sensory attributes using nine-point hedonic scale, from like extremely (9) to dislike extremely (1) as described by Larmond (1977).

Preparation of fruit drinks

Fruit drinks were prepared by mixing all ingredients as shown in Table 1. The synthesized steviolbioside was used for the substitution of sucrose at ratio 1:1 and 2:1 in fruit drinks. The fruit drink was filled into glass bottles of 200 mL capacity. The bottles were closed tightly and autoclaved at 105 °C for 15 min, 1.5 bar. The glass bottles were cooled and stored for 4 months at 5 ± 2 °C in the refrigerator. All samples were analysed for chemical and microbiological analysis every month. During sensory evaluation, the panellists rated the samples for taste (sweetness or bitterness), flavour and overall acceptability using nine point hedonic scales as mentioned above.

Chemical analysis

Moisture content and total acidity of samples were determined according to the Association of Official Agricultural Chemists (AOAC 2000). The pH value was recorded using a pH meter (pH MVx 100 Beckman, USA). Cake volume was measured by the rapeseed displacement method. Specific volumes of the respective cakes were calculated after measuring their weight according to the American

Association of Cereal Chemists (AACC 1983). Compression measurements were performed with a Texture Analyser (Texture Pro CT3 V1.2, Brookfield, Middleboro, USA) equipped with a 5 kg load cell at a speed of 1 mm/s to a distance of 20 mm with a 4 g trigger load for 30 s. The viscosity was measured with a rotary viscometer (Brookfield Model DV-II + Pro, USA) at 30 rpm and 0.5 rpm at temperature 23 ± 2 °C for apple and mango juices respectively (Suwonsichon and Peleg 1999). The colour values of all samples were evaluated by a Hunter Lab Ultra Scan VIS model, colorimeter (USA). The lightness (L^*), redness (a^*), yellowness (b^*), chroma (C^*) and hue angle (h) were calculated from the colour primaries (Santipanichwong and Supphantharika 2007). Total colour difference (ΔE) between reference and samples with sweeteners was calculated from the respective L^* , a^* and b^* differences using the following equation:

$$\Delta E = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2 \right]^{1/2}$$

Microbiological analysis

Microbiological analysis was carried out following American Public Health Association (APHA 1992) using different selective media to enumerate different viable microorganism groups; Oxytetracycline—glucose—yeast extract agar for yeasts and moulds; Violet red bile lactose agar for *Coliform* bacteria and Clostridien- Differential Bouillon (DRCM) Merck, Germany was used to enumerate *Clostridium* and plate count. Tryptophane glucose yeast agar was also used to enumerate the total plate count of bacteria.

Statistical analysis

Data were evaluated by the analysis of variance and Student–Newman–Keuls Test using a Co-Stat Software computer program (2004).

Results and discussion

The HPLC analysis of the commercial stevioside **1** indicated that the sample was 97.8% pure.

Hydrolysis of stevioside **1**

Stevioside (St) **1** was hydrolysed using sodium hydroxide in methanol as solvent, to the corresponding acid **2** (Scheme 1). The structure of the desired product (steviolbioside, **2**) was characterized by spectroscopic analysis, IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and elemental analysis. The purity of

Table 1 Ingredients (%) used in preparing fruit drinks

Ingredients %	Natural drink	
	Apple	Mango
Pulp or concentrate	5.00	15
Sucrose or steviolbioside 2	11.00	12
Carboxymethylcellulose (CMC)	–	0.33
Citric acid	0.30	0.14
β -carotene 10% CWS	–	0.005
Sodium benzoate	0.01	0.01
Ascorbic acid	0.03	0.03
Flavouring agent	0.04	0.03
Caramel colour	0.001	–
Water	83.619	72.455

Steviolbioside **2** was calculated according to its sweetness equivalent

Scheme 1 Synthesis of steviolbioside (Sb, **2**) from stevioside (St, **1**)

steviolbioside **2** was further confirmed by HPLC analysis (Fig. 2).

Physical characteristics of steviolbioside sweetener

The melting point of steviolbioside **2** sweetener was determined on a Mel-Temp apparatus and was found to be 198–199 °C, which was lower than the melting point of stevioside **1** (200–201 °C).

The solubility results indicated that steviolbioside **2** sample was soluble in water, methanol and ethanol and insoluble in acetone, chloroform and ether. Stevioside **1** was similarly soluble in methanol and ethanol, less soluble in water and insoluble in acetone, chloroform and ether (Moussa et al. 2003; Soejarto et al. 1983; Abou-Arab et al. 2010).

The aqueous solution of steviolbioside **2** showed good stability over a wide range of pH values and temperatures. The thermal treatment in a pH range of 2–10 for 2 h showed good stability and no decomposition occurred at 60 and 100 °C. This feature is an advantage for steviolbioside **2** sweetener's utility in foods and beverages. Steviolbiosides are commonly used as natural sweeteners in beverages and

foods (Geuns 2003). Their main characteristics include their thermal stability up to 238 °C, resistance to acid hydrolysis, they are not fermentable, and most importantly nontoxic. These properties make them quite attractive candidates as additives in food and clinical applications (Awney et al. 2011; Gasmalla et al. 2014).

Sensory properties of the steviolbioside **2**

Sweetness equivalency and potency

The edulcorante properties of the steviol-glycosides vary from one another in terms of sweetness and quality of the taste. Accordingly, the organoleptic property of steviolbiosides depend on the ratio of the steviolbiosides obtained using different extraction and purification methodologies. The sweetness equivalency values for steviolbioside **2** are shown in Table 2 as evaluated by the trained panellists. Almost 11.36 mg/100 mL of steviolbioside **2** is 44 times more potent than 0.5% sucrose solution. Moreover, it is obvious that 5% sucrose equivalency, represented (194 mg/100 mL) of Sb **2** which act 25.77 times more potent than sucrose. Meanwhile, sweetness of 830 mg/100 mL of Sb **2**

Fig. 2 HPLC analysis of steviolbioside **2** sample. Conditions: HPLC Linear gradient system 84–55% CH₃CN/H₂O, H₃PO₄, pH = 5), over 15 min, Agilent 1200 PDA detector, detection at $\lambda = 230$ nm, A Zorbax NH₂ column (5 μ m 4.6 $\times$ 250 mm); flow rate 2.0 mL/min. Injection volume: 70 μ L. t_R [Steviolbioside] = 5.519 min (100%)

Table 2 Comparison between the sweetness potency and concentrations of the tested sweeteners

Concentration of sucrose (%)	Steviolbioside 2		Stevioside 1	
	Concentration (%)	Sweetening potency*	Concentration (%)	Sweetening potency*
0.5	0.011	44	0.003	200
2	0.062	32.36	0.012	166.9
5	0.194	25.77	0.04	125
7	0.365	19.17	0.058	120
10	0.540	18.51	0.086	116
15	0.83	18.07	0.145	103

* Potency was defined as the number of times sweeter a compound, on a weight basis, was than an isosweet concentration of sucrose

is 18.07 times more potent than 15% sucrose solution, while 145 mg/100 mL (0.15%) of St **1** is equal to the sweetness of 15% sucrose solution, but exhibits other taste attributes such as (e.g. bitterness and black liquorice). In addition, sweetness potency is system dependent. Therefore, it is important to define the medium (e.g. water, phosphoric acid at pH 2.5, etc.). The results agreed with previous reported work (Moussa et al. 2003; Massoud and Amin 2005; Yoshikawa et al. 1979), which indicates that stevioside had slight bitterness and some astringency aftertaste. Among the 4 diterpene glycosides, stevioside showed low general acceptability while rebaudioside A showed the lowest bitterness and astringency, and highest acceptability. The sweetness fold of these glycosides compared to sucrose is 100–125 in steviolbioside **2**, and 300-fold in stevioside **1** (Crammer and Ikan 1986).

Taste profile

Trained panellists did not detect any significant sour, salty, savoury, metallic or bitter or licorice off taste when evaluated the synthesized steviolbioside **2** in water at approximately 15% sucrose levels. Sweetness and bitterness

aftertaste were evaluated for different concentrations of steviolbioside **2** in aqueous solutions and compared with the same concentration of stevioside **1** and aqueous sucrose solution, Fig. 3. The sweet aftertaste time for 0.54% steviolbioside **2** which give the sweeteners intensity for the sucrose concentration 10% and stevioside **1** 0.086% was 35 s. Further increasing of steviolbioside **2** concentration up to 830 mg/100 mL, increased the sweet aftertaste time to 58 s without any other taste. While, the aftertaste of stevioside **1** was a mixed taste of sweetness and bitterness, and the aftertaste time increased by increasing the stevioside **1** concentration. Increasing the stevia sweeteners concentration more than 0.10%, gave a mixed aftertaste of bitterness and astringency and the aftertaste time increased up to 80 s (Moussa et al. 2003). Stevioside **1** exhibits sweetness taste at low sucrose equivalency (SE) levels, but exhibit other taste attributes such as (e.g. bitterness and black liquorice) at higher SE levels (Young and Wilkens 2007).

Acute toxicity

Steviolbioside **2** was further evaluated for its oral acute toxicity in male mice using a previously reported method

Fig. 3 Comparison of sensory attributes of steviolbioside (Sb, 2) and stevioside (St, 1) in water

(Verma et al. 1994). The results indicated that the compound under investigation was nontoxic and well tolerated by experimental animals up to 300 mg/kg. Moreover, this compound was tested for its toxicity through the parenteral route (Bekhit and Fahmy 2003). The results revealed that the test compound was non-toxic up to 150 mg/kg.

Food applications

Physical properties and sensory evaluation of baked shortened cake prepared with steviolbioside 2 sweetener

As shown in Table 3, moisture content of baked shortened cake varied between 15.32 and 26.92%. Moisture content and water absorption have direct impact on the texture attributes of products and strong correlation was found between moisture content and hardness (He and Hosney 1990). The obtained results indicated that the use of texturized inulin increased the moisture content as compared with the control sample in the different studied products, Table 3. The increment in moisture content may be attributed to the formation of a gel network, thus increasing the water holding ability, which is a typical characteristic of carbohydrate or protein-based fat replacers (Zahn et al. 2010).

However, specific volume is an important parameter, as it measures the cake density and quality, especially when it is linked with acceptance, appearance, crumb texture and grain. The specific volumes of cake containing steviolbioside 2 and stevioside 1 showed a lower value as compared to the control ones indicating that a lower amount of air remained in the cake. Higher gas retention and higher expansion of the product lead to a higher specific volume (Gomez et al. 2008).

Colour data were expressed by Hunter L*, a* and b* values corresponding to lightness, redness, and yellowness, respectively. In general, the crust and crumb colour of samples was affected by the replacement of cake sugar with sweeteners, Table 3. The crust colour, a* and b* values decreased, and the L* values increased, by increasing the substitution levels of steviolbioside 2 and inulin. On the contrary, in crumb cake the replacement of sugar by steviolbioside 2 led to an increase of the a*, b*, L*, and chrome (C*) values and the decrease of hue (h). The sample containing 50% stevioside 1 had the highest L* value and lowest a* value for crust cake and had the highest b* and a* values of crumb cake compared with steviolbioside 2 sample. These results might be due to the hydrolysis of low molecular weight fructan to fructose during the baking process, which may also favour non enzymatic browning reactions (Peressini and Sensidoni 2009; Purlis 2010). The ΔE parameters were higher than 3 in cake containing steviolbioside 2 or stevioside 1 which

are obvious for the human eye in comparison to the control (Table 3).

Textural parameters of the studied samples are tabulated and summarized in Table 3 and Fig. 4. It can be noted that hardness, hardness work cycle and total work cycle values increased in shortened cake with sweeteners compared to the control, where these values shown a decrease in cake made with 100% steviolbioside 2. No differences were shown in deformation of hardness among all treatments compared with the control samples. The texture modifier change of cake texture may be due to the ratio of texturized inulin in cake, which decreases the number of air bubbles and the existence of a denser matrix of muffin. The addition of Fibruline (1–5%) did not have any impact on the dough resistance to deformation (Collar et al. 2007).

Sensory rating scores of cake made with steviolbioside 2 or stevioside 1 as replacer for sucrose are presented in Table 3. Cakes prepared with 50% steviolbioside 2 had higher mean scores for shape, colour and overall acceptability as compared to the control sample. Increasing the level of replacer to 100% steviolbioside 2 resulted in significant decrease in all sensory properties rating scores compared to that of the control. For sweetness and after-taste, cakes replaced by steviolbioside 2 and stevioside 1 exhibited no significant difference as compared to their controls. Texture of such cakes was extremely liked by panellists. Thus, the use of inulin in cakes was effective for technological and nutritional advantages of cookies and may have additional health benefits including prebiotic effect and enhanced mineral absorption. However, when sugar was substituted with steviolbioside 2, at 80% level, the flavour component was found to be most affected as indicated by the lower score as compared to control (Table 3). Inulin or fructooligosaccharides (FOS) enriched cookies had a golden sheen which led to higher score on the colour parameter. Moderate reducing power of FOS can give rise to slight browning reaction during baking, which could impart better colour to such products. In an earlier study (Gennaro et al. 2000), Raftilose P® was found to have reducing capacity which may be expected to be susceptible to Maillard reaction.

Physico-chemical, Microbiological properties and Sensory evaluation of apple and mango natural drink prepared with steviolbioside 2 sweetener

Table 4 summarized some of the physico-chemical properties namely pH, titratable acidity, total soluble solids, and viscosity of apple and mango natural drinks prepared with different sweeteners. It can be noted that the synthesized alternative sweetener had no effect on the pH value. Total soluble solids (TSS) of all drinks decreased as the ratio of the steviolbioside 2 added increased compared to fruit

Table 3 Physico-chemical and organoleptic properties of shortened cake

Parameters	Control	Replacing sugar with 50% St	Replacing sugar with Sb		
			(50)	(75%)	(100%)
Moisture (%)	15.32 ± 0.22	23.28 ± 0.66	24.60 ± 0.52	24.44 ± 1.09	26.92 ± 0.73
Specific volume	1.96 ± 0.15	1.68 ± 0.42	1.72 ± 0.33	1.85 ± 0.50	1.86 ± 0.46
Colour values					
L*	60.96	70.41	67.42	66.88	56.2
Crumb	79.66	76.64	82.97	81.39	81.07
a*	17.91	11.76	13.52	13.59	20.14
Crumb	−0.11	2.88	1.67	1.73	2.35
b*	47.09	47.67	46.18	46.31	43.42
Crumb	31.77	37.17	33.94	34.9	36.36
C*	50.39	49.10	48.12	48.26	47.86
Crumb	31.77	37.28	33.98	34.94	36.49
h	69.18	76.14	73.68	73.65	65.12
Crumb	90.20	85.57	87.19	87.16	86.30
ΔE	–	11.29	7.86	6.12	6.41
Crumb	–	6.87	4.34	4.02	5.39
Textural analysis					
Hardness cycle (g)	690	1033	1073	1854	1593
Deformation at Hardness (mm)	9.87	9.84	9.84	9.84	9.86
Hardness work cycle1 (mJ)	37.1	53	56.5	112.4	76
Recoverable deformation cycle1 (mJ)	3.52	2.49	2.49	3.47	3.1
Recoverable work cycle1 (mJ)	2.6	2.2	2.4	2.7	3.3
Total work cycle1 (mJ)	39.7	55.2	58.9	119.4	80.3
Load (g) at target	318	449	451	910	773
Deformation at target (mm)	10	10	10	10.01	10.02
Sensory evaluation					
Surface appearance					
Shape	8.56 ^a	8.50 ^a	8.65 ^a	8.23 ^a	7.77 ^b
Colour	8.50 ^a	8.58 ^a	8.67 ^a	8.15 ^{ab}	7.38 ^b
Eating properties					
Texture	8.23 ^a	8.10 ^a	8.17 ^a	7.35 ^b	7.09 ^b
Taste (sweetness)	8.50 ^a	8.04 ^b	8.29 ^{ab}	7.69 ^c	7.32 ^d
Flavour	8.22 ^a	8.00 ^a	8.00 ^a	7.38 ^b	6.85 ^c
Aftertaste (sweetness)	7.72 ^a	7.62 ^{ab}	7.80 ^a	7.43 ^b	5.00 ^c
Overall acceptability	8.60 ^a	8.50 ^a	8.68 ^a	7.90 ^b	7.20 ^c

Means in a row not sharing the same letter are significantly different at $P \leq 0.05$

St stevioside **1**; Sb: steviolbioside **2**; L* lightness; a* redness; b* yellowness; C* chroma; h hue angle; $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$

drinks containing sucrose only. This reduction in TSS may be caused by the used quantities of the alternative sweeteners. It was obvious that viscosity decreased as sugar replacement level increased and the control sample has the highest viscosity value for mango (344 cP) and for apple (8 cP) juices (Table 4). The storage period (2–5 °C) for 4 months on all the tested samples shows a slight decrease of both pH and viscosity. It is evident that the pasteurization followed by storage at low temperature was sufficient to keep the sample for 4 months. In addition, stevia

sweeteners inhibit the growth and reproduction of some bacteria (Geuns, 2003).

The data presented in Table 4 indicates a diversity in colour values (Hunter L*, a* & b*) of the studied products. The results showed that the L*, a* and b* values were influenced by the substitution levels of steviolbioside **2** and the type of juice. The L*, a* and b* values increased in case of the mango juice containing steviolbioside **2** compared to the control ones. On the other hand, in apple juice containing steviolbioside **2**, L* and b* values decreased

Fig. 4 Brookfield graph for textural analysis of cakes, at a speed of 2 mm/s to a distance of 10 mm for 30 s using different concentrations of steviolbioside **2**

and slight increase in a^* values was noticed. According to Cadena et al. (2013), the browning alterations in mango nectar sweetened with different sweeteners such as neotame, sucralose, stevia with 97% rebaudioside A, or acesulfame-K could be associated with nonenzymatic processes with the formation of caramel colored pigments. Where Rocha and Bolini (2015) found that the passion fruit juice sweetened with different sweeteners such as aspartame, stevia extract, sucralose and neotame, cyclamate or saccharin were lighter in colour, and the values for the parameter of luminosity (L^*) retracting and the intensity of the yellow colour (b^*) were lower than in case of the sample with sucrose.

It is clear that ΔE parameters were lower than 3 in juice containing steviolbioside **2** and sucrose which is not obvious for the human eye in comparison to the control drinks.

Microbiological properties of fresh or stored fruit juices for all treatments showed that yeasts and moulds, coliform bacteria and clostridium are not detected in 0.1 gm samples. This is due to the good hygienic conditions during manufacture and during storage period.

Sensory evaluation: The data indicated that all drinks prepared and sweetened with steviolbioside **2**/sucrose blends (2:1), had the higher score of sweetness, flavour, overall acceptability and absence of bitterness compared with control samples. The blend samples (1:1) showed the lowest intensity of mango flavour as compared to the other samples, probably influenced by the greater intensity of sweet taste in relation to the control sample. The steviolbioside **2** and sucrose blend showed a greater intensity of residual sweetness than the control. Schiffman et al. (2003), reported that sweetener blends have become important in the production of foods and beverages, making use of the benefits of multiple sweeteners, as synergistic taste enhancement. Sweeteners profile modifications offer advantages over the use of single sweetener as well as economic and stability advantages. The results indicated that the panellists preferred the mango samples sweetened with these blend. In addition, after 120 days of storage, the steviolbioside **2**/sucrose (2:1) blend showed a sensory profile closer to that of sucrose samples.

Table 4 Physico-chemical and organoleptic properties of natural mango and apple drinks prepared with steviolbioside **2**

Juice Parameter	Mango			Apple		
	Control	T1 Suc:Sb2 (1:1)	T2 Suc:Sb2 (1:2)	Control	T1 Suc:Sb2 (1:1)	T2 Suc:Sb2 (1:2)
T. S. S. (%)	13.1	9.5	7.1	13.1	9.1	8.1
Titrateable acidity (%)	0.21	0.21	0.22	0.33	0.34	0.34
pH	3.88	3.87	3.87	2.81	2.81	2.82
Viscosity	344	320	316	8	7.2	7.2
Colour values						
L*	45.56	47.5	48.04	29.29	28.57	27.42
a*	6.38	8.01	8.04	0.89	1.31	1.91
b*	31.26	33.78	34.67	6.07	6	5.28
ΔE	–	3.58	4.53	–	0.84	2.27
Sensory evaluation						
Taste						
Sweetness	8.20 ^a	8.14 ^a	8.65 ^a	8.10 ^a	8.25 ^a	8.50 ^a
Bitterness	–	–	–	–	–	–
Flavour	8.28 ^{ab}	8.08 ^b	8.52 ^a	8.05 ^b	8.10 ^a	8.37 ^a
Overall-acceptability	8.10 ^{ab}	7.90 ^b	8.38 ^a	8.19 ^a	8.13 ^a	8.22 ^a

Means in a row not sharing the same letter are significantly different at $P \leq 0.05$

Suc sucrose; Sb **2** steviolbiodide; L* lightness; a* redness; b* yellowness

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

Conclusion

From the aforementioned results steviolbioside **2** has many beneficial properties and abundant potential as a sweetener in beverage and food products. This work illustrates that steviolbioside **2** has a clean sweet taste. It can be used as a sucrose substitute and a natural non-nutritive source of sweeteners in low calorie products to many consumers. It is also well-suited for blending with other non-calorie or carbohydrate sweeteners.

Cakes sweetened with 50% steviolbioside **2** as sugar replacer in presence of inulin as a bulking agent had no significant effect on the organoleptic properties, but improved the scores of the overall acceptability. All drinks replacing sugar with Sb at 66% level had the highest score of sweetness, flavour, overall acceptability and absence of bitterness comparable to those prepared using sugar alone.

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