Research Article

Extraction, preparation, sensory evaluation and sweet perception of *Stevia rebaudiana* based food products

Tehneyat Fatima[1], Fazia Ghaffar[1*] and Aurang Zeb[2]

1. Department of Food & Nutrition Sciences, College of Home Economics, University of Peshawar, KPK, 2512-Pakistan
2. Nuclear Institute for Food and Agriculture (NIFA) Tarnab, Peshawar. KPK, 24330-Pakistan
Corresponding author's email: faziaghaffar@uop.edu.pk

Abstract

Stevia rebaudiana (*S. rebaudiana)* is a shrub is a naturally sourced zero-calorie sweetener. The present study aimed at extracting sweetness components, preparation, standardization, & sweet perception of *S. rebaudiana* grown under the existing climatic conditions of Tarnab, Peshawar. The *S. rebaudiana* extract was prepared by dissolving 74 grams of *stevia* leaves powder in1000ml of distilled water and purification with calcium hydroxide. A total of twelve food products were developed by using sucrose as control and *stevia* extract as an experimental sweetener. Sensory evaluation for overall acceptability of the products were evaluated by 10 trained panelists using a 10 points hedonic scale. On the basis of sensory tests three products were rejected due to strong flavor and taste of chlorophyll still present. The data showed the overall acceptability score for control halwa was 8.12 ±0.67 as against 6.78 ±0.37 for *stevia* halwa with highly significant value of 0.000 and percent difference of 16.5%. Overall acceptability for control pancakes were 7.71 ±0.58 as against 6.62 ±0.64 for *stevia* sweetened pancakes and for control cookies 7.25 ±0.33 as against 6.93 ±0.96 for stevia cookies with non-significant difference. Perception of sweetness for *stevia* extract in comparison of sugar solution was done by threshold test. Highest perception was reported for 5mL/1000 mL water being equivalent to a sugar solution of 10 grams/100mL water. The duration of sweetness stimulus was greater for *stevia (lasted 40 seconds)* than for sucrose.
Keywords: Product development*; Stevia rebaudiana;* Sensory evaluation; Sweet perception; Sweet perception duration

Introduction

The sixth most important risk factor which contributes to the overall burden of the diseases is the excessive body weight. The ratio is approximately 1.1 billion adults and 10% of children come in that range [1]. Out of 194 countries, Pakistan ranked 165 in overweight category with 22.2% of individuals over the age of 15 crossing the threshold of obesity [2]. The other studies also showed that one in four Pakistani adults are being overweight [3].

Due to increase number of diabetic patients in the world, the need for alternative sweeteners of low caloric values have been increased. Saccharin, aspartame, cyclamates

etc are used as alternative sugars. The use of some of these sweeteners is strictly banned because of their toxic effects [4]. Natural sweeteners include honey, sucrose (table sugar), and glucose [5]. Some sweeteners are called intense sweeteners which are several hundred times sweeter than sucrose (table sugar). They contain calories thus used in very small amounts because of their high sweetening properties [6]. Thus there is a continuous research for the alternative sweeteners which has low toxicity and calorific value with high intensity sweetening effect [7].

S. rebaudiana is a shrub indigenous to Brazil and Paraguay belonging to the compositae family in which a carbohydrate present, called Steviosides which is 300 times sweeter than sucrose [8]. *S. rebaudiana* is used for hundreds of years because of its naturally sweetening substance and flavoring ingredients [9]. It has lower calorie and Glycemic index compared to common sugar. It contains good source of protein, ash and crude fiber which are necessary for good health [6]. It contains anti carcinogenic and anti periodontophatic properties [10]. *Stevia* is cardio tonic and has antihypertensive effects [11]. It has ant hyperglycemic effect and is glucagnostatic that shows is safe for diabetics [12]. It is anti-oxidant and has anti-obesity effects [13]. Furthermore, studies have shown that its extract can actually improve blood sugar levels [8]. Pancreatic beta cells produces insulin which helps in insulin secretion, steviosides in it directly act on them for this purpose [14]. The objectives of the current study was to prepare *stevia* extract and its comparison with sugar based products to access their sensory evaluation, perception of sweetness, duration of perception.

Materials and methods

Preparation of *S. rebaudiana* extract & purification

Preparation of *stevia* powder and *stevia* extract

Preparation of *stevia* powder

The sun dried leaves of *S. rebudiana* were grounded in a lab grinder (Dawlance-DWBL 600MS). The powder was weighed again on measuring scale.

Preparation of *stevia* solution and removal of chlorophyll

About 74 grams of *S. rebudiana* leaves were dissolved in 1000 ml of distilled water. Dried Calcium hydroxide (approx. 05gm) was added in the *Stevia* solution after some time chlorophyll settled down to the bottom.

Filtration and neutralization of pH of the solution

The solution was filtered on the filter paper (Whattman-42). The filtrate obtained was a clear solution devoid of chlorophyll up to a large extent. Due to the high pH of the extract solution, citric acid was added to the level until the pH was adjusted to 7.0

Condensation and storage of the solution:

The solution was condensed for 15 minutes on hot water bath. The condensation process was done to enhance the sweetening quality of *stevia*. The clear solution of *stevia* was stored in freezer to prevent the extract from any foreign matter and was used within 2 weeks.

Preparation of food products

In order to standard the recipe of local foods made with *stevia* extract against sucrose as a control sweetening agent the following products were prepared.

a. Halwa, b. Pancakes, c. Cookies (Meethi Tikki), d. Cup Cakes, e. Black Tea, f. Green Tea

Sensory evaluation of the products

A total of 12 food items (six prepared with *stevia* extract and six with sucrose i.e. table sugar) were presented to ten trained panelists. Sensory attributes evaluated were the degree of liking (DOL) for taste, appearance, texture, odor, and overall acceptability. All panelists evaluated the samples using a 10-

point category hedonic scale (1 = dislike extremely; 5 = neither like nor dislike; 10 = like extremely).

Perception of sweetness sensation to *stevia* extract

About three solutions were prepared by dissolving 5ml, 10ml and 15ml of *stevia* extract in 1000 ml water while 5 gm, 10gm, and 15 gm of table sugar was added to 100ml of water respectively. Sweetness equivalence of *stevia* to sugar was carried out on sweetness threshold test. A panel of 20 judges (age ranging from 18 to 35 years) was asked to match the sweetness of the test solution to table sugar.

Duration of perception of the sweetening solutions

Perception test was also done, on the above mentioned solutions, in order to assess the duration of sweetness of the *stevia* and sugar. The test was done in order to note the duration of sweet stimulus of *stevia* extract and the sugar solution. A panel of 20 judges with age ranging from 18 to 35 years was asked to taste the solution and time duration in minutes was noted with stop watch till the point of disappearance of sweetness stimulus felt on tongue.

Results and discussion

Comparison of sensory evaluation of the food products

The sensory evaluation of the all the six products prepared from sugar and *stevia* Extract is given in (Table 1). Overall acceptability of *stevia* based products was lower when compared with sugar based products. In these conventionally prepared products the lower color and appearance score for *stevia* halwa was due to lack of desirable brownish appearance. This may be due to lower level of sucrose which is required for the development of desirable brown color by caramelization. The judges also noted the bitter taste in *stevia* products. This was because of the chlorophyll that was still present in the extract. The findings of the current study are in strong agreement with overall acceptability of carbonated beverages and kulfi sweetened with *stevia* extract powder [15, 16]. Salama also proposed that the physical properties of the products get enhanced when stevia replaced 20, 40 and 60% sucrose in an ice cream mix [17]. Similarly overall acceptability scores were also reported by other investigators for stevia as a bio sweetener and as a sweetening agent in soft ice creams [18-20].

Table 2 showed the sensory evaluation of the all the six rejected products prepared from sugar and *stevia* extract. The products had strong flavor and bitter taste due to pronounced flavor of chlorophyll which was still present in *stevia* based products. As reported by other investigators this bitter after taste associated with stevia can be a limiting factor that might affect the overall acceptability of the food product [21, 22]. As suggested this sourness may also exert a negative impact on the degree of liking in carbonated beverages [15].

Perception of sweetness

The threshold test for sweet perception (Table 3) showed that the highest combination was 5ml/1000mL water based *stevia* solution with 10gm/100ml water based sugar solution. The above results of the current study are in compliance with another study which reported that 1 gm of stevia in 100 ml water was equivalent to a sucrose solution containing 20 grams of sucrose [6].

Duration of sweet perception

The test was for the duration of sweet stimulus of *stevia* extract and the Sugar solution (Table 4) showed that taste stimulus lasted for more than 40 seconds and the duration was greater for *stevia* than for sucrose as perceived by 20 of the judges. The results are in conformity with the study of Savita et al who reported similar results for *Stevia* solution as perceived by 50 percent judges [6].

Table 1. Comparison of sensory evaluation of accepted food products

Parameters	Halwa		Pancakes		Cookies	
	Sugar based	*Stevia* **based**	**Sugar based**	*Stevia* **based**	**Sugar based**	*Stevia* **based**
1.Appearance Mean ± SD % Difference P- Level	8.40 ±0.84	6.60 ±0.69 21.4% 0.001	8.10 ±0.87	6.40 ±0.69 20.9% 0.000	7.30 ±1.05	7.20 ±0.91 1.3% 0.591
2.Texture Mean ± SD % Difference P- Level	7.80 ±0.63 11.5% 0.019	6.90 ±0.73	7.40 ±0.96 12.1% 0.041	6.50±0.52	6.60 ±1.17 0.1% 1.000	6.60 ±1.17
3.Taste Mean ± SD % Difference P- Level	8.10 ±0.73	6.40 ±1.17 20.9% 0.001	7.90 ±0.56	7.00 ±0.94 11.3% 0.019	7.10 ±1.10	6.30 ±1.25 11.2% 0.182
4.Odor Mean ± SD % Difference P- Level	7.40 ±0.51	6.50 ±1.17 12.1% 0.029	7.30 ±0.48	6.70 ±0.95 8.2% 0.081	7.30 ±0.67	6.90 ±1.10 5.4% 0.309
5.Acceptability Mean ± SD % Difference P- Level	8.12 ±0.67	6.78 ±0.37 16.5% 0.000	7.71 ±0.58	6.62 ±0.64 14.1% 0.000	7.25 ±0.33	6.93 ±0.96 32% 0.343

Table 2. Comparison of sensory evaluation of the rejected food products

Parameters	Cup Cakes		Black Tea		Green Tea	
	sugar based	*stevia* **based**	**sugar based**	*stevia* **based**	**sugar based**	*stevia* **based**
1.Appearance Mean ± SD % Difference P- Level	7.30 ±1.05	7.21±0.92 1.2% 0.591	8.40 ±0.84	6..60±0.69 21.4% 0.000	8.10 ±0.87	6.40±0.69 20.9 % 0.001
2.Texture Mean ± SD % Difference P- Level	6.63 ±1.19	6.62 ±1.18 0.15% 0.019	7.80 ±0.63	6.40±1.17 17.91% 0.061	7.90 ±0.56	7.00±0.94 11.3% 0.018
3.Taste Mean ± SD % Difference P- Level	7.71 ±0.58	6.50 ±1.17 15.6% 0.051	8.10 ±0.73	6.40 ±1.17 20.9% 0.001	7.40 ±0.96	6.50 ±0.52 12.1% 0.182
4.Odor Mean ± SD % Difference P- Level	7.90 ±0.56	6.70 ±0.95 15.1% 0.029	7.40 ±0.51	6.50 ±1.17 12.1% 0.081	7.71 ±0.58	6.62 ±0.64 14.1% 0.309
5.Acceptability Mean ± SD % Difference P- Level	7.71 ±0.58	6.62 ±0.64 14.1% 0.018	8.12 ±0.67	6.78 ±0.37 16.5% 0.089	7.30 ±0.48	6.70 ±0.95 8.2% 0.343

Table 3. Perception of sweetness

S. No.	Number of Judges	Percentage of Judgment Responses	*Stevia* Extract concentration (in ml)/1000ml	Sugar (grams) Equivalency /100ml
1	1	5	5ml	5
2	11	55	5ml	10
3	2	10	5ml	15
4	0	0	10ml	5
5	4	20	10ml	10
6	1	5	10ml	15
7	0	0	15ml	5
8	0	0	15ml	10
9	1	5	15ml	15

Table 4. Duration of perception

Sweet stimulus (sec)	5ml *stevia* extract (No. of judges)	10 gm sucrose (No. of judges)
<20	0	1
20-40	3	15
>40	17	4

Conclusion

It is concluded from the study that *S. rebudiana* can successfully be utilized as a natural sweetener in the preparation of indigenous bakery products. Further investigations are needed to remove the inherent green color and chlorophyll flavor left in the extract to improve its utility, and preparation of variety of food products and beverages.

Authors' contributions

Conceived and designed the experiments: F Ghaffar & A Zeb, Performed the experiments: T Fatima & F Ghaffar, Analyzed the data: F Ghaffar, Contributed materials/ analysis/ tools: A Zeb, Wrote the paper: T Fatima & F Ghaffar.

References

1. Haslam, DW & James WP (2005). Obesity. *Lancet* 366: 1197-1209.
2. Streib L (2007) World's Fattest Countries. *Forbes*. cited at www.google.com.pk
3. Gulf News (2006). One in four adults is overweight or clinically obese. Cited at www.gulfnew.com/news/paksitan.
4. Weihrauch MR & Diehl V (2004) Artificial sweeteners- do they bear a carcinogenic risk. *J of Annals of Oncolog* 15: 1460–1465.
5. Crammer B & Ikan R (1977). Properties of synthesis of sweetening agents. *Chemical Society Reviews* 6: 431–465.
6. Savita SM, Sheela K, Sharan SAG, Shankar S, & Ramakrishna P (2004). Stevia Rebaudiana – A Functional Component for Food Industry. *J Hum Ecol* 15(4): 261-264.
7. Adari BR, Goka R, Reddy R, Prasad E, Sundergopal S, Yerrapragada VL & Ravikumar R. (2012) An improvised process of isolation, purification of steviosides from Stevia rebaudiana Bertoni leaves and its biological activity. *International J of Food Science & Technology* 47(12): 2554-2560.

8. Curi R, Alvarez M, Bazotte RB, Botion LM, Godoy & Bracht A (1986) Effect of Stevia rebaudiana on glucose tolerance in normal adult humans. *Brazil J Med Biology Resource* 19: 771-774.

9. Margaret A (2015). Stevia, Nature's Zero-Calorie Sustainable Sweetener: A New Player in the Fight against Obesity. *Journal Nutrition Today* 50(3): 129-134.

10. Contreras S (2013). Anticariogenic properties and effects on periodontal structures of *S. rebaudiana* Bertoni: Narrative review. *J Oral Res* 2(3): 158-166.

11. Hossain AB, Islam M, Saw R & Mandal, AK (2011). A review on natural sweetener plant – Stevia having medicinal and commercial importance. *J of Croatian Agronomy* 73(1-2): 75-91.

12. Goyal SK, Shamsher & Goyal RK (2010). Stevia (Stevia rebaudiana) a bio-sweetener: a review. *International Journal of Food Sci and Nutrition* 61(1): 1–10.

13. Arora E, Khajuria V, Kumar, S, Gillani, Z, Sadiq S & Tandon, VR (2010). Stevia: A Promising Herbal Sweetener. *JK Science J of Medical Education and Res* 12(4): 212-213.

14. Jeppesen P B, Gregersen S, Poulsen CR & Hermansen K. (2000). Stevioside acts directly on pancreatic beta cells to secrete insulin: actions independent of cyclic adenosine monophosphate and adenosine triphosphate-sensitive K+-channel activity. *Metabolism* 49: 208–214.

15. Saniah, KC & Sharifah SM (2012). The application of Stevia as sugar substitute in carbonated drinks using Response Surface Methodology. *J Trop Agric and Fd Sci* 1: 23–34.

16. Giri A, Rao R & Ramesh V (2012). Effect of partial replacement of sugar with Stevia on the quality of kulfi. *J Food Sci Technology* 51(8): 1612-1616.

17. Salama FMM (2004). The use of some natural sweeteners in ice-cream manufacturing. *Egypt J Dairy Sci* 322: 355-366.

18. Alizadeh M, Lalabadi MA & Kheirouri S (2014). Impact of using Stevia on physicochemical, sensory, rheology and Glycemic index of soft ice cream. *Food and Nutrition Sci* 5: 390-396.

19. Schiffman SS, Booth BJ, Carr BT, Losee ML, Miller EAS & Graham BG (1995). Investigation of synergism in binary mixtures of sweeteners. *Brain Research Bulletin* 38: 105-120.

20. Cardello HM, Dasilva MA & Damasio MH (1999). Measurement of the relative sweetness of Stevia extract, Aspartame and cyclamate/saccharin blends as compared to sucrose at different concentration. *Plant Foods for Human Nutrition* 54: 119-130.

21. Tan SL (2009). Stevia-satisfying Malaysia's sweet tooth. *Proceedings of National Conference on New Crops and Bio Resource* 43-51.

22. Saniah k, Samsiah SM, Lip MJ, Nazrul MH & Azizah I (2009). The potential of Stevia as a herbal sugar substitute in a non-carbonated drink. *Proceedings of National Conference on New Crops and Bio Resource* 220-222.