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EFFECT OF *STEVIA REBAUDIANA* BASED FOOD PRODUCTS ON POST PRANDIAL GLYCEMIC RESPONSES AMONG HEALTHY YOUNG FEMALES

FATIMA T[1], GHAFFAR F[1*], AURANGZEB[2]

[1]Department of Food & Nutrition Sciences, College of Home Economics, University of Peshawar, 25120, Peshawar, Khyber Pakhtunkhwa, , Pakistan

[2]Nuclear Institute for Food and Agriculture (NIFA) Tarnab, Peshawar, Postal Code: 24330, KPK, Pakistan

For correspondence; Cell: +92- 0333-9225753, Email: faziaghaffar@upesh.edu.pk

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ABSTRACT

Stevia Rebaudiana is a shrub that belongs to the *compositae* family. Naturally scored as a zero-calorie sweetener, it can be used in a variety of products. The current study aimed at developing indigenous food products sweetened with *Stevia Rebaudiana* and later were compared with sucrose for Glycemic responses among young females (18-22 years). The products developed were tested on hedonic scale for acceptability for five basic parameters. The products were analyzed for proximate composition. Of the evaluated products, pancakes scored highest sweetened with *Stevia.* The addition *Stevia* altered the proximate composition. Major differences found were in carbohydrates, fats and caloric value. Glycemic indices showed significantly difference among all the *Stevia* and sucrose sweetened products. *Stevia Rebaudiana* sweetened products kept post prandial blood glucose low throughout the test period. Threshold test was done to compare the sweetness of *Stevia* extract and sugar solution. The highest combination was found to be 5ml of *Stevia* extract in 1000 ml water equivalent to a Sugar solution containing 10 grams of Sugar .The duration of the sweet stimulus for *Stevia* lasted for 40 seconds as perceived by 20 of the judges. It is concluded from the present study that *Stevia Rebaudiana* has a greater potential in the preparation of low sucrose and low calorie products.

Keywords: *Stevia Rebaudiana*, Proximate Analysis, Nutritional Composition, Glycemic Responses, Sweetness Threshold, Sweetness Duration

INTRODUCTION

One of the six basic taste sensations known by humans for centuries is sweetness [1]. Primary sources of natural sweeteners include glucose, fructose and sucrose [2]. These sweeteners have very high caloric value which increases the risk of Diabetes and Obesity being undesirable for human health [1]. Due to these risk factors, there is an increasing demand for substitute sweeteners which are low in calories [3].

Stevia Rebuadiana is a sweet herb and was included in sunflower (*Asteraceae*) family in 1905 which is 130-300 times sweeter than sucrose [4-6]. The plant was originated from Paraguay and Brazil and was used in teas and beverages [7]. This plant has also been called as sweet leaf or sugar leaf [8]. Different glycosides extracted from this plant were named as Steviosides, Rebaudiosides and Dulcosides [9]. Among these; Rebaudiosides are most suitable for human health because of their least bitter taste properties [7]. The Rebaudiosides-B, Rebaudiosides-C, Rebaudiosides-D and Rebaudiosides-E differ from each other by their glucose side chain alignment [10]. The steviosides are the major glycosides which are responsible for its sweetness [4]. The sweetness is taken out from leaves by extraction [11]. The leaves of *Stevia* are green in color and used in huge variety of foods [12]. *Stevia* leaves also contain protein, ash and crude fiber and a good source of iron, calcium, potassium, magnesium, phosphorus, zinc, flavonoids, Vitamin A and C [13, 14].

Besides these qualities, *Stevia* is also heat stable and has good solubility and light stability [7]. Recently, it becomes an important non caloric sweetener in Japan and Brazil and has been used in several different products like fruit juices, tobacco products, chewing gums, processed foods etc [15, 16]. Different researchers found that the natural constituent of *Stevia* is beneficial for human health [16]. It helps to prevent diabetes, reduce weight, prevents tooth decays and increases the process of digestion [17]. By increasing water excretion in the body, it also helps to improve kidney functions [18]. Further studies showed that *Stevia* has cardiotonic, contraceptive, and hypoglycemic properties [19, 20].

Among these protective uses, the main use of *Stevia* is as a sweetener [21]. It improves the blood sugar [22]. Insulin (a hormone responsible for decreasing the sugar levels in blood) is stimulated by steviosides which boast the insulin production of pancreatic cells and increases the glucose tolerance as well [23]. Globally about 6 percent of the

world's total population is affected by Diabetes which is due to high glycemic Index foods [4]. Glycemic Index shows how much carbohydrates containing food raise the blood sugar level. The greater the number of available carbohydrates, the higher will be the blood sugar level in the body [24]. The glycemic control can be done by taking low glycemic diets and is strictly recommended for the patients of Diabetes [25, 26]. Thus steviosides are the food additives can be used to improve the glucose regulation in diabetes and has lower calorie and Glycemic index as compared to sugar [27, 3].

The current study aimed to prepared and purify the *Stevia* extract, its comparison with sugar based products to analyze their organoleptic acceptability, nutritional composition, sweetness equivalence, duration of sweetness stimulation and the effect on the glycemic responses of selected food products among young females.

2. METHODS AND PROCEDURES

2.1 Preparation and Purification of *Stevia* Extract:

The sun dried leaves of *Stevia Rebaudiana* were grinded in a lab grinder (Dawlance-DWBL 600MS). The weighed powder was then dissolved in 1000ml of distilled water. Approximately 5 grams of dried Calcium hydroxide was added in the s*tevia* solution so that chlorophyll is separated from the leaves. Filtration was done on the filter paper (Whattman-42). The obtained filtrate was a clear solution devoid of chlorophyll up to a greater extent. The pH of the solution was adjusted up to 7.0 by adding some crystals of citric acid. Condensation of the solution was done to enhance the sweetening quality of *Stevia* for 15 minutes on the hot water bath. After cooling, the cleared solution was stored in refrigerator at 5°C to protect against any spoilage.

2.2 Preparation of the Products:

Three different types of local foods were prepared from s*tevia* extract with simultaneous products with sucrose to be considered as controls. The products were suji halwa, pancakes, cookies (Meethi Tikki) with the standardized recipes.

2.3 Sensory Evaluation of the products:

A total of six food items (three prepared with s*tevia* extract and three 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).

3.4 Proximate Analysis of the food products:

The nutritional analysis of the food products was done by AOAC method [28]. Percent moisture, ash, proteins, fats and fiber were determined by these standard procedures while carbohydrates was determined by the formula as weight by difference between 100 and the summation of other proximate parameters as Nitrogen free Extract (NFE)

3.5 Perception of Sweetness Sensation to *Stevia* Extract:

Sweetness equivalence of Stevia to sugar was carried out for the threshold test. A panel of 20 judges (age ranging from 18 to 35 years) was asked to match the sweetness. The solutions were prepared by 5ml, 10ml; 15ml of *stevia* extract in1000ml of water and 5, 10, 15, 20, 25 gm sucrose in 100 ml water respectively.

3. 6 Duration of Perception of the Sweetening Solutions

Duration of sweet perception was also done in order to generalize the sweetness of *Stevia* and sugar. The test was done to note the duration of sweet stimulus of *Stevia* extract and the Sugar solution on taste buds. A panel of 20 judges with age ranging from 18 to 45 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.

3.7 **Glycemic Responses of the young females to Products**

After the overall acceptability of the products, a group of 30 healthy young females at the Department of Food and Nutritional Sciences, College of Home Economics, University o Peshawar were recruited based on written content. Self constructed questionnaires were provided to the volunteers and received a brief medical evaluation to identify physical or psychological contradictions. Participants were required to meet the following inclusion criteria: Age ranges between 18 to 22 years, height varies from 4.9- 5.8 inches, weight lies between 45- 60 kg and BMI ranges from 18 – 25.8 Participants having history of diabetes, anemia, hormonal imbalances, allergies, acute infections, caffeine addictions, dislike of the selected food products were excluded.

7.1 Determination of Blood Glucose

The food products were tested for glycemic responses. Intravenous blood (approx.5cc) was drawn to estimate blood glucose. The volunteers were divided into control and experimental groups and were first tested for fasting glucose .Afterwards the females, in the experimental group consumed *stevia*

based products while those in the control group consumed similar products made with sugar. Post prandial blood glucose was estimated at 30, 60, 90 and 120 minutes intervals. Blood glucose was analyzed with Tinder's method.

3. RESULTS AND DISCUSSIONS

3.1 Proximate Analysis

Proximate analysis of all the six samples is given in ***Table-1.*** The moisture level of the *Stevia* products is high as compared with the sugar based products. Some studies showed that water absorption capacity of *Stevia* is due to its greater level of protein content [13]. The products made with s*tevia* might have low shelf life and can't be placed for longer time at room temperature. The mean content of fats showed lowered values for s*tevia* based products suggesting *stevia* based products absorb less oil than sugar products. The current study suggested that along with less sugar based calories the products made with s*tevia* extract might be lower in fat. This lowered caloric contribution will be of benefit in the context of public health. The lower percent carbohydrates in the products seem to be due to lowered values sugars conventionally to these products. Such products can be more beneficial to the patient of diabetes due to the lower sucrose level. The findings are in agreement with those of Salem and Massoud [29]. These researchers reported that *Stevia* sweetened frozen yogurt contained 33.86% less calories than sucrose based yogurt. The energy values of all the *Stevia* based products were low due to the absence of sucrose being added for sweetness that thought to be a major contributor to calorie level as reported by Apurba *et al* [6].

3.2 Sensory Properties of the Products:

Sensory properties of all the six products prepared from Sugar and *Stevia* extract are given in ***Table 2.*** The scores of *Stevia* products were less when compared with Sugar products. The products showed less brownish appearance and were lighter in color. This could be due to the absence of sucrose level which helps to enhance the color of finished products as a result of caramelization. Similar findings were also reported by other investigators where the color and appearance score of control samples were significantly higher than those of their s*tevia* treated Kulfi samples [6].

Non significant differences were found in texture and odor of the control and experimental samples. Taste and overall acceptability of the *stevia* based products were lower when compared with sugar based products. The judges attributed this to the bitter after taste in the *stevia* products. This might be due to the presence of chlorophyll

that was still present in the extract. The finding of the current study is in contrary with other researcher which reported that the physical properties of the products improved when 50% *Stevia* was added with sucrose [30].

3.3 Perception of Sweetness

Sweetness equivalence of Stevia in comparison to sugar is shown in **Table-3.** Threshold test was done in order to match the sweetness of *Stevia* extract with the Sugar solution. The results showed that the highest combination was that of 5mlof Stevia extract in 1000ml water being equivalent to 10gm/100ml sugar in water. The current findings are in compliance with Savita et al who proposed that 1 gm of Stevia in 100 ml water was equivalent to a sucrose solution containing 20 grams of sucrose solution [13].

3.4 Duration of Perception

The duration perception test was done to note the duration of sweet stimulus of s*tevia* extract on tongue **(Table-4).** As perceived by 20 judges, the duration was highest for s*tevia* as compared to sugar solution which lasted for more than 40 seconds. The results are in conformity with the study of other researchers who reported similar results for *Stevia* solution as perceived by 50 percent judges in their study [13].

3.5 Glycemic Responses of Sugar and *Stevia* Based Products

The six samples (three sugar based and three *stevia* based) tested for Glycemic responses among 30 female subjects are presented in **Table-5** and **Figure-1**. The fasting blood glucose was normal in both groups. Following ingestion of tested products peak value of blood glucose were observed after 30 minutes in both groups. The blood glucose values in control group remained higher after ingestion of all the sucrose based products throughout the test period. Contrarily with the Stevia based products, the blood glucose levels significantly remained lowered throughout the post prandial test period (i.e. 60, 90 and 120 minutes). The spike in the blood glucose after 30 minutes was attributed to the entry glucose into the after circulation after the consumption of carbohydrates based meals [31]. The post prandial lowered glycemic indices with *stevia* based products in the current study are in strong agreement with another study [15].

Table 1: Proximate Analysis of the Products

Parameters	Halwa		Pancakes		Cookies	
	Sugar	*Stevia*	Sugar	*Stevia*	Sugar	*Stevia*
Ash% Mean ± SD %Difference P- Level	0.29 ±0.05	0.21 ±0.02 -27.5% 0.195	0.61±0.01	0.31±0.01 -49.1 0.001*	0.75 ±0.04	0.82 ±0.026 +9.3 0.089
Moisture% Mean ± SD % Difference P- Level	29.44±0.65	35.37±0.60 -20.1 0.11	16.45 ±0.01	18.46±0.20 -12.2 0.000*	41.95±0.56	51.92±0.25 -23.7 0.001*
Crude Fiber% Mean ± SD % Difference P- Level	1.27 ±0.14	1.2 ±0.10 -5.5 0.935	1.1±0.01	0.56 ±0.40 49.0 0.575	0.33 ±0.03	0.28±0.105 15.1 0.26
Fats (g/100g) Mean ± SD % Difference P- Level	19.51±0.13	13.43±0.37 31.1 0.002*	16.22±0.79	15.36±0.95 5.3 0.189	20.81±0.79	16.74 ±0.57 -19.5 0.008
Proteins (g/100g) Mean ± SD % Difference P- Level	8.60±0.14	8.66±0.32 -0.69% 0.168	15.50 ±0.43	11.93 ±0.04 23.0% 0.014*	13.46 ±0.08	11.44 ±0.57 15.0% 0.019*
CHO (g/100g) Mean ± SD % Difference P- Level	40.89±0.91	41.13±0.67 -0.58 0.003*	50.12±0.63	53.38±0.08 -6.5 0.004*	22.7±0.55	18.8±0.03 17.1 0.002*
Energy (Kcal) Mean ± SD % Difference P- Level	373.55±4.12	320.03±3.44 14.3 0.005*	408.46±8.95	399.48±7.07 2.1 0.002*	331.98±1.90	271.62±7.55 18.1 0.003*

*** Differences are significant P≤0.05**

Table-2: Sensory Evaluation of Food Products

	Halwa		Pancakes		Cookies	
Parameters	Sucrose	*Stevia*	Sucrose	*Stevia*	Sucrose	*Stevia*
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
Texture Mean ± SD % Difference P- Level	7.80 ±0.63	6.90±0.73 -11.5 0.019*	7.40 ±0.96	6.50±0.52 -12.1 0.041*	6.60 ±1.17	6.60 ±1.17 -0.1 1.000
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
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
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

*** Differences are significant at P≤0.05**

Table 3: Perception of Sweetness

S. No.	No. of judges	Percentage	*Stevia* extract concentration 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

Table 5: Glycemic Indices Responses of Sugar and *Stevia* based products

Parameters	Halwa		Pancakes		Cookies	
	Sugar	*Stevia*	Sugar	*Stevia*	Sugar	*Stevia*
Fasting Mean ± SD % Difference P- Level	70.6 ±0.54	70.6±0.89 0.00 1.00	71.6 ±1.14	71.4±1.67 0.27 0.749	71.4 ±0.54	71.2 ±1.30 0.28 0.704
30 minutes Mean ± SD % Difference P- Level	134.0 ±10.9	112.8 ±4.81 15.8 0.026	129.8 ±2.58	118.4±3.8 8.7 0.009	126.0 ±4.30	119.4±2.88 5.2 0.008
60 minutes Mean ± SD % Difference P- Level	121.0 ±3.67	105.4 ±2.07 - 12.8 0.001*	126.2±3.34	113.8±6.94 9.8 0.002*	121.2 ±4.65	113.2±4.60 6.6 0.003*
90 minutes Mean ± SD % Difference P- Level	116.8 ±1.64 102.2±2.48 12.5%	0.000*	115.2 ±4.81	104.6±5.02 9.20 0.001*	114.2±4.96	105.6 ±2.79 7.5 0.004*
120 minutes Mean ± SD % Difference P- Level	110.2 ±3.56	95.0±2.91 -13.7 0.003*	107.2 ±3.19	95.6±3.64 10.8 0.004*	106.2 ±3.89	97.2 ±1.30 8.4 0.001*

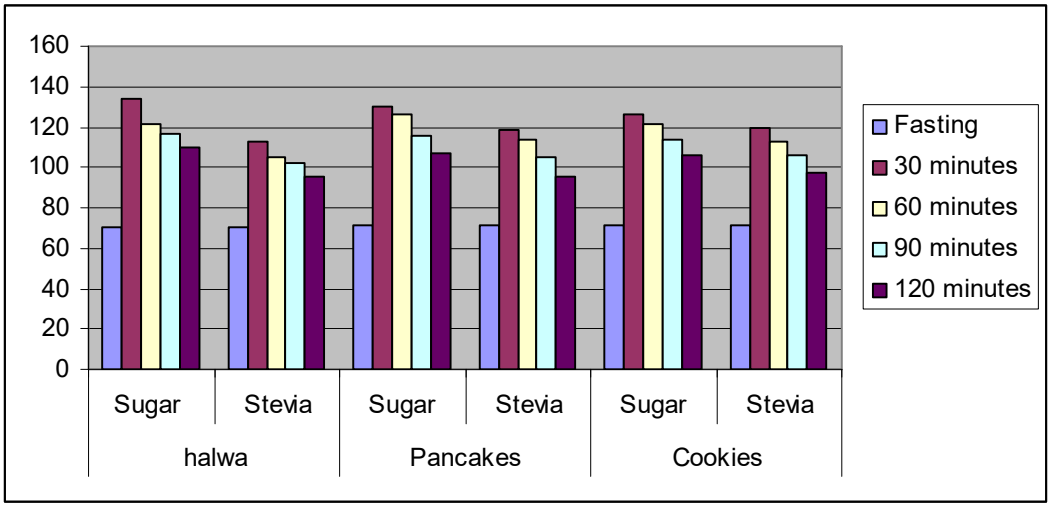

Figure-1: Mean Glycemic Indices of the Respondents

CONCLUSION

It is concluded from the current study that the addition of *Stevia Rebaudiana* into the food products can help in weight reduction and management of glycemic responses among diabetic patients. Further investigations are needed to remove the inherent green color and chlorophyll flavor left in the extract to improve its utility in a variety of food products and beverages.

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