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Research Article

Utilization of *Stevia rebaudiana* Bertoni (Chini Biruwa) as Replacement of Sucrose in Yoghurt

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Abstract

Objective: The study was conducted to utilize the water extract of *Stevia rebaudiana bertoni* (chini biruwa) as the replacement of sucrose in yoghurt. The specific objectives are to calculate the proximate composition of Stevia leaf powder, to extract the sweet glycosides or steviol glycosides from Stevia powder, to conduct the acceptability test of stevia extract with sugar solution of different concentration, to compare sweetness of stevia extract with sucrose solution, to optimize the Stevia concentration in yoghurt on the basis of sensory evaluation and to carry out physicochemical analysis of the formulated yoghurt.

Materials and Methods: The entire ingredient like Stevia leaf powder, Yoghurt ingredients like milk, SMP and yoghurt culture were procured from Kathmandu valley. Other necessary facilities were provided by college. Proximate calculation was done as per [1]. Extraction of sweet glycosides from stevia powder was done by boiling in water at different ratios as by Abou-Arab., *et al* [2]. Optimization of extract and comparison of sweetness of stevia extract with sucrose solution was done by sensory method [3]; optimizations of the yoghurt formulation with stevia extract (15°Bx) of different concentrations were carried out.

Results: The proximate composition of dried Stevia leaf was found to be as; Moisture 8.06 ± 1.2%, Protein 2.4 ± 1.3%, Fat 5.2 ± 2.1%, Crude fiber 15.21 ± 2.1%, Carbohydrates 68.2 ± 6.8%, Ash 8.34 ± 1.7% and Reducing sugar 1.52 ± 0.2%. It was found that the TSS of extract decreased as the water proportion increased during extraction. At 5% level of significance, there was significant difference between the extract. The best optimization of extract was found at the ratio of 1:30 by sensory method. While the sweetness of the 2°Bx extract was found to be equivalent to 30°Bx sucrose solution. The best optimization for the yoghurt formulation was found by incorporating 0.15% of 15°Bx extract at ($P < 0.05$). The physicochemical analysis of stevia incorporated yoghurt shows higher amount of protein, SNF and acidity than sucrose incorporated yoghurt at ($P < 0.05$).

Conclusions: Yoghurt prepared by incorporating 0.15% of 15°Bx extract was found to be best in terms of sensory analysis. This shows that the normal sugar or sucrose can be replaced by the proper use of stevia leaf extract as the extract ratio of 1:30 of stevia to water was accepted by people from sensory analysis.

Keywords: *Stevia rebaudiana Bertoni; Sucrose; Yoghurt*

Introduction

Nepal, a landlocked country located in the central part of main Himalayan range is floristically rich and diversified. Nepal possesses 2.80 percent plants of the total number of species found globally. The Himalayas are famous for medicinal plants and are used in traditional healing systems like Ayurvedic, Homoeopathic, Amchi, etc. Some of these plants are even used for allopathic medicine. Stevia,

botanically known as *Stevia rebaudiana* Bertoni is a genus of about 240 species of herbs and shrubs in the sunflower family (Asteraceae), native to subtropical and tropical regions from western North America to South America. Today its cultivation has spread to other regions of the world, including Canada and some parts of Asia and Europe [4,5].

The species *Stevia rebaudiana*, commonly known as sweet leaf, honey leaf or simply stevia, is widely grown for its sweet leaves. The compounds in the leaves are called stevioside and rebaudioside and they can be more than 200 times sweeter than sucrose and the leaf by itself is about 20 to 30 times sweeter than sucrose. Presently, Stevia is well known for its high content of sweet diterpene (about 4 - 20%) in dry-leaf matter [6]. This plant is totally innocuous, posing no threat to human life and health, holding out in fact great hope of the production of a non-calorie sweetener with health benefits [7]. The study was conducted to utilize the water extract of *Stevia rebaudiana* (chini biruwa) as the replacement of sucrose in yoghurt. Yoghurt is one of the most popular fermented dairy products widely consumed all over the world. It is considered a healthy food due to high digestibility and bioavailability of its protein, calcium and energy. The sugar present in it is milk-sugar i.e. lactose and table sugar i.e. sucrose is incorporated to enhance the sweet taste. Sucrose is highly avoided by diabetic and health concern individuals hence decreasing the consumption of nutritious yoghurt by significant amount. The intake of yoghurt can be increased with more health benefits by substituting the sucrose with stevia glycosides. Yoghurt with various flavoring and aroma additives is very popular. Cardamom is also used as to enhance the flavor. Incorporating cardamom and stevia can enhance flavor and nutrition value of yoghurt at the same time.

Material and Methods

Stevia leaf powder was collected from Palm Agrotech and Bio-energy Nepal (PABEN), Kathmandu. Yoghurt ingredients like milk, SMP, yoghurt culture and additives as cardamom essence were procured from Nepal Dairy Pvt. Ltd., Kathmandu. All the equipments and chemicals were used of analytical grade available in laboratory.

Methodology of the research is given below.

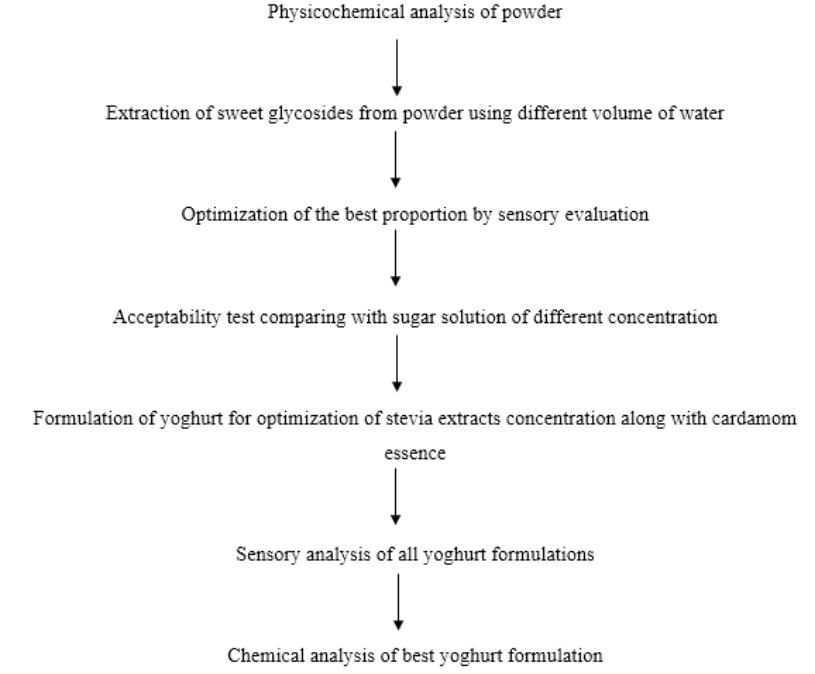

Figure: *Research Methodology.*

Proximate analysis of the stevia powder was carried out as per [1]. Extraction of stevia glycosides was done as 5 grams of stevia powder was boiled in water in different ratio like 1:15, 1:20, 1:25, 1:30 and 1:40 for 1 hour in conical flask fitted with condenser. More recent processing methods used water filtration procedures and do not use methanol and so produce a natural product. Newer facilities in Brazil used only water extraction procedures and claims 96% purity of the product [8]. After extraction, all six liquids were evaluated with respect to their total soluble solid. The total soluble solid of each extract were noted and sensory evaluation of the extract was carried out. The mass were filtered through Whatman no.1 in vacuum filter. The brown colored extracts were collected in clean bottles and labeled. Prepared extracts were subjected to sensory evaluation for comparison of sweetness of stevia extract with standard sugar solution. Samples were given to 32 semi-trained panelists. They were asked to compare the sweetness of the given sample i.e. 2°Bx stevia extract with different standard sugar solutions i.e. 10°Bx, 20°Bx, 30°Bx, 40°Bx and 50°Bx. Formulation of yoghurt was done as per 15°Bx stevia in different concentration with milk. Milk having 4% fat and 8% SNF was taken for the yoghurt preparation. The milk was standardized to 15% total solids by adding skim milk powder and it was heat treated at 85°C for 30 minutes. It was cooled to 40 - 45°C spontaneously and about 1% thermophilic lactic culture was inoculated which was followed by incorporating 0.2% cardamom essence. Different concentration of 15°Bx stevia extract i.e. 0.15%, 0.25%, 0.3% and 0.35% (0.3, 0.5, 0.6 and 0.7 mL respectively) was calculated and measured using pipette and added to respective 200mL cup which was labeled. The inoculated milk was poured equally into each cup giving all together four formulation of stevia incorporated yoghurt and one plain yoghurt. All the samples were incubated at 44°C for 24 hours and the yoghurt was cooled to 4°C spontaneously. Moisture, fat, protein, total solids, SNF and acidity of all the yoghurt samples was determined according to AOAC [1]. Sensory test of yoghurt samples were carried by 9-point hedonic rating test [3], in the participation of 40 semi-trained panelists comprising of faculties and undergraduate students of National College of Food Science and Technology, Kathmandu. Statistical analysis was performed using Microsoft excel 2007.

Results and Discussion

The physicochemical properties of stevia powder were determined. Sweet glycoside from stevia powder was then extracted in water in different ratio. Optimization of the total soluble solid and acceptability test of extract was carried out. The best ratio was then used for the yoghurt preparation. Yoghurt was prepared by using different formulation of stevia extract and its physicochemical properties (TS, acidity, fat, SNF, moisture, protein) and sensory quality were evaluated.

Parameters	Stevia Powder
Moisture (%)	8.06 ± 1.2
Ash (g/100g)	8.34 ± 1.7
Fat	5.72 ± 2.4
Protein	2.4 ± 1.3
Reducing Sugar	1.5 ± 0.2
Crude fiber	15.21 ± 1.3
Carbohydrate	68.28 ± 6.8

Table 1: *Physicochemical properties of dry stevia powder.*

Values are Mean ± Standard Deviation obtained from the triplicate data.

The moisture content of stevia power was found to be 8.06 ± 1.24 which correspond to moisture content determined by Savita., *et al.*[9], Mishra., *et al.* [10] and Kaushik., *et al*. [11] such as 7, 7 and 7.7 respectively. The ash content and fat content of the stevia was found to be 8.34 ± 1.7 and 5.72 ± 2.4 respectively which is parallel to the value determined by Abou-Arab., *et al.* [2] as 7.4 and 3.7 respectively. Similarly, crude fiber and carbohydrate value obtained 15.21 ± 1.3 and 68.28 ± 6.8 respectively correspond to the value determined by Savita., *et al* [9]. Protein content in this study was found to be lower than previous studies which may be due to difference in the cultivation area and land. From this study and investigation, stevia leaves are a good source of carbohydrates and crude fiber which are essential factors for maintenance of health. On the other hand, high ash content indicates that the stevia leaves are a good source of inorganic minerals [2].

Sample No.	Extraction ratio (powder/water)	TSS°Bx	Extract from 5 grams
1.	1:15	3.2 ± 0.0	2.51^{a} ± 0.0
2.	1:20	2.4 ± 0.01	2.47^{a} ± 0.01
3.	1:25	1.8 ± 0.01	2.28^{b} ± 0.01
4.	1:30	1.3 ± 0.1	2.0^{c} ± 0.1
5.	1:35	1.0 ± 0.0	1.76^{d} ± 0.0
6.	1:40	0.6 ± 0.01	1.22^{e} ± 0.01
	LSD at 5%		0.075

***Table 2**: Effect of extraction ratio by hot water on extract and total soluble solid.*

Values are Mean ± Standard Deviation obtained from the triplicate data. Superscript with different alphabets in the column differ significantly (P < 0.05).

Extraction of sweet glycosides was done by heating the powder in water for an hour at different ratios such as 1:15, 1:20, 1:25, 1:30, 1:35 and 1:40 (w/v). Total soluble solid of the extract was determined and the presence of significant difference between the extract was analyzed using Analysis of Variance (ANOVA) at 5% level of significance.

The above table shows that the more water used for extraction, the concentration of stevioside in the extract decrease. Similar results were obtained by Abou-Arab., *et al* [2]. The statistical analysis showed that there is significant difference between extracts of different ratio as P < 0.05. In order to find the variation between individual samples, the mean differences of each sample with each other were calculated and were compared with Least Significant Difference LSD. It was found that there is significant difference between all samples with each other except sample 1 and 2.

Optimization of extract on the basis of total soluble solid and sensory evaluation was done according to sensory analysis by 30 panelist. 1:30 proportion of stevia powder to water was observed to be best. The proportion 1:30 was found to be best than other proportion because at higher proportion steviol concentration may be low but also at low proportion the concentration of bitter component may be relatively higher than steviol to affect the taste. According to Soejarto., *et al*. [12] the bitter taste common to many stevia species was probably due to sesquiterpene lactones. It was also suggested that volatile aromatic or essential oils, tannins and flavonoids contribute to the unpleasant flavors associated with stevia.

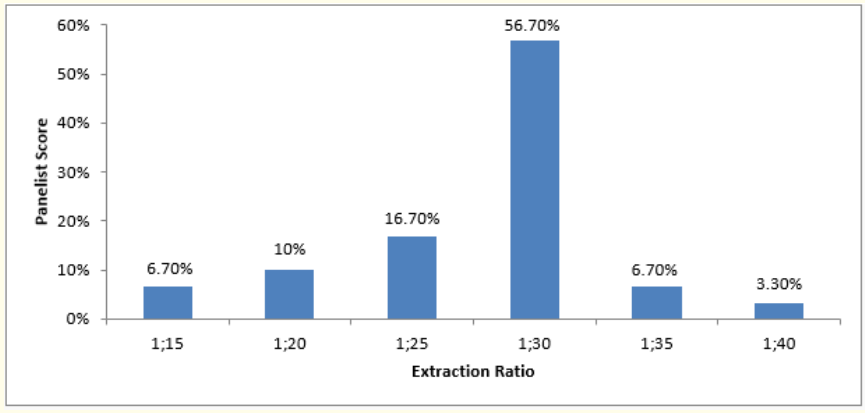

***Figure 1:** Optimization chart of extract.*

Comparison of sweetness of stevia with sucrose was done as the extracted sample of 2°Brix and standard sugar solutions of 10, 20, 30, 40 and 50°Brix was provided to 32 semi- trained panelists and were asked to compare the sweetness of the given sample with different standard sugar solutions. The standard sugar solution of 30° Bx obtained the highest score by 60%. This shows that the stevia extract of 2°Brix is equivalent to 30° Brix sugar solution. According to Ghanta., et al. [6], steviol glycosides are approximately 300 times sweeter than sucrose. Similarly, 10°Brix, 20°Brix, 40°Brix and 50°Brix sugar solution obtained 6.7%, 13.3%, 16.7% and 3.3% respectively.

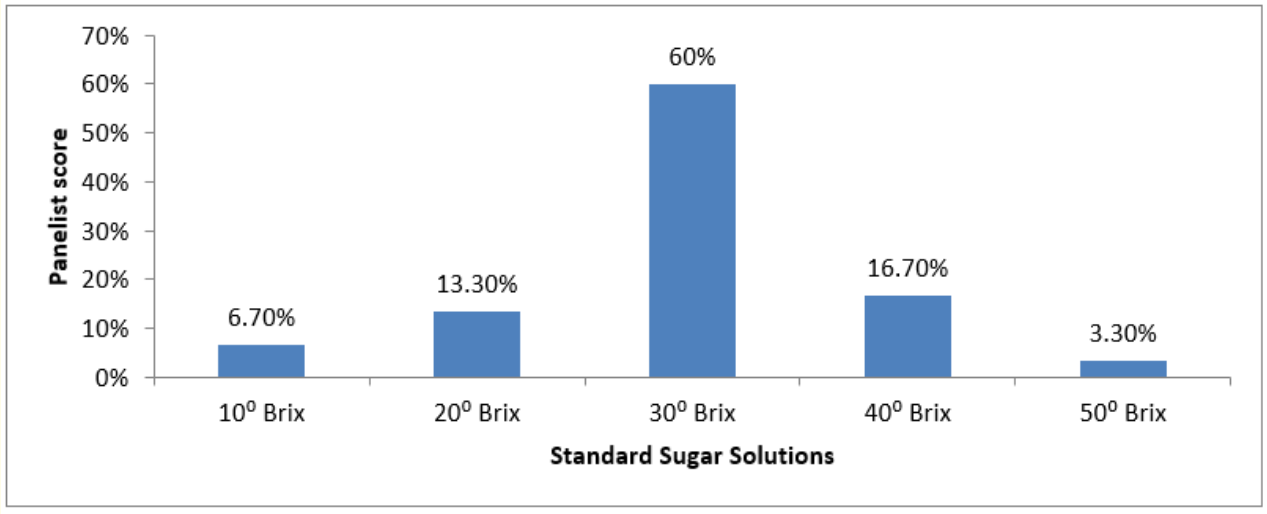

Figure 2: *Comparison chart of sweetness of Stevia extract with standard sugar solution.*

Sensory evaluation was carried out for color, texture, taste, flavor, appearance and overall acceptance by 30 semi trained panelists using 9 point hedonic method described by Rangana [3]. Formulation of yoghurt was optimized by adding different concentration of stevia extract 15° Brix such as 0%, 0.15%, 0.25%, 0.3% and 0.35% in 200 mL cup of yoghurt and pouring of inoculated pasteurized and cooled (45°C) milk flavored with cardamom essence of 0.2%, which was incubated for 24 hours at 44°C. It was then cooled to 5°C and was subjected to sensory evaluation by hedonic method. Panelists of 30 comprising the faculties and students of National College of Food Science and Technology performed the test.

The order of superiority of Sample A, B, C, D and E representing 0%, 0.15%, 0.25%, 0.3% and 0.35% Stevia extract in yogurt respectively can be summarized as:

- Color: Sample B > Sample C > Sample A > Sample D > Sample E
- Texture: Sample B = Sample C = Sample D > Sample E > Sample A
- Taste: Sample B = Sample C > Sample D > Sample E > Sample A
- Flavor: Sample B > Sample C = Sample D > Sample E > Sample A
- Appearance: Sample B > Sample C = Sample D = Sample E > Sample A
- Overall acceptance: Sample B > Sample C > Sample D > Sample E > Sample A

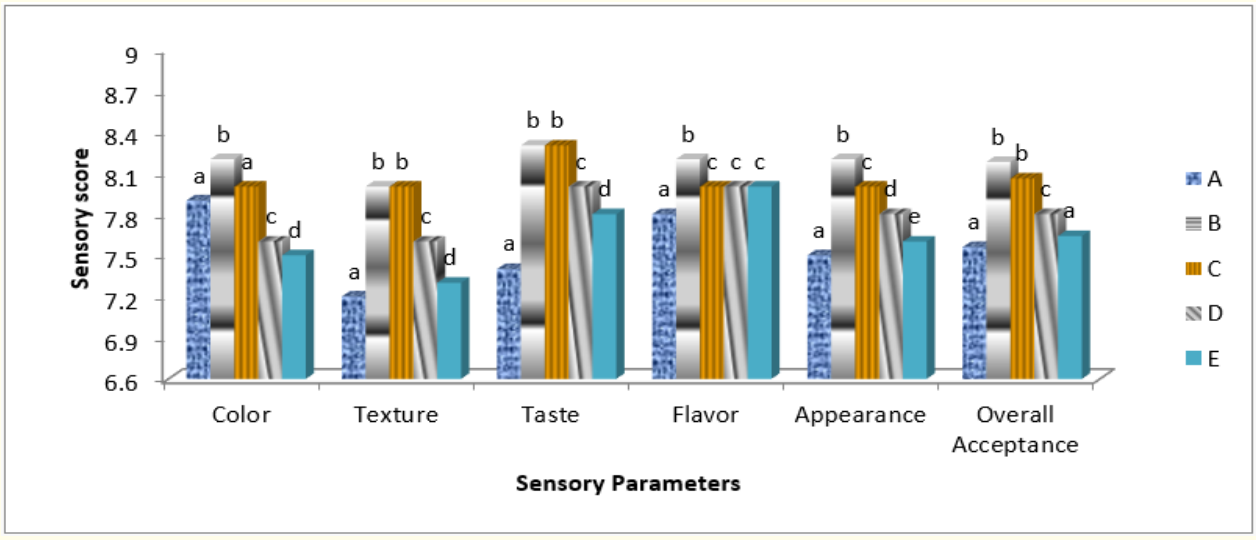

Figure 3: *Mean Scores of Sensory Attributes*

From the above result it was found that sample B scored highest in terms of color, texture, taste, flavor and appearance in comparison to other sample whereas sample E scored lowest highest in terms of color and sample A scored lowest in texture, taste, flavor and overall acceptance.

Parameters	Best Formulation (Sample B)
Moisture (%)	85 ± 0.0
Total Solid	15.03 ± 0.05
SNF	12.07 ± 0.05
Fat	3.97 ± 0.03
Acidity	1.0 ± 0.01
Protein	5.33 ± 0.05

Table 3: *Chemical composition of stevia incorporated yoghurt.*

Physicochemical analysis of best formulation of stevia incorporated yoghurt was done. The values were found according to Tamime and Robinson [13] and CAC [14]. The result shows that the highly accepted formulation contained significant amount of protein, fat and SNF and since the SNF do not contain any sucrose the yoghurt can be considered as nutritional product. It was also found that range of bioactive peptides encrypted within the sequence of the native proteins is released which makes yoghurt more healthy [15].

Conclusion

The study found that normal table sugar can be effectively substituted by incorporating 0.15% steviol glycosides of 15°Bx in yoghurt. Yoghurt prepared by incorporating 0.15% of 15°Bx extract was found to be best in terms of sensory analysis. This work shows that steviol glycosides i.e. water extract of stevia leaf powder is at least thirty times sweeter than sucrose. Highly nutritious, sweet and full of health

benefits aromatic yoghurt can easily replace mostly-avoided, sucrose incorporated sweet yoghurt by the utilization of steviol glycosides in experimentally calculated amount. Cardamom essence also plays important role as the desirable sensory attributes in yoghurt.

Bibliography

1. AOAC. "Official method of analysis of AOAC International". (A. of O. A. Chemists, Ed.) (18th edition). Washington, DC (2005).
2. Abou-Arab A., *et al*. "Physico-chemical assessment of natural sweeteners steviosides produced from Stevia rebaudiana Bernoti plant". *African Journal of Food Science* 4.5 (2011): 269-281.
3. Rangana S. "Handbook of analysis and Quality control for fruit and vegetable products". New Delhi, India: Tata Mc Graw Hill Company Ltd (2007).
4. Amzad-Hossain M., *et al*. "Chemical composition of the essential oils of stevia rabaudiana Bertoni leaves". *Asian Journal of Traditional Medicines* 5 (2010): 56-61.
5. Gardana C., *et al*. "Evaluation of steviol and its glycosides in Stevia rebaudiana leaves and commercial sweetener by ultra-high-performance chromatography-mass spectrometry". *Journal of Chromatography A* 1217.9 (2010): 1463-1470.
6. Ghanta S., *et al*. "Oxidative DNA damage preventive activity and antioxidant potential of stevia rebaudiana bertoni, a natural sweetener". *Journal of Agricultural and Food Chemistry* 55.26 (2007): 10962-10967.
7. Roberto LM., *et al*. "Stevia rebaudiana Bertoni, source if high-potency natural sweetener. A comprehensive review on the biochemical, nutritional and functional aspects". *Journal of Food Chemistry* 132.3 (2012): 1121-1132.
8. Sharma V. "Stevia: Prospects as an emerging natural sweetener, International Food Division". *Journal of Agricultural and Food Chemistry* 23 (2007): 231-135.
9. Savita SM., *et al*. "Stevia rebaudiana- A functional component for food industry". *Journal of Human Ecology* 15.4 (2004): 261-264.
10. Mishra P., *et al*. "Stevia rebaudiana- A magical sweetener". *Global Journal of Biotechnology and Biochemistry Research* (2010): 62-74.
11. Kaushik R., *et al*. "Nutrient composition of cultivated stevia leaves and the influence of polyphenols and plant pigments on sensory and antioxidant properties of leaf extracts". *Journal of Food Science and Technology* 47.1 (2010): 27-33.
12. Soejarto DD., *et al*. "Potential sweeting agents of plant origin-II. Organoleptic evaluation of Stevia leaf Herbarium samples for sweetness". *Journal of Natural Products* 45.5 (1982): 590-599.
13. Tamime A and Robinson RK. "Yoghurt: Science and Technology (2nd edition)". Florida: CRC press (1999).
14. CAC. Codex standard for fermented milks (2008).
15. Aneja RP., *et al*. "Technology of Indian milk products" (2002): 158-182.

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