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PHYSICO-CHEMICAL CHARACTERISTICS AND NUTRITIONAL PROFILE OF PROCESSED AND RAW STEVIA POWDERS: A COMPARATIVE STUDY

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ABSTRACT
Stevia (*Stevia rebaudiana* Bertoni), a natural, non-caloric sweetener, has gained significant attention in the food industry due to its potential health benefits and nutritional value. This study aims to investigate the physico-chemical properties and nutritional composition of processed and non-processed Stevia powders to evaluate the effects of processing on its quality. Stevia samples were subjected to various processing methods including drying and milling, while non-processed samples were analyzed in their natural form. Physico-chemical parameters such as moisture content, ash content, pH, and bulk density were measured. Additionally, the nutritional composition was assessed through the determination of macronutrients (protein, carbohydrate, fat) and micronutrients (vitamins and minerals) using standard analytical methods.
The results indicate significant differences between processed and non-processed Stevia powders in terms of moisture content and nutritional components. Processed Stevia exhibited reduced moisture and ash content, while also showing a slight decrease in protein levels compared to non-processed samples. However, the processing led to an enhanced concentration of carbohydrates, making it potentially more suitable for use as a natural sweetener in food applications. These findings highlight the impact of processing on the quality and functionality of Stevia, suggesting that processing methods can optimize Stevia's application in the food industry. Further research is recommended to explore the long-term stability and health benefits of processed Stevia powders.

KEYWORDS: *Stevia powder, Nutritional composition, Physico- chemical properties, Processing methods, Food science, Bioactive compounds.*

INTRODUCTION
Stevia (Stevia rebaudiana L.) is popularly known as sweet leaf, sugar leaf, honey leaf and candy leaf. It is also known as “Calorie free Bio sweetener of High Quality” (Balaswamy et al., 2014). It is native of Paraguay (South America), where it grows as a perennial at an elevation of 200 to 500 m with a mean temperature of 23 degrees (range, 6 to 43 degrees) and annual rainfall of 1500 to 1800 mm and belongs to Asteraceae (Compositae) family. The indigenous Guarani people had long been aware of the special sweetening ability of its leaves as well as other therapeutic characteristics. They used the plant, which they termed "kaa he-he" (which translates to "sweet herb"), as a taste enhancer and sweetener for their green herbal tea "mate" and other household uses (Singh and Rao et al., 2005).
Stevioside is 150 to 300 times sweeter than sucrose and possesses a strong residual flavour. Rebaudioside A has a sweetness level of about 250 to 400 times that of sucrose which possesses less residual flavor and has a wide range of food application (Balaswamy et al., 2014).
The utilization of steviol glycosides (SGs) is now encouraged as food additive in order to minimize the dependence of masses on calorie rich sugar like from sugarcane, beet sugar, honey, etc. that would ultimately lessen the incidence of diabetes and its related diseases (Jahangir Chughtai et al., 2020).
Stevia can be regarded as safe for human consumption because no harm has been linked to it throughout these years (Chranioti et al., 2016). It was found to have a significant impact on controlling blood pressure, glucose levels, renal functioning, managing obesity and treating dental disorders (Ahmad et al., 2018; Jahangir Chughtai et al., 2020). It also has a strong ability to scavenge free radicals (Ibrahem et al., 2020).
The Joint Food and Agriculture Organization / World Health Organization Expert Committee on Food Additives reviewed the safety of steviol glycosides and an acceptable daily intake (ADI) for steviol glycosides (expressed as steviol equivalents) of 4mg/kg body weight/day was recommended by European Food Safety Authority in 2010 (Balaswamy et al., 2014).

MATERIAL AND METHODS

Raw Materials

The Stevia rebaudiana plant and commercially available processed stevia used for the study were procured from Department of Horticulture, GKVK, UAS, Bengaluru and Stevia World, Peenya, Bangalore respectively. Other ingredients for development of value-added products were procured from local markets of Bangalore.

Study location and study period

Study was carried at Department of Food Science and Nutrition, UAS, GKVK, Bengaluru during the year 2021-2023.

Physico-chemical properties of stevia powders

Physical properties of *Stevia rebaudiana* plant, Dehydration parameters of *Stevia rebaudiana* leaves, Relative sweetness and sweetness retention time of stevia powders, Physico-chemical properties of stevia green leaves powder and processed white stevia powder were recorded using standard procedures.

Nutrient analysis of stevia powders.

Stevia green leaves powder and processed white stevia powder were analyzed for nutritional composition which includes proximate composition such as moisture, ash, protein, fat and crude fiber (AOAC,2005). Carbohydrate was obtained by differential method and energy by computation method.

Micronutrients viz., calcium, iron, zinc, copper, manganese, sodium, potassium, magnesium and phosphorus (AOAC,2005) were analyzed. Antioxidant activity and Bio active compounds such as total phenols and total flavonoids were determined using the standard laboratory procedures. Suitable statistical analysis was carried out such as Means of triplicates, Students t test, to find the significant difference between the samples analyzed ($p< 0.05$).

RESULTS AND DISCUSSION

Physical properties of (*Stevia rebaudiana)* plant

Determining physical characteristics of *Stevia rebaudiana* plants helps to describe the type of the plant. *Stevia rebaudiana*'s physical features, including plant height, weight and leaf weight for plants that were 18 months old, are listed in Table 1. The plant was typically between 22 & 28 cm tall, with leaves ranging in length from 3 to 5.2 cm and weighing 10.5 g (per ten plants).

Table 1: Physical properties of *Stevia rebaudiana* plant

Parameter	Values
Age of plant (Range in years)	1-2
Age of plant (Average in years)	1.5
Plant height (Range in cm)	22-28
Plant height (Average in cm)	23.5
Leaf length (Range in cm)	3-5.2
Leaf length (Average in cm)	3.8
Whole plant weight (Range in g)	8.9-15
Whole plant weight (Average in g)	10.5

Note: Values represented are an average of ten plants

The outcomes were consistent with Sardar et al. (2022) study where it was recorded as depending on the agroclimatic conditions and the soil composition, the physical properties change.

Dehydration parameters of *Stevia rebaudiana* leaves

The process of dehydrating stevia leaves plays a pivotal role in concentrating and preserving the sweetening compounds found in the leaves, primarily steviol glycosides, while extending the shelf life of the plant material. This dehydration process involves the removal of moisture from freshly harvested stevia leaves to prevent microbial growth, enzymatic degradation and deterioration of the leaves' quality. By reducing the water content, the leaves become more compact, easier to store and suitable for various applications, including sweetener production, herbal teas etc. The Fig1: Shows Process of Dehydration of Stevia leaves.

In a tray dryer, plants were dried out until they achieved the desired constant weight. The results are shown in Table 2. After 10 hours of dehydration at 60 °C, the starting weight of fresh plants decreased from 100 g to 37 g (constant weight), indicating significant water loss throughout the drying process. Dehydration ratio for the entire facility was 2.70.

Dehydration caused, a 63 per cent weight reduction as a result of the water loss. Additionally, dehydration causes plant components to shrink and acquire a mildly crunchy feel, making them simpler to be powdered.

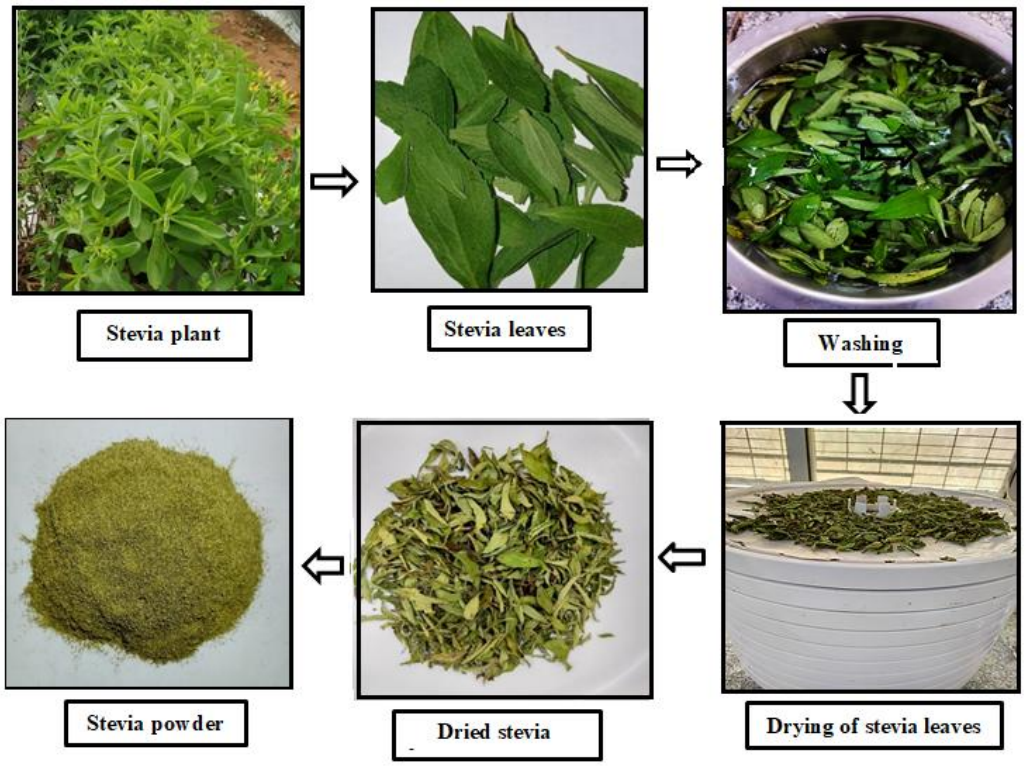


Fig1: Shows Process of Dehydration of Stevia leaves

Table 2: Dehydration parameters of *Stevia rebaudiana* leaves

Parameters	Values
Temperature (°C)	60
Drying duration (hours)	10
Dehydration ratio	2.7
Fresh leaves weight(g)	100
Weight after drying(g)	37
Moisture (%)	63

Similar results were obtained by Samsudin and Aziz et al. (2013) which revealed that drying of stevia leaves at temperatures of 50-60 °C was an effective method in removing moisture content. At this temperature, the quality of dried leaves produced in terms of colour, sweetness and nutrient content was better compared with drying at 70 °C.

It was determined that the cabinet tray drying method proved to be the most effective approach for dehydrating drumstick leaves. This method exhibited superior nutrient retention, including protein, carbohydrates, crude fiber and minerals, as well as enhanced preservation of the dehydrated characteristics when compared to alternative drying techniques such as oven drying, shade drying and sun drying (Satwase et al., 2013).

Relative sweetness and sweetness retention time of stevia powders

The relative sweetness and sweetness retention time of sweet are important concepts in the field of food science and sensory analysis. These factors play a significant role in determining the perception and overall sensory experience of sweetness in food and beverages.

Relative sweetness refers to the sweetness level of a substance compared to a reference substance, which is often sucrose (table sugar) in the context of sweetness evaluation. Sweetness retention time, on the other hand, refers to the duration or persistence of the sweet taste sensation after consumption.

Sweetness equivalence of Stevia green leaves powder and commercially available processed white stevia powder to sugar was carried out for threshold test. 1gm of both stevia powders was asked to be matched with 10gm, 15gm, 20gm and 25gm sugar. Perception of duration of Sweet Stimulus was measured in seconds in comparison to sweet stimulus of sugar.

These parameters are useful in understanding the sweetness intensity and persistence of different stevia products compared to sugar, which can be valuable in formulating and designing food and beverage products to achieve the desired level of sweetness and taste profile. Additionally, the processing of stevia can significantly impact its sweetness and sensory attributes, as evidenced by the differences between stevia green leaves powder and processed white stevia powder.

Table 3 depicts the results regarding the sweetness equivalence and sweetness retention duration of the two types of stevia powders. The data reveals that a majority of participants (65%) perceived that 1 gram of Stevia Green Leaves Powder (SGLP)

is equivalent to 15 grams of sucrose. In contrast, a majority (72%) found that 1 gram of Processed White Stevia Powder (PWSP) is equivalent to 25 grams of sucrose

Table 3: Relative sweetness and sweetness retention time of stevia powders

Quantity of stevia (g)	Sucrose (g)	Perception (%)		Sweet stimulus (min)	Perception (%)		
		SGLP	PWSP		Sucrose (1g)	SGLP (1g)	PWSP (1g)
1	10	10	5	<1	82	13	2
1	15	65	8	1-2	11	77	5
1	20	18	15	2-3	5	6	12
1	25	7	72	>3	2	4	81

* Values are average of 25 replications; SGLP: stevia green leaves powder; PWSP: processed white stevia powder

In terms of sweetness retention time, the results indicate that SGLP predominantly provides a sweetness that lasts for 1-2 minutes, whereas PWSP offers a longer-lasting sweetness, with the majority (81%) of subjects experiencing a retention time of more than three minutes. This suggests that when compared to SGLP, PWSP not only imparts a higher relative sweetness but also offers a longer-lasting sweetness sensation.

Physico-chemical properties of stevia green leaves powder and processed white stevia powder. The physico-chemical properties of powders play a fundamental role in numerous fields, from food and pharmaceuticals to materials science and industrial processes. These properties encompass a wide range of characteristics, including bulk density, water absorption and oil absorption. The specific combination of these properties determines the behavior and performance of powders in various applications.

Table 4: Physico-chemical properties of stevia green leaves powder and processed white stevia powder

Physico-chemical properties	Stevia green Leaves powder		Processed white stevia powder
Moisture (%)	11.09		6.56
pH	5.6		4.3
Bulk density (g/mL)	0.48		0.6
Water absorption capacity (%)	6.6		9.6
Oil absorption capacity (%)	5.4		8.5
Colour	L*	49.96	97.7
	a*	-4.27	-0.11
	b*	26.15	1.01

Note: Mean of triplicate samples L* [L* = 0 (black) and L* = 100 (white)]; a* (-a* = greenness and +a* = redness); b* (-b* = blueness and + b* = yellowness)

Table 4 provides an overview of the physico-chemical properties of both stevia green leaves powder and processed white stevia powder. Specifically, it was observed that stevia green leaves powder has a bulk density of 0.48 g/ml, while processed white stevia powder has a slightly higher bulk density of 0.6 g/ml. In addition, when it comes to water and fat absorption capabilities, stevia green leaves powder exhibited values of 6.6 and 5.4, respectively, whereas processed white stevia powder demonstrated higher absorption capacities, with values of 9.6 for water and 8.5 for fat. Consequently, processed white stevia powder surpasses stevia green leaves powder in terms of bulk density and its ability to absorb water and oil.

Processed white stevia powder comprises primarily steviol glycoside and isomalt, serving as a bulking agent. The significant physicochemical properties, such as the increased bulk density, oil absorption and water absorption, might be due to the presence of isomalt in the formulation. The present study results are in line with the study conducted by Savita et al. (2004)

Nutrient composition of stevia green leaves powder and processed white stevia powder

Nutrient composition, a fundamental aspect of nutritional science and food analysis, involves the quantitative assessment of the various essential components that make up the nutritional content of foods and dietary products. Nutrient composition analysis provides a detailed understanding of the macro and micro-nutrient content of a given food item, revealing the presence and concentration of elements such as carbohydrates, proteins, fats, vitamins, minerals and fiber.

Estimation of proximate and selected vitamins and minerals were performed on stevia green leaves powder and processed white stevia powder and the results are depicted in Table5. When comparing the proximate composition of Stevia green leaves powder to processed white stevia powder, notable differences were observed. Stevia green leaves powder contained11.09 per cent moisture, 12.08 g of protein, 8.86 g of total ash, 2.94 g of fat, 14.8 g of crude fiber, 50.23 g of carbohydrates, 275.70 kcal of energy, 15.26 mg of vitamin C and 370 µg of β- carotene per 100 g of the sample. In contrast, processed white stevia powder exhibited different values, including 6.02 mg of vitamin C, 75 µg of β-carotene, 7.2 g of protein, 0.04 g of fat, 6.56 per cent moisture and 1.23 g of crude fiber per 100 grams. It's worth noting that Stevia green leaves powder outperformed processed white stevia powder in nearly all proximate parameters except for energy content**.**

Stevia green leaves powder contains higher amounts of vitamin C and ß-carotene, compared to processed white stevia powder.

Stevia green leaves powder contains more calcium (32mg), magnesium (84mg), phosphorus (318mg), potassium(1770mg), zinc(1.26mg), copper (2.6mg), iron (30.6mg) and manganese (5.8mg) than processed white stevia powder (28mg), (16mg), (112mg), (478.4mg), (0.78mg), (1.03mg), (6.23mg) and (2.6mg) respectively. At 5 per cent level of significance (p 0.05), the difference in all nutrients, including proximate and minerals was shown to be statistically significant between the two stevia powders. The results were in line with the study conducted by Khiraoui *et al*. (2017) who gave the proximate and minerals profile of stevia green leaves powder on dry weight basis.

Gasmalla *et al*. (2014) conducted a study on the assessment of the nutritional Composition of *Stevia rebaudiana* Bertoni Leaf where the results obtained were on par with stevia green leaves powder. Buyuk *et al*. (2022) investigated the mineral composition of stevia leaves. The results were found lower than the present study. It may be due to different location and harvesting time.

Table 5: Nutrient composition of *Stevia rebaudiana* green leaves powder and processed white stevia powder

Parameters	Stevia green leaves power	Processed white stevia powder	t-test
Proximate			
Moisture (%)	11.09	6.56	129.4*
Protein (g)	12.08	7.2	143.5*
Total ash (g)	8.86	1.03	653.4*
Crude fiber (g)	14.8	1.23	407.1*
Fat (g)	2.94	0.04	615.8*
Carbohydrates#(g)	50.23	85	105.8*
Energy## (Kcal)	275.70	369.16	315.5*
Vitamins			
Vitamin C (mg)	15.26	6.02	1240.1*
β- carotene (µg)	370	75	625.78*
Minerals (mg)			
Calcium	32	28	8.40*
Magnesium	84	16	144.9*
Phosphorus	318	112	436.9*
Potassium	1770	478.4	3323.3*
Zinc	1.26	0.78	101.8*
Copper	2.6	1.03	48.65*
Iron	30.6	6.23	729.2*
Manganese	5.8	2.6	68.58*

Note: *significant @ 5%; NS- non-significant; # By difference; ## By computation

Antioxidant activity and bioactive components of stevia green leaves powder and processed white stevia powder

Antioxidant activity and bioactive components in plants include alkaloids, terpenoids, flavonoids, nitrogen-containing compounds, organosulfur compounds, phenolics, *etc.* that not only provide nutritional value but can also present therapeutic potential.

Estimation of antioxidant activity and bioactive compounds like, total polyphenols and flavonoids was performed on stevia green leaves powder and processed white stevia powder. Table 6 depicts the antioxidant activity and bioactive components of stevia green leaves powder and processed white stevia powder. The antioxidant activity and bioactive components like, total polyphenols and flavonoids were recorded highest in stevia green leaves powder, which were 2744.06 mg AAE/100g, 1397.19mg GAE/100g and 54.75 mg RE/100g respectively.

Table 6: Antioxidant activity and bioactive components of stevia powders on dry weight basis (mg/100g)

Particulars	Stevia green leaves powder	Processed white stevia powder	t-test
Anti-oxidant activity (µg AAE)	27744.06	1017.55	2673.43*
Total Polyphenols (µGAE)	1397.19	143.90	2608.96*
Flavonoids (µRE)	92	54.75	209.24*

Note: *significant @ 5%; NS- non-significant

The bioactive component of the processed white stevia powder is as follows: antioxidant activity was 1017.55 µg AAE/100 g, total polyphenols was 143.90 µg GAE/100 g and flavonoids were 54.756 µg RE /100g. The statistical analysis indicates that there is a significant difference, at 5 per cent level of significance ($p < 0.05$), between stevia green leaves powder and processed white stevia powder.

Rao et al. (2014) studied on Antioxidant Activity of Stevia (*Stevia rebaudiana* L.) Leaf Powder and Commercial Stevioside Powder. The results revealed that both Polyphenol and total antioxidant contents were higher in Stevia green leaves powder than in Commercial Stevioside Powder.

CONCLUSION

The study revealed that the physical and nutritional characteristics of processed stevia powder was significantly different from that of non-processed stevia powder. It was found that green stevia powder boasts the highest nutritional content, including moisture (11.09%), protein (12.0g), fat (2.94g), crude fiber (14.8g), carbohydrates (50.23g), and energy (275.70 kcal). Additionally, it has the highest mineral content when compared to processed white stevia powder. In terms of antioxidant activity and bioactive compounds, the green powder also outperforms the processed version, showing superior antioxidant activity and higher levels of total polyphenols and flavonoids. Thus, these stevia powders could be used as alternative additives to sugar based on the potential results exhibited in its physico-chemical properties and the nutritional composition.

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