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Thermal and Morphological Properties of Waste Product and Leaf Powder of Stevia Rebaudiana Bertoni

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Abstract **– Stevia rebaudiana Bertoni, belonging to the Compositae family, is a sweet herb contains diterpene glycos- -ides, namely, stevioside, rebaudiosides A–F, steviolbioside, and dulcoside A, which are responsible for the typical sweet taste. Waste product and dried leaf of stevia rebaudiana were analyzed heavy metals, scanning electron microscopy, Thermal properties (DSC) and FT-IR spectrum analysis. (Mercury) Hg, (Selenium) Se, (Cadmium) Cd and (Lead) Pb were determined in waste stevia (0.07µg /g, 0.8µg / g, 0.11µg /g and 0.19µg /g) respectively. (SEM) were scanned for both samples and clear difference was observed in samples. Thermal characteristics (DSC) was investigated and results showed slight various between waste and leaf powder of stevia, after that FTIR was studied and appeared different chemical groups in waste and leaf sample of stevia rebaudiana respectively.**

Keywords **– Stevia Rebaudiana, DSC, Heavy Metals, FTIR Spectrum, Morphological Properties.**

I. Introduction

Stevia rebaudiana Bertoni is a branched bushy shrub of the Asteraceae family, native to the Amambay region in the north east of Paraguay. It also occurs in the neighboring parts of Brazil and Argentina. The plant Stevia rebaudiana Bertoni (compositae) has been widely cultivated in the world for the sweet diterpene glycosides that are mainly contained in its leaves [1]. Today planting and cultivation have spread to other regions of the world, including Canada and some parts of Asia (China, Korea and Japan) and Europe [2-3]. Presently, Stevia is well-known for its high content of sweet diterpene (about 5 – 30 %) in dry-leaf matter [4], and it is the source of a huge number of sweet diterpenoid compounds which are known glycosides [5] and the stevia glycosides are the compounds responsible for the sweet taste. Among the 230 species in the genus Stevia, only the species rebaudiana and phlebophylla produce steviol glycosides [6]. Historic- -ally, low caloric sweeteners have been investigated as a possible substitute for sugar; one important class of low caloric sugar substitutes is known as a high-intensity sweetener, which is at least 100–150 times sweeter than sucrose. Nowadays, the most common high-intensity sweeteners in the world market are made of synthetic compounds in addition, high concentrations of some types of synthetic sweeteners, such as saccharin, have been reported as being hazardous to health [7-8]. On the other hand, reports published by the European Food Safety Authority indicate that low concentrations of low-calorie sweeteners are safe to be consumed by humans. Further research is needed to relate the results obtained from animal studies in order to assess the risk for human health [9]. A lot of work has been done on the ecology, importance of the plant, its production requirements and the agronomic and management aspects of the plant to be grown as a crop, the current status of understanding of the plant and its potential as an alternate source of sweetening to cane sugar [10]. The sweet principle was first isolated in 1909 and only in 1931 was the extract purified to produce stevioside, the chemical structure of which was established in 1952 as a diterpene glycoside. Stevioside is described as a glycoside comprising three glucose molecules attached to an aglycone, the steviol moiety. During the 1970s, other compounds were isolated, including rebaudioside A, with a sweetening potency even higher than stevioside [11]. Stevioside is extracted from the leaves of S. rebaudiana Bertoni (a plant widely cultivated in the world such as northeastern Paraguay, South America, Central America, and Mexico). It is a white, crystalline, odorless powder, which is approximately 300 times sweeter than sucrose [12].

A number of studies have suggested that, besides sweetness, stevioside, along with related compounds which include rebaudioside A, steviol and isosteviol, may also offer therapeutic benefits, as they have anti-hyperglycemic, antihypertensive, anti-inflammatory, anti-tumour, anti-diarrhoeal, diuretic, and immunomodulatory effects [13]. The leaves of Stevia has functional and sensory properties superior to those of many other high-potency sweeteners, and is likely to become a major source of high-potency sweetener for the growing natural food market in the future [14]. At present times sweet diterpene glycosides of the shrub S. rebaudiana Bertoni have gained importance in food, cosmetic, and pharmaceu- -tical industries [15], and Steviol glycosides are noncaloric sweeteners, which makes them an attractive sugar substitute for food industry [16]. Glycosides are compounds containing a carbohydrate molecule (sugar) bound to a non-carbohydrate moiety. These compounds are mainly found in plants, and they can be converted, by hydrolytic cleavage, into a sugar and a non-sugar component (aglycone). They are named specifically by the type of sugar that they contain, as glucosides (glucose), pentosides (pentose), fructosides (fructose), etc [17]. Stevia, the common name for the extract stevioside from the leaves of S. rebaudiana Bertoni, is new promising renewable raw food stuff on the world market and is a natural, sweet-tasting calorie-free botanical that may also be used as a sugar substitute or as an alternative to artificial sweeteners [18]. The natural sweeteners of Stevia leaves, called steviol glycosides, are diterpenes, isolated and identified as stevioside, steviolbioside, rebaudioside A, B, C, D, E, F and dulcoside [19].

Our study aimed to determine and evaluate heavy metal concentration in waste product and leaf powder of stevia rebaudiana Bertoni. On the other hand studied comparing between waste and leaf powder of stevia rebaudiana in scanning electron microscopy, differential scanning calorimetry and Fourier transform infrared (FTIR) Spectroscopy which give us efficient acknowledgement about some physic-chemical, thermal and morphological characteristics in stevia materials.

II. Materials and Methods

A. *Materials*

Dried stevia leaves and waste stevia product were obtained from the Yancheng Xiaguang Stevioside Trading Company (Jiangsu, China), drying conditions were under sun and temperature around 25-30 °C for 10 days then put dried leaves in plastic bags and stored at room temperature until use. Waste stevia was a white powder product which it had some treatment after extraction some compounds (steviol glycosides) from stevia leaves, High-speed blender (25000/ min), type WK–1000A (Qingzhou Jing cheng Machinery Co., LTD - Shandong - China), pH -meter FE20 Mettler-Toledo Instruments (Shanghai, China), Centrifuge CT14D Shanghai Techomp Bio Equipment LTD (Shanghai, China). Sodium hydroxide, (H2SO4), Hydrochloric acid (HCL), KMNO4, solvents and others chemical were obtained from Sigma Chemical Co. (Shanghai, China), all other chemicals and reagents used were of analytical grade.

B. *Sample Pretreatments*

The dried leaves were blended to powder using a high speed blender (25000 /min). The powder samples were stored in polyethylene bags at 4 °C until used [20]. There was no pretreatment for waste stevia caused was ready powder to use in experiments.

C. *Analysis of Heavy Metal Concentration*

Waste stevia product and Powder stevia leaf (1 g) placed in a 100 mL beaker, and 8 mL concentrated nitric acid (68 %) and 4 mL of perchloric acid (72 %) were added. The mixture was heated gently at 55°C for 20 min until a light yellow coloured solution was obtained. The samples solutions were not allowed to dry during digestion. Then sample filtered into a 50 mL standard flask, two 10 mL portions of distilled water were used to wash the beaker and the contents filtered into a 50 mL flask. The filtered mixture was allowed to cool at room temperature before dilution to the mark and then it was mixed notably by shaking. The solution was analyzed on an atomic absorption spectrometry for mercury (Hg), arsenic (As), lead (Pb) and cadmium (Cd).

D. *Scanning Electron Microscopy (SEM) Analysis*

Scanning electron microscopic (SEM) studies were carried out using a scanning electron microscope SEM (HITACHI - SU1510 - JAPAN) to detect morphological properties for waste and leaf powder of stevia rebaudiana respectively. The samples were coated before loading to the scanning electron microscopy. The coated samples were loaded into the system and the image was viewed under 1.0 KV potential using secondary electron image. The image was captured using 11.20 mm Ricoh Camera of 600x Mag.

III. Statistical Analysis

Analysis of variance (ANOVA) was applied and significant difference (at $p< 0.05$) between mean values was evaluated by LSD test at using SPSS version 19.0 (SPSS, Chicago, IL, USA).

IV. Results and Discussion

A. *Heavy Metal Content*

The results of heavy metals contents in waste and leaves stevia rebaudiana showed in Table 1. The values of heavy metals were different according to stevia (Waste and dried leaf) respectively, according to results (Pb) value was higher in dried leaf than waste that was (1.2 µg/g and 0.19 µg/g) respectively, in addition that Table 1 showed that the level of Cd was significantly ($p \leq 0.05$) higher in leaf powder than waste product. However, Hg level was much higher in waste product compared with leaf powder (0.07 µg/g and 0.01 µg/g) respectively, for Se the study showed that waste product has much amount of Selenium comparing with leaf powder. The values of heavy metals in stevia rebaudiana which grown and produce in China match with international accepted range [21]. The limits of heavy metals should be 10(Pb), 1(Cd), 5(As) and 0.1(Hg) µg/g as reported by [22].

Table 1 Heavy metal contents (µg/g) of waste product and dried leaf of stevia rebaudiana.

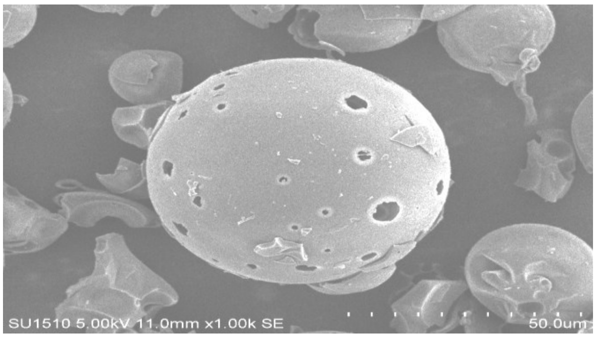
Heavy metals	Waste product	Leaf powder
(Cadmium) Cd	0.11 ± 0.01a	0.86 ± 0.04b
(Lead) Pb	0.19 ± 0.02a	1.2 ± 0.06b
(Selenium) Se	0.8 ± 0.02b	0.01 ± 0.00b
(Arsenic) As	0.11 ± 0.02a	0.06 ± 0.02a
(Mercury) Hg	0.07 ± 0.04a	0.01 ± 0.00a

Mean values in the same column with different letters are significantly different ($p \leq 0.05$).

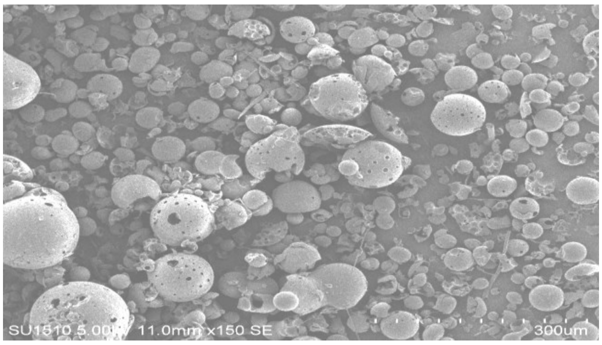
B. *SEM (Scanning Electron Microscopy) Analysis in Waste and Leaf Powder of Stevia*

Waste product and dried leaf powder of stevia rebaudiana bertoni were subjected for morphological structural analyses using (SEM) scanning electron microscopy. Under SEM treatment the surface of samples showed significant variations in size of particles and shape of it. The surface of waste stevia sample was distributed unorganized particles and appeared deteriorated particles due to long process during extraction and production some compounds from stevia, in addition to shape of particle in waste stevia was circular that having form of circles which was notably different from dried powder leaf. O the other hand the surface of dried leaf powder was similar to bricks with different anomalistic, compact and rough and particles were aggregated closely together.

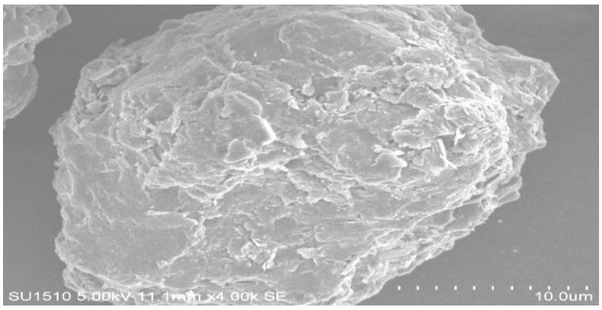
The reasons in difference of surfaces in waste product and leaf powder respectively caused that waste stevia subjected to many process during production steviol compounds which caused degradation in shape surface of particles. On the other side leaf powder the main reason which effect on leaf powder particles shape was drying process under sun [23-24].



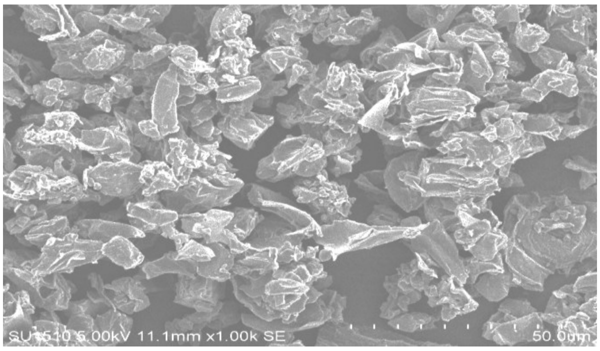
A



B



C



D

Fig. 1. Scanning electron microscopy (SEM) for waste product and dried leaf of stevia rebaudiana.
(A) Waste product of stevia (5k) -------------- (B) waste product of stevia (3k).
(C) Dried leaf of stevia (5k) --------------------- (D) dried leaf of stevia (3k).

Fig. 1. shown morphological structural surfaces for waste product and dried leaf of stevia rebaudiana Bertoni which observed that there was significant different destruction on shape and size of particles due to different process subjected for waste and dried leaf powder of stevia respectively.

V. Conclusion

Stevia rebaudiana Bertoni leaves generally have important roles in food applications. Steviol glycosides have a zero calories and can be used in nutritious diet. This research revealed that Waste product and dried leaf of stevia have different shape morphologically, DSC shown slight variance between samples and FTIR spectrum observed little difference between waste and dried leaves respectively. However this study showed that can do further experiments in waste stevia to investigate and get more knowledge about waste product.

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References

[1] Soejarto, D. (2002). Botany of Stevia and Stevia rebaudiana. In A. Kinghorn (Ed.), Stevia: The genus Stevia (pp. 18–39). London, New York: Taylor and Francis.
[2] Amzad-Hossain, M., Siddique, A., Mizanur-Rahman, S., & Amzad-Hossain, M. (2010). Chemical composition of the essential oils of Stevia rebaudiana Bertoni leaves. Asian Journal of Traditional Medicines, 5, 56–61.
[3] Gardana, C., Simonetti, P., Canzi, E., Zanchi, R., & Pietta, P. (2003). Metabolism of stevioside and rebaudioside A from Stevia rebaudiana extracts by human microflora. Journal of Agricultural Food Chemistry, 51, 6618–6622.
[4] Ghanta, S., Banerjee, A., Poddar, A., & Chattopadhyay, S. (2007). Oxidative DNA damage preventive activity and antioxidant potential of Stevia rebaudiana (Bertoni) Bertoni, a natural sweetener. Journal of Agricultural Food Chemistry, 55, 10962–10967.
[5] Prakash, I., Dubois, G., Clos, J., Wilkens, K., & Fosdick, L. (2008). Development of rebiana, a natural, non-caloric sweetener. Food and Chemical Toxicology, 46, S75–S82.
[6] Brandle, J., & Telmer, P. (2007). Steviol glycoside biosynthesis. Phyto chemistry, 68, 1855–1863.
[7] Fukushima S, Arai M, Nakanowatari J, Hibino T, Okuda M, Ito N (1983) Differences in susceptibility to sodium saccharin among various strains of rats and other animal species. Gann 74:8–20.
[8] Whysner J, Williams GM (1996) Saccharin mechanistic data and risk assessment: urine composition, enhanced cell proliferation, and tumor promotion. Pharmacol Ther 71:225–252.
[9] Authority EFS (2011) Statement of EFSA on the scientific evaluation of two studies related to the safety of artificial sweeteners. EFSA Journal 9: 16 www.efsa.europa.eu/efsajournal.
[10] Skarla BP, Josaph R, Mathew G and Joy PP, Stevia - A sweet herb, Indian J Arecanut, Spices Med Plants, 2004, 6, 24-27.
[11] Barriocanal, L., Palacios, M., Benitez, G., Benitez, S., Jimenez, J., Jimenez, N., et al. (2008). Apparent lack of pharmacological effect of steviol glycosides used as sweeteners in humans, a pilot study of repeated exposures in some normatensive and hypotensive individuals and in type 1 and type 2 diabetics. Regulatory Toxicology and Pharmacology, 51, 37–41.

[12] Allam A, Nassar A, Besheit S (2001) Nitrogen fertilizer requirements of Stevia rebaudiana bertoni, under Egyptian conditions. Egypt J Agric Res 79:1005–1018.
[13] Chatsudthipong, V., & Muanprasat, C. (2009). Stevioside and related compounds: Therapeutic benefits beyond sweetness. Pharmacology & Therapeutics, 121, 41–54.
[14] Goyal, S., Samsher & Goyal, R. (2010). Stevia (Stevia rebaudiana) a bio-sweetener: A review. International Journal of Food Sciences and Nutrition, 61, 1–10.
[15] Kroyer G (2010) Stevioside and Stevia-sweetener in food: application, stability and interaction with food ingredients. J Verbr Lebensm 5:225–229.
[16] Crammer B, Ikan R (1986) Sweet glycosides from the stevia plant. Chem Br 22:915.
[17] Bernal, J., Mendiola, J., Ibáñez, E., & Cifuentes, A. (2011). Advanced analysis of nutraceuticals. Journal of Pharmaceutical and Biomedical Analysis, 55, 758–774.
[18] Anton, S., Martin, C., Han, H., Coulon, S., Cefalu, W., Geiselman, P., et al. (2010). Effects of Stevia, aspartame, and sucrose on food intake, satiety and postprandial glucose and insulin levels. Appetite, 55, 37–43.
[19] Geuns, J. (2003). Stevioside. Phytochemistry, 64, 913–921.
[20] Gasmalla MAA, Yang RA, Amadou I, Hua X (2014) Nutritional composition of Stevia rebaudiana bertoni leaf: effect of drying method. Trop J Pharm Res 13:61–65.
[21] Choi YY (2011) International /National Standards for Heavy Metals in Food. Government Laboratory (Australia).
[22] Kabelitz L, SieVer SH (2004) Contaminates of medicinal and food herbs with a view to EU regulations. Inn Food Technol 1: 25-27.
[23] Lin SD, Sung JM, Chen CL (2011) Effect of drying and storage conditions on caffeic acid derivatives and total phenolics of Echinacea Purpurea grown in Tai Food Chem. 125: 226-231
[24] Hossain MB, Barry – Ryan C, Martin Diana AB, Brunton NP (2010) Effect of drying method on the antioxidants capacity of six Lamiaceae herbs. Food Chem. 123: 85-91.

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