

Compare Spectroscopy and Thermal 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 glycosides, 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]. Historically, 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 pharmaceutical 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. Thermal Characterization (Differential Scanning Calorimetry)

Thermal properties of waste and leaf powder of stevia rebaudiana were characterized using the differential scanning calorimeter DSC (DSC - Q200 - V24.8 Build 120, TA instrument, USA). Waste and leaf powder samples (10± 1 mg) were hermetically closed in aluminum pans and heated in a calorimeter from 20 to 200 °C at constant rate 10 °C /min. Then, they were kept for 5 min at 200 °C, and cooled back to 20 °C at the same rate 10 °C /min. Empty aluminum pan was used as a reference sample.

D. Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra were obtained using (NEXUS, Thermo., US) with a single - reflection diamond attenuated total reflection (ATR) and a mercury - cadmium telluride (MCT) detector was used. For all samples spectra were collected in the 4000–500 cm⁻¹. The samples were blended with potassium bromide powder (KBr) and then pressed into tablets. The measurements were done at a resolution of 4 cm⁻¹ with 64 scans. Waste product and leaf powder of stevia were placed onto the surface of the crystal and

contact of ATR crystal with the sample was provided Screwdrivers. Experiments were carried out at room temperature on each replicates. All spectra obtained were subjected to a multipoint linear base-line correction using the OMNIC software (version 7.3, Thermo Electron Co-operation).

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. Thermal Characterization (DSC) Analysis

(DSC) thermal characterization is used to detect changes in chemical compounds during heating process. DSC experiments subject samples to heat under environmental conditions, and the aim of this test is measuring temperature effect on samples compounds during heat flow. During heat flow could occurs loss of some compounds such as volatile compounds and some amino acids.

Fig. 2. showed thermal characteristics of stevia rebaudiana samples (waste product and dried leaf powder) respectively, two samples (waste and leaf powder) showed under (DSC) treatment a big response which appearing that steviol glycosides (main compounds in stevia) were notably exposed and denatured in both samples respectively. On the other side Fig. 2. Showed little bit difference in thermal retrogression temperature during operation. The temperature decomposition value showed that waste product stevia sample was slightly higher compared with dried leaf sample.

The two curves of (DSC) analysis observed that samples were stable with little degradation up to 80°C after that, above 80°C until 200°C thermal degradation and decomposition appeared in different areas. Our results showed that first big degradation in waste product sample was between 90 - 120°C and endothermic peak in this area was wide and at 120 °C exactly. On the other side result in dried leaf powder was similar with slight different which observed sharp decomposition at 130°C which explain that decompose and oxidase organic material occurs in the range (100 - 130°C).

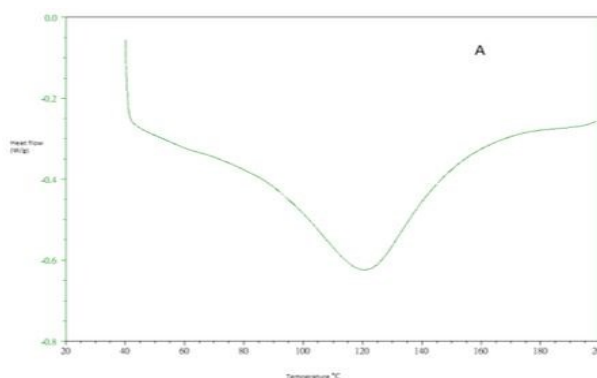


Fig. 2. A. DSC curve for dried leaf powder.

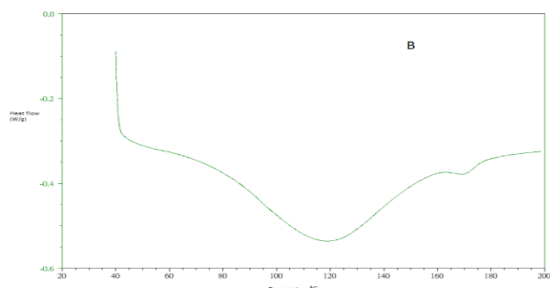


Fig. 2. B. DSC curve for waste stevia product

B. FT-IR Spectrum Analysis

FT-IR spectra were obtained to investigate the molecular properties of steviol glycosides in waste product and leaf powder of stevia rebaudiana and evaluate the variance between samples. The FT-IR spectrum of Waste product and leaf powder was observed in Fig.3. The sample of dried leaf powder showed a typical broad peak at (3303 cm^{-1}) caused the stretching vibration of O-H in the sugar ring, while it was (3359 cm^{-1}) in waste product. The peak at (2919 cm^{-1}) in dried leaf and peaks at (2927 cm^{-1} , 2873 cm^{-1}) in waste stevia suggested due to C-H stretching vibration such (CH₂) or (CH₃-CH₂-) [25]. The absorption beak at (1602 cm^{-1}) in dried leaf was due to presence of (C=O) amide group with bending vibration, on the other side in peaks in waste stevia were at (1725 cm^{-1} and 1654 cm^{-1}) caused (C=O) with ester and amide groups respectively. However peaks at (1258 cm^{-1} , 1020 cm^{-1}) in dried leaf showed that (C-O) bending stretching vibration exists on the ring which observed glycosides bonds in this area [26], whereas in waste stevia the peaks were at (1067 cm^{-1} , 1027 cm^{-1}) which also give similar result as leaf with slight difference between two samples.

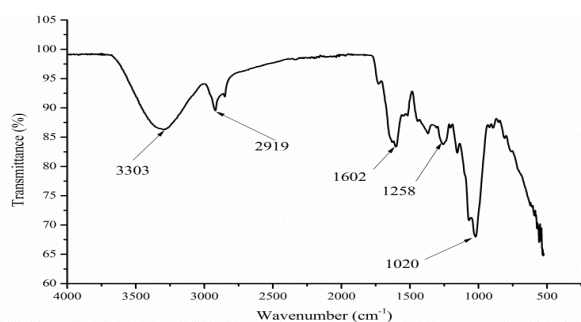


Fig. 2. (a) FT-IR spectrum of dried leaf powder of stevia rebaudiana.

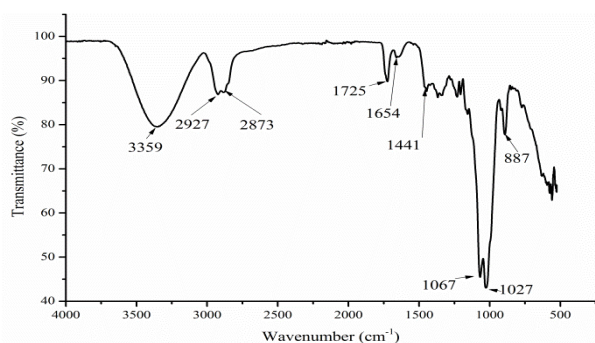


Fig. 2. (b) FT-IR spectrum of waste product of stevia rebaudiana.

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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