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Chemical Composition and Functional Properties Assessment of Stevia Rebaudiana Molasses

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Abstract **— The purpose to determine the chemical composition, nutritional and functional properties of waste product (molasses) of stevia, Fiber, ash, fat and protein values were evaluated in the waste stevia. Carbohydrate (glycosides) content was higher in the waste (87.44 %) compared with other contents. The waste stevia had high concentration of Na (109.21 mg/100g), However Potassium (K) and Calcium (Ca) in waste product were determined (7.78 mg/100g and 85.46 mg/100g) respectively. Total essential amino acids in waste were (0.5 g/100g). Acetic acid, Propionic acid; ethyl esterand LINALOOL L were higher in waste stevia (10.11%, 11.15% and 1.55%) respectively. Vitamin C and folic acid were showed in waste stevia (0.07 mg/100g and 0.09 mg/100g) respectively. Waste product had a low water and oil absorption capacity (1.02 ml/g and 0.91ml/g) respectively. Bulk density was 0.36g/ml in waste stevia, Solubility(S %) showed high value78.83% in waste. Waste product of stevia rebaudiana was analyzed heavy metals, scanning electron microscopy, Thermal properties (DSC) and FT-IR spectrum analysis.**

Keywords **— Waste Product of Stevia Rebaudiana, Nutritional Composition, Volatile Compound, Amino Acids, Minerals and Vitamins, SEM, DSC and FT-IR Spectrum.**

I. Introduction

Stevia, botanically known as Stevia rebaudiana Bertoni (Family-Asteraceae) is a sweet herb. A perennial herb, Stevia is a member of the daisy family. The leaves are mid green and intensely sweet. The compounds in the leaves are called stevioside and rebaudioside and they can be more than 200 times sweeter than sugar. The plant bears greenish cream flowers in autumn, native to the Amambay region in the northeast of Paraguay [1]. Today its cultivation has spread to other regions of the world, including Brazil, Argentina Canada and some parts of Asia and Europe [2-3]. Presently, Stevia is known as its high content of sweet diterpene (about 8–25%) in dry-leaf materials. These days Stevia Rebaudiana is the source of a number of sweetent- diterpenoid glycosides [4]. Nowadays, the most common high-intensity sweeteners in the world market are made of synthetic compounds. On the other hand, high concentrations of some types of synthetic sweeteners, such as saccharin, have been reported as being hazardous to health and may cause some risks such cancer or other diseases. Among the (200-220) species in the genus Stevia, the species of rebaudiana and phlebophylla can produce steviol glycosides [5]. Steviol is the common aglycone backbone of the sweet stevia glycosides that have been analyzed by liquid chromatography coupled with UV, MS and ELS detection [6].The powder extract of stevia rebaudiana leaves and its waste contain some chemical and nutritional compounds such alkaloids, chlorophylls, xanthophyll's, flavonoids, hydroxyl-cynnamic acids, tannin, oligosaccharides, free sugars, amino acids, lipids, vitamins and the most value compound is glycosides[7] .

As known the chemical and nutritional characteristics of waste and extracts of the leaves powder are dependent on the applied conditions of plant cultivation, and manufacturing process respectively.Stevia sweeteners which produced from fresh leaves and waste stevia are used in yogurt, soft drink, beverages, hygiene products and various delicacies which are common in Europe, Canada and Asia [2]. Modern medical researches show that increased use of products enriched with sugar (sucrose) and artificial sweeteners are harmful for human health such Obesity and diabetes which cause heart attack and many risk diseases. Therefore, using low caloric products as a substitute for sugar(sucrose) such stevia rebaudiana sweeteners [8]. In this work we compared chemical and nutritional properties between leaves powder and waste of stevia rebaudiana, first time in this research we have been investigated waste stevia chemical composition to reuse again in producing nutritional compounds.

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

II. Experimental

A. Materials

The waste stevia obtained from Yancheng Xiaguang Stevioside Trading Company Ltd, (Jiangsu, China) after processing, then deposited at school of food science, Jiangnan University, Wuxi, China.

Reagents solution, NAOH, HNO3, H_2SO_4, HCL, was obtained from Sigma Chemical Reagent Co, Ltd (Shanghai, China). Water purified was obtained from MilliQ water purification system (Millipore, France). All solutions, solvents and chemicals reagents were in analytical grade.

B. Sample preparation

Waste stevia rebaudiana obtained as powder from com-

-pany with slight modification.

C. Chemical composition

The ash, protein, fiber,fat and moisture contents wereanalyzed and determined according to methods in AOAC [9].The moisture content was determined by weigh 3 gram of samples in clean preheated crucible. The crucible was heated in the oven for two hours at 105ºC. For ash content was analyzed using 2 gram of samples were put in cruciblethen placed in muffle furnace at 550 0C For 5 h until a white grey or reddish ash was obtained. The fat content was estimated using soxhlet machine (SZC-101 Fat Meter, Xian Jian Instruments Co., Ltd, Shanghai, China) for 6 h extracted by hexane with slight modification. Crude protein was determined using Kjeldahl method (DK-3400, FOSS, Hillerød, Denmark), with some modification. The carbohydrate content was estimated by sum of the (moisture, fat, protein, fiber and ash contents) was subtracted from 100.

D. Mineral analysis

The composition of minerals was analyzed in the samples which prepared and obtained from ash according to ash method [10] with some modification. One gram of each sample from waste powder of stevia rebaudiana was put in crucibles and put in muffle furnace until obtained the ash. The residue was dissolved in 10 ml of (HNO_3: HCl, 2:3, v/v) then heated until fumes disappeared. Solution was transferred separately in 50 ml volumetric flasks by filtration using whatman filter paper No 42, then volume made up to 50 ml with distilled water. After that minerals (Ca, Mg, Na, Fe, Cu, K, and Zn) were analyzed separately, using an Atomic Absorption Spectrophotometer of Spectra AA 220, USA Varian). The results were calculated as mg/100 g dry sample of waste stevia product.

E. Analysis of heavy metal concentration

Waste stevia product powder (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).

F. Amino acid determination

Amino acids were determined according to the method describing in AOAC. Dried samples were incubated and digested with HCl (6 M) at 110°C for 24 h under vacuum. (RP-HPLC) Reversed phase high performance liquid chromatography, analysis was achieved into an Agilent 1100 analytical HPLC column (4.6×250 mm) (Agilent Technologies, Palo Alto, CA, USA) system with o phthaldialdehyde (OPA) precolumn derivatization [11]. The samples were injected onto a Zorbax 80A C18 column (4.6×250 mm, Agilent Technologies) at 40°C with detection at 338nm. The mobile phase A was 7.35mM/L sodium acetate/ triethylamine/ tetrahydrofuran (500:0.12: 2.5, v/v/v) respectively, adjusted to pH 7.2 with acetic acid, while mobile phase B (pH 7.2) was 7.35 mM/L sodium acetate/m ethanol/acetonitrile (1:2:2, v/v/v) assembly system Agilent technologies. The results of amino acids analysis showed as g per 100 g of the waste stevia product (molasses).

G. Determination of volatile compounds

The volatile compounds were identified using gas chromatography-mass spectrometry (GC–MS) [12].the volatile compound were carried out and separated on a CP-Sil-8CB (Varian, Walnut Creek, CA, USA) combined with silica capillary column (30 m length, 0.25 mm, id, and 0.25 μm film thickness) in Varian (model 3800, Varian, Palo Alto, CA, USA) gas chromatography.

The injection port was maintained at 220 ºC, and the interface of flame ionization detector (FID) was maintained at 250ºC, the volatile compounds were achieved and separated on a capillary column {DB WAX} (30 × 0.25 μm, J and W Scientific, Folsom, CA, USA). The compounds separation was programmed as follows: the oven temperature was set at 40 ºC for 3 min, 80-100ºC for 6 ºC/min, 100-230ºC for10 ºC/min, and 230 ºC for 10 min. Helium was applied as the carrier gas, a constant column flow was 0.8 ml/min. The injector was maintained at 250°C. The parameters of (GC– MS) included: ionization mode: EI+; emission current: 200μA; electron energy: 70eV; interface temperature: 250°C; source temperature: 200°C; and detector voltage: 350 V. Identification of volatile compounds were considered experimentally by comparing the compounds with the mass spectra of standard compounds which found in the Wiley and NIST libraries. The relative contents were defined based on retention indices.

H. Determination of vitamin contents

Vitamins contents were analyzed using the method according to [13] with slight modification. (2 g) of sample were mixed with 3 ml n-hexane and 10 ml HPLC grade water. The solution placed in a sonication device for 30 min at room temperature. The mixture was centrifuged at 10,000 rpm for 30 min ,then The aqueous solution was filtered through filter paper and 0.45μm filter before HPLC analysis. The filtered solution (10 μl) was injected into HPLC system (Agilent 1100 Technologies, USA), C18 (4.6 mm×250 mm) column was applied for the separation of water-soluble vitamins in sample at room temperature. The solvents which used were applied as follows: Solvent A consisted of methanol, solvent B was sodium 1-heptanesulfonat; the mobile phase flow rate was maintained at 0.9 ml/min, vitamins content were determined by adding the standard vitamins to samples and individual peak area was calculated according to the peak area corresponding standard vitamin, then Results were calculated based on dry weight of powder sample.

III. Analysis of Functional Properties

A. Bulk density (BD)

Bulk density was determined according to method [14] with slight modifications. 10 g of sample was measured an and placed gently in a graduated cylinder (25 ml) after tapping the cylinder on a laboratory bench several times until no visible decrease in volume was noticed. The apparent bulk density BD based on the weight (g) and volume (ml) was calculated as follows:

Bulk density (g/ml) = Weight of sample/
Volume of sample after tapping

B. Water and Oil Absorption Capacity (WAC & OAC)

Water absorption index was measured by the method describing by [15] with minor modification. A 1.0 g of sample was mixed with 15 ml of distilled water or 10 ml of vegetable oil in a 25 ml centrifuge tube and agitated on a vortex mixer for 2 min, and then centrifuged at 5000 rpm for 20 minutes. The supernatant was decanted and discarded. The adhering drops of water were removed and the tube reweighed again.

{WAC&OAC g/g} = (Weight tube + sediment) - weight of empty tube/ Weight of sample

C. Solubility

Solubility was determined according to the method described by [16] with slight modifications. A 15.0 ml of distilled water was added to 1.0 g of powder sample in centrifuge tube, and heated in a water bath at 95 °C for 40 min, with constant stirring. The resulting slurries was cooled to room temperature and centrifuged at 5000 rpm for 20 min. The supernatant was decanted into an evaporating dish, dried in an oven at 105 ° C for 2h. The dried supernatant and the sediment were weighed. The percentage of solubility based on dry sample was calculated as follows:

Solubility (S %) = dry supernatant weight/ weight of dry sample * 100%

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 product of stevia rebaudiana. 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 and Areas were ranging from approximately 1 cm to 5 microns (scanning rate).

E. Thermal characterization (Differential Scanning Calorimetry)

Thermal properties of waste product of stevia rebaudiana were characterized using the differential scanning calorimeter DSC (DSC- Q200- V24.8 Build 120, TA instrument, USA). Waste and raw material 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.

F. Fourier transform infrared (FTIR) Spectroscopy

FTIR spectra were obtained using (NEXUS, Thermo., US) with a single-reflection diamond attenuated total reflection (ATR) and a mercurye-cadmiume 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 of stevia was 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).

IV. Results

A. Chemical composition analysis

The proximate chemical composition analysis of waste was shown in Table 1. The different values in the proximal components analysis of waste product stevia rebaudiana explained that waste contained less values comparing with other studies in raw materials of stevia rebaudiana because waste was got from raw material after processing and most of value chemical components was lost during prosess.as table 1.shown the most value of waste analysis was carbohydrates 87.44 and less were fat and protein (1.60-2.63) respectively. On the other hand ash and fiber were (3.61 and 6.72 g/mg) respectively.

Table 1: Chemical composition analysis of waste product *S.*rebudiana

Components	Waste
Moisture	4.36±0.55
Ash	3.61±0.65
Fat	0.90±0.36
Protein	2.63±0.35
Fiber	3.72±0.81
Carbohydrates (glycosides)	87.44±1.20

B. Mineral contents

The results of minerals contents were achieved in Table. Macro minerals (calcium, Magnesium and Potassium) and micro minerals (iron, zinc, copper and manganese) detected in waste productof stevia rebaudiana Bertoni. Results in waste product showed less values except Sodium (Na) which determined in waste stevia remarkably (109 mg/100g).on the other hand Fe, Zn, K and Ca were so low and decreased noticeably in waste stevia.

C. Heavy metal content

The results of heavy metals contents in waste product of stevia rebaudiana showed in Table 3.The values of heavy metals were different according to heavy elements in waste stevia. However the result of (Pb) value in waste product was (0.19 µg/g), in addition that Table 3 showed that the level of Cd was significantly ($p \leq 0.05$) high in waste product. However, Hg level was much higher in waste product compared with other studies in raw material of stevia which showed (0.02 µg/g) in waste product.

Table 2. Minerals content of waste product of stevia rebaudiana, (calculated as mg /100g).

Minerals	waste
Iron (Fe)	2.06±0.08
Copper (Cu)	0.16±0.01
Zinc (Zn)	0.29±0.04
Sodium (Na)	109.21±3.32
Magnesium (Mg)	39.14±1.53
Potassium (K)	7.78±1.83
Calcium (Ca)	85.46±1.66

Table 3. Heavy metal contents (µg/g) of waste product and raw material of stevia rebaudiana

Heavy metals	Waste product
(Cadmium) Cd	0.11±0.01[a]
(Lead) Pb	0.19±0.02[a]
(Selenium) Se	0.03±0.02[b]
(Arsenic) As	0.01±0.02[a]
(Mercury) Hg	0.02±0.04[a]

D. Amino acid contents

The contents of amino acids in the waste product of S.rebudiana are shown in Table 4. According to the results, data in table 4, showed that waste stevia rebaudiana contained essential amino acids (Histidine, Thereonine, Thereonine, Valine, Phenylalanine, Isoleucine, Leucine and Lysine), the amounts of amino acids in waste stevia rebaudiana have been less than those recorded by FAO&WHO[17]. Tyrosine is non- essential amino acid was showed high amount in waste stevia (0.04 g/100g) comparing previous studies in raw material of stevia rebaudiana.

E. Volatile compounds

The volatile components of waste product of S.rebudiana are represented and summarized in Table 5. Around 45 of volatile compounds were identified in the waste stevia whereas between(55-57) components were appeared in previous studies in raw material of stevia rebaudiana. The results in waste stevia showed efficient amounts of {Acetic acid Propionic acid, ethyl ester and Decanal (CAS)}.On the other hand wasteproduct of stevia rebaudiana was observed low amounts of (Hexanal (CAS), Dodecanal and 3-Tridecanone). Some volatile compounds such (l-Limonene, TRANS-2-UNDECEN-1-OL and LINALOOL L) were showed in waste samples in rich amounts compared with other volatile compounds.

F. Vitamins contents

Vitamins compounds results for waste product of stevia rebaudiana were obtained and showed in table 6.regarding to results waste stevia showed less amounts of vitamins in general. Vitamin C and Folic acid(B9) were 0.07 and 0.09 mg/100g in waste stevia product respectively, in addition to very low amounts of B2 and B6 were presented in waste stevia. Niacin (B3) and Thiamine (B1) were not determined in the waste sample of stevia rebaudiana.

Table 4. Essential and non-essential amino acids composition in waste product of S.rebudiana, (calculated g/100g dry powder sample)

Amino acids	Waste	FAO/WHO
Histidine	0.003	0.18
Thereonine	0.004	0.34
Arginine	0.02	0.35
Valine	0.007	0.35
Methionine	0.002	0.25
Phenylalanine	0.004	0.63
Isoleucine	0.003	0.28
Leucine	0.008	0.66
Lysine	0.004	0.58
Total essential Amino acids	0.20	3.55
Aspartic acid	0.02	--
Glutamic acid	0.005	--
Serine	0.02	--
Glycine	0.009	--
Alanine	0.008	--
Tyrosine	0.004	--
Cysteine	ND	--
Proline	0.003	--
Total Non-essential Amino acids	0.23	--

G. Bulk density (BD)

The BD result of waste product stevia rebaudiana was arranged and shown in Figure 1, which was 0.36 g/ml.

H. Functional properties (WAC & OAC)Water and oil absorption capacity

Figure 1, showed functional properties (WAC, OAC) of stevia rebaudiana waste product varieties. The data appeared that the water absorption capacity in waste stevia product (WAC) was higher than oil absorption capacity (OAC) which was (1.02 ml/g and 0.91 ml/g) respectively

K. Solubility (S %)

In this study results of (S) indicate that waste stevia has the highest value of solubility which recorded around (78.83 %).

L. SEM (Scanning electron microscopy) analysis in waste product of stevia rebaudiana

Waste product sample of stevia rebaudiana bertoni was 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 respectively. The results of waste product using scanning electron microscopy (SEM) showed in figure 2.

Table 5. Volatile compounds identified in waste product of stevia using gas chromatography-mass spectrometry (GC–MS).

Contents	Area (%)Waste
Acetic acid	10.11
Propionic acid, ethyl ester	11.15
Chloroform	0.71
Benzene, methyl- (CAS)	1.12
Hexanal (CAS)	1.09
Tetradecane (CAS)	1.01
2-Propanol, 1-methoxy-	1.23
l-Limonene	2.53
Ethanol, 2-ethoxy- (CAS)	0.45
Furan, 2-pentyl- (CAS)	0.16
Tetradecane,2,6,10-trimythyl-	ND
Dodecanal	2.72
Docosane (CAS)	2.20
Decanal (CAS)	7.32
2-Propanone, 1-hydroxy-	2.07
Cyclohexasiloxane, dodecamethyl- (CAS)	0.11
Butanoic acid, 3-methyl- (CAS)	1.12
Decane,2,6,7-trimethyl- (CAS)	ND
Phenol, 2,4-bis(1,1-dimethylethyl)-	0.57
Cyclohexane, nitro- (CAS)	ND
9-Octadecenoic acid (Z)-(CAS)	ND
Heptaflourobutric acid, n-pentadecyl es	ND
Decane,2,5,6-trimethyl-(CAS)	ND
TRANS-2-UNDECEN-1-OL	0.97
Junipene	ND
Benzyl oxy tridecanoic acid	1.51
Byrimidine,4-methyl- (CAS)	ND
2,5-dimethyl-5-nitrohexnal	ND
Pseudosolasodine diacetate	ND
Propanoic acid, 2-methyl-, 3-hydroxy-2,4	0.42
LINALOOL L	1.55
3-Tridecanone	0.32
3-Cyclohexene-1-methanol, .alpha.,.alpha	ND
Cyclopentadecanone,4-methyl	ND
Triacetin	0.58
7-Octen-2-ol, 2,6-dimethyl-	0.54
Caryophyllene oxide	ND
2-Pentadecanone,6,10,14-trimythl-	ND
(+)Spathulenol	ND
Benzyl oxy tridecanoic acid	1.50
Heptadecane	ND
3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,	0.42
1-Azabicyclo[3.2.1]octan-6-ol,	ND
4-Pentenal,2-ethyl- (CAS)	ND

M. 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.3. showed thermal characteristics of stevia rebaudiana samples (waste product).

N. FT-IR spectrum analysis

FT-IR spectra were obtained to investigate the molecular properties of steviol glycosides in waste product of stevia rebaudiana and evaluate the variance between samples. The FT-IR spectrum of Waste product of stevia rebaudiana was observed in Fig.4

Table 6. Vitamins composition of S. rebaudiana waste products extracts (mg /100g of dry sample)

Vitamin	Waste
Vitamin C	0.07±0.01
Vitamin B2	0.10±0.01
Vitamin B6	0.05±0.01
Folic acid	0.09±0.01
Niacin	0.00±0.00
Thiamin	0.00±0.00

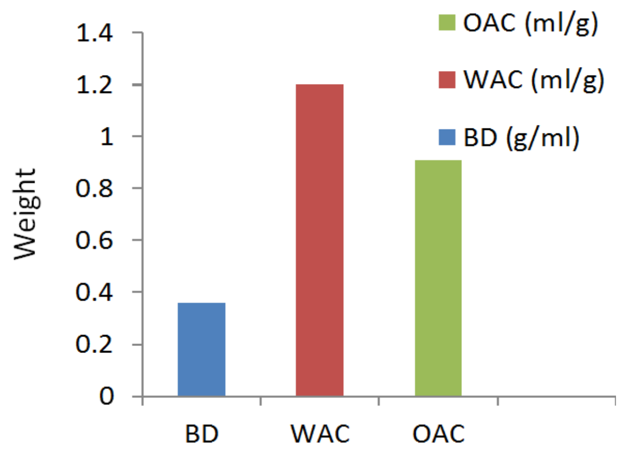

Figure 1: functional properties of stevia rebaudiana waste product (Molasses). Bulk density (BD) Water absorption capacity (WAC) and oil absorption capacity (OAC).

V. Discussion

According to previous researches and studies we found that chemical compositions of waste stevia have not previously been reported. The chemical composition results of waste product of S.rebaudiana indicate that moisture in waste stevia product reported lowest value due to effect of processing on stevia leaves during extraction , purification and production steviol glycosides such (stevioside and Rebaudioside A)in addition to spray drying which effect on moisture in waste product . Moisture value was lessthan moisture in raw materials of stevia rebaudiana according to previous studies [18-19].in our research ash value in waste stevia recorded 3.62 g/100g which was very low comparing with ash analysis in stevia leaves in previous studies [20].Table 1 shown that crude fiber content in waste stevia product also was less than those that reported in raw material of stevia rebaudiana byMishra et al [18] and Serio[21].Protein and fat in waste stevia were in low amounts compared with others which found high values of protein and crude fat in raw material

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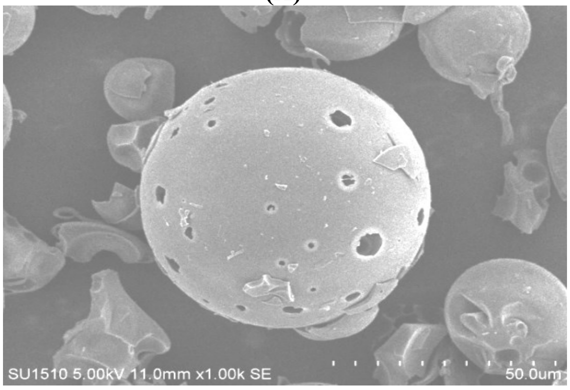
of stevia [20-22]. Highvalues of carbohydrates were found in waste product of stevia rebaudiana due to existence amounts of steviol glycosides in the waste after treatment and processing. Our results in Table.1 shown significantly different in chemical composition values between waste product and raw material of stevia which were studied by other researchers.

(A)



(B)

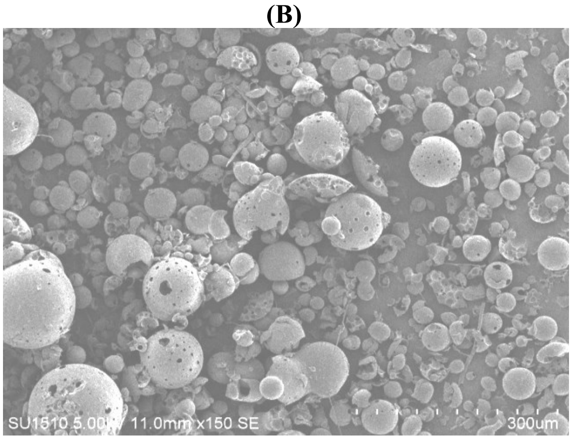


Fig.2. Scanning electron microscopy (SEM) for waste product of stevia rebaudian; (A) Waste product of stevia (5k), (B) Waste product of stevia (3k).

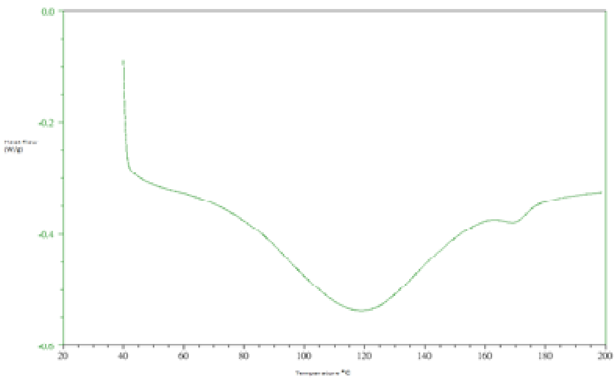


Fig.3. Differential scanning calorimetry (DSC) curve for waste stevia product

Minerals have many important functions in human body, minerals elements are needed in every small amounts in our diets which called essential trace elements [23], the levels of potassium (K) and calcium (Ca) were higher than other minerals in waste stevia product. Table 2, shown less amounts values of minerals in waste product, except Sodium (Na) which showed high value in the waste. Calcium, Magnesium, Iron and Zinc that are so important nutritionally were found in good amounts in waste product of stevia, these elements could be used in many products as additive to improve some nutritional properties [24].Heavy metals in Table 2 showed that the level of Cd was less in waste product compared with those resulted reported in previous studies in raw material of stevia. However, Hg level was much higher in waste product compared with raw material in stevia rebaudiana [19]. For Se the study showed that waste product has much amount of Selenium comparing with raw material. The values of heavy metals in stevia rebaudiana which grown and produce in China match with international accepted range [31]. The limits of heavy metals should be 10(Pb), 1(Cd), 5(As) and 0.1(Hg) µg/g as reported by [32].

The values of essential amino acids were compared with those reported by (FAO&WHO), the results of essential amino acids in waste stevia was less than those found by FAO report (FAO&WHO) [17] and less than recorded in raw material of stevia leaves. This study indicates that after extraction steviol glycosides, stevia could be used as a good source of essential amino acids which improve many functions in human body and maintain good health.

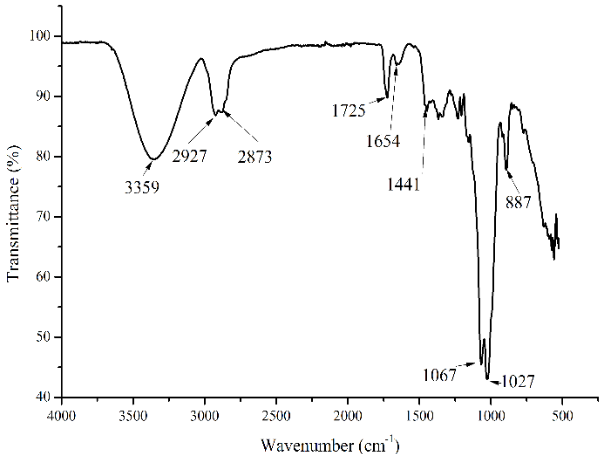


Fig.4. FT-IR spectrum of waste product of stevia rebaudiana

The volatile compound sin waste product of S.rebaudiana shown different results, Acetic acid and propionic acid were abundant amount in waste while Docosane, Tetradecane and Triacetin reported low amounts in waste product. In our study we found some compounds higher in waste stevia than other compounds and when compared with volatile compounds in raw material of stevia in previous study in Japan and Serbia [25-26] showed high different amount between waste product and raw material of stevia rebaudiana .However waste product of S.rebaudiana can be used as a good source for some volatile compounds and add to many food and beverage to improve and support flavor and taste.

Vitamins as known divided into two groups: the fat-soluble vitamins and water-soluble vitamins. Water soluble vitamins include B group like B1, B2 and B6. Vitamins play important roles in our life and it's necessary for many functions in metabolism [27], Vitamin C found in food and used as a dietary supplement. As a supplement it is used to treat and prevent scurvy and some diseases such as cancer [28]. In our study we found low amounts of vitamin C and folic acid in waste product of stevia which was less than those studied in raw material [29], whereas there was no detected for Niacin and Thiamine in waste stevia. Bulk density is, basically, is how a material compact under various loads and can also be an indicator of flow.BD in cereal can be used to determine the conditions of storage and package [14].

The WAC in waste stevia showed low absorption capacity, result of wastewas less than that study on raw material of stevia Mexico [30]. In previous study on stevia leaves shoed that stevia has good water absorption capacitywhich give it role to possess a sufficient water absorption capacity, that allowing it to play an important role in food processing. The OAC showed also different values in waste and previous study in stevia leaves, AOC could play important role in an industrial food production and could effect on flavor retainers and increases mouth feel of food products. In the area of food processing (S%) consider one of the most important function in food industries which has effect in other properties, in our study waste stevia showed higher solubility than raw material of stevia rebaudiana .

Under SEM treatment the surface of waste sample showed significant variations in size of particles and shape compared with previous study in raw material of stevia. 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 raw material. On the other hand in many studies showed that the surface of raw material 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 raw material respectively caused that waste stevia subjected to many process during production steviol compounds which caused degradation in shape surface of particles [23-24].

Waste sample showed under (DSC) treatment a big response which appearing that steviol glycosides (main compounds in stevia) was notably exposed and denatured in waste sample of stevia. On the other side Fig.3. Showed little bit difference in thermal retrogression temperature during operation. The temperature decomposition value showed that waste product stevia sample was notablyless compared with raw material in last studies. The curve of (DSC) analysis observed that waste sample was 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 in previous study on raw material of stevia the temperature of thermal decomposition occurs up to 200°C in different positions [19]. This study was found little variance different between waste product and last studies in raw material of stevia in DSC test which observed that waste product effected by long process during extraction steviol glycosides compared with raw material.

The sample of waste product showed a typical broad peak at (3359 cm^{-1}) caused the stretching vibration of O−H in the sugar ring. The peaks at (2927 cm^{-1}, 2873 cm^{-1}) in waste stevia suggested due to C−H stretching vibration such (CH2) or (CH3−CH2−) [25]. On the other side peaks in waste stevia at (1725 cm^{-1} and 1654 cm^{-1}) caused (C=O) with ester and amide groups respectively. However in waste stevia the peaks were at (1067 cm^{-1}, 1027 cm^{-1}) showed that (C−O) bending stretching vibration exists on the ring which observed glycosides bonds in this area [26].

VI. Conclusion

The key of our knowledge that chemical composition and functional properties of waste stevia have not previously been reported, This study is shown that the chemical composition, amino acids, vitamin and functional properties of waste stevia product were different significantly compared with studies in raw materials of stevia. The waste product of stevia rebaudiana can be used as source of carbohydrates (glycosides) steviol glycosides which can be as alternative Sugar and could apply in Food Industry. For waste stevia can reused again for extraction and production steviol compounds and extract some volatile compounds which effect on taste and flavor.

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