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Original Research Article

Nutritional Composition of *Stevia rebaudiana* Bertoni Leaf: Effect of Drying Method

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Abstract

***Purpose**: To determine the effect of three methods of drying, viz, sun, oven and microwave, on Stevia rebaudiana Bertoni leaf's nutritional composition.*
***Methods:** Fresh Stevia rebaudian bertoni leaves were dried separately by sun, oven and microwave. The chemical composition was determined by Association of Official Agricultural Chemists (AOAC) method. Tannin content was measured by titrimetric method while heavy metals were analyzed by atomic absorption spectrometry.*
***Results:** The following data were obtained for the plant when the three drying methods were employed: moisture content, 4.45 – 10.73 %; ash, 4.65 – 12.06 %; protein, 12.44 – 13.68 %; fat, 4.18 – 6.13 %; total dietary fiber, 4.35 – 5.26 % and total carbohydrates, 63.10 – 73.99 %. The pH value was 5.96, 5.95 and 6.24 for sun, oven and microwave drying, respectively. Total energy of the plant material was in the range 362.3 – 384.2 kcal/100 g while tannin content was in the range 5.43 – 5.91 %. Moreover, reducing sugar was 4.5, 4.8 and 5.3 %, respectively, for sun, oven and microwave drying. Heavy metals content varied in stevia leaves, with lead was detected in high concentration in sun-, oven- and microwave–dried materials with values of 4.77, 0.14 and 2.16 µg/g, respectively, while the corresponding values for cadmium were 0.49, 0.44 and 0.33 µg/g, Arsenic level was 0.30, 0.09 and 0.10 µg/g for sun, oven and microwave drying, respectively. Mercury level was the same (0.1 µg/g) irrespective of the drying method.*
***Conclusion**: The results obtained indicate that Stevia leaves is a good source of carbohydrate and other nutrients and hence a substitute for sugar in processed drinks. Furthermore, drying reduces nutritional values with the exception to fiber content.*

***Keywords:** Stevia rebaudiana Bertoni , Nutritional composition, Protein, Heavy metals, Drying, Total energy*

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INTRODUCTION

Stevia rebaudiana Bertoni is a branched bushy shrub of the Asteraceae family and originated from South America [1], and its cultivation has spread worldwide [2]. Stevia is well-known for its high content of sweet components. The dry extract of its leaves contains flavonoids, alkaloids, chlorophylls, xanthophylls, hydroxyl-cynnamic acids (caffeic, chlorogenic, etc), oligosaccharides, free sugars, amino acids, lipids and trace elements [3]. Stevia crude leaf extract is used to sweeten soft drinks, soju, soy sauce,

yogurt, and other foods in Japan, Korea and Brazil [2,4].

Stevia rebaudiana has become rather widespread over a wide range of climatic regions around the world and can apparently be successfully grown under different conditions [5]. Since the chemical composition of extracts of the leaves is dependent on the applied conditions of plant cultivation, they have become the subject of several research topics. Stevia is known to be at least 50 - 100 times sweeter than sucrose [6] and is a frequent alternative to many synthetic sweeteners that do not provide the realistic taste of sugar as well as some types of synthetic sweeteners, such as saccharin which is associated with the potential risk of bladder cancer [2]. Stevia sweeteners are used in beverages, tabletop sweeteners, salty and processed food, personal hygiene products and various delicacies that are popular in Asia.
The increasing consumption of sugar (sucrose) has resulted in several nutritional and medical problems, such as obesity, diabetes and heart disease. Therefore, low caloric sweeteners have been investigated as a substitute for sugar. In view of this, the objective of this work was to study the effect of three different drying methods on the chemical composition and nutritional value of the leaves of *Stevia rebaudiana* Bertoni grown in China.

EXPERIMENTAL

Materials

The fresh green leaves of *Stevia Rebaudiana* Bertoni were purchased in September 2011 (Jiangsu, China) from Yancheng Xiaguang Stevioside Trading Company Ltd. Dr Tan Ya Li of School of Medicine and Pharmaceutics, Jiangnan University, Wuxi, China authenticated the the plant material and a specimen (voucher no. 3-YXSTC-29/09/11) deposited in the herbarium of School of Food Science and Technology, Jiangnan University, Wuxi, China). Indigo solution, H_2SO_4, NaOH, HCl, $KMNO_4$ were obtained from Sinopharm Chemical Reagent Co, Ltd (Shanghai, China). All other chemicals and reagents used were of analytical grade.

Sample preparation

The green fresh leaves were allowed to dry using three different methods of drying; one part by direct sun light for about 5 days, second part using an oven (Oven DGG-9070A: Shanghai, China) at 60 ^oC for 16 h and the third part in an microwave (Midea MG720FC8-NS: Foshan, China) set at 2450 MHz and 700 W microwave for 6 min. The dried leaves were then blended to powder using a high-speed blender (25000/min), WK–1000A; Qing Zhou Jing Cheng Machinery Co., Ltd. (Shandong, China). The powder samples were stored in polyethylene bags at 4 ^oC until used.

Proximate analysis

The prepared samples were analyzed for moisture, protein, fat, crude fiber and ash according to the methods described in the AOAC [7]. The carbohydrate content was determined by subtracting the total crude protein, crude fiber, ash and fat from the total dry weight (100 g) of the food sample differences.

Total energy (Calorific value)

Energy was determined according to the method described by Sukkar [8] using the Atwater factor. By this determination, 1 g of carbohydrate provides 4 kcal; 1 g of protein provides 4 kcal and 1 g fat provides 9 kcal.

Reducing sugar

Reducing sugars content were determined according to a previous study [9] using 4 g of Stevia leaf powder. The sample was placed in a 200 mL flask, and the volume made up to mark with distilled water. The mixture was filtered using a Whatman (no. 1) filter paper and titrated with Fehling's solution to determine reducing sugars content.

Determination of tannin content

Three grams of Stevia leaf powder was extracted with 250 mL distilled deionized water (dd H_2O) in a 250 mL volumetric flask, left without shaking for 4 h at room temperature and filtered. The analysis of tannins content was performed according to International Pharmacopoeia and AOAC [7] method. The extract (25 mL) was mixed with 25 mL of indigo carmine solution in a 1 L flask and 750 mL of dd H_2O was added. The mixture was titrated with aqueous solution of $KMNO_4$ (0.1 N) until the blue colour of the solution changed to green. A few drops of $KMNO_4$ (0.1 N) were again used to change the green colour to golden yellow. Blank titration was also carried out using a mixture of 25 mL indigo carmine solution and 750 mL dd H_2O [7]. All the samples were analyzed in triplicate, and tannin content (%T) computed as in Eq 1.

$$T(\%) = \frac{(V - V^0) \times 0.004157 \times 250 \times 100}{g \times 25} \quad \ldots\ldots\ldots (1)$$

where V = volume of 0.1 N aqueous solution of $KMNO_4$ titrated for the test material; V_0 the volume of 0.1 N aqueous solution of $KMNO_4$ titration forthe blank; 0.004157 the tannin equivalent in 1 mL of 0.1 N aqueous solution of $KMNO_4$; g the mass of the sample used in the analysis; 250 the volume of the volumetric flask (mL); and 100 the percentage factor..

Determination of heavy metal concentration

Powdered stevia leaf powder (0.5 g) placed in a 100 mL beaker, and 5 mL concentrated nitric acid (68 %) and 2 mL of perchloric acid (72 %) were added. The mixture was heated at 70 ^oC for 15 min until a light coloured solution was obtained. The sample solution was not allowed to dry during digestion. The sample was then filtered into a 50 mL standard flask, two 5 mL portions of distilled water were used to rinse the beaker and the contents filtered into a 50 mL flask. The filtered mixture was allowed to cool to room temperature before dilution to the mark and then it was mixed thoroughly by shaking. The solution was analyzed on an atomic absorption spectrometry for lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg).

Statistical analysis

The experiments were conducted at least in triplicate. Analysis of variance (ANOVA) was performed and significant difference (at $p < 0.05$) between mean values was evaluated by Fisher LSD test at using SPSS version 19.0 (SPSS, Chicago, IL, USA).

RESULTS

Proximate chemical compositions

The proximate chemical composition of dried Stevia *rebaudiana* leaf is shown in Table 1. The results using sun drying method exhibited significant ($p < 0.05$) values in moisture, ash and fat contents of stevia leaves. On the other hand, the study showed that both fiber and protein contents were not significant ($p < 0.05$) in the dried leaves obtained by the three drying methods. The microwave drying appeared to have significant effect ($p < 0.05$) on the values of reducing sugar and carbohydrate (Table 1). In addition microwave drying seemed to increase the value of pH by reducing the leaves acidity as shown in Table 1. Table 2 shows the total energy values of sun, oven and microwave drying were 362.29, 368.04 and 384.23 Kcal/100g, respectively.

The tannin content of Stevia leaves obtained by three different drying methods is presented in Figure 1, and it ranged from 5.2 – 6.0 % with no significant difference ($p \leq 0.05$). The heavy metal concentrations of stevia leaves are presented in Table 3. The values of different metals in stevia leaves samples vary according to drying method. Highest values of Pb, Cd, and As are found in the sun dried sample, with significant differences ($p \leq 0.05$) when compared to microwave and oven drying. Table 3 showed that the levels of Cd and As were still significantly ($p \leq 0.05$) higher in sun-dried than in oven- and microwave-dried samples. However, Hg level remained the same in all the samples, irrespective of the drying method (Table 3).

Table 1: Chemical composition (mean ±SD) of dried *S. rebaudiana* leaf

Parameter	Sun-dried	Oven dried	Microwave dried
pH	5.96	5.95	6.24
Reducing sugar	4.50±0.10^c	4.80±0.05^b	5.30±0.03^a
Moisture	10.73±1.33^a	7.46±0.05^b	4.45±0.92^c
Ash	12.06±1.33^a	8.06±0.65^b	4.65±1.08^c
Crude fiber	5.03±0.16^a	5.26±1.17^a	4.35±0.39^a
Crude protein	13.68±1.86^a	12.44±0.81^a	12.83±0.17^a
Fat	6.13±0.63^a	4.39±0.30^b	4.18±0.45^b
Carbohydrate	63.10±1.20^c	69.85±0.05^b	73.99±0.02^a

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

Table 2: Total energy (mean ±SD) of dried *S. rebaudiana* leaf

Drying method	Carbohydrate	Protein	Fat	Total energy(cal/100g)
Sun	252.4±4.8^c	54.72±7.44^a	55.17±5.67^a	362.29±8.31^b
Oven	279.4±0.2^b	49.13±3.22^a	39.51±2.7^b	368.04±5.70^b
Microwave	295.29±1.22^a	51.32±27.89^a	37.62±4.05^b	384.23±2.36^a

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

Table 3: Heavy metal contents (µg/g) of dried *S. rebaudiana* leaf

***Stevia* leaf**	**Pb**	**Cd**	**As**	**Hg**
Sun-dried	4.77±0.09[a]	0.49±0.01[a]	0.30±0.02[a]	0.01±0.01[a]
Oven-dried	0.14±0.01[c]	0.44±0.01[b]	0.09±0.04[b]	0.01±0.00[a]
Microwave-dried	2.16±0.02[b]	0.33±0.02[c]	0.10±0.02[b]	0.01±0.02[a]

Note: Pb = lead; Cd = cadmium; As = arsenic and Hg = mercury

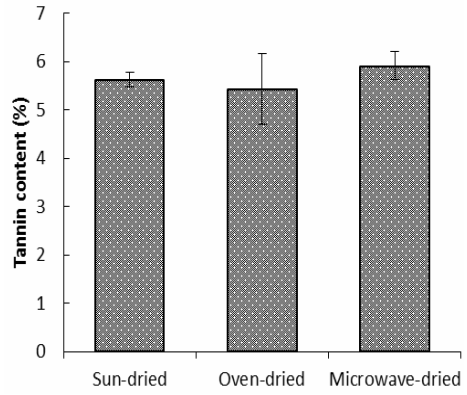

Figure 1: Tannin content of dried *S. rebaudiana* leaf

DISCUSSION

The composition data of dried *S. rebaudiana* leaf indicate that sun-dried method extract with higher moisture content due to the intensity of sun heat. In our work ash content was similar to reported by Mishra *et al* [10]. Tadhani and Subhash [6] reported a slightly higher amount of ash content and substantial amount of crude fiber [2, 11, 12]. Our study have shown the more protein content as compare to Kaushik *et al* [11] and Abou-Arab *et al* [13]; whereas, the fat content value was higher than that reported by Serio [4], and a valuable carbohydrate was found. Gisleine *et al* [3] reported that protein, fat, crude fiber, ash, and carbohydrates were present in dried sample. Our results demonstrate the same figures in Table 1. The results of moisture and ash content of stevia leaves were significantly different between the three methods of drying. There was no significant difference observed in crude fiber and proteins. The fat content in sun drying tends to be higher than that of oven and microwave drying, this shows that the drying methods has significant effect on the leaves. However, microwave drying exhibited higher amount of carbohydrate compare to sun and oven drying (Table 1). The values of reducing sugar were significantly different with lower effect on microwave drying (Table 1). Stevia leaves are a good source of nutritional values regardless of methods of drying. It has been used as a substitute for sugar in place of pure stevioside in different food preparations and its high ash content indicates that the stevia leaves are good source of inorganic minerals [2, 11].

The changing in total energy refers to the fact that biochemical and physiological changes occurred during the time of different drying methods. The total energy of microwave drying was significantly higher than its counterpart sun and oven drying. It was also found that microwave drying resulted in higher amount of tannins than the sun drying whereas, oven drying exhibited the lowest value. There is no significant difference between the three drying methods for tannins contents. Our value is lower than that of Rai [2] who found 7.8 %. Tannins possessing useful properties such as antioxidant, anti-apoptosis, anti-aging, anti carcinogenic, anti-inflammatory, as well as anti atherosclerosis and cardiovascular protection [14].

The results of heavy metals in *Stevia* leaves grown in China fall within internationally accepted range [15] where vegetables should not exceed 6, 0.1, 1.4 and 0.5 µg/g respectively for Pb, Cd, As and Hg. However, Kabelitz [16] reported the limits of heavy metals to be 10 (Pb), 1 (Cd), 5 (As) and 0.1 (Hg) µg/g.

CONCLUSION

Drying method can affect the nutritional composition of *Stevia* leaves and cause a serious decline in the content of standard phytochemical constituents. The plant leaf can be used as raw material for the extraction and production of functional food ingredients, qs well as act as a source of carbohydrates, protein, crude fiber, minerals, which are valuable for human nutrition.

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