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# EXTRACTION OF TOTAL STEVIOSIDE CONTENT FROM DRIED *STEVIA REBAUDIANA* BERTONI LEAVES

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***Abstract:*** *In the present study, some main components of dried Stevia leaves were determined such as protein, lipid, fiber, sugar content, etc. Total stevioside content was extracted by conventional method with hot water as solvent and all parameters in extraction process affect significantly the yield of stevioside. The results indicated the optimal extraction conditions were an extraction temperature of 85°C, sample/solvent ratio of 1/75 (w/v) and an extraction time of 120 min with the highest total stevioside content of 8.36%.*

***Keywords:*** *extraction, steviosides, sugar, sweetener, water.*

## 1. Introduction

In recent years, consumers have been more interested in health products and have started using natural products as well as diet products. In particular, there is a significant increase in human awareness to alternative sweeteners to sugars. Steviosides have been widely used commercially as the healthy, noncariogenic, zero calorie, natural sweeteners in several conventional food products such as bakery products like muffins, cake [7], [11] and different foodstuffs such as soft drinks with cola- and citrus-like carbonated beverages [5] on the ground that they are non-caloric, high content in sweet-tasting without affecting on textural, appearance and flavor properties of these products. Therefore, it is can suggest that *Stevia* extracts can substitute sugars in food products to reduce the amount of sugar intake for health reasons. In researches of health safety, *Stevia* leaves have been proven as a safe material by EC (European Commission), JECFA (Joint FAO/WHO Expert Committee on Food Additives) and FSANZ (Food Safety Australia and New Zealand). Moreover, the acceptable daily intake (ADI) is 0-4, this means that people can consume 4 mg of steviosides equivalent per day and per kg bodyweight [10].

Steviosides have been extracted from *Stevia rebaudiana* Bertoni leaves. They are about 300 times sweeter than sucrose tastes [8]. Extracts of *Stevia* plants contain a high level of total glycoside, at least 14% [3].

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*Stevia rebaudiana* Bertoni is the member of the genus *Stevia* and *Asteraceae* (Compositae) family. Besides, it is one of only two members which contain glycosides. This plant has been widely cultivated in the world (Egypt, Japan, Brazil, Paraguay and other countries) for the sweet glycosides that are almost contained in its leaves [1, 3, 8]. The eight glycosides have been recognized in *Stevia* leaves where they may account for 10 to 20% of the leaf dry weight. In which, four major glycosides are 0.3% dulcoside, 0.6% rebaudioside C, 3.8% rebaudioside A and 9.1% stevioside, respectively. Other glycosides also have been identified in *Stevia* like rebaudioside B, C, and E; dulcosides A and B [3].

Although disadvantages of the conventional methods for extraction of steviosides consist of long extraction and difficult purification procedures but these methods illustrate the simple, produce savings and environmentally friendly process in collecting steviosides. This process show "green" method for isolation of steviosides to enhance the final yield [16]. Hot water was preferred solvent for steviosides extraction as it could help to remain the better-tasting rebaudioside A which was more soluble than stevioside in water [13].

Therefore, traditional separation and isolation of natural-compounds substances from plant using various techniques with aqueous extraction still remains the potential solutions. So, the major aim of this research is to study the effect of different parameter factors on the steviosides content with aqueous extraction in which the total steviosides were extracted through hot water extractor from the dried leaves of *Stevia rebaudiana* Bertoni.

## 2. Materials and Methods

### 2.1. Sample Preparation

The dried *Stevia* leaves (*S. rebaudiana* Bertoni) were purchased from Stevia Ventures Company, Ha Noi, Vietnam. Moisture of samples is under 5%. The dried samples were ground into small pieces that could pass through a sieve of 4 mm, packed by polyethylene bags, stored at 4°C.

### 2.2. Chemicals and Reagents

Anthrone was obtained from HiMedia Laboratories (India) and stevioside (purity: ≥95.0%) was supplied by Sigma Chemical Co. (Germany). All other chemicals and solvents were of analytical reagent grade.

### 2.3. Determination of Some Components of *Stevia* Leaves

The samples were analyzed for moisture, protein, fat, fiber and ash according to the methods described in the AOAC [2].

Reducing sugars were estimated by 3,5-dinitrosalicylic acid (DNS) method using D-glucose as standard and total sugars were determined by Bertrand's method.

### 2.4. Extraction of Stevioside

The dried samples were extracted by water according to Abou-Arab et al. [1] with slight modifications. The dried sample of *Stevia* plant (2 g) was extracted by deionized water as solvent with uninterrupted stirring at various material/solvent ratios of 1/25, 1/50, 1/75, 1/100, 1/125, 1/150 and 1/175 (w/v). The extraction temperatures vary between 55 and 100°C, with extraction

time from minutes to hours. After the treatment, the obtained extracts were vacuum-filtered and used for analysis.

### 2.5. Determination of Total Stevioside Content (TSC)

Total stevioside content in dried leaves was determined by Anthrone-sulphoric acid method as described by Wei [19] with some modifications. Anthrone reagent was prepared by dissolving 0.2 g of anthrone in 100 mL $H_2SO_4$. This reagent was freshly prepared whenever needed. After that, 6 mL of anthrone solution was mixed with 2 ml of extract sample and shaken vigorously. The tube was placed into an ice-bath to prevent the loss of water by evaporation. After cooling, the tube was heated to boiling for 10 minutes and was cooled again in running tap water. The test tubes were incubated for 30 minutes, at room temperature (27°C). Blank was maintained with distilled water and reagents. The changes in the absorbance of the green solution were measured at 630 nm. TSC were estimated by comparing the absorbance of sample with the absorbance of stevioside standard.

### 2.6. Statistical Analysis

Experimental results were analyzed by the one-way analysis of variance (ANOVA) method and significant differences among the means from triplicate analyses at $p<0.05$ were determined by Fisher's least significant difference (LSD) procedure using Statgraphics software (Centurion XV). The values obtained were expressed in the form of a mean±standard deviation (SD).

## 3. Results and Discussion

### 3.1. Determination the Components of Dried Material

The moisture makes up the small component in dried sample (4.84%); it was lower than other studies (5.37%-7%) [1, 14] because of the different dried conditions. This result shows that it is very easy to preserve sample for a long time in normal condition. In addition, protein (11.5%), lipid (3.53%) and ash (7.04%) content (Table 1) are similar with study of Abou-Arab et al. [1] and lower than that of Tadhani and Subhash [18] (protein (20.4%), lipid (4.34%) and ash (13.1%). These differences of chemical components of sample from different places have close link to soil, climate, harvesting season, gene, etc. In this study, fiber content is quite low (9.2%); cellulose is the main component of fiber and constitutes the cell wall of plant, thus, the solvent can easily penetrate into the cell wall and the extraction yield can be improved. Besides, total sugar and reducing sugar are high, 16% and 6.23%, respectively. These results are also similar with that of Abou-Arab et al. [1] (15.54%). From received results of this study, *Stevia* leaves are the rich nutritious material, which consist of the essential factors for maintenance of health such as protein, fiber, carbohydrates, lipid, etc. with different contents. Specially, this raw material can be used as an alternate source of cane sugar in different food preparations.

### 3.2. Effect of Material/Solvent Ratio on the TSC Extraction

The dried samples were extracted with distilled water under the various sample/solvent ratios during 3 hours at

65°C. The results showed that TSC have significant differences from different material/solvent ratios ($p<0.05$). TSC increased rapidly from material/solvent ratios of 1/25 to than of 1/100, then decreased slowly during the rest of scale. This pointed that the solvent volume affects significantly TSC. The best TSC value was 6.61±0.1% at the sample/solvent ratio of 1/75 (Table 2).

According to experimental data, as the volume used for extraction increases, the TSC of the extracts also increases, leading to an increase in the diffusion rate. However, if the large volume of solvent is used, the received results will point out the negative yield. The extraction process is stopped when the diffusion process obtained the equilibrium [4]. Besides, water is high polar solvent and it has a positive effect on solubility of bioactive compounds and other solutes, especially steviosides. In addition, adding volume of solvent (water) accelerates the mass-transfer process between solid and liquid by increasing the permeability of the plant tissues [15] and breaks the bounding between solutes and plant matrix. The optimum result of this case at material/solvent ratio of 1/75 was lower than that of Abou-Arab et al. [1] at material/solvent ratio of 1/35 (7.53%), this showed that the extraction yield depends on many factors, especially source of material and extraction method. Therefore, the material/solvent ratio of 1/75 was used for further experiments.

*The main components of the dried Stevia leaves* Table 1

| N$^o$ | Chemical components | Content [%] |
|---|---|---|
| 1 | Moisture | 4.84 |
| 2 | Protein | 11.5 |
| 3 | Fiber | 9.2 |
| 4 | Ash | 7.04 |
| 5 | Total sugar | 16.0 |
| 6 | Reducing sugar | 6.23 |
| 7 | Lipid | 3.53 |

*TSC of extracts at various material/solvent ratios* Table 2

| Material/solvent ratio [w/v] | Total stevioside content [%] |
|---|---|
| 1/25 | $5.52\pm0.09^{b}$ |
| 1/50 | $5.72\pm0.08^{c}$ |
| 1/75 | $6.61\pm0.1^{de}$ |
| 1/100 | $6.66\pm0.13^{e}$ |
| 1/125 | $6.55\pm0.09^{de}$ |
| 1/150 | $6.46\pm0.1^{d}$ |
| 1/175 | $5.04\pm0.14^{a}$ |

Different superscript letters in the same column denote significant differences ($p<0.05$).

### 3.3. Effect of the Extraction Temperature on the TSC Extraction

Table 3 showed that there are the significant differences of TSC between various extraction temperatures ($p<0.05$) but the extraction temperature of 85°C had the best results and the TSC obtained the maximum values (8.15±0.17%). The extraction temperature is the most important factor of extraction process; it can affect strongly the yield of TSC. The present investigation indicates that there were an increase in temperature (55°C-85°C), an increase in TSC were noticed. When the extraction temperature was too high; it led to the decrease in yield (over 85°C for this case).

According to Dacome et al. [6], using hot-water treatment was a classical extraction method. The high temperature extraction can accelerate the diffusion of organic compounds, reduces the viscosity of the solvents, opens cell matrix and easily releases these compounds into solvent. However, the disadvantage of method is associated high temperature, long extraction time and consumes a large of energy. The optimum temperature of this study was higher than that of study of Rouhani [17], who extracted TSC from *Stevia rebaudiana* by ultrasound-assisted extraction at 70°C with glycerol as solvent or higher than received result of Jaitak et al. [9], they extracted TSC from this material by microwave-assisted extraction at 50°C with distilled water, methanol and ethanol as solvents. These results proved that extraction temperature closely depended on extraction methods and solvents. From the results obtained, the extraction temperature of 85°C was chosen as the extraction temperature for the next experiments.

### 3.4. Effect of the Extraction Time on the TSC Extraction

Extraction time is also the most important factor, which affects directly the yield of TSC. TSC in extracts were significantly different for all extraction times ($p<0.05$). The yield obtained the optimum value at extraction time of 120 minutes and TSC value was 8.36±0.05% (Table 4). In general, the yield of extraction process increased quickly with increasing extraction time from 10 to 120 minutes. Then, it dropped sharply for rest of time.

Table 3

*TSC of extracts at various extraction temperatures*

| Extraction temperature [$^oC$] | Total stevioside content [%] |
|---|---|
| 55 | $6.27±0.13^a$ |
| 65 | $7.32±0.34^b$ |
| 75 | $7.78±0.19^c$ |
| 85 | $8.15±0.17^d$ |
| 95 | $7.94±0.1^{cd}$ |
| 100 | $7.18±0.07^b$ |

Different superscript letters in the same column denote significant differences ($p<0.05$).

*TSC of extracts at various extraction time* Table 4

| Extraction time [minutes] | Total stevioside content [%] |
|---|---|
| 10 | $7.19\pm0.04^{b}$ |
| 20 | $7.29\pm0.04^{bc}$ |
| 40 | $7.41\pm0.07^{c}$ |
| 60 | $7.74\pm0.08^{de}$ |
| 80 | $7.84\pm0.1^{ef}$ |
| 100 | $7.97\pm0.06^{fg}$ |
| 120 | $8.36\pm0.05^{h}$ |
| 140 | $8.23\pm0.08^{h}$ |
| 160 | $8.05\pm0.1^{g}$ |
| 180 | $7.77\pm0.08^{de}$ |
| 200 | $7.62\pm0.13^{d}$ |
| 220 | $7.22\pm0.12^{b}$ |
| 240 | $6.9\pm0.16^{a}$ |

Different superscript letters in the same column denote significant differences ($p<0.05$).

In fact, the classical extraction methods have some disadvantages such as low yield, extraction time longer than modern extraction methods (for instance microwave-assisted extraction, ultrasound- assisted extraction, supercritical fluid extraction, etc.) but they can be inexpensive, simple and efficient. The results show the effects of the extraction time on the yield of the extracts at the temperature of 85°C. Extending extraction time could improve the yield from 7.19% to 8.36%. However, the yield varied slowly and there was no significant difference when the time was over 120 minutes. Depending on various extraction methods leads to the significant difference of extraction time, for instance, Liu et al. [12] extracted TSC from *Stevia rebaudiana* Bertoni by ultrasound-assisted extraction for 32 min, while Jaitak et al. [9] also extracted TSC from this material by microwave-assisted extraction for 1 min. Based on the achieved results, the optimal treatment condition in this study was extraction time of 120 minutes.

## 4. Conclusions

In this study, the traditional extraction method of stevioside from *Stevia rebaudiana* was carried out by changing various extraction process factors such as material/solvent ratio, extraction time and extraction temperature. All these factors strongly affected TSC. The results indicated that maximum value of the three output parameters was 8.36% TSC under optimum conditions (material/solvent ratio of 1/75, extraction temperature of 85°C and extraction time of 120 min).

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