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STEVIOSIDES COMPOSITION IN *STEVIA REBAUDIANA BERTONI*: EFFECT OF DRYING METHODS

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Abstract

A field experiment was carried out in the field of experiments of the Medical and Aromatic Plants Research Unit at the College of Agriculture, University of Baghdad, Iraq in 2017 on *Stevia rebaudiana* plant as a comparison guide of eight drying methods on total glycoside composition in leaves and branches. A complete randomized block design (RCBD) was used with three replications. The drying treatments were: solar drying, shade, oven (40°C, 50°C, and 60°C), and microwave (for 1, 2, and 3 minutes). The results showed that the highest dry matter percentage in leaves reached at drying by shade and microwave for 2 minutes (23.52% and 22.81%, respectively) and the best composition of total glycoside sweetener including of stevioside (St) and rebaudioside A (Reb A) reached by solar drying and oven at 50°C was 7.86% and 7.84%, respectively. In the branches, the highest percentage was at drying by shade and microwave for 1 minute 20.14% and 20.09%, respectively, while the highest percentage of sweetener was by drying in oven (60°C) and microwave for 2 minutes 0.355% and 0.350%, respectively, followed by the same content by solar drying and oven at 50°C (0.255%).

Key words: Stevia; drying method; dry matter; stevioside.

Introduction

Stevia rebaudiana Bertoni is a bushy branched plant of the Asteraceae family and originated from South America (Gisieine *et al*., 2006). The steviosides (St) are the main sweet compounds that can be extracted from stevia plant to be used as sweeteners to sugars in several countries as a calorie-free sweetener such as Japan, China, and Brazil, of benefit to diabetics and those wishing to reduce sugar intake health reasons (Midmore and Ran, 2002). Several natural products have been extracted from *Stevia rebaudiana*, the best known of them are the steviol glycosides (SGs) such as rebaudioside (Reb) (A–F), stevioside, steviol bioside, dihydroisosteviol, dulcoside A, and rubusoside, with St being the most represented (4%–14% w/w) followed by Reb A (2%–4% w/w) (Savita *et al*., 2004 and Guleria, 2012). The content of SGs depends on cultivar, basic agricultural techniques, and growing conditions (Brandle *et al*., 1992; Geuns, 2000). St is the main sweet component in stevia, it tastes about 300 times sweeter than sucrose (0.4% solution) with calorie-free, maintains good dental health as well as an antibacterial, antihypertensive, antihyperglycemic, anti-inflammatory, antidiarrheal, antitumor, diuretic, and immunomodulatory effects and many other benefits (Mandan *et al*., 2010 and Kumar, 2013). Stevia leaves have functional and sensory properties superior to those of many other sweeteners (Gupta *et al*., 2013). Additionally, stevia is a good source of carbohydrates, protein, fiber, and antioxidant compounds.

The drying process is the primary means of keeping the plant from rapid degradation by inhibiting microbiological activity, the chemical, and biological reactions that lead to it, as well as reducing the size and weight of the product and the ease of storage, transport, and circulation (Chakraborty and Dey, 2016). Muller and Heindl (2006) indicated that the drying costs may reach between 30%–50% of the total costs of the product of medicinal plants. Drying is one of the most important procedures in post-harvest handling process of stevia. Freshly harvested stevia contained about 80% moisture

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and will deteriorate easy if not properly dried. Hot air drying for stevia was recommended to be carried out at 43°C or sun drying at ambient condition of less than 60% relative humidity (Hatter, 2010). The drying of stevia leaves is an effective method that increases the shelf-life of the leaves. The choice of drying method depends on several factors such as plant type, leave moisture, and type of active compounds (steviol or antioxidant) as they are affected by drying temperature, but the costs of the drying method should also be determined. Therefore, based on the overlap of several factors in the selection of suitable drying method of the dried plant part, many studies have pointed to this meaning through the relevant studies. Periche *et al.* (2015) conducted a study on the application of different drying methods (freeze drying, hot air drying at 100°C and 180°C, and shade drying) on SGs in stevia leaves, so he found that the ideal method for stevia leaves drying depends on their final use of being sweetener by shade drying or antioxidant by 180°C hot air drying. Abdalbasit *et al*., (2014) determined the effect of three methods of drying such as sun, oven, and microwave on *Stevia rebaudiana* Bertoni leaves nutritional composition, the results obtained indicate that reducing sugar was 4.5%, 4.8%, and 5.3% for sun, oven, and microwave, respectively, furthermore, drying reduces nutritional values with the exception to fiber content drying. The content of eight natural sweeteners found in stevia leaves was also determined and an increase in the content of seven of the sweeteners, excluding steviol bioside, was found at drying temperature up to 50°C (Lemus-Mondaca *et al*., 2016). Charikleia *et al*., (2016) studied the test quality in the final products, so they found that the bitter aftertaste of SGs can be reduced by applying spray, freeze, or oven drying. Different drying methods were applied to *Stevia rebaudiana* Bertoni leaves, in sun-dried samples the total glycoside content was higher, with no significant differences compared with shade or convection drying, whereas radiation drying adversely affected the content of Reb A and Reb C and was therefore a method of lowering the composition of total glycoside, while shadow or sun drying may be a low-cost alternative for farmers (Aranda-González, 2017). The present research was carried out to study the content of total steviol which included St and Reb A as affected by different drying methods.

Materials and Methods

An experiment was carried out in the laboratories of Medical and Aromatic Plants Research Unit/College of Agriculture/University of Baghdad to research the influence of drying methods on steviol content of stevia plant. The variety of *Stevia rebaudiana* Bertoni named Spanti obtained from Fito Co. the Spanish seeds company was planted in March 1, 2017 and harvested before flowering in May (12/6/2017). A complete randomized block design was used with three replications; a sample of 10 plants was taken from each plot. The fields samples were cleaned to determine fresh leaves and branches weight (first season, 1st cut). The fresh leaves and branches samples of each treatment divided randomly into eight treatments (D1, D2, D3, D4, D5, D6, D7, and D8) of drying methods were studied. Solar drying, shade drying, oven at 40°C, 50°C, 60°C drying, and microwave at 1, 2, 3 minutes drying, respectively, with total number of 24 experimental units.

Stevia green fresh plant was dried by using eight different methods, first was dried by direct sun light (solar drying) (44°C) in open air for 24 hours, second part was dried at the shade place in open air (33°C as average) for 72 hours, third part was dried in the microwave (Samsung—Model ME731K, Malaysia) set at 2450 MHZ and 800 W for three different times 1, 2, and 3 minutes, and the fourth part was dried by a fan oven (model-binder) at 40°C, 50°C, and 60°C for 24 hours for leaves and 48 hours for branches, respectively. The dried leaves were blended by using a high blender power (1,400 rpm) model 100 Pulverizer. The powdered samples were stored in polyethylene bags at 4°C–5°C until used.

The total steviol included St and Reb A in dried powder leaves and branches were extracted separately by mixing each one with hot water at 65°C at 1: 25 w/v for 3 hours (Galal, 2002). While both St and Reb A contents in leaves and branches were determined using HPLC method (Vanìk*et al.* 2001). The HPLC (Agilen 1200 model) was used for the determination of glycoside sweeteners content in stevia leaves and branches extract by using analytical standards supplied from Sigma-Aldrich Co. The retention time and sample area for the standard solution of St and Reb A (Fig.s 1 and 2) and the samples solution were calculated. The mobile phase, which started with (15: 75 acetonitril: water), ended with 50: 50 acetonitrile: water in 30 minutes, and with a flow rate of 1.0 ml min^{-1} was measured by a wavelength of 205 nm and a temperature of 35°C. The concentration of each steviol compounds in the model was calculated according to the calibration equation according to Vanìk *et al.*.

The dry matter ratio was calculated according to the following equation:

$$\text{Dry matter ratio (D.M.\%)} = \frac{\text{Dry weight of sample}}{\text{Fresh weight of sample}} \times 100$$

The data were analyzed by Genstat program Version 4.0. The means were compared by the test of least significant difference (LSD) at 0.05 level of probability (LSD 5%) and the correlation coefficient (*r*) was calculated between some studied traits (Steel *et al.*, 1997).

Results

Dry matter ratio of stevia plant affects by different drying methods

The results in table 1 indicate that there were significant differences between drying treatments in dry matter ratio in leaves and branches, the highest dry matter percentage in leaves reached at drying by shade (D2) and microwave for 2 minutes (D7) was 23.52% and 22.81%, respectively. In the branches, the highest percentage was at drying by shade and microwave for 1 minute 20.14% and 20.09%, respectively, compared with the lowest percentage recorded at oven 60°C in leaves and branches 18.41% and 16.42%, respectively.

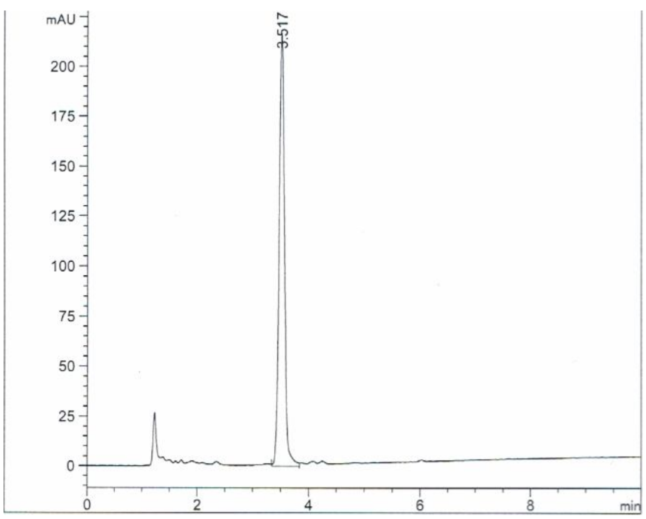

Fig. 1: HPLC chromatogram for Stevia (Stevioside standard).

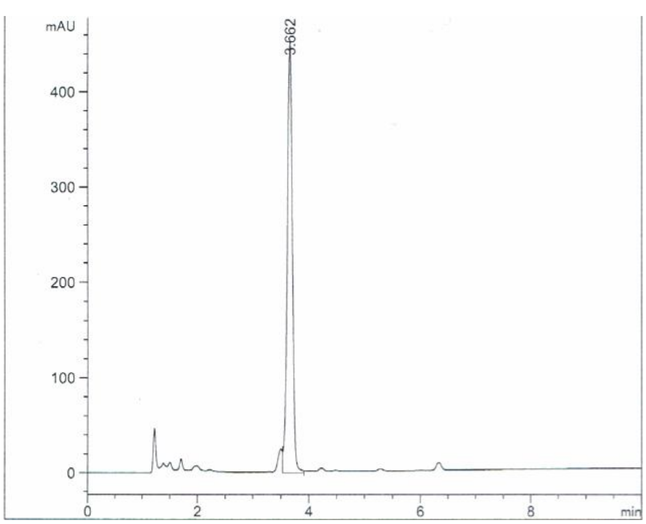

Fig. 2: HPLC chromatogram for Stevia (Reb A standard).

Total stevioside affects by different drying methods

Fig.s 3 and 4 showed the HPLC chromatogram of St in leaves affected by the sun and oven at 50°C as the best two methods for stevia leaves drying.

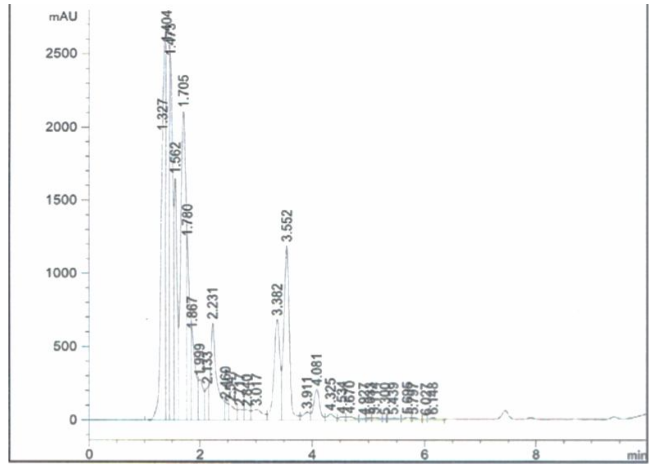

Fig. 3: HPLC chromatogram for stevia (sun drying sample).

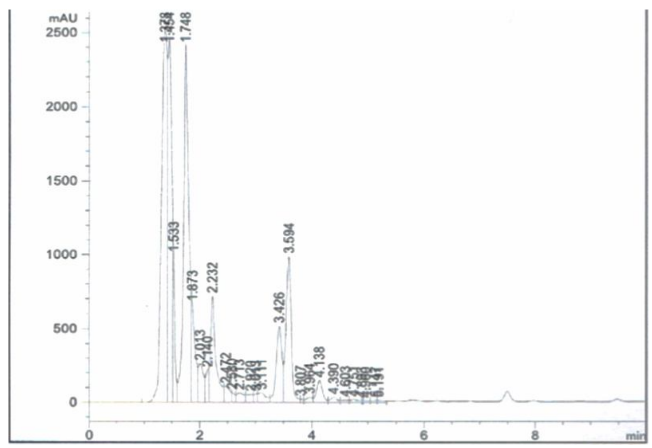

Fig. 4: HPLC chromatogram for stevia (oven 50°C drying sample).

The data in Fig. 5, showed that highest percentage of St in the leaves due to the drying methods obtained in the drying by oven treatment at 50°C, microwaves for 3 minutes, and solar drying was 5.32%, 5.29%, and 5.28%, respectively, without any significant differences between them, while the Reb A had the highest percentages in drying by microwave for 1 minute and solar drying 2.77% and 2.58%, respectively, without significant difference between them and the other treatments fig. 6.

In Fig. 7, the treatment of solar drying recorded the highest percentage in total steviol (St and Reb A) were as 7.86% followed the drying by oven at 50°C, microwave for 3 minutes and the oven at 60°C (7.84%, 7.77%, and 7.52%, respectively). The results indicate that there is a positive economic value in reducing the costs of sun and shade drying, especially when there is correlated with no

Table 1: Leaves and branches dry matter ratio affects by drying methods.

Plant parts	Drying methods								Mean
	D1	D2	D3	D4	D5	D6	D7	D8	
Leaves	21.51	23.52	22.36	22.81	22.79	21.55	21.43	18.41	21.79
Branches	18.95	20.14	20.09	19.02	17.57	17.64	16.72	16.42	18.31

LSD 5% leaves: 0.75. LSD 5% branches: 0.64.

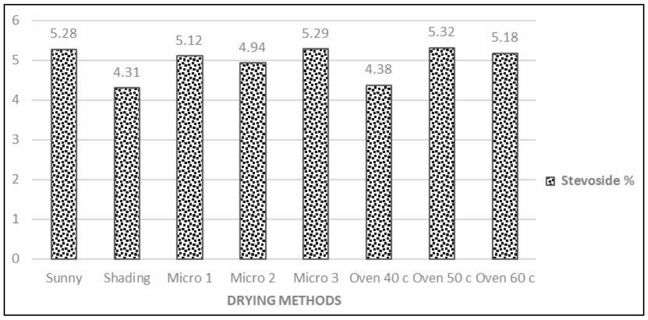

Fig. 5: The percentage of Stevioside in leaves of *Stevia rebaudiana* according to the drying methods

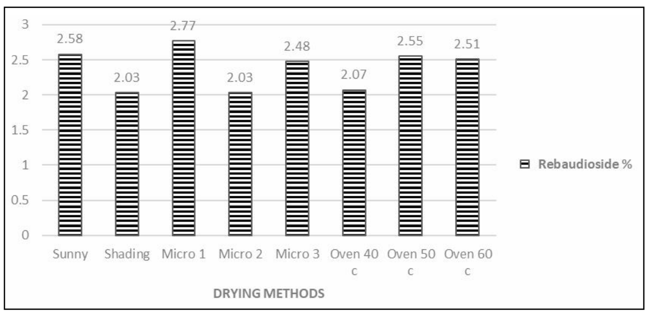

Fig. 6: Percentage of Reb A in leaves of *Stevia rebaudiana* according to the drying methods.

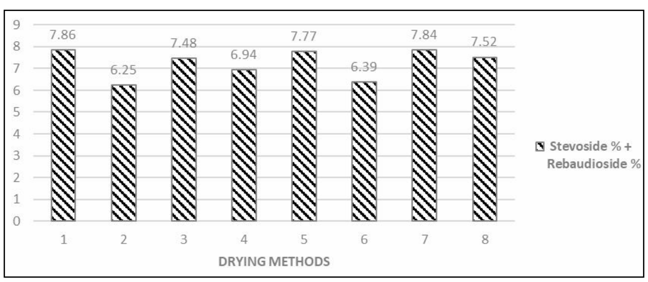

Fig. 7: Percentage of St and Reb A in leaves of *Stevia rebaudiana* according to the drying methods.

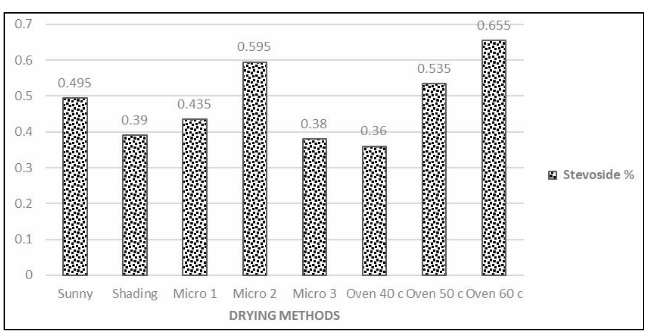

Fig. 8: The percentage of St in branches of *Stevia rebaudiana* according to the methods of drying.

significant decrease in total steviol of these treatment, moreover, the solar drying showed the high sweet glycosides content (total steviol) compared with the most treatments. This may be due to the relatively high temperatures in the solar drying conditions in the country during the summer months (June) which were suitable for drying while maintaining a high level of the ratio of treatment mentioned.

The results of fig. (8) show that the percentage of St was significantly affected by the drying method in the branches. The highest percentage was recorded at drying by oven (60°C) and drying by microwave for 2 minutes reached 0.655% and 0.595% respectively followed by solar drying and oven at 50 °C 0.535 and 0.495% respectively) while the lowest percentage of St was recorded at oven drying (40°C) and microwave for 3 minutes 0.36% and 0.38 % respectively.

Fig. (9) shows the significant effect of the percentage of Reb A in the branches and for the same treatments in Fig. 8 with a slight difference in their order. The highest percentage reached at drying by oven (60°C) and microwave for 2 minutes 0.355% and 0.35%, respectively, followed by solar drying and oven at 50°C was the same ratio (0.255%) compared with the lowest percentage of the drying by oven (40°C) which showed (0.175%), furthermore, the same St content were appeared at drying by shade, microwave for 1 and 3 minutes (0.185%).

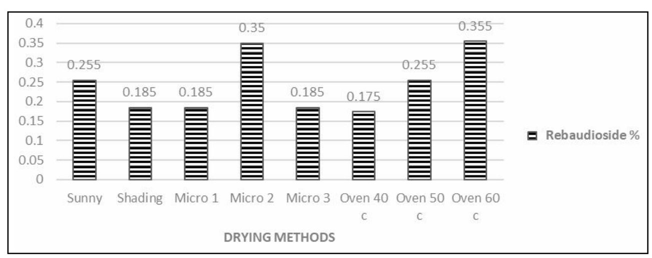

Fig. 9: The percentage of Reb A in the branches of *Stevia rebaudiana* according to the methods of drying.

Correlation Between Studied Traits

According to simple correlation for drying treatments (table 2) show a positive significant correlation between the both natural sweeteners in stevia plant in different drying methods. The D.W. leaves were correlated positively and significantly with D.W. branches and correlated negatively with St branches, the St leaves were high correlated positively and significantly with Reb leaves. The same high positive response between St branches and Reb in branches may be due to positive significant response of D.W. leaves.

Table 2: Correlation coefficient (*r*) between studied traits under the effect of drying methods.

Traits studied	(D.W.%) branches	(Reb A) leaves	(St) branches	(D.W.%) leaves	(Reb A) branches
(St) leaves	0.128	0.885*	-0.144	0.207	-0.0170
(Reb A) branches	-0.316	-0.191	0.905*	-0.480	
(D.W.%) leaves	0.697*	0.018	-0.597*		
(St) branches	-0.382	-0.140			
(Reb A) leaves	-0.090				

*Significant at $P = 0.05$.

The steviol glycosides under studying, St and Reb have the same pathway in the plant (Brandle and Telmer, 2007), so the St increases without decreasing the concentration of Reb A, which means their increasing was parallelly. The accumulation of dry matter in the leaves and branches may be attributed to glycoside distribution in the plant and the efficiency of the leaves as the source of carbohydrate production while the branches were considered as the sink to transfer the elements within the plant (Taiz and Zeiger, 2010).

Discussion

The reduction of dry matter ratio at drying in the oven 60°C may be due to the high temperature during drying, which evaporates a higher percentage of moisture from vegetables part, therefore, a decrease was observed in the dry matter ratio with higher the temperature of the oven, while in the shade, the temperature was about 33°C as average.

The change in plant content from sweeteners and carbohydrates is subjected to biochemical and physiological changes which occurring during the drying period (Abdlebasit *et al.* 2014). The SGs content depends on the drying method and plant species (Okoh *et al.* 2008) as well as the growth conditions that can be modified in the glycosides content in the plant's (Gonzalez *et al.* 2017). When drying conditions of temperature and humidity are appropriate, a high level of active compound can be obtained in the dried plant.

The results cleared that the methods of natural drying by solar and shade was preserving the color of the dried vegetable product which saves and increases the marketing value of the costumer. A high level of sweetener has been obtained was important to the food quality especially when considering the solar methods as a short drying period (24 hours) which leads to reduce the drying costs compared to microwaves and ovens. Furthermore, it saves time compared to the shade drying method (3 days or 72 hours). It is more important to have a good sweeteners proportion in the branches which provides a greater opportunity to get more benefit from the all plant instead of leaves only, as a source of sweetener that is more economical in sweeteners production through two things: the first is production increase and the second is very important to avoid the problem of separating leaves from the branches and to save time.

The drying process of medicinal plants is one of the most expensive processes and the cost varies according to the method of drying. Therefore, the method used should be as efficient and low cost as possible (Ghasemi *et al.* 2013). The method of solar drying is low in cost and without the need for any energy source (Gonzalez *et al.* 2017). The only advantage of drying by ovens is that the dried plant is not exposed to external conditions such as dust, etc. However, pollution can be overcome by sterilization of the plant product after drying. So, the choice, between solar drying and drying by oven at 50°C methods, depends on production costs and the availability of food safety conditions which achieve the highest ratio of sweeteners in leaves and branches together as shown in fig. 7 especially when there are no significant differences between them.

Conclusion

We conclude that the best way to dry the *Stevia rebaudiana* is the solar drying specially in hot, dry summer countries like Iraq , which was characterized by giving the highest ratio of sweetener as well as the low drying costs, followed drying by oven at 50°C.

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