J. Food and Dairy Sci., Mansoura Univ., Vol. 6 (10): 591 - 611, 2015

PRODUCTIVITY AND QUALITY OF STEVIA AND THE EFFECTS OF DRYING ON STEVIOSIDES AND ITS USAGE IN BAKERY

Soha R.A. Khalil*; Eman S. Mohamed and A.E. Attia ***
*Sugar crops. and **Food Tech Res. Inst., Agric. Res. Center, Giza, Egypt.

ABSTRACT

Stevia rebaudiana, Bertoni have been investigated as a possible substitute for sugar; one important class of no caloric sugar substitutes and as natural source of antioxidants

This investigation aimed to evaluate the productivity traits and quality of three Stevia varieties named (Spanti, Egy1 and China1). Also, the impact of some drying methods (open air, microwave and oven-dried leaves) on the stevioside sweetener, total phenolic and chemical compositions was studied.

Data combined analysis of two filed experiment during season 2013/2014 and 2014/2015 showed that Stevia has good productivity under Egypt conditions. It's planted during March and gives four cuts annually by ranging from 1.698 to 2.606 ton/fed dry leaves with ratio of stevioside (St) content from 6.72 to 8.67 and rebaudioside A (Reb-A) A% from 4.23 to 8.67 according to variety.

China1 variety surpassed the other two varieties in fresh and dry weight of leaves g/plant, followed by Spanti and Egy1 varieties respectively, as well as in all quality parameters like annual total leaves fresh and dry yield and rebaudioside A% content in dry leaves. Moreover its good sensory of sweetens and bitter acceptability in water extract. It was rich in total phenolic and nutritional value.

Regarding the impact of drying methods, it is worthy to mentions that total steviosides, total phenolic compounds and pigments were decreased in dry leaves for all varieties especially drying by oven at 60 °C but, in fact the chemical composition of dry leaves was more influenced by the varieties than different drying methods.

In accordance with previous results china1variety was selected in the form of syrup steviosides for preparing cup cake as a replacer of sugars and food additive by substituting levels 25, 50, 75 and 100% with sucrose.The results of sensory evaluation of cake prepared with equal ratio of sugar to stevioside syrup (50:50) exhibited insignificant difference between control (sucrose) where, it had a high score for appearance, flavor, taste, color, sweet acceptability, texture, and overall acceptability.

These results suggest that stevioside syrup can substitute sugar and be used in sweet manufactures.

Keywords: Cake, drying, productivity, quality parameter, *Stevia rebaudiana*, Stevioside syrup and total phenols.

INTRODUCTION

Stevia rebaudiana, Bertoni is a branched bushy shrub of the Asteraceae family and originated from south America (Gisleine *et al.,* 2006). Stevia is cultivated in different places of the world, it is expected that in the Egyptian agriculture environment one Fadden of stevia may produce up to 400 Kg of Stevia sugar annually (Allam, 2007). The leaves have been traditionally used for hundreds of years in Paraguay and Brazil to sweeten local teas, medicines and as a ‘sweet treat’.

Development of new varieties of *S. rebaudiana* with a higher content of rebaudioside-A (Reb-A) and a reduced content of stevioside (ST) is the main aim of plant breeders concerned with the improvement and utilization of this source of natural sweeteners. Conventional plant breeding approaches is the best method for improving quality traits in a highly cross-pollinated crop like stevia.(Yadav *et al.*, 2011).

Plant leaf yield is proportional to branch number, leaf number and (not always) plant height (Buana, and Goenadi, 1985, Buana, 1989 and Shyu *et al.*, 1994). Total stevioside content is positively correlated with leaf/stem ratio (Tateo *et al.*, 1998) Leaf thickness is positively correlated with Reb-A/St ratio (Shyu *et al.*, 1994). High rebaudiosid A content is linked to high net photosynthetic rate, high chlorophyll and protein content (Weng *et al*., 1996).

Drying is one of the most important activities in postharvest handling of *Stevia*. Freshly harvested *Stevia* leaves contained about 80% moisture content and will deteriorate easily if not properly dried. *Stevia* leaves have to be dried within 8 h after harvest in order to retain the high level of sweetness. Hot air drying was recommended to be carried out at 43 °C or sun drying at an 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 However, drying causes changes in the product mainly associated with fragrance. The effect of a particular drying method on the release or retention of volatile compounds is not predictable and depends on the compound and the product being dried (Di-Cesare *et al.,* 2003).

Beben *et al.*, (2015) reported that stevia extracts prepared in different solvents contain significant amounts of biologically active photochemical with antioxidant activity and might be used as ingredients of food. Polyphenols content and antioxidant activity are presented in both stevia leaf powder, and commercial stevioside according to Rao *et al.,* (2014), but the higher polyphenols was (5.6%) in the leaf powder, while it was 2.3% in commercial stevioside powder as mentioned by Taleie *et al.* (2012)

The steviosides are compounds that can be extracted and used as alternative sweeteners to sugars, of particular benefit to diabetics and those wishing to reduce sugar intake for health reasons (Midmore and Rank, 2002). Stevioside is present with an average of 4-20% in the dry matter of the plant leaves, which primarily depends on cultivar characteristics of plants and basic agricultural techniques (Brandle *et al.,* 1992; Geuns, 2000). Taking the sweetening powder of the stevia sugar into consideration, these 400 Kg of Stevia sugar are equivalent to about 80,000 sweetening units. Note that one feddan of sugar cane produces about 5,000 sweetening units and one feddan of sugar beet produces about 3,500 sweetening units. A sweetening units is equivalent to the sweetness of one kilogram of sucrose (Allam, 2007).

One of the most interesting sugar alcohols constitutes is rebaudiosid A(Reb A).*This* natural component has a clean taste and very influence on the product characteristics since only small amounts of the ingredient need to be added to the product(Parkash and DuBois, 2008).

Several studies performed on substituting stevia in sugar containing bakery product such as pound cake (Schirmer *et al.,*2012) muffina (Zhan *et al*., 2012),yoghurt cake (Abdel-Salam *et al.,* 2009) , bread (Parimalavalli, 2007) and biscuits Vatankhah *et al.,* (2015).

The present research was carried out to study the content of total steviosides and antioxidants content for three varieties of *S. rebaudiana Bertoni* (Spanti, Egy1 and China1)as affected by three drying methods (open air, microwave and oven-dried leaves). The study includes evaluation of productivity for each variety (fresh and dry leaf weight kg/fed). The extracted steviosides sweetener from the variety with the highest stevioside content was utilized in the preparation of low-energy bakeries.

MATERIALS AND METHODS

Materials:

Three varieties of Stevia. rebaudiana Bertoni named Spanti, Egy1 and China1 obtained from Sugar Crops Research Institute (SCRI), Agricultural Research Centre (ARC), Giza, Egypt,. were planted and harvested during the two successive season 2013/2014 and 2014/2015. A complete randomized block design was used with three replications. Each plot was 2 m by 2.5 m containing 33 plants. A sample of 5 plants was taken from each plot to determine yield components. **:** Wheat flour 72%, Fresh egg, Sugar, Vegetable oil, Fresh milk, Baking powder and Vanilla were obtained from local market at Giza city, Egypt.

Method:

Yield components included: Plant height, number of branches/plant, fresh and dry leaves weight/plant and fresh and dry leaves yield/fed (four cuts per year where first cut was taken three months after transplanting, March 15 and followed by one cut each 3 months). One sample of leaves (First season, second cut) was collected for sweetener analyses.

Cup cake formula: Wheat flour 72% - Fresh egg – Sugar –Stevioside syrup – Vegetable oil – Fresh milk – Baking powder – Vanilla.

Sample preparation for drying methods

The field's samples were cleaned and divided randomly into 5 portions (fresh leaves, air drying, microwave drying and two oven drying, 40^oC and 60^oC) for each variety with a total number of 15 samples. The green fresh leaves were allowed to dry using three different methods; first part was dried by direct open air for 3 days, second and third parts using an oven (Oven DGG-9070A: Shanghai,China) at 60 & 40 ^oC for 16 &14 h respectively and the forth part were dried in thew 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.

Gross Chemical composition:

The prepared samples were analyzed for moisture, protein, fat, crude fiber and ash according to the methods described in the AOAC (2005). The carbohydrate content was determined by subtracting the total crude protein, ash and fat from the total dry weight (100 g) of the food sample differences.

Specific gravity and pH were determinate according to AOAO (2005).

Total chlorophylls and carotenoids:

Both chlorophylls (A and B) and carotenoids were determined in Stevia leaves before and after dehydration with different methods according to Wettestein (1959).

Total stevioside(TS): Total stevioside in dried powder leaves extracted by the water method was determined by Anthrone-sulphoric acid method as described by Wei (1984). While both stevioside (ST) and rebaudioside A (Reb A) contents were determined using HPLC method (Nishiyama *et al*. (1992).

Total phenolic content in the methanolic extract:

Total phenolic content in extract were assayed Spectrophotometrically using the Folin- Ciocalteu method (Gamez-Meza *et al .,* 1997).

Extraction and purification of stevioside from stevia plant

Stevia sweetener (stevioside) was extracted from the dried ground leaves of Stevia plant by using water with extraction efficiencies up to 98% achievable removed of stevioside and so produce "natural product" (Nishiyama, 1991).

Extraction with water:

The dried ground leaves of stevia plant were extracted by water according to Nishiyama *et al*. (1992).The dried ground leaves were mixed with hot water (65°C) at 1:50 (w/v). Stevia leaves were extracted by using hot water for 3 h

Purification Steps and stevioside syrup water extracted:

The crude extract containing stevioside was filtered through Whatman No. 4 filter paper (filtrate A) and purified by addition of 5% Ca (OH)2 (based on wt. of dried leaves). and the filtrate were collected, passing through ion exchange column (packed with Amberlite IR-4B resin) to remove the undesirable colors at a rate of 1 ml/sec at 25°C. The elute (clear and colourless solution) containing stevioside was collected (in which pigments were adsorbed on resin) and then concentrated by using rotary evaporator at 45°C to the maximum concentration value. At each step total soluble solids (TSS), pH, Stevioside and de-pigmentation were determined according to method described by Cheng *et al*., (1985).

Determination Total soluble carbohydrate (TC) in the water extract it was modified by used total stevioside content (TS) in the extract and calculated by using the equation reported by (Nishiyama *et al*. (1991) as follow TC=7.56+0.96 TS

Determination Color: was determined by the color Wesson method using Lovibond glasses calibrated in accordance with Gillett (1960). A 5.25 inch color cell was used.

Determination of Viscosity of the stevioside samples was measured by using Brookfield viscometer, according to method described by Hayta *et al*. (2002).

Determination of Total soluble solids (TSS) according to AOAC (2005).

Determination of stevioside by HPLC

The stevioside content of water extract was determined using (HPLC) as described by Nishiyama *et al*. (1992).

Formulation of cup cake: were prepared according the procedure of A.A.C.C., (1983) and tabulated as follows:

Table (1) Replacement of sugar by surup Steviosides in the tested cup cake formula:

Ratio of syrup stevioside replacement sugar	control	25%	50%	75%	100%
Materials amounts in formula					
Wheat flour 72% (g)	200	200	200	200	200
Fresh egg (g)	180	180	180	180	180
Sugar (g)	160	120	80	40	0
Syrup Stevioside (ml)	0	0.80	1.60	2.40	3.2
Vegetable oil (ml)	100	100	100	100	100
Fresh milk (ml)	240	240	240	240	240
Baking powder (g)	9.0	9.0	9.0	9.0	9.0
Vanilla (g)	2.0	2.0	2.0	2.0	2.0

Organoleptic porperties:

Organoleptic characteristics were evaluated for bakers cake. Sweet taste, flavor, color, texture , appearansce, sweet acceptability , bitter acceptability according to DuBois and Stephenson (1985).

Statistical analysis:

Data were analyzed using an ANOVA of RCBD (the analytical package MSTAT-c v 2.1. (1988). Data from each year (combined over years) were analyzed and Barteltt's test for heterogeneity of error variances across years indicated that error terms were homogeneous. Treatment means were compared by using Duncun test at 0.05 level of probability according to Waller and Duncan (1969). A combined analysis for the seasons was done according to Gomez and Gomez (1984).

Statistical analyses of chemical compositions, extraction purification and organoleptical were carried out by SPSS program SPSS (2000).Data were expressed as mean ± SE and the statistical analysis was performed using one way analysis of variance followed by Duncan's test to specify the variation level at 0.05 according to Steel and Torrie (1980)

RESULTS AND DISCUSSION

Growth parameters of three varieties of stevia leaves:

Data presented in Table (2) show that the tested stevia varieties differed significantly in their plant height cm and number of branches/plant. Spanti variety surpassed the other two varieties. The difference among the three stevia varieties could be due to the variation in their gene make-up and their response to the environmental condition. There were a significantly increase among varieties in plant height by 9.1 and 36.2 % and a rise from 26.9 and 36.25 % in number of branches/plant while increased the same traits with ascending increase from the first to the fourth cut by 26.03 and 63.8 cm in plant height and from 12.5 and 22.7 in number of branches/plant.

Factorial ANOVA for the factors						
K value	**Source**	**Degree of freedom**	**Sum of Squares**	**Mean square**	**F value**	**Prob**
1	Replication	2	0.042	0.021	0.0615	
2	Factor A (cuts)	3	491.761	163.920	483.5194**	0.0000
4	Factor B (variety)	2	516.508	258.254	761.7770**	0.0000
6	AB	6	194.923	32.487	95.8281**	0.0000
7	Error	22	7.458	0.339		

Table(2) Growth parameter of three Stevia varieties

Var	**Plant height cm**					**No of branches/plant**				
	1st cut	**2nd cut**	**3rd cut**	**4th cut**	**Mean**	**1st cut**	**2nd cut**	**3rd cut**	**4th cut**	**Mean**
Spanti	24.5^{k}	25.5^{J}	31.5^{g}	66.5^{b}	37.00^{b}	12.5^{e}	11.1^{F}	10.5^{F}	16.5^{d}	12.6^{c}
Egy1	23.5^{L}	26.2^{I}	31.9^{g}	54.0^{c}	33.9^{c}	12.5^{e}	16.5^{d}	13.34^{e}	21.83^{c}	16.0^{b}
China1	30.1^{h}	32.8^{e}	50.8^{d}	70.9^{a}	46.2^{a}	12.5^{e}	21.5^{c}	23.5^{b}	29.8^{a}	21.8^{a}
Mean	26.03^{d}	28.15^{c}	38.11^{b}	63.8^{a}	-	12.5^{d}	16.37^{b}	15.7^{c}	22.7^{a}	-

Each mean value, within the same column, followed by the same letter is not significantly different at 0.05 level.

The interaction between cuts and varieties had a significant effect on plant height cm and number of branches/plant whereas Spanti variety recorded the highest value in two traits with fourth cut. These results are in agreement with those obtained with Taleie *et al* (2012) who showed that Maximum plant height (70 cm) and number of branches/plant from 12 to 30 /plant during the fourth cuts. These results may be due to the role of quantitative genetic composition which could be influenced by environmental factors. It is reported that increasing the growth period, is associated with increasing plant height as mentioned by Shamskia *et al*., (2006). Moreover, the positive effect of (environments X genetic) factors which could be directly correlated to the fact that conveniently environmental factor had increased number of leaf bearing points in terms of increased number of branches per plant (Maheshwar, 2005).

Productivity traits of three varieties:

Data in Table (3) show that the three stevia varieties differed significantly in productivity traits. China variety showed the superiority over the other two varieties followed by Spanti and Egy1 varieties respectively in leaves fresh and dry weight g/plant. The variation among the tested stevia varieties in these traits might be due to their gen make-up and their response to the environmental conditions. China1variety recorded a significant increased over two varieties in leaves fresh and dry weight g/plant by 19 and 4 % in fresh and rising by 9 and 53 % in dry respectively while increased the same traits with the first cut showed marked increase over the other three cuts. Regarding the interaction between Stevia cuts and varieties had a significant effect on leaves fresh and dry weight g/plant whereas Spanti variety recorded the highest value in two traits with first cut. These results may be attributed to the role of different factors affected on plant growth and environment climatic conditions which play an important role in the efficiency of crops.

Table (3) Productivity traits of three stevia varieties

Var	Leaves fresh weight/plant g					Leaves Dry weight/plant g				
	1st cut	2nd cut	3rd cut	4th cut	Mean	1st cut	2nd cut	3rd cut	4th cut	Mean
Spanti	274.44^{b}	34.44^{e}	27.00^{h}	31.00^{f}	91.72^{b}	24.4^{c}	12.2^{F}	9.20^{j}	10.80^{i}	19.80^{b}
Egy1	242.1^{c}	30.3^{fg}	23.00^{i}	29.6^{g}	81.26^{c}	48.00^{a}	13.00^{d}	5.40^{L}	12.78^{g}	14.16^{c}
China1	312.44^{a}	17.00^{j}	12.52^{k}	42.00^{d}	96.00^{a}	47.00^{b}	20.38^{j}	5.80^{k}	13.78^{e}	21.72^{a}
Mean	276.32^{a}	27.24c	20.84^{d}	42.00^{b}	-	39.8^{h}	15.20^{b}	6.80^{d}	12.44^{c}	-

Each mean value, within the same column, followed by the same letter is not significantly different at 0.05 level.

Quality parameters of three varieties of stevia leaves:

Data presented in Table (4) show that tested stevia varieties differed significantly in their total leaves fresh and dry weight/plant as well as yield of leaves fresh and dry weight kg/fed/year also, their content of stevioside % and rebaudioside A% as well as organoleptic of sweet and bitter acceptability in dry leaves. The results showed that Shina1 variety was superior over the other two varieties in all quality parameters whereas its recorded significantly increased in total of leaves fresh and dry weight/plant by (18.85 and 5.88 %) and (53.53 and 9.75%) respectively, while it increased by 18.77 and 5.87% in yield of leaves fresh weight Kg/fed/year as well as increased by 9.77and 53.47% in yield of leaves dry weight g/fed/year. These results can be attributed to the superiority of china1 variety in plant height and number of branches/plant also in fresh and dry of leaves for total cuts therefore evaluation of dry matter production and dispensation to various plant parts is important for designation of total yield of crop by Maheshwar, (2005).

Table (4) Quality parameters of three varieties of Stevia leaves

Quality parameter	varieties of stevia		
	Spanti	Egy1	China1
Total of leaves fresh weight g/plant	375.54^{b}	334.54^{c}	397.62^{a}
Yield of leaves fresh weight ton/fed/year	11.266^{b}	10.036^{c}	11.928^{a}
Total of leaves dry weight g/plant	79.18^{b}	56.6^{c}	86.9^{a}
Yield of leaves drying weight ton/fed/year	2.375^{c}	1.698^{b}	2.606^{a}
Mean Steviosid/100g leaves	8.67^{a}	7.67^{b}	6.72^{c}
Mean rebaudioside –A/100g leaves	4.23^{c}	4.26^{b}	8.67^{a}
Organoleptic mean of sweet acceptability in dry leaves (Score: 10)	5.0^{c}	6.3^{b}	9.3^{a}
Organoleptic mean of bitter acceptability in dry leaves (Score: 10)	9.2^{a}	6.0^{b}	5.0^{c}

Each mean value, within the same column, followed by the same letter is not significantly different at 0.05 level

On the other hand, Andolfi *et al*., (2006) found that the highest quantity of leaf dry matter produced was approximately 3.6 t ha^{-1} for the most productive genotype in the first year. Antonella *et al.,* (2008), found that yield of fresh and dry leaves per year ranged from 12 to 14 t ha^{-1} and 2 to 3 t ha^{-1} respectively, when drying of the soft green leaf material is completed immediately after harvesting utilizing a drying wagon or a kiln depending on weather conditions and density of loading, it generally takes 24 to 48 hours to dry stevia at 40°C to 50°C.

Moreover, data in Table (4) show that the content of rebaudioside A% in dry leaves was markedly increase in China1 variety more than other two varieties and on the contrary, its content decreased from stevioside%. These results may be lead to improve the taste of sweet and bitter less of organoleptic of sweet and bitter acceptability in dry leaves. The variation among the tested stevia varieties in these traits might be due to their gen make-up, but the improvement of taste lead to increasing ratio of rebaudioside A% as was mentioned by Carakostas *et al* (2008) and Schiffman (2000) They reported that the main sweetening components are stevioside and rebaudioside A with an amount of about 90 % w/w. of all sweet glycosides in the leaves.

DuBois (2000) noted that rebaudioside-A is usually present as 30-40% of total sweetener and has the sweetest taste, assessed as 180 to 400 times sweeter than sugar with no bitter aftertaste (licorice taste or lingering effect). While Oddone (1999) who observed that the ratio of rebaudioside-A to stevioside is the accepted measure of sweetness quality; the more rebaudioside-A the better. If rebaudioside-A is present in equal quantities to stevioside, it appears that the aftertaste is eliminated. These results are in line with those mentioned by Singh and Rao, (2005) who reported that rebaudioside A contributes to the typical sweet taste similar to sucrose but differences in taste are caused by more polar groups in rebaudioside A that enable better solubility and ultimately more similarity to taste of sucrose, unlike the molecule of stevioside. These results are in agreement with those of Midmore and Rank (2002).

Effect of Chlorophyll (A and B), Carotenoids and Total stevioside by different drying methods:

Data in Table (5) show that leaves of stevia varieties differed significantly in Chlorophyll traits and total stevioside in the all drying methods under study. As shown in Table (4) fresh leaves of China1 variety recorded the highest value with Chlorophyll traits and total stevioside these results are in agreement with those of Weng, *et al*. (1996) who mentioned that high R/A content is linked to high net photosynthetic rate, high chlorophyll and protein content.

Significant decrease is observed of dried leaves in chlorophyll A, B, carotenoids, total pigment and total steviosides in all drying methods compared to fresh leaves. It is worthy to mention that drying in an oven at $60^{o}C$ recorded the lowest mean in chlorophyll A,B, carotenoids, total pigment and total stevioside content compared to other drying methods under study. Spanti variety gave the lowest mean of chlorophyll A and carotenoids content while China1 recorded the lowest mean in chlorophyll B, Egy1 variety achieved lowest mean in total stevioside content compared to the other two varieties. Whereas traits of total pigment spanti and china1 varieties obtained the lowest mean with no significant difference between them.

Table (5) Effect of different drying methods on Chlorophyll (A and B), Carotenoids and Total stevioside

Varieties	Drying method					
	Fresh	**Open air**	**Microwave**	**Oven °C 40**	**Oven °C 60**	**Mean**
Chlorophyll A mg/L						
Spanti	102.87c	56.20 h	67.78 e	48.98 j	41.05 k	63.37 c
Egy1	109.14 b	61.03 f	74.97 d	57.84 g	41.03 k	68.80 a
China1	119.85 a	51.75 i	60.48 f	48.94 j	40.93 k	64.39 b
Mean	110.62 a	56.33 c	67.74 b	51.92 d	41.00 e	-
Chlorophyll B mg/L						
Spanti	62.70 b	26.00 h	31.97 e	27.70 fg	25.15 i	34.70 a
Egy1	61.03 c	27.13 fg	33.00 d	27.88 f	22.05	34.22 b
China1	65.87 a	25.28 hi	27.29 fg	26.99 g	20.44 k	33.17 c
Mean	63.20 a	26.14 d	30.75 b	27.52 c	22.55 e	-
Carotenoids mg/L						
Spanti	29.75 c	8.30 e	10.20 d	6.6hi	5.95j	12.16 c
Egy1	38.88 b	8.25 e	7.58 f	6.85 gh	6.40i	13.59 b
China1	41.75 a	7.25 fg	8.50 e	7.28fg	6.20ij	14.20 a
Mean	36.79 a	7.93 c	8.76 b	6.91 d	6.18 e	
Total pigment mg/L						
Spanti	195.32c	90.50ef	109.94d	83.27gh	79.18 fgh	111.64 b
Egy1	209.05 b	96.41e	115.55d	92.57e	83.57fg	119.63 a
China1	227.47 a	84.28fg	96.26e	83.20 fgh	79.36h	113.511 b
Mean	210.61 ef	90.39 c	107.25 b	86.35 d	80.04 e	
Total steviol %						
Spanti	15.57cd	15.18ef	15.17f	15.05f	14.05h	15.00 b
Egy1	14.59g	14.44g	14.42g	14.45g	14.06h	14.39 c
China1	16.38a	15.77bc	16.00b	15.49cde	15.30def	16.79 a
Mean	15.511 a	15.13 bc	15.19 b	15.00 c	14.47 d	-

Each mean value, within the same column, followed by the same letter is not significantly different at 0.05 level.

These results might be associated with the negative thermal effective of on the pigments as a mentioned by Abou-Arab *et al.,* (2010) who found that sun dried plants showed reduction in the pigments content being 47.40, 41.32, 74.85 and 50.99% (on dry basis) with chlorophyll A and B, carotenoids and total pigments, respectively.

On the other hand, stevioside content was 1.77% in the fresh leaves and reduction percent of stevioside with sun drying by 0.81%.

These results are in agreement with those Brandle *et al*. (1992), Singh and Rao (2005); Braz de Oliveira *et al*., 2011) who mentioned that specifically, green leaves contain greater amount of chlorophyll A and B located in the chloroplasts of plant cells. The precursors of steviol glycosides are synthesized in chloro-plasts so the tissues without chlorophyll pigment do not contain or contain only minor amounts of the sweet steviol glycosides.

The content and distribution of sweet glycosides, primarily stevioside and rebaudioside, considerably varies depending on genotype of stevia. Chloroplasts are important precursors for the synthesis of stevioside and steviol glycosides. In respect of the interaction among varieties and drying methods the highest reduction of chlorophyll A,B, carotenoids and total pigment were obtained by China1 variety with use of oven drying at 60^{o}C compared to fresh leaves. Regarding total steviosides the reduction was achieved by Spanti and Egy1 varieties with no significant difference between them moreover the highest percentage observed was for china1 variety with Microwave drying method.

Gross chemical composition of stevia leaves varieties under different dry method:

Data in Table (6) show that gross chemical composition of stevia dry leaves (on dry weight basis). The obtained results indicate that the percentage of moisture content in Spanti variety was 6.2 and 6.5% dried in oven 60 ◦C and open air respectively. These results are in agreement with those mentioned by Hatter (2010), who reported that hot air drying was recommended to be carried out at 43 °C or sun drying at an ambient condition of less than 60% relative humidity. While, Rajab *et al.,* (2009) noted that drying at a temperature of 60-70°C for 8 hours significantly contributes to the preservation of the stevia leaves quality. The main requirement to be achieved in dehydration of stevia leaves is short and conducted period of drying at non-invasive temperatures these results also confirmed by Mishra *et al*., (2010); Lasekan *et al*., (2013), who mentioned that after harvest, leaves should be air dried (natural drying) at lower temperature (40-50 °C) during 24 to 48 hours and others about stevia leaves dehydration was conducted in a laboratory drier using a temperature of 55 °C with constant air flow for a period of 2 hours.

Data in Table (6) also show that crude protein slightly affected by drying method in all varieties while among varieties the highest amount of protein content in dried leaves was observed in China1 variety followed by Egy1 and Spanti varieties under all drying methods as well as crude fat content showed the same trend the highest ratio of fat was detected in Spanti variety followed by Egy1 and China1 varieties (4.19, 6.10 and 3.69%) respectively these results are in agreement with those of Goyal *et al.* (2010) who reported that proteins ranged from (10 to 20 g dry matter).

At the same time study reveled that crud fiber and ash content had no affect by drying method and in the contrary by varieties. In the general considerable amount were (16.28, 12.22 and 11.54) in crud fiber % and (9.80, 9.31 and 8.65) in ash with Spanti, Egy1 and China1 respectively. These results are in line with mentioned that high ash content indicates that the Stevia leaves are good source of inorganic minerals (ash) (Lemus-Mondaca *et al*., 2012) found that high ash content (6 -13 g/100 g dry matter) and also dietary fibers (11.2 to 18.5 g/100 g dry matter), which are very important in maintaining of human health (Goyal *et al.,* 2010)

Table (6) Chemical composition of stevia leaves varieties under different dry method

Varieties	Drying method				
	Open air	**Microwave**	**Oven ^{o}C 40**	**Oven ^{o}C 60**	**Mean**
	Moisture%				
Spanti	6.48^{f} ± .0351	6.34^{h} 0.0577	6.29^{i} ±0.0273	6.27^{j} ±0.0667	6.32^{c}±0.015
Egy1	6.63^{d} ±0.0176	6.43^{g} ± .012	6.45^{f} ± 0.0145	6.25^{k} ± .0153	6. 45^{a}±0.023
China1	6.72^{c} ±0.0176	6.64^{d}± 0.0882	6.56^{e}±0.0145	6.24^{k} ±0.0882	6.42^{b}±0.033
Mean	6.61^{b}±0.03	6.47^{c}±0.017	6.43^{c}±0.02	6.25^{d}±0.023	
	Protein%				
Spanti	8.39^{c} ± 0.0333	8.36^{c} ± .0333	8.28^{c} 0.088	8.13^{c} ± 0.0115	8.31^{c}± 0.033
Egy1	9.12^{b} ± 0.0577	9.09^{b} ± 0.033	9.01^{b} ± 0.033	8.96^{b} ± 0.0285	9.07b± 0.055
China1	15.4^{a} ± 0.0577	15.3^{ab} ± 0.057	15.2^{bc} ± 0.033	15.1^{c} ± 0.0882	15.27^{a}± 0.033
Mean	10.97^{a}±0.0333	10.92^{a}± 0.0355	10.83^{ab}±0.0335	10.73^{b}± 0.0537	
	Fat%				
Spanti	4.26^{b} ± 0.088	4.23^{b} ± 0.0088	4.15^{b} ± 0.057	4.02^{b} ± 0.057	4.19^{b} ± 0.057
Egy1	6.15^{a} ± 0.057	6.12^{a} ± 0.088	6.06^{a} ± 0.057	5.99^{a} ± 0.057	6.10^{a} ± 0.012
China1	3.76^{c} ± 0.057	3.72^{c} ± 0.057	3.63^{c} ± 0.057	3.57^{c} ± 0.012	3.69^{c} ±0.0115
Mean	4.72^{a}± 0.012	4.69^{b} ± 0.057	4.61^{b} ± 0.057	4.52^{d} ± 0.012	
	Crud fiber%				
Spanti	16.3^{a} ± 0.0882	16.3^{a} ± 0.0233	16.2^{a} ± 0.012	16.2^{a} ± 0.012	16.28^{a} ± 0.033
Egy1	12.3^{b} ± 0.0133	12.2^{b} ± 0.012	12.2^{b} ± 0.0176	12.1^{b} ± 0.0186	12.22^{b} ± 0.057
China1	11.6^{c} ± 0.012	11.6^{c} ±0.0577	11.5^{c} ± 0.0115	11.3^{c} ± 0.0882	11.54^{c} ± 0.033
Mean	13.40^{a} ± 0.057	13.37^{a} ± 0.088	13.30^{a} ± 0.033	13.20^{a} ± 0.057	
	Ash%				
Spanti	9.99^{a} ±0.0115	9.87^{a} ± 0.0115	9.48^{a} ±0 .0333	9.41^{a} ±0 .0333	9.80^{a}±0.0123
Egy1	9.44^{b} ± 0.0577	9.33^{b} ±0 .0882	9.21^{b} ± 0.0333	9.11^{b} ±0 .0577	9.31^{b}±0 .0115
China1	8.76^{c} ±0 .0577	8.65^{c} ± 0.0577	8.58^{c} ± 0.012	8.46^{c} ± 0.0153	8.65^{c}±0.0577
Mean	9.40^{a}± 0.0133	9.28^{a} ±0.0115	9.09^{ab} ± 0.0333	8.99^{b}± 0.0115	
	Carbohydrate %				
Spanti	71.94^{ab}±0.057	71.25^{b}± 0.0115	70.98^{d}±0. 0577	71.09^{c}± 0.0333	71.50^{a} ± 0.057
Egy1	69.17^{f}± 0.0088	69.87^{e}± 0.0333	68.64^{h}± 0.0577	68.84^{g}± 0.0577	69.21^{b} ± 0.0882
China1	66.00^{j}± 0.0115	65.75^{jk}± 0.0577	65.52^{k}± 0.0088	65.71^{j}± 0.0115	65.92^{c}± 0.0088
Mean	69.04^{a} ± 0.088	68.96^{b}± 0.0577	68.55^{c} ± 0.0333	68.38^{c}± 0.0577	

Each mean value, within the same column, followed by the same letter is not significantly differentat 0.05 level. (Each mean value is followed ± SE)

The obtained results in Table (6) indicate that the percentage of total carbohydrate content of dry leaves varied significantly affect among drying method but it was more affected by varieties than drying methods. Total carbohydrate contents were 71.5, 69.21 and 65.92 in Spanti , Egy1 and China1 varieties, respectively. It can be concluded that the dried stevia leaves are a good source of carbohydrates 35-69 g/100 g dry matter depending on genotype (Mishra *et al.*, 2010 and Goyal *et al.,* 2010).

Gasmalla *et al*., (2014) found that drying method can affect the nutritional composition of *Stevia* leaves and cause a serious decline in the content of standard phytochemical constituents. Moreover were obtained that when dried the plant leaves by three drying methods total carbohydrates contents were 63.10, 69.85 and 73.99 % for air, oven at 55°C and microwave drying respectively and others mentioned that Stevia dry leaves rich in 61.93, protein 11.54% and crud fiber 15.52 % dry weight (Sharma *et al*., 2006).

Effect of drying methods on total phenolic content:

Data in Table (7) indicate that percentage of total phenolic content as a gallic acid equivalents in methanol extracts of dry stevia leaves varied significantly depending on drying method. The obtained results in Table (7) also indicate that the percentage of Total phenolic of dry leaves varied significantly among drying method but it was markedly more affected by varieties. Total phenolic content were 13.15, 15.67 and 17.94% in Spanti, Egy1 and China1 varieties, respectively. The highest ratio of total phenolic compounds (18.8%) was present in China1 variety dried by open air, while the lowest ratio (13.2 %) was in Spanti variety when drying with used oven at 60 ◦C.

Total phenolic content ranged between 2.53 – 6.52% gallic acid equivalents in ethanol extracts of stevia leaf (Jahan *et al.,* 2010). The lower polyphenol content of 4.2% (weight on dry basis) was reported in stevia leaf by Kaushik *et al.* (2010). The study indicated that the high polyphenols (5.6%) present in SLP can be of importance for food and medicinal uses The phenolic compounds present in the herbs and spices have been Hydrodistillation yielded very high total phenols 14.7% in the extract, which is equivalent to 3.62% polyphenols in herbs (Hinneburg *et al.,* 2006). The hydrodistilled extracts are reported to be used in the fictionalization of foods and beverages as phenolic compounds have been ascribed with health-promoting properties (Harborne and Williams, 2000). Polyphenols are known to protect lipids, carbohydrates and proteins from degradation (Halliwell, 1997) as well as Rao *et al.,* (2014) studied that revealed the polyphenol content and antioxidant activity of both stevia leaf powder and commercial stevioside. The higher polyphenols (15.6%) in the leaf powder were observed compared to the commercial stevioside powder (2.3%) (Taleie *et al.,* 2012).

Therefore, stevia plant has significant potential for use as a natural antioxidant (Shukla *et al*., 2009; Ahmad *et al.,* 2010; Kim *et al.,* 2011). These results suggested that the highest levels of antioxidant activity were due to the presence of phenolic compounds. Previous data have demonstrated that water extract *S. rebaudiana* leaf is rich in polyphenols (Shukla *et al.,* 2012).

From previous results it was clear that China1 variety had the highest content of total phenolic compounds Therefore it was selected to prepare syrup steviosedes from it and used as a substitute for sugar in the cup cake because of its high ratio of rebaudioside A and low stevioside which leads to preferentially sweet and least bitter tasting of stevia extract

Table (7) Effect of drying methods on total phenolic content for three varieties of stevia

Varieties	Drying method				
	Open air	Microwave	Oven °C 40	Oven °C 60	Mean
	Total phenolic content in dry leaves %				
Spanti	14.8 ^{f}± .0233	13.7^{fg} ± 0.0333	13.7^{fg} ±0.0115	13.0 ^{g} ± 0.012	13.15^{c} ±0.0123
Egy1	16.3^{d} ± 0.0882	15.3^{de} ± 0.0882	15.2^{e} ± 0.012	15 .10^{e}± 0.0153	15.67^{b} ± 0.057
China1	18.8^{b} ± 0.0882	17.5^{c} ± 0.026	17.3^{c} ± 0.0577	17.00^{c} ± 0.0333	17.94^{a} ±0.0285
Mean	16.69 ^{b}± 0.057	15.54^{c}±0.0115	15.40^{d}± 0.0233	15.21^{e}±0.0115	

Each mean value, within the same column, followed by the same letter is not significantly different at 0.05 level (Each mean value is followed ± SE)

Extraction and purification of stevioside form china1 variety:

Data in Table (8) it could be noticed that TSS was 6.9% in water extract declined to 4.2% after Ca(oH)2 filtration and 3.2 after ion exchange while Stevioside and Reb –A contents were also decreased in water extract from 6.22 and 7.13 % to 5.0 and 6.13 after Ca(oH)2 filtration and ion exchange. These results are in line with those mentioned by (Galal, 2002).

Meanwhile de-pigmentation was increased through the purification process. This means in the same time results that the natural stevioside sweetener was extracted from the dried ground leaves by water was rich in pigments. The difference in pigment according to TSS and de-pigmentation could be attributed to the ability of water as a solvent to extract more soluble solids as a mentioned by (Nishiyama *et al.,* 1992).

Table (8) Effect of water extraction (1:50) and purification Steps on de-pigmentation% and content of stevioside and Rab-A form china1 variety

purification Steps	pH	TSS	St/100g leaves%	Reb –A /100g leaves %	Carotenoilds mg/L per-moves at 420 nm	Chlorophyll A&B mg/L per- moves at 670 nm	de-pigmentation %
Crude water extraction ratio 1:50	5.5^{c}	6.9^{a}	6.22^{a}	7.13^{a}	6.7^{a}	60.03^{a}	-
After Ca(oH)2 filtration	8.5^{a}	4.2^{b}	5.6^{b}	6.85^{b}	6.00^{b}	30.25^{b}	45.68^{b}
After ion exchange	6.0^{b}	3.2^{c}	5.0^{c}	6.13^{c}	5.3^{c}	9.30^{c}	59.72^{a}

Each mean value, within the same column, followed by the same letter is not significantly different at 0.05 level.

Therefore, it was found that the purification needed more steps in case of water extraction However, in respect to safety and it is of economically a great interest to use water for extraction than methanol. These results are in agreement with those of Abou- Arab *et al.,* (2010) who noticed that extraction of more pigments during water extraction was occurred compared to methanol and methanol/water extraction. This indicated that methanol and ethanol/water extraction were much easier and simple than water extraction.

Physical and chemical characteristics of stevioside syrup:

Data in Table (9) show that stevioside syrup contained total soluble carbohydrate and total stevioside 58.2 and 52.7%, respectively. These results are in agreement with those of Lasekan and Naidu (2013) stated that there is a very close correlation between stevioside content and soluble carbohydrate content that can allow for simple estimation of steviosides content in dried leaves also about stevioside syrup.

Table (9) Physical and chemical characteristics of stevioside china1 syrup variety:

Characteristics	Stevioside syrup water extracted
Total soluble carbohydrate%	58.2
Total Sevioside%	52.7
TSS%	72.3
Purity related to TSS%	72.89
pH	6.2
Viscosity (c.p)	4.7
Color (as a inch color cell)	20.40
Specific gravity w/w	0.875

Syrup rescored and TSS stevioside syrup recorded 72.3 and purity related to TSS 72.89% as well as pH value 6.2 .These results are in harmony with those recorded by (DuBios and Stephenson, 1985) and (Nishiyama *et al.,* 1992) and Buckenhuskers and Omran (1997).

Concerning to the viscosity of stevioside syrup, it was 4.7 centipoises (c.p) this means that the viscosity of the syrup increases with increasing the brix values. These results are in agreement with those obtained by (Galal, 2002).

Meanwhile color recorded 20.40 intensity was higher in sample of stevioside syrup than sample of extracted water. This might be due to using high temperatures during the processing of syrup in rotary evaporator, particularly at the end of concentration process, whereas, this step causes the increasing of dark color or browning of the final syrup as well as heating stevioside of water extract during syrup processes leads to coagulate some non-sugar substances such as the proteins and some plant pigments as a mentioned by (Abou-Arab *et al.,* 2010).Whereas stevioside syrup recorded specific gravity 0.875. These results are in accordance with those obtained by (DuBios and Stephenson 1985)

Organoleptic characteristics of cup cake treated with different levels of stevioside

Data presented of Table (10) showed that, in general, stevioside shows no significant difference in all parameters, up to 50% stevioside replacement of sucrose. These results are in agreement with those of Galal, (2002) who indicate that the availability of utilizing the natural steviosids sweetener with low calorie value in the production of some bakery products and therefore could be incorporated with other bakery products for diabetes and other consumers.

(Singh and Rao, 2005) reported that rebaudioside A contributes to the typical sweet taste (similar to sucrose Differences in taste are caused by more polar groups in rebaudioside A that enable the rebaudioside A better solubility and ultimately more similarity to taste of sucrose, unlike the molecule of stevioside (Mitchell, 2006; Carakostas *et al.,*2008).

In general, the formulation containing steviosid had a lower hardness than to samples produced with sugar. Sensory evaluation of biscuits prepared with equal ratio of sugar to stevioside (50:50) exhibited the highest sensory scores for flavor color, taste and overall acceptability (Vatankhah *et al*., 2015) and for pound cake (Schirmer *et al.,*2012).

Stevioside and rebaudioside A are thermally stable at higher temperatures, and have wide application in the food and in the bakery industry (Tanaka, 1988) quoted termo-stability of stevioside even at temperature of 200°C, which allows a wide range of stevia commercial use.

Table (10) Mean values of the organoleptic characteristics of bakers cake with different percent stevioside replacement sucrose

Characteristics	Score(5)				
	percent of Stevioside replacement sucrose				
	control	25%	50%	75%	100%
Taste	9.0 ± 0.224a	9.0 ± 0.302a	8.9 ± 0.296a	8.6 ± 0.263b	8.2 ± 0.211c
Flavore	9.5 ± 0.167a	9.3 ± 0.250a	9.2 ± 0.289a	9.1 ± 0.318a	8.8 ± 0.364a
color	9.1 ± 0.167a	9.1 ± 0.283a	9.0± 0.267a	8.8 ± 0.208a	8.8 ± 0.296b
Texture	9.4 ± 0.204a	9.3 ± 0.202a	9.3 ± 0.269a	9.0 ± 0.230b	8.5± 0.021c
Appearance	9.3 ± 0.176a	9.2 ± 0.203a	9.2 ± 0.270a	9.0 ± 0.218a	8.9± 0.260b
Sweet acceptability	9.5 ± 0.224a	9.4 ± 0.471a	9.3 ± 0.494a	7.0 ± 0.407b	5.5 ± 0.427c
Bitter acceptability	9.5 ± 0.024a	9.2 ± 0.071a	9.2 ± 0.295a	7.3 ± 0.417b	5.0 ± 0.227c
Over all acceptability	9.3 ± 0.124a	9.1 ± 0.401a	9.1 ± 0.440a	7.1 ± 0. 047b	5.3 ± 0.0227c

Each mean value, within the same column, followed by the same letter is not significantly different at 0.05 levels.
Each mean value is followed ± SE

CONCLUSION

Finally, it could be concluded that, one feddan of Stevia produces annually from 10.03 to 11.928 ton fresh leaves and from 1.698 to 2.606 ton dry leaves with an average content of stevioside from 6.72 % to 8.67 % and rebaudioside A from 4.23% to 8.67% according to variety. 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, as well as act as a source of carbohydrates, protein, crude fiber and antioxidant especially polyphenols.

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إنتاجية وجودة الاستيفيا وتأثيرالتجفيف على الاستيفوزيد واستخدامه فى صناعة المخبوزات

سها رمضان ابوالعلا خليل* ، ايمان صلاح محمد و احمد السيد عطيه ***
*** معهد بحوث المحاصيل السكرية، **معهد بحوث تكنولوجيا الاغذية، مركز البحوث الزراعية، الجيزة، مصر.**

بعتبر الاستيفوزيد واحد من اهم البدائل الطبيعية للسكر الخاليه من السعرات الحراريه وكمصدر طبيعى لمضادات الاكسدة. إشتملت هذه الدراسة على تقييم انتاجية و جودة ثلاث اصناف من الاستيفيا (China1 وEgy1 وSpanti) وايضا تقيم تأثير طرق التجفيف المختلفة (التجفيف الهوائى ، الميكروويف ، الفرن) على الاستيفوزيد والفينولات والقيمة الغذائية لتلك الاصناف .

إتضح من التحليل التجميعى لتجربتين حقليتين خلال موسمى ٢٠١٣ /2014 و 2014/2015 جودة انتاجية الاستيفيا تحت الظروف المصريه حيث تم زراعته خلال شهر مارس وحصد اربع مرات سنويا بمتوسط إنتاجية تراوح من 1.698الى 2.606 طن للفدان ،بنسبه استيفوزيد تراوحت من 6.72 الى 8.67ونسبه rebaudioside A% تراوحت من 4.23 الى8.67 على حسب الصنف .

اظهر الصنف China1 تفوقا فى صفة وزن الاوراق الطازجة والجافة بالجرام لكل نبات وتلاه الصنف Spanti ثم Egy1 على التوالى وكذلك كان متفوقا فى صفات الجودة مثل محصول الاوراق الطازج والجاف للفدان والنسبه المئويه rebaudioside A لمحتوى الاوراق الجافة علاوة على جودة وتقبل الطعم السكرى والمرارة فى المستخلص المائى وارتفاع الفينولات والقيمة الغذائيه .

أظهرت طرق التجفيف ان النسبة المئويه للاستيفوزيد والفينولات والصبغات انخفضت فى اوراق الاصناف الثلاثه مع كل طرق التجفيف وخاصة التجفيف بالفرن على درجة ٦٠ درجة مئويه كما تبين التحليل الكيماوى لاوراق الاستبقيا الجافه انها تأثرت بدرجة اكبر بالاصناف عنها بطرق التجفيف المختلفه.

وفقا للنتائج السابقة تم اختيار الصنف china1 واستخدامه فى صورة شراب الاستيفوزيد لصناعة الكيك كبديل للسكر بنسب استبداليه ٢٥، ٥٠، ٧٥، ١٠٠% .

أظهر التذوق الحسى انه لايوجد اى فروق معنويه من حيث المظهر ، والنكهه ، والطعم ، واللون ، وقبول الحلاوة ، والاحساس بالمرارة للكيك المصنوع بشراب الاستيفيوزيد بنسبة (٥٠:٥٠)% مقارنه بالمصنوع من السكر لذلك توضح الدراسة بإمكانية استخدام شراب الاستيفوزيد كبديل للسكر فى صناعة المخبوزات والحلويات.