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The Impact of Natural Stevia Extract (Stevioside) as a Sucrose Replace on Quality Characteristics of Selected Food Products

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ABSTRACT

The study was conducted to produce low caloric sweeteners from stevia leaves (Stevia rebaudiana Bertoni) as a natural source, and determined its effect at different levels (25, 50, 75 and 100%) on quality and texture characteristics of different food products (cake, biscuit and jam) as well as products' nutritive values (carbohydrates, fat, protein, ash , moisture and minerals).Stevia leaf contains (on dry weight basis)12.89 g protein, 11.96 g ash, 12.41 g crude fiber, 410.25mg calcium, 60.03mg iron and 51.12 mg sodium. The refined extract syrup is approximately 250 times sweeter than sucrose. The results of the study showed that cakes weight increased slightly after baking upon addition of different levels of stevioside compared to the control (with sucrose only), the products had golden-red color (with stevia extract up to75%) and contained high moisture values . Better sensory characteristics scores and acceptability were given to cake with 25and 50% extract. Stevia as a sucrose replacement (up to 75%) had a better impacts on Biscuit physical quality (weight, height, volume and color and general acceptability). Jam prepared with stevioside (25%) had better sensory quality and general acceptability. In general, the prepared products with stevioside showed to contain higher values of iron and sodium. It can be recommended that sativa extract (as natural sweeteners) can be used as sucrose substitute in different food products (cake, biscuit and Jam), however, the appropriate percentage that be added should be determined according to products characteristics and the quality that need to be achieved. Food industries should be encourage to include stevioside extract as sucrose replacer in their strategic plan for development of low caloric food products.

Keywords: Natural sweeteners, *Stevia rebaudiana*, Stevioside Extraction, bakery product, Jam, quality characteristics.

Introduction

Sucrose is a principal ingredient in several food products (such as cakes, biscuits, and Jam) its role extends beyond providing energy and sweetness, it acts as a tenderizer by retarding and restricting gluten formation, increasing temperatures of egg protein denaturation , starch gelatinization , and contributing to bulk and volume (Kulp *et al*., 1991 and Shukla, 1995). Low / non-caloric sweeteners may offer some hope to those who desire to avoid the debilitating diseases associated with excessive sugar consumption (Malik *et al*., 2006). Due to high intake of sugars, especially sucrose, global trends in food processing have encouraged producers to use sweeteners, to a wide extent (Małgorzata and Anna 2015).The overconsumption of refined sugars, especially sucrose, promotes inappropriate positive caloric balance, excessive weight gain and obesity. Both xylitol and saccharine have been linked to tumor development and aspartame continues to prompt controversy in its reported wide range of negative side effects. Therefore, finding other alternative sweeteners especially from natural sources would be of a great value in this area. Stevia, a natural sweetener plant having medicinal and commercial importance is being used all over the world. *Stevia rebaudiana* Bertoni is the botanical name of stevia. The leaves of the stevia shrub contain specific glycosides which produce a sweet taste but have no caloric value. It is estimated to be 300 times sweeter than cane sugar. Stevia (*Steviarebaudiana Bertoni*) is a low-calorie plant, because it provides the body with only 2.7 kcal/g. Biotechnology companies are commercially producing stevia through tissue culture and marketing stevia in different form such as leaf powder, liquid and fresh leaves (Aswin *et al*., 2015).

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The Stevia plant (Figure 1) is a small shrub originally grown in South America, particularly in Brazil and Paraguay where it is known as stevia or honey leaf, Kaa-he-e (Hanson and De Oliveira, 1993). According to Alaam (2007) the Stevia plant was recently introduced to Egyptian agriculture, one feddan of Stevia may produce up to 400 kg of Stevia sugar, annually. According to Geuns (2000) and Anita *et al*. (2008) stevia and stevioside (Figure 2)are safe when used as sweeteners. Both are suited for diabetics and phenylketonuria patients, as well as for obese persons intending to lose weight by avoiding sugar supplements in the diet. It was the stevioside and steviol, stimulate insulin secretion by direct action on beta cells and these compounds have potential role as anti hyperglycemic agent in the treatment of type 2 diabetes mellitus. Moreover, the Stevia Sweetener is stable under dry conditions and in aqueous food systems, also it is heat stable up to 200°C (Apurba et al., 2014). Currently, in the European Union have been approved steviol glycosides with the E 960 symbol for use in 31 food categories, and it would be beneficial for food industries to develop the novel formulations for cakes and biscuits with stevia as sucrose substitute and produce products with good quality and achieve consumer acceptability (Małgorzata and Anna 2015).

Sucrose is a principal ingredient in several food products : at high percentage (in products such as Jam) , at moderate percentage (in product such as Cake) and at low percentage (in product such as Biscuit) .The present study was undertaken to investigation the impact of different levels of stevia sweetener as a sucrose replacer on quality characteristics of some food products (Cake, Biscuit and Jam).

Fig. 1 : The photograph of stevia plant and its leaves

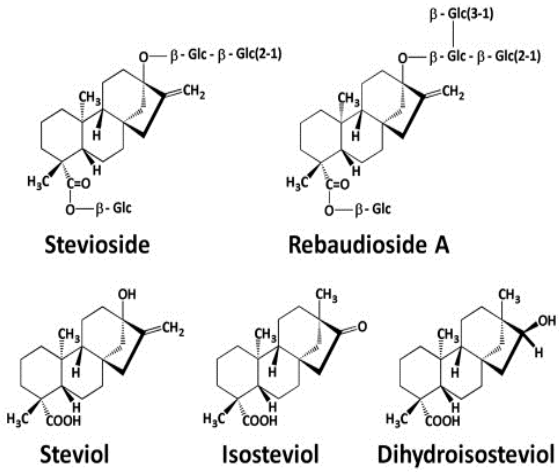

Fig. 2 : Chemical structures of stevioside and its related compounds

Materials and Methods

Materials:

Dried Stevia Leaves :

Dried stevia leaves (Stevia rebaudiana Bertoni) were obtained from the Institute of Sugar Crops, Agriculture Research Center, Giza, Egypt. The dried leaves milled and kept in closed plastic bags in the laboratory until analyzed. Wheat flour (72%), strawberry, pectin, sugar, oil, butter milk, whole eggs, Vanillin and baking powder were obtained from the local market.

Chemicals: were obtained from Sigma Chemical Company, Egypt.

Methods:

Preparation of Extraction of stevioside:

Two different procedures were used to extract the stevia sweeteners from dried stevia leaves. The first procedure, stevia leaves were extracted by hot water, however, the second, leaves were extracted by methanol extraction as the following:

Extraction with water:

Stevia leaves were extracted by water according to Starrat *et al*., (2002) using two pre-treatment methods soaking in chloroform and hexane Phillips, (1987) for 20 h. Stevia leaves were mixed with water at different

percentage (W/V) of powder leaves/ water ratios (1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, 1:50 and minerals content (Ca - K - and Fe) 1:60) at 60-65 °C for 3 h.

Determination of stevioside content:

The stevioside content were determined according to the method described by Nishiyama *et al*., (1992) using a correlation between soluble carbohydrate and stevioside content, analyzed by the classic method of phenol-sulphuric acid method. The stevioside content was calculated using an equation [ST = 1.041 (TC - 7.51) + 0.86, ST: Stevioside content, TC: Total carbohydrate)].

Proximate Compositions of Stevia Leaves and the prepared food products were determined for moisture content, fat, ash, protein, crude fiber and minerals content (Ca, K, Fe, Na and K) according to A.O.A .C. (2005). Also, Total Anthocyanins contents was determined according to Abou-Arab, *et al*., (2010).

Physical and chemical characteristics of sweeteners:

Total soluble solids (T.S.S) were measured with a Hand refractometer (ATA G0N-1E Birx, Japan), acidity was determined by titration with 0.1N NaOH and expressed as of citric acid; phenolphthalein was used as the indicator, the pH measured with using pH meter (Model 646 Digital, USA), specific gravity of syrup was calculated using the following equation: Weigh of 100ml. of syrup / Weigh of 100ml. of distilled water, ash content and refractive index of sweeteners was determined according to the method A.O.A.C. (2005). Determination the viscosity of syrups was done using Hoppler viscometer at 25ºC.

Rheological properties of the prepared dough:

The rheological properties of wheat flour dough's were assessed in the National Research Center, Food Technology Dep., Dokki, by farinograph to determine: water absorption (%), dough development time (minute), dough stability (minute) and dough weakening (minute). Also, extensograph test was used to determine dough (millimeter), dough resistance to extension (elasticity) (Barbender Unit) (B.U.) dough energy (cm^2) and proportional number (B.U./ millimeter) according to AACC, (2000).

Preparation of different products using extraction of stevioside :

The Stevia extracts were used as sucrose replacement at different levels (0, 25, 50, 75 and 100 %), the amount of liquid (milk and/or water) in the standard recipe was modified to keep the amount of total liquids and sugar in balance .The substitution vary somewhat between different recipes (jam, cake , biscuit) for producing better characteristics. In addition, modifications was done with regard to the type of leaving agents used in the standard recipes of shortening cake and biscuit. Consistency of the produced batter and Jam were determined by line spread measurement (cm) according to Penfield and Camphell (1990).

Cake was prepared according to the method described by Abd-Elreheim,(1997) with some modifications: with increasing the levels of stevia extracts there was a need for decreasing amount of liquid (milk) to reach the desirable quality of cake butter (viscosity).Cake butter was weighed (180g) and put in a pan then baked in an oven at 180ºc .

Biscuit were prepared as the procedures of Penfield and Camphell (1990) with some modifications: the developed dough was kept in refrigerator for 10 min., then flattened manually using wooden roller to about ½ cm. thickness and cut into spherical pieces and baked in oven at 180ºc.

Strawberry Jam preparation was followed according to the Abbas (2007) procedures.

Products quality Evaluation:

All the prepared products with different percentages of stevia extracts were subjected to objective evaluated to determine the percentage of stevia extracts that would maintain better quality and acceptability to the final products. The evaluated parameters for bakery products (cake and biscuit) included: percentage change (%) in weight after baking, height, volume, index to volume, density, product tendernes according to Penfield and Camphell (1990) and color by hunter colorimeter according to Hunter (1975). For Jam evaluation, Total soluble solids (% TSS) were expressed as ºBrix (0 - 90) for syrup samples using Digital Refractometer (ATAGO Japan) at room temperature (25 ºC ± 1) according to A.O.A.C. (2005).

Organoleptic evaluation:

The prepared products with different percentages of stevia extract were subjected to sensory evaluation by trained panelists (stuff members in the faculty of Home Economics, Helwan university) using score sheet according to Penfield and Camphell (1990). Panelist were asked to evaluate bakery products (shortening cake and biscuit) for characteristics included (taste, flavor, internal and external color, texture, cell uniformity and over all acceptability). While, sensory evaluation of jam were carried out according to (taste, flavor, color,

degree of sweetness, intensity, consistency and over all acceptability). The results were calculated (as means), data was statistically analyzed and tabulated in comparison to control.

Statistical Analysis:

The statistical analysis was carried out using SAS, PC statistical software for sensory evaluation results. The results were expressed as (mean ± SE). Data was analyzed by one way analysis of variance (ANOVA).The differences between means were tested for significance using least significant difference test (LSD) at ($P < 0.05$) (SPSS, 1986).

Results and Discussions

The present study was carried out to investigate the effect of different levels of stevioside (25,50,75 and 100%) from different products (Cake, Biscuit and Jam) as a replacement of sucrose on the nutritive value and quality properties of some products.

Proximate chemical composition of stevia leaves are summarized in table (1) on dry weight basis. The results (g/100g) showed that leaves contained (7.98, 12.89, 8.95, 11.96, 12.41, 57.12 and 9.10%) for moisture, protein, fat, ash, crude fiber, total carbohydrate and stevioside, respectively. Also, minerals composition (mg/100g) of stevia leaves which were (410.25, 12.95, 60.03, 51.12 and 695.25) for calcium, phosphorus, iron, sodium and potassium, respectively. Therefore, the present findings indicate that stevia considered a good source of protein, ash , crude fiber ,iron, calcium and potassium. The present results were agree with that reported by Apurba *et al*., (2014) and Ramakrishnan *et al*., (2010).

Mean values of stevioside (%) extracted from stevia leaves by hot water (60-65 °C) after soaking in chloroform or hexane are presented in table (2). the extraction yield was significantly ($P< 0.05$) increased with the sample/water ratio in the range of (1:30-1:40), but it was slightly decreased in ratio (1:60) sample/water ratio without soaking in solvents. Generally, the superlative ratios of steviosed extraction in (1:35 and 1:40). While extraction after soaking leaves in chloroform or hexane gave small amount of stevioside content comparable to water extraction without soaking. Extraction by hot water after soaking in chloroform or soaking in hexane produced better result at ratio of (1:40).

Table 1: Proximate chemical composition of stevia leaves (g/100g on dry weight basis).

Proximate chemical composition of Stevia leaves	Constituents Mean ±S.E
Moisture	7.98 ± 0.42
Protein	12.89 ± 0.56
Fat	8.95 ± 0.12
Ash	11.96 ± 0.08
Crude fiber	12.41 ± 0.11
Total carbohydrates	57.12 ± 1.25
Stevioside	9.10 ± 0.35
Mineral (mg / 100 g)	
Ca	410.25
P	12.95
Fe	60.03
Na	51.12
K	695.25

Rheological properties results of wheat flour (control) and wheat flour with different percentage of stevia extracts (25, 50,75 and100%) according to farinograph test are presented in (Table 3). It was found that water absorption (%) and mixing to tolerance index (B.U) decreased in all prepared samples with stevia extracts than that of the control. Arrival time (min) and time development (min)were decreased in all the substitution samples than that of the control, however, it had higher value with regard to dough weakling (B.U) as compared to control and the other samples. The values of arrival time (min) and develop in time (min) were found to be lower as compared to that of the control, but dough weakling (B.U) and mixing to tolerance index (B.U) were higher as compared to control. Rheological properties results of wheat flour (control) and wheat flour with different percentage of stevia extracts (25, 50,75 and100%) according to extensograph test are presented in (table 4) it was found that extensibility (cm) increased and dough energy (cm^2) and resistance extension (B.U) also increased in all the prepared samples with stevia extracts than that of the control. While, sample with 25% extracts had a lower value for resistance extension (B.U) than the other. Baltsavias *et al*., (1999) found that, extensional viscosity decreased with increasing sucrose content. The stress-stranin curves for the sugar-free dough's indicated a stronger elastic contribution to deformation than did those for the sucrose and syrup dough's, sucrose delays mixing time.

These results agreed with the reported observations by Gallagher *et al*., (2003) who stated that sugar inhibits gluten development during dough mixing by competing with the flour for the recipe water, resulting in less tough and more crumbly biscuits. Manoher and Rao (1997) found that, increasing sugar level in biscuits dough resulted a decrease of extrusion time, consistency hardness and gluten recovery of dough. Moreover, Baltsavias *et al*., (1999) found that, extensional viscosity decreased with increasing sucrose content.

Table 2: Mean values of stevioside (%) extracted from stevia leaves by hot water (60-65 °C) after soaking in chloroform or hexane.

Extraction ratio	Extraction by hot water	Extraction by hot water after(soaking in chloroform)	Extraction by hot water after(soaking in hexan)
	Extraction Efficiency	Extraction efficiency	Extraction Efficiency
1:10	28.01^{C}	14.06^{D}	17.16^{E}
1:15	29.2^{C}	16.57^{D}	19.9^{E}
1:20	33.73^{C}	33.13^{C}	22.41^{DE}
1:25	41.36^{BC}	44.81^{BC}	25.74^{D}
1:30	64.96^{B}	62.7^{B}	33.37^{C}
1:35	88.8^{A}	77.71^{AB}	48.51^{B}
1:40	86.53^{A}	81.4^{A}	58.05^{A}
1:50	35.76^{C}	33.85^{C}	34.92^{BC}
1:60	31.23^{CD}	31.47^{CD}	34.09^{BC}

Means with different the same letters in column are significantly different at ($P \leq 0.05$).

Table 3: Effect of stevia extract on wheat flour according to farinograph test.

Samples	Water absorption%	Arrival time (min)	Develop in time (min)	Dough Stability time (min)	Mixing to tolerance index(B.U)	Dough Weakling (B.U)
100% wheat flour(control)	62.5	1.2	4.5	5.5	21	90
25% stevia ex. (10.22ml)	51.75	1.1	4.0	5.5	25	100
50% stevia ex. (20.45ml)	41.95	1.1	4.0	5.0	25	100
75% stevia ex. (30.68ml)	32.80	1.0	3.5	5.0	22	110
100% stevia ex. (40.90ml)	21.75	1.0	3.5	5.0	24	120

Table 4 : Effect of stevia extract on wheat flour according to extensograph test.

Samples	Extensibility (mm)(E)	Resistance extension (B.U)(R)	Proportion No (R/E)	Dough Energy(cm^2)
100% wheat flour(control)	110	460	4.18	80
25% stavia ex.	115	480	4.17	90
50% stavia ex.	120	510	4.25	100
75% stavia ex.	130	540	4.15	91
100% stavia ex.	130	540	4.15	85

In general, it was found that as the replacement levels of stevioside extract increased in cake batter (for 50% replacement level), the obtained line spread values (after three min.) tended to be higher according to the results of line spread as presented in table (5), which indicated the effect of stevioside extract on cake batter texture by making it less consistency at that level . This observation required concern about cake recipe modifications when stevioside extract is used with regard to the amount of liquid required for cake batter formation (reduce the amount of liquid required for cake batter formation).

Table 5: Results of Line spread test for cake batter prepared with different levels of stevia extract as compared to the control value.

Samples / Cake	Treatments				
	Control	25% stevia	50% stevia .	75% stevia	100% Stevia
Medium space (cm)	Based on liquid required for batter cake formation				
After 1 minute	8.12 cm	8.12 cm	10 cm	6.7 cm	7.1 cm
2 min	8.5 cm	8.8 cm	10.5 cm	7.3 cm	7.5 cm
3 min	9.5 cm	9.3 cm	11 cm	8.1 cm	7.8 cm
Medium space (cm)	Based on liquid required for control batter cake formation				
After 1 minute	8.12 cm	10.3 cm	10.1 cm	10.7 cm	11.3 cm
2 min	8.5 cm	11.2 cm	10.5 cm	11.5 cm	12 cm
3 min	9.5 cm	11.7 cm	11.3 cm	12.5 cm	12.3 cm

The tabulated results (Table 6 and Figure 3) show the characteristics of cake products with different levels of stevia extract in comparison to control sample**.** Cakes weight (%) relative to control values were (95.6, 88.01, 77.4 and 83.27% respectively) upon stevioside extract substitution (25, 50, 75 and 100 % respectively). In addition, index to volume values (as % relative to control) were found to decrease as substitution level

increases, with the best value (90%),which was close to the control, was obtained with 25% stevioside extract. Lower cakes baking quality was observed in cake with 75 and 100% stevia extract, similar findings were observerd by Peck (1994) and Ludewig and Laukamp (1994).

Hunter colorometer results for baked cakes with different levels of steviosideat are presented in Table (7), the results indicated that, lightness (L) values for cake which prepared with all replacements of stevioside extract were lower than the control. In general, cakes prepared with stevioside extract with25and 50% extract of stevioside tended to be golden-red in color while cake samples prepared with levels 75 and 100% extract were found to be red- brown in color. These results are agree with Attia, *et al*., (1993) and Yaseen, *et al*., (2005).In this trend Lin *et al*. (2010) reported that, cakes without sucrose have a lighter color crust, this corresponds with a visually detectible variation in lightness of crust as the amount of sucrose in formulation was reduced. The visual lightening of samples as more sucrose was replaced with rebaudioside-A and erythritol may be attributed to the higher erythritol content. These substance does not caramelize or participate in carbonyl-amine browning reactions.

Table 6 : Physical properties of shortening cakes prepared with different percentage of stevioside as sucrose substitute.

Physical properties.	Cake Samples				
	Control	+ 25% stevioside	+50 % stevioside	+75% stevioside	100 % stevioside
Dough Water uptake (ml milk)	30	28	26	24	22.5
Weight Before baking (gm)	180	180	180	180	180
Weight After baking (gm)	177.10	169.47	155.87	137.16	147.48
Changes in weight (%)	1.6	5.85	13.40	23.8	18.06
Cake's weight relative to control (%)	-	95.6	88.01	77.4	83.27
Baking Time (min)	35	35	35	35	35
Height (cm	5.3	4.6	4.2	3.8	4.2
Height relative to control (%)	-	86.7	79.2	71.6	79.2
Volume(cm3)	370	350	330	270	300
Volume relative to control (%)	-	94	89.1	79.9	81.08
Index to volume(cm)	6	5.4	5.02	4.74	4.76
Relative to control (%)	-	90	83.6	79	79.3
Specific volume(cm3/g)	2.08	2.06	2.11	1.9	2.03
Relative to control (%)	-	99	100.9	91.3	97.5

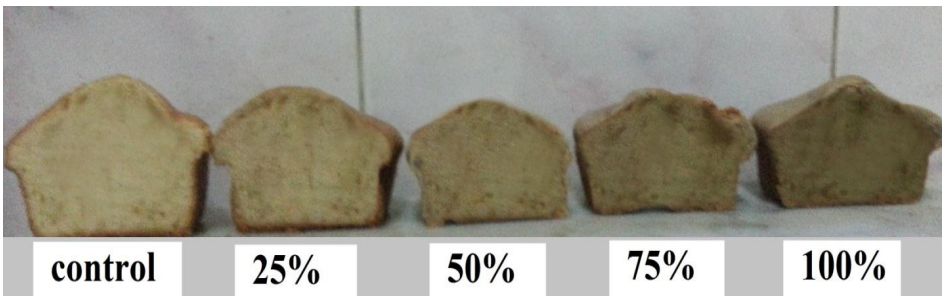

Fig. 3: Photograph of longitudinal section of cakes prepared with of stevioside extract.

Table 7: Hunter color values of cake with different levels of stevioside extract.

Samples	L*	a*	b*	a/b	Saturation	Hue
Control	56.51	2.64	16.03	2.09	4.32	25.50
Sample with stevioside 25%	54.03	3.15	13.38	1.15	4.30	68.94
Sample with stevioside 50%	52.01	3.75	11.78	0.31	3.94	72.34
Sample with stevioside 75%	49.09	4.25	10.81	0.39	3.88	68.53
Sample with stevioside 100%	46.33	5.91	2.1	2.47	2.83	19.56

L = lightness, a*= redness, b*= yellowness*

Biscuit quality evaluation:

As presented in table (8), Biscuit with stevioside extract levels (50,75 and100%) had significant decrease in all physical characteristics, it is evident, that the addition of stevia extracts up to 25% caused gradually decreased in physical characteristics. However, values of physical parameters of biscuit with 25% stevia extract was significantly ($p<0.05$) higher as compared to control. This means that the presence of stevia sugar replacer in the biscuit formula under the present study condition should not exceed 25% to keep the product quality in the acceptable level. The present findings agreed with the reported study by Saxena *et al*., (1992).

Results of Hunter colorimeter for biscuit (Table 9 and Figure 4) indicated that redness (a) values for baked biscuit prepared with stevioside extract at different level were approximately similar to the control. On the other hand, yellowness (b) values for biscuit samples were higher than the control. In this trend Yaseen *et al*., (2005) reported that, a decrement in color differences was observed by using stevia sugar in biscuit formula in comparison with fructose biscuit, however, mixtures of sugar substitutes minimized such differences in color biscuit than when it used separately.

Table 8: Physical properties of biscuit prepared with different levels of stevia extract.

Physical properties.	Biscuit Samples				
	Control	Stevioside 25%	Stevioside 50%	Stevioside 75%	Stevioside 100%
Water absorption (ml)	30	30	30	30	30
Weight Before backing (gm)	21.2	20.2	19.1	18.1	17.11
Weight After backing (gm)	16.62	17.2	16.20	16.89	13.85
Change in weight (gm)	29.3	17.4	17.9	7.16	23.5
Backing Time (min)	20	20	20	20	20
Height (cm)	1.25	1.4	1	0.75	0.75
Volume(cm^3)	42	45	36	34	35
Specific volume(cm^3/g)	2.52	2.6	2.2	2.01	2.5
Relative to control (%)	-	103	98.6	79.8	100

Table 9: Results of Hunter colorimeter values for biscuit with different levels of steviosideat extract.

Samples	L*	a*	b*	a/b	Saturation	Hue
Control	66.93	3.54	10.2	0.34	3.70	70.86
With stevioside 25%	56.62	3.50	14.01	0.46	4.52	65.11
With stevioside 50%	62.81	3.83	12.82	0.29	4.08	73.36
With stevioside 75%	60.71	3.90	12.51	0.31	4.05	72.68
With stevioside 100%	58.33	3.83	12.82	0.29	4.08	73.36

L = lightness, a*= redness, b*= yellowness*

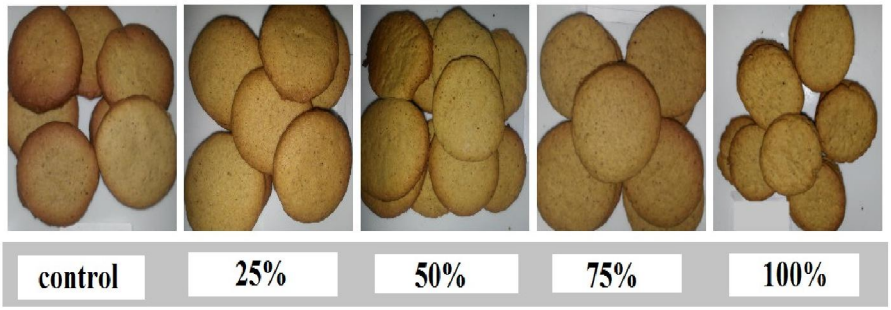

Fig. 4 : photograph of biscuits prepared with different levels of stavia extract.

Quality evaluation of Jam:

Line spread test is useful in such product because it refers to degree of viscosity (the value decreased as the viscosity increases and become more consistency) as presented in table (10). Values (cm/min) of Jam samples prepared with different levels of stevia extract (25, 50, 75 and 100 %) were found to be (1.1, 2.1, 2.6 and 3.7cm, respectively) as compared to the control (1 cm). As the level of stevia extract increased above 25% jam consistency was found to decrease.

Table 10: Line spread test (cm) of jam prepared with different levels of stevia extract in comparison to the control sample.

Jam / Samples	Treatment				
	Control	25% ex. stevia	50% ex. stevia	75% ex. stevia	100% ex. Stevia
Medium space (cm)					
After: 1 minute	1 cm	1.1 cm	2.1 cm	2.6 cm	3.7 cm
2 min	1.5 cm	1.3 cm	2.2 cm	3 cm	4.2 cm
3 min	1.5 cm	1.7 cm	2.3 cm	3.8 cm	4.8 cm

According to physical characteristics results (Table 11), slight differences were found in PH values among jam samples (25, 50, 75 and 100% of stevia extract) which were (3.65, 3.69, 3.71 and 3.75, respectively)

as compared to the control value (3.60). All the investigated parameters (total soluble solids, acidity, Brix/acid ratio and anthocyamin contents) were found to be lower than the control values.

Table 11: physical properties of jam prepared with different levels of stevia extract.

Samples / Constituents	Control	100% stevia.ex	75% stevia.ex	50% stevia.ex	25% stevia.ex
T.S.S (total soluble solids)	$76.74^{A}\pm0.92$	$25.9^{D}\pm0.11$	$40.9^{C}\pm0.12$	$59.8^{BC}\pm0.14$	$61.9^{B}\pm0.18$
PH value	$3.60^{D}\pm0.13$	$3.75^{A}\pm012$	$3.71^{AB}\pm0.11$	$3.69^{B}\pm0.12$	$3.65^{BC}\pm0.12$
Acidity	$1.51^{A}\pm0.11$	$1.48^{B}\pm0.15$	$1.45^{BC}\pm0.16$	$1.40^{C}\pm0.17$	$1.38^{D}\pm0.13$
Brix /acid ratio	$50.12^{A}\pm0.57$	$17.50^{D}\pm0.12$	$28.20^{C}\pm0.17$	$42.71^{B}\pm0.13$	$44.85^{B}\pm0.51$
Anthocyamin mg/100gm	$34^{A}\pm0.18$	$33.81^{BC}\pm0.19$	$33.79^{C}\pm0.22$	$33.86^{B}\pm0.31$	$33.91^{AB}\pm0.28$

Value represent means ± standard deviations calculated from three replicates.

Organoleptic evaluation and chemical composition of products with stevia extract:

-Cake product:

It was noticed (table 12) that, scores (mean ± SE) given to cake appearance made with 25% stevia extracts was higher than that of the other samples. Scores (mean ± SE) given for cake's volume prepared with different levels of stevia extract (25, 50, 75 and 100%) decreased (4.4±0.516, 4.0 ±0.667, 3.8 ± 0.633 and 3.9 ± 0.568, respectively) as compared to the control (4.6±041639). However the difference was not significant (P< 0.05). The highest score given for internal color was for cakes prepared with 25% stevia extracts. The differences in external color among cake prepared with (25 and 50% stevia extracts) were not significantly low (P< 0.05). The scores for taste of cakes prepared with (25 and 50%) stevia extract were significantly (P< 0.05) higher as compared to those prepared with (75 and 100%) stevia extract. These results showed that complete replacement with stevia extract was undesirable in cake product. Abou-Arab *et al*., (2010) reported that bitter taste, common to many stevia species, was probably due to volatile aromatic or essential oils, tannins and flavonoids, which contributed to the implant flavor associated with stevia.

Table 12: Organoleptic evaluation (score) of cake prepared with different levels of extracts in sativa extract.

Samples / Characteristics	Cake					LSD
	Sample(control) with stevioside 0%	Sample with stevioside 25%	Sample with stevioside 50%	Sample with stevioside 75%	Sample with stevioside 100%	
Volume	$4.6^{A}\pm0.416$	$4.4^{AB}\pm0.516$	$4.0^{BC}\pm0.666$	$3.8^{C}\pm0.632$	$3.9^{BC}\pm0.567$	0.5324
Internal color	$4.8^{A}\pm 0.421$	$4.6^{A}\pm 0.699$	$3.8^{B}\pm1.032$	$3.2^{B}\pm0.918$	$2.4^{C}\pm 0.966$	0.765258
External color	$4.7^{A}\pm 0.483$	$4.2^{A}\pm 0.788$	$3.2^{B}\pm1.316$	$3.0^{BC}\pm1.054$	$2.2^{C}\pm0.918$	0.87061
Cell uniformity	$4.6^{A}\pm 0.516$	$3.9^{A}\pm0.875$	$2.8^{B}\pm .918$	$2.3^{B}\pm 0.948$	$2.1^{B}\pm0.737$	0.74438
Taste	$4.6^{A}\pm0.516$	$4.1^{AB}\pm0.737$	$3.6^{BC}\pm0.843$	$2.9^{C}\pm 1.286$	$2.4^{C}\pm 1.821$	0.89786
Odor	$4.9^{A}\pm 0.316$	$4.7^{A}\pm 0.483$	$3.7^{B}\pm 0.44$	$3.2^{BC}\pm 0.632$	$2.8^{C}\pm0.918$	0.54961
Softness	$4.4^{A}\pm0.699$	$4.0^{A}\pm0.666$	$3.6^{BC}\pm0.699$	$3.0^{C}\pm0.666$	$2.2^{C}\pm0.632$	0.61486
Degree of sweetener	$4.5^{A}\pm0.660$	$3.9^{A}\pm0.95$	$3.0^{B}\pm1.080$	$2.6^{B}\pm0.960$	$2.5^{B}\pm1.330$	0.8172
General acceptability	$4.7^{A}\pm0.485$	$4.2^{B}\pm0.485$	$3.5^{C}\pm0.437$	$3.1^{D}\pm0.316$	$2.7^{D}\pm0.632$	0.44007

Different superscript letters in the same row denotes significant differences (p ≤ 0.05).

Cakes prepared with 25% stevia extract had higher scores (mean ± SE) with regard to softness (4.0±0.667) as compared to other cake samples. cakes prepared with 25% stevia extract had significantly (p<0.05) higher score for general acceptability (4.2± 0.486) as compared to the other cakes sample. Data in Table (13) showed nutritive values of cake products. It was found that cake with all studied levels of stevia extract, contained approximately the same value of protein and higher value, of moisture and ash while it had lower contents of total value of fat and carbohydrates as compared the control. Minerals composition (mg /100g) of cake which had better characteristics (cake 50%) compared to the control values. It was found that, cake made with stevia extract had higher contents of iron and sodium, while it had lower calcium values than the control.

Biscuit product:

Data of Table (14) showed scores (mean ± SE) given to biscuit product by the panelist, no significant differences (P ≤ 0.05) was found among all the prepared samples as compared to control in all the evaluated characteristics (appearance, external and internal color, taste, degree of sweetness, odor, firmness, s softness and overall acceptability) of sensory evaluation, similar findings were observed by Yaseen, *et al*.(2005).

Biscuit prepared with (25, 50, 75 and 100%) sativa extracts contained (Table 15) approximately the same values of protein, fat and ash, while lower content of carbohydrates and higher value of moisture than that of control. Iron (Fe), calcium (Ca) and sodium (Na) content of products with better characteristics [biscuit (75%)] compared to the control values are presented in table (15). It was found that, biscuits made with stevia extract

had higher contents of (iron and sodium) than that of control. Whereas, biscuits prepared with stevia extract had lower values calcium.

Table 13: Nutritive value of shortening cake prepared with different levels of stevoside extract.

Nutrient / Samples	Moisture (g/100g)	Ash (g/100g)	Protein (g/100g)	Fat (g/100g)	Carbohydrates (g/100g)	Mineral (mg/100g)		
						Ca	Na	Fe
Control	33^{D}.24±0.41	0.98^{D}±0.11	10.22^{C}±0.35	26.51^{A}±0.22	29.25^{A}±1.17	0.071	0.52	0.062
25% stevia.ex	34^{C}.72±0.49	1.38^{C}±0.13	10.54^{B}±0.31	24.11^{B}±0.24	25.22^{B}±1.14			
50% stevia.ex	35^{B}.03±0.48	1.46^{C}±0.14	10.66^{AB}±0.28	23.56^{BC}±0.26	24.18^{BC}±1.13	0.061	0.56	0.22
75% stevia.ex	35^{B}.95±0.51	1.71^{B}±0.12	10.74^{A}±0.33	22.68^{C}±0.28	22.11^{C}±1.15			
100% stevia.ex	37^{A}.03±0.38	1.90^{A}±011	10.81^{A}±0.38	21.83^{D}±0.24	14.98^{D}±1.11			

**Means with the same superscripts at the same column are not significant at (p<0.05).*

Table 14: Organoleptic evaluation of biscuits prepared with different levels of stevia extract.

Samples / Characteristics	Biscuit samples					LSD
	Control	25% stevioside	50% stevioside	75% stevioside	100% stevioside	
Appearance	3.7 ±1.1	3.4±0.96	3.76±0.72	3.53±0.96	2.92±0.95	NS
Internal color	3.61± 1.0	3.5± 0.78	3.23± 1.09	3.23 ±0.92	3.07 ± 0.86	NS
External color	3.62±1.2	3.4± 0.96	3.46 ± 0.88	3.23 ± 0.83	3.0±0.91	NS
Softness	3.4 ± 1.5	3.23 ± 1.1	3.07 ± 1.16	3.61± 0.87	3.38 ± 1.12	NS
Taste	3.5 ± 1.3	3.0 ± 1.08	3.09 ± 1.14	3.23 ± 1.01	3.0± 1.08	NS
Odor	3.7 ±1.03	3.5± 0.97	3.38 ± 0.87	3.61 ± 1.26	2.84±1.07	NS
Degree of sweetener	3.53±1.2	3.23±1.30	3.61±0.77	3.30±0.85	3.15±1.28	NS
Firmness	3.5±1.1	3.2±0.87	3.07±1.26	3.23±0.73	2.92±1.19	NS
General acceptability	3.7±0.95	3.1±0.86	3.23±0.83	3.53±0.88	2.76±0.83	NS

Different superscript letters in the same row denotes significant differences (p ≤ 0.05).

Table 15: Nutritive value of Biscuit fortified with different levels of stevia extract.

Samples	Moisture (g/100g)	Ash (g/100g)	Protein (g/100g)	Fat (g/100g)	Carbohydrates (g/100g)	Mineral (mg/100g)		
						Ca	Na	Fe
Control	4.91^{D}±0.43	1.16^{C}±0.11	6.11^{C}±0.28	16.81^{A}±0.31	74.57^{A}±1.14	0.067	0.60	0.066
25% steviaex.	4.97^{D}±0.39	1.18^{C}±0.12	6.13^{C}±0.25	16.71^{B}±0.44	73.11^{A}±1.31			
50% stevia ex.	5.11^{C}±0.38	1.21^{B}±0.13	6.18^{B}±0.29	16.64^{C}±0.41	69.84^{B}±1.22			
75% stevia ex.	5.17^{B}±0.41	1.23^{B}±0.15	6.22^{B}±0.31	16.39^{C}±0.33	63.14^{C}±1.21	0.061	0.68	0.16
100% stevia ex.	5.22^{A}±0.48	1.26^{A}±013	6.28^{A}±0.22	16.24^{D}±0.38	54.71^{D}±1.18			

Different superscript letters in the same row denotes significant differences (p ≤ 0.05).

Jam product:

Sensory evaluation results (score) for jam which was prepared with stevia extract are presented in table (16). Jam prepared with 100% of stevia extract had significant lower color scores (2.6363±1.286) as compared to control (4.09±0.943). With regard to taste of prepared jam with (50,75 and 100%) levels of stevia extract ,there were significant decrease in the given scores (3.45±1.035, 2.81±1.078 and 1.63±0.674, respectively) in comparison with control (4.36±0.924). Degree of sweetener was decreased as the stevia extract level increased, and the lower significant value was observed with (75 and 100%) stevia extract.

Chemical constituents of jam by different level of stevioside were presented in Table (17). Jam samples had significant decreased ($P \leq 0.05$) in protein, fat and ash contents for all jam prepared with different levels of stevia extract than the control jam. However, moisture contents of jam with different levels of stevia extract were found to be higher (39.10±1.11, 40.21±0.14, 59.10±0.91 and 74.10±1.12 respectively) as compared to jam control (23.26±0.28). Also, Jam prepared with 50% (which achieved better characteristics) contained higher values of Ca, Na and Fe as compared to the control sample.

The overall findings of the present study indicated that the percentage of stevia extract as natural sucrose replacer varied among food products depend on the characteristics that need to achieve for high sugar product such as jam, medium sugar product such as shortening cake and low sugar product such as biscuits. It would be beneficial for food industries to develop the novel formulations for cakes, biscuits, jam and other food products with stevia as a natural sucrose substitute in appropriate percentages to develop low caloric products with good quality and better consumer acceptability.

Table 16: Organoleptic evaluation score of jam prepared with different levels of stevia extracts

Samples / Characteristics	Jam					LSD
	(control) 0%	Sample 25%	Sample 50%	Sample 75%	Sample 100%	
Viscosity	4.36 ^{A} ± 0.9244	3.81 ^{A} ± 1.167	3.40 ^{AB} ± 1.341	3.72 ± 0.9045^{A}	2.45 ^{B} ±1.572	1.0527
Color	4.09 ^{A} ± 0.943	3.63 ^{A} ± 1.206	3.90 ^{A} ± 0.831	3.50 ^{AB} ±01.322	2.63 ^{B} ± 1.286	0.9882
Taste	4.36 ^{A} ± 0.924	4.00 ^{AB} ± 1.000	3.45 ^{BC} ± 1.035	2.81 ^{C} ± 1.078	1.63 ^{D} ± 0.674	0.8301
Odor	4.09 ^{A} ± 0.943	3.90 ^{A} ± 1.415	3.81 ^{A} ± 0.750	3.45 ^{A} ± 1.035	2.18 ^{B} ±1.167	0.9493
Degree of sweetener	4.45 ^{A} ±0.934	3.90 ^{AB} ±0.943	3.81 ^{AB} ±0.981	3.09 ^{B} ±0.943	1.81 ^{C} ±0.981	0.8335
General acceptability	4.72 ^{A} ±0.646	4.18 ^{AB} ±0.750	3.81 ^{BC} ±0.873	3.27 ^{C} ±1.103	2.27 ^{D} ±0.904	0.7572

Different superscript letters in the same row denotes significant differences ($p \leq 0.05$).

Table 17: Chemical constituents of jam by different level of stevioside .

Samples / Constituents	Control	25% stevia.ex	50% stevia.ex	75% stevia.ex	100% stevia.ex
Moisture %	23.26D±0.28	9.10BC±1.11	40.21C±0.14	59.10B±0.91	74.10A±1.12
Total sugar %	67.80A±0.51	50.97B±1.24	33.87C±1.20	16.89D±1.14	N.D
Non reducing sugar %	34.06A	24.65B	17.08C	8.54D	N.D
Reducing sugar %	33.74A±0.41	26.32B±1.23	16.79C±1.32	8.35D±1.17	N.D
Protein	3.14A±0.13	2.75B±0.31	2.69C±0.20	2.61CD±0.18	2.41D±0.27
Fat	0.91A±0.10	0.89B±0.16	0.86D±0.17	0.88BC±0.14	0.87C±0.12
Ash	3.11A±0.12	2.95C±0.16	2.91CD±0.12	2.87D±0.11	2.99B±0.13
V.C mg/100gm	119.56B±1.41	117.41D±1.19	117.57D±1.21	118.96C±1.15	121.33A±1.16
Mineral (mg/100g: Ca	0.055		0.077		
Na	0.28		0.32		
Fe	0.073		0.14		

Value represent means ± standard deviations calculated from three replicates

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