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Influence of sugar replacement by stevioside on physicochemical and sensory properties of biscuit

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RESEARCH ARTICLE

Abstract

Physiochemical and sensory evaluation of the reduced-sugar biscuits containing 0, 50 and 100% stevioside (*Stevia rebaudiana* Bertoni*)* as sugar replacement were investigated. Results showed that the stevioside substitution in biscuit formulation had no significant effects ($P<0.05$) on ash, fat and protein contents of biscuit but the pH and moisture content significantly increased. Complete replacement of sucrose with stevioside reduced calorie levels up to 15% in biscuit formulation. Specific volume, volume and bulk density had no considerable change with increasing stevioside level but diameter and expansion coefficient significantly decreased. The stevioside replacement increased the thickness and water activity. Addition of stevioside in the various biscuit formulations reduced the texture hardness, while their flexibility significantly increased. Stevioside incorporation resulted in the production of lighter biscuits with higher L* and a* and lower b* colour values. Sensory evaluation of biscuits prepared with equal ratio of sugar to stevioside (50:50) exhibited the highest sensory scores for flavour, colour, taste and overall acceptability.

Keywords: biscuit, stevioside, calorie, texture, sensory evaluation

1. Introduction

Biscuits are flour-based bakery product which attract consumers owing to their various tastes, long shelf life and relatively low cost (Manley, 2000). Due to competition in the market and increased demand for health-promoted natural products, attempts are being made to improve biscuits' nutritional value as well as functionality by modifying their nutritive composition (Rahaie *et al.*, 2012). Since a very important aspect of biscuits is its sugar content, the granulated sugar can have desirable effects on flavour, dimensions, colour, hardness and surface finish of biscuits. However, today a high level of sugar is not desirable (Vitali *et al.*, 2009).

Consumption of excessive quantities of granulated-sugar increases the energy intake which can lead to harmful effects on the body, including obesity and chronic diseases. Nowadays serious health care and social concerns regarding excess weight, has raised the importance of sugar-free products (Cross, 2007). Replacement of natural materials and artificial components has been employed to provide sweetness for the diet. Sorbitol, mannitol, xylitol, erythritol, lactitol, maltitol, stevioside, thaumatins, etc. are natural sweeteners which exhibit an appropriate potential for food applications. Also, artificial sweeteners including sucralose, aspartame and acesulfame-K are frequently used as sugar substitutes (Manisha *et al.*, 2012).

Stevioside, the major component of *Stevia rebaudiana* Bertoni, is extensively used as a non-caloric natural sweetener in food industry which is approximately 300 times sweeter than sucrose (Manisha *et al.*, 2012; Wang *et al.*, 2012). Lowering blood pressure, treating obesity, decreasing blood sugar and potentiating insulin secretion are exclusive therapeutic characteristics of stevioside (Wang *et al.*, 2012). Stevioside provides zero calories in a wide range of beverages and foods and well-suited for blending with other non-calorie or carbohydrate sweeteners. The stevioside molecule is stable under dry conditions and in aqueous food systems. Its stability is significantly better than other artificial sweeteners like aspartame (Hamzah *et al.*, 2013).

Several studies performed on substituting stevia in sugar-containing bakery products such as pound cake (Schirmer *et al.*, 2012), muffins (Zhan *et al.*, 2012), yoghurt cake (Abdel-Salam *et al.*, 2009) and bread (Parimalavalli, 2007) but no extensive research have been done on high caloric sugar-contain biscuits. Therefore in this study we sought to address the effect of stevioside substituting on some physical, chemical and sensory properties of biscuit.

2. Materials and methods

Materials

Wheat flour prepared from Khorasan Co (Mashhad, Iran) and its chemical properties. The moisture, ash, acid soluble ash, gluten, protein, pH, and acidity contents were determined by approved AACCI (2000) methods 44-01.01, 08-01.01, 8-21.01, 56-61.02, 46-10.01, 02.52.01 and 02-31.01, respectively (Table 1). Stevioside powder was purchased from Serva (St Louis, MO, USA). Shortening was obtained from Partodane-khazar Ltd. (Mazandaran, Iran). All other reagents used in this study were of analytical grade quality. The pH was measured using a digital pH meter (Model Toptronic, Milan, Italy).

Biscuit formulation

The formulations used for the preparation of biscuits were named A, B and C as presented in Table 2. Wheat flour, ammonium bicarbonate (NH_4HCO_3), salt, milk powder, vanilla and baking powder were mixed thoroughly at 60 rpm for 2 min in a dough mixer (model ESTM 4600, type A10; Electrolux GmbH, Nürnberg, Germany). Sugar and/or stevioside were poured into warm water (50±1 °C) and these solutions were added to the powdered ingredients. Instantaneously, vegetable oil and shortening were added to the mixture along with sugar and/or stevioside. Finally all of the ingredients were mixed at 80 rpm for 3-4 min until gaining an homogeneous mix. The temperature of the dough was kept at 28 °C for 15 min. Then the biscuit dough was flattened and moulded uniformly so that dough diameter was 2 mm. Biscuits were eventually transferred onto a tray and baked at 170 °C for 20 min using the rotary oven (Zuccheli Forni, Trevenzuolo, Italy). After baking, the biscuits were cooled for 10 min at room temperature, packaged and maintained in a dry place to carry out various experiments.

Table 1. Chemical properties of wheat flour. Data are expressed as mean ± standard deviation (n=3).

Characteristics	Amount
Moisture content (%)	14.0±0.01
Ash (%)	0.45±0.02
Acid-insoluble ash (%)	0.02±0.006
Gluten (%)	25.0±0.31
Protein (%)	9.33±0.23
pH	5.6±0.12
Acidity	1.3±0.12

Physical characteristics

Diameter and thickness of biscuits from each formulation (e.g. A, B and C) were measured randomly using calibrated electronic calipers (accuracy 0.01) edge to edge and an average of six selected individual determinations was considered. Biscuit expansion coefficient was determined from ratio of diameter to thickness (Krishnan *et al.*, 2011; Zoulias *et al.*, 2000).

Biscuit volume was measured by rapeseed displacement method (Manisha *et al.*, 2012). Apparent density was calculated using mass to volume ratio (Garavand *et al.*, 2013) and specific volume of biscuits was determined from the volume to mass ratio. After milling the biscuits, the water activity of samples were determined using water activity meter (model MS1; Novasina, Lachen, Switzerland) (Zoulias *et al.*, 2000).

Colour evaluation

Lightness (L*), redness (a*) and yellowness (b*) values of the biscuit were measured using the HunterLab colour measuring system (Labscan XE system; HunterLab, Reston, VI, USA). The positive and negative values of a* indicate the reddishness and greenishness of samples, respectively. On the other hand, a positive value of b* indicates yellowishness and its negative indicates bluishness of the biscuit samples.

Texture measurement

In order to investigate biscuit hardness and flexibility, a texture analyser (CNS Farnell, Borehamwood, UK) was used following a triple beam snap technique depicted by Sudha *et al.* (2007). The force required for 25% compression was recorded using the following condition: a probe with 12 mm diameter and 10 cm height and crosshead speed of 1 mm/s. This experiment conducted 3 h after the biscuit baking at room temperature (25 °C).

Table 2. The ingredients (g) of various biscuit formulations.

Ingredients (g)	Biscuit samples		
	A (0% stevioside)	B (50% stevioside)	C (100% stevioside)
Wheat flour	300	300	300
Sugar	90	45	0
Stevioside (after the equivalent)	0	0.675	1.350
Shortening	45	45	45
Vegetable oil	30	30	30
Salt	3.3	3.3	3.3
Milk powder	5.4	5.4	5.4
Baking powder	1.2	1.2	1.2
Ammonium bicarbonate	1.2	1.2	1.2
Water	90	90	90
Vanilla	0.9	0.9	0.9

Chemical analysis

The moisture, crude protein, crude fat and total ash contents of biscuits were determined by approved AACCI (2000) methods 44-01.01, 46-10.01, 30-10.01 and 08-01.01. The nitrogen conversion factor used for crude protein calculation was 6.25. The carbohydrate content (%) was calculated by subtracting the contents of crude protein, fat, ash, moisture. The total sugar determined according to Bolten *et al.* (1979). Results were expressed on a wet basis.

Calorie calculation

The calories of protein, carbohydrate and fat are 4, 4, and 9 kcal/g, respectively. The calories of biscuits were calculated per 100 g of the different samples (Lin *et al.*, 2003).

Sensory evaluation

The sensory attributes (maximum evaluation score = 5), colour, flavour, taste, texture, chewiness and overall acceptability were assessed by a trained panel including fifteen members of the Department of Food Science and Technology of Islamic Azad University, Sabzevar Branch, and the data were analysed statistically (Reddy *et al.*, 2005).

Statistical analysis

The data were subjected to analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's multiple range test ($P<0.05$) using SAS statistical software (version 9.2; SAS Inc., Chicago, IL, USA).

3. Results and discussion

Evaluation of physical properties of biscuits

Analysis of variance showed that substitution of stevioside with sugar had significant effects ($P<0.05$) on the diameter, thickness and expansion coefficient in biscuit formulations (Table 3). As is obvious from Table 3, increasing stevioside contents led to meaningful increase of biscuit thickness but decreased the biscuit diameter and expansion coefficient significantly ($P<0.05$). There are significant differences ($P<0.05$) between thickness and expansion coefficient of different formulations, although there is no significant difference between B and C formulations for diameter. The maximum values for diameter and expansion coefficients are related to formulation A (0% stevioside), while maximum thickness attributed to formulation C

Table 3. Dimensional parameters of different biscuit formulations. Data are expressed as mean ± standard deviation (n=3).[1]

Biscuit formulations	Thickness (mm)	Diameter (mm)	Expansion coefficient
A (0% stevioside)	5.11±0.23^a	57.83±2.31^a	11.30±0.43^a
B (50% stevioside)	5.43±0.21^b	54.16±3.31^b	9.98±0.82^b
C (100% stevioside)	5.80±0.26^c	51.00±2.60^b	8.81±0.76^c

[1] In each column, values with different superscript letters are significantly different ($P<0.05$).

(100% stevioside). The trend observed in this study was in accordance with the results of Taylor *et al.* (2008), who investigated replacement of sugar with tagatose in cookies. Also Savitha *et al.* (2008) reported increased levels of sucralose and maltodextrin in biscuit formulation reduced the diameter and increased thickness of the final product which is the same trend observed in our study. Gluten network development was limited by increasing the amount of sugar in the formulations, hence, the dough loses its elasticity, but maintains its plasticity. Reducing sugar in biscuit formulations caused water absorption through gluten and its further development resulted in increased dough elasticity, which is the main reason during rolling, increasing thickness and reducing diameter of dough (Lazaridou *et al.*, 2007). Furthermore, Savitha *et al.* (2008) stated that reduced viscosity due to the elimination of sugar in dough formulations could be one of the possible mechanisms for reduced diameter and increased thickness in biscuit.

The results of physical characteristics assessment of biscuit formulations are represented in Table 4. They indicate that with increasing stevioside substitution with sugar in the formulations, the water activity of biscuits increased significantly (P<0.05). Formulation C (containing 100% stevioside) had the highest water activity while formulation A had the lowest (without any stevioside), likewise, significant differences were found for all three formulations in the light of water activity (Table 4). Zoulias *et al.* (2000) found a similar trend for water activity of cookies that had been supplemented with sugar alcohols. They noted that sucrose exhibited the least water activity during baking due to the affinity towards water and reduction the amount of free water. In another study, Akesowan (2009) reported that reduction of sugar significantly increased the water activity of cake, probably due to the fact that sweeteners do not provide water binding properties as that of sugar.

As indicated in Table 4, the results of the statistical analysis of volume, specific volume and bulk density showed no significant effect of stevioside on the physical properties of biscuits. However, with the increasing percentage of stevioside in biscuit formulations, the volume and specific volume decreased and the bulk density increases partially, however, no significant differences were observed. This gas formation potential is limited due to the inadequate development of gluten network structure in short-type recipe biscuits and cookies (Maache-Rezzoug *et al.*, 1998). Sugar hindered the starch gelatinisation during biscuit baking and permitted the air bubbles to expand by water vapour and carbon dioxide. As a result, higher volume was obtained. Gelatinisation of starch was delayed by the sugar solution and was related to the abilities of sugar to bind available water to starch granule, lowered the water activity, formation of sugar bridges between starch chains and formation of an anti-plasticising effect, relative to water (Faridah and Aziah, 2012). Thus, lowering sugar by substituting stevioside resulted in early gelatinisation of starch during baking and limited the volume of biscuit. Schirmer *et al.* (2012) noted that sucrose improve the stabilisation of protein foams and their interfaces.

Colour assessment

The lightness (L* value) of biscuits was increased significantly (P<0.05) by the stevioside replacement. The L* value for the A formulation (containing 0% stevioside) was about 57, which with increasing stevioside substitution to 100% (in C formulation) increased to about 67. It can be seen from Table 5 that a* value (redness) decreased significantly with increase in stevioside levels. Thus, the addition of stevioside reduced the red colour of biscuits. As the stevioside level boosted from 0 to 100%, a* value declined from about 7 to 3.3. Statistical analysis showed that increase in stevioside in biscuit formulations from 0 to 50% increased b* value (yellowness) significantly (P<0.05),

Table 5. Colorimetric parameters of different biscuit formulations. Data are expressed as mean ± standard deviation (n=3).[1]

Biscuit formulation	L*	a*	b*
A (0% stevioside)	56.97±1.52^{c}	6.97±0.37^{a}	22.73±0.26^{b}
B (50% stevioside)	62.23±1.69^{b}	5.76±0.34^{b}	23.57±0.03^{a}
C (100% stevioside)	66.64±2.28^{a}	3.28±0.26^{c}	23.72±0.36^{a}

[1] In each column, values with different superscript letters are significantly different (P<0.05).
L* = lightness; a* = redness; b* = yellowness

Table 4. Various physical characteristics of biscuit formulations. Data are expressed as mean ± standard deviation (n=3).[1]

Biscuit formulation	Water activity	Volume (ml)	Specific volume (cm^3/gr)	Bulk density (gr/cm^3)
A (0% stevioside)	0.308±0.001^{c}	25.86±0.55^{a}	1.930±0.03^{a}	0.517±0.009^{a}
B (50% stevioside)	0.332±0.003^{b}	25.23±0.37^{ab}	1.873±0.03^{ab}	0.526±0.015^{a}
C (100% stevioside)	0.349±0.005^{a}	24.90±0.36^{b}	1.858±0.01^{b}	0.530±0.016^{a}

[1] In each column, values with different superscript letters are significantly different (P<0.05).

while b* value did not change notably when the stevioside level was raised from 50 to 100%. It is obvious that A and C formulations have the highest and lowest b* values. These results were similar to the study of Lin *et al.*, (2003) who found that the L* values of chiffon cakes gradually increased with higher levels of erythritol. Due to the thermal stability, stevioside does not degrade and reacts with amino acids by Maillard reaction (Akesowan, 2009). Hence, the alteration of L* value occurred with increasing stevioside levels. Similar results have been reported by Zoulias *et al.* (2000) with additions of acesulfame-K (an artificial sweetener) which had no significant effect on the colour of low-fat cookies.

Texture assessment

Sugar delivers sweetness, affects structure formation and improves crumb texture and tenderness of bakery products. Baking with reduced sugar or even sugar-free, results in loss of viscosity and bulk owing to low solids, poor aeration and cell structure (Manisha *et al.*, 2012). Table 6 shows the influence of stevioside on the texture of biscuits. The hardness value for formulation A without stevioside was about 35.0 N. Replacement of sugar with stevioside significantly decreased the hardness values from 34.5 to 15.0 N with increasing loading of stevioside from 0 to 100%. Sugar can prevent gluten network development during dough mixing by competing with the flour for recipe water (Gallagher *et al.*, 2003). As well, sugar recrystallisation after biscuit cooling improve the texture hardness (Mushtaq *et al.*, 2010; Taylor *et al.*, 2008). Pareyt *et al.* (2009) stated that lowering sugar content from 31.2 to 17.6% result in reduction of cookies hardness from 20.5 to 16.1 N. In general, the formulations containing stevioside had a lower hardness than to the sample produced with sugar. This fact can probably be attributed to the low inherent solubility of composing units of stevioside in comparison to sucrose which can lead to an open inhomogeneous network microstructure in the developed biscuits and thus a low resistance to load forces.

Evaluation of biscuits flexibility or fracturability is demonstrated in Table 6. According to results of this study, with increase in levels of stevioside in biscuit formulations no significant differences (P<0.05) were found in flexibility. The reason can be attributed to the brittle nature and the inherent fragility of biscuits. Flexibility of biscuit samples were in the range of 0.364 to 0.497 mm. In biscuits containing sugar, limitation of gluten network development, glass formation and sugar recrystallisation are the main reasons for changes in biscuit fracturability and tenderness (Lara *et al.*, 2011).

Table 6. Texture parameters of different biscuit formulations. Data are expressed as mean ± standard deviation (n=3).[1]

Biscuit formulation	Hardness (N)	Flexibility (mm)
A (0% stevioside)	34.467±2.60^{a}	0.364±0.108^{a}
B (50% stevioside)	24.667±2.06^{b}	0.411±0.450^{a}
C (100% stevioside)	14.933±4.21^{c}	0.497±0.112^{a}

[1] In each column, values with different superscript letters are significantly different (P<0.05).

Chemical analysis of prepared biscuits

The impact of the addition of stevioside on the chemical properties of biscuit is shown in Table 7. Statistical analysis indicated that the addition of stevioside to biscuit formulation did not have any significant effect total ash, fat and protein, the probable reason being that the same type and amount of flour and fat used in each of the three biscuit formulations (Manisha *et al.*, 2012). The moisture content of the biscuits increased significantly (P<0.05) with increasing stevioside levels in formulations so that the maximum moisture content was related to samples in which the sugar has been replaced completely with stevioside (formulation C). Sugar raises protein denaturation and starch gelatinisation temperatures and helps remove moisture from the product (Kocer *et al.*, 2007). Similar results have been revealed by Zoulias *et al.* (2000), Pareyt *et al.* (2009) and Manisha *et al.* (2012). The results of the pH measurement showed that with the increasing replacement with stevioside in biscuit formulations, pH increased significantly so that formulation C had the highest pH value. The pH increment in stevioside rich biscuit formulations

Table 7. Chemical analysis of different biscuit formulations. Data are expressed as mean ± standard deviation (n=3).[1]

Biscuit formulation	Moisture content (%)	Ash (%)	pH	Protein (%)	Fat (%)	Total sugar (%)	Carbohydrate (%)
A (0% stevioside)	3.506±0.07^{c}	1.083±0.04^{b}	6.55±0.02^{c}	9.740±0.03^{b}	23.043±0.21^{a}	17.72±0.16^{a}	60.43±0.01^{a}
B (50% stevioside)	3.837±0.06^{b}	1.106±0.04^{ab}	6.62±0.02^{b}	9.846±0.05^{ab}	23.000±0.16^{a}	9.88±0.22^{b}	52.32±0.03^{b}
C (100% stevioside)	4.093±0.03^{a}	1.117±0.03^{a}	6.81±0.03^{a}	9.913±0.12^{a}	22.980±0.10^{a}	1.79±0.13^{c}	44.17±0.03^{c}

[1] In each column, values with different superscript letters are significantly different (P<0.05).

is mainly due to the fact that non-enzymatic browning reactions (Maillard browning) did not occur in the absence of reducing sugars. In the presence of sugar (sucrose) and high temperatures, reduction of sugars such as glucose and fructose are formed and the reaction between these reducing sugars and amino acids results in a pH drop due to the non-enzymatic browning reactions (Stadler *et al.*, 2002). The results for the pH and moisture content showed exhibited that all formulations are specified in the standard range for biscuits (Manley, 2000).

The results of ANOVA showed that there are significant differences (P<0.05) between the total sugar content of all biscuit formulations. As the stevioside content of formulations increased, the total sugar of biscuits decreased significantly due to the reduction of sucrose levels in biscuit formulation.

Generally, low calorie sweeteners are ingredients added to foods and drinks to provide sweet taste without calories, or with very few calories. Most low calorie sweeteners are several hundred times sweeter than table sugar, meaning that only small quantities need to be added to achieve a sweetening effect (Kroger *et al.*, 2006). Our results are consistent with those of Manisha *et al.* (2012) and Lin *et al.* (2003) for substituting sweeteners in Danish cookies and cake, respectively. Also the chemical analysis indicated that increasing replacement with stevioside in formulations from 0 to 100% leads to lowering of the carbohydrate content of biscuits from 60 to 44%. A reduction of carbohydrate levels ascribed to decreasing total sugars in formulations. These results are similar to those reported by Akesowan (2009) and Lin *et al.* (2010).

Calorie assessment

Figure 1 illustrates the changes in biscuit calories during replacement of sugar with stevioside. The results show that increasing the stevioside ratio in the mixture reduced calorie levels significantly (P<0.05) so that sample C has the least amount of calories. According to the results, it was found that the complete replacement of sugar with stevioside reduced calorie by 15% and that this drop associated to the absence of sugar in the formulation of biscuits and zero-calorie sweetener stevioside. The highest amount of calories belongs to the sample A with about 488 kcal/100 g, while the lowest amount of calories observed in sample C with 423 kcal/100 g. Lin *et al.* (2003) reported that replacement of erythritol with sucrose in chiffon cake from 0 to 100% reduced the cake calorie content considerably so that it declined from 540 to 478 kcal/100 g because of the elimination of sugar in cake preparation. Our findings are in accordance with the results of Pasha *et al.* (2002) and Akesowan (2009).

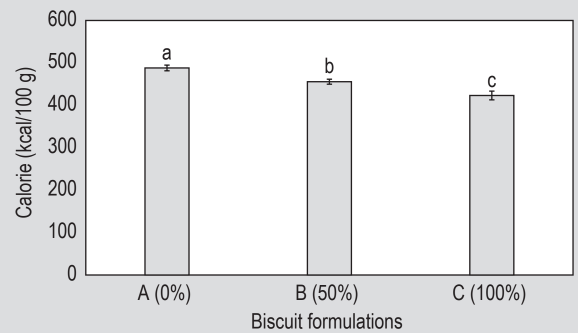

Figure 1. Effect of stevioside substituting on calorie content of various biscuit formulations (A-C) containing 0, 50 or 100% stevioside. Bars with different letters mean significant differences (*P*<0.05).

Sensory assessment

Table 8 demonstrates the sensory profile of the biscuits prepared from various formulations. It is evident that the sensory scores of the biscuits in terms of flavour and aroma were higher in A formulation (containing 100% sugar) compared to stevioside containing formulations B and C. The occurrence of such a phenomenon is strongly related to melanoidins generated by the Maillard reaction (Wang *et al.*, 2011). Nevertheless, in terms of colour, taste and overall acceptability, the biscuits based on both sugar and stevioside (B formulation) scored higher than the other two formulations. The sensory rating for the biscuits based on 100% stevioside (C formulation) was higher in terms of texture and chewiness quality than those made from 100% sugar or stevioside, these findings are consistent with texture analysis profile mentioned above. Overall, biscuits prepared with 50% sugar and 50% stevioside (B formulation)

Table 8. Sensory evaluation (maximum = 5) of different biscuit formulations. Data are expressed as mean ± standard deviation (n=3).[1]

Biscuit formulation	Colour	Taste	Flavour and aroma	Texture and chewiness	Overall acceptability
A (0% stevioside)	3.13±0.02^{c}	4.06±0.70^{a}	4.26±0.70^{a}	3.20±0.77^{a}	3.73±0.53^{ab}
B (50% stevioside)	4.40±0.02^{a}	4.53±0.63^{a}	3.93±0.70^{a}	3.93±0.79^{a}	4.33±0.31^{a}
C (100% stevioside)	3.60±0.03^{b}	3.33±0.47^{b}	3.26±0.79^{b}	4.46±0.74^{a}	3.53±0.46^{b}

[1] In each column, values with different superscript letters are significantly different (*P*<0.05).

exhibited the highest sensory scores and desirability by the panellists.

4. Conclusions

The evaluation of sucrose replacement by stevioside in biscuits and the impact on the product quality were evaluated. Physicochemical and sensory experiments for analysing the structural and chemical changes during stevioside substituting were conducted. Stevioside containing biscuits have an appropriate potential in preventing diabetes and obesity because of reduced sugar and calorie contents. On the other hand, the low-calorie biscuits (especially formulation B) not only have no adverse effects on the quality of the biscuits but also improve some quality attributes of biscuit including colour, chewiness, flavour and overall acceptability.

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