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Formulation, Senso-chemical Analysis and Shelf-life Study of Biscuits using Stevia Leaf as the Substitute for Sugar

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ABSTRACT

Background: Biscuits are the most popular cereal-based bakery and sugar is an important element of biscuits which provides unique taste, color and texture. However, the consumption of sugar is linked to potential health risks. Therefore, there is a scope to substitute sugar with natural non-caloric sweetener, maintaining all sensory and storage qualities of the biscuits.

Methods: The present study was conducted to find an acceptable formulation to manufacture biscuits using stevia leaf. Dried leaf powder and fresh juice were used as a substitute for sugar in biscuit manufacturing. After baking, biscuits were tested for sensory qualities. In addition, biscuits were packaged with different packaging materials and organoleptic changes were assessed.

Result: Biscuits made with 18 g stevia powder and 55 ml stevia juice per kg of flour showed the best results comparing all aspects of consumer preferences. The organoleptic and microbiological evaluations of stevia powder biscuit and stevia juice biscuit showed no significant changes and unsafe measures for one month of storage in glass, plastic jar and tin can. Therefore, sugar-free biscuits can be made following either stevia powder biscuit or stevia juice biscuit formulations and stored in either glass, plastic jar, or in tin can to remain safe for human consumption.

Key words: Biscuit formulation, Stevia juice biscuit, Stevia powder biscuit, Sugar substitute.

INTRODUCTION

The sweetest glycosides in stevia leaves are 'stevioside' and 'rebaudioside' which are 250-300 times sweeter than sucrose and chemically and thermally stable (Boileau *et al*., 2012). Besides, stevia provides a wide range of health benefits and is likely to become the ultimate natural sweetener in the food industry (Samuel *et al*., 2018). It is anti-hyperglycemic, anti-hypertensive, anti-caries and decreases LDL, increases HDL, cures inflammatory bowel diseases, decreases obesity as well as maintains cardiovascular health (Gandhi *et al*., 2018). Recently stevia leaf extracts have been approved for use in foods and beverages in more than 150 countries across the globe.

Several attempts have been taken to formulate stevia-based biscuits (Gupta *et al*., 2017, Paridhi *et al*., 2017). Stevia juice has recently been incorporated in soft drinks as a substitute for sugar (Agulló *et al*., 2022). However, it is not well known how stevia juice and stevia powder affect the sensory attributes and shelf life of biscuits. Researchers are trying to find out the consumer acceptance of such novel products (Cieslinski, 2019). Under these circumstances, this study was designed to formulate biscuits incorporating stevia powder and juice, compare their sensory qualities and study the shelf life of the formulated biscuits under various storage conditions.

MATERIALS AND METHODS

Preparation of stevia powder and juice

For powder preparation, stevia (collected from Bangladesh Sugarcrop Research Institute (BSRI), Gazipur) samples were dried at 40°C at a constant air velocity of 2.0±0.2 m/s, until the equilibrium condition was reached (±0.01 g). The dried stevia leaf was ground into a fine powder using-a pulverizer (F-P400E miniature all-round planetary ball mill; Hunan Fukas Test Instrument Co., Ltd.) at 2000 rpm for 5 min (Cao *et al*., 2021). The stevia powder (moisture content 4.5±0.01%) was poured into glass jar and stored at room temperature (28±2°C).

Stevia juice was prepared from the fresh leaf manually using a mortar-and-pestle and filtered through Whatman filter paper No 4 (20-25 µm). The stevia juice was also stored in glass jars at room temperature (28 ± 2°C) for further uses. The total soluble solids (TSS) of the stevia juice was determined using a digital hand refractometer (Islam *et al*., 2018).

Preparation of standard biscuit

The standard biscuit (SB) was prepared according to the standard formulation (Davidson, 2018) which has shown in Table 1.

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All the ingredients were mixed according to the standard method of biscuit manufacturing. The mixture was baked at 204°C for 10 min. The baked biscuits were cooled to room temperature (28 ± 2°C) and preserved for further utilization.

Preparation of stevia biscuit

The stevia powdered biscuit (SPB), and stevia juiced biscuit (SJB) was prepared according to the procedure mentioned for SB. To obtain an acceptable formulation for SPB, and SJB, only the sugar (Table 1) was substituted by stevia powder and stevia juice at different concentrations, as shown in Table 2. To avoid excessive liquidity, the amount of added oil was slightly reduced (91 ml) in the formulation of SJB.

Due to the organic odor of stevia leaf, the vanilla essence was added to the dough at a concentration of 2.0 ml/kg flour instead of 1.6 ml/kg flour.

Sensory evaluation of SPB and SJB

The sensory evaluation of prepared SPB and SJB was carried out according to "generic sensory descriptive analysis" shown by Cao *et al*. (2019) with little modification. The panel consisted of 10 panelists (5 women, age 25-50 years. The panelists were agreed to use a hedonic rating for taste, flavor, color, texture and overall acceptability of the products, from 9, to 1 (9 = Like extremely, 8 = Like very much, 7 = Like moderately, 6 = Like slightly, 5 = Neither like nor a dislike, 4 = Dislike slightly, 3 = Dislike moderately, 2 = Dislike very much and 1 = Dislike extremely).

Table 1: The formulation for standard biscuit.

Ingredients	Quantity
Wheat flour (kg)	1
Sugar (powder) (g)	470
Milk (powder) (g)	67
Egg (pcs)	6
Oil (Soybean) (ml)	105
Ghee (ml)	270
Baking powder (g)	9.5
Vanilla essence (ml)	1.6
Table salt (g)	6.25

Table 2: The concentration of stevia powder and juice in SPB and SJB as the substitute for sugar.wc

SPB Formulation	Concentration of stevia powder (g/kg flour)	SJB Formulation	Concentration of stevia juice (mL/kg flour)
SPB1	2.0	SJB1	10.0
SPB2	4.0	SJB2	15.0
SPB3	6.0	SJB3	20.0
SPB4	8.0	SJB4	25.0
SPB5	10.0	SJB5	30.0
SPB6	12.0	SJB6	35.0
SPB7	14.0	SJB7	40.0
SPB8	16.0	SJB8	45.0
SPB9	18.0	SJB9	50.0
SPB10	20.0	SJB10	55.0
SPB11	22.0	SJB11	60.0
SPB12	24.0	SJB12	65.0

Proximate composition analyses

The prepared biscuits were analyzed for moisture content (Islam *et al*., 2010), ash content and fat content (Zhou *et al*., 2021), total sugars (Cao *et al*., 2020a), protein contents (Xu *et al*., 2021) and fiber contents (Sharmin *et al*., 2021). All analyses were carried out maintaining standard official methods of analysis.

Shelf life study of biscuits

The prepared biscuits packaged in six different packaging systems (in a glass jar, tin can, zipper bag, aluminium wrapper, plastic jar and vacuum packs) were hermetically sealed and stored at room temperature (28±2°C). These biscuits were evaluated for color, texture, flavor and overall acceptability at an interval of three days for one month.

Microbiological assessment

Among various methods, total plate count is the most popular method of evaluating microbial load. Total plate count was carried out according to the method shown by Cao *et al*. (2020b).

Statistical analysis

Analysis of variance (ANOVA) tests was applied to identify differences between different formulated biscuits. Duncan's multiple range test (DMRT) at $P<0.05$ was applied for multiple comparisons of the mean values. Principal component analysis and all other statical analysis was carried out using R software (version 3.4.4, R Development Core Team, 2018).

RESULTS AND DISCUSSION

Sensory evaluation

The biscuits prepared using stevia powder and juice were analyzed and compared with the standard formulation. To find out the acceptable formulation, the SPB and SJB were evaluated for their sensory attributes. The results of the sensory evaluation of stevia powder biscuits have presented in Table 3.

The color, flavor, taste, texture and overall acceptability were significantly different among different formulations. According to Table 3, the formulation SPB1 got the significantly highest scores for color (6.95) and flavor (6.58), indicating 'Like moderately'; on the other hand, SPB1 received significantly lowest scores against taste (5.59), texture (5.87) and overall acceptability (5.62) indicating 'Like slightly'. Formulation SPB9 shows the highest scores for taste (6.88), texture (6.79) and overall acceptability (6.86) that represent SPB9 as 'Like moderately'. The sensory values for color and flavor of SPB9 was (6.10) and (6.22) respectively which reveal the formulation as 'Like slightly'. Based on overall scores against individual sensory attributes, results indicate that formulation SPB9 has more consumers' preferences.

The results of the sensory evaluation of stevia juice biscuits have presented in Table 4.

No significant differences were found for color and flavor in the stevia juice biscuit formulations (Table 4). The highest scores for color, flavor, taste, texture, and overall acceptability achieved by the SJB were 7.22 (SJB2), 7.06 (SJB10), 7.62 (SJB10), 7.31 (SJB10) and 7.22 (SJB10) respectively. The SJB10 secured 7.01 for color attribute, which means 'Like moderately'. The highest score (7.62) secured by taste attribute reveals that the SJB formulated with 55 ml/kg flour stevia juice (SJB10) tastes 'Like very much', indicating all other attributes as 'Like moderately'. Therefore, the acceptable formulation for SJB with maximum consumers' preferences is SJB10 that contains 55 mL stevia juice in 1 kg flour (Table 4).

Principal component analysis

To overview the sensory evaluation, data were subjected to principal component analysis (PCA). The data were auto-scaled before analysis. A two component PCA model was built. From the score plot it can be seen that principal component (PC) 1, explains 75% of the variation while PC2 explains 21% of the variation. Interestingly two clusters were formed for stevia powder biscuits and stevia juice biscuits (Fig 1a). Sample with lower numbers attributed to better color properties while higher numbers attributed to better texture, taste and acceptability (Fig 1b). SPB9 and SJB10 are the two biscuits highly preferred by the panelists (Fig 1).

Based on the results of sensory evaluation, for further proximate analysis, formulation SPB9 (Table 3) and SJB10 (Table 4) were taken into consideration, and obtained results were compared with that of SB.

Proximate composition analysis

The obtained proximate composition of acceptable formulation SPB9, SJB10, and SB is presented in Fig 2.

Moisture, ash, fiber, protein, fat and carbohydrate contents were significantly ($p<0.05$) different among SB,

Table 3: Sensory evaluation of stevia powdered biscuits.

*Samples	Scores secured by individual attributes				
	Color	Flavor	Taste	Texture	Overall acceptability
SB	6.95^{a}	6.58^{a}	6.88^{a}	5.87^{b}	6.86^{a}
SPB1	6.95^{a}	6.58^{a}	5.59^{c}	5.87^{b}	5.62^{c}
SPB2	6.86^{a}	6.49^{ab}	5.81^{c}	5.96^{b}	5.73^{c}
SPB3	6.78^{a}	6.44^{b}	5.87^{bc}	6.01^{b}	5.82^{bc}
SPB4	6.70^{ab}	6.37^{b}	5.93^{b}	6.08^{b}	5.86^{b}
SPB5	6.48^{b}	6.08^{bc}	5.98^{b}	6.33^{ab}	5.90^{b}
SPB6	6.40^{b}	6.30^{b}	6.21^{b}	6.40^{ab}	5.95^{b}
SPB7	6.01^{bc}	6.15^{b}	6.10^{b}	6.23^{b}	6.23^{b}
SPB8	6.20^{bc}	6.02^{bc}	6.08^{b}	6.11^{b}	6.50^{a}
SPB9	6.10^{bc}	6.22^{b}	6.88^{a}	6.79^{a}	6.86^{a}
SPB10	5.96^{bc}	5.92^{bc}	6.18^{b}	6.52^{a}	6.59^{a}
SPB11	5.92^{bc}	5.87^{c}	6.01^{b}	6.02^{b}	5.91^{b}
SPB12	5.87^{c}	5.80^{c}	5.98^{b}	5.71^{b}	5.80^{bc}

Similar superscripts in the same column indicate no significant difference between the means ($p < 0.05$). *See Table 2 for details.

Table 4: Sensory evaluation of stevia juiced biscuits.

*Samples	Scores secured by individual attributes				
	Color	Flavor	Taste	Texture	Overall acceptability
SB	6.95^{a}	6.58^{a}	6.88^{b}	5.87^{c}	6.86^{ab}
SJB1	7.07^{a}	6.98^{a}	6.41^{cd}	6.70^{b}	5.96^{c}
SJB2	7.22^{a}	7.01^{a}	6.48^{c}	6.77^{b}	6.13^{c}
SJB3	7.12^{a}	7.02^{a}	6.52^{c}	6.82^{b}	6.38^{bc}
SJB4	7.21^{a}	7.03^{a}	6.59^{c}	6.87^{b}	6.52^{b}
SJB5	7.14^{a}	7.04^{a}	6.63^{bc}	6.91^{ab}	6.66^{b}
SJB6	7.11^{a}	7.01^{a}	6.82^{b}	6.97^{a}	6.83^{ab}
SJB7	7.02^{a}	6.98^{a}	6.88^{b}	7.11^{a}	6.92^{a}
SJB8	6.98^{a}	6.97^{a}	6.92^{b}	7.02^{a}	6.96^{a}
SJB9	6.99^{a}	6.96^{a}	6.97^{b}	7.03^{a}	6.98^{a}
SJB10	7.0^{a}	7.06^{a}	7.62^{a}	7.31^{a}	7.22^{a}
SJB11	6.98^{a}	6.87^{a}	7.46^{ab}	6.99^{a}	6.68^{b}
SJB12	6.98^{a}	6.88^{a}	6.92^{b}	6.91^{ab}	6.62^{b}

Similar superscripts in the same column indicate no significant difference between the means ($p < 0.05$). *See Table 2 for details.

SPB9 and SJB10. As shown in Fig 2, the moisture contents in SB, SPB9, and SJB10 were 3.43%, 1.61% and 4.04% respectively, which are consistent with a previous study that reported 1-5% moisture in biscuits/cookies (Miller, 2016). The moisture content in the control sample (SB) was higher than that of SPB9 (Fig 2), which might be due to the higher moisture-binding capacity of sugar, on the other hand, SPB9 and SJB10 (Fig 2) did not contain any sugar. Since the stevia juice had 10% TSS only, it might result in excessive free water in SJB10 as compared to SB and SPB9. Previous report showed that moisture content of biscuits varies with their formulations (Schober *et al*., 2003). The higher amount of ash content 1.19%, fat content 32.68%, protein conten15.56% and fiber content 3.53% in SPB9 (Fig 2) might be induced from stevia powder prepared from the stevia leaf. Besides, the control sample (SB) did not contain any stevia component rather contained only sugar as a sweetening agent, and the sugar obviously supplied no ash, fat, protein, and fiber in the SB. These results are in accordance with the findings of Yildiz and Gocmen (2021).

Shelf-life evaluation

The control and formulated biscuits (SB, SPB, SJB) packed in various packaging materials were stored at room temperature (28±2°C). The shelf life of the packaged biscuits was studied in terms of organoleptic changes and microbial proliferation (Ranganna, 1986).

In general, hermetically sealed packages for biscuits provide longer shelf life than non-hermetically sealed packages (Alabakan, 1997). The results of the organoleptic study have shown in Fig 3. Most of the products did not show any changes over the storage period. Slight color changes in SB were observed on day 30 stored in a zipper bag. Interestingly all the vacuum-packed biscuits showed slightly changed color on day 30. No changes were observed for flavor properties. According to Fig 3, by 30 day's storage period at room temperature (28±2°C) the texture of SB became 'moderately soft' while color of all biscuits (SB, SPB9 and SJB10) turned to 'slightly changed' which might be due to the evaporation of free water present in biscuits inside the vacuum packs (Heldman, 2012). Odd flavor induces in the biscuits during storage due to oxidation of fat (Rani *et al*., 2020). The reason of no off flavor might be due to the antioxidative properties of stevia (Kim *et al*., 2011).

Different packaging systems greatly affected the texture of the biscuits. On days 21 and 24, 27 and 30, slight changes in textural properties of SB were observed for vacuum, zipper, and aluminum foil pack, respectively. However, moderate textural change was observed for vacuum packs and zipper bags only. SB was not acceptable on day 30 packed with a vacuum pack and zipped bag. SPB9 and SJB10 did not show any changes over the storage; hence were acceptable after 30 days of storage regardless of

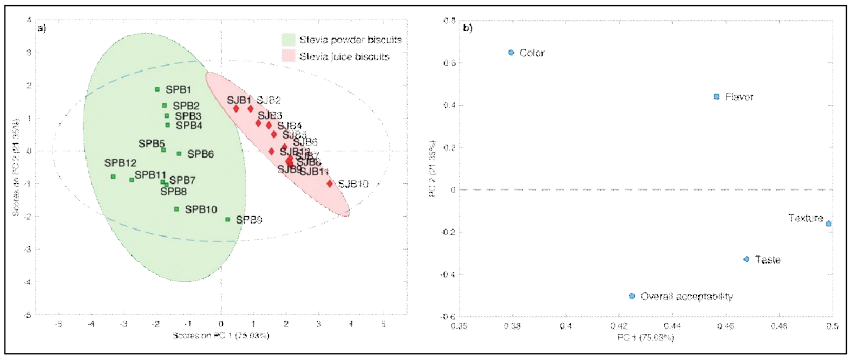

Fig 1: Scores (a) and loadings (b) plot of the sensory evaluation of stevia biscuits. Data presented in the score plot is the mean value. See Table 2 for details of the product.

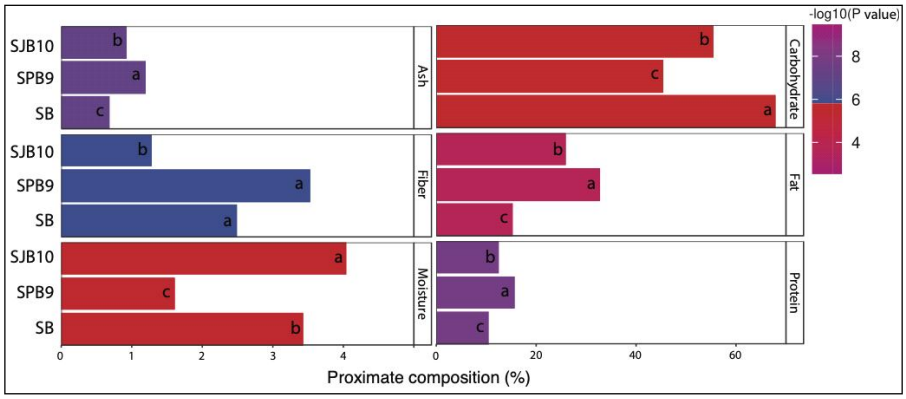

Fig 2: Per cent composition of the stevia biscuits. Different letters in the same group represent significant differences. For details of SB, SPB9, and SJB10, see Table 1 and Table 2.

packaging materials. Both stevia biscuits performed well in terms of textural quality, the reason could be due to lees moisture absorption in the stevia biscuits (Araste *et al.*, 2015). Results suggest that zipper bag, aluminium foil pack and vacuum pack was not suitable for biscuits.

Microbial evaluation

A considerable amount of microbial colony in SB was found at 24 and 27 days of storage (8.9×10^3, 1.4×10^4 and CFU/g respectively) (Fig 4). Compare to the control sample, SJB10 showed lower microbial growth in all the packaged biscuits except for aluminium foil. On day 29 and 31, most product showed a level of 1-4 $\times 10^4$ CFU/g. However, biscuits packaged in aluminium foil showed a significant increase in microbial load (2.14×10^6 CFU/g). Vacuum pack however showed best results in term of microbial activity. The results provide an understanding of which formulation and packaging combination is suitable. The FAO/WHO experts reported that maximum 10^6 mesophilic aerobic bacteria per gram egg product are safe for human consumption (Frazier and Westhoff, 2003). Therefore, based on results obtained in organoleptic assessment and microbiological evaluation, the shelf life of formulated biscuits (SPB9 and SJB10) could be extended to 30 days by hermetically packaging them in

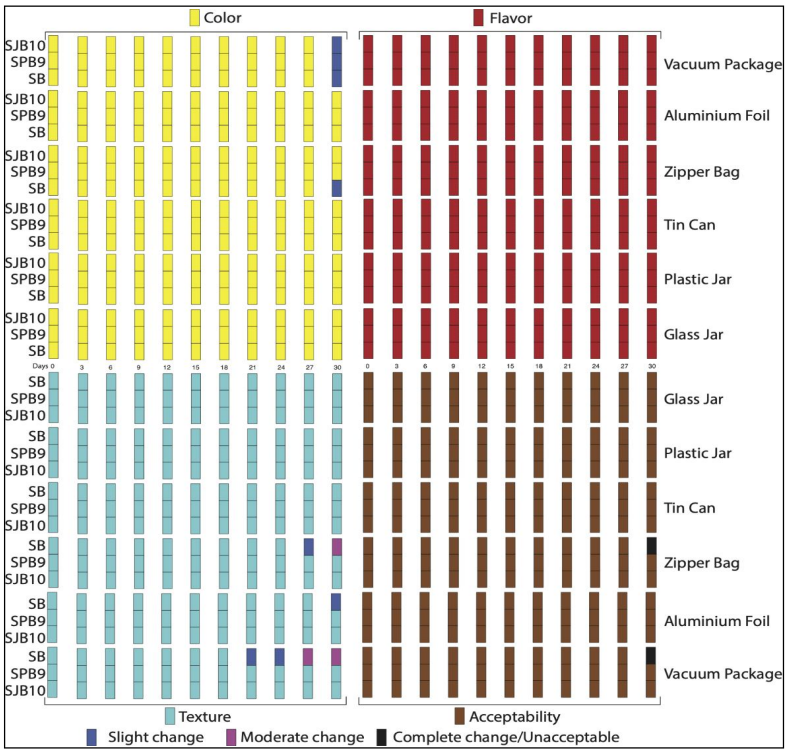

Fig 3: Colormap of the organoleptic evaluation of formulated biscuits stored in different packaging materials. For details of SB, SPB9, and SJB10, see Table 1 and Table 2.

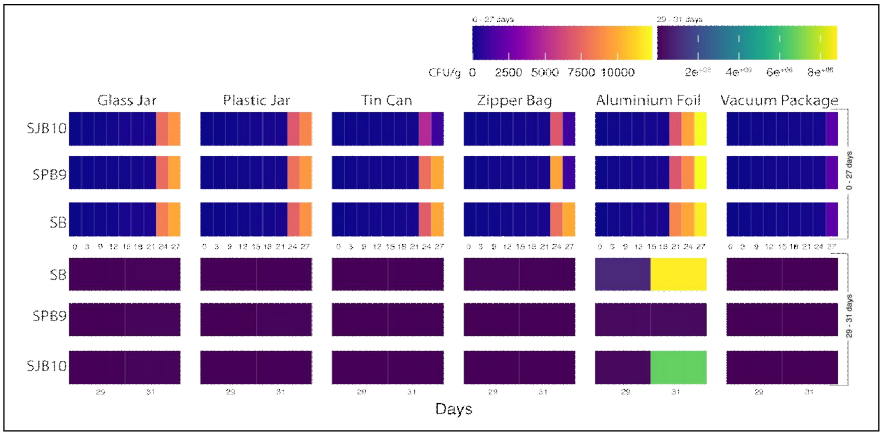

Fig 4: Colormap of the microbial load evaluation of formulated biscuits stored in different packaging materials. For details of SB, SPB9, and SJB10, see Table 1 and Table 2.

glass jar, plastic jar, tin can and vacuum pack using no added preservatives. Moreover, aseptic packaging during industrial production would increase the shelf-life of the products (Galić *et al*., 2009).

CONCLUSION

The risk of coronary heart diseases and diabetes is increasing day by day. To get rid of these health threats, sugar or sugar products should be avoided. This study revealed that sugar can be successfully replaced with stevia in biscuits. Both the stevia powder biscuits and stevia juice biscuits achieved satisfactory sensory scores. The formulated products were microbiologically safe during storage either in tin can, plastic and glass jar. Moreover, they did not change quality during the storage period.– In this study, the storage life of biscuits was checked for one month. Further study for longer time in tin can, plastic and glass jar with cost analysis would help the baking industries to produce stevia-based biscuits.

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Conflict of interest

Authors declare no conflict of interest.

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