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DEVELOPMENT AND QUALITY EVALUATION OF LOW - CALORIE WHITE FINGER MILLET BISCUIT USING STEVIA REBAUDIANA

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ABSTRACT

The rising demand for healthier snack options has prompted the development of low-calorie food products. This study focuses on the formulation and quality evaluation of a low-calorie biscuit made from white finger millet (*Eleusine coracana*) and sweetened with *Stevia rebaudiana*, a natural, zero-calorie sweetener. The primary objective was to create a biscuit that retains sensory appeal while reducing its caloric content and enhancing nutritional value.

White finger millet flour was used as the main ingredient, replacing complete refined wheat flour, and sugar was completely substituted with *Stevia rebaudiana* at varying concentrations (1, 1.25 and 1.5 g/recipe). A comprehensive nutritional analysis was conducted to assess the caloric reduction, macronutrient composition, and overall nutrient profile of the biscuits. Physical characteristics such as texture, moisture content, and color were also analyzed. Sensory evaluation by a trained panel assessed taste, texture, and overall acceptability.

Results showed a significant reduction in calories, with up to a 40 – 50 kcal per 100g of biscuits compared to traditional sugar-sweetened biscuits. Sensory evaluations indicated that biscuits with complete sugar replacement with 1.25 g/recipe of stevia green leaves powder incorporation and with 1 g/recipe of processed white stevia powder were the most acceptable, with minimal differences in taste and texture compared to the control sample. Furthermore, the inclusion of white finger millet enhanced the nutritional profile, notably increasing dietary fiber, calcium, and essential micronutrient content.

In conclusion, the developed biscuits provide a nutritious, low-calorie snack option for health-conscious consumers. The product's potential for commercial success, alongside its long-term shelf life, warrants further investigation.

KEYWORDS: *White finger millet, Stevia rebaudiana, Low-calorie biscuit, Sugar substitute, Nutritional evaluation, Dietary fiber, Healthy snacks, Obesity and diabetes, Biscuit formulation*

INTRODUCTION

The food industry is actively seeking solutions to reduce the levels of certain ingredients related to health concerns, such as salt, fats, and sugars, in processed foods. This effort aims to create healthier options that align with consumer expectations. Over the past few decades, there has been a growing recognition of the role food plays in promoting human health. Many consumers now view food as a critical factor in improving their quality of life. It is widely acknowledged that low-calorie diets are essential for maintaining a healthy lifestyle and may also help prevent lifestyle-related diseases (Savita et al., 2004). As part of this movement, there is an increasing demand for alternative sweeteners to replace traditional sugars. While artificial sweeteners offer the advantage of fewer calories, they may also pose certain health risks, including potential weight imbalances, toxic effects, allergic reactions, and metabolic disturbances. High concentrations of these additives may even lead to genetic and proliferative disorders (Alizadeh et al., 2014).

Finger millet grains vary in size, shape, and color, with brown being the most common. However, the dark color caused by flavonoids and tannins in brown finger millet can reduce its acceptability. White finger millet (KMR-340), developed from the parent varieties WRT-14 and GE2924 by the Zonal Agricultural Research Station (UAS, Bengaluru) at V.C. Farm, Mandya, Karnataka, India, offers a solution. This hybrid millet boasts excellent nutritional properties, containing 11.98 g of protein, 392 mg of calcium, and 4.72 mg of iron per 100 g. With its superior nutritional content and appealing white flour, white finger millet has significant potential in the bakery industry. Studies have shown that bakery products made with KMR-340 are more acceptable to consumers compared to those made with brown finger millet (GPU 28) (Navyashree et al., 2022).

MATERIAL AND METHODS

Raw materials

The *Stevia rebaudiana* plant and commercially available processed stevia used for the Study were procured from Department

of Horticulture, GKVK, UAS, Bengaluru and Stevia World, Peenya, Bangalore respectively. Other ingredients for development of value-added products were procured from local markets of Bangalore.

Formulation and standardization of stevia-based biscuits.

The study was carried out at Department of Food Science and Nutrition, UAS, GKVK, Bengaluru. The biscuit was prepared by using white finger millet, stevia green leaves powder, processed white stevia powder, fat, milk powder and fructo-oligosaccharides. Control biscuit was developed with the same composition using sugar instead of stevia powders and refined wheat flour instead of white finger millet.

Sensory evaluation

Biscuits developed with different levels of stevia green leaves powder, processed white stevia powder and control biscuit were evaluated for sensory characteristics (appearance, flavour, colour, consistency, taste, and overall acceptability) by a panel of 21 semi-trained judges on a nine-point hedonic scale to select the best acceptable variation.

Nutrient and bioactive component analysis of stevia-based biscuits

Stevia based white finger millet biscuit was analyzed for nutritional composition which includes proximate composition such as moisture, ash, protein, fat and crude fiber (AOAC, 2005). Carbohydrate was obtained by differential method and energy by computation method. Antioxidant activity and Bio-active compounds such as total phenols and total flavonoids were determined using the standard laboratory procedures.

Storage quality evaluation

Control and stevia-based biscuits were packed in aluminum sliver foil pouches with nitrogen flush (modified atmospheric packaging technique) and without nitrogen flush and stored at ambient temperature (30±10 ºC). Samples were drawn every 15th day (0- 90th day) and analyzed for quality parameters viz., sensory evaluation, moisture content, free fatty acid, peroxide value and microbial load.

Microbial load

The microbial analysis of the developed stevia-based biscuit was carried out by standard plate count method using Nutrient Agar (NA) for bacteria, Eosin Methylene Blue Agar (EMBA) for coliforms and Martin's Rose Bengal Agar (MRBA) for fungi.

Statistical analysis

Suitable statistical analysis was carried out such as Means of triplicates, f test, DMRT to find the significant difference between the samples analyzed ($p< 0.05$).

RESULTS AND DISCUSSION

Formulation of stevia based sweet and salt biscuits

Sweet and salt biscuits were prepared by incorporating Stevia green leaves powder and commercially available processed white stevia powder at different levels (1, 1.25 and 1.5 g/recipe) and compared with its control. Sugar was completely replaced with two different stevia powders and refined wheat flour was completely replaced with white finger millet flour. The flow chart for preparation of Stevia based sweet and salt biscuits has been depicted in Fig.1. In GSB1 and WSB1, 30g of sugar was replaced with 1g of dehydrated stevia green leaves powder and commercially available processed white stevia powder respectively.

In GSB2 and WSB2, 30g of sugar was replaced with 1.25g of dehydrated stevia green leaves powder and commercially available processed white stevia powder and in GSB3 and WSB3, 30g of sugar was replaced with 1.5g of dehydrated stevia green leaves powder and commercially available processed white stevia powder respectively and all other ingredients were kept constant. The following table1depicts formulation of stevia -based biscuits. The products are shown in Plate 1.

Table 1. Formulation of stevia based sweet and salt biscuit

Sl. No.	Ingredients (g)	Control	GSB1	GSB2	GSB3	WSB1	WSB2	WSB3
1	Refined wheat flour	50	-	-	-	-	-	-
2	White Finger millet flour	-	50	50	50	50	50	50
3	Fat	25	20	20	20	20	20	20
4	Stevia	-	1	1.25	1.5	1	1.25	1.5
5	Sugar	30	-	-	-	-	-	-
6	Fructooligosaccharides	-	15	15	15	15	15	15
7	Carom and cumin	2	2	2	2	2	2	2
8	Milk	10	10	10	10	10	10	10
9	Salt	1.2	1.2	1.2	1.2	1.2	1.2	1.2
10	Baking soda	0.1	0.8	0.8	0.8	0.8	0.8	0.8

GSB1= stevia green leaves powder-based biscuit (1 g/recipe)
GSB2= stevia green leaves powder-based biscuit (1.25 g/recipe)
GSB3= stevia green leaves powder-based biscuit (1.5 g/recipe)
WSB1= processed white stevia powder-based biscuit (1 g/recipe)
WSB2= processed white stevia powder- based biscuit (1.25 g/recipe)

WSB3= processed white stevia powder-based biscuit (1.5 g/recipe)

Organoleptic evaluation of stevia based sweet and salt biscuits

Stevia green leaves powder and processed white stevia powder were used to make sweet and salt biscuits in varying proportions (1, 1.25and 1.5 g/recipe) and then compared with the control. Sugar was replaced with stevia powder at different levels of incorporation with two variations such as stevia green leaves powder and processed white stevia powder to develop sweet and salt biscuits. The formulated stevia based sweet and salt biscuits was subjected for sensory evaluation and results are presented in Table 2

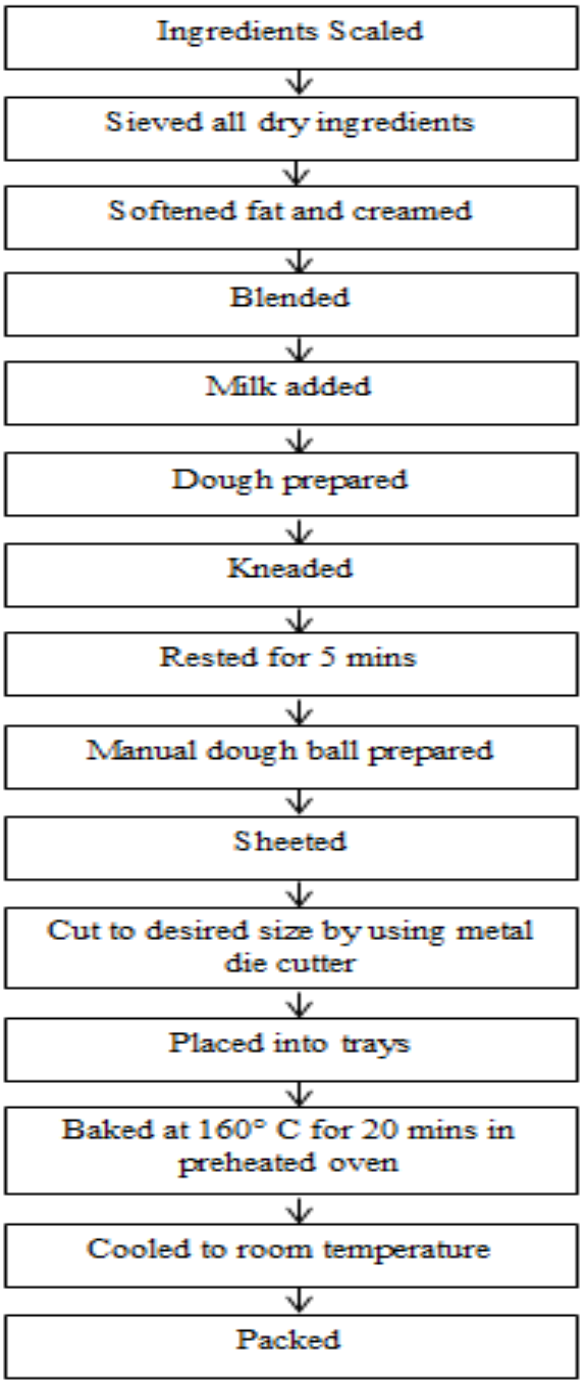


Fig. 1: Flow chart -Preparation of stevia based sweet and salt biscuit

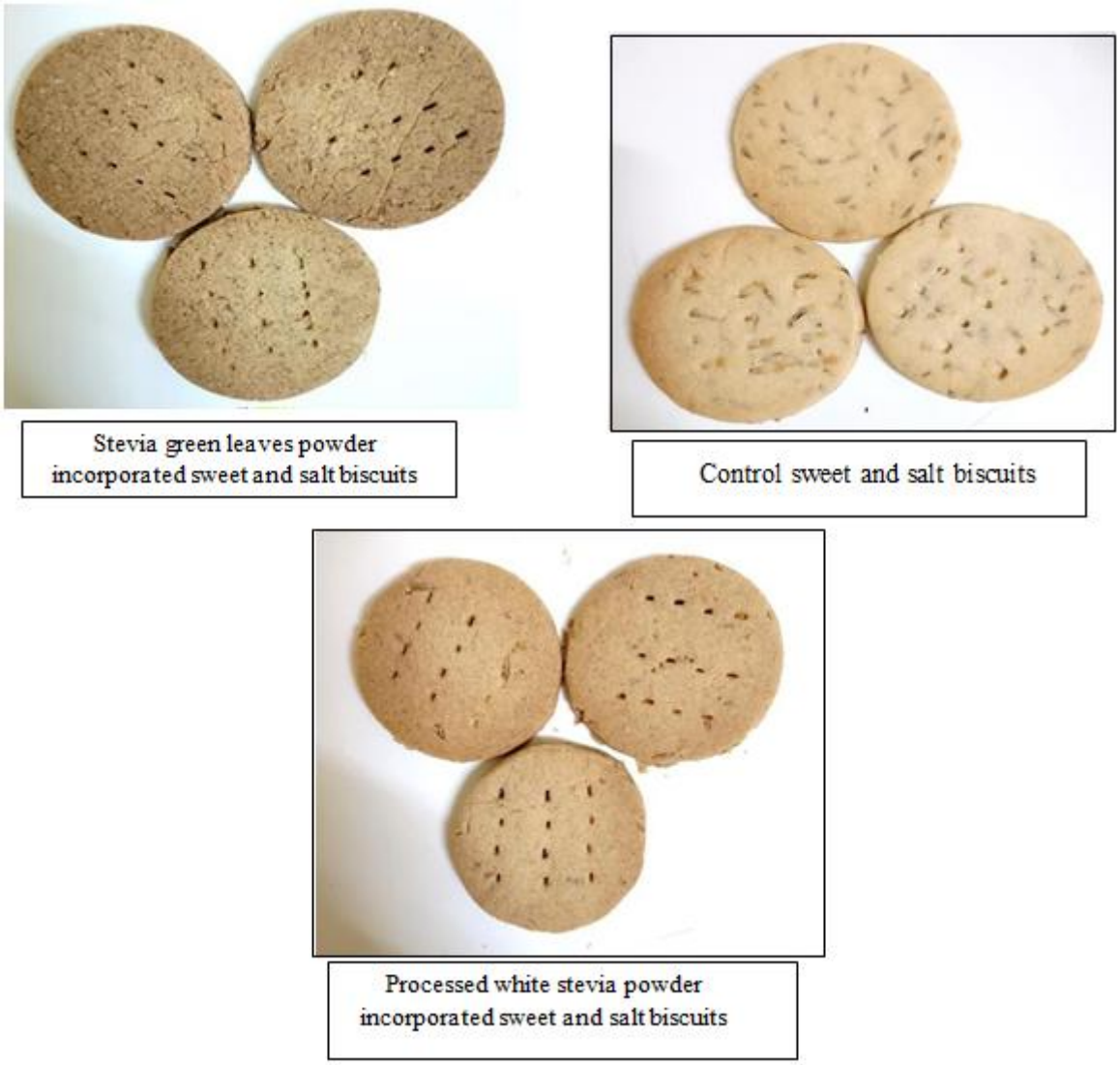


Plate 1: Stevia based sweet and salt biscuit

Sweet and salt biscuits were developed from a unique blend of ingredients to achieve their distinct flavor and texture. The primary components include refined wheat flour for control and white finger millet flour for experimental product providing the biscuit with its foundation structure. The addition of fat contributes to the biscuit's tenderness, while Stevia and sugar (control) were used for sweetness, catering to different taste preferences. Fructo-oligosaccharides are included as a source of prebiotic fiber, promoting gut health. The biscuits feature a delightful blend of carom and cumin seeds, enhancing their aroma and taste with a tint of spicy flavor. Milk was used to bind the ingredients together, further enriching the biscuit's texture and flavor profile. Salt and baking soda were included to balance the sweetness and facilitate the leavening process, resulting in a biscuit that combines the contrasting flavors of sweet and salt, with a satisfying crunchy texture.

Table 2: Organoleptic evaluation of stevia based sweet and salt biscuits

Variations	Organoleptic Characteristics				
	Appearance	Flavour	Taste	Texture	OAA
Control	8.50^{a}	8.58^{a}	8.62^{a}	8.62^{a}	8.60^{a}
GSB1	8.08^{ab}	7.45^{c}	7.50^{de}	7.52^{de}	7.37^{c}
GSB2	7.81^{b}	7.41^{c}	7.33^{de}	7.20^{e}	7.45^{c}
GSB3	7.9b	7.27^{c}	7.12^{e}	7.29^{e}	7.16^{c}
WSB1	8.47^{a}	8.45^{a}	8.41^{ab}	8.31^{ab}	8.35^{a}
WSB2	8.41^{a}	8.18^{ab}	8.08^{bc}	8.12^{bc}	7.97^{b}
WSB3	8.18^{ab}	7.91^{b}	7.75^{cd}	7.83^{cd}	7.85^{b}

Note: Similar superscripts indicate non-significant difference as per DMRT ($p = < 0.05$).
Control = Control sweet and salt biscuits
GSB1= stevia green leaves powder-based biscuit (1 g/recipe)

GSB2= stevia green leaves powder-based biscuit (1.25 g/recipe)
GSB3= stevia green leaves powder-based biscuit (1.5 g/recipe)
WSB1= processed white stevia powder-based biscuit (1 g/recipe)
WSB2= processed white stevia powder- based biscuit (1.25 g/recipe)
WSB3= processed white stevia powder-based biscuit (1.5 g/recipe)
All sensory qualities were higher for the control biscuits. The levels of incorporation with the highest scores were GSB2 with 1.25 g/recipe of stevia green leaves powder incorporation and WSB1 with 1 g/recipe of processed white stevia powder inclusion. GSB2 had appearance 7.81, flavour 7.41, taste 7.33, texture 7.20 and overall acceptability 7.45 which means like moderately whereas WSB1 had scores like appearance 8.47, flavour 8.45, taste 8.41, texture 8.31 and overall acceptability 8.35 which means like very much and the lowest scores were for GSB3 with 1.5 g/recipe of incorporation of stevia green leaves powder. At 5 percent level, it was determined that there was a statistically significant difference in the sensory attributes of appearance, flavour, taste, consistency and overall acceptability except for control.
Gupta et al. (2017) formulated low-calorie Biscuits from different level of stevia powder (0 per cent i.e., control sample and 5 to 7 g stevia powder) incorporation and were evaluated for their nutritional value and organoleptic properties. The product had the maximum approval, according to the sensory and nutritive analysis.

Nutritional composition of best accepted stevia based sweet and salt biscuits

Value-added stevia based sweet and salt biscuits were developed using six variations with different levels and two types of stevia powders. Different ratios of stevia green leaves powder and processed white stevia powder were used. Based on sensory evaluation, the products with the highest overall acceptability scores for 1.25 g/recipe in case of stevia green leaves powder and for 1 g/recipe in case of processed white stevia powder incorporation—were identified and studied in the lab for nutritional characteristics like moisture, ash, protein, fat, carbohydrate and energy. The results are shown in (Table 3).

Table 3: Nutrient composition of best accepted stevia-based sweet and salt biscuits per 100g

Nutrients	Sweet and salt Biscuits			F- value	CD at 5%
	Control	GSB	WSB		
Moisture (%)	5.10	6.65	5.95	*	0.30
Protein (g)	8.32	14.79	12.02	*	0.12
Fat (g)	21.2	13.15	12.95	*	0.84
Ash (g)	1.10	1.85	1.24	*	0.07
Crude fiber (g)	3.20	3.68	2.90	*	0.28
Carbohydrates[#] (g)	61.08	59.88	64.94	*	3.44
Energy[##] (kcal)	468.4	417.03	424.39	*	3.09

Note: *significant @ 5%; NS-Non-Significant; # By difference; ## By computation GSB: stevia green leaves powder biscuit; WSB: processed white stevia powder biscuit

When compared to products incorporated with the processed white stevia powder, the stevia green leaves powder-incorporated products (at 1.25 g/recipe) had the highest nutrient composition. According to Garcia–Serna *et al.* (2014) substitution of 100 percent sucrose with Stevia significantly ($p<0.05$) increased the percentage moisture of the biscuits.
Stevia green leaves powder biscuit contained 6.65 percent moisture, 14.79g of protein,1.85 g of total ash, 13.15 g of fat, 3.68 g of crude fiber, 59.88 g of carbohydrates and 417.03 kcal of energy per 100 g of the sample. When comparing the proximate composition of Stevia green leaves powder biscuit, processed white stevia powder biscuit and control, notable differences were not observed.
The results of the current study were consistent with the results of the study conducted by Gupta et al. (2017). According to statistical analysis, the difference in the nutritional composition of the control and between the developed samples was shown to be statistically significant at 5 percent level. Antioxidant activity and bioactive components of best accepted sweet and salt biscuits
The table 4 presents data on antioxidant activity and bioactive components, including total polyphenols and flavonoid content, found in Sweet and Salt Biscuits. These values are measured in milligrams per 100 grams (mg/100g) and are compared to control.

Table 4. Antioxidant activity and bioactive components of best accepted stevia-based sweet and salt biscuits per 100g

Particulars	Sweet and salt biscuit			F- value	CD at 5%
	Control	GSB	WSB		
Antioxidant (mg AAE) (mg/100g)	1.82	2.81	1.99	*	0.147
Total Polyphenols (mg GAE) (mg/100g)	1.73	3.76	2.80	*	0.16
Flavonoids (mg/100g)	71.7	93.6	78.5	*	0.98

Note: *significant @ 5%; NS-Non-Significant

In case of antioxidant activity, the stevia green leaves powder biscuit stands out with the highest levels of antioxidants at 2.81 mg AAE per 100g, followed by the processed white stevia biscuit at 1.99 mg AAE per 100g, while the control has the lowest antioxidant activity at 1.82 mg AAE per 100g.

Similar trends were observed in terms of total polyphenols, with the stevia green leaves powder biscuit having the highest concentration at 3.76 mg GAE per 100g, followed by the processed white stevia biscuit at 2.80 mg GAE per 100g and the control biscuit had 1.73 mg GAE per 100g.

Regarding flavonoids, the stevia green leaves powder biscuit leads with the highest content at 93.6 mg per 100g, followed by the processed white stevia biscuit at 78.5mg per 100g and the control depicting the lowest at 71.7 mg per 100g. The differences in antioxidant activity, flavonoid content and polyphenols content among the biscuits are statistically significant at 5% level of significance. The results of the current investigation into the bioactive component of biscuits were consistent with those of Islam (2021) study.

Shelf -life study of the best accepted stevia-based sweet and salt biscuits

Table 5 represent the Organoleptic properties of stevia based sweet and salt biscuits during shelf- life study using different packaging techniques. The best-accepted stevia-based biscuit and control samples were subjected to a shelf-life study by being stored in two different packages, namely aluminium silver foil pouches without nitrogen flush and aluminium silver foil pouches with nitrogen flush at room temperature and were observed for changes in quality parameters over a three-month storage period, including moisture, peroxide value, free fatty acid, microbial load and sensory attributes.

The Stevia-based biscuit underwent a comprehensive storage study involving sensory evaluation by a panel of 21 semi-trained members, utilizing a 9-point hedonic scale. Evaluations were conducted at various time points, including the 1st, 15th, 30th, 45th, 60th, 75th and 90th day of storage. The results of this study revealed a notable trend of decreasing sensory scores across multiple attributes, including appearance, flavor, taste, consistency and overall acceptability, as the storage period extended.

When comparing products packed in standard aluminium silver foil pouches without nitrogen flush and to those packed in aluminium silver foil pouches with a nitrogen flush, it becomes evident that the latter consistently achieved the highest sensory scores across all variations as well as the Control product. Further, biscuits have lost their visual appeal and flavour in both packaging condition on 75th day of storage studies. On the first day, the overall acceptability for the control biscuit was 8.60 without nitrogen flush packing and 8.60 with nitrogen flush packaging; however, on the 60th day, these scores dropped to 7.37 and 7.79, respectively.

The stevia green leaves powder-incorporated biscuit, on the other hand, received scores of 7.44 and 7.44 on the first day and 7.12 and 7.20 on the 60th day for packaging without nitrogen and packaging with nitrogen, respectively. The processed white stevia powder incorporated biscuit received scores of 8.35 and 8.35 on the first day and 7.89 and 7.99 on the 60th day respectively, for packaging without nitrogen and packaging with nitrogen. The significant decrease in the overall acceptability after 3 months of storage was mainly because of loss of crispiness of biscuits due to increase in the moisture content (kumarand Barmanray et al., 2007). The aesthetic attractiveness and flavour of the biscuits in both packing conditions started to fade after 75 days.

Table 5: Organoleptic properties of stevia based sweet and salt biscuits during shelf- life study using different packaging techniques

Products	Duration (day)	Appearance		Flavour		Taste		Aroma		Overall Acceptability	
		Without NF	With NF	Without NF	With NF	Without NF	With NF	Without NF	With NF	Without NF	With NF
Control	1st	8.50	8.50	8.58	8.58	8.62	8.62	8.62	8.62	8.60	8.60
	15th	8.25	8.31	8.12	8.28	8.22	8.31	8.11	8.25	8.26	8.39
	30th	8.02	8.12	7.84	8.10	8.12	8.25	8.03	8.16	8.06	8.11
	45th	7.92	8.06	7.28	7.99	8.03	8.11	7.98	8.00	7.95	7.85
	60th	7.88	7.95	7.10	7.62	7.53	7.94	7.63	7.88	7.37	7.79
	75th	Product spoilage has been noticed									
	90^{th}										
	F-value	*	*	*	*	*	*	*	*	*	*
	SEm±	0.14	0.11	0.15	0.13	0.15	0.14	0.14	0.12	0.15	0.13
	CDat5%	0.42	0.40	0.43	0.45	0.46	0.44	0.42	0.38	0.34	0.22
GSB	1st	7.83	7.83	7.41	7.41	7.33	7.33	7.37	7.37	7.44	7.44
	15th	7.55	7.81	7.32	7.35	7.29	7.30	7.19	7.22	7.40	7.43
	30th	7.46	7.65	7.22	7.26	7.23	7.26	7.13	7.19	7.39	7.41
	45th	7.23	7.36	7.19	7.20	7.11	7.13	7.02	7.08	7.33	7.35
	60th	6.89	7.10	7.03	7.16	7.02	7.10	7.00	7.03	7.12	7.20
	75th	Product spoilage has been noticed									

	90th										
	F-value	*	*	*	*	*	*	*	*	*	*
	SEm±	0.14	0.12	0.15	0.13	0.14	0.11	0.15	0.13	0.14	0.12
	CDat5%	0.41	0.40	0.42	0.33	0.48	0.22	0.45	0.42	0.42	0.28
WSB	1st	8.47	8.47	8.45	8.45	8.41	8.41	8.33	8.33	8.35	8.35
	15th	8.40	8.43	8.40	8.42	8.32	8.39	8.27	8.30	8.24	8.33
	30th	8.31	8.39	8.23	8.38	8.14	8.20	8.20	8.24	8.12	8.25
	45th	8.25	8.33	8.13	8.20	8.01	8.11	8.16	8.18	8.05	8.12
	60th	7.96	8.01	7.92	8.02	7.89	7.96	7.88	8.00	7.89	7.99
	75th	Product spoilage has been noticed									
	90th										
	F-value	*	*	*	*	*	*	*	*	*	*
	SEm±	0.15	0.15	0.14	0.13	0.14	0.15	0.15	0.14	0.13	0.11
	CDat5%	0.44	0.42	0.36	0.28	0.42	0.44	0.43	0.41	0.45	0.40

GSB: stevia green leaves powder biscuits WSB: processed white stevia powder biscuit NF: nitrogen flush

Moisture content of best accepted stevia-based sweet and salt biscuit during shelf-life study

Table 6 presents the moisture content of stevia-based sweet and salt biscuits stored under two different conditions: in aluminium foil pouches without a nitrogen flush and in aluminium foil pouches with a nitrogen flush.

Table 6: Moisture content of stevia-based sweet and salt biscuits during storage period using different packaging techniques

Duration (days)	**Aluminium foil pouch without nitrogen flush**			**Aluminium foil pouch with nitrogen flush**		
	Control	**GSB**	**WSB**	**Control**	**GSB**	**WSB**
1st	5.10^{e3}	6.65^{e1}	5.95^{e2}	5.10^{e3}	6.65^{e1}	5.95^{e2}
15th	6.25^{d3}	7.69^{d1}	6.99^{d2}	5.96^{d2}	7.23^{c3}	6.02^{d1}
30th	7.78^{c1}	8.71^{c2}	8.01^{c3}	7.08^{c3}	8.28^{b1}	7.33^{c2}
45th	8.59^{b1}	9.78^{b2}	9.61^{b3}	8.68^{b1}	9.36^{a3}	8.98^{b2}
60th	9.81^{a3}	10.98^{a2}	10.29^{a1}	9.00^{a3}	9.45^{a1}	9.21^{a2}

Note: Mean of triplicate samples, similar superscripts indicate non-significant difference as per DMRT ($p = < 0.05$), i.e., 1, 2, 3, depicts between samples and a, b, c depicts duration. GSB: stevia green leaves powder biscuits. WSB: processed white stevia powder biscuits

The duration of the storage period ranges from the 1st day to the 60th day. For biscuits in the aluminium foil pouch without nitrogen flush, the moisture content started at 5.10% on the 1st day for Control and 5.95% for WSB variation, while the GSB variation had a slightly higher moisture content of 6.65%. As the storage period progressed, there was a steady increase in moisture content for all three types of biscuits, reaching 9.81% for Control, 10.98% for GSB and 10.29% for WSB on the 60th day. There is a significant difference in moisture levels both among storage days and between the control and developed stevia-based biscuits.

On the other hand, biscuits stored in aluminium foil pouches with a nitrogen flush exhibited initial moisture content on the 1st day, with the Control, GSB and WSB variations having values of 5.10%, 6.65%and 5.95%, respectively. Over the period of 60 days, these biscuits showed a more gradual increase in moisture content, with the Control reaching 9.00%, GSB at 9.45% and WSB at 9.21%, by the 60th day.

The obtained data revels that the nitrogen flush packaging method effectively helps to reduce the rate of moisture absorption in the biscuits compared to without nitrogen flush. Managing moisture content is essential to ensure that the products remain fresh and appealing to consumers throughout their intended shelf life.

Islam. (2021) conducted a study on quality evaluation of therapeutic biscuits from red kidney bean, potato flour and stevia leaf. The result showed that the moisture content of formulated biscuits ranges between 3.29 and 3.88 percent and which is lowest compare to the current study.

Peroxide value (PV) and free fatty acid (FFA) of best accepted stevia-based sweet and salt biscuits during shelf-life study.

Table 7 depicts the peroxide values (mEqO2/kg) of different samples stored in different packaging conditions over a period of time. The packaging conditions include aluminum foil pouches without nitrogen flush and aluminum foil pouches with nitrogen flush. The duration of storage ranges from the 1st day to the 60th day.

In products packaged with both packaging material (nitrogen flush and without nitrogen flush), there is a notable increase in peroxide values with the extension of storage duration, indicating a significant difference in peroxide levels over time .In the absence of nitrogen flush packaging, the peroxide value for control increases from an initial value of 1.84 to 2.56. In contrast, with nitrogen flush packaging, it shows a relatively lower increase from 1.84 to 2.10. For both GSB and WSB products, without nitrogen flush packaging leads to an increase from initial values of 1.88 to 2.52 and 1.83 to 2.50, respectively. When nitrogen

flush packaging is employed, the increase is comparatively less, with values rising from 1.88 to 2.23 for GSB and from 1.83 to 2.16 for WSB.

According to the statistics, the products with aluminum foil pouches that have been nitrogen flushed typically have lower peroxide values than the products with no nitrogen flush packing. This suggests that the presence of nitrogen flush in the packaging can help reduce the oxidative degradation of the product.

A study by Chugh et al. (2015) reported similar findings, where the peroxide values of control biscuits and those formulated with polydextrose and guar gum ranged from 1.44 to 5.11 mEqO2/kg of fat and 1.24 to 3.43 mEqO2/kg of fat, respectively, over a 90-day storage period. The study also revealed that the free fatty acid (FFA) content was higher in control biscuits compared to those where fat had been partially replaced. Although the peroxide values increased with storage time, they remained within the permissible limits. Likewise, in the current study, the peroxide values were found to be within the standards set by FSSAI (2018), which specifies a maximum of 10 mEqO2/kg for oils.

Changes in free fatty acid content of biscuit, specifically the percentage of oleic acid, over a period of 60 days under different packaging conditions were presented in Table 7. The packaging conditions included an aluminum foil pouch without nitrogen flush and aluminum foil pouch with a nitrogen flush.

Significant differences in peroxide values and free fatty acid values are observed between the 1st and 60th days of storage. Additionally, there is a significant difference in peroxide values between the control products and the developed products.

For free fatty acid levels, there was no significant difference observed between the control and developed biscuits up to the 30th day. However, from the 45th day to the 60th day, a significant difference emerged in both packaging techniques, which included nitrogen flush and nitrogen-free packaging.

In case of nitrogen flush packing, at the beginning of the study (1st day), the free fatty acid content in the Control group was 0.28%, which remained relatively stable over the first 60 days, with values of 0.29%, 0.32%, 0.36%and 0.41% on the 15th, 30th, 45thand 60th day, respectively. In contrast, the GSB group, initially at 0.27% on the 1st day, displayed a different pattern. The free fatty acid content increased gradually over the first 30 days, with values of 0.28%, 0.30%, 0.34%and 0.40% on the respective days for 15^{th}, 30^{th}, 45^{th}, 60^{th} day for GSB group.

The WSB group started at 0.26% on the 1st day and showed a similar trend of increasing free fatty acid content over time, with values of 0.27%, 0.29%, 0.32%and 0.38% on the same 15th, 30th, 45th and 60th day, respectively.

In contrast, when packaging is done without nitrogen flush, the free fatty acid values in both variations (GSB and WSB), as well as the control group, are higher compared to products that are packaged with nitrogen flush.

During storage even though increased FFA value did not cause any rancid odour or off flavour. All the values were under permissible limit. However free fatty acid value of present study was within the standard specified by IS 1011: 2002 (1.2 % FFA) fourth revision.

The current research results pertaining to peroxide and free fatty acid levels align closely with those reported in the study conducted by Umesha et al. (2015).

Table 7: Peroxide value (mEqO2/kg) and Free fatty acid content (% oleic acid) of stevia based sweet and salt Biscuits during shelf-life study using different packaging techniques

Duration (days)	**Peroxide value (mEqO2/kg)**						**Free fatty acid content (% oleic acid)**					
	Aluminium foil pouch without nitrogen flush			**Aluminium foil pouch with nitrogen flush**			**Aluminium foil pouch without nitrogen flush**			**Aluminium foil pouch with nitrogen flush**		
	Control	**GSB**	**WSB**	**Control**	**GSB**	**WSB**	**Control**	**GSB**	**WSB**	**Control**	**GSB**	**WSB**
1st	1.84^{c1}	1.88^{b2}	1.83^{b1}	1.84^{c1}	1.88^{b2}	1.83^{b1}	0.28^{c2}	0.27^{c2}	0.26^{b2}	0.28^{c2}	0.27^{c2}	0.26^{b2}
15th	1.87^{c1}	1.92^{b2}	1.86^{b1}	1.85^{cd1}	1.90^{c2}	1.85^{cd1}	0.30^{c2}	0.30^{c2}	0.28^{b1}	0.29^{cd2}	0.28^{cd2}	0.27^{cd2}
30th	1.96^{c1}	1.97^{b1}	1.98^{b1}	1.88^{bc1}	1.94^{c3}	1.90^{c2}	0.34^{c2}	0.33^{c2}	0.31^{b2}	0.32^{bc2}	0.30^{bc2}	0.29^{bc2}
45th	2.20^{b3}	2.37^{b2}	2.10^{b1}	1.92^{b1}	2.01^{b3}	1.99^{b2}	0.42^{b1}	0.40^{b1}	0.38^{a2}	0.36^{ab2}	0.34^{b1}	0.32^{b1}
60th	2.56^{a1}	2.52^{a1}	2.50^{a2}	2.10^{a3}	2.23^{a1}	2.16^{a2}	0.50^{a1}	0.49^{a1}	0.42^{a2}	0.41^{a2}	0.40^{a2}	0.38^{a1}

Note: Mean of triplicate samples, similar superscripts indicate non-significant difference as per DMRT ($p = < 0.05$), i.e., 1, 2, 3, depicts between samples and a, b, c depicts duration. GSB: stevia green leaves powder biscuits WSB: processed white stevia powder biscuits

Microbial load during storage of stevia based sweet and salt biscuits

The microbial load, which refers to the quantity and diversity of microorganisms present in a product, can significantly affect its quality and safety. The introduction of microorganisms into products can occur at various stages of production and packaging. During storage, these microorganisms can multiply and potentially cause spoilage or even pose health risks to

consumers. Factors such as temperature, humidity, packaging materials and storage conditions play crucial role in determining the rate of microbial growth and product stability.

Table 8 depicts, the microbial population of stevia-based sweet and salt biscuits, evaluated over a period of 60 days under different storage conditions, with a focus on the effects of packaging materials with and without nitrogen flush. The microbial counts were expressed in colony-forming units per gram (cfu×10^2 /g).

Aluminium foil pouch packing without nitrogen flush

Among aluminium foil pouch without nitrogen flush packed products, initially the microbial counts were not detected in the control, stevia green leaves powder based (GSB) biscuits and processed white stevia powder-based biscuit (WSB). After 15 days of storage, bacterial counts were recorded. The control had a count of 1.92 cfu×10^2 /g, while GSB and WSB had counts of 1.35 and 1.23 cfu×10^2 /g, respectively. On the 30th day, bacterial counts increased. The control had 2.75 cfu×10^2 /g, GSB had 1.55 cfu×10^2 /g and WSB had 1.36 cfu×10^2 /g. Further, the microbial counts continued to rise. On 45th day, the control had 2.93 cfu×10^2 /g, GSB had 1.91 cfu×10^2 /g and WSB had 1.52 cfu×10^2 /g for bacteria. On the 60th day, bacterial counts further increased where, the control had 3.85 cfu×10^2/g, GSB had 1.98cfu×10^2 /g and WSB had 1.77 cfu×10^2 /g.

Among aluminium foil pouch without nitrogen flush packed products, initially fungal counts were not detected up to 30 days. On 30th day, the fungal counts were 0.30, 0.42 and 0.52 cfu×10^2 /g for control, GSB and WSB, respectively. Further, the microbial counts continued to rise and on 45th day, the fungal counts were 0.44, 0.57and 0.66 cfu×10^2 /g, and on 60th day, the fungal counts increased to 0.51, 0.62and 0.78 cfu×10^2 /g, respectively.

Coliform counts were not detected during the whole storage period among aluminium foil pouch without nitrogen flush packed products

Aluminium foil pouch packing with nitrogen flush

Among aluminium foil pouch with nitrogen flush packed products, initially similar to that of aluminium foil pouch without nitrogen flush packed products, the microbial counts were not detected in all categories. After 15 days, microbial counts remained not detected in all categories due to nitrogen flush packing technology. On the 30th day, bacterial counts started to appear. Bacterial counts were 1.6 cfu×10^2 /g for control, 1.53 cfu×10^2 /g for GSB and 1.2 cfu×10^2 /g for WSB. On the 45th day, bacterial counts slightly increased, the control had 1.8 cfu×10^2 /g, GSB had 1.73 cfu×10^2 /g and WSB had 1.50 cfu×10^2/g. By the 60th day, bacterial counts increased further and the control had 2.3 cfu×10^2 /g, GSB had 1.59 cfu×10^2 /g and WSB had 1.60 cfu×10^2 /g.

Among aluminium foil pouch with nitrogen flush packed products, on 30th day the fungal counts appeared and were recorded at 0.2, 0.18 and 0.19 cfu×10^2 /g, and further on 45th day, the fungal counts remained relatively low at 0.35, 0.19 and 0.23 cfu×102 /g, in case of control, GSB and WSB respectively. On 60th day, the fungal counts also increased, to 0.48, 0.32 and 0.53 cfu×10^2 /g for control, GSB and WSB, respectively

Coliform counts were not detected during the whole storage period among aluminium foil pouch with nitrogen flush packed products similar to that of aluminium foil pouch without nitrogen flush packed products

The developed products exhibit lower bacterial and fungal populations compared to the control. Additionally, across all three products and in both packaging techniques, the bacterial population was higher than the fungal population. Overall, the results indicate that the microbial population increased during storage in both packaging conditions, with higher counts in biscuits without nitrogen flush. The presence of nitrogen flush in the packaging appeared to delay microbial growth.

Table 8: Effect of storage on microbial population of best accepted stevia based sweet and salt biscuit using different packaging techniques

Packaging material	Storage days	Total Bacterial population (cfu×10^2/g)			Fungi population (cfu×10^2/g)			Coliform population (cfu/g)		
		Control	GSB	WSB	Control	GSB	WSB	Control	GSB	WSB
Aluminium foil pouch without nitrogen flush	Initial	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)
	15th	1.92(1.55)	1.35(1.36)	1.23(1.31)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)
	30th	2.75(1.80)	1.55(1.43)	1.36(1.36)	0.30(0.89)	0.42(0.95)	0.52(1.00)	0.00(0.71)	0.00(0.71)	0.00(0.71)
	45th	2.93(1.85)	1.91(1.55)	1.52(1.42)	0.44(0.96)	0.57(1.03)	0.66(1.07)	0.00(0.71)	0.00(0.71)	0.00(0.71)
	60th	3.85(2.85)	1.98(1.57)	1.77(1.51)	0.51(1.00)	0.62(1.05)	0.78(1.13)	0.00(0.71)	0.00(0.71)	0.00(0.71)
Aluminium foil pouch with	Initial	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)
	15th	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)	0.00(0.71)
	30th	1.60(1.	1.53(1.	1.20(1.	0.20((0.	0.18(0.	0.19(0.	0.00(0.	0.00(0.	0.00(0.

nitrogen flush		44)	42)	30)	83)	82)	83)	71)	71)	71)
	45th	1.80(1.51)	1.73(1.49)	1.50(1.41)	0.35(0.92)	0.19(0.83)	0.23(0.85)	0.00(0.71)	0.00(0.71)	0.00(0.71)
	60th	2.30(1.67)	1.81(1.59)	1.60(1.44)	0.48(0.98)	0.32(0.90)	0.53(1.01)	0.00(0.71)	0.00(0.71)	0.00(0.71)

GSB: stevia green leaves powder biscuits. WSB: processed white stevia powder biscuits
Values in parenthesis are transformed by $\sqrt{(x+0.5)}$

The absence of coliform counts throughout the study suggests that the products maintained good hygiene standards, especially in the presence of nitrogen flush. This simply indicated that total viable count of biscuits was within the microbial limit of 10^4 to less than 10^6 cfu/g of ready to eat food product (ICMSF, 2002). Stevia -based biscuits packaged in aluminium foil showed a significant increase in microbial load (2.14 × 106 cfu/g) for 30th day. Vacuum pack however showed best results in term of microbial activity by Ali *et al*. (2023).

CONCLUSION

People nowadays are more concerned about their health and are looking for natural alternatives that are both pleasant and nutritious. The development of low-calorie biscuits using white finger millet instead of refined wheat flour and stevia instead of sugar represents a significant step forward in the realm of healthier food options. The replacement of sugar with stevia, a natural sweetener with low calories, not only reduces the overall calorie count but also caters to the rising concerns over the negative health impacts of excessive sugar consumption. Furthermore, by replacing the refined wheat flour with white finger millet, a whole grain rich in dietary fibre, essential minerals and protein, which not only reduced the calorie content but have also enhanced the nutritional value of these products. In addition to the health benefits, the use of white finger millet and stevia could be the sustainable options in food industry.

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