Nutritional and Sensory Qualities of Cookies with the Incorporation of Blue Green Algae (*Spirulina platensis*), Cassava (*Manihot esculenta*) and Stevia (*Stevia rebaudiana*)

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Seema et al.: Development of Nutrient-Rich, Sugar-Free Cookies Using Spirulina, Cassava, and Stevia

Nutritional and health promoting products are in high demand in our daily lives, as many diseases can be prevented and managed by the use of therapeutic diets. Among the food items, cookies are easy to carry, store, and consume by individuals of all ages. These are also considered a preferred vehicle for enriching macro- and micronutrients. Cookies prepared in the present study contain nutritionally rich proteins and carbohydrates, i.e., *Spirulina platensis* and *Manihot esculenta*, respectively. A sugar-free herb, i.e., *Stevia rebaudiana*, is added as a substitute for table sugar. Varying amounts of *Spirulina platensis*, *Cassava esculenta*, and *Stevia rebaudiana* have been tested in cookies, with the other two ingredients held constant. Organoleptic studies revealed that cookies with 5.0 % *Spirulina platensis*, 10 % cassava powder, and 10 % stevia powder are acceptable. Nutritional analysis revealed that cookies containing these 3 ingredients in optimal concentrations are richer in protein and iron compared to those without them.

Key words: Cookies, *Manihot esculenta*, nutritional analysis, organoleptic evaluation, *Spirulina platensis*, *Stevia rebaudiana*

Health is a major concern in our daily lives, so items that promote good health are in high demand. Among the food products, cookies have a longer shelf life, are easy to transport, and are ready to eat at any time. The common ingredients in cookies include refined wheat flour, sugar, and butter. Wheat is generally used because of its rheological properties^[1]. Spirulina platensis, an edible algae, is considered a valuable raw material for producing nutritious products with significant health benefits. It contains sufficient amounts of single cells proteins (60 %-70 %), and all other essential nutrients like iron, magnesium, zinc, provitamin A, and the essential fatty acid gamma (γ) -linolenic acid. Cassava (Manihot esculenta), a staple food, is a root crop and a rich source of carbohydrates, primarily cultivated for human consumption^[2]. The plant is characterized by the presence of various secondary metabolites, including terpenoids, flavonoids, and polyphenolic compounds. Their many medicinal benefits include the inhibition of digestive enzymes such as lipases, glucosidase, and amylase, as well as anti-inflammatory and antiarthritic properties. These benefits could be useful

in the prevention and management of cardiovascular diseases, type 2 diabetes, and related comorbidities, such as metabolic syndrome^[3]. Cassava (Manihot esculenta) contains 89.9 % carbohydrates, 0.5 % protein, 8.7 % moisture, 0.34 % fat, 0.32 % ash (minerals), and 0.10 % crude fiber, highlighting its status as a carbohydrate-rich staple^[4]. The high sugar content of bakerygoods has been implicated to diabetes and obesity. Due to this factor, sugar is being replaced with substitute like stevia that tastes sweet^[5]. Stevia is being used in dental pastes, mouthwashes, herbal medications, tonics for diabetics, and daily use items. The leaves of Stevia rebaudiana have zero caloric value, are non-nutritive, and contain the highly potent polyol, sorbitol. When used as a sugar alternative in food, the diabetic individuals may be benefitted. Given the high nutritional value of spirulina, cassava and stevia, these ingredients

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are used as part of the raw materials for preparing cookies, replacing a portion of the wheat flour. The present study details the process of preparation, organoleptic and nutritional analysis of cookies using spirulina, cassava, and stevia powder as ingredients.

MATERIALS AND METHODS

Procurement of raw materials:

The authentic powders of blue green algae (*Spirulina platensis*), cassava (*Manihot esculenta*), were purchased from Healthy Hey Organic Store. The stevia powder (*Stevia rebaudiana*) was produced by Council Of Scientific And Industrial Research-Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP), Lucknow. The other ingredients like whole wheat flour, butter, baking powder, sugar, and table salt were purchased from local market of Lucknow. Mixture of butter and table sugar or stevia powder was mixed using multi mix hand mixer until creamy texture appeared. The fluffy cream was thoroughly mixed with the dry ingredients and kneaded to make soft dough. The

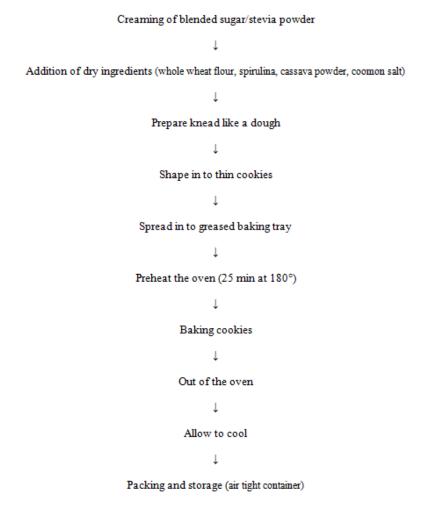
dough was then rolled on a platform and cut into round shape biscuit. The biscuits were placed in preheated oven (180°) for 25 min and after that cooled for 30 min at ambient temperature. The cooled biscuits were kept in air tight containers (fig. 1).

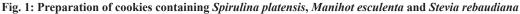
Physical appearance of prepared cookies:

Physical appearance of the cookies, including height, width, and thickness, were measured with digital vernier calipers with an accuracy of 0.01 mm, and the spread ratio was computed using the technique provided^[6].

Organoleptic analysis of prepared cookies:

Organoleptic or sensory evaluation was conducted using a panel consisting of 10 trained members, 10 semi-trained members, and 10 laypersons. The 9-point hedonic scale was employed for the hedonic rating^[7]. Sensory evaluation was based on various attributes such as color, flavor, texture, taste, and overall acceptability of the cookies (Table 1).





	Hedonic rating
9	Like extremely
8	Like very much
7	Like moderately
6	Like slightly
5	Neither like nor dislike
4	Dislike slightly
3	Dislike moderately
2	Dislike very much
1	Dislike extremely

Proximate analysis of prepared cookies:

Proximate analysis of the prepared cookies, including moisture content, carbohydrate content, ash content, fat content, protein content, fiber content, and sugar content, was determined according to standard methods^[8,9].

Statistical analysis:

The mean and Standard Deviations (SD) were used to express the data. One-way Analysis of Variance (ANOVA) was performed to compare the data using GraphPad Prism version 5.

RESULTS AND DISCUSSION

Table 2 shows the concentrations of cassava (Manihot esculenta) powder and other common ingredients at fixed levels in the dough for preparing biscuits T1, T2, and T3, with cassava powder amounts of 5 g, 10 g, and 15 g corresponding to 63 g, 58 g and 53 g of whole wheat flour, respectively. Sample T0 contains only 60 g of wheat flour. A fixed amount of stevia powder (10 g), spirulina powder (1 g), butter (20 g), baking powder (0.90 g), table sugar (19.00 g), and table salt (0.10 g) was added to the dough for samples T0-T3. An organoleptic evaluation of prepared samples T0-T3 was conducted, with sample T2 scoring the highest in all observed parameters as rated by the 15 panelists. Table 3 shows the mean score for each parameter assessed. The scores for the most acceptable sample, T2 were 8.50 ± 0.51 for color, 8.28 ± 0.61 for texture, 8.14 ± 0.36 for flavor, 8.21±0.57 for taste, and 8.35±0.49 for overall acceptability. In comparison, the scores for color, texture, flavor, taste, and overall acceptability in the other samples were 7.28±0.61, 7.07±0.26, 7.14±0.36, 7.07±0.61, and 7.00±0.55, respectively.

Table 4 presents the concentrations of spirulina (*Spirulina platensis*) powder and other common ingredients at fixed amounts in the dough for preparing biscuits T1, T2, and T3, with spirulina powder levels of 0.5 g, 1 g, and 1.5 g corresponding to 55.5 g, 55.0 g, and 54.5 g of whole wheat flour. Sample T0 contains only 60.00 g of wheat flour. A fixed amount of stevia powder (13 g), cassava powder (10 g), butter (20 g), baking powder (0.90 g), table sugar (19.00 g) and table salt (0.10 g) was added to the dough for samples T0 to T3 respectively.

An organoleptic evaluation of the prepared samples T0-T3 was conducted, with sample T1 scoring the highest across all observed parameters, as rated by the 15 panelists. Table 5 shows the mean score for each parameter assessed. The scores for the most acceptable sample, T1 were 7.92 ± 0.73 for color, 7.28 ± 0.72 for texture, 8.07 ± 0.82 for flavor, 8.06 ± 0.47 for taste, and 8.14 ± 0.36 for overall acceptability. In comparison, the scores for color, texture, flavor, taste, and overall acceptability in the other samples were 7.57 ± 0.51 , 7.14 ± 0.53 , 7.92 ± 0.61 , 7.28 ± 0.46 , and 7.07 ± 0.47 , respectively.

Table 6 presents the concentrations of stevia (*Stevia rebaudiana*) powder and other common ingredients in fixed amounts in the dough for preparing biscuits T1, T2 and T3, with stevia powder levels of 5 g, 10 g and 15 g corresponding to 63 g, 58 g and 53 g of whole wheat flour. Sample T0 contains only 60 g of wheat flour. A fixed amount of spirulina powder (1 g), cassava powder (10 g), butter (20 g), baking powder (0.90 g), table sugar (19 g) and table salt (0.10 g) was added to the dough for samples T0-T3 respectively.

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TABLE 2: VARYING CONCENTRATION OF CASSAVA IN FOOD PRODUCT

	Ingredients							
Sample code	Whole wheat flour (g)	Butter (g)	Baking powder (g)	Sugar (g)	Table salt (g)	Stevia powder (g)	Spirulina powder (g)	Cassava powder (g)
T0 (control)	60	20	0.9	19	0.1			
T1	63	20	0.9		0.1	10	1	5
T2	58	20	0.9		0.1	10	1	10
Т3	53	20	0.9		0.1	10	1	15

TABLE 3: ORGANOLEPTIC EVALUATION OF PREPARED SAMPLES

Sample code	Color	Texture	Flavor	Taste	Overall acceptability
ТО	7.28±0.61	7.07±0.26	7.14±0.36	7.07±0.61	7.00±0.55
T1	7.35±1.00	7.21±0.42	7.28±0.58	7.14±0.86	7.07±0.82
T2	8.50±0.51	8.28±0.61	8.14±0.36	8.21±0.57	8.351±0.49
Т3	6.42±0.51	6.92±0.47	6.50±0.51	6.42±0.51	6.50±0.51

TABLE 4: VARYING CONCENTRATION OF SPIRULINA IN FOOD PRODUCT

	Ingredients								
Sample code	Whole wheat flour (g)	Butter (g)	Baking powder (g)	Sugar (g)	Table salt (g)	Stevia powder (g)	Spirulina powder (g)	Cassava powder (g)	
T0 (control)	60	20	0.9	19	0.1	-	-	-	
T1	55.5	20	0.9	-	0.1	13	0.5	10	
T2	55	20	0.9	-	0.1	13	1	10	
Т3	54.5	20	0.9	-	0.1	13	1.5	10	

TABLE 5: ORGANOLEPTIC EVALUATION OF PREPARED COOKIES

	Parameters							
Sample code	Color	Texture	Flavor	Taste	Overall acceptability			
то	7.57±0.51	7.14±0.53	7.92±0.61	7.28±0.46	7.07 ±0.47			
T1	7.92±0.73	7.28±0.72	8.07±0.82	8.06±0.47	8.14 ±0.36			
Т2	6.50±0.51	6.28±0.46	6.64±0.63	6.14±0.53	6.21 ±0.80			
Т3	6.21±0.57	6.07±0.47	6.50±0.51	5.78±0.42	6.07 ±0.82			

TABLE 6: VARYING CONCENTRATION OF STEVIA IN FOOD PRODUCT

	Ingredients							
Sample code	Whole wheat flour (g)	Butter (g)	Baking powder (g)	Sugar (g)	Table salt (g)	Stevia powder (g)	Spirulina powder (g)	Cassava powder (g)
T0 (control)	60	20	0.9	19	0.1	-	-	-
T1	63	20	0.9	-	0.1	5	1	10
T2	58	20	0.9	-	0.1	10	1	10
Т3	53	20	0.9	-	0.1	15	1	10

An organoleptic evaluation of prepared samples T0-T3 was conducted with sample T2 scoring the highest in all observed parameters as rated by the 15 panelists. Table 7 shows the mean score for each parameter assessed. The scores for the most acceptable sample, T2, were 8.42±0.51 for color, 8.35±0.49 for texture, 8.57±0.51 for flavor, 8.50±0.51 for taste and 8.57±0.51 for overall acceptability. In comparison, the scores for color, texture, flavor, taste and overall acceptability in the other samples were 7.07±0.61, 7.00±0.67, 7.07±0.61, 7.07 ± 0.73 and 7.14 ± 0.66 respectively. The proximate analysis of the prepared cookies is shown in Table 8. The results indicate that the total carbohydrate content in the most acceptable sample (T2) was approximately 55.9 g, protein content around 8.47 g, total fiber about 6.62 g, and total fat content around 18 %, compared to 55.61 g, 8.20 g, 6.52 g, and 18 % in the control sample (T0) which lacked cassava powder. However, both calcium and iron levels were significantly higher in T2 compared to T0. The calcium and iron contents in T2 were calculated to be around 0.35 mg and 5.92 mg, respectively, while in T0, they were approximately 0.24 mg and 4.98 mg. The energy content in both T2 and T0 was nearly the same, at 360 kcal and 366 kcal, respectively. These observations reveal that cookies containing cassava are richer in calcium and iron.

The present study outlines the preparation of a biofortified cookie rich in protein, calcium, and iron. India has one of the largest food industries in the world, with its biscuit and cookie production ranking second only to the United States in global output^[10]. In developing nations, including India, people primarily consume starchy foods, which are a leading cause of Protein-Energy Malnutrition (PEM), contributing to disorders like kwashiorkor and marasmus in children. The growing population has created a significant demand for alternative sources of food, nutrition, and energy^[11]. Previous studies have demonstrated that spirulina (Spirulina platensis), cassava (Manihot esculenta), and stevia (Stevia rebaudiana) possess therapeutic properties that are beneficial to human health.

Sample code	Color	Texture	Flavor	Taste	Overall acceptability
Т0	7.07±0.61	7.00±0.67	7.07±0.61	7.07±0.73	7.14±0.66
T1	7.14±0.86	7.14±0.66	7.71±0.46	7.42±0.51	7.42±0.75
T2	8.42±0.51	8.35±0.49	8.57±0.51	8.50±0.51	8.57±0.51
Т3	6.85±0.53	6.50±0.51	6.64±0.49	6.57±0.51	6.71±0.46

TABLE 8: NUTRIENT ANALYSIS OF ACCEPTABLE PRODUCT

Nutrients/100 g	T0 (control)	TA (accepted)
Carbohydrate (g)	56.14	55.9
Protein (g)	8.2	8.47
Total Fats (g)	18.08	18.07
Total Fiber (g)	6.52	6.62
Iron (mg)	0.24	0.35
Calcium (mg)	498	5.92
Moisture (%)	3.2	9.17
Energy (Kcal)	360.8	366.57

In a study reported by Kumar et al.[12] stated that spirulina when used as a protein supplement (4.0 %)w/w) in the diet alleviated iron and protein deficiencies in albino rats. Individually supplementing malnourished rats with spirulina-fortified foods resulted in increased body weight, restored Hemoglobin (HB) levels, serum protein, albumin, serum iron, and hepcidin levels, and decreased iron-binding capacity. It also alleviated oxidative stress caused by malnutrition in the liver, spleen, and kidneys by reducing lipid peroxidation and enhancing the activities of superoxide dismutase and glutathione-metabolizing enzymes. In another study, the addition of spirulina-fortified foods reversed pathological changes such as fatty liver, thinning of cardiac muscle fibers, and degradation of intestinal villi caused by protein and iron deficiencies. Recently, Karizi et al.^[13] reported that Spirulina platensis demonstrated significant anti-atherogenic effects in treating type-2 diabetes individuals. In a study involving 60 individuals, participants were randomly assigned to either the Spirulina platensis group (2 g/d) or a placebo group for 3 mo while continuing metformin as part of their regular treatment. The study monitored HbA1c, fasting blood sugar, total cholesterol, and Low-Density Lipoprotein (LDL) levels, all of which decreased, while High-Density Lipoprotein (HDL) levels showed a slight increase, highlighting the anti-atherogenic potential of Spirulina platensis. Gogna et al.^[14] conducted a study which shows that Spirulina platensis is known to have a number of promising effects on the prevention of cancer, oxidative stress, obesity, diabetes, cardiovascular diseases, and anemia. Additionally, spirulina has been shown to be effective in alleviating muscle cramps due to its high protein and mineral content. Researchers have determined that the recommended safe dosage of spirulina for adults is approximately 3 g/d. Given its rich protein content and high nutrient bioavailability, spirulina has great potential for use in the development of value-added products and supplemental foods^[15]. According to Albtoosh et al.[16] showed through their research that using 30 young adult male albino rats that were randomly separated into 3 groups of 10 each, it was found that spirulina has a protective effect against cardiotoxicity induced by sodium arsenite. The histological changes linked to arsenite-treated animals are significantly decreased in spirulina treated groups. A study conducted by Daki et al.[17] found that phycocyanobilin and phycocyanin derived from spirulina and other plant extracts have been proven to enhance HDL cholesterol and reduce blood sugar, total cholesterol, and triglycerides in diabetic rats and also prevent the osteoporosis in diabetic individuals. The experiment done by cassava fibers are effective in preventing the onset of diabetes and obesity in rats induced by a High-Sugar Diet (HSD), suggesting their potential as a dietary intervention for managing metabolic disorders. Cassava fibre supplementation given to Swiss albino male mice either with or without a high sugar diets improved intraperitoneal Glucose Tolerance Test (ipGTT) following a glucose load (2 g/kg body weight). Studies have also shown that consuming a diet high in cassava fibers helps maintain glucose homeostasis and prevents the development of metabolic syndromes associated with diabetes and obesity. Prostate cancer cell proliferation and migration were significantly reduced when treated with a combination of stevia extracts and enzalutamide, but not when treated with enzalutamide alone, highlighting the potential synergistic effect of stevia in enhancing cancer treatment efficacy^[18]. Research has shown the beneficial role of stevia in reducing blood glucose levels in chronic diabetes, demonstrating its potential as a natural treatment option. In this experiment, a diabetic rat was administered 400 mg/kg body weight of dry Stevia rebaudiana extract orally. Throughout the trial, blood glucose levels were monitored monthly for 84 d, while urine production was examined on 1st d and 84th d. On 84th d, the lipid profile was also assessed. Significant hyperglycemia was observed in the diabetic rats compared to normal rats. Although rats treated with Stevia rebaudiana extract tended to have lower blood glucose levels than the diabetic control group, a significant difference was noted on 84th d of the trial^[19].

In the current study, cookies were standardized by replacing cane sugar with crude powder of *Stevia rebaudiana* and substituting highly saturated vegetable oils with butter from buffalo milk. The carbohydrate and protein contents were modified by partially replacing them with the crude powder of *Manihot esculenta* and *Spirulina platensis*, which are rich in protein, calcium, and iron. This formulation could be highly beneficial for the large diabetic population.

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Conflict of interest:

The authors declared no conflict of interests.

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