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Quality Evaluation of Antioxidants, and Calcium-rich Sucrose-free Biscuit Based on Spirulina and Unutilized Eggshell

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Abstract

In many low and middle-income countries, particularly in Africa and South Asia, calcium intake is still inadequate even below half of the requirements. Obesity and diabetes related complications and antioxidants deficiency are also increasing worldwide. Therefore, an experiment has been done to formulate antioxidant and calcium-enriched biscuit with spirulina and eggshell powder at varying proportions. Spirulina is a protein and antioxidant enriched algae, and eggshell is a calcium enriched unutilized food leftover. Five different biscuit samples S1, S2, S3, S4, and S5 were developed with different combinations of the mentioned ingredients. All five biscuit formulations were evaluated concerning their sensory characteristics along with the proximate and microbial analysis. Biscuits with spirulina powder and eggshell powder contain more protein, ash, fiber, antioxidants, and mineral contents in contrast to the control biscuit sample. The proximate analysis indicated that the incorporation of spirulina and eggshell powder enhanced the protein content from 10.91% to 12.78%, ash 3.35% to 3.90%, fat 11.05% to 11.52%, fiber 0.15% to 0.32%, calcium content from 755 mg to 2785.5 mg/100 g and total antioxidant contents from 9.6% to 27.3% of the biscuit samples in a significant ($p < 0.05$) way. Microbial analysis indicates that the incorporation of the ingredients does not affect the shelf-life of the produce. Moreover, the sensory evaluation of this study indicated that the 3% spirulina and 6% eggshell powder induced biscuit (S4) was more accepted. In conclusion, the incorporation of conjugated spirulina and eggshell powder has the potential to enhance the nutritional composition of conventional biscuits, especially calcium and antioxidants.

Keywords

Spirulina powder, Eggshell powder, Protein, Calcium, Functional food, Food security

Introduction

Biscuits are ubiquitous bakery products globally esteemed for their crisp texture and golden-brown color. Traditionally, they are crafted from wheat flour, butter, and sugar, often incorporating additional ingredients such as dried fruits, nuts, and food coloring. Biscuits are an economical, traditional, and convenient ready-to-eat snack favored by individuals across various age groups [1].

The world market production of wheat, the major raw material of biscuits, is 760 million tons in 2017 and expanding by 4.5% annually up to 2020 [2]. Concurrently, the global biscuit market, expanding at a compound annual growth rate of 5%, is projected to attain a valuation of USD 135 billion by 2023 [3]. This growth signifies robust industry potential, ensuring a stable supply of raw materials, thereby stabilizing costs and prices.

A critical environmental issue pertains to eggshell waste, a by-product of the egg industry, ranked as the 15th largest food-based pollution source by the environmental protection agency [4]. In 2018, global chicken egg production resulted in approximately 25,000 tons of eggshell waste [5]. They may contribute to a variety of environmental issues, including fungal growth [4]. This waste, frequently disposed of in landfills, contributes to soil contamination and fungal proliferation [6]. Notably, eggshells, rich in calcium carbonate (85% - 95%), can meet 50% of an adult female's daily calcium requirement, underscoring their potential as a dietary calcium source [7-9].

Calcium is indispensable for numerous physiological functions, including skeletal structure, muscle contraction, neural transmission, and hormone regulation [10]. Inadequate calcium intake can precipitate conditions such as rickets in children and osteopenia and osteoporosis in adults, affecting approximately 200 million individuals globally [10, 11]. Moreover, calcium deficiency accounts for around 29,000 maternal deaths annually, making it a significant contributor to maternal mortality [12-14].

Spirulina, a blue-green algae, is acclaimed for its high protein content and a plethora of nutrients, including vitamins (A, B, and C), carbohydrates, iron, calcium, and other essential minerals [15]. Along with pigments like phycobiliproteins (allophycocyanin, C-phycocyanin, and beta-carotene) linolenic acid (an important fatty acid) and chlorophyll A, is also a significant part of its excellent dietary content [16]. It possesses potent antioxidant properties, enhancing the body's antioxidant capacity and offering various health benefits, such as tumor suppression, anti-inflammatory effects, and cardiovascular health improvement [17].

Spirulina-enriched biscuits provide a higher protein content compared to commercial biscuits [18]. Regular consumption of spirulina can bolster the immune system, reduce inflammation, and enhance overall health, making it particularly beneficial for individuals with conditions like AIDS/HIV and arthritis [19, 20].

This research addresses the dual challenges of diabetes and obesity by developing sucrose-free biscuits suitable for diabetic and overweight individuals. In the USA, 30.7% of the population is overweight, 42.4% are obese, and 11.3% have diabetes [21]. Dietary interventions are crucial for managing these conditions, and our biscuits aim to provide a nutritious and palatable option without sugar [22, 23]. As dietary patterns significantly influence glycemic control, there is an increasing call for innovative and palatable food options tailored for individuals navigating the complexities of diabetes. Sugar has a direct influence on glycemic control [24].

Bangladesh, a developing country, grapples with significant issues related to protein-energy malnutrition and calcium deficiency [25]. Vulnerable groups, including pregnant women, children, and the elderly, necessitate increased protein and calcium intake. The production of protein and calcium-rich products industrially is costly [26]. Consequently, we utilize naturally grown spirulina and waste product eggshells to create affordable, nutrient-rich biscuits.

Previous research has investigated antioxidant-rich biscuits using various plant sources. Our study uniquely combines spirulina and eggshells to produce biscuits rich in both antioxidants and calcium. While other studies have utilized insects and fish as calcium sources, our approach leverages food waste, addressing both nutritional and environmental concerns.

Therefore, this study aims to augment the nutritional value and functionality of biscuits by incorporating spirulina and eggshells, rendering them rich in antioxidants and calcium. This innovation not only fulfills consumer demands and energy requirements but also contributes to waste management.

Materials and Methods

Materials

Bangladesh Council of Scientific and Industrial Research (BCSIR), in Dhaka, provided the spirulina powder. Wheat flour, powder milk, soya oil, salt, sugar, and baking soda were collected from the local market of Jashore district. Eggshells were collected from the nearest restaurant of Jashore University of Science and Technology. Eggshells were then grounded into powdered form by using standard procedure.

Methods

Preparation of eggshell powder

Collected eggshells were washed, sun-dried, and sterilized using an autoclave at 121 ˚C, Dried in the microwave-oven at 180 ˚C. Then grinded using grinders and stored in sealed poly bags between 25 - 27 ˚C for further analysis. The process is illastarated on figure 1.

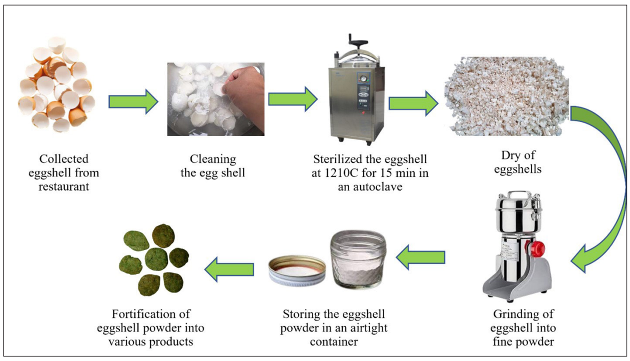

Figure 1: Procedure for eggshell powder preparation.

The manufacturing process of biscuit

The flour was mixed to prepare biscuits according to the ratios as presented in table 1. The ratio of the ingredients was taken in considering the product meets a significant portion of daily calcium and antioxidant requirements as well as it becomes delicious. The procedure outlined by Krystyjan et al. [27] was used to make biscuit dough and baked it with some modifications. The biscuit dough was baked at 180 ˚C for 25 - 30 min in a convection oven (TIN - TN 50). The resulting

Table 1: Compositional ratio of five formulations of biscuit.

Ingredient	S1	S2	S3	S4	S5
Flour (g)	100	94	93	91	96
Spirulina (g)	0	3	1.5	3	1.5
Eggshell (g)	0	3	6	6	3
Milk (g)	5	5	5	5	5
Soyabean oil (ml)	16	16	16	16	16
Water (ml)	50	50	50	50	50
Sugar substitute* (g)	7	7	7	7	7
Salt	0.5	0.5	0.5	0.5	0.5
Baking soda (g)	0.6	0.6	0.6	0.6	0.6

Note: * = Zero calory sugar substitute (sucralose, lactose, and crospovidone), commercially available as zero Cal tablet i.e., used for diabetic patient.

bread samples were allowed to cool in pan for 10 min, then inverted onto a cooling plate before being placed in polyethylene bags and kept at room temperature until future testing. The process is shown in figure 2.

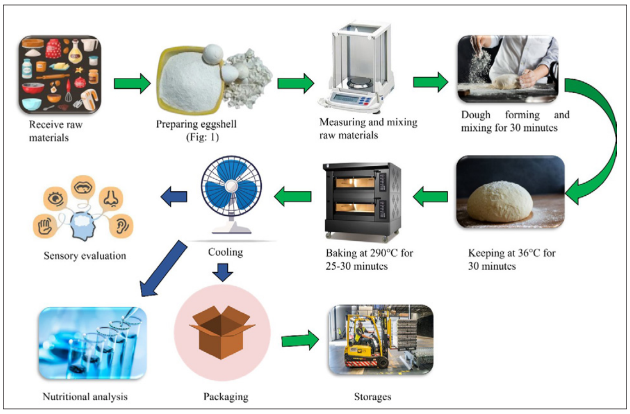

Figure 2: Procedure of biscuits preparation.

Compositional ratio of five formulations of biscuit

Using different proportions of wheat flour, spirulina, eggshell, milk, soya oil, water, sugar (Zero calories), salt and baking soda along with spirulina powder, we have developed five biscuits (S1, S2, S3, S4, and S5).

Determination of physical characteristics

After 3 h of cooling at room temperature, a digital micrometer (0.001 mm, Mitutoyo, Minoto-Ku, Tokyo, Japan) was used to measure the diameter (mm) and thickness (mm) [28]. The spread ratio was estimated by dividing diameter by the number of using the accepted procedure (AACCI 74 - 09.01), the biscuits' hardness and fracturability were assessed by texture analyzer (TA-XT plus, Stable Microsystems, England) [29].

Proximate compositions of developed five different biscuits samples

Proximate composition and biochemical determinations were done in the laboratory of Nutrition and Food Technology, Jashore University of Science and Technology which is cross-checked by Institute of Food Science and Technology (IFST), Bangladesh Council for Scientific and Industrial Research (BCSIR), Dhaka.

Determination of moisture contents

By using oven drying techniques, the moisture content of samples is identified. Following the oven drying process, the following equation was used to determine the samples' moisture contents [30].

$$\%Moisture\ content = \frac{W1-W2}{W}\times 100$$

Where: W_1 = Initial weight of the Petri dish containing sample; W_2 = Final weight of the Petri dish containing sample; and W = Sample weight.

Determination of protein content

The amount of protein was measured by the basic AOAC Kjeldhal procedure. The total organic nitrogen was determined. To convert percent nitrogen to percent crude protein, a factor was used.

Since nitrogen makes up 16% of most proteins, the conversion factor is 6.25 (100/16 = 6.25). The percentages of nitrogen as well as protein in the samples were determined by the following equation:

$$\%\ Nitrogen = \frac{(S-B)\times N\times 0.014\times 6.25\times 100}{W}$$

Where: S = Titration reading for sample; B = Titration reading for blank; N = Strength of hydrochloric acid; and W = Sample weight.

$$\%Crude\quad protein = \%Nitrogen\times 6.25$$

Determination of fat content

The total fat content of the samples was identified by (dry weight basis) organic solvent extraction methods followed by AOAC [30]. Using a Soxhlet extractor in this method, the crude fat content was determined $1-\frac{As}{Ac}\times 100$ by calculating as follows:

$$\%Fat = \frac{(A-B)}{W}\times 100$$

Where: A = Weight of conical flask + extracted oil; B = Weight of conical flask; and W = Sample weight.

Determination of ash content

The ash content of a sample is usually determined by the official method of ash [30]. In this method, the loss of weight of the samples is used to determine the ash contents by the following equation.

$$\%\ Ash\ content = \frac{W2-W1}{W}\times 100$$

Where: W_1 = Weight of the crucible + ash; W_2 = Weight of the crucible + sample; and W = Weight of the sample.

Determination of fiber content

The crude fiber content of a dry sample was measured by using the method of AOAC [30]. In this method, fiber content was calculated as follows:

$$\%Fiber = \frac{A - B}{W} \times 100$$

Where: A = Weight of crucible + residue; B = Weight of crucible + residue after putting in muffle furnace; and W = Sample weight.

Determination of total carbohydrate content

The content of available carbohydrates in the samples was calculated using subtraction, which involves taking the sample's total values for moisture, ash, protein, and fat out (per 100 gm) as followed.

$$\%Carbohydrate = 100 - (Moisture + protein + ash)$$

Determination of antioxidant activity

Total antioxidant: Total antioxidant can be determined by using a 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical-scavenging assay. DPPH of the sample was determined spectrophotometrically according to an updated technique that Espin, Soler-Rivas, and Wichers [31] detailed. It is a discoloration assay, and to evaluate it, antioxidant is added to an ethanol-based DPPH solution, and then the absorbance at 517 nm measured. The capability to scavenger the DPPH radicals was measured using the following equation [31].

The DPPH scavenging effect (%) = $1 - \frac{As}{Ac} \times 100$

Where: As = The absorbance in the presence of sample; and Ac = The absorbance of the control (0.5 ml DPPH solution of 0.1 M without sample).

Total phenolic content (TPC): TPC of biscuits samples was identified using the Folin–Ciocalteu method with some modifications [32]. A discoloration assay that is assessed by at a wavelength of 765 nm, absorbance was measured spectrophotometrically (Spark 10 M; Tecan, Männedorf, Switzerland). A calibration curve was created using gallic acid solutions (GA Avantor Performance Materials, Gliwice, Poland). The amount of a TPC present in the biscuits was measured in terms of gallic acid equivalents (GAE), which were represented as mg of GAE per 100 g of sample.

Mineral analysis of developed biscuit samples

After wet digestion, minerals such as phosphorus, calcium, magnesium, zinc and iron were measured using an atomic absorption spectrophotometer (Varian AA240; Varian Inc., Australia) in accordance with the pertinent procedures outlined in Official Methods of Analysis AOAC. Model 410 flame photometer (Sherwood Scientific Ltd., UK) was used to measure the amounts of sodium and potassium.

Microbial analysis

The S4 sample was subjected to the Fawole and Oso technique for the aerobic plate count. The aerobic plate count was performed on the S4 sample using the method described by Fawole and Oso. Ten grams of each sample were obtained and homogenized aseptically for about two minutes in a blender (Japan, India) with 90 ml of sterile distilled water. Test tubes were filled with serial dilutions (using 9 ml of sterile distilled water and 1 ml of homogenates). Plate count agar (PCA, Oxoid) was used to plate one milliliter of each dilution on sterile Petri dishes, and then, the dishes were kept at 37 °C for 24 - 36 h and count by the following formula.

$$Cfu/g = \frac{Number\ of\ colonies\ counted}{Dilution \times Volume\ of\ sample}$$

Log cfu/g samples were used to express counts of the visible colonies. The study was carried out on the first, 30th, and 60th days of production. The samples of the 30th, and 60th days were preserved in air-tight polyethylene packages.

Sensory evaluation

A customer's Hedonic rating test was used to evaluate the acceptance of developed items by consumers using the results of a testing panel of 20 people (45% men, and 55% women). Before evaluation of the samples, participants were asked for any allergies to commonly used ingredients, and written consent to participate in the study was taken. On a scale of typically nine points, from "like extremely" to "dislike excessively," the panelists were asked to score their acceptance of the product. Using a nine-point hedonic scale, samples were evaluated for appearance, flavor, texture, taste, color, mouth feel, crispiness, and overall quality. Each point on the scale is given a numerical value to examine the outcome. The scores received by each sample are then compared with the scores received by other samples in the series. The scale was set up in a way that: 9 = Like extremely; 1 = Dislike extremely.

Statistical analysis

The data was examined using IBM SPSS Statistics version 22 software. The mean and standard error of the mean were used to display the data of three replicates in the tables. Analysis of variance (ANOVA) was used to evaluate whether statistically significant differences existed between the sample means at a 5% level of significance.

Results

Physical and textural properties of biscuits

Weight, diameter, thickness, and spread ratio values were significantly affected by spirulina and eggshell powder addition. Table 2 presents the physical properties of biscuits prepared with different levels of spirulina and eggshell.

Table 2: Physical properties of biscuit samples enriched with spirulina powder and eggshell powder.

Physical properties	Weight (g)	Diameter (mm)	Thickness (mm)	Spread ratio
S1	8.80 ± 0.45	45.87 ± 0.32	5.40 ± 0.12	8.494444
S2	9.01 ± 0.22	46.12 ± 0.31	5.80 ± 0.33	7.951724
S3	8.13 ± 0.26	46.82 ± 0.33	5.10 ± 0.16	9.180392
S4	7.54 ± 0.68	46.36 ± 0.35	4.94 ± 0.17	9.384615
S5	8.97 ± 0.39	47.21 ± 0.45	5.36 ± 0.13	8.807836

Proximate analysis of developed biscuits

The proximate compositions (moisture content, lipid, fiber, protein, carbohydrate, total antioxidants, TPC) of five different biscuits were analyzed. Each experiment was conducted three times to get an accurate result. The mean value of three experiments is given below in table 3. Here our selected sample's (S4) percentages for moisture, ash, carbohydrate, protein, fat, fiber, total antioxidant status (DPPH), and TPC were 7.52, 3.66, 64.81, 12.49, 11.52, 0.32, 27.32% and 0.42 (mg GAE/g of dm) and for control sample (S1) they were 7.01, 2.90, 78.45, 8.74, 10.91, 0.13, 9.63%, and 0.16 (mg GAE/g of dm) respectively. Figure 3 illustrates the major nutrients in the pic chart.

Mineral composition of biscuit samples

Table 4 displays the results for the mineral profiles of wheat flour and eggshell powder. The results showed that calcium made up most of the eggshell powder (2785 mg/100 g) followed by sodium, potassium, magnesium, iron, zinc, and manganese.

Microbial load analysis

Average concentrations of total microbial growth of sample S1 and S4 were observed at 0 day, 30th, and 60th day. Both samples (control and selected sample) show similar results. The detailed result is given below in table 5.

Sensory characteristics

The sensorial evaluations for appearance, crispness, taste, odor, texture, mouth feels, and overall acceptability of biscuits are shown in table 6. From table 6 it is seen that sample S4 got the highest overall acceptability score. It is also seen that product S4 secured the highest mean score for appearance, odor, texture, and overall acceptability are 8.60, 8.50, 8.70, and 8.90 respectively whereas the scores for product S1 are 8.60, 8.50, 8.50, and 8.64 respectively.

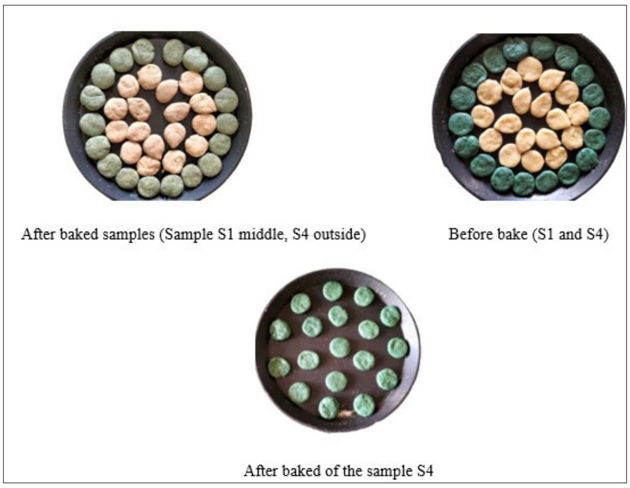

Figure 3: Biscuits samples before and after baking.

Table 5: Total microbial growth of developed biscuit samples.

Biscuit sample	Dilution	Microbial counts on different days (cfu/g)		
		0 day	30th day	60th day
S1	1/10	NG	3.3×10^2	8.4×10^3
	1/100	NG	2.8×10^2	7×10^3
	1/1000	NG	2×10^2	8×10^3
S4	1/10	NG	3×10^2	7×10^3
	1/100	NG	2.6×10^2	6.7×10^3
	1/1000	NG	2×10^2	10^4

Note: NG = No growth.

Discussion

Composite flour is commonly used in a variety of foods to boost the quantity of key micronutrients, improve the nutritional quality of the food, and offer greater health benefits

Table 3: Proximate composition (mean ± SEM) of the five developed biscuit samples.

Biscuit	Moisture (%)	Ash (%)	Carbohydrate (%)	Protein (%)	Fat (%)	Fiber (%)	Antioxidant status (%)	TPC (mg GAE/g dm)
S1	7.01 ± 0.45	2.90 ± 0.1	70.3 ± 0.26	8.74 ± 0.25	10.91 ± 0.11	0.13 ± 0.11	9.63 ± 1.15	0.16 ± 0.03
S2	7.12 ± 0.35	3.35 ± 0.06	64.99 ± 0.47	12.78 ± 0.14	11.05 ± 0.1	0.32 ± 0.05	17.40 ± 0.72	0.45 ± 0.03
S3	7.83 ± 0.35	3.90 ± 0.1	66.72 ± 0.76	10.91 ± 0.12	11.16 ± 0.15	0.15 ± 0.01	25.36 ± 0.17	0.27 ± 0.02
S4	7.52 ± 0.43	3.66 ± 0.06	64.52 ± 0.65	12.49 ± 0.13	11.52 ± 0.05	0.29 ± 0.05	27.32 ± 0.61	0.42 ± 0.04
S5	7.87 ± 0.60	3.51 ± 0.1	64.35 ± 1.06	12.85 ± 0.25	11.17 ± 0.10	0.25 ± 0.01	18.06 ± 0.11	0.31 ± 0.02

Table 4: Mineral composition of biscuit samples; (mg/100 g; mean ± SD; n = 3).

Minerals	S1	S2	S3	S4	S5
Calcium	755 ± 224	1378.11 ± 358	2645.52 ± 706	2785.52 ± 721	1264.12 ± 137
Sodium	201.1 ± 61	487.50 ± 11.30	486.32 ± 17.30	501.1 ± 14.30	488.50 ± 15
Potassium	86.47 ± 4.21	125.49 ± 8.60	118.85 ± 11.36	136.47 ± 9.43	105.91 ± 6.81
Magnesium	28.71 ± 11.54	41.09 ± 18.63	40.83 ± 28.57	68.83 ± 39.20	38.19 ± 8.71
Iron	1.10 ± 0.07	1.16 ± 0.16	0.89 ± 0.03	1.30 ± 0.03	0.96 ± 0.2
Zinc	0.95 ± 0.03	0.92 ± 0.13	1.01 ± 0.03	1.81 ± 0.04	1.12 ± 0.30
Manganese	0.83 ± 0.03	1.04 ± 0.05	1.13 ± 0.02	1.13 ± 0.02	1.02 ± 0.04
Copper	0.18 ± 0.06	0.21 ± 0.06	0.26 ± 0.02	0.35 ± 0.02	0.21 ± 0.08

Table 6: Sensory analysis of biscuits with different proportions of spirulina powder and eggshell powder.

Biscuit	Appearance	Taste	Odor	Texture	Mouth feels	Crispiness	Overall acceptability
S1	8.60 ± 0.51	8.50 ± 0.85	8.40 ± 0.51	8.50 ± 0.70	8.44 ± 0.51	8.51 ± 0.65	8.64 ± 0.52
S2	7.53 ± 0.52	7.90 ± 0.56	7.47 ± 0.70	7.92 ± 0.73	7.52 ± 0.80	7.52 ± 0.63	7.71 ± 0.48
S3	7.62 ± 0.51	6.80 ± 0.63	7.32 ± 0.48	7.44 ± 0.70	7.36 ± 0.58	7.40 ± 0.71	7.20 ± 0.42
S4	8.67 ± 0.37	8.30 ± 0.52	8.50 ± 0.67	8.70 ± 0.48	8.02 ± 0.27	8.16 ± 0.46	8.90 ± 0.31
S5	7.40 ± 0.84	7.30 ± 0.67	7.00 ± 0.81	7.22 ± 0.66	7.17 ± 0.41	7.11 ± 0.61	7.33 ± 0.67

Note: Data presented as mean ± standard deviation.

with low risk. However, the food made from composite flour must be compatible, and ingredients must be readily available and accessible without significantly affecting the sensory properties or consumer acceptance of the food.

The application of spirulina and eggshell powder considerably changed the figures for diameter, thickness, and spread ratio (Table 2). The diameter of the biscuit increased after the spirulina and eggshell were added. The high protein and mineral content of spirulina as well as the flour mixes' eggshell content when compared to the control, which increases the diameter, may be responsible for this outcome. Sunflower seed flour was employed in other trials that used food additives and revealed an increase in diameter [33], spirulina powder [34], and mandarin peel powder [35]. Spread ratio is a key factor in determining the quality of biscuits. For superior biscuits, higher spread ratio values are preferred [36]. It was shown that when thickness decreased, the spreading ratio of biscuit samples rose. The biscuits sample S4 had the highest spread ratio (9.38). In comparison to the control biscuits, all concentrations of spirulina and eggshell enhanced the spread ratio values (8.49).

Proximate analysis data shows that the intended biscuit with spirulina and eggshell powder has a greater nutritional profile (protein, ash, fat, fiber, and antioxidants) than conventional. The moisture content of control and developed sample was analyzed as 7.01% and 7.52%, respectively. A slight increase was observed in moisture content of biscuits with increased eggshell powder. Ndife et al. [37] found similar results in their study which is from 8.55 to 6.95%. The high moisture content in bread products is affected by the ingredients used and the water added to the dough. Because of its better storage stability, the biscuit sample's lower moisture level is preferred. The high ability of flours employed in formulations to bind water determines the moisture content of biscuits. A decline was observed from previous studies in moisture content of biscuits with increased eggshell powder and vice versa for spirulina. Food shelf life is greatly influenced by its moisture level; the lower the moisture content, the greater the food's storage stability [38]. The study's findings were consistent with those found in the results of Marcinkowska-Lesiak et al. [39].

Product S4 also contained more protein (12.49%) than control S1 product (8.74%) due to the aid of spirulina powder with it. Ndife et al. [37] found decrease of protein for 5% eggshell flour was 12.14% to 11.43% and Singh et al. [40] found protein increase for 7% spirulina addition from 5.60% to 13.72%. Their findings are quite similar. The increased protein content of biscuits is influenced by the higher protein content of spirulina powder as well as the protein content of the raw materials used. By adding 3.0% spirulina powder to the biscuit's protein formula, both the protein content and the amino acid content (such as alanine, lysine, aspartic acid, glutamic acid, arginine, threonine, serine, valine, and tryptophan) increased. The most significant amino acids among these were isoleucine, leucine, methionine, lysine, threonine, phenylalanine, valine, and tryptophan [41].

Khan et al. [9] reported an increase in ash levels in biscuit samples enriched with spirulina and eggshell powder. Product S4 contained more ash than control S1 due to adding spirulina, and eggshell powder with it. In comparison to conventional or controlled biscuits, the preferred intended biscuit S4 prepared with spirulina, and eggshell powder has a high concentration of fat i.e., 10.91%, 11.52% respectively. Ndife et al. [37] found a slight increase of ash i.e., 4.73% to 5.27% for 5% eggshell flour and Singh et al. [40] got from 1.50% to 2.50% increase for using 7% spirulina flour. The results in their study are like our findings. This is more significant because fats in baked goods have a variety of uses, including lubricating the mass, increasing calorie content, enhancing flavor and color, and encouraging gas retention in biscuits to increase volume and softness.

The fiber content increased to 0.32% on the desired S4 biscuit with spirulina and eggshell when compared to the control S1 (0.13%). Ndife et al. [37] found decrease of fiber content i.e., 7.83% to 7.65% for 5% eggshell flour and according to Singh et al. [40] findings from 7% spirulina flour is 2.20% and 4.50% fiber for control and spirulina used sample. Spirulina is a good source of dietary fiber. Which supports the findings.

Phycocyanin, carotene and xanthophyll pigments, tocopherol, and phenolic compounds are among the compounds present in spirulina which are responsible for the antioxidant enriched our biscuits with a higher antioxidant ratio. Our preferred product, S4 contained more antioxidants compared to control biscuits i.e., DPPH and TPC were 27.32% and 0.42 (mg GAE/g of dm) and for control sample (S1) they were 9.63% and 0.16 (mg GAE/g of dm) respectively. Marcinkowska-Lesiak et al. [39] found the same results in their study i.e., DPPH and TPC were 29.25% and 0.42 (mg GAE/g of dm) for 3% spirulina powder and control sample were 8.27% and 0.14 (mg GAE/g of dm) respectively, and 9.59% DPPH by using crude date fibers. Arnold et al. [42] found there's no significant change in antioxidants for using eggshell powder in bread which supports our findings. Antioxidant activity reduces free radicals in the human body and has effects on health benefits. Antioxidants are substances that neutralize free radicals and defend against diseases that harm people, including cancer, cholesterol, arthritis, Parkinson's, Alzheimer's, and other age-related issues. Complex anti-radical detoxification defenses are also present in mammalian cells.

This variation in calcium content in the present study is mainly due to the addition of eggshell powder in different ratios. Results for the mineral content of biscuit samples are displayed in table 3. The results represented that calcium made up most of its composition (2785.52 mg/100 g) followed by sodium, potassium, magnesium, zinc, iron, and manganese i.e., 501.10 mg, 136.47 mg, 68.83 mg, 1.81 mg, 1.30 mg, and 1.13 mg respectively. While compared to S4 sample, the control sample had the lowest amounts of these minerals. The mineral composition of the powdered eggshell has been identified by numerous researchers.

Average concentrations of total microbial growth of sample S4 in airtight package biscuit were not found at 0 day. The sample's colony count was 3×10^2 cfu/g on the 30th day and 7×10^3 cfu/g on the 60th day for 10 times dilution. The same result was shown for 100 and 1000-times dilution. This indicates the airtight package keeps it contribution to shelf life increasing at a desired time duration. These counts are low and within safe ranges, thus they cannot be considered health risks. The overall microbe count of blended foods typically ranges from 0 to 10^4 cfu/g, which is still below permissible limits when compared to microbial criteria. It has been well reported that grain and soybean products with total plate counts greater than 10^6 cfu/g are regarded as microbially hazardous [43-45]. And according to WHO guidelines, the maximum allowed limits for baked goods (cake, biscuit, and bread) for total plate count is 2.0 × 105 cfu/g. Based on our experiment, no treatment surpassed 10^6 cfu/g.

Our exploration into the creation of a biscuit tailored for both diabetic and obese individuals address the intricate challenge of harmonizing nutritional requirements. The careful selection of ingredients becomes paramount to strike this delicate balance, guiding us towards crafting a biscuit that is not only palatable but also aligned with the dietary needs of a dual demographic.

Our focus on nutritive ingredients and the incorporation of non-nutritive sweeteners aligns with established strategies for diabetic-friendly nutrition. Simultaneously, our commitment to addressing the needs of individuals dealing with obesity involves a keen awareness of caloric density. Our biscuit aims to be a satisfying option without contributing excessively to the overall caloric intake, thereby facilitating weight management in obese individuals.

Simultaneously, the formulation considers the needs of individuals managing obesity by providing a snack option that, while satisfying, does not compromise their efforts toward caloric restriction. This dual approach is vital, recognizing the interplay between glycemic control and weight management in the overall health and well-being of individuals with comorbid diabetes and obesity. Our study signifies a step forward in the development of a biscuit that serves as a nutrient-dense and satisfying option for individuals managing both diabetes and obesity. By addressing the nutritional needs of this dual demographic, we aspire to contribute to the broader discourse on health-conscious food innovation.

The mean scores for appearance, taste, odor, texture, mouth feels, crispiness, and overall acceptability of developed different types of biscuits are given in table 4. Using a hedonic scale rating, it was decided that the product S4 was satisfactorily accepted. In the case of all perimeter preferences among the products showed that there was considerable ($p < 0.05$) difference in acceptability among the products. The highest obtained mean scores for taste, mouth feels, and crispiness were 8.50, 8.44, and 8.51 respectively, for control product S1, whereas 8.30, 8.02, and 8.16 for product S4.

The picture of the samples S1 and S4 is shown in figure 3. It is also seen that product S4 secured the highest mean score for appearance, odor, texture, and overall acceptability, which were 8.60, 8.50, 8.70, and 8.90, respectively, which are almost like control product S1 and followed by other products.

Conclusion

Biscuit Products enriched with spirulina powder and eggshell powder showed improved protein, fiber, ash, calcium, iron, sodium, zinc, magnesium, and antioxidant content than the control product. An increase in these bioactive ingredients promoted its qualities as a functional food especially for the calcium deficient obese and diabetic people. The addition of spirulina at 3% level and eggshell at 6% level, according to sensory evaluation, produced the top marks in terms of appearance, texture, odor, and general acceptability in all biscuit samples produced.

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Conflict of Interest

None.

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