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Development and Analysis of Cookies Enriched with Soy Flour and Oats for Enhanced Protein, Fiber and Antioxidant Activity

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ABSTRACT

The study aimed to develop protein- and fiber-enriched cookies by partially substituting wheat flour with soy flour and oats. Four formulations (S1–S4) were prepared with varying substitution levels to assess their nutritional, functional, sensory, and microbiological qualities. The formulation S4 exhibited the highest nutrient composition, including protein (16.79%), fat (12.65%), and fiber (4.82%), along with elevated mineral contents zinc (2.35 mg/100 g), iron (5.79 mg/100 g), magnesium (116.20 mg/100 g), and calcium (91.73 mg/100 g). Phytochemical analysis revealed that S4 also contained the greatest flavonoid (0.54 mg QE/g) and phenolic (0.18 mg GAE/g) concentrations, as well as the highest DPPH radical scavenging activity (55.04%). Among sensory parameters, S2 was most preferred for color, flavor, taste, and overall acceptability. After one month of storage, S4 demonstrated the lowest total viable count, indicating good microbial stability. Overall, the incorporation of soy flour and oats significantly enhanced the nutritional and antioxidant properties of cookies, suggesting their potential as functional and health-promoting snack alternatives.

1- Introduction

Cookies are among the most widely consumed bakery products globally due to their convenience, affordability, and extended shelf stability. However, conventional cookies are predominantly formulated using refined wheat flour, which is poor in phytochemicals and dietary fiber yet rich in rapidly digestible carbohydrates, rendering them nutritionally inadequate [1-4]. Given the increasing consumer preference for foods that combine convenience with health benefits, the reformulation of traditional bakery items has emerged as a significant focus within the functional food industry [5]. Such reformulations aim to enhance nutritional value by modifying macronutrient composition, incorporating bioactive components, or replacing refined ingredients with nutrient-dense alternatives [6].

Recent dietary recommendations advocate a higher intake of dietary fiber approximately 20–35 g per day to prevent and manage chronic non-communicable diseases such as type 2 diabetes, hypertension, and colorectal cancer [7]. Accordingly, substituting refined wheat flour with fiber-rich and protein-dense materials offers a practical approach to developing healthier baked products. Soy flour represents an ideal supplement due to its rich composition of high-quality proteins, essential amino acids, lipids, vitamins, minerals, and dietary fiber [8-9]. Moreover, soy protein exhibits digestibility and amino acid balance comparable to animal proteins and has been associated with reduced risks of cardiovascular disease, osteoporosis, and metabolic disorders [10]. From both nutritional and functional perspectives, soy flour incorporation enhances the protein content, emulsification capacity, and water-binding properties of baked goods [11].

Oats are another valuable cereal ingredient with well-established functional and health-promoting attributes [12]. They possess a balanced amino acid profile, complex carbohydrates, dietary fiber, and bioactive constituents such as phenolic compounds, carotenoids, phytic acid, and sterols [13-15]. Particularly, β-glucan a soluble fiber unique to oats has been extensively documented for its physiological benefits, including attenuation of hypercholesterolemia, glycemic regulation, and weight management [16-17]. The European Food Safety Authority (EFSA) recognizes β-glucan for its scientifically substantiated role in maintaining normal blood cholesterol levels [18-19].

Although numerous studies have examined the incorporation of either soy or oat flour into bakery formulations, limited investigations have explored their combined application and synergistic effects on the functional, phytochemical, and microbiological properties of cookies. Addressing this gap, the present study was designed to develop composite cookies by partially substituting wheat flour with varying proportions of soy flour and oats. The primary objective was to evaluate the influence of these substitutions on the proximate composition, phytochemical content, antioxidant activity, and microbial stability of the final products. This work contributes novel insights into the formulation of nutritionally enhanced and functionally improved cookies that align with contemporary health-oriented dietary trends.

2-MATERIALS AND METHODS

2.1 Sample collection

Soyabean, oatmeal, sesame seeds, egg, wheat flour, sugar, butter, milk powder, baking powder, salt, and ammonium bicarbonate were collected from the local market (Dinajpur Bangladesh).

2.2 Chemical and reagents

For various experiments, the following analytical grade reagents are required: sodium carbonate, hydrochloric acid, sodium nitrite, aluminum chloride, DPPH, sodium dihydrogen phosphate dehydrate, potassium ferricyanide, ferric chloride, disodium hydrogen phosphate dehydrate, trichloroacetic acid, ferrous sulfate heptahydrate, sulfuric acid, phenol, sodium potassium tartrate, and methanol.

2.3 Preparation of soybean flour

Soybean flour was prepared following a modified procedure of Mugabo et al. [20]. Mature soybean seeds were sorted to remove broken seeds and foreign materials, then rinsed under potable tap water for 2 min and drained. The cleaned seeds were cooked in boiling water (100 °C) for 25 min in a stainless-steel cooking vessel (Model SW-45, IKAWerke GmbH & Co. KG, Germany). After cooking, the seeds were spread on a stainless-steel tray and pan-roasted. Roasting was carried out in an electric roasting pan (Model RTP-600, LabTech Instruments, India) preheated to 65 °C (60–70 °C range). The cooked seeds were added, and the pan temperature was gradually raised and maintained such that the seed surface temperature reached approximately 120 °C. The seeds were stirred continuously using a stainless-steel spatula to ensure uniform roasting for about 12 min, until a uniform light-brown colour developed, indicating complete roasting. The internal temperature of representative seeds was monitored using a digital probe thermometer (Model HI98509 Checktemp, Hanna Instruments, Italy) and recorded at 2-min intervals. After roasting, the seeds were transferred to a desiccator (Model D-420, Thermo Fisher Scientific, USA) and cooled to room temperature (25 ± 2 °C) for 30 min. The cooled seeds were milled into fine powder using a high-speed blender (Model BL2216, Kenwood, UK) for 2 min and sieved through a 60-mesh sieve (250 µm) using a mechanical sieve shaker (Model AS 200 basic, Retsch GmbH, Germany). The resulting flour was packed in moisture-resistant, high-density polyethylene (HDPE) bags, sealed, and stored at 25 ± 5 °C in a dark, dry environment until further use.

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Table 1: Formulation of the cookies

Ingredients	S1	S2	S3	S4
Wheat flour (gm)	100	80	70	60
Soy Flour (gm)	-	10	15	20
Oat’s flake (gm)	-	10	15	20
White sesame Seed (gm)	-	1	1	1
Baking soda (gm)	0.4	0.4	0.4	0.4
Ammonium bicarbonate (gm)	1	1	1	1
Milk powder (gm)	10	10	10	10
Sugar (gm)	20	20	20	20
Butter (gm)	20	20	20	20
Egg (gm)	25	25	25	25

2.4 Preparation of cookies

Four different cookie formulations were prepared using varying proportions of soybean flour and oat flakes, as shown in Table 1. The required quantities of each flour were weighed using a digital balance (Model BL-220H, Citizen Scale India Pvt. Ltd., India) and thoroughly mixed in a stainless-steel bowl. To this mixture, 0.4 g sodium bicarbonate (baking soda), 1.0 g ammonium bicarbonate, 1.0 g sesame seeds, and 10.0 g milk powder were added to form the dry blend. Separately, one fresh whole egg (50 g) and 20.0 g granulated sugar were beaten together using an electric hand mixer (Model HM-133, Bajaj Electricals Ltd., India) until the mixture became uniform and slightly frothy. Next, 20.0 g unsalted butter was added and blended for approximately 3 min at medium speed to form a smooth, creamy paste. The wet mixture was then gradually incorporated into the dry mixture with continuous mixing until a homogeneous dough was obtained. Oat flakes were then gently folded into the dough for even distribution. The dough was rolled to a uniform thickness of 1.0 cm using a stainless-steel rolling pin and cut into circular shapes (diameter 5.0 cm) using a cookie cutter. Additional sesame seeds (0.5 g per cookie) were sprinkled on the upper surface. The shaped cookies were placed on a greased baking tray lined with parchment paper and baked in a preheated laboratory convection oven (Model DGH-9420A, Desco Medical India, India) at 160 °C for 12 min. After baking, cookies were cooled to room temperature (25 ± 2 °C) for 30 min on a wire rack. The cooled cookies were wrapped in high-density polyethylene (HDPE) film (0.05 mm thickness) and stored at ambient laboratory conditions (25 ± 5 °C; relative humidity 50 ± 5 %) until further analyses.

2.5 Proximate Composition Analysis

The proximate composition of both the soybean–oat flour blends and the baked cookies including moisture, crude protein, crude fat, crude fiber, and total ash contents was determined following the standard methods described by the Association of Official Analytical Chemists (AOAC, 2016) [21]. Specifically, moisture content was determined by the oven-drying method (Method No. 925.10), crude protein by the Kjeldahl method (Method No. 920.87) using a nitrogen conversion factor of 6.25, crude fat by the Soxhlet extraction method (Method No. 920.85), ash by incineration in a muffle furnace at 550 °C (Method No. 923.03), and crude fiber by acid–alkali digestion (Method No. 962.09). To determine the total carbohydrate, the amounts of moisture, ash, fiber, protein, and fat were subtracted from 100. The color characteristics of the flour and cookie samples were measured using a portable colorimeter (Model BC-200, Biaobase Biodustry Co. Ltd., Shandong, China). Before measurement, the instrument was calibrated using a standard white tile (L* = 100, a* = 0, b* = 0). The CIE Lab* color space parameters were recorded, where L* indicates lightness (0 = black, 100 = white), a* represents redness/greenness (+a = red, –a = green), and b* represents yellowness/blueness (+b = yellow, –b = blue).

2.6 Preparation of Extract

The extraction of bioactive compounds was performed with slight modifications to the procedure described by Halim et al. [22]. Approximately 1.0 g of finely powdered sample was weighed using a digital analytical balance (Model BL-220H, Citizen Scale India Pvt. Ltd., India) and extracted with 20 mL of 80% methanol (v/v), maintaining a solid-to-solvent ratio of 1:20 (w/v). The mixture was placed in a 100 mL conical flask, tightly covered with aluminum foil to minimize solvent evaporation, and stirred continuously at room temperature (25 ± 2 °C) for 20 min using a magnetic stirrer (Model 78-1, Remi Instruments, India). After extraction, the suspension was centrifuged at 4000 rpm for 15 min using a centrifuge (Model TD5A, Changsha Xiangzhi

Centrifuge Instrument Co., Ltd., China). The resulting supernatant was filtered through Whatman No. 1 filter paper (GE Healthcare, UK) to obtain a clear extract. The filtrate was collected into 15 mL falcon tubes and stored at 4 ± 1 °C in a laboratory refrigerator (Model BCD-90, Haier Group Corp., China) until further analysis

2.7 Determination of total phenolic content (TPC)

Halim et al. [23] made minor adjustments to the TPC of the sample extracts. We measured the absorbance of the clarified liquid at 725 nm (UV-1800, Shimadzu, Japan). The TPC was indicated in gallic acid equivalents per gram of material.

2.8 Determination of total flavonoid content (TFC)

TFC was calculated using the colorimetric technique outlined by Rahman et al. [24], with a few minor adjustments. The absorbance (UV-1800, Shimadzu, Japan) was measured at 510 nm. The TFC, which is measured in milligrams of quercetin equivalents (mg QCE) per gram of material, was determined using a standard curve for quercetin.

2.9 Determination of DPPH activity

The method by Tahosin et al. [25] was utilized to evaluate the samples' ability to neutralize free radicals. The antioxidant activity was indicated as the percentage of DPPH radical inhibition. It was determined using this formula: DPPH scavenging capacity (%) = (A control - A sample) / (A control) × 100 [Measured absorbance at 515 nm]

2.10 Determination of FRAP Activity

To determine the FRAP activity, 0.5 mL of the extracted sample was mixed with 2.5 mL of potassium ferricyanide (1% w/w) and phosphate buffer (2.5 mL, 0.2M, pH 6.6) in a test tube. A spectrophotometer was used to measure the absorbance of the mixture at 700 nm, and the FRAP activity was expressed as micro MTrolox Equivalents (TE) er g of material [24].

2.11 Microbial analysis

The total viable count (TVC) of microorganisms in the samples was determined using the standard spread plate technique as described by Hartman [26] with minor modifications. A nutrient agar medium (Hi Media Laboratories Pvt. Ltd., India) was prepared according to the manufacturer's instructions and sterilized by autoclaving at 121 °C for 15 min under a pressure of 103 kPa (15 lb in^{-2}) using an autoclave (Model MLS-3751L, Sanyo Electric Co. Ltd., Japan). Serial dilutions of each sample were prepared aseptically by transferring 1 mL of the original sample into 9 mL of sterile distilled water in a test tube to obtain a 10^{-1} dilution. Further serial dilutions were made up to 10^{-6} as required. From each dilution, 0.1 mL aliquots were pipetted onto sterile Petri dishes (90 mm diameter) containing solidified nutrient agar. The sample was evenly spread across the agar surface using a flame-sterilized glass spreader to ensure uniform distribution. The inoculated plates were inverted and incubated at 37 ± 1 °C for 24 h in a laboratory incubator (Model MIR-154, Panasonic Healthcare Co., Japan). After incubation, plates exhibiting 30–300 colonies were selected for enumeration, and the total viable count was calculated as colony-forming units per gram (CFU/g) of sample. Plates that were overgrown or contaminated were excluded from the count.

2.12 Storage study

After being freshly prepared, the microbiological load of the cookies was examined. Subsequently, the cookies were stored at ambient temperature (25 ± 5°C) to ensure no defects.

2.13 Sensory Evaluation of the Cookies

Twenty panelists from Hajee Mohammad Danesh Science and Technology University in Bangladesh, who were semi-trained, evaluated the cookies based on their sensory perception. Every food sample received a unique three-digit random number that was assigned to it through a random coding process. The sample's randomized order was shown to each panelist one at a time. Panelists were requested to evaluate the coded samples based on their enjoyment level for each sensory attribute, including color, aroma, texture, taste, and general acceptability.

2.14 Statistical analysis

All experiments were performed in triplicate (n = 3), and the results are expressed as mean ± standard deviation (SD). The experimental data were subjected to one-way analysis of variance (ANOVA) to determine statistically significant differences among the different formulations at a 95% confidence level. When significant differences were detected ($p < 0.05$), Duncan's Multiple Range Test (DMRT) was applied as a post hoc test to identify pairwise differences between treatment means. Statistical analyses were carried out using SPSS statistical software (Version 22.0, IBM Corp., Armonk, NY, USA).

3- RESULTS AND DISCUSSION

3.1 Physical characteristics of cookies

Table 2 presents the physical characteristics of the soy–oat composite cookies, including weight, thickness, width, and spread ratio, which differed significantly among formulations ($p < 0.05$). Cookies containing 20% soybean flour and 20% oat flakes were the thickest (8.87 mm) and widest (50.57mm), while their spread ratio (5.70–5.79) was slightly lower than that of the control (5.88), indicating reduced dough flow during baking. These differences can be attributed to the partial substitution of wheat flour with protein- and fiber-rich soybean and oat flours, which diluted gluten content and altered dough viscosity, limiting gas retention and restricting spread [27-28]. Additionally, the higher water-binding capacity of the added flours likely contributed to increased thickness and dimensional stability, consistent with observations in other composite cookies [29-30]. From a functional perspective, the interplay between reduced gluten cross-linking, increased protein denaturation, and fiber-induced water absorption during baking explains the thicker, moderately fewer spread cookies, suggesting that soy–oat incorporation can enhance structural integrity and textural quality.

Table 2. Physical characteristics of cookies

Parameters	S1	S2	S3	S4
Weight (g)	18.17±0.06^{c}	18.63±0.15^{b}	18.80±0.10^{ab}	19.00±0.10^{a}
Width (mm)	50.17±0.06^{c}	50.37±0.12^{b}	50.40±0.10^{b}	50.57±0.06^{a}
Thickness (mm)	8.53±0.06^{c}	8.70±0.10^{b}	8.73±0.06^{b}	8.87±0.06^{a}
Spread ratio	5.88±0.04^{a}	5.79±0.07^{ab}	5.77±0.04^{b}	5.70±0.04^{b}

Here, S1=Control, S2=10% Soy flour+10% Oat, S3=15% Soy flour+15% Oat, S4=20% Soy flour+20% Oat.$^{a-c}$ Different superscript alphabets indicate significant differences among the formulations ($p < 0.05$)

3.2 Color values

Table 3 presents the color characteristics (L*, a*, b*) of the baked cookies, which differed significantly among formulations ($p < 0.05$). Cookies containing 20% soybean flour and 20% oat flakes exhibited slightly lower lightness (L* = 34.76–43.46) and higher redness (a* = 9.97–10.82) and yellowness (b* = 19.23–21.94) compared to the control, resulting in a visibly darker hue. The observed color changes can be attributed to Maillard reactions between reducing sugars and proteins during baking, as well as starch caramelization and dextrinization, which are accelerated by heat and contribute to browning [31]. Factors such as water activity, pH, sugar content, and types of amino compounds further modulate these reactions, affecting the final color intensity [32]. Similar patterns were reported by El Salous et al. [33] and Suriya et al. [30] for cookies formulated with wheat flour and pitaya (Hylocereus undatus) peel flour, indicating that the incorporation of protein- and fiber-rich non-wheat ingredients can consistently enhance redness and yellowness while reducing lightness in baked products.

Table 3. Colorimetry values for prepared cookies

Sample	L*	a*	b*
S1	41.97±0.60^{b}	9.54±0.03^{c}	19.23±0.02^{d}
S2	43.46±0.24^{a}	10.82±0.03^{a}	21.09±0.03^{c}
S3	37.35±0.02^{c}	9.97±0.02^{b}	21.94±0.02^{b}
S4	34.76±0.03^{d}	10.83±0.02^{a}	23.13±0.02^{a}

Here, S1=Control, S2=10% Soy flour+10% Oat, S3=15% Soy flour+15% Oat, S4=20% Soy flour+20% Oat. $^{a-d}$ Different superscript alphabets indicate significant differences among the formulations ($p < 0.05$)

3.3 Proximate compositions

The proximate composition of the developed cookies is presented in Table 4, showing significant variation ($p < 0.05$) among samples. Moisture content ranged from 3.61 ± 0.01% (S4) to 4.33 ± 0.02%, highlighting the influence of ingredient composition on water retention. The lower moisture content observed in S4 is likely due to the higher proportion of oats and soybeans, which have substantial water-binding capacities through protein-water interactions and fiber hydration [34]. Proteins form hydrogen bonds with water, whereas dietary fibers, particularly soluble fibers, trap water within their matrix, collectively reducing free water in the dough [35]. These differences were statistically significant ($p < 0.05$), confirming that formulation plays a critical role in moisture retention. Comparable studies report similar trends Aly et al. [36] observed moisture levels between 3.07% and 3.52% in cookies with varying oat content, with the highest moisture in 50% oat cookies and the lowest in 75% oats plus 5% cinnamon. Giram et al. [37] reported maximum moisture of 4.23%, while Asadi et al. [38] documented up to 5% in cream-free cookies. Variations among studies may reflect differences in flour types, fat content, sugar composition, and baking parameters, illustrating the combined effect of ingredients and processing on water retention.

Mechanistically, the water absorption of flour is influenced by protein concentration and structure, starch characteristics (amylose content and damaged starch proportion), and pentosan levels, all of which affect dough viscosity and water-binding capacity [34]. Protein-starch-fiber interactions limit free water availability, reducing moisture content in the baked product. From a functional perspective, all cookie samples had moisture below 10%, which is favorable for prolonged shelf life [39]. Low moisture content limits microbial growth, decreases rates of chemical reactions such as lipid oxidation and Maillard browning, and improves physical stability, thereby enhancing overall product quality [40]. These findings emphasize that careful formulation, particularly optimizing protein and fiber ratios, is essential for controlling moisture content and ensuring cookie stability.

Table 4. Proximate composition of cookies

Sample	%Moisture	%Ash	%Fiber	%Protein	%Fat	% Carbohydrate
S1	4.33 ± 0.02^{a}	2.69 ± 0.02^{d}	2.78 ± 0.03^{d}	9.85 ± 0.04	10.21 ± 0.03^{d}	70.14 ± 0.03^{a}
S2	4.13 ± 0.02^{b}	3.23 ± 0.02^{c}	3.71 ± 0.03^{c}	13.33 ± 0.06^{c}	11.19 ± 0.02^{c}	64.41 ± 0.1^{b}
S3	3.81 ± 0.02^{c}	3.64 ± 0.02^{b}	4.23 ± 0.02^{b}	14.97 ± 0.02^{b}	11.85 ± 0.02^{b}	61.51 ± 0.02^{c}
S4	3.61 ± 0.01^{d}	4.23 ± 0.02^{a}	4.82 ± 0.01^{a}	16.79 ± 0.02^{a}	12.65 ± 0.02^{a}	57.89 ± 0.03^{d}

Here, S1=Control, S2=10% Soy flour+10% Oat, S3=15% Soy flour+15% Oat, S4=20% Soy flour+20% Oat. $^{a-d}$ Different superscript alphabets indicate significant differences among the formulations ($p < 0.05$)

Ash content represents the inorganic mineral fraction remaining after complete combustion of organic matter in food, encompassing essential elements such as calcium, magnesium, iron, and phosphorus, which are vital for enzymatic regulation, bone formation, and other physiological processes [23]. In this study, ash content ranged from 2.69 ± 0.02% to 4.23 ± 0.02%, with S4 exhibiting the highest value, likely due to the higher proportion of oats and soybeans. Soybeans inherently possess elevated mineral content, which, combined with their high protein levels and larger molecular weight components, contributes to increased ash in flour blends [8]. The observed differences in ash content were statistically significant ($p < 0.05$), demonstrating that ingredient composition directly influences mineral content in the final product. These findings are consistent with Aly et al. [36], who reported a positive correlation between the percentage of soybean flour and ash content in cookies, and with Giram et al. [37], who observed the highest ash level (1.53%) in cookies formulated with 50% oats and 10% finger millet. The slightly higher ash values in the present study may also reflect the inclusion of other minor components during cookie preparation, such as leavening agents or fortifying ingredients, which contribute additional minerals.

From a nutritional perspective, cookies enriched with soy and oat flour blends offer a meaningful source of dietary minerals. Minerals in these cookies can support hormonal function, enhance bone mineralization, and facilitate nerve impulse transmission, emphasizing their functional significance in human diets [41]. Consequently, the incorporation of soybean and oat flours not only improves the proximate composition but also enhances the mineral value of the cookies, making them a nutrient-dense snack option.

Table 4 displays data showing that the mean percentage of fiber content of the cookies varied from 2.78±0.03 % to 4.82±0.01 %. The high fiber in S4 is attributed to the higher percentage of oats and soybeans in that sample. A previous study found that the highest percentage of crude fiber was found in soybean flour (5.06 %) and oats (6.09 %), while the lowest percentage was found in wheat flour (3.04 %) [8]. Nevertheless, high fiber content is required for food products because it aids in peristalsis, adds bulk to food, and protects against several gastrointestinal disorders in humans [42]. The research findings indicate that the fat content of the cookies prepared in this study is slightly lower than that of previous studies [43]. The fat content of cookies was found to increase with an increase in blending proportion, ranging from 10.21% to 12.65%.

The elevated fat content in the cookies not only increases their caloric value but also improving the flavor and texture of the final product. Furthermore, fat is an important source of energy and plays a crucial role in transporting fat-soluble vitamins A, D, E, and K [44]. In the current study, S4 has a significantly greater mean value of protein content. In previous studies, Aly et al. [36] and Roger [39] reported measured values of 5.38–8.52% and 13.08%, respectively; the current values are higher. Nonetheless, changes in ingredient sampling may be the cause of the discrepancies shown in the current study's outcomes.

The samples showed a substantial variation in their carbohydrate content ($p < 0.05$), ranging from 57.89±0.03 to 70.14±0.03%. Sample S4 had the maximum carbohydrate content, whilst Sample S1 displayed the lowest. When compared to maize-tiger nut flour [45], the carbohydrate content in the whole wheat-soy okara-tiger nut residue biscuits ranged from 55.52% to 66.27%. This was lower than the carbohydrate levels of 74.70% to 80.80% found in plantain-sorghum biscuits that were fortified with soy okara [46]. The variations in the raw materials utilized could be the cause of this. The energy value of food compositions is influenced by the amount of carbohydrates present, as per Okache et al. [47]. These biscuits are perfect for all ages, but especially for babies, who need the high carbohydrate content to support their fast growth.

3.4 Mineral content

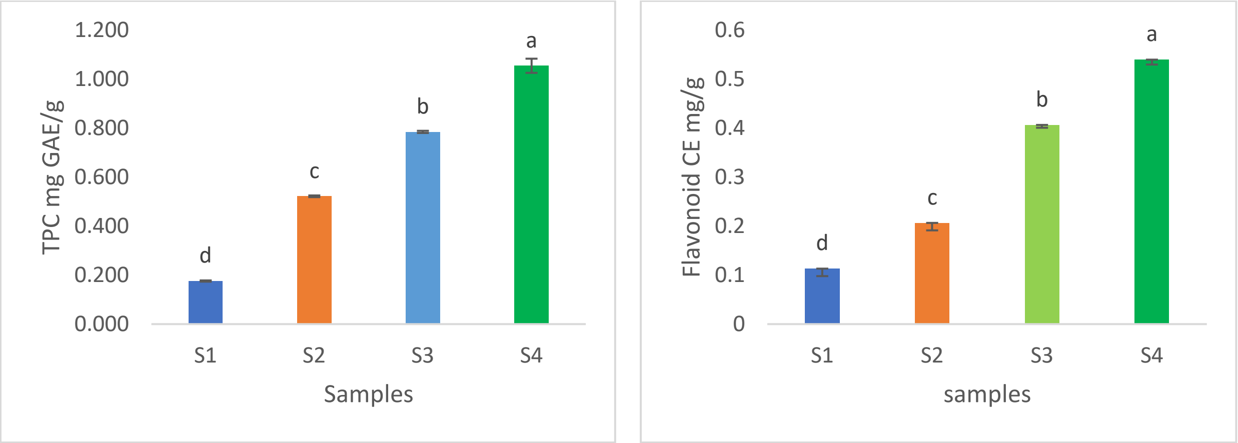
Overall, all formulations showed significant levels of essential metals for the organism, including Ca, K, Zn, and Mg, which are metals that have crucial roles in metabolic processes [48]. Table 5 displays the mineral composition of the baked cookies. All the minerals Ca, Fe, Mg, and Zn generally differ significantly ($p < 0.05$) from one sample to another. According to the study Ca, Mg, Zn, and Fe varied from 13.03- 91.7 mg/100g, $58.32 - 116.20$ mg/100g, $1.55 - 2.35$ mg/100g, and 2.72-5.79 mg/100g respectively are higher than those obtained by Maia et al. [49] for cookies prepared from legumes green grain. The result variation of the existing study could be due to the choice of ingredients being different from another research works.

Calcium is essential not only for the proper functioning of the heart and neuromuscular systems, but also for the healthy development and maintenance of bones. It is essential for proper bone development and mineralization throughout childhood [50]. Potassium is an essential mineral for the body to sustain appropriate cellular functions, maintain acid and electrolyte equilibrium, and regulate the volume of all bodily fluid [51]. Foods's rich in potassium can mitigate the adverse impact of sodium, which may help in managing blood pressure [52]. Zinc is one of the most important trace elements that has considerable effects on public health. Zinc plays a significant role in healing wounds, regulating blood pressure, and ensuring the immune system functions properly [53]. The primary causes of zinc deficiency in infants and children include parenteral nutrition, undernourishment, malnutrition, or insufficient zinc levels in breast milk [54]. Many essential biological functions depend on magnesium, a crucial element. For optimal growth of its metabolic processes, the human body requires a steady intake of magnesium through diet [55-56]. Mg plays a crucial role in the formation of a child's skeletal framework. According to [57], maintaining a favorable balance of magnesium is essential to ensure adequate amounts for growth and metabolic needs. Iron deficiency, the most widespread nutritional shortfall globally, is considered a major factor contributing to anemia, particularly in infants and young children, with elevated needs occurring during the weaning phase [58].

Table 5. Mineral content of cookies

Formulation	Ca (mg/100g)	Fe (mg/100g)	Mg (mg/100g)	Zn (mg/100g)
S1	13.03 ± 0.07^{d}	2.72 ± 0.01^{d}	58.32 ± 0.01^{d}	1.55 ± 0.02^{d}
S2	58.11 ± 0.01^{c}	4.29 ± 0.02^{c}	87.38 ± 0.01^{c}	1.96 ± 0.02^{c}
S3	74.69 ± 0.01^{b}	5.10 ± 0.03^{b}	104.33 ± 2.08^{b}	2.14 ± 0.02^{b}
S4	91.73 ± 0.02^{a}	5.79 ± 0.02^{a}	116.20 ± 0.08^{a}	2.35 ± 0.03^{a}

Here, S1=Control, S2=10% Soy flour+10% Oat, S3=15% Soy flour+15% Oat, S4=20% Soy flour+20% Oat. $^{a-d}$ Different superscript alphabets indicate significant differences among the formulations ($p < 0.05$)



Here, S1=Control, S2=10% Soy flour+10% Oat, S3=15% Soy flour+15% Oat, S4=20% Soy flour+20% Oat. $^{a-d}$ Different superscript alphabets indicate significant differences among the formulations ($p < 0.05$)

Figure1. The phytochemical content of the prepared cookies

3.5 Bioactive compounds

Naturally occurring antioxidants and phenolic chemicals enhance the oxidative stability of food and are very probable due to their positive health effects [23]. Food products originating from plants are abundant in polyphenols, which include antioxidant and nutraceutical qualities. The cookies made showed a notable difference ($p > 0.05$) in overall phenolic content. As shown in Figure 1, the total phenolic content ranged from 0.18 to 1.06 mg GAE/g. Sample S4, incorporating 20% soybean flour and 20% oat flour, showed the greatest phenolic content, whereas sample S1, lacking these flours, exhibited the lowest phenolic level. In a similar investigation, Uriarte-fr et al. [59] discovered that functional cookies created with flours from oyster mushrooms, nopal, and amaranth exhibited phenolic levels between 0.68 and 1.37 mg GAE/g, whereas a control cookie using wheat flour had a phenolic content of 0.272 mg GAE/g.

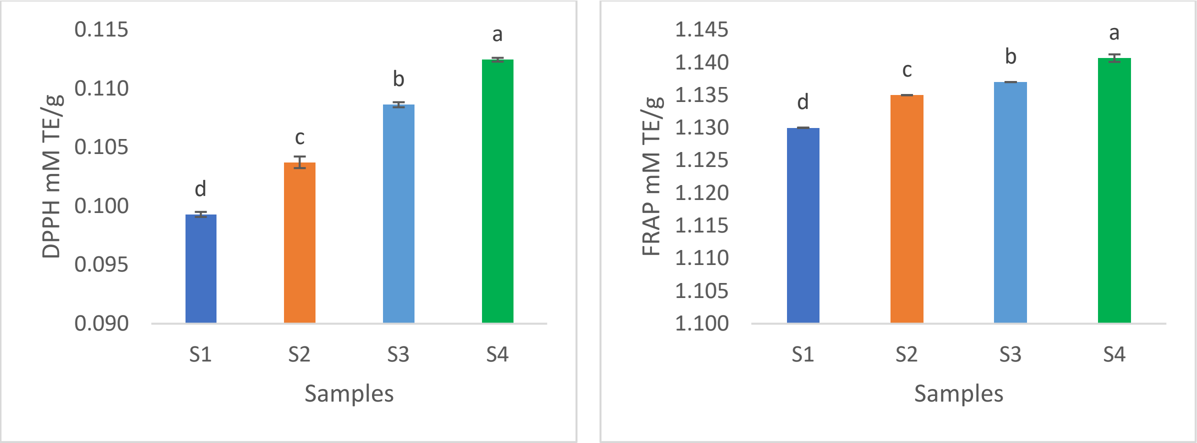
Flavonoids, a significant category of phytochemicals, are acknowledged for their ability to counteract free radicals and act as antioxidants [60]. In this study, natural ingredients were used to create antioxidant-rich products that had certain functional qualities. The flavonoid content of the prepared cookies was significantly ($p>0.05$) different.

The functional cookies enhanced with soybean and oats showed a total flavonoid content (TFC) of 0.54 mg QE/g, suggesting that these cookies could offer a significant quantity of flavonoids to consumers. This value was considerably higher ($p < 0.05$) than the TFC of the control cookies, recorded at 0.11 mg QE/g. Sample S4 contains the highest amount of flavonoid because it contains 20% soybean flour and 20% oat flour whereas sample S1 contains no soybean flour and oat flour so S1 is low in flavonoid content. The TFC of the samples of the present study was low because oat grain contains very little amount of flavonoid content. Recently, Pinto et al. [61] reported 0.515 mg CE/g Cookies TFC in chestnut shells -extract-enriched cookies while 0.163 mg CE/g Cookies TFC in control cookies. However, flavonoids and polyphenols both make significant contributions to human health. Whereas flavonoids can prevent low-density lipoproteins from oxidizing and interfering with the production and spread of free radicals, polyphenols are recognized for their anti-inflammatory, anticarcinogenic, and antioxidant properties. The specific flavonoid's structure determines how different their therapeutic potential can be. The findings suggest that adding date seeds to cookies can effectively increase their antioxidant content [24].

3.6 Antioxidant activity

To evaluate the capability of antioxidants to neutralize free radicals, FRAP and DPPH tests are commonly used methods. Halim et al. [23] state that the capacity of the DPPH radical to decolorize when antioxidants are present is what enables it to eliminate free radicals. Nonetheless, FRAP's core capability to convert Fe^{3+} to Fe^{2+} was evaluated to determine its antioxidant effectiveness [22]. There was a significant difference ($p>0.05$) in the inhibitory activity of DPPH and FRAP in the cookies. Sample S1 had the lowest inhibition rates (0.099 mM TE/g and 1.13 mM TE/g, respectively), whereas sample S4 had the highest inhibitory activity for DPPH (0.113 mM TE/g) and FRAP (1.1407 mM TE/g).

Pinto et al. [61] stated DPPH (0.672mg TE/g) and FRAP (7.30 mg TE/g) activity in chestnut shells -extract-enriched cookies while DPPH (0.306 mg TE/g) and FRAP (2.63 mg TE/g) activity in control cookies (without extract). In comparison to the current study, the earlier study's greater results may have been attained by using chestnut shells to make the cookies, which may have created synergistic effects between the various antioxidants found in chestnut shells. However, the differences between the results of the present study could be the consequence of variations in the extraction method utilized in each study, or the solvent employed. All materials have the potential to act as an efficient source of antioxidants during the manufacturing process of high-value goods, consistent with the study's findings [24].



Here, S1=Control, S2=10% Soy flour+10% Oat, S3=15% Soy flour+15% Oat, S4=20% Soy flour+20% Oat. $^{a-d}$ Different superscript alphabets indicate significant differences among the formulations ($p < 0.05$)

Figure 2. Antioxidant activity of cookies

3.7 Microbiological study of the prepared cookies

The overall viable bacterial count differed among the samples. Table 6 depicts the count of viable bacteria found in every sample. The peak count was noted in S1, varying from 3.42 to 4.86 log cfu/g, whereas the minimum count was found in S4, with measurements between 3.23 and 4.81 log cfu/g following two months of storage. The lower moisture content in S4 may explain the decreased microbial count. Cookies usually show little microbial spoilage because their moisture content is about 1% to 5%. As noted by Kumari et al. [53], the total plate count for control whole wheat flour cookies ranged from 3.20 to 4.32 log cfu/g during a storage duration of 75 days. Types I and II whole grain flour biscuits had overall plate counts ranging from 3.44 to 4.25 log cfu/g and 3.55 to 4.20 log cfu/g, respectively, but Type-III whole wheat flour biscuits had counts ranging from 3.67 to 4.21 log cfu/g.

Table 6. Change of log. cfu/g in samples during storage

Storage period	Sample no.	Total count(cfu/ml)	Total count (log. cfu/ml)
Day 0	S1	2700	3.43 ± 0.01^{a}
	S2	2400	3.38 ± 0.01^{a}
	S3	2100	3.32 ± 0.01^{a}
	S4	1700	3.23 ± 0.01^{a}
Day 15	S1	8200	3.91 ± 0.04^{a}
	S2	8000	3.90 ± 0.02^{a}
	S3	7800	3.89 ± 0.01^{a}
	S4	7400	3.87 ± 0.02^{a}
Day 30	S1	9400	3.97 ± 0.02^{a}
	S2	8900	3.95 ± 0.03^{a}
	S3	8500	3.93 ± 0.04^{a}
	S4	8200	3.91 ± 0.01^{a}
Day 45	S1	26000	4.41 ± 0.03^{a}
	S2	23000	4.36 ± 0.03^{a}
	S3	20000	4.30 ± 0.03^{a}
	S4	16000	4.20 ± 0.04^{a}
Day 60	S1	73000	4.86 ± 0.03^{a}
	S2	70000	4.85 ± 0.02^{a}

S3	68000	4.83 ± 0.03^{a}
S4	65000	4.81 ± 0.02^{a}

Here, S1=Control, S2=10% Soy flour+10% Oat, S3=15% Soy flour+15% Oat, S4=20% Soy flour+20% Oat. $^{a-d}$ Different superscript alphabets indicate significant differences among the formulations ($p < 0.05$)

3.8 Sensory evaluation of the prepared cookies

The samples underwent sensory assessment to assess various characteristics. A panel of 30 judges evaluated the color, flavor, taste, and overall acceptability of the freshly baked cookies. The typical sensory scores for the cookies showed significant variations ($p>0.05$). Table 6 presents the sensory evaluation results for the control cookies as well as those made with soybean flour and oats, which were stored in LDPE packaging for a duration of two months. Despite this storage time, the panelists evaluated the sensory attributes including appearance, color, flavor, texture, taste, and overall acceptability as satisfactory. The sample that scored the highest for all sensory attributes was S2, containing 10% soybean flour and 10% oats. The darker color of sample S4 may be associated with its higher content of phenolics and ash [62]. It was noted that the sensory evaluations for both the control cookies and those prepared with soybean flour and oats decreased as the storage duration increased (0–60 days). The cookies created received largely favorable assessments, with scores in all categories exceeding five, which is the minimum acceptable threshold on a nine-point hedonic scale. Overall, cookies made with 10% soybean flour and oats were deemed 'well accepted' in terms of color, flavor, texture, and overall appeal. A similar observation was made by Suriya et al. [30] concerning cookies made with flour from Amorphophallus paeoniflorin.

Table 7. Sensory attributes of prepared cookies based on the mean score

Day	Sample	Color	Flavor	Taste	Texture	Overall acceptability
0	S1	8.27 ± 0.45^{b}	7.90 ± 0.80^{b}	8.27 ± 0.45^{b}	8.03 ± 0.89^{b}	8.40 ± 0.50^{b}
	S2	8.87 ± 0.35^{a}	8.50 ± 0.51^{a}	9.00 ± 0.00^{a}	8.63 ± 0.49^{a}	9.00 ± 0.00^{a}
	S3	7.77 ± 0.43^{c}	7.40 ± 0.50^{c}	7.77 ± 0.43^{c}	7.40 ± 0.50^{c}	7.77 ± 0.43^{c}
	S4	6.77 ± 0.43^{d}	6.40 ± 0.50^{d}	6.77 ± 0.43^{d}	6.40 ± 0.50^{d}	6.77 ± 0.43^{d}
30	S1	8.20 ± 0.48^{b}	7.83 ± 0.79^{b}	8.20 ± 0.48^{b}	7.97 ± 0.89^{b}	8.33 ± 0.55^{b}
	S2	8.87 ± 0.35^{a}	8.50 ± 0.51^{a}	9.00 ± 0.00^{a}	8.63 ± 0.49^{a}	9.00 ± 0.00^{a}
	S3	7.77 ± 0.43^{c}	7.40 ± 0.50^{c}	7.77 ± 0.43^{c}	7.40 ± 0.50^{c}	7.77 ± 0.43^{c}
	S4	6.77 ± 0.43^{d}	6.40 ± 0.50^{d}	6.77 ± 0.43^{d}	6.40 ± 0.50^{d}	6.77 ± 0.43^{d}
60	S1	8.17 ± 0.46^{b}	7.80 ± 0.76^{b}	8.17 ± 0.46^{b}	7.93 ± 0.87^{b}	8.30 ± 0.53^{b}
	S2	8.83 ± 0.38^{a}	8.47 ± 0.51^{a}	8.97 ± 0.18^{a}	8.60 ± 0.50^{a}	8.97 ± 0.18^{a}
	S3	7.77 ± 0.43^{c}	7.40 ± 0.50^{c}	7.77 ± 0.43^{c}	7.40 ± 0.50^{c}	7.77 ± 0.43^{c}
	S4	6.77 ± 0.43^{d}	6.40 ± 0.50^{d}	6.77 ± 0.43^{d}	6.40 ± 0.50^{d}	6.77 ± 0.43^{d}

Here, S1=Control, S2=10% Soy flour+10% Oat, S3=15% Soy flour+15% Oat, S4=20% Soy flour+20% Oat. $^{a-d}$ Different superscript alphabets indicate significant differences among the formulations ($p < 0.05$)

4-Conclusion

The study's findings unequivocally show that combining soy flour and oat flour with wheat flour enhances the nutritional value of cookies. Soybean and oat flours could therefore be used with wheat flour to create cookies that have improved physicochemical qualities and palatable sensory qualities. The study also showed that the protein, fiber, ash, energy, and bioactive component contents of the cookies rise with a higher proportion of soybean and oat flour in the composite. Particularly, the addition of about 20% soybean flour and 20% oat flour produced cookies with a high level of antioxidant capacity and nutrition. Once more, it was noted that the cookies were "well-accepted" in terms of color, flavor, texture, and general acceptability at 10% soybean flour and oats integration level. To address the issue of malnutrition, soybean, and oat flour should be investigated effectively and efficiently in the creation of diverse nutritionally enhanced value-added healthier products.

Conflict of interest

The authors have no conflicts of interest to disclose that are compatible with the subject matter of this article.

Consent to participate

All authors have expressed their authorization to engage in this publication.

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Ethical Statement

This is to inform you that in this study, we have not been involved in any animal and human studies, except sensory evaluation. In the case of sensory evaluation, the participants provided their verbal agreement

Author Contribution

Md. Abdul Halim: Formal analysis, Investigation, Methodology, Software, writing – original draft, Writing – review & editing. **Fahriha Nur A Kabir:** Writing – review & editing. **Anika Tahosin:** Methodology, Software, writing – original draft, **Farhana Afrose Fariha:** Methodology, Software, writing – original draft. **Md Shohel Rana Palleb:** Writing – review & editing, **Md. Ashraful Islam:** Writing – review & editing. **Eliza Parvin Alo**: Writing – original draft & **Anwara Akter Khatun:** Conceptualization, Investigation, Resources, Supervision, writing – original draft, Writing – review & editing.

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