

Research Article

Enhancing Cookie Quality Through the Incorporation of Germinated Chia Seed (*Salvia hispanica* L.) Flour: A Sustainable Bioprocessing Strategy

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The rising demand for nutrient-dense, functional food products has urged the expansion of sustainable bioprocessing techniques. One such method, germination, involves the controlled application of biological processes to transform seeds into germinated seeds. This process improves composite flours' nutritional and technological properties, making them a promising component for innovative food products. Chia seeds, a popular superfood, are a possible substitute raw material in the food industry due to their high nutritive value. Additionally, they can produce a substantial amount of nutrient-rich green matter, which can be utilized as a source of biologically active substances. Considering the rising popularity of germination and the known health benefits of chia seeds, this study explored the impact of germinated chia seed flour (GCSF) and nongerminated chia seed flour (NCSF) on wheat flour-based cookies. The GCSF was prepared by sprouting chia seeds for 5 days, followed by drying and grinding into a fine powder. The study evaluated the impact of GCSF on the cookies' nutritional profile, physicochemical properties, technological features, and organoleptic attributes. Cookies were prepared by formulating wheat flour with 10% GCSF (T₁), 10% NCSF (T₂), and 5% GCSF + 5% NCSF (T₃), while the control sample (T₀) was prepared using 100% wheat flour. The addition of GCSF into cookies notably ($p < 0.05$) enhanced their WAC (0.94 g H₂O/g dry sample), OAC (0.97 g oil/g dry sample), crude protein (13.48%), and crude fiber (5.24%) content. Conversely, cookies formulated with NCSF exhibited elevated levels ($p < 0.05$) of crude fat (23.92%) and total carbohydrates (54.07%). However, the overall consumer acceptance of chia seed-based cookies was slightly diminished when using GCSF, as evidenced by the principal component analysis (PCA).

Keywords: bioprocessing techniques; flour-based cookies; functional quality; sensory attributes; Spanish sage

Summary

- Chia seed flour is used as a nutritious ingredient to enhance cookie quality.
- The study compares germinated and nongerminated chia seed flour in cookies.

- Germinated chia seed flour (GCSF) improves key quality parameters in cookies.
- GCSF-formulated cookies are healthier with less fat and carbohydrate.
- GCSF cookies are less preferred but offer superior nutritional benefits.

1. Introduction

Salvia hispanica L., also known as chia seed, chia sage, or Spanish sage, is grown annually and it is a member of the Lamiaceae family [1]. Historically cultivated in tropical and subtropical regions, chia seed has gained global significance as a dietary supplement [2]. Recent studies emphasizing its high nutritional and nutraceutical value have renewed interest in its cultivation [1, 3]. Chia seeds contain 15%–25% protein, 18%–35% dietary fiber, 15%–35% fat, 4%–6% ash, and 18%–31% carbohydrates [4]. Their high fiber content can contribute to satiety and reduced calorie intake. By acting as a prebiotic, soluble fiber feeds the beneficial food bacteria for a balanced and healthy gut microbiome which facilitates optimal digestion and overall well-being. Vitamins and minerals play vital roles as regulators and activators [5]. Chia seeds are also gluten-free, rich in vitamins and minerals, and have a healthy ratio of omega-6 to omega-3 (0.3–0.35). They mainly consist of α -linolenic acid (60%) out of all polyunsaturated fatty acids [6]. Several research studies have been conducted claiming that chia usage may provide various health benefits, including reductions in blood triglycerides and cholesterol, appetite suppression, improvements in blood glucose control, improved intestinal health, as well as decreased risks of colon cancer and cardiovascular diseases [7].

The European Commission classifies chia seeds as a novel food ingredient in baked products, with a maximum inclusion level of 10% due to potential allergenic effects [8]. Germination is a green food development technique that transforms seeds into seedlings. Altering their chemical composition can increase the availability of vitamins, minerals, amino acids, fiber, and other nutrients, offering a simple and cost-effective method to enhance the nutritional value of seeds [9]. Germination of chia seeds breaks down complex macromolecules like proteins, fatty acids, and carbohydrates into simpler forms, improving digestibility, bioavailability, and reducing the presence of antinutritional components [2]. Roasting is a dry heat cooking technique that cooks food by radiating or forced air convection heat, usually at a high temperature (150°C–400°C) and a short time (< 20 min) [10]. In the baking industry, various food products are often roasted using conventional methods, but these techniques can result in prolonged cooking times, high temperatures, and inconsistent heating. These factors can result in undesirable qualities such as harshness, phenolicity, tarry and smoky flavors, off-putting acidity, and the development of unpleasant flavor and aroma profiles [11]. Microwave roasting is a rapid, efficient, and affordable method for uniform roasting, expanding the global accessibility of roasted foods. It enhances food quality, including color, sensory appeal, and bioactive compounds [1].

Cookies are a popular bakery item with an excellent source of energy, often lacking significant nutritional value due to the use of refined wheat flour [12]. Adding chia seed flour can enhance their nutritional profile, texture, and

sensory properties, while also contributing to body weight management and satiety [13]. Previous studies have shown that incorporating chia seed flour (up to 20% w/w) into a nutri bar can increase mineral content, protein, fiber, and antioxidant activity [14]. Additionally, chia seed flour can improve the texture and reduce baking loss in wheat bread [4]. Furthermore, incorporating mucilage-rich chia flour up to 10% (w/w) into bread formulations can enhance both nutritional and textural properties. This is attributed to chia flour's mucilage, which has superior water absorption capacity (WAC) and dough-strengthening properties [15]. A comprehensive literature review revealed a significant gap in research on the use of microwave-roasted chia seed flour in composite flour cookie dough. This innovative approach rapidly enhances the nutritional profile of chia seeds through microwave roasting, making it a sustainable and promising ingredient for cookie production [6]. Considering this research gap, this study aimed to utilize chia seed flour combined with wheat flour as a nutritious ingredient to develop a novel, sustainable, and wholesome cookie product while exploring its nutritional profile, sensory, and overall quality.

2. Materials and Methods

Chia seed (*Salvia hispanica* L.), whole wheat flour (containing 11.35% protein, 1.51% fiber, 0.85% ash, 1.20% fat, and 82.59% carbohydrate), sugar, salt, butter, eggs, baking powder, and vanilla extract were used as ingredients for cookie preparation. All ingredients were purchased from a local market located at Churamonkati Union of Jashore district, Bangladesh. Analytical-grade chemicals used in this experiment were purchased from Sigma-Aldrich (USA) and Merck (Germany).

2.1. Germination and Preparation of Chia Seed Flour. The chia seeds were cleaned with distilled water to remove any dirt and unwanted particles. According to Ghafoor et al. [1] with minor modifications, germination was carried out by soaking the seeds in distilled water at a 1:2 (w/v) ratio and refrigerating for 3 h. Subsequently, the seeds were transferred to a 2–2.5-mm-thick cotton cloth and sprayed with distilled water 4–5 times daily to maintain moisture. Germination continued at $25 \pm 1^\circ\text{C}$ until the seedlings reached 2–3 cm in length on the 5th day (Figure 1).

After germination, the seeds were air-dried at room temperature for 30 min, according to Saeed et al. [16]. Both germinated and nongerminated chia seeds (300 g each) were roasted in a microwave oven (MW5000H, Samsung, South Korea) at 450 W for 30 min. The roasted seeds were then ground using a domestic mixer grinder (JP_HMG 550-Watt, Jaipan, India) and sieved to obtain fine flour. The resulting germinated chia seed flour (GCSF) and nongerminated chia seed flour (NCSF) were packaged and kept at 4°C for subsequent analysis. The microwave dosage (2700 J/g) received by the chia seeds was estimated using equation (1), following the method of Oyeyinka et al. [17].

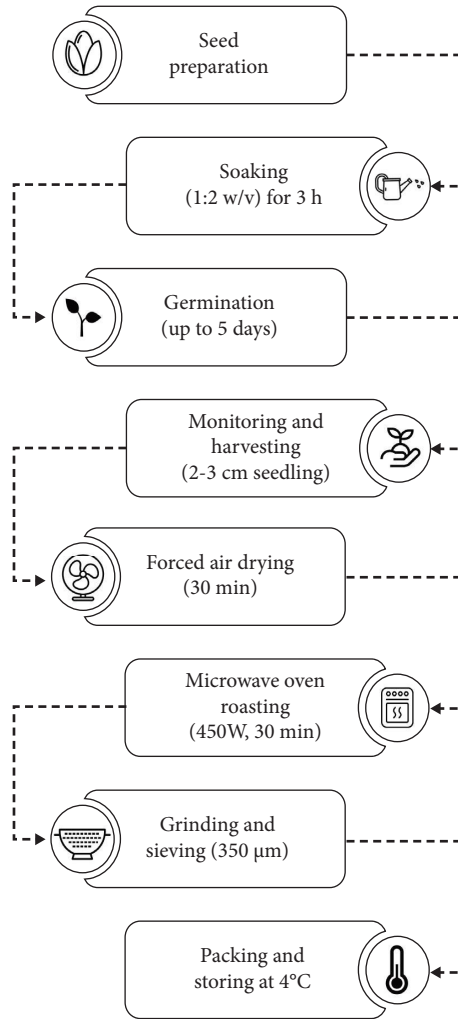


FIGURE 1: Process flow diagram for chia seed germination and flour preparation.

$$\text{Microwave dosage (J/g)} = \frac{\text{Microwave power (watt)} \times \text{Microwave exposure time (sec)}}{\text{Initial weight of sample (g)}} \quad (1)$$

2.2. Preparation of Functional Cookies. According to the method of Bello et al. [18] with minor changes, cookies were prepared using 100% wheat flour (T_0), wheat flour with 10% (w/w) GCSF (T_1), 10% (w/w) NCSF (T_2), and 5% (w/w) GCSF + 5% (w/w) NCSF (T_3). Butter (35 g), sugar (30 g), and an egg (medium size) were mixed in a clean bowl with a domestic mixer until fluffy. Salt (1 g), vanilla culinary essence (5 mL), baking powder (1.5 g), and 100 g of uniformly mixed flour (T_0 , T_1 , T_2 , T_3) were added and mixed thoroughly for 5 min. Each dough, formulated from the different flour blends, was thoroughly kneaded and then sheeted to a uniform thickness of 1 cm on dry, clean baking trays. Round cookies with a 4.50-cm diameter were cut out using a cookie cutter. The shaped doughs were subjected to

a baking process in a preheated convection oven (EO-42K3, Esquire Electronics Ltd, Japan) at a temperature of $180 \pm 5^\circ\text{C}$ for 25 min. Cooled cookies were individually bagged (Ziploc) and kept at ambient temperature (Figure 2).

2.3. Proximate Analysis of Functional Cookies

2.3.1. Moisture Content. The determination of moisture content was carried out according to Kumari et al. [19]. In brief, 2 g of finely powdered cookie samples were taken into a dry Petri dish and dried in a hot-air oven at $105 \pm 2^\circ\text{C}$ for 2 h. Moisture content was determined by weighing the cookie samples before and after drying and applying the obtained values to equation (2).

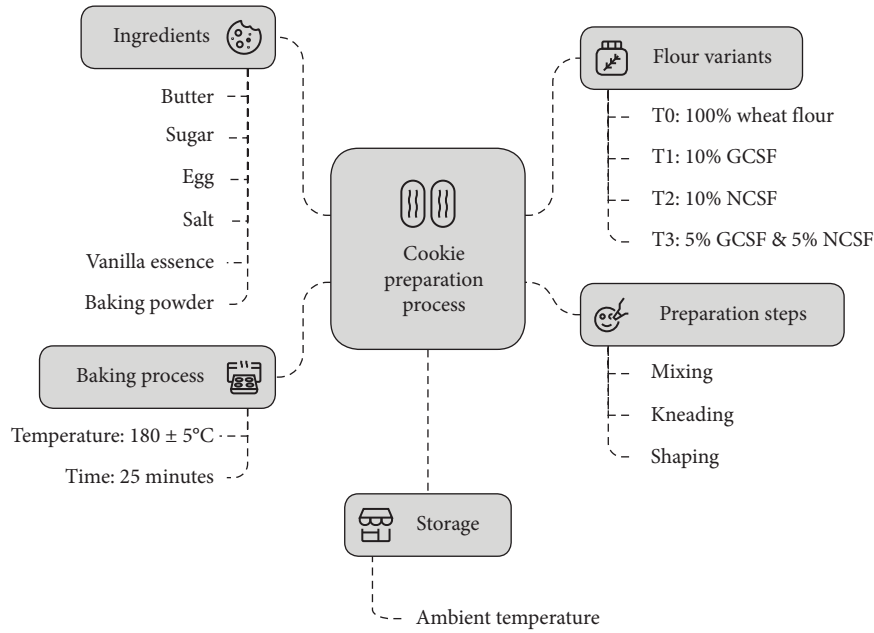


FIGURE 2: Technological flowchart for the preparation of functional cookies.

$$\text{Moisture (\%)} = \frac{\text{Weight of fresh sample with Petri dish (g)} - \text{Weight of dried sample with Petri dish (g)}}{\text{Weight of sample taken for estimation (g)}} \times 100. \quad (2)$$

2.3.2. Crude Fiber Content. The crude fiber content was estimated following the modified method of Gebremariam et al. [20]. In brief, 2 g of the dried powdered cookie samples was digested in 0.26 N H₂SO₄ (200 mL) for 30 min, filtered through a double-layered muslin cloth, and rinsed with deionized water. Subsequently, the precipitate was further

digested in 0.07 N NaOH (200 mL) for 30 min. Again, the filtered solution was rinsed with deionized water, and the resulting residue was dried out in a hot-air oven at 180°C for 6 h. Subsequently, it was ashed in a muffle furnace (1100 LHM01, SnolTherm, Lithuania) at 600°C for 1.5 h. The crude fiber content was calculated using equation (3).

$$\text{Crude fiber (\%)} = \frac{\text{Weight of ash with crucible (g)} - \text{Weight of crucible (g)}}{\text{Weight of sample taken (g)}} \times 100. \quad (3)$$

2.3.3. Crude Fat Content. According to Kumari et al. [19], the crude fat content was ascertained. Two grams of powdered cookie samples was packed in a cellulose thimble and put in a previously weighted solvent cup filled with hexane and inserted in a fat analyzer (LSFA-A10, Labtron, UK). The

extraction process was continued for 6 h. Then the sample was collected and dried at 70°C to facilitate the complete evaporation of hexane. Crude fat content was determined using equation (4).

$$\text{Crude fat (\%)} = \frac{\text{Weight of solvent cup after extraction (g)} - \text{Weight of solvent cup before extraction (g)}}{\text{Weight of sample taken (g)}} \times 100. \quad (4)$$

2.3.4. Ash Content. The determination of ash content was conducted using a methodology outlined by Kumari et al. [19] with minor modification. Two grams of dried samples was

placed in a crucible and heated in a muffle furnace at 600°C for 6 h followed by the weighing of the obtained residue, and the ash content was calculated using equation (5).

$$\text{Ash (\%)} = \frac{\text{Weight of crucible with ash (g)} - \text{Weight of crucible (g)}}{\text{Weight of sample taken (g)}} \times 100. \quad (5)$$

2.3.5. Crude Protein Content. The crude protein content was estimated by digesting 1 g of each sample with 8 mL of H₂SO₄ (98%) and the mixture of K₂SO₄ and CuSO₄ at a ratio of 1:9. After thorough mixing, the mixture was digested in the digestion chamber until it became clear, then cooled for 2 h. Then, distilled water (30 mL) and 35% NaOH (10 mL) were added to the mixture followed by the distillation using

the Kjeldahl distillation unit (KA-9830, Scitek Global, Inc., USA). In a conical flask of 2% H₃BO₃ (20 mL), NH₃ was collected in the form of NH₄OH as it was produced during the distillation process. The distillate was titrated with 0.1 N HCl using methyl red as an indicator. Nitrogen and crude protein (%) were calculated using equations (6) and (7), respectively [20].

$$\text{Nitrogen content, N (\%)} = \frac{\text{Titer value for sample (mL)} - \text{Titer value for blank (mL)} \times 0.1\text{N} \times 14}{\text{Weight of sample (g)}} \times 100, \quad (6)$$

$$\text{Crude protein content (\%)} = \%N \times 6.25. \quad (7)$$

2.3.6. Total Carbohydrate Content. Following the method of Kabir et al. [21], total carbohydrate content was estimated by subtracting the individual percentages of protein, fat, fiber, moisture, and ash from 100.

2.4. Mineral Content of Cookies. According to Yalcin et al. [22] with minor changes, the mineral content of cookie samples was determined. First, 5 g of each sample was digested with 15 mL of 65% HNO₃ and 3 mL of 30% H₂O₂ at 120°C for 15 min using a hot-plate magnetic stirrer (MS7-H550-S, DLAB, USA). The samples were then filtered using Whatman-42 filter paper, diluted to 100 mL with ultra-deionized water, and analyzed for mineral content (Na, Ca, Zn, Fe) using atomic absorption spectroscopy (AA-7000, Shimadzu, Japan). The results were expressed as % (w/w).

2.5. Physical Properties of Cookies. The dimensions of the cookies, including thickness and diameter, were determined using a Fowler vernier caliper (USA) and expressed in mm. The spread ratio was determined by dividing the diameter of a well-prepared cookie by its thickness. The hardness was measured using a texture analyzer (TA1SH-203V, LLOYD Materials Testing, UK) equipped with a load cell of 50 kg and a trigger force of 5 g at room temperature. The hardness of the cookies was estimated by implementing a cutting-shear test with Warner Bratzler Blade (HDP/WBR) and a fixed speed of 2 mm/s. The acceptable hardness value (g force) was

recorded when the cookies were completely broken into two pieces [23]. The measurements were taken after 24 h of storage in airtight glass jars.

2.6. Color Measurement. The color parameters of the flour and cookie samples were determined by a precision colorimeter (BCM-110, Biobase, China). The powdered samples (10 g) were placed in a Petri dish to measure the color parameters and expressed as L* (lightness), a* (redness/greenness), and b* (yellowness/blueness). Before measurements, demineralized water was used to calibrate the colorimeter (L* = 100, a* = 0, b* = 0) [24].

2.7. Technological Properties

2.7.1. WAC and Oil Absorption Capacity (OAC). The WAC and OAC were determined following the modified methodology of Saeed et al. [16]. A 0.5-g aliquot of each sample was suspended in 10 mL of distilled water, vortexed for 1 min using an MS-M200V vortex mixer (China), incubated at room temperature for 10 min, and centrifuged (DSC-200T, Digisystem, Taiwan) at 1066 × g for 25 min. Following centrifugation, the supernatant was removed, and the sediment was weighed and analyzed for WAC. Distilled water was replaced with soybean oil to analyze OAC. Equations (8) and (9) were employed to calculate the WAC and OAC of the samples.

$$\text{WAC (g H}_2\text{O/g dry sample)} = \frac{\text{Weight of H}_2\text{O absorbed sample (g)} - \text{Weight of dry sample (g)}}{\text{Weight of dry sample (g)}}, \quad (8)$$

$$\text{OAC (g oil/g dry sample)} = \frac{\text{Weight of oil absorbed sample (g)} - \text{Weight of dry sample (g)}}{\text{Weight of dry sample (g)}}. \quad (9)$$

2.7.2. Swelling Capacity (SC). The SC of the flour and cookie samples was estimated according to the method of Saeed et al. [16] with minor changes. In brief, 1 g of each sample was added to 10 mL of distilled water in a Falcon tube. After thoroughly vortexed, the mixture was heated in a thermostatic water bath (HH-4, Wincom, China) at 80°C for 30 min with occasional stirring to prevent agglomeration. Then, the mixture was cooled at ambient temperature and centrifuged at $1066 \times g$ for 15 min. The supernatants were carefully removed, followed by the weighing of the swollen samples. The SC of the samples was calculated by equation (10).

$$SC \text{ (g/g)} = \frac{\text{Weight of swollen particles (g)}}{\text{Weight of sample (g)}}. \quad (10)$$

2.7.3. Bulk Density (BD) and Tapped Density (TD). The BD and TD of the flour and cookie samples were estimated according to the method of Muala et al. [25] with minor alterations. Briefly, 1-g sample was transferred to a 10-mL graduated cylinder and gently shaken to flatten the top surface of the flour within. Then, the cylinder was gently tapped by a glass rod for 10 min until no visual change in the volume was seen. The BD and TD were calculated according to the following equations (11) and (12):

$$BD \text{ (g/mL)} = \frac{\text{Weight of sample (g)}}{\text{Volume of sample (mL)}}, \quad (11)$$

$$TD \text{ (g/mL)} = \frac{\text{Weight of sample (g)}}{\text{Volume of sample after tapping (mL)}}. \quad (12)$$

2.8. Sensory Evaluation. The sensory evaluation of the cookies was performed by 20 semitrained panelists, including students and faculty from the Department of Food Engineering, Jashore University of Science and Technology, Jashore-7408, Bangladesh. The cookies were served to the panelists on a white plate coded with three random digits. The panelists were allowed to assess the samples in bright daylight and suggested drinking mineral water between samples to avoid aftertaste [26]. Each cookie samples were evaluated for texture, color, flavor, taste, and overall acceptance using a nine-point hedonic scale, where 9 represents “like extremely” and 1 represents “dislike extremely” [27].

2.9. Statistical Analysis. All experiments were conducted in triplicates. SPSS Version 25 (IBM, USA) was utilized for the analysis of variance (ANOVA) of the obtained data. Analyzed data of the experiments were reported as mean $\pm$ SD. Tukey’s Honestly Significant Difference (HSD) test was used to compare means and determine statistically significant differences at $p < 0.05$. The principal component analysis (PCA) of the sensory analysis, the Pearson correlation, and the hierarchical cluster analysis (HCA) were conducted using Origin software (Version 10.1.0.170, OriginLab, USA).

3. Results and Discussion

3.1. Proximate Composition of Functional Cookies

3.1.1. Moisture Content. The moisture content of the formulated cookies is expressed in Table 1. According to the results, the germination process significantly improved ($p < 0.05$) the moisture content of the cookies, so that the moisture content increased from 3.22% (T_0) to 5.02% (T_1) of cookie samples, which is within an acceptable limit ($< 10\%$) to ensure prolonged shelf life [28]. The elevated moisture content in T_1 is likely due to increased water absorption by chia seeds. This is associated with enhanced metabolic activity, leading to a higher proportion of hydrated cells and the activation of endogenous enzymes [19, 28]. These findings align with previous research demonstrating increased moisture content in cookies formulated with germinated grain flour [29].

3.1.2. Crude Fiber Content. The addition of GCSF notably improved ($p < 0.05$) the crude fiber content of the formulated cookies. T_1 revealed the highest fiber content at 5.24%, while the control exhibited 1.46% (Table 1). This increment of crude fiber in formulated cookies might be due to the high crude fiber content (24.72%) of chia seed [30]. Concurrently, the germination process enhances cell wall biosynthesis, leading to new dietary fiber formation [31]. Hettiarachchi and Gunathilake [9] and Sofi et al. [32] also observed a similar trend of increasing crude fiber content in germinated legume flours and germinated desi chickpea flours, respectively, which supports the present study. Foods containing at least 3 g/100 g and 6 g/100 g of dietary fiber can be classified as a source of dietary fiber and are considered high in dietary fiber, respectively. In the present findings, the fiber content of cookies incorporated with GCSF and NCSF falls within this range (4.37%–5.24%). Consequently, consuming 100 g of cookies incorporated with 10% GCSF can contribute approximately 21% of the WHO’s recommended daily dietary fiber intake of 25 g for adults [33].

3.1.3. Crude Fat Content. A significant reduction ($p < 0.05$) was observed in the crude fat content of cookies formulated with GCSF compared to the control samples whose value ranged from 21.08% (T_0) to 23.92% (T_2). GCSF-formulated cookies (T_1) exhibited the lowest crude fat content (22.84%) among the treated sample while the nongerminated one (T_2) showed the highest (23.92%). The higher fat content of chia seed (15%–35%) might have increased the crude fat content of formulated cookies [4]. In addition, the observed reduction in crude fat content of T_1 suggests that fatty acids were catabolized to CO_2 and H_2O during germination, providing the necessary energy for seed expansion and the synthesis of new structural components in the developing seedling [19, 34]. Similar findings were also reported for germinated minor millet flour cookies and germinated common buckwheat flour by Sharma and Riar [35] and Shreeja et al. [34], respectively.

TABLE 1: Proximate composition of functional cookies.

Sample	Moisture (%)	Crude fiber (%)	Crude fat (%)	Ash (%)	Crude protein (%)	Total carbohydrate (%)
T ₀	3.22 ± 0.02 ^d	1.46 ± 0.02 ^d	21.08 ± 0.01 ^d	1.14 ± 0.01 ^c	11.16 ± 0.01 ^d	61.95 ± 0.05 ^a
T ₁	5.02 ± 0.07 ^a	5.24 ± 0.02 ^a	22.84 ± 0.01 ^c	1.65 ± 0.01 ^a	13.48 ± 0.03 ^a	50.77 ± 0.02 ^d
T ₂	4.08 ± 0.02 ^c	4.37 ± 0.01 ^c	23.92 ± 0.01 ^a	1.59 ± 0.02 ^b	12.79 ± 0.01 ^c	54.07 ± 0.02 ^b
T ₃	4.83 ± 0.02 ^b	4.68 ± 0.02 ^b	23.09 ± 0.01 ^b	1.64 ± 0.01 ^a	13.41 ± 0.01 ^b	53.02 ± 0.03 ^c

Note: Means within a column followed by different superscripts are significantly different ($p < 0.05$). T₀: 100% wheat flour cookies; T₁: 90% wheat flour and 10% GCSF cookies; T₂: 90% wheat flour and 10% NCSF cookies; T₃: 90% wheat flour, 5% GCSF, and 5% NCSF cookies.

3.1.4. Ash Content. Ash content offers an estimation of the mineral percentage present in food. The incorporation of chia seed flours significantly increased ($p < 0.05$) the ash content of cookies, and the values fluctuated from 1.14% to 1.65% (Table 1). The higher mineral content of chia seed flour compared to wheat flour might increase the ash content of the resulting cookies [4]. Similarly, the germinated chia seed cookie (T₁) showed higher ash content (1.65%) compared to the nongerminated chia seed cookie (T₂, 1.59%). This increased ash content can be attributed to the hydrolysis of phytic acid by activated phytase during germination, which enhances mineral bioavailability as reflected in the ash content [36]. Supposedly, germination induced a loss of dry matter, including carbohydrates and crude fat, which consequently upscaled the ash content of chia seeds. Based on the study by Demir and Bilgiçli [37] and Ghafoor et al. [1], germination significantly boosted the ash content, reflecting higher mineral levels of quinoa and chia seed flours, respectively, aligning with the present findings.

3.1.5. Crude Protein Content. The germination process resulted in an overall increase ($p < 0.05$) in the protein content of chia seed-incorporated cookies. The protein content of cookies ranged from 11.16% (T₀) to 13.48% (T₁) (Table 1). The observed growth in protein content may be due to enzymatic reactions in the seed during germination which released the free amino acids from the endosperm [38]. Furthermore, it is consistent with our findings that chia seed germination involves the catabolism of carbohydrates and fats via respiratory processes, leading to the biosynthesis of new protein structures [9]. A similar trend was reported in bread formulated with germinated rice flour [39] and rice noodles supplemented with sprouted chickpea flour [40]. The recommended daily allowance (RDA) for protein for an average Asian adult male is 46.16 g (calculated as $0.8 \text{ g kg}^{-1} \text{ day}^{-1}$) [41]. Consuming 100 g of cookies formulated with 10% GCSF provides approximately 29.20% of RDA.

3.1.6. Total Carbohydrate Content. The addition of chia seed flour resulted in a significant reduction ($p < 0.05$) in the total carbohydrate content of cookies, which decreased from 61.95% (T₀) to 50.77% (T₁). The germination process significantly reduced the carbohydrate content of the cookies, with the highest reduction observed in cookies prepared with GCSF in T₁, as compared to those incorporated with NCSF in T₂. The observed decrease in

carbohydrate content in chia seeds during germination may be attributed to elevated amylase activity, which catalyzes starch breakdown [42]. Concomitantly, these carbohydrate resources may be utilized for protein synthesis during germination [9]. According to WHO, the recommended daily carbohydrate intake is 130 g for adults and children aged 1 year and older. This recommendation is based on the brain's reliance on glucose, derived from sugars and starches, as its primary energy source [43]. Consuming 100 g of GCSF-incorporated cookies can provide approximately 41.6% of the daily carbohydrate requirement. Lower-carbohydrate diets may offer potential benefits for the elderly population due to age-related reductions in gastric acidity, small intestinal microbial balance, and digestive enzyme capacity [31]. A similar pattern of decreasing carbohydrate content was observed in gluten-free noodles made with sprouted chickpea flour [40] and biscuits prepared with germinated pumpkin seed flour [44].

3.2. Mineral Content. Minerals are indispensable for human physiological functions, including immune system regulation, cardiac function, hormone production, bone mineralization, and neural transmission [45]. Table (2) represents the mineral composition (Ca, Na, Zn, and Fe) of chia seed-based cookies. The incorporation of chia seeds significantly enhanced ($p < 0.05$) the mineral content of the cookies. Among the formulations, T₁ exhibited the highest Ca levels (0.012503% w/w), while T₃ demonstrated the highest concentrations of Na (0.692664% w/w), Zn (0.002529% w/w), and Fe (0.002250% w/w). High mineral content of chia seed could explain the increased mineral levels in these formulated cookies [1].

The findings revealed that chia seed flour significantly enhanced the Ca content of cookies, which offers potential benefits for bone health, blood coagulation, muscle function, and metabolic processes [20]. Goyat et al. [46] demonstrated that adding up to 15% chia seed flour to cookies led to a notable rise in Ca (from 36.06 to 108.90 mg/100 g), Zn (from 0.99 to 1.24 mg/100 g), and Fe (from 1.36 to 1.97 mg/100 g). Conversely, the study also found a decrease in Na levels, from 304.55 to 285.94 mg/100 g. A similar increase in mineral content after adding chia seed flour was also observed in fat-replaced, chia seed-based biscuits [22]. The varying mineral content of chia seed-based cookies reported in previous studies could be due to factors like soil composition, climate, geographical location, and seed maturity [1, 47].

TABLE 2: Mineral composition of chia seed–formulated cookies.

Sample	Mineral content (% w/w)			
	Ca	Na	Zn	Fe
T ₀	0.005787 ± 0.000081 ^c	0.510332 ± 0.003102 ^d	0.001171 ± 0.000001 ^d	0.001745 ± 0.000011 ^b
T ₁	0.012503 ± 0.000205 ^a	0.598763 ± 0.003491 ^b	0.001224 ± 0.000003 ^c	0.001590 ± 0.000016 ^d
T ₂	0.008164 ± 0.000163 ^b	0.575324 ± 0.003213 ^c	0.001808 ± 0.000005 ^b	0.001708 ± 0.000002 ^c
T ₃	0.012155 ± 0.000130 ^a	0.692664 ± 0.003104 ^a	0.002529 ± 0.000003 ^a	0.002250 ± 0.000016 ^a

Note: Means within a column followed by different superscripts are significantly different ($p < 0.05$). T₀: 100% wheat flour cookies; T₁: 90% wheat flour and 10% GCSF cookies; T₂: 90% wheat flour and 10% NCSF cookies; T₃: 90% wheat flour, 5% GCSF, and 5% NCSF cookies.

3.3. Physical Properties of Cookies. As summarized in Table 3, the addition of GCSF notably influenced ($p < 0.05$) the physical characteristics of the cookies. According to the findings, T₂ exhibited the highest diameter (47.89 mm) and thickness (12.05 mm), while T₁ showed the highest spread ratio (4.14) as compared to other cookies. The reduced diameter (46.38 mm) and thickness (11.19 mm) of T₁ can be attributed to the higher fiber content of GCSF, which likely decreased dough viscosity, thus increasing the spread ratio of T₁ during baking [48]. Comparable observations were reported by Bolarinwa et al. [49], who observed that germinated brown rice flour and germinated white rice flour also increase the spread ratio of formulated cookies.

On the other hand, Saeed et al. [16] reported that cookies made with germinated chickpea flour had significantly lowered the spread ratio compared to roasted flour. While a higher spread ratio is often considered desirable in cookies, the conflicting results regarding the spread ratio observed in different studies can be attributed to variations in the chemical composition of the raw materials and the specific formulations used in each study [50, 51].

The breaking strength of a cookie can be quantified by its hardness, which represents the highest force applied before it fractures. The hardness of a cookie significantly influences its overall texture, affecting its perception as either tough or crumbly. The significant effect ($p < 0.05$) of adding chia seed flour on the hardness of cookies is shown in Table 3. Cookies containing GCSF (23,057.24 g force, T₁) had higher hardness than other cookies. This significant increase in the hardness of T₁ could be due to the lower fat content of the cookies. Fat content enhances lubrication and decreases the strength of gluten bonds within the dough matrix, which eventually softens the cookies [17]. Furthermore, the gluten protein within the dough is coated by solid fat crystals during kneading, inhibiting the formation of an extensive gluten network, thus contributing to the softer texture of the cookies [16]. Concurrently, the elevated fiber content may have contributed to the increased hardness observed in cookies enriched with GCSF [52]. The findings in this study are consistent with Jan et al. [53], who reported that the incorporation of chia seed flour significantly increased the hardness of the functional cookies.

3.4. Color Parameter. Germination significantly influenced ($p < 0.05$) the color properties of chia seed flour and the formulated cookies (Figure 3). Consistent with the color changes observed in the chia seed flour itself during

germination, cookies formulated with 10% GCSF (T₁) exhibited a more pronounced shift in color, with a 61.62% decrease in lightness (L^*), a 95.6% increase in redness (a^*), and 39.82% increase in yellowness (b^*) compared to cookies made with 10% NCSF (T₂) (Figure 4).

A similar observation was noticed in pasta enriched with 10%–30% germinated quinoa flour [31] and bread containing 5%–20% germinated lupine flour [54]. The darker color tone of cookies formulated with chia seed flour can be attributed to the presence of naturally occurring pigments in the chia seeds. However, the germination process may alter the composition of carbohydrates and proteins, resulting in a reduction of color properties in cookies made with GCSF [55].

3.5. Technological Properties of Flour and Cookies

3.5.1. WAC and OAC. There were noticeable changes ($p < 0.05$) in the WAC and OAC of chia seed flours and cookie samples as depicted in Figure 5(a). The WAC and OAC of chia seed flours were significantly ($p < 0.05$) increased from 1.63 to 2.30 g H₂O/g dry sample and from 0.87 to 1.14 g oil/g dry sample, respectively, after germination. Similar observations of the positive impact of germination on WAC and OAC for germinated legumes and cereals were also reported by Singh Sibian and Singh Riar [29], and for finger millet varieties by Yenasew and Urga [56]. This incremental behavior of WAC and OAC could be due to the increase of water-loving biopolymers and aquaphobic amino acids in germinated chia seeds [32]. Another reason for the rise in WAC and OAC could be the alteration of starch granules in germinated chia seed, which might have positively impacted its WAC and OAC value [28, 56]. The same reason could be considered for the similar pattern of increase in WAC and OAC values in chia seed–formulated cookies. Among other formulated cookies, the highest WAC (0.94 g H₂O/g dry sample) and OAC (0.97 g oil/g dry sample) were seen in T₁. The observed trend of increase in WAC and OAC in cookie samples might be beneficial in preventing staling by moisture loss and improving flavor as well as mouth feel of the cookies [57].

3.5.2. SC. SC refers to the capability of starch in the flour to absorb water, which results in the enlargement of starch granules, thus changing the internal structure of the flour [58]. From the results of this study (Figure 5(a)), the SC was significantly improved ($p < 0.05$) in GCSF (4.33 g/g) than the

TABLE 3: Physical properties of chia seed–formulated cookies.

Sample	Diameter (mm)	Thickness (mm)	Spread ratio	Hardness (g force)
T ₀	45.70 ± 0.31 ^c	11.93 ± 0.02 ^b	3.83 ± 0.02 ^c	17,769.33 ± 18.61 ^d
T ₁	46.38 ± 0.03 ^b	11.19 ± 0.06 ^d	4.14 ± 0.02 ^a	23,057.24 ± 28.59 ^a
T ₂	47.89 ± 0.11 ^a	12.05 ± 0.05 ^a	3.97 ± 0.06 ^b	18,067.71 ± 16.48 ^c
T ₃	46.09 ± 0.04 ^{bc}	11.53 ± 0.04 ^c	3.99 ± 0.02 ^b	20,856.03 ± 38.88 ^b

Note: Means within a column followed by different superscripts are significantly different ($p < 0.05$). Abbreviations: T₀: 100% wheat flour cookies; T₁: 90% wheat flour and 10% GCSF cookies; T₂: 90% wheat flour and 10% NCSF cookies; T₃: 90% wheat flour, 5% GCSF, and 5% NCSF cookies.

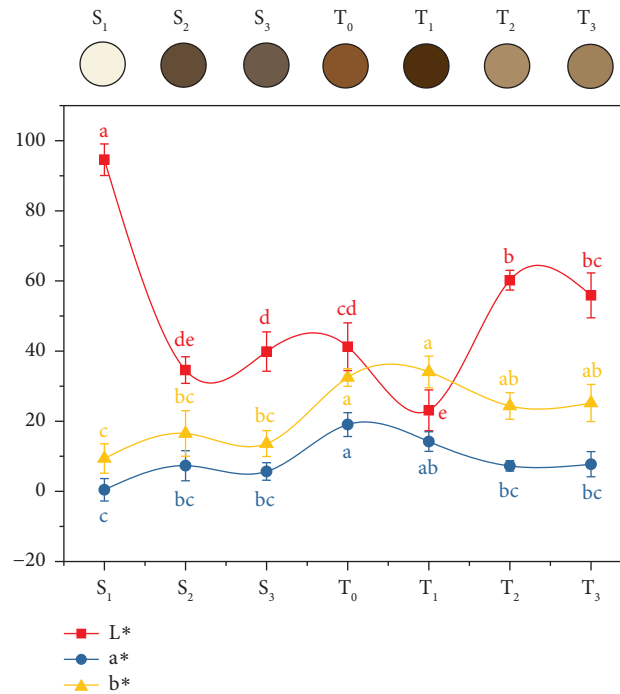


FIGURE 3: Color parameters of treated chia seed flours and chia seed–formulated cookies. Mean with different superscripts along each treatment denotes significant differences ($p < 0.05$). Abbreviations: S₁: wheat flour; S₂: germinated chia seed flour (GCSF); S₃: non-germinated chia seed flour (NCSF); T₀: 100% wheat flour cookies; T₁: 90% wheat flour and 10% GCSF cookies; T₂: 90% wheat flour and 10% NCSF cookies; T₃: 90% wheat flour, 5% GCSF, and 5% NCSF cookies.



FIGURE 4: Chia seed–formulated cookies.

NCSF (3.14 g/g). The germination process might increase the hydrophilic portion of chia seed flour which could facilitate the interaction of flour with water [32]. Again, the enhanced solubility of proteins and the conversion of polysaccharides into monosaccharides may be responsible for the higher SC values for GCSF [16]. According to the researchers, different factors (e.g., starch content, post-harvest treatment, and

processing history) could affect the SC of flour [48]. Germinated wheat (3 g/g) and sorghum (4.94 g/g) flour demonstrated higher SC than the nongerminated one which supports the present study [59]. Similarly, the cookies formulated with 10% GCSF (T₁) showed higher SC (3.76 g/g), as expected. The reduced fat content of the T₁ sample due to the germination of chia seed obstructed the development of

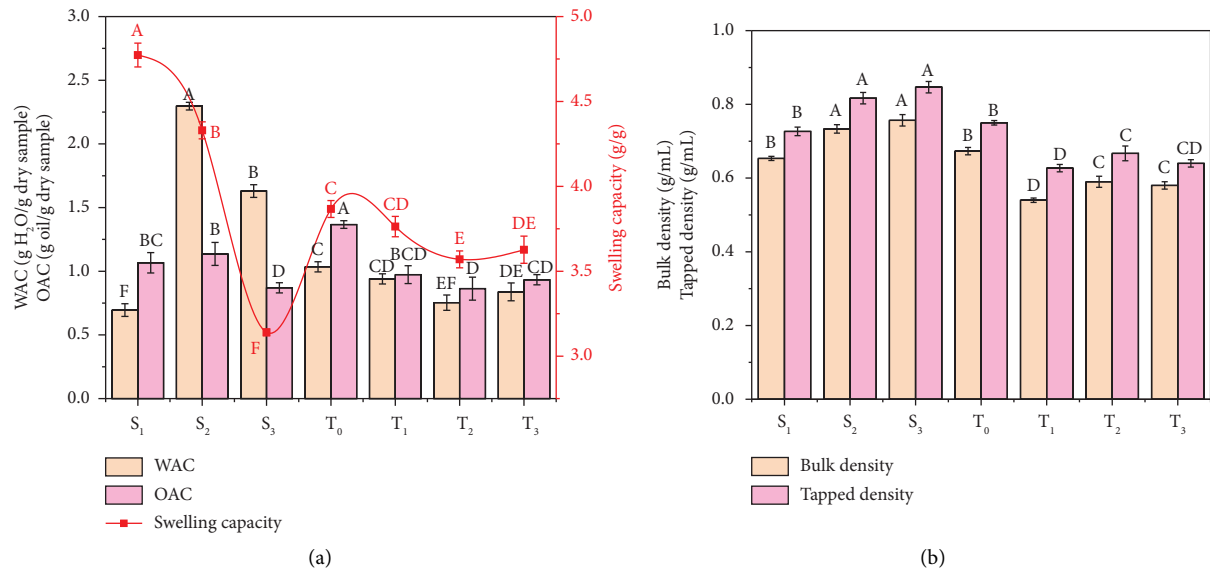


FIGURE 5: Technological properties of treated chia seed flours and chia seed-formulated cookies. Mean with different superscripts along each treatment denotes significant differences ($p < 0.05$). Abbreviations: S₁: wheat flour; S₂: germinated chia seed flour (GCSF); S₃: non-germinated chia seed flour (NCSF); T₀: 100% wheat flour cookies; T₁: 90% wheat flour and 10% GCSF cookies; T₂: 90% wheat flour and 10% NCSF cookies; T₃: 90% wheat flour, 5% GCSF, and 5% NCSF cookies.

starch-lipid complexes, which is responsible for the reduction of SC of food ingredients [16, 56]. The higher SC might help prevent cookies from drying out quickly.

3.5.3. BD and TD. Figure 5(b) shows the changes in BD and TD of GCSF and NCSF, as well as their corresponding cookie flours. The results indicated that the germination process significantly ($p < 0.05$) reduced the BD and TD of analyzed samples. BD of flour measures how tightly packed the flour particles are within a given volume, while TD represents the maximum achievable compactness of flour after applying external pressure [60, 61]. BD and TD of GCSF reduced from 0.76 to 0.73 g/mL and 0.85 to 0.82 g/mL, respectively. This reduction in BD and TD of chia seeds after germination could be attributed to the alteration of carbohydrates or enzyme hydrolysis during germination [55, 62]. The porous structure of flours caused by microwave roasting might be another reason for the decrease in BD and TD [10]. Similar results were reported in sprouted black chickpea flour [55] and sprouted chickpea flour [62]. In the same way, the BD and TD of GCSF-formulated cookies (T₁) were also reduced (0.54 g/mL and 0.63 g/mL, respectively) compared to the nongerminated one (0.59 g/mL and 0.67 g/mL, respectively). This could be due to the reduced BD and TD of GCSF. Reduced BD and TD may reduce packaging costs as well as help in the formulation of complementary foods and enhance the digestibility of food products, particularly for children with underdeveloped digestive systems [58, 63].

3.6. Sensory Analysis. As depicted in Figure 6(a), the sensory assessment of chia seed-based cookies revealed a significant impact of chia seed flour addition on key sensory parameters. Notably, the textural quality of the cookies was

markedly improved with the T₃ sample exhibiting the highest textural quality (8.35) among all formulations. Panelists claimed that GCSF resulted in cookies with a slightly harder texture and NCSF led to a less crunchy texture, which is accountable for the reduced texture score. The observed variations in texture can be attributed to the differential fiber and fat content present in the GCSF and NCSF [17, 52].

Based on the panelist's report, T₂ scored highest in color parameter (7.43), while T₁ (5.10) scored the lowest because of its darker appearance. The enzymatic hydrolysis of chia seed during germination may facilitate the migration and percolation of pigments from the outer layer to the inner layer which could potentially impacted in overall visual appeal of formulated cookies [64]. Additionally, the germination process may have altered the composition of carbohydrates and proteins which could have contributed to the darker appearance of the cookies [55]. The highest score on flavor (8.43) and taste (8.33) was obtained by T₀. According to Atudorei and Codină [65], the unpleasant aroma after germination could decreased the flavor and taste scores of formulated cookies. Furthermore, panelists might subconsciously associate plain cookies (T₀) with a desired taste and flavor profile, potentially influencing their ratings.

3.7. PCA. PCA was implemented to investigate the impact of chia seed flour on the key sensory characteristics of the cookies (Figure 6(b)). The first two principal components (PC1 and PC2) collectively accounted for 88.27% of the total variation in the data. PC1, explaining 63.17% of the variance, was primarily influenced by color (0.40511), flavor (0.53042), taste (0.50677), and overall acceptance (0.49652). Conversely, texture (0.75826) was the only dominant factor

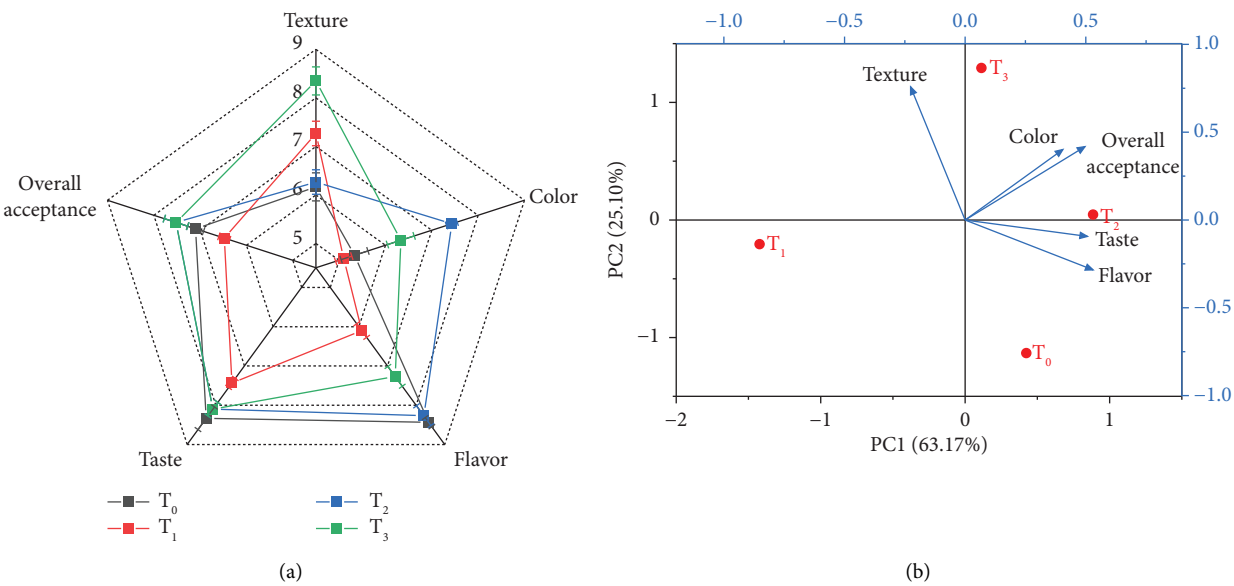


FIGURE 6: (a) Sensory analysis and (b) principal component analysis (PCA) of formulated cookies. Abbreviations: T₀: 100% wheat flour cookies; T₁: 90% wheat flour and 10% GCSF cookies; T₂: 90% wheat flour and 10% NCSF cookies; T₃: 90% wheat flour, 5% GCSF, and 5% NCSF cookies.

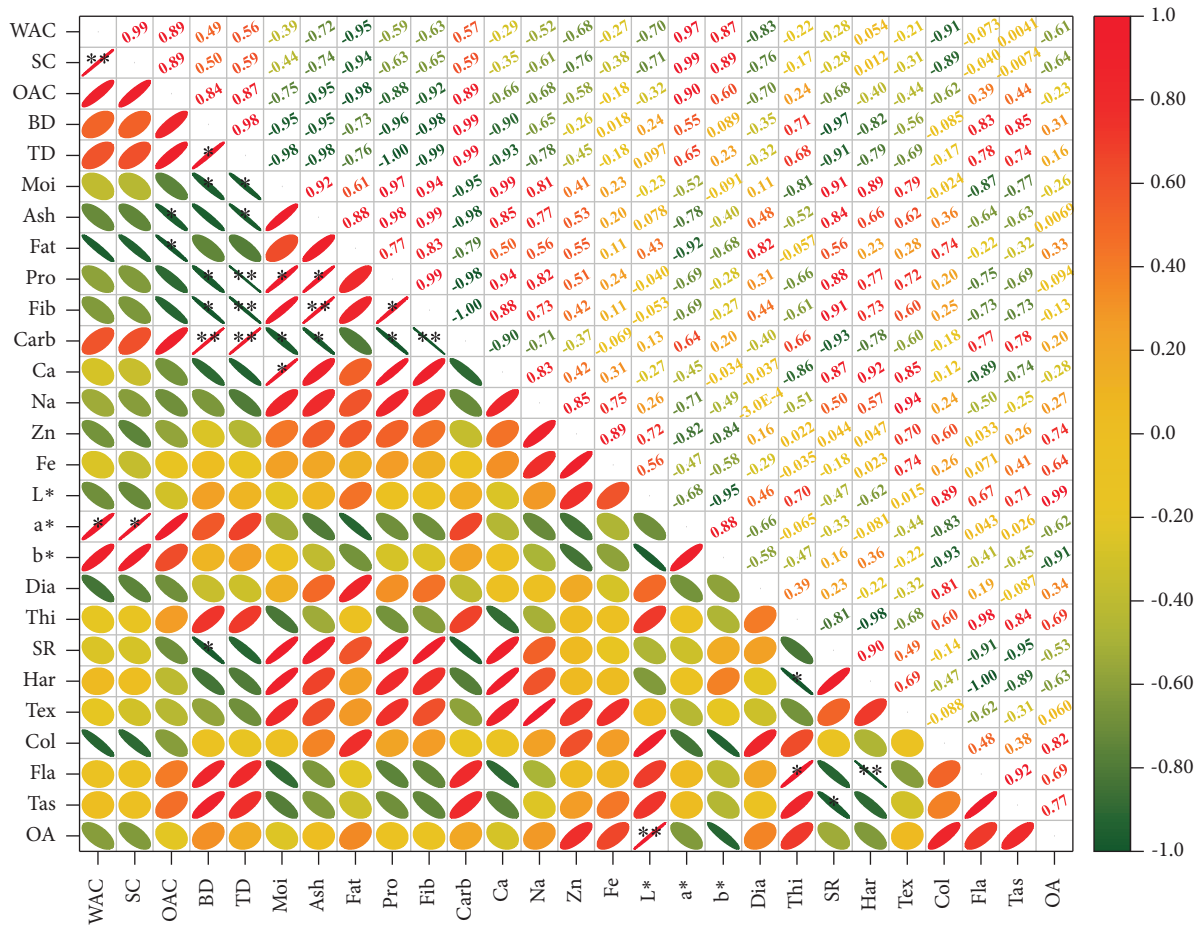


FIGURE 7: Pearson correlation coefficient of chia seed-formulated cookies. * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$.

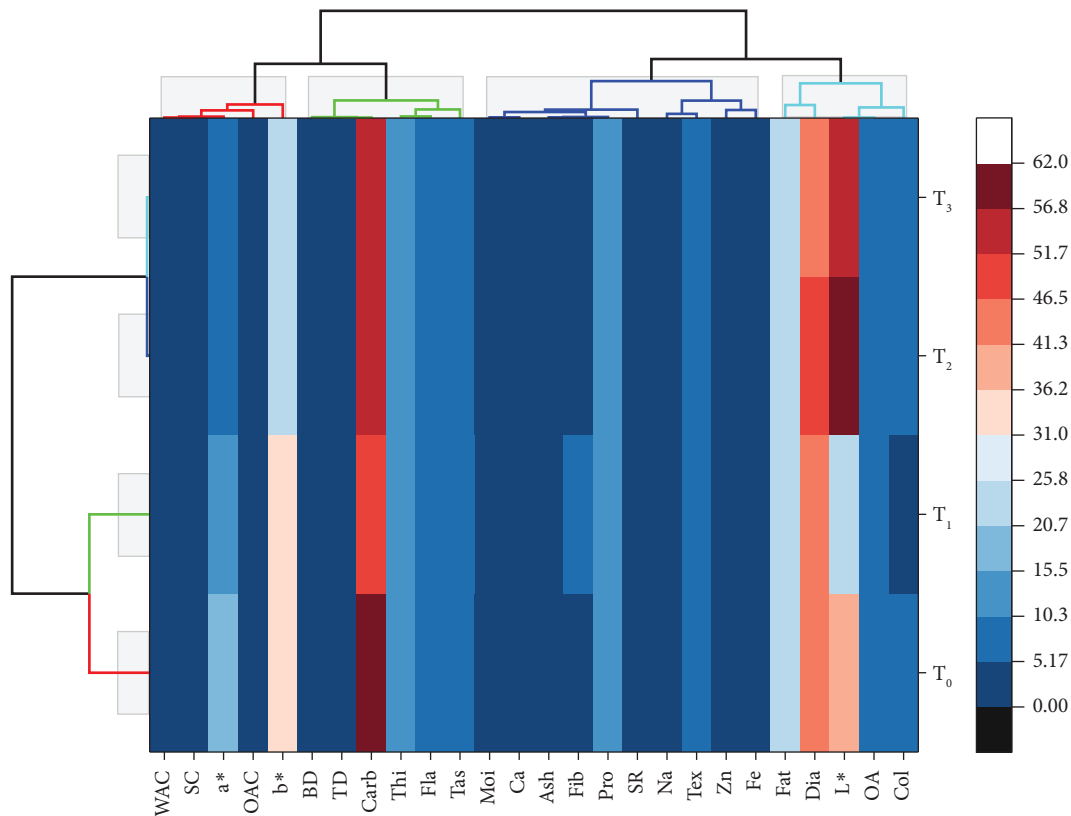


FIGURE 8: Hierarchical cluster analysis (HCA) performed on chia seed-formulated cookies with distance measurement based on the Pearson correlation distance and group average method of clustering.

contributing to PC2, which explained 25.10% of the variability. The overall acceptance of the formulated cookies was primarily influenced by color, taste, and flavor. According to the PCA biplot, T_1 was distinctly separated from T_0 , T_2 , and T_3 suggesting that its darker appearance and unpleasant earthy flavor, associated with GCSF, negatively impacted consumer acceptance [40, 66]. Concurrently, T_2 exhibited a strong association with flavor and taste, while T_3 was closely linked to texture and color. These findings suggest that cookies made with NCSF were perceived to have superior taste and flavor [65], whereas those containing a combination of GCSF and NCSF were favored for their texture and visual appeal, aligning with the panelists' assessments.

3.8. Multivariate Analysis. To investigate potential relationships among the variables in chia seed-formulated cookies, a Pearson correlation analysis was conducted (Figure 7). This statistical technique quantifies the strength and direction of linear relationships between variables, assessing whether changes in one variable are systematically associated with changes in another. A significant correlation is established between two variables when their correlation coefficient exceeds the threshold of ± 0.75 , indicating a substantial positive or negative association [67, 68]. The correlation showed a significant positive correlation ($r = 0.99$) between protein (Pro) and fiber content (Fib) of

cookies. In alignment with previous findings by Yang et al. [69], the present result demonstrates a positive correlation between germination and increased protein and fiber content. Concurrently, SC was positively associated with WAC ($r = 0.99$) and OAC ($r = 0.89$). Similar findings were also observed by Saeed et al. [16], who reported that this direct relationship might be attributed to the positive alteration of starch granules in cookies due to germination.

Furthermore, the lightness (L^*) positively correlates with overall acceptance (OA; $r = 0.99$) which indicates that the light brown color of cookies was more appreciated than the darker one. Chikpah et al. [51] confirmed the present findings, noting that high chia fruit pulp concentrations (over 15%) could darken cookies and affect their sensory perception. Conversely, protein (Pro) demonstrated an inverse correlation with total carbohydrate (Carb; $r = -0.98$), which could be attributed to the newly formed protein compounds and enzymatic breakdown of starch during germination [17, 42]. Again, cookie hardness (Har) was positively correlated with protein (Pro; $r = 0.77$) and fiber content (Fib; $r = 0.73$). These findings were consistent with Giuberti et al. [70], suggesting that protein and fiber can enhance the structural integrity of cookie dough, leading to a more compact and resilient matrix. Furthermore, calcium (Ca) exhibited a strong positive correlation with hardness (Har; $r = 0.92$) and protein content (Pro; $r = 0.94$) of cookies. This relationship suggests a potential interaction between calcium and protein, which may contribute to the structural

integrity of cookies, ultimately influencing their hardness [71]. In summary, these results shed light on the interconnectedness between different quality parameters in chia seed cookies, contributing to a deeper understanding of their functional property, sensory attributes, and overall acceptability.

In Figure 8, the HCA of chia seed cookies identified four distinct clusters based on their quality attributes. The vertical clusters were primarily distinguished by the experimental treatments applied to the cookies and the horizontal clusters were primarily differentiated by the measured attributes of the cookies. Cluster 1 primarily comprised properties related to WAC, SC, redness (a^*), OAC, and yellowness (b^*), suggesting a strong association with the control sample (T_0). Cluster 2 included BD, TD, total carbohydrate (Carb), thickness (Thi), flavor (Fla), and taste (Tas), indicating a significant influence from GCSF on cookies (T_1).

Cluster 3 focused on moisture (Moi), Ca, ash, fiber (Fib), protein (Pro), spread ratio (SR), Na, texture (Tex), Zn and Fe, and highlighting the impact of NCSF on cookies (T_2). Finally, Cluster 4 encompassed fat, diameter (Dia), lightness (L^*), overall acceptability (OA), and color (Col) of the cookies, suggesting a strong influence of the mixture of GCSF and NCSF (T_3) into cookies. These findings collectively demonstrate that the germination of chia seed notably affects the quality characteristics of chia seed cookies.

4. Conclusion

The research findings concluded that incorporating chia seed flour into cookie recipes is a viable and practical method for producing cookies with unique composite flour. By utilizing germinated chia seed as a key ingredient in the baking process offers a viable solution to combat malnutrition in developing countries and could offer a valuable market opportunity, as consumers seek healthier and more nutritious food options. The observed improvements in nutritional properties, including protein, fiber, and mineral content in chia seed-formulated cookies, may be attributed to the synthesis of new compounds via metabolic reactions during the germination process. The germination process significantly improved the functional and physicochemical properties of chia seed-based cookies. This positive impact can likely be attributed to the physiological and biochemical transformations that occur during chia seed germination. Based on the findings, incorporation of GCSF is recommended to produce nutritionally enhanced, technologically sound, and organoleptically acceptable functional cookies. This recommendation is supported by both chemical analysis and sensory evaluation data. Hence, in light of the empirical evidence, GCSF emerges as a promising ingredient for the development of functional baked products.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Tanjim Ahmed: conceptualization (equal), formal analysis (lead), methodology (lead), investigation (lead), visualization (equal), writing—original draft (lead), and writing and review—editing (equal); **Md. Rakibul Islam:** conceptualization (lead), investigation (lead), methodology (lead), supervision (lead), validation (lead), visualization (equal), writing—original draft (equal), and writing and review—editing (lead); **Md. Hasan Tarek Mondal:** conceptualization (equal), formal analysis (equal), methodology (equal), and writing and review—editing (equal); **Mrityunjoy Biswas:** conceptualization (equal), project administration (equal), supervision (equal), and writing and review—editing (equal); **Qazi Shabab Mahmood:** formal analysis (equal), methodology (equal), investigation (equal), and writing—original draft (equal); **Pallab Biswas:** formal analysis (equal), methodology (equal), and writing—original draft (equal); **Md. Akhtaruzzaman:** conceptualization (equal), project administration (lead), supervision (lead), and writing and review—editing (equal).

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