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*Research article*

## **Enhancing the functional qualities of soy flour cookies through solid-state fermentation with *Streptococcus thermophilus***

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**Abstract:** In the present study, *Streptococcus thermophilus* was utilized as a starter culture for the solid-state fermentation of soybeans. The fermentation was conducted on soybeans (with and without hull) at 5, 10, and 15 h intervals. The results indicate that the 15 h fermentation significantly reduced antinutritional factors and enhanced nutritional components. Fermented soy flour from both hull (HSF) and dehulled soybeans (DSF) was then used to develop cookies with varying concentrations. The formulations included: control (100% wheat flour), DSF-25 (soy flour 25%; wheat flour 75%), DSF-50 (soy flour 50%, wheat flour 50%), DSF-75 (soy flour 75%, wheat flour 25%), and DSF-100 (soy flour 100%). Blends of fermented soy flour and wheat flour were analyzed for functional properties, which were influenced by increasing soy flour concentration. Key findings included a decrease in

moisture content with higher soy flour concentrations, the highest water and oil absorption capacities in DSF-100 (239.6%), and the lowest in DSF-50 (123.63%). The weight and thickness of the cookies decreased as soy flour concentration increased. Textural analysis revealed increased hardness in DSF-25, DSF-50, and DSF-75, but decreased hardness in DSF-100 due to the high soy flour concentration. Sensory analysis showed the highest acceptability for DSF-25 and DSF-50 cookies, while DSF-100 cookies were less preferred. This study demonstrated the potential of using *Streptococcus thermophilus* for solid-state fermentation of soybeans to enhance their nutritional profile and reduce antinutritional factors. It provides insights into developing nutritionally and phenolic-enriched baked products, catering to consumer demand for functional foods with added health benefits.

**Keywords:** soybean; *Streptococcus thermophilus*; fermentation; antinutritional; cookies

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## 1. Introduction

Bakery products are globally popular due to their distinctive flavors, wide availability, and affordable cost. Cookies, prepared from composite flour, stand out as versatile snacks, frequently consumed as protein fortification vehicles, with prolonged shelf-stability and higher acceptability to satisfy hunger cravings [1,2]. With the aim of protein fortification, the impact of composite flours is being evaluated on the nutritional, sensory, and technological properties of product development [3]. Different ingredients are used in the manufacturing of cookies, including different flours (wheat, maize, plantain, sorghum, soybean, and pearl millet), oils, and additives, with health-promoting effects [4].

Soybean is an important crop with good nutritional qualities, including a rich source of edible oil (20%–22%) and a high concentration of protein (40%–45%), polysaccharides, vitamin E, vitamin B, and minerals. Soy proteins contain various bioactive peptides with anti-hypertensive, anti-carcinogenic, anti-hyperglycemic, immune-boosting, antioxidant, and weight-reducing properties. However, soybeans contain some antinutritional factors and complex hydrocolloids, limiting its utilization in various food products. These limiting factors can be reduced by fermentation, as healthy microbes enhance the quality of soybean protein and decrease antinutritional inhibitory factors [5,6].

Fermented foods promote health and maintain stable societies through bioconversion. Fermentation enhances foods' taste, nutritional content, and shelf life; ultimately, it can increase immunity, protect against pathogens, reduce blood cholesterol levels, and combat the effects of diabetes mellitus, cancer formation, allergic reactions, atherosclerosis, and excess weight [7]. Solid-state fermentation (SSF) is a process that occurs in a solid medium with minimal or no free water, relying on humidity to support the growth and metabolic activities of microorganisms on adherent cells. SSF has garnered significant research interest due to its higher yields and efficiency, superior product characteristics, reduced costs, and low energy requirements. SSF of soybean enhances soy protein and reduces its antinutritional potential and other limiting factors. During this process, complex macromolecular compounds such as fats, proteins, and carbohydrates are converted into polypeptides, oligopeptides, and fatty acids by microbial enzymes, thereby enhancing the nutritional availability of soybeans [6,8,9].

*Streptococcus thermophilus* is widely used as a starter culture in the dairy industry and has attracted attention as a beneficial bacterium due to its health-promoting functionalities in humans. It can survive harsh gastrointestinal (GI) conditions, modulate the immune system by producing

advantageous compounds (folate, bacteriocins), and release antioxidant enzymes and bioactive peptides that can quench free radicals (superoxide dismutase, NADH peroxidase), modulating pro-inflammatory mediators and metabolizing food components. It has demonstrated a positive effect on reducing cholesterol levels, proving to be a potential functional component for food and pharmaceutical applications [10,11].

The incorporation of probiotics in the development of diverse bakery food products has been perceived to enhance several physico-chemical, technological, and sensory aspects. Due to the synbiotic (probiotic and prebiotic) relation, the potential baked goods have improved qualitative features [12]. Fermented soybean flour cookies can be made using a blend of wheat flour and fermented soy flour. However, challenges include masking unpleasant tastes, maintaining a high protein/efficiency ratio, and preserving texture while reducing water absorption capacity. Soy flour, being a rich protein source, holds potential for mitigating malnutrition in developing countries. The current study used SSF with *Streptococcus thermophilus* to evaluate the compositional and phenolic components, the suppression of antinutritional factors, the bioavailability, and the digestibility of soy protein. Furthermore, the fermented soybean flour in combination with wheat flour was used to develop protein-enriched functional cookies. The cookies were subsequently examined for their physical, textural, and sensory attributes to ensure good quality.

## 2. Materials and methods

### 2.1. Compositional analysis

The compositional analysis (moisture, fat, and protein) of soybeans was evaluated according to a standard procedure [13].

### 2.2. Fermented soybean flour

Soybeans [*Glycine max* (L.) Merr.] were processed to remove stones, metal particles, dirt, dust, and damaged and immature seeds. Seeds were washed to remove contamination, soaked overnight, and then dehulled. Soaked hulled and dehulled soybean seeds were boiled (5 min) and supplemented with sucrose (2 g). Soybean seeds were sterilized in an autoclave for 20 min and inoculated with *Streptococcus thermophilus* [CHR-Hansen, Denmark; 5% v/m in sterilized phosphate buffer saline (PBS)] in sterile conditions in a laminar air flow (Figure 1). Inoculated seeds were placed in an incubator for fermentation at 37 °C for 5, 10, and 15 h in anaerobic conditions [14]. Fermented soybeans were dried in oven trays at 40–45 °C for 12 h. Fermented seeds were ground, sieved through a 250- $\mu$ m mesh size sieve, and packed in air-tight bags until use [4].

### 2.3. Antinutritional factors

#### 2.3.1. Phytic acid

The sample (2 g) was mixed with 100 mL of H<sub>2</sub>SO<sub>4</sub> (2%); the mixture was left to stand for 3 h and then filtered. Distilled water was added to the flask containing 50 mL of filtrate. A solution of ammonium thiocyanate (10 mL, 0.3%) was used as an indicator. A standard solution of ferric chloride

was used for titration, and the value was recorded when the color slightly changed into brownish yellow and persisted for 5 min [15].

$$\text{Phytic acid (\%)} = \frac{\text{Titrate value} \times 0.00195 \times 1.19 \times 3.55}{\text{Weight of sample}} \times 100 \quad (1)$$

### 2.3.2. Saponin

The sample (0.5 g) was defatted with 10 mL of petroleum ether; residues were extracted twice using methanol (80%) for 4 h and stored in the dark for further analysis. The extract (0.1 mL), 80% methanol (0.4 mL), 72% H<sub>2</sub>SO<sub>4</sub> (5.0 mL), and 8% valine solution (0.5 mL) were mixed well in a water bath at 60 °C and cooled. The absorbance of the sample and a blank was read at 544 nm with a UV-visible spectrophotometer [16].

### 2.3.3. Tannin

A sample size of 0.25 g was dissolved with 10 mL of HCl (4% in methanol). The sample was centrifuged at 4500 rpm for 10 min. HCl (5 mL, 1% in methanol) was mixed with the residues of the first extraction and diluted. Catechin was prepared at concentrations from 100 to 1000 ppm in methanol as the standard. The extract and standard solution, 1 mL each, were mixed with 4 mL of vanillin-HCl. Absorbances for blank, sample, and standard solutions were assessed at 500 nm using a spectrophotometer (UV-visible) [17]. The catechin equivalent (CE) was calculated as follows:

$$CE (\%) = \frac{CC \times VM}{VE \times Wt} \times 100 \quad (2)$$

CC, catechin concentration; VM, volume; VE, volume of extract; Wt, weight of the sample.

### 2.3.4. Total phenolic contents

The ground sample was extracted with ethanol (80%) and centrifuged to obtain a supernatant. Folin-Ciocalteu reagent (5 mL) was added to the 0.2 mL of sample extract and incubated at 25 °C for 4 min. Sodium carbonate (1.5 mL, 0.566 M) was mixed with the aliquot and incubated at 25 °C for 95 min. A UV-visible spectrophotometer was used to measure the absorbance of the sample at 725 nm [18].

## 2.4. Estimation of *in vitro* protein digestibility

The sample (2 g) was soaked, homogenized, and diluted to 20 mL. The homogenized sample was digested with pepsin (14 mg) in HCl (14 mL of 0.1 mol/L) at 37 °C for 2 h. Trichloroacetic acid (TCA) (15 mL, 10%) was added. The blend was filtered, and TCA-soluble fractions were assessed for nitrogen using Kjeldahl's method [19]. Digestibility was determined as follows:

$$\text{Protein Digestibility (\%)} = \frac{N \text{ in supernatant} - N \text{ in pepsin}}{N \text{ in sample}} \times 100 \quad (3)$$

## 2.5. Functional properties of fermented soybean flour

### 2.5.1. Bulk density (BD)

The tested flour (10 g) sample was gently poured into a graduated cylinder (25 mL) by gentle tapping. The final volume occupied by the flour was then measured and recorded as g/mL [20].

$$\text{Bulk Density} \left( \frac{\text{g}}{\text{mL}} \text{ or } \frac{\text{g}}{\text{cm}^3} \right) = \frac{\text{weight of sample}}{\text{Volume of sample after tapping}} \quad (4)$$

### 2.5.2. Swelling capacity (SC)

The powder sample was poured up to the 5 mL mark in a 50 mL graduated measuring cylinder, and distilled water was added (25 mL). The mixture was mixed homogeneously by inverting, allowing it to rest, and inverting again after 2 min to mix. Then, it was allowed to rest for 8 min. Finally, the volume occupied by the sample was estimated [21].

### 2.5.3. Water (WAC) and oil absorption capacity (OAC)

WAC was measured as per the method proposed by [22] with some modifications. The flour solution (1 g/10 mL) was mixed for 1 min at high speed on a vortex mixer, allowed to stand for 30 min, and centrifuged (3000 rpm, 30 min). For OAC, oil was used instead of water and calculated as oil bound per gram of flour.

$$\text{WAC, OAC (g/g)} = \frac{\text{Weight after centrifugation} - \text{Initial weight of sample}}{\text{Initial weight of sample}} \quad (5)$$

### 2.5.4. Emulsion activity (EA) and stability (ES)

Flour (2.0 g) was placed in a 50 mL Falcon tube and mixed with 20 mL of cold distilled water (4 °C) and 20 mL of refined vegetable oil. The contents were centrifuged at 4500 rpm for 10 min, and the height of the emulsified layer was measured for emulsion activity. To evaluate emulsion stability, the same conditions were applied after heating the emulsion at 80 °C for 30 min in a water bath. The aliquot was allowed to cool at room temperature, and the levels of emulsified layers were examined accordingly [23].

$$\text{Emulsion Activity (\%)} = \frac{\text{Height of emulsion layer}}{\text{Height of whole layer}} \times 100 \quad (6)$$

### 2.5.5. Foam capacity (FC) and foam stability (FS)

FC and FS of flour blends were measured by mixing 2 g of the sample with 100 mL of distilled water. The suspension was blended (5 min, 13,000 rpm) at room temperature and transferred immediately to a measuring cylinder (250 mL). Foam volume was examined. FC was calculated as the percentage increase in volume, whereas FS was assessed by measuring the fall in FC over 1 h [20].

$$\text{Foam capacity (\%)} = \frac{\text{volume after whipping} - \text{volume before whipping}}{\text{volume before whipping}} \times 100 \quad (7)$$

$$\text{Foam stability (\%)} = \frac{\text{volume after standing} - \text{volume before whipping}}{\text{volume before whipping}} \times 100 \quad (8)$$

## 2.6. Cookie production

Cookies were prepared using the method reported by [24,25] with some modifications. Based on qualitative features, the best treatments selected were DSB-15 (dehull soybean with 15 h fermentation time) and HSB-15 (hull soybean with 15 h fermentation time). These two varieties were ground to fine powder (DSF, dehull soy flour; HSF, hull soy flour) and eight treatments (Figure 2) were prepared using different concentrations of flours (DSB-15, HSB-15, and wheat flour): T<sub>0</sub>: wheat flour (100%), DSF-25: DSB-15 (25%) and wheat flour (75%), DSF-50: DSB-15 (50%) and wheat flour (50%), DSF-75: DSB-15 (75%) and wheat flour (25%), DSF-100: DSB-15 (100%), HSF-25: HSB-15 (25%) and wheat flour (75%), HSF-50: HSB-15 (50%) and wheat flour (50%), HSF-75: HSB-15 (75%) and wheat flour (25%), HSF-100: HSB-15 (100%).

## 2.7. Physical properties

Physical parameters of cookies, namely weight, thickness, diameter, and spread ratio, were evaluated according to previous methods [26]. The weight of cookies was measured with a digital weighing balance in triplicate. Thickness was examined using vernier calipers by stacking six cookies; average values were expressed in millimeters. The diameter of the six cookies was also estimated using vernier calipers. As a check determinant, cookies were turned 90°, and the mean value of the diameter of six cookies was examined. The spread ratio was calculated by determining the ratio between the mean diameter value (D) and thickness (T).

## 2.8. Color

A colorimeter was used to determine color properties. The device was calibrated using standard white and black bodies. The various color parameters (L\*, a\*, b\*) were estimated in triplicate [27].

L\* specified black to white colors (0 to 100), a\* designated red to green (+/- red/green), and b\* indicated yellow to blue (+/- yellow/blue). Other parameters [chroma (c) and hue] were also derived.

## 2.9. Textural properties

The hardness of the cookies was determined using a universal testing machine (UTM) (model Zwick Roell, USA) with a wedge-shaped cutting probe in compression mode. The speed of the probe was adjusted to 10 mm/s, the data was collected at a resolution of 500 Hz, and the load cell used was 5 kg. Each cookie was compressed in terms of peak force, and its hardness was noted [28].

## 2.10. Sensory evaluation

Panelists were directed to assess the cookies' sensory features, including color, taste, appearance,

crispness, and overall acceptability, as endorsed by the ethical committee of the university. A hedonic scale (9-point) [1.0: extremely dislike; 5.0: neither like nor dislike; and 9.0: extremely like] was used [29]. Developed cookies were labeled with code numbers and offered to 15 panelists (male and female) in a randomized order from the Faculty of Food Science & Nutrition, BZ-University. Water was used as a palate cleanser, and panelists were instructed to refrain from discussing their assessments to limit influencing each other. Questionnaires were provided to the panelists to freely provide suggestions.

### 2.11. Statistical analysis

The obtained results were evaluated statistically by analysis of variance (ANOVA) under a completely randomized design (CRD). Mean values were compared at a 5% significance level using Statistix Software version 8.1 [30].

## 3. Results and discussion

### 3.1. Compositional analysis of raw and fermented soybean flour

The effect of fermentation on the compositional analysis (moisture, protein, and fat) of soybean seeds was evaluated (Table 1). The statistical analysis showed that fermentation has a significant positive effect on the protein content of soybeans. However, for fat and moisture concentrations, the effect of fermentation was non-significant.

**Table 1.** Compositional evaluation of raw and fermented soybeans.

Treatments	Protein %	Fat %	Moisture %
T <sub>0</sub>	33.170 ± 0.206 <sup>g</sup>	17.623 ± 0.107 <sup>f</sup>	11.033 ± 0.035 <sup>a</sup>
DSB-5	34.967 ± 0.035 <sup>e</sup>	18.343 ± 0.030 <sup>e</sup>	9.59 ± 0.517 <sup>d</sup>
DSB-10	39.747 ± 0.050 <sup>b</sup>	18.750 ± 0.02 <sup>c</sup>	10.113 ± 0.015 <sup>c</sup>
DSB-15	43.010 ± 0.085 <sup>a</sup>	19.030 ± 0.060 <sup>a</sup>	10.910 ± 0.036 <sup>a</sup>
HSB-5	33.890 ± 0.079 <sup>f</sup>	18.270 ± 0.017 <sup>e</sup>	8.850 ± 0.04 <sup>e</sup>
HSB-10	37.077 ± 0.068 <sup>d</sup>	18.487 ± 0.015 <sup>d</sup>	9.780 ± 0.06245 <sup>d</sup>
HSB-15	37.890 ± 0.115 <sup>c</sup>	18.910 ± 0.02 <sup>b</sup>	10.567 ± 0.025 <sup>b</sup>

Mean values (± SD,  $n = 3$ ) with different letters show significant differences ( $p < 0.05$ ) column-wise. T<sub>0</sub>, raw soybean without fermentation; DSB-5, dehull soybean with 5 h fermentation time; DSB-10, dehull soybean with 10 h fermentation time; DSB-15, dehull soybean with 15 h fermentation time; HSB-5, hull soybean with 5 h fermentation time; HSB-10, hull soybean with 10 h fermentation time; HSB-15, hull soybean with 15 h fermentation time.

The moisture contents of fermented soybeans (hull and dehull) were lower than that of raw soybeans (11.033%) but increased gradually with the fermentation time. In a previous study, the moisture present in raw soybeans was 8.07%, while in the current study, moisture was 11.033% [31]. Multiple extrinsic or intrinsic factors are responsible for moisture variations. Regarding fat evaluation, the fat contents of fermented soybeans (hull and dehull) were higher than those of raw soybeans (17.623%) and increased gradually with the fermentation time. The fat contents were comparable to those found in a previous study (in the range of 18.43%) [14].

In DSB, an increasing protein percentage was observed in DSB-5 (34.967%), reaching 39.747%

in DSB-10 and 43.010% in DSB-15, compared to the control (33.170%). In HSB, protein increase was found to be 33.890% (HSB-5), reaching 37.077% and 37.890% in HSB-10 and HSB-15, respectively. Recent findings show that the 33.170% protein value is comparable to the soybean protein content of 33.10% [14]. Solid state fermentation enhanced the protein and essential amino acids contents of soybeans. Also, the presence of higher protein contents may be attributed to a reduction in antinutritional factors that bind with proteins and other compounds, and reduce the digestibility and bioavailability of proteins. Still, fermentation plays a pivotal role in reducing these contributing factors [32,33].



**Figure 1.** Soybean seeds (H, with hull; DH, without hull) fermented for 5, 10, and 15 h.

### 3.2. Antinutritional factors

The mean antinutritional factor values of non-fermented and fermented soybean flours are presented in Table 2. Significant differences were found between different treatments. The concentration of phytic acid decreased with an increase in fermentation time. A greater reduction in concentration was found in DSB-15 (0.1398 mg/g) and HSB-15 (0.2071 mg/g) when compared to the control (0.4180 mg/g). A gradual decrease was found in DSB flour after 5, 10, and 15 h of fermentation, at 25%, 37.60%, and 66.55%, while for HSB flour it was 13.70%, 31.29% and 50.47%, respectively. As such, fermentation showed a greater impact on the decrease of phytic acid in HSB (66.55%). In previous studies, legume fermentation by various strains, like *Bacillus pumilus*, for 72 h, caused a reduction in phytic acid content; similarly, fermentation with *L. plantarum* for 5 days significantly reduced phytic acid from 1.16 to 0.04 mg/g [34,35].

**Table 2.** Antinutritional factors, phenolics, and in vitro digestibility of native and fermented soybeans.

Treatments	Phytic acid (mg/g)	Saponin (mg/g)	Tannin (mg/g)	TPC (mg/g)	In vitro protein digestibility (%)
T <sub>0</sub>	0.4180 ± 0.0010 <sup>a</sup>	3.97 ± 0.000023 <sup>a</sup>	0.0953 ± 0.0002 <sup>a</sup>	0.1090 ± 0.0012 <sup>c</sup>	75.053 ± 0.0404 <sup>g</sup>
DSB-5	0.3135 ± 0.0002 <sup>c</sup>	1.28 ± 0.000008 <sup>c</sup>	0.0441 ± 0.0009 <sup>c</sup>	0.1072 ± 0.0001 <sup>d</sup>	80.077 ± 0.8963 <sup>e</sup>
DSB-10	0.2608 ± 0.0003 <sup>e</sup>	0.11 ± 0.000006 <sup>f</sup>	0.0392 ± 0.0004 <sup>d</sup>	0.1077 ± 0.0001 <sup>d</sup>	86.267 ± 0.1067 <sup>b</sup>
DSB-15	0.1398 ± 0.0001 <sup>g</sup>	0.106 ± 0.000005 <sup>g</sup>	0.0274 ± 0.0002 <sup>f</sup>	0.1100 ± 0.0001 <sup>c</sup>	89.150 ± 0.1323 <sup>a</sup>
HSB-5	0.3607 ± 0.0002 <sup>b</sup>	1.3 ± 0.00004850 <sup>b</sup>	0.0579 ± 0.0013 <sup>b</sup>	0.1122 ± 0.0014 <sup>b</sup>	76.940 ± 0.0265 <sup>f</sup>
HSB-10	0.2872 ± 0.0038 <sup>d</sup>	1.25 ± 0.0000070 <sup>d</sup>	0.0418 ± 0.0002 <sup>c</sup>	0.1128 ± 0.0018 <sup>b</sup>	81.233 ± 0.0153 <sup>d</sup>
HSB-15	0.2071 ± 0.0001 <sup>f</sup>	1.17 ± 0.0000046 <sup>e</sup>	0.0327 ± 0.0002 <sup>e</sup>	0.1146 ± 0.0004 <sup>a</sup>	83.167 ± 0.1478 <sup>c</sup>

Mean values (± SD,  $n = 3$ ) with different letters show significant differences ( $p < 0.05$ ) column-wise. T<sub>0</sub>, raw soybean without fermentation; DSB-5, dehull soybean with 5 h fermentation; DSB-10, dehull soybean with 10 h fermentation; DSB-15, dehull soybean with 15 h fermentation; HSB-5, hull soybean with 5 h fermentation; HSB-10, hull soybean with 10 h fermentation; HSB-15, hull soybean with 15 h fermentation.

Higher decreasing saponin contents were observed in DSB-15 (0.106 mg/g) and HSB-15 (1.17 mg/g) flour when compared to the control (3.97 mg/g). The gradual decrease in DSB flour after 5, 10, and 15 h of fermentation was 67.75%, 71.78%, and 97%; for HSB flour, it was 67.2%, 68.51%, and 70.52%, respectively. Fermentation showed a greater impact on the decrease of saponin in DSB (97%). According to a previous study, raw soybeans contained 5 mg/g saponin; in another recent study, 3.97 mg/g saponin content was observed in untreated soybeans [36]. Saponin decreases significantly with fermentation using *Bifidobacterium infantis* and *Streptococcus thermophilus* at 37 °C for 24 h [37]. The tannin contents also decreased in DSB-15 (0.0274 mg/g) and HSB-15 (0.0327 mg/g) flour when compared to the control (0.093 mg/g). The gradual decrease in DSB flour after 5, 10, and 15 h of fermentation was 53.72%, 58.86%, and 71.24%; for HSB flour, it was 39.24%, 58.13%, and 65.68%, respectively. Fermentation showed a greater impact on the decrease of tannin in DSB (71.24%). Other works found 10–30 mg/kg of tannin in soybeans [38], but *Lactobacillus plantarum* reduced its impact and caused a significant reduction in tannin contents after 24 h of fermentation [35].

### 3.3. *In vitro* protein digestibility

Fermentation time greatly influenced protein digestibility. Higher values were recorded in DSB-15 (89.150%) and HSB-15 (83.167%) flour when compared to the control (75.053%) (Table 2). In the current study, *in vitro* protein digestibility of soybeans was enhanced to 89.150% after 15 h of fermentation; previously, protein digestibility was found to be significantly improved (up to 83.9%) after 17.5 h of fermentation using *Lactobacillus plantarum* [14].

### 3.4. Total phenolic content (TPC)

TPC was significantly affected by increased fermentation time for DSB and HSB flour (Table 2). Both the DSB and HSB flours showed a gradual increase in TPC with increasing fermentation time; the TPC values at 5 h fermentation were lower (0.1072 mg/g) and higher (0.1122 mg/g), respectively, for DSB and HSB flour when compared to the control (0.1090 mg/g). TPC in HSB flour was higher than in DSB flour, due to the presence of total phenolics in the hull. The statistical tests showed that an increase in fermentation time impacted the TPC in HSB flour more than in DSB flour. While our work found 0.1090 mg/g, raw soybeans were found to contain 0.11–1.75 mg/g [39]. Lactic acid bacteria, including *Bifidobacterium infantis* and *Streptococcus thermophilus*, influenced the enhancement of TPC in soymilk after 24 h fermentation at 37 °C [37]. The total polyphenol (235.9%) and aglycone isoflavone (324.12%) contents were significantly enhanced by the solid-state fermentation process, resulting in higher antioxidant potential [32].

### 3.5. Functional properties of fermented blended soybean flour

The functional properties of fermented blended soybean flour depicted higher significance for all treatments (Table 3). Moisture content revealed variable behavior for DSF and HSF flour blends, but a decrease in moisture content was found for both HSF and DSF when compared to the control. The presence of higher moisture in HSF was attributed to the dietary fiber and protein, which may cause a reduction in water-soluble starch, generating partial humidity in flours [21].

In DSF, bulk density was found to be higher in DSF-75 (0.88 g/cc); in HSF, HSF-50 contained the highest value (1.00 g/cc), while the lowest density (0.769 g/cc) was observed in DSF-25. An increase in bulk density has been linked with an increase in DSF fermented flour. Similar results were found in previous research [40]. The incorporation of wheat flours with DSF flour led to an increase in bulk density, but it decreased (non-significantly) when HSF flour was mixed with wheat flour. Bulk density was greatly influenced by particle size and moisture content. High bulk density of flour was preferred for the food formulations and their desirability. Additionally, lower bulk density can be an advantage while preparing supplemental foods [41,42].

The swelling capacity of DSF and HSF was significantly affected ( $p < 0.5$ ). The highest value, observed in the control treatment, was 15 mL, followed by 13 and 9.33 mL in DSF-25 and HSF-25, respectively. In both DSF and HSF, SC decreased gradually with an increase in soy flour concentration, but DSF showed higher values than HSF. HSF-100 exhibited the lowest value of SC (7.4 mL). This reduction was associated with the increase in soy flour concentration. The lower SC in HSF may be due to a higher concentration of insoluble dietary fiber.

In HSF, maximum and minimum WAC were observed in HSF-75 (281.93%) and HSF-25 (140.4%),

respectively. In DSF, maximum and minimum WAC were found for DSF-75 (239.63%) and DSF-25 (123.63%), respectively, being significantly influenced by the enhancement in soy flour. It was hypothesized that more water would be absorbed due to the presence of fiber in HSF but not in DSF [21]. In another study, a variation in functional features could be noted in flour blends (wheat and soy fermented flour) [43]. In DSF, maximum and minimum OAC occurred in DSF-75 (140.3%) and DSF-100 (119.20%), respectively. In HSF, maximum and minimum OAC were observed in HSF-75 (248.27%) and HSF-25 (104.93%), respectively. HSF-75 presented higher OAC than the others due to the presence of fiber concentrations, in accordance with a previous study [44]. There is a possible, valuable molecular interaction that specifically increases the sustainability of flavors and improves the palatability as well as the shelf life of different products, especially cereal-based bakery products or others that require fat absorption [40]. The presence of protein is considered to influence OAC due to hydrophobic interactions with lipid hydrocarbon chains; as a result, non-polar amino acid side chains can be generated [45].

Higher FC and FS were observed in HSF-75 (34.65%) and HSF-25 (73.73%), while lower FC and FS were shown in DSF-100 (13.04%) and HSF-50 (27.27%), respectively. The variations in FC and FS found here were in line with previous findings [46,47]. Flours with higher FC may produce more air bubbles that bond with the elastic protein film. Air bubbles might be broken down easily, therefore contributing to lower foam stability [45].

EA and ES were found to be lower in DSF-100 (6.00%) and HSF-100 (23.30%) and higher in the control, HSF-75 (18.88%), and DSF-75 (53.77%). These findings are in agreement with other studies, which found proteins to be a good surface-active agent, influencing emulsification and stabilization impact [44,48].

### 3.6. Qualitative properties of cookies

#### 3.6.1. Physical properties

The presence of DSF and HSF led to significant differences ( $p \leq 0.05$ ) in various parameters such as weight, diameter, height, and spread ratio of cookies (Table 4). Higher weight was found in DSF-25 (11.475 g), and lower weight was found in DSF-100 (10.123 g), compared to the control (11.12 g). These results show that higher concentrations of HSF decrease cookie weight.

Maximum and minimum mean weight of cookies in HSF were found in HSF-25 (11.479 g) and HSF-100 (10.746 g), respectively. Increased cookie weight in HSF compared to DSF is clearly due to the presence of fiber content in HSF [49]. In DSF, DSF-50 and DSF-100 exhibited higher (42.66 mm) and lower (35 mm) diameters, while the control treatment ( $T_0$ ) exhibited a diameter of 45.00 mm. When compared with cookies with 100% soy flour concentration, the diameter of DSF-100 was significantly reduced. In HSF cookies, HSF-25 and HSF-100 possessed the higher (43 mm) and lower (40 mm) diameters. Column-wise comparisons revealed that cookies' diameter decreased with an increase in soy flour concentration. The diameter of HSF cookies was higher than that of DSF cookies, as HSF contained a higher amount of hull (fiber content) [50].

**Table 3.** Functional properties of soy fermented flour blends.

Flour	Moisture %	BD (g/cc)	SC (mL)	WAC %	OAC %	FC %	FS %	EA %	ES %
T <sub>0</sub>	12.4 ± 0.02 <sup>a</sup>	1.157 ± 0.05 <sup>a</sup>	15.0 ± 0.5 <sup>a</sup>	140.03 ± 0.25 <sup>f</sup>	121.03 ± 0.80 <sup>f</sup>	25.45 ± 0.90 <sup>b</sup>	71.57 ± 6.13 <sup>a</sup>	18.88 ± 1.92 <sup>a</sup>	46.2 ± 1.38 <sup>bc</sup>
DSF-25	10.407 ± 0.05 <sup>e</sup>	0.769 ± 0.01 <sup>f</sup>	13.00 ± 0.5 <sup>b</sup>	123.63 ± 4.72 <sup>g</sup>	116.57 ± 1.58 <sup>f</sup>	17.33 ± 1.15 <sup>d</sup>	71.52 ± 8.41 <sup>a</sup>	12.2 ± 1.924 <sup>bc</sup>	44.0 ± 0.66 <sup>bc</sup>
DSF-50	10.1 ± 0.03 <sup>g</sup>	0.78 ± 0.0 <sup>ef</sup>	12.0 ± 0.5 <sup>c</sup>	138.90 ± 6.58 <sup>f</sup>	130.07 ± 6.14 <sup>e</sup>	23.40 ± 1.06 <sup>c</sup>	47.66 ± 3.6 <sup>b</sup>	10.44 ± 3.42 <sup>bcd</sup>	48.66 ± 0.66 <sup>b</sup>
DSF-75	9.9 ± 0.3 <sup>h</sup>	0.88 ± 0.0 <sup>c</sup>	10.0 ± 0.5 <sup>d</sup>	239.60 ± 4.35 <sup>c</sup>	140.43 ± 5.06 <sup>d</sup>	35.47 ± 0.85 <sup>a</sup>	47.05 ± 4.49 <sup>b</sup>	8.88 ± 3.84 <sup>cd</sup>	53.77 ± 2.14 <sup>a</sup>
DSF-100	9.3 ± 0.04 <sup>i</sup>	0.85 ± 0.00 <sup>cd</sup>	9.0 ± 0.62 <sup>ef</sup>	219.20 ± 0.7 <sup>d</sup>	119.20 ± 2.59 <sup>f</sup>	13.04 ± 1.08 <sup>f</sup>	69.40 ± 9.52 <sup>a</sup>	6.00 ± 1.15 <sup>d</sup>	27.778 ± 1.92 <sup>c</sup>
HSF-25	11.4 ± 0.01 <sup>b</sup>	0.98 ± 0.0 <sup>b</sup>	9.33 ± 0.15 <sup>de</sup>	140.4 ± 4.45 <sup>f</sup>	104.93 ± 5.18 <sup>g</sup>	18.62 ± 0.98 <sup>d</sup>	73.73 ± 4.80 <sup>a</sup>	15.778 ± 4.23 <sup>ab</sup>	41.7 ± 4.43 <sup>c</sup>
HSF-50	11.1 ± 0.14 <sup>c</sup>	1.00 ± 0.02 <sup>b</sup>	8.40 ± 0.1 <sup>f</sup>	198.2 ± 4.92 <sup>e</sup>	228.70 ± 5.18 <sup>b</sup>	23.958 ± 1.04 <sup>bc</sup>	27.27 ± 2.28 <sup>c</sup>	12.22 ± 5.09 <sup>bc</sup>	45.55 ± 5.09 <sup>bc</sup>
HSF-75	10.8 ± 0.04 <sup>d</sup>	0.99 ± 0.11 <sup>b</sup>	8.70 ± 0.1 <sup>ef</sup>	281.93 ± 0.68 <sup>a</sup>	248.57 ± 0.15 <sup>a</sup>	34.651 ± 0.83 <sup>a</sup>	29.96 ± 3.13 <sup>c</sup>	18.88 ± 5.09 <sup>a</sup>	43.7 ± 3.00 <sup>c</sup>
HSF-100	10.3 ± 0.07 <sup>f</sup>	0.81 ± 0.01 <sup>de</sup>	7.4 ± 0.1 <sup>g</sup>	268.97 ± 14.18 <sup>b</sup>	207.63 ± 1.52 <sup>c</sup>	25.03 ± 0.85 <sup>e</sup>	44.76 ± 1.90 <sup>b</sup>	8.88 ± 1.92 <sup>cd</sup>	23.3 ± 3.33 <sup>d</sup>

Mean values (± SD,  $n = 3$ ) containing different letters significantly differ from each other when compared column-wise. T<sub>0</sub>, 100% wheat flour or control; DSF-25: DSB-15 (25%) + wheat flour (75%); DSF-50: DSB-15 (50%) + wheat flour (50%); DSF-75: DSB-15 (75%) + wheat flour (25%); DSF-100: DSB-15 (100%); HSF-25: HSB-15 (25%) + wheat flour (75%); HSF-50: HSB-15 (50%) + wheat flour (50%); HSF-75: HSB-15 (75%) + wheat flour (25%); HSF-100: HSB-15 (100%). BD, bulk density; SC, swelling capacity; WAC, water absorption capacity; OAC, oil absorption capacity; FC, foaming capacity; FS, foaming stability; EA, emulsion activity; ES, emulsion stability.

The findings regarding cookie thickness depicted non-significant differences between the DSF and HSF-based cookies, but there were small variations in treatments. In DSF and HSF cookies, higher thickness was found in HSF-50 (10.970 mm) and lower in DSF (9.91 mm). In HSF and DSF cookies, decreased thickness was observed in the treatment with 100% soy flour, since wheat flour was not present. The variation in cookie thickness with the enhancement in soy flour increased their quality, which could be defined by protein concentration [51].

In both HSF and DSF cookies, the maximum mean spread ratio was measured in HSF-25 (4.067); the minimum was observed in DSF-100 (3.53), when compared with the control (4.9055). A decrease in the spread ratio of cookies might be due to the higher concentration of soy rather than wheat flour [52]. An increase in protein concentration reduces the spread ratio of cookies. The higher protein content used in this analysis for soy flour may have led to a decreased distribution. A higher soy flour substitution of wheat flour resulted in decreased basic parameters. This is probably due to fibers in soy flour that interfere with the matrix composition, limiting the dough's gas-retaining ability [53].

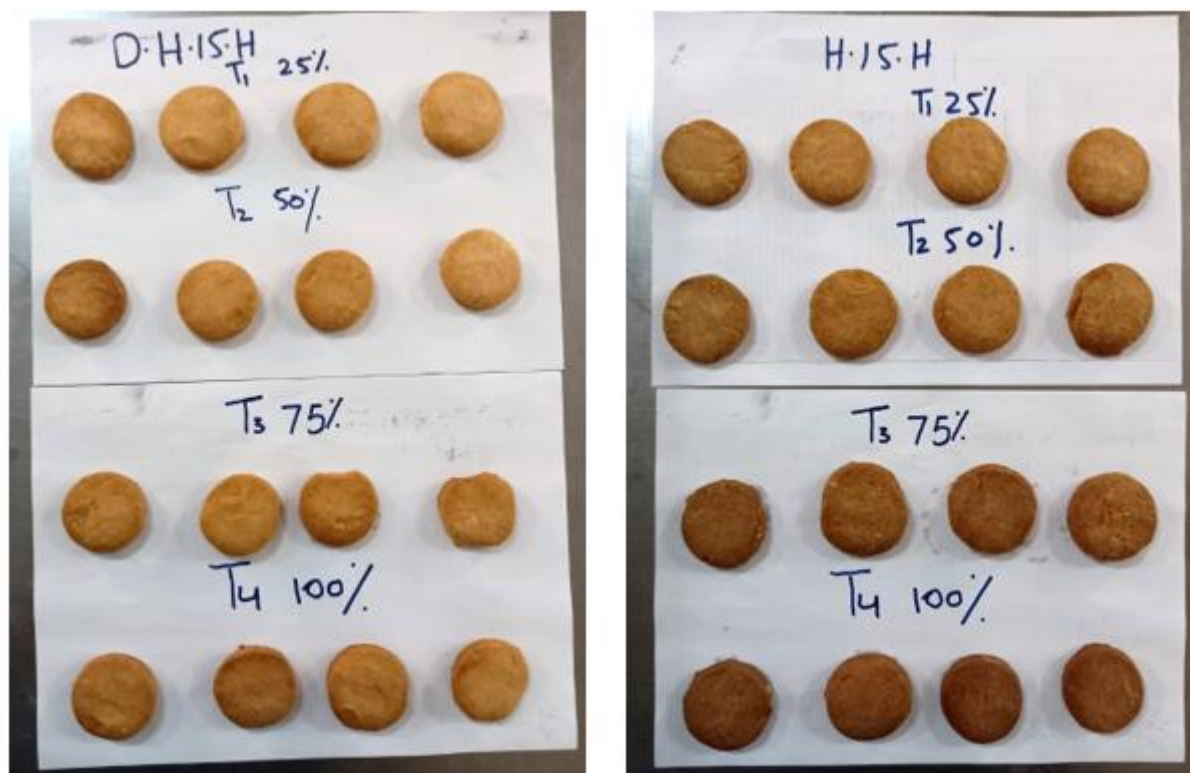
### 3.6.2. Color analysis of cookies

Statistical analysis of cookies revealed a highly significant effect of dehull and hull fermented soybean flour on crust brightness, redness, and yellowness of all cookies (Table 4). A higher value of L\* was found in DSF-50 (75.43), while a lower value was measured in DSF-100 (64.14) when compared to the control (73.51). Results revealed that the higher concentration of soy flour in DSF-100 reduced brightness. In HSF cookies, the maximum mean value of L\* was observed in DSF-50 (70.55), and the minimum mean value of L\* was observed in HSF-75 (58.25). This reveals that a higher concentration of fiber in the HSF cookies significantly decreased their brightness. This decrease in cookie brightness might also be due to the fermentation of HSF. In addition to soy protein isolate and non-enzymatic browning reactions, Maillard products also result in darker cookies and increased saturation due to the high protein and carbohydrate contents of soy flour [54,55]. In another study, bread samples containing 15% soy exhibited darker crust and crumb color, as compared to other bread samples [56].

**Table 4.** Qualitative features of soy flour fermented cookies.

Flour conc	Weight	Diameter	Height	Spread ratio	L*	a*	b*	Hardness
T <sub>o</sub>	11.12 ± 1.8 <sup>a</sup>	45.00 ± 1 <sup>a</sup>	9.173 ± 0.03 <sup>b</sup>	4.9055 ± 0.10 <sup>a</sup>	73.51 ± 5.40 <sup>ab</sup>	10.52 ± 0.28 <sup>c</sup>	38.11 ± 1.74 <sup>a</sup>	13.55 ± 0.21 <sup>d</sup>
DSF-25	11.475 ± 0.3 <sup>a</sup>	42.333 ± 1.5 <sup>b</sup>	10.437 ± 0.02 <sup>ab</sup>	4.055 ± 0.10 <sup>cd</sup>	68.43 ± 12.92 <sup>abc</sup>	8.037 ± 0.83 <sup>d</sup>	33.85 ± 3.65 <sup>c</sup>	21.85 ± 1.90 <sup>a</sup>
DSF-50	10.814 ± 0.7 <sup>a</sup>	42.66 ± 1.5 <sup>b</sup>	10.890 ± 0.6 <sup>a</sup>	3.919 ± 0.32 <sup>bcd</sup>	75.43 ± 5.36 <sup>a</sup>	10.77 ± 1.08 <sup>c</sup>	34.52 ± 1.65 <sup>c</sup>	21.46 ± 1.61 <sup>a</sup>
DSF-75	10.734 ± 0.009 <sup>ab</sup>	38.333 ± 0.5 <sup>d</sup>	10.363 ± 0.08 <sup>ab</sup>	3.698 ± 0.06 <sup>abc</sup>	70.36 ± 3.24 <sup>abc</sup>	12.64 ± 0.69 <sup>bc</sup>	35.65 ± 1.88 <sup>ab</sup>	16.60 ± 0.14 <sup>bc</sup>
DSF-100	10.123 ± 0.02 <sup>a</sup>	35.000 ± 1 <sup>e</sup>	9.91 ± 2.5 <sup>b</sup>	3.53 ± 1.4 <sup>ab</sup>	64.14 ± 3.21 <sup>cd</sup>	14.70 ± 0.67 <sup>ab</sup>	35.10 ± 1.27 <sup>abc</sup>	14.05 ± 1.48 <sup>cd</sup>
HSF-25	11.479 ± 0.2 <sup>ab</sup>	43.000 ± 1 <sup>c<sup>d</sup></sup>	10.163 ± 0.2 <sup>ab</sup>	4.067 ± 0.07 <sup>abcd</sup>	70.55 ± 1.10 <sup>abc</sup>	12.46 ± 0.99 <sup>c</sup>	33.85 ± 0.70 <sup>bc</sup>	17.55 ± 0.92 <sup>b</sup>
HSF-50	11.295 ± 0.3 <sup>a</sup>	42.000 ± 1 <sup>b<sup>c</sup></sup>	10.970 ± 0.56 <sup>a</sup>	3.82 ± 0.14 <sup>bcd</sup>	64.50 ± 1.55 <sup>bcd</sup>	15.61 ± 3.50 <sup>a</sup>	32.42 ± 0.60 <sup>cd</sup>	18.20 ± 0.28 <sup>b</sup>
HSF-75	10.945 ± 0.5 <sup>a</sup>	41.000 ± 1 <sup>a</sup>	10.85 ± 0.4 <sup>ab</sup>	3.77 ± 0.10 <sup>cd</sup>	58.25 ± 3.06 <sup>d</sup>	15.19 ± 0.41 <sup>a</sup>	29.78 ± 1.17 <sup>d</sup>	23.55 ± 0.14 <sup>a</sup>
HSF-100	10.746 ± 0.4 <sup>ab</sup>	40.00 ± 1.7 <sup>cd</sup>	10.80 ± 0.08 <sup>ab</sup>	3.70 ± 0.09 <sup>d</sup>	68.55 ± 2.54 <sup>abc</sup>	15.56 ± 0.26 <sup>a</sup>	30.59 ± 1.62 <sup>d</sup>	18.25 ± 2.19 <sup>b</sup>

Mean values (± SD, n = 3) containing different letters are significantly different from each other when compared column-wise. T<sub>o</sub>, 100% wheat flour or control; DSF-25: DSB-15 (25%) + wheat flour (75%); DSF-50: DSB-15 (50%) + wheat flour (50%); DSF-75: DSB-15 (75%) + wheat flour (25%); DSF-100: DSB-15 (100%); HSF-25: HSB-15 (25%) + wheat flour (75%); HSF-50: HSB-15 (50%) + wheat flour (50%); HSF-75: HSB-15 (75%) + wheat flour (25%); HSF-100: HSB-15 (100%).



**Figure 2.** Cookies developed with various concentrations of wheat and soybean flour (HSF and DSF).

In describing the color of DSF cookies, higher and lower values of  $a^*$  were found in DSF-100 (14.70) and DSF-25 (8.037) compared to the control (10.52). These findings show that the redness of cookies increased as the concentration of soy flour increased. In hull fermented soy flour cookies, the higher mean value of  $a^*$  was found in HSF-50 (15.61), and a lower mean value of  $a^*$  was observed in HSF-25 (12.46). In comparison with DSF cookies, the redness in HSF cookies was higher. This might be due to either a higher amount of fiber present in hull flour cookies or to the Maillard reaction during baking [55]. In DSF cookies, a higher value of  $b^*$  was found in DSF-75 (35.65), and a lower value was found in DSF-25 (33.85). The control showed a higher value (38.11). In HSF cookies, an increased value of  $b^*$  was detected in HSF-25 (33.85), and a decreased value was observed in HSF-75 (29.78). These results showed that yellowness in HSF cookies was lower than in dehulled soy flour cookies. A significant impact of the addition of soy flour was observed on the color development of cookies. A decrease in luminosity and saturation during baking indicates that the major reason for the color development was the Maillard reaction, although the caramelization of sugar may have had some influence [57]. Fermentation affected the optical properties of soybean meals, with significant changes in lightness ( $L^*$ ), redness ( $+a^*$ ), and yellowness ( $+b^*$ ). The process of autoclaving induced a significant lightness ( $L^*$ ) decrease in the samples. Milling processes and thermal drying alter the physical properties, which may also contribute to the darkening of fermented soybean meal powders [32].

### 3.6.3. Textural profile analysis

Statistical analysis revealed that the hardness of cookies increased significantly with an increase in concentration of DSF and HSF used for cookie development (Table 4).

The maximum force applied to break the cookies increased with increased soy flour concentration. In DSF cookies, the highest hardness was observed in DSF-25 (21.85 N), and the lowest hardness was observed in DSF-100 (14.05 N). The mean hardness of  $T_0$  was 13.55 N. In HSF cookies, the highest hardness value was recorded in HSF-75 (23.55 N), and the lowest was recorded in HSF-25 (17.55 N). Hardness increased from dehulled cookies to hull cookies, as these contained fiber in the form of hull [22]. This variation might be due to an increase in soy flour concentration. The addition of soy flour with other additives in cookies seems to be responsible for the increase in the hardness value and strength of cookies [58]. The cookies' toughness is attributed to the development of the protein matrix, which was facilitated by the presence of moisture, enhancing gluten network formation with starch [59,60,61]. Solid-state fermentation significantly enhanced the nutritional features, especially protein contents, of soy flour, and also promoted its digestibility. The slightly greater hardness of the soy flour cookies can be credited to a higher protein level, which led to the development of an enhanced structure with other hydro-colloids in the cookies [32,33,62].

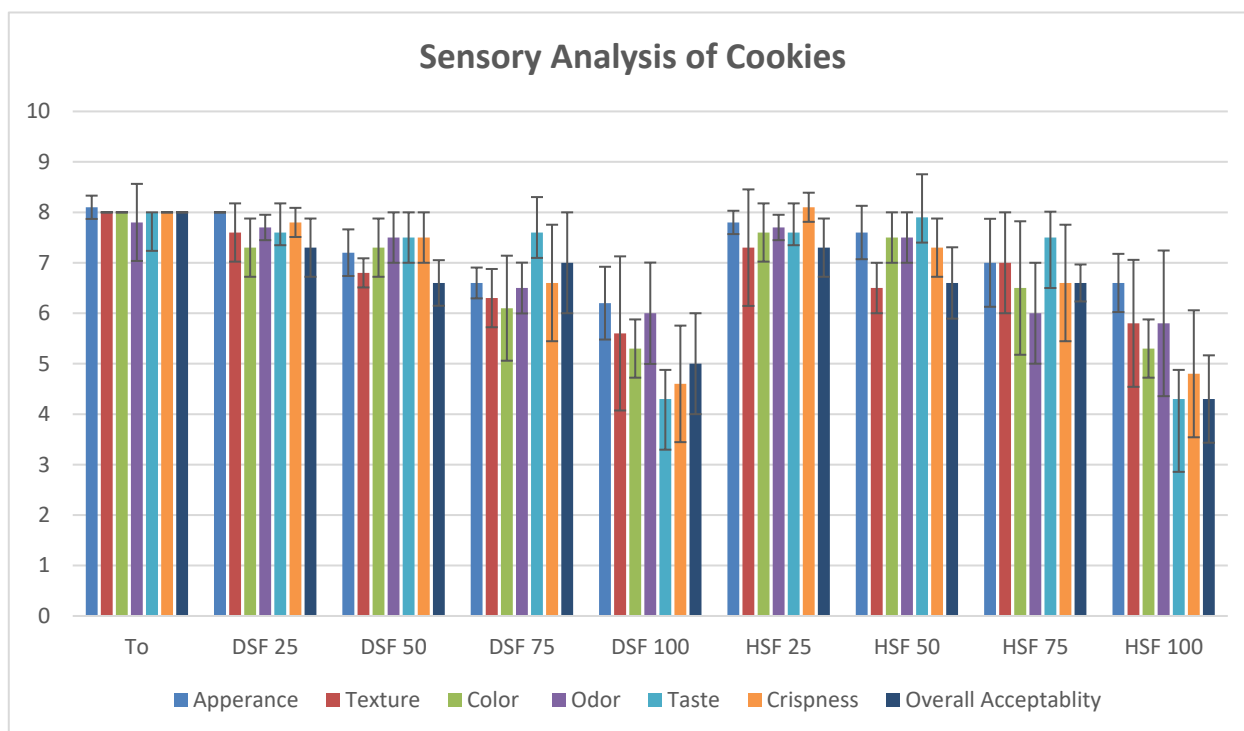
#### 3.6.4. Sensory evaluation of cookies

Cookies with DSF and HSF exhibited significant differences in sensory parameters (Figure 3). The highest appearance score was observed to be 8.1 in  $T_0$ ; the minimum score was 6.2 in DSF-100. In DSF cookies, the sensory evaluation score for appearance was 8, 7.2, 6.6, and 6.2 in DSF-25, DSF-50, DSF-75, and DSF-100, respectively. For HSF cookies, the score was 7.8, 7.6, 7, and 6.6 in HSF-25, HSF-50, HSF-75, and HSF-100, respectively. Overall, DSF-25 got the highest appearance score, comparable to the control (8.1). The sensory score for texture was 7.6, 6.8, 6.3, and 5.6 in DSF-25, DSF-50, DSF-75, and DSF-100, respectively. A good sensory hedonic score (7.6) was observed in DSF-25, but a lower score (5.6) was found in DSF-100. Furthermore, the sensory score for texture was 7.3, 6.5, 7, and 5.8 in HSF-25, HSF-50, HSF-75, and HSF-100, respectively.

In the color sensory parameter, a higher hedonic score (8.0) was found in  $T_0$ , and a lower (5.3) was detected in both DSF-100 and HSF-100. Both DSF-25 and DSF-50 got a sensory color score of 7.3, while DSF-75 and DSF-100 had scores of 6.1 and 5.3, respectively. The sensory score was 7.6 in HSF-25, 7.5 in HSF-50, and 6.5 in HSF-75. The color of  $T_0$  cookies was appealing, as compared to other HSF and DSF cookies, therefore obtaining higher scores.

Regarding the odor parameter, a good score (7.8) was observed in  $T_0$ ; the lowest (5.8) was found in HSF-100. DSF-25 had an odor score of 7.7; this decreased progressively to 7.5, 6.5, and 6 in DSF-50, DSF-75, and DSF-100, respectively. HSF-25 cookies had a score of 7.7, compared to 7.5, 6, and 5.8 for HSF-50, HSF-75, and HSF-100, respectively.

The lowest taste score (4.3) was recorded in both DSF-100 and HSF-100; a score of 7.9 was observed in HSF-50. The change in taste was due to the enhancement of soy flour concentration and fermentation. The maximum score for overall acceptability of cookies was 8 in  $T_0$ ; the minimum score was 4.3 in HSF 100. The sensory hedonic score for overall acceptability observed was 7.3 for DSF-25 and 6.6 for DSF-50, HSF-50, and HSF-75, while 7 was recorded for DSF-75 and 5 for DSF-100.



**Figure 3.** Sensory analysis of soy flour cookies.

Higher acceptability was recorded in the control treatment for all sensory parameters. In treated samples, up to 25% and 50% replacement of DSF led to a higher sensory perception as compared to HSF cookies. Perceptions decreased with the enhancement in soy flour concentration. In another study, cookies developed with 10%–15% soy flour exhibited excellent sensory traits; however, as soon as flour concentration increased to 20%, sensory parameters decreased, including taste, appearance, and overall acceptability [63]. Solid-state fermentation seems to enhance nutritional parameters, such as polyphenol and flavonoid contents, of soy flour. Clearly, these components present suitable health impacts, but in product development, a gradual increase in soy flour concentration leads to a strong beany flavor. This explains the lower sensory perceptions of bakery products, as shown by the favoring of the product by the consumer [32,59].

#### 4. Conclusions

Solid-state fermentation with *S. thermophilus* for 15 h represented a sustainable technology that enhanced the nutritional quality of soy flour (DSB and HSB) by reducing antinutritional factors and improving polyphenols, protein availability, and digestibility. The various fermented flour blends possessed better functional properties (WAC and OAC) than the control. Subsequently, the application of fermented soy flour in cookie production demonstrated potential for creating nutritionally enriched baked products. Comparatively, cookies developed from DSF presented excellent qualitative properties; DSF-25 and DSF-50 presented the highest sensory acceptability. This study confirmed that soybean-fermented cookies offer significant nutritional benefits and can contribute effectively in combating malnutrition across different age groups, as a fiber, protein, and polyphenolic food source, playing a pivotal role in curtailing various health disorders. Incorporating fermented soy flour into

bakery products increases their digestible protein and fiber content and contributes to a more nutritious product for consumers. Also, it reduces the burden on conventional wheat flour utilization for cookie development.

### Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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### Conflict of interest

The authors declare no conflicts of interest.

### Author contributions

Majid Hussain: Conceptualization, Data curation, Writing-review and editing, Formal analysis; Taha Rababah, Vaida Bartkutė-Norkūnienė: Funding acquisition, Data curation, Writing-review and editing; Muhammad Azam: Formal analysis, Conceptualization, Writing-review, and editing; Bandar Hamadneh, Numan AL-Rayyan: Conceptualization, Funding acquisition, Data curation, Writing-review, and editing; Ali Almajwal, Rania Mohamed Ali Jammal: Writing-review and editing, Data curation, Funding acquisition, and Conceptualization. All authors have approved the final version of the manuscript.

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