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## Quality Characteristics of Healthy Bread Produced from Germinated Brown Rice, Germinated Mung Bean, and Germinated White Kidney Bean

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### Abstract

Germinated grains and pulses are known as sources of bioactive compounds that provide beneficial effects for human health. Moreover, germination improves their protein digestibility and biological properties. This research aimed to investigate the physicochemical properties of the composite flour made by blending germinated brown rice (GBR), germinated mung bean (GMB), and germinated white kidney bean (GWKB) at the ratio of 1:1:1. Additionally, the quality of bread made by substituting the composite flour for wheat flour at 25%, 30%, 35%, and 40% (w/w) was assessed. The results showed that the composite flour had significantly higher amounts of fat, protein, crude fiber, ash, GABA, and total phenolic content than wheat flour. The antioxidant capacity, calculated as vitamin C equivalent antioxidant capacity (VCEAC), of the composite flour was 42.86 mg/100 g, which was twice that of wheat flour. The pasting properties of the GBR, GMB, and GWKB flours, including the composite flour were considerably different from those of wheat flour. Based on this study, the optimized level of the composite flour substitution was 25 g per 100 g of wheat flour. The wheat composite flour bread showed higher levels of beneficial components, such as protein, minerals, gamma-aminobutyric acid (GABA) and polyphenols, and antioxidant capacity, than the control bread. Bread made with a 25% substitution of composite flour had a more yellow hue than the control wheat bread, but there was no discernible difference in the loaf's specific volume, density, and firmness.

**Keywords:** *germinated brown rice; germinated mung bean; germinated white kidney bean; composite flour; pasting properties; healthy bread*

### 1. Introduction

One of the most popular bakery items for human nutrition is bread. The most popular type of bread in much of the industrialized world is white bread, which has a low nutritional value. White bread also has a high score on the glycemic index (GI) which can cause rapid spikes in blood glucose levels that, over time, can harm the body. In recent years, there has been a trend towards healthier baked goods in Thailand, with many bakeries offering a range of low-fat, low-sugar, and whole grain products. This trend has been driven by the increasing awareness of the importance of a balanced diet and the need to address the growing

rates of obesity and chronic diseases in the country (BBM Magazine, 2023). A mixture of flours, starches, and other ingredients intended to replace wheat flour either partially or entirely in bakery and pastry products is called composite flour (Noorfarahzilah et al., 2014). It has been shown that using composite flour by adding pulse flour to wheat flour in bread making is a viable means of enhancing the nutritional profile of the products (Olanami et al., 2022). Pulse legumes are a rich source of protein, dietary fiber, some vitamins and minerals. They provide a well-balanced essential amino acid profile when consumed with cereals and other foods (Klupšaitė, & Juodeikienė, 2015). Pulse

legumes, especially common beans, were also reported as rich in zinc and iron (Philipo et al., 2020). Cereal-based foods can be fortified with pulse flours to increase their protein content and quality while compensating for the lysine and threonine inadequacies in wheat flour (Millar et al., 2019). The bran layers and embryo of brown rice (BR) include a variety of nutritional and bifunctional components, including dietary fibers,  $\gamma$ -oryzanol, vitamins, and minerals. (Zhao et al., 2020). Gamma-aminobutyric acid (GABA), phenolic acids, flavonoids, and  $\gamma$ -oryzanol are the bioactive substances found in BR. Additionally, BR, GBR, and their derived fractions or extracts have demonstrated a variety of biological properties and potential health advantages, including antioxidant, antidiabetic, and anticancer activities (Saleh et al., 2019).

Nowadays, research on sprouted grains and legumes, especially their advantages for producing nutritious food products, is of current interest to food manufacturers. When a seed germinates, it undergoes a number of metabolic processes that change its chemical composition compared to that of a raw seed. Sibian et al. (2017) reported that in the seeds of brown rice, wheat, and triticale, germination increased protein-based quality criteria such the essential amino acid index, biological value, protein efficiency ratio, and nutritional index. Brown rice was shown to have the highest essential amino acid index among the grains following germination. During the germination of brown rice, the content of GABA, total phenolic compounds, and antioxidant activities were reported to increase (Cáceres et al., 2017). The taste of brown rice, nutritional content, and health benefits can all be increased through the process of germination (Wu et al., 2013). GBR flour made from germinating brown rice for 48 hours provided bread superior nutritional quality in terms of higher content of protein, lipids, and bioactive compounds (GABA and polyphenols), higher antioxidant activity, lower phytic acid content, and a lower glycemic index (Cornejo et al., 2015). In addition, during germination of BR, the activities of hydrolytic enzymes that hydrolyze starches, non-starch polysaccharides, and proteins were significantly increased, leading to an increase in the number of glycopeptides with few amino acids (Wunthunyarat et al., 2020).

In consideration of germinated pulses, germination increased the nutritional values of pulses by increasing protein/ starch digestibility and vitamin content and by decreasing antinutritional

compounds (Sozer et al., 2017; Avezum et al., 2022). Tiansawang et al. (2016) reported that the highest GABA content was found in germinated mung bean and dietary fiber content also increased during germination. Mung bean flour's components, physiochemical, and functional characteristics were all significantly impacted by germination. With longer germination times, protein content and functional properties such as water absorption index, water solubility index, oil absorption capacity, and water retention capacity of the flour improved, but ash and fat content, as well as the viscosity of the paste, declined (Liu et al., 2018). The improvement of protein digestibility and bioaccessibility which depend on the hydrolysis of the indigestible protein, deactivation of protease inhibitors, and improvement of protein solubility were found in germinated legume seeds (Ohanenye et al., 2020). The intensification of metabolites, which are health-beneficial bioactive compounds was reported in mung beans during the germination process. These compounds are attributed to antioxidant, anti-diabetic, antimicrobial, anti-hyperlipidemia, anti-hypertension, anti-inflammatory, anti-cancer, anti-tumor, and anti-mutagenic properties in germinated mung bean (Ganesan, & Xu, 2018). The anticancer and immunomodulatory activities of mung bean sprouts were also reported by Hafidh et al. (2012). In kidney beans, germination led to a relative increase in melatonin content and significant antioxidant activity (Aguilera et al., 2014).

In this study, the composite flour made from germinated brown rice (GBR), germinated mung bean (GMB), and germinated white kidney bean (GWKB) flours in a ratio of 1:1:1 was used to substitute 25-40% of the wheat flour used in bread making. The physical and chemical properties of the composite flour, including bread quality made from the composite flour, were also analyzed.

## 2. Objectives

To investigate the suitable amount of the composite flour made from GBR, GMB, and GWKB as a replacement for wheat flour in the production of healthier bread as well as to examine the physical and chemical properties of the composite flour.

## 3. Materials and methods

### 3.1 Raw materials

Organic mung bean, organic white kidney bean, and Hom Mali brown rice were purchase from SiamPrana Co., Ltd., Thailand.

### 3.2 Chemicals and reagents

GABA and all HPLC grade solvents (acetonitrile, trifluoroacetic acid (TFA), and water including gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Folin-Ciocalteu's phenol reagent were purchased from Sigma-Aldrich (St. Louise, MO, USA). A fluorescence-labeling reagent, 9-fluorenylmethyl chloroformate (FMOC-Cl) was purchased from Fluka (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade and purchased from commercial suppliers.

### 3.3 Preparation of GBR, GMB, GWKB flour and composite flour

GBR flour: Brown rice grains were rinsed with water 3-4 times to remove impurities. Then germination was carried out by soaking the grains in slightly acidic water with a pH of 5.6 (adjusted with 1 N citric acid) with a grain-to-water ratio of 1:3 w/v at room temperature for 12 hours to reduce rancidity in GBR flour (Loikeao et al., 2014) and to increase the GABA content of the germinated grains (Zhang et al., 2014). Afterward, the grains were rinsed with water and placed on a germination tray for the germination process in a dark environment at room temperature for 18 hours according to Lin et al. (2015) to increase antioxidative properties and bioactive compounds of GBR. After germination, GBRs were steamed by water vapor for 15 minutes to terminate the germination process, and then they were dried in a hot air oven (Memmert, model UM600, USA) at 60 °C until their moisture content was 10-12% (wet basic). The dried grains were ground in a hammer mill (Crompton, model 3000 series, England) and sifted through an 80-mesh sieve. The GBR flour was kept in an aluminum foil bag for use throughout the experiment.

GMB flour: Mung bean seeds were rinsed with water 3-4 times to remove impurities, then germination was carried out by soaking the seeds in 1mM CaCl<sub>2</sub> solution at pH 5.0 (adjusted with citrate buffer) with a seed-to-water ratio of 1:3 w/v at room temperature for 12 hours. Pretreatment of common bean seeds with CaCl<sub>2</sub> was reported to improve the germination (Silveira et al., 2020) and pH 5.0 was found to be the optimum condition enhancing GABA content in germinated mung bean (Chen et al., 2018). Afterward, the seeds were rinsed with water and placed on a germination tray for the germination process in a dark environment at room temperature for 10 hours. Germination of mung

bean under dark conditions significantly improved the nutritional values and functional characteristics of mung bean seeds (Huang et al., 2020). Then, the seeds were submerged in hot water at 85 °C to terminate germination and were subsequently dried in a hot air oven (Memmert, model UM600, USA) at 60 °C until their moisture content reached 10-12% (wet basic). The dried seeds were ground and kept with the same method as GBR flour.

GWKB flour: White kidney bean seeds were germinated, dried, ground to flour, and stored using the same method of GMB flour except their germination time was 9 hours.

The composite flour was made by blending GBR flour, GMB flour, and GWKB flour at a ratio of 1:1:1 and it was subjected to further analyses and used for bread making.

### 3.4 Physical and chemical properties of GBR, GMB, GWKB flour and composite flour

#### 3.4.1 Determination of water content and $a_w$

The moisture content of GBR, GMB, GWKB flour and composite flour were measured followed the standard method (AOAC, 2000) and  $a_w$  was measure by water activity meter (AQUALAB 3 TE, USA).

#### 3.4.2 Color measurement

Color of GBR, GMB, GWKB flour and the composite flour were measured using a colorimeter (Minolta CR-400, Tokyo, Japan) and were compared to wheat flour. Analysis was done on the values L\* (darkness/brightness), a\* (red/green shade), and b\* (blue/yellow shade).

#### 3.4.3 Pasting properties

The pasting characteristics of GBR, GMB, GWKB flour and the composite flour were measured by a Rapid Visco Analyzer (RVA, model 4V, Newport Scientific, Warriewood, Australia) and compared to wheat flour in accordance with the AACC procedure 61-02.01 (AACC 2000). Direct weighing of the samples (1.5 g, dry basis) into an RVA canister was followed by the addition of distilled water (25 mL). Before running the RVA, the slurry was uniformly mixed with plastic paddle to prevent lump formation. The RVA test profile included (1) a stirring speed of 960 rpm for the initial 10 seconds and 160 rpm for the remainder of 12.5 minutes test, and (2) a temperature program equilibrating at 50 °C for 1.0 minute, ramping up to 95 °C in 4.8 minutes, holding at 95 °C until 7.5 minutes, ramping down to 50 °C at 11 minutes, and

holding at 50 °C until 12.5 minutes. The pasting properties were examined in triplicate.

#### 3.4.4 Proximate analysis

Moisture, fat, protein, crude fiber, ash, and carbohydrate (by difference) contents of the composite flour compared to wheat flour were determined following the standard method of AOAC (2002).

#### 3.4.5 Determination of GABA content

The GABA content of the composite flour was analyzed compared to that of wheat flour. Firstly, GABA The GABA content of the composite flour was analyzed and compared to that of wheat flour. Firstly, GABA was extracted following the modified method of Oh, & Choi (2001). Two grams of flour samples were dissolved in 8 mL of a 12:5:3 (v/v/v) mixture of methanol, chloroform, and water. After vortexing, the mixture was centrifuged at 12,000 g for 15 minutes at 4 °C. After collecting the supernatant, the pellet was dissolved in 2 mL of chloroform and 4 mL of water. The mixture was then vortexed and centrifuged at 12,000 g for 15 minutes. To obtain the upper phase containing GABA, the supernatant was collected, mixed with the initial supernatant, and recentrifuged. The collected samples were thoroughly dried in a vacuum oven at 60 °C and redissolved in 5 mL deionized water. The samples containing GABA were passed through 0.45 µm filters and analyzed using HPLC after FMOC-Cl derivatization.

Analysis of GABA content was carried out using HPLC following the modified method of Khwanchai et al. (2014). A total volume of 5.0 mL was made by combining aliquots of 0.4 mL of sample extracts with 2.6 mL of 0.1 M borate buffer solution (pH 10), and 2.0 mL of an FMOC-Cl solution in acetonitrile, then vortex mixing for 30 seconds, followed by leaving for 15 minutes to immediately derivatize. HPLC analyses were conducted on a Hewlett-Packard 1090 control system (Agilent Technologies, Inc., USA). Derivatized GABA was separated using a reverse-phase C18 column (5 µm; 3.9 × 150 mm). Acetonitrile (mobile phase A) and 0.5% TFA (mobile phase B) were used as mobile phases for gradient elution. At first, 35% A and 65% B were run, then 20% A and 80% B for 3 minutes, followed by 45% A and 55% B for 10 minutes, and 35% A and 65% B for 20 minutes. The analysis was conducted using a flow rate of 1.0 mL/minute. The fluorescence excitation ( $\lambda_{ex}$ ) and emission ( $\lambda_{em}$ )

wavelengths were set at 270 and 315 nm, respectively. This procedure enabled the separation of GABA at 22.02 minutes. The GABA quantification was calculated through the standard curve of GABA (a linear range over 5 – 70 mg/L).

#### 3.4.6 Determination of total phenolic content and antioxidant capacity

To determine total phenolic content (TPC) and antioxidant capacity of the composite flour and wheat flour, the samples were extracted as the modified method of Ji-u, & Apisittiwong (2022). Two grams of the flour were extracted with 10 mL of 70% ethanol by vortexing for 30 seconds and then sonicating for 20 minutes. Before being analyzed, the extracts were kept in the dark after being filtered using Whatman No. 1 filter paper. The total phenolic content of the extracts was determined by the modified method of Onsrisawat et al. (2022) and Ji-u, & Apisittiwong (2022). One milliliter of the sample was mixed vigorously with 5 mL of the 5% Na<sub>2</sub>CO<sub>3</sub> solution and 0.5 mL of the Folin-Ciocalteu reagent. The solution was then quickly diluted with distilled water to 25 mL and well mixed. After incubation in the dark at room temperature, the absorbance was measured at 765 nm, and its value was compared to a standard curve of a gallic acid solution. Total phenolic content was calculated as milligrams of gallic acid equivalents (mg GAE/100 g).

Antioxidant capacity of the extracts by DPPH scavenging assay were analyzed using a modified method of Kim et al. (2002). The mixture of 0.1 mL of sample and 2.9 mL of 1 mM DPPH that was dissolved in 80% aqueous methanol was incubated in the dark for 30 minutes with the foil covering on. Decreasing absorbance was measured at 517 nm. A control was made up of 2.9 mL of DPPH solution and 0.1 mL of 50% aqueous methanol. L-ascorbic acid solution was utilized as the standard for the DPPH technique analysis. In order to determine the vitamin C equivalent antioxidant capacity (VCEAC) in mg/100 g, the sample's absorbance reduction was compared to that of the standard vitamin C concentration.

### 3.5 Bread making from replacement of wheat flour with the composite flour: sensory and quality evaluation

#### 3.5.1 Bread preparation

Breads were prepared by replacing wheat flour with the composite flour at 25, 30, 35, and 40% (w/w). One hundred grams flour, 10 grams

sugar, 1.75 grams salt, 1 gram yeast, 4 grams milk powder, 6 grams butter, and 60 grams water were mixed and fermented for 45 - 60 minutes at 37 °C in an incubator until the loaf volume increased 2 times. Subsequently, breads were baked in an electric oven at 200°C for 30 minutes. After cooling, the breads were stored in plastic bags.

### 3.5.2 Specific loaf volume and density evaluation

The cooling loaves were weighed immediately. Sesame seed displacement was used to calculate loaf volume (Hasmadi et al., 2014). Specific loaf volume was calculated by dividing loaf volume by loaf weight, and density of crumb was calculated by dividing loaf weight by loaf volume.

### 3.5.3 Crumb color measurement

Bread slices of thickness 2.5 cm were prepared and color ( $L^*$ ,  $a^*$ , and  $b^*$  values) was measured from the middle of each slice using a colorimeter (CE-10, Minolta Inc., Japan). Three replications of each batch and five measurements per slice of bread were recorded for the replication process.

### 3.5.4 Firmness evaluation

Bread firmness was determined using a Texture analyzer (Stable Micro System; TA.XT.plus C, UK) according to the standard method of AACC (Method 74-09, AACC 1986). Bread slices with a thickness of 2.5 cm were prepared after being stored for a day. Using a 3.6-cm diameter cylindrical probe (P/36R) with a pre-test speed of 1.0 mm/second, a test speed of 1.7 mm/second, and a post-test speed of 10.0 mm/second, the force required to compress the sample to 25% of its height was recorded. Replications were conducted three times per batch with three measurements taken per loaf. According to this method, "firmness" is defined as the amount of force (measured in grams) needed to compress the product by a predetermined distance (25 mm at a 25% compression).

### 3.5.5 Sensory evaluation

The bread samples prepared with the replacement of wheat flour with the composite flour at 25, 30, 35, and 40% (w/w) were served to 30 semi-trained panelists comprised of students and

staff of Rangsit University. A 9-point hedonic scale was used to grade the samples' appearance, color, flavor, taste, texture, and overall acceptability.

### 3.5.6 Nutritional evaluation

The proximate compositions of bread made with wheat flour and bread made with wheat flour substituted with composite flour, which had acceptable quality in terms of specific loaf volume, color, texture, and sensory evaluation were determined using the standard method of AOAC (2002). Additionally, GABA content, total phenolic content, and antioxidant activity of the samples were examined as 3.4.5 and 3.4.6

### 3.5.7 Statistical analysis

Experiments were conducted in triplicate. The information is shown as mean standard deviation. Using statistical software (SPSS 11 for window, SPSS Inc., USA). Each parameter from the completely randomized experimental design underwent Analysis of Variance (ANOVA). Using Duncan's New Multiple Range Test (DMRT), a difference in results was deemed significant at  $p < 0.05$ .

## 4. Results and Discussion

### 4.1 Physical and chemical properties of GBR, GMB, GWKB flour and the composite flour

Color characteristics,  $a_w$ , and moisture content of the individual and composite flours compared to wheat flour were shown in Table 1. There were differences in moisture content and  $a_w$  of the individual flours, which were significantly lower than those of wheat flour. The moisture content in the sample flours was within the recommended moisture level of 14% for safe storage, and their  $a_w$  was below 0.6, which guarantees the flour's safety during storage (Shahzadi et al., 2005; Wang et al., 2017). The color of wheat flour was significantly whiter but less red than that of the individual flours and the composite flour. All of the flours and wheat flour in this study were more green as shown by the negative value of  $a^*$ . The color of the individual flour was more yellow than that of composite flour. The composite flour produced with cassava, rice, potato, soy bean flour, and potato starch also had a negative value of  $a^*$  (Tharise et al., 2014).

**Table 1** Color ( $L^*$ ,  $a^*$ ,  $b^*$ ),  $a_w$ , and moisture content of GBR, GMB, GWKB flour and the composite flour compared to wheat flour

Type of Flour	$L^*$	$a^*$	$b^*$	$a_w$	% MC
GBR flour	87.30 <sup>c</sup> ± 0.10	-5.97 <sup>c</sup> ± 0.06	16.73 <sup>b</sup> ± 0.06	0.21 ± 0.00	5.00d ± 0.41
GMB flour	86.80 <sup>d</sup> ± 0.36	-7.90 <sup>c</sup> ± 0.00	16.50 <sup>b</sup> ± 0.10	0.37 ± 0.01	5.89c ± 0.07
GWKB flour	88.67 <sup>b</sup> ± 0.25	-6.80 <sup>d</sup> ± 0.10	18.30 <sup>a</sup> ± 0.10	0.24 ± 0.01	5.19d ± 0.34
Composite flour	88.97 <sup>b</sup> ± 0.15	-3.33 <sup>b</sup> ± 0.15	13.40 <sup>c</sup> ± 0.26	0.25 ± 0.00	6.41b ± 0.30
Wheat flour	93.47 <sup>a</sup> ± 0.15	-2.27 <sup>a</sup> ± 0.42	8.73 <sup>d</sup> ± 0.12	0.45 ± 0.01	10.70a ± 0.04

Different letters in superscript of each column indicate a significant difference at  $p < 0.05$ .

GBR: germinated brown rice, GMB: germinated mung bean, GWKB: germinated white kidney bean

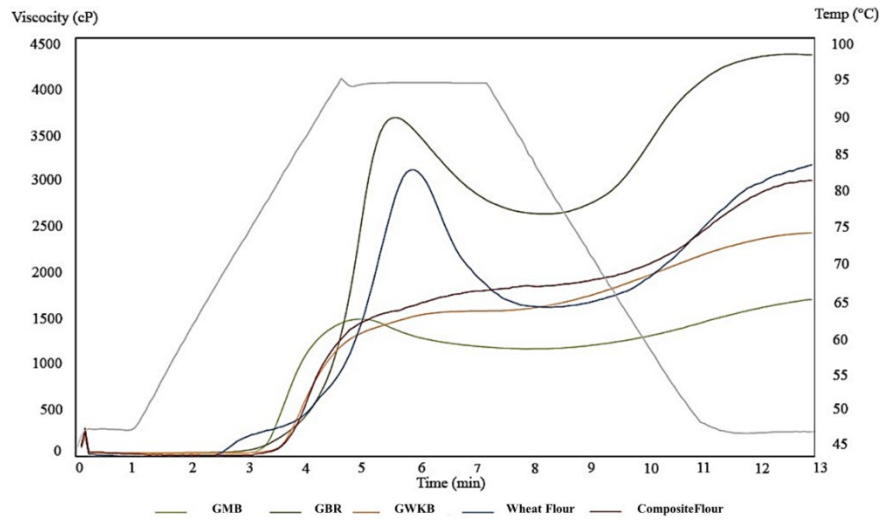
The pasting curve of the individual and composite flours is presented in Figure 1 and the results are shown in Table 2. The pasting profile of flour, which impacts the texture, digestibility, and ultimate usage of starch-based food commodities, is one of the most significant factors impacting the quality and aesthetic evaluation of food. (Onweluzo, & Nnamuchi, 2009; Ajanaku et al., 2012). Our results showed that pasting profile of the individual and composite flour were considerably different from that of wheat flour. High peak viscosity indicated a high starch content (Ragaee, & Abdel-Aal, 2006; Ekunseitan et al., 2017). In addition, a higher fat content in the composite flour (Table 3) may prevent the interaction of starch molecules, reducing the swelling of starch molecules, and the protein content in legume flours can inhibit starch granule swelling and decrease viscosity; therefore, the viscosity of starch is changed (Ratnawati et al., 2019). According to Stone et al. (2019), legume flours have lower starch content and higher levels of protein than cereal flours. Furthermore, whole waxy wheat and substituted flours were observed to have lower peak viscosities than commercial white wheat flour due to their higher dietary fiber and lower total carbohydrate contents (Hung et al., 2007). This explains why wheat flour's peak viscosity was higher than that of GMB, GWKB, and the composite flour. Nevertheless, wheat flour's peak viscosity was lower than that of GBR flour, which may be due to the grain's high protein level (13–14%; utilized for manufacturing bread).

The breakdown and final viscosity of the individual and composite flours were significantly lower than that of wheat flour. The lower the breakdown viscosity, the higher the stability of starches during heating and mechanical agitation

(Colussi, et al., 2020). The final viscosity, frequently used to assess a sample's capacity to form a gel after cooking and cooling, ranged from 1,716 – 2,966 cPs for pulse flour, 2,966 cPs for the composite flour, and 3,183 cPs for wheat flour. The higher setback viscosity of the composite flour indicated increased tendency of the flour for retrogradation. Both the composite flour made from wheat, malted sorghum, and soy bean flour (Aluge et al., 2016) and the composite flour made from germinated kidney beans, chickpeas, and wheat (Sibian, & Riar, 2020) showed a higher retrogradation tendency.

A higher pasting temperature indicates a higher water binding capacity, a higher gelatinization tendency, and lower swelling properties of starch-based flour owing to high degree of association between starch granules (Ekunseitan et al., 2017). The results obtained from this study indicated that wheat flour had the lowest pasting temperature, which was consistent with Wani et al. (2016), where wheat flour had a lower pasting temperature than wheat-composite flour.

In germinated brown rice and edible seeds, a number of chemical compositions and bioactive substances underwent considerable alterations as a result of germination. It has been shown that GABA, vitamins, polyphenols, and other phytochemicals increase during germination, significantly enhancing the nutritional content of edible seeds (Moongngarm, & Sactung, 2010; Idowu et al., 2020). As shown in Table 3, the amount of fat, protein, crude fiber, ash which is an index of mineral content of food, GABA, TPC, and antioxidant activity of the composite flour made from GBR, GMB, and GWKB were significantly higher than those of wheat flour.



**Figure 1** Pasting profiles of GBR, GMB, GWKB flour and the composite flour compared to wheat flour

**Table 2** Pasting properties of GBR, GMB, GWKB flour and the composite flour compared to wheat flour

Type of flour	Viscosity (cPs)					Peak Time	Pasting Temperature
	Peak	Trough	Breakdown	Final	Set Back from Peak		
GBR flour	3,691.67a ± 18.58	2,630.67a ± 18.77	1,061.00b ± 4.58	4,351.33a ± 27.79	659.67c ± 32.04	5.67c ± 0.07	80.08b ± 0.80
GMB flour	1,485.00e ± 2.65	1,158.67c ± 4.93	326.33c ± 7.09	1,716.67e ± 14.74	231.67d ± 16.26	4.98d ± 0.04	77.72c ± 0.03
GWKB flour	1,585.00d ± 10.15	N/A	N/A	2,445.33d ± 12.90	860.33b ± 5.51	7.00a ± 0.00	81.70a ± 0.00
Composite flour	1,788.67c ± 15.04	N/A	124.00d ± 22.87	2,966.33c ± 39.02	1,177.67a ± 33.38	6.98a ± 0.00	80.90ab ± 0.52
Wheat flour	3,155.67b ± 40.51	1,653.67b ± 113.53	1,502.00a ± 80.07	3,183.33b ± 62.17	51.00e ± 5.66	6.03b ± 0.20	70.77d ± 0.94

Different letters in superscript of each column indicate a significant difference at  $p < 0.05$ .

GBR: germinated brown rice, GMB: germinated mung bean, GWKB: germinated white kidney bean

**Table 3** Proximate composition, GABA, TPC, and antioxidant capacity of the composite flour compared to wheat flour

Composition	Composite flour	Wheat flour
Moisture (%)	6.41 ± 0.30 <sup>b</sup>	10.70 ± 0.04 <sup>a</sup>
Fat (%)	3.51 ± 0.62 <sup>a</sup>	2.07 ± 0.33 <sup>b</sup>
Protein (%)	19.34 ± 0.36 <sup>a</sup>	16.56 ± 0.19 <sup>b</sup>
Crude fiber (%)	33.45 ± 1.43 <sup>a</sup>	24.36 ± 0.65 <sup>b</sup>
Ash (%)	3.02 ± 0.02 <sup>b</sup>	0.55 ± 0.00 <sup>a</sup>
Carbohydrate (%)	67.72 ± 0.68 <sup>b</sup>	70.12 ± 0.20 <sup>a</sup>
GABA (mg/100 g)	19.53 ± 0.16 <sup>a</sup>	1.97 ± 0.01 <sup>b</sup>
TPC (mg GAE/100 g)	170.25 ± 3.32 <sup>a</sup>	49.34 ± 0.71 <sup>b</sup>
VCEAC (mg/100 g)	42.86 ± 2.08 <sup>a</sup>	21.38 ± 0.46 <sup>b</sup>

Different letters in superscript of each row indicate a significant difference at  $p < 0.05$ .

GABA: Gamma-aminobutyric acid, TPC: total phenolic content; VCEAC: vitamin C equivalent antioxidant capacity

#### 4.2 Quality characteristics and sensory evaluation of breads prepared by varying the proportion of the composite flour substituted for wheat flour.

Quality attributes, i.e., color, specific volume, density, and firmness, including sensory evaluation of bread samples and a control (0% composite flour), are summarized in Table 4. The L\* value, which represents brightness, significantly decreased as the amount of composite flour increased. However, as the proportion of composite flour rose, the a\* and b\* values increased. Therefore, the color of the bread samples became more yellow, as shown in Figure 2. This occurrence may be explained by the reducing sugar produced during germination in the composite flour, which interacted with free amino acids, resulting in the formation of brown pigments. Bread containing 5% to 20% of germinated cowpea flour has also been found to cause a darkening of the bread crumb. (Hallén et al., 2004). The data presented in Table 4 also showed that the specific volume of bread samples slightly decreased, while their density increased as the amount of composite flour increased. Since the unique breadmaking properties of wheat flour are attributed mainly to the ability of its gluten network when mixing with water. The lower specific volume of the composite flour bread can be explained as a reduced capacity of the gluten network to enclose air (Menon et al., 2015). The increase in fiber content in the composite flour bread (Table 4) also led to a reduction in loaf volume and specific volume (Menon et al., 2015). The firmness of composite flour breads was significantly higher than that of the control bread and was the highest at 40% composite flour substitution. This may be due to a higher fiber content in the composite flour bread compared to

the control bread. Moreover, it is known that bread with a firmer crumb has a lower specific volume, suggesting a more compressed cell structure (Charoenthaikij et al., 2010). Kotsiou et al. (2022) also reported that substituting roasted chickpea flour considerably enhanced the viscosity and elasticity of the dough, indicating a higher resistance to flow

a much lower specific volume and a tougher texture. Atudorei, & Codină (2020) studied the use of germinated legumes in the bread making process, and concluded that the quantity and type of germinated legumes added to wheat flour determined every change in the bread-making process and bread quality. In terms of the pasting profile of wheat flour and the composite flour, variations in characteristics such as size and shape, rigidity, other functional properties, and amylose and amylopectin content are known to influence pasting properties that impact the textural quality of food products (Patil et al., 2020).

Table 5 indicates that semi-trained panelists equally liked the 25% substitution of composite flour and the wheat control bread in all attributes. Increasing the percentage of composite flour substitution more than 25% significantly lower the liking score in all attributes. The wheat control bread had a higher liking score on appearance than the composite wheat flour bread. This may be explained by the fact that substitution of wheat flour with the composite flour led to breads with a more observable porous crumb structure. Therefore, among the composite wheat flour breads, the 25% substitution bread was selected for further bread quality evaluation owing to its higher scores in appearance, color, flavor, taste, texture, and overall acceptability.

**Table 4** Physical properties of breads prepared by varying the proportion of the composite flour substituted for wheat flour

% Composite flour (w/w)	Color value			Specific volume (cm <sup>3</sup> /g)	Density (g/cm <sup>3</sup> )	Firmness (g)
	L*	a*	b*			
0	74.38 ± 0.91 <sup>a</sup>	-4.93 ± 0.23 <sup>c</sup>	18.85 ± 0.15 <sup>d</sup>	3.58 ± 0.03 <sup>a</sup>	0.28 ± 0.00 <sup>c</sup>	202.56 ± 36.14 <sup>c</sup>
25	71.10 ± 0.72 <sup>b</sup>	-4.37 ± 0.23 <sup>b</sup>	21.78 ± 0.72 <sup>c</sup>	3.46 ± 0.13 <sup>ab</sup>	0.29 ± 0.01 <sup>bc</sup>	212.76 ± 15.96 <sup>c</sup>
30	71.75 ± 0.65 <sup>b</sup>	-4.35 ± 0.13 <sup>b</sup>	21.35 ± 0.82 <sup>c</sup>	3.43 ± 0.12 <sup>ab</sup>	0.29 ± 0.01 <sup>bc</sup>	269.63 ± 9.90 <sup>ab</sup>
35	66.23 ± 0.65 <sup>c</sup>	-3.90 ± 0.10 <sup>a</sup>	22.67 ± 0.75 <sup>b</sup>	3.13 ± 0.19 <sup>bc</sup>	0.32 ± 0.02 <sup>ab</sup>	314.29 ± 52.12 <sup>b</sup>
40	64.43 ± 0.42 <sup>d</sup>	-3.63 ± 0.38 <sup>a</sup>	23.60 ± 0.20 <sup>a</sup>	3.03 ± 0.28 <sup>c</sup>	0.33 ± 0.03 <sup>a</sup>	415.37 ± 47.50 <sup>a</sup>

Different letters in superscript of each column indicate a significant difference at  $p < 0.05$





**Figure 2** Appearance of bread made from 25-40% (w/w) composite flour compared to wheat flour bread

**Table 5** Sensory evaluation of breads prepared by varying the proportion of composite flour substituted for wheat flour

% Composite flour	Attributes					
	Appearance	Color	Flavor	Taste	Texture	Overall Acceptability
0	7.97 ± 1.05 <sup>a</sup>	7.97 ± 1.05 <sup>a</sup>	7.33 ± 1.14 <sup>a</sup>	7.64 ± 1.14 <sup>a</sup>	7.64 ± 1.14 <sup>a</sup>	7.79 ± 1.14 <sup>a</sup>
25	7.58 ± 1.23 <sup>ab</sup>	7.33 ± 1.29 <sup>ab</sup>	7.00 ± 1.27 <sup>a</sup>	6.70 ± 1.21 <sup>b</sup>	7.18 ± 1.1 <sup>ab</sup>	7.18 ± 1.10 <sup>ab</sup>
30	7.03 ± 1.42 <sup>b</sup>	6.91 ± 1.59 <sup>b</sup>	6.88 ± 1.22 <sup>a</sup>	6.61 ± 1.62 <sup>b</sup>	6.67 ± 1.51 <sup>b</sup>	6.79 ± 1.43 <sup>b</sup>
35	6.91 ± 1.72 <sup>b</sup>	7.11 ± 1.45 <sup>b</sup>	6.68 ± 1.46 <sup>a</sup>	6.52 ± 1.35 <sup>b</sup>	6.64 ± 1.45 <sup>b</sup>	6.76 ± 1.56 <sup>b</sup>
40	7.06 ± 1.39 <sup>b</sup>	6.88 ± 1.36 <sup>b</sup>	6.88 ± 1.36 <sup>a</sup>	6.91 ± 1.42 <sup>b</sup>	6.76 ± 1.35 <sup>b</sup>	7.00 ± 1.58 <sup>b</sup>

Different letters in superscript of each column indicate a significant difference at  $p < 0.05$

**Table 6** Proximate composition, GABA, TPC, and antioxidant capacity of the composite flour bread compared to control bread

Composition (%)	Composite flour bread	Control bread
Moisture (%)	30.571 ± 0.50 <sup>ns</sup>	30.33 ± 0.19 <sup>ns</sup>
Fat (%)	15.27 ± 0.49 <sup>ns</sup>	15.60 ± 0.14 <sup>ns</sup>
Protein (%)	11.35 ± 0.10 <sup>a</sup>	10.87 ± 0.01 <sup>b</sup>
Crude fiber (%)	0.34 ± 0.03 <sup>a</sup>	0.23 ± 0.02 <sup>b</sup>
Ash (%)	1.00 ± 0.03 <sup>a</sup>	0.76 ± 0.01 <sup>b</sup>
Carbohydrate (%)	41.82 ± 0.82 <sup>ns</sup>	42.44 ± 0.82 <sup>ns</sup>
GABA (mg/100 g)	79.47 ± 0.02 <sup>a</sup>	15.05 ± 0.01 <sup>b</sup>
TPC (mg GAE/100 g)	0.87 ± 0.00 <sup>a</sup>	0.68 ± 0.01 <sup>b</sup>
VCEAC (mg/100 g)	64.33 ± 0.82 <sup>a</sup>	50.01 ± 0.33 <sup>b</sup>

Different letters in superscript of each column indicate a significant difference at  $p < 0.05$ .

GABA: Gamma-aminobutyric acid, TPC: total phenolic content; VCEAC: vitamin C equivalent antioxidant capacity

#### 4.3 Nutritional composition, GABA, TPC, and antioxidant capacity of the 25% composite flour bread compared to control bread.

As was shown in Table 6, bread produced by substituting 25% composite flour for wheat flour had had significantly higher protein, crude fiber, and ash contents than control bread. It was because the composite flour had higher levels of protein, crude fiber, and ash. The 25% composite flour bread had a greater GABA and TPC content than the control bread, which led to a higher antioxidant capacity. Many reports have indicated that bread made from composite-wheat flour, with composite flour made from cereal pulse-fruit seed, malted finger millet and red kidney bean, white kidney bean, germinated bean, also had higher protein, crude fiber, ash, and total phenolic content than

control wheat bread (Menon et al., 2015; Bhol, & Bosco, 2014; Ukeyima et al., 2019; Atudorei et al., 2021).

#### 5. Conclusion

The composite flour prepared by blending GBR flour, GMB flour, and GWKB flour at a ratio of 1:1:1 can be substituted for wheat flour in bread formulation. Increasing the amount of the composite flour relative to wheat flour decreased the specific volume and increased the yellowness and firmness of the bread. The level of the composite flour, up to 25% by weight substitution, was optimized to produce acceptable bread with comparable overall acceptability when compared to wheat flour. The developed bread using the 25% composite flour had improved nutritional qualities

i.e. protein, crude fiber, and mineral content including GABA, phenolic content, and antioxidant capacity. Therefore, this study demonstrated the feasibility of the composite flour made from GBR, GMB, and GWKB as substitutes for wheat flour to complement the protein, minerals, bioactive compounds, and antioxidant capacity of bread products.

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