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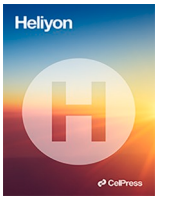

A study on the use of sorrel seed flour (Hibiscus sabdariffa) for improving functionality of wheat flour bread

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ABSTRACT

Bread presents one of the easiest opportunities as a food vehicle for delivery of nutritional and health-promoting benefits to large segments of the world population. However, its low nutritional status due to lack of balance of essential amino acids and inadequate macro- and micronutrients has necessitated recent interest in the development of high-protein hybrid breads (HPHB). Sorrel seed, an underutilized, neglected protein-rich seed holds promising nutritional and antioxidant potentials as source of good quality protein, dietary fibre and bioactive compounds. Furthermore, germination of plant seeds increases the bioavailability of these nutritional and bioactive compounds. Hence, this study has investigated the influence of germination time on nutritional, and functional properties of sorrel seed flour. Further, the amino acid profile, dietary fibre and rheological functionality of wheat-germinated defatted sorrel seed bread were assessed. The sorrel seed was germinated for 24–48 h and defatted. Thereafter, the germinated defatted sorrel seed flours were used to partially replace wheat flour using a linear replacement (w/w) of 95-80% wheat (W) and 5–20% germinated defatted sorrel seed (GS) flours to obtain W95:GS5; W90:GS10, W85:GS15 and W80:GS20. These composite flours and 100% wheat flour (control) were used to produce breads using standard recipe and methods. Results showed significant increase ($P < 0.05$) in crude protein, dietary fibre and mineral contents after 24 and 48 h germination of sorrel seed. While 24 h germination significantly ($P < 0.05$) increased WAC from 93.75% to 103.13%, further germination (48 h) caused a reduction of 26.67% (from 93.75 to 68.75%). *In vitro* protein digestibility of wheat flour decreased significantly ($P < 0.05$) as supplementation of germinated defatted sorrel seed flour increased. Supplementation of wheat flour with germinated defatted sorrel seed flour in bread production resulted in 51.84–121.42% significant ($p < 0.05$) increase in the protein content of wheat bread. Similarly, total essential amino acids, dietary fibre, mineral, and ash contents followed the same increasing trend. The *in-vivo* biological value which ranged from 82.10 to 89.40% was significantly higher ($p < 0.05$) than 58.30% obtained for the control (100% wheat bread) Thus, inclusion of germinated defatted sorrel seed flour in bread production may serve as a low-cost nutritional supplement for enhancing the nutritional profile and functional benefits of wheat bread.

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1. Introduction

Recent global increases in mortality rates resulting from increasing incidences of chronic, diet-related diseases have made consumer health a key focus for both the food industry and consumers. Particularly, in developing countries like Nigeria, protein energy malnutrition has become a menace that threatens the health and survival of large segment of the population. Hence, cereal-legume complementation is being strongly promoted by both governmental and non-governmental agencies as an effective and sustainable strategy for tackling this challenge [32,75,76,120]. Usually, cereal-legume composite flours made into porridge serve as commonly consumed breakfast for toddlers in many African countries. This is because cereals serve as rich sources of energy and oligosaccharides, however, they are limiting in essential amino acids such as lysine [114]. On the other hand, legumes are rich in protein, dietary fibre, essential amino acids, micronutrients, and polyphenolic contents [29,102,105]. Several studies have linked consumption of plant-based functional foods high in dietary fibre and polyphenols to lowered risks to many diet-related diseases such as diabetes, cardiovascular disease, obesity, hypertension, and gastrointestinal disorder [42,54,90]. Thus, legume proteins have become the central focus towards achieving a more sustainable plant-based human diet [57,88].

Cereal-based staple foods such as bread present one of the easiest opportunities for cereal-legume complementation and delivery of available nutritional and health-promoting benefits to large segments of the population since it is an important staple consumed globally by all age groups [54,87]. Moreover, bread prepared solely from wheat and refined wheat flour has low nutritional quality due to lack of balance of essential amino acids and inadequate macro- and micronutrients [29,102]. This justifies recent interest towards development of high-protein hybrid breads (HPHB) which are breads prepared from composite flours in which wheat has been partially replaced with legume flours. These hybrid breads have shown promising balance of amino acid composition and an upgraded nutritional value, thereby serving as low-cost, value-added, high-quality protein sources and carriers of health-promoting bioactive compounds into the body [33,57,107]. This is especially important for developing countries where malnutrition is prevalent and for many Sub-Saharan African countries that have accumulated huge debt burden and economic losses due to their dependence on imported wheat [33,43].

In recent times, there has been increasing research interest in low-income developing countries on the possible exploitation of underutilized neglected legumes (jack bean, scarlet bean, winged bean, sword bean, velvet bean, sorrel seeds) as a low-cost strategy to improve food security in the region by reducing hunger and malnutrition and enhancing utilization of local produce. This is because these legumes are available and cheap as compared to conventional legumes (such as soybean and groundnut) which are already overextended and expensive. Sorrel, also known as Roselle (*Hibiscus sabdariffa* var. *sabdariffa* Linn.) seed is considered as an underutilized oil producing seeds with nutritional and antioxidant potentials as source of good quality protein, dietary fibre and bioactive compounds [19,22,83]. A member of the *Malvaceae* family, it grows widely in both tropical and subtropical regions of the world, with China and Thailand being the largest producers and suppliers. However, Sudan and Nigeria are reported as the world's best sorrel producers. Other countries which cultivate the crop extensively for its pleasant red-coloured calyx (has both commercial and export values) include Mexico, India, Egypt, Senegal, Tanzania, Mali, East Indies, Iran, and Jamaica [45,92]. The calyces have entered international trade, being used by the herbal tea and beverage industry for production of hot and cold herbal beverages, jellies, confectionaries, and other products. Furthermore, due to its potential to serve as cheap alternative to imported black currant, large scale production of the seeds is promoted by both government and non-governmental parastatals. It is therefore expected that large quantities of the seed (which is a by-product from the calyx removal) will consequently be produced [92]. Unfortunately, these seeds are being discarded in the producing areas, with very little quantity often kept aside as animal feed [45,71,82]. Despite being one of the major producers in the world, the seed is only consumed in the northern parts of Nigeria where it is fermented into a condiment (known as *mungzantusa*). However, it has shown potential as a valuable food resource owing to its protein content of 32.28–34% (reported to be higher than melon, cowpea, and groundnut seeds) and essential amino acids comparable to those in soybeans [71]. Also, its high dietary fibre (14% dry weight fibre) enhances its potential as a high-fibre dietary supplement as compared with other high-fibre plants like oats, rice, etc. It is also a rich source of cellulose, valuable micro-nutrients including phosphorus, calcium, magnesium, vitamins C and E, and possesses high phenolic and flavonoid contents and radical scavenging activities. Its cholesterol-free vegetable oil is rich in unsaturated and essential fatty acids like linoleic acid, phytosterols and tocopherols, especially beta–cytosestrol and gamma–tocopherol [10,19,53,103]. As such, this seed possesses potentials to meet sustainable food and nutrition security by serving as cheap source of good quality protein, dietary fibre and phenolic compounds for production of high-protein hybrid bread (HPHB), especially in tropical and subtropical regions. However, a major challenge hampering its use in human food is the presence of a too-hard-to-remove seed coat, bitter taste, and high amounts of antinutrients which affect its functionality, palatability and nutrient digestibility.

In our previous study [19], attempts were made at removing the seed coat. Although this study reported significant improvement in the nutritional composition and antioxidative properties and reduced antinutrient contents of dehulled sorrel seed flour, the drudgery involved in the process (except if automated) may discourage its utilization. Furthermore [105], proposed the utilization of sorrel seed protein isolate for composite bread production. This study similarly reported significant nutritional improvement in bread. However, the technology, scientific expertise and costs involved in the process of protein isolation may have far-reaching economic impact for its applicability especially in low-income and developing countries like Nigeria where indigenous, low-cost technologies such as germination is easily practiced. This is based on recent increasing interest on indigenous processing techniques as adoptable technologies for reducing antinutritional factors (ANFs) and enhancing nutritional profile and antioxidative properties of leguminous seeds and grains with the aim of attaining sustainable food and nutrition security in the region [3,31]. Germination is one of the indigenous food processing technologies used for enhancement of the nutritional, antioxidative and functional properties of seeds and grains. It is

an effective bioprocessing technology which commences when quiescent dry seeds absorb water and terminates when the embryonic axis elongates [59,63]. During germination, the structure of the endosperm of grains/seeds is modified through the secretion of gibberellic acid resulting from synthesis of several hydrolytic enzymes including α-amylase, β-amylase, proteases, glucanases and oligosachharidases. These processes bring about a release of bound nutrients, reduction of ANFs, elaboration of phenolic compounds and improved functional and pasting properties, thereby transforming the seeds and grains to sources of functional ingredients for formulation of diverse healthy foods [27,31,32,70,108]. Currently, short term germination (24–72 h) is more commonly used as compared to long term germination of 72–168 h. According to Ref. [106]; short term germination reduces chances of microbial contamination and low raw material yield. Significant improvement in nutritional, functional and antioxidant properties, macronutrients digestibility and reduced ANFs have been reported in short-term germinated African yam bean seeds, Bambara groundnut, amaranth and quinoa [31,32]; Cornejo et al., 2019; [36]. The suitability of some germinated seed flours in the production of value-added functional bread have also been reported [32,34,70,100].

[64] revealed that the enhancement of 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) scavenging potential of germinated sorrel seed extract could be harnessed in the production of value-added functional wheat-based foods that would provide both nutritional and health benefits. Furthermore [14], review on use of germinated legumes in composite bread making recommended the addition of flour from germinated seeds due to improvement in the nutritional and quality characteristics of the composite bread and rheological properties of the dough [24,44,98]. However, till date, no study has reported the use of germinated sorrel seed flour in bread. Therefore, the present study was aimed at investigating the effect of short-term germination (24–48h) on the nutritional composition, antinutrient content and antioxidant activity of sorrel seed and the effect of blending wheat with germinated sorrel seed flour on the nutritional composition, functional and rheological properties of the flour blends. The effect of partial replacement of wheat flour with germinated sorrel seed flour on the nutritional composition, dietary fibre, amino acid profile, *invitro* digestibility, and acceptability of wheat bread were also evaluated. Furthermore, protein quality and haematological properties of the breads were assayed using *in vivo* studies.

2. Materials and methods

2.1. Materials

About 10 kg of red sorrel (*Hibiscus sabdariffa* L.) seeds were purchased from Commercial Mall, Sokoto, Nigeria. Bakers' hard wheat flour (Golden Penny Plc. Lagos, Nigeria), instant dry yeast (*Saccharomyces cereisiae*; Puratos, Belgium), salt and sugar (Dangote Refinery Plc, Lagos, Nigeria), and shortening (Jubi Margarine, PT Citra NutrindoLanggeng, JL RungkutIndustrii/21, Surabaya JI 60293, Indonesia) were all obtained from the Commercial Mall, Akure, Nigeria.

2.2. Preparation of germinated sorrel seed flour

The methods of [23,65] with some modifications were adopted for the germination process. The seeds were sorted to remove bad ones and foreign materials and washed severally using clean tap water. Thereafter, they were soaked in 1% sodium hypochlorite solution (NaClO) for 30 min to decontaminate the seeds. Thereafter, they were drained and then steeped in clean tap water at room temperature for 12 h (to attain a moisture level of 42–46%), drained, and spread on a clean moistened jute bag to germinate. The sorrel seeds were allowed to undergo germination under controlled atmosphere (25 ± 2 °C; for 24 and 48 h) with intermittent wetting at intervals of 8 h (to keep the relative humidity high to support seed growth up to 0.5 cm sprouting height). At the end of 48 h, non-germinated seeds were manually selected and discarded, while the germinated seeds were oven dried (Model 320, Gallenkamp, UK) at 50 °C for 8h, milled (Asiko Attrition Mill, Lagos, Nigeria; Serial No A11) and sieved (sieve size 0.4 mm).

The germinated sorrel seed flours were divided into two parts; the first part was used for analyses to determine effect of germination on the sorrel seed. Based on the high crude fat content, the second part was defatted by Soxhlet (Model 98-1-B, Serial No. 201806300309, Huanghua Faithful Instrument Co. Ltd. Huanghua City, China) extraction method using normal hexane for 8 h. Thereafter, the defatted flour was oven dried at 60 °C for 1 h, packaged and stored in a sealed container at -4 °C.

2.3. Formulation of composite blends and bread preparation

Three (3) independent batches of composite flour blends were formulated using a linear replacement (w/w) of 95-80% wheat and 5–20% germinated defatted sorrel seed flour to obtain four (4) blends of W95:GS5; W90:GS10, W85:GS15 and W80:GS20. The control flour samples were W100 and GS100 (W- 100% wheat flour; GS – 100% germinated defatted sorrel seed flour). Bread preparation was done using the modified straight-dough method by measuring farinograph water absorption of flour as reported by Ref. [16]. Thereafter, three independent batches of dough were mixed for bread preparation. Each batch consisted of a recipe of 100g flour, 6.5 g sugar, 1.5 g iodized salt, 3.0 g bakers' yeast (*Saccharomyces cerevisiae*), 3.9 g shortening and 150 mL water. The dry ingredients, apart from yeast, were mixed and poured into the mixer (Omega spiral mixer, Model OMJ-25, China Omega Baking Machinery Co. L. No. 88, East Taishan Road, Shenzhou City, Hengshui, Hebei, China). The yeast and water were mixed and added into the mixer and the mixer was set initially to run at speed 1 for 1 min. Thereafter, the speed was increased to No. 2 and mixing was allowed for 10 min, after which the dough was divided into smaller pieces, covered, and allowed to ferment initially for 15 min at ambient temperature (28 °C ± 2). The dough was manually kneaded, molded, placed into greased baking pans, and further allowed for secondary fermentation (proofing) for another 50–60 min at 37 °C ± 2 to allow the dough to rise to the brim of the pans. The pans were packed into a

thermostatically controlled oven (Model GP-OV-100-F–SS–DIG, St Helens, Merseyside, Great Britain) and baked at 230 °C for 25–30 min until golden brown. The loaves were cooled on a wire rack in the cooling room at ambient temperature for 12 h, packaged and labelled appropriately prior to further analysis.

2.4. Determination of proximate composition of sorrel seed flour, flour blends and wheat-germinated defatted sorrel seed bread

The crude protein (Kjeldahl method; method no. 950.36), total ash (method no. 930.22), crude fibre (method no. 950.37), crude fat (using soxhlet extraction method no. 950.36) and moisture (air oven method) were evaluated using [12] standard methods. Carbohydrate was calculated by difference. Energy value was estimated using the Atwater factor (protein × 4) + (fat × 9) + (carbohydrate × 4). Dietary fibre was determined according to Ref. [12] methods.

Micronutrients were determined by the dry ashing method using flame photometer to evaluate sodium (Na) and potassium (K) contents, while atomic absorption spectrophotometer was used to evaluate iron (Fe), magnesium (Mg), calcium (Ca) and Zinc (Zn). Phosphorus was evaluated spectrophotometrically (Model LUS-A22, LABTRON Equipment Ltd, UK) using the phospho-Vanadomolybdate method [12]. Molar ratios – Ca/P, Na/K, Ca/Mg, and K/Ca + Mg were thereafter calculated as described by Ref. [47].

2.5. Determination of antinutritional factors (ANFs) of sorrel seed

Phytate content was evaluated accoridng to the modified method of [67] as reported by Ref. [114]. Eight grams (8 g) of each sample was dispersed in 200 mL of 2% HCl and extracted. Following extraction, the dispersion was filtered, and 50 mL of the filtrate was mixed with 10 cm^3 of 0.3% ammonium cyanide (NH_4SCN) and diluted with 107 mL of distilled water. The extract was titrated against 0.00195 g/mL of Ferric chloride solution until a brownish yellow colour persisted. Phytate content was then calculated in mg/g.

Tannin was evaluated using [114] protocol. The sample was measured (1.0 g) and dispersed in distilled water (10 mL), shaken vigorously, and centrifuged at 3000×*g* for 20 min. The filtrate (2.5 mL) and standard tannic acid solution (2.5 mL) were separately dispersed into 50 mL flask, respectively, and Folin-Denis reagent (1.0 mL) and saturated Na_2CO_3 solution (2.5 mL) were poured into each of the volumetric flasks. Thereafter, the mixture was diluted with distilled water to mark in the volumetric flask (50 mL) and incubated for 60 min at room temperature. The absorbance was measured at 250 nm in an electronic spectrophotometer (Genway model 6000i). Readings were taken with the reagent blank at zero. The tannin content was calculated.

$$Tannin\left(\frac{mg}{g}\right) = \frac{Absorbance\ of\ sample\ x\ Concentration\ of\ standard\ x\ Total\ volume\ of\ extract}{Absorbance\ of\ standard\ solution\ x\ Weight\ of\ sample\ used\ x\ Volume\ of\ extract}$$

Oxalate was determined according to the methods of [115]. The sample (2.5 g) was digested with 10 mL 6 M HCl at a temperature of 60 °C for 60 min with continuous stirring using a magnetic stirrer, and then filtered. To 5.0 mL of the filtrate, 1.0 mL of 5 M ammonium hydroxide solution was added (to adjust the pH) until the colour of the solution changed from salmon pink to faint yellow. Phenolphthalein indicator (2 drops), glacial acetic acid (3 drops) and 5% calcium chloride (5.0 mL) were added to the solution to precipitate insoluble oxalate, and the solution was allowed to incubate for 120 min at room temperature. It was thereafter centrifuged at *2500*×*g* for 20 min. The precipitate was washed with distilled hot water, 5.0 mL of 3 M tetraoxosulphate (VI) acid was added and incubated in a water bath at 60 °C for 20 min. Freshly prepared 0.01 M potassium permanganate ($KMnO_4$) was titrated against 12.5 mL of the filtrate until a faint pink colour appeared and persisted for about 30 s. The volume of $KMnO_4$ used was read from the burette reading and oxalate content was calculated (mg/g) as:

$$Oxalate\left(\frac{mg}{g}\right) = volume\ of\ KMnO_4\ used\ (ml) x\ 0.9004$$

Evaluation of trypsin inhibition activity was done by adopting the modified method of [69]. One gram (1 g) of the sample was mixed with 50 ml of 0.01 M NaOH at pH between 8.4 and 10.00. The mixture was allowed to stand for 3 h with intermittent stirring. Two (2) ml of diluted extract was then dispensed into test tubes with 2 ml of cold trypsin solution (4 mg in 200 ml of 0.001 M HCl) added into each tube. The tubes were then placed in water bath at 37 °C and 5 ml of Benzoyl-DL-Arginine-P-nitro anilide hydrochloride (BAPNA) (40 mg was dissolved in 1 ml of dimethyl sulfoxide and diluted to 100 ml with tris buffer 0.05 M, pH 8 and diluted to 100 ml with tris buffer 0.05 M, pH 8.2, pre warmed to 37 °C) was added as substrate to each tube. After 10 min, the reaction was terminated by adding 30% acetic acid and the content of each tube was thoroughly mixed. Thereafter the content of each tube was centrifuged at 3000×*g* and filtered. Absorbance of the filtrate was measured at 410 nm against reagent blank. The reference was prepared similarly as the sample except that 2 ml of distilled water was used in place of extract.

2.6. Calculation of mineral molar ratios and bioavailability index of sorrel seed

Predicted bioavailability of dietary minerals was determined by dividing the mole of the antinutrient with the mole of the mineral to obtain molar ratio of phytate and oxalate to calcium, iron, or zinc [48]. Phytate:Zn, Phytate:Ca and Phytate:Ca/[Zn] molar ratios were calculated. Critical values of calcium:phytate <6, phytate:iron >1, phytate:zinc >15, and phytate:calcium/zinc >0.5 were used to predict the bioavailability [50].

2.7. In vitro protein digestibility (IVPD) of wheat:germinated defatted sorrel seed flour blends

The method of [86] was adopted for IVPD determination. A known weight of the samples (2:1 w/v) was dispensed into clean beakers. Thereafter, sodium dehydrate citrate buffer of pH 2 and 10% molarity was added (35 mL) followed by inclusion of digestibility enzyme – pepsin (1.5 g/L) and incubated at 37 °C for 120 min after incubation, the mixtures were centrifuged at 1500×*g* for 5 min and filtered. The filtrate was discarded while the precipitate was washed twice and frozen dried. The nitrogen content of the precipitate was then determined [12]. IVPD was calculated using the Equation below:

$$Protein\ digestibility = \frac{Nitrogen\ in\ supernatant - Nitrogen\ in\ blank}{Nitrogen\ in\ sample}\ x\ 100$$

2.8. Determination of functional properties of wheat-germinated defatted sorrel seed flour blends

The methods of [104] were adopted for determination of oil and water absorption capacity as reported by Ref. [93]. Bulk density, swelling index and capacity were determined as described by Ref. [93]; while foaming capacity (FC) was determined using the methods of [110]. Emulsifying capacity was done using [12] protocol.

2.9. Rheological measurement of wheat-germinated defatted sorrel seed flour blends

The rheological properties of the dough were measured using the Brabender Farinograph (Model: E−380, Brabender OHG, Diusburg, Germany) according to the approved methods of American Association of Cereal Chemists [9]. The amount of water for mixing was automatically determined based on the moisture content of the flour. The farinograph was equipped with a 300g capacity mixer and mixing was done for 15 min while the graph was being generated automatically.

2.10. Determination of amino acid profile of wheat-germinated defatted sorrel seed bread

Amino acids of samples were determined using Automated Amino Acid Analyzer (Model 6300; Beckman Coulter Inc., Fullerton, Calif., USA) [12], while tryptophan was determined as described by Ref. [55]. The protein quality indices like Branched chain amino acids (BCAAs), Limiting amino acid (LAAs), Essential amino acid index (EAAI) and Abundant amino acids (AAAs) (Ijarotimi et al., 2022a) were calculated using the amino acid profile.

2.11. Nutritional quality evaluation of wheat-germinated defatted sorrel seed bread

Animal right: The use of animal right was obtained from ethical committee, Federal University of Technology Akure and study was carried out in accordance with the national safety rules governing the use of animal (FUTA/SAAT/2022/0099; Ethical permit number).

Animal design: Six groups (n = 5) of Wistar rats (weight 30 g of male and female gender) obtained from the animal colony unit of the Department of Biochemistry, Federal University of Technology Akure, were used. The animals were subjected to 7 days acclimatization (metabolic cage of 12 h light and 12 dark illuminations at relative humidity of 35–40%) with water and feeding in excess [91]. Thereafter, the animals were fed with experimental bread samples for 28 days and in the last 7 days of the experiment, animal faeces and urine were collected and analyzed for nitrogen content. Prior to that, daily feed intake, feed left over, and weekly weight gains were recorded. Feed efficiency (FE), biological value (BV), protein efficiency ratio (PER), net protein utilization (NPU), true digestibility (TD), and nitrogen retention (NR) were all calculated using the protocols of [90].

$$\textbf{Feed efficiency}\ (\textbf{FE}) = \frac{\text{Weight gained (g)}}{\text{Food intake (g)}}$$

$$\textbf{Biological value}\ (\textbf{BV}) = \frac{\text{Ni} - (\text{Nf} - \text{Nef}) - (\text{Nu} - \text{Neu})}{\text{Ni} - (\text{Nf} - \text{Neu})} x\ 100$$

$$\textbf{\textit{Protein efficiency ratio}}\ (\textbf{\textit{PER}}) = \frac{\text{Weight gained (g)}}{\text{Protein intake (g)}}$$

$$\textbf{\textit{Net protein utilization}}\ (\textbf{\textit{NPU}}) = \frac{\text{Ni} - (\text{Nf} - \text{Nef}) - (\text{Nu} - \text{Neu})}{\text{Ni}} x\ 100$$

$$\textbf{\textit{True protein digestibility}}\ (\textbf{\textit{TPD}}) : = \frac{\text{Ni} - (\text{Nf} - \text{Nef})}{\text{Ni}} x\ 100$$

$$\textbf{\textit{Nitrogen retention}}\ (\textbf{\textit{NR}}) = \text{Ni} - (\text{Nf} - \text{Nef}) - (\text{Nu} - \text{Neu})$$

Where, *Ni: Nitrogen intake in proteins on the test diet; Nf: faecal nitrogen on the test diet; Nef: nitrogen excreted on nitrogen free diet (metabolic*

Table 1
Proximate, dietary fibre, minerals composition and antinutrient content of sorrel seed as affected by germination time.

parameters	Samples		
	Raw ungerminated	Germinated sorrel seed	
		24 h	48 h
Proximate composition (g/100 g DW)			
Moisture	5.50 ± 0.45^{c}	10.01 ± 0.91^{a}	6.93 ± 0.11^{b}
Crude protein	32.32 ± 0.51^{c}	38.01 ± 1.01^{a}	35.49 ± 0.83^{b}
Crude fat	25.39 ± 0.31^{ab}	26.11 ± 0.54^{a}	24.18 ± 0.07^{c}
Total ash	11.16 ± 0.85^{a}	6.39 ± 0.07^{c}	6.98 ± 0.14^{b}
Carbohydrate	18.43 ± 1.12^{a}	8.59 ± 0.69^{c}	16.16 ± 0.71^{b}
Dietary fiber (g/100 gDW)			
Soluble dietary fibre	5.29 ± 0.21^{c}	5.99 ± 0.04^{b}	6.38 ± 0.36^{a}
Insoluble dietary fibre	19.69 ± 0.08^{c}	20.04 ± 0.10^{b}	20.74 ± 0.21^{a}
Total dietary fibre	24.98 ± 1.01^{c}	26.03 ± 0.64^{b}	27.12 ± 0.32^{a}
Mineral composition (mg/100gDW)			
Cu	0.04 ± 0.01^{b}	0.16 ± 0.03^{a}	0.14 ± 0.01^{a}
Fe	0.19 ± 0.05^{c}	0.38 ± 0.01^{b}	0.47 ± 0.01^{a}
Zn	1.15 ± 0.11^{c}	2.04 ± 0.24^{b}	2.95 ± 0.31^{a}
P	3.96 ± 0.03^{c}	5.09 ± 0.11^{b}	5.47 ± 0.01^{a}
Ca	21.79 ± 3.54^{c}	35.17 ± 1.34^{b}	39.53 ± 2.01^{a}
Mg	19.01 ± 1.01^{c}	26.14 ± 0.71^{a}	24.26 ± 0.32^{b}
K	110.89 ± 0.05^{c}	119.87 ± 0.53^{b}	121.74 ± 0.95^{a}
Na	5.84 ± 0.12^{bc}	5.97 ± 0.01^{b}	6.04 ± 0.01^{a}
Mineral molar ratios			
Na/K	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}
Ca/P	5.50 ± 0.02^{c}	6.91 ± 0.02^{b}	7.23 ± 0.02^{a}
Ca/Mg	1.15 ± 0.01^{c}	1.35 ± 0.01^{b}	1.63 ± 0.02^{a}
K/Ca + Mg	2.72 ± 0.02^{a}	1.96 ± 0.01^{b}	1.91 ± 0.01^{c}
Antinutritional factors[a]			
Oxalate (mg/g)	4.04 ± 0.19^{a}	3.47 ± 0.01^{b} (14.11%)	2.93 ± 0.10^{c} (27.48%)
Phytate (mg/g)	32.75 ± 0.26^{a}	22.01 ± 2.01^{b} (32.79%)	15.04 ± 1.27^{c} (54.08%)
Tannins (mg/g)	7.03 ± 0.14^{a}	6.60 ± 0.37^{b} (6.12%)	4.84 ± 0.71^{c} (31.15%)
Saponin (mg/g)	33.0 ± 1.00^{a}	24.01 ± 0.57^{b} (27.24%)	17.73 ± 1.00^{c} (46.27%)
Millimolar ratios of minerals to ANFs			
[Phy]/[Zn]	28.05 ± 1.00^{a}	10.63 ± 1.00^{b}	5.02 ± 1.00^{c}
[Phy]/[Ca]	0.91 ± 0.01^{a}	0.38 ± 0.01^{b}	0.23 ± 0.01^{c}
[Phy]/[Fe]	146.25 ± 1.00^{a}	49.15 ± 1.00^{b}	27.15 ± 1.00^{c}
[Phy × Ca]/[Zn]	15.29 ± 1.00^{a}	9.34 ± 1.00^{b}	4.96 ± 1.00^{c}
[Ox]/[Ca]	4.69 ± 1.01^{a}	1.96 ± 0.05^{b}	1.12 ± 0.02^{c}
Functional properties			
WAC (%)	93.75 ± 2.11^{b}	103.13 ± 6.71^{a}	68.75 ± 7.93^{c}
OAC (%)	67.14 ± 7.34^{c}	90.29 ± 3.04^{b}	102.7 ± 5.21^{a}
Bulk density (g/cm^3)	0.63 ± 0.01^{a}	0.59 ± 0.02^{a}	0.62 ± 0.01^{a}
Swelling index	4.08 ± 1.01^{b}	ND	7.03 ± 0.81^{a}
Foaming capacity (%)	16.00 ± 0.35^{a}	12.00 ± 0.11^{c}	14.01 ± 1.21^{b}

Values are Means ± Standard deviation of three determinations. Values in the same row with the same superscript alphabet are not significantly different ($p < 0.05$). ND – Not determined.

[a] Values in parenthesis are percentage reduction of antinutrient in the germinated samples in comparison with the control (raw ungerminated sorrel seed flour).

nitrogen); Nu: urinary nitrogen whilst on the test diet, Neu: urinary nitrogen excreted on nitrogen-free diet (endogenous nitrogen).

2.12. Hematological determination of rats fed with wheat-germinated defatted sorrel seed bread

After 28 days of feeding, blood samples were collected from the experimental animals after anaesthetization with chloroform. Animal carcasses were handled in accordance with safety policy detailed by ethical committee. The packed cell volume, white blood cell, red blood cell, neutrophils and lymphocytes were calculated using the medical laboratory method detailed by Ref. [90].

2.13. Sensory evaluation of wheat-germinated defatted sorrel seed bread

The methods of [16,20] were adopted for selection of a 30-member panel made up of staff and students at the Federal University of Technology, Akure, Nigeria, for the evaluation of sensory properties of the bread samples. Panelists (males and females, ranging from ages 22–64 years) were selected, after an oral interview, using parameters such as: familiarity with the control sample (100% wheat bread), good health, non-smoker, non-allergic to wheat/sorrel seed, willingness to participate, and passion/likeness for bread. Bread samples were evaluated 9 h after baking for properties including crumb colour, crust, crumb texture, aroma, taste, and general acceptability using a 9-point Hedonic scale rating where 9 represented like extremely and 1, dislike extremely for each attribute

evaluated. The evaluation was done under adequate lightning, no noise, no odour and well-ventilated sensory room with water made available to the panelists for mouth rinsing in-between analysis. Consent to participate was obtained using verbal communication from panelists while ethical approval was obtained from ethical committee, Federal University of Technology Akure. The study was carried out in accordance with the national food safety rules (FUTA/SAAT/2022/00100).

2.14. Statistical analysis of data

All determinations were carried out in triplicate and data subjected to analysis of variance using IBM SPSS V.20 (USA). Data are presented as mean ± standard deviation after being subjected to Duncan's Multiple Range Test at 5% significant difference.

3. Results and Discussion

3.1. Proximate composition and dietary fibre of sorrel seed as affected by germination time

The proximate composition, dietary fibre, mineral elements, mineral molar ratios, antinutritional factors (ANFs), bioavailability of mineral (as expressed by the ratios of minerals to ANFs) and functional properties of sorrel seed flour as affected by short term germination for 24–48 h are presented in Table 1.

The initial 24h germination resulted in significant ($p < 0.05$) increase in the moisture content of sorrel seed flour from 5.5 g/100 g–10.01 g/100 g. This may be due to the water absorbed during soaking before germination commenced. According to Refs. [38,59,81]; absorption of water is an important phenomenon that stimulates the germination process by activating seeds from their dormancy. This restores the seed's metabolic activities, prompting the synthesis of enzymes that bring about changes in the biochemical, nutritional, and sensorial properties of the seeds. However, the decrease from 10.01 g/100 g–6.93 g/100 g at 48 h of germination indicates that the seed moisture was utilized during the germination process, thus suggesting that water is one of the important factors necessary for true germination to take place [26,59]. The low moisture content of the germinated seed flour is within the safe level for prolonged storage of flours (≤10 g/100 g). Hence, during prolonged storage, the flour may not be susceptible to moisture-dependent deteriorative processes.

Crude protein content of 32.32 g/100 g reported here for the raw seed flour is higher than 24.93–26.62g/100 g previously reported [19,40,123] but falls within the range (32.28–33.5g/100 g) reported by Refs. [2,52]. Germinating the seed for 24 h resulted in a significant ($P < 0.05$) percentage increase of 17.61% (from 32.32 to 38.01g/100 g) while further germination up to 48h caused a drop in the protein content from 38.01 to 35.49g/100 g. Although, in comparison with the control, this value still indicates a significant increase of 9.81% (from 32.32 to 35.49g/100 g). Protein increase during germination has been attributed to synthesis of enzymes by germinating seeds, resulting in degradation of macronutrients including protein with subsequent release of free amino acids that may increase protein content. Also, the synthesis of newly formed protein may contribute to protein increase during germination [31,122]. Therefore, the incorporation of germinated sorrel seed to staple food products may be a low-cost strategy for enhancing the protein content of staple foods and reducing protein energy malnutrition reported to be predominant in developing countries such as Nigeria.

Results obtained showed a high fat content of 25.39g/100 g for the raw ungerminated sorrel seed. This value is higher than 21.03g/100 g previously reported by Ref. [123] but similar to 26.24–27.22g/100 g reported by Refs. [19,52]. Although there was an initial increase in fat content during 24 h germination from 25.39 to 26.11g/100 g, this increase was insignificant ($P > 0.05$) and continued germination up to 48 h significantly ($P < 0.05$) decreased it to 24.18g/100 g. Lipid reduction during germination has been attributed to its utilization as energy source for seed growth and increased lipolytic activity in which about 25 g/100 g of lipids in the seed can be hydrolyzed so as to promote respiratory activity and meet energy requirements during germination [31,121]. Similar reduction has been reported in germinated Bambara groundnut and amaranth species [31,35].

Carbohydrate content of the sorrel seed significantly ($P < 0.05$) decreased from 18.43 to 8.59g/100 g at 24 h germination. However, as germination progressed up to 48 h, it increased to 16.16g/100 g. The initial decrease may be due to its utilization as an energy source by hydrolytic enzymes. Subsequent increase as germination progressed may be due to amylase activity resulting in the degradation of starch to increased sugar contents. This agrees with the findings of [35,74] who reported increased carbohydrate and sugar contents during germination of amaranth species and peanut, kidney, mung, and soybeans. According to Refs. [95,116]; as soon as resting seeds are hydrated prior to germination, there is an increased metabolic activity stimulating increased activity of hydrolytic enzymes which hydrolyze starch with increased total sugar levels [72]. also reported that increased α-galactosidase activity causes breakdown of α-1,6-galatosidic linkages, thus, increasing the number of total sugars.

Dietary fibre contents of the ungerminated and germinated sorrel seed flours ranged from 5.29 to 6.38g/100 g, 19.69–20.74g/100 g and 24.98–27.12g/100 g (dry weight) for soluble, insoluble, and total dietary fibres, respectively. Values and ratios of 1.0:3.72 for soluble to insoluble fibre reported here are comparable with those (5.89, 19.59, 25.48 and 1.0:3.3, respectively) previously reported [52]. Germination significantly ($P < 0.05$) increased these values by 4.20–8.57%, with the insoluble fibre (from 19.69g/100 g in the raw ungerminated sorrel seed flour to 20.04g/100 g and 20.74g/100 g in germinated samples) being predominantly higher than the soluble fibre (from 5.29% to 5.99% and 6.38%) (Table 1). This indicates that germination could be an important process for increasing dietary fibre content of legumes including sorrel seed. These findings agree with previous studies on some non-conventional and conventional legumes including cowpea, jack, velvet and hyacinth beans, African yam bean, and peanut, mung, kidney, and soybeans [21,32,74]; respectively). Increase in the soluble dietary fibre may be due to cleavage of inter-molecular bonds, breakdown of protein structures, and solubilization of macromolecules [62]. However, changes in cellular structure and generation of compounds, such as celluloses and hemi-celluloses as structural elements of the growing sprout have been reported to cause increased insoluble dietary

fibre [58], while total dietary fibre increase during germination has been attributed to formation of new primary cell walls and resistant starch [68].

This increase in the germinated sorrel seed can help in improving the texture of food products to which germinated sorrel seed flour has been incorporated [83] such as composite bread with reduced wheat content since this is one of the major challenges of gluten-free breads. High dietary fibre content reported in the present study corroborates previous reports that sorrel seed is a rich source of dietary fibre [51,92]. Hence, consumption of sorrel seed may have physiological and health benefits for its consumers by lowering serum cholesterol, producing short chain fatty acids from fibre fermentation in colon, altering concentration of insulin and hormones, and reduction of risk to colon cancer [83].

3.2. Mineral composition, antinutritional factors, mineral bioavailability and functional properties of sorrel seed as affected by germination time

There was significant ($P < 0.05$) increase in Fe, Zn, P, Ca, Mg, K and Na contents during germination of sorrel seed. Previous studies have reported increased mineral contents in sorrel seed during processing and attributed this to improved hydrochloric acid extractability of minerals which occurs because of the destructive effects of processing (including germination) on antinutritional factors (ANFs) and subsequent release of bound minerals [2,52,80]. The relatively high content of Ca reported in the present study is very advantageous based on the importance of Ca in the prevention of osteoporosis. This may have influenced the increase in the Ca/P (5.50–7.23) and Ca/Mg (1.15–1.63) molar ratios during germination (Table 1). These values which are higher than the FAO recommended ratios of >1.0 and are an indication of the potential of germinated sorrel seed to aid in the prevention of osteoporosis in adults and rickets in children when consumed or incorporated into food products [61]. Furthermore, the Ca/P values which are above 2 indicate increased absorption of calcium in the small intestine [78]. As such, consumption of this seed may aid in supporting calcium absorption in the small intestine. Similarly, the low Na/K ratio (0.05) reported in the present study indicates the suitability of the seed for individuals with high blood pressure.

There was progressive percentage decrease of 14.11–27.48%, 32.79–54.08%, 6.12–31.15% and 27.24–46.27% in oxalate (from 4.04 mg to 3.47–2.93 mg), phytate (from 32.75 mg to 22.01–15.04 mg) tannin (from 7.03 mg to 6.60–4.84 mg) and saponin (from 33.0 mg to 17.73 mg) contents, respectively as germination progressed. The highest reduction of 54.08% was recorded in the phytate content of sorrel seed germinated for 48 h, followed by saponin (46.27%), while the least decrease of 6.12% was observed in tannin content of 24h-germinated seeds. This may explain the relative increase in the Ca content of the seed [117]. noted that germination aids in disintegrating the phytate:Ca bond by increased activity of gibberellic acid and phytase, thereby promoting bioavailability of minerals especially Ca. These findings are comparable with reduction rates of 27–51% reported during progressive germination of Bambara groundnut [31]. Tannin reduction may be attributed to high activity of polyphenol oxidase and other catabolic enzymes during germination, while reduction of phytate contents results from increasing activities of endogenous phytases which degrade phytate into inorganic phosphorous and inositol [73,112]. Although ANFs are known to form complexes with macro and micronutrients thereby altering their availability, recent reports suggest that their antioxidant and anti-carcinogenic properties confer health benefits [4,25]. Furthermore, the millimolar ratio of minerals to ANFs which is an indication of the chelation activities between phytate-zinc-calcium, and oxalate-calcium shows a significant reduction between raw ungerminated sorrel seed (28.05; 0.91; 146.25; 15.29 and 4.69) compared with germinated sorrel seeds (10.63–5.02; 0.38–0.23; 49.15–27.15; 9.34–4.96 and 1.96–1.12) with 48 h germination having the least millimolar ratio for Phy/Zn; Phy/Ca; Phy/Fe; Phy/Ca/Zn and Ox/Ca respectively. The decrease observed in germinated samples compared with ungerminated samples indicates increased mineral bioavailability. Hence, germination helps in improving digestibility of mineral elements.

Functional properties are important parameters that determine the utilization of flours in product development and germination has been reported to cause significant modification of functional properties of seed, hence influencing their functionality in product development [8,63]. Additionally, the functionality of plant proteins facilitates their use as ingredients in product development because they confer important attributes such as appearance, flavor, colour, odour, texture, and structure to food products [6]. In the present study, water absorption capacity (WAC) varied with germination time. While 24 h germination significantly ($P < 0.05$) increased WAC from 93.75% to 103.13%, further germination (48h) caused a reduction of 26.67% (from 93.75 to 68.75%). These variations are an indication that the changes that take place in seeds during germination are time-dependent and influence the ultimate processing conditions that the seed would be subjected to meet specific demands for product development. Hence, the initial increase in WAC may be attributed to modification which exposed the hydrophilic domains and amino acid residues of proteins and other macromolecules that have high affinity for aqueous medium thereby increasing the water binding sites [28].

The high WAC of germinated sorrel seed flour suggests its suitability in bakery products where hydration property is important to improve dough handling characteristics and enhance functional and sensory properties [119]. On the other hand, oil absorption capacity consistently increased with germination time from 67.14% to 90.29–102.7%. This agrees with previous reports on germinated sorrel seed [19] but is contrary to the decrease reported in germinated Bambara groundnut, mung bean, pea, and lentil [31,41]. These differences may be related to differences in the seeds' composition and longer germination times (between 72 and 120 h) reported in these previous studies. The high WAC and OAC reported here further emphasizes the suitability of sorrel seed flour in composite formulations with wheat for production of gluten-free, value-added baked foods since water and fat binding properties are important functional properties desired in dough making [39,118]. This is because the water/fat binding capacity of proteins is an important index for absorption and retention of oil (a property required in flours for bakery products) which influences flavour retention, texture and mouth feel of food products like ground meat formulations, doughnuts, pancakes, baked goods, and soups [7].

Bulk density obtained in present study decreased but not significantly ($P > 0.05$) (Table 1). This slight decrease may be attributed to

Table 2
Proximate composition, dietary fibre, *in vitro* protein digestibility and mineral composition of wheat:germinated defatted sorrel seed flour blends.

	Samples					
	W100	W95:GS5	W90:GS10	W85:GS15	W80:GS20	GS100
Proximate composition (g/100g DW)						
Moisture	8.41 ± 0.01^{e}	8.23 ± 0.02^{f}	8.56 ± 0.01^{d}	9.30 ± 0.03^{b}	9.42 ± 0.04^{a}	8.97 ± 0.01^{c}
Crude protein	8.05 ± 0.17^{f}	11.52 ± 0.02^{e}	15.45 ± 0.13^{d}	17.77 ± 0.02^{c}	19.49 ± 0.15^{b}	37.46 ± 0.90^{a}
Crude fat	2.38 ± 0.03^{f}	2.73 ± 0.01^{e}	3.99 ± 0.11^{d}	4.53 ± 0.11^{c}	5.07 ± 0.07^{b}	6.37 ± 0.21^{a}
Total ash	1.75 ± 0.02^{e}	1.75 ± 0.02^{e}	1.85 ± 0.03^{d}	2.11 ± 0.01^{c}	2.91 ± 0.01^{b}	4.89 ± 0.01^{a}
Carbohydrate	85.94 ± 2.02^{a}	80.84 ± 1.11^{b}	77.97 ± 0.97^{c}	73.35 ± 2.06^{d}	69.66 ± 0.13^{e}	46.44 ± 1.08^{f}
Total dietary fibre (%)	11.46 ± 0.52^{f}	19.79 ± 0.27^{e}	21.23 ± 0.32^{d}	22.99 ± 0.02^{c}	25.37 ± 2.18^{ab}	27.12 ± 0.33^{a}
IVPD (%)	62.24 ± 1.85^{a}	61.70 ± 2.12^{a}	58.98 ± 2.06^{b}	48.67 ± 1.09^{c}	43.28 ± 1.05^{d}	39.20 ± 1.08^{e}
Mineral composition (mg/kg)						
K	405.05 ± 5.05^{f}	440.03 ± 13.05^{e}	476.0 ± 3.5^{d}	654.10 ± 7.10^{c}	690.15 ± 8.15^{b}	1980.50 ± 17.50^{a}
Ca	34.05 ± 3.11^{f}	51.05 ± 6.05^{e}	74.05 ± 1.51^{d}	79.89 ± 4.10^{c}	98.20 ± 12.02^{b}	329.07 ± 10.10^{a}
P	346.07 ± 5.26^{f}	371.33 ± 4.50^{e}	378.21 ± 2.02^{d}	405.26 ± 4.00^{c}	420.31 ± 9.03^{b}	602.58 ± 20.01^{a}
Na	5.01 ± 0.04^{f}	5.81 ± 0.05^{e}	6.61 ± 1.01^{d}	8.51 ± 0.81^{c}	10.41 ± 1.51^{b}	18.80 ± 3.30^{a}
Mg	138.31 ± 3.01^{f}	143.33 ± 3.81^{e}	171.71 ± 6.02^{d}	210.61 ± 10.01^{c}	289.62 ± 12.02^{b}	477.05 ± 9.15^{a}
Fe	3.81 ± 0.01^{f}	4.21 ± 0.31^{e}	5.11 ± 0.01^{d}	7.32 ± 0.05^{c}	8.30 ± 0.90^{b}	11.60 ± 2.01^{a}
Na/K	0.012 ± 0.001^{a}	0.013 ± 0.001^{a}	0.014 ± 0.001^{a}	0.013 ± 0.001^{a}	0.015 ± 0.001^{a}	0.009 ± 0.001^{b}
Ca/P	0.098 ± 0.001^{e}	0.137 ± 0.001^{d}	0.196 ± 0.001^{c}	0.197 ± 0.001^{c}	0.233 ± 0.001^{b}	0.545 ± 0.001^{b}

Values are Means ± Standard deviation of three determinations. Values in the same row with the same superscript alphabet are not significantly different ($p < 0.05$).
Key: W100 - 100% wheat flour; W95:GS5 - 95% wheat:5% germinated defatted sorrel seed flour; W90:GS10 - 90% wheat:10% germinated defatted sorrel seed flour; W85:GS15 - 85% wheat:15% germinated defatted sorrel seed flour; W80:GS20 - 80% wheat:20% germinated defatted sorrel seed flour; GS100 - 100% germinated defatted sorrel seed flour; IVPD – *in vitro* protein digestibility.

possible modification during germination resulting from breakdown of complex compounds such as starch and proteins [61]. This low bulk density may positively influence the possible utilization of germinated sorrel seed flour for enriching protein quality and content of cereal-based complementary diets in developing countries which are high in energy and starch. This is because low bulk density is desirable in the formulation of complementary diets since it ensures high nutrient density [1,18].

Swelling index was observed to significantly ($P < 0.05$) increase (from 4.08 to 7.03). The increase in swelling index may be due to an increase in soluble solids brought about by the breakdown of lipid, fiber, and larger amount of amylose–lipid complex in the sample [84]. Since swelling affects changes in hydrodynamic properties of food and impacts characteristics such as body, thickening and increased viscosity to foods [18], the increase recorded in the present study may aid in improving the viscosity of the wheat-germinated sorrel seed composite bread which is a challenge associated with wheat-supplemented breads. According to Ref. [99]; swelling power is positively related to the number of soluble solids. On the other hand, foaming capacity decreased significantly ($P < 0.05$) from 16% to 12% at 24 h germination but increased to 14% by 48 h, although this was still lower than the control. Values observed here are higher than those (8–10%) reported in a previous study which reported contrarily that germination increased foaming capacity [64]. The reduction in foaming capacity may be linked to modification of macromolecules during germination which may have resulted in denaturation and diminution of protein, thereby decreasing foaming capacity [15].

3.3. Proximate composition, dietary fibre, in vitro protein digestibility and mineral composition of wheat: germinated defatted sorrel seed flour blends

Based on higher values of protein content, dietary fibre and functional properties and lower values of ANFs obtained for the for the 48h-germinated sorrel seed flour, this sample was selected, defatted, and used for further investigations in this study. Table 2 presents the proximate composition, dietary fibre, *in vitro* protein digestibility and mineral composition of wheat flour as affected by substitution of 48h-germinated defatted sorrel seed flour. The moisture content of the flours ranged from 8.23 to 9.42g/100g, with increasing substitution of germinated defatted sorrel seed flour resulting in higher values. Observed low moisture contents which are below the safe moisture control (≤10%) for flours indicate suitability of the flour blends for prolonged storage [17].

There was significant ($P < 0.05$) increase in the protein content of wheat as increasing levels of germinated defatted sorrel seed flour was added. Initial protein content of wheat flour (W100) was 8.05g/100g; addition of 5% sorrel seed flour increased it to 11.52g/100g (an increase of 43.11% in W95:GS5), while up to 20% sorrel seed in the blend increased the value to 19.49g/100g (an increase of 142.11% in W80:GS20). This is consistent with previous studies of wheat flours substituted with leguminous seed flours [5]. This observation may present a low-cost alternative for protein enrichment of starchy staples, which predominantly make up most diets in developing countries like Nigeria where animal protein sources are largely unaffordable for most families due to low income and poverty. Invariably, this may help to curb protein energy malnutrition prevalent among low-income households [17]. Furthermore, this will enhance the utilization of sorrel seed, reduce wastage and the environmental menace usually associated with the discarded seeds. Additionally, it will help to increase farmers' and national economies, and lessen total dependence on imported wheat, thereby reducing the debt burden usually accumulated from wheat importation [61].

Similarly, crude ash and crude fat contents increased with increasing addition of germinated defatted sorrel seed flour. This may be attributed to the addition effect of sorrel seed which is a micronutrient-, oil-rich seed. However, the fat contents of 2.73–6.37g/100g

Table 3
Functional and rheological properties of wheat:germinated defatted sorrel seed flour blends.

	Samples					
	W100	W95:GS5	W90:GS10	W85:GS15	W80:GS20	GS100
Functional properties						
WAC (%)	116.00 ± 4.21^{f}	178.00 ± 12.06^{e}	203.00 ± 6.03^{d}	213.00 ± 2.05^{c}	216.00 ± 1.04^{b}	240.00 ± 5.01^{a}
OAC (%)	182.12 ± 0.33^{c}	180.1 ± 0.85^{b}	160.03 ± 0.91^{d}	160.51 ± 1.16^{d}	199.33 ± 4.32^{a}	140.05 ± 5.21^{e}
BD (g/cm^{3})	0.77 ± 0.02^{a}	0.77 ± 0.01^{a}	0.74 ± 0.01^{a}	0.66 ± 0.01^{c}	0.70 ± 0.01^{b}	0.59 ± 0.02^{d}
Swelling Index	2.03 ± 0.11^{c}	2.03 ± 0.06^{c}	3.00 ± 0.10^{b}	3.00 ± 0.01^{b}	3.00 ± 0.02^{b}	7.03 ± 0.06^{a}
Swelling capacity (g/g)	2.37 ± 0.02^{c}	2.51 ± 0.15^{b}	2.56 ± 0.04^{b}	2.45 ± 0.11^{b}	2.57 ± 0.2^{b}	3.41 ± 0.03^{a}
Foaming capacity (%)	34.11 ± 1.03^{a}	22.22 ± 0.24^{c}	21.90 ± 0.13^{d}	18.33 ± 1.01^{e}	16.27 ± 0.97^{f}	31.26 ± 1.22^{b}
Emulsifying capacity (%)	2.22 ± 0.21^{f}	4.55 ± 1.01^{e}	6.67 ± 0.54^{d}	11.36 ± 0.33^{c}	21.74 ± 2.43^{b}	26.67 ± 2.08^{a}
Rheological properties						
Dough Extensibility (cm)	4.78 ± 0.03^{a}	4.05 ± 0.21^{b}	2.40 ± 0.01^{c}	1.19 ± 0.01^{d}	Nil	Nil
Resistance to extensibility	8.69 ± 0.21^{a}	3.93 ± 0.10^{b}	2.00 ± 0.04^{c}	Nil	Nil	Nil
Dough stability time (min)	11.30 ± 0.14^{a}	10:00 ± 0.10^{b}	9.30 ± 0.00^{c}	8.30^{d} ± 0.20^{d}	8.00 ± 0.21^{e}	Nil
Water Absorption (%)	3.20 ± 0.32^{f}	5.84 ± 0.45^{e}	14.59 ± 5.10^{d}	33.23 ± 6.03^{c}	71.25 ± 8.01^{b}	88.66 ± 6.87^{a}

Values are Means ± Standard deviation of three determinations. Values in the same row with the same superscript alphabet are not significantly different ($p < 0.05$).
Key: W100 - 100% wheat flour; W95:GS5 - 95% wheat:5% germinated defatted sorrel seed flour; W90:GS10 - 90% wheat:10% germinated defatted sorrel seed flour; W85:GS15 - 85% wheat:15% germinated defatted sorrel seed flour; W80:GS20 - 80% wheat:20% germinated defatted sorrel seed flour; GS100 - 100% germinated defatted sorrel seed flour; WAC – Water absorption capacity; OAC – Oil absorption capacity; BD – Bulk density.

Table 4
Proximate composition, energy value and dietary fibre of wheat:germinated defatted sorrel seed bread.

	Bread samples				
	W100	W90:GS10	W85:GS15	W80:GS20	GS100
Proximate composition (g/100g DW)					
Moisture	10.28 ± 0.04^{d}	10.62 ± 0.06^{c}	11.16 ± 0.04^{a}	10.98 ± 0.03^{b}	9.47 ± 0.04^{e}
Crude protein	7.33 ± 0.03^{e}	11.13 ± 0.10^{d}	14.89 ± 0.10^{c}	16.23 ± 0.07^{b}	36.01 ± 0.01^{a}
Crude fibre	3.11 ± 0.01^{e}	3.87 ± 0.05^{d}	4.09 ± 0.08^{c}	4.25 ± 0.01^{b}	5.56 ± 0.03^{a}
Crude fat	4.27 ± 0.01^{e}	4.63 ± 0.02^{d}	5.59 ± 0.02^{c}	6.65 ± 0.01^{b}	7.43 ± 0.02^{a}
Total Ash	2.99 ± 0.03^{e}	3.13 ± 0.01^{d}	3.38 ± 0.01^{c}	4.28 ± 0.03^{b}	5.30 ± 0.03^{a}
carbohydrate	82.3 ± 0.03^{a}	77.24 ± 0.04^{b}	72.05 ± 0.04^{c}	68.59 ± 0.09^{d}	45.7 ± 0.03^{e}
Energy value (Kcal)	396.95 ± 0.23^{c}	395.15 ± 0.05^{d}	398.07 ± 0.12^{b}	399.13 ± 0.33^{a}	393.71 ± 0.57^{e}
Dietary fibre (%)	11.47 ± 0.52^{e}	22.73 ± 0.32^{d}	24.99 ± 0.2^{c}	27.32 ± 0.18^{b}	29.12 ± 0.33^{a}

Values are Means ± Standard deviation of three determinations. Values in the same row with the same superscript alphabet are not significantly different ($p < 0.05$).
Key: W100 - 100% wheat bread; W90:GS10 - 90% wheat:10% germinated defatted sorrel seed bread; W85:GS15 - 85% wheat:15% germinated defatted sorrel seed bread; W80:GS20 - 80% wheat:20% germinated defatted sorrel seed bread; GS100 - 100% germinated defatted sorrel seed bread.

reported here (are comparatively lower than 24.18g/100g earlier reported in Table 1 for the full-fat germinated sorrel seed due to defatting) are not high enough to promote any fat-dependent oxidative changes in the flour samples during prolonged storage. On the other hand, carbohydrate content decreased significantly ($P < 0.05$) with progressive addition of germinated sorrel seed flour. This may be due to carbohydrate-dilution effect contributed by increasing addition of the protein-rich sorrel seed flour [61].

There was significant increase in the total dietary fibre content as germinated defatted sorrel seed flour level increased in the blend formulations, with up to 20% sorrel addition (W80:GS20) resulting in an increase of 25.37% (Table 2). The importance of dietary fibre in both consumer's health and quality of products is vital for promoting its addition in composite blend formulations. Hence, products made from these flour blends with significantly improved dietary fibre content will on one hand, promote consumer's health based on the physiological and health benefits of dietary fibre. Secondly, it will ensure improved product quality, especially in gluten-free bakery products such as wheat-supplemented breads. Several studies have reported improvement in the overall qualities including biochemical composition, cooking properties, textural characteristics, and taste of products such as bread, biscuits, cookies, and pasta, due to addition of dietary fibre components [54,77,83,113].

There was progressively consistent decrease in the *in vitro* protein digestibility (IVPD) of wheat flour as germinated defatted sorrel seed supplementation increased. While 5% germinated sorrel seed flour (W95:GS5) inclusion had no significant ($P > 0.05$) effect, increasing levels of 10, 15, and 20% germinated defatted sorrel seed flour inclusion resulted in significant ($P < 0.05$) IVPD decrease of 5.24% [from 62.24% in the control (W100) to 58.98% in W90:GS10], 21.80% (from 62.24% in W100 to 48.67% in W85:GS15) and 30.46% (from 62.24% in W100 to 43.28% in W80:GS20). Thus, indicating decreasing protein digestibility as germinated defatted sorrel seed flour increased in the flour blends. This may be attributed to the low digestibility often associated with leguminous seeds contributed by their high contents of antinutritional factors (that form complexes with micro and macronutrients) and enzyme inhibitors, thereby reducing their digestibility [66]. reported that trypsin inhibitor activities in leguminous seeds are responsible for reduced bioavalability of bound amino acids and proteins. However, since simple food processing techniques involving heat

application significantly improve digestibility of protein because of heat on trypsin inhibitor activity, it is expected that the digestibility of these flour blends will improve significantly during food preparation processes such as cooking and baking [17,61].

The K, Ca, P, Na, Mg and Fe contents of the composite flour blends ranged from 405.05 to 690.15 mg/kg, 34.05–98.2 mg/kg, 346.07–420.31 mg/kg, 5.01–10.41 mg/kg, 138.31–289.62 mg/kg and 3.81–8.30 mg/kg, respectively. Mineral contents of the flour blends increased significantly ($P < 0.05$) with increasing substitution of germinated defatted sorrel seeds, with the highest values observed at 20% substitution. This increase may be due to the addition of sorrel seed flour, reported as a rich source of micronutrients, which is higher than in wheat flour. This may be advantageous and a low-cost approach for meeting micronutrient needs of consumers of the composite bread since micronutrient deficiency is one of the health challenges prevalent in developing countries [30,60,61].

3.4. Functional and rheological properties of wheat: germinated defatted sorrel seed flour blends

The functional and rheological properties of wheat flour and dough varied significantly ($P < 0.05$) as substitution with germinated defatted sorrel seed flour increased in the blends. WAC and OAC ranged from 116.00 to 216.00% and 182.12–199.33%, respectively with increasing levels of germinated defatted sorrel seeds increasing WAC but decreasing OAC (except in W80:GS20 which increased) (Table 3). The increase may be attributed to an increase in protein content due to germinated defatted sorrel seed inclusion in the blends. WAC influences the hydration properties of flours and high WAC is desirable in bakery products for improvement in yield, consistency and body and enhancement of functional and sensory properties [94,119]. Hence, the high WAC recorded in these flour blends will aid in improving the overall functional and sensory properties of the composite breads. On the other hand, the high OAC may be due to the positively charged amino acid composition which caused its protein to bind oil [13]. The foaming capacity increased with reduction in the amount of germinated defatted sorrel seeds incorporated in the flour blends. However, the high values (16.27–22.22%) recorded here may be a positive attribute for these flour blends intended for production of composite bread. This is because good foaming capacity (which depends on the interfacial film formed by proteins) is a desirable attribute for flours intended for use in the production of various baked products based on its ability to maintain the suspension of air bubbles [15]. Thus, it may aid in entrapping more air bubbles, thereby resulting in increased volume and enhanced crumb formation of the composite bread (see Table 4).

Emulsifying and swelling capacities significantly increased. Although, bulk density decreased, the decrease was not significant ($P > 0.05$) (Table 3). The increase in the swelling capacity of the flour blends corroborates the increase in the swelling index of the germinated defatted sorrel seed flour and may have been influenced by possible interaction between protein and starch-related structures of the blends.

The rheological behaviour to ascertain the baking potential of wheat: germinated defatted sorrel seed flour blends as presented in Table 3 revealed that dough extensibility significantly decreased ($p < 0.05$) from 4.78 cm to 1.19 cm with increase in germinated defatted sorrel seed flour inclusion, while up to 20% sorrel seed inclusion resulted in null extensibility [64]. reported similar reduction in the extensibility of wheat flour supplemented with sprouted and decorticated sorrel seed flour. Farinograph water absorption significantly ($p < 0.05$) increased as germinated defatted sorrel seed flour inclusion increased in the blends from 3.20 to 71.25%. This increase may be attributed to the increase in protein content of the flour on addition of the germinated sorrel seed flour. This may indicate that the germinated defatted sorrel seed protein has high water absorption capacity. This is in line with the report of [11] that cereals and legumes proteins may exhibit high water absorption capacity.

3.5. Proximate composition, energy value and dietary fibre of wheat: germinated defatted sorrel seed bread

The substitution of wheat flour with germinated defatted sorrel seeds in bread production resulted in 51.84–121.42% significant ($p < 0.05$) increase in protein content as compared to the control (W100 - 100% wheat bread). The protein content for the composite bread samples W85:GS15, W80:GS20 were within the recommended 25% protein daily intake [79,90]. Increased protein content of wheat:germinated defatted sorrel seed bread samples may promote growth and repair of worn-out tissues. It may further be used as a diet for prevention and/or management of protein energy malnutrition prevalent in developing countries including Nigeria [61,89].

Similarly, crude, and dietary fibre contents of wheat bread increased significantly ($p < 0.05$) as germinated defatted sorrel seed flour inclusion increased. Results obtained ranged from 3.11 to 4.25 g/100 g and 11.47–27.32% in W100 to W80:G20, respectively. Comparatively, the crude fibre content observed in this study is higher compared with 1.19 g/100 g reported in wheat bread fortified with Bambara groundnut (Oguntuase *et al*., 2022) and 1.49 g/100 g in wheat bread enriched with acha and amaranth [87]. This increase is of immense health benefits because dietary fibre plays an important role in human health upon consumption such as weight loss, lowering cholesterol and blood sugar level [96,97]. This composite wheat-germinated defatted sorrel seed bread may present an opportunity for delivery of these functional benefits to consumers of conventional wheat bread. In addition, the significantly high dietary fibre contents in the composite bread play important roles in promoting laxation and aid in preventing and/or managing the prevalence and severity of constipation and hemorrhoids which are conditions commonly associated with bread prepared from refined wheat flour [56,101,109].

Energy is the number of calories released when the body breaks down (digests and absorbs) food. The energy value obtained in W80:GS20 (399.13 kcal/100 g) was observed to be slightly higher than 396.95 kcal/100 g obtained in control W100. This is beneficial as it implies that W80:GS20 may provide more calories/energy. Hence, it may serve as a substitute to W100 bread sample and as a cheap source of energy for the tropical region in Africa [89].

Table 5
Amino acid (g/100g protein) profile and predicted nutritional qualities of wheat-germinated defatted sorrel seed breads.

Amino acids	W100	W90:GS10	W85:GS15	W80:GS20	GS100	WHO/FAO/UN (2007)
Essential amino acids						
Histidine	1.43 ± 0.02^{d}	2.71 ± 0.01^{c}	2.53 ± 0.03^{d}	3.26 ± 0.04^{b}	5.57 ± 0.01^{a}	1.60
Isoleucine	1.02 ± 0.02^{e}	3.23 ± 0.02^{c}	3.10 ± 0.11^{cd}	3.73 ± 0.02^{b}	6.71 ± 0.01^{a}	4.20
Leucine	3.15 ± 0.05^{e}	7.35 ± 0.04^{d}	7.92 ± 0.12^{c}	8.72 ± 0.31^{b}	11.24 ± 0.94^{a}	1.90
Lysine	1.56 ± 0.05^{e}	3.53 ± 0.02^{d}	3.72 ± 0.02^{c}	4.72 ± 0.12^{b}	7.73 ± 0.52^{a}	1.60
Methionine	1.22 ± 0.02^{e}	2.21 ± 0.01^{d}	2.71 ± 0.03^{c}	2.85 ± 0.05^{b}	3.43 ± 0.13^{a}	2.80
Phenylalanine	2.32 ± 0.32^{e}	5.05 ± 0.05^{ab}	4.73 ± 0.10^{d}	4.84 ± 0.04^{c}	5.18 ± 0.21^{a}	2.80
Threonine	2.16 ± 0.12^{e}	3.31 ± 0.11^{d}	3.68 ± 0.03^{c}	4.05 ± 0.11^{b}	4.89 ± 0.15^{a}	0.90
Tryptophan	0.10 ± 0.01^{d}	1.21 ± 0.01^{b}	1.11 ± 0.01^{c}	1.11 ± 0.01^{c}	1.52 ± 0.02^{a}	0.50
Valine	1.13 ± 0.30^{e}	6.14 ± 0.04^{c}	5.62 ± 0.00^{d}	6.72 ± 0.13^{b}	10.51 ± 0.83^{a}	1.30
∑EAA	14.09^{e}	34.74^{d}	35.12^{c}	40.00^{b}	56.78^{a}	
Non essential amino acids						
Alanine	4.22 ± 0.00^{e}	5.11 ± 0.11^{a}	4.62 ± 0.02^{c}	4.31 ± 0.01^{d}	4.72 ± 0.02^{b}	–
Arginine	5.85 ± 0.05^{c}	6.15b±0.0^{5}	5.60e±0.02^{e}	5.66d±0.0^{4}	10.61 ± 0.03^{a}	–
Aspartic acid	6.85 ± 0.05^{e}	6.95 ± 0.05^{d}	7.15 ± 0.05^{c}	7.40 ± 0.10^{b}	10.80 ± 0.10^{a}	–
Cysteine	2.61 ± 0.01^{c}	2.82 ± 0.02^{b}	2.63 ± 0.02^{c}	2.61 ± 0.01^{c}	5.15 ± 0.02^{a}	–
Glycine	2.89 ± 0.01^{d}	3.42 ± 0.04^{b}	3.15 ± 0.05^{c}	3.51 ± 0.01^{a}	3.42 ± 0.02^{b}	–
Serine	5.90 ± 0.10^{d}	7.15 ± 0.15^{a}	6.85 ± 0.06^{b}	6.25 ± 0.05^{c}	4.43 ± 0.15^{e}	–
Tyrosine	2.85 ± 0.05^{d}	4.07 ± 0.08^{a}	3.13 ± 0.03^{c}	2.64 ± 0.03^{e}	3.39 ± 0.01^{b}	–
∑NEAA	31.17 ± 0.08^{e}	35.67 ± 0.03^{b}	33.13 ± 0.02^{c}	32.38 ± 0.03^{d}	42.52 ± 0.02^{a}	–
∑AA	45.26 ± 0.03^{e}	70.41 ± 0.06c	68.25 ± 0.05^{d}	72.38 ± 0.03^{b}	99.30 ± 0.08^{a}	–
Predicted nutritional qualities						
PER	1.11 ± 0.01^{e}	2.43 ± 0.02^{d}	2.42 ± 0.01^{c}	2.73 ± 0.01^{b}	4.11 ± 0.01^{a}	2.50
EAAI (%)	25.40 ± 0.02^{d}	65.29 ± 0.04^{c}	65.86 ± 0.04^{c}	81.19 ± 0.08^{b}	98.18 ± 0.03^{a}	65
Predicted BV (%)	35.99 ± 0.085^{d}	59.46 ± 0.03^{c}	60.09 ± 0.03^{c}	76.79 ± 0.05^{b}	97.49 ± 0.08^{a}	70
Nutritional index (%)	1.86 ± 0.01^{e}	7.27 ± 0.02^{d}	9.81 ± 0.02^{c}	13.18 ± 0.08^{b}	36.08 ± 0.07^{a}	–
ΣSAA(Meth + Cys)	3.83 ± 0.01^{e}	5.03 ± 0.03^{d}	5.34 ± 0.02^{c}	5.46 ± 0.02^{b}	8.58 ± 0.06^{a}	–
ΣArAA(Phe + Tyr)	5.17 ± 0.02^{e}	9.12 ± 0.02^{b}	7.86 ± 0.03^{c}	7.48 ± 0.03^{d}	8.57 ± 0.02^{a}	–
Arginine/Lysine	3.75 ± 0.02^{a}	1.74 ± 0.01^{b}	1.51 ± 0.01^{c}	1.19 ± 0.01^{e}	1.37 ± 0.01^{d}	–
Essential Amino Acid Scores						
1st LAA	Tryptophan	Lysine	Lysine	Lysine	Lysine	–
AAA	Arginine	Arginine	Arginine	Arginine	Arginine	–

Values are Means ± Standard deviation of three determinations. Values in the same row with the same superscript alphabet are not significantly different ($p < 0.05$).
Key: W100 - 100% wheat bread; W90:GS10 - 90% wheat:10% germinated defatted sorrel seed bread; W85:GS15 - 85% wheat:15% germinated defatted sorrel seed bread; W80:GS20 - 80% wheat:20% germinated defatted sorrel seed bread; GS100 - 100% germinated defatted sorrel seed bread.

3.6. Amino acid profile and predicted nutritional qualities of wheat: germinated defatted sorrel seed bread

The total essential amino acids value obtained in the present study (Table 5) increased across the composite breads from 34.74 g/100 protein in W90:GS10 to 40.00 g/100 g/100 g of protein in W80:GS20 as germinated defatted sorrel seed flour inclusion increased. These values were, however, significantly lower ($p < 0.05$) as compared to 56.78 g/100 g of protein obtained for sample GS100 but higher significantly ($p < 0.05$) than 14.09 g/100 g of protein obtained for the control wheat bread (W100). The values obtained in the present study are within the range of 30.16–40.53 g/100 g protein reported for wheat-bambara groundnut bread [85]. Essential amino acids are amino acids that the body cannot synthesize, thus required to be consumed via diet. The high contents of essential amino acids recorded in the composite wheat:germinated defatted sorrel seed bread are advantageous due to their health benefits such as promoting proper functioning of body cell, tissues repair, protein synthesis and nutrient absorption [60]. Similar trend was observed in total non-essential amino acids with values ranging from 35.67 g/100 g of protein in W90:GS10 to 32.38 g/100 g of protein in W80:GS20 but lower compared with 42.52 g/100 g of protein obtained in GS100 and higher compared with 31.17 g/100 g obtained in W100. Non-essential amino acids include aspartic acid, cysteine, arginine among others.

Biological value is a measurement index used in assessing the quantity of protein present in a diet, that is, bioavailable for the body to utilize [60]. Although, the predicted biological value obtained in W80:GS20 (76.79%) was lower compared with 97.49% obtained in GS100, it still falls within the [46] recommended >70% for good protein quality diet and higher than 35.99% obtained in W100. This is an indication that W80:GS20 may provide adequate amino acids (proteins) that may be biologically metabolized and utilized by the body to promote growth and development as well as repairs of worn-out tissues [46,89]. Food products with predicted biological value greater than 70% are usually recommended as this high biological value aids adequate metabolism of the protein chain reaction, thereby making it easy to be reabsorbed into the blood stream for health benefits [60]. The results (>70%) reported in the present study compare favourably with similarly high values reported in several legume-enriched food products [60,61,88,89].

Aromatic amino acids (including phenylalanine and tyrosine) are important amino acids which help in secretion of neurological bioactive compounds that promote maintenance of neurobiological activities of the body [111]. The aromatic amino acid content of the wheat:germinated defatted sorrel seed bread (7.48 g/100 g of protein in W80:GS20–9.12 g/100 g of protein in W90:GS10) were comparable with 8.57 g/100 g of protein in G100 but higher than 5.17 g/100 g of protein in W100. The increase in the aromatic amino

Table 6
Protein quality and haematological parameters of rats fed with wheat-germinated defatted sorrel seed breads.

Parameters	Experimental diets				
	W100	W90:GS10	W85:GS15	W80:GS20	GS100
Protein quality of experimental diets (bread)					
FE	0.16 ± 1.40^{d}	0.19 ± 1.30^{c}	0.22 ± 2.40^{b}	0.23 ± 1.70^{b}	3.40 ± 1.40^{a}
BV (%)	58.30 ± 0.03^{d}	82.10 ± 0.21^{c}	82.20 ± 0.01^{c}	89.40 ± 0.02^{b}	95.40 ± 0.05^{a}
PER	0.16 ± 0.03^{a}	0.14 ± 0.01^{a}	0.07 ± 0.08^{a}	0.14 ± 0.02^{a}	0.24 ± 0.10^{a}
NPU (%)	22.21 ± 0.39^{e}	40.10 ± 0.05^{d}	50.15 ± 0.23^{c}	57.97 ± 0.71^{b}	67.97 ± 0.47^{a}
TD (%)	72.13 ± 0.71^{d}	72.89 ± 0.02^{d}	81.62 ± 1.05^{c}	89.36 ± 0.76^{b}	96.36 ± 0.19^{a}
NR	0.42 ± 0.11^{d}	1.13 ± 0.03^{c}	1.13 ± 0.02^{c}	1.40 ± 0.15^{b}	2.40 ± 0.05^{a}
Feeding indices(g) of rats fed the experimental diets					
Food intake	82.75 ± 5.00^{c}	82.63 ± 5.40^{c}	92.94 ± 4.00^{b}	93.63 ± 4.60^{b}	97.89 ± 5.40^{a}
Weight gain	17.65 ± 2.40^{c}	17.83 ± 3.00^{c}	25.35 ± 3.50^{b}	30.55 ± 5.30^{a}	20.11 ± 5.40^{b}
Relative organ weight (g)					
Liver	3.87 ± 1.10^{b}	4.60 ± 0.40^{ab}	5.47 ± 0.42^{a}	5.93 ± 0.61^{a}	3.61 ± 0.20^{b}
Kidney	1.01 ± 0.01^{a}	0.93 ± 0.23^{a}	1.13 ± 0.31^{a}	1.20 ± 0.20^{a}	1.00 ± 0.01^{a}
Haematological properties of rats fed the experimental diets					
PCV (%)	40.00 ± 1.00^{a}	32.00 ± 3.00^{c}	30.67 ± 1.15^{c}	36.00 ± 2.00^{b}	26.00 ± 2.00^{d}
WBC (x10^{3}mm^{3})	3.63 ± 0.06^{d}	5.56 ± 0.02^{ab}	6.63 ± 0.08^{a}	4.07 ± 0.09^{c}	2.37 ± 0.02^{e}
RBC (x 10^{6}mm^{3})	4.53 ± 0.12^{a}	3.57 ± 0.25^{c}	3.30 ± 0.20^{c}	4.13 ± 0.15^{b}	3.00 ± 0.40^{c}
Neutrophils (%)	54.67 ± 2.89^{c}	62.33 ± 4.93^{ab}	67.33 ± 2.31^{a}	60.00 ± 1.00^{bc}	59.00 ± 2.00^{d}
Lymphocytes (%)	45.00 ± 2.65^{a}	37.33 ± 4.62^{bc}	32.67 ± 2.31^{c}	39.67 ± 1.53^{bc}	41.00 ± 2.23^{b}

Values are Means ± Standard deviation of three determinations. Values in the same row with the same superscript alphabet are not significantly different ($p < 0.05$).
Key: W100 - 100% wheat bread; W90:GS10 - 90% wheat:10% germinated defatted sorrel seed bread; W85:GS15 - 85% wheat:15% germinated defatted sorrel seed bread; W80:GS20 - 80% wheat:20% germinated defatted sorrel seed bread; GS100 - 100% germinated defatted sorrel seed bread; FE: Feed efficiency; BV: Biological value; PER: Protein efficiency ratio; NPU: Net protein utilization; TD: True digestibility; NR: Nitrogen retention; PCV: Pack cell volume; WBC: White blood cell; RBC: Red blood cell.

acid content which may be attributed to inclusion of the germinated defatted sorrel seed in the composite bread, could be beneficial for the consumers.

The arginine/lysine ratio obtained in the present study were all significantly greater ($p < 0.05$) than 1 (Table 5). This indicates that the samples contained more arginine than lysine and could be of health benefit. Arginine assists in the relaxation of blood arteries by secreting nitric oxides which aid easy flow of blood through the arteries to the heart. Thus, helping to reduce risk of high blood pressure and other cardiovascular diseases [61,89]. Furthermore, since arginine was reported as the most abundant amino acid in the bread samples, this implies that consumer of these breads may be at lower risk of hypertension.

3.7. Protein quality of the experimental diets (bread), and haematological parameters of rats fed with the experimental diets

Results obtained in the *in-vivo* study (Table 6) further establish the protein quality of the experimental bread developed from wheat: germinated defatted sorrel seed flour blends. The *in-vivo* biological value of the wheat: germinated defatted sorrel seed breads further established the results from the *in-vitro* study (Table 5). The *in-vivo* biological value ranged from 82.10% in W90:GS10 to 89.40% in W80:GS20 which is significantly higher ($p < 0.05$) as compared to 58.30% obtained in W100 but lower than 95.40% obtained in GS100. This is an indication that the amino acids (proteins) of the developed wheat: germinated defatted sorrel seed bread may be easily biologically available for absorption into the body system to promote adequate maintenance and repair of worn-out tissues.

The protein efficiency ratio (PER) of rats fed with the experimental diets of wheat: germinated defatted sorrel seed bread (W90: GS10 – W80:GS20) and the control samples (W100 and G100) ranged between (0.07–0.24) which did not differ significantly ($p > 0.05$). Meanwhile, the net protein utilization (NPU) and true digestibility (TD) (40.10–57.97% and 72.89–89.36%) obtained in rats fed with the composite wheat: germinated defatted sorrel seed bread samples were comparable with those of rats fed with GS100 (67.97 and 96.36%) but higher than those (22.21 and 72.13%) of rats fed with W100. The true digestibility obtained in the present study were all greater than 70%. This is an indication that the bread samples may be able to provide physiological needs since they are highly digestible [88].

The feeding indices of rats fed with the experimental diets increased with increasing proportion of germinated defatted sorrel seed flour. Values obtained ranged from 82.63 g in W90:GS10 to 93.63 g in W80:GS20 and 17.83 g in W90:GS10 to 30.55 g for food intake and weight gain, respectively. The feed intake obtained in wheat: germinated defatted sorrel seed bread were lower compared to 97.89 g obtained in GS100 but higher than 82.75 g obtained in W100. Meanwhile, the weight gained in wheat: germinated defatted sorrel seed bread is significantly higher (17.83 g–30.55 g) compared with 17.65 g and 20.11 g obtained in W100 and GS100, respectively. The feeding indices are an indication of the ability of the feed to nourish the body system in respect to the nutrient content of the feed [91]. This implies that the wheat: germinated defatted sorrel seed bread, especially W80:GS20 may provide adequate nutrients to promote proper growth and development.

The relative organ weight of the rats (5.93 g and 1.20 g) obtained in W80:GS20 is significantly higher ($p < 0.05$) as compared to 3.87 g and 1.01 g; 3.61 g and 1.00 g obtained in W100 and GS100 for liver and kidney weights of the experimental rats, respectively.

Table 7
Sensory attributes of wheat-germinated defatted sorrel seed breads.

Parameters	Bread samples				
	W100	W90:GS10	W85:GS15	W80:GS20	GS100
Crumb texture	7.40 ± 0.52^{a}	7.80 ± 0.42^{a}	6.00 ± 0.12^{b}	5.80 ± 0.02^{c}	4.00 ± 0.82^{d}
Aroma	6.20 ± 0.79^{b}	7.50 ± 0.53^{a}	5.90 ± 0.57^{b}	4.40 ± 0.53^{c}	4.90 ± 0.32^{d}
Taste	7.60 ± 0.56^{a}	7.90 ± 0.74^{a}	5.70 ± 0.67^{b}	4.90 ± 0.74^{c}	3.20 ± 1.23^{d}
Crumb and crust colour	7.80 ± 0.12^{b}	8.00 ± 0.17^{a}	6.70 ± 0.28^{b}	5.00 ± 0.31^{c}	5.70 ± 0.48^{d}
Overall acceptability	7.30 ± 0.48^{b}	7.90 ± 0.57^{a}	6.00 ± 0.42^{c}	5.90 ± 0.57^{c}	4.60 ± 0.52^{d}

Values are Means ± Standard deviation of three determinations. Values in the same row with the same superscript alphabet are not significantly different ($p < 0.05$).
Key: W100 - 100% wheat bread; W90:GS10 - 90% wheat:10% germinated defatted sorrel seed bread; W85:GS15 - 85% wheat:15% germinated defatted sorrel seed bread; W80:GS20 - 80% wheat:20% germinated defatted sorrel seed bread; GS100 - 100% germinated defatted sorrel seed bread.

This further establishes the ability of the composite wheat: germinated sorrel seed breads to enhance development of internal tissues and organs within the body. The high values obtained in W80:GS20 further establish that the sample contains adequate minerals and vitamins that can promote organ development [60,61].

The packed cell volume (PVC), White blood cell (WBC), and red blood cell (RBC) obtained in wheat: germinated defatted sorrel seed bread (32) recommended6.63×10^{3} mm^{3} and 3.30–4.13×10^{6} mm^{3}), as well as those obtained for W100 (40.00%; 3.63×10^{3} mm^{3} and 4.53×10^{6} mm^{3}) and GS100 (26%; 2.37×10^{3} mm^{3} and 3.00×10^{6} mm^{3}) are within the safe limits (40%; 6.6–12.6×10^{3} mm^{3} and 6.76–9.76×10^{6} mm^{3}) recommended by Refs. [37,49]. PVC is an index for measurement of the proportion of blood in internal cell, a depletion below recommended value is an indication of inflammatory diseases which results in an abnormal increase in red blood cell higher than the recommended value. Comparatively, the value obtained in the present study is an indication that the wheat: germinated defatted sorrel seed bread may be safe for consumption with no implication or risk of inflammatory diseases.

3.8. Sensory attributes of bread from wheat: germinated defatted sorrel seed flour blends

The sensory attributes presented in Table 7 indicate that the panelists ranked W90:GS10 comparably with the control (W100). Furthermore, W90:GS10 was ranked slightly higher than other composite bread samples in crumb texture, aroma, taste, crumb, and crust colour, and overall acceptability. However, there was no significant difference ($p > 0.05$) in the crumb texture, aroma, and taste of the control sample (W100) and W90:GS10. This implies that 10% inclusion of germinated defatted sorrel seed flour in wheat flour may be suitable for production of organoleptically acceptable wheat-protein enriched composite bread.

4. Conclusion

This study proposes the utilization of germinated sorrel seed flour as a low-cost alternative for reducing protein energy malnutrition associated with starchy staples, which predominantly forms bulk of the diet in low-income developing countries like Nigeria. The research findings in the present study have established the possibility of producing acceptable wheat-supplemented bread with higher nutritional quality and health-promoting benefits as compared to 100% wheat bread using wheat-germinated defatted sorrel seed composite flour blends. The composite flour blend W80:GS20 (wheat 80% and germinated defatted sorrel seed flours 20%) may be a potential substitute to the relatively expensive starchy foods fed to toddlers. Hence, the use of sorrel seed for food-to-food fortification in cereal-legume foods may provide low-cost alternative to expensive food fortification, enhance the seed's utilization, and reduce wastage and the negative health impacts on both the environment and citizens due to large quantities of seeds discarded annually. Ultimately, this will help in boosting both the farmers' income and national economy by reducing dependence on imported wheat.

Author contribution statement

Helen Nwakego AYO-OMOGIE, Timilehin David Oluwajuyitan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Emem Imeobong OKORIE, Odunayo Opeyemi, Naomi Damilare AWOSANMI: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics statement

Consent to participate was obtained using verbal communication from panelists while ethical approval was obtained from ethical committee, Federal University of Technology Akure. The study was carried out in accordance with the national food safety rules (FUTA/SAAT/2022/00100).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] F.M.A. Abass, R. Saifullah, M.E. Azhar, Assessment of physical properties of ripe banana flour prepared from two varieties: Cavendish and dream bananas, Int. Food Res. J. 16 (2009) 183–189.
[2] A.Y. Abu El Gasim, M.A. Mohammed, Fururndu, a meat substitute from fermented roselle (*Hibiscus sabdariffa* L.) seed: investigation on amino acids composition, protein fractions, minerals content and HCl-extractability and microbial growth, Pakistan J. Nutr. 7 (2) (2008) 352–358.
[3] J.A. Adebiyi, A.O. Obadina, A.F. Mulaba-Bafubiandi, O.A. Adebo, E. Kayitesi, Effect of fermentation and malting on the microstructure and selected physicochemical properties of pearl millet (*Pennisetum glaucum*) flour and biscuit, J. Cereal. Sci. 70 (2016) 132–139.
[4] O.A. Adebo, P.B. Njobeh, J.A. Adebiyi, S. Gbashi, J.Z. Phoku, E. Kayitesi, Fermented pulse-based food products in developing nations as functional foods and ingredients, in: M.C. Hueda (Ed.), Functional Food - Improved Health through Adequate Food, InTech, Croatia, 2017, pp. 77–109.
[5] O.R. Adegbanke, D.O. Ojo-Uyi, T.D. Oluwajuyitan, Application of Bambara groundnut in the production of cookies, Food Sci. Qual. Manag. 83 (2019) 56–60.
[6] F.U. Akharume, R.E. Aluko, A.A. Adedeji, Modification of plant proteins for improved functionality: a review, Comp Rev Food Sci Food Saf 20 (2021) 198–224.
[7] A.I. Akinyede, I.A. Amoo, A.F. Eleyinmi, Chemical and functional properties of full fat and defatted Dioclearefiexa seed flours, J. Food Agric. Environ. (2005) 112–115. http://www.world-food.net.
[8] K.E. Alawode, M.A. Idowu, A.A. Adeola, E.K. Oke, S.A. Omoniyi, Some quality attributes of complementary food produced from flour blends of orange-fleshed sweet potato, sorghum, and soybean, Croat. J. Food Sci. Technol. 9 (2) (2017) 122–129.
[9] American Association of Cereal Chemists (AACC), Approved methods of the American association of cereal chemists, Retrieved from, eleventh ed., American Association of Cereal Chemists, St. Paul: MN, 2000, http://www.aaccnet.org/approvedmethods/toc.aspx.
[10] I. Amin, E.K.I. Hainida, S.M.N. Halimatul, Roselle (*Hibiscus sabdariffa* L.) seeds-nutritional composition, protein quality and health benefits, Food 2 (2008) 1–16.
[11] A.A. Anton, K.A. Ross, O.M.R. Lukow, G. Fulcher, S.D. Arntfield, Influence of added bean flour (*Phaseolus vulgaris* L.) on some physical and nutritional properties of wheat Flour tortillas, Food Chem. 109 (2008) 33–41.
[12] AOAC, Official methods of analysis, in: Association of Official Analytical Chemist. AOAC International, Suite 500, 481 North Frederick Avenue, Gaithersburg, Maryland 20877-2417, USA, nineteenth ed., 2012.
[13] F. Appiah, J. Asibuo, P. Kumah, Physicochemical and functional properties of bean flours of three cowpea (*Vigna unguiculata* L. Walp) varieties in Ghana, Afr. J. Food Sci. 5 (2011) 100–104.
[14] D. Atudorei, G.G. Codina, Perspectives on the use of germinated legumes in the bread making process - a review, Appl. Sci. 10 (2020) 6244, https://doi.org/10.3390/app10186244.
[15] C.G. Awuchi, V.S. Igwe, C.K. Echeta, The functional properties of foods and flours, Int. J. Adv. Acad. Res. Sci. Technol. Engin. 5 (11) (2019).
[16] H.N. Ayo-Omogie, Gluten-reduced sweet potato-wheat bread: influence of fermented sweet potato flour addition on bread quality and dough rheology, J. Culin. Sci. Technol. (2021), https://doi.org/10.1080/15428052.2020.1738297.
[17] H.N. Ayo-Omogie, O.O. Johnson, O.O. Awolu, T.D. Oluwajuyitan, Physicochemical, functional, pasting properties and Fourier Transform Infrared Spectroscopy of pure and modified Cardaba banana (*Musa* ABB) starches, Food Chem. Adv. 1 (2022), 100076, https://doi.org/10.1016/j.focha.2022.100076, 1-10.
[18] H.N. Ayo-Omogie, R. Ogunsakin, Assessment of chemical, rheological and sensory properties of fermented maize-cardaba banana complementary food, Food Nutr. Sci. 4 (2013) 844–850, https://doi.org/10.4236/fns.2013.48110.
[19] H.N. Ayo-Omogie, A.A. Osanbikan, Comparative influence of dehulling on the composition, antioxidative and functional properties of sorrel (*Hibiscus sabdariffa* L.) seed, Food Nutr. Sci. 10 (2) (2019) 148–173, https://doi.org/10.4236/fns.2019.102012.
[20] A.H. Bakare, O.F. Osundahunsi, J.O. Olusanya, Rheological, baking and sensory properties of composite bread dough with breadfruit (*Artocarpus communis* Forst) and wheat flours, Food Sci. Nutr. 4 (4) (2016) 573–587, https://doi.org/10.1002/fsn3.2016.4.issue-4.
[21] V. Benitez, S. Cantera, Y. Aguilera, E. Molla, R.M. Esteban, M.F. Diaz, M.A. Martin-Cabrejas, Impact of germination on starch, dietary fiber and physicochemical properties in non-conventional legumes, Food Res. Int. 50 (1) (2013) 64–69.
[22] S.M.M. Bertagnolli, M.L.R. Silveira, A.D.O. Fogaca, L. Umann, N.G. Penna, Bioactive compounds and acceptance of cookies made with Guava peel flour, J. Food Sci. Technol. 34 (2014) 303–308.
[23] I.F. Bolarinwa, S.A. Olaniyan, L.O. Adebayo, A.A. Ademila, Malted sorghum-soy composite flour: preparation, chemical and physico-chemical properties, J. Food Process. Technol. 6 (2015) 8, https://doi.org/10.4172/2157-7110.1000467.
[24] F. Boukid, E. Zannini, E. Carini, E. Vittadini, Pulses for bread fortification: a necessity or a choice? Trends Food Sci. Tech. 88 (2019) 416–428.
[25] R. Campos-Vega, G. Loarca-Pina, B.D. Oomah, Minor components of pulses and their potential impact on human health, Food Res. Int. 43 (2010) 461–482.
[26] C. Chaiyasut, B.S. Sivamaruthi, N. Pengkumsri, M. Saelee, P. Kesika, S. Sirilun, S. Peerajan, Optimization of conditions to achieve high content of gamma amino butyric acid in germinated black rice and changes in bioactivities, Food Sci. Technol. 37 (2017) 83–93.
[27] A. Chauhan, D.C. Saxena, S. Martin-Cabrejas, Total dietary fiber and antioxidant activity of gluten free cookies made from raw and germinated amaranth (*Amaranthus* spp.) flour, LWT"Food Sci. Technol. 63 (2) (2015) 939–945, https://doi.org/10.1016/j.lwt.2015.03.115.
[28] P. Chawla, L. Bhandari, P.K. Sadh, R. Kaushik, Impact of solid-state fermentation (*Aspergillus oryzae*) on functional properties and mineral bioavailability of Black-eyed pea (*Vigna unguiculata*) seed flour, Cereal Chem. 94 (3) (2017) 437–442, https://doi.org/10.1094/CCHEM-05-16-0128-R.
[29] C.E. Chinma, J.C. Anuonye, O.C. Simon, R.O. Ohiare, N. Danbaba, Effect of germination on the physicochemical and antioxidant characteristics of rice flour from three rice varieties from Nigeria, Food Chem. 185 (2015) 454–458.
[30] C.E. Chinma, M. Ilowefah, B. Shammugasamy, Y. Ramakrishnan, K. Muhammad, Chemical, antioxidant, functional and thermal properties of rice bran proteins after yeast and natural fermentations, Int. J. Food Sci. Technol. 49 (10) (2014) 2204–2213, https://doi.org/10.1111/ijfs.12533.
[31] C.E. Chinma, J.O. Abu, B.N. Asikwe, T. Sunday, O.A. Adebo, Effect of germination on the physicochemical, nutritional, functional, thermal properties and *in vitro* digestibility of Bambara groundnut flours, LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 140 (2021), 110749.
[32] C.E. Chinma, O.E. Adedeji, I.I. Etim, G.I. Aniaka, E.O. Mathew, U.B. Ekeh, N.L. Anumba, Physicochemical, nutritional, and sensory properties of chips produced from germinated African yam bean *(Sphenostylisstenocarpa)*, LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 136 (2021) (2021), 110330.
[33] C.E. Chinma, S.O. Azeez, H.T. Sulayman, K. Alhassan, S.N. Alozie, H.D. Gbadamosi, N. Danbaba, H.A. Oboh, J.C. Anuonye, O.A. Adebo, Evaluation of fermented African yam bean flour composition and influence of substitution levels on properties of wheat bread, J. Food Sci. 85 (12) (2020) 4281–4289.

[34] C.E. Chinma, V.C. Ezeocha, O.E. Adedeji, H.N. Ayo-Omogie, B.C. Oganah-Ikujenyo, N.L. Anumba, G.E. Enimola, D.O. Adegoke, R. Alhassan, O.A. Adebo, Germinated Bambara groundnut (Vigna subterranean) flour as an ingredient in wheat bread: physicochemical, nutritional and sensory properties of bread, J. Food Sci. (2023), https://doi.org/10.1111/1750-3841.16585.

[35] F. Cornejo, G. Novillo, E. Villacres, C.M. Rosell, Evaluation of the physicochemical and nutritional changes in two amaranth species (Amaranthus quitensis and Amaranthus caudatus) after germination, Food Res. Int. 121 (2019) (2019) 933–939.

[36] A.M.G. Darwish, H.O.A. Al- Jumayi, H.A. Elhendy, Effect of germination on the nutritional profile of quinoa (*Cheopodium quinoa* Willd.) seeds and its anti-anemic potential in Sprague-Dawley male albino rats, Cereal Chem. (2020) 1–13.

[37] N.C. Diana, Appendix: therapeutic drug monitoring and laboratory reference ranges, in: J.M. Stephen, A.P. Maxine (Eds.), Current Medical Diagnosis and Treatment, 46th ed, McGraw Hill, 2007, pp. 1767–1775.

[38] V. Dogra, P.S. Ahuja, Y. Sreenivasulu, Change in protein content during seed germination of a high altitude plant *Podophyllum hexandrum* Royle, J. Proteonomics 78 (2013) 26–38.

[39] A. Drakos, G. Doxastakis, V. Kiosseoglou, Functional effects of lupin proteins in comminuted meat and emulsion gels, Food Chem. 100 (2) (2007) 650–655, https://doi.org/10.1016/j. foodchem.2005.09.088.

[40] H. Duwa, E.O. Oyawoye, A.A. Njidda, Effect of processing methods on the utilization of sorrel seed meal by broilers, Pakistan J. Nutr. 11 (2012) 38–46, https://doi.org/10.3923/pjn.2012.38.46.

[41] T.A. El-Adawy, E.H. Rahma, A.A. El-Bedawey, A.E. El-Beltagy, Nutritional potential and functional properties of germinated mung bean, pea and lentil seeds, Plant Foods Hum. Nutr. 58 (2003) 1–13.

[42] C.O. Eleazu, P.N. Okafor, A. Ikpeama, Total antioxidant capacity, nutritional composition and inhibitory activity of unripe plantain (*Musa paradisiaca*) on oxidative stress in alloxan-induced diabetic rabbits, Pak J Nutr 9 (2010) 1052–1057.

[43] C. Eleazu, K. Eleazu, C. Aniedu, J. Amajor, A. Ikpeama, I. Ebenezer, Effect of partial replacement of wheat flour with high quality cassava flour on the chemical composition, antioxidant activity, sensory quality, and microbial quality of bread, Preventive Nutrit. Food Sci. 19 (2) (2014) 115–123.

[44] N. Ertas, N.E. Bilgicli, Effect of different debittering processes on mineral and phytic acid content of lupin (*Lupinus albus* L.) seeds, J. Food Sci. Tech. 51 (2014) 3348–3354.

[45] FAO, Hibiscus – Post-harvest Opertaions. INPhO – Post-harvest Compendium, Food and Agriculture Organization of the United Nations, 20pp, 2020.

[46] FAO/WHO/UNU, Protein and Amino Acid Requirements in Human Nutrition, WHO Press, 2007.

[47] E.L. Ferguson, R.S. Gibson, L.U. Thompson, M. Berry, Phytate, zinc, and calcium contents of 30 East African foods and their calculated phytate:Zn, Ca:phytate, and [Ca][phytate]/[Zn] molar ratios, J. Food Compos. Anal. 1 (4) (1988) 316–325, https://doi.org/10.1016/0889-1575(88)90031-2.

[48] H.F. Gemede, G.D. Haki, F. beyene, A.Z. Woldegiorgis, S.K. Rakshit, Proximate, mineral, and antinutrient compositions of indigenous Okra (*Abelmoschus esculentus*) pod accessions: implications for mineral bioavailability, Food Sci. Nutr. 4 (2) (2015) 223–233.

[49] E. Giannini, F. Botta, A. Fasoli, P. Ceppa, D. Risso, P.B. Lantieri, G. Celle, R. Testa, Progressive liver functional impairment is associated with an increase in AST/ALT ratio, Dig. Dis. Sci. 44 (6) (1999) 1249–1253.

[50] A.A. Hailu, G. Addis, The content and bioavailability of mineral nutrients of selected wild and traditional edible plants as affected by household preparation methods practiced by local community in Benishangul Gumuz Regional State, Ethiopia, Int. J. Food Sci. 2016 (2016) 1–7, https://doi.org/10.1155/2016/7615853. Article ID 7615853.

[51] E.K.I. Hainida, I. Amin, H. Normah, N. Mohd-Esa, Z.A.B. Ainul, Effects of defatted dried roselle (*Hibiscus sabdariffa* L.) seed powder on lipid profiles of hypercholesterolemia rats, J. Sci. Food Agric. 88 (2008) 1043–1050.

[52] E.K.I. Hainida, I. Amin, H. Normah, N. Mohd-Esa, Nutritional and amino acid contents of differently treated Roselle (*Hibiscus sabdariffa* L.) seeds, Food Chem. 111 (2008) (2008) 906–911.

[53] S.M.N. Halimatul, I. Amin, N. Mohd-Esa, A.G. Nawalyah, M. SitiMuskinah, Protein quality of roselle (*Hibiscus sabdariffa* L.) seeds, ASEAN Food J. 14 (2007) 131–140.

[54] L. Han, J. Zhang, H. Cao, Effects of orange peel powder on rheological properties of wheat dough and bread aging, Food Sci. Nutr. 9 (2021) 1061–1069.

[55] M. Hariharan, S. Naga, T. VanNoord, Systematic approach to the development of plasma amino acid analysis by high-performance liquid chromatography with ultraviolet detection with precolumn derivatization using phenyl isothiocyanate, J. Chromatogr. B Biomed. Sci. Appl. 621 (1) (1993) 15–22.

[56] Y.H. Ho, M. Tan, F. Seow-Choen, Micronized purified flavonidic frac- tion compared favorably with rubber band ligation and fiber alone in the management of bleeding hemorrhoids: Randomized controlled trial, Dis. Colon Rectum 43 (2000) 66–69.

[57] A. Hoehnel, J. Bez, I.L. Petersen, R. Amarowicz, J. Juskiewicz, E.K. Arendt, E. Zannini, Enhancing the nutritional profile of regular wheat bread while maintaining technological quality and adequate sensory attributes, Food Funct. 11 (2020) 4732–4751, https://doi.org/10.1039/DOFOO671H.

[58] F. Hubner, T. O'Neil, K.D. Cashman, E.K. Arendt, The influence of germination conditions on beta-glucan, dietary fibre and phytate during the germination of oats and barley, Eur. Food Res. Technol. 231 (1) (2010) 27–35.

[59] A.T. Idowu, O.O. Olatunde, A.E. Adekoya, S. Idowu, Germination: an alternative source to promote phytonutrients in edible seeds, Food Quality and Safety 4 (2020) 129–133.

[60] O.S. Ijarotimi, M.R. Fatiregun, T.D. Oluwajuyitan, Nutritional, antioxidant and organoleptic properties of therapeutic-complementary-food formulated from locally available food materials for severe acute malnutrition management, Bull. Natl. Res. Cent. 46 (1) (2022) 1–15.

[61] O.S. Ijarotimi, T.G. Ogunmola, T.D. Oluwajuyitan, Effect of some traditional processing operations on the chemical, functional, antioxidant, glycaemic index and glycaemic load of groundnut (*Arachis hypogea* L.) seed flour, J. Food Meas. Char. 16 (3) (2022) 2024–2040.

[62] R. Jan, D.C. Saxena, S. Singh, Comparative study of raw and germinated Chenopodium (*Chenopodium album*) flour on the basis of thermal, rheological, minerals, fatty acid profile and phytocomponents, Food Chem. 269 (2018) 173–180.

[63] M.D. Jimenez, M. Lobo, N. Samman, Influence of germination of quinoa (*Chenopodium quinoa*) and amaranth (*Amaranthus*) grains on nutritional and techno functional properties of their flours, J. Food Compos. Anal. 84 (2019), 103290.

[64] R.B. Karma, U.D. Chavan, Antioxidant activity and nutritional value of roselle seeds flour, Int. J. Curr. Microbiol. Appl. Sci. 6 (4) (2017) 2654–2663, https://doi.org/10.20546/ijcmas.2017.604.309.

[65] I.D. Kwari, S.S. Diarra, A.O. Raji, S.B. Adamu, Egg production and egg quality laying hens fed raw or processed sorrel (*Hibiscus sabdariffa*) seed meal, Agric. Biol. J. N. Am. 2 (2011) 616–621, https://doi.org/10.5251/abjna.2011.2.4.616.621.

[66] A. Lopez-Martinez, V. Azuara-Pugliese, A. Sanchez-Macias, G. Sosa-Mendoza, E. Dibildox- Alvarado, A. Grajales-Lagunes, High protein and low-fat chips (snack) made out of a legume mixture, CyTA - J. Food 17 (1) (2019) 661–668.

[67] M. Latta, M. Eskin, A simple and rapid colorimetric method for phytate determination, J. Agric. Food Chem. 28 (6) (1980) 1313–1315.

[68] C.K. Lee, R. Karunanithy, Effects of germination on the chemical composition of glycine and phaseolus beans, J. Sci. Food Agric. 51 (1990) 437–445.

[69] K. Liu, P. Markakis, Trypsin inhibition assay as related to limited hydrolysis of inhibitors, Anal. Biochem. 178 (1) (1989) 159–165, https://doi.org/10.1016/0003-2697(89)90373-4.

[70] E. Maetens, N. Hettiarachchy, K. Dewettinck, R. Horax, K. Moens, D.O. Moseley, Physicochemical and nutritional properties of a healthy snack chip developed from germinated soybeans, LWT"Food Sci. Technol. 84 (2017) 505–510.

[71] T.G. Maffo, E.E. Agbor, N.H.S. Mekoudjou, S.C.N. Kengne, I. Gouado, Proximate and mineral composition, protein quality of *hibiscus sabdariffa* l. (roselle) seeds cultivated in two agro ecological areas in Cameroon, Int. J. Nutr. Food Sci. 3 (4) (2014) 251–258.

[72] M.A. Martin-Cabrejas, N. Ariza, R. Esteban, E. Molla, K. Waldron, F.J. Lopez-Andreu, Effect of germination on the carbohydrate composition of the dietary fiber of peas (*Pisum sativum* L.), J. Agric. Food Chem. 51 (5) (2003) 1254–1259.

[73] S. Mbithi-Mwikya, C.J. Van, Y. Yiru, A. Huyghebaert, Nutrient and antinutrient changes in finger millet (*Eleusine coracan*) during sprouting, LWT Food Sci Technol 33 (2000) 9–14.

[74] R.M.R. Megat, A. Azrina, M.E. Norhaizan, Effect of germination on total dietary fibre and total sugar in selected legumes, Int. Food Res. J. 23 (1) (2016) 257–261.

[75] K.A. Millar, E. Gallagher, R. Burke, S. McCarthy, C. Barry-Ryan, Proximate composition and anti-nutritional factors of fava-bean (*Vicia faba*), green-pea and yellow-pea (*Pisum sativum*) flour, J. Food Compos. Anal. 82 (2019), 103233.

[76] O. Muller, M. Krawinkel, Malnutrition and health in developing countries, CMAJ (Can. Med. Assoc. J.) 173 (2005) 279–286, https://doi.org/10.1503/cmaj.050342.

[77] A.G. Nassar, A.A. AbdEl-Hamied, E.A. El-Naggar, Effect of citrus by-products flour incorporation on chemical, rheological and organoleptic characteristics of biscuits, World J. Agric. Sci. 4 (5) (2008) 612–616.

[78] D.C. Nieman, D.E. Butterworth, C.N. Nieman, Nutrition. WM.C, Brown Publishers, Dubuque, USA, 1992, pp. 237–312.

[79] NIS, Nigerian Industrial Standard (NIS 344: 2004), Standard for Edible Cassava Flour, 2004.

[80] S.G. Nkhata, E. Ayua, E.H. Kamau, J.B. Shingiro, Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes, Food Sci. Nutr. 6 (2018) 2446–2458.

[81] H. Nonogaki, G.W. Bassel, J.D. Bewley, Germination—still a mystery, Plant Sci. 179 (2010) 574–581.

[82] K.L. Nyam, C.P. Tan, O.M. Lai, K. Long, Y.B. Che Man, Some physicochemical properties and bioactive compounds of seed oils, LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 42 (2009) 1396–1403.

[83] Kar-Lin Nyam, Sod-Ying Leao, Chin-Ping Tan, Kamariah Long, Functional Properties of Roselle *(Hibiscus sabdariffa* L.) seed and its application as bakery product, J. Food Sci. Technol. 51 (12) (2014) 3830–3837, https://doi.org/10.1007/s13197-012-0902-x.

[84] O.B. Ocheme, O.E. Adedeji, G. Lawal, U.M. Zakari, Effect of germination on functional properties and degree of starch gelatinization of sorghum flour, J. Food Res. 4 (No. 2) (2015) 2015.

[85] S.O. Oguntuase, O.S. Ijarotimi, T.D. Oluwajuyitan, G. Oboh, Nutritional, antioxidant, carbohydrate hydrolyzing enzyme inhibitory activities and glyceamic index of wheat bread as influenced by Bambara groundnut substitution, SN Appl. Sci. 4 (4) (2022) 1–16.

[86] A.O. Ojokoh, W. Yimin, Effect of fermentation on chemical composition and nutritional quality of extruded and fermented soya products, Int. J. Food Eng. 7 (4) (2011). *Art. 6*.

[87] A.I. Olagunju, T.D. Oluwajuyitan, S.I. Oyeleye, Multigrain bread: dough rheology, quality characteristics, *in vitro* antioxidant and antidiabetic properties, J. Food Meas. Char. (2021) 1–14, https://doi.org/10.1007/s11694-020-00670-3.

[88] T.D. Oluwajuyitan, S.O. Ijarotimi, T.N. Fagbemi, G. Oboh, Blood glucose-lowering, glycaemic index, carbohydrate-hydrolysing enzyme inhibitory activities of potential functional food from plantain, soy-cake, rice-bran and oat-bran flour blends, J. Food Meas. Charact. 15 (4) (2021) 1–10.

[89] T.D. Oluwajuyitan, O.S. Ijarotimi, T.N. Fagbemi, Plantain-based dough meal: nutritional property, antioxidant activity and dyslipidemia ameliorating potential in high-fat-induced rats, Food Frontiers 3 (3) (2022) 489–504.

[90] T.D. Oluwajuyitan, O.S. Ijarotimi, T.N. Fagbemi, Nutritional, biochemical and organoleptic properties of high protein-fibre functional foods developed from plantain, defatted soybean, rice-bran and oat-bran flour, Nutr. Food Sci. (2020), https://doi.org/10.1108/NFS-06-2020-0225.

[91] T.D. Oluwajuyitan, O.S. Ijarotimi, Nutritional, antioxidant, glycemic index and antihyperglycaemic properties of improved traditional plantain-based (*musa* abb), dough meal enriched with tigernut (Cyperus esculentus) and defatted soybeans (*Glycine max*) cake for diabetic patients, Heliyon e1504–9 (2019), https://doi.org/10.1016/j.heliyon.2019.e01504.

[92] T.O. Omobuwajo, L.A. Sanni, Y.A. Balami, Physical properties of sorrel (*Hibiscus sabdariffa*) seeds, J. Food Eng. 45 (1) (2000) 37–41.

[93] O.A. Omowaye-Taiwo, T.N. Fagbemi, E.M. Ogunbusola, A.A. Badejo, Effect of germination and fermentation on the proximate composition and functional properties of full-fat and defatted *Cucumeropsis mannii* seed flours, J. Food Sci. Technol. 52 (2015) 5257–5263, https://doi.org/10.1007/s13197-014-1569-2.

[94] O.F. Osundahunsi, T.N. Fagbemi, E. Kesselman, E. Shimoni, Comparison of the physicochemical properties and pasting characteristics of flour and starch from red and white sweet potato cultivars, J. Agric. Food Chem. 51 (2003) 2232–2236.

[95] J. Pallavi, K. Varma, Effect of germination and dehulling on the nutritive value of soybean, Nutr. Food Sci. 46 (4) (2016) 595–603.

[96] K. Pame, B. Daimary, S. Borah, Utilization of dietary fibre in meat products as functional foods, Int. J. Agric. Environ. Biotechnol. 15 (1) (2022) 147–151.

[97] S. Pathania, N. Kaur, Utilization of fruits and vegetable by-products for isolation of dietary fibres and its potential application as functional ingredients, Bioactive Carbohydrates and Dietary Fibre 27 (2022), 100295.

[98] L. Patrascu, I. Aprodu, M. Garnai, I. Vasilean, Effect of germination and fermentation on the functionality of wheat-pulses flour mixtures, J. Biotechnol. 280 (2018) S52–S53.

[99] N. Phattanakulkaewmorie, T. Paseephol, A. Moongngarm, Chemical compositions and physico-chemical properties of malted sorghum flour and characteristics of gluten free bread, World Acad. Eng. Technol. 57 (2011) 9–27.

[100] H. Polat, T.D. Capar, C. Inanir, L. Ekici, H. Yalcin, Formulation of functional crackers enriched with germinated lentil extract: a response surface methodology box-behnken design, LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 123 (2020), 109065.

[101] N.V.P. Prasadi, I.J. Joye, Dietary fibre from whole grains and their benefits on metabolic health, Nutrients 12 (2020) 3045, https://doi.org/10.3390/nu12103045.

[102] S. Rai, A. Kaur, C.S. Chopra, Gluten-free products for celiac susceptible people, Front. Nutr. 5 (2018) 116.

[103] P.U. Rao, Nutrient composition and biological evaluation of Mesta (Hibiscus sabdariffa) seeds, Plant Foods Hum. Nutr. 49 (1996) 27–34, https://doi.org/10.1007/BF01092519.

[104] S. Rodriguez-Ambriz, A. Martinez-Ayala, F. Millan, G. Davila-Ortiz, Composition and functional properties of *Lupinus campestris* protein isolates, Plant Foods Hum. Nutr. 60 (2005) 99–107, https://doi.org/10.1007/s11130-005-6835-z.

[105] T.A. Sanni, E.M. Ogunbusola, C.N. Jaiyeoba, K.T. Araoye, Nutritional and sensory characteristics of bread produced from wheat and cassava flour, fortified with sorrel seed protein isolate, FUOYE J. Eng. Technol. (FUOYEJET) 5 (1) (2020) 6–10.

[106] R. Setia, Z. Dai, M.T. Nickerson, E. Sopiwnyk, L. Malcolmson, Y. Ai, Impacts of short-term germination on the chemical compositions, technological characteristics and nutritional quality of yellow pea and faba bean flours, Food Res. Int. 122 (2019) 263–272.

[107] C. Shrivastava, S. Chakraborty, Bread from wheat flour partially replaced by fermented chickpea flour:Optimizing the formulation and fuzzy analysis of sensory data, LWT 90 (2018) 215–223, https://doi.org/10.1016/j.lwt.2017.12.019.

[108] C.W. Simons, C. Hall, Consumer acceptability of gluten-free cookies containing raw, cooked and germinated pinto bean flours, Food Sci. Nutr. 6 (2017) 77–84.

[109] G.A. Soliman, Dietary fiber, atherosclerosis, and cardiovascular disease, Nutrients 11 (2019) 1155.

[110] K.W. Sze-Tao, S.K. Sathe, Functional properties and in vitro digestibility of almond (Prunus dulcis L.) protein isolate, Food Chem. 69 (2000) 153–160, https://doi.org/10.1016/S0308-8146(99)00244-7.

[111] M.A. Tayab, M.N. Islam, K.A.A. Chowdhury, F.M. Tasnim, Targeting neuroinflammation by polyphenols: a promising therapeutic approach against inflammation-associated depression, Biomed. Pharmacother. 147 (2022), 112668.

[112] T. Traore, C. Mouquet, C. Icard-Verniere, A. Traore, S. Treche, Changes in nutrient composition, phytate and cyanide contents and α-amylase activity during cereal malting in small production units in Ouagadougou (Burkina Faso), Food Chem. 88 (2004) 105–114.

[113] C.M. Tudoric, V. Kuri, C.S. Brennan, Nutritional and physicochemical characteristics of dietary fibre enriched pasta, J. Agric. Food Chem. 50 (2) (2002) 347–356.

[114] P. Udomkun, C. Tirawattanawanichb, J. Ilukorc, P. Sridonpaid, E. Njukwea, P. Nimbonae, B. Vanlauwef, Promoting the use of locally produced crops in making cereal-legume-based composite flours: an assessment of nutrient, antinutrient, mineral molar ratios, and aflatoxin content, Food Chem. 286 (2019) (2019) 651–658.

[115] A. Ukpabi, E.O. Ejidoh, Experimental Procedures for Food and Water Analysis, San Press Publishers, Enugu, Nigeria, 1989, p. 89.

[116] G. Urbano, M. Lopez-Jurado, S. Frejnagel, E. Gomez- Villalva, J.M. Porres, J. Frias, C. Vidal-Valverde, P. Aranda, Nutritional assesment of raw and germinated pea (*Pisum Sativum* L.) protein and carbohydrate by *in vitro* and *in vivo* techniques, Nutrition 21 (2) (2005) 230–239.

[117] B. Venkidasamy, D. Selvaraj, A.S. Nile, S. Ramalingamb, G. Kai, S.H. Nile, Indian pulses: a review on nutritional, functional and biochemical properties with future perspectives, Trends Food Sci. Technol. 88 (2019) 228–242.

[118] C.B.J. Villarino, V. Jayasena, R. Coorey, S. Chakrabarti-Bell, S.K. Johnson, Nutritional, health, and technological functionality of lupin flour addition to bread and other baked products: benefits and challenges, Crit. Rev. Food Sci. Nutr. 56 (5) (2016) 835–857, https://doi.org/10.1080/10408398.2013.814044.
[119] I.A. Wani, S.N. Andrabi, D.S. Sogi, I. Hassan, Comparative study of physicochemical and functional properties of flours from kidney bean (*Phaseolus vulgaris* L.) and green gram (*Vigna radiata* L.) cultivars grown in Indian temperate climate, Legume Science 2 (2020) 11.
[120] WHO (World Health Organization), Fact sheets: Noncommunicable diseases. http://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases, 2018.
[121] L. Xu, L. Chen, B. Ali, N. Yang, Y. Chen, F. Wu, X. Xu, Impact of germination on nutritional and physicochemical properties of adlay seed (Coixlachryma-*jobi* L.), Food Chem. 229 (2017) 312–318, https://doi.org/10.1016/j.foodchem.2017.02.096.
[122] M. Xu, Z. Jin, S. Simsek, C. Hall, J. Rao, B. Chen, Effect of germination on the chemical composition, thermal, pasting, and moisture sorption properties of flours from chickpea, lentil, and yellow pea, Food Chem. 295 (2019) 579–587.
[123] N. Zohreh-Mokhtari, Z. Soheila, G. Ali, Evaluation of chemical, nutritional and antioxidant characteristics of Roselle (*Hibiscus sabdariffa* L.) seed, Nutr. Food Sci. 5 (No 1) (2018) 41–46.