



ISSN (E): 2277-7695  
ISSN (P): 2349-8242  
NAAS Rating: 5.23  
TPI 2023; 12(3): 4763-4772  
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[www.thepharmajournal.com](http://www.thepharmajournal.com)

Received: 20-01-2023

Accepted: 23-02-2023

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## Effect of germination on the physicochemical and anti-nutritional properties of finger millet (*Eleusine coracana*), pearl millet (*Pennisetum glaucum*), and sorghum (*Sorghum bicolor*)

Shikha Sharma, Anurag and Amar P Garg

### Abstract

Millet is a small, seeded cereal crop, and it plays an important role in nutrient and food security. The purpose of this study was to see the effect of the germination process on the physicochemical, functional properties, phyto-chemical, and antioxidant properties (DPPH and total phenol) of millets flour. The results showed that the sprouting process showed a decrease in pH with a corresponding increase in TTA during the germination of millets flour. The proximate composition of the millets increases in protein (7.15%-10.0%) and fiber (1.39%-6.23%) with a fat reduction (6.25%-1.31%) after the germination of pearl millet, finger millet, and sorghum flour. Mineral analysis of germinated millet flour revealed that calcium, zinc, and phosphorus content increased significantly. The highest iron content obtained in finger millet flour was increased after germination while decreased in pearl millet and sorghum flour. Magnesium content decreased in GSF and increased in GPMF and GFMF. In terms of functional properties, the Germination process significantly increased the water/oil absorption capacity, emulsion activity & stability of all millet flours but it decreased the bulk density and swelling power of finger millet and sorghum. The result of the phyto-chemical composition revealed that during germination, saponin content was found to significantly increase while there was reduction in tannin and phytate content of the flour. It also increased the DPPH radical scavenging activity of all germinated millet flour. The total phenol range increased during germination from 125.6-150.1 mgGAE/100 g and 80.11-112.41 mgGAE/100 g of FMF and PMF respectively while decreasing in sorghum. Finding from these results showed that germination increased the nutritional profile, and functional and antioxidant properties and decreased the anti-nutritional content in millet flours which improved the acceptability, digestibility, bioavailability of nutrients and the production of gluten-free food products for people who suffer from celiac disease.

**Keywords:** Millets, germination, physicochemical, antioxidant activity, phyto-chemical

### Introduction

In the present scenario, people are more conscious about the health and nutritional value of food. Nutrient availability of food depends on a range of factors like the type of food, raw material, and processing of food. Low nutritional value and non-availability of nutrients from a food source is the major cause of malnutrition in underdeveloped countries. WHO/UNICEF (2012) reported inadequate complementary food as a major cause of child malnutrition in many developing countries. Various nations organize campaigns/programs to spread awareness for use of the maximum potential of its nutrition using some cooking or other processing techniques. Alternative ways for the enhancement of nutritional profiles are explored daily in food research and development. Sometimes fortification of essential nutrients or other techniques is used to enhance the nutritional proportion of food. Other approaches that have been made to increase and improve the nutritional quality of food grains include biotechnological aspects (hybrids or gene modification), fortification and processing methods [1]. Traditional methods like soaking, germination, and fermentation are also used nowadays to enhance the nutritional value of food. Germination of grains is of immense importance both from a nutritional as well as ease of operation point of view [2].

Soaking and Germination could serve as a traditional approach to improve and increase the bioavailability of nutrient components. Before the processes like germination, cooking, canning, and fermentation, grains are hydrated to the level where they reach the maximum weight due to the absorption of water. The absorption process might be affected by numerous factors like legume genus, species/variety, process duration, temperature, pH, the salinity of

the soaking media, and the storage conditions undergone before processing [3]. The metabolic activity of grain initiates as soon as it starts absorbing water, which is accompanied by the reduction of various anti-nutritional factors, loss of water-soluble vitamins [4], reduction of soluble carbohydrate and riboflavin [5]. Followed by soaking, the germination of grains takes place under the best condition. During germination reserve materials are degraded, commonly used for respiration and synthesis of new cells before developing an embryo [3].

Germination of most cereals and legumes has shown a positive effect on nutrients in the human diet compared to raw food grains [7]. It improves the nutritional profile of the grains and reduces some anti-nutritional factors. Apart from the enhancement of nutritional profile, bioavailability is also of important concern for nutrition to contribute to health. Lowering anti-nutritional factors during germination improves the bioavailability of the various minerals, vitamins, and dietary fibers which are of immense significance from both health as well as nutritional point of view [8].

Therefore, the consumption of sprouted legumes has many advantages over raw legumes, including reduction of anti-nutritional factors and enhanced *in-vitro* protein digestibility are dandiest. Overall nutritional quality of legumes improved significantly during sprouting. The main problem associated with developing countries is the modification of food at the household level to increase the bioavailability of nutrients [8]. Traditional infant foods made of un-processed raw cereals or tubers may be low in several nutrients which are of utmost importance due to their impact on physical and cognitive development.

Infants lack the full functioning of the digestive tract; thus, improvement of digestibility is one of the prominent reasons to incorporate the technique for household utilization of grains for infant foods. There is a decrease in the caloric content of the sprouted seed and thus comparatively the nutrient: energy ratio is higher than the original seed, therefore, sprouted legumes are now part of health enthusiasts. Sprout production is a simple process of germination under controlled conditions and can be achieved in any season and serves as a good alternative to vegetables [11].

## Materials and Methods

This research aimed to evaluate the changes in the chemical composition of grains after germination. Germinated grains were milled to powder form and changes in the protein, ash, carbohydrates, fiber, and other parameters were compared to the raw grains.

## Sample collection

Millet samples of pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*), and sorghum (*Sorghum bicolor*) were purchased from the local market of Meerut, U.P. The fresh grains were germinated and processed into flour using the standard method [6]. All reagents used for the study are of analytical grade.

## Preparation of raw and germinated flour

Collected cleaned grains were washed, drained, and dried in an oven dryer (Model No. DHG-9101 ISA) at 40 °C for 24 h (Figure 1). The dried pearl, finger, and sorghum millet grains were milled and sieved (screen diameter 100 µm) to produce raw pearl millet (RPMF), raw finger millet flour (RFMF), and

raw sorghum flour (RSF) which served as control. For the germination process, cleaned grains were washed and soaked in water at room temperature for 24 hours. Water was drained from millet grains after soaking. Millet grains were spread individually on clean jute bags covered with a damp cotton cloth and left for 48 hours at 35 °C to germinate. Millet grains were sprinkled with water at 4 h break to stimulate the germination process. The optimum germination temperature of pearl, finger, and sorghum millet grains have been reported at 28-35 °C [6] and the germination rate usually increases until the time reaches 42-72 hours [7]. After germination, germinated grains were washed with distilled water, drained, and dried in an oven dryer at 40 °C for 24 hours. Dried germinated millet grains were milled and sieved (100 µm mesh sieve) to obtain germinated pearl millet flour (GPMF), germinated finger millet flour (GFMF), and germinated sorghum flour (GSF). Those flours were packed in airtight polyethylene bags. The packed germinated flour was stored at room temperature for further analysis.

## Determination of pH and Total Titratable acidity (TTA) of millet flour

The pH of the flour was analyzed by homogenizing 10 g of each sample of germinated and non-germinated flour (control) was mixed with 90 ml distilled water and determined using a calibrated pH meter (PHS-25, Techmel, USA). The mixture was left at room temperature for 30 min. the pH meter was then used to measure the pH values of the supernatants [8].

Total titratable acidity was determined following the method of the Association of Official Analytical Chemists [9]. Ten milliliters of an aliquot of millet sample were mixed with two drops of phenolphthalein indicator in a test tube and thoroughly shaken. The mixture was titrated against 0.1 M NaOH until there was a change in color to the persistent pink endpoint and acidity was calculated [10].

## Proximate analysis of millet flour

The raw and germinated flour samples were examined for proximate composition such as moisture, protein (total N × 6.25), ash (incineration in a muffle furnace for 24 h at 550 °C), fat (Soxhlet extraction method), and crude fiber (sample digestion with dilute acid and alkali) by using the standard method of Association of Official Analytical Chemists (AOAC) [9].

## Determination of mineral content of millet flour

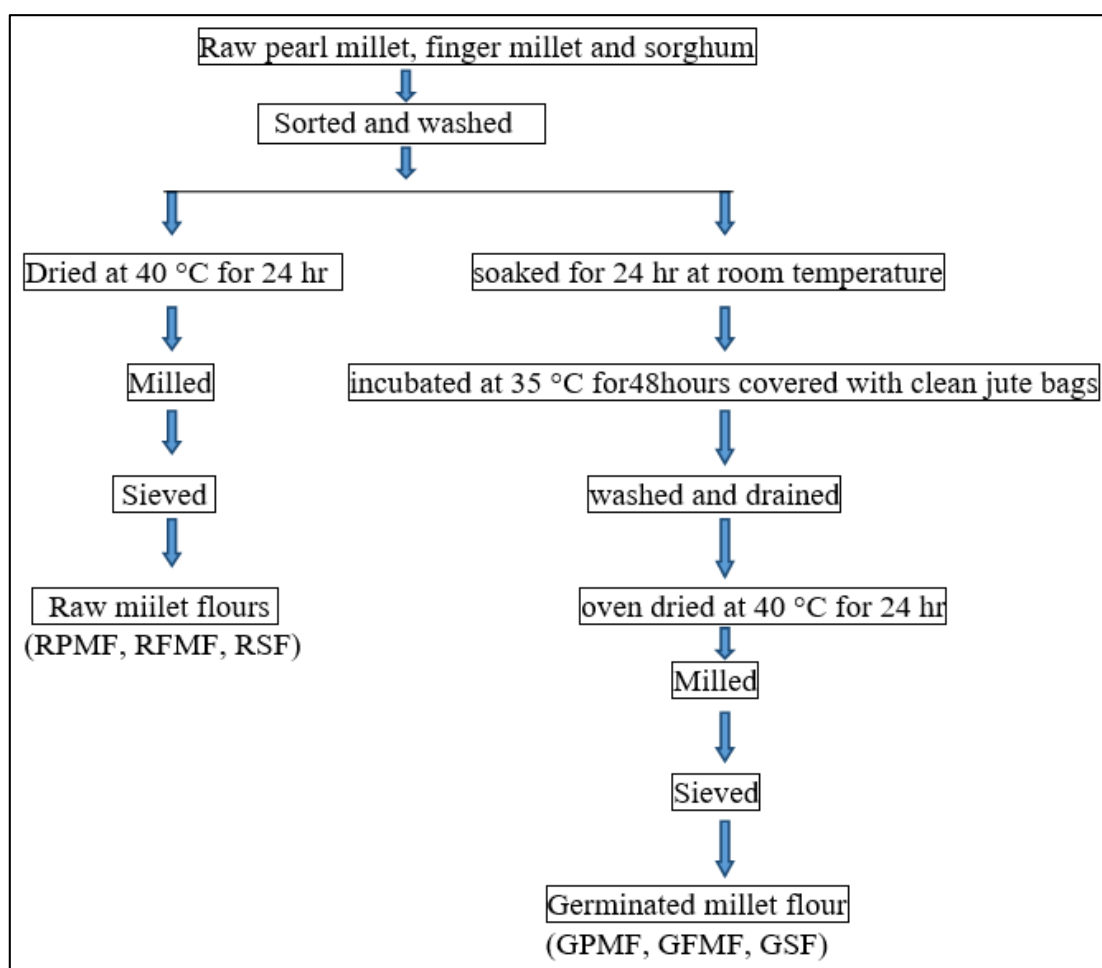
The mineral composition (calcium, magnesium, potassium, zinc, and iron) of the millet flour samples was determined using the Association of Official Analytical Chemists (AOAC) methods [9].

## Determination of functional properties

The raw and germinated flour samples were examined for functional properties such as bulk density (BD, g/ml), water absorption capacity (WAC, g/g), oil absorption capacity (OAC, g/g), swelling capacity (SC, g/g), foaming capacity (FC, %), foaming stability (FS, %), emulsion activity (EA, %) and emulsion stability (ES, %). The bulk density for flour samples was determined according to the method described by [11]. Water and oil absorption capacities were investigated by the method of Beuchat *et al.* [12], while the emulsion activity and emulsion stability were determined by the method developed by Yasumatsu *et al.* [13]. Foam capacity and foam

stability were measured using the method described by Narayana *et al.* [14]. The swelling capacity of the flour samples

was assessed by modifying the methods of Williams *et al.* [15].



**Fig 1:** Preparation of germinated and raw millet flours (pearl, finger and sorghum)

#### Determination of phytochemical properties of millet flour

The method was used to determine the tannin content of millet flour describe by Owheruo *et al.* [6]. A sample of millet was weighed, mixed with 10 ml of 70% aqueous acetone, properly covered, and placed in an ice bath shaker at room temperature for 2 hr. After this, the solution was centrifuged, and the supernatant was stored in ice. About 0.2 ml of the solution was introduced into the test tube that contained 0.8 ml of distilled water, while 0.5 ml Folin–Ciocalteu reagent and 2.5 ml of 20% Na<sub>2</sub>CO<sub>3</sub> were added. The mixture was vortex and allowed to incubate for 40 min at room temperature, while absorbance was read at 725 nm.

The saponin content of millets flour samples was determined following the spectrophotometric method of Brunner [16]. 2 g of the finely ground sample was weighed into a 250 ml beaker and 100 ml of isobutyl alcohol or (But-2-ol) was also added. Shaker was used to shake the mixture for 5 hrs to ensure uniform mixing. The mixture was then filtered with No 1 Whatman filter paper into a 100 ml beaker containing 20 ml of 40% saturated solution of magnesium carbonate (MgCo<sub>3</sub>). The mixture obtained was again filtered through No 1 Whatman filter paper to obtain a clean colorless solution. One ml of the colorless solution was taken into a 50 ml volumetric flask using the pipette, and 2 ml of 5% iron (iii) chloride (FeCl<sub>3</sub>) solution was added and made up to the mark with distilled water. It was allowed to stand for 30 min for the

color to develop. The absorbance was read against the blank at 380 nm.

Phytate was determined following Wheeler and Ferrel [17]. 4 gm of millets flour were soaked in 100 ml of 2% HCL for 3 hr and then filtered through a No. 1 Whatman filter paper. 25 ml of the filtrate was mixed with 5 ml of 0.3% ammonium thiocyanate solution as an indicator after which 53.5 ml of distilled water was added to give it proper acidity. This was titrated against standard iron (III) chloride solution that contained about 0.00195 g of iron per milliliter until a brownish-yellow color persist for 5 min.

#### Determination of antioxidant properties

The free radical scavenging ability of the extract against DPPH (1,1- diphenyl-2- picrylhydrazyl) using the Gyamfi *et al.* [18] method. One 1 ml of the extract was mixed with 1 ml of the 0.4 Mm methanolic solution of the DPPH the mixture was left in the dark for 30 min before measuring the absorbance at 516 nm. The activity was expressed as a percentage of DPPH scavenging relative to control using the following equation (1).

$$\text{DPPH Scavenging Activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad (1)$$

Total phenol content was carried out according to the method described by Singleton *et al.* [19]. About 0.2 ml of the extract was mixed with 2.5 ml of 10% folic-coculture agent and 2 ml of 7.5% sodium carbonate. The reaction mixture was subsequently incubated at 45 °C for 40 mins, and the absorbance was measured at 700 nm in the spectrophotometer (Spectrum 23A Spectrophotometer, Gulfex medical and scientific, England). Total phenol content was expressed as mg gallic acid equivalents (GAE)/g samples, gallic acid will be used as standard phenol.

### Statistical analysis

The experiments were conducted in triplicate and results were expressed as mean  $\pm$  standard deviation (SD) of three independent replications by using MS excels- 2007.

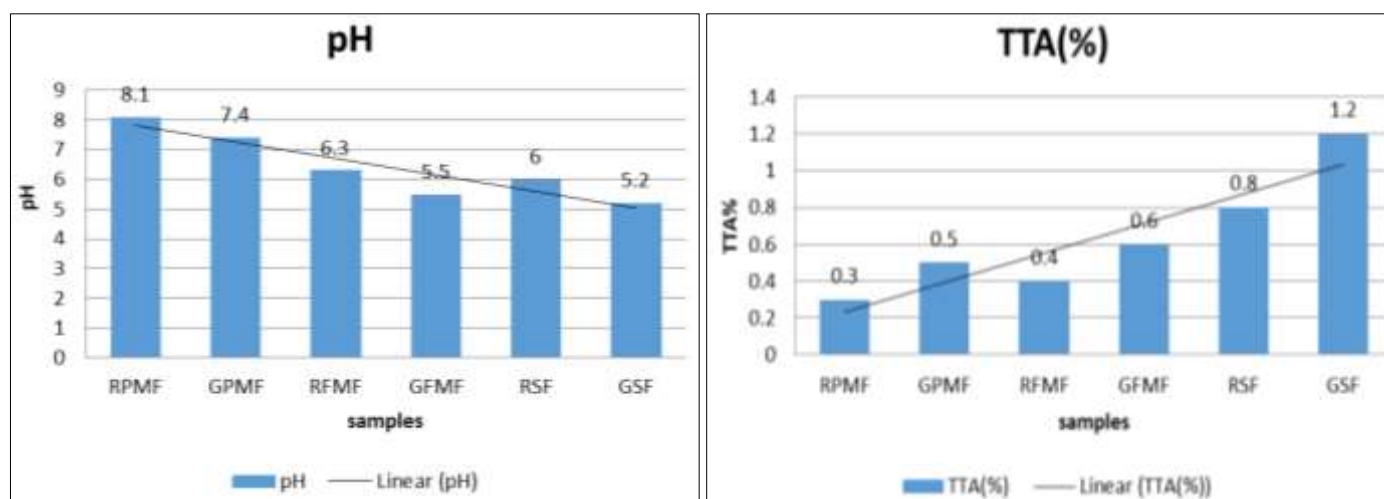
## Results and Discussion

### pH and TTA of raw and germinated millet flour

The effect of germination value on the pH of pearl millet, finger millet, and sorghum flour samples shown in figure 3. The maximum pH value (8.1) was obtained from the raw pearl millet (RPMF). Whereas the minimum pH value (5.2) was obtained in the germinated sorghum flour (GSF). Germination significantly ( $p < 0.05$ ) decreases the pH from raw millet (pearl, finger, and sorghum) flour to germinated millet (pearl, finger, and sorghum) flour 8.1- 7.4, 6.3- 5.5 and 6.0- 5.2 respectively. Similar results were reported by Vijaya Kumar [20] of a decrease in pH values of non-germinated and germinated millet flours. The decreases in pH values of germinated millet flour samples may be a result of the production of organic acids during germination. The results obtained in this present study is the similar to the finding by Shweta [21] who reported a reduction in pH and increased

acidity as sprouting progressed in finger and pearl millet and in this study finger millet pH value observed was (8.5-7.2) lower than the value of pearl millet (8.3-7.7). Studies have shown that germination induces the synthesis of hydrolytic enzymes, such as starch-degrading enzymes and proteases with the release of sugars and amino acids and also reported that pearl millet could raise the hydrogen ion content of the stomach to an alkaline state and thus reduce the occurrence of ulcer [22].

The effect of germination value on the total to table acidity of pearl, finger, and sorghum millet flour is shown in Figure 3. The highest total titrable acidity (1.2%) was recorded in the germinated sorghum flour (GSF), but the lowest content (0.3%) was recorded in ungerminated raw pearl millet flour (RPMF). Decreased pH and increased TTA might be due to the degradation of some complex organic molecules such as lipids, phytin, and protein to simpler compounds. So, the increase in acidity as germination took place could be related to the rate at which complex compounds were hydrolyzed to fatty acids, phosphates, and amino acids and therefore improving the digestibility of germinated millets flour [23]. Similar trends were observed in previous studies that reported an increase in TTA after germination [24]. Shweta [21] observed that pearl millet could raise the hydrogen ion content of the stomach to an alkaline state and thus reduce the occurrence of ulcers. The germination time has a positive correlation with the TTA of finger millet flour. Similar results were reported by Nefale and Mashau [25] on the content of total titrable acidity of finger millet, which increased as the germination period increased. The result obtained in the present studies by Owhero *et al.* [6] also indicated that the content of total titrable acidity increased as the germination period increased both in African finger millet and pearl millet.



**Note:** Values stand for a mean of triplicate  $\pm$  standard deviation. Means with no common letters within a row differed ( $p < 0.05$ ). RPMF- raw pearl millet flour, GPMF- germinated pearl millet flour, RFMF- raw finger millet flour, GFMF- germinated finger millet flour, RSF- raw sorghum flour, GSF- germinated sorghum flour.

**Fig 1:** pH and Total Titrable Acidity in millet flours during germination

### Proximate compositions of raw and germinated flour

The proximate composition of germinated and non-germinated pearl, finger, and sorghum flour is shown in Table 1. The moisture content of pearl, finger, and sorghum flour samples before germination was 8.50%, 8.47%, and 9.50% which increased by 8.62%, 8.65% and 9.72% after germination respectively mean moisture content increased significantly after germination same as in case of sorghum [26].

The moisture content of grains was  $\leq 10\%$  recommended as a safe limit, for extended preservation of flours. Dry grains absorb water rapidly, influenced by the structure of the grains, and the increase in water uptake with time is due to the increasing number of cells within the seed becoming hydrated as reported by Nonogaki [27].

The protein contents in millet flour as affected by soaking and germination are given in Table 1. The highest protein content



(10.71 g/100 g) was recorded in the germinated pearl millet flour (10.0 g/100 g) while the lowest protein content (7.15 g/100 g) was in raw sorghum flour (RSF). The protein content was significantly increased in all three-millet flour after germination reported by Nakata *et al.* [28] that the protein content of finger millet flour increases as the germination time increased due to the activity of the protease, an increase that degraded peptides into amino acids. The current research study result is consistent with the research finding of Ashwani Kumar *et al.* (2021) which showed that the protein content increased after germination. The increased concentration of protein in the bioprocess finger millet flour samples could be attributed to the synthesis of enzymes by the fermenting or germinating grains, synthesis of newly formed proteins, and degradation of other constituents such as anti-nutritional factors (ANFs) [30].

Ash content varied after germination. It is significantly increased from RPMF (1.84 g/100 g) and RFMF (2.35 g/100 g) to GPMF (1.94 g/100 g) and GFMF (2.84 g/100 g). Germination of sorghum flour led to a decrease in ash content of RSF (1.89 g/100 g) than GSF (1.53 g/100 g) as shown in Table 1. Ojokoh *et al.* [31] reported in pigeon peas that Ash content is a measure of the mineral content of grain; therefore, after germination due to a decrease in metal-binding water-soluble secondary metabolites, there might be an increment in the ash content. The decrease in ash content represents the loss of minerals due to rootlet and washing of the sorghum in water to reduce the sour smell during the period of germination. These studies are reported by Wardle, *et al.*, [26]. Raw pearl, finger, and sorghum millet flour contained 4.12, 2.05, and 6.25 g/100 g) of fat, which decreased by 26.9%, 36.09%, and 19.05% respectively after germination. The significant reduction in fat content could be due to the increased activity of lipase enzymes and severed as an energy source during germination. The fat content decrease in germinated flour might increase the shelf life by decreasing

rancidity, which is most likely due to enzymes released in the flour (Owhero *et al.* 2019). Similarly, the results of germination related to a decrease in fat and an increase in protein were reported by Dogra *et al.* [32].

Cure fiber was increased in germinated pearl, finger, and sorghum flour from 1.39% to 2.11%, 3.75% to 4.55%, and 4.21% to 6.23% respectively in Table 1. This is due to the synthesis of structural compounds like cellulose and hemicellulose and the breakdown of starch during germination [33]. In the present study, Owhero *et al.* [6] reported that an increase in germinated FMF may stem from the formation of the new primary cell whereas the observed reduction in crude fiber value after fermentation could be to the degradation of crude fiber by enzymes. Wardle, *et al.* [26] showed that crude fiber was decreased in soaked peanut and mung bean but increased in soaked rice and soybean. This study expresses that the germination process affects the level of crude fiber during the period of soaking before the actual phase of germination. Similar research has been reported by Chinma *et al.* [34] and Obadina *et al.* [33] in finger millet, pearl millet, and sorghum respectively.

The carbohydrate content of GPMF, GFMF, and GSF decrease significantly ( $p < 0.05$ ) in germination as compared to non-germinated flour from 74.99% to 74.32%; 76.01% to 74.14% and 71.00% to 68.48% respectively. This means the highest carbohydrate content was found in RFMF, whereas the lowest carbohydrate content was observed in GSF. The variation in carbohydrate content could be to the increasing and decreasing of food components such as moisture, fat, protein, ash, and fiber during germination [35]. The result of the current study especially during germination is similar to the suggestion of Aserse *et al.* [36] in finger millet and Obadina *et al.* [33] in pearl millet. The result of germination with the decrease in total carbohydrates agrees with those of the germination effect and this study on sorghum by Derbew and Moges [35].

**Table 1:** Proximate compositions (g/100 g) of raw and germinated millets flour

So. No.	Moisture	Protein	Ash	Fat	Fiber	Carbohydrate
RPMF	8.50±0.12	9.16±0.24	1.84±0.14	4.12±0.02	1.39±0.12	74.99±0.002
GPMF	8.03±0.11	10.00±0.18	1.94±0.18	3.01±0.14	2.11±0.25	74.32±0.21
RFMF	8.47±0.32	7.36±0.13	2.35±0.17	2.05±0.18	3.75±0.02	76.01±0.18
GFMF	8.65±0.25	8.51±0.25	2.84±0.12	1.31±0.17	4.55±0.02	74.14±0.13
RSF	9.50±0.11	7.15±0.11	1.89±0.11	6.25±0.15	4.21±0.24	71.00±0.12
GSF	9.72±0.25	8.95±0.4	1.53±0.07	5.06±0.06	6.23±0.14	68.49±0.25

**Note:** Values represent the mean of triplicate ± standard deviation. Means with no common letters within a row differed ( $p \leq 0.05$ ). RPMF- raw pearl millet flour, GPMF- germinated pearl millet flour, RFMF- raw finger millet flour, GFMF- germinated finger millet flour, RSF- raw sorghum flour, GSF- germinated sorghum flour.

### The mineral content of raw and germinated millet flour

Calcium content of RPMF and RFMF were observed to increase during germination from 35.76 to 71.21 mg/100 g and 225.15 to 280 mg/100 g respectively, while it decreased in sorghum flour from 31.09 to 25.12 mg/100 g (Table 2). Chauhan & Sarita [29] reported that the calcium content increased during germination with the value were 342.4 mg/100 g for raw finger millet flour and 359.6 mg/100 g for germinated finger millet flour. Due to decreases in oxalic acid during germination, the appropriate increases in calcium content in finger and pearl millet because oxalic acid is known to interfere with calcium absorption [36]. This result recommended that the germinated millet flour may be a good source of calcium. Abd El-Moneim, *et al.* [37] observed that

Calcium content decreased after germination from 33.09 to 12.50 mg/100 g. The magnesium content of RPMF and RFMF were detected to increase after germination from 95.12 to 97.43 mg/100 g and 1231.5 to 1589.2 mg/100 g while it decreased in the sorghum flour from 112.31 to 101.5 mg/100 g. Owhero, *et al.* [6] reported that increase in magnesium content on germinated pearl and finger millet flour whereas Luo, *et al.* [38] reported a decrease during the germination of sorghum.

The potassium content of pearl millet (375.45 to 523.02 mg/100 g), finger millet (550.12 to 565.09 mg/100 g), and sorghum flour (298.45 to 221.72 mg/100 g) was found to increase during germination. The mineral content increases in the processed finger millet flours are vital for the provision of

these nutrients for body functions. The significant increase ( $p \leq 0.05$ ) in mineral content of the bioprocesses finger millet samples could be ascribed to a reduction in ANFs during germination [39]. Aserse *et al.*, [36] found that raw sorghum contains 198.80 to 387.78 mg/100 g potassium and 5.17 to 11.26 mg/100 g calcium after germination. Potassium content is important in the diet due to the roles they perform in blood sugar regulation and nerve function in the body.

The zinc content of GPMF, GFMF and GSF were increased significantly ( $p < 0.05$ ) in germination as compared to non-germinated flour from 4.04 to 4.80 mg/100 g, 15.10 to 17.54 mg/100 g and 3.19 to 3.49 mg/100 g. This result is obtained from the previous report where there was an increase in zinc content after germination of pearl and finger millet and it is a multifunctional nutrient needed in glucose and lipid metabolism, hormone functionality, and wound healing [39, 40]. Of the three varieties of germinated millet flour, GFMF contained the highest zinc content (17.54 mg/100 g), whereas GSF had the least (3.49 mg/100 g) zinc. From further discussion, there is a significant increment in the mineral content of millet flour after germination Iswarya *et al.* [40] reported that, in millet flour, a greater concentration of minerals is in the covering layers and germ than in the endosperm portion of the kernel. So, millet was used without removing the outer covering.

The iron content of non-germinated millet flours was 5.98 mg/100 g for pearl, 12.39 mg/100 g for finger, and 4.13

mg/100 g for sorghum flour. However, at germination, the iron content decreased, and the value was 5.23 mg/100 g for pearl millet and 2.98 mg/100 g for sorghum flour. The highest iron content obtained in finger millet flour was increased after germination from 12.39 to 40.12 mg/100 g (Table 2). This could be due to an increase in the activity of phytase enzymes during germination. The enzyme will hydrolyze the link between the protein and the enzyme minerals, releasing the minerals and increasing their availability [36]. The finding of this result was like Shakirah *et al.* [39], who indicated that the iron content was 181.75 mg/100 g for non-germinated finger millet flour and 406.4 mg/100 g for germinated finger millet flour. Minerals are needed for the normal functioning of nerve and muscle cells, to maintain a healthy immune system, and help to make the bone strong. The decrease in the iron content during the germination of sorghum flour could be due to the presence of anti-nutritional factors. Abd El-Moneim *et al.*, [37] reported that sorghum iron content was decreased after germination. Ebisa *et al.* [41] reported that millets are rich in minerals, but the bioavailability of these minerals is usually low due to the presence of anti-nutritional factors. Therefore, more minerals could be released during soaking due to the release of bounded minerals with phytate because of the solubility of phytate in water [41]. The decrease in the mineral contents during malting could be due to the use of the minerals in the process of germination by the growing embryo [35].

**Table 2:** Mineral composition (mg/100 mg) of raw and germinated millets flour

So. No.	Calcium	Magnesium	Potassium	Zinc	Iron
RPMF	35.76±0.02	95.12±0.125	375.45±0.25	4.04±0.214	5.98±0.14
GPMF	71.21±0.36	97.43±0.11	523.02±0.21	4.80±0.21	5.23±0.78
RFMF	225.15±0.14	1231.5±5.36	550.12±0.214	15.10±0.21	12.39±0.21
GFMF	280.4±1.25	1589.2±0.125	565.09±0.5	17.54±0.14	40.12±0.14
RSF	31.09±0.14	112.31±0.21	298.45±0.02	3.19±0.14	4.13±0.17
GSF	25.12±0.05	101.5±0.14	321.72±0.12	3.49±0.58	2.98±0.21

**Note:** Values stand for the mean of triplicate ± standard deviation. Means with no common letters within a row differed ( $p \leq 0.05$ ). RPMF- raw pearl millet flour, GPMF- germinated pearl millet flour, RFMF- raw finger millet flour, GFMF- germinated finger millet flour, RSF- raw sorghum flour, GSF- germinated sorghum flour.

### Effect of germination on functional properties of raw and germinated millets flour

The bulk density for GPMF, GFMF, and GSF was found to be 0.79 g/cm<sup>3</sup>, 0.65 g/cm<sup>3</sup>, and 0.75 g/cm<sup>3</sup> respectively while the bulk density for non-germinated RPMF, RFMF, and RSF were reported 0.75 g/cm<sup>3</sup>, 0.97 g/cm<sup>3</sup> and 0.83 g/cm<sup>3</sup> respectively (Table 3). The study results indicated that the value of bulk density increased due to germination for pearl millet flour but decreased in the case of germinated finger and sorghum flour. This study was similar to previous studies [11, 39]. The bulk density of flour samples influences the energy, density, texture, and mouth feel. Bulk density can also be affected by different factors such as the preparation and storage of flours. Lower bulk density might be for softening the grains during soaking which produced flour particles after milling [11]. During germination, the breakdown of fat, starch, and protein might be another cause of lower bulk density. Elkhalfa *et al.* [43] reported a reduction in density after the germination and fermentation of sorghum.

The study result showed that the water absorption capacity of germinated millet flour samples was higher than raw millet flour samples (Table 3). The highest water absorption (WAC) was found in GFMF (4.12 g/g) and the lowest was found in

RSF (1.89 g/g). WAC represents the volume occupied by the starch after swelling in excess water, which maintains the integrity of starch in aqueous dispersion. The result was obtained by Shakirah *et al.* [39]. Siddiqua *et al.* [11] reported that higher water absorption capacity helps to improve the softness, bulkiness, and consistency of products. Elkhalfa *et al.* [43] also reported that germination increases the WAC of sorghum flour after three days of germination.

The trend of oil absorption capacity (OAC) differed from those of WAC (Table 3). The OAC was significantly ( $p \leq 0.05$ ) increased in all germinated millet flour samples. An increase in the OAC of sorghum flour after two days of germination by 19%. and Higher OAC improves the taste, flavor, and lipophilicity of food products as well as it might be due to solubilization and dissociation of protein leading to exposure of non-polar constituents from within the protein molecules during germination [43]. Higher OAC of germinated millet flour suggests that the flour could be used to produce gluten-free bakery products where a high amount of oil is required. WAC and OAC are useful indicators of the ability of the protein in the material to prevent fluid loss during food storage [40].

The swelling capacity (SC) was found as higher in GPMF while in the case of GFMF and GSF, it was found lower (Table 3). Similar results have been reported after germination by Shakirah *et al.* [39]. SC was increased which might be due to the degradation of fat, fiber, and starch-lipid complex of flour during germination. Starch is a major constituent of millet flour, and its structure influences the functional properties of flour such as swelling power [31]. Due to the degradation of starch, the presence of amylase and amylopectin might be also responsible for increasing the SC of germinated grains. High SC makes millet flour useful to act as a thickener in liquid food products.

The foaming capacity (FC) and foaming stability (FS) of GPMF, GFMF, and GSF were increased (Table 3). The highest value of FC was found in RSF (17.56%) while RPFM (8.65%) had the lowest value. This study was similar to the results reported by Elkhalfifa *et al.* [43]. But in the case of GFMF, foaming capacity and foaming stability were decreased. A similar result was considered Ashwani *et al.* [44]. Elkhalfifa *et al.* [43] observed the increment in the FC of

germinated sorghum to the fact that during germination, the number of solubilized proteins increased, resulting in improved FC. Both FC and FS can be affected by protein, pH, surface tension, and viscosity. According to Siddiqua *et al.* [11], germination may have led to surface-denatured protein and reduced the surface tension of molecules, which gave good formability. Millet flours with high FC and FS can be used for bakery products and improves the shelf life, consistency, and appearance of products and are also applicable for gluten-free products.

During germination emulsion activity (EA) and emulsion stability (ES) were found as highest in GSF (49.03% and 38.56% respectively) and lowest in RFMF (19.24% and 15.21% respectively). Elkhalfifa *et al.* (2018) reported that during germination increase in the EA and ES of sorghum flour and emulsion properties could be due to an increase in stabilized oil droplets at the interface which is a function of food components [43]. Siddiqua *et al.*, [11] also absorbed that EA and ES were increased by the action of hydrophobic protein activity.

**Table 3:** Effect of germination on the functional properties of raw and germinated millets flour

Functional parameters	RPMF	GPMF	RFMF	GFMF	RSF	GSF
Bulk density (g/cm <sup>3</sup> )	0.75±0.03	0.79±0.52	0.97±0.78	0.65±0.85	0.83±0.98	0.75±0.525
Water absorption capacity (g/g)	2.25±0.25	2.65±0.11	3.33±0.89	4.12±0.14	1.89±0.21	2.23±0.25
Oil absorption capacity (g/g)	1.55±0.44	2.50±0.21	2.17±0.25	2.56±0.84	1.34±0.24	1.79±0.35
Swelling capacity (g/g)	4.21±1.25	4.65±0.12	5.09±0.15	4.78±0.14	4.90±0.21	3.87±0.32
Foaming capacity (%)	8.65±2.50	9.43±1.25	13.65±0.25	10.89±0.001	12.0±0.002	17.56±0.12
Foaming stability (%)	6.69±0.36	10.31±0.25	15.0±0.001	12.7±0.22	10.45±0.001	18.0±0.124
Emulsion activity (%)	25.31±0.01	30.12±0.36	19.24±0.25	23.45±0.002	35.0±0.88	49.03±1.25
Emulsion stability (%)	18.04±0.05	24.30±0.38	15.21±0.36	21.03±0.33	25.87±0.77	38.56±0.25

**Note:** Values represent the mean of triplicate ± standard deviation. Means with no common letters within a row differed ( $p \leq 0.05$ ). RPMF- raw pearl millet flour, GPMF- germinated pearl millet flour, RFMF- raw finger millet flour, GFMF- germinated finger millet flour, RSF- raw sorghum flour, GSF- germinated sorghum flour.

#### Phyto chemical properties of raw and germinated millet flour

Table 4 shows the phytochemical content of raw and germinated millet flours. The tannin content in raw millets flour (0.61 mg/g; 1.61 mg/g and 0.79 mg/g) was observed to reduce significantly during germination (0.45 mg/g; 1.04 mg/g and 0.47 mg/g) for pearl millet, finger millet, and sorghum, respectively. A decrease in tannin during germination can be attributed to the leaching of polyphenols in soaking water and increased enzymatic action [44]. These results were more related to the report on finger millet by Aserse Yenasew *et al.* [45]. A similar observation has been documented by Iswarya *et al.* [40], who reported a reduction of tannin content in pearl millet after germination. Sorghum (53%) had the largest reduction of tannin content after germination, whereas pearl millet (26%) had the lowest percentage reduction of tannin content during germination.

From the table given below (Table 4), GFMF recorded the highest amount of saponin followed by GPMF and GSF during germination. Saponin content significantly ( $p \leq 0.05$ ) increased from 0.55 to 0.96 mg/g in pearl millet flour, 2.07 to 4.45 mg/g in finger millet flour and 2.11 to 3.93 mg/g in sorghum flour after germination. A similar result from the other studies was observed in finger and pearl millet by Chauhan *et al.* [29]. This result was possible because of the displacement of stored phyto-chemicals from the germinated grains. Saponin is beneficial for the human function of several

organs and also for treating various diseases [45]. The abundance of phyto-chemicals in the millets enhances nutraceutical potential, so making them a reliable source of functional foods [6]. The indigestion status of saponin content has been related to a decrease in overall blood sugar. This is the only reason that the saponin content is high during germination, when millets are soaked and germinated under ambient temperature, then both the endogenous and newly synthesizing enzyme being to modify the millet and increase the phyto-chemical constituents present in the millets.

Phytate content in pearl millet, finger millet, and sorghum flour significantly ( $p \leq 0.05$ ) decreased from 15.11 to 8.34 mg/g, 15.98 to 9.77 mg/g, and 18.37 to 15.76 mg/g respectively. The highest percentage reduction of phytate content was found in pearl millet flour (40.9%) while the lowest reduction of phytate content was in sorghum flour (14.2%) after germination. The reason for the reduction of phytate content after germination may be due to enhancing the phytase activity that hydrolysis of phytate phosphorus into inositol monophosphate [36]. A similar reduction of phytate content was observed during the germination of finger and pearl millet [6, 36] and sorghum grains [41]. Decreasing in phytate content during germination could be attributed to leaching out during hydration as well as activation of phytase after germination. Similar trends in the phytic acid content of finger millet reduced during germination [46].

**Table 4:** Effect of germination on the phyto-chemical properties of raw and germinated millet flour

Phyto-chemicals	RP MF	GP MF	RF MF	GF MF	RS F	GS F
Tannin (mg/g)	0.61±0.20	0.45±0.07	1.61±0.61	1.04±0.52	0.79±0.01	0.37±0.001
Saponin (mg/g)	0.55±0.01	0.96±0.44	2.07±1.21	4.45±1.02	5.11±0.04	2.93±0.027
Phytate (mg/g)	15.11±0.22	6.34±0.47	15.98±2.85	9.77±0.08	18.37±1.25	15.76±2.89

**Note:** Values represent the mean of triplicate ± standard deviation. Means with no common letters within a row differed ( $p \leq 0.05$ ).

#### Antioxidant properties of raw and germinated millet flour

DPPH and total phenol content of raw and germinate millet flour samples are presented in Table 5. Comparing the non-germinated and germinated pearl millet, finger millet, and sorghum, free radical scavenging activity (DPPH) content increased significantly ( $p \leq 0.05$ ) from 55.25% to 82.42%, 71.34% to 80.0% and 41.11% to 60% respectively (Table 5). Both raw and germinated millets have enzymatic antioxidant activity, in which GPMF was noted to have the highest increment of DPPH (27.17%); this is followed by GFMF and GSF. The reducing ability of a substance may explain its potential antioxidant activity [39]. From Table 5, it is clear that pearl millet, finger millet, and sorghum possess the ability to DPPH and therefore can be employed as a source of antioxidants to prevent the accumulation of unwanted substances in the system [6]. Similar results have been reported to increase antioxidant activities in pearl and finger millet which could be attributed to the activities of enzymes produced by microbial activities, the metabolism of phenolic compounds by fermenting microorganisms, and the release of previously bound phenol [6].

**Table 5:** Effect of germination on the Antioxidant properties of raw and germinated millet flour

Antioxidant	RP MF	GP MF	RF MF	GF MF	RS F	GS F
DPPH (%)	55.25±0.02	82.42±1.25	71.34±0.23	80.0±2.5	41.11±0.24	60±0.78
Total phenol (mg GAE/100 g)	80.11±0.15	112.4±1.0	125.6±0.14	150.1±0.25	180±0.98	140.5±3.5

**Note:** Values represent the mean of triplicate ± standard deviation. Means with no common letters within a row differed ( $p \leq 0.05$ ).

#### Conclusion

Germination is a traditional processing technique that shows positive effects on some physicochemical, functional, phytochemical, and antioxidant properties of pearl, finger millet, and sorghum flour. Germination caused a significant decrease in pH but an increase in the TTA value of millet flours. In this study, it was observed that the germination process increased protein, fiber, and ash content in millet flour, but fat and carbohydrate decreased significantly. Moisture content increased with germination. Germinated finger millet was the highest millet in calcium and iron content. It was concluded that the germination process can increase the availability of minerals and help to fulfill the nutritional needs of infants and young children to support their early growth and development. There was a significant increase in the water/oil absorption capacity, emulsion activity, and stability of the germinated flour samples making the flours good during the manufacturing of bakery products and thickening foods while reducing the bulk density and swelling power except for GPMF which is suitable for weaning foods or nutritionally dense food. Germination treatment significantly increased the mineral content but decreased the concentration of phytochemicals except for saponin. In addition, germination had increased DPPH and total phenolic content in all millet flours except sorghum. This improved antioxidant activity suggests developing a nutrient-rich product with germinated millet flours for therapeutic diet and this finding will encourage the use of germinated flour to

The total phenol content (TPC) in pearl and finger millet flour samples significantly ( $p \leq 0.05$ ) increased after germination from 80.11 to 112.4 mg GAE/100 g and 125.6 to 150.1 mg GAE/100 g respectively, while sorghum flour decreased TPC during germination from 120-81.5 mg GAE/100 g. Similar results were also reported by Ebisa *et al.* [41] during the germination of sorghum, the TPC was decreased. A similar finding was obtained with pearl, finger, and foxtail millet during germination, phenolic compounds in millets grain increased due to cell wall-degrading enzymes, which became active and modified the cell wall structure of the grain [6, 41]. According to Owheruo *et al.* [6], after germination, an increase in TPC may be due to the enzymatic release of bound phenolic compound. Phenols play a role in the antioxidative potential of grains and contribute to the extension of the shelf-life of cereal/millet products. Shakirah *et al.* [39] observed that germination and fermentation of finger millet at different temperatures increased the methanol extractable TPC and was associated with the synthesis of hydrolytic enzymes resulting in the modification of cell-wall structure, and synthesis of new compounds with bioactive potentials.

produce gluten-free food products for people who suffer from celiac disease.

#### Conflict of Interest

All the authors declare that there are no conflicts of interest.

#### Acknowledgments

We would like to sincerely thank the Department of School of Biological Engineering & Life Sciences, Shobhit Institute of Engineering & Technology for all their help and coordination. We are indebted to every person who is directly or indirectly involved in the completion of this research.

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