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Germination-induced changes in physicochemical, nutritional, antioxidant, and mineral properties of seven millet species

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ABSTRACT

Millets are rich in nutrients, and proper processing methods could further improve their nutritional value. Controlled germination at various durations affects the physicochemical properties of seven millet varieties, and their correlation was studied. A significant increase in grain length was observed, where sorghum had a length of 4.68 mm at 24 hours, little millet had a length of 1.70 mm at 48 hours after germination, and the breadth of finger millet had risen to 1.41 mm at 60 hours after germination. Morphological changes with softening of the seed coat and swelling of the grains were observed during germination. Germination had a great effect on nutrient composition in millets ($p < 0.05$). Finger millet had the maximum phenolic content at 25.54 µg CAE/g (12 hours), its protein content decreased to 5.47% (36 hours), and barnyard and little millet had significant drops in radical scavenging activity to 6.00% and 22.49%, respectively. The Pearson correlation analysis revealed species-specific relationships between phenolics, antioxidant activity, and the selected minerals, as well as varying mineral-protein interactions across the evaluated millets. This result provides a scientifically robust basis for selecting the raw millets, and further, it is used for developing value-added functional foods with improved bioactive and mineral profiles.

1. INTRODUCTION

There is a growing recognition of millets as nutrient-dense, climate-resilient cereals that are critical to food and nutritional security, especially in the semi-arid and resource-scarce regions of the world [1]. Millets are believed to be promising functional foods because of their richness in nutritional constituents such as dietary fiber, essential amino acids, phenolic compounds, and valuable nutrients such as calcium, iron, and zinc [2]. However, the bioavailability of these elements is often reduced by varietal variations, anti-nutritional effects, polyphenolic complexes, and processing conditions that may chelate divalent minerals and limit their intestinal absorption [3]. Thus, scientific and technological interest in streamlining the processing methods to improve the nutrient accessibility and bioavailability without affecting the product quality is increasing [4,5]. The use of millets as processed foods is an ancient process that involves soaking, germination, fermentation, and cooking. Germination has so far emerged as one of the simplest, cost-efficient, and biologically efficient bioprocessing methods [6].

Germination of grains is triggered by water uptake and enzymatic activity that initiates a coordinated metabolic reprogramming. This effect activates hydrolytic enzymes, including α-amylase, proteases, and phytases, which leads to the hydrolysis of starch, the release of proteins, and the reduction of antinutritional compounds [5]. Moreover, it has been demonstrated that germination of cereals augments bioactive molecules and has an impact on mineral composition that depends on the time-dependent metabolic changes during the process of germination [7]. Besides primary metabolism, germination activates the secondary metabolic pathways and, in particular, the phenylpropanoid pathway, which increases the production and release of phenolic compounds [6,8]. Mechanisms of enhanced antioxidant activity during germination include partial depolymerization of phenolics bound and enzyme hydrolysis of cell wall polymers [2].

Mineral dynamics are not always favorable in the germination process. To balance the enzymatic nutrient release and mineral retention, soaking and early germination should be maximized to minimize the release of soluble minerals that may occur during soaking and early germination [3]. These metabolic modifications are also related to structural changes of the germinated grains, such as protein-starch matrix remodeling, porosity, and erosion of starch grains on the surface. Grain shape, physicochemical behavior, and nutrient accessibility are all directly impacted by these modifications [4,9,10]. Several studies have concentrated on individual millet varieties or particular nutritional parameters that incorporate morphometric traits during germination, proximate composition, antioxidant qualities, mineral profiling, and multivariate interactions across multiple millet species. However, the relationship of nutrients during germination in small millets remains limited. Therefore, the primary objective of the present study is to investigate the effects of different germination

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durations on selected millet species by integrating physical, selected nutrients, antioxidant, mineral, and correlation-based statistical modelling analyses to explain the coordinated biochemical and structural responses associated with germination.

2. MATERIALS AND METHODS

2.1. Materials

Fully mature and clean samples of seven different species of millet, such as bajra (*Pennisetum glaucum),* sorghum (*Sorghum bicolor*), foxtail millet (*Setaria italica*), kodo millet (*Paspalum scrobiculatum*), little millet (*Panicum sumatrense*), barnyard millet (*Echinochloa spp*.), and finger millet (*Eleusine coracana*), were procured from the local market in Coimbatore, Tamil Nadu, India. After careful cleaning to remove damaged grains and other debris, the samples were refrigerated for further use.

2.2. Germination process

The germination process was carried out following the procedure from an earlier study [11]. The cleaned samples were soaked for 6 hours in deionized water at 32°C ± 2°C. The samples were wrapped in muslin cloth to promote aeration and regular moisture maintenance throughout germination after the excess water was drained. The germination process was conducted in an incubator (M/s Lark Instruments, Chennai) at 38°C ± 2°C for various durations (0, 12, 24, 36, 48, and 60 hours). Following each germination phase, the samples were dried for 7 hours at 60°C in a cabinet dryer until the moisture content was around 10%. The control (non-germinated) sample was also dried under the same conditions to achieve a comparable moisture content. In addition, morphological observations were carried out on the fresh samples, and the dried samples were kept in a refrigerator for further physicochemical studies.

2.3. Physical characteristics

Physical characteristics such as 100-grain weight, grain length, and breadth were measured before and after germination. Three random samples were chosen at random for each type of millet. A digital Vernier caliper (precision: 0.01 mm; M/s Zhart) was used to measure the grain dimensions, and the average of 10 measurements was noted for each parameter.

2.4. Morphological characteristics

Morphological characteristics of raw and germinated millet grains were examined using a stereomicroscope equipped with a color camera (Nikon, Y-TV55, Japan). Images were captured at 10× magnification with a camera zoom of 0.55×. Fresh samples were used to observe germination-induced structural changes.

2.5. Nutritional analysis

Crude protein and ash contents of raw and germinated millet samples were determined according to standard methods of the American Association of Cereal Chemists (AACC; methods 46–13.01 and 08–01.01, respectively) [12]. Total phenolic content (TPC) was estimated using the Folin–Ciocalteu method and expressed as catechin acid equivalents (µg CAE/ g) as per the method [13]. Antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, and the results were expressed as % radical scavenging activity (RSA) of methanolic extracts [14]. Nutrients were analyzed in the samples on a dry weight basis.

2.6. Mineral analysis

Mineral composition was determined as per the method [15]. A 1 g sample was subjected to tri-acid digestion using nitric acid, sulfuric acid, and perchloric acid. The digested material was diluted to 50 ml with double-distilled water and filtered using Whatman No. 1 ashless filter paper. The concentrations of calcium (Ca), magnesium (Mg), manganese (Mn), zinc (Zn), and iron (Fe) were quantified using an atomic absorption spectrophotometer (AAS; PerkinElmer Pinnacle 500 series) at their respective wavelengths. Mineral concentrations were calculated using calibration curves prepared from standard solutions.

2.7. Statistical analysis

All measurements were performed in one independent biological replicate, with three analytical measurements per replicate where applicable. Results are expressed as mean ± SD. Differences among germination durations were evaluated using one-way analysis of variance, followed by the post-hoc test, with statistical significance set at $p < 0.05$ using SPSS software (version 21.0; IBM Corp., Chicago, USA). Pearson correlation analysis was performed in R software using the mean value of each parameter of the individual millet species. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were conducted using OriginPro 2024.

3. RESULTS AND DISCUSSIONS

3.1. Physical characteristics

The physical traits of specific millet grains were determined in relation to the duration of germination of the grains (0–60 hours) to examine morphological changes (Table 1). There were significant differences in grain length, breadth, length-to-breadth (L/B) ratio, and 100-grain weight ($p < 0.05$), indicating progressive structural changes in structure throughout germination in the selected samples. The 100-grain weight of bajra grains dropped from 7.22 g at 0 hours to 6.85 g at 60 hours. During the first 24 hours of germination, grain length and breadth measured 3.13 mm and 1.90 mm, respectively, but thereafter decreased slightly. In contrast, sorghum exhibited a slight increase in 100-grain weight from 5.09 g at 24 hours to 5.66 g at 60 hours; the maximum length of the grain (4.68 mm) and breadth (2.77 mm) were recorded. Barnyard millet had highly consistent grain weight, but a large change in dimension values, with the maximum length and breadth being 1.53 mm and 0.85 mm, respectively, at 36 hours. Similarly, little millet had a steady grain weight (~2.7 g), but the grain length gradually increased to 1.70 mm at 48 hours, and the grain breadth was at its maximum of 1.13 mm; hence, the grain size was highly expanded. The grain weight of foxtail millet remained relatively constant, whereas the length and breadth both grew significantly to reach the highest breadth of 1.19 mm after 60 hours. The 100-grain weight of kodo millet was comparatively stable (~ 3.4 g), resulting in the maximum value of the L/B ratio of 2.18 at 60 hours, but the grain sizes increased slowly. Finger millet showed substantial changes in dimensions and a slight increase in grain weight. The grain width increased to 1.41 mm at 60 hours compared to 0.92 mm at 0 hours, and the L/B ratio slightly varied to 1.11.

Similar results were observed from a previous study in 10 green gram cultures and finger millets [11,16]. Changes in grain length and width might be largely attributed to the swelling of starch granules during soaking, as water gradually penetrates the grains and leads to irreversible expansion [17]. In some millet varieties during different germination period in various millets, the apparent increase in

Table 1. Physical characteristics of the non-germinated and germinated millet samples.

Sample name	Germination Time (Hours)	100-grains weight (g)	Length (mm)	Breadth (mm)	L/B
Bajra	0	7.22 ± 0.04^{d}	2.46 ± 0.07^{a}	1.64 ± 0.03^{a}	1.50
	12	7.28 ± 0.18^{cd}	2.62 ± 0.03^{b}	1.81 ± 0.04^{b}	1.45
	24	7.04 ± 0.04^{bc}	3.13 ± 0.04^{e}	1.90 ± 0.01^{c}	1.65
	36	6.57 ± 0.04^{a}	2.81 ± 0.01^{d}	1.84 ± 0.04^{b}	1.53
	48	6.91 ± 0.16^{b}	2.72 ± 0.08^{c}	1.83 ± 0.04^{b}	1.49
	60	6.85 ± 0.01^{b}	2.71 ± 0.02^{c}	1.79 ± 0.01^{b}	1.51
Sorghum	0	5.12 ± 0.08^{a}	4.29 ± 0.11^{a}	2.48 ± 0.09^{a}	1.73
	12	5.08 ± 0.05^{a}	4.39 ± 0.10^{a}	2.63 ± 0.02^{ab}	1.67
	24	5.09 ± 0.08^{a}	4.68 ± 0.20^{b}	2.77 ± 0.10^{c}	1.69
	36	5.34 ± 0.13^{b}	4.27 ± 0.07^{a}	2.75 ± 0.11^{c}	1.55
	48	5.51 ± 0.04^{bc}	4.37 ± 0.09^{a}	2.64 ± 0.11^{ab}	1.66
	60	5.66 ± 0.22^{c}	4.46 ± 0.00^{a}	2.54 ± 0.04^{a}	1.76
Barnyard millet	0	4.08 ± 0.14^{a}	1.21 ± 0.05^{a}	0.41 ± 0.00^{a}	2.95
	12	4.11 ± 0.13^{a}	1.23 ± 0.02^{b}	0.65 ± 0.02^{b}	1.89
	24	4.25 ± 0.08^{ab}	1.35 ± 0.00^{c}	0.83 ± 0.01^{d}	1.63
	36	4.06 ± 0.10^{a}	1.53 ± 0.03^{d}	0.85 ± 0.02^{d}	1.80
	48	4.38 ± 0.11^{b}	1.40 ± 0.03^{c}	0.84 ± 0.02^{d}	1.67
	60	4.21 ± 0.09^{ab}	1.35 ± 0.00^{c}	0.73 ± 0.00^{c}	1.85
Little millet	0	2.73 ± 0.12^{a}	1.21 ± 0.02^{a}	0.51 ± 0.01^{a}	2.37
	12	2.72 ± 0.12^{a}	1.28 ± 0.05^{ab}	0.76 ± 0.03^{b}	1.68
	24	2.65 ± 0.10^{a}	1.32 ± 0.05^{b}	0.75 ± 0.02^{b}	1.76
	36	2.75 ± 0.01^{a}	1.40 ± 0.05^{c}	0.73 ± 0.02^{b}	1.92
	48	2.73 ± 0.03^{a}	1.70 ± 0.04^{d}	1.13 ± 0.04^{d}	1.50
	60	2.70 ± 0.08^{a}	1.47 ± 0.04^{c}	0.87 ± 0.00^{c}	1.69
Foxtail millet	0	3.92 ± 0.14^{ab}	1.15 ± 0.03^{a}	0.64 ± 0.00^{a}	1.80
	12	3.91 ± 0.11^{ab}	1.48 ± 0.05^{b}	0.93 ± 0.03^{b}	1.59
	24	3.84 ± 0.02^{ab}	1.57 ± 0.03^{c}	1.09 ± 0.02^{c}	1.44
	36	3.73 ± 0.01^{a}	1.46 ± 0.04^{b}	1.13 ± 0.04^{cd}	1.29
	48	3.96 ± 0.06^{b}	1.44 ± 0.02^{b}	1.15 ± 0.04^{cd}	1.25
	60	3.75 ± 0.13^{a}	1.42 ± 0.05^{b}	1.19 ± 0.05^{d}	1.19
Kodo millet	0	3.47 ± 0.15^{a}	1.02±0.04^{a}	0.51 ± 0.01^{a}	2.00
	12	3.48 ± 0.09^{a}	1.32±0.05^{b}	0.68 ± 0.01^{b}	1.94
	24	3.45 ± 0.15^{a}	1.51±0.03^{c}	0.82 ± 0.04^{e}	1.84
	36	3.46 ± 0.13^{a}	1.51±0.06^{c}	0.78 ± 0.01^{d}	1.94
	48	3.43 ± 0.01^{a}	1.56±0.04^{cd}	0.76 ± 0.01^{cd}	2.05
	60	3.42 ± 0.03^{a}	1.61±0.02^{d}	0.74 ± 0.01^{c}	2.18
Finger millet	0	4.73 ± 0.09^{bc}	0.98±0.01^{a}	0.92 ± 0.03^{a}	1.07
	12	4.73 ± 0.12^{bc}	1.23±0.03^{b}	0.94 ± 0.01^{a}	1.31
	24	4.81 ± 0.10^{c}	1.27±0.04^{b}	1.24 ± 0.04^{b}	1.02
	36	4.54 ± 0.11^{a}	1.34±0.06^{c}	1.31 ± 0.05^{c}	1.02
	48	4.60 ± 0.05^{ab}	1.38±0.00^{c}	1.33 ± 0.02^{c}	1.04
	60	4.80 ± 0.09^{c}	1.56±0.04^{d}	1.41 ± 0.01^{d}	1.11

Values are expressed as mean ± SD (n = 3); L/B – Length and Breadth ratio; Different superscript letters within the same column of the each millet sample indicate significant differences ($p < 0.05$) among different germination times.

grain length may also be influenced by the partial retention of dried epicotyl and hypocotyl tissues after drying of the samples, as these structures are difficult to remove completely. However, with extended germination, a reduction in grain dimensions such as length and width is often observed. This decline may be linked to structural alterations, including the development of surface pits and cavities in starch granules, along with the breakdown of larger particles into smaller fragments [18]. Furthermore, variations in these physical

characteristics among different millet samples are likely influenced by factors such as genetic differences between varieties, cultivation conditions, and the moisture content of grains during postharvest handling.

3.2. Morphological characteristics

Comparative visual evaluation of single millet grains across all germination periods revealed progressive morphological alterations across species (Fig. 1). Due to water absorption during germination, which initiates metabolic reactivation, the early phases (0–24 hours) are marked by softening of the seed coat and swelling of the grain. In intermediate stages (24–48 hours), radicle protrusion and axial elongation were observed, with finger millet emerging rapidly and pearl millet showing slower shoot emergence. Germination of the millets (particularly foxtail and finger millet) occurs at an advanced stage (after 60 hours) and is indicated by the development of shoot primordia, increased translucency of the grain, and rupture of the seed coat. The first in a series of morphological changes during germination is water absorption, which softens the seed coat, increases grain turgidity, and triggers cellular expansion [19]. Observable markers of active tissue differentiation and growth during germination are radicle emergence, coleoptile production, and axial elongation. These structural variations depend on the natural permeability of the seed coat, grain structure, and species-specific metabolic activity. Surface erosion and pore formation occurred in millet starch granules that they ascribed to structural reorganization and enzyme hydrolysis during the germination process [18]. Sprouting changes the microstructure of seeds, making them more porous and less hard, which positively affects processing characteristics and digestibility [4]. Combined of these, the comparative visualization of physical features in millet samples offers a phenotypic basis to improve food applications based on germination, since it highlights the differences in the germination reactions among millet samples.

3.3. Nutritional characteristics

The nutrient levels of the chosen millets were compared during germination periods of 0–60 hours, and significant ($p < 0.05$) variations in nutrients were observed among samples and across germination times (Table 2). Ash content remained relatively consistent across most millet varieties, with foxtail millet having the highest value at 48 hours (2.31% ± 0.01%) and kodo millet having the lowest value at 12 hours (0.99% ± 0.01%). Protein content responses varied among species. Finger millet recorded a sharp decrease after germination, going from 10.87% ± 0.21% at 0 hours to 5.47% ± 0.11% at 36 hours, while foxtail millet peaked at 36 hours (11.41% ± 0.38%).

The highest TPC was consistently found in finger millet, reaching 25.54 ± 0.28 µg CAE/g at 12 hours. Barnyard millet exhibited a mid-germination decline to 7.24 ± 0.14 µg CAE/g at 24 hours, followed by recovery in later stages. Species-specific trends in RSA were observed. Finger millet exhibited the highest initial RSA (72.27 ± 1.95 % at 0

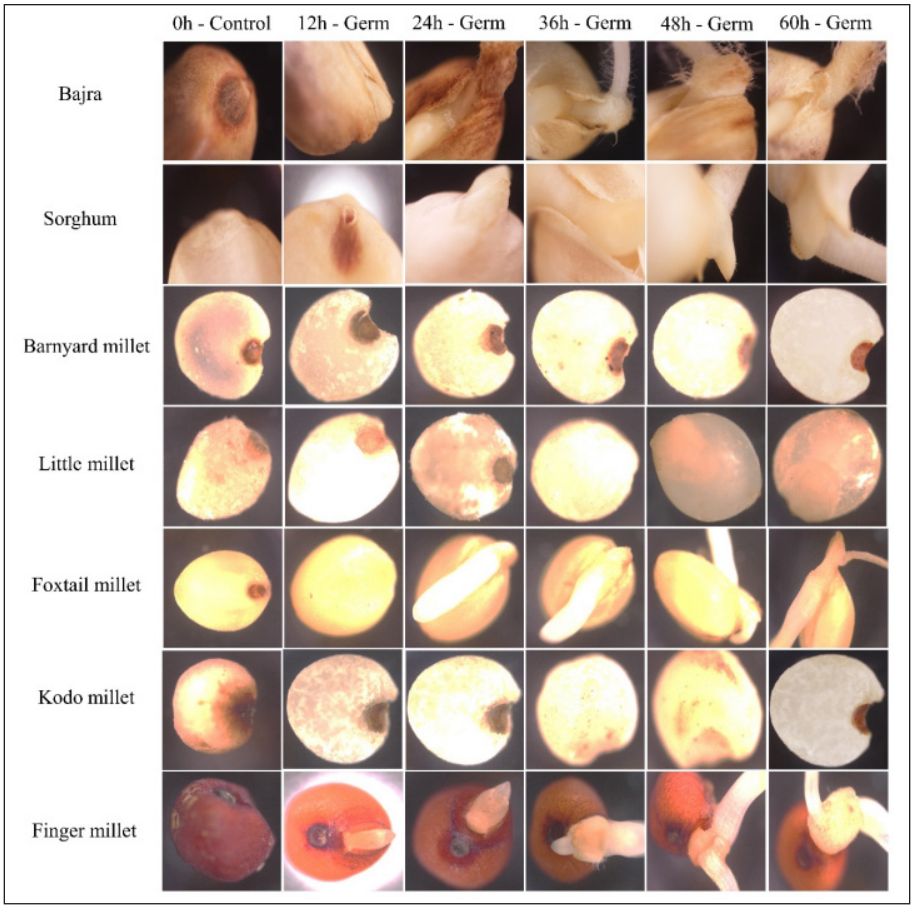

Figure 1. Morphological changes of the selected millets during different germination periods. Samples were examined using a stereomicroscope equipped with a colour camera; images were captured at 10× magnification with a camera zoom of 0.55×; Germ—Germination.

Table 2. Nutritional characteristics of non-germinated and germinated millet samples.

Sample name	Germination Time (Hours)	Ash (%)	Protein (%)	Total phenolic content (µg CAE/g)	% RSA
Bajra	0	1.99 ± 0.06^{b}	9.54 ± 0.32^{c}	17.43 ± 0.70^{c}	71.62 ± 3.06^{c}
	12	1.98 ± 0.08^{b}	9.63 ± 0.24^{c}	18.95 ± 0.07^{d}	68.89 ± 0.37^{bc}
	24	1.33 ± 0.03^{a}	8.05 ± 0.15^{b}	12.37 ± 0.42^{b}	66.38 ± 2.33^{a}
	36	1.99 ± 0.02^{b}	7.61 ± 0.27^{a}	1.55 ± 0.01^{a}	66.23 ± 0.25^{bc}
	48	2.00 ± 0.03^{b}	9.22 ± 0.20^{c}	1.49 ± 0.06^{a}	66.01 ± 0.98^{bc}
	60	1.99 ± 0.01^{b}	10.16 ± 0.15^{d}	1.50±0.02^{a}	68.01 ± 2.64^{bc}
Sorghum	0	1.65 ± 0.03^{b}	7.87 ± 0.18^{cd}	7.27 ± 0.05^{d}	35.26 ± 0.16^{c}
	12	1.32 ± 0.00^{a}	7.62 ± 0.01^{bc}	7.74 ± 0.24^{e}	48.91 ± 1.72^{e}
	24	1.33 ± 0.03^{a}	7.85 ± 0.10^{cd}	2.07 ± 0.07^{a}	49.56 ± 0.54^{e}
	36	2.00 ± 0.01^{c}	7.43 ± 0.17^{ab}	5.09 ± 0.03^{b}	10.59 ± 0.22^{a}
	48	1.32 ± 0.04^{a}	8.12 ± 0.34^{d}	6.38 ± 0.03^{c}	31.55 ± 0.65^{b}
	60	1.66 ± 0.02^{b}	7.22 ± 0.29^{a}	6.38 ± 0.02^{c}	40.94 ± 1.03^{d}
Barnyard millet	0	1.66 ± 0.06^{c}	9.2 ± 0.41^{b}	10.98 ± 0.35^{c}	25.55 ± 0.02^{e}
	12	1.66 ± 0.00^{c}	9.78 ± 0.12^{c}	10.92 ± 0.20^{c}	13.86 ± 0.21^{d}
	24	1.33 ± 0.05^{b}	9.72 ± 0.24^{c}	7.24 ± 0.14^{a}	13.54 ± 0.33^{d}
	36	1.00 ± 0.00^{a}	9.51 ± 0.21^{bc}	7.64 ± 0.26^{a}	12.88 ± 0.22^{c}
	48	1.97 ± 0.02^{d}	8.33 ± 0.20^{a}	9.05 ± 0.22^{b}	8.19 ± 0.03^{b}
	60	1.67 ± 0.03^{c}	7.92 ± 0.05^{a}	11.39 ± 0.36^{c}	6.00 ± 0.03^{a}
Little millet	0	1.67 ± 0.05^{b}	8.31 ± 0.13^{ab}	7.56 ± 0.26^{d}	38.54 ± 1.70^{e}
	12	1.66 ± 0.07^{b}	8.61 ± 0.36^{bc}	5.14 ± 0.17^{b}	34.72 ± 0.28^{d}
	24	1.33 ± 0.03^{a}	8.82 ± 0.19^{c}	4.59 ± 0.17^{a}	29.37 ± 0.77^{c}
	36	1.67 ± 0.01^{b}	8.11 ± 0.13^{a}	5.18 ± 0.02^{b}	26.64 ± 0.86^{b}
	48	1.33 ± 0.00^{a}	8.27 ± 0.16^{ab}	4.56 ± 0.04^{a}	23.80 ± 0.30^{a}
	60	1.67 ± 0.03^{b}	8.00 ± 0.03^{a}	7.11 ± 0.31^{c}	22.49 ± 0.65^{a}
Foxtail millet	0	1.66 ± 0.00^{b}	9.97 ± 0.34^{a}	5.43 ± 0.08^{c}	16.81 ± 0.50^{f}
	12	1.32 ± 0.01^{a}	9.70 ± 0.05^{a}	5.45 ± 0.14^{c}	14.96 ± 0.65^{e}
	24	1.33 ± 0.02^{a}	10.62 ± 0.41^{b}	2.88 ± 0.00^{a}	13.54 ± 0.34^{d}
	36	1.67 ± 0.07^{b}	11.41 ± 0.38^{c}	3.99 ± 0.11^{b}	10.9 ± 0.03^{c}
	48	2.31 ± 0.01^{c}	10.72 ± 0.01^{b}	6.13 ± 0.06^{d}	9.83 ± 0.17^{b}
	60	1.67 ± 0.02^{b}	10.57 ± 0.03^{b}	6.21 ± 0.06^{d}	8.41 ± 0.31^{a}
Kodo millet	0	1.33 ± 0.04^{b}	7.48 ± 0.18^{b}	5.69 ± 0.05^{b}	25.98 ± 0.75^{d}
	12	0.99 ± 0.01^{a}	7.79 ± 0.17^{c}	5.77 ± 0.02^{b}	19.21 ± 0.76^{c}
	24	1.65 ± 0.03^{c}	8.33 ± 0.01^{d}	7.05 ± 0.16^{c}	19.43 ± 0.11^{c}
	36	1.65 ± 0.02^{c}	8.01 ± 0.20^{c}	5.38 ± 0.01^{a}	19.21 ± 0.29^{c}
	48	1.65 ± 0.05^{c}	9.40 ± 0.05^{e}	5.36 ± 0.18^{a}	18.23 ± 0.25^{b}
	60	2.00 ± 0.02^{d}	7.01 ± 0.03^{a}	5.33 ± 0.08^{a}	15.50 ± 0.25^{a}
Finger millet	0	1.99 ± 0.05^{a}	10.87 ± 0.21^{d}	23.32 ± 1.03^{d}	72.27 ± 1.95^{c}
	12	1.98 ± 0.05^{a}	6.26 ± 0.14^{c}	25.54 ± 0.28^{e}	65.28 ± 0.76^{a}
	24	1.99 ± 0.03^{a}	5.80 ± 0.16^{b}	21.35 ± 0.58^{c}	64.96 ± 0.00^{a}
	36	1.99 ± 0.04^{a}	5.47 ± 0.11^{a}	22.50 ± 0.67^{d}	64.41 ± 1.63^{a}
	48	2.33 ± 0.07^{b}	5.76 ± 0.10^{b}	14.44 ± 0.07^{b}	65.07 ± 1.82^{a}
	60	2.66 ± 0.09^{c}	5.58 ± 0.16^{ab}	12.94 ± 0.06^{a}	67.69 ± 1.16^{ab}

Values are expressed as mean ± SD (n = 3); %RSA—radical scavenging activity; CAE—catechin acid equivalents; Different superscript letters within the same column of the each millet sample indicate significant differences ($p < 0.05$) among different germination times.

hour). RSA decreased during the early stages of germination, reaching a minimum of 64.41 ± 1.63 % at 36 hours, followed by an increase to 67.69 ± 1.16 % at 60 hours. These results indicate a time-dependent response rather than a consistent increase in antioxidant activity throughout germination. Likewise, little millet showed a consistent decline in % RSA (from 38.54% ± 1.70% to 22.49% ± 0.65%; $p < 0.05$), with the lowest total phenolic concentration recorded at 24 hours (4.59 ± 0.17 µg CAE/g).

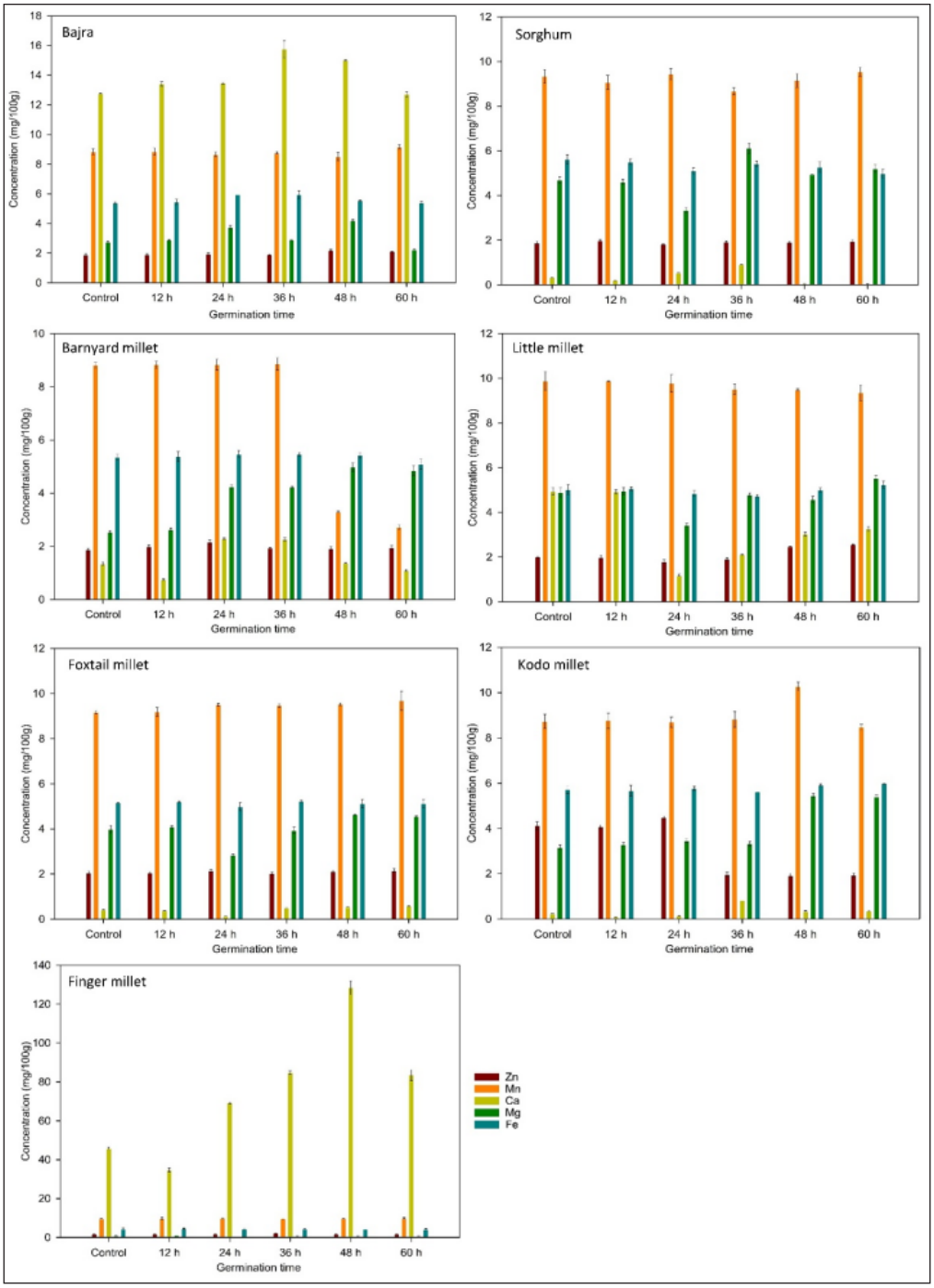

Figure 2. Changes in mineral content of the samples during different germination periods. Values expressed as mean ± SD from three replication of each sample.

The enzymatic hydrolysis and mobilization of stored macronutrients during the germination process result in changes to the nutritional profile. Proteases break down stored proteins into peptides and free amino acids, improving digestibility and bioavailability, while amylases break down starch into simpler sugars, increasing the amount of soluble carbohydrates [11,20]. During germination of finger millet (0–96 hours), there are decreases in starch and protein content and increases in reducing sugars, total sugars, crude fiber, ascorbic acid, and antioxidant activity [16]. After 48 hours, total phenols and tannins increased following an initial decline. Similar trends have been documented in pearl millet, where germination improved mineral bioaccessibility by lowering antinutritional elements, including phytic acid and oxalates [21,22].

The reduction in antioxidant activity during long term germination is associated with dynamic metabolism such as biosynthesis, transformation, and utilization of phenolic compounds. In the early stages, activation of the phenylpropanoid pathway and increased phenylalanine ammonia-lyase activity promote the release and synthesis of phenolics, resulting in a temporary increase in TPC. As germination progresses, phenolics are increasingly utilized to scavenge

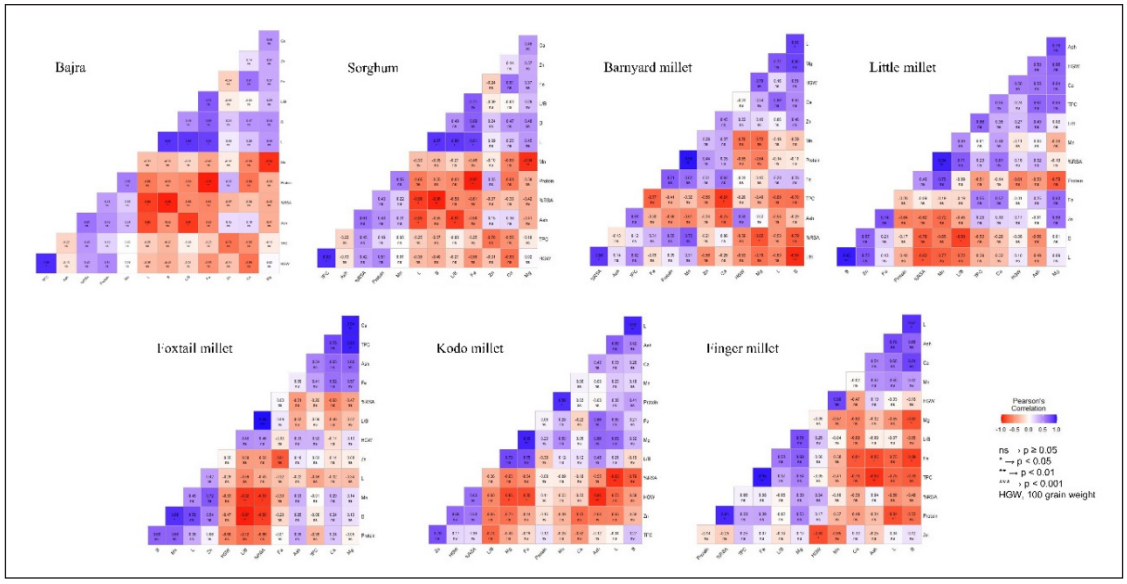

Figure 3. Pearson correlation between analytical characteristics during different germination periods for germinated samples.

reactive oxygen species generated during enhanced respiration, while oxidative enzymes such as polyphenol oxidase and peroxidase further convert phenolics into less active oxidation products, leading to reduced antioxidant activity [23,24].

In addition, the absence of a direct correlation between TPC and antioxidant activity is expected because the Folin–Ciocalteu assay is used to estimate total reducing capacity rather than the antioxidant efficacy of individual phenolic compounds of the sample. Differences in phenolic composition, structural modifications during germination, and the varying antioxidant capacities of individual phenolic compounds can result in inconsistent relationships between TPC and radical-scavenging activity. Moreover, antioxidant activity in the sample is also influenced by non-phenolic antioxidants, such as ascorbic acid, tocopherols, carotenoids, and antioxidant peptides formed during germination, which collectively contribute to the species-specific responses among the millet varieties [11,25,26]. Additionally, glutamate decarboxylase activity and activation of the phenylpropanoid pathway have been linked to increased γ-aminobutyric acid and phenolic content during germination [8]. Overall, findings in nutritional characteristics indicate that nutrient composition modifications during germination are both time-dependent and species-specific, highlighting the potential for optimizing germination conditions to enhance the nutritional quality of millets.

3.4. Mineral characteristics

Germination affected the mineral composition of the selected millet samples in a highly diverse and mineral-specific manner, indicating complex interactions between enzymatic activation, metabolic demand, and potential leaching losses (Table S1; Fig. 2). In most of the millet samples, Zn and Mn exhibited relatively similar trends. In millets such as bajra, sorghum, and barnyard millet, Zn contents did not significantly alter germination intervals ($p > 0.05$). However, compared to the starting value (2.01 mg/100 g), little millet showed a significant rise ($p < 0.05$) at 48 hours (2.47 ± 0.01 mg/100 g) and 60 hours (2.57 ± 0.03 mg/100 g), but kodo millet showed a reduction after 36 hours.

The Mn content remained comparatively consistent in the majority of samples except for barnyard millet, which showed a notable and substantial decrease after 48 hours (3.30 ± 0.03 mg/100 g) and 60 hours (2.72 ± 0.09 mg/100 g) compared to its original level (8.81 mg/100 g). The overall stability of Zn and Mn indicates low metabolic utilization and decreased leaching susceptibility during germination. These results are in line with those of [27], who noted insignificant variations in Zn extractability in pearl millet, and [16], who showed limited variation in trace mineral content during millet germination.

In contrast, Ca and Mg showed species-specific differences among the samples. Finger millet showed a significant ($p < 0.05$) rise in Ca, which peaked at 128.46 ± 3.59 mg/100 g at 48 hours, more than 180% higher than the starting value of 45.87 mg/100 g. The Ca concentration of other millets, such as little millet and bajra, also showed significant variations. Sorghum and kodo millet exhibited substantial increases in magnesium concentration at later stages of germination (6.12 and 5.45 mg/100 g, respectively). These changes in germinated kodo millet may be the consequence of decreased chelation and enzymatic release of bound minerals [3].

In most samples, Fe levels either slightly decreased or remained constant. For example, at 60 hours, sorghum and barnyard millet showed significant decreases ($p \leq 0.05$) to 4.98 and 5.09 mg/100 g, respectively. This decrease might be due to soluble iron being leached during soaking and germination [3]. Kodo millet, on the other hand, showed a significant increase in the Fe concentration (6.00 mg/100 g after 60 hours), suggesting possible genotypic variations in iron mobilization and retention [3].

3.5. Correlation of physicochemical characteristics

Pearson's correlation analysis across all the millet samples during selected periods of germination consistently demonstrated strong interrelationships among grain morphology, proximate composition, antioxidant properties, and mineral distribution (Fig. 3) and scatterplot matrix values of the samples presented in Figure S1. Dimensional traits such as length, breadth, and L/B ratio were tightly coupled in all cereals ($r \approx 0.81–0.95$, $p < 0.05$), confirming coordinated structural expansion

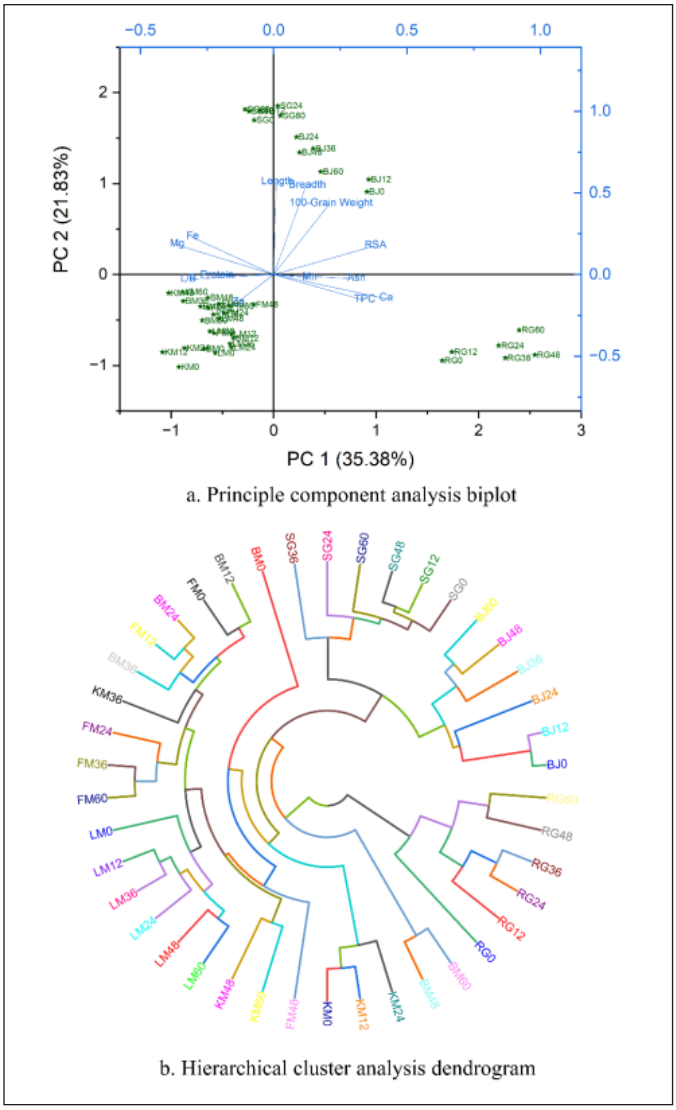

Figure 4. Multivariate analysis of physicochemical, nutritional, antioxidant, and mineral characteristics of germinated millet samples. **a.** Principal component analysis biplot and **b.** Hierarchical cluster analysis dendrogram

BJ, Bajra; SG, Sorghum; KM, Kodo millet; FM, Foxtail millet; BM, Barnyard millet; LM, Little millet; R, Ragi (Finger millet), The number presented in the nearby sample is representing for germination time.

during sprouting. Such morphometric synchronization aligns with hydration-induced cellular elongation and enzymatic softening of endosperm matrices reported during finger millet germination [5]. The 100-grain weight of the samples showed positive associations with TPC in bajra, whereas sorghum exhibited a weak and non-significant correlation (r = 0.16), and with ash or calcium in several millets (r ≈ 0.66–0.78), indicating that biochemical enrichment accompanied physical changes. Similar increases in phenolics and mineral-linked functional improvements during germination have been reported in quinoa, proso/barnyard millets, and kodo millet, where prolonged germination enhanced bioactive compounds, nutrient density, and mineral availability [4,6,7].

TPC showed variable links to antioxidant activity, with barnyard and kodo millets exhibiting weak correlations (r = 0.12 and r = 0.16, respectively), while little millet exhibited a very strong association between %RSA and zinc (r = 0.94, p < 0.01). This agrees with findings that germination increased phenolics alongside antioxidant potential in kodo millet [8], and with quinoa germination studies reporting time-dependent increases in bioactive compounds [7]. In little millet, the very strong association between % RSA and zinc (r = 0.94, p < 0.01) suggests coordinated micronutrient mobilization and antioxidant defense, possibly reflecting zinc's structural role in antioxidant enzymes. In finger millet, protein and TPC showed a moderate correlation (r = 0.38), suggesting that proteolysis releases amino acids during germination that may serve as precursors or regulatory signals for phenolic biosynthesis, a phenomenon consistent with functional changes reported in finger millet flour during germination [7,11].

Mineral interaction changes were noted across samples, reflecting dynamic nutrient redistribution. Strong positive correlations were observed between calcium and magnesium (foxtail millet, r = 0.94), manganese and iron (barnyard millet, r = 0.96, p < 0.001), and magnesium and iron (kodo millet, r = 0.93, p < 0.01), indicating coordinated mobilization of divalent cations during sprouting. Similar mineral shifts have been reported in pearl millet, brown rice, and amaranth during germination, where enzymatic hydrolysis of phytate enhanced mineral extractability and bioavailability [11,28,29]. In addition, the reduction of phytic acid during germination improves the digestibility of minerals, particularly iron and zinc, as previously demonstrated in pearl millet [27]. Conversely, pronounced antagonistic relationships were detected between protein and manganese in sorghum (r = –0.97, p < 0.01) and bajra (r = –0.94, p < 0.01), as well as between manganese and magnesium in bajra (r = –0.94, p < 0.01). These inverse relationships of minerals may reflect competitive redistribution during enzymatic activation phases. Negative correlations between zinc and TPC (barnyard millet, r = –0.91, p < 0.01) and between ash and zinc in sorghum (r = –0.91, p < 0.01), this further supports selective micronutrient mobilization during sprouting. Enhanced iron and calcium bioavailability during germination has been previously attributed to phytate degradation and mineral release from bound complexes [21].

3.6. Multivariate analysis of physicochemical characteristics

3.6.1. Principal component analysis

PCA was performed to investigate the multivariate relationships among the physicochemical, nutritional, antioxidant, and mineral characteristics of germinated millet samples (Fig. 4a). The first two principal components explained 57.21% of the total variation, with PC1 accounting for 35.38% and PC2 for 21.83%. The loading plot indicated that 100-grain weight, grain length, grain breadth, antioxidant activity, ash, TPC, calcium, and manganese were positively associated with PC1, whereas other parameters, including protein, Fe, Mg, and the L/B ratio were positioned on the opposite side, suggesting contrasting contributions to sample discrimination. Finger millet samples at germination times of 0 and 60 hours were distinctly separated along the positive side of PC1 and were closely associated with higher calcium, TPC, RSA, and ash contents, confirming their unique nutritional characteristics among the evaluated millet species. In contrast, sorghum and bajra samples were distributed mainly in the upper quadrants because of their greater grain dimensions and grain weight, while little millet, foxtail millet, kodo millet, and barnyard millet formed a relatively compact group, indicating similar physicochemical responses during germination. The close association between TPC and RSA suggests that phenolic compounds contributed substantially to antioxidant activity, whereas the separation of protein from these variables reflects the differential metabolic pathways involved in storage protein mobilization and phenolic biosynthesis during germination. Similar multivariate relationships between

phenolics, antioxidant activity, and mineral composition have been reported in germinated cereals and millets, where genotype and germination duration jointly influence nutritional quality [24,30,31].

3.6.2. Hierarchical cluster analysis

HCA further supported the PCA results by classifying millet samples according to their overall similarity in physicochemical and nutritional characteristics (Fig. 4b). The dendrogram revealed distinct clustering patterns, with finger millet, sorghum, and bajra forming relatively independent groups, whereas little millet, foxtail millet, kodo millet, and barnyard millet clustered more closely, indicating comparable biochemical responses during different periods of germination. Finger millet remained clearly separated from the other millet species across all germination durations, reflecting its distinctive nutritional profile, particularly its high calcium concentration and antioxidant potential. Sorghum samples also clustered closely because of their consistently larger grain dimensions, while bajra formed a separate subgroup associated with higher grain weight. Within individual millet species, samples from adjacent germination durations generally clustered together, suggesting that germination induced gradual rather than abrupt biochemical changes. These clustering patterns demonstrate that genotypic differences among the samples exerted a stronger influence than germination duration on the overall nutritional and physicochemical characteristics, although progressive metabolic modifications during germination also contributed to sample differentiation. Similar clustering behavior has been reported for cereal grains, where varietal characteristics predominantly determine multivariate nutritional profiles, while germination acts as a secondary factor enhancing biochemical and functional attributes [30–32].

4. CONCLUSION

Germination significantly altered the structural, nutritional, and mineral properties of selected millets, demonstrating that sprouting is an intensive metabolic reprogramming process. The length of the grain in finger millet was considerably high between 0.98 mm at 0 hour and 1.56 mm at 60 hours, whereas the length of the little millet was 1.70 mm at 48 hours and that of sorghum was 4.68 mm at 24 hours, showing that the morphology changed. Reductions in bajra 100-grain weight (7.22–6.85 g) and an increase in sorghum (5.12–5.66 g) indicated a species-specific response to germination. A significant increase in calcium enrichment was observed (up to 128.46 mg/100 g) at 48 hours and, in phenolic content, particularly in finger millet (maximum 25.54 µg CAE/g) at 12 hours. Correlation analysis revealed species-dependent relationships between phenolic content, antioxidant activity, and selected minerals, along with varying interactions between mineral content and protein, demonstrating that metabolic changes throughout germination were complex and varied among millet varieties. PCA and HCA supported the distinct characteristics of finger millet, sorghum, and bajra. However, these responses are affected by the varietal characteristics and the maturity of the grain, and this should be taken into consideration when analyzing the effects of germination. Overall, the results indicate a time-dependent improvement in the functional and nutritional quality of germinated millets. This provides a scientifically robust basis for selecting raw millets and developing value-added functional foods with improved bioactive and mineral profiles. These findings support the development of nutrient-dense functional foods from climate-resilient millets, contributing to Sustainable Development Goal (SDG) 2 (Zero Hunger) through improved nutritional security, SDG 3 (Good Health and Well-being) by promoting healthier diets, and SDG 12 (Responsible Consumption and Production) through sustainable, low-input food processing technologies.

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6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All authors are eligible for authorship as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

All the data are available with the authors and shall be provided upon request.

10. PUBLISHER'S NOTE

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11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI) tools for writing or editing the manuscript, and no images were manipulated using AI.

12. SUPPLEMENTARY MATERIAL

The supplementary material can be accessed at the journal's website: Link here [https://jabonline.in/admin/php/uploadss/1538_pdf.pdf]

REFERENCES

1. Thiyagamoorthy UM, Palanisamy S, Packialakshmi JS, Sadayandi G, Lakshmanan K, Karunakaran K, *et al.* Deciphering the significance of millets as a superfood in promoting gut health: a review. Food Rev Int 2025; 42: 1–24; doi: https://doi.org/10.1080/87559129.2025.2584335
2. Wang Y, Zhang Y, Li S, Huang K, Cao H, Qiu W, *et al.* Mechanism of the release and transformation of polyphenols during germination and fermentation in millets: profile and metabolomics based analysis. Food Funct 2025;16(5):2004–17; doi: https://doi.org/10.1039/d4fo05625f
3. Lestienne I, Icard-Vernière C, Mouquet C, Picq C, Trèche S. Effects of soaking whole cereal and legume seeds on iron, zinc and phytate contents. Food Chem 2005;89(3):421–5; doi: https://doi.org/10.1016/j.foodchem.2004.03.040
4. Sofi SA, Rafiq S, Singh J, Mir SA, Sharma S, Bakshi P, *et al.* Impact of germination on structural, physicochemical, techno-functional, and digestion properties of desi chickpea (*Cicer arietinum* L.)

flour. Food Chem 2023;405:135011; doi: https://doi.org/10.1016/j.foodchem.2022.135011
5. Yenasew A, Urga K. Effect of the germination period on functional properties of finger millet flour and sensorial quality of porridge. Food Sci Nutr 2023;11(5):2336–43; doi: https://doi.org/10.1002/fsn3.3240
6. Bhavadharani PV, Gurumoorthi P. Impact of germination on nutritional components, antinutritional, and functional properties of proso and barnyard millets. Food Chem Adv 2025;6:100896; doi: https://doi.org/10.1016/j.focha.2025.100896
7. Nazih A, Maatougui A, Eseghir L, Houmy N, Aboukhalid K, Bazile D, *et al*. Effect of germination time on the content of nutritional and bioactive compounds of Chenopodium quinoa Wild. seeds cultivated in Eastern Morocco. Pol J Food Nutr Sci. 2025;75(2):97–107; doi: https://doi.org/10.31883/pjfns/203331
8. Sharma S, Saxena DC, Riar CS. Changes in the GABA and polyphenols contents of foxtail millet on germination and their relationship with *in vitro* antioxidant activity. Food Chem 2018;245:863–70; doi: https://doi.org/10.1016/j.foodchem.2017.11.093
9. Sibian MS, Saxena DC, Riar CS. Effect of germination on chemical, functional and nutritional characteristics of wheat, brown rice and triticale: a comparative study. J Sci Food Agric 2017;97(13):4643–51; doi: https://doi.org/10.1002/jsfa.8336
10. Ganapathyswamy H, Subramani T, Kalaivanan K, Pannerselvam A, Sundararajan A. Effect of germinated brown rice supplementation on prediabetes markers: a 120-day dietary intervention study in selected female groups. Rice Sci 2025;32(1):11–4; doi: https://doi.org/10.1016/j.rsci.2024.08.006
11. Subramani T, Ruckmangathan S, Ganapathyswamy H, Palanisamy S, Palaniappan M, Sankar SL, et al. Effect of optimized germination on nutritional functional and phytochemical characteristics of green gram. Sci Rep. 2026;16:13821; doi: https://doi.org/10.1038/s41598-026-42908-y
12. AACC. Approved Methods of the American Association of Cereal Chemists. Vol. 1. Amer Assn of Cereal Chemists, St. Paul, MN, 2000.
13. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 1999;299:152–178; doi: https://doi.org/10.1016/S0076-6879(99)99017-1
14. Lim YY, Lim TT, Tee JJ. Antioxidant properties of several tropical fruits: a comparative study. Food Chem 2007;103(3):1003–8; doi: https://doi.org/10.1016/j.foodchem.2006.08.038
15. Bongjo NB, Aondoaver AS, Gbertyo JA, Fulai AMA, Nchung L. Nutritional and functional properties of wheat-defatted peanut-orange peel composite flour. Eur J Nutramp; Food Saf. 2022;74–86; doi: https://doi.org/10.9734/ejnfs/2022/v14i121283
16. Kumar A, Kaur A, Gupta K, Gat Y, Kumar V. Assessment of germination time of finger millet for value addition in functional foods. Curr Sci 2021;120(2):406–413; doi: https://doi.org/10.18520/cs/v120/i2/406-413
17. Mir SA, Bosco SJD. Effect of soaking temperature on physical and functional properties of parboiled rice cultivars grown in temperate Region of India. Food Nutr Sci 2013;04(03):282–8; doi: https://doi.org/10.4236/fns.2013.43038
18. Li C, Oh SG, Lee DH, Baik HW, Chung HJ. Effect of germination on the structures and physicochemical properties of starches from brown rice, oat, sorghum, and millet. Int J Biol Macromol 2017;105:931–9; doi: https://doi.org/10.1016/j.ijbiomac.2017.07.123
19. Upretee P, Bandara MS, Tanino KK. The role of seed characteristics on water uptake preceding germination. Seeds 2024;3(4):559–74; doi: https://doi.org/10.3390/seeds3040038
20. Subramani T, Ganapathyswamy H, Sampathrajan V, Thiyagamoorthy U. Study on various green aqueous extraction techniques for extracting cottonseed milk to improve nutritional quality. J Cott Res 2026;9(1): 1–15; doi: https://doi.org/10.1186/s42397-025-00248-8
21. Suma PF, Urooj A. Nutrients, antinutrients & bioaccessible mineral content (invitro) of pearl millet as influenced by milling. J Food Sci Technol. 2014;51(4):756–761; doi: https://doi.org/10.1007/s13197-011-0541-7
22. Krishnan R, Meera MS. Pearl millet minerals: effect of processing on bioaccessibility. J Food Sci Technol 2018;55(9):3362–72; doi: https://doi.org/10.1007/s13197-018-3305-9
23. Dueñas M, Hernández T, Estrella I, Fernández D. Germination as a process to increase the polyphenol content and antioxidant activity of lupin seeds (*Lupinus angustifolius* L.). Food Chem 2009;117(4):599–607; doi: https://doi.org/10.1016/j.foodchem.2009.04.051
24. Gan RY, Lui WY, Wu K, Chan CL, Dai SH, Sui ZQ, *et al.* Bioactive compounds and bioactivities of germinated edible seeds and sprouts: an updated review. Trends Food Sci Technol 2017;59:1–14; doi: https://doi.org/10.1016/j.tifs.2016.11.010
25. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem 2005;53(10):4290–302; doi: https://doi.org/10.1021/jf0502698
26. Shahidi F, Ambigaipalan P. Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects—a review. J Funct Foods 2015;18:820–97; doi: https://doi.org/10.1016/j.jff.2015.06.018
27. Badau MH, Nkama I, Jideani IA. Phytic acid content and hydrochloric acid extractability of minerals in pearl millet as affected by germination time and cultivar. Food Chem 2005;92(3):425–35; doi: https://doi.org/10.1016/j.foodchem.2004.08.006
28. Owolade SO, Ezekiel OO, Aderibigbe OR. Effects of germination periods on proximate, mineral, and antinutrient profiles of pearl millet (*Pennisetum glacum*) and grain amaranth (*Amaranthus cruentus*) flours. Biol Life Sci Forum. 2024;40(1):9; doi: https://doi.org/10.3390/blsf2024040009
29. Li W, Lai X, Gu T, Wang H, Zhang Z. Seed germination ecology of eclipta (*Eclipta prostrata*) in dry direct-seeded rice fields from China. Weed Sci 2025;73(1): 1–8; doi: https://doi.org/10.1017/wsc.2025.10045
30. Shahidi F, Yeo J. Insoluble-bound phenolics in food. Molecules 2016;21(9):1216; doi: https://doi.org/10.3390/molecules21091216
31. Saleh ASM, Zhang Q, Chen J, Shen Q. Millet Grains: nutritional quality, processing, and potential health benefits. Compr Rev Food Sci Food Saf 2013;12(3):281–95; doi: https://doi.org/10.1111/1541-4337.12012
32. Devi PB, Vijayabharathi R, Sathyabama S, Malleshi NG, Priyadarisini VB. Health benefits of finger millet (*Eleusine coracana* L.) polyphenols and dietary fiber: a review. J Food Sci Technol 2014;51(6):1021–40; doi: https://doi.org/10.1007/s13197-011-0584-9

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