

Effect of Soaking, Germination, and Roasting on Physical, Nutritional, Bioactive, and Antinutritional Properties of Underutilized Grains.

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Abstract

Some of the underutilized grains that received a lot of scientific attention are foxtail millet (*Setaria italica*), kodo millet (*Paspalum scrobiculatum*) and amaranth (*Amaranthus* spp.) as nutrient dense alternatives which may improve food and nutritional reliability. In the existing study, the physical, functional, optical, nutritional, bioactive and antinutritional properties of three grains in raw, soaked (12 and 24 h), germinated (24, 48 and 72 h) and roasted (180°C, 10 s) states were systematically evaluated. Among the raw grains, amaranth showed highest crude protein (13.04%), total phenolic content (53.84 mg GAE/100 g), anthocyanin content (322.78 mg CGE/kg) and antioxidant activity (18.11%) while foxtail millet showed highest crude fat (3.42%) and calorific value (351.24 kcal/100 g) and kodo millet showed highest crude fiber (7.18%). Progressive germination (72 h) resulted in a substantial ($p < 0.05$) surge in crude protein (17.88%, 25.88% and 32.98%) in foxtail millet, kodo millet and amaranth respectively with decline in antinutritional factors such as phytic acid (48.50-57.43%), tannins (60.00-70.83%) and trypsin inhibitor activity (19.10-34.90%). Germination enhanced the total phenolic content, flavonoids and DPPH radical scavenging antioxidant action significantly in all the three grains. Roasting at 180°C led to an effective reduction in moisture content and antinutritional factors, especially trypsin inhibitor activity (4.27-48.04%) with maximum reduction in foxtail millet. The results offer comprehensive evidence based on the application of germination and roasting as low cost and scalable processing interventions to expand the nutritional quality and bioavailability of underutilized grains with major implications for food product development, functional food formulation, and dietary divergence strategies in food-insecure regions.

Keywords: underutilized grains, germination, soaking, roasting, antinutritional factors, bioactive compounds

Introduction

One of the leading concerns of the twenty-first century is food safety. It is assessed that there are 735 million people that endure chronic undernourishment in the world (Food and Agriculture Organization [FAO], 2023). Cereal crops such as rice, wheat and maize are still the mainstay of agricultural production and diet, but their nutritional constraints, particularly in terms of protein quality, micronutrient density and bioactive compounds, have prompted scientists to seek alternative grains (Saleh et al., 2013). Millet and pseudocereals are underutilized grains that contain a reservoir of nutritional and functional diversity that might be a key role in combating micronutrient deficiencies and diet-related chronic diseases (Chandrasekara & Shahidi, 2012).

Foxtail millet (*Setaria italica*) is an ancient cereal, among the oldest cultured crops in the world, with domestication in China dated to approximately 8,000 years ago rendering to archaeological attestation (Lu et al., 2009). It is a hardy C4 cereal that is resilient to drought and grows well in semi-arid and arid environments with limited agricultural inputs and therefore is an ecologically sustainable crop for resource-limited farming systems (Diao, 2007). Nutritionally, foxtail millet is characterized by its protein content of 11–12%, a relatively high crude fat content (~3.5%) and a loaded profile of dietary fiber and vital minerals (Sharma & Niranjana, 2018). Moreover, it has a remarkable antioxidant activity owing to its polyphenolic concentration that consists phenolic acids and flavonoids (Chandrasekara & Shahidi, 2011).

Kodo millet (*Paspalum scrobiculatum*) is mainly cultivated in the Indian subcontinent of Africa and is a staple grain for many rural communities (Deshpande et al., 2015). Kodo millet is abundant in crude fibre (~7%) which

is higher than most of the major cereals and is accountable for its desirable glycaemic index (Annor et al., 2017). It also provides to be an excellent source of B-complex vitamins, iron and calcium (Hegde et al, 2005). However, the existence of antinutritional factors which include phytic acid and tannins confines the bioavailability of its minerals and proteins, a challenge that is common to many millet species (Kumar et al., 2018).

Amaranth (*Amaranthus* spp.) pseudocereal that has been millennia in Mesoamerica and Andean region (Rastogi & Shukla, 2013). Amaranth differs from true cereals as it is a dicotyledonous plant with a distinct amino acid profile and relatively high lysine content, which compensates for the amino acid deficiencies of traditional cereal grains (Bressani, 2003). Amaranth is further characterized by its elevated total phenolic concentration, anthocyanin levels and antioxidant activity, all of which contribute to significant health-promoting benefits (Pasko et al., 2009). It contains 13-15% protein which is higher than most millets and thus a very valuable crop in the fight against protein-energy malnutrition (Venskutonis & Kraujalis, 2013).

These grains have a great nutritional potential but their use is restrained by the presence of antinutritional factors (ANFs) such as phytic acid, tannins, trypsin inhibitors and saponins. Phytic acid (myo-inositol hexakisphosphate) forms insoluble complexes using divalent cations such as iron, zinc and calcium which lowers the intestinal absorption and bioavailability of these cations (Gupta et al., 2015). Tannins are polyphenolic compounds that bind proteins and are resistant to enzymatic digestion decreasing the digestibility of protein (Samtiya et al., 2020). Trypsin inhibitors are protease inhibitors that inhibit pancreatic trypsin activity, resulting in a decrease in the efficiency of protein hydrolysis in the gastrointestinal tract (Gilani et al., 2012). All these ANFs combined reduce the nutritional value of underutilized grains and require processing strategies to overcome their effects.

For millennia, various traditional food treating methods like soaking, germination, fermentation and roasting have been applied in different cultures to refine palatability, digestibility and nutritional standard of cereal grains and legumes (Hotz & Gibson, 2007). The simplest kind of pre-treatment is soaking. This involves soaking the grains in water for extended durations. This process stimulates endogenous phytase and other hydrolytic enzymes, leading to the breakdown of phytic acid and other ANFs (Liang et al., 2008). During germination, the biochemical processes initiated during soaking are intensified with the increase of seed metabolism, including the synthesis of hydrolytic enzymes, mobilization of storage proteins and de novo biosynthesis of bioactive compounds such as vitamins, phenolics and gamma-aminobutyric acid (GABA) (Gan et al., 2017). Roasting is a dry-heat treatment that causes Maillard reactions, thermal degradation of thermolabile ANFs and structural changes of starch and protein, which concurrently alter the nutritional and sensory characteristics of grains (Jogihalli et al., 2017).

Specific processing treatments have been examined for their effects on specific grain types in individual studies. However, there is a major gap in the literature for comprehensive, comparative assessments that systematically evaluate multiple processing modalities across several underutilized grain species under standardized experimental conditions. Such comparative data are critical for the identification of optimal processing strategies tailored to the specific compositional characteristics of individual grains plus for directing the progress of value-added food products. Thus, the present study was carried out to (a) regulate the physical, functional, optical, nutritional, bioactive and antinutritional properties of foxtail millet, kodo millet and amaranth in raw form; (b) investigate the effects of soaking (12 and 24 h), germination (24, 48 and 72 h) and roasting (180°C, 10 s) on the nutritional, bioactive and antinutritional profiles of the three grains; and (c) suggest the best processing interventions for improving the nutritional quality along with reducing antinutritional factors in these underutilized grain species.

2. Materials and Methods

2.1 Materials and Chemicals

Three underutilized grain species selected for this investigation: foxtail millet (*Setaria italica*), kodo millet (*Paspalum scrobiculatum*), and amaranth (*Amaranthus* spp.). All grain samples were procured from local markets and cleaned by removing foreign matter, broken seeds, and other impurities. Analytical-grade chemicals and reagents, including Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, rutin, sodium phytate, tannic acid, aluminum chloride, and benzoyl DL-arginine para-nitroanilide hydrochloride, were procured from Hi-Media Laboratories (Mumbai, India) and Sigma-Aldrich (St. Louis, MO, USA).

2.2 Processing Treatments

2.2.1 Soaking

Soaking was performed according to the method described by Egli et al. (2002). Cleaned raw seeds were immersed in distilled water at a grain-to-water ratio of 1:5 (w/v) and maintained at ambient room temperature for 12 and 24 h. Following soaking, the water was decanted and the grains were dried in a hot air oven at 40°C for 24 h until a constant weight was achieved. Dried samples were stored in airtight polyethylene pouches at 4°C until further analysis.

2.2.2 Germination

For germination, cleaned raw seeds were first soaked in distilled water (1:5, w/v) for 16 h as a pre-treatment. Following decantation of the soaking water, the hydrated seeds were spread on trays lined with moist muslin cloth and incubated at 25°C for 24, 48, and 72 h to facilitate germination (Egli et al., 2002). The muslin cloth was periodically sprinkled with distilled water to maintain adequate moisture. After each germination period, the germinated seeds were dried at 40°C for 24 h, ground into flour, and stored at 4°C in airtight pouches.

2.2.3 Roasting

Roasting was carried out following the method of Sindhu et al. (2019). Raw grain seeds were placed on a preheated iron plate maintained at 180°C and roasted for 10 seconds with continuous stirring to ensure uniform heat distribution. The roasted grains were cooled to ambient temperature, pulverized using a laboratory grinder, sieved through a 60-mesh sieve, and stored at 4°C in heat-sealable pouches.

2.3 Physical and Functional Property Analysis

Thousand grain weight was determined according to AACC (2000) approved methods. Bulk density was measured using the volumetric displacement method described by Huang et al. (2005), while tap density was determined following Jones et al. (2000). Swelling capacity was estimated according to Williams et al. (1983) by measuring volumetric changes after 24 h of hydration. Water absorption capacity (WAC) was determined using the centrifugation method described by Sosulski (1962), and oil absorption capacity (OAC) was measured following Kaur et al. (2015). Water solubility index (WSI) was estimated from the dried supernatant obtained during WAC determination, following Stojceska et al. (2008). Color parameters (*L*, *a*, *b*) were measured using a Konica Minolta chromameter (CR-400, Konica Minolta, Japan), where *L* represents lightness (0 = black, 100 = white), *a* represents the green (–) to red (+) axis, and *b* represents the blue (–) to yellow (+) axis.

2.4 Proximate Composition Analysis

Moisture content was determined by the air-oven drying method at 105°C (Association of Official Analytical Chemists [AOAC], 1990). Total ash content was estimated by dry ashing in a muffle furnace at 525°C for 4–6 h (Ranganna, 1986). Crude fat was extracted using a Soxhlet apparatus (Soxoplus, Pelican Instruments) with acetone as the solvent (Ranganna, 1986). Crude fiber was determined by sequential acid–alkali digestion using a Fibroplus system (Pelican Instruments) with 1.25% H₂SO₄ and 1.25% NaOH, followed by ashing at 550°C (Ranganna, 1986). Crude protein was estimated by the Kjeldahl method (digestion, distillation, and titration) using a Kjeloplus system (Pelican Instruments) with a nitrogen-to-protein conversion factor of 6.25 (Ranganna, 1986). Total carbohydrate content was calculated by the difference method (World Health Organization [WHO], 1973), and calorific value was computed using Atwater factors of 4.0, 9.10, and 4.2 kcal/g for protein, fat, and carbohydrates, respectively (WHO, 1973).

2.5 Antinutritional Factor Analysis

Phytic acid content was determined by the modified colorimetric method using Wade reagent, as described by Gao et al. (2007), with sodium phytate as the standard. Tannin content was determined by the Folin-Ciocalteu method (Saxena et al., 2013) with tannic acid as a reference standard, and absorbance was measured at 700 nm. Trypsin inhibitor activity (TIA) was determined using benzoyl DL- arginine para-nitroanilide hydrochloride as substrate according to the modified method of Kakade et al. (1974), and absorbance was read at 410 nm.

2.6 Bioactive Compound and Antioxidant Analysis

Total phenolic content (TPC) was determined by the Folin–Ciocalteu method (Ainsworth and Gillespie, 2007) with gallic acid as the standard, and results were expressed as mg gallic acid equivalent (GAE) per 100 g of sample. Total flavonoid content was determined by the aluminum chloride colorimetric method (Lahlou et al., 2014) using rutin as the standard, and results were expressed as mg quercetin equivalent (QE) per 100 g. Anthocyanin content was quantified spectrophotometrically and reported as mg cyanidin-3-glucoside equivalent (CGE) per kg. Antioxidant activity was evaluated using the DPPH radical scavenging assay modified by Bouaziz et al. (2008) by measuring the percentage inhibition of the DPPH radical at 520 nm.

2.7 Statistical Analysis

All analyses were conducted in triplicates and results were expressed as mean ± standard deviation (SD). Data were analyzed using the SPSS version 16.0 (IBM Corp., Armonk, NY, USA) for one-way and two-way analysis of variance (ANOVA). Post hoc comparisons were carried out using Duncan's least significant difference (LSD) test. Statistical significance was set at $p \leq 0.05$.

3. Results and Discussion

3.1 Physical, Functional, and Optical Properties of Raw Grains

Table 1 summarizes physical, functional and optical assets of the three underutilized grains. Significant ($p \leq 0.05$) inter-species variances were observed in all the parameters which are indicative of the inherent contrasts in the morphology and composition of foxtail millet, kodo millet and amaranth.

Table 1 Physical, Functional, and Optical Properties of Underutilized Grains

Parameters	Amaranth	Kodo Millet	Foxtail Millet
Thickness (mm)	1.18±0.20 ^c	2.01±0.15 ^c	1.79±0.60 ^d
Width (mm)	1.48±0.08 ^c	2.08±0.07 ^d	2.65±0.49 ^c
Length (mm)	1.76±0.15 ^c	4.02±0.20 ^d	5.81±0.54 ^c
1000 kernel weight (g)	3.92±0.19 ^c	4.15±0.18 ^d	11.45±0.48 ^c
Bulk density (g/ml)	0.97±0.02 ^a	0.92±0.01 ^b	0.94±0.02 ^b
Tap density (g/ml)	1.11±0.10 ^b	1.05±0.09 ^c	1.06±0.10 ^c
WAC (ml/g)	2.02±0.62 ^a	1.01±0.11 ^d	1.21±0.12 ^c
OAC (ml/g)	0.97±0.14 ^c	1.52±0.14 ^a	1.17±0.18 ^d
Swelling capacity (%)	58.84±2.01 ^d	62.45±2.18 ^b	56.21±3.12 ^c
Solubility index (%)	7.28±0.12 ^b	6.61±0.25 ^c	5.81±0.48 ^d
L*	64.78±2.05 ^b	71.86±2.34 ^a	46.95±1.39 ^c
a*	2.36±0.06 ^b	3.65±0.23 ^a	1.95±0.10 ^d
b*	0.42±0.19 ^a	0.29±0.28 ^b	-0.21±0.29 ^d

Note. Values are presented as mean $\pm$ SD ($n = 3$). Values within rows sharing the same superscript letters are not significantly different according to Duncan's LSD post hoc test at $p \leq 0.05$. WAC = water absorption capacity; OAC = oil absorption capacity.

Foxtail millet had largest kernel dimensions of the three grains, with length 5.81 ± 0.54 mm, width 2.65 ± 0.49 mm, and thickness 1.79 ± 0.60 mm, whereas amaranth had the smallest kernels (length 1.76 ± 0.15 mm, width 1.48 ± 0.08 mm, thickness 1.18 ± 0.20 mm). Similarly, 1000-kernel weight was also found to follow similar pattern in which foxtail millet (11.45 ± 0.48 g) was significantly heavier than kodo millet (4.15 ± 0.18 g) and amaranth (3.92 ± 0.19 g). These differences in dimensions are due to genotypic differences and have practical implications for the design of grain processing equipment and milling characteristics (Balasubramanian and Viswanathan, 2010).

Bulk density varied from 0.92 ± 0.01 g/ml (kodo millet) to 0.97 ± 0.02 g/ml (amaranth) and tap density from 1.05 ± 0.09 g/ml (kodo millet) to 1.11 ± 0.10 g/ml (amaranth). Amaranth has higher bulk and tap densities due to its smaller, more spherical grain morphology, which allows for tighter packing arrangements. These density parameters are important to the food industry with regard to packaging, storage and transportation logistics (Shimelis et al., 2006).

Water absorption capacity (WAC) was maximum in amaranth (2.02 ± 0.62 ml/g) which was 2 times higher than kodo millet (1.01 ± 0.11 ml/g). The higher WAC of amaranth might be due to its higher protein content and the availability of more hydrophilic amino acid side chains, which interact with water molecules by hydrogen bonding (Kaur & Singh, 2005). In contrast, kodo millet showed the highest oil absorption capacity (OAC) (1.52 ± 0.14 ml/g), suggesting a greater ratio of non-polar amino acid residues and hydrophobic surface area in the kodo millet flour particles. OAC is a functional property of importance in the formulation of food products, especially in fried foods and emulsion systems (Kinsella, 1979). The highest swelling capacity was found in kodo millet ($62.45 \pm 2.18\%$) which is in agreement with its high starch content and the capability of its starch granules to imbibe water and swell in the process of hydration.

Color analysis showed maximum lightness for kodo millet ($L = 71.86 \pm 2.34$) followed by amaranth ($L = 64.78 \pm 2.05$) and foxtail millet ($L = 46.95 \pm 1.39$). The very low L value of foxtail millet indicates darker grain color, which may be attributed to the higher concentrations of pigments like carotenoids and phenolic compounds in the pericarp (Dykes & Rooney, 2006). In kodo millet, the a values of the red–green axis were positive for all the grains with the maximum redness ($a = 3.65 \pm 0.23$). The b values showed slight yellowness for amaranth and kodo millet and slight blueness for foxtail millet ($b = -0.21 \pm 0.29$), which agrees with the presence of anthocyanin-type pigments.

3.2 Nutritional, Bioactive, and Antinutritional Properties of Raw Grains

Moisture content ranged from $\pm 0.05\%$ (foxtail millet) to $10.86 \pm 0.95\%$ (amaranth). All the three grains had moisture levels within safe storage limits of $\leq 14\%$, indicating good drying and storage conditions (Brooker et al., 1992). Crude protein contents were highest in amaranth ($13.04 \pm 0.89\%$), which agrees with the well-established

better protein status of pseudocereals than true millets. Amaranth protein is especially known for its high lysine content (5.2 g/100 g protein), which is almost double that of wheat and three times that of maize (Bressani, 2003). Foxtail millet had the second highest protein content ($11.52 \pm 0.05\%$) and kodo millet had the lowest ($9.12 \pm 0.85\%$). The protein contents are like those stated by Saleh et al. (2013) and Deshpande et al. (2015) for millets grown in similar agroclimatic conditions. Table 2 exhibits the proximate composition, bioactive compound content, and antinutritional factor levels of the three raw grains.

Table 2: Nutritional, Bioactive, and Antinutritional Properties of Underutilized Grains

Parameters	Foxtail Millet	Kodo Millet	Amaranth
Moisture content (%)	8.74 ± 0.05^c	9.21 ± 0.04^b	10.86 ± 0.95^a
Crude fat (%)	3.42 ± 0.03^c	2.28 ± 0.05^b	2.73 ± 0.88^d
Crude fiber (%)	5.26 ± 0.29^c	7.18 ± 0.04^b	3.71 ± 0.03^d
Ash (%)	2.91 ± 0.65^b	2.74 ± 0.57^d	2.95 ± 0.79^c
Crude protein (%)	11.52 ± 0.05^d	9.12 ± 0.85^e	13.04 ± 0.89^b
Carbohydrates (%)	68.15 ± 0.92^c	69.07 ± 1.01^b	66.71 ± 1.15^d
Calorific value (kcal/100 g)	351.24 ± 2.12^c	336.48 ± 2.25^d	342.86 ± 2.94^b
Tannins (mg/g)	0.45 ± 0.19^a	0.24 ± 0.10^c	0.21 ± 0.11^c
Phytic acid (mg/g)	1.67 ± 0.10^a	1.48 ± 0.12^b	1.18 ± 0.14^c
Antioxidant activity (%)	12.74 ± 0.28^d	13.22 ± 0.26^c	18.11 ± 1.95^b
TPC (mg GAE/100 g)	33.45 ± 3.01^d	25.08 ± 2.98^c	53.84 ± 3.18^b
Anthocyanins (mg CGE/kg)	98.36 ± 1.12^d	86.45 ± 1.15^c	322.78 ± 3.42^c
Flavonoids (mg QE/100 g)	12.41 ± 1.08^d	13.74 ± 1.55^c	11.82 ± 1.95^c
TIA (mg TIA/100 g)	25.12 ± 1.43^a	18.76 ± 1.02^c	14.01 ± 1.06^c

Note. Values are presented as mean $\pm$ SD ($n = 3$). Values within rows sharing the same superscript letters are not significantly different at $p \leq 0.05$. TPC = total phenolic content; TIA = trypsin inhibitor activity.

Foxtail millet had crude fat ($3.42 \pm 0.03\%$) while amaranth ($2.73 \pm 0.88\%$) and kodo millet ($2.28 \pm 0.05\%$) had lower values. The lipid fraction in millets and amaranth contains a high amount of unsaturated fatty acids, particularly oleic and linoleic acid, which are correlated with cardiovascular health benefits (Sharma & Niranjana, 2018). The crude fiber content was significantly higher in kodo millet ($7.18 \pm 0.04\%$) than in foxtail millet ($5.26 \pm 0.29\%$) and amaranth ($3.71 \pm 0.03\%$). Kodo millet with its relatively high fiber content is a suitable ingredient for dietary formulations targeting glycemic control and gut health. Dietary fiber can regulate postprandial glucose response and stimulate colonic fermentation to generate beneficial short-chain fatty acids (Annor et al., 2017). Amongst the bioactive compounds, amaranth had significantly higher levels of total phenolic content (53.84 ± 3.18 mg GAE/100 g), anthocyanins (322.78 ± 3.42 mg CGE/kg) and DPPH antioxidant activity ($18.11 \pm 1.95\%$). The anthocyanin content of amaranth was around 3.3 and 3.7 times higher than foxtail millet (98.36 ± 1.12 mg CGE/kg) and kodo millet (86.45 ± 1.15 mg CGE/kg) respectively. Anthocyanins are the water-soluble pigments of the flavonoid class that have occurred exhibiting anti-inflammatory, anti-carcinogenic and cardioprotective impacts in epidemiological and experimental studies (Li et al., 2017). Amaranth has a very high content of anthocyanins, which is due to betacyanins and other pigmented polyphenols present in the seed coat (Pasko et al., 2009).

With respect to antinutritional factors, foxtail millet proved to have the highest content of tannins (0.45 ± 0.19 mg/g), phytic acid (1.67 ± 0.10 mg/g) and trypsin inhibitor activity (25.12 ± 1.43 mg TIA/100 g). On the contrary, amaranth recorded the lowest phytic acid (1.18 ± 0.14 mg/g) and trypsin inhibitor activity (14.01 ± 1.06 mg TIA/100 g). Foxtail millet has a relatively high amount of phytic acid, which is a major concern for nutrition because phytic acid diminishes the bioavailability of essential minerals by 25–60% depending on the molar ratio

of phytate to the specific mineral (Gupta et al., 2015). These results underline the necessity to apply appropriate processing treatments to reduce ANF levels before consumption or incorporation in food products.

3.3 Effect of Soaking and Germination on Foxtail Millet

Table 3 reports the influence of soaking (12 and 24 h) and germination (24, 48, and 72 h) on the nutritional, bioactive, and antinutritional properties of foxtail millet. The germination process brought significant modifications in the nutritional profile of foxtail millet. The crude protein increased from 11.52 ± 0.05 % in the raw grain to 13.58 ± 0.08 % after 72 h of germination (17.88%). The rise in protein content is primarily ascribed to the de novo synthesis of proteins during seedling growth, the accumulation of nitrogenous compounds as dry matter is broken down during respiration, and the hydrolysis of storage proteins to soluble forms (Lemmens et al., 2019). At the same time, crude fiber increased significantly from 5.26 ± 0.29 % to 7.86 ± 0.19 % (49.43% increase), due to the biosynthesis of structural cell wall polysaccharides, such as cellulose and hemicellulose during elongation of radicle and plumule (Gan et al., 2017).

Table 3 Effect of Soaking and Germination on Nutritional, Bioactive, and Antinutritional Properties of Foxtail Millet

Parameters	Raw (0 h)	Soaking 12 h	Soaking 24 h	Germ. 24 h	Germ. 48 h	Germ. 72 h
Moisture (%)	8.74 ± 0.05^a	8.31 ± 0.04^b	8.15 ± 0.03^b	7.96 ± 0.04^c	7.71 ± 0.05^c	7.45 ± 0.06^d
Crude fat (%)	3.42 ± 0.03^a	3.31 ± 0.04^a	3.24 ± 0.05^b	3.16 ± 0.04^b	3.05 ± 0.05^c	2.91 ± 0.06^c
Crude fiber (%)	5.26 ± 0.29^c	5.91 ± 0.21^d	6.08 ± 0.18^d	6.58 ± 0.17^c	7.22 ± 0.15^b	7.86 ± 0.19^a
Ash (%)	2.91 ± 0.65^a	2.82 ± 0.54^a	2.75 ± 0.47^b	2.70 ± 0.43^b	2.66 ± 0.39^b	2.62 ± 0.41^b
Crude protein (%)	11.52 ± 0.05^c	11.86 ± 0.07^c	12.14 ± 0.08^d	12.66 ± 0.09^c	13.11 ± 0.07^b	13.58 ± 0.08^a
Carbohydrates (%)	68.15 ± 0.92^a	67.89 ± 0.85^a	67.52 ± 0.81^a	66.94 ± 0.76^b	66.18 ± 0.69^c	65.58 ± 0.72^c
Cal. value (kcal)	351.24 ± 2.12^a	349.96 ± 2.05	348.84 ± 2.10^b	347.23 ± 2.18^b	344.95 ± 2.01^c	342.88 ± 2.15^c
Tannins (mg/g)	0.45 ± 0.19^a	0.43 ± 0.15	0.40 ± 0.14^c	0.28 ± 0.10^d	0.22 ± 0.09^e	0.18 ± 0.08
Phytic acid (mg/g)	1.67 ± 0.10^a	1.49 ± 0.09^b	1.35 ± 0.08	1.14 ± 0.07^c	0.98 ± 0.05^d	0.86 ± 0.04^d
Antioxidant (%)	12.74 ± 0.28^d	13.46 ± 0.21^c	13.88 ± 0.25^c	14.39 ± 0.27^c	15.12 ± 0.31^b	17.28 ± 0.35^a
TPC (mg GAE)	33.45 ± 3.01^d	33.92 ± 2.78	34.58 ± 2.54^c	35.26 ± 2.31^c	36.48 ± 2.18^b	38.92 ± 2.11^a
Flavonoids (mg QE)	12.41 ± 1.08^c	13.26 ± 1.01^d	13.95 ± 0.96^d	14.88 ± 0.91^c	16.97 ± 0.88^b	19.12 ± 0.92^a
TIA (mg/100g)	25.12 ± 1.43^a	22.96 ± 1.38^b	21.54 ± 1.32^c	19.32 ± 1.25^d	18.04 ± 1.21^d	16.82 ± 1.18^e

Note. Values are presented as mean $\pm$ SD ($n = 3$). Values within rows sharing the same superscript letters are not significantly different at $p \leq 0.05$.

Crude fat decreased 3.42 ± 0.03 % to 2.91 ± 0.06 % during germination period, which was a reduction of 14.91 %. This reduction is owed to the activation of lipase during germination, which mobilizes stored lipids as energy sources for embryonic growth through the β -oxidation of fatty acids (Chung et al., 1989). The carbohydrate content was also reduced gradually from 68.15 ± 0.92 % to 65.58 ± 0.72 % that is indicative of the use of starch reserves through amylolytic activity to provide energy for metabolic activities during germination (Hubner & Arendt, 2013).

Phytic acid decreased from 1.67 ± 0.10 mg/g to 0.86 ± 0.04 mg/g after 72 h germination, corresponding to 48.50% decrease. This decrease is mainly mediated by the activation of endogenous phytase (myo-inositol hexakisphosphate phosphohydrolase, EC 3.1.3.26) that catalyzes the sequential dephosphorylation of phytic acid to lower inositol phosphates and inorganic phosphate (Liang et al., 2008). Likewise, the tannin content diminished by 60.00% (from 0.45 ± 0.19 to 0.18 ± 0.08 mg/g) possibly because of the loss of water-soluble tannins through leaching during soaking, enzymatic oxidation by polyphenol oxidase, and polymerization of tannins into insoluble

high-molecular-weight complexes during germination (Samtiya et al., 2020). The trypsin inhibitor activity was reduced by 33.04% (from 25.12 ± 1.43 to 16.82 ± 1.18 mg TIA/100 g) because of the proteolytic degradation of Kunitz-type trypsin inhibitor proteins during germination (Gilani et al., 2012).

The levels of bioactive compound generally increased with the germination time. Total phenolic content rose from 33.45 ± 3.01 to 38.92 ± 2.11 mg GAE/100 g (16.35 % increase), flavonoids increased from 12.41 ± 1.08 to 19.12 ± 0.92 mg QE/100 g (54.07 % increase), and DPPH antioxidant activity elevated from 12.74 ± 0.28 % to 17.28 ± 0.35 % (35.64 % increase). The rise in phenolic compounds during germination corresponds to the activation of phenylalanine ammonia-lyase (PAL), the key enzyme of the phenylpropanoid pathway, and release of bound phenolics from cell wall matrices by esterases and cell wall-degrading enzymes (Ti et al., 2014). Notably, the anthocyanin content showed a slight but significant decrease from 98.36 ± 1.12 to 95.76 ± 1.06 mg CGE/kg (−2.64%) which might be due to oxidative degradation and enzymatic conversion of anthocyanins during the metabolically active germination process (Kaukovirta-Norja et al., 2004).

3.4 Effect of Soaking and Germination on Kodo Millet

Table 4 summarizes the outcomes of soaking and germination treatments on kodo millet. Kodo millet exhibited similar trends to that of foxtail millet for soaking and germination treatments albeit the extent of adjustment was different for several parameters. The crude protein increased from 9.12 ± 0.85 % to 11.48 ± 0.59 % after 72h germination which was enhancement of 25.88 % . The higher protein content in kodo millet equated to foxtail millet might be caused by the higher initial starch content and higher amylolytic activity of kodo millet, which results in a greater relative loss of carbohydrate dry matter and thereby concentration of protein (Deshpande et al., 2015). Crude fiber significantly increased by 22.01% (7.18 ± 0.04 to 8.76 ± 0.09 %) and crude fat significantly decreased by 22.81% (2.28 ± 0.05 to 1.76 ± 0.06 %) with 3.03% reduction in carbohydrate content.

Table 4 Effect of Soaking and Germination on Nutritional, Bioactive, and Antinutritional Properties of Kodo Millet

Parameters	Raw (0 h)	Soaking 12 h	Soaking 24 h	Germ. 24 h	Germ. 48 h	Germ. 72 h
Moisture (%)	9.21 ± 0.04^a	9.05 ± 0.05^a	8.88 ± 0.04^a	8.71 ± 0.03^b	8.48 ± 0.04^b	8.24 ± 0.05^c
Crude fat (%)	2.28 ± 0.05^a	2.18 ± 0.04^b	2.08 ± 0.05^b	1.92 ± 0.04^c	1.85 ± 0.05^c	1.76 ± 0.06^d
Crude fiber (%)	7.18 ± 0.04^d	7.62 ± 0.06^c	7.84 ± 0.05^c	8.09 ± 0.07^b	8.58 ± 0.08^a	8.76 ± 0.09^a
Ash (%)	2.74 ± 0.57^b	2.82 ± 0.49^a	2.76 ± 0.45^b	2.71 ± 0.43^b	2.65 ± 0.41^c	2.58 ± 0.39^c
Crude protein (%)	9.12 ± 0.85^c	9.58 ± 0.78^d	10.04 ± 0.72^d	10.42 ± 0.68^c	10.91 ± 0.64^b	11.48 ± 0.59^a
Carbohydrates (%)	69.07 ± 1.01^a	68.72 ± 0.96^b	68.38 ± 0.91^b	68.01 ± 0.88^b	67.45 ± 0.82^c	66.98 ± 0.79^c
Cal. value (kcal)	336.48 ± 2.25^a	335.82 ± 2.11^a	334.96 ± 2.06^a	332.88 ± 2.02^b	331.45 ± 1.98^c	330.12 ± 2.01^c
Tannins (mg/g)	0.24 ± 0.10^a	0.23 ± 0.09^a	0.21 ± 0.08^b	0.15 ± 0.07^c	0.09 ± 0.03^d	0.07 ± 0.02^c
Phytic acid (mg/g)	1.48 ± 0.12^a	1.24 ± 0.10^b	1.05 ± 0.09^c	0.96 ± 0.08^d	0.81 ± 0.06^e	0.63 ± 0.05
Antioxidant (%)	13.22 ± 0.26^c	13.88 ± 0.24^d	14.26 ± 0.27^d	14.61 ± 0.28^c	15.74 ± 0.31^b	16.52 ± 0.35^a
TPC (mg GAE)	25.08 ± 2.98^c	26.12 ± 2.74^d	26.84 ± 2.56^d	27.45 ± 2.38^c	31.02 ± 2.15^b	33.18 ± 2.09^a
Flavonoids (mg QE)	13.74 ± 1.55^d	14.22 ± 1.42^d	14.88 ± 1.33^c	15.04 ± 1.25^c	17.48 ± 1.11^b	17.92 ± 1.08^a
TIA (mg/100g)	18.76 ± 1.02^a	17.21 ± 0.94^b	17.05 ± 0.91^b	16.38 ± 0.86^c	15.82 ± 0.83^c	15.18 ± 0.79^d

Note. Values are presented as mean $\pm$ SD ($n = 3$). Values within rows sharing the same superscript letters are not significantly different at $p \leq 0.05$.

Reduction in antinutritional factors was more pronounced in kodo millet. The highest percentage reduction of phytic acid was found in the three grains (from 1.48 ± 0.12 to 0.63 ± 0.05 mg/g, 57.43%). This finding shows that

kodo millet has a very active endogenous phytase system which is well induced during germination (Kumar et al., 2018). Tannin content reduced by 70.83% (0.24 ± 0.10 to 0.07 ± 0.02 mg/g) and trypsin inhibitor activity decreased by 19.10% (18.76 ± 1.02 to 15.18 ± 0.79 mg TIA/100 g). The lower percentage reduction of TIA as compared to phytic acid and tannins indicates that the trypsin inhibitors of kodo millet may be more thermostable or structurally resistant to proteolytic degradation as compared to those of foxtail millet and amaranth (Gilani et al., 2012).

The bioactive compound improvement was significant in total phenolic content (32.30%, 25.08 ± 2.98 to 33.18 ± 2.09 mg GAE/100 g), antioxidant activity (24.96%, $13.22 \pm 0.26\%$ to $16.52 \pm 0.35\%$) and flavonoids (30.42%, 13.74 ± 1.55 to 17.92 ± 1.08 mg QE/100 g). These improvements are consistent with the reported activation of phenolic biosynthesis pathways during germination and the release of matrix bound phenolics through disruption of cell wall (Chandrasekara & Shahidi, 2012).

3.5 Effect of Soaking and Germination on Amaranth

The impacts of soaking and germination on amaranth are exhibited in Table 5. Among the three grains, amaranth showed the most dramatic changes in nutrition upon germination. The crude protein content increased by 32.98% from $13.04 \pm 0.89\%$ in the raw grain to $17.34 \pm 0.62\%$ after 72 h germination. This rise in protein content is consistent with the high metabolic activity of germinating amaranth seeds, which produce large amounts of storage, enzymatic and structural proteins in the rapid growth of the seedlings (Colmenares de Ruiz & Bressani, 1990). The final protein content of 17.34% was higher than most of the common cereal foods like wheat (~12%), rice (~7%) and maize (~9%) suggesting the potential of germinated amaranth as a high protein ingredient for nutritional supplementation (Bressani, 2003).

Table 5 Effect of Soaking and Germination on Nutritional, Bioactive, and Antinutritional Properties of Amaranth

Parameters	Raw (0 h)	Soaking 12 h	Soaking 24 h	Germ. 24 h	Germ. 48 h	Germ. 72 h
Moisture (%)	10.86 ± 0.95^a	10.68 ± 0.88^b	10.52 ± 0.84^b	10.60 ± 0.79^b	10.08 ± 0.73^b	9.62 ± 0.68^c
Crude fat (%)	2.73 ± 0.88^a	2.61 ± 0.81^b	2.50 ± 0.75^b	2.16 ± 0.68^c	2.12 ± 0.65^c	2.08 ± 0.61^c
Crude fiber (%)	3.71 ± 0.03^d	3.92 ± 0.04^c	4.78 ± 0.05^a	4.15 ± 0.04^b	4.58 ± 0.05^b	4.74 ± 0.06^a
Ash (%)	2.95 ± 0.79^a	2.89 ± 0.71^a	2.84 ± 0.65^b	2.79 ± 0.61^b	2.75 ± 0.58^c	2.68 ± 0.55^c
Crude protein (%)	13.04 ± 0.89^d	13.36 ± 0.82^c	13.58 ± 0.76^c	14.29 ± 0.71^b	14.95 ± 0.66^b	17.34 ± 0.62^a
Carbohydrates (%)	66.71 ± 1.15^a	66.54 ± 1.08^a	65.62 ± 1.02^b	65.96 ± 0.95^b	65.48 ± 0.88^c	63.42 ± 0.82^d
Cal. value (kcal)	342.86 ± 2.94^a	342.42 ± 2.71^a	338.35 ± 2.58^c	339.78 ± 2.45^c	340.02 ± 2.31^c	341.68 ± 2.26^b
Tannins (mg/g)	0.21 ± 0.11^a	0.19 ± 0.10^b	0.17 ± 0.09^c	0.13 ± 0.08^d	0.10 ± 0.06^c	0.07 ± 0.05
Phytic acid (mg/g)	1.18 ± 0.14^a	1.10 ± 0.13^b	1.07 ± 0.12^b	1.07 ± 0.11^b	0.93 ± 0.10^c	0.59 ± 0.08^c
Antioxidant (%)	18.11 ± 1.95	23.24 ± 1.74^c	29.58 ± 1.42^d	30.62 ± 1.28^c	32.88 ± 1.11^b	38.12 ± 1.06^a
TPC (mg GAE)	53.84 ± 3.18^c	54.92 ± 2.95^d	55.46 ± 2.78^d	58.82 ± 2.51^c	62.05 ± 2.26^b	66.48 ± 2.12^a
Flavonoids (mg QE)	11.82 ± 1.95^d	12.31 ± 1.78^d	12.62 ± 1.62^c	13.68 ± 1.48^b	14.24 ± 1.35^b	16.28 ± 1.21^a
TIA (mg/100g)	14.01 ± 1.06^a	12.82 ± 0.98^b	12.65 ± 0.91^c	11.08 ± 0.85^d	10.82 ± 0.79^d	9.12 ± 0.72^c

Note. Values are presented as mean $\pm$ SD ($n = 3$). Values within rows sharing the same superscript letters are not significantly different at $p \leq 0.05$.

Amaranth also had the biggest improvement in bioactive compounds. The antioxidant activity increased significantly from $18.11 \pm 1.95\%$ to $38.12 \pm 1.06\%$, an increase of 110.49%, which was the highest among all grain-treatments in this study. The total phenolic content was increased by 23.48 % (from 53.84 ± 3.18 to 66.48 ± 2.12 mg GAE/100 g) and flavonoids by 37.73 % (from 11.82 ± 1.95 to 16.28 ± 1.21 mg QE/100 g). Specifically,

the content of amaranth anthocyanins increased slightly during germination (from 322.78 ± 3.42 to 328.16 ± 2.51 mg CGE/kg, +1.67%) compared to foxtail millet and kodo millet. This unique response implies that amaranth has an active anthocyanin biosynthetic pathway during germination, likely involving the upregulation of chalcone synthase and other flavonoid biosynthesis genes in response to light exposure and metabolic activation during seedling development (Pasko et al., 2009).

The decrease of antinutritional factors followed similar trends to the other grains. The phytic acid was reduced by 50.00% (1.18 ± 0.14 to 0.59 ± 0.08 mg/g), tannins by 66.67% (0.21 ± 0.11 to 0.07 ± 0.05 mg/g) and trypsin inhibitor activity by 34.90% (14.01 ± 1.06 to 9.12 ± 0.72 mg TIA/100 g). Moreover, the mineral analysis of germinated amaranth showed a significant increase in the bioavailable fractions of copper (+100.00%), manganese (+155.17%), iron (+31.47%), zinc (+68.48%), and calcium (+5.20%) which suggests that the decrease in phytic acid during germination directly translates to improved mineral bio-accessibility (Gupta et al., 2015).

3.6 Effect of Roasting on Nutritional and Antinutritional Properties

Table 6 displays the relative effects of roasting at 180°C for 10s on the nutritional, bioactive, and antinutritional properties of the three grains. Roasting showed a significant loss in moisture content of all the three grains. The losses were foxtail millet (−37.99%, from $8.74 \pm 0.05\%$ to $5.42 \pm 0.08\%$), kodo millet (−37.46%, from $9.21 \pm 0.04\%$ to $5.76 \pm 0.07\%$) and amaranth (−32.60%, from $10.86 \pm 0.95\%$ to $7.32 \pm 0.82\%$). Flash evaporation at high temperature during roasting results rapid moisture loss, and at the same time, enhanced the shelf life of the grain by reducing the water activity below the level for microbial growth and enzymatic degradation (Jogihalli et al., 2017).

Table 6 Effect of Roasting on Nutritional, Bioactive, and Antinutritional Properties of Foxtail Millet, Kodo Millet, and Amaranth

Parameters	Raw	Roasted	Raw	Roasted	Raw	Roasted
Moisture (%)	8.74±0.05	5.42±0.08	9.21±0.04	5.76±0.07	10.86±0.95	7.32±0.82
Crude fat (%)	3.42±0.03	4.24±0.05	2.28±0.05	2.61±0.06	2.73±0.88	3.38±0.74
Crude fiber (%)	5.26±0.29	5.01±0.24	7.18±0.04	6.95±0.05	3.71±0.03	3.42±0.04
Ash (%)	2.91±0.65	2.88±0.58	2.74±0.57	2.70±0.51	2.95±0.79	2.99±0.68
Crude protein (%)	11.52±0.05	11.26±0.07	9.12±0.85	8.88±0.74	13.04±0.89	12.28±0.81
Carbohydrates (%)	68.15±0.92	71.34±0.88	69.07±1.01	72.38±0.95	66.71±1.15	71.02±1.08
Calorific value	351.24±2.12	369.18±2.04	336.48±2.25	355.92±2.18	342.86±2.94	364.75±2.65
Tannins (mg/g)	0.45±0.19	0.35±0.15	0.24±0.10	0.15±0.08	0.21±0.11	0.16±0.08
Phytic acid (mg/g)	1.67±0.10	1.28±0.08	1.48±0.12	1.09±0.09	1.18±0.14	0.95±0.10
Antioxidant (%)	12.74±0.28	12.91±0.31	13.22±0.26	13.42±0.29	18.11±1.95	21.92±1.78
TPC (mg GAE/100g)	33.45±3.01	34.12±2.84	25.08±2.98	25.78±2.76	53.84±3.18	55.96±2.95
TIA (mg/100g)	25.12±1.43	13.06±1.18	18.76±1.02	17.95±0.94	14.01±1.06	9.18±0.82

Note. Values are presented as mean $\pm$ SD ($n = 3$).

A noteworthy observation was an increase in apparent carbohydrate content on roasting (foxtail millet: +4.68%, kodo millet: +4.79%, amaranth: +6.46%) along with an increase in apparent crude fat content. These changes are mostly artifacts of the concentration effect due to loss of moisture, and not true biosynthesis of macronutrients (Obadina et al., 2013). In all the grains, the crude protein content was slightly decreased (foxtail millet: −2.26%, kodo millet: −2.63%, amaranth: −5.83%), due to Maillard type reactions between reducing sugars and amino acids at high temperature leading to the formation of Amadori and Maillard compounds which are not detected by Kjeldahl nitrogen assay (Jogihalli et al., 2017). The crude fiber content also decreased slightly, likely due to the thermal degradation of hemicelluloses and other heat labile fiber components.

Roasting was efficient in lowering antinutritional factors, but generally to a lesser extent than 72 h germination. The reduction in phytic acid was 23.35% in foxtail millet, 26.35% in kodo millet and 19.49% in amaranth. Phytic acid roasting results in thermal degradation that cleaves ester bonds between the phosphate groups and the inositol ring, resulting in formation of lower inositol phosphates with reduced capacity to chelate minerals (Siddhuraju & Becker, 2001). Thermal depolymerization and oxidative degradation reduced the tannin content by 22.22%, 37.50% and 23.81% in foxtail millet, kodo millet and amaranth, respectively (Duodu et al., 2003). Roasting had the most significant influence on trypsin inhibitor activity, with foxtail millet exhibiting a reduction of 48.01% (from 25.12 ± 1.43 to 13.06 ± 1.18 mg TIA/100 g) and amaranth and kodo millet exhibiting 34.48 and 4.32% reductions, respectively. Kunitz-type serine protease inhibitors are thermolabile trypsin inhibitors, which are irreversibly denatured when exposed to heat, explaining the large reductions observed (Gilani et al., 2012).

Roasting affected the levels of bioactive compounds differently. All the grains exhibited a slight increase in total phenolic content (foxtail millet: +2.00%, kodo millet: +2.79%, amaranth: +3.94%) which may be ascribed to the release of bound phenolics from the food matrix and formation of Maillard reaction products with antioxidant activity (Chandrasekara & Shahidi, 2011). The antioxidant activity also slightly increased (amaranth: +21.04%; foxtail millet: +1.33%; kodo millet: +1.51%). In all the grains, the flavonoids increased (Foxtail millet: +21.19%, Kodo millet: +11.43%, amaranth: +10.66%) while the anthocyanin levels decreased slightly, which is consistent with the thermal sensitivity of anthocyanin pigments (Patras et al., 2010).

3.7 Comparative Evaluation of Processing Treatments

A critical comparative assessment of the three processing approaches demonstrated that germination (72 h) was the most effective treatment to simultaneously enhance the nutritional quality and decrease antinutritional factors in all three grain species. This germination superiority is due to the unique nature of the process, which is biologically mediated and exploits the metabolic machinery of the seed itself, including the coordinated expression of hydrolytic enzymes (phytases, proteases, lipases and amylases), the biosynthesis of bioactive secondary metabolites via the phenylpropanoid pathway, and the structural remodeling of seed tissues during embryogenesis (Gan et al., 2017).

Soaking, although effective as a preliminary treatment, resulted in relatively small changes in most parameters. The primary mechanisms of action during soaking involve the passive diffusion of water-soluble antinutrients (primarily tannins and some heat-stable trypsin inhibitors) into the soaking medium and some limited enzymatic activation caused by imbibition (Hotz & Gibson, 2007). The incremental nature of soaking effects, as demonstrated by the often non-significant differences between 12 and 24 h soaking for many parameters, suggests that extended soaking beyond 12 h provides marginal benefits and may not be worth the additional processing time for practical applications.

Roasting gave the particular advantage of rapid processing (10 s at 180°C) and was most efficient for thermal inactivation of trypsin inhibitors especially in foxtail millet. However, the roasting process was not as effective as germination to reduce the phytic acid and tannin levels and did not lead to the same degree of protein enrichment or improvement in bioactive compounds. Also, the loss of moisture and slight decrease in protein during roasting are acceptable but represent a nutritional compromise that needs to be considered in product formulation (Sindhu et al., 2019).

Among the three grains, amaranth was the most nutritionally responsive to the processing treatments and showed the greatest absolute increases in protein content, antioxidant activity and total phenolic content on germination. This responsiveness makes germinated amaranth a very promising functional food ingredient for applications that require high protein content, potent antioxidant activity and low levels of antinutritional factors. Kodo millet, due to its high fiber content and substantial reduction in phytic acid upon germination, is suitable for inclusion in fiber-enriched low-glycemic-index food products. Foxtail millet showed the highest initial antinutritional factor load but showed significant reductions after germination and roasting, thus making it suitable for multiple processing strategies depending on the end-use application

4. Conclusion

The present study is a comprehensive and systematic assessment of the physical, nutritional, bioactive, and antinutritional properties of three underutilized millets namely foxtail millet, kodo millet and amaranth and the effects of soaking, germination and roasting on their compositional profiles. The unpolished grains had complementary nutritional strengths: amaranth had highest protein and bioactive compounds, kodo millet had highest dietary fiber and foxtail millet had highest caloric density. However, all three grains had high levels of antinutritional factors that limit the bioavailability of their nutrients.

The best processing intervention was germination for 72 h which resulted in significant increases in protein content (17.88–32.98% increase), crude fiber (22.01–49.43% increase), total phenolic content (16.35–32.30% increase), flavonoids (30.42–54.07% increase) and antioxidant activity (24.96–110.49% increase) while achieving considerable reductions in phytic acid (48.50–57.43%), tannins (60.00–70.83%) and trypsin inhibitor activity

(19.10–34.90%). Roasting was an effective and rapid complementary treatment, especially for thermal inactivation of trypsin inhibitors. These findings support the strategic utilization of germination as a primary processing intervention, combined with roasting for synergistic reduction in anti-nutritional factors for the development of nutritionally enhanced food products from underutilized grains.

Future research should examine the effects of combined processing treatments (e.g., germination followed by roasting), the implications of these treatments on in vitro and in vivo protein digestibility and mineral bioavailability, and the techno-functional properties of processed grain flours for use in specific food product categories like infant complementary foods, sports nutrition products and gluten-free formulations. Furthermore, consumer acceptance studies and economic feasibility analyses would enable the translation of these findings into commercially viable food products that contribute to dietary diversification and nutritional security.

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