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Effects of processing methods on nutritional enrichment, digestibility, and shelf-life of foxtail millet (*Setaria italica* (L.) P. Beauvois) flour

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Abstract

Foxtail millet (*Setaria italica* (L.) P. Beauvois) is nutritionally rich with significant potential as an alternative to conventional cereal grains, particularly in addressing food security challenges in India. The present study investigated the influence of various processing methods (roasting, germination, boiling, and pressure cooking) on the nutrient composition, protein digestibility, and shelf stability of foxtail millet flour. Statistical analysis revealed significant differences (p=0.05) in protein digestibility and nutrient composition among the various processing methods. Germination led to the highest protein digestibility (7.39 ± 0.13), whereas boiling and pressure cooking resulted in moderate but acceptable digestibility. Mineral analysis revealed that essential micronutrients (iron, zinc, and calcium) were retained post-processing, underscoring the nutritional value of the processed flours. Among the processing methods, germination increased protein content, *in vitro* protein digestibility, fiber, and ash levels, followed by roasting, boiling, and pressure cooking. Germination also enhanced the levels of soluble (21.65%), insoluble (10.05%), and total dietary fiber (10.7%). Shelf-life analysis of raw, roasted, and germinated flours over six months, with sampling at 30-day intervals, demonstrated that roasting extended shelf stability for up to five months with a gradual increase in moisture content (10.38% to 15.62%), peroxide value (3.21 to 16.03 meq/kg), acid value (3.24 to 14.85 KOH/g), and free fatty acid content (0.97% to 5.45% as oleic acid). Sensory evaluation of chapati prepared from roasted flour indicated its acceptability for up to 150 days of flour storage, whereas raw and germinated flour remained acceptable for 90 days.The study highlights that a simple processing method significantly enhances the nutritional profile, digestibility, bioavailability, and storage stability of foxtail millet flour. It also emphasisesthe potential of foxtail millet as a nutrient-dense, sustainable food source, contributing to improved dietary quality and food security.

1. Introduction

India has made substantial progress in economic growth and education since its independence. Additionally, achieving universal healthcare has remained a longstanding national priority (Priya *et al*., 2023). Following the Green Revolution, the focus of agricultural and nutritional policies has shifted from merely increasing food production and achieving self-sufficiency in food grains to promoting more balanced and healthier diets (Minocha *et al*., 2019). In alignment with the sustainable development goals (SDGs), global initiatives aim to eradicate hunger and ensure year-round access to safe, nutritious, and sufficient food by 2030 (Jain *et al*., 2016). Despite these efforts, Indian dietary surveys demonstrated that a high proportion of the population remains at risk ofmultiple nutrient deficiencies, with protein deficiency being a major concern. The primary sources of protein in the Indian diet are cereals and pulses; however, their protein quality is suboptimal due to the presence of limiting amino acids such as lysine and methionine. Furthermore, the digestion, absorption, and bioavailability of these proteins are not always efficient. Given India's escalating malnutrition burden characterized by both undernutrition (including vitamin, mineral, and protein deficiencies) and overnutrition (obesity, metabolic syndrome, and lifestyle-related diseases). There is a growing awareness of the need to transition towards healthier, more accessible, sustainable, and cost-effective dietary options that include millet (Seetha *et al*., 2024).

Considering the changing climatic conditions, rising global food insecurity, and persistent malnutrition, there is an urgent need to diversify staple crop production and consumption. Traditional, nutrient-dense, and climate-resilient crops must be reintegrated into modern agricultural systems to enhance food security and sustainability (Dhaka *et al*., 2024). Among these, millet crops belonging to the grass family Poaceae hold significant potential due to their adaptability to harsh environmental conditions (Sivagamy *et al*., 2024). Millets are primarily cultivated in rainfed regions of the semi-arid tropics, where they serve as a critical food source due to their inherent drought tolerance (Kamal *et al*., 2024). Traditionally perceived as "poor man's grains, often referred to as "crops of the future," are inherently resistant to pests and insects, making them highly suitable for cultivation in diverse agro-climatic conditions. Compared to conventional staples like wheat (*Triticum aestivum*), rice (*Oryza sativa*), and maize (*Zea mays*), millets require minimal

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water inputs and exhibit greater resilience to climate variability, positioning them as a sustainable alternative for agricultural stability and food security. Millets have demonstrated potential in preventing cancer, reducing cardiovascular disease risk, lowering cholesterol and blood pressure, and improving gastrointestinal health, while being gluten-free (Durairaj *et al*., 2019). The health benefits of millets are attributed to bioactive compounds such as oligosaccharides, resistant starch, lipids, antioxidants (flavonoids, phenolic acids), phytosterols, lignans, and anti-nutritional factors like tannins and phytic acid (Edge *et al*., 2005). Milletsare capable to enhance nutritional value without compromising palatability, which can be utilized as a functional ingredient in food products and as a partial ingredient for the substitution of wheat flour (Kaur *et al*., 2025).

Foxtail millet (*Setaria italica* (L.) *P. Beauvois*), a significant minor millet, offers a superior nutritional profile compared to other cereal grains (Muthamilarasan and Prasad, 2020). It contains 11% protein, 3.9% fat, 19.1% dietary fiber, 59.1% starch, 7.0% ash, and 106.6 mg of phenol per 100 g (Das *et al*., 2019). Its high protein content and quality amino acid profile make it a viable alternative to animal-based protein sources (Sachdev *et al*., 2021). Rich in magnesium, foxtail millet exhibits antibacterial properties, supports detoxification, and is recognised for promoting heart health (Saini *et al*., 2021). Traditionally consumed in northern China by pregnant and nursing women, it is valued for its role in reducing the risk of chronic diseases such as diabetes and aiding cholesterol metabolism (Zhang and Liu, 2015). Additionally, foxtail millet has shown antiulcer activity, antioxidant properties, and gastric mucosal protection (Lin *et al*., 2020). It is gluten-free and provides vitamins A, E, and B complex vitamins (Devi *et al*., 2014). Due to its functional components, it can serve as a nutraceutical (Pradeep and Guha, 2011), with bioactive compounds that inhibit proteolytic and amylolytic enzymes (Luithui *et al*., 2021).

However, there are challenges in processing and storing millet flour. The small grain size can lead to oil-rich germ separation during milling, exposing lipids to oxidation, and resulting in rancidity and off-flavours, especially under high-moisture and oxygen-exposure conditions. The high-fat content and lipase activity further accelerate rancidity, impacting millet flour's quality and shelf-life (Sruthi and Rao, 2021). Factors such as moisture and humidity contribute to rancidity, and shelf-life evaluation often involves monitoring peroxide values, acid values, and microbial load (Jalgaonkar *et al*., 2016; Geetha *et al*., 2020). Appropriate processing techniques can reduce rancidity and antinutritional factors, improving both sensory and nutritional properties (Saleh *et al*., 2013). Different processing techniques, such as fermentation, germination, roasting, and soaking, exist on household and industrial scales to enhance micronutrient bioavailability, improve palatability, and reduce antinutritional components (Hotz and Gibson, 2007; Budhwar *et al*., 2020). Thus, the present study aims to examine the effect of various household processing methods on nutritional composition, digestibility, and storage stability of the foxtail millet flour.

2. Materials and Methods

2.1 Raw materials

The present study was conducted in the Department of Food and Nutrition, College of Community Science, PAU. A foxtail millet germplasm panel comprising 1,000 lines was evaluated over a period of four consecutive years.Two lines having a short duration of 60-65 days, giving moderate yield, were shortlisted for multilocation trials in Punjab during the summer season. These two promising germplasm lines of foxtail millet; namely, F1=R_1F_4; F2=R3F3 seeds, were selected for the study and were obtained from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab. The seeds were thoroughly cleaned to remove impurities and subjected to four common processing methods: roasting, germination, boiling, and pressure cooking.

2.2 Flour preparation

After processing, the cleaned raw grains of the selected germplasm line were ground to flour using an electric grinder with a 20-mesh size and stored at ambient conditions for further analysis. Flour of both unprocessed (raw) andprocessed grains was also made using the same techniques as processed grains. A detailed procedure for making the flour is given in Figure 1.

2.3 Chemical analysis

Unprocessed and processed foxtail millet flour from both germplasm lines was stored in LDPE pouches under ambient conditions for subsequent analysis. The flour samples were analysed in triplicate for the following parameters namely, proximate composition, ash content, protein content (the protein content was calculated applying a conversion factor of 6.25), crude fat content, moisture content (AOAC, 2000), crude fiber (AOAC, 2006), minerals-iron, zinc and calcium using an atomic absorption spectrophotometer (Analyst 200, Perkin Elmer), antinutritional factors such as phenols (Singleton *et al*., 1999), tannins (AOAC, 1985), phytic acid (Haug and Lantzsch, 1983) and trypsin inhibitor activity (Roy and Rao, 1971).

2.4 Dietary fibre

Dietary fibre (soluble, insoluble, and total) was quantified in the samples using the Megazymetotal dietary fibre assay kit (K-TDFR-200A), following the standard protocol (AOAC, 2000), which involves enzymatic hydrolysis of starch, protein, and other non-fibre components, leaving dietary fibre for quantification. 1 g of dried and defatted sample was suspended in 40 ml of MES-TRIS buffer (pH 8.2) in a beaker and gelatinised in a boiling water bath. Subsequently, 50 µl of α-amylase enzyme was added, and the mixture was incubated in a shaking water bath at 95°C for 30 min. The sample was then cooled to 60°C, and 100 µl of protease enzyme was added, followed by incubation for 30 min at 60°C. The pH of the sample was adjusted to 4.1-4.8 using 0.5 M hydrochloric acid. Following this, 200 µl of amyloglucosidase enzyme was introduced, and the sample was incubated at 60°C for an additional 30 minutes. The hydrolysed mixture was filtered under vacuum through a pre-weighed crucible containing celite using Whatman No. 1 filter paper. The insoluble dietary fibre (IDF) residue retained on the filter paper was thoroughly washed with 78% ethanol, 95% ethanol, and acetone to remove residual sugars and proteins. The residue was then dried in an oven at 105°C to constant weight to determine the IDF content. The combined filtrate and washings were treated with four volumes of preheated (60°C) 95% ethanol to precipitate soluble dietary fibre (SDF). The precipitated SDF was filtered through another crucible containing celite, following the same washing procedure with 78% ethanol, 95% ethanol, and acetone. The crucible containing the SDF was dried at 105°C overnight to constant weight, cooled in a desiccator, and weighed to determine the SDF content. A reagent blank (without sample) was prepared using the same procedure to account for any contributions of the enzymes to the total fibre mass. The crucibles containing the dried IDF and SDF residues were subsequently placed in a muffle furnace at 525°C for 5 h to remove organic matter. The remaining ash content was weighed, and the ash mass was subtracted from the dried fibre residues to obtain the corrected fibre content. The total dietary fibre was calculated using the formula given and expressed in a percentage.

Total dietary fiber (TDF) = (IDF + SDF) 6 Blank * 100/Sample weight

2.5 *In vitro* protein digestibility

The protein digestibility of the flour samples was determined using the method described by Akeson and Stahmann (1964). A 0.5 g sample was suspended in 50 ml of pepsin solution within a conical flask and incubated at 37°C for 24 h. Incubated samples were neutralised with 30 ml of 0.2 N sodium hydroxide solution. Subsequently, 50 ml of pancreatin solution was added to the neutralised sample, further incubating for 24 hat 37°C. An enzyme blank, without the protein sample, was prepared under the same conditions. To ensure aseptic conditions, a few drops of toluene were added to each sample. The resulting mixtures were centrifuged at high speed and filtered using Whatman No. 44 filter paper. The nitrogen content of the residual material was determined using the Micro-Kjeldahl method. The digestibility coefficient of the sample, based on a 100 g sample, was calculated by subtracting the residual protein from the initial protein content.

2.6 Shelf-life analysis

The flour prepared from unprocessed and processed foxtail millet grains of both the germplasm lines was packed in low-density polyethene (LDPE) pouches and stored at ambient temperatures (30°C). The flours were analysed for changes in moisture content (AOAC, 2000), peroxide (AOAC, 2000), acid values (Cox and Pearson, 1962), and free fatty acid (Tarladgis *et al*., 1960). The shelf-life of the flour was also assessed by making chapati (Indian flat bread, as it is the most common way of consuming any flour in the Indian population) of the stored flour for 6 months and evaluating its organoleptic characteristics at an interval of 30 days using 9-point Hedonic rating scale (Heymann and Lawless, 2013).

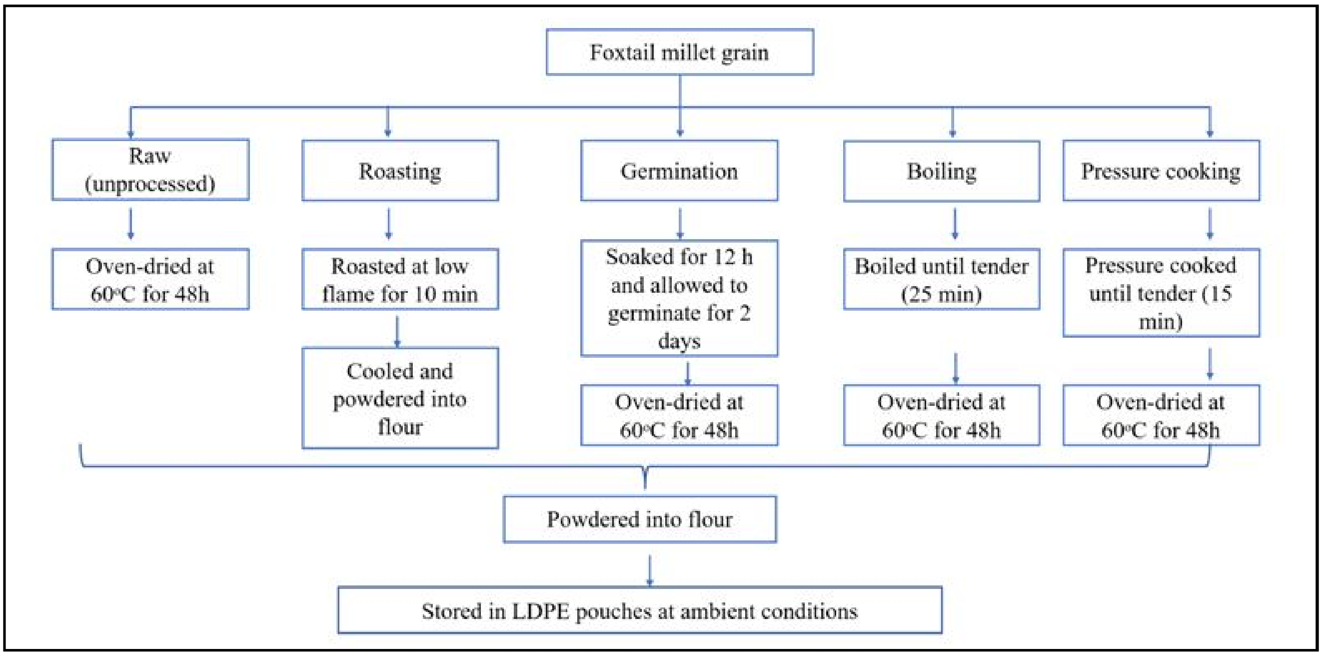

Figure 1: Flow chart for flour preparation from the millet grains.

2.7 Statistical analysis

The data was analyzed by a complete block design using SPSS software (26 versions). The mean and standard deviation for various parameters were computed. Analysis of Variance (One-way and Two-way ANOVA) and post hoc tests were employed to assess the influence of common processing methods on the nutritional profile and shelf-life of Foxtail millet flour.

3. Results

Table 1 and Figure 2 represent the proximate composition and mean percent change of unprocessed and processed foxtail millet flour of both the germplasm lines, respectively. Unprocessed foxtail millet flour of F1 and F2 had a moisture content of 10.82 ± 0.44 and 9.94 ± 0.11%, respectively. Roasting resulted in the highest moisture loss. Germination increased protein content, while it was reduced in roasting. The crude fat content of foxtail millet flour decreased significantly ($p \leq 0.05$) by all processing techniques, with the maximum reduction observed in F1 (2.48 ± 0.01) and F2 (3.25 ± 0.07) flours prepared after roasting. Germination also resulted in a significant reduction in crude fat content, with 9.1% in F1 and 9.3% in F2. Boiling and pressure cooking also reduced crude fat content, with a significant reduction in boiling compared to pressure cooking. Unprocessed millet flour of both the lines, F1 and F2 millet,has varying crude fibre content, whereas processing techniques reduced the fibre content significantly ($p \leq 0.05$). The total ash content in foxtail millet flour increased significantly during germination and roasting. However, boiling and pressure cooking reduced the total ash content. The highest ash concentration was found in germination, followed by roasting, with boiling and pressure cooking resulting in a reduction in total ash content. The carbohydrate content of raw and treated foxtail millet varied between 52.54 ± 0.28 and 71.38 ± 0.31 g/100 g.

Figure 3 represents the mineral composition of the unprocessed and processed millet flour from both lines. The current study found that the mineral content (iron, zinc and calcium) increased significantly ($p \leq 0.05$) in germinated flour. However, flour from other processing methods such as boiling and pressure cooking in both lines iron, zinc and calcium content decreased significantly. Roasting foxtail millet decreased the iron and zinc content by 4.9 and 7.8% in F1, 5.9 and 2.2% in F2 whereas, it increased the calcium content by 12.7 and 7.5% in F1 and F2, respectively.

The effects of the various treatments, on the antinutritional factors of foxtail millet flour have been presented in Table 2. The total phenolic content of processed millet flour in the F1 line ranged from 58.38 ± 0.62 to 112.59 ± 0.93 mg GAE/100 g, while in the F2 line, it ranged from 61.29 ± 0.85 to 127.57 ± 0.98 mg GAE/100 g. All processing methods, except germination, resulted in a significant reduction in phenolic content compared to the unprocessed flour. The greatest phenolic reduction was observed in pressure cooking (up to 50%), followed by boiling and roasting (20-40%). Similarly, all processing methods effectively reduced tannin content. Unprocessed flour from both foxtail millet lines exhibited the highest tannin levels, but processing led to significant reductions ($p \leq 0.05$), with germination showing the greatest effect, reducing tannins by 50%. Unprocessed millet flour exhibited phytic acid contents of 564.72 ± 3.51 and 544.17 ± 3.68 mg/100 g in the F1 and F2 lines, respectively. Pressure cooking and boiling led to notable decreases in phytic acid content, with reductions of 35.8% and 29.7% during boiling, and 38.7% and 32.8% during pressure cooking. While boiling and pressure cooking were effective in reducing phytates, roasting showed the least impact on phytic acid content.

Table 1: Proximate composition of unprocessed and processed foxtail millet flour (g/100 g on dry weight basis)

Processing technique	Germplasm lines	Moisture	Protein	Fat	Fiber	Ash	Carbohydrate
Raw (unprocessed)	F1	10.82 ± 0.04^{a}	12.38 ± 0.68^{e}	3.29 ± 0.08^{e}	7.06 ± 0.12^{e}	3.77 ± 0.21^{e}	58.91 ± 0.72^{g}
	F2	09.94 ± 0.11^{b}	13.59 ± 0.05^{c}	4.17 ± 0.07^{a}	7.83 ± 0.07^{b}	4.20 ± 0.1^{c}	60.27 ± 0.24^{f}
Roasted	F1	05.11 ± 0.05^{g}	11.12 ± 0.21^{g}	2.48 ± 0.01^{i}	5.87 ± 0.09^{i}	4.01 ± 0.09^{d}	71.38 ± 0.31^{a}
	F2	04.83 ± 0.21^{h}	12.24 ± 0.22^{e}	3.25 ± 0.07^{e}	6.44 ± 0.14^{f}	4.58 ± 0.04^{b}	68.66 ± 0.22^{c}
Germinated	F1	07.39 ± 0.13^{d}	15.27 ± 0.44^{b}	2.75 ± 0.21^{g}	7.58 ± 0.04^{c}	4.50 ± 0.07^{b}	52.54 ± 0.28^{h}
	F2	06.74 ± 0.09^{f}	16.09 ± 0.38^{a}	3.78 ± 0.01^{c}	8.26 ± 0.11^{a}	5.11 ± 0.07^{a}	60.02 ± 0.27^{f}
Boiled	F1	07.56 ± 0.07^{c}	12.01 ± 0.41^{f}	2.51 ± 0.05^{h}	6.18 ± 0.03^{h}	2.46 ± 0.02^{i}	69.31 ± 0.46^{b}
	F2	06.91 ± 0.05^{e}	13.41 ± 0.26^{d}	3.56 ± 0.03^{d}	7.24 ± 0.08^{d}	3.38 ± 0.05^{g}	65.50 ± 0.42^{e}
Pressure cooked	F1	07.58 ± 0.15^{c}	12.24 ± 0.31^{e}	2.88 ± 0.11^{f}	6.25 ± 0.08^{g}	2.85 ± 0.03^{h}	67.93 ± 0.23^{d}
	F2	06.95 ± 0.09^{e}	13.37 ± 0.18^{c}	3.82 ± 0.05^{b}	7.31 ± 0.09^{d}	3.52 ± 0.18^{f}	64.33 ± 0.34^{e}

where, F1 = R_1F_4 and F2 = R_3F_3; Values are expressed as mean ± S.D. Values having different alphabetical superscripts represent a significant ($p \leq 0.05$) difference between the processing treatments and between the lines of the foxtail millet flour

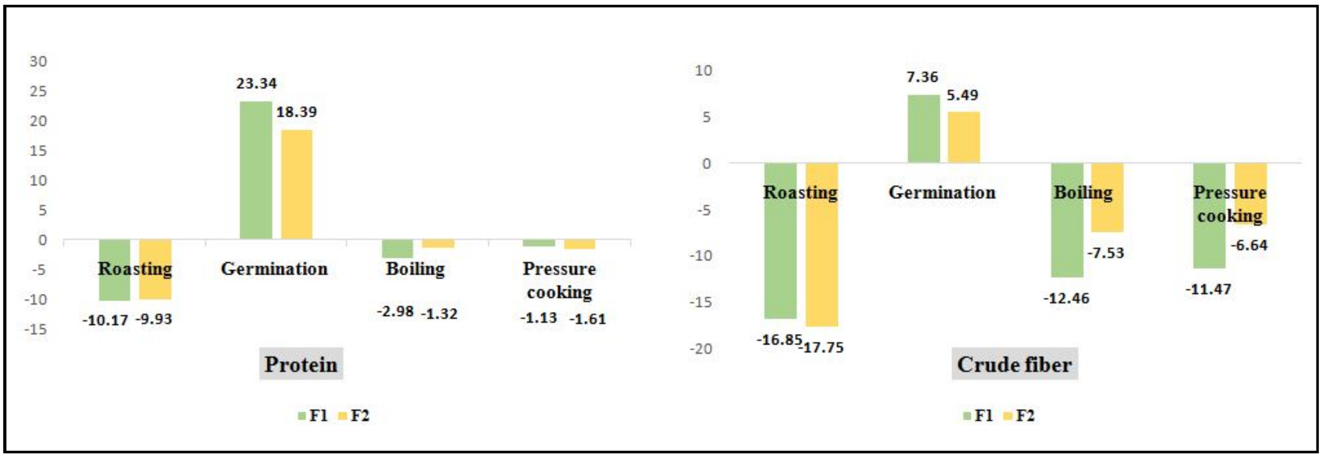

Figure 2: Mean per cent change in proximate composition of foxtail millet flour after processing compared to unprocessed.

Trypsin inhibitors were found to be highest in the raw millet flour, 9.98 ± 0.01 and 10.34 ± 0.01 mg/g of protein in F1 and F2, respectively, which decreased to 5.67 ± 0.01 and 6.54 ± 0.11 mg/g of protein after the treatment, where grains were germinated before making flour. Processing exhibited a significant effect on dietary fibre content also. Germination increased both soluble and insoluble fibre content by 22.2% and 11% in F1 and 21.1% and 9.1% in F2, respectively. Other processing methods significantly ($p<0.05$) reduced both fibre types, with the maximum reduction occurring during boiling, followed by roasting and pressure cooking. The dietary fibre content of raw and processed foxtail millet flour showed significant variation depending on the processing technique. Total dietary fibre in raw and processed foxtail millet ranged from 12.40 ± 0.03 to 15.71 ± 0.04 g/100 g for F1 and 11.84 ± 0.04 to 14.93 ± 0.07 g/100 g for F2. A significant reduction ($p<0.05$) in dietary fibre content was observed with all processing methods except germination, which led to an 11.7% and 9.7% increase in total dietary fibre in F1 and F2, respectively. Boiling resulted in the greatest reduction in total dietary fibre, with values dropping to 12.4 g/100 g for F1 and 11.84 g/100 g for F2, followed by pressure cooking and roasting.

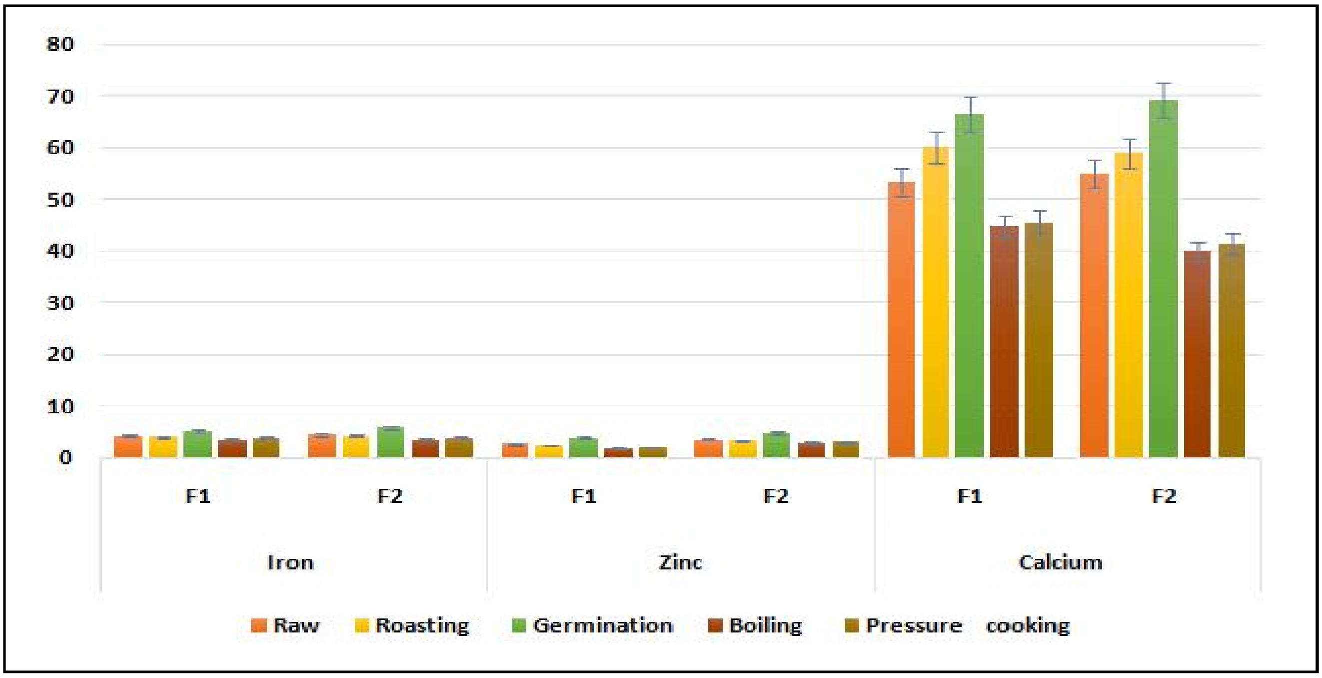

Figure 3: Mineral composition of foxtail millet flours (mg/100 g, dry weight basis).

Table 2: Antinutritional factors of unprocessed and processed foxtail millet flour

Processing technique	Variety	Phenols (mg GAE/ 100 g)	Tannins (mg TAE/ 100 g)	Phytic acid (mg phytic acid/ 100 g)	Trypsin inhibitor activity (mg/g protein)
Raw (unprocessed)	F1	091.48 ± 1.21^{c}	746.68 ± 4.32^{b}	564.72 ± 3.51^{a}	09.98 ± 0.01^{b}
	F2	111.59 ± 0.91^{b}	812.61 ± 1.42^{a}	544.17 ± 3.68^{b}	10.34 ± 0.01^{a}
Roasted	F1	069.52 ± 0.52^{d}	487.27 ± 4.15^{de}	388.51 ± 2.74^{d}	08.42 ± 0.01^{c}
	F2	076.87 ± 1.39^{b}	505.34 ± 3.81^{de}	419.85 ± 2.65^{c}	08.13 ± 0.02^{d}
Germinated	F1	112.59 ± 0.93^{b}	457.31 ± 3.05^{e}	269.36 ± 3.62^{h}	05.76 ± 0.01^{j}
	F2	127.57 ± 0.98^{a}	468.03 ± 3.98^{e}	310.74 ± 6.67^{g}	06.54 ± 0.11^{g}
Boiled	F1	065.57 ± 0.79^{de}	547.20 ± 4.31^{cd}	362.95 ± 2.99^{e}	06.43 ± 0.01^{h}
	F2	067.31 ± 1.61^{d}	574.35 ± 1.57^{c}	382.36 ± 4.11^{d}	06.83 ± 0.04^{f}
Pressure cooked	F1	058.38 ± 0.62^{e}	571.52 ± 2.19^{c}	345.99 ± 2.58^{f}	06.04 ± 0.02^{i}
	F2	061.29 ± 0.85^{e}	554.32 ± 3.45^{c}	365.45 ± 2.99^{e}	07.57 ± 0.09^{e}

where, F1 = R_1F_4 and F2 = R_3F_3; Values are expressed as mean ± S.D. Values having different alphabetical superscripts represent significant ($p\leq0.05$) difference between the processing treatments and between the lines of the foxtail millet flour

The *in vitro* protein digestibility (IVPD) of raw foxtail millet flour was 51.87 ± 1.1% in line F1 and 56.75 ± 2.07% in line F2. All processing methods-germination, boiling, roasting, and pressure cooking led to a significant improvement in IVPD ($p\leq0.05$). Among these, germination exhibited the greatest enhancement, increasing digestibility by 76.5% in F1 and 55% in F2. Roasting also contributed to improved IVPD, with increases of 21.8% and 25.5% in F1 and F2, respectively. Overall, germination proved to be the most effective method for enhancing protein digestibility in foxtail millet (Figure 4). The shelf-life of was analysed for unprocessed (raw) and only two processing techniques (roasting and germination) that gave the best nutritional components by assessing their susceptibility to oxidative deterioration measured through moisture content, peroxide value, acid value, and free fatty acids during storage in low-densitypolyethene (LDPE) pouches over six months and is presented in Table 4. The moisture content of foxtail millet flours on the 0^{th}day was 10.82 ± 0.31% and 9.94 ± 0.42% in raw flour, 5.11 ± 0.28% and 4.83 ± 0.36% in roasted flour, and 7.39 ± 0.06% and 6.74 ± 0.08% in germinated flour for F1 and F2, respectively. As the storage period progressed, the moisture content increased in all flour samples. By day 90, the raw flour had reached moisture levels of 12.06% and 12.14% in F1 and F2, respectively. Germinated flour exhibited moisture levels of 12.92% and 12.23% by day 120, while roasted flour-maintained moisture content at 12.05% and 12.15% on day 180. The initial peroxide value of raw foxtail millet flour was 2.95 ± 0.06 and 3.48 ± 0.05 meq/kg in lines F1 and F2, respectively. During storage, the peroxide value significantly ($p \leq 0.05$) increased in all samples. However, treated flours exhibited lower peroxide values compared to raw flours, ranging from 2.16 to 3.02 meq/kg in foxtail millet on the 0^{th} day. By the 90^{th} day, the peroxide value of raw flours increased to 10.05 ± 0.004 and 10.14 ± 0.06 meq/kg in F1 and F2. Germinated flours reached a similar peroxide value at the end of 4 months, while roasted flours remained stable until150 days. Overall, roasting and germination significantly reduced the peroxide value in both lines of foxtail millet flours compared to their raw counterparts, effectively improving shelf-life. It has been observed that the acid value of raw, roasted, and germinated foxtail millet flour significantly ($p \leq 0.05$) increased over the storage period in the current investigation. On day 0, the acid values for raw foxtail millet in F1 and F2 were 3.12 ± 0.04 and 3.36 ± 0.04 mg KOH/g, respectively, which increased to 14.39 ± 0.11 and 15.32 ± 0.18 mg KOH/g by day 180. The FFA content significantly ($p \leq 0.05$) increased over the storage period for all samples.

Table 3: *In vitro* protein digestibility (%) and dietary fibre of millet flour (g/100 g on dry weight basis)

Processing technique	*In vitro* protein digestibility		Soluble dietary fiber		Insoluble dietary fiber		Total dietary fiber	
	F1	F2	F1	F2	F1	F2	F1	F2
Raw	51.87 ± 1.17^b	56.75 ± 2.07^a	0.81 ± 0.03^a	0.71 ± 0.03^b	13.25 ± 0.05^a	12.89 ± 0.06^b	14.06 ± 0.04^a	13.60 ± 0.08^b
Roasting	63.20 ± 1.83^b	71.25 ± 1.41^a	0.75 ± 0.03^a	0.65 ± 0.03^b	13.18 ± 0.05^a	12.21 ± 0.05^b	13.68 ± 0.09^a	12.86 ± 0.04^b
Germination	91.60 ± 1.63^a	87.97 ± 1.48^b	0.99 ± 0.03^a	0.86 ± 0.04^b	14.72 ± 0.05^a	14.07 ± 0.03^b	15.71 ± 0.04^a	14.93 ± 0.07^b
Boiling	77.48 ± 1.44^a	73.19 ± 0.95^b	0.73 ± 0.04^a	0.62 ± 0.03^b	11.67 ± 0.04^a	11.22 ± 0.04^b	12.40 ± 0.03^a	11.84 ± 0.04^b
Pressure cooking	82.75 ± 0.87^a	77.52 ± 0.96^b	0.79 ± 0.03a	0.68 ± 0.04^b	12.19 ± 0.04^a	11.68 ± 0.06^b	12.98 ± 0.06^a	12.36 ± 0.11^b

where, F1= R_1F_4 and F2= R_3F_3; Values are expressed as mean ± S.D. Values having different alphabetical superscripts represent significant ($p \leq 0.05$) differences between the processing treatments and between the lines of the foxtail millet flour

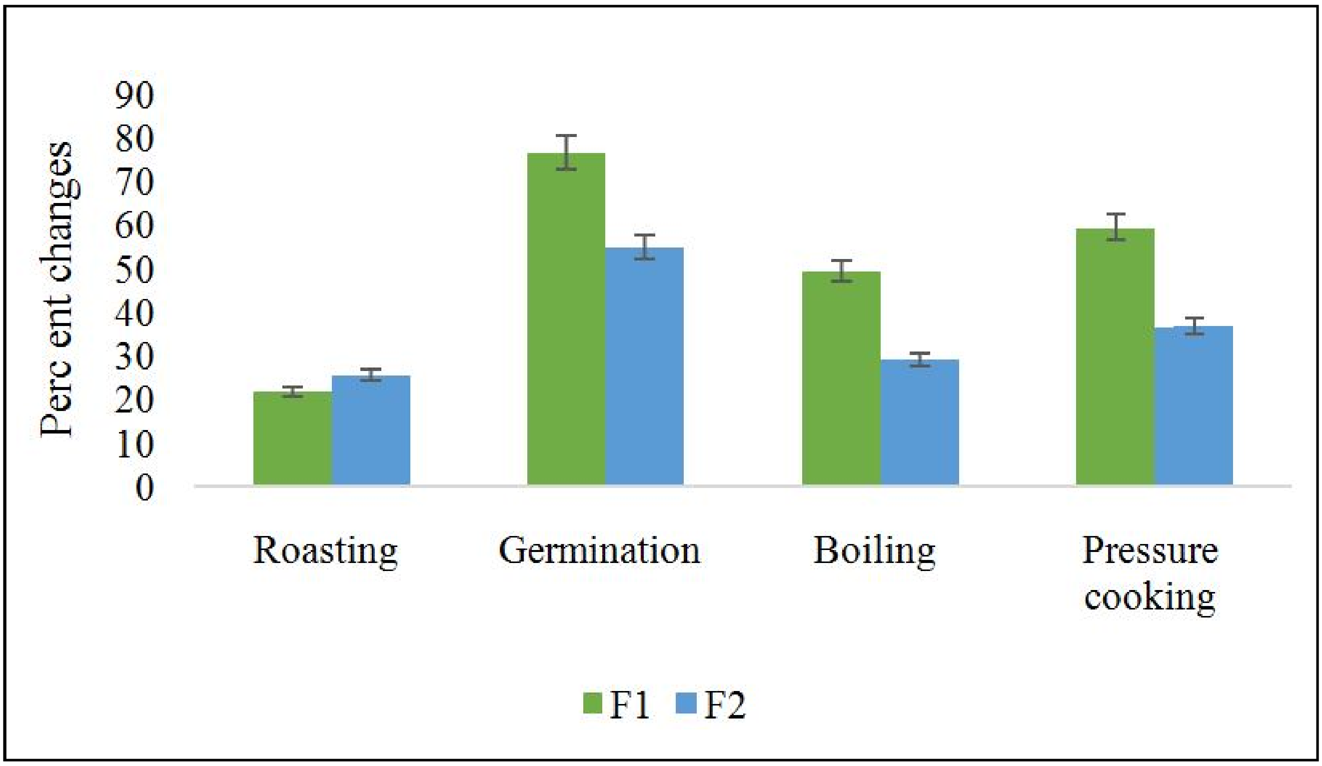

Figure 4: Mean per cent change in the *in vitro* protein digestibility (IVPD) of foxtail millet flour after treatments.

Table 4: Shelf-life stability of millet flour based on physicochemical and oxidative parameters

Storage period (days)	Processing method	Moisture content (%)		Peroxide value (meq/kg)		Acid value (KOH/ g of oil)		Free fatty acid (%)	
		F1	F2	F1	F2	F1	F2	F1	F2
0	Raw	10.82 ± 0.31^{a}	09.94 ± 0.42^{b}	02.95 ± 0.06^{b}	03.48 ± 0.05^{a}	03.12 ± 0.04^{b}	03.36 ± 0.04^{a}	00.90 ± 0.01^{b}	01.05 ± 0.02^{a}
	Roasting	05.11 ± 0.28^{a}	04.83 ± 0.36^{b}	02.16 ± 0.05^{b}	02.79 ± 0.05^{a}	02.11 ± 0.03^{b}	02.27 ± 0.03^{a}	00.69 ± 0.01^{b}	00.76 ± 0.02^{a}
	Germination	07.39 ± 0.06^{a}	06.74 ± 0.08^{b}	02.42 ± 0.04^{b}	03.02 ± 0.04^{a}	02.78 ± 0.02^{b}	03.11 ± 0.04^{a}	00.74 ± 0.02^{b}	00.88 ± 0.01^{a}
30	Raw	11.24 ± 0.03^{a}	10.73 ± 0.18^{b}	05.77 ± 0.06^{b}	05.75 ± 0.03^{a}	05.97 ± 0.05^{a}	05.22 ± 0.06^{b}	01.98 ± 0.01^{a}	02.29 ± 0.01^{b}
	Roasting	05.89 ± 0.11^{a}	05.76 ± 0.07^{b}	02.46 ± 0.15^{b}	02.88 ± 0.06^{a}	02.52 ± 0.05^{b}	03.64 ± 0.06^{a}	01.00 ± 0.02^{b}	01.03 ± 0.01^{a}
	Germination	08.56 ± 0.43^{a}	07.54 ± 0.26^{b}	04.38 ± 0.04^{a}	04.24 ± 0.06^{b}	03.81 ± 0.07^{b}	04.71 ± 0.32^{a}	01.86 ± 0.01^{a}	01.75 ± 0.01^{b}
60	Raw	11.68 ± 0.05^{a}	11.28 ± 0.38^{b}	07.25 ± 0.08^{b}	08.28 ± 0.05^{a}	07.84 ± 0.05^{b}	08.92 ± 0.06^{a}	02.67 ± 0.01^{b}	02.81 ± 0.01^{a}
	Roasting	06.48 ± 0.43^{b}	06.81 ± 0.08^{a}	04.78 ± 0.06^{b}	05.48 ± 0.08^{a}	03.28 ± 0.06^{b}	03.90 ± 0.06^{a}	01.21 ± 0.01^{b}	01.48 ± 0.01^{a}
	Germination	09.62 ± 0.27^{a}	08.73 ± 0.02^{b}	06.25 ± 0.07^{b}	07.83 ± 0.01^{a}	05.72 ± 0.05^{b}	07.41 ± 0.08^{a}	02.28 ± 0.004^{a}	02.29 ± 0.02^{a}
90	Raw	12.06 ± 0.00^{b}	12.14 ± 0.25^{a}	10.05 ± 0.004^{b}	10.14 ± 0.06^{a}	10.05 ± 0.05^{b}	10.76 ± 0.05^{a}	03.07 ± 0.01^{b}	03.15 ± 0.02^{a}
	Roasting	07.18 ± 0.16^{b}	08.23 ± 0.36^{a}	05.13 ± 0.06^{b}	06.52 ± 0.05^{a}	04.77 ± 0.06^{b}	04.92 ± 0.05^{a}	01.48 ± 0.01^{b}	01.74 ± 0.01^{a}
	Germination	10.84 ± 0.10^{a}	09.85 ± 0.51^{b}	08.39 ± 0.06^{b}	08.98 ± 0.06^{a}	08.09 ± 0.01^{b}	09.14 ± 0.04^{a}	02.79 ± 0.01^{a}	02.78 ± 0.01^{b}
120	Raw	12.57 ± 0.46^{b}	12.77 ± 0.04^{a}	12.14 ± 0.06^{b}	12.16 ± 0.12^{a}	11.85 ± 0.07^{a}	11.79 ± 0.06^{b}	03.28 ± 0.07^{b}	03.68 ± 0.01^{a}
	Roasting	09.94 ± 0.23^{a}	09.06 ± 0.17^{b}	06.45 ± 0.08^{b}	06.84 ± 0.11^{a}	06.69 ± 0.09^{b}	06.73 ± 0.06^{a}	02.17 ± 0.01^{b}	02.59 ± 0.01^{a}
	Germination	12.92 ± 0.06^{a}	12.23 ± 0.24^{b}	10.17 ± 0.07^{b}	10.36 ± 0.06^{a}	10.12 ± 0.07^{b}	10.83 ± 0.11^{a}	03.17 ± 0.01^{b}	03.19 ± 0.01^{a}
150	Raw	13.86 ± 0.09^{b}	14.78 ± 0.43^{a}	13.82 ± 0.09^{b}	14.06 ± 0.09^{a}	13.61 ± 0.09^{a}	13.13 ± 0.24^{b}	04.31 ± 0.04^{a}	04.27 ± 0.03^{b}
	Roasting	10.73 ± 0.51^{b}	11.14 ± 0.28^{a}	09.16 ± 0.12^{a}	08.87 ± 0.09^{b}	08.30 ± 0.17^{b}	09.84 ± 0.12^{a}	02.84 ± 0.04^{a}	02.78 ± 0.03^{b}
	Germination	13.64 ± 0.06^{b}	13.75 ± 0.36^{a}	12.21 ± 0.06^{b}	12.33 ± 0.14^{a}	12.91 ± 0.15^{a}	12.63 ± 0.32^{b}	03.03 ± 0.02^{b}	03.86 ± 0.04^{a}
180	Raw	15.58 ± 0.31^{b}	15.67 ± 0.35^{a}	15.72 ± 0.29^{b}	16.35 ± 0.29^{a}	14.39 ± 0.11^{b}	15.32 ± 0.18^{a}	05.80 ± 0.07^{a}	05.09 ± 0.07^{b}
	Roasting	12.05 ± 0.62^{b}	12.15 ± 0.04^{a}	10.86 ± 0.11^{a}	10.86 ± 0.22^{a}	10.35 ± 0.17^{b}	11.26 ± 0.09^{a}	03.09 ± 0.06^{b}	03.12 ± 0.04^{a}
	Germination	15.15 ± 0.27^{b}	15.24 ± 0.18^{a}	14.08 ± 0.35^{b}	14.34 ± 0.16^{a}	13.71 ± 0.18^{b}	14.02 ± 0.16^{a}	03.27 ± 0.07^{b}	04.87 ± 0.04^{a}

where F1= R_1F_4 and F2= R_3F_3; Values are expressed as Mean ± S.D. Values having different alphabetical superscripts represent significant ($p \leq 0.05$) differences between the processing treatments and between the lines of the foxtail millet flour

Figure 5: Sensory scores of chapati prepared from raw and processed foxtail millet flour at an interval of 30 days.Trends in sensory attributes throughout the storage period arepresented in Figure 5. Chapatis prepared using flour fromthe initial days received acceptability scores above 8 for both raw and processed flour. However, sensory scores declined with increased storage time. Chapatis made from raw flour remained acceptable only for two months, with overall scores of 7.20 ± 0.24 and germinated flour remained acceptable for up to three months, with scores of 6.80 ± 0.26. Roasting proved the most effective treatment, extending the chapatis' acceptability made from the flour stored for four months and obtained with scores of 7.00 ± 0.26.

4. Discussion

A comprehensive evaluation of the present study highlights the effects of various processing techniques on the nutritional composition, protein digestibility, and shelf-life of foxtail millet flour, providing critical insights into optimising its nutritional quality and storage stability for broader dietary applications. Moisture content is crucial in food preparation, directly influencing quality and shelf stability (Wilson *et al*., 2024). The processing methods applied in this study, such as roasting, germination, boiling, and pressure cooking, resulted in significant reductions in moisture content, with roasting causing the highest loss due to direct heat exposure (Yousaf *et al*., 2016). Germination led to a significant increase in protein content, whereas roasting resulted in a decline. This reduction in protein content during roasting is likely due to protein degradation caused by high temperatures (Singh *et al*., 2018). Previous studies have also demonstrated that while germination enhances protein concentration in foxtail millet flour, roasting can contribute to protein loss (Chauhan and Sarita, 2018; Sharma *et al*., 2018). Crude fat content decreased across all processing methods, which can be attributed to the formation of resistant starch-lipid complexes (Singh *et al*., 2018).

Crude fibre, a complex polysaccharide primarily found in the bran of millet grains, exhibited an increase in germinated flour due to starch depletion and the synthesis of structural components such as cellulose and hemicellulose during germination (Panda *et al*., 2020; Yousaf *et al*., 2021). Conversely, roasting resulted in the most pronounced reduction in crude fiber content, likely due to the loss of outer grain fiber layers (Gowda *et al*., 2022).Total ash content increased significantly following germination and roasting, possibly due to phytase activation, which degrades phytates and releases orthophosphate and essential minerals (Bindra and Manju, 2019). However, boiling and pressure cooking led to a reduction in ash content, which may be attributed to mineral leaching from the seed coat into the cooking medium (Singh *et al*., 2018). Variations in carbohydrate content across processing methods could be due to starch hydrolysis, which converts complex carbohydrates into simpler sugars (Kavitha and Parimalavalli, 2014).

In the present study, germinated foxtail millet flour exhibited the highest mineral composition, which can be attributed to phytase-mediated phytate degradation. This process hydrolyses phytates into inositol and releases orthophosphate, thereby elevating mineral content (Yousaf *et al*., 2021) and enhancing bioavailability (Proietti *et al*., 2009). Conversely, boiling and pressure cooking led to a significant reduction in mineral content, likely due to the leaching of water-soluble minerals into the cooking medium during processing.

Antinutritional factors (ANFs), such as phytic acid and tannins, are organic and inorganic compounds that function as a chemical defence system against fungi, insects, and predators. While beneficial for plant protection, these compounds reduce nutrient bioavailability in humans and animals, thereby lowering the overall nutritional value of food (Sinha and Khare, 2017). Various processing methods can effectively mitigate ANFs, thereby enhancing nutrient bioavailability. Phenolic compounds, a class of phytochemicals with antioxidant properties, have been associated with reduced risks of chronic diseases, including diabetes, cancer, and cardiovascular disorders (Xiang *et al*., 2019). In the current investigation, all processing methods, except germination, significantly reduced phenolic content compared to unprocessed flour. Germination enhanced phenolic release due to the action of cell wall-degrading enzymes, which break ester and ether bonds linking phenolics to non-starch polysaccharides in the grain cell walls (Duodo, 2014). The most substantial phenolic loss was observed in pressure cooking (up to 50%), followed by boiling and roasting (20-40%), likely due to tissue disruption (Siah *et al*., 2014). Tannins, water-soluble phenolic compounds found in cereals such as barley, sorghum, and millets, interfere with protein digestion by forming tannin-protein complexes *via* hydrogen bonding (Joye, 2019; De Camargo *et al*., 2019). All processing methods effectively reduced tannin content. This reduction may be attributed to the hydrophobic interactions between tannins and seed proteins or enzymes (Abioye *et al*., 2018), as well as tannin leaching during germination, boiling, and pressure cooking (Sharma *et al*., 2016). Additionally, roasting was particularly effective in reducing tannin content due to the heat-labile nature of tannins.

Phytic acid, or phytates, is a common antinutrient in cereals, legumes, nuts, and oilseeds, with its levels increasing as grains mature. It forms complexes with positively charged metal ions, reducing mineral bioavailability (Fuster *et al*., 2017). In this study, all processing methods led to a reduction in phytic acid content, likely due to phytase enzyme activation. The enhanced hydrolytic activity of phytase under elevated temperature and pressure conditions further facilitated phytic acid degradation (Liu *et al*., 2019). Trypsin inhibitors (TIs) are another class of ANFs that impair protein digestion by inhibiting trypsin, a key proteolytic enzyme (Adeyemo and Onilude, 2013). Unprocessed millet flour exhibited high trypsin inhibitor activity, which significantly declined following processing. Germination was the most effective method for TI reduction, while boiling, pressure cooking, and roasting also contributed to its degradation due to the heat-labile nature of these inhibitors. Similar reductions in TIs have been reported in rice bran (31% reduction after boiling), attributed to deamidation and disulfide bond degradation (Irakli *et al*., 2020). Likewise, roasting led to a 13% reduction in buckwheat TIs (Deng *et al*., 2015) and a 26.3% reduction in finger millet TIs, likely due to the denaturation of protease inhibitors, thereby improving protein digestibility (Chauhan and Sarita, 2018).

Dietary fiber is classified into soluble and insoluble types based on its solubility in water. Soluble fiber forms a gel-like matrix in the digestive tract, facilitating smoother food passage and aiding in nutrient absorption (Cheng *et al*., 2017). In contrast, insoluble fiber increases intestinal bulk, promoting bowel movement and enhancing gut motility (Akinola *et al*., 2017). In this study, all processing methods, except germination, led to a reduction in both soluble and insoluble dietary fibre. The decrease in insoluble fiber can be attributed to the partial degradation of cellulose and hemicellulose into simpler carbohydrates during heat treatments (Benitez *et al*., 2011). Additionally, high-temperature processing may cause thermal degradation of dietary fibers, further contributing to fiber loss (Gowda *et al*., 2022; Krishnan *et al*., 2012). Conversely, germination increased dietary fiber, likely due to structural modifications in the polysaccharide components of the cell wall. These germination-induced changes, which enhance fiber content, align with previous findings (Sharma *et al*., 2016).

The Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO) recommend the protein digestibility corrected amino acid score (PDCAAS) as a standardised method for assessing protein quality in human nutrition (Schaafsma, 2005). Protein digestibility is influenced by intrinsic factors, such as protein structure and amino acid composition, as well as extrinsic factors, including pH, temperature, and the presence of antinutritional compounds. Processing techniques play a crucial role in modifying these factors, thereby influencing protein digestibility. In this study, all processing methods enhanced *in vitro* protein digestibility (IVPD). Heat treatment during roasting led to protein denaturation, improving enzymatic hydrolysis and digestibility, consistent with previous findings (Pushparaj and Urooj, 2011). Boiling and pressure cooking also significantly improved IVPD by reducing antinutritional factors and facilitating protein-protein interactions that enhance digestibility. These findings align with prior research on various millet species, where cooking increased protein digestibility by over 26% (Annor *et al*., 2017; Duodu *et al*., 2003). Additionally, germination was particularly effective in enhancing IVPD, as previously observed in pearl millet (Pushparaj and Urooj, 2011). Among the processing methods examined, germination proved to be the most efficient in improving protein digestibility in foxtail millet.

Millets, due to their relatively high-fat content, are prone to oxidation when exposed to air, leading to a decline in both nutritional value and sensory quality. The shelf-life of millet flour is significantly affected by oxidative and hydrolytic deterioration, which accelerates rancidity and limits its storage stability (Bekele *et al*., 2020). This instability is primarily attributed to the presence of lipase and lipoxygenase enzymes, which catalyse lipid oxidation, resulting in off-flavours and rancidity-one of the major drawbacks of millet consumption (Rani *et al*., 2018). To ensure storage stability, the moisture content of millet flour should not exceed 12%, as higher levels promote rancidity (Anonymous, 2012). In the present study, roasted foxtail millet flour-maintained moisture levels below this threshold throughout the 180-day storage period, whereas raw and germinated flours exceeded this limit by the 90^{th} and 120^{th} days, respectively, making them more susceptible to quality degradation.

Lipid oxidation, measured through peroxide value, increased in all samples over the storage period. However, roasted flours exhibited greater resistance to oxidative deterioration, likely due to the breakdown of primary oxidation products during roasting, which enhances shelf-life (Goszkiewicz *et al*., 2020). These findings align with prior research demonstrating that roasting and germination reduce fat content and oxidative deterioration, thus improving product stability. For instance, roasting at 97°C has been shown to lower crude fat content in finger millet, thereby extending its shelf-life, while fat catabolism during germination similarly reduces the risk of rancidity (Sruthi and Rao, 2021). Acid value, a critical marker of oxidative deterioration, quantifies the free fatty acid (FFA) concentration resulting from triglyceride hydrolysis (Shahidi and Zhong, 2005). According to the Codex Alimentarius Commission, the permissible acid value in food products is 10 mg KOH/g.

In this study, raw and germinated millet flour exceeded this limit by the 90th and 120th days, respectively, whereas roasted flour remained within the acceptable range for up to 150 days. The reduced acid value in roasted flour is likely due to the inactivation of lipase and lipoxygenase during roasting, which slows oxidation and extends shelf-life (Rani *et al*., 2018). Germination also contributed to a lower acid value by reducing fat content through lipolysis and loss of solids during soaking (Sruthi *et al*., 2021). These findings emphasise the superior effectiveness of roasting in mitigating oxidative deterioration and enhancing millet flour storage stability. FFA content, a key indicator of secondary lipid oxidation and fat hydrolysis, also increased significantly ($p \leq 0.05$) over the storage period for all samples. The Codex Alimentarius Commission sets a permissible FFA limit of 3% (as oleic acid). Roasting at high temperatures effectively inactivated lipase, the enzyme responsible for FFA production, thereby preventing excessive FFA accumulation and extending the flour's shelf-life (Bhati *et al*., 2016). Germination similarly slows FFA increases by metabolising released FFAs via glyoxylate and oxidation pathways, converting triacylglycerols into carbohydrates (Sruthi and Rao, 2021). These results indicate that dry heat treatments, such as roasting and germination, significantly improve the storage stability of foxtail millet flour by minimising FFA accumulation and delaying oxidative degradation. Overall, the study demonstrated that processing techniques such as roasting and germination significantly prolonged the shelf-life of foxtail millet flour to approximately 4-5 months, whereas untreated flour exhibited stability for only two months. The millet-chickpea combination can be used for developingvalue-added, cost-effective products with high organoleptic scores, nutritional profile, shelf-life, and consumer acceptability (Rana *et al*., 2025). These processing methods not only mitigated oxidative deterioration and moisture accumulation but also improved sensory attributes, thereby enhancing the acceptability and convenience of millet-based products for wider consumer adoption.

5. Conclusion

Minor millets, such as foxtail millet, are highly nutritious and offer superior nutrient profiles compared to other cereal grains. These millets are also rich in antioxidants. However, the oil-rich germ in millet makes processing and storage of millet flour challenging, as it tends to separate from the endosperm. To address these issues, various processing methods commonly used at household levels,such as roasting, fermentation, germination, and soaking can be employed to reduce rancidity and minimise antinutritional factors. The present study aimed to assess the impact of commonly used processing techniques on the nutritional composition and shelf-life of foxtail millet cultivars. Four methods-roasting, germination, boiling, and pressure cooking were applied to the millet grains, and their effects on nutritional attributes were analysed. The findings revealed that processing methods had a significant influence on the nutritional profile of foxtail millet. *In vitro* protein digestibility improved after the treatments, while soluble, insoluble, and total dietary fiber levels decreased following roasting, boiling, and pressure cooking when compared to raw flour. Antinutritional components like phytic acid and tannins, present in foxtail millet, were significantly reduced by these processing methods. The shelf-life of flour derived from roasted and germinated grains was monitored over 6 months, with measurements taken every 30 days. All evaluated parameters increased significantly as storage time progressed, though the increase was slower in roasted flour. In contrast, germination resulted in a marked increase in total phenol content over time. In conclusion, simple processing procedures such as germination (soaking grains for 12 h, followed by germination for 48 h and drying in an oven at 60ÚC for 48 h and grinding into flour) and roasting can be used to boost the nutritional profile of foxtail millet flour by making the nutrients more digestible and accessible. The general population and food industries must be made aware of the use of common processing techniques before making products from Foxtail millet to make more nutritious products with a longer shelf-life.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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