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Research Article

Effect of Processing Techniques on Techno-Functional, Thermal, Pasting, and Digestibility Characteristics of Selected Millet Flours

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Abstract: Millets such as proso, foxtail, and finger millet are nutrient-dense grains with potential as functional food ingredients. Processing methods like fermentation, germination, and roasting can significantly enhance their nutritional and functional properties, making them suitable for incorporation into a wide range of food products. This study investigated the structural and functional modifications in millet flours induced by fermentation, germination, and roasting. Functional properties (water and oil absorption capacities, solubility), pasting behavior, thermal characteristics, starch digestibility, and protein digestibility were analyzed. Fourier Transform Infrared Spectroscopy (FTIR) was used to confirm changes in functional groups associated with processing. All processing methods improved the functional and digestibility properties of millet flours. Water absorption index ranged from 4.4 to 5.7 g/g and solubility index from 2.0 to 3.5 g/100 g. Oil and water absorption capacities were 1.5–1.9 g/g and 1.6–2.8 g/g, respectively. Pasting viscosities showed peak values between 74.8 and 125.4 cP, final viscosities of 83.4–141.7 cP, setback viscosities of 12.9–62.5 cP, and breakdown viscosities of 4.4–20.7 cP. Thermal analysis indicated onset, peak, and conclusion temperatures of 63.4–85.7 °C, 72.3–92.5 °C, and 79.2–102.3 °C, respectively. Readily digestible starch (14.3–40.7%), slowly digestible starch (38.9–57.7%), and resistant starch (14.7–28.9%) levels were significantly influenced by processing. Protein digestibility improved, ranging from 68.4 to 83.6%. FTIR confirmed the presence of functional groups, including alkane (-CH), hydroxyl (-OH), alkene (=CH), carbonyl (-C=O), and amine (-NH), indicating biochemical modifications. These findings support the potential use of processed millet flours as functional and nutritional ingredients in the development of diverse food products.

***Keywords*:** proso millets, finger millets, foxtail millets, digestibility, roasting, fermentation, germination

1. Introduction

Millets were cultivated even before the cultivation of rice and wheat, in the regions of Africa and Asia, especially in China, Pakistan, Bangladesh, and India. The major varieties of millets include proso (*Panicum miliaceum*), pearl (*Pennisetum glaucum*), foxtail (*Setaria italica*), and finger (*Eleusine coracana*). Millets are a nutritionally rich source of dietary fiber (12–20%), protein (6–19%), carbohydrates (60–70%), minerals (2–4%), and polyphenols [1]. Consumption

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of millets controls the release of blood glucose and is ultimately good for diabetic patients [2]. Millets are utilized on a large scale for the manufacturing of many food and beverage items, including non-alcoholic/alcoholic drinks, fermented/non-fermented flatbreads, and porridge [3]. The quality and production of these products highly depend on the composition, structure, and techno-functional characteristics of the major components of millets (starches and proteins). Millets contain a good amount of resistant starch, which is good for colon health [4].

The functional, rheological, and digestibility characteristics of millet flours determine the quality of the product. These characteristics play a crucial role in evaluating the physical and nutritional quality of raw material and final products and in predicting the processing behavior. It is also important to understand and predict how proteins, fats, and starches behave in a food system [5]. However, the rheological characteristics are pivotal for developing the recipe, flow process, predicting texture, optimization, processing, and storage stability, and to understand the conformational changes in the food matrix. Rheological behavior, particle size, shape, viscosity, rigidity, and swelling behavior of starches also affect the digestibility characteristics of the food matrix [6]. The concentration of resistant starch, protein, and anti-nutritional factors significantly influences the digestion rate due to complex interactions with each other. The compositional variation in structure and pretreatments such as fermentation, germination, and roasting of millet flours to change the composition has shown a significant influence on the functional, rheological, and digestibility characteristics of flours [5, 7]. Moreover, these characteristics of flours depend highly on the variation and changes in different millet varieties, which can be monitored to understand the end behavior of the product [1].

Dry heat treatment had a more pronounced impact on the pasting viscosity of flour compared to starch. It led to an increase in the pasting viscosity of the flour, whereas for starch, only the final viscosity was elevated [8, 9]. Unroasted flaxseed exhibited significantly higher foaming capacity and stability. In contrast, roasted flour demonstrated the highest values for water absorption capacity, bulk density, solubility, ash, fiber, and carbohydrate content. It also showed the lowest levels of moisture, protein, fat, oil absorption capacity, tap density, porosity, angle of repose, and water absorption index [10]. In some previous studies, the influence of fermentation, germination, and steeping on antinutritional elements, functional and chemical properties (tannin and phytic acid) of millets has been studied. However, the effect of these pretreatments on the structural changes of millet flour samples from Thailand has not been studied yet [11, 12]. Furthermore, how these structural changes are responsible for changes in techno-functional, thermal, digestibility, and pasting characteristics of flour samples needs to be addressed. This study aims to address the influence of fermentation, germination, and roasting on the structural changes of flour samples from major millet varieties (finger, foxtail, and proso millets) and how these structural changes affect the flour characteristics. However, all three selected varieties have different compositions, which can be compared to analyze the results and the influence of these differences on the properties of millet flours. Foxtail millet is rich in dietary fiber and has a low glycemic index, making it ideal for diabetic-friendly diets [13]. Proso millet is high in protein and easy to digest, while finger millet is exceptionally rich in calcium and iron, supporting bone health and anemia prevention [14]. This knowledge could be useful to understand the mechanism that governs these properties during processing and can be used to modify these characteristics according to the required need.

2. Materials & methods

2.1 *Materials and reagents*

Proso Millet (PM, BR-7), Foxtail Millet (FoM, PS 4), and Finger Millet (FM, VL 124) were supplied by Darich Green Co., Ltd., Thailand. All chemicals, such as phosphate buffer, HCl, NaOH, 3,5-dinitrosalicylic acid, pancreatic amylase, and trypsin, were procured from Sigma Aldrich Co. Ltd, USA. The starch kit was procured from Mega-zyme International, Ireland. Soybean oil was purchased from the local market.

2.2 *Sample preparation*

After cleaning and washing seeds, 300 g of each millet sample was used for fermentation, germination, and roasting. Samples were steeped in distilled water for 72 h at 25 °C to obtain the fermented seeds. Samples were soaked in distilled water at 25 °C for 12 h and were spread on muslin cloth for 18–24 h with continuous watering to maintain the germination of seeds. Each millet sample was roasted in acid-washed sand for 8–10 min at around 80 °C. Samples

were collected from sand using a sieve of 200 µm and washed in distilled water [15]. The germinated, roasted, and fermented samples were oven dried at 55–60 °C for 24 h using a hot air oven to obtain a moisture content of 10–12%. The dried samples were milled into powders using a high-speed grinder (DXFILL, DXM-400, Thailand) at 18,000 rpm for 5 min and sifted through an 80 mesh sieve.

2.3 *Proximate compositional analysis*

The protein, fat, ash, carbohydrate, and moisture contents were estimated according to Association of Official Analytical Chemists (AOAC) protocols [16]. Protein content was estimated using the Kjeldahl method, where the nitrogen content of the sample is measured and then multiplied by a conversion factor (6.25) to estimate total protein. Fat content was estimated using the Soxhlet method, in which fat is extracted from the sample using a non-polar solvent (petroleum ether) under continuous reflux, and the extracted fat is weighed after solvent removal. Ash content was estimated using a muffle furnace at 550 °C for 2 h, and crude fiber was determined by digesting the sample with HCl, washing with distilled water, digesting again with NaOH, and washing with distilled water. The residue was dried to estimate the fiber concentration. Starch content was assessed following the method outlined by Wongsagonsup et al. [17] through a starch kit (Mega-zyme International).

2.4 *Techno-functional characteristics*

Water Solubility Index (WSI) and Water Absorption Index (WAI) were determined according to the method outlined by Umar et al. [18] with minor changes. The sample (2.5 g) was dissolved in 30 mL of distilled water and then cooked at 90 °C for 15 min. Samples were centrifuged at 1,200 × g for 10 min using a lab-scale centrifuge (Hettich, EBA 8S, Germany), and the supernatant was collected. The weight of residue and dried supernatant was assessed by drying the sample at 110 °C till no weight change. The WAI and WSI were estimated using equations 1 and 2, respectively.

$$\text{WAI (g/g)} = \frac{Weight\ of\ sediments}{Weight\ of\ sample} \tag{1}$$

$$\text{WSI (g/100 g)} = \frac{Weight\ of\ dried\ supematent}{Weight\ of\ sample} \times 100 \tag{2}$$

To determine the Water Absorption Capacity (WAC), 1 g of the sample was combined with 10 mL of distilled water in a centrifuge tube. The mixture was stirred thoroughly for 30 sec and then incubated at 25 °C for 30 min. Samples were centrifuged (EBA, Hettich, Germany) for 25 min at 1,200 × g to discard the supernatant to measure the weight of the sample. WAC was estimated using equation 3.

$$\text{WAC (g/g)} = \frac{Weight\ of\ water\ absorbed}{Weight\ of\ sample} \tag{3}$$

Oil absorption was determined by dispersing the sample (1 g) in 10 mL of soybean oil in a centrifuge tube using a vortex mixer and allowing it to stand for 30 min. The samples were centrifuged (Hettich, EBA 8S, Germany) at 1,200 × g for 15 min. An excess amount of oil was removed [19]. Oil Absorption Capacity (OAC) was assessed using equation 4.

$$\text{OAC (g/g)} = \frac{Weight\ of\ oil\ absorbed}{Weight\ of\ sample} \tag{4}$$

2.5 *Pasting characteristics*

Pasting properties of flours were estimated following the method of Tangsrianugul et al. [20] with slight modification. A 2.5 g flour was added to an empty canister, and 25 mL of water was added and stirred well just before the evaluation of the viscosity profile. The canister was fitted into the Rapid Visco-Analyzer (RVA-4C, Newport Scientific Pty. Ltd., Australia) and heated at a prespecified heat cycle. Samples were held at 50 °C for 1 min and heated with a uniform heat rate to 95 °C within 5 min, held at this temperature for 3 min. The samples were cooled to 50 °C in 3 min and held for 1 min. Peak Viscosity (PV), Final Viscosity (FV), Trough Viscosity (TV), Setback Viscosity (SBV = FV − TV), and Breakdown Viscosity (BDV = PV − TV) were determined using Thermocline software.

2.6 *Thermal analysis of powders*

A 2 mg portion of homogenized millet powder was blended with 9 mg of distilled water and hermetically sealed in an aluminum pan. The pans were sealed to prevent moisture loss and then kept at room temperature (25 °C) for 1 hour to ensure a stable moisture content. The sealed pans were heated from 25 °C to 150 °C at a rate of 10 °C per minute, with a nitrogen purge flow of 50 mL/min, using a Differential Scanning Calorimetry (DSC) instrument (Model-214, NETZSCH, Germany). Transition temperatures such as Onset Temperature (T_o), Peak Temperature (T_p), Conclusion Temperature (T_c), and Gelatinization Enthalpy (ΔH, J/g) were analyzed [21].

2.7 *Structural changes in samples*

Structural changes in the powder samples were analyzed using Fourier Transform Infrared spectroscopy to assess their impact on functional properties. Approximately 2 mg of each powder sample was pressed onto an optical crystal cell, and spectra were recorded in the range of 4,000 to 500 cm^{-1} using a FTIR Spectrometer (Nicolet iS50, Thermo Scientific, USA) at 25 °C [22].

2.8 *Starch fractionation*

Samples (50 mg/mL in 0.2 M phosphate buffer, pH 6.9) were mixed with 0.5 mL of pancreatic amylase suspension (1.5 mg/mL, 40 U/mg) and incubated at 25 °C for 2 h. After incubation, 2 mL of 3,5-Dinitrosalicylic Acid (DNS) reagent was added, the volume was adjusted to 25 mL with distilled water, and the mixture was boiled for 5 min. Aliquots (0.25 mL) were collected at 20 and 120 min of incubation and mixed with 10 mL of 70% (v/v) ethanol to halt enzymatic activity. The mixtures were then centrifuged at 1,500 × g for 10 min and filtered to remove large particles. The resulting supernatants were used to determine glucose concentration using the Glucose Oxidase-Peroxidase (GOPOD) assay kit [17]. Rapidly Digestible Starch (RDS), Slowly Digestible Starch (SDS), and Resistant Starch (RS) content were estimated using equations 5, 6, and 7, respectively.

$$\text{RDS (\%)} = \%G20 \times 0.9 \tag{5}$$

$$\text{SDS (\%)} = (\%G120 - \%G20) \times 0.9 \tag{6}$$

$$\text{RS (\%)} = \%TS - (\%RDS + \%SDS) \tag{7}$$

2.9 *Protein digestibility*

Protein digestibility was evaluated by incubating 1 g of the sample with pepsin solution (3,000 U/mL, pH 2.5) at 37 °C and 150 Revolutions Per Minute (RPM) in a shaking incubator. After 2 h of incubation, 30 mL of trypsin (2,500 U/mL) was added to the solution, and the pH was adjusted to 7 using 0.5 M NaOH. Samples were further incubated at 37 °C with shaking (150 RPM) for 2 hours, then digestion was halted by adding 10% Trichloroacetic Acid (TCA), followed by centrifugation at 503 × g for 20 minutes, and the supernatant's protein content was measured using the Bradford assay [23]. Protein digestibility was estimated using equation 8.

$$\text{Digestibility (\%)} = \frac{\textit{Protein content in digesta}}{\textit{Protein content in sample}} \times 100 \quad (8)$$

2.10 *Statistical analysis*

Statistical analysis of triplicate measurements was performed using Statistical Package for the Social Sciences (SPSS) software (Version 16, Chicago, IL, USA). Analysis of Variance (ANOVA) (One-way) followed by Tukey's post hoc test was applied to identify significant differences among samples at a 95% confidence level.

3. Results & discussion

3.1 *Nutritional analysis of millet flours*

Table 1. Nutritional composition of millet flours after germination, fermentation, and roasting

Composition	FMC	FMF	FMR	FMG	FoMC	FoMF	FoMR	FoMG	PMC	PMF	PMR	PMG
Moisture (%)	8.15 ± 1.2^{b}	8.5 ± 0.9^{b}	8.2 ± 1.1^{b}	8.4 ± 0.7^{b}	8.53 ± 1.3^{b}	8.5 ± 0.8^{b}	7.8 ± 0.9^{ab}	8.9 ± 0.6^{b}	7.8 ± 0.9^{ab}	8.1 ± 1.3^{b}	6.4 ± 1.2^{a}	8.3 ± 0.9^{b}
Total Carbs (%)	79.0 ± 2.4^{bc}	78.1 ± 2.1^{bc}	78.5 ± 1.7^{bc}	77.4 ± 1.2^{bc}	73.0 ± 1.4^{b}	70.2 ± 1.5^{a}	76.3 ± 1.3^{b}	70.2 ±1.8^{a}	73.2 ± 1.9^{b}	68.3 ± 1.4^{a}	75.4 ± 1.3^{b}	69.5 ± 1.5^{a}
Starch (%)	59 ± 3.1^{ab}	56.2 ± 1.2^{a}	58.3 ± 1.1^{ab}	57.5 ± 1.4^{ab}	59 ± 2.2	57.4 ± 1.4^{ab}	58.4 ± 1.2^{ab}	56.4 ± 0.9^{a}	56.0 ± 1.5^{a}	54.7 ± 1.6^{a}	58.3 ± 1.3^{ab}	55.1 ± 0.9^{a}
Fiber (%)	3.8 ± 0.2^{c}	3.44 ± 0.6^{c}	3.54 ± 0.5^{c}	4.25 ± 0.8^{d}	7.6 ± 0.4^{g}	5.6 ± 1.1^{e}	6.2 ± 1.4^{f}	6.4 ± 0.7^{f}	2.2 ± 0.2^{ab}	2.4 ± 0.9^{b}	1.8 ± 2.2^{a}	2.1 ± 0.3^{ab}
Protein (%)	8.6 ± 0.3^{a}	9.6 ± 0.9^{ab}	8.4 ± 1.3^{ab}	9.5 ± 1.1^{ab}	10.8 ± 0.4^{b}	11.7 ± 1.2^{bc}	8.7 ± 1.1^{a}	12.3 ± 0.6^{c}	12.2 ± 0.6^{c}	13.3 ± 0.6^{d}	10.9 ± 1.1^{b}	12.4 ± 1.2^{c}
Fat (%)	4.6 ± 0.4^{c}	4.1 ± 1.1^{c}	4.2 ± 0.5^{c}	4.2 ± 0.3^{c}	4.2 ± 0.3^{c}	3.5 ± 0.7^{b}	3.1 ± 0.4^{b}	3.7 ± 2.2^{b}	1.0 ± 0.1^{a}	0.9 ± 0.5^{a}	0.9 ± 0.2^{a}	1.1 ± 0.4^{a}
Ash (%)	2.3 ± 0.2^{c}	2.37 ± 0.3^{c}	2.1 ± 0.7^{c}	2.96 ± 0.6^{cd}	0.47 ± 0.1^{a}	0.3 ± 0.4^{b}	0.37 ± 0.4^{b}	1.7 ± 0.5^{b}	1.7 ± 0.2^{b}	1.4 ± 0.7^{b}	1.2 ± 2.2^{b}	1.3 ± 1.2^{b}

Each treatment value is given as the mean of triplicate ± SD. Means with no common letters within a row significantly differ ($p < 0.05$).
FMC: Finger Millet-Control, FMF: Finger Millet-Fermented, FMR: Finger Millet-Roasted, FMG: Finger Millet-Germinated, FoMC: Foxtail Millet-Control, FoMF: Foxtail Millet-Fermented, FoMR: Foxtail Millet-Roasted, FoMG: Foxtail Millet-Germinated, PMC: Proso Millet-Control, PMF: Proso Millet-Fermented, PMR: Proso Millet-Roasted, PMG: Proso Millet-Germinated.

The nutritional composition of millet flours after processing was estimated and listed in Table 1. These nutritional values for all varieties were in close range with values obtained in previous studies for finger millet [24, 25], foxtail millet [26], and proso millet flours [27]. The moisture content was below the recommended safe limit (10%) for each variety, even after each treatment [25]. A higher amount of carbohydrate content (79.0%) with the lowest protein content (8.6%) was observed in FMC as compared to other varieties. Starch content was almost the same for all types of millet flours, while the fiber content (7.6%) was significantly higher in FoMC as compared to other varieties. The minimum amount of fat and ash was observed for FoMC and FMC, respectively. The protein content was increased in the case of fermentation and germination, which could be due to the growth of enzymes, the synthesis of new proteins, and the degradation of antinutritional factors during these processes [25, 28]. A slight increase in the protein concentration of FM due to germination has also been observed in a previous study [1]. The decrease in the concentration of fat and ash content was observed due to germination and fermentation. The lipolytic hydrolysis of lipids by the lipase enzyme and consumption of lipids as an energy source decrease the fat content during these processes [29]. The reduction in ash content can also be related to enzymatic activities, which reduce the dry matter in grains [30]. However, after roasting of grains, a decrease in moisture, fat, ash, and protein content was observed for all varieties. A similar trend

was observed in a previous study for foxtail millet flour after roasting the grains [26]. The heat treatment removes the moisture content from grains, indicating a decrease in moisture content [31]. An increase in carbohydrate concentration after roasting was observed in this study, which was also observed in a previous study after cooking treatment [31]. The higher amount of carbohydrate in flour samples could serve as a source of energy and minerals [32]. Roasting reduces moisture and sometimes volatile compounds or heat-sensitive components. As a result, the remaining nutrients, including carbohydrates, become more concentrated on a per-weight basis. Additionally, Maillard reactions or partial starch gelatinization during roasting may alter carbohydrate structure [15]. The decline in protein content resulted from the elimination of certain amino acids. The decrease in fat and moisture content stemmed from the breakdown of fat or the formation of starch-lipid complexes. However, ash and crude fiber content increased due to a decrease in phytic acid content during roasting [33].

3.2 *Functional properties of millet flours*

The WAC, solubility, and WAI are crucial parameters in food matrix and affect the functional characteristics of food formulation. Different flour samples exhibit varying affinities for water molecules, which is influenced by the differences in the polar amino acid residues of their proteins [34]. The WAI of all the selected flour samples was in the range of 4.4–5.7 g/g; the highest WAI (5.7 g/g) was observed for FMR, and the lowest WAI (3.8 g/g) was observed for FoMG (Table 2). The WAI values for all varieties were slightly different from the control sample due to fermentation, roasting, and germination. The WAI of flour samples is influenced by the hydrophilicity of proteins and starches [35]. Fermentation and germination increase the secondary and tertiary structures of proteins by breaking down primary structures, which increases the hydrophilicity [31]. The WSI values for all the samples were in the range of 2–3.5 g/100 g, the highest values were for FMF, while the lowest was for PMC. All the processing methods showed an increase in WSI for all the millet flours. The increase in WAI and WSI of millet flour was also observed by Sudha et al. [26] due to the roasting process. Roasting showed greater influence on WSI and WAI as compared to germination and fermentation due to glucose and fructose being reducing sugars that react with amino acids in thermal processes to generate specific hydrophilic compounds [36].

Table 2. Functional properties of millet flours after processing

Properties	FMC	FMF	FMR	FMG	FoMC	FoMF	FoMR	FoMG	PMC	PMF	PMR	PMG
WAI (g/g)	4.8 ± 0.3^{ba}	4.5 ± 0.1^{a}	5.7 ± 1.1^{c}	4.4 ± 0.5^{a}	4.5 ± 0.7^{a}	4.5 ± 0.9^{a}	5.2 ± 1.1^{b}	4.4 ± 0.6^{a}	4.5 ± 0.4^{a}	4.7 ± 0.5^{a}	5.1 ± 0.6^{b}	4.8 ± 0.3^{ba}
WSI (g/100 g)	2.9 ± 0.1^{c}	3.5 ± 0.1^{bc}	3.2 ± 0.9^{cd}	3.3 ± 0.2^{cd}	3.2 ± 0.3^{cd}	3.3 ± 0.2^{cd}	3.3 ± 0.2^{cd}	3.2 ± 0.3^{cd}	2.0 ± 0.2^{a}	2.8 ± 0.3^{a}	3.0 ± 0.4^{c}	3.0 ± 0.2^{c}
OAC (g/g)	1.6 ± 0.1^{a}	1.5 ± 0.1^{a}	1.8 ± 0.2^{ab}	1.9 ± 0.1^{b}	1.6 ± 0.2^{a}	1.7 ± 0.1^{a}	1.9 ± 0.4^{ab}	1.6 ± 0.1^{a}	1.7 ± 0.2^{a}	1.6 ± 0.2^{a}	1.8 ± 0.2^{ab}	1.7 ± 0.1^{a}
WAC (g/g)	1.9 ± 0.2^{ab}	2.8 ± 0.2^{c}	2.2 ± 0.4^{b}	2.2 ± 0.4^{b}	2.1 ± 0.1^{b}	2.1 ± 0.2^{b}	2.3 ± 0.2^{b}	1.8 ± 0.2^{a}	1.6 ± 0.1^{a}	2.1 ± 0.1^{b}	1.8 ± 0.1^{a}	2.1 ± 0.2^{b}

Each treatment value is given as the mean of triplicate ± SD. Means with no common letters within a row significantly differ ($p < 0.05$).

OAC of flour refers to the protein's ability to bind oil through physical capillary attraction, involving the entrapment of oil within protein molecules via non-covalent bonds [32]. The OAC and WAC values were in the range of 1.5–1.9 g/g and 1.6–2.8 g/g, respectively. The values of OAC and WAC were found to be almost similar, whereas germinated finger millet flour showed OAC (1.9 g/g) significantly higher ($p < 0.05$) than other flours. The fermented finger millet flour also showed significantly higher ($p < 0.05$) WAC (2.8) value from other flour samples. WAC increased due to fermentation, which reduced the insoluble fraction of flour and increased the soluble starch and amino acids [37] as well as the secondary and tertiary structures of proteins by breakdown of primary structures, which increases the hydrophilicity [38]. The OAC increased for all the selected flour samples after roasting, germination, and fermentation because variation is generated in the non-polar sides of protein subunits, which bind with the oil side chain of hydrocarbon [31]. The higher OAC is suitable for improving the mouthfeel and flavor of food formulations such as

sausages, chiffon, deserts, sponge cakes, and toppings [39].

3.3 *Pasting characteristics*

Pasting properties are influenced by the stiffness of starch particles, which impacts the swelling capacity of granules and the ability of amylose to leach into the solution [40]. This is essential for choosing appropriate binders and thickeners in food products [41]. Pasting properties of all varieties after fermentation, germination, and roasting are shown in Table 3. Peak, final, setback, and breakdown viscosities were in the range of 74.8–125.4 cP, 83.4–141.7 cP, 12.9–62.5 cP, and 4.4 to 20.7 cP, respectively, for all millet flour samples. Peak viscosity values for all processed samples of finger millets (FMF, FMR, and FMG) were lower than FMC and were statistically insignificant ($p > 0.05$). Peak viscosities for all foxtail millet flour samples (FoMF, FoMR, and FoMG) were insignificant ($p > 0.05$) due to germination, roasting, and fermentation. Proso Millet flour had significantly ($p < 0.05$) lower peak viscosities due to Roasting (PMR) and Germination (PMG), while the Fermentation (PMF) showed nonsignificant ($p > 0.05$) results. Similar results for pasting properties of finger millet flour [42], proso millets [43], and foxtail millets [44] were also reported by previous studies. Pasting temperatures for finger, foxtail, and proso millet flours were in the range of 78.5 to 89.2 °C and values for processing treatment of each variety were statistically nonsignificant ($p > 0.05$). The rise in pasting temperature may result from the formation of double-helical, highly structured amylopectin clusters [45]. The pasting temperature and peak viscosity had a negative relationship due to increased interactions between starch molecules after processing [46]. A comparable increase in pasting temperature was observed in heat-treated proso millet flours, where protein denaturation enhances interactions with starch [39]. The PV, TV, FV, and SBV values were significantly decreased ($p < 0.05$) due to fermentation, germination, and roasting, while the BDV values were increased as compared to the control sample for all varieties of millet flours. Due to these processing techniques, the structure of proteins and carbohydrates changes [31]. Starch particles that swell less tend to break down more easily, causing an increase in Breakdown Viscosity (BDV) and a decrease in amylose complexes with lipids and proteins, which in turn lowers peak viscosity [20].

Table 3. Pasting properties of millet flours after no treatment, fermentation, roasting, and germination.

Properties	FMC	FMF	FMR	FMG	FoMC	FoMF	FoMR	FoMG	PMC	PMF	PMR	PMG
PV (cP)	104.2 ± 1.8^{d}	91.3 ± 3.1^{c}	89.8 ± 2.1^{c}	88.9 ± 2.3^{c}	74.8 ± 1.5^{a}	78.5 ± 1.2^{ab}	77.6 ± 1.4^{a}	72.3 ± 1.1^{a}	124.2 ± 0.7^{e}	125.4 ± 2.6^{e}	117.2 ± 1.7^{c}	110.4 ± 1.7^{d}
TV (cP)	97.6 ± 2.1^{de}	84.1 ± 2.1^{c}	82.5 ± 2.2^{c}	84.5 ± 3.1^{c}	55.1 ± 1.7^{a}	57.8 ± 2.4^{a}	61.2 ± 1.5^{a}	54.9 ± 1.6^{a}	115.4 ± 0.6	111.3 ± 3.2	99.2 ± 1.2^{bc}	91.5 ± 0.8^{d}
FV (cP)	121.9 ± 1.1^{e}	98.6 ± 2.3^{c}	96.7 ± 1.2^{ab}	97.4 ± 2.2^{c}	83.4 ± 2.1^{a}	87.6 ± 1.8^{ab}	88.3 ± 1.2^{ab}	89.5 ± 0.9^{ab}	131.3 ± 0.4^{f}	136.4 ± 2.4^{fg}	124.7 ± 0.8^{g}	127.6 ± 2.2^{ef}
BDV (cP)	6.6 ± 0.2^{ab}	7.2 ± 0.8^{ab}	7.3 ± 1.4^{ab}	4.4 ± 1.5^{a}	19.7 ± 1.3^{de}	20.7 ± 1.5^{de}	16.4 ± 0.8^{cd}	17.4 ± 0.6^{cd}	8.8 ± 0.5^{ab}	14.1 ± 1.1^{c}	18.0 ± 0.5^{d}	18.9 ± 1.3^{d}
SBV (cP)	24.3 ± 0.2^{c}	14.5 ± 0.3^{ab}	14.2 ± 1.1^{ab}	12.9 ± 1.2^{a}	28.3 ± 0.9^{d}	29.8 ± 1.7^{d}	27.1 ± 1.3^{d}	34.6 ± 0.8^{e}	15.9 ± 0.3^{ab}	25.1 ± 1.9^{c}	25.5 ± 1.2^{c}	36.1 ± 0.8^{ef}
Peak T (min)	6.4 ± 0.4^{bc}	6.47 ± 0.6^{bc}	5.20 ± 0.5^{ab}	6.39 ± 0.7^{bc}	6.4 ± 0.3^{bc}	4.5 ± 0.6^{a}	4.8 ± 0.3^{a}	5.7 ± 0.2^{ab}	6.3 ± 0.5^{bc}	6.2 ± 0.6^{bc}	5.1 ± 0.8^{a}	5.8 ± 0.4^{ab}
Pasting T (°C)	84.85 ± 2.4^{b}	87.3 ± 3.5^{c}	88.4 ± 3.2^{c}	89.2 ± 1.7^{c}	79.5 ± 2.1^{a}	81.5 ± 1.7^{a}	83.6 ± 1.2^{ab}	85.2 ± 1.1^{ab}	78.5 ± 2.4^{a}	78.9 ± 2.5^{a}	82.4 ± 3.1^{ab}	80.7 ± 1.8^{ab}

Each treatment value is given as the mean of triplicate ± SD. Means with no common letters within a row significantly differ ($p < 0.05$). Peak Viscosity (PV), Trough Viscosity (TV), Final Viscosity (FV), Breakdown Viscosity (BDV), and Setback Viscosity (SBV).

3.4 *Thermal characteristics*

Whole flour is a complex system of protein, starch, fat, fiber, and other compounds that affect the heating behavior of the flour sample [41]. The gelatinization factors, including T_o, T_p, T_c, and ΔH of flour samples after processing,

were listed in Table 4. The T_o, T_p, and T_c values for all the selected flour samples were in the range of 63.4–85.7 °C, 72.3–92.5 °C, and 79.2–102.3 °C, respectively. The highest values of T_o, T_p, and T_c were for FoMR, FMC, and FMC, respectively, while the lowest values were for PMF, PMG, and PMF, respectively. It was observed that the fermentation and germination decreased the T_o, T_p, and T_c values for finger, proso, and foxtail millet flours. However, roasting treatment of grains increased T_o, T_p, and T_c values for foxtail and finger millet flours, while decreasing for finger millet flours with the decrease in ΔH values. The higher T_o values due to roasting of foxtail and proso millet flours were due to structural changes and alteration of the inter-crystalline amorphous form of starch granules [47]. Due to exposure to high temperatures during roasting, higher gelatinization temperatures were also observed in a previous study for chestnut flour [31]. Different values for gelatinization temperatures were found for different flour samples due to variation in form, size, and distribution of starch granules [48]. Since the ΔH value reflects the amount of double-helical structures, heat treatment can reduce ΔH due to starch gelatinization and rearrangement, which enhances the ideal double-helical order and raises the gelatinization temperature [49]. These results for thermal properties were similar to results obtained in previous studies for finger millet [42], foxtail millet [50], and proso millet [39]. Changes in T_o, T_p, and T_c values reflect the thermal behavior of starch in millet flours, affecting how easily and uniformly the starch gelatinizes during processing. Higher gelatinization temperatures typically enhance texture stability and water retention, improving the quality and shelf life of baked or extruded products.

Table 4. Thermal properties of millet flour samples after different processing treatments

Properties	FMC	FMF	FMR	FMG	FoMC	FoMF	FoMR	FoMG	PMC	PMF	PMR	PMG
T_o (°C)	82.4	75.4	80.2	74.9	84.5	80.2	85.7	81.3	67.2	63.4	66.9	64.2
T_p (°C)	92.5	86.9	90.3	88.7	86.7	85.5	91.4	84.5	75.4	74.5	78.4	72.3
T_c (°C)	102.3	97.6	99.9	95.4	89.3	92.3	94.5	93.4	82.3	79.2	84.3	81.3
T_c-T_o (°C)	19.9	22.2	19.7	20.5	4.8	12.1	8.8	12.1	15.1	15.8	17.4	17.1
ΔH (J/g)	3.25	3.11	2.16	2.97	3.12	2.43	2.35	2.93	3.95	3.47	3.13	5.13

T_o: Onset gelatinization Temperature, T_p: Peak gelatinization Temperature, T_c: Conclusion Temperature, and ΔH: Change in Enthalpy.

3.5 *Structural changes in flour samples after processing*

The Fourier Transform Infrared Spectroscopy (FTIR) analysis using the functional group identification was used to observe the effect of fermentation, germination, and roasting on the millet flours. FTIR spectra for millet flours were collected after processing and illustrated in Figure 1 to observe the peaks in specific regions of the samples. These peaks showed hydroxyl (-OH), alkane (-CH), amine(-NH), carbonyl (-C=O), and alkene (=CH) functional groups, respectively, in the flour samples [31, 51]. The peak regions of 1,200–900 cm^{-1} and 1,700–1,500 cm^{-1} represented carbohydrates and proteins in the samples, respectively. The amide I region (1,700–1,600 cm^{-1}) primarily corresponds to C=O stretching vibrations of the polypeptide backbone, while the amide II region (1,600–1,500 cm^{-1}) is associated with N-H bending and C-N stretching vibrations of proteins [52]. While the number of peaks remained consistent across all selected flour samples, noticeable variations were observed in the intensity of peak absorption. The fermentation reduced the peak intensity in the region of 3,300–3,200 cm^{-1} for finger and foxtail millet flours. The peak at 1,746 cm^{-1} for FoMC moved to 1,700 cm^{-1} because of germination on the C=C group. The peak at 2,850 cm^{-1} due to -CH_2 of lipids vanishes after Roasting (PMR), and the peak at 1,707 cm^{-1} also moves to 1,740 due to Fermentation (PMF) of proso millet flours. These results suggest that the molecular composition of the flour samples remained largely unchanged, highlighting how the processing methods primarily enhance functional and physicochemical properties.

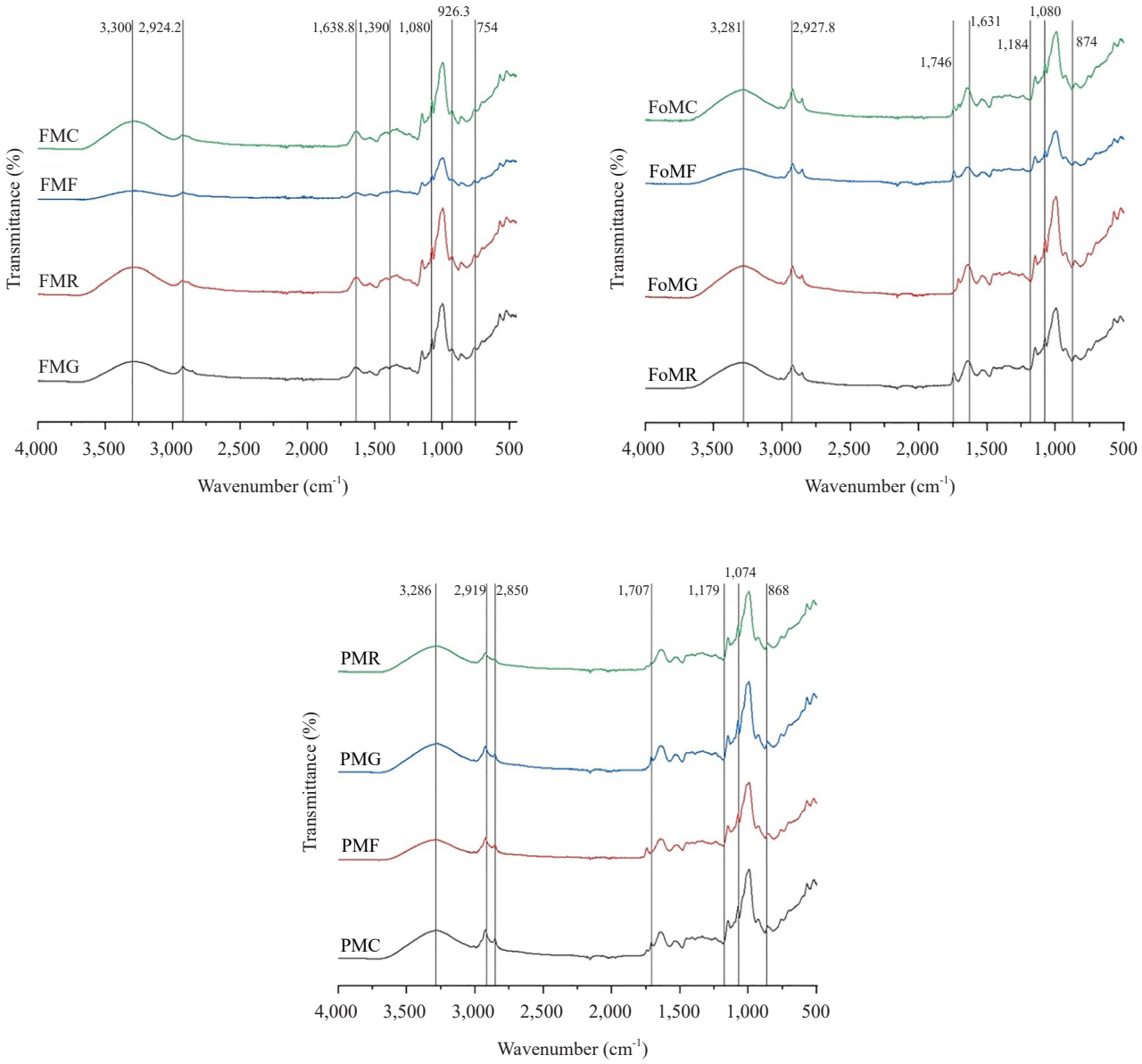

Figure 1. The structural changes observed in the millet flours after processing. FM: Finger Millet, FoM: Foxtail Millet, and PM: Proso Millet prepared after Frmentation (F), Germination (G), and Roasting (R), while the sample without any pretreatment was used as a Control (C)

3.6 *Starch digestibility*

Starch digestion is a key nutritional indicator in millets, reflecting their product value and health benefits. Higher levels of Resistant Starch (RS) and Slowly Digestible Starch (SDS) are associated with numerous health advantages, including a lower glycemic index, reduced fat and cholesterol levels, and potential protection against colon cancer [53]. The digestibility of starch fractions was estimated and presented in Table 5. The RDS, SDS, and RS values were in the range of 14.3–40.7%, 38.9–57.7%, and 14.7–28.9%, respectively. Digestion of millet starch is affected by many factors, such as millet types, processing operation, lipids, protein fractions, amylose content, starch morphology, amylose lipid complexes, fiber, and antinutrients in the flour, which can influence the enzyme activity on molecules [39, 54]. RDS fraction increased due to fermentation and germination, while the RS fraction decreased significantly for all types of flour samples. SDS fraction for finger and proso millet flours decreased while remaining statistically unchanged ($p > 0.05$) for foxtail millet flours due to fermentation and germination. However, the roasting process showed a significant

($p < 0.05$) increase in RS and SDS fractions and a decrease in RDS fraction for all the selected flour samples, which is good for many health benefits. Processing methods such as germination, fermentation, and removal of proteins and lipids reduce the RS content due to increased susceptibility of the enzyme. Other techniques, including autoclaving and roasting, enhance the RS content of millet flour [2]. Similar effects of germination and fermentation on the digestible starch of finger millets [25], proso millets [55], and foxtail millets [56] have been observed in previous studies.

Table 5. Starch digestion from millet flours after processing treatments

Properties	FMC	FMF	FMR	FMG	FoMC	FoMF	FoMR	FoMG	PMC	PMF	PMR	PMG
RDS (%)	15.6 ± 0.6^{a}	23.4 ± 0.3^{b}	14.3 ± 0.4^{a}	34.4 ± 0.4^{c}	33.4 ± 1.2^{c}	38.5 ± 1.1^{cd}	28.2 ± 1.4^{bc}	35.4 ± 2.0^{c}	32.4 ± 0.2^{c}	34.4 ± 0.3^{c}	29.2 ± 0.4^{bc}	40.7 ± 0.8^{d}
SDS (%)	57.7 ± 1.2^{d}	56.3 ± 0.5^{d}	56.8 ± 0.3^{d}	50.9 ± 0.9^{c}	44.2 ± 0.8^{b}	41.4 ± 1.2^{a}	45.9 ± 1.8^{b}	44.9 ± 0.9^{b}	39.1 ± 1.1^{a}	38.9 ± 0.2^{a}	40.6 ± 0.6^{a}	39.1 ± 0.5^{a}
RS (%)	26.7 ± 0.7^{cd}	20.3 ± 0.1^{b}	28.9 ± 0.5^{cd}	14.7 ± 0.1^{a}	22.4 ± 0.4^{bc}	20.1 ± 0.7^{b}	24.3 ± 0.4^{c}	18.8 ± 1.1^{b}	28.5 ± 1.3^{d}	26.7 ± 0.8^{cd}	30.2 ± 0.7^{d}	20.2 ± 0.4^{b}

Each treatment value is given as the mean of triplicate ± SD. Means with no common letters within a row significantly differ ($p < 0.05$). RDS: Rapidly Digestible Starch, SDS: Slowly Digestible Starch, and RS: Resistant Starch.

3.7 *In vitro protein digestibility*

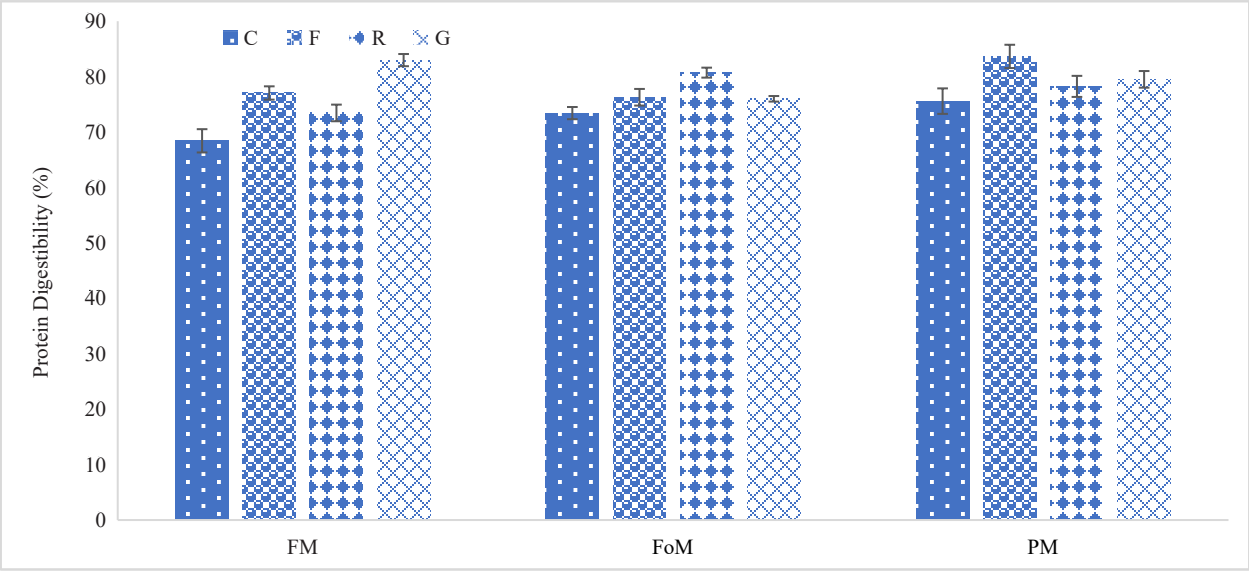

Figure 2. *In vitro* protein digestibility of millet flour samples. FM: Finger millet, FoM: Foxtail millet, and PM: Proso millet prepared after fermentation (F), germination (G), and roasting (R), while the sample without any pretreatment was used as a control (C)

Many processing methods, such as cooking, fermentation, soaking, roasting, microwave, and germination, have been found to improve the nutritional value of millet proteins by decreasing the antinutritional factors. However, these processing methods, along with antinutritional factors, can also decrease the micronutrients and generate unwanted chemical changes in flour [57]. The *in vitro* protein digestibility results are demonstrated in Figure 2. The protein digestibility was improved due to all processing techniques, and values were in the range of 68.4–83.6% for all millet flours. The FMC, FoMC, and PMC showed protein digestibility of 68.4, 73.4, and 75.6%, which increased to 77.1, 76.3, and 83.6% due to fermentation, 73.5, 80.7, and 78.2% due to roasting, and 82.9, 76, and 79.5% due to the germination

process. Poor digestibility of FMC protein was due to interactions of proteins with starch, fiber, and phytic acids [58]. Improvement in protein digestibility of finger millet was also observed due to heating [58] and fermentation [59] in previous studies. Germination improves the protein digestibility by reducing the phytic acid and polyphenols in seedlings and increasing the soluble protein fraction due to proteolytic enzymes [50]. Phytic acid reduces *in vitro* protein digestibility by forming insoluble complexes with proteins and inhibiting digestive enzymes. When phytic acid levels are reduced through processing methods like fermentation or germination, these inhibitory interactions are minimized. This enhances the accessibility of enzymes to protein substrates, allowing for more efficient hydrolysis during digestion [60]. The heating process increases the protein susceptibility to enzyme attack and reduces the interaction of proteins with other complex compounds, thus improving the digestibility [61].

4. Conclusion

The investigation into the effect of processing methods on the pasting, techno-functional, and digestibility characteristics of major millet flours underscores the intricate interplay between processing techniques and the resultant physicochemical characteristics of these nutritious grains. The carbohydrate, ash, and fiber content decreased while protein content increased due to this processing technique. Functional properties such as WSI, WAI, WAC, and OAC improved because of the structural breakdown of complex molecules, along with an increased concentration of hydrophilic components and protein content. Increased WAC and OAC improved the functional properties of flour, which can be used in the food formulation of healthy products without gluten. The pasting and gelatinization temperatures were decreased in all samples due to processing, indicating a reduction in complex compounds and antinutritional factors. *In vitro* protein digestibility was enhanced for all samples, while roasting increased the SDS and RS fractions of starch. These processing techniques can be used to improve the nutritional quality of these millets, offering significant potential for innovative food product development with improved functional, pasting, and digestibility characteristics. Nevertheless, the optimal processing method should be carefully selected based on the desired outcomes, considering both nutritional enhancement and technological functionality.

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Conflict of interest

The authors declare no competing financial interest.

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