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Recent Progress in Nutrition

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Research Article

Influences of Household Processing Methods on Nutritional Composition, Anti-nutritional Factors and Antioxidant Activities of Foxtail Millet

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Academic Editor: Costantino Paciolla

Special Issue: Nutritional Quality Improvement Of Cereals and Their Derived Products

Recent Progress in Nutrition
2023, volume 3, issue 4
doi:10.21926/rpn.2304020

Received: July 17, 2023
Accepted: October 25, 2023
Published: November 09, 2023

Abstract

The present investigation aims to study the influence of household processing methods, such as soaking, germination, and steam cooking, on nutritional composition (proximates, amino acids, minerals, and vitamins), antinutrient factors (phytochemicals: tannins, total phenols, phytic acid; enzyme inhibitors: trypsin and α-amylase inhibitors) and antioxidant properties of whole grains of foxtail millet. The contents of total ash (1.42%), fat (3.02 g/100 g), and mineral chromium (12.83 mg/100 g) were found to be high upon soaking. Trypsin inhibitors were reduced from 26.84% (steam cooking) to 12.45% upon soaking. Germination enhanced the contents of protein (11.57 g/100 g), minerals, i.e., Ca (32.48 mg/100 g), P (5.82 mg/100 g), Fe (5.81 mg/100 g), amino acid tyrosine and vitamin B_1. A significant

decrease in the contents of tannins from 218.93 mg GAE/g (soaking) to 93.93 mg GAE/g), phytic acid from 307.5 mg/g (soaking) to 97.63 mg GAE/g) and α-amylase inhibitors from 35.20% (steam cooking) to 5.61% were recorded upon germination. The TPCs were reduced from 13.63 mg GAE/g (soaking) to 6.81 mg GAE/g upon steam cooking. Germinated grains recorded the highest antioxidant properties based on the scavenging capacity of DPPH (0.59 mg AScE/g), $ABTS^{+}$ (10.77 mg TE/g) radicals and RPA (0.89 mg AScE/g). Overall, different household processing methods altered the composition of nutrients, antinutrients, and antioxidant properties. Germination has enriched the bioavailability of protein, carbohydrates, minerals, amino acids, and antioxidant properties and reduced the anti-nutritional factors, especially phytic acid, tannins, and α- amylase inhibitors.

Keywords
Antinutrients; cooking; foxtail millet; germination; nutrients; soaking

1. Introduction

Foxtail millet [*Setaria italica* (L.) P. Beauv], often known as "Italian or German millet," is a cereal from the subfamily Panicoideae and Poaceae. The crop has been cultivated in semiarid regions of Asia as a staple food and forage in Europe and other parts of the World [1]. Foxtail millet ranks second in world production, about 2.29×10^6 t from an area of 10.57×10^6 ha [2]. Like other millets, foxtail millet can withstand heat, drought, salt, and poor soil quality, making it suitable for dryland and hilly agriculture. Foxtail millet is considered a model crop to study the genetics and genomics of other cereals and biofuel crops. Recently, this crop caught global attention due to its unique nutritional composition, agronomical, climate resilient features, and economic benefits [3].

Compared to staple cereals, foxtail millet is nutritionally rich with higher concentrations of starch, protein, minerals, fat, dietary fiber, vitamins, and phytochemicals with diverse therapeutic applications. It is a key ingredient in preparing pancakes, porridge, noodles, cookies, bread, drinks, and instant powder [4]. Foxtail millet grains contain anti-nutritional factors like phytochemicals e.g., tannins, phytates, phenols, and enzyme inhibitors e.g., α-amylase and trypsin inhibitors [5]. Phytates mitigate oxidative stress by binding iron in Fenton's reaction. Phenolics and tannins are potential natural sources of antioxidants in food [6]. The polyphenols and high fiber in foxtail millet have adjuvant therapeutic effects on cardiovascular disease, cancer prevention, and body weight regulation [7]. Foxtail millet flour is a functional food for people with diabetes due to its slow starch digestibility and median glycemic index [8]–enzymes like α-amylase and α-glucosidase help to regulate carbohydrate metabolism and reduce blood sugar levels and obesity. However, the multifunctional activities of these enzymes were inhibited by some specific inhibitors [9].

Foxtail millet has an indigestible seed coat, which needs to be removed before the grain is fit for consumption. The primary processing methods, such as dehusking, soaking, germination, roasting, drying, polishing, and milling, are used to consume foxtail millet grains. In addition, these processes minimize the inhibitory effect of anti-nutritional factors, increase mineral bioavailability and protein digestibility, prolong the shelf-life, and enhance sensory characteristics [10]. Simultaneously, secondary processing methods such as fermenting, parboiling, cooking, puffing,

popping, malting, baking, flaking, and extrusion were employed to prepare value-added foxtail millet food [11]. Much attention has been given to the studies on the nutritional composition [12], antioxidant, and sensory properties of foxtail millet [6, 13-15] Various researchers have also studied the effect of processing methods such as extrusion, super fine grinding, microbial fermentation, germination, and heat-moisture treatment on nutrient composition [10, 16], and anti-nutritional factors [9].

The processing techniques will inevitably have favorable or unfavorable effects on nutritional composition, anti-nutritional factors, and antioxidant activities of foxtail millet [17]. However, the studies on the impact of household processing methods on antinutrient factors are limited [18]. Comparative analysis for nutritive compounds and anti-nutritional elements is also scarce [19]. Therefore, the present investigation aims to study how commonly used household methods, such as soaking (hydration), germination, and steam cooking (thermal treatment) affect nutritional composition (proximates, amino acids, minerals, and vitamins), anti-nutritional factors, and antioxidant properties of the flour of the whole grains of foxtail millet variety (SIA 3222).

2. Materials and Methods

2.1 Materials

The grains of one certified foxtail millet variety SIA 3222 were procured from the Regional Agricultural Research Station (RARS), Nandhyal, Andhra Pradesh, India. Grains were graded by sieving to obtain uniform-sized seeds, cleaned, and stored in a cool and dry place until further use.

2.2 Sample Preparation and Processing of Foxtail Millet

2.2.1 Soaking

Grains were soaked in distilled water in a 1:3 (w/v) ratio for 16 h. Afterward, the soaked grains were evenly distributed on a tray and dried at room temperature (26°C) in the shade for 3 days (Figure 1a). The processed grains were ground to fine flour to pass through a B.S.S sieve (300 µm) using a laboratory model electric grinder (Butterfly company, 750 W) at room temperature (26°C). The flour of soaked (Figure 1d) grains was air-dried in the shade for 8 h and stored at 20°C in polythene pouches until further use.

Figure 1 Foxtail millet grain and flour. (a) Soaked grain. (b) Germinated grain. (c) Steam cooked grain. (d) Flour of soaked grain. (e) Flour of germinated grain. (f) Flour of steam-cooked grain.

2.2.2 Germination

Grains were soaked in distilled water in a 1:3 (w/v) ratio for 16 h. After that, the soaked grains were placed on wet muslin cloth and germinated at dark conditions for 2 days with regular

wetting using distilled water. The grown grains were air-dried in the shade for 3 days and ground to fine flour to pass through a B.S.S sieve (300 µm) using a laboratory model electric grinder at room temperature (26°C). The flour of germinated (Figure 1e) grains were air-dried in the shade for 8 h and stored at 20°C in polythene pouches until further use.

2.2.3 Steam Cooking

Grains were soaked in distilled water in a 1:3 (w/v) ratio for 16 h. After that, the soaked grains were then cooked in a domestic pressure cooker for 20 min. Steam-cooked (Figure 1c) grains were air-dried in the shade for 3 days and ground to fine flour to pass through a B.S.S sieve (300 µm) using a laboratory model electric grinder at room temperature (26°C). The steam-cooked grain flour (Figure 1f) was air-dried in the shade for 8 h and stored at 20°C in polythene pouches until further use.

2.3 Nutritional Analysis

2.3.1 Proximate Analysis

Moisture Content. Moisture content (%) was determined using a hot-air oven following the standard Association of Official Analytical Chemists (AOAC) method [20]. The result of moisture content (%) was calculated using the following equation.

% moisture = $(W_2 - W_3 / W_2 - W_1) \times 100$, where W_1 = weight of the empty crucible, W_2 = weight of crucible + flour before drying, W_3 = final weight of crucible + flour after drying.

Ash Content. The ash content was determined using the AOAC (975.03) official analysis method [21]. The samples of known weight were held at 600°C in a muffle furnace for 3 h.

Crude Fiber. The flour (5 g) was boiled with H_2SO_4 and KOH under reflux. After filtering the solution, the residue was boiled again with NaOH. The dried residue was incinerated at 600°C in a muffle furnace. The flour of the crude fiber (g/100 g) was determined using the method described by Raghuramulu et al. [22]. The results were reported as grams (g) per 100 g of flour (g/100 g).

Fat Content. The flour (5 g) was subjected to hexane and shaken before collecting the upper layer. The pellet was extracted with hexane three times, with all the upper layers being collected and filtered each time. Filtrate was contained in a pre-weighed beaker and evaporated until the solvent was fully evaporated. The fat content of the flour was estimated according to the method described by Hara and Radin [23]. The results were reported as g/100 g.

Carbohydrates. Total carbohydrates were determined according to the method described by Ramakrishna et al. [24]. The flours of soaked, germinated, and steam cooking were individually mixed with 5 mL HCl (2.5 N) for 3h in a boiling water bath. Subsequently, the mixtures were cooled to room temperature. Na_2CO_3 was used to neutralize it until the effervescence stopped. The mixture was diluted using distilled water and centrifuged at 3000 rpm for 5 min. The supernatant was carefully collected, and separate aliquots (0.5 and 1.0 mL) were prepared. Subsequently, 4.0 mL of ice-cold anthrone reagent was added. The reaction mixture was incubated in a water bath at boiling temperature for 8 min, followed by subsequent cooling. The absorbance was measured at

630 nm with a UV/vis spectrophotometer (Shimadzu UV-1800) against the blank. The amount of carbohydrates was determined using the D-glucose calibration curve, and the results were expressed as g/100 g.

Proteins. The protein content (%) of the flour was analyzed using the method of Lowry et al. [25] using Folin-Ciocalteu Reagent (FCR). The optical density (OD) was measured at 660 nm using a spectrophotometer. The protein content was calculated from the linear equation of a stranded curve prepared with Bovine serum albumin (BSA), and the results were reported as g/100 g.

2.4 Mineral Analysis

A microwave vessel was used to digest the flours of soaked, germinated, and steam-cooked grains (2 g) using a combination of HNO_3 and H_2O_2 at a 10:4 (v/v) ratio. The vessels were tightly capped and placed into the microwave digestion (Milestone ETHOS) system. The samples were digested in two steps. In the first step, the samples were subjected to a power of 1000 W, a temperature of 110°C, a ramp duration of 15 min, and a hold time of 5 min. In the second step, the samples were exposed to a power of 1000 W, a temperature of 190°C, a ramp time of 10 min, and a hold time of 20 min. The vessels were extracted from the digestor and then let to cool down to ambient temperature. The vessels were cautiously opened, and the contents were transferred into an acid-cleaned 50 mL standard measuring flask. The contents were diluted with grade-1 water to a final volume of 50 mL. The digested samples were subjected to analysis using the Optimal Emission Spectrometer (OES) equipped with the Inductively Coupled Plasma (ICP) (Perkinelmer, Optima 5300 DV). The concentrations of potassium (K), calcium (Ca), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), phosphorus (P), sodium (Na), and magnesium (Mg) contents were determined by measuring their absorbance at 766.40, 317.933, 259.939, 213.857, 257.61, 589.592, 279.077, 324.752 and 213.617 nm, respectively. The mineral contents were quantified using yttrium as an internal standard. The minerals were quantified by comparing their concentrations to known concentrations in the working standard solutions, which were used for calibrating the device. The concentration of macro and microelements was conveyed as mg/100 g of sample dry matter.

2.5 Vitamin B Complex Analysis

The B-group vitamins, such as vitamins B_1, B_2, B_3 and B_6 were extracted by mixing 25 g samples with 0.1 N HCl. The samples were kept in a water bath maintained at 95°C for 30 min and cooled to room temperature. For vitamin B_2, the extract was vortexed and centrifuged for 10 min. The supernatant obtained was filtered through a 0.45 µm filter before injection. For vitamin B_1, the samples were subjected to derivatization for oxidation of thiamine to trichrome by potassium ferry cyanide in an alkaline medium. Afterward, the models were vortexed and centrifuged for 10 min. The supernatant obtained was filtered through a 0.45 µm filter before injection. For vitamin B_3, 25 g sample was extracted with pre-heated (45°C) water. The extract was vortexed and centrifuged for 10 min. The supernatant obtained was filtered through a 0.45 µm filter before injection. For vitamin B_6, the 25 g sample was extracted with 5% meta-phosphoric acid and vortexed for 5 min. The extract was centrifuged for 10 min, and the supernatant obtained was filtered through a 0.45 µm filter before injection.

HPLC system (Shimadzu, UFLC Prominence, Japan) equipped with an autosampler and fluorescence detector (Waters 23695) used to analyze B-group vitamins. The Inertsil, Octadecylsilane column with the dimension of 250 × 4.6 mm with 5 µm particle size was used for the analysis. The temperature of the column was adjusted to match the ambient room temperature.

Vitamin B_1: The mobile phase comprised 300 mL of methanol and 700 mL of sodium acetate buffer in 1 L of water. The elution of the samples was conducted at a flow rate of 1.0 mL/min. The process lasted for 20 min. Vitamin B_1 was detected using fluorescence with excitation at 368 nm and emission at 440 nm. The results were reported as mg/100 g.

Vitamin B_2: The mobile phase consisted of 300 mL of methanol and 700 mL of sodium acetate buffer in 1 L of water. The elution of the samples was conducted at a flow rate of 1.0 mL/min. The process lasted for 25 min. Vitamin B_2 was detected using fluorescence with excitation at 468 nm and emission at 520 nm. The results were reported as mg/100 g.

Vitamin B_3: The mobile phase consisted of sodium acetate-phosphate buffer (pH 5.2) and acetonitrile. The elution of the samples was conducted at a flow rate of 1.0 mL/min. The process lasted for 25 min. Vitamin B_3 was detected using fluorescence with excitation at 322 nm and emission at 380 nm. The results were reported as mg/100 g.

Vitamin B_6: The mobile phase consisted of potassium-phosphate buffer (pH 3.2) and acetonitrile. The elution of the samples was conducted at a flow rate of 1.0 mL/min. The process lasted for 20 min. Vitamin B_6 was detected using fluorescence with excitation at 290 nm and emission at 395 nm. The results were reported as mg/100 g.

2.6 Amino Acid Analysis

A clean sample tube was used to collect 1 g flour and subjected to hydrolysis using 10 mL of 6 N HCl solution containing 1% phenol. The hydrolysis process was carried out at 110°C for 24 h, ensuring that no air was introduced throughout the procedure. Subsequently, the hydrolysates were cooled, followed by vacuum drying under and filtration using a 0.45 µm syringe filter. Then, 0.5 mL aliquots were diluted with 7 mL of water, followed by the pH adjustment of the resulting solution to 7-8 with NaOH (6 N). The final volume was then adjusted to 10mL using water. The reconstituted sample underwent pre-heating in a water bath at 55°C. To a book of 20 µL of the reconstituted selection, 140 µL of AccQ*Flour Borate Buffer and 40 µL of AccQ*Flour reagent were subsequently added. The mixture was vortexed for several seconds and hydrolyzed the surplus reagent to form 6- aminoquinoline. The contents were transferred from the tube to a vial labeled as Ria. The vials were sealed with a septum lined with silicone, followed by heating in a water bath for 10 minutes at 55°C. The vials were subjected to cooling, after which the contents were transferred to a high-performance liquid chromatography (HPLC) vial. The blank was subjected to derivatization without heat, as previously reported.

The amino acid derivatives were separated on a Waters (e2695 module) HPLC system equipped with an autosampler and fluorescence detector. A sample of 5 µL was injected into a Waters hydrolysate amino acid column (Diameter 3.9 mm × 150 Length). The temperature of the column was adjusted to 45°C. The mobile phase consisted of a buffer solution (containing 11.48 g of sodium acetate anhydrous and 1.722 g of triethylamine in 800 mL water). The pH was adjusted to 5.02 using orthophosphoric acid, and the volume was 1000 mL. Additionally, the mobile phase

included a combination of water and acetonitrile. The elution of samples was performed at a flow rate of 1.0 mL/min, with a total duration of 45 min. The amino acids such as aspartic acid (Asx), serine (Ser), glutamic acid (Glx), glycine (Gly), histidine (His), arginine (Arg), threonine (Thr), alanine (Ala), proline (Pro), cysteine (Cys), tyrosine (Tyr), valine (Val), methionine (Met), lysine (Lys), isoleucine (Iseu), leucine (Leu), phenylalanine (Phe) were detected using fluorescence detection at 250 nm (excitation) and 395 nm (emission). Identifying the individual amino acid peaks corresponding to amino acids was accomplished by comparing their retention times with those of standards. The results were reported as grams of amino acid per 100g (g/100 g).

Tryptophan (Tyr) was determined by a base hydrolysis process using 10 mL of 6 N NaOH at 110°C for 24 h while ensuring no air was introduced. The sample was reconstituted using concentrated hydrochloric acid (HCl) at a pH of 3.0 in a water bath at 55°C. Subsequently, the piece was derivatized using methanol. The model underwent vortexing, centrifugation, and filtration using a 0.45 µm syringe filter. The separation of amino acid derivatives was conducted using a Waters (e2695 module) HPLC system equipped with an autosampler and fluorescence detector. A sample of 5 µL was injected into an Inertsil, Octadecysilane column with dimensions of Diameter 4.6 mm × 250 Length. The temperature of the column was adjusted to match the ambient room temperature. The mobile phase comprised 1 mL of trifluoroacetic acid in 1 L of water and acetonitrile. The elution of the samples was conducted at a flow rate of 1.0 mL/min, and the process lasted for 10 min. Tryptophan (Try) was detected using fluorescence with excitation at 280 nm and emission at 356 nm. The results were reported as grams (g) of amino acid per 100 g (g/100 g).

2.7 Anti-nutritional Factors

2.7.1 Phytochemicals

Condensed Tannins. The condensed tannin content in the flour was determined according to the method described by Arora and Kaur [26] using $K_4Fe(CN)_6$ and $FeCl_3$ (prepared in 0.1 N HCl). The optical density was read at 605 nm against a blank using a spectrophotometer. For calibration, Gallic acid (GAE) was used as the standard. The tannin content was estimated using the equation below, and the result was represented as mgGAE/g of the flour. The tannin content = C × 10 × 100 / 200, where C is the concentration that corresponds to the OD value.

Total Phenolic Content (TPC). The total phenol content of flour extract was estimated according to the method described by Raghuramulu et al. [22] using GAE as the standard. The flour (1 g) was subjected to 80% ethanol. The ethanolic flour extracts were added with Folin-Ciocalteu reagent (FCR). Subsequently, the reaction was neutralized with Na_2CO_3 solution. The resulting blue solution's optical density (OD) was read at 765 nm using a spectrophotometer (Shimadzu UV-1800). The results were expressed as mg GAE/g.

Phytic Acid. Phytic acid was estimated using sodium phytate as standard by Davies and Reid method [27]. The flour (1 g) was extracted with HNO_3 before adding $Fe(NH_4)_2(SO_4)_2$ and NH_4SCN. The OD values were taken at 465 nm using a spectrophotometer. The results were expressed as mg/g.

2.7.2 Enzyme Inhibitors Activities

Trypsin Inhibitors. The trypsin inhibitor activity of the flours was analyzed using the method described by Roy and Rao [28] using BSA as a substrate. The flour (1 g) was subjected to 80% ethanol. The activity of the enzyme trypsin was assayed in the reaction mixture (RM). The RM consisted of trypsin solution (0.5%), flour extract, BSA (1%), HCl, and phosphate buffer (0.1 M, pH 7.5). The reaction was stopped by adding ice-cold TCA. The absorbance was measured at 280 nm using a UV-Vis spectrophotometer against a blank. The experiment proceeded without flour extract, considered 100% trypsin enzyme activity. Results expressed as a percentage of trypsin enzyme enhancement or inhibition concerning control. The results are expressed as a percentage (%) of enhancement or inhibition of trypsin activity calculated concerning management using the following equation.

(%) of relative enzyme activity (REA) = (enzyme of test/enzyme activity of control) × 100 and the % of inhibition in the trypsin activity = (100 - % REA).

α-Amylase Inhibitors. The inhibitory properties of flour extracts against α-amylase were determined according to the method described by Sudha et al. [29]. The flour (1 g) was dissolved with distilled water. The enzyme activities of α- amylase were assayed using amylase enzyme suspended in sodium phosphate buffer, followed by the addition of flour extract and soluble starch solution. The reaction was stopped by adding concentrated HCl and iodine solution. The OD values of the reaction mixture were measured at 580 nm using a UV-Vis spectrophotometer. The experiment proceeded without flour extract, considered 100% enzyme activity. The results are expressed as a percentage (%) of enhancement or inhibition of α-amylase activity calculated concerning control using the following equation.

(%) of relative enzyme activity (REA) = (enzyme activity of test/enzyme activity of control) × 100 and the % of inhibition in the α-amylase activity = (100 - % REA).

2.8 Antioxidant Activities

Antioxidant activities of the flours were estimated based on the scavenging capacity of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals, 2, 2-azan-bi-(3-ethylbenzothiazoline-6-sulphonate) (ABTS) radical cation and Reducing power assay (RPA).

2.8.1 DPPH Radical Scavenging Activity

DPPH assay was performed according to the method of Wong-Paz et al. [30] using ascorbic acid (ASC) as standard. The flour (1 g) dissolved in 80% acetone (10 mL) before adding freshly prepared DPPH methanol solution. The absorbance was recorded at 517 nm. The values were expressed as the amount of scavenged DPPH radical per gm of foxtail millet dry weight (mg AScE/g).

2.8.2 $ABTS^+$ Radical Cation Scavenging Capacity

ABTS radical scavenging assay was performed following the method described by Re et al. [31] with slight modifications. $ABTS^+$ radical cation was generated by reacting ABTS with potassium persulfate ($K_2S_2O_8$). The flour extract was mixed with $ABTS^+$ radical. The absorbance was

immediately read at 734 nm. Trolox (TE) was used as a standard antioxidant for calibration, and values were expressed as the amount of scavenged $ABTS^{+}$ radicals as mgTE/g.

2.8.3 Reducing Power Assay (RPA)

The ability of flour extracts to reduce iron (III) to iron (II) was assessed by the method of Ferreira et al. [32] using ascorbic acid as the standard. The flour (1 g) was subjected to the mixture before centrifugation at 3000 rpm. Finally, $FeCl_3$ was added to the supernatant solution and the absorbance was measured at 700 nm. The values were expressed as mg AScE/g.

2.9 Statistical Analysis

All experiments were repeated thrice, and the results were presented as mean ± standard deviation (SD). Tukey's Multiple Comparison Test using a one-way analysis of variance (ANOVA) at $p > 0.05$ significant level was used to assess the difference in mean values. GraphPad software (version 9.0, Dotmatics, California, USA) was used for all statistical analyses.

3. Results and Discussion

3.1 Proximate Analysis

The proximate compositions of flours of soaked, germinated, and steam-cooked grains of foxtail millet on a dry weight basis have been reported in Table 1. The mean moisture content of flours significantly ($p > 0.05$) varied from 42.4 to 78.1%. Valli Pasha et al. [13] reported moisture content ranging from 30 to 32 g/kg. The flours of soaked (42.4%) grains showed lower moisture content, followed by germinated grains (47.1%) and steam-cooked grains (78.1%). Low moisture levels are beneficial for storing grain flour for extended periods [33]. The total ash of flours significantly ($p > 0.05$) varied from 1.12 to 1.42%. The soaked grain had the highest total ash content (1.42 g/100 g) among the flours, followed by steam-cooked (1.36 g/100 g) and germinated grains (1.12 g/100 g). Soaked grain flour has the greatest ash concentration compared to other flours, indicating its high mineral content.

Table 1 Effect of household processing methods on proximate composition of flours of foxtail millet grains*.

Proximate Composition	Soaking	Germination	Steam cooking
Moisture (%)	42.4 ± 0.1^{a}	47.1 ± 0.1^{a}	78.1 ± 0.1^{b}
Total ash (%)	1.42 ± 0.1^{c}	1.12 ± 0.1^{b}	1.36 ± 0.1^{b}
Total fat (g/100 g)	3.02 ± 0.1^{ns}	2.53 ± 0.1^{ns}	2.77 ± 0.1^{ns}
Protein (g/100 g)	7.32 ± 0.1^{c}	11.57 ± 0.1^{b}	8.80 ± 0.1^{b}
Carbohydrates (g/100 g)	70.5 ± 0.6^{ns}	73.28 ± 1.7^{ns}	66.13 ± 0.1^{ns}
Crude fiber (g/100 g)	5.13 ± 0.1^{ns}	5.26 ± 0.3^{ns}	5.84 ± 0.1^{a}

*Values are mean ± standard deviation of three replicates. Comparison between the flours was performed by one-way ANOVA followed by Tukey's multiple comparison test. Different letters (a, b and c) indicate statistically significant ($p > 0.05$) differences between the flours

within the group concerning the parameters analyzed. (ns) indicates that there was no significant difference detected in the parameters.

The total fat content of the investigated flours ranged between 2.53 to 3.02 g/100 g (Table 1). Valli Pasha et al. [13] reported fat content ranging from 36 to 39 g/kg in foxtail millet. Geervani and Eggum [12] reported the fat content ranged from 3.5 to 4.74%. Yang et al. [34] reported the values of crude fat content were 2.82 to 4.47 g/100 g. Yang et al. [34] aid a range of 4.4 to 7.3 g/100 g for crude fat. The total fat content was highest in the flour of soaked grains (3.02 g/100 g), followed by steam-cooked grains (2.77 g/100 g) and germinated grains (2.53 g/100 g). Similar observations were made in soaked grains compared to processed samples in foxtail millet [35]. Germination reduces fat content in flour due to lipid breakdown and fatty acid oxidation [4]. Another research by Gowda et al. [11] ocusing on steam cooking revealed lower fat content in the germinated grains of different millets.

The range for the mean protein content of flours was 7.32 to 11.53 g/100 g. According to Valli Pasha et al. [13], the protein content ranged from 113 to 129 g/kg on a dry weight basis. The average protein content in 14 different varieties of foxtail millet varied from 11.13 to 18.75% [36]. According to Yang et al. [34], the protein content ranged from 11.85 to 20.58 g/100 g. In contrast, Chen et al. [33] observed a protein content ranging from 9.5 to 18.9 g/100 g. Of the three flours tested, protein content was significantly ($p > 0.05$) the highest in the flour of germinated grains (11.57 g/100 g) as compared to steam-cooked grains (8.81 g/100 g) and soaked grains (7.32 g/100 g). The protein content of grains rises during germination due to the activation of hydrolytic enzymes [10]. Flour's protein content is reduced due to the breakdown of amino acids during cooking [37].

The carbohydrate content of flour ranged from 66.13 to 73.28 g/100 g. Chen et al. [38] reported a range of 71.5 to 83.8 g/100 g for total carbohydrates. The highest content was found in the flour of germinated grains (73.28 g/100 g), and the lowest value was observed in the flour of steam-cooked grains (66.13 g/100 g), followed by the flour of soaked grains (70.5 g/100 g). The increase in the carbohydrate content may be due to enzymatic degradation of starch molecules into small molecules of soluble sugars upon germination [11].

Crude fiber of flours was significantly ($p > 0.05$) ranged from 5.13 to 5.84 g/100 g (Table 1). Geervani and Eggum [12] reported the fiber content of whole grain went from 19.11 to 30.81%. Valli Pasha et al. [13] noted total dietary fiber content ranging from 176 to 208 g/Kg. Crude fiber content was significantly ($p > 0.05$) highest in the flour of steam-cooked grains (5.84 g/100 g), followed by the flour of germinated grains (5.26 g/100 g) and the flour of soaked grains (5.13 g/100 g). Fiber consists mainly of cellulose, hemicellulose, and lignin. Cooking brings about several changes in physical characteristics and structural changes in fiber components [39]. The high temperature breaks the glycosidic bonds of polysaccharides, which can lead to oligosaccharides and thus increase the quantity of fiber [40]. Similarly, cooking has increased finger millet's total fiber content [41]. Another study reports that the fiber content increases with germination [42].

3.2 Mineral Analysis

The content of four macro elements [potassium (K), calcium (Ca), phosphorus (P) and magnesium (mg)] and seven microelements [chromium (Cr), iron (Fe), molybdenum (Mo), manganese (Mn), zinc (Zn), copper (Cu) and sodium (Na)] were analyzed in the flours of soaked

grains, germinated grains and steam-cooked grains of foxtail millet. The results are presented in Table 2. Of the four macro elements analyzed, Ca and Mg showed significant ($p > 0.05$) differences in all three-grain flours. However, the other two macroelements and all the microelements analyzed had no significant ($p > 0.05$) difference. The present study identified macro elements with the highest concentration were K (225.89 to 240.42 mg/100 g), Ca (28.85 to 32.48 mg/100 g), P (5.64 to 5.82 mg/100 g) and Mg (2.72 to 2.89 mg/100 g) (Table 2). The variations in the minerals upon processing may be due to the different processing conditions adopted [9].

Table 2 Effect of household processing methods on the mineral content of flours of foxtail millet grains*.

Mineral elements (mg/100 g)	Soaking	Germination	Steam cooking
Macro elements			
Potassium (K)	236.38 ± 0.87^{ns}	225.89 ± 1.52^{ns}	240.42 ± 0.80 ^{ns}
Calcium (Ca)	31.40 ± 0.25^{a}	32.48 ± 0.54^{ns}	28.85 ± 0.52^{a}
Phosphorous (P)	5.64 ± 0.16^{ns}	5.82 ± 0.01^{ns}	5.81 ± 0.01^{ns}
Magnesium (Mg)	2.89 ± 0.01^{ns}	2.83 ± 0.01^{ns}	2.72 ± 0.01^{b}
Micro elements			
Chromium (Cr)	12.83 ± 0.31^{ns}	11.86 ± 0.12^{ns}	11.63 ± 0.18^{ns}
Iron (Fe)	5.5 ± 0.02^{ns}	5.81 ± 0.10^{ns}	4.62 ± 0.06^{ns}
Molybdenum (Mo)	2.4 ± 0.05^{ns}	2.16 ± 0.03^{ns}	2.33 ± 0.03^{ns}
Manganese (Mn)	2.29 ± 0.01^{ns}	2.62 ± 0.06^{ns}	2.26 ± 0.01^{ns}
Zinc (Zn)	2.06 ± 0.03^{ns}	2.27 ± 0.02^{ns}	1.92 ± 0.01^{ns}
Selenium (Se)	1.16 ± 0.03^{ns}	0.86 ± 0.03^{ns}	1.06 ± 0.03^{ns}
Copper (Cu)	0.75 ± 0.02^{ns}	0.91 ± 0.02^{ns}	0.76 ± 0.01^{ns}
Sodium (Na)	0.55 ± 0.01^{ns}	0.56 ± 0.01^{ns}	0.54 ± 0.01^{ns}

*Values are mean ± standard deviation of three replicates. Comparison between the flours was performed by one-way ANOVA followed by Tukey's multiple comparison test. Different letters (a and b) indicate a statistically significant ($p > 0.05$) difference between the flours within the group with respect to the parameters analyzed. (ns) indicates that there was no significant difference detected in the parameters.

The flours of steam-cooked grains (240.42 mg/100 g) showed the highest K content, followed by soaked grains (236.38 mg/100 g) and germinated grains (225.89 mg/100 g). The flours of sprouted grains (32.48 mg/100 g) had significantly ($p > 0.05$) highest content of Ca as compared to soaked (31.40 mg/100 g) and steam-cooked (28.85 mg/100 g) grain flours. The highest content of P was found in the flours of germinated grain (5.82 mg/100 g), while the lowest range was in the flours steam-cooked (5.81 mg/100 g) and soaked grain (5.64 mg/100 g) flours. The highest Mg content was found in the flours of soaked grain (2.89 mg/100 g), and the lowest content was 2.72 mg/100 g in the flour of steam-cooked grains. Minerals play an essential role in signal transduction, cell energy production, and oxygen transport, act as co-enzymes, and help the nervous system to work correctly [43]. The results are in agreement with a previous study by Valli Pasha et al. [13], who reported that foxtail millet contained an amount of Ca (0.2 g/kg), P (5.65 g/kg), K (7.14 g/kg) and Mg (2.59 g/kg).

Chromium (Cr) was reported to be higher in content, followed by Fe, Mo, Mn, Zn, Se, Cu, and Na. The flour's mean chromium (Cr) content ranged from 11.63 to 12.83 mg/100 g. Among three flours, soaked grain (12.83 mg/100 g) flour had the highest Cr content (Table 2). Trivalent chromium is considered an essential trace element for humans. Chromium contributes to the metabolism of primary macromolecules, whereas hexavalent chromium is considered a poor resource for chromium compared to vegetables and cereals. The upper limit for adult chromium supplementation may be 240 µg/day. In the present study, a higher level of chromium may be due to the milling process, as reported in wheat flour [44]. The flour's mean iron (Fe) content ranged from 4.62 to 5.5 mg/100 g. The Fe content was found to be highest (5.81 mg/100 g) in the flour of germinated grains, followed by steam-cooked grains (4.62 mg/100 g) and soaked grain (5.5 mg/100 g) flours. The flour's mean molybdenum (Mo) content ranged from 2.16 to 2.4 mg/100 g. The highest content of Mo was higher in the flours of soaked grain (2.4 mg/100 g), while the lowest content was found in the flours of germinated grain and steam-cooked grain (2.33 mg/100 g) flours.

The mean manganese (Mn) content of flours ranged from 2.26 to 2.62 mg/100 g. The highest Mn content was observed in the flour of germinated grains (2.62 mg/100 g), and the lowest content was found in the flours of soaked grain (2.29 mg/100 g) and steam-cooked (2.26 mg/100 g) grains. Flour's mean zinc (Zn) content ranged from 1.92 to 2.27 mg/100 g. The highest Zn content was found in the flour of germinated grain (2.27 mg/100 g) compared to other flours. The mean selenium (Se) content was observed from 0.86 to 1.06 mg/100 g. The highest content of Se was marked in the flours of soaked grain (1.16 mg/100 g), and the lowest content was found in the flours of germinated grain (0.86 mg/100 g). Flour's mean copper (Cu) content ranged from 0.75 to 0.91 mg/100 g. The highest content of copper was reported in the flours of germinated grain (0.91 mg/100 g), while the lowest content was found in the flours of soaked (0.75 mg/100 g). The mean sodium (Na) content flours varied from 0.54 to 0.56 mg/100 g. The highest sodium content of flours was observed in germinated grain (0.56 mg/100 g), while the lowest range of Na was found in the flours of steam-cooked grain (0.54 mg/100 g) and soaked grain (0.55 mg/100 g) (Table 2).

The flour of germinated grain contained the highest content of mineral elements such as Ca, P, Fe, Mn, Zn, Cu, and Na followed by the flour of soaked grains with the highest concentration of four elements: Mg, Cr, Mo, and Se. The flour of steam-cooked grains has only one part with the highest concentration i.e. K. Soaking millet grains in the water boosts the leaching of minerals and their *in vitro* solubility [45]. According to Sharma et al. [46], germinated foxtail millet showed a comparable rise in mineral content. Results also indicate that foxtail millet grain flours are an excellent source of K, Ca, P, Mg, Cr, Fe, Mo, Mn, Zn, Se, and Cu. If utilized correctly, this cereal can potentially address the problem of mineral nutrient deficiency in developing nations. Additionally, it can be used to fortify flours with low mineral content.

3.3 Vitamin B Complex Composition

The B complex vitamins, including vitamin B_1, vitamin B_2, vitamin B_3, and vitamin B_6, are an essential group of micronutrients that contribute to human health. The contents of B complex vitamins in the flours of soaked, germinated, and steam-cooked grains of foxtail millet on a dry weight basis have been estimated. The flour's vitamin B_1 (thiamine) content ranged from 0.33 to 0.51 mg/100 g. The flour of germinated grains (0.51 mg/100 g) significantly recorded higher values

of vitamin B_1 than the flour of soaked grain (0.48 mg/100 g), followed by steam-cooked grains (0.33 mg/100 g). Other vitamins, such as vitamin B_2, vitamin B_3, and vitamin B_6 were not detected in all three flours. Ramashia et al. [33] reported that the concentration of vitamin B_1 in the range of 0.6 to 0.27 mg/100 g in finger millet and other vitamins, such as vitamin B_2, vitamin B_3, and vitamin B_6 were not detected in the flour. These B- vitamins may be sensitive to processing conditions challenging to see. However, vitamin B_1 might have less reduction due to its resistance to household processing methods [47]. Among the methods tested, germination increased the concentration of vitamin B_1. Steam cooking of grain might have altered the structural configuration of vitamin B_1, which may be the reason for the reduction in the content [17].

3.4 Amino Acids Composition

Amino acids (AAs) are essential for various biological and chemical processes in different parts of the human body, including tissue development and repair, enzyme synthesis and function, and molecular transport [4]. A total 18 amino acids (AA) were analyzed in three flours of foxtail millet grains. The total amino acid content of the flours ranged from 13.59 to 14.37 g/100 g. The highest content of total amino acids was recorded in the flours of steam cooked grains (14.37 g/100 g) followed by soaked grains (14.21 g/100 g) and germinated (13.59 g/100 g) grains (Table 3). AAs were classified as essential (EAA) or non-essential amino acids (N-EAA). The leucine, valine, phenylalanine, isoleucine, threonine, histidine, lysine, tryptophan and methionine belong to the group of essential amino acids (EAA), whereas glutamic acid, tyrosine, proline, alanine, aspartic acid, serine, arginine, glycine and cysteine belongs to the group of non-essential amino acids (N-EAA). Of the nine EAAs analyzed, tryptophan, leucine, proline, isoleucine showed significant (p > 0.05) differences, and lysine, valine, phenylalanine, methionine and cysteine showed a non-significant difference in all three flours of grains. Among the nine N-EAAs analyzed, glutamic acid, tyrosine, proline and alanine showed significant (p > 0.05) differences. The aspartic acid, serine, arginine, glycine and cysteine showed non-significant differences in all three-grain flours (Table 3).

Table 3 Effect of household processing methods on amino acids composition of flours of foxtail millet grains*.

Amino acids (g/100 g)	Soaking	Germination	Steam cooking
Essential Amino Acids			
Leucine	1.68 ± 0.01^{d}	1.09 ± 0.01^{d}	1.67 ± 0.01^{ns}
Valine	0.73 ± 0.01^{ns}	0.73 ± 0.01^{ns}	0.72 ± 0.01^{ns}
Phenylalanine	0.71 ± 0.01^{ns}	0.69 ± 0.01^{ns}	0.72 ± 0.01^{ns}
Isoleucine	0.61 ± 0.01^{ns}	0.58 ± 0.01^{a}	0.61 ± 0.01^{ns}
Threonine	0.45 ± 0.01^{d}	0.44 ± 0.01^{ns}	0.45 ± 0.01^{ns}
Histidine	0.26 ± 0.01^{d}	0.25 ± 0.01^{ns}	0.24 ± 0.01^{ns}
Lysine	0.23 ± 0.01^{d}	0.22 ± 0.01^{ns}	0.22 ± 0.01^{ns}
Tryptophan	0.18 ± 0.01^{ns}	0.18 ± 0.01^{ns}	0.18 ± 0.01^{ns}
Methionine	0.16 ± 0.01^{ns}	0.15 ± 0.01^{ns}	0.16 ± 0.01^{ns}
Non Essential Amino Acids			
Glutamic acid	2.72 ± 0.01^{ns}	2.55 ± 0.01^{c}	2.63 ± 0.01^{b}
Tyrosine	1.75 ± 0.01^{a}	2.02 ± 0.01^{d}	1.95 ± 0.01^{c}

Proline	1.23 ± 0.01^{d}	1.19 ± 0.01^{a}	1.26 ± 0.01^{a}
Alanine	1.18 ± 0.01^{ns}	1.12 ± 0.01^{b}	1.14 ± 0.01^{a}
Aspartic acid	1.02 ± 0.01^{ns}	1.06 ± 0.01^{ns}	1.1 ± 0.01^{ns}
Serine	0.53 ± 0.01^{ns}	0.54 ± 0.01^{ns}	0.54 ± 0.01^{ns}
Arginine	0.35 ± 0.01^{ns}	0.35 ± 0.01^{ns}	0.34 ± 0.01^{ns}
Glycine	0.33 ± 0.01^{d}	0.34 ± 0.01^{ns}	0.36 ± 0.01^{a}
Cysteine	0.06 ± 0.01^{ns}	0.05 ± 0.01^{ns}	0.06 ± 0.01^{ns}
Total Amino Acids	14.21 ± 0.01^{a}	13.59 ± 0.01^{d}	14.37 ± 0.01^{c}

*Values are mean ± standard deviation of three replicates. Comparison between the flours was performed by one-way ANOVA followed by Tukey's multiple comparison test. Different letters (a, b, c, and d) indicate statistically significant (p > 0.05) differences between the flours within the group and the parameters analyzed. (ns) indicates that there was no significant difference in the parameters.

The flours of foxtail millet grain contained highest concentration of amino acids such as glutamic acid (2.55 to 2.77 g/100 g), tyrosine (1.75 to 2.02 g/100 g), leucine (1.09 to 1.68 g/100 g), proline (1.10 to 1.26 g/100 g), alanine (1.12 to 1.18 g/100 g) and aspartic acid (1.02 to 1.1 g/100 g). Flours were very low in lysine (0.22 to 0.23 g/100 g), methionine (01.5-0.16 g/100 g), cysteine (0.05-0.06 g/100 g), and tryptophan (0.18 g/100 g and histidine (0.24-0.26 g /100 g). Geervani and Eggum [12] reported higher content of glutamic acid, tyrosine, leucine, proline, alanine, and aspartic acid in the foxtail millet flour. An earlier study concluded that foxtail millet flour has lower lysine, tryptophan, methionine, and cysteine [16]. All other EAAs and non-EAAs are considerably available: phenylalanine (0.69-0.72 g/100 g), valine (0.72-0.73 g/100 g), isoleucine (0.58-0.61 g/100 g), serine (0.53-0.54 g/100 g), threonine (0.44-0.45 g/100 g), glycine (0.33-0.36 g/100 g) and arginine (0.34-0.35 g/100 g). A similar pattern of AA composition was also observed in whole grains of 14 Indian foxtail millet varieties [37].

Soaked millet flour contained higher levels of four EAAs (glutamic acid, alanine, arginine, and histidine) and two non-EAAs (leucine and lysine). Germinated millet flour contained higher levels of non-EAA Tyrosine. Steam-cooked millet flour had a higher concentration of two EAAs (aspartic acid and glycine) and two non-EAAs (proline and phenylalanine). Soaking and steam cooking influenced the concentration of threonine, isoleucine, methionine, and cysteine. Arginine and valine concentrations were influenced by soaking and germination. In the present study, the content of amino acid species in the germinated flour was lower than in soaked millet flour and steam-cooked flour. Similarly, Saleh et al. [4] found that the cooked composite flour had a more outstanding total amino acid content than the cooked native pearl millet flour. These findings suggest that soaking might improve the protein content of foxtail millet grains.

3.5 Anti-nutritional Factors (ANF)

3.5.1 Phytochemicals

The tannin content in foxtail millet flours ranged between 93.93 to 218.93 mg GAE/g (Table 4). According to Panwar et al. [19], the tannin content of barnyard millet seeds ranged from 3.25 to 3.96 mg/g, whereas the tannin content of finger millet seeds ranged from 2.05 to 2.62 mg/g. Contrarily, Giridhar et al. [9] reported tannin content in the sequence of finger millet (42.1) >

barnyard millet (35.5) > foxtail millet (28.7). The dark-colored grains contain higher tannin content than light-colored grains, possibly due to the higher tannin content in finger millet than in foxtail millet [9]. Significant (p > 0.05) differences were found in the tannin content was found in the flours of germinated grains (93.93 mg GAE/100 g) followed by steam-cooked grains (116.6 mg GAE/g) and soaked (218.93 mg GAE/g) grains. Similarly, processing methods reduced tannin content to 50% in little and finger millet [18].

Table 4 Effect of household processing methods on anti-nutritional factors of flours of foxtail millet grains*.

Anti-nutritional Factors	Soaking	Germination	Steam cooking
Phytochemical Factors			
Phytic acid (mg/g)	307.5 ± 1.2^{d}	97.63 ± 2.58^{d}	145.87 ± 0.1^{d}
Tannins (mg GAE/g)	218.93 ± 1.3^{b}	93.93 ± 0.2^{b}	116.6 ± 2.58^{b}
TPC (mg GAE/g)	13.63 ± 3.3^{ns}	8.83 ± 2.8^{ns}	6.81 ± 0.61^{ns}
Enzyme inhibitors			
α-Amylase inhibitors (%)	23.59 ± 0.1^{a}	5.61 ± 0.1^{c}	35.20 ± 0.1^{a}
Trypsin inhibitors (%)	12.45 ± 0.1^{ns}	25.29 ± 0.1^{ns}	26.84 ± 0.1^{ns}

*Values are mean ± standard deviation of three replicates. Comparison between the flours was analyzed by one-way ANOVA followed by Tukey's multiple comparison test. Different letters (a, b, c, and d) indicate statistically significant (p > 0.05) differences between the flours within the group to the parameters analyzed. (ns) indicates that there was no significant difference in the parameters.

Polyphenols are phytochemicals and antioxidants that have been shown to significantly reduce the risk of cancer, cardiovascular disease, and type II diabetes [4]. The foxtail millet's total phenolic content ranged from 6.81 to 13.63 mg GAE/g (Table 4). According to Kim et al. [48], the full phenolic content of proso millet varied from 18.0 to 26.5 mg GAE/g and foxtail millet from 12.0 to 26.7 mg GAE/g. Total polyphenols in barnyard millet ranged from 20.30 to 27.80 mg GAE/g. The current research found that foxtail millet had a more excellent polyphenolic content than proso millet, barnyard millet, and other foxtail millet varieties. Low levels of TPC were found in the flours of steam-cooked (6.81 mg GAE/g) grains as compared to germinated (8.83 mg GAE/g) and soaked (13.63 mg GAE/g) grains. The steam cooking reduced the concentrations of polyphenols in the flours of foxtail millet. Similar findings revealed that the longer the cooking time, the higher the loss of total phenolic content evaluated. The applied heat may considerably affect phenolic compounds' stability during steam cooking by breaking esterified and glycosylated bonds. Free or bound phenols may change their molecular structure or degrade upon steam cooking [49].

The phytic acid content in foxtail millet flours ranged between 97.63 to 307.5 mg/g (Table 4). Phytic acid levels in barnyard millet seeds varied from 3.3 to 3.7 mg/g, ranging from 5.54 to 5.58 mg/g in finger millet seeds [17]. Phytates or phytic acid, also known as inositol hexa phosphatase (IP_6), have a considerable propensity to chelate different metal ions, such as zinc and calcium, leading to low mineral bioactivity. Phytic acid also inhibits the activity of various digestive enzymes, including pepsin, α-amylase, and trypsin [46]. The processing method significantly (p > 0.05) decreased the phytic acid content in the flours of foxtail millet grains. The lowest values were

recorded with the flour of germinated grains (97.63 mg/g), followed by steam-cooked grains (145.87 mg/g) and soaked grains (307.5 mg/g). Germination reduced the phytate levels, possibly due to the hydrolytic action of phytase and possible degradation of IP_6 into its monomeric forms of IP_2, IP_3, IP_4, and IP_5 [50].

Three anti-nutritional factors, phytic acid, tannins, and total phenols, were determined in the flours of soaked, germinated, and steam-cooked grains (Table 4). The distribution of phytates, tannins, and polyphenols in various grains varies greatly. Antinutrient concentrations are more significant in bran than in other parts of the grain [51]. Foxtail millet flours demonstrated the highest content of phytic acid followed by tannin (93.93 to 218.93 mg GAE/100 g) and total phenolic contents (6.81 to 13.63 mg GAE/g), indicating that considerable variation exists for anti-nutritional factors based on the processing method. The lowest contents of anti-nutritional elements were found in the germinated and steam-cooked grain flour. As reported in finger millet, this indicates that anti-nutritional factors can be eliminated using processing methods like germination. Anti-nutritional factors may be reduced during germination owing to polyphenol leaching in the soaking water and enhanced activity of hydrolytic enzymes [52].

3.5.2 Enzyme Inhibitor Activities

Inhibitor activities for α-amylase and trypsin enzymes were estimated in the flours of soaked, germinated, and steam-cooked grains (Table 4). The processing method significantly *(p* > 0.05) decreased the α-amylase enzyme inhibitors activity in the flours. The lowest values were recorded in the flour of germinated grains (5.61%), followed by soaked (23.59%) and steam-cooked grains (35.20%). The enzyme α-amylase hydrolyzes α-(1, 4)-glycosidic bonds in starch or polysaccharides. α-amylase inhibitors, commonly known as starch blockers, may lower endogenous α-amylase activity [53]. The inhibition of α-amylase enzyme activity is known to play a vital role in regulating blood glucose levels [54]. Tashiro et al. [55] reported that whole grain of foxtail millet contains at least one type of α-amylase inhibitor.

Trypsin enzyme inhibitory activity of flours ranged from 12.45 to 26.84%. The lowest trypsin inhibitor activity was found in the flours of soaked (12.45%) grains, followed by the flour of germinated (25.29%) grains and steam-cooked grains (26.84%) (Table 4). Trypsin is an enzyme involved in the breakdown of many different proteins. Trypsin inhibitors alter trypsin enzyme activity, rendering it unable to bind with proteins. Tashiro et al. [55] reported that the whole grain of foxtail millet includes at least three different kinds of trypsin inhibitors. The reduced activity of trypsin inhibitors in the flour of soaked grains may be accounted for leaching during soaking, as reported in grain legumes [56]. Foxtail millet flours demonstrated the highest content of α-amylase inhibitors (5.61 to 35.20%) than trypsin inhibitors (12.45 to 26.84%), indicating considerable variation for the enzyme inhibitors based on the processing method. The differences in the values reported for α-amylase and trypsin inhibitors could be the enzyme's origin, enzyme concentration, type of substrate, and processing conditions [56]. Diabetes mellitus type II may be controlled by carbohydrate-digesting enzymes such as α-amylase and α-glucosidase, which regulate glucose absorption in the intestine [9].

3.6 Antioxidant Activities

The antioxidant activities of three types of flours were evaluated based on measuring scavenging activity for ABTS, DPPH radicals, and RPA assay (Table 5). Flours' ABTS radical scavenging activity ranged between 0.66 to 0.889 mg TE/g. The flour of germinated grains had the highest scavenging capacity (10.77 mg TE/g) towards quenching of ABTS, whereas the flours of soaked (7.85 mg TE/g) and steam-cooked (4.17 mg TE/g) grain showed inhibition. However, ABTS activity showed a significant ($p > 0.05$) difference in the three flours tested. The ABTS test involves ABTS reaction with metmyoglobin and H_2O_2 at 37°C to produce $ABTS^+$. In the presence of an antioxidant such as trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) or prospective antioxidants in material extracts, the $ABTS^+$ exhibits a relatively steady blue-green color. The $ABTS^+$ blue color will be reduced to some level in proportion to the concentration of antioxidants [57].

Table 5 Effect of household processing methods on antioxidant activities of flours of foxtail millet grains*.

Antioxidant Activities	Soaking	Germination	Steam cooking
$ABTS^+$ (mg TE/g)	7.85 ± 0.73^{ns}	10.77 ± 0.38^{a}	4.17 ± 0.58^{a}
DPPH (mg AScE/g)	0.54 ± 0.1^{ns}	0.59 ± 0.01^{ns}	0.58 ± 0.03^{ns}
RPA (mg AScE/g)	0.88 ± 0.01^{ns}	0.89 ± 0.01^{ns}	0.66 ± 0.02^{b}

*Values are mean ± standard deviation of three replicates. Comparison between the flours was performed by one-way ANOVA followed by Tukey's multiple comparison test. Different letters (a and b) indicate a statistically significant ($p > 0.05$) difference between the flours within the group and the parameters analyzed. (ns) indicates that there was no significant difference in the parameters.

The flour of germinated grains (0.59 mg AScE/g) has recorded the highest DPPH activity, followed by steam-cooked grains (0.58 mg AScE/g) and soaked grains (0.54 mg AScE/g). In the DPPH assay, the purple-colored DPPH radical is reduced by an antioxidant or hydrogen donor into yellow-colored and non-radical α-α-diphenyl-β-picryl hydrazine. The hydrogen-donating properties of antioxidants are attributable to their DPPH scavenging activities. The discoloration level represents the antioxidant extract's scavenging ability [58].

The RPA content ranged between 0.66 to 0.89 mg AScE/100 g. The highest RPA content was observed in the flour of germinated grains (0.89 mg AScE/100 g), while the lowest content was recorded in the flour of steam-cooked grain (0.66 mg AScE/100 g). However, RPA content showed a significant ($p > 0.05$) difference in the three flours tested. The RPA approach reduces the ferric-ferrocyanide complex to ferrous dependent on antioxidants [59]. This study suggests that germinated grain flour has a stronger ferric-reducing antioxidant capacity than other flours. Three assays, ABTS, DPPH, and RPA, have shown considerable variation in antioxidant activities based on the type of flour and the processing method applied. The ABTS, DPPH, and RPA assays showed the highest antioxidant activity in the flour of germinated grains, followed by the soaked and steam-cooked grains. In the present study, ABTS, DPPH, and RPA scavenging levels were negatively correlated with the total phenolic content of the flour (Table 4 & Table 5). Not only phenolics but also protein hydrolysates, insoluble fiber, carotenoids, and polysaccharides were shown to have

antioxidant potential in foxtail millet [60]. It is also important to note that the antioxidant activity might vary depending on the processing technique used [4].

3.7 Summary of the Effect of Three Processing Methods on Nutritional Composition, Anti-nutritional Factors and Antioxidant Activities

The changes in nutritional composition, anti-nutritional factors, and antioxidant activities concerning different household processing methods are summarized in Table 6. Soaking grains is a traditional domestic food processing procedure used to reduce the antinutrient content of food. This method requires soaking grains in distilled water for 16 h or longer at room temperature before milling them into flour [61]. Soaking foxtail millet grains in water increased moisture content, total ash and fat, minerals (Mg, Cr, Mo, and Se), and amino acids (glutamic acid, alanine, arginine, and histidine) (Table 4). Trypsin inhibitor activity in foxtail millet became lower after soaking. Similar observations were made by Patel et al. [62]. Soaking causes several nutritional and functional changes in the flours, presumably due to mineral leaching in an aqueous solution [45]. All food processors may adopt this basic processing procedure, particularly in rural regions where millet crops are mostly farmed [52].

Table 6 Summary of the effect of three processing methods on nutritional composition, anti-nutritional factors, and antioxidant activities.

Parameters	**Processing Methods**		
Proximate Composition	**Soaking**	**Germination**	**Steam cooking**
Moisture (%)	↓	↓	↑
Total ash (%)	↑	↓	↓
Total fat (g/100 g)	↑	↓	↓
Protein (g/100 g)	↓	↓	↓
Carbohydrates (g/100 g)	↓	↑	↓
Crude fibre (g/100 g)	↓	↓	↑
Mineral Composition (mg/100 g)			
Potassium (K)	↓	↓	↑
Calcium (Ca)	↓	↑	↓
Phosphorous (P)	↓	↑	↓
Magnesium (Mg)	↑	↓	↓
Chromium (Cr)	↑	↓	↓
Iron (Fe)	↓	↑	↓
Molybdenum (Mo)	↑	↓	↓
Manganese (Mn)	↓	↑	↓
Zinc (Zn)	↓	↑	↓
Selenium (Se)	↑	↓	↓
Copper (Cu)	↓	↑	↓
Sodium (Na)	↓	↑	↓
Vitamin B_1	↓	↑	↓
Amino Acids Composition (g/100 g)			
Glutamic acid	↑	↓	↓

Alanine	↑	↓	↓
Aspartic acid	↓	↓	↑
Serine	↓	↑	↑
Threonine	↑	↓	↑
Arginine	↑	↑	↓
Glycine	↓	↑	↑
Histidine	↑	↓	↓
Tryptophan	---	---	---
Tyrosine	↓	↑	↓
Leucine	↑	↓	↓
Proline	↓	↓	↑
Valine	↑	↑	↓
Phenylalanine	↓	↓	↑
Isoleucine	↑	↓	↑
Lysine	↑	↓	↓
Methionine	↑	↓	↑
Cysteine	↑	↓	↑
Total Amino Acids	↓	↓	↑
Anti-nutritional Factors			
Phytic acid (mg/g)	↑	↓	↓
Tannins (mgGAE/g)	↑	↓	↓
TPC (mgGAE/g)	↑	↓	↓
α-Amylase inhibitors (%)	↓	↓	↑
Trypsin inhibitors (%)	↓	↓	↑
Antioxidant Activities			
ABTS (mgTE/g)	↓	↑	↓
DPPH (mgAScE/g)	↓	↑	↓
RPA (mgAScE/g)	↑	↓	↓

Note: (↑): Increases, (↓): decreases, (---): not detectable.

Germination, sprouting, and malting are all synonymous terms. Germination is a biological process that occurs in seeds and includes the interconversion and formation of new molecules. This leads to improvement of nutritional composition, functional properties, and reduction in the anti-nutritional factors of grains [4]. It was observed that during germination of foxtail millet grains, protein, carbohydrates, minerals (Ca, P, Fe, Mn, Zn, Cu, and Na), amino acids tyrosine, vitamin B_1, and antioxidant properties were improved. Phytic acid and tannins were decreased in grains of foxtail millet after germination. The germination process stimulates the activity of hydrolyzing enzymes, which are responsible for the breakdown of macromolecules such as carbohydrates and lipids. Germination also eliminates the protein and fiber matrix and decreases the concentration of antinutrients such as phytic acid and tannins [50]. Moreover, endogenous enzymes enriched the foxtail millet via germination with higher antioxidant activity [63]. The results suggest that foxtail millet can scavenge free radicals and be employed as a potential source to fight free radicals formed in the human body.

Cooking is a process of preparing staple food at the household level. It is linked to alternations in the nutrient composition of millet [64]. The present study indicates that steam cooking increases the contents of fiber, mineral K, and amino acids (aspartic acid, glycine, proline, and phenylalanine). Cooking also resulted in a decrease in total phenolics and antioxidant activities. The loss in antioxidant content and exercise may also be related to oxidation during thermal treatments such as heating, boiling, and roasting [4].

4. Conclusion

The present study's findings show that soaking, germination, and steam cooking have altered the composition of foxtail millet grain's nutritional, antinutrient, and antioxidant properties. Germination enriched the bioavailability of protein, carbohydrates, minerals, amino acids, and antioxidant properties and reduced the anti-nutritional factors, especially phytic acid, tannins, and α- amylase inhibitors. Except for vitamin B_1, the other B complex vitamins were not investigated in the three tested flours, possibly due to their sensitivity to household processing conditions. This study also gives an insight into the α-amylase and trypsin inhibition activity of foxtail millet grains. There is still a need to focus on optimizing the other primary processing methods to make foxtail millet more acceptable for consumption by the majority of the population. Further studies are required to investigate the molecular interaction between specific phytochemicals and key amylolytic enzymes such as α-glucosidase and α-amylase in carbohydrate metabolism.

Acknowledgments

Ms. Suneetha Vallepu (SV) and Mrs. Naseem Shaik (NS), express their thanks to the Department of Science and Technology, Govt. of India, New Delhi, for awarding INSPIRE research fellowships.

Author Contributions

Ms. V. Suneetha conducted experimental design, data collection and statistical analysis. Ms. Naseem, and Mr. Khadar Basha assisted as team members to Ms. V. Suneetha. Prof. P.S. Sha Valli Khan participated in the supervision of experimental design, data collection and statistical analysis, writing and revision of the manuscript. Both Dr. V. Ramakrishna and Dr. Narasimhulu helped with data discussion, editing and revision of the manuscript.

Funding

SV (IF 180840/2019) and NS (IF 190462/2020).

Competing Interests

All authors declare that they have no conflict of interest in this paper.

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