

Comparative Evaluation of Fermentation and Emerging Processing Technologies for Enhancing Nutritional Quality and Bioactive Phytochemicals in Finger Millet Flour

Muhammad Amir

Bahauddin Zakariya University

Hind A. Abu-Hiamed

Princess Nourah bint Abdulrahman University

Saeed Akhtar

Bahauddin Zakariya University

Muhammad Sameem Javed

Bahauddin Zakariya University

Furukh Faiz

Karakoram International University

Kanza Saeed

Lahore University of Biological and Applied Sciences

Muhammad Jawad

Ohio University

Wisha Saeed

Bahauddin Zakariya University

Wafa Almarkhan

Princess Nourah bint Abdulrahman University

Muhammad Zubair Khalid

zubairkhalid730@gmail.com

Government College University, Faisalabad

Tawfiq Alsulami

King Saud University

Article

Keywords: Finger millet, anti-nutrients, fermentation, sonication, hydration, GC-MS

Posted Date: August 18th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10521708/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Finger millet (*L.*) is a nutrient-dense, stress-resilient cereal widely cultivated in tropical and subtropical regions; however, its nutritional potential is limited by anti-nutrients such as tannins and phytic acid. This study comparatively evaluated fermentation, sonication, sonication-assisted hydration (SAH), and alkaline soaking (Na_2CO_3 and NH_4OH) for their effects on nutritional composition, anti-nutrient reduction, color, antioxidant activity, and in vitro starch and protein digestibility of finger millet flour. Processing significantly ($p < 0.05$) influenced all parameters. Fermentation enhanced protein content (11.83%) compared to control (9.03%), while SAH increased fat content. Na_2CO_3 soaking most effectively reduced tannins (– 72.99%), whereas NH_4OH soaking and fermentation markedly decreased phytic acid. Fermented flour exhibited the highest antioxidant activity, while alkaline treatments reduced antioxidant potential. Fermentation and SAH significantly improved protein digestibility and resistant starch content. Overall, fermentation and SAH emerged as the most promising treatments, enhancing nutritional quality, bioavailability, and functional attributes while minimizing anti-nutritional factors.

Introduction

In the changing climate (droughts and floods), food security is a serious concern of developing and underdeveloped nations and has sensitized policymakers to take steps towards sustainable agriculture. The primary focus is to adopt drought-resistant and climate-resilient crops instead of merely relying on genetic modifications. Finger millet (*Eleusine coracana* L) is an internationally captivated crop due to its nutritional and phytochemical profile and adaptability to varying climatic zones, ranging from high hills to coastal plains. Finger millet needs minimum inputs, resists pathogens, and yields good, even in harsh conditions [1]. The nutritional composition of finger millet showed that total carbohydrates are present in the range of 71.90–81.5% while moisture, fat, protein, fiber, and ash are 7.5–11.75%, 1.2–1.7%, 5–9.6%, 15–20%, and 2.5–3.5% respectively [2, 3]. Finger millet is well known for its nutritional, phytochemical, and low glycemic index, but over the years there has been a rapid decline in the production and consumption of millet. The reason for being underutilized is the high dependency on wheat as a staple cereal, its year-round availability and the presence of intrinsic toxins in finger millet have the potential to reduce sensory acceptability [4].

With the advancement in scientific knowledge, finger millet is gaining much popularity as a health-promoting cereal. Micro-nutrient profiling of the crop revealed that its Ca, Zn, Fe, K, Mn, and P contents are in the range of 2.40–3.40, 0.013–0.018, 0.041–0.048, 3.80–5.20, 0.051–0.062 and 1.60–2.90 mg/g respectively [5, 6]. Calcium is the predominant mineral in finger millet with levels up to 10 times higher than those in other cereals and approximately three times greater than the calcium content of milk [3, 7]. Phytochemical profiling indicates that finger millet contains total phenols i.e., 122 mg/GAE/100g [8] while the tannins were 1.64 mg/g [9]. Other compounds like flavonoids, phytic acid, oxalates, and hydrogen cyanide are present in the range of 62.23–74.05, 210.75–302.75, 19.80–26.23, and 2.45–2.80mg/100g respectively [3, 6]. Seeds of finger millet are also rich in essential amino acids i.e., tryptophan, cystine, methionine, and aromatic amino acids when compared to the rest of the cereals [10]. The crop is a good source of vitamins from the B group like thiamine, folic acid, niacin, and riboflavin [9]. However, the availability of these nutrients is limited by the presence of anti-nutritional factors (ANFs) especially phytic acid, tannins, and oxalates inherent in the grains [8].

Reduced dietary diversity is a major cause of chronic diseases and mortality in both the developing and developed worlds. In developing countries, including Pakistan, the second leading cause of death for children under five is protein-energy malnutrition [11]. These children rely on starchy weaning foods like wheat and other staples that are deficient in essential amino acids. Finger millet (*Eleusine coracana* L) contains an appreciable amount of these amino acids but has poor protein digestibility and ultimately reduced protein availability [12]. The biochemical composition of finger millet can help reduce the risk of metabolic conditions, high blood pressure, and digestive tract illnesses. However, anti-nutrients such as tannins and phytates can reduce protein digestibility and bind to minerals, which may limit its nutritional benefits. So, these anti-nutrients need to be removed for maximum health benefits [13, 14]. Traditionally, fermentation, malting, popping, roasting and soaking in water were practiced to remove anti-nutrients but these treatments remained a challenge at the community level as they result in compromised nutritional quality and sensory attributes. Nowadays, ultrasonication is widely used to improve the nutritional quality and reduce the anti-nutrient burden in cereals and legumes [15]. Conventional processing is time-consuming and often results in poor-quality grains. This research was designed to find a solution that will reduce anti-nutrients in a short period of time, thus improving the nutritional profile and preserving sensory attributes. Ultra-sonication during hydration of finger millet could reduce the processing time and anti-nutrient contents in finger millet [16]. Nexus to the above, the present study aims to explore the impact of *Lactiplantibacillus plantarum* fermentation, sonication, sonication-assisted hydration and alkaline soaking on the nutritional quality, anti-nutrient reduction, antioxidant activity, improvement of sensory attributes and in vitro protein and starch digestibility.

Materials and Methods

2.1. Sample procurement, cleaning, and milling

The grains of brown variety finger millet were procured from the local farming community of District Muzaffargarh, Pakistan in the harvest year 2022. The procured grain samples were de-hulled manually by the pounding method and cleaned by winnowing. The cleaned grain samples were then categorized into six sub-samples (T_0 : raw grains, T_1 : finger millet (FM) flour for fermentation, T_2 : FM grains for sonication, T_3 : FM grains for sonication assisted hydration, T_4 : soaking of FM grains in sodium carbonate solution, T_5 : soaking of FM grains in ammonium hydroxide solution). Each sub-sample was treated accordingly and converted into flour using a laboratory mill (LM-120) Perkin Elmer US. The flour was then stored in air tight polyethylene bags at 10°C for further analysis.

2.2. Fermentation of finger millet grain flour

For the fermentation of finger millet flour, inoculum was prepared by taking 0.2 mg of *Lp. plantarum* V299 lyophilized cells preserved at -80 °C in 20 ml falcon tube containing 10 mL of MRS broth. The cells were revived by incubating (Memmert BE500 incubator, Malente, Germany) the falcon tube at 32 °C for 48 hours. After the completion of incubation, vegetative cells were transferred to MRS (DeMann Ragosa Sharpe) agar containing Petri dishes followed by incubation at 32 °C for 48 hours under anaerobic condition. On completion of 48 hours, *Lp. plantarum* colonies from Petri dishes were harvested, transferred into pre-sterilized 1.5 mL falcon tubes and centrifuged (Model Hermle, Z326-K, Malente, Germany) at 4000 rpm for 5 minutes at 4 °C, supernatant was discarded and bacterial pellet was resuspended in 0.9% sterilized ringer solution [17]. Now 750 µL from saline solution was added to 1 L fermentation jars containing 1:4 flour to water and incubated at 37 °C for 48 hours to allow proper fermentation. pH and acidity of the samples were monitored immediately to check fermentation progress by taking 10 mL from each jar after 6 hours. The samples were taken using a sterile pipette and all the sterile conditions were maintained in a laminar airflow chamber. After the completion of fermentation, the samples were dried in a dehydrator at 50 °C. The dried sample was again ground to flour using a laboratory mill (LM-120) Perkin Elmer US and flour was stored in air tight polyethylene bags at 10 °C for further analysis [18].

2.3. Sonication and sonication-assisted hydration of finger millet grains

Sonication of the cleaned sample was done by taking 50 g of grains and 200 mL of distilled water in a 500 mL beaker. Sonication conditions were set to 70 % ultrasound amplitude, 20 kHz frequency, 60 °C temperature, and 700 W input power for 15 minutes. Process conditions were kept the same for the entire period of treatment [19].

Ultrasound-assisted hydration of finger millet grains was done by taking 50 g of cleaned grains and 225 mL of distilled water in a 500 mL beaker. The grains were soaked for 90 minutes and ultra-sonication conditions were set according to the previous optimization study by [16] at various time-temperature combinations. The sonicator (PRO650, LABMAN Private Limited, India) was set to 50 % amplitude for 90 minutes' hydration time with a 10-second off cycle after every 90 seconds at 40 °C. The treated grains were washed with distilled water and dried at 50 °C in the dehydrator. The dried sample was converted into flour using a laboratory mill (LM-120) Perkin Elmer US and kept in air tight polyethylene bags at 10 °C for further analysis.

2.4. Soaking with Sodium Carbonate (Na₂CO₃) and Ammonium Hydroxide Solution

The de-hulled and finally cleaned samples (100 g) were soaked in 8% Na₂CO₃ and 5 % ammonium hydroxide solution for 10 hours in separate glass jars. After soaking the grains were washed with distilled water and dried in a vertical tunnel dehydrator (Pamico Technologies Limited Pakistan) at 50 °C. The dried grains were converted into flour and stored in air tight polyethylene bags at 10 °C for further analysis [20].

2.5. Determination of Proximate, pH, and titratable acidity

Proximate analysis of the raw and processed grain flour was carried out by following the AOAC [21] in triplicate. For the determination of moisture content, the AOAC method number 925.10 specified for food was followed, while the solvent extraction method 948.22 was used for crude fat. Crude protein content was quantified by acid digestion followed by steam distillation as detailed in 2001.11 and crude fiber (978.10) by removing sugars, proteins, lipids, starches, lignin and other acid base digestible components. Ash contents were determined by AOAC 942.05, pH (981.12) and titratable acidity by AOAC-947.05.

2.6. Color measurement

Instrumental color analysis based on light transmission or reflection provides an objective measure of color variations in different treatments. The CIE Lab scale represents color values, where L* indicates lightness (black to white), a* (green to red) and b* (blue to yellow) spectrum. The instrumental color of the samples was measured using hand-held CIE LAB CH Chroma Meter (CDME-2506) by calibrating against the black and white standard colors based on L*, a* and b* values. The readings were taken in triplicates for each of the flour sample in the presence of white background [22].

2.7. Estimation of Tannin Contents of finger millet grains flour

Accurately weighed 0.5 g of finger millet flour sample from each treatment and 75 mL of distilled water were taken in a 250 mL conical flasks. Then flasks were heated for 30 minutes to boil the contents followed by 20 minutes' centrifugation at 1000 RCF and 22 cm radius (2000 rpm). The supernatant was harvested in a 100 mL volumetric flask and volume was made up to the mark with distilled water. In separate 100 mL conical flasks, 10 mL sodium carbonate solution, 5 mL of Folin-Denis reagent, 75 mL distilled water and 1 mL of the sample extract were added. The contents were shaken well and volume was made to 100 mL mark with distilled water. UV-Vis spectrophotometer (V-3000; VWR, Darmstadt, Germany) was used to take absorbance of the sample and blank after 30 minutes' stay time at 700 nm [23], results were measured against the tannic acid standard curve and presented in mg/100g.

2.8. Mineral profiling of finger millet grain flour

Mineral contents of the raw and treated finger millet grain flour samples were determined by following the method of George, Mildred [24]. For that purpose, moisture free sample (0.5 g) from each treatment were taken in 100 mL Erlenmeyer flasks and digested with 5 mL perchloric acid and 10 mL nitric acids at 80 °C. The digestion was stopped when white fumes of acid disappeared. The flasks were cooled to room temperature and digestate was transferred to 50 mL conical flask and diluted up to the mark using deionized water. The diluted samples were filtered by Wattman filter paper No. 42. The resulting filtrate was subjected to mineral analysis by ICP spectroscopy (Agilent 5800 ICP-OES).

2.9. Determination of Phytic acid of finger millet grains flour

Finger millet flour samples (0.06 g from each treatment) were extracted for 30 minutes with 10 mL of 0.2 N HCl solution and filtered using Whatman No. 41 filter paper. An aliquot of 0.5 mL of the extracts was transferred to 10 mL test tubes and capped properly followed by addition of 2 mL distilled water and 1 mL ferric solution (0.2 g of ammonium iron (III) sulfate dodecahydrate in 1000 mL of 2 N HCl). The test tubes were heated for 30 minutes in water bath at boiling, cooled for 15 minutes in ice water and placed to adjust at room temperature. Then 0.25 mL of potassium thiocyanate solution (15 % w/v) was added to each test tube and mixed properly. UV-Vis spectrophotometer (V-3000; VWR, Darmstadt, Germany) was used to record absorbance after a stay time of 25-30 minutes at 480 nm [25].

2.10. Extract preparation and phytochemical evaluation of finger millet grains flour

Sequential extraction was performed for the phytochemical evaluation of the finger millet flour samples. Methanol, ethanol, and aqueous solvent were used with a sample to solvent ratio of 1:10. The sample and the solvent were shaken overnight in a 250 mL conical flask using an orbital shaker. The contents were allowed to settle and were then filtered with Whatman filter paper no. 41. The filtrate was subsequently evaporated to one third of its volume using a rotary evaporator. The temperature range was set to 34 °C for organic solvents and 50 °C for the aqueous solvent.

2.11. Antioxidant Activity Assays

2.11.1. DPPH Radical Scavenging Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the extracts was determined by taking 2 mL of the 0.1 mM DPPH and 2 mL of the extracts in test tubes and placed in dark for 30 minutes. Readings were taken at 517 nm using a UV-Vis spectrophotometer (V-3000; VWR, Darmstadt, Germany). To compare the results, DPPH solution in methanol without extract was taken as control [26].

$$\% \text{ DPPH inhibition} = (\text{absorbance of the control} - \text{absorbance of the test extracts}) / (\text{absorbance of the control}) \times 100 \quad (\text{i})$$

2.11.2. Ferric Reducing Antioxidant Power (FRAP) Assay

Ferric reducing antioxidant potential was measured by taking 10 mM TPTZ, 20 mM FeCl₃·6H₂O and 3.7 pH sodium acetate buffer at a ratio of 1:10:1 and the contents were vortexed properly. A 100 µL of the extract and 3 mL of the FRAP solution were taken in test tube and incubated for 30 minutes in the dark. Absorbance of control and samples was taken at a 593nm using UV-Vis spectrophotometer (V-3000; VWR, Darmstadt, Germany). Results were expressed as µMol of FeSO₄ equivalents per gram defatted samples [27].

2.11.3. Hydrogen Peroxide (H₂O₂) Scavenging Activity

To assess the scavenging activity of hydrogen peroxide (H₂O₂), a 40 mM phosphate buffer at pH 7.4 and a 2 mM H₂O₂ solution were prepared. In an Eppendorf tube, 0.1 mL of the extract was combined with 0.3 mL of the phosphate buffer, bringing the total volume to 0.4 mL. Following this, 0.6 mL of the H₂O₂ solution was added. The reaction mixture was allowed to stand for 10 minutes, after which the absorbance was measured at 230 nm. The hydrogen peroxide scavenging activity was calculated using the equation provided by Wong et al. [28].

$$\text{H}_2\text{O}_2 \text{ scavenging activity } \% = A^\circ - A / (A^\circ) \times 100 \quad (\text{ii})$$

A° is the absorbance of control whereas A is absorbance of the standards or samples.

2.11.4. Total Flavonoid and Phenolic Content Determination

Total flavonoid content (TFC) was determined using the method of Xu and Chang [29]. Briefly, 1 mL of the extract was mixed with 0.3 mL of 5 % sodium nitrite solution and incubated for 5 minutes. Then, 0.3 mL of 10 % aluminum chloride (AlCl₃) solution was added, followed by 2 mL of 1 M sodium hydroxide. The final volume was adjusted with distilled water, and absorbance was measured at 510 nm using a UV-Vis spectrophotometer. A calibration curve was prepared using standard quercetin solutions (10-100 µg/mL), and the results were expressed as milligrams of quercetin equivalents per 100 grams of sample (mg QE/100 g).

Total phenolic content (TPC) was determined following the method of Yadav, Mishra [16]. A total of 0.5 mL of the extract was mixed with 6 g/L sodium carbonate and 20-fold diluted Folin-Ciocalteu reagent to develop a molybdenum blue color. The mixture was incubated in the dark for 90 minutes, and absorbance was measured at 725 nm. A calibration curve was prepared using gallic acid, and results were expressed as mg gallic acid equivalents (GAE) per 100 g of sample.

2.12. In vitro starch digestibility of finger millet flour samples

The in vitro starch digestibility of the raw and treated finger millet grain flour samples was determined by the method of Ren, Chen [30]. In short, free glucose (FG) was obtained by hydrolyzing the samples in a boiling water bath with invertase enzyme. Then conditions were simulated according to in vivo digestion by mixing the pancreatin, pepsin, invertase and amyloglucosidase at 37 °C. After setting the experiment, glucose was obtained at 20 min and 120 min (G20, G120 respectively). Then residues present in the test tubes were incubated in a boiling water bath followed by subjecting to alkaline hydrolysis. The total glucose (TG) was obtained by digesting the hydrolyzed samples with amyloglucosidase. The total digestible starch and resistant starch contents were measured by glucose oxidase-peroxidase method using a GOD-POD diagnostic kit (Applygen Technologies, Beijing, China).

2.13. In-vitro protein digestibility (IVPD) of finger millet flour samples

In-vitro protein digestibility of the finger millet flour samples was determined by taking 200 mg from each treatment into 100 mL Erlenmeyer flask having pepsin (1.5 g/L) and sodium citrate tribasic dehydrate (35 mL, 0.1 mol/L, pH 2.0). The mixture containing Erlenmeyer flasks were incubated in a water bath at 37°C for 2 hours and centrifuged at 10,000×g for 15 min. The supernatant was decanted and residues were washed followed by drying and nitrogen content determination by AOAC, (2005). The % IVPD was calculated by dividing the supernatant protein percent by total protein of the corresponding sample [8].

2.14. Phytochemical analysis by GC-MS

GC-MS analysis of the finger millet flour samples was done by preparing plant extract and running the sample according to the method described by Nsofor, Nwaoguikpe [31]. The sample extract was prepared by taking 10 g of samples and in 100 ml HPLC grade acetonitrile in 250ml conical flasks. The contents were shaken using an orbital shaker for 24 hours with 20 minutes stay time after every 4 hours and then allowed to settle. The supernatant was filtered using Wattman filter paper No. 41 and again by vacuum-assisted membrane filtration. The filtrate was concentrated by rotary evaporator to 1/5 volume. The achieved concentrate was then filtered by a 0.22 µm syringe filter and used for GC-MS analysis. A capillary column (DB-5ms) was used in the GC-MS. Initial temperature of the column was 40 °C which gradually raised to 150 °C at the rate of 20 °C per minute. Then temperature was raised to 230 °C at the rate of 5 °C/minute. The operation proceeded till the temperature increased to 280 °C at 10°C/minutes and remain the same for 8 minutes. Injector port temperature stayed at 280 °C while detector temperature was 250 °C at the same time with a 1ml/min flow rate. Helium was used as carrier gas. The set value of ionization voltage 70 eV and split ratio 110.1 eV. Standard reference compounds representing common phytochemicals were analyzed alongside the samples to verify retention times and mass spectra. A solvent blank was analyzed between sample runs to confirm there was no contamination or carryover. The GC-MS instrument was calibrated at the start of each analytical run using a manufacturer-provided calibration mixture, ensuring mass accuracy, retention time stability, and consistent detector sensitivity.

2.15. Statistical analysis

Each experiment was performed in triplicate to ensure precision and reproducibility of the results. Statistical analysis of the data was performed by arranging all the data on Excel sheets and taking means and standard deviation using Excel commands while compared for level of significance using Statistix 8.1. ANOVA (analysis of variance) was performed by factorial design and results were compared to check the differences among treatments at the $p \leq 0.05\%$. Means of the results were compared by Latin square design following the modified method of [32].

Results

3.1.1. Proximate composition of flour made from raw and treated finger millet grains.

The fermentation of finger millet grain flour significantly increased the protein content by 22.33 % while non-significantly decreasing when sonicated, sonication-assisted hydrated and soaked in sodium carbonate and ammonium hydroxide solutions (Table 1). However, a slight decrease in fat was observed in fermented, sodium carbonate and ammonium hydroxide-soaked grain flour compared to the control, but increased significantly ($p<0.05$) in the sonication-assisted hydrated (22.27 %) and sonicated grain flour (12.30 %). The fiber contents were not affected by sonication, sonication-assisted hydration, sodium carbonate and ammonium hydroxide soaking, but reduced by 6.52 % in the fermented samples. Almost a non-significant increase ($P > 0.05$) in ash content (4.11 %) was divulged for fermented flour, while sodium carbonate and ammonium hydroxide soaking reduced the ash content by $\approx 7.79\%$.

Table 1: Proximate composition of flour made from raw and treated finger millet grains (%).

Treatment	Moisture	Fat	Protein	Fiber	Ash
T ₀	10.92±0.95 ^a	1.71±0.12 ^{bc}	9.03±0.20 ^{bc}	14.11±0.06 ^a	5.13±0.17 ^{ab}
T ₁	9.86±0.28 ^b	1.69±0.07 ^{bc}	11.83±0.28 ^a	13.19±0.18 ^b	5.35±0.16 ^a
T ₂	9.96±0.26 ^{ab}	1.95±0.16 ^b	9.02±0.81 ^{bc}	14.08±0.13 ^a	4.99±0.12 ^b
T ₃	9.90±0.25 ^{ab}	2.20±0.19 ^a	9.07±0.17 ^b	13.97±0.16 ^{ab}	4.68±0.15 ^c
T ₄	10.07±0.27 ^{ab}	1.58±0.40 ^c	8.74±0.18 ^c	14.10±0.07 ^a	4.73±0.61 ^{bc}
T ₅	10.08±0.42 ^{ab}	1.58±0.14 ^c	8.81±0.21 ^c	14.08±0.24 ^a	4.74±0.88 ^{bc}

T₀ = Untreated finger millet grains flour, T₁ = Fermented finger millet flour, T₂ = Sonicated finger millet grains flour, T₃ = Sonication assisted hydrated finger millet grains flour, T₄ = Sodium carbonate (8 %) soaked grains flour, T₅ = Ammonium hydroxide (5 %) soaked grains flour; Values are presented as mean ± standard deviation. Different superscript letters (a–z) within the same row and column indicate statistically significant differences ($p < 0.05$), while identical superscripts indicate non-significant differences

3.1.2. Mineral and heavy metal contents of flour made from raw and treated finger millet grains.

The mineral and heavy metal contents of raw and treated finger millet flour revealed that the effect of treatments was highly significant ($p \approx 0.00$) as presented in Table 2. Fermentation enhanced the calcium (Ca), iron (Fe), zinc (Zn), phosphorous (P) and magnesium (Mg) contents by 10.44, 22.44, 19.80, 5.76 and 14.10 % respectively, compared to control sample. Conversely, reductions were observed in potassium (K) (8.41 %), lead (Pb) (8.22 %), cadmium (Cd) (0.96 %) and Mercury (Hg) (10.53 %) in fermented samples in comparison to the control sample. Sonication led to a pronounced decrease in Fe, Zn and Ca concentrations by 62.80, 71.18 and 55.06 % respectively, but this reduction rate was quite lower for Pb (27.40 %), Cd (0.96 %) and Hg (43.16 %). Similarly, sonication-assisted hydration markedly reduced the concentrations of Fe (77.76 %), Zn (68.66 %), Mg (24.85 %), P (50.68 %), Na (39.41 %), K (26.28 %), Ca (71.48 %), Pb (28.08 %), Cd (23.33 %) and Hg (50.00 %). A comparable decline was observed for mineral contents of the sodium carbonate (Na_2CO_3) and ammonium hydroxide (NH_4OH) soaked samples. In sodium carbonate soaked grain flour, Fe, Zn and Ca contents were reduced by 51.97, 62.39 and 50.90 % respectively. Ammonium hydroxide soaking lowered iron (39.17%), Zn (55.00 %), Mg (11.36 %), P (41.94 %), Na (67.94 %), K (32.31 %), Ca (40.10 %), Pb (30.14 %), Cd (27.53 %) and Hg (62.11 %). The results for the heavy metal contents were well below the safe limits set by Food and Drug Administration i.e., < 20 ppm of lead, < 0.30 ppm of cadmium and 1 ppm of mercury.

Table 2: Mineral and heavy metal contents of flour made from raw and treated finger millet grains

Treatments	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅
Fe (mg/100g)	5.08±0.08 ^b	6.22±0.12 ^a	1.89±0.045 ^e	1.13±0.03 ^f	2.44±0.03 ^d	3.09 ± 0.02 ^c
Zn (mg/100g)	2.422±0.032 ^b	2.904±0.063 ^a	0.698±0.042 ^e	0.759±0.067 ^e	0.911±0.034 ^d	1.090±0.015 ^c
Mg (mg/100g)	139.05±0.67 ^b	158.65±1.20 ^a	119.42±0.83 ^e	104.50±1.05 ^f	129.21±0.99 ^c	123.25±0.85 ^d
P (mg/100g)	274.27±0.759 ^b	290.08±1.053 ^a	157.20±0.667 ^d	135.26±0.609 ^f	149.42±0.494 ^e	159.23±0.760 ^c
Na (mg/100g)	3.40±0.012 ^c	22.40±0.936 ^a	1.93±0.03 ^{de}	2.06±0.046 ^d	13.29±0.24 ^b	1.09±0.02 ^e
K (mg/100g)	394.20±3.55 ^a	361.03±0.88 ^b	294.61±1.16 ^c	290.59±1.64 ^d	257.60±1.09 ^f	266.85±0.53 ^e
Ca (mg/100g)	179.83±0.65 ^b	198.61±0.52 ^a	80.82±1.09 ^e	51.29±0.82 ^f	88.29±0.43 ^d	107.72±1.08 ^c
Pb (ppm)	0.0146±0.00 ^a	0.0134±0.00 ^b	0.0106±0.00 ^c	0.0105±0.001 ^c	0.0094±0.00 ^d	0.0102±0.00 ^c
Cd (ppm)	0.0523±0.00 ^a	0.0518±0.001 ^b	0.0401±0.00 ^c	0.0209±0.00 ^e	0.0361±0.016 ^d	0.0379±0.003 ^d
Hg (ppm)	0.0021±0.00 ^a	0.0019±0.00 ^b	0.00108±0.00 ^c	0.00095±0.00 ^d	0.00085±0.00 ^e	0.00072±0.00 ^f

T₀ = Untreated finger millet grains flour, T₁ = Fermented finger millet flour, T₂ = Sonicated finger millet grains flour, T₃ = Sonication assisted hydrated finger millet grains flour, T₄ = Sodium carbonate (8 %) soaked grains flour, T₅ = Ammonium hydroxide (5 %) soaked grains flour; Values are presented as mean ± standard deviation. Different superscript letters (a–z) within the same row and column indicate statistically significant differences ($p < 0.05$), while identical superscripts indicate non-significant differences

3.1.3. Effect of fermentation on pH and titratable acidity of finger millet flour

The impact of fermentation time on pH and titratable acidity of finger millet flour was found to be highly significant. As indicated in Table 3, the pH of the flour decreased as fermentation time increased while titratable acidity exhibited a direct relationship with the duration of fermentation. At the start of fermentation, pH of the flour samples was 6.71 ± 0.03 , which dropped to 3.39 ± 0.012 after 48 hours. Similarly, the initial titratable acidity of the flour was 0.49 ± 0.004 %, which increased to 0.97 ± 0.005 % after 36 hours and further rose to 1.04 ± 0.002 % by the end of the fermentation period.

Table 3: Effect of fermentation on pH and titratable acidity of finger millet flour

Fermentation Time (hours)	pH	Titrateable Acidity (%)
0	6.71±0.03 ^a	0.49±0.004 ⁱ
6	6.03±0.08 ^b	0.55±0.002 ^h
12	5.64±0.02 ^c	0.60±0.002 ^g
18	5.35±0.01 ^d	0.66±0.003 ^f
24	5.15±0.02 ^e	0.73±0.004 ^e
30	4.89±0.03 ^f	0.84±0.004 ^d
36	4.73±0.01 ^g	0.96±0.005 ^c
42	4.59±0.05 ^h	1.01±0.002 ^b
48	3.38±0.02 ⁱ	1.04±0.002 ^a

For fermentation whole finger millet grains were taken, converted to flour and fermented by *Lactiplantibacillus plantarum*; Values are presented as mean ± standard deviation. Different superscript letters (a–z) within the same row and column indicate statistically significant differences ($p < 0.05$), while identical superscripts indicate non-significant differences

3.1.4. Instrumental color of finger millet grains and flour (L^* , a^* and b^* value)

The L^* values of raw and treated finger millet grains (Figure 1A) reveal that control exhibited a darker tonality compared to treated samples. Grain soaked in ammonium hydroxide showed the lightest color followed by sonication-assisted hydrated, sodium carbonate soaked and sonicated. A similar trend was observed for flour (Figure 1B), where ammonium hydroxide-treated grain flour displayed the lightest color, trailed by sonication-assisted hydrated, sodium carbonate soaked and sonication. The L^* values of fermented flour were comparable to those of the control sample. In the a^* color coordinate, which shifts from green (negative) to red (positive), the effects of different treatments on finger millet grains (Figure 1C) and flour (Figure 1D) were notable. Sonication-assisted hydration resulted in the most intense reddish hue, followed by sonication alone, whereas ammonium hydroxide and sodium carbonate-soaked grains shifted towards the greenish spectrum. A similar pattern was observed in the flour, where ammonium hydroxide and sodium carbonate treatments led to the lowest a^* values, while fermented flour remained comparable to the control. For the b^* coordinate, representing the blue (–) to yellow (+) spectrum, the results (Figure 1E and Figure 1F) indicate that raw and fermented flour exhibited stronger yellow tones compared to untreated grains. However, flour from sonicated, sonication-assisted hydration, ammonium hydroxide and sodium carbonate-treated grains showed reduced yellowness, contributing to a lighter appearance. These findings suggest that chemical and physical treatments significantly influence the color characteristics of both grains and flour, which may have implications for their sensory appeal and consumer preference in food applications.

3.1.5. Tannins and phytate contents of flour made from raw and treated finger millet grains (mg/100g).

Comparative effect of different treatments showed that sodium carbonate soaking was the most effective treatment and reduced tannin contents by 72.99 % followed by sonication assisted hydration (69.56 %), sonication (61.51 %), ammonium hydroxide soaking (60.89 %) and fermentation (39.00 %). The above mentioned treatments also reduced the phytic acid contents but not proportionate to reduction in tannins contents (Table 4). Maximum reduction in phytic acid was noted for ammonium hydroxide soaked grain flour (27.87 %) and a similar was observed for fermented (27.64 %) and sonication assisted hydrated (25.89 %) samples. The least reduction in phytic acid contents was observed for sonicated (10.74 %) and sodium hydroxide soaked (20.88 %) grain flour samples.

Table 4: Tannins and phytate contents of flour made from raw and treated finger millet grains.

Treatment	Tannins (mg/100g)	Phytic acid (mg/100g)
T ₀	54.51±0.051 ^a	50.593±0.035 ^a
T ₁	33.25±0.133 ^b	36.607±0.017 ^e
T ₂	20.98±0.129 ^d	45.157±0.039 ^b
T ₃	16.593±0.056 ^e	37.547±0.021 ^d
T ₄	14.72±0.086 ^f	40.027±0.434 ^c
T ₅	21.32±0.230 ^c	36.503±0.320 ^e

T₀ = Untreated finger millet grains flour, T₁ = Fermented finger millet flour, T₂ = Sonicated finger millet grains flour, T₃ = Sonication assisted hydrated finger millet grains flour, T₄ = Sodium carbonate (8 %) soaked grains flour, T₅ = Ammonium hydroxide (5 %) soaked grains flour; Values are presented as mean ± standard deviation. Different superscript letters (a–z) within the same row and column indicate statistically significant differences ($p < 0.05$), while identical superscripts indicate non-significant differences

3.1.6. Antioxidant potential of various extracts of flour made from raw and treated finger millet grains (mg/100g)

Results for the antioxidant potential (DPPH, FRAP, TPC, H₂O₂ scavenging activity, and flavonoid contents) of raw and treated finger millet grain flour are presented in Table 5. The effects of treatments and solvents were highly significant on the antioxidants of finger millet flour extracts. The outcomes of the study highlighted that maximum DPPH, FRAP, TPC, and flavonoid contents were observed in the methanolic extract, followed by ethanolic and aqueous extracts, while the highest H₂O₂ scavenging activity was noted for the aqueous extract compared to the ethanolic and methanolic extracts. However, fermentation was the most effective treatment, followed by sonication-assisted hydration, sonication, and soaking in sodium and ammonium hydroxide. The methanolic extract of the fermented sample yielded the highest TPC, 467.53±6.3 mg/100g GAE, compared to the control sample (386.97±2.8 mg/100g GAE), while the lowest TPC (45.13±11.8 mg/100g GAE) was found in the aqueous extract of sonication-assisted hydrated grain flour. The results indicated that the maximum DPPH activity (86.97±0.70 mg/100g GAE) was observed in the methanolic extract of the fermented flour, while the minimum (11.01±1.89 mg/100g GAE) was noted for the aqueous extract of the ammonium hydroxide-soaked grain flour. The data for FRAP also signifies that the methanolic extract of fermented finger millet flour yielded the maximum (89.93±0.62 mg/100g TE) compared to ethanolic (50.93±0.99 mg/100g TE) and aqueous (38.95±0.40 mg/100g TE) extracts of the same sample, but the lowest activity was found for the aqueous extract of the sodium hydroxide-soaked grain flour, which was 10.93±0.83 mg/100g TE.

Maximum H₂O₂ scavenging activity was observed in the aqueous extract of the fermented finger millet flour sample (209.12±3.68 mg/100g), followed by the ethanolic (194.40±1.95 mg/100g) and methanolic (191.18±1.75 mg/100g) extracts of the same sample. The minimum H₂O₂ scavenging activity was found in the methanolic extract of the ammonium hydroxide-soaked grains flour extract (153.11±1.23 mg/100g). The effects of different treatments and solvents were significant ($p < 0.05$) on the hydrogen peroxide scavenging activity of the finger millet flour samples, indicating that the aqueous extract yielded maximum H₂O₂ scavenging activity, followed by the ethanol and methanol extracts. However, the fermented and sonication-assisted hydrated grains flour extracts exhibited the highest H₂O₂ scavenging activity among other samples. When evaluating the outcomes of different treatments (solvents), the methanolic extract of the fermented finger millet flour yielded the maximum flavonoid content (FC), at 87.087±0.46 mg QE/100g, compared to the methanolic extract of the control sample (81.057±0.17 mg QE/100g). In addition to fermentation, the methanolic extract of the flour prepared from sonication-assisted hydrated grains also yielded an appreciable FC (73.377±0.24 mg QE/100g). The lowest FC values were observed for the extract of sodium carbonate and ammonium hydroxide-soaked grains flour.

Table 5: Antioxidant potential of various extracts of flour made from raw and treated finger millet grains (mg/100g)

Parameters	Solvents	Treatments					
		T ₀	T ₁	T ₂	T ₃	T ₄	T ₅
TPC	Aqueous	126.9 ± 0.5 ^{bN}	144 ± 2.8 ^{aM}	62.2 ± 8.2 ^Q	45.13±11.8 ^{fR}	94.63±0.6 ^Q	74.27±0.6 ^{dP}
	Ethanol	247.47±0.5 ^{bE}	327.53±0.8 ^{aC}	183.77±5.3 ^{dI}	222.63±0.5 ^{cG}	164.97±0.8 ^{eK}	158.00±8.6 ^{fL}
	Methanol	386.97±2.8 ^{bB}	467.53±6.3 ^{aA}	219.63±3.7 ^{eH}	283.83±5.2 ^{cD}	241.17±1.1 ^{dF}	180.80±1.1 ^{fJ}
DPPH	Aqueous	22.68±0.86 ^{cK}	33.78±1.25 ^{aH}	20.61±1.02 ^{dL}	31.45±1.41 ^{bI}	14.32±0.85 ^{eM}	11.01±1.89 ^{fN}
	Ethanol	53.58±0.99 ^{bF}	64.99±1.52 ^{aD}	48.53±0.59 ^{dG}	51.88±0.82 ^{cE}	29.99±1.65 ^{eIJ}	24.30±0.84 ^{fK}
	Methanol	74.33±0.57 ^{bC}	86.97±0.70 ^{aA}	67.94±0.89 ^{dC}	71.17±0.94 ^{cB}	35.03±1.51 ^{eH}	28.84±0.19 ^{fJ}
FRAP	Aqueous	26.78±0.32 ^{bG}	38.95±0.40 ^{aD}	21.62±0.90 ^{cH}	22.77±0.57 ^{cH}	10.93±0.83 ^{eK}	16.58±0.63 ^{dI}
	Ethanol	29.66±0.34 ^{cF}	50.93±0.99 ^{aC}	33.43±0.39 ^{bE}	22.96±0.87 ^{dH}	14.36±0.72 ^{eJ}	12.80±0.53 ^{fK}
	Methanol	60.28±0.72 ^{bB}	89.93±0.62 ^{aA}	48.92±0.15 ^{bCD}	51.46±0.12 ^{cC}	38.63±0.26 ^{dD}	37.27±0.27 ^{dD}
H ₂ O ₂	Aqueous	190.58±0.51 ^{bABC}	209.12±3.68 ^{aA}	180.87±2.92 ^{dBCD}	185.29±2.43 ^{cBC}	177.43±3.14 ^{eBCD}	173.47±13.63 ^{fB-E}
	Ethanol	185.13±1.74 ^{bBCD}	194.40±1.95 ^{aAB}	172.17±1.35 ^{cDE}	172.80±1.55 ^{cDE}	166.25±1.25 ^{dDE}	163.73±1.14 ^{eDE}
	Methanol	176.32±1.65 ^{bBCD}	191.18±1.75 ^{aABC}	174.64±1.41 ^{bB-E}	164.77±2.54 ^{cE}	165.08±2.11 ^{cDE}	153.11±1.23 ^{dE}
Flavonoids	Aqueous	15.067±0.32 ^{bK}	26.783±0.33 ^{aJ}	15.330±0.54 ^{bK}	13.240±0.19 ^{cL}	10.207±0.04 ^{dM}	5.277±0.70 ^{eN}
	Ethanol	68.347±0.23 ^{aD}	59.453±0.11 ^{cdG}	62.280±0.20 ^{cF}	64.583±0.34 ^{bE}	58.860±0.32 ^{cdG}	56.423±0.20 ^{dH}
	Methanol	81.057±0.17 ^{bB}	87.087±0.46 ^{aA}	62.143±0.16 ^{dF}	73.377±0.24 ^{cC}	56.367±0.31 ^{eH}	49.163±0.35 ^{fI}

T₀ = Untreated finger millet grains flour, T₁ = Fermented finger millet flour, T₂ = Sonicated finger millet grains flour, T₃ = Sonication assisted hydrated finger millet grains flour, T₄ = Sodium carbonate (8 %) soaked grains flour, T₅ = Ammonium hydroxide (5 %) soaked grains flour; Values are expressed as mean ± standard deviation. Different lowercase superscripts within the same row indicate significant differences among treatments, while different uppercase superscripts within the same column indicate significant differences among solvents at $p < 0.05$. Similar superscripts denote non-significant differences.

Note:

Capital lettering in the table show the comparative effect of treatments and solvents on antioxidant contents of treated finger millet grains flour extract while small letters indicate the differences among treatment

3.1.7. Phytochemistry of raw and treated finger millet grains flour

The retention time, peak area, molecular formula and chemical name of raw and treated finger millet grain flour extracts are presented in Tables 6, 7, 8, 9, 10 and 11. The fermented flour extract exhibited a maximum of 141 peak hits, followed by the control sample with 38 peak hits, while the minimum was observed in sodium carbonate with 8 peak hits and ammonium hydroxide with 3 peak hits in the soaked grain flour. The compounds were listed based on the highest probability for each peak hit. In the control sample, the majority of the compounds were phytosterols, terpenoids, fatty acids, and methyl esters. However, fatty acids were found as the major component in the fermented finger millet flour extract, followed by methyl esters and phytosterols. Results from the NIST20.L indicated that phytosterols and fatty acids were derived from the sonicated, sonication-assisted hydrated, sodium carbonate, and ammonium hydroxide grain flour extracts.

Table 6: Chemical constituents present in the Acetonitrile extract of fermented (T1) finger millet flour

Sr. No.	Peak No.	Retention Time	Area %	Molecular Formula	Name
1	1	9.045	0.03	C ₁₅ H ₂₄ O	Caryophyllene oxide
2	2	11.487	0.00	C ₁₅ H ₂₄ O	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene
3	5	13.984	0.01	C ₁₇ H ₃₂ O	9-Hexadecenoic acid, methyl ester, (Z)
4	5	13.984	0.01	C ₁₇ H ₃₂ O	Methyl hexadec-9-enoate
5	6	14.115	0.01	C ₁₇ H ₃₂ O	9-Hexadecenoic acid, methyl ester
6	7	14.818	0.13	C ₁₇ H ₃₄ O ₂	6-Pentadecenoic acid, 13-methyl-, methyl ester
7	12	17.751	2.92	C ₁₇ H ₃₄ O ₂	Methyl palmitate
8	12	17.751	2.92	C ₁₇ H ₃₄ O	Hexadecanoic acid, methyl ester
9	15	18.28	0.72	C ₁₇ H ₃₄ O ₂	Pentadecanoic acid, 14-methyl-, methyl ester
10	19	20.271	0.07	C ₁₆ H ₃₀ O ₂	Methyl (Z)-10-pentadecenoate
11	20	20.744	0.11	C ₁₆ H ₃₂ O ₂	Methyl 13-methyltetradecanoate
12	21	20.879	0.03	C ₁₈ H ₃₂ O ₂	9,12-Octadecadienoic acid (Z,Z)-
13	23	21.227	0.12	C ₁₈ H ₃₂ O ₂	10E,12Z-Octadecadienoic acid
14	24	21.404	0.16	C ₁₈ H ₃₄ O	cis-10-Heptadecenoic acid methyl ester
15	25	21.668	0.17	C ₁₆ H ₃₂ O	n-Hexadecanoic acid
16	26	22.425	0.19	C ₁₈ H ₃₂ O	9,17-Octadecadienal, (Z)-
17	27	22.602	0.16	C ₁₈ H ₃₆ O ₂	Hexadecanoic acid,15-methyl-, methyl ester
18	39	25.317	9.39	C ₁₉ H ₃₄ O ₂	8,11-Octadecadienoic acid, methyl ester
19	40	25.377	1.80	C ₁₉ H ₃₄ O ₂	10,13-Octadecadienoic acid, methyl ester
20	44	25.763	1.72	C ₁₉ H ₃₄ O ₂	methyl 10-trans,12-cis-octadecadienoate-Molbase
21	49	26.177	1.06	C ₁₉ H ₃₆ O	11-Octadecenoic acid, methyl ester
22	50	26.220	1.03	C ₁₉ H ₃₆ O ₂	10-Octadecenoic acid, methyl ester
23	51	26.289	0.57	C ₁₉ H ₃₆ O	9-Octadecenoic acid, methyl ester
24	52	26.321	1.02	C ₁₉ H ₃₆ O	cis-13-Octadecenoic acid, methyl ester
25	53	26.532	2.86	C ₁₉ H ₃₈ O ₂	Methyl stearate
26	64	28.002	0.67	C ₁₈ H ₃₂ O ₂	Linoelaidic acid
27	66	28.319	2.12	C ₁₈ H ₃₄ O ₂	Oleic Acid
28	66	28.319	2.12	C ₁₈ H ₃₄ O ₂	cis-Vaccenic acid
29	67	28.487	0.51	C ₁₈ H ₃₆ O	Octadecanoic acid
30	73	29.285	0.13	C ₁₈ H ₃₂ O ₂	Linoelaidic acid
31	75	29.481	0.21	C ₇₅ H ₁₃₄ O	Linoleyl methyl ketone
32	80	30.102	1.02	C ₂₁ H ₄₀ O ₂	cis-Methyl 11-eicosenoate
33	81	30.185	0.17	C ₂₁ H ₄₀ O ₂	cis-13-Eicosenoic acid, methyl ester
34	84	30.525	0.63	C ₁₉ H ₃₆ O ₃	Glycidyl palmitate
35	85	30.617	1.14	C ₂₁ H ₄₂ O	Eicosanoic acid, methyl ester
36	86	30.682	0.14	C ₂₁ H ₄₀ O ₂	Methyl 9-eicosenoate

37	87	30.830	0.17	C ₁₉ H ₃₆ O	2-Methyl-Z,Z-3,13-octadecadienol
38	88	31.038	0.69	C ₂₁ H ₄₂ O	Eicosanoic acid, methyl ester
39	94	31.936	0.11	C ₂₃ H ₃₂ O ₂	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-
40	95	32.048	0.06	C ₁₈ H ₃₂ O	9,17-Octadecadienal, (Z)-
41	97	32.368	0.18	C ₂₂ H ₄₄ O ₂	Heneicosanoic acid, methyl ester
42	100	33.059	0.48	C ₁₃ H ₂₂	Bicyclo[10.1.0]tridec-1-ene
43	105	33.833	2.93	C ₂₃ H ₄₄ O ₂	Methyl 11-docosenoate
44	110	34.756	0.07	C ₁₉ H ₃₆ O	Cyclopropaneoctanal, 2-octyl-
45	113	35.251	0.05	C ₂₃ H ₄₄ O ₂	22-Tricosenoic acid
46	125	36.920	0.28	C ₂₅ H ₄₈ O ₂	15-Tetracosenoic acid, methyl ester
47	131	38.350	0.03	C ₃₀ H ₅₀	Squalene
48	136	39.519	0.08	C ₂₄ H ₄₃ ClO ₄	Fumaric acid, 2-chloroethyl octadecyl ester
49	140	40.452	0.06	C ₂₇ H ₄₆ O ₂	delta.-Tocopherol

The above listed compounds were identified from the Database Front | Demo - Live, MassHunter\LIBRARY\NIST20.L, MassHunter\LIBRARY\RTLPEST3. with > 95% probability)

Table 7: Chemical constituents present in the Acetonitrile extract of raw (T₀) finger millet flour

Sr. No.	Peak No.	Retention Time	Area %	Molecular Formula	Name
1	1	24.285	1.01	C ₁₆ H ₃₂ O	n-Hexadecanoic acid
2	2	24.462	0.56	C ₁₈ H ₃₂ O	9,17-Octadecadienal, (Z)-
3	3	25.138	14.16	C ₁₄ H ₂₄ O	13-Tetradec-11-yn-1-ol
4	3	25.138	14.16	C ₂₂ H ₃₈ O ₂	Butyl 9,12-octadecadienoate
5	4	25.392	11.55	C ₁₈ H ₃₄ O	9-Octadecenal, (Z)-
6	5	25.691	1.18	C ₁₈ H ₃₂ O	9,12-Octadecadienoic acid (Z,Z)-
7	6	26.46	1.42	C ₂₁ H ₄₀ O ₃	Glycidol stearate
8	7	26.814	0.11	C ₁₈ H ₃₂ O ₂	Linoelaidic acid
9	8	26.950	0.18	C ₁₉ H ₃₆ O ₂	11-Octadecenoic acid, methyl ester
10	10	27.685	11.32	C ₁₈ H ₃₄ O ₂	Oleic Acid
11	12	29.454	4.91	C ₂₉ H ₅₀ O ₂	dl.alpha.-Tocopherol
12	12	29.454	4.91	C ₂₉ H ₅₀ O ₂	Vitamin E
13	14	31.382	0.40	C ₇₅ H ₁₃₄ O	Linoleyl methyl ketone
14	15	32.308	0.31	C ₁₈ H ₃₂ O	9,12-Octadecadienoic acid (Z,Z)-
15	17	32.966	0.22	C ₁₉ H ₃₆ O	Cyclopropaneoctanal, 2-octyl-
16	18	33.231	0.66	C ₇₅ H ₁₃₄ O	Linoleyl methyl ketone
17	21	34.561	0.06	C ₂₁ H ₄₀ O ₄	1-cis-Vaccenoylglycerol
18	22	35.290	6.73	C ₂₈ H ₄₈ O	Campesterol
19	26	36.552	6.29	C ₂₉ H ₄₈ O	Stigmasterol
20	30	38.231	0.76	C ₁₅ H ₂₆ O	Ginsenosol
21	31	38.907	21.52	C ₂₉ H ₅₀ O	gamma.-Sitosterol
22	31	38.907	21.52	C ₂₉ H ₅₀ O	beta.-Sitosterol
23	32	39.223	1.78	C ₁₃ H ₂₂ O Si	Thymol, TMS derivative
24	33	39.443	1.25	C ₂₉ H ₄₈ O	Fucosterol
25	33	39.443	1.25	C ₂₄ H ₃₄ O ₄	3,5-Proggesterol acetate
26	35	39.913	0.59	C ₁₆ H ₂₈ N ₂ O ₂	2-Myristynoyl-glycinamide

The above listed compounds were identified from the Database Front | Demo - Live, MassHunter\LIBRARY\NIST20.L, MassHunter\LIBRARY\RTLPEST3. with > 95% probability)

Table 8: Chemical constituents present in the Acetonitrile extract of sonicated (T₂) finger millet grains flour

Sr. No.	Peak No.	Retention Time	Area %	Molecular Formula	Name
1	1	27.515	6.41	C ₁₈ H ₃₂ O ₂	10E,12Z-Octadecadienoic acid
2	2	27.626	58.63	C ₁₈ H ₃₄ O ₂	Oleic Acid
3	3	27.972	1.26	C ₁₈ H ₃₂ O ₂	9,12-Octadecadienoic acid (Z,Z)-
4	5	37.842	4.66	C ₁₄ H ₂₂ O	4-tert-Octylphenol, TMS derivative
5	6	38.217	26.25	C ₄₂ H ₆₃ O ₄ P	Tris(2,4-ditert-butylphenyl)phosphate

The above listed compounds were identified from the Database Front | Demo - Live, MassHunter\LIBRARY\NIST20.L, MassHunter\LIBRARY\RTLPEST3. with > 95% probability)

Table 9: Chemical constituents present in the Acetonitrile extract of sonication assisted hydrated (T₃) finger millet grains flour

Sr. No.	Peak No.	Retention Time	Area %	Molecular Formula	Name
1	1	24.294	1.25	C ₁₆ H ₃₂ O ₂	n-Hexadecanoic acid
2	2	26.955	0.54	C ₁₉ H ₃₆ O	9-Octadecenoic acid (Z)-, methyl ester
3	3	27.517	3.18	C ₁₈ H ₃₂ O ₂	Linoelaidic acid
4	4	27.633	19.78	C ₁₈ H ₃₂ O ₂	10E,12Z-Octadecadienoic acid
5	5	28.054	1.98	C ₁₈ H ₃₄ O ₂	Oleic Acid
6	6	34.605	4.42	C ₁₈ H ₃₄ O	Z,E-3,13-Octadecadien-1-ol
7	8	34.868	9.67	C ₂₉ H ₄₈ O	Stigmast-4-en-3-one
8	9	35.728	10.60	C ₃₀ H ₅₀ O	9,19-Cyclolanost-25-en-3-ol, 24-S
9	10	35.954	2.77	C ₃₀ H ₅₀ O	alpha.1-Sitosterol
10	11	35.997	2.22	C ₁₃ H ₂₂ O Si	Thymol, TBDMS derivative
11	12	38.904	3.48	C ₆ H ₁₈ O ₃ Si ₃	Cyclotrisiloxane, hexamethyl-
12	14	39.904	11.97	C ₁₃ H ₁₀ F ₃ N ₃ O	Pyridine-3-carboxamide, oxime, N

The above listed compounds were identified from the Database Front | Demo - Live, MassHunter\LIBRARY\NIST20.L, MassHunter\LIBRARY\RTLPEST3. with > 95% probability)

Table 10: Chemical constituents present in the Acetonitrile extract of sodium carbonate soaked (T₄) grains finger millet flour

Sr. No.	Peak No.	Retention Time	Area %	Molecular Formula	Name
1	1	27.622	1.55	C ₁₈ H ₃₄ O	9-Octadecenoic acid, (E)-
2	3	36.994	27.50	C ₁₇ H ₃₀	1,8,11-Heptadecatriene, (Z,Z)-
3	5	37.959	1.28	C ₁₄ H ₂₂ O ₂	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-
4	8	39.487	2.5	C ₁₄ H ₂₂ O ₂	2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)

The above listed compounds were identified from the Database Front | Demo - Live, MassHunter\LIBRARY\NIST20.L, MassHunter\LIBRARY\RTLPEST3. with > 95% probability)

Table 11: Chemical constituents present in the Acetonitrile extract of ammonium hydroxide soaked (T₅) grains finger millet flour

Sr. No.	Peak No.	Retention Time	Area %	Molecular Formula	Name
1	1	27.622	52.98	C ₁₈ H ₃₄ O	6-Octadecenoic acid
2	2	37.173	26.89	C ₁₃ H ₂₂ O Si ₂	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl
3	2	37.173	26.89	C ₁₃ H ₂₂ O Si	Thymol, TMS derivative
4	3	37.955	20.14	C ₁₃ H ₂₂ O Si	4-tert-Butylphenol, TMS derivative

The above listed compounds were identified from the Database Front | Demo - Live, MassHunter\LIBRARY\NIST20.L, MassHunter\LIBRARY\RTLPEST3. with > 95% probability)

3.1.8. *In vitro* digestion of starch and protein

The starch composition and its digestibility play an important role in human nutrition and health, as higher percentages of resistant starch control glycemic index, reduce fat and cholesterol and prevent colon cancer. The comparative results presented in Figure 2 divulge that the total starch of the raw sample was 53.21 % which reduced to 50.31 % when fermented, but remained stable for the sonicated (52.69 %), sonication-assisted hydrated (52.93 %), sodium carbonate (52.78%) and ammonium hydroxide soaked (52.85 %) grain flour. However, these treatments substantially influenced the in vitro digestibility by

altering the starch structural accessibility to enzymatic hydrolysis. Results for in vitro starch digestibility are presented on a percentage basis of the total starch. In vitro starch digestion depicted that maximum resistant starch (23.99 %) was noted for sonication-assisted hydrated samples while minimum for the sodium carbonate and ammonium hydroxide soaked samples i.e., 11.59 and 11.99 %. The highest in vitro starch digestibility percentage was found for sodium carbonate (88.40 %) and ammonium hydroxide soaked (88.00%) samples and the lowest for sonication-assisted hydrated (76.00 %) samples. The in vitro starch digestibility of raw flour was 85.99 % while that of fermented samples was 83.99 %.

Figure 3 presents the results regarding in vitro protein digestibility (IVPD) of raw and treated finger millet flour samples. The fermented finger millet flour contains maximum crude protein contents (11.83 %) followed by raw (9.07 %), sonicated (9.03 %), sodium carbonate soaked (9.03 %), ammonium hydroxide soaked (8.81 %) and sonication-assisted hydrated (8.74 %). The highest IVPD was observed for fermented samples followed by sonication assisted hydrated i.e., 87.63 % and 79.33 % respectively of the crude protein. Minimum IVPD (59 %) was noted for raw samples.

Discussion

The proximate analysis of control (T0), fermented (T1), sonicated (T2), sonication assisted hydrated (T3), sodium carbonate (T4) and ammonium hydroxide (T5) soaked finger millet grains flour revealed that fat contents were decreased in fermented, sodium carbonate and ammonium hydroxide soaked grains flour. In fermented flour, this reduction may be due to the utilization of lipids by microbial lipases for metabolic activities. In sodium carbonate- and ammonium hydroxide-soaked samples, the decrease in fat content can be attributed to the leaching of lipids into the soaking solution and partial hydrolysis of triglycerides caused by the alkaline conditions, which enhance lipid solubilization and breakdown. Mutshinyani, Mashau [33] suggested that fat content during fermentation may be reduced due to the utilization of fats by fermenting microflora for their metabolic activities. However, Nouska, Hatzikamari [34] reported an improvement in textural properties, such as taste and aroma, in fermented flour. Interestingly, a highly significant increase in fat content of the sonication-assisted hydrated (22.27 %) and sonicated finger millet grains (12.30 %) flour samples was associated with increased extractability of fat due to the rupturing of the cellular matrix. Contrarily, the fermentation process significantly ($P < 0.05$) enhanced the protein contents (22.33 %) which is attributed to the reduction of anti-nutrient bound nitrogen during fermentation. Furthermore, protein contents may increase due to microbial metabolism i.e., the breakdown of complex proteins into peptides and the conversion of carbohydrates into metabolic products yielding nitrogenous compounds [35]. Jan, Kumar [9] reported an increase in protein contents of the finger millet flour when fermented with diverse fermentation cultures (*Lactiplantibacillus plantarum*, *Lactobacillus brevis*, *Saccharomyces cerevisiae* L. and with yeast + ammonium sulfate). A negligible decrease in protein contents was observed in all other treatments compared to the control group, which may be attributed to the leaching of soluble nitrogenous compounds such as free amino acids, peptides, and small proteins during sonication-assisted hydration and soaking. The combined effect of cellular disruption by ultrasound and solubilization in the soaking medium likely enhanced the loss of these nitrogenous constituents. The results indicated that the reduction in fiber content during fermentation might be due to the action of α -amylases and cellulolytic enzymes produced by *Lactiplantibacillus plantarum*. α -Amylases cleave α -1,4 glycosidic bonds in starch and other polysaccharides, while cellulases hydrolyze β -1,4 linkages in cellulose and hemicellulose. These enzymatic activities break down the complex carbohydrate matrix, converting fiber into short-chain fatty acids, organic acids, and gases such as CO_2 , H_2 , and CH_4 , thereby reducing the overall dietary fiber content. Kambabazi, Hitayezu [36] stated that yeast and yeast mold fermentation reduced the fiber contents of finger millet flour samples through microbial metabolism of complex polysaccharides. Similar results were observed by Samtiya, Badgujar [37] who demonstrated that fermentation with diverse microbial cultures influenced the nutrient composition, anti-nutritional factors, and bioactive compounds of finger millet flour. In contrast, alkali-soaked (sodium carbonate and ammonium hydroxide) malted millet flour showed minimal reduction in fiber content, likely due to partial hydrolysis of hemicellulose and cellulose and leaching of soluble fiber into the soaking medium [38]. Sonication and sonication-assisted hydration treatments caused mechanical disruption of the cellular matrix, increasing the porosity and solubilization of fiber, which led to slight reductions in fiber content. Regarding ash content, fermentation increased ash by degrading phytic acid and tannins, thereby releasing bound minerals, whereas soaking, sonication, and sonication-assisted hydration slightly altered ash content due to leaching of soluble minerals or structural modifications in the seed matrix [9]. These observations indicate that the effects on fiber and ash depend on the nature of the treatment, with fermentation primarily modifying the composition enzymatically, while soaking and sonication act through physical and chemical mechanisms.

The increase in mineral content of the fermented flour was due to microbial metabolism, production of organic acids and release of enzymes that break anti-nutrient (myo-inositol hexakisphosphate) and prevent the formation of insoluble mineral complexes (phytates) thus liberating quantifiable minerals. A comparable increase in mineral content of the fermented samples was also observed by Jan, Kumar [9] during assessing the effects of various treatments on bioactive, nutritional and anti-nutritional compounds of finger millet. Mudau, Mashau [39], explicated that spontaneous fermentation enhanced the mineral contents of the finger millet biscuits and a similar result was reported by Kiczorowski, Kiczorowska [40] for heavy metal contents of the fermented vegetables. A highly significant reduction in iron, zinc, magnesium, phosphorous and other mineral contents may be associated with the disintegration of the seed coat deep into the aleurone layer and cavitation in the seed. While working on ultrasound-assisted extraction of minerals from sorghum seeds, Curti, Cora Jofre [41] divulged similar results. Chemical treatments, i.e., soaking in sodium carbonate and ammonium hydroxide solution also resulted in reduced mineral contents. This reduction in mineral contents may be attributed to corrosion of the seed coat and ultimately leading to the leaching of minerals, as majority of the minerals are present in the seed coat. On the other hand, leaching is the principal aspect of reduction in heavy metal contents during sonication, sonication-assisted hydration and soaking in sodium carbonate and ammonium hydroxide solutions.

The effect of *Lactiplantibacillus plantarum* fermentation on pH and titratable acidity was highly significant as resulted in decrease in pH and increase in titratable acidity which were due to enzymatic hydrolysis of complex nutrients into simpler compounds and the production of organic acids (i.e., lactic acid, acetic acid, and propionic acid) by the fermenting microflora. Similarly, logarithmic growth of fermenting microflora is also responsible for converting free sugars into pyruvate and subsequently lactic acid which is the primary contributor to the observed increase in titratable acidity during fermentation. The results were similar to the findings of Mutshinyani, Mashau [33] who claimed a decline in pH from 5.97 to 3.79 due to reduction of soluble organic acids during LAB fermentation of flour. Similarly, Yadav, Mishra [42] reported a drop in pH while assessing in-vitro digestibility and quality characteristics of finger

millet milk fermented by *Lactiplantibacillus plantarum* (MCC 2974). Similar findings were reported by Mudau, Mashau [39] of the spontaneously fermented flour.

The shift in color from darker to lighter tone (L^* value), brown to reddish green (a^* value), and white (b^* value) was due to the degradation of coloring compounds in the aleurone layer. Comparatively, maximum color was removed by ammonium hydroxide soaking, indicating a highly corrosive action and physiological modification of the seed coat. Ammonium hydroxide interacts with complex carbohydrates and leaches colorants [43]. Improvement in the whitish color of the sodium carbonate-soaked grains flour was attributable to the formation of sodium phytate and disintegration of mineral anti-nutrient complexes. The degradation of colors of the sonication-assisted hydrated grains was because of ultrasound waves on the complexes, which resulted in the leaching of colors. Das, Nayak [44] revealed that ultrasound-assisted extraction enhances the recovery of colorants. A lesser improvement in the color of fermented and sonicated samples was noted than ammonium hydroxide soaking and sonication-assisted hydration treatment. The degradation of coloring substances during fermentation was due to the breakdown of phytate minerals complexes. Martín-Gómez, García-Martínez [45] reported that yeast fermentation is not associated with color breakdown while bacterial fermentation degrades colorants of the foods being fermented. Onipe and Ramashia [46] reviewed the finger millet seed coat behavior by various treatments and reported that colorants are degraded by chemical and non-thermal treatments. In the present study color profile of the flour made from treated seeds was comparatively lighter than whole grains and its flour.

The reduction in tannin and phytate contents during fermentation was due to the activity of polyphenol oxidase enzymes released by *Lactiplantibacillus plantarum* in the fermentation medium and other catabolic enzymes like phytase. Similar results were reported by Abioye, Babarinde [5] when finger millet flour was fermented to check the effect of fermentation on nutrients, anti-nutrients, antioxidants, phenols, and flavonoids. George, Mildred [24] reported a 52 % decline in tannin contents of the fermented finger millet flour while working on improving the bio-accessibility of processed finger millet. Tannin and phytate contents were reduced during soaking and sonication as a result of leaching and degradation of the aleurone layer and color compounds. The maximum reduction of tannin contents in sodium hydroxide-soaked samples can be correlated to the deprotonation of the $-OH$ group of the tannins by sodium hydroxide, resulting in high solubility of the tannins in the water phase. That's why in pharmacognosy practices, tannin contents are extracted by using sodium hydroxide to get maximum recovery [47]. The decrease in tannin contents of the sonication-assisted hydration and sonicated samples might be the result of the degradation of the aleurone layer and increased porosity of the cellular matrix. Reduction in tannin contents by ultrasound-mediated hydration of the finger millet and kodo millet grains was also observed by several groups of researchers [16, 48, 49]. The decline in phytic acid was linked with phytase activity thus hydrolyzing phytic acid, releasing inorganic phosphate, and improving the availability of minerals and proteins.

Finger millet is a promising health-promoting cereal due to its antioxidant properties, which play a crucial role in health and disease. The recovery of antioxidants varies with processing methods, solvents used, and the extraction process. Ultrasound waves create pores in the cellular matrix and enhance the dissolving power of phytochemicals. However, with an increased extraction rate, there is a slight adverse effect of ultrasound waves on FRAP activity due to the deprotonation of phenolic compounds [39]. Increased FRAP activity of the fermented samples may be attributed to the release of bound phenolics metabolic and enzymatic cleavage of chelated phytochemicals [8]. However, decreased FRAP activity in ammonium hydroxide and sodium carbonate-soaked grains flour may be associated with the leaching of colored compounds reducing minerals, anti-nutrients, and other phytochemicals in the system and chelation with mineral complexes [5].

The enhanced TPC of fermented samples may be due to the breakdown of complexes and the release of bound phenolics. Microbial metabolism also results in the production of antioxidants. Jan, Kumar [9] reported an increase in TPC of fermented finger millet flour using *Lactobacillus plantarum*, *Lactobacillus brevis*, *Saccharomyces cerevisiae* L. with ammonium sulfate activator as a potential fermenting microbiota. The higher levels of TPC in sonication-assisted hydrated grains flour were attributed to ultrasound waves, leading to increased release of organic compounds due to cavitation and increased porosity of cells. Nkhoma [50], reported reduced TPC of sodium carbonate and ammonium hydroxide-soaked grains due to chemical reactions with sodium and ammonium ions. Leaching has been a major contributor to reduced total phenolic contents. Abdelseed [51] found that decortication reduces ash, protein, fat, crude fiber, tannin contents, phytate contents, phytochemicals (DPPH, FRAP), and total phenolic contents.

Fermented samples showed the highest DPPH activity while the lowest DPPH activity was found for ammonium hydroxide and sodium carbonate-soaked grains flour. Comparative evaluation of the effects of different solvents on DPPH activity revealed that methanolic extract had the highest DPPH activity followed by ethanolic and aqueous extracts. Sonication-assisted hydration also showed an enhanced DPPH activity. This may be due to the polarity of each solvent used for extraction purposes [52]. Similarly, the hydrogen peroxide scavenging activity of fermented flour is maximized due to the release of bound phenolic compounds through microbial metabolism. Some studies showed that fermenting microbes produce free radical scavengers when they use minerals like selenium [15]. The H_2O_2 scavenging activity of sonication-assisted hydrated grains flour is higher due to the breakdown of mineral complexes, which are chelated by anti-nutrients. The release of chelated phytochemicals and structural modification is the basis of increased hydrogen peroxide scavenging activity. However, reduced H_2O_2 scavenging activity of sodium carbonate and ammonium hydroxide-soaked grains flour extracts can be linked to the leaching of phytochemicals due to corrosive action and seed coat disintegration [53]. Ammonium ions form ammonium hydrate complexes and bind antioxidants, reducing the antioxidant potential of plant extracts. In conclusion, fermentation improves the hydrogen peroxide scavenging activity of finger millet grains flour compared to other methods. Flavonoid, a yellow-colored secondary metabolite of polyphenolic compounds found in flower petals, is a potential medicinal chemistry compound. Its antiviral, antioxidant, anti-cancer, and anti-inflammatory properties make it a valuable resource. The extraction efficiency of flavonoids in ethanol is higher than in water due to their neutral nature, making them more soluble in the organic phase [54].

GCMS screening of the control sample (T_0) delineated that dl-alpha.tocopherol, vitamin E, campesterol, stigmasterol, ginsenoside, gamma.-sitosterol, beta.-sitosterol, thymol, TMS derivative, fucosterol, 3,5-progesterone acetate and 2-Myristinoyl-glycinamide were common constituents. These phytochemicals are linked with number of health benefits making finger millet a promising health promoting cereal. Phyto-sterols are strong antioxidants and prevent cellular oxidative damage thus fostering cardiovascular health [15]. These sterols possess anticancer and anti-inflammatory activity along with supporting

immune functions. The other fractions like fatty acids present in Table 4.12 are linked with improved cardiovascular and gut health. While discussing the health promotion potential of each phytochemical is far beyond the approach as these are linked with hundreds of metabolic pathways. However, a brief insight suggest that tocopherols inhibit the production of free radicles and prevent the proliferation of cancer cells, reduce aging process and promote cardiovascular health [27]. Campesterol reduce cholesterol absorption in luminal cells of large intestine and enhance blood flow so used in skin care products while stigmasterol reduce the occurrences of parasitemia and improve T. congolense induced anemia.

The reduction in in-vitro starch digestibility (IVSD) of the sonication-assisted hydrated samples suggests that hydrothermal treatments result in mild structural rearrangements, such as retrogradation and the formation of amylose lipid complexes. However, a slight decline in IVSD of the fermented samples may be due to molecular rearrangements during drying of the fermented flour. While non-thermal treatments, i.e., sodium carbonate and ammonium hydroxide soaking of the finger millet grain are responsible for the structural modification of the starch, thus promoting enzymatic hydrolysis. The study conducted by Demirkesen-Bicak, Arici [55] reported that sourdough fermentation increased resistant starch, reduced IVSD of the prepared bread and linked it with retrogradation of the amylose contents. There is no comparative data on IVSD of the sonicated and sonication-assisted hydration of the finger millet but some studies reported that IVSD was improved by sonication and sonication-assisted hydration of the cereal grains [16, 56]. Similarly, heat-moisture treatment and extrusion processing of the finger millet starches have shown enhanced digestible starch fractions and reduced RS due to destruction of double helices and crystalline structure [57, 58]. Vidhyalakshmi and Meera [59] reported that ultrasonication of pearl millet modified the starch and increased the resistant starch content. The increase in resistant starch content is responsible for the comparatively low IVSD of the finger millet. From a nutritional perspective, fermentation and sonication-assisted hydration were the most effective treatments to control glycemic responses and serve as potential prebiotics.

Before in-vitro protein digestion (IVPD), the crude protein contents of the fermented samples were comparatively higher than those of raw, sonicated, sonication-assisted hydrated, sodium carbonate and ammonium hydroxide soaked grain flour. This suggests that fermentation enhanced nitrogen availability, possibly due to microbial hydrolysis of the tannin and phytates (protein-polyphenol complexes) and the release of bound amino acids [9]. This supports the higher IVPD of the fermented samples as free amino acids are readily available for in-vitro digestion. Such an increase in digestibility after fermentation was reported by Azeez, Chinma [8] and Narayanasamy [60], who found that microbial fermentation enhances proteolytic enzyme activity, reduces anti-nutrients (tannins and phytates), and improves the accessibility of peptide bonds to digestive enzymes. Conversely, the lowest IVPD noted for the raw samples reflects that unprocessed finger millet contains protein-polyphenol and protein-carbohydrate complexes that hinder enzymatic breakdown. Sonication and hydrothermal treatments also showed a moderate improvement in IVPD, suggesting partial denaturation of the protein structures improves digestibility. Similar outcomes were reviewed by Bangar, Suri [61], who reported that physical treatments like sonication and mild hydrothermal processing enhance protein solubility and digestibility through structural modification and reduced cross-linking. Soaking in sodium carbonate and ammonium hydroxide solution also improved IVPD, which was due to the activation of proteolytic enzymes during soaking of the finger millet grains. Overall, fermentation proved to be the most effective treatment for enhancing the IVPD of finger millet, while hydrothermal treatments had a limited impact. These findings emphasize the potential of controlled fermentation and physical modification as promising strategies to improve the nutritional quality and bioavailability of millet proteins for functional food applications.

Conclusion

Under changing climate, dietary diversity and nutritional security are the needs of recent era to ensure good health of the populations. This is only possible by adopting climate-resilient crops and optimizing processing techniques to ensure the availability of food and maximum delivery of nutrients. All the processing techniques i.e., fermentation, sonication, sonication-assisted hydration, sodium carbonate, and ammonium hydroxide soaking have their own merits and demerits. Sonication, sodium carbonate, and ammonium hydroxide solution soaking reduced maximum antinutrients and improved color but resulted in poor nutritional and phytochemical profile. However, improvement in color and lowering of antinutrients was comparatively less prominent for the fermented and sonication-assisted hydrated grains flour but preserved nutritional and phytochemical properties. Conclusively, fermentation and sonication-assisted hydration of finger millet grains flour can be further investigated and used at an industrial scale to promote dietary diversity and ultimately health of the community. This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

Declarations

Funding:

Not applicable

Author Contribution

M.A. conceived and designed the study, performed the investigation, conducted the formal analysis, and wrote the original manuscript draft. H.A.A.-H. contributed to the methodology, validation, and critical revision of the manuscript. S.A. supervised the study, contributed to the study design, and critically reviewed and edited the manuscript. M.S.J. performed data curation, formal analysis, and visualization. F.F. conducted laboratory experiments, investigation, and data collection. K.S. contributed to the investigation, validation, and manuscript review. M.J. performed statistical analysis, software application, and data visualization. W.S. assisted with the investigation, data curation, and validation. W.A. contributed resources and critically reviewed and edited the manuscript. M.Z.K. interpreted the data, contributed to formal analysis, substantially revised and edited the manuscript, and improved the

scientific language. T.A. supervised the project, secured resources, contributed to the interpretation of the results, and critically reviewed and edited the manuscript. All authors read and approved the final manuscript and agree to be accountable for all aspects of the work.

Acknowledgments:

The authors would like to thank the Faculty of Food Science and Nutrition, Bahauddin Zakariya University (BZU), Multan, Pakistan, for providing research facilities and academic support. The authors gratefully acknowledge the financial support provided by the Princess Nourah bint Abdulrahman University through the Researchers Supporting Project (PNURSP2026R252). The authors also extend their sincere appreciation to the King Saud University for funding this research under the Ongoing Research Funding Program (ORF-2026-641).

Conflicts of Interest: The authors declare no conflicts of interest.

Data Availability

The major finding has been provided in this article and the data presented in this study are available on request from the corresponding author.

References

1. Mbinda, W. & Mukami, A. A review of recent advances and future directions in the management of salinity stress in finger millet. *Front. Plant Sci.* **12**, 734798 (2021).
2. Kuruburu, M. G. et al. Variations in the anticancer activity of free and bound phenolics of finger millet (*Eleusine coracana* (L.) Gaertn; variety KMR-301) seeds. *Phytomedicine plus.* **2** (2), 100276 (2022).
3. Nakarani, U. M. et al. Nutritional and phytochemical profiling of nutraceutical finger millet (*Eleusine coracana* L.) genotypes. *Food Chem.* **341**, 128271 (2021).
4. Sood, S. et al. Phenomics and genomics of finger millet: current status and future prospects. *Planta* **250** (3), 731–751 (2019).
5. Abioye, V. et al. Varietal and processing influence on nutritional and phytochemical properties of finger millet: A review. *Heliyon*, **8**(12). (2022).
6. Jayawardana, S. A. S. et al. Dietary fibers, starch fractions and nutritional composition of finger millet varieties cultivated in Sri Lanka. *J. Food Compos. Anal.* **82**, 103249 (2019).
7. David, B. M. et al. Proximate composition, mineral and phytochemical constituents of *Eleusine coracana* (finger millet). *Int. J. Adv. Chem.* **2** (2), 171–174 (2014).
8. Azeez, S. O. et al. Impact of germination alone or in combination with solid-state fermentation on the physicochemical, antioxidant, in vitro digestibility, functional and thermal properties of brown finger millet flours. *Lwt* **154**, 112734 (2022).
9. Jan, S. et al. Effect of diverse fermentation treatments on nutritional composition, bioactive components, and anti-nutritional factors of finger millet (*Eleusine coracana* L.). *J. Appl. Biology Biotechnol.* **10** (1), 46–52 (2022).
10. Maharajan, T., Ceasar, S. A., Ajeesh, T. P. & Krishna Finger millet (*Eleusine coracana* (L.) Gaertn): Nutritional importance and nutrient transporters. *CRC. Crit. Rev. Plant Sci.* **41** (1), 1–31 (2022).
11. Zaheer, A. et al. Malnutrition in Children of Growing Age and the Associated Health Concerns. one health perspective In: (eds Abbas, R. Z., Saeed, N. M., Younus, M., Aguilar-Marcelino, L. & Khan, A.) One Health Triad. Unique Scientific, Faisalabad, Pakistan. (2023).
12. Syeunda, C. O. et al. In vitro protein digestibility of finger millet complementary porridge as affected by compositing precooked cowpea with improved malted finger millet. *J. Food Sci. Technol.* **58** (2), 571–580 (2021).
13. Tripathi, M. K. et al. *Economic, nutritional, and health importance of finger millet*, in *The Finger Millet Genome* p. 13–33 (Springer, 2022).
14. Puranik, S. et al. Harnessing finger millet to combat calcium deficiency in humans: challenges and prospects. *Front. Plant Sci.* **8**, 1311 (2017).
15. Wang, H. et al. Effect of different processing methods on the millet polyphenols and their anti-diabetic potential. *Front. Nutr.* **9**, 780499 (2022).
16. Yadav, S., Mishra, S. & Pradhan, R. C. Ultrasound-assisted hydration of finger millet (*Eleusine Coracana*) and its effects on starch isolates and antinutrients. *Ultrason. Sonochem.* **73**, 105542 (2021).
17. Lanza, B. et al. Single and multiple inoculum of *Lactiplantibacillus plantarum* strains in table olive lab-scale fermentations. *Fermentation* **6** (4), 126 (2020).
18. Kitum, V. C. et al. Effect of *Lb. plantarum* BFE 5092 fermentation on antinutrient and oligosaccharide composition of whole red haricot bean (*Phaseolus vulgaris* L.). *Int. J. Food Sci.* **2020** (1), 8876394 (2020).
19. Hassan, S. et al. Phytochemical characterization of ultrasound-processed sorghum sprouts for the use in functional foods. *Int. J. Food Prop.* **23** (1), 853–863 (2020).
20. Novitasari, E. & Novita, D. Physical quality and tannin content of sorghum (*Sorghum bicolor* L.) at different temperature and soaking immersion. in IOP Conference Series: Earth and Environmental Science. IOP Publishing. (2022).
21. AOAC. Official Method of Analysis of Analytical Chemists, 14th edition. (2015).
22. Onyango, C. et al. Physico-chemical properties of flour, dough and bread from wheat and hydrothermally-treated finger millet. *J. Cereal Sci.* **93**, 102954 (2020).

23. Younis, M. et al. Lactic acid fermentation ameliorates intrinsic toxicants in *Brassica campestris* L. leaves harvested at different growth stages. *Foods* **13** (12), 1826 (2024).
24. George, N. et al. Nutritional composition and anti-nutrient levels in raw and processed varieties of finger millet promoted for nutritional security. *Food Nutr. Sci.* **14** (12), 1183–1205 (2023).
25. Zeb, A. et al. Modification of rapid method for phytic acid determination in wheat and its production. *Biol. Sciences-PJSIR.* **47** (4), 322–324 (2004).
26. Leong, L. & Shui, G. An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chem.* **76** (1), 69–75 (2002).
27. Lee, J. H. et al. Comparative analyses of total phenols, flavonoids, saponins and antioxidant activity in yellow soy beans and mung beans. *Int. J. food Sci. Technol.* **46** (12), 2513–2519 (2011).
28. Wong, S. P., Leong, L. P. & Koh, J. H. W. Antioxidant activities of aqueous extracts of selected plants. *Food Chem.* **99** (4), 775–783 (2006).
29. Xu, B. J. & Chang, S. K. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *J. Food Sci.* **72** (2), S159–S166 (2007).
30. Ren, X. et al. In vitro starch digestibility, degree of gelatinization and estimated glycemic index of foxtail millet-derived products: Effect of freezing and frozen storage. *J. Cereal Sci.* **69**, 166–173 (2016).
31. Nsofor, W. N. et al. FTIR and Amino acid profile of methanol extract of *Tetrapleura tetraptera* fruit. *J. Drug Deliv Ther.* **13**, 61–69 (2023).
32. Navyashree, N. et al. White Finger Millet (KMR-340): A comparative study to determine the effect of processing and their characterisation. *Food Chem.* **374**, 131665 (2022).
33. Mutshinyani, M., Mashau, M. E. & Jideani, A. I. O. Bioactive compounds, antioxidant activity and consumer acceptability of porridges of finger millet (*Eleusine coracana*) flours: Effects of spontaneous fermentation. *Int. J. Food Prop.* **23** (1), 1692–1710 (2020).
34. Nouska, C. et al. Enhancement of textural and sensory characteristics of wheat bread using a chickpea sourdough fermented with a selected autochthonous microorganism. *Foods* **12** (16), 3112 (2023).
35. Niazifar, M. et al. Slow-release non-protein nitrogen sources in animal nutrition: A review. *Heliyon*, **10**(13). (2024).
36. Kambabazi, M. R. et al. Assessment of microbiological changes during production of malted and fermented finger millet flour. *Rwanda J. Agricultural Sci.* **1** (1), 31–35 (2019).
37. Samtiya, M. et al. Effect of selective fermentation on nutritional parameters and techno-functional characteristics of fermented millet-based probiotic dairy product. *Food Chemistry: X.* **22**, 101483 (2024).
38. Bello, F., Inyang, U. & Umoh, A. Effect of alkaline steeping on the nutritional, antinutritional and functional properties of malted millet (*Pennisetum glaucum*) flour. *Int. J. Innov. Food Nutr. Sustain. Agric.* **5**, 17–23 (2017).
39. Mudau, M., Mashau, M. E. & Ramashia, S. E. Nutritional quality, antioxidant, microstructural and sensory properties of spontaneously fermented gluten-free finger millet biscuits. *Foods* **11** (9), 1265 (2022).
40. Kiczorowski, P. et al. Effect of fermentation of chosen vegetables on the nutrient, mineral, and biocomponent profile in human and animal nutrition. *Sci. Rep.* **12** (1), 13422 (2022).
41. Curti, M. I. et al. Greening Ultrasound-Assisted Extraction for Sorghum Flour Multielemental Determination by Microwave-Induced Plasma Optical Emission Spectrometry. *J. Anal. Methods Chem.* **2021** (1), 9201094 (2021).
42. Yadav, S., Mishra, S. & Pradhan, R. C. Effect of fermentation with probiotic *Lactiplantibacillus plantarum* (MCC 2974) on quality characteristics and in vitro digestibility of finger millet Milk. *ACS Food Sci. Technol.* **4** (1), 152–160 (2023).
43. Gomes, S. A., Reghu, V. & Ramaswamy, P. Sprouting in Seeds Aided by Nitrogen Sourced from Ammonia Fumes Leached from Aluminum Dross. *Materials Today: Proceedings*, (2023).
44. Das, P., Nayak, P. K., Krishnan, R. & Kesavan Ultrasound assisted extraction of food colorants: Principle, mechanism, extraction technique and applications: A review on recent progress. *Food Chem. Adv.* **1**, 100144 (2022).
45. Martín-Gómez, J. et al. Phenolic compounds, antioxidant activity and color in the fermentation of mixed blueberry and grape juice with different yeasts. *Lwt* **146**, 111661 (2021).
46. Onipe, O. O. & Ramashia, S. E. Finger millet seed coat—a functional nutrient-rich cereal by-product. *Molecules* **27** (22), 7837 (2022).
47. Abubakar, A. R. & Haque, M. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *J. Pharm. Bioallied Sci.* **12** (1), 1–10 (2020).
48. Dubey, A. & Tripathy, P. P. Ultrasound-mediated hydration of finger millet: Effects on antinutrients, techno-functional and bioactive properties, with evaluation of ANN-PSO and RSM optimization methods. *Food Chem.* **435**, 137516 (2024).
49. Dey, S. et al. Optimizing the effect of ultrasonication and germination on antinutrients and antioxidants of kodo (*Paspalum scrobiculatum*) and little (*Panicum sumatrense*) millets. *J. Food Sci. Technol.* **60** (12), 2990–3001 (2023).
50. Nkhoma Maize (*Zea mays*) bran as a biorefinery feedstock for the co-extraction of nutraceuticals and functional food compounds, in Stellenbosch University. South Africa. (2024).
51. Abdelseed, B. H. H., Adiamo, H. A., Ahmed, O. Q. & Babiker, E. E. S.O. and Impact of decortication methods on total phenolic content, antioxidant activity and oxidative stability of two pearl millet cultivars during storage. *Annals Food Sci. Technol.*, **19**(4). (2018).
52. Ajiboye, A. A. D. & Adeyemo, O. A. Investigation on antioxidants, free radical scavenger and lipid peroxidation activities of whole grains finger millet (*Eleusine coracana* L). *Int. J. Plant. Biol.* **8** (1), 6684 (2017).

53. Kumar, G. P., Sudheer, D., Zhang, K. P. & Dhital, P. Leaching of Phytochemicals from Beans during Hydration, Kinetics, and Modeling. *Foods* **12** (2), 354 (2024).
54. Bitwell, C. et al. A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. *Sci. Afr.* **19**, e01585 (2023).
55. Demirkesen-Bicak, H. et al. Effect of different fermentation condition on estimated glycemic index, in vitro starch digestibility, and textural and sensory properties of sourdough bread. *Foods* **10** (3), 514 (2021).
56. Kumari, S., Kaur, B. P. & Thiruvalluvan, M. Ultrasound modified millet starch: Changes in functional, pasting, thermal, structural, in vitro digestibility properties, and potential food applications. *Food Hydrocoll.* **153**, 110008 (2024).
57. Umar, M., Hassan, M. & Rungraeng, N. In vitro digestibility and physicochemical analysis of heat-moisture treated finger millet flour and starch. *Sustainable Food Technol.* **3** (1), 215–226 (2025).
58. Adebowale, K., Afolabi, T. & Olu-Owolabi, B. Hydrothermal treatments of Finger millet (*Eleusine coracana*) starch. *Food Hydrocoll.* **19** (6), 974–983 (2005).
59. Vidhyalakshmi, R. & Meera, M. Correction: Dry heat and ultrasonication treatment of pearl millet flour: effect on thermal, structural, and in-vitro digestibility properties of starch. *J. Food Meas. Charact.* **17** (4), 4249–4249 (2023).
60. Narayanasamy Efficacy of fermentation parameters on protein quality and microstructural properties of processed finger millet flour. *J. Food Sci. Technol.* **58** (8), 3223–3234 (2021).
61. Bangar, S. P. et al. Influence of processing techniques on the protein quality of major and minor millet crops: A review. *J. Food Process. Preserv.* **46** (12), e17042 (2022).

Figures

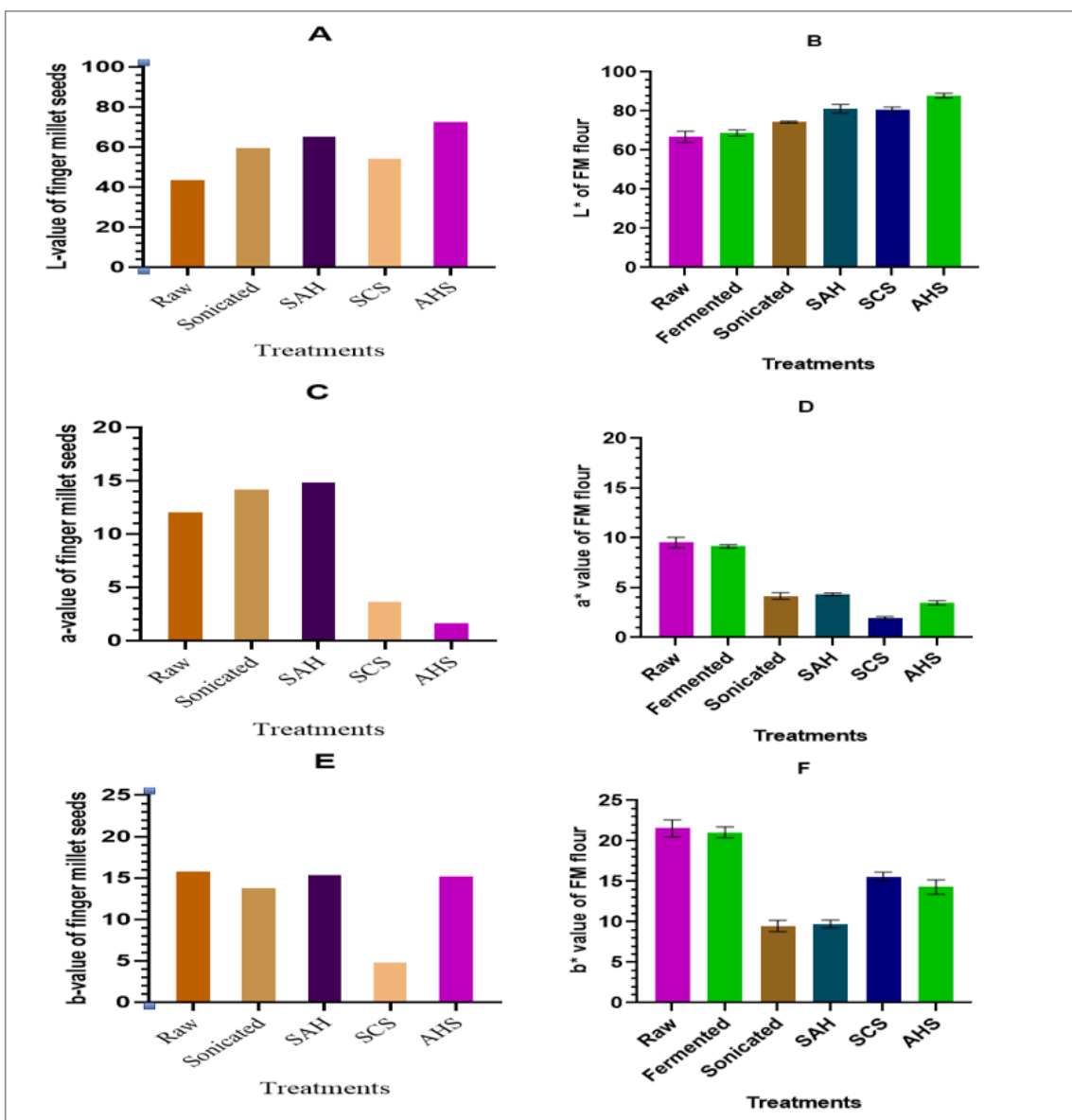


Figure 1

Instrumental color of raw and treated finger millet grains and flour (L*, a*and b* value)

Raw = control sample (T0)

Fermented = Fermented finger millet flour with Lactiplantibacillus plantarum (T1)

Sonicated = Sonication of finger millet grains for 15 minutes at 70 % amplitude and 20kHz frequency (T2)

SAH = Sonication assisted hydration of finger miller grains for 90 minutes with 10 seconds off cycle after 90 s at 50 % amplitude and 20kHz frequency (T3)

SCS = Soaking of finger millet grains in 8 % solution of sodium hydroxide for 10 hours (T4)

AHS= Soaking of finger millet grains in 5 % solution of ammonium hydroxide for 10 hours (T5)

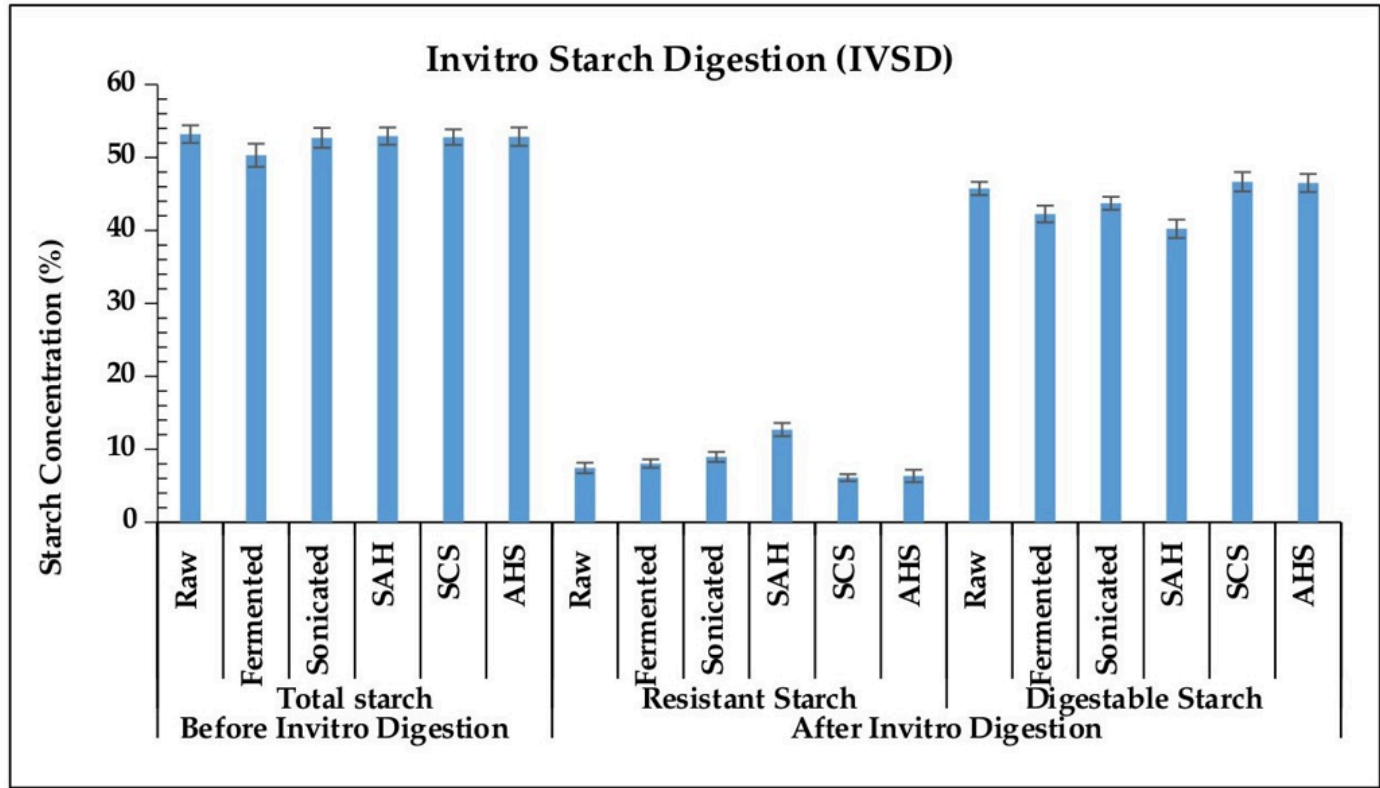


Figure 2

Effect of in vitro Digestion on starch of different treated flour samples

Raw = control sample (T0)

Fermented = Fermented finger millet flour with Lactiplantibacillus plantarum (T1)

Sonicated = Sonication of finger millet grains for 15 minutes at 70 % amplitude and 20kHz frequency(T2)

SAH = Sonication assisted hydration of finger miller grains for 90 minutes with 10 seconds off cycle after 90 s at 50 % amplitude and 20kHz frequency (T3)

SCS = Soaking of finger millet grains in 8 % solution of sodium hydroxide for 10 hours (T4)

AHS= Soaking of finger millet grains in 5 % solution of ammonium hydroxide for 10 hours (T5)

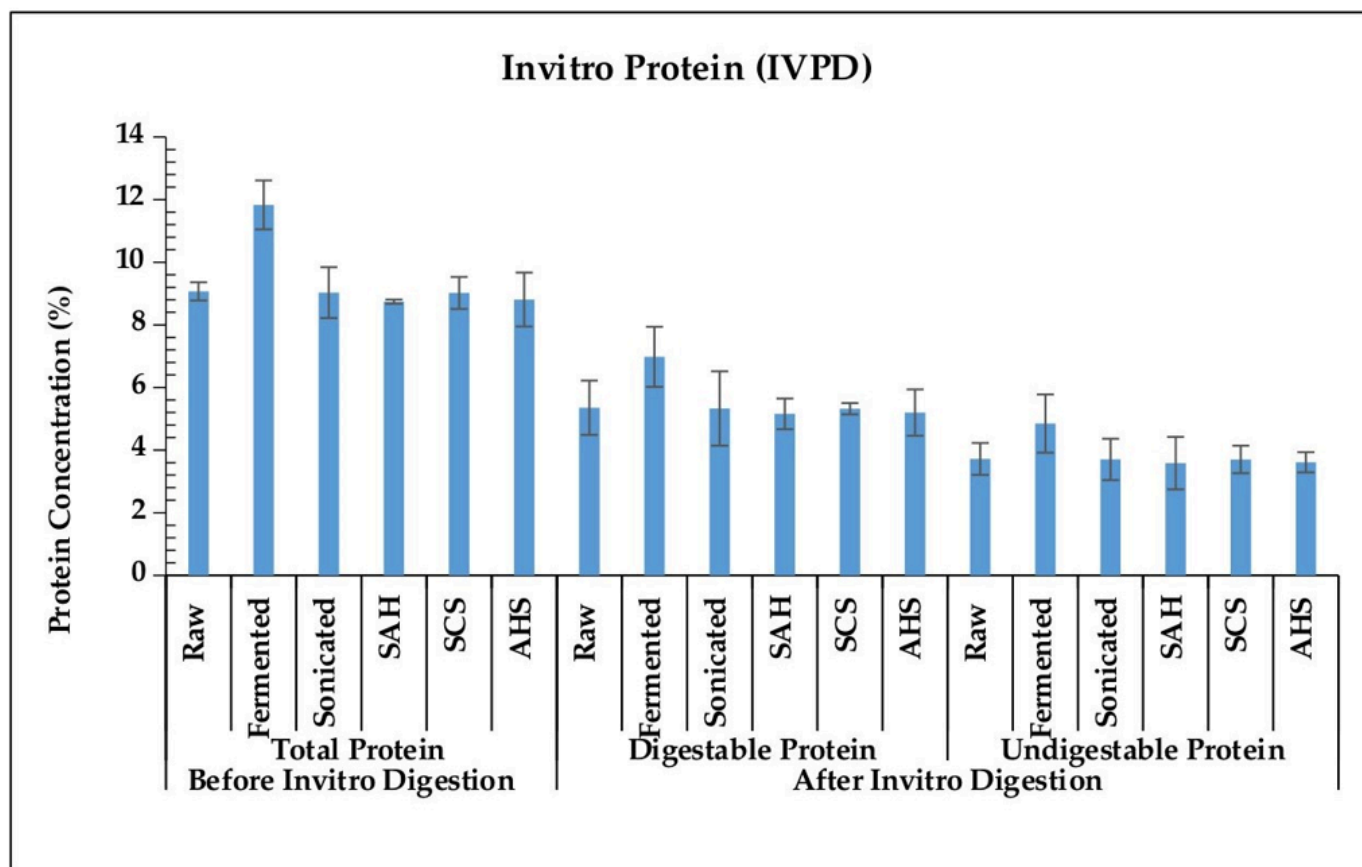


Figure 3

Effect of in vitro Digestion on Protein of different treated flour sample

Raw = control sample (T0)

Fermented = Fermented finger millet flour with *Lactiplantibacillus plantarum* (T1)

Sonicated = Sonication of finger millet grains for 15 minutes at 70 % amplitude and 20kHz frequency (T2)

SAH = Sonication assisted hydration of finger miller grains for 90 minutes with 10 seconds off cycle after 90 s at 50 % amplitude and 20kHz frequency (T3)

SCS = Soaking of finger millet grains in 8 % solution of sodium hydroxide for 10 hours (T4)

AHS= Soaking of finger millet grains in 5 % solution of ammonium hydroxide for 10 hours (T5)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Graphicalabstract.docx](#)