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Original Research

Unraveling the effect of nixtamalization on the nutritional, functional and crystallinity of sorghum and finger millet flours

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Research highlights

- Nixtamalization led to peak shifts towards higher theta values, indicating reduced crystallinity of flour particles.
- Differential scanning calorimetry (DSC) analysis indicated higher onset, peak, and end temperatures in nixtamalized flours than in native grains.
- Nixtamalization enhanced the functional, nutritional, and thermal properties of millet flours.

Abstract: The alkaline processing method of nixtamalization increases mineral absorbability and functional characteristics, and product development capabilities when applied to cereal grains. The research on maize has produced extensive findings about the plant, yet researchers have not studied how climate-resilient small millets such as sorghum and finger millet, which contain nutritional benefits and functional advantages, respond to this treatment. This study investigates how nixtamalization with 2% food-grade $Ca(OH)_2$ affects the different characteristics of sorghum and finger millet flours. The process involved treating whole grains by cooking and steeping in 2% $Ca(OH)_2$, followed by washing, drying, and milling. Native and nixtamalized flours were assessed for pH, ash and mineral content, and their functional properties, total phenolic content, and antioxidant activity using the DPPH method, and their thermal behavior. Nixtamalization caused significant changes in the properties of the flour compared to its original state. The treatment raised the pH value to 12.00 for sorghum and 11.32 for finger millet from their natural pH range of 6.5 to 7.0, while excess alkali was removed through the steps of washing and drying, ensuring safe residual levels and compliance with established food-processing practices. The ash content increased from 1.08% to 1.93% in sorghum and from 1.76% to 3.61% in finger millet, showing a significant mineral enhancement. Finger millet exhibited the highest enrichment levels, with 301.43 mg of calcium and 191.9 mg of magnesium per 100 grams of product, while sodium content increased slightly from 4.44 mg to 4.71 mg per 100 grams. The functional properties of the product improved, particularly with increased oil absorption capacity, while the water solubility index showed a slight reduction of 2.02%. The total phenolic content in finger millet increased by 16%, while sorghum showed an 11.4% increase, and antioxidant activity remained unchanged. Thermal analysis revealed higher end temperatures, suggesting enhanced thermal stability. The nixtamalization process enhanced mineral availability, fat-holding capacity, and processing functionality of both millet flours. The research demonstrates that nixtamalized millet flours serve as functional ingredients, which provide nutritional value to weaning mixes, elderly nutrition products, gluten-free products, and traditional fortified cereal-based foods.

Keywords: Nixtamalization, Calcium hydroxide, Sorghum, Finger millet, Mineral bioavailability

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1. Introduction

India dominates global millet production, contributing approximately 40.6% of total output, with an estimated production of 10.91 million tonnes during 2018–2019. India ranks first in nutri-rich millet production and second in rice and pulses worldwide [1]. However, the country also bears a high burden of malnutrition, ranking second globally in child malnutrition, with more than one-third of the world's malnourished children residing in India. This disparity highlights the urgent need to improve the utilization and nutritional quality of locally available staple crops such as millets.

Millets are a diverse group of small-seeded cereals belonging to the Poaceae family, cultivated predominantly in arid and semi-arid regions owing to their drought tolerance, low input requirements, and adaptability to marginal soils [2]. Major species include finger millet (Eleusine coracana), pearl millet (Pennisetum glaucum), foxtail millet (Setaria italica), and sorghum (Sorghum bicolor). Finger millet is particularly valued for its exceptionally high calcium content (up to 344 mg/100 g), dietary fibre, essential amino acids, and micronutrients such as iron and zinc [3] , while sorghum provides substantial protein (up to 18%) and dietary fibre but exhibits low lysine content and reduced protein digestibility in conventional preparations [4]. Despite their nutritional richness, the presence of antinutrients and limited nutrient bioavailability restricts their full dietary potential, necessitating appropriate processing interventions.

Nixtamalization using calcium hydroxide [$Ca(OH)_2$] is a traditional cereal-processing technique widely applied to maize in Central America and Mexico [5]. Wood ash or plant ashes were not utilized as native alkali sources in this study because of issues that would affect standard testing procedures, their ability to produce safe results, and compliance with regulatory requirements. The chemical composition and alkalinity of ash-based materials vary according to the plant species, combustion temperature, and environmental conditions. The different processing conditions cause changes in starch gelatinization, protein modification, calcium uptake, and overall product quality, which makes it difficult to repeat experiments and compare results. Ash-based alkalis contain unwanted elements, which include heavy metals, polycyclic aromatic residues, and insoluble particulates, and these elements pose food safety risks that are hard to measure. These materials do not belong to the category of standardized food-grade additives according to regulatory requirements, and they are not recognised as Codex/JECFA controlled food processing materials.

Cooking and steeping grains in $Ca(OH)_2$ solution induces several physicochemical modifications, including pericarp removal, partial starch gelatinization, protein denaturation, enhanced hydration, and diffusion of calcium into the endosperm matrix [6]. These changes improve milling characteristics, increase calcium content and niacin bioavailability, enhance protein digestibility, and reduce mycotoxins and antinutritional factors [5,7]. Consequently, nixtamalization improves both the nutritional quality and functional properties of cereal flours, such as water/oil absorption, dough handling, and thermal stability. Such improvements make nixtamalized flours suitable for diverse food applications, including fortified complementary foods, weaning mixes, nutrition beverages for the elderly, gluten-free bakery products, extruded snacks, and other value-added functional formulations [8,9]. The use of calcium hydroxide [$Ca(OH)_2$] as the nixtamalization agent and its health effects, based on its chemical properties, received your valuable input, which we appreciate. The selection process requires our explanation and justification according to the current understanding of the selected item. The worldwide food industry employs calcium hydroxide (food-grade slaked lime; INS 526) as the standard alkali for nixtamalization because it improves processing operations, while enhancing nutritional values and ensuring food safety. The alkaline treatment process enables removal of the pericarp, and the conversion of starch into its gelatinous form, and the transformation of protein structures, which results in better dough production and improved digestibility [10]. The compound $Ca(OH)_2$ enhances the product's calcium content, releases bound niacin to enable better nutrient absorption, and has functioned as a preventive measure against pellagra in maize-eating communities [11]. Existing studies have confirmed that the lime nixtamalization process results in decreased levels of mycotoxins, which includes fumonisins, thus making food products safer to consume. The study used only food-grade $Ca(OH)_2$, which met Codex and JECFA standards, according to [12]. The study maintained traditional concentration levels, while the cooking process enabled complete removal of extra alkali through thorough kernel washing, which resulted in reduced free lime content in the final product. The controlled environment allows $Ca(OH)_2$ to function as a processing aid without any risk of chemical contamination. The current method operates according to recognized cereal processing standards, which have established their safety through extensive documentation. Concentrations around 1–2% are frequently considered optimal, as they provide efficient processing and improved nutritional quality without excessive solids loss or residual alkalinity. In preliminary trials conducted prior to the main study, lower lime levels (<1%) resulted in incomplete pericarp removal and poor dough cohesiveness, whereas higher concentrations (>2–2.5%) did not yield additional technological benefits and occasionally produced overly soft kernels and increased nejayote losses [13,14]. Therefore, 2% $Ca(OH)_2$ was selected as a mid-range, reproducible, and technologically appropriate concentration, consistent with traditional and industrial nixtamalization conditions reported in the literature.

Although extensively studied in maize, the application of $Ca(OH)_2$-based nixtamalization to millets remains limited. Therefore, the objective of this study was to evaluate the effect of 2% $Ca(OH)_2$ treatment on the physicochemical, mineral, functional, antioxidant, and thermal properties of sorghum and finger millet flours compared with their native counterparts, with the aim of enhancing their nutritional quality and suitability for value-added food development.

2. Materials and methods

Sorghum (Sorghum bicolor L.) and finger millet (Eleusine coracana L.) grains (local commercial varieties) were procured from the Mysore market, Karnataka, India (12.3°N, 76.6°E), a semi-arid tropical region characterized by average annual temperatures of 24–32 °C and rainfall of approximately 700–800 mm.

All reagents were of analytical grade. Calcium hydroxide [$Ca(OH)_2$, food grade, ≥95% purity], Folin–Ciocalteu reagent, gallic acid, sodium carbonate, methanol, and DPPH were purchased from Sigma-Aldrich (St. Louis, USA). Hydrochloric acid and other acids were obtained from Merck (Mumbai, India).

Major equipment used included a rice huller, aspirator, and abrasive polisher (Indosaw Pvt. Ltd., India), pH meter (Eutech Instruments, Singapore), water activity analyzer (Novasina LabSwift, Switzerland), particle size analyzer (Microtrac S3500, USA), color spectrophotometer (CM-5, Konica Minolta, Japan), SEM (JEOL, Japan), DSC (TA Instruments, USA), and X-ray diffractometer (Bruker D2 Phaser, Germany).

Grains and flours were stored in airtight polyethylene pouches at 4 °C to minimize enzymatic activity, lipid oxidation, insect infestation, and microbial growth, thereby preserving the functional and nutritional quality of cereal flours.

2.1 Sample preparation

2.1.1 Native flours

Grains were cleaned using a mechanical cleaner and destoner to remove foreign materials. Sorghum was tempered with 5% water and rested for 10 minutes to reach approximately 11% moisture, while finger millet was tempered with 3% water for 20 minutes to achieve 13–14% moisture. Dehusking/decortication was performed using a rice huller operating at 1425 rpm. The grains were pulverized, passed through a 60-mesh sieve, and stored at 4 °C until analysis.

2.1.2 Dry milled nixtamal flours

Dry-milled nixtamal sorghum millet flour (DMNSMF) and dry-milled nixtamal finger millet flour (DMNFMF) were prepared using the method of Gopika and Joshi [15]. Nixtamalization was performed using 2% (w/w, grain basis) food-grade $Ca(OH)_2$. Grains were cooked in a lime solution (1:1 grain-to-solution ratio) at 75-80 °C for 30 minutes, steeped for 4 hours, washed thoroughly to remove excess alkali and pericarp (nejayote removal), dried at 50 °C for 6 hours, milled, and sieved (60-mesh). The resulting dry-milled nixtamal flours were stored at 4 °C. This concentration was selected based on literature indicating optimal pericarp removal, calcium uptake, and functional modification without excessive kernel damage.

2.2 Physical properties of flours

2.2.1 Density

The bulk density (ρb) of the flour was determined using a modified method based on Fraser et al., [16], which relies on the mass volume relationship. 2 grams of flour were poured into a 10 ml measuring cylinder and the initial volume was recorded as the bulk density. The cylinder was then tapped 50 times and the final volume was recorded as the tapped density. Two grams of flour were gently poured into a 10 mL graduated cylinder and the unsettled volume (V_0) recorded. The cylinder was tapped 50 times until a constant volume (Vt) was obtained. Fifty taps were sufficient to achieve stable packing for fine cereal flours while preventing the over-compaction reported with excessive tapping.

$$\text{Bulk density}(\rho b\quad) = m\quad /V0 \qquad \text{Eq 1}$$

$$\text{Tapped density}(\rho_t) = \frac{m}{V_t} \qquad \text{Eq 2}$$

$$\text{Hausner ratio} = \frac{\rho t}{\rho b} \qquad \text{Eq 3}$$

$$\text{Carr Index}(\%) = \frac{\rho t - \rho b}{\rho t} * 100 \qquad \text{Eq 4}$$

$$\text{Porosity }(\%) = (1 - \frac{\rho b}{\rho t})*100 \qquad \text{Eq 5}$$

2.2.2 pH

Flour (1 g) was dispersed in 10 mL distilled water (1:10 w/v), stirred for 10 minutes, and equilibrated for 30 minutes at room temperature. The pH was measured using a calibrated digital pH meter (buffers 4.0 and 7.0). Measurements were performed in triplicate [17].

2.2.3 Water activity (aw)

Water activity was measured using a Novasina water activity analyzer, which operates based on electrolysis. The instrument has a measurement range of 0.030 to 1.000 aw, a resolution of 0.001 aw, and a precision of ±0.003 aw within the calibration range.

2.3 Proximate analysis

The proximate composition analysis of nixtamalized and native sorghum millet and finger millet was conducted in accordance with the AOAC [17] guidelines. Mineral composition was analyzed via Plasma Atomic Absorption Spectroscopy following acid digestion of the ash with concentrated HCl.

2.4 Hydration and lipid interaction properties

The Water Absorption Capacity (WAC) and Water Solubility Index (WSI) were analyzed using the method described by Anderson et al., [18]. Oil Absorption Capacity was measured following the procedure outlined by Sosluski et al., [19] with oil absorption expressed as the percentage of oil retained per g of flour. The swelling power and solubility index were measured at temperatures of 30°C, 50°C, 70°C, and 90°C following the procedure described by Singh et al. [20].

2.5 Particle size distribution

The Microtrac S3500 determined particle size using three precisely placed red laser diodes to accurately characterize particles. Water activity was measured using a Novasina analyzer based on an electrolytic principle, with a range of 0.030–1.000 aw.

2.6 Colour analysis of the flours

The colour parameters (L*, a*, b*) of flour samples were measured using a bench-top spectrophotometer (CM-5, Konica Minolta, Japan) with a pulsed xenon lamp as the light source (wavelength range: 360–740 nm). The instrument was calibrated automatically using the internal standard before measurements.

2.7 Scanning electron microscopy (SEM)

The morphological properties of finger and sorghum millet flours were examined using a scanning electron microscope (SEM) at ×5000 magnification. The flour samples were mounted on an aluminum stub with double-sided cellophane tape and coated with a gold-palladium (60:40, g/g) layer using an auto fine coater before imaging.

2.8 Determination of total phenolic content and antiradical potential

2.8.1 Extraction of phenolic compounds

Phenolic compounds were extracted following the method of Singleton et al. [21] with slight modifications. Flour samples (1 g) were mixed with 10 mL of 75% ethanol (v/v) and extracted under continuous shaking (150 rpm) for 3 hours at room temperature. The mixture was centrifuged at 5000 × g for 10 minutes, and the supernatant was collected. The residue was re-extracted under the same conditions, and both extracts were combined and stored at 4 °C in amber bottles until analysis.

2.8.2 Total phenolic content (TPC)

The extract was diluted 2-fold with distilled water, and 0.5 mL of Folin-Ciocalteu reagent was added to the diluted extract, followed by 1.5 mL of 20% Na_2CO_3 solution. The mixture was diluted to a final volume of 10 mL with distilled water. Gallic acid was used as the standard, and the TPC was expressed as mg gallic acid equivalents per gram (mg GAE/g) [21]. The total phenolic content was determined using the provided formula.

$$\text{Total phenol content (mg GAE/g)} = C*V/M$$

2.8.3 Total antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay: The antioxidant activity was measured using 40 µL of the extract was mixed with 2.9 mL of DPPH solution in methanol. The reduction in DPPH radical was monitored by measuring absorbance at 515 nm [22].

$$\% \text{ inhibition} = (A_0 - A_s)/A_o \times 100$$

where
A_0 = control absorbance
A_s = sample absorbance

IC_{50} values (mg mL^{-1}) were determined from plots of percentage inhibition versus extract concentration using linear regression. Lower IC_{50} values indicate higher anti-radical potential.

2.9 Differential scanning calorimetry (DSC)

A small amount of sample was placed in an aluminum pan, with a matching pan containing an inert or baseline material as a reference. After calibrating the DSC with standard materials, both pans were positioned in the instrument. The pans were heated or cooled at a constant rate while measuring heat flow versus temperature.

2.10 X-ray diffraction studies

A Bruker D2 Phaser System X-ray Diffractometer (XRD) was employed following the method of Gopika and Joshi [15] to analyze the crystalline and amorphous characteristics of millet samples. The interplanar distance was calculated following the method described by Figueroa et al. [23].

2.11 Statistical analysis and data presentation

All analyses were performed in triplicate, and results were expressed as mean ± standard deviation. Data were analyzed using one-way ANOVA followed by Tukey's test

($p < 0.05$) using SPSS software version 3.2. Physicochemical and functional parameters were presented in both tabular and graphical formats to facilitate comparison and visualization of treatment effects. The principle component analysis was conducted using R software (version 4.4.2) with the FactoMineR and factoextra packages.

3. Results and discussion

3.1 Physical properties of nixtamalized flours

The physical properties of native and $Ca(OH)_2$-nixtamalized sorghum and finger millet flours are presented in Table 1. Nixtamalization significantly influenced pH, particle packing behavior, and powder flow characteristics, while bulk density and water activity remained largely unaffected. A pronounced increase in pH was observed in nixtamalized flours, with values rising from near-neutral conditions in native sorghum (7.21) and finger millet (6.67) to highly alkaline conditions in DMNSMF (12.00) and DMNFMF (11.32). This increase is attributed to diffusion and retention of calcium hydroxide within the grain matrix, which dissociates into Ca^{2+} and OH^- ions, creating an alkaline environment. Similar pH elevations have been widely reported for lime-treated maize and sorghum and are associated with structural softening of the pericarp, protein unfolding, and starch matrix loosening during nixtamalization [14,24]

Table 1. Physico chemical and proximate composition of native and nixtamal PM flour

Parameters assessed	SMF	DMNSMF	FMF	DMNFMF
Bulk Density(g/mL)	0.601^{a} ±0.13	0.592^{a} ±0.04	0.680^{a} ± 0.14	0.749^{a} ±0.16
Tapped Density(g/mL)	0.646^{a} ±0.11	0.639^{a} ±0.11	0.857^{a} ±0.15	0.794^{a} ±0.10
pH	7.21^{a} ±0.09	12.00^{b} ±0.08	6.67^{a} ±0.11	11.32^{b} ±0.09
Water Activity	0.351^{a} ±0.07	0.345^{a} ±0.07	0.353^{a} ±0.07	0.383^{a} ±0.08
Proximate composition (db.)				
Moisture (%)	8.35^{a} ±0.07	7.30^{b} ±0.84	9.80^{a} ±0.14	7.10^{b} ±0.28
Protein (%)	10.56^{a} ±0.13	10.83^{a} ±0.06	8.46^{a} ±0.13	8.73^{a} ±0.05
Ash (%)	1.08^{a} ±0.12	1.93^{b} ±0.07	1.76^{a} ±0.09	3.61^{b} ±0.15
Fat (%)	3.65^{a} ±0.12	4.69^{b} ±0.11	3.78^{a} ±0.05	4.03^{a} ±0.11
Mineral estimation (db.)				
Calcium (mg/100g)	32.25^{a} ±0.15	133.30^{b} ±0.12	134.93^{a} ±0.11	301.43^{b} ±0.17
Potassium (mg/100g)	509.70^{a} ±0.29	489.82^{a} ±0.24	411.25^{a} ±0.09	380.88^{a} ±0.21
Magnesium (mg/100g)	253.45^{a} ±0.34	314.15^{b} ±0.28	178.7^{a} ±0.15	191.9^{a} ±0.24
Sodium(mg/100g)	3.89^{a} ±0.13	4.44^{b} ±0.16	4.21^{a} ±0.14	4.71^{b} ±0.11

SMF –Sorghum millet flour, DMNSMF –Dry milled nixtamal Sorghum millet flour, FMF –Finger millet flour, DMNFMF –Dry milled nixtamal Finger millet flour. Values are mean ± Standard deviation of three determinations (n = 3). Values with the different superscript within rows are significantly different at $p < 0.05$ compared to respective controls SMF and FMF

Bulk densities remained statistically similar across native and nixtamalized samples (approximately 0.59–0.68 g/mL), consistent with reports indicating that nixtamalization can maintain or slightly alter density depending on grain type and processing conditions [8]. Finger millet showed a comparatively higher tapped density in the native state (0.857 gmL^{-1}), which may be attributed to its finer particle size and higher dietary fibre content that enable closer particle packing [15,24]. After nixtamalization, tapped density decreased (0.794 gmL^{-1}), suggesting partial disruption of compact structures and reduced interparticle cohesion due to removal of surface mucilage and modification of fibre–starch interactions. Such structural loosening following alkaline cooking has also been reported for other cereal flours [25].

Flowability parameters provided further insight into powder handling characteristics. Hausner ratio and Carr's index values indicated excellent flow for SMF (HR 1.07; CI 6.97%), DMNSMF (HR 1.08; CI 7.35%), and DMNFMF (HR 1.06; CI 5.67%), whereas native finger millet flour exhibited comparatively poor flow (HR 1.26; CI 20.65%). According to standard classification, CI values below 10% indicate excellent flow, while values above 20% suggest cohesiveness and reduced flowability [26]. The poor flow of native finger millet may be attributed to its small particle size, higher fibre content, and increased surface friction, which promote interparticle adhesion and hinder free movement. Notably, nixtamalization markedly improved the flow behavior of finger millet flour, likely due to partial degradation of surface polysaccharides, reduction in fines, and increased particle smoothness, which collectively reduce cohesion and enhance powder mobility. Therefore, the enhanced flow characteristics of DMNFMF, in particular, suggest superior applicability in ready-to-mix formulations, fortified health blends, complementary foods, and nutrition powders for the elderly, where rapid dispersibility and ease of handling are essential.

Water activity measurements showed no significant difference, suggesting that moisture content and microbial stability were not adversely affected by nixtamalization. Water activity values remained low and statistically unchanged (0.34–0.38) across treatments, indicating that nixtamalization did not increase free moisture or compromise storage stability. Maintenance of low aw values (<0.6) ensures microbiological safety and prolonged shelf life of cereal flours, consistent with earlier observations in nixtamalized cereal products [27].

Overall, $Ca(OH)_2$-nixtamalization preserved bulk density and moisture stability while significantly enhancing the flowability of finger millet flour without adversely affecting sorghum. These modifications improve powder handling and expand the suitability of nixtamalized millet flours for industrial processing and value-added food applications.

3.2 Proximate composition of the flours

Nixtamalization with $Ca(OH)_2$ significantly influenced the proximate composition of both sorghum and finger millet flours, primarily through moisture removal, mineral diffusion, and matrix restructuring. Moisture content decreased in nixtamalized flours (DMNSMF 7.30%; DMNFMF 7.10%) compared with native samples (8.35–9.80%). This reduction is attributed to the combined effects of alkaline cooking followed by drying, which removes free and loosely bound water. Lower moisture (<10%) is desirable for cereal flours, as recommended by FAO/WHO and Codex guidelines for safe storage stability, since reduced water availability limits microbial growth and enzymatic deterioration [24].Similar moisture reductions after nixtamalization have been reported for maize and sorghum, where drying enhances shelf-life and milling efficiency.

Protein content remained largely unchanged in both cereals. The slight increase observed in nixtamalized sorghum (10.56 → 10.83%) is likely a concentration effect resulting from moisture loss rather than true protein synthesis. However, alkaline treatment can improve protein digestibility by disrupting disulfide bonds and weakening starch–protein matrices, thereby enhancing enzyme accessibility. Recent studies on millet processing have similarly shown improved in vitro digestibility without substantial quantitative protein losses [11]. Ash content increased markedly after treatment, especially in finger millet (1.76 → 3.61%), confirming significant mineral incorporation from $Ca(OH)_2$. Correspondingly, calcium levels increased nearly threefold in sorghum and more than twofold in finger millet, demonstrating effective fortification. This enrichment is a hallmark of nixtamalization and aligns with reports that lime treatment improves dietary calcium intake and mineral bioavailability [15,11] From a public health perspective, such mineral enhancement is highly relevant in India, where calcium deficiency and childhood malnutrition remain prevalent. Thus, nixtamalized finger millet flour (DMNFMF), with calcium levels exceeding 300 mg 100 g^{-1}, has strong potential as a nutrient-dense ingredient for complementary and elderly nutrition foods. Fat content increased slightly in nixtamalized sorghum (4.69% vs. 3.65%), possibly due to liberation of bound lipids and concentration effects. Alkaline heat disrupts lipid–protein complexes, enhancing extractable fat fractions. Comparable trends have been noted in processed cereal flours where structural breakdown increases lipid accessibility [29].

3.3 Hydration and lipid interaction properties

Nixtamalization significantly affects the functional properties of sorghum and finger millet flours, which are critical for their applications in food formulation. The oil absorption capacity (OAC) of nixtamalized sorghum flour (DMNSMF) increased markedly to 3.19 g/g compared to 2.21 g/g in the native flour (SMF), as shown in Table 2. This enhancement suggests improved fat-binding ability, which can positively impact the mouthfeel, flavour retention, and palatability of food products such as bakery items and meat analogues. The increase in OAC is likely due to protein unfolding and the exposure of hydrophobic sites during alkaline treatment, facilitating better lipid interactions [15,24,11]. However, the water solubility index (WSI) exhibited a slight decline, particularly in nixtamalized finger millet flour (DMNFMF 2.43% vs. FMF 2.48%), representing a 2.02% decrease. This aligns with observations by Khatoniar and Das, who reported reduced solubility due to starch-protein network formation and amylose-lipid complexation during alkaline treatment [28].This reduction is attributed to starch retrogradation and the formation of cross-links within starch and protein matrices, which reduce solubility [30]. The swelling power (SP) of millet flours generally increases with temperature due to enhanced starch granule hydration and gelatinization. In this study, nixtamalized finger millet flour (DMNFMF) exhibited the highest swelling power, reaching 12.73% at 90°C, which was significantly greater than that of native finger millet flour (FMF, 9.44%). Similar trends were observed in sorghum flours. This increase confirms the structural disruption caused by nixtamalization, which facilitates greater water uptake and starch granule expansion. Similarly, physical and functional properties analysis of finger millet by Khatoniar and Das [29] reported swelling powers ranging from 8% to 12%, with alkaline processing increasing swelling capacity by disrupting the grain matrix and facilitating hydration [28].

Table 2. Functional properties of native and nixtamal flours

Parameters assessed	SMF	DMNSMF	FMF	DMNFMF
Functional Properties				
Water absorption index	2.68 ^{a} ±0.21	2.89 ^{a} ±0.13	2.55 ^{a} ±0.07	2.96 ^{b} ±0.04
Oil absorption index	2.21 ^{a} ±0.09	2.21 ^{a} ±0.17	2.29 ^{a} ±0.01	2.15 ^{a} ±0.16
Water solubility index	5.50 ^{a} ±0.42	2.48 ^{b} ±0.13	3.37 ^{a} ±0.06	2.43 ^{b} ±0.07
Solubility (%)				
30°C	5.54 ^{a} ±0.09	2.78 ^{b} ±0.09	4.09 ^{a} ±0.09	2.79 ^{b} ±0.10
50°C	5.68 ^{a} ±0.08	4.63 ^{b} ±0.12	4.54 ^{a} ±0.11	3.72 ^{b} ±0.11
70°C	7.84 ^{a} ±0.11	5.09 ^{b} ±0.13	5.91 ^{a} ±0.06	4.19 ^{b} ±0.13
90°C	9.23 ^{a} ±0.07	8.34 ^{a} ±0.12	12.73 ^{a} ±0.06	5.59 ^{b} ±0.09
Swelling(%)				
30°C	2.94 ^{a} ±0.05	3.48 ^{b} ±0.13	3.15 ^{a} ± 0.09	3.57 ^{b} ±0.09
50°C	3.19 ^{a} ±0.09	4.42 ^{b} ±0.12	3.40 ^{a} ±0. 12	4.55 ^{b} ±0.11
70°C	4.69 ^{a} ±0.12	4.83 ^{a} ±0.05	6.90 ^{a} ±0. 13	5.91 ^{b} ±0.09
90°C	9.34 ^{a} ±0.13	9.98 ^{a} ±0.07	9.44 ^{a} ±0. 14	8.69 ^{a} ±0.08
Colorimetric analysis				
L*	84.24 ^{a} ±0.23	81.36 ^{a} ±0.23	73.45 ^{a} ±0.23	54.04 ^{b} ±0.09
a*	1.2 ±0.01	-1.045 ±0.00	4.53 ^{a} ±0.07	5.43 ^{b} ±0.07
b*	11.84 ^{a} ±0.09	19.1 ^{b} ±0.012	9.28 ^{a} ±0.13	17.35 ^{b} ±0.24
ΔE*	15.37 ^{a} ±0.10	22.70 ^{b} ±0.07	23.20 ^{a} ±0.15	43.97 ^{b} ±0.14
Particle size analysis				
MV (µm)	139.4 ^{a} ±0.23	127.9 ^{a} ±0.23	98.86 ^{b} ±0.13	151.9 ^{c} ±0.19
MN(µm)	19.85 ^{a} ±0.19	20.32 ^{c} ±0.12	17.06 ^{a} ±0.23	15.55 ^{b} ±0.1
MA(µm)	65.15 ^{b} ±0.12	65.06 ^{b} ±0.19	50.88 ^{a} ±0.17	72.26 ^{b} ±0.27

SMF –Sorghum millet flour, DMNSMF –Dry milled nixtamal Sorghum millet flour, FMF –Finger millet flour, DMNFMF –Dry milled nixtamal Finger millet flour. Values are mean ± Standard deviation of three determinations (n = 3). Values with the different superscript within rows are significantly different at $p < 0.05$ compared to respective controls SMF and FMF.

Figure 1. Visual appearance of native and nixtamalized finger millet and sorghum flours.
a. native finger millet flour (FMF); b. dry milled nixtamal finger millet flour (DMNFMF);
c. native sorghum millet flour (SMF); d. dry milled nixtamal sorghum millet flour (DMNSMF)

3.4 Colour analysis

Color parameters (L*, a*, b*, and total color difference ΔE*) indicate significant changes in millet flours after nixtamalization. Native sorghum flour (SMF) showed higher lightness (L* = 84.24) compared to nixtamalized sorghum flour (DMNSMF, L* = 81.36), reflecting slight darkening due to lime treatment. Nixtamalized sorghum also exhibited an increase in yellowness (b*) and a shift towards a greenish hue (negative a*). Finger millet flours exhibited more pronounced changes, where nixtamalization caused substantial darkening. Redness (a*) and yellowness (b*) values increased, indicating intensified reddish-yellow hues attributed to Maillard browning and pigment transformations during alkaline cooking [6]. These color shifts align with studies reporting that nixtamalization alters pigment composition through non-enzymatic browning and chemical changes, affecting flour appearance and potentially consumer acceptance [31]. Figure 1 illustrates the flour samples, with nixtamalized flours appearing lighter and more homogeneous.

3.5 Particle size analysis

For sorghum, native flour (SMF) showed an MV of 139.4 μm, MN of 19.85 μm, and MA of 65.15 μm. Nixtamalized sorghum flour (DMNSMF) displayed a slightly reduced MV (127.9 μm) and MA (65.06 μm), but an increased MN (20.32 μm), indicating a shift towards finer particles with a more uniform distribution. This reduction in particle size aligns with effects observed by Paliwal et al., where alkaline treatment and milling lead to starch granule disruption and particle size refinement, enhancing flour surface area and hydration rate [31]. However, nixtamalized finger millet flour (DMNFMF) demonstrated significant increase in particle size, suggesting agglomeration or particle aggregation due to starch gelatinization and protein cross-linking during alkaline cooking [32].

3.6 Scanning electron microscopy (SEM)

The SEM images provide a magnified view (3.00 KX, 15.00 kV) of nixtamalized pearl millet, sorghum, and finger millet flours, revealing detailed surface morphology (Figure 2). Native flours (SMF and FMF) show relatively smooth, and compact starch granules with uniform sizes, characteristic of minimally processed millets. The starch granules in finger millet appear smaller and more uniform than those in sorghum, consistent with their intrinsic botanical differences. In contrast, nixtamalized flours (DMNSMF and DMNFMF) exhibit significant structural disruption. The alkaline treatment during nixtamalization causes partial gelatinization of starch granules, breaking down the rigid surface and resulting in swelling, fusion, and agglomeration of starch-protein matrices. This process creates a more porous and heterogeneous structure with irregular granules and void spaces, facilitating improved

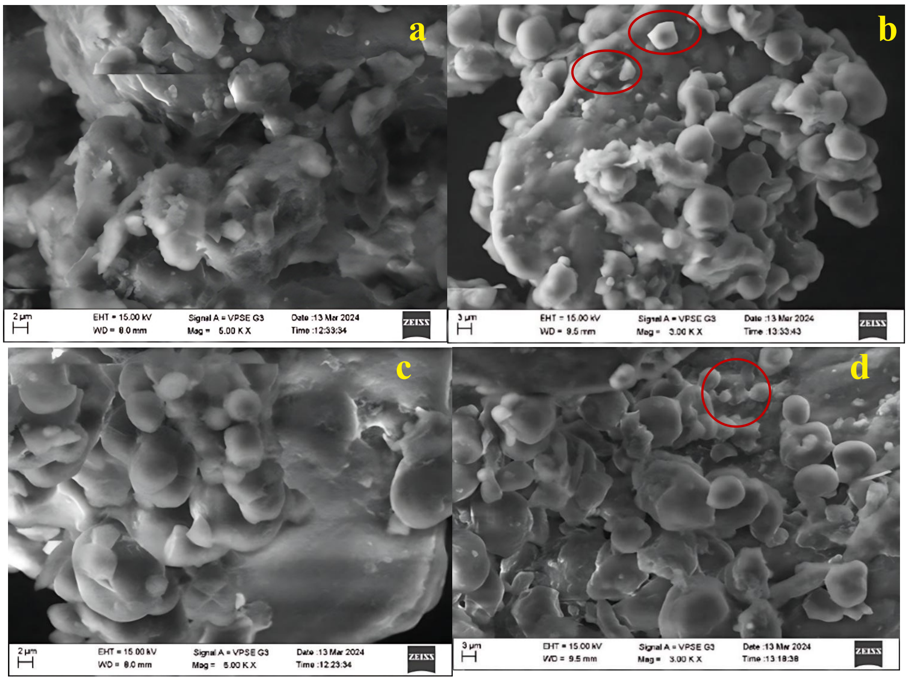

Figure 2. Scanning Electron Micrographs (SEM) of Sorghum and Finger Millet Flours at High Magnification Micrographs show the surface morphology of (a) native sorghum millet flour (SMF), (b) dry milled nixtamal sorghum millet flour (DMNSMF), (c) native finger millet flour (FMF), and (d) dry milled nixtamal finger millet flour (DMNFMF). Images were captured at 3,000x and 5,000x magnification.

water absorption and enzymatic digestibility [15,24,11,33]. Furthermore, the increased surface roughness and disrupted morphology in nixtamalized samples align with enhanced functional properties such as swelling power and oil absorption observed experimentally. These microstructural modifications also correlate with improved mineral incorporation, as calcium ions from lime interact with starch and protein components, stabilizing the altered granule structure.

3.7 Antioxidant profile of flours

The antioxidant activity of sorghum and finger millet flours was evaluated using DPPH radical scavenging activity and total phenolic content (TPC) assays (Table 3). Regarding antioxidant potential, no significant changes ($p > 0.05$) in DPPH radical scavenging activity were observed across treatments. DPPH radical scavenging activities remained statistically unchanged, with both native and nixtamalized flours showing high values (92.61% to 96.81%), indicating strong free radical scavenging capacity retained even after alkaline treatment. This persistence suggests that nixtamalization does not significantly degrade the overall antioxidant capacity of millet flours, consistent with reports that phenolic compounds may be partially protected or transformed rather than lost during alkaline cooking [15]. However, total phenolic content slightly increased in DMNFMF (5.44 mg GAE/100 g) compared to FMF (4.69 mg GAE/100 g), though this difference was not significant. The increase in TPC may result from the release of bound phenolic compounds facilitated by the breakdown of cell wall structures and liberation of phenolics during nixtamalization [15,11]. However, some phenolics are also sensitive to alkaline degradation, which may explain the lack of significant changes in DPPH activity.

Table 3. Thermal characteristics and antioxidant parameters

Parameters assessed	SMF	DMNSMF	FMF	DMNFMF
Antioxidant Profile				
DPPH Radical Scavenging Activity (%)	93.26 ^{a} ±1.7	92.61 ^{a} ±0.15	96.81 ^{a} ±0.29	93.72 ^{a} ±1.51
Total Phenolic content (mg GAE/100g)	5.54 ^{a} ±0.14	5.28 ^{a} ±0.97	4.69 ^{a} ±0.26	5.44 ^{b} ±1.86
Differential Scanning Calorimetry				
Onset Temperature (°C)	72.08 ^{a} ±1.89	77.37 ^{a} ±1.82	71.80 ^{a} ± 1.26	77.05 ^{a} ± 1.06
Peak Temperature (°C)	75.30 ^{a} ±1.66	80.67 ^{a} ±1.92	75.27 ^{a} ±0.26	80.68 ^{a} ±0.35
End Temperature (°C)	78.73 ^{a} ±1.45	85.00 ^{a} ±1.65	79.16 ^{a} ±0.19	89.89 ^{b} ±0.26

SMF –Sorghum millet flour, DMNSMF –Dry milled nixtamal Sorghum millet flour, FMF –Finger millet flour, DMNFMF –Dry milled nixtamal Finger millet flour. Values are mean ± Standard deviation of three determinations ($n = 3$). Values with the different superscript within rows are significantly different at $p < 0.05$ compared to respective controls SMF and FMF.

3.8 Differential scanning calorimetry (DSC)

The thermal behavior of millet flours, studied through Differential Scanning Calorimetry (DSC), provides insights into starch gelatinization, protein denaturation, and structural stability, all of which affect processing and cooking quality. DMNSMF exhibited onset, peak, and end temperatures of 77.37°C, 80.67°C, and 85.0°C, respectively, while DMNFMF showed values of 77.05°C, 80.68°C, and 89.89°C, as presented in Table 3. The increase in thermal transition temperatures suggests that nixtamalization induces molecular rearrangements in starch and protein matrices, resulting in enhanced thermal stability. The significantly higher end temperature in DMNFMF indicates a more extensive gelatinization range, likely due to interactions between calcium ions introduced during nixtamalization and starch granules, which strengthen intermolecular bonds and delay complete gelatinization. Additionally, protein denaturation during alkaline cooking can affect the thermal properties by altering starch-protein complexes, influencing heat absorption profiles [34,35]. Literature with similar findings reports that alkaline treatments, including nixtamalization and fermentation, generally increase gelatinization temperatures and modify enthalpy changes, reflecting changes in crystallinity and molecular order within starch structures [35].

3.9 X- ray diffraction studies

The XRD patterns of nixtamalized flours—finger millet flour (Sample 1), dry-milled nixtamalized finger millet flour (Sample 2), sorghum millet flour (Sample 3), and dry-milled nixtamalized sorghum millet flour (Sample 4) were revealed in Figure 3. The XRD patterns of nixtamalized flours (Samples 1–4) revealed polycrystalline structures with both crystalline and amorphous phases. Major diffraction peaks were observed at 2θ values of

14.2°, 15.41°, 17.90°, 20.18°, and 21°, alongside broad amorphous halos at 16° and 35°. Sample 1 showed the highest intensity at 14.2°, while Samples 2, 3, and 4 displayed similar, lower intensities. At 15.41° and 17.90°, Samples 2 and 4 had weaker peaks compared to Samples 1 and 3. Peaks at 20.18° and 21° were similar across all samples, with 21° being more prominent. Samples 1 and 3 showed higher crystallinity. The observed peaks suggest the presence of small crystallites embedded in an amorphous matrix. The amorphous halo at 35° was consistent across all four samples, indicating a significant non-crystalline fraction. Overall, all samples demonstrated a polycrystalline structure with a dominant amorphous phase, as indicated by the strong background centered at approximately 15°. Despite similarities in background intensity and amorphous fraction across all samples, background subtraction would be necessary for a more accurate crystallinity assessment. Currently, no XRD studies specifically on these flours have been reported in the literature. However, previous research suggests that amylopectin contributes to an A-type diffraction pattern, as proposed by [36]. It has been hypothesized that in starch granules, amylopectin forms crystalline regions, while branch points reside in amorphous domains [37]. Additionally, amylose, being a linear polymer, is thought to be primarily incorporated within amorphous regions. Further validation of these findings would require synchrotron-based XRD studies for enhanced structural resolution.

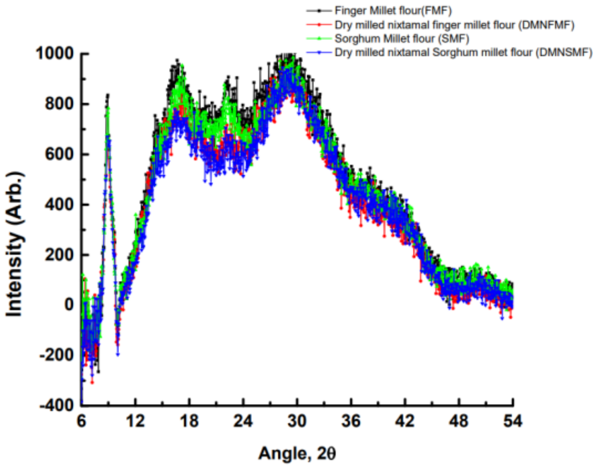

Figure 3. X-ray diffraction profiles of native and nixtamalized millet flours

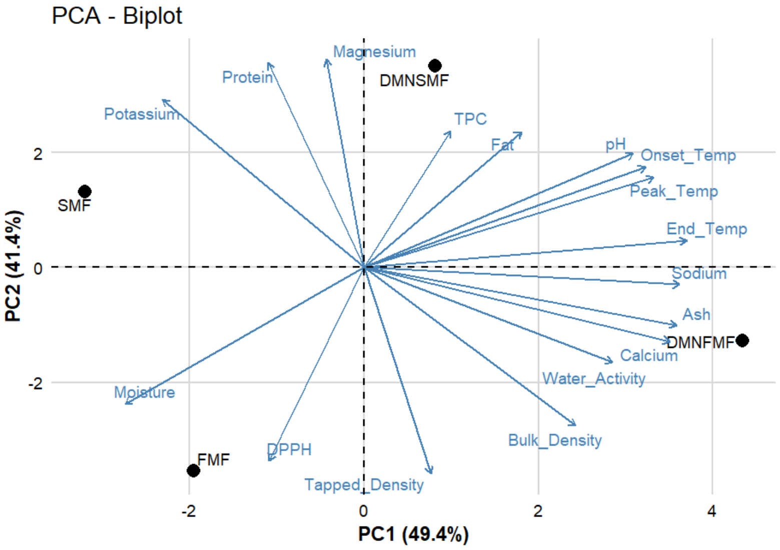

Figure 4. Principal Component Analysis (PCA) biplot illustrating the multivariate relationship among physicochemical, mineral, antioxidant, and thermal properties of native and nixtamalised millet flours

3.10 Multivariate principle component analysis

Principal Component Analysis demonstrated that PC1 and PC2 together explained 90.8% of the total sample variation, because they accounted for 49.4% and 41.4% of the total variance, respectively (Figure 4). The results showed that all attributes of the samples tested through alkaline processing techniques displayed changes, as PC1 examined mineral content (calcium, sodium, and ash), pH, bulk density, water activity, and thermal parameters (which included onset, peak, and end temperatures). DMNFMF showed complete strength across the positive PC1 axis due to its elevated levels of calcium, ash, sodium, and thermal transition measurements. The native SMF sample showed a negative PC1 axis position, which indicated its connection to potassium and protein. Protein, magnesium, potassium, and antioxidant-related parameters served as the primary elements that created the PC2 profile. DMNSMF showed a positive PC2 clustering pattern due to increased magnesium and protein content, while FMF showed a negative PC2 pattern, associated with DPPH activity and tapped density. The distinct classification between native samples (SMF and FMF) and nixtamalised samples (DMNSMF and DMNFMF) demonstrates that alkaline processing produces major alterations in both compositional and functional elements, and thermal characteristics.

4. Conclusions

The objective of this study was to evaluate the effect of $Ca(OH)_2$-based nixtamalization on the physicochemical, nutritional, structural, thermal, antioxidant, and techno-functional properties of sorghum and finger millet flours, and to assess their suitability for value-added food applications.

Nixtamalization significantly modified flour characteristics by increasing alkalinity (pH), ash content, and mineral concentrations, particularly calcium and magnesium, confirming effective mineral fortification through lime diffusion. Moisture content decreased after processing, enhancing storage stability and shelf life, while protein and lipid contents showed minimal or concentration-related changes. Structural analyses (particle size, SEM, and XRD) revealed starch disruption, partial gelatinization, and the formation of porous, heterogeneous matrices, which directly influenced functional behavior.

Distinct functional trends were observed. As a direct effect of nixtamalization, oil absorption capacity increased and water solubility slightly decreased due to starch–protein interactions and complex formation, indicating improved matrix stability. With increasing temperature, swelling power and solubility progressively increased because of enhanced starch hydration and gelatinization. Thus, nixtamalization altered the baseline functionality of the flours, whereas temperature primarily governed the extent of hydration and swelling during cooking. These complementary effects explain the improved thickening, binding, and processing performance of the treated flours.

Thermal analysis showed elevated gelatinization temperatures, suggesting enhanced structural stability through calcium–starch interactions. Antioxidant capacity remained largely preserved, with slight increases in total phenolics likely due to the release of bound compounds. Collectively, these modifications improved handling, hydration, and nutritional attributes.

From an application perspective, nixtamalized sorghum flour demonstrated suitability for extrusion and bakery systems, while nixtamalized finger millet flour, due to its high calcium content and improved flowability, is particularly promising for fortified complementary foods, elderly nutrition products, gluten-free formulations, and functional health mixes. Therefore, $Ca(OH)_2$-nixtamalization represents an effective and scalable strategy to enhance both the nutritional quality and techno-functional performance of millet-based ingredients.

Limitations of the study

This study was limited to in vitro physicochemical, functional, and thermal evaluations. In vivo nutrient bioavailability, protein digestibility, glycemic response, and long-term storage stability were not assessed. Sensory acceptance and consumer preference studies were also beyond the scope of this work. Future investigations addressing these aspects, along with process optimization at pilot or industrial scale, are necessary to fully establish the commercial and nutritional potential of nixtamalized millet flours.

Abbreviations

DSC: Differential Scanning Calorimetry
DMNSMF: Dry Milled Nixtamal Sorghum Millet Flour
DMNFMF: Dry Milled Nixtamal Finger Millet Flour
WAC: Water Absorption Capacity
WSI: Water Solubility Index
SEM: Scanning Electron Microscopy
TPC: Total Phenolic Content
DPPH: 2,2-diphenyl-1-picrylhydrazyl
XRD: X-ray Diffractometer
SMF: Sorghum Millet Flour
FMF: Finger Millet Flour
OAC: Oil Absorption Capacity

Authors' contributions

Sneha: Investigation and Data Analysis; Naomi: Data Analysis and writing original draft; Shruti Joshi-

Conceptualization , writing(Review and editing).

Conflict of interest

The authors verify that they do not possess any conflicts of interest regarding the research detailed in this manuscript.

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