EEET ECOLOGICAL ENGINEERING & ENVIRONMENTAL TECHNOLOGY

Ecological Engineering & Environmental Technology, 2026, 27(9), 284–302
https://doi.org/10.12912/27197050/226397
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Received: 2026.07.13
Accepted: 2026.08.03
Published: 2026.08.15

Comparative assessment of eco-friendly priming strategies for enhancing the nutritional, phytochemical, and antioxidant properties of germinated *Moringa oleifera* Lam. seeds

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ABSTRACT

Sustainable and environmentally friendly technologies are increasingly required to improve the nutritional quality of plant resources while supporting sustainable food systems. Moringa oleifera seeds are a nutrient-dense resource with considerable potential for food and nutraceutical applications; however, their utilization remains limited by the presence of antinutritional compounds. This study aimed to compare the effectiveness of three seed priming techniques hydropriming (distilled water, HG), chemopriming (ascorbic acid, CG), and biopriming (*M. oleifera* leaf extract, MG) to identify the most effective treatment for simultaneously improving the nutritional composition, monosaccharide profile, bioactive compound content, and antioxidant capacity while reducing antinutritional factors in germinated *M. oleifera* seed flour. Treated seeds were evaluated for proximate composition, monosaccharide profile, antinutritional factors, extraction yield, phenolic compounds, and antioxidant activity. Our results demonstrate a significant improvement ($p < 0.05$) in both nutritive and bioactive features. MG produced the greatest increases in protein (+7.62%), fiber (+11.52%), ash (+14.41%), total phenolics (+82.62%), and flavonols (+103%), while markedly reducing alkaloids (−29.03%), oxalates (−55.97%), saponins (−41.18%), and phytates (−61.78%). Concurrently, priming treatments altered the monosaccharide composition by introducing additional sugars and increasing soluble sugar concentrations. Furthermore, antioxidant capacity was significantly enhanced in all treated samples, with CG exhibiting the strongest DPPH, ABTS, and FRAP scavenging activities. These findings demonstrate that eco-friendly priming-assisted germination represents an effective green biotechnological strategy for upgrading the nutritional and functional value of *M. oleifera* seeds. The proposed approach contributes to the sustainable valorization of plant resources and supports the development of functional ingredients for environmentally responsible food and nutraceutical systems.

Keywords: *Moringa oleifera*, seed priming, germination, antioxidant activity, antinutritional factors.

INTRODUCTION

In contemporary society, the shift toward health-conscious dietary habits has stimulated a growing demand for functional foods rich in bioactive compounds (Carović-Stanko et al., 2016). *Moringa oleifera* stands as a prime example, as nearly every part of the plant holds nutritional, medicinal, and industrial value (Ijarotimi et al., 2013). Due to its notable climatic adaptability, this species, originally from the sub-Himalayan region, is now widely distributed and cultivated throughout tropical and subtropical regions (León-López et al., 2019). This plant offers a strategic alternative to address nutritional deficiencies and sustainably improve food security amid worldwide cereal shortages (El-Anoos et al., 2022). Despite their richness, *Moringa* seeds require further processing, as raw flour lacks the functional and nutritional properties needed by the food industry (Chinma et al., 2017).

Nutritional properties can be modulated through technological and biotechnological processes, with germination representing a sustainable biotechnological strategy to reduce antinutritional factors while enhancing the bioavailability of health-promoting metabolites (León-López et al., 2019; Santos et al., 2020). Over the past decade, sprout-based foods have gained growing attention, driven by plant-based dietary trends and health awareness (Frimpong et al., 2025). Germination can be enhanced through seed pretreatments such as priming, a widely adopted technique for improving seed vigor and germination performance (Duary, 2020). Depending on the desired outcome, different priming methods, including hydropriming, chemopriming, and other approaches, may be employed (Koushal et al., 2024). While hydropriming remains the simplest method, chemical priming, using natural or synthetic protective compounds, more effectively accelerates germination and enhances seed tolerance to environmental stresses (Duary, 2020; Frimpong et al., 2025). Ascorbic acid is a promising priming agent that mitigates oxidative stress and regulates plant growth via phytohormone biosynthesis (Soares et al., 2023). Biological priming successfully stimulates plant development by using naturally occurring nourishing compounds (Soares et al., 2023). Moringa leaf extract (MLE) is a notable eco-friendly example, readily applicable by farmers (Nouman et al., 2014). Its rich content of antioxidants, vitamins, minerals, and phytohormones enhances seed biochemical properties and promotes plant growth under both normal and stress conditions (Khan et al., 2017).

Although seed priming has been extensively investigated as a strategy to improve germination, seedling establishment, and subsequent plant growth and productivity (Khan et al., 2022; Soares et al., 2023), its potential to enhance the nutritional and functional quality of seeds intended for human consumption has received far less attention. In *M. oleifera*, previous food-oriented studies have mainly focused on germination or conventional hydropriming (Matthew et al., 2022; Ndife et al., 2020; Rayan and Embaby, 2016; Saa et al., 2022), whereas the effects of other environmentally friendly priming techniques on the biochemical and functional quality of germinated seeds remain largely unexplored. The physiological changes induced by seed priming, including de novo protein and nucleic acid synthesis, may improve seed quality and offer promising opportunities for food applications (Ellouzi et al., 2021). In this regard, combining different priming techniques with germination represents a sustainable strategy for producing protein-rich flours with lower levels of antinutritional compounds (Frimpong et al., 2025).The present study addresses this gap by systematically comparing hydropriming, chemopriming, and plant-based biopriming combined with germination, focusing on the nutritional and phytochemical quality of *M. oleifera* seeds rather than plant growth performance. This work highlights seed priming as a sustainable metabolic strategy to enhance seed nutritional value for food applications.

MATERIAL AND METHODS

Plant material and seed collection

Fully matured *Moringa oleifera* Lam. seeds were collected in January 2024 from healthy, locally grown trees in the Blida region, Algeria. After harvest, seeds were manually separated from the pods and carefully cleaned to remove dust, plant debris, damaged seeds, and other foreign materials. Only healthy, fully developed seeds of uniform maturity were selected for the experiments. The cleaned seeds were sealed in airtight polyethylene bags and stored at 4 °C until use to preserve their viability, minimize moisture uptake, and prevent microbial contamination.

Preparation and processing of *M. oleifera* seeds

Seed dehulling and constitution of experimental batches

M. oleifera seeds were manually dehulled, retaining only the kernels for all experimental treatments, while the hulls were retained for subsequent valorization. Kernels were then carefully sorted and cleaned to eliminate impurities, including sand, plant debris, damaged kernels, and foreign particles. Only healthy, intact, and uniformly sized kernels were selected for the experiments. The overall experimental workflow, including seed dehulling, priming treatments, germination, drying, milling, and flour production, is outlined in Figure 1. To minimize experimental variability, kernels were randomly assigned to the four treatment groups, ensuring comparable seed size and visual quality among batches:

- (UG): Raw, untreated, and ungerminated kernels used as the control.
- (HG): Kernels subjected to hydropriming by soaking in distilled water prior to germination.
- (CG): Kernels subjected to chemopriming by soaking in an aqueous ascorbic acid solution prior to germination.
- (MG): Kernels subjected to biopriming by soaking in an aqueous *M. oleifera* leaf extract prior to germination.

Priming treatment of samples

For each treatment, 100 g of dehulled kernels were surface sterilized by immersion in 1 L (1:10 w/v) of 0.5% sodium hypochlorite solution for 10 min to inhibit microbial contamination and mold growth. The kernels were then thoroughly rinsed three times with distilled water to remove residual sodium hypochlorite (León-López et al., 2019). The control kernels (UG) were dried at 40 °C for 24 h to determine their initial dry weight, then ground.

Prior to the main experiment, preliminary germination trials were conducted to determine the optimal concentration of ascorbic acid for chemopriming. Three concentrations (20, 50, and 80 mg/L) were evaluated based on germination percentage and germination capacity. The concentration of 50 mg/L was selected for the subsequent experiments because it provided the highest germination performance, whereas the highest concentration (80 mg/L) resulted in a slight reduction in germination capacity, suggesting a concentration-dependent inhibitory effect.

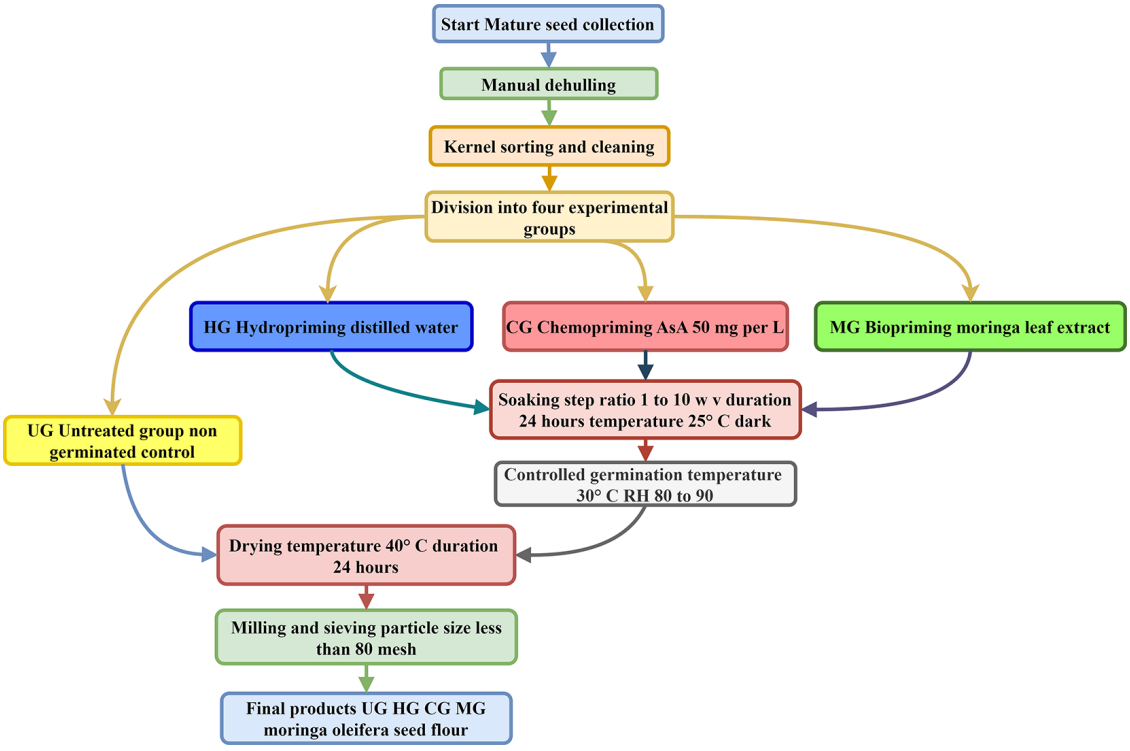

Figure 1. Integrated experimental design and processing workflow for priming treatments and germination of *M. oleifera* seeds toward functional flour production

- Hydropriming (HG) was carried out following Matthew et al. (2022), with minor modifications. The kernels were soaked in distilled water. Hydropriming was chosen because it is a simple, cost-effective technique whose efficacy in stimulating seed vigor has been widely demonstrated in the literature (Koushal et al., 2024).
- Chemopriming (CG) was adapted from Soares et al. (2023). An aqueous ascorbic acid (AsA) solution of 50 mg/L was prepared, based on preliminary trials. AsA was chosen for its antioxidant and protective properties, which enhance seed tolerance and physiological performance (Duary, 2020).
- Biopriming (MG) was adapted from Soares et al. (2023). An aqueous *M. oleifera* leaf extract (MLE) was prepared according to Basra et al. (2011). Briefly, young fresh leaves were frozen overnight, ground using a laboratory blender, filtered through muslin cloth, and the filtrate was diluted 30-fold (1:30, v/v) with distilled water before use. MLE was chosen for its nutrient richness, bioactive compounds, and eco-friendly, cost-effective nature (Soares et al., 2023).

For each treatment, the kernels were soaked in their respective priming solutions at a seed-to-solution ratio of 1:10 (w/v) for 24 h at 25 ± 1 °C in the dark. Following priming, the kernels were drained, rinsed with distilled water, and immediately subjected to the germination process.

Germination of pretreated kernels

After soaking, the kernels were drained and evenly distributed in a single layer on moistened standard filter paper placed in three independent plastic trays per treatment (Kacem, 2024). Germination was carried out for 72 h at 30 ± 1 °C in the dark. Moisture was maintained throughout the germination period by spraying the kernels with distilled water every 24 h to keep the filter paper uniformly moist while preventing waterlogging. At the end of the germination period, the germinated kernels were immediately collected for subsequent drying and flour preparation. The germination conditions were adapted from León-López et al. (2019), with minor modifications.

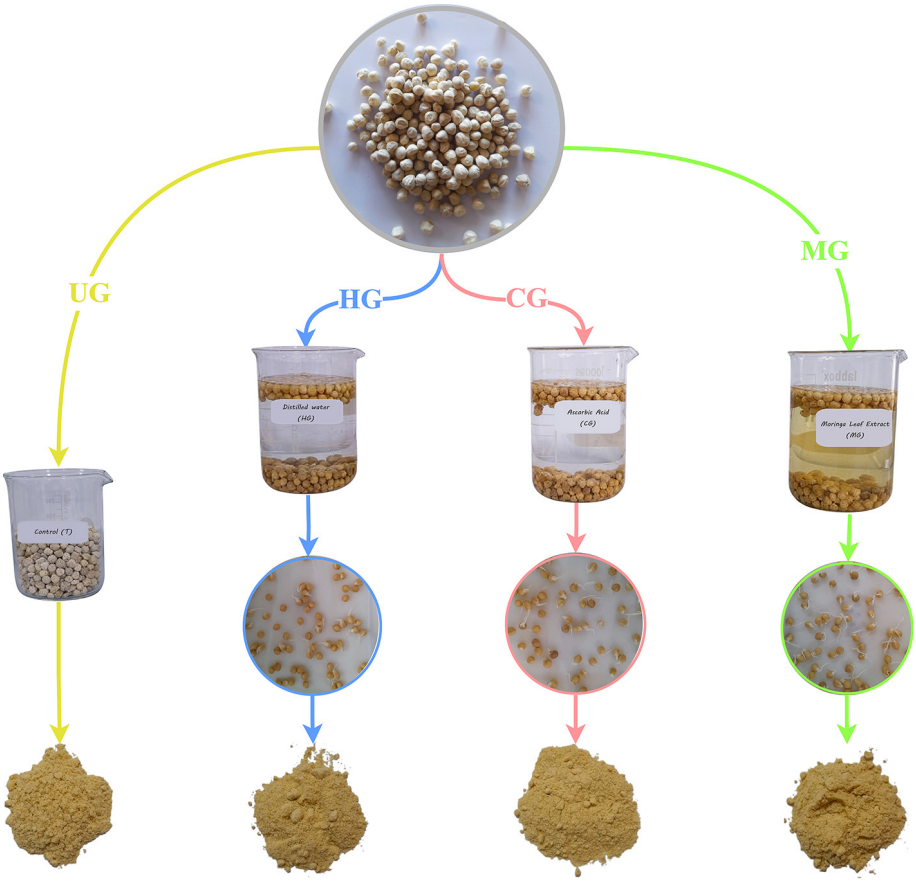

Figure 2. M. oleifera seed flour obtained from different priming treatments: UG – raw, ungerminated seeds; HG – hydropriming; CG – chemopriming; MG – biopriming

Drying and flour production

Following germination, the kernels were dried in a forced-air convection oven at 40 ± 1 °C for 24 h. The dried kernels were then cooled to room temperature in a desiccator to prevent moisture reabsorption before being ground using a laboratory knife mill and sieved through an 80-mesh (180 µm) sieve to obtain homogeneous flour samples, as illustrated in Figure 2 (Servín de la Mora-López et al., 2018). The resulting flours were immediately sealed in airtight polyethylene bags and stored at 4 °C until subsequent physicochemical, phytochemical, and antioxidant analyses.

Proximate chemical composition analysis

Moisture, ash, crude protein, crude fat, and crude fiber contents were determined according to the official methods of AOAC International (2019). Moisture was determined using Method 925.10, ash using Method 923.03, crude protein by the Kjeldahl method (Method 979.09), crude fat by Soxhlet extraction (Method 920.39), and crude fiber using Method 962.09. Total carbohydrate content was calculated by difference. The Atwater general factors were used to determine the samples' energy value, with proteins and carbs contributing 4 kcal/g and lipids contributing 9 kcal/g (FAO, 2003).

Analysis and identification of monosaccharides

Extraction and hydrolysis of polysaccharides

Polysaccharides from *M. oleifera* flour samples were extracted using hot water and ethanol precipitation, then purified and lyophilized. For monosaccharide analysis, they were acid-hydrolyzed with TFA under nitrogen, centrifuged, and vacuum-dried (Lv et al., 2009).

HPLC equipment and analytical conditions

Carbohydrate separation was performed using a Thermo Dionex Ultimate 3000 UHPLC system coupled with an RID refractometer (ERC RefractoMax 520 Refractive Index Detector), with a Thermo Scientific™ HyperREZ™ XP Carbohydrate H^+ column, length 300 mm, diameter 7.7 mm, particle size 8 µm. The mobile phase was ultrapure H_2O, flow rate 0.4 mL/min in isocratic mode, separation temperature 30 °C, separation time: 45 min, 35 °C at the RID at 5 Hz. Before HPLC analysis, all extracts were filtered through a 0.22 µm filter and transferred to 2 mL HPLC vials. The injection volume was 20 µl. Peak identification was performed using Chromeleon software with a standard curve ranging from 0.01 to 1 mg/mL of the following carbohydrates: ribose, arabinose, fructose, glucose, galactose, mannose, and sucrose (with $R^2 > 0.98$).

Identification of antinutritional factors

Determination of alkaloid content

Total alkaloid content was determined according to Waseem et al. (2022). Briefly, 5 g of the sample was extracted with 10% (v/v) acetic acid in ethanol, filtered, and treated with ammonium hydroxide to precipitate alkaloids. The precipitate was collected, oven-dried, and quantified gravimetrically. Results were expressed as mg/100 g DW.

Determination of oxalate content

Oxalate content was determined by titration (Adeleke et al., 2017). Dry samples were acid-digested in HCl, centrifuged, and filtered. The filtrate was treated with ammonia, precipitated with $CaCl_2$, dissolved in H_2SO_4, and titrated with 0.1 M $KMnO_4$. The results were expressed as mg/100 g DW.

Determination of total saponins

Total saponin content was determined following Ejikeme et al. (2014) as adapted by Ibraheem et al. (2019). Samples were extracted with aqueous ethanol, purified by organic solvent extraction and salting-out, and dried. Results were expressed in g/100 g DW.

Determination of phytates

Phytate content was determined spectrophotometrically according to Haug and Lantzsch (1983) Haug and Lantzsch (1983), as described by Waseem et al. (2022). Briefly, 1 g of the sample was extracted with 10 mL of 0.2 N HCl. The extract was reacted with ferric solution, centrifuged, and the supernatant was mixed with 2,2′-bipyridine reagent. Absorbance was measured at 519 nm, and phytate content was quantified using a phytic acid standard curve. Results were expressed as mg/100 g DW.

Extraction and quantification of bioactive compounds

Preparation of extracts

M. oleifera flour samples (03 g) were extracted twice with 70% ethanol, sonicated, and centrifuged. Rotary evaporation was used to concentrate the mixed supernatants at 40 °C, and they were then chilled until analysis (Owon et al., 2021).

Extraction yield

According to Falleh et al. (2008), the extraction yield (%) was estimated as the ratio of the dry weight of the evaporated extract to the dry weight of the sample, then multiplied by 100.

Total phenolic content (TPC)

The Folin-Ciocalteu technique was used to calculate the total phenolic content (Singleton et al., 1999). After mixing the extract with diluted Folin reagent, it was incubated, treated with Na_2CO_3, and measured at 725 nm. The results were given in milligrams of gallic acid equivalents per one hundred grams of dry matter (mg GAE/100 g DM).

Total flavonoid content (TFC)

The $AlCl_3$ technique was used to figure out the total flavonoid content (Woisky and Salatino, 1998). The absorbance was measured at 430 nm after the extract and 2% methanolic $AlCl_3$ were combined and incubated for 15 min. The results were given in milligrams of quercetin equivalents per one hundred grams of dry matter (mg QE/100 g DM).

Total flavonols content (TFLC)

The corresponding method was used to calculate the total flavonol content (Mbaebie et al., 2012). After mixing the extract with sodium acetate and $AlCl_3$ and incubating it for 2.5 h, the absorbance at 440 nm was measured. The results were given in milligrams of quercetin equivalents per one hundred grams of dry matter (mg QE/100 g DM).

Hydrolysable tannin content (HTC)

The $FeCl_3$ method (Mole and Waterman, 1987) was used to determine the amount of hydrolysable tannin. After the extract and $FeCl_3$ solution were combined, the absorbance was observed at 660 nm, and the results were reported in milligrams of tannic acid equivalents per one hundred grams of dry matter (mg TAE/100 g DM).

Evaluation of antioxidant activity

DPPH radical scavenging activity

According to Afify et al. (2012), the DPPH radical-scavenging capacity (RSC) was assessed. After combining the extract with DPPH methanolic solution and letting it sit in the dark for 30 minutes, the absorbance at 515 nm was measured.

$$RSC\ (\%) = (Acontrol - Asample) / Acontrol \times 100 \quad (1)$$

ABTS radical scavenging activity

ABTS radical scavenging activity was evaluated in line with (Afify et al., 2012). $ABTS^{\bullet+}$ was generated with potassium persulfate, diluted to $A_{734}{}^{7} = 0.70 \pm 0.02$, and reacted with the extract for 30 min in the dark.

$$AA\ (\%) = (Acontrol - Asample) / Acontrol \times 100 \quad (2)$$

Ferric reducing antioxidant power (FRAP)

The reducing power was determined according to Tesfay et al. (2016). FRAP reagent (acetate buffer, Fe (II)-TPTZ, $FeCl_3$) was freshly prepared. A sample (30 µL) was mixed with 900 µL FRAP reagent, and absorbance was read at 593 nm after 10 min. The results were expressed as mg ascorbic acid equivalents per one hundred grams of dry matter (mg AAE/100 g DM).

Statistical analysis

All experiments were conducted using three independent biological replicates, and the results are expressed as mean ± standard deviation (SD). Statistical analyses were performed using XLSTAT 2016. Differences among treatments were assessed by one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post hoc test for multiple comparisons. Differences were considered statistically significant at $p < 0.05$. To investigate the relationships among treatments and measured variables, principal component analysis (PCA) was performed using the Pearson correlation matrix in XLSTAT 2016. In addition, hierarchical cluster

analysis (HCA) and the corresponding heatmap were generated from mean-centered and standardized (z-score) data using Euclidean distance and Ward's hierarchical agglomerative clustering method to evaluate similarities among treatments and clustering patterns of the analyzed variables.

RESULTS AND DISCUSSION

Effects of priming treatments on the nutritional composition of *M. oleifera* seed flour

The chemical composition of *M. oleifera* seed flour was evaluated to assess how various priming strategies combined with germination influence nutritional quality. Results are summarized in Table 1.

Moisture content

Priming treatments induced a slight but statistically significant reduction in moisture content ($p < 0.05$) compared to raw seeds (UG). This decrease may be attributed to the activation of pregerminative metabolism during priming, which facilitates water removal during drying (Liu et al., 2025). All values fall within the moisture range (3.5–10.6%) reported for M. oleifera seeds (Saa et al., 2019). The downward trend after processing is consistent with Ijarotimi et al. (2013), who reported lower moisture content in germinated flours (9.43%) compared to ungerminated ones (10.60%). Similarly, Matthew et al. (2022) reported that priming followed by drying contributes to reducing the free water fraction in processed seeds. This reduction is beneficial for shelf-life, as moisture levels below 10% inhibit microbial growth and enhance physicochemical stability during storage (Matthew et al., 2022; Saa et al., 2022), thereby improving the technological stability and storage potential of the resulting flour.

Ash content

Ash content varied significantly among treatments ($p < 0.05$). Hydropriming (HG) led to a 13.51% decrease, likely reflecting the leaching of water-soluble minerals into the soaking medium (Lemmens et al., 2019). In contrast, biopriming (MG) significantly increased ash content (+14.41%), suggesting an enrichment effect from the *Moringa oleifera* leaf extract, whose mineral constituents may have contributed to increasing the mineral fraction of the treated kernels (Khan et al., 2022; Yasmeen et al., 2013). Chemopriming also resulted in a slight increase in ash content compared with the untreated control, indicating that the nature of the priming solution influences mineral retention during seed processing. Chinma et al. (2017) reported increased ash content in germinated *Moringa* seeds, from 3.85% to 4.92%, supporting the mineral enrichment observed in the MG and CG treatments. Overall, these findings

Table 1. Chemical composition, monosaccharide content, and energy value of M. oleifera seed flour under different treatment conditions (UG – raw, ungerminated seeds; HG – hydropriming; CG – chemopriming; MG – biopriming)

Parameter (%)	UG	HG	CG	MG
Moisture	5.19 ± 0.03a	4.45 ± 0.04c	4.52 ± 0.02c	4.80 ± 0.02b
Ash	3.33 ± 0.12b	2.88 ± 0.08c	3.49 ± 0.13ab	3.81 ± 0.09a
Crude fiber	3.56 ± 0.12b	3.59 ± 0.04b	3.65 ± 0.09ab	3.97 ± 0.14a
Crude protein	36.96 ± 0.47c	38.16 ± 0.26b	39.20 ± 0.24ab	39.77 ± 0.13a
Crude fat	39.87 ± 0.11a	39.38 ± 0.46a	38.31 ± 0.09b	37.17 ± 0.15c
Carbohydrates	11.09 ± 0.76a	11.54 ± 0.72a	10.83 ± 0.27a	10.49 ± 0.52a
Energy (Kcal/100 g DW)	558.16 ± 0.96a	560.38 ± 2.37a	552.22 ± 0.58b	543.49 ± 0.75c
Monosaccharides (g/100g DW); n.d.				
Sucrose	ND	0.36 ± 0.00	0.22 ± 0.00	0.75 ± 0.04
D+ Glucose	0.35 ± 0.05	0.52 ± 0.02	0.46 ± 0.01	0.57 ± 0.01
D+ Fructose	0.45 ± 0.00	0.65 ± 0.01	0.53 ± 0.02	ND
Xylose	ND	ND	ND	0.36 ± 0.01
Ribose	ND	ND	0.03 ± 0.03	ND

Note: Values are expressed as mean ± standard deviation (n = 3); values with different letters within the same row are significantly different ($p < 0.05$) according to Tukey's test. ND – not detected.

support biopriming-assisted germination as an effective strategy to improve the mineral quality of *M. oleifera* seed flour for functional food development (Calizaya-Milla et al., 2022).

Fiber content

The different types of priming caused changes in fiber content compared to raw, ungerminated seeds (UG). HG and CG showed a slight increase, at 0.84% and 2.53%, respectively, while MG exhibited the highest content (+11.52%) compared to UG ($p < 0.05$). These variations are attributed to cell wall remodeling during germination, which increases structural polysaccharide synthesis and the relative proportion of dietary fiber through starch reserve degradation (Servín de la Mora-López et al., 2018). The greater increase in MG may reflect the growth-promoting activity of phytohormones present in *M. oleifera* leaf extract, which stimulate cell division (Soares et al., 2023). Our results are consistent with those of Saa et al. (2022), who reported increased crude and total dietary fiber in *Moringa* seeds after soaking and germination. Similarly, Servín de la Mora-López et al. (2018) reported an approximately 12% increase in total dietary fiber following optimized germination, closely matching the improvement observed in the MG treatment. The observed increase confirms the positive effect of priming, particularly biopriming, on fiber enrichment and the improvement of the seeds' functional properties, suggesting beneficial health effects, including a reduced risk of type 2 diabetes, obesity, and cardiovascular disease (Coello et al., 2020).

Protein content

All priming treatments promoted a significant increase in protein content, reaching a maximum of 39.77 % in MG (+7.62% vs UG). This increase may result from the metabolic activation induced by priming-assisted germination, which promotes protein synthesis and the depletion of carbohydrate and lipid reserves, thereby increasing the relative protein concentration (Liu et al., 2025; Abdalla et al., 2019). This enrichment is consistent with the metabolic stimulation triggered by phytohormones and nutrients present in leaf extracts, which enhance protein synthesis during the early stages of germination (Soares et al., 2023; Yasmeen et al., 2013). Matthew et al. (2022) reported higher protein content in germinated *Moringa* seed flour (32.13%) than in raw flour, and although our initial value is higher, the same enrichment trend is confirmed. Similarly, Frimpong et al. (2025) reported an approximately 9% increase in crude protein following hydropriming-assisted germination, supporting the beneficial effect of priming on seed protein content. Overall, biopriming with *M. oleifera* leaf extract further enhanced the nutritional value of the seed flour, highlighting its potential as a protein-rich functional ingredient for food fortification.

Fat content

The fat content decreased progressively following the applied treatments compared to raw, UG. A non-significant reduction was observed with HG (−1.23%), followed by a significant decrease for CG (−3.91%), with the greatest reduction for MG (−6.78%). This decline reflects the enhanced mobilization of triacylglycerol reserves during priming-assisted germination, providing energy and carbon skeletons required for seedling development (Liu et al., 2025; Servín de la Mora-López et al., 2018). Our findings agree with those of León-López et al. (2019) and Calizaya-Milla et al. (2022), who likewise reported significant reductions in lipid content during *Moringa* seed germination, confirming the preferential mobilization of lipid reserves. The present findings suggest that lipids, rather than carbohydrates, served as the primary energy substrate during *Moringa* seed germination, consistent with observations reported for other oil-rich seeds (Servín de la Mora-López et al., 2018). Although fatty acid composition was not determined in the present study, previous studies have shown that germination may increase the relative proportion of beneficial polyunsaturated fatty acids despite a reduction in total lipid content, thereby enhancing the nutritional value of germinated seeds (Liu et al., 2025). From a technological perspective, the lower lipid content may also improve flour oxidative stability, thereby reducing the risk of rancidity during storage (Ndife et al., 2020).

Carbohydrate content

Carbohydrate content remained generally stable following priming treatments, although slight, non-significant variations suggest that carbohydrate metabolism was modulated according to the priming strategy applied. Starch, the major carbohydrate reserve in *Moringa* seeds, is hydrolyzed by α-amylase into soluble sugars such as

glucose and sucrose to support embryo growth (Lemmens et al., 2019). The slight increase after hydropriming may reflect transient sugar accumulation, whereas the slight decreases after chemo- and biopriming suggest enhanced carbohydrate utilization to support seedling growth (Gul et al., 2025; Liu et al., 2025; Yasmeen et al., 2013). Baltazar et al. (2023) demonstrated that hydro- and nutripriming significantly reduce total starch content through enhanced starch-degrading enzyme activity. This carbohydrate stability enhances the potential of these flours for use in the formulation of healthy food products with controlled energy release (Liu et al., 2025; Saa et al., 2019).

Energy content

The energy content of *M. oleifera* seeds was influenced by priming treatments, with an overall downward trend following chemopriming and biopriming, reflecting changes in the caloric balance of the samples. This decrease is consistent with the observed depletion of lipid reserves, which serve as the primary energy reserve in oil-rich seeds. During germination, reserve compounds are mobilized to sustain the metabolic processes required for embryo development and seedling growth (Servín de la Mora-López et al., 2018). Priming, particularly biopriming, appears to accelerate reserve mobilization, resulting in lower energy density (Frimpong et al., 2025). Accordingly, the greater decrease observed in the MG treatment is consistent with its more pronounced reduction in lipid content. Our findings agree with the mechanism proposed by León-López et al. (2019), who reported that intensive germination preferentially mobilizes storage lipids, thereby reducing the overall caloric value of *Morinwga* seeds.

Monosaccharides

The monosaccharide profile underwent significant qualitative and quantitative shifts. The emergence of sucrose and the increase in D-glucose across all treatments reflect the hydrolysis of reserve polysaccharides by α-amylase to support embryo respiration. The detection of xylose and ribose specifically in primed samples (MG and CG) provides biochemical evidence of active cell wall remodeling and accelerated RNA synthesis, confirming that priming activates metabolic pathways earlier than in dormant seeds (Liu et al., 2025; Saa et al., 2022). Tesfay et al. (2016) reported increased amylase activity after 24 h of imbibition, leading to higher simple sugars for radicle respiration, consistent with our observed increase in free sugars compared to the dormant control. Also, Frimpong et al. (2025) identified a similar HPLC profile in *Moringa*, with glucose as the dominant monosaccharide and variations in xylose and arabinose. These results are generally significant and indicate a change in the simple carbohydrate profile of *M. oleifera* seeds, characterized by the emergence of new sugars and a redistribution of monosaccharides under the influence of priming and germination treatments.

Effects of priming treatments on certain antinutritional factors

Alkaloids

Alkaloid content in raw seeds (8.34 ± 0.19 mg/100 g DM) significantly decreased after priming (Figure 3a), with reductions of 10.79% (CG), 19.90% (HG), and 29.03% (MG), indicating a progressive effect of priming on alkaloid reduction. This result is comparable to that of Frimpong et al. (2025), who observed a 33% decline in *M. oleifera* seeds after hydropriming and germination. Seed soaking promotes alkaloid leaching, while germination further reduces their levels through enzymatic degradation and their utilization for new tissue synthesis (Liu et al., 2025). The greater reduction after biopriming may be attributed to the enhanced germination-related metabolism stimulated by phytohormones and bioactive compounds in *Moringa* leaf extract (Khan et al., 2022).

Oxalates

Priming treatments led to a significant decrease in oxalate content compared to control seeds (1.91 ± 0.15 mg/100 g DM). While HG and CG showed comparable reductions (34.55% and 38.22%, respectively), MG proved most effective, lowering the content by 55.95% (0.84 ± 0.12 mg/100 g DM) (Figure 3b). These results reinforce the observations of Matthew et al. (2022), who also reported a significant decline in oxalate content following germination of *M. oleifera* seeds, confirming that biological treatments significantly improve the nutritional profile of seeds by mitigating oxalate levels. Germination triggers metabolic reactivation that promotes reserve

mobilization and the degradation of antinutritional factors, while biopriming further enhances these processes through the action of phytohormones present in *Moringa* leaf extract (Liu et al., 2025; Soares et al., 2023).

Saponins

Saponin reduction showed high sensitivity to the type of priming agent (Figure 3c). While hydropriming (HG) and biopriming (MG) induced significant decreases (reaching 2.01 ± 0.13 g/100 g DM and1.20 ± 0.14 g/100 g DM, respectively), chemopriming (CG) resulted in a marginal, non-significant decrease (−1.47%). As suggested by El-Anoos et al. (2022), the primary mechanism for this reduction is the leaching of saponins into the soaking medium. Our results are consistent with Saa et al. (2022), who highlighted the synergistic effect of soaking and germination on saponin depletion in *Moringa* flour.

Phytates

Phytate levels varied significantly across all treatments ($p < 0.05$), with a distinct dependency on the priming method (Figure 3d). The strongest reduction was observed under biopriming (61.78%), followed by CG (50.13%) and HG (31.84%). This trend suggests that biopriming may further enhance the phytate degradation induced by germination, possibly through the stimulatory effects of phytohormones present in *Moringa* leaf extract on early metabolic activity (Liu et al., 2025; Soares et al., 2023). These findings corroborate previous research (Frimpong et al., 2025; Saa et al., 2022) identifying soaking and sprouting as a robust method for antinutrient degradation. From a nutritional perspective, reducing phytate content may improve mineral bioavailability by limiting mineral chelation, thereby enhancing the nutritional quality of *M. oleifera* seed flour (Ebert, 2022).

Effects of priming treatments on the extraction yield and profile of bioactive phenolic compounds

Extraction yield

Priming treatments significantly enhanced the extraction yield ($p \leq 0.05$). Compared to the ungerminated control (UG), hydropriming (HG) induced a moderate, non-significant increase (+9.93%), whereas chemopriming (CG) showed a more pronounced effect (+26.36%). The highest yield was observed in biopriming (MG: 14.99 ± 0.56%, +43.17%), underscoring the superior efficacy of this treatment in promoting the release of extractable compounds (Figure 4). These results align with Owon et al. (2021), who emphasized that the extraction yield in *M. oleifera* seeds is highly dependent

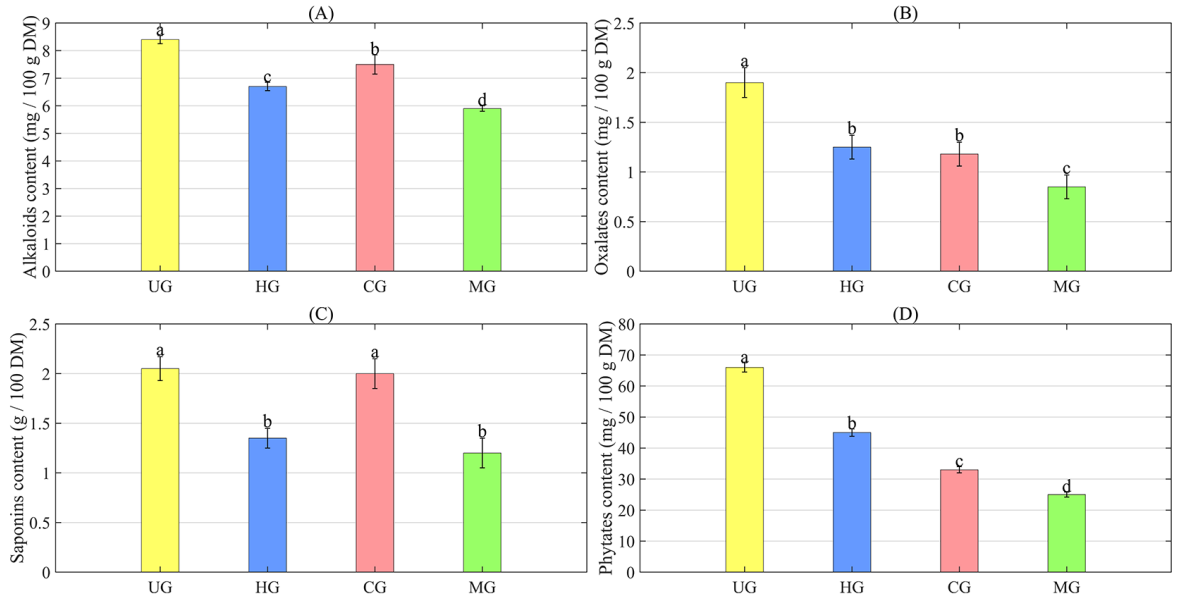

Figure 3. Antinutritional factors in various M. oleifera seed flours, including (A) Alkaloids, (B) oxalates, (C) saponins, (D) phytates. UG: ungerminated seeds; HG: hydropriming; CG: chemopriming; MG: biopriming. Values are expressed as mean ± standard deviation (n = 3); values with different letters are significantly different ($p \leq 0.05$) according to Tukey's test.

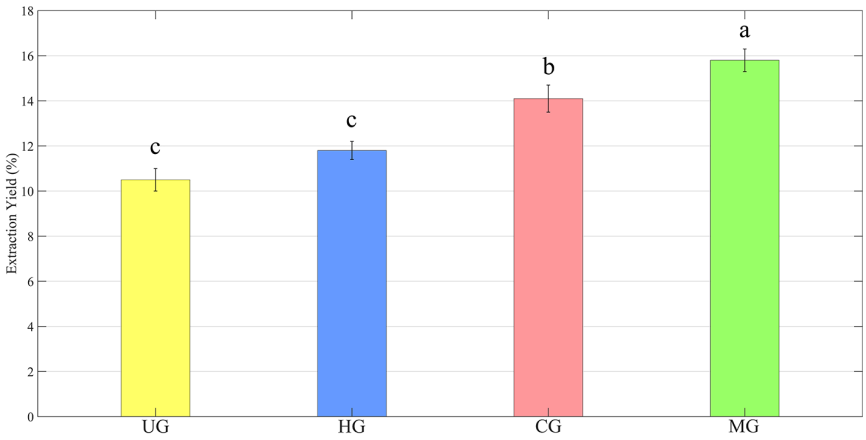

Figure. 4. Variation in extraction yield (%) of M. oleifera seed flour as a function of priming treatments: UG: raw, ungerminated seeds; HG: hydropriming; CG: chemopriming; MG: biopriming. Values are expressed as mean ± standard deviation (n = 3); values with different letters are significantly different ($p \leq 0.05$) according to Tukey's test

on pre-treatment conditions. The substantial increase observed in MG confirms the potential of priming and germination to synergistically enhance compound extractability. This improvement likely stems from a synergistic mechanism: germination activates endogenous hydrolytic enzymes that degrade the cell matrix (Liu et al., 2025); hydropriming optimizes this process through controlled hydration and cell wall remodeling (Frimpong et al., 2025); and biopriming with *Moringa* leaf extract further accelerates nutrient reserve mobilization and tissue structural changes (Yasmeen et al., 2013).

Total phenolic compounds

Priming treatments significantly enhanced the total phenolic content (TPC) compared to raw seeds (Figure 5a). A progressive increase was observed from hydropriming (HG) to chemopriming (CG) and biopriming (MG), with the latter achieving the highest value (388.13 ± 11.82 mg GAE/100 g DM, an 82.62% increase). This suggests a priming-dependent stimulation of phenolic biosynthesis. The increase may be attributed to the release of bound phenolics during germination, which is further promoted by hydropriming through enhanced water uptake and hydrolytic enzyme activity (Frimpong et al., 2025; Liu et al., 2025). The superior performance of biopriming may reflect the stimulatory effect of bioactive compounds and phytohormones in *Moringa* leaf extract on secondary metabolite accumulation during germination (Mashamaite et al., 2021; Soares et al., 2023). Similarly, León-López et al. (2019), reported a significant increase in TPC during germination of *Moringa* seeds, confirming that germination promotes phenolic accumulation. While Servín de la Mora-López et al. (2018) reported a 64% increase in TPC in germinated *Moringa* seeds, the 82.62% increase obtained with MG indicates that biopriming further enhances this response beyond germination alone. Given the established role of phenolics in mitigating oxidative stress, cardiovascular diseases, and metabolic disorders, these findings underscore the potential of MG-primed *Moringa* seeds as high-value functional food ingredients (Liu et al., 2025).

Flavonoids

Priming induced a significant rise in flavonoid content compared to raw seeds, particularly under CG and MG treatments (Figure 5b). The comparable performance of CG and MG suggests that biological elicitation can be as effective as chemical priming. The increase in flavonoids is mainly attributed to enhanced de novo biosynthesis via the phenylpropanoid pathway during germination (Miyahira et al., 2021). The higher flavonoid levels after biopriming may reflect the stimulatory effect of phytohormones and bioactive compounds in *Moringa* leaf extract on flavonoid biosynthesis (Soares et al., 2023; Yasmeen et al., 2013). Our findings align with Gu et al. (2020), confirming the extractable potential of *Moringa* flavonoids. Furthermore, Soares et al. (2023) demonstrated that *Moringa* leaf extract is rich in flavonoids and other bioactive

compounds, supporting the greater flavonoid accumulation observed after biopriming. Given their recognized antioxidant properties, the increased flavonoid content further supports the use of primed germinated *Moringa* seeds as functional food ingredients (Liu et al., 2025).

Flavonols

Flavonol content increased significantly across all priming treatments compared to raw seeds (50.99 ± 3.91 mg QE/100 g DM). The highest accumulation was observed in MG-treated seeds (103.62 ± 3.62 mg QE/100 g DM, +103%), followed by CG (+83.50%) and HG (+37.46%) (Figure 5c). This highlights the effectiveness of MLE-based biopriming in enhancing flavonol biosynthesis, likely through activation of the phenylpropanoid pathway and phenylalanine ammonia-lyase (PAL) activity (Frimpong et al., 2025). The greater accumulation after biopriming may reflect the stimulatory effect of phytohormones and bioactive compounds in *Moringa* leaf extract on secondary metabolism (Soares et al., 2023; Yasmeen et al., 2013). This observation is consistent with Król et al. (2015), who highlighted that elicitor-based priming induces systemic defense mechanisms, resulting in the upregulation of secondary metabolites. Similarly, Márton et al. (2010) reported that flavonol accumulation in sprouts is strongly influenced by germination conditions, supporting the present findings that optimizing priming conditions further enhances flavonol biosynthesis. Because flavonols are among the most potent antioxidant flavonoids, their marked increase further strengthens the functional and nutraceutical value of primed germinated *Moringa* seed flour (Liu et al., 2025).

Hydrolysable tannins

Priming treatments promoted the accumulation of hydrolysable tannins (Figure 5d). Specifically, HG (+45.43%) and MG (+67.52%) showed significant increases, whereas CG elicited the highest content (165.45 ± 8.75 mg TAE/100 g DM). The superior performance of chemopriming may be attributed to the regulatory role of ascorbic acid during germination, promoting the accumulation of bioactive phytochemicals (Zrig et al., 2023). These results are in close agreement with León-López et al. (2019), who observed a similar range (169.98 ± 6.98 mg TAE/100 g DM) in germinated *Moringa* seeds. The observed increase suggests that, unlike some antinutrients, tannins may be synthesized or released during metabolic reactivation to bolster antioxidant defense. However, because tannins can form complexes with proteins (Matthew et al., 2022),

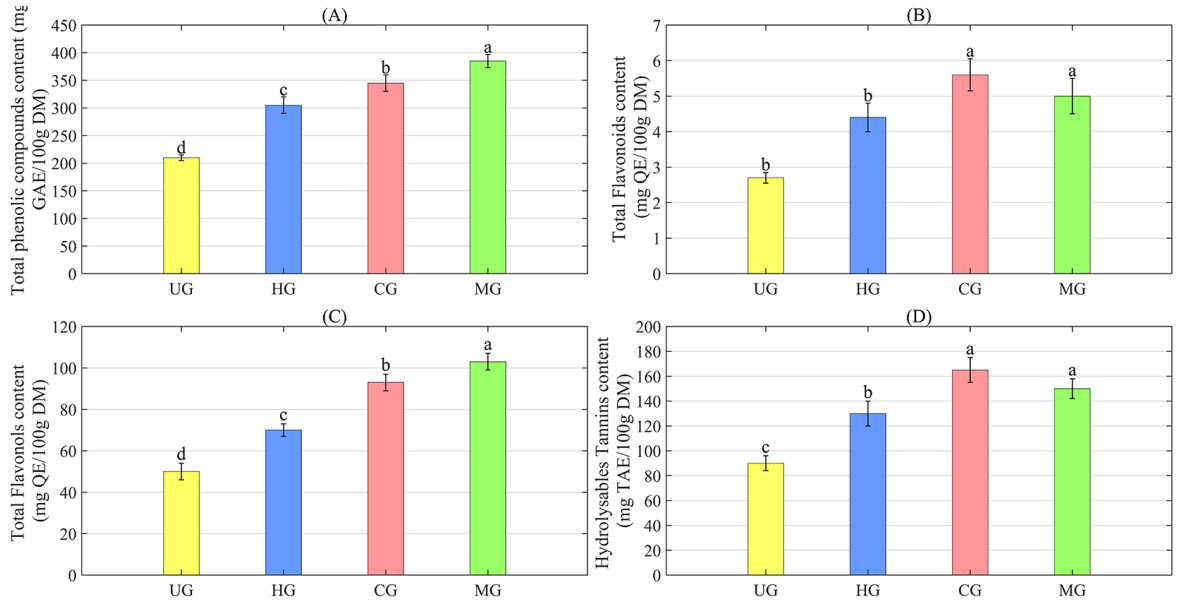

Figure 5. Effects of priming treatments on the bioactive phenolic compounds in *M. oleifera* seed flour, including (A) total phenolic compounds, (B) flavonoids, (C) flavonols, and (D) hydrolysable tannins. UG: ungerminated seeds; HG: hydropriming; CG: chemopriming; MG: biopriming.Values are expressed as mean ± standard deviation (n = 3); values with different letters are significantly different ($p \leq 0.05$) according to Tukey's test

further studies are needed to evaluate the nutritional balance between increased antioxidant capacity and antinutritional potential (León-López et al., 2019; Saa et al., 2022). Nevertheless, the marked accumulation of hydrolysable tannins may substantially contribute to the enhanced antioxidant capacity and functional value of primed *Moringa* seed flour.

Effect of priming treatments on antioxidant activity

Priming treatments significantly enhanced the antioxidant activity of *M. oleifera* seed flour across all assessed assays (DPPH, ABTS, and FRAP; Figure 6). Chemopriming (CG) proved the most effective, yielding the highest values in every assay. Notably, CG nearly doubled the DPPH (+94.11%) and FRAP (+101.35%) activities compared with the control, while also producing the highest ABTS scavenging activity (40.90 ± 0.44%). Biopriming (MG) displayed intermediate effects, while hydropriming (HG) showed the least impact, establishing an efficacy order of HG < MG < CG. This superior antioxidant performance is consistent with the marked accumulation of flavonoids and hydrolysable tannins observed in CG-treated seeds, indicating that antioxidant activity depends not only on the total amount of phenolic compounds but also on their composition and reducing capacity. Beyond its intrinsic radical-scavenging properties, ascorbic acid maintains cellular redox homeostasis during germination and promotes the biosynthesis of antioxidant metabolites and enzymes, thereby contributing to the preservation and accumulation of phenolic compounds and enhancing the overall antioxidant capacity of germinated seeds (Soares et al., 2023; Zrig et al., 2023). These findings agree with Zrig et al. (2023), who demonstrated that ascorbic acid priming improves the antioxidant potential of germinated seeds through redox regulation and stimulation of secondary metabolism.

Principal component analysis, hierarchical clustering heatmap, a nd hierarchical cluster analysis of *M. oleifera* seed flours

To obtain an integrated overview of the physicochemical, nutritional, antinutritional, and antioxidant changes induced by the different priming treatments, multivariate analyses including PCA, hierarchical clustering heatmap, and hierarchical cluster analysis (HCA) were performed (Figures 7–9). The PCA biplot (Figure 7) explained 81.01% of the total variance on the first two principal components (F1 = 68.39%; F2 = 12.62%),

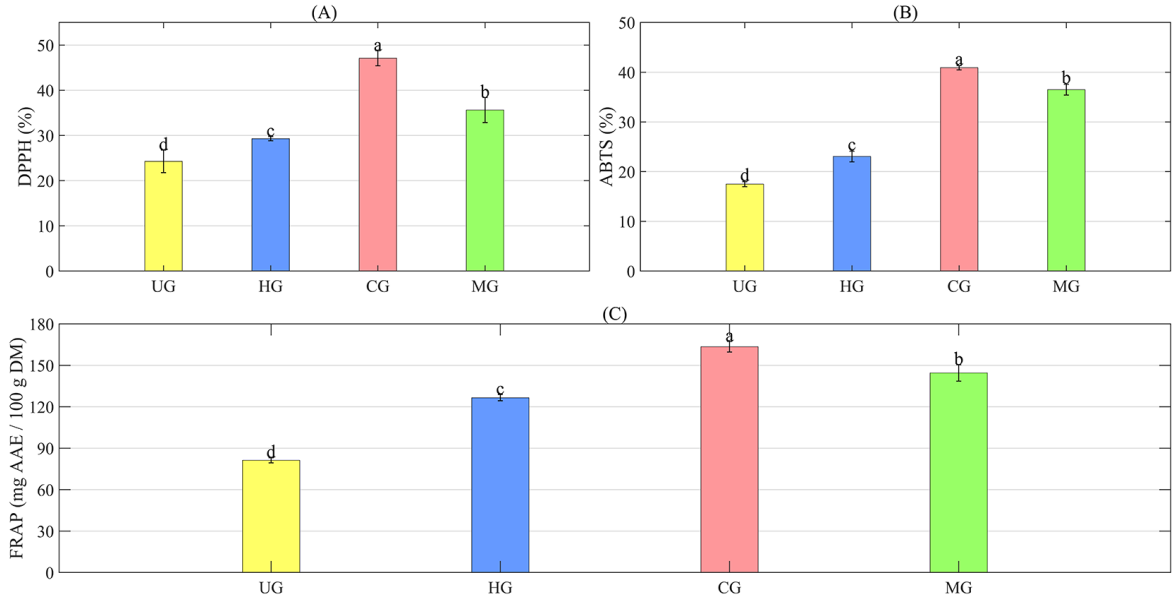

Figure 6. Effects of priming treatments on the antioxidant activity of M. oleifera seed flour, as assessed by three methods: (A) DPPH radical scavenging activity, (B) ABTS radical scavenging activity, and (C) FRAP reducing power. UG: raw, ungerminated seeds; HG: hydropriming; CG: chemopriming; MG: biopriming. Values are expressed as mean ± standard deviation (n = 3); values with different letters are significantly different ($p \leq 0.05$) according to Tukey's test

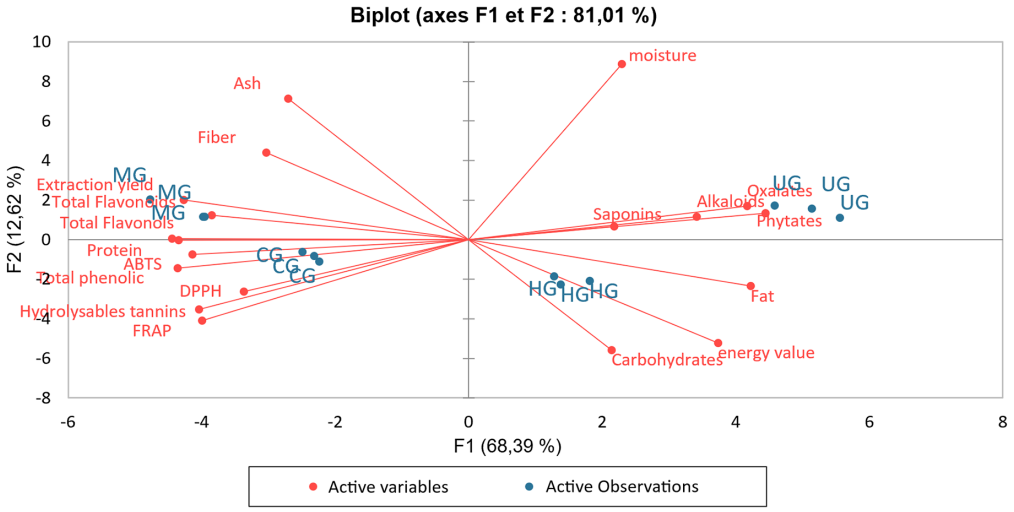

Figure 7. Principal component analysis (PCA) biplot of *M. oleifera* seed flours subjected to different priming treatments. Arrows represent the contribution and direction of the variables, whereas points correspond to the biological replicates of each treatment

indicating that the principal plane adequately represented the relationships among variables and treatments. The first principal component (F1) clearly separated two contrasting groups of variables. On the positive side, fat, energy value, carbohydrates, and antinutritional compounds (phytates, oxalates, alkaloids, and saponins) were closely associated with the ungerminated control (UG). Conversely, protein, fiber, ash, extraction yield, total phenolics, flavonoids, flavonols, hydrolysable tannins, and antioxidant activities (DPPH, ABTS, and FRAP) were negatively correlated with F1 and associated with primed and germinated samples. The score plot revealed a progressive discrimination of treatments along F1 following the sequence UG → HG → CG → MG, suggesting a gradual metabolic shift induced by priming and germination. The second component (F2) accounted for 12.62% of the total variance and was mainly influenced by moisture, ash, and fiber, providing additional discrimination between HG and the remaining treatments.

The hierarchical clustering heatmap (Figure 8) confirmed the PCA results by revealing two well-defined clusters of variables. The first cluster comprised fat, energy value, carbohydrates, and antinutritional factors, whereas the second grouped protein, fiber, ash, extraction yield, phenolic compounds, and antioxidant activities. Accordingly, UG exhibited higher standardized levels of lipids, energy-related components, and antinutritional compounds but lower levels of phenolic compounds and antioxidant activity. In contrast, MG displayed the opposite profile, characterized by enhanced protein, fiber, extraction yield, and phenolic compounds together with reduced antinutritional factors. CG was primarily associated with superior antioxidant activity, while HG showed an intermediate profile between the ungerminated control and the two advanced priming treatments. The HCA dendrogram (Figure 9) further supported these observations by clearly separating the samples into four clusters corresponding to UG, HG, CG, and MG, with all biological replicates clustering tightly within their respective treatments. Moreover, the dendrogram revealed two major branches: one grouping CG and MG, and the other HG and UG, indicating that chemopriming and biopriming induced more pronounced biochemical modifications than hydropriming. The close clustering of biological replicates also demonstrates the high reproducibility and robustness of the experimental data.

The strong agreement among the PCA, heatmap, and HCA revealed a treatment-dependent metabolic gradient extending from the UG to the primed treatments, with MG representing the most advanced stage of this transition. The progressive decrease in fat, energy value, and antinutritional factors, together with the increase in protein, phenolic compounds, and antioxidant activities, reflects the metabolic reprogramming associated with germination, characterized by reserve mobilization and the activation of hydrolytic enzymes (Liu et al., 2025). Similar responses have been reported in several germinated

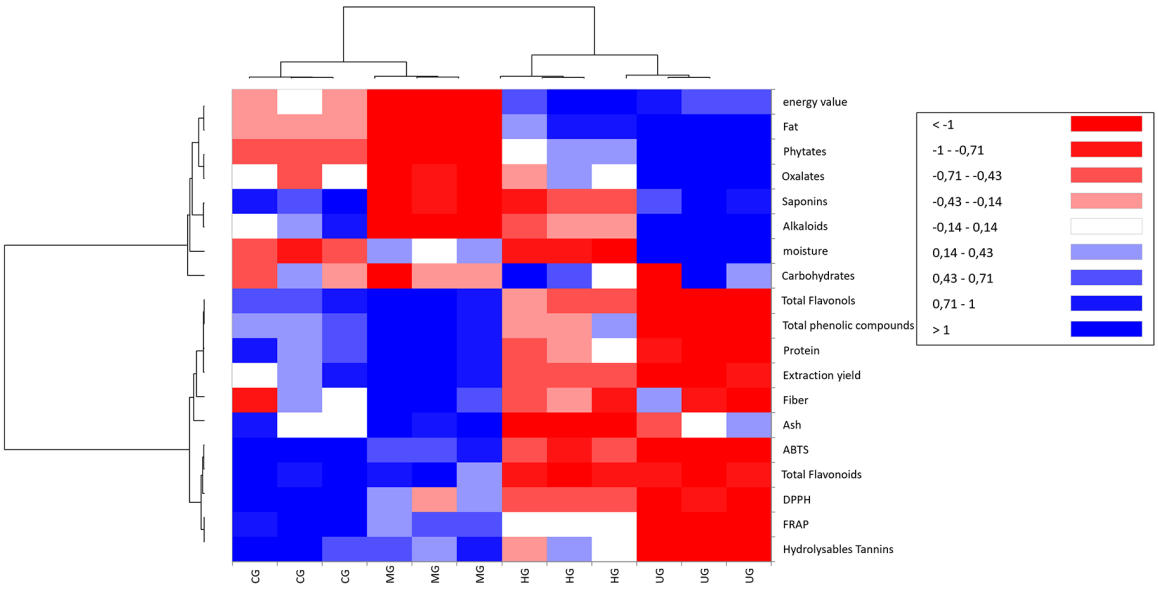

Figure 8. Hierarchical clustering heatmap of *M. oleifera* seed flours subjected t different priming treatments. Columns represent the biological replicates of the four treatments (UG, HG, CG, and MG), whereas rows correspond to the analyzed variables. The color scale represents standardized values (z-scores), illustrating similarities and differences among treatments

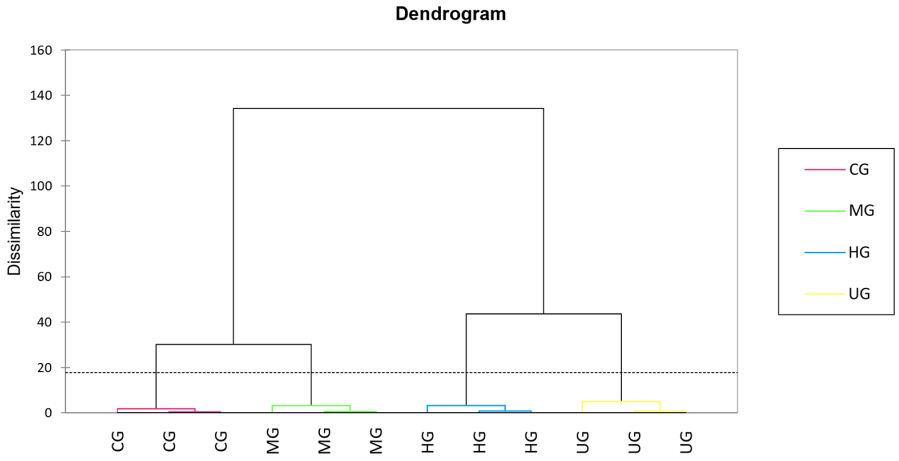

Figure 9. Hierarchical cluster analysis (HCA) dendrogram of *M. oleifera* different seed flours based on nutritional composition, antinutritional factors, bioactive compounds, and antioxidant activities. Samples were clustered according to their similarity, allowing discrimination of the four treatments

cereals and legumes, including substantial reductions in phytates (up to approximately 75%) and concomitant increases in phenolic compounds and antioxidant capacity (Yılmaz Tuncel et al., 2025). The superior performance of MG is likely attributable to the biostimulant properties of *M. oleifera* leaf extract, which is rich in phytohormones, antioxidants, minerals, amino acids, and soluble sugars capable of stimulating seed metabolism and secondary metabolite biosynthesis (Khan et al., 2022). Similar improvements in the nutritional quality and antioxidant potential of bioprimed seeds have also been reported by Nawaz et al. 2024), supporting the effectiveness of Moringa leaf extract as a natural priming agent. In contrast, CG showed the strongest association with antioxidant activity, particularly DPPH, ABTS and FRAP, which is consistent with the regulatory role of ascorbic acid in the ascorbate–glutathione cycle and its ability to stimulate antioxidant metabolism and defense enzymes such as superoxide dismutase, catalase and peroxidase (Han et al., 2025; Mi et al., 2025). Hydropriming (HG) exhibited intermediate characteristics, reflecting the activation of early germination metabolism through water uptake alone, without the

additional hormonal and antioxidant stimulation provided by biopriming or chemopriming.

Overall, the three complementary multivariate approaches consistently demonstrate that priming substantially modifies the biochemical profile of *M. oleifera* seed flour. Their combined interpretation confirms that *Moringa* leaf extract biopriming is the most effective strategy for improving nutritional quality, phenolic composition, and reducing antinutritional factors. In contrast, ascorbic acid priming provides the greatest enhancement of in vitro antioxidant capacity. These findings are consistent with previous chemometric studies on germinated seeds, in which the combined use of PCA, heatmap and HCA has proven effective for discriminating treatments and characterizing biochemical changes during germination (Guo et al., 2021).

CONCLUSIONS

This study demonstrated that hydro-, chemo-, and biopriming treatments combined with germination constitute effective eco-friendly strategies for improving the nutritional, phytochemical, and antioxidant profiles of *M. oleifera* seeds while reducing their antinutritional factors. Among the treatments evaluated, biopriming was the most effective for enhancing the accumulation of bioactive compounds, whereas chemopriming produced the greatest improvement in antioxidant activity. Beyond confirming the benefits of germination, this comparative study highlights the value of selecting specific priming strategies to tailor the nutritional and functional quality of *M. oleifera* seeds. These findings highlight the potential of priming-assisted germination as a sustainable biotechnological approach for valorizing *M. oleifera* seeds and promoting their application in functional foods and nutraceutical products. Further studies should investigate the bioaccessibility of key bioactive compounds together with the techno-functional and sensory properties of primed germinated flours in real food systems to support their industrial application.

REFERENCES

1. Adeleke, O., Adiamo, O. Q., Fawale, O. S., Olamiti, G. (2017). Effect of soaking and boiling on antinutritional factors, oligosaccharide contents and protein digestibility of newly developed bambara groundnut cultivars. *Turkish Journal of Agriculture - Food Science and Technology*, *5*(9), 1006–1014. https://doi.org/10.24925/turjaf.v5i9.1006-1014.949
2. Afify, A. E. M. M. R., El-Beltagi, H. S., Abd El-Salam, S. M., Omran, A. A. (2012). Biochemical changes in phenols, flavonoids, tannins, vitamin E, β -carotene and antioxidant activity during soaking of three white sorghum varieties. *Asian Pacific Journal of Tropical Biomedicine*, *2*(3), 203–209. https://doi.org/10.1016/S2221-1691(12)60042-2
3. AOAC International. (2019). *Official methods of analysis of AOAC International* (21st ed.). AOAC International.
4. Baltazar, M., Oppolzer, D., Carvalho, A., Gouvinhas, I., Ferreira, L., Barros, A., Lima-Brito, J. (2023). Hydropriming and nutripriming of bread wheat seeds improved the flour's nutritional value of the first unprimed offspring. *Plants*, *12*(2). https://doi.org/10.3390/plants12020240
5. Calizaya-Milla, Y. E., Saintila, J., García-García, J. P., Dávila Villavicencio, R., Vásquez-Vásquez, J. E., Pacheco-Espinoza, J. I., Sotelo-Méndez, A., Pampa-Quispe, N. B. (2022). Macronutrients and micronutrients in germinated and non-germinated seed flour and moringa leaves *(Moringa oleifera* L.). *Food Research*, *6*(4), 138–145. https://doi.org/10.26656/fr.2017.6(4).448
6. Carović-Stanko, K., Petek, M., Grdiša, M., Pintar, J., Bedeković, D., Ćustić, M. H., Satovic, Z. (2016). Medicinal plants of the family lamiaceae as functional foods-A review. In *Czech Journal of Food Sciences 34*(5), 377–390. Czech Academy of Agricultural Sciences. https://doi.org/10.17221/504/2015-CJFS
7. Chinma, C. E., Lata, L. J., Chukwu, T. M., Azeez, S., Ogunsina, B. S., Ohuoba, E., Yakubu, C. (2017). Effect of germination time on the proximate composition and functional properties of moringa seed flour. *African Journal of Agriculture, Technology and Environment*, *6*(2), 117–133. http://repository.futminna.edu.ng:8080/jspui/handle/123456789/15872
8. Coello, K. E., Frias, J., Martínez-Villaluenga, C., Cartea, M. E., Abilleira, R., Peñas, E. (2020). Potential of germination in selected conditions to improve the nutritional and bioactive properties of moringa (*Moringa oleifera* l.). *Foods*, *9*(11). https://doi.org/10.3390/foods9111639
9. Duary, S. (2020). Seed priming: A comprehensive approach to alter the biotic and abiotic stresses of field crops. *International Journal of Chemical Studies*, *8*(5), 1705–1708. https://doi.org/10.22271/chemi.2020.v8.i5w.10545
10. Ebert, A. W. (2022). Sprouts and microgreens—novel food sources for healthy diets. In *Plants* (Vol. 11, Number 4). MDPI. https://doi.org/10.3390/plants11040571
11. Ejikeme, C. M., Ezeonu, C. S., Eboatu, A. N. (2014). Determination of physical and phytochemical

constituents of some tropical timbers indigenous to Niger delta area of Nigeria. *European Scientific Journal*, *10*(18), 247–270.

12. El-Anoos, E., Fahmy, H., Mahmoud, E., Sharaf, A. (2022). Effect of soaking process on chemical characteristics and anti nutritional factors of some moringa seed varieties. *Journal of Food and Dairy Sciences*, *13*(1), 9–15. https://doi.org/10.21608/jfds.2022.117048.1035
13. Ellouzi, H., Oueslati, S., Hessini, K., Rabhi, M., Abdelly, C. (2021). Seed-priming with H_2O_2 alleviates subsequent salt stress by preventing ROS production and amplifying antioxidant defense in cauliflower seeds and seedlings. *Scientia Horticulturae*, *288*, 110360. https://doi.org/10.1016/J.SCIENTA.2021.110360
14. Falleh, H., Ksouri, R., Chaieb, K., Karray-Bouraoui, N., Trabelsi, N., Boulaaba, M., Abdelly, C. (2008). Phenolic composition of *Cynara cardunculus* L. organs, and their biological activities. *Comptes Rendus - Biologies*, *331*(5), 372–379. https://doi.org/10.1016/j.crvi.2008.02.008
15. FAO. (2003). *Food energy : methods of analysis and conversion factors : report of a technical workshop, Rome, 3-6 December 2002*. FAO.
16. Frimpong, R. A. Y., Affrifah, N. S., Amissah, J. G. N., Agyei-Amponsah, J., Kerler, J., Saalia, F. K. (2025). Impact of priming and sprouting on the quality and functionality of *Moringa oleifera* seed flour. *International Journal of Food Science*, *2025*(1). https://doi.org/10.1155/ijfo/3909494
17. Gu, X., Yang, Y., Wang, Z. (2020). Nutritional, phytochemical, antioxidant, α-glucosidase and α-amylase inhibitory properties of *Moringa oleifera* seeds. *South African Journal of Botany*, *133*, 151–160. https://doi.org/10.1016/j.sajb.2020.07.021
18. Gul, P., Khan, J., Li, Y., Li, Q., Zhang, H., Liu, K. (2025). Drying characteristics and quality enhancement of *Moringa oleifera* seeds through ultrasound and microwave-assisted germination combined with infrared vacuum drying. *Ultrasonics Sonochemistry*, *120*. https://doi.org/10.1016/j.ultsonch.2025.107432
19. Guo, S., Klinkesorn, U., Lorjaroenphon, Y., Ge, Y., Na Jom, K. (2021). Effects of germinating temperature and time on metabolite profiles of sunflower (*Helianthus annuus* L.) seed. *Food Science & Nutrition*, *9*(6), 2810–2822. https://doi.org/10.1002/fsn3.1983
20. Han, P., Ma, H., Lu, L., Zhu, J., Nie, X., Xu, J., Li, Z. (2025). Ascorbic. *Plants*, *14*(20). https://doi.org/10.3390/plants14203122 acid priming boosts cotton seed chilling tolerance via membrane stability and antioxidant cycles
21. Haug, W., Lantzsch, H. (1983). Sensitive method for the rapid determination of phytate in cereals and cereal products. *Journal of the Science of Food and Agriculture*, *34*(12), 1423–1426. https://doi.org/10.1002/jsfa.2740341217
22. Ibraheem, A. S., Nuhu, A., Musa, M., Ashade, N. O. (2019). Evaluation of proximate content and vitamin profile of *Moringa oleifera* seed hull. *International Journal of Livestock Production*, *10*(8), 188–191. https://doi.org/10.5897/ijlp2018.0512
23. Ijarotimi, O. S., Adeoti, O. A., Ariyo, O. (2013). Comparative study on nutrient composition, phytochemical, and functional characteristics of raw, germinated, and fermented Moringa oleifera seed flour. *Food Science & Nutrition*, *1*(6), 452–463. https://doi.org/10.1002/fsn3.70
24. Kacem, N. S. (2024). Assessment of the germination potential of *Moringa oleifera* seeds under different treatment conditions. *Ecological Engineering and Environmental Technology*, *25*(11), 321–325. https://doi.org/10.12912/27197050/192836
25. Khan, S., Basra, S. M. A., Afzal, I., Nawaz, M., Rehman, H. U. (2017). Growth promoting potential of fresh and stored Moringa oleifera leaf extracts in improving seedling vigor, growth and productivity of wheat crop. *Environmental Science and Pollution Research*, *24*(35), 27601–27612. https://doi.org/10.1007/s11356-017-0336-0
26. Khan, S., Ibrar, D., Bashir, S., Rashid, N., Hasnain, Z., Nawaz, M., Al-Ghamdi, A. A., Elshikh, M. S., Dvořáčková, H., Dvořáček, J. (2022). Application of *Moringa leaf* extract as a seed priming agent enhances growth and physiological attributes of rice seedlings cultivated under water deficit regime. *Plants*, *11*(3). https://doi.org/10.3390/plants11030261
27. Koushal, S., Mankar, A. A., Anbarasan, S., Kumar, V., Kumari, J., S., N., Jahan, R., R, K. K., Satapathy, S. N. (2024). Mechanism and methodologies of seed priming: Enhancing germination and crop resilience. *Plant Cell Biotechnology and Molecular Biology*, *25*(11–12), 185–194. https://doi.org/10.56557/pcbmb/2024/v25i11-128944
28. Król, P., Igielski, R., Pollmann, S., Kepczyńska, E. (2015). Priming of seeds with methyl jasmonate induced resistance to hemi-biotroph *Fusarium oxysporum* f.sp. lycopersici in tomato via 12-oxophytodienoic acid, salicylic acid, and flavonol accumulation. *Journal of Plant Physiology*, *179*, 122–132. https://doi.org/10.1016/J.JPLPH.2015.01.018
29. Lemmens, E., Moroni, A. V., Pagand, J., Heirbaut, P., Ritala, A., Karlen, Y., Lê, K., Van den Broeck, H. C., Brouns, F. J. P. H., De Brier, N., Delcour, J. A. (2019). Impact of cereal seed sprouting on its nutritional and technological properties: A critical review. *Comprehensive Reviews in Food Science and Food Safety*, *18*(1), 305–328. https://doi.org/10.1111/1541-4337.12414
30. León-López, L., Bañuelos-Piña, A. M., Reyes-Moreno, C., Milán-Carrillo, J. Contreras-Andrade,

Sánchez-Magaña, L. M., Cuevas-Rodríguez, E. O. (2019). Optimisation of temperature and time for the dark germination bioprocess of *Moringa oleifera* seeds to boost nutritional value, total phenolic content and antioxidant activity. *International Food Research Journal*, 831–839. http://www.ifrj.upm.edu.my

31. Liu, Y., Kai, Y., Huang, D., Liu, S. Q., Lu, Y. (2025). A Comprehensive review of germination impact on Moringa seeds and sprouts: Physiological and biochemical changes, bioactive compounds, health benefits, and food applications. In *Comprehensive Reviews in Food Science and Food Safety* (Vol. 24, Number 6). John Wiley and Sons Inc. https://doi.org/10.1111/1541-4337.70296
32. Lv, Y., Yang, X., Zhao, Y., Ruan, Y., Yang, Y., Wang, Z. (2009). Separation and quantification of component monosaccharides of the tea polysaccharides from *Gynostemma pentaphyllum* by HPLC with indirect UV detection. *Food Chemistry*, *112*(3), 742–746. https://doi.org/10.1016/j.foodchem.2008.06.042
33. Márton, M., Mándoki, Z., Csapó-Kiss, Z., Csapó, J. (2010). The role of sprouts in human nutrition. A review. In *Alimentaria* (Vol. 3).
34. Mashamaite, C. V., Pieterse, P. J., Mothapo, P. N., Phiri, E. E. (2021). *Moringa oleifera* in South Africa: A review on its production, growing conditions and consumption as a food source. *South African Journal of Science*, *117*(3/4). https://doi.org/10.17159/sajs.2021/8689
35. Matthew, O. J., Saidu, A. N., Jigam, A. A., Ocheme, O. B. (2022). Nutritional, antinutritional, and functional properties of different processed (soaking, germination, and boiling) flour from *Moringa oleifera* lam (moringa) seed. *Journal of Food Processing and Preservation*, *46*(9). https://doi.org/10.1111/jfpp.16052
36. Abdalla, M. M. F., Abdelhamid, M. T., Ahmad, M. (2019). Priming and Pretreatment of Seeds and Seedlings. In H. Mirza, F. Vasileios (Eds.), *Priming and Pretreatment of Seeds and Seedlings* (Springer). Springer Singapore. https://doi.org/10.1007/978-981-13-8625-1
37. Mbaebie, B. O., Edeoga, H. O., Afolayan, A. J. (2012). Phy to chemical analysis and antioxidants activities of aqueous stem bark extract of *Schotia latifolia* Jacq. *Asian Pacific Journal of Tropical Biomedicine*, *2*(2), 118–124. https://doi.org/10.1016/S2221-1691(11)60204-9
38. Mi, C., Hong, L., Sun, S., Zhao, S., Dou, L., Mao, P. (2025). Ascorbic acid priming restores the seed vigor by enhancing the mitochondrial AsA-GSH cycle and related gene expression in the aged oat seeds. *Physiologia Plantarum*, *177*(2). https://doi.org/10.1111/ppl.70190
39. Miyahira, R. F., De, J., Lopes, O., Elisabete, A., Antunes, C. (2021). The use of sprouts to improve the nutritional value of food products: A brief review. *Plant Foods for Human Nutrition*. https://doi.org/10.1007/s11130-021-00888-6/Published
40. Mole, S., Waterman, P. G. (1987). A critical analysis of techniques for measuring tannins in ecological studies. *Oecologia*, *72*(1), 148–156. https://doi.org/10.1007/BF00385059
41. Nawaz, H., Rehman, H., Yousef, M. M., Ali, L., Hussain, N., Abdullah, M., Iqbal, R., Alharbi, S. A., Ansari, M. J., Kriaučiūnienė, Z., Ayaz, M. (2024). Zinc-priming via Moringa leaf extract (MLE30) achieved Zn biofortified wheat grains and drought tolerance by efficient anti-oxidant status. *Plant Stress*, *12*, 100501. https://doi.org/10.1016/j.stress.2024.100501
42. Ndife, U., Onwuzuruike, Ofia-Olua, J. (2020). Effects of different processing methods on the quality of *Moringa oleifera* seed flours. In *Science & Sustainable Tech* (Vol. 2, Number 1). www.mouau.edu.ng.
43. Nouman, W., Basra, S. M. A., Yasmeen, A., Gull, T., Hussain, S. B., Zubair, M., Gul, R. (2014). Seed priming improves the emergence potential, growth and antioxidant system of *Moringa oleifera* under saline conditions. *Plant Growth Regulation*, *73*(3), 267–278. https://doi.org/10.1007/s10725-014-9887-y
44. Owon, M., Osman, M., Ibrahim, A., Salama, M. A., Matthäus, B. (2021). Characterisation of different parts from Moringa oleifera regarding protein, lipid composition and extractable phenolic compounds. *OCL - Oilseeds and Fats, Crops and Lipids*, *28*. https://doi.org/10.1051/ocl/2021035
45. Rayan, A. M., Embaby, H. E. (2016). Effects of dehulling, soaking, and cooking on the nutritional quality of Moringa oleifera seeds. *Journal of Agroalimentary Processes and Technologies*, *22*(3), 156–165.
46. Saa, R. W., Fombang, E. N., Ndjantou, E. B., Njintang, N. Y. (2019). Treatments and uses of Moringa oleifera seeds in human nutrition: A review. In *Food Science and Nutrition* (Vol. 7, Number 6, pp. 1911–1919). Wiley-Blackwell. https://doi.org/10.1002/fsn3.1057
47. Saa, R. W., Fombang Nig, E., Radha, C., Ndjantou, E. B., Njintang Yanou, N. (2022). Effect of soaking, germination, and roasting on the proximate composition, antinutrient content, and some physicochemical properties of defatted *Moringa oleifera* seed flour. *Journal of Food Processing and Preservation*, *46*(3). https://doi.org/10.1111/jfpp.16329
48. Santos, C. S., Silva, B., Valente, L. M. P., Gruber, S., Vasconcelos, M. W. (2020). The effect of sprouting in lentil (*Lens culinaris*) nutritional and microbiological profile. *Foods*, *9*(4). https://doi.org/10.3390/foods9040400

49. Servín de la Mora-López, G., López-Cervantes, J., Gutiérrez-Dorado, R., Cuevas-Rodríguez, E. O., Milán-Carrillo, J., Sánchez-Machado, D. I., Reyes-Moreno, C. (2018). Effect of optimal germination conditions on antioxidant activity, phenolic content and fatty acids and amino acids profiles of *Moringa oleifera* seeds. *Revista Mexicana de Ingeniera Quimica*, *17*(2), 547–560. https://doi.org/10.24275/10.24275/uam/izt/dcbi/revmexingquim/2018v17n2/Servin
50. Singleton, V. L., Orthofer, R., Lamuela-Ravent6s, R. M. (1999). *52 Polyphenols and Flavonoids [14] [14]* Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent.
51. Soares, T. F. S. N., Muniz, E. N., Sousa, J. P. S., Oliveira Júnior, L. F. G. de, Barbosa, A. M., Silva, A. V. C. da. (2023). Seed priming as a strategy to increase the performance of drumstick tree. *South African Journal of Botany*, *157*, 279–286. https://doi.org/10.1016/j.sajb.2023.03.037
52. Tesfay, S. Z., Modi, A. T., Mohammed, F. (2016). The effect of temperature in moringa seed phytochemical compounds and carbohydrate mobilization. *South African Journal of Botany*, *102*, 190–196. https://doi.org/10.1016/j.sajb.2015.07.003
53. Waseem, M., Akhtar, S., Ahmad, N., Ismail, T., Lazarte, C. E., Hussain, M., Manzoor, M. F. (2022). Effect of microwave heat processing on nutritional indices, antinutrients, and sensory attributes of potato powder-supplemented flatbread. *Journal of Food Quality*, *2022*. https://doi.org/10.1155/2022/2103884
54. Woisky, R. G., Salatino, A. (1998). Analysis of propolis: Some parameters and procedures for chemical quality control. *Journal of Apicultural Research*, *37*(2), 99–105. https://doi.org/10.1080/00218839.1998.11100961
55. Yasmeen, A., Basra, S. M. A., Wahid, A., Nouman, W., Hafeez-ur-Rehman. (2013). Exploring the potential of *Moringa oleifera* leaf extract (MLE) as a seed priming agent in improving wheat performance. *Turkish Journal of Botany*, *37*(3), 512–520. https://doi.org/10.3906/bot-1205-19
56. Yılmaz Tuncel, N., Polat Kaya, H., Sakarya, F. B., Andaç, A. E., Korkmaz, F., Ozkan, G., Tuncel, N. B., Capanoglu, E. (2025). The effect of germination on antinutritional components, in vitro starch and protein digestibility, content, and bioaccessibility of phenolics and antioxidants of some pulses. *Food Science and Nutrition*, *13*(5). https://doi.org/10.1002/fsn3.70103
57. Zrig, A., Yousif Sidahmed Elsheikh, S., Hamouda, F., Najar, B., A. Alsherif, E., Magdy Korany, S., Hassan, A. H. A., AbdElgawad, H. (2023). Potassium nitrate and ascorbic acid priming improved tissue chemical composition and antioxidant and antimicrobial activities of linseed (*Linum usitatissimum* L.) Sprouts. *ACS Omega*, *8*(39), 35975–35987. https://doi.org/10.1021/acsomega.3c03002