

Comparative effects of germination and cooking processes on the functional and therapeutic properties of horse gram (*Macrotyloma uniflorum*)

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ABSTRACT

The study evaluated the effects of germination and cooking on the chemical composition, functional properties, antioxidant activity, and antidiabetic potential of horse gram (*Macrotyloma uniflorum*) flour. Morphological and structural changes were analyzed using Field Emission Scanning Electron Microscopy (FE-SEM) and Fourier Transform Infrared Spectroscopy (FTIR). Germinated horse gram (HG-G) exhibited significantly higher levels of crude protein, crude fiber, phenolics, and flavonoids compared to cooked horse gram (HG-C) and raw horse gram (HG-R) flour. FTIR analysis revealed substantial structural alterations in carbohydrates, proteins, and lipids in both HG-G and HG-C, while FE-SEM imaging showed marked morphological differences in HG-G and HG-C compared to HG-R. Germination significantly enhanced key functional properties including gelation capacity, foaming ability, emulsification capacity, pasting behavior, thermal stability, and rheological characteristics compared to cooking and raw forms. Notably, HG-G demonstrated superior antioxidant capacity (DPPH, FRAP, and reducing power assays), and greater inhibition of α -amylase and α -glucosidase, indicating stronger antidiabetic potential. These results support the use of germinated and cooked horse gram flours as functional ingredients in therapeutic diets and nutraceutical formulations.

1. Introduction

Food legumes have served as a cornerstone of human nutrition for millennia, prized for their high nutritional value and environmental sustainability. Globally, they are considered one of the important sources of plant-based protein and essential nutrients, second only to cereals in terms of consumption and agricultural significance (Singh et al., 2020). India remains the world's largest producer and consumer of pulses (FAO, 2016). Among these, underutilized legumes hold significant potential as nutrient-dense foods, offering various health benefits while supporting both global and regional food security (Goyal et al., 2018).

Horse gram (*Macrotyloma uniflorum*), a climate-resilient legume native to the Indian subcontinent, remains underutilized and

underexplored despite its considerable potential (Handa et al., 2017). With increasing interest in nutritious, health-oriented diets, researchers are turning their attention to neglected crops like horse gram, which is a rich source of calcium, molybdenum, and iron (Prasad and Singh, 2015). This legume also boasts a nutrient-dense profile, abundant in protein, carbohydrates, and essential amino acids, offering substantial nutritional and energy benefits (Bhartiya et al., 2015). In addition, horse gram has been associated with several health benefits, including relief from asthma, ulcer healing, kidney stone prevention, and improved insulin sensitivity, earning it recognition as a "superfood" (Chauhan, 2022).

Despite these advantages, horse gram contains certain anti-nutritional factors that hinder mineral absorption, reduce protein digestibility, and contribute to the common "hard-to-cook" nature of

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many legumes (Pal et al., 2016; Prasad and Singh, 2015). These limitations restrict its broader adoption. To overcome such challenges, conventional processing methods like soaking, germination, and cooking are widely employed to reduce anti-nutritional components (Pal et al., 2016). Among them, germination has been particularly effective in enhancing the nutritional quality of legumes by reducing anti-nutrients, increasing bioactive compounds, and improving the bioavailability of proteins and minerals. It also reduces phytate levels, thereby enhancing iron and calcium absorption, while increasing dietary fiber, free amino acids, and other functional constituents (Frias et al., 2002; Vidal-Valverde et al., 2002). Cooking further improves the palatability and digestibility of legumes by deactivating enzyme inhibitors and hemagglutinins, leaching polyphenols, and gelatinizing starch (Wood, 2017).

While extensive studies have explored the effects of soaking and germination on the nutritional quality of horse gram (Ghumman et al., 2016; Handa et al., 2017; Moktan and Ojha, 2016; Pagar et al., 2021; Pal et al., 2016), limited comparative research exists on how both germination and cooking influences its nutritional value. Therefore, this study aimed to evaluate and compare the effects of germination and cooking on the chemical composition, structural characteristics, functional properties, antioxidant activity, and antidiabetic potential of horse gram.

2. Materials and methods

2.1. Processing of horse gram

2.1.1. Source of horse gram

Horse gram was procured from a local supplier in Pune, India. To ensure cleanliness, the grains were thoroughly rinsed with distilled water to remove any impurities or extraneous particles. After washing, the grains were dried at 40 °C for 8 h, stored in airtight containers, and maintained at 25 °C until further use.

2.1.2. Flour sample preparation

A total of 400 g of horse gram seeds were rinsed thoroughly. Of these, 200 g were soaked in 600 mL of distilled water at 25 °C for 6 h, and then allowed to germinate in a dark chamber at the same temperature for 48 h. The remaining 200 g were soaked in 500 mL of distilled water for 4 h and then cooked at 100 °C until no white core remained when crushed between glass slides (Seena and Sridhar, 2005). Following germination and cooking, the seeds were dehydrated separately in an oven at 50 °C for 10 h. Raw, germinated, and cooked seeds (200 g each) were ground into flour and passed through a No 40 U.S. Standard Testing Sieve to achieve a uniform particle size of 300 µm. The samples were labeled as horse gram raw (HG-R), horse gram germinated (HG-G) and horse gram cooked (HG-C), respectively. The flour samples were stored in airtight glass jars at 25 °C until further analysis.

2.1.3. Extract preparation

Ultrasound-assisted extraction was used to prepare extracts of HG-R, HG-G, and HG-C using with a UP200 Ultrasonic processor, following the method described by Peng et al. (2008). Acidified ethanol (40 %, adjusted to pH 1.5) was used as the solvent at a solvent-to-sample ratio of 20:1 (ethanol:sample). Extraction was performed at 100 % amplitude with a 0.5-second pulse cycle for 10 min. The extract was centrifuged 3500 × g for 15 min, filtered, and evaporated at 50 °C. The resulting extract was then freeze-dried and stored at -20 °C for subsequent analysis of total phenolic and flavonoid contents, as well as antioxidant and antidiabetic activities.

2.2. Chemical composition

2.2.1. Proximate composition

The proximate composition of the flours was determined to assess

moisture, crude fat, ash, and crude protein contents, following the standard procedures outlined by AOAC (2019). Carbohydrate content was calculated by difference.

2.2.2. Total phenolic content (TPC)

The total phenolic content of the flour extracts was measured using the Folin-Ciocalteu method (Hu et al., 2016). A working solution of the extract was prepared at a concentration of 1 mg/mL. For the assay, 0.5 mL of the extract was mixed with 2 mL of Folin-Ciocalteu reagent (diluted 1:1 with distilled water), vortexed thoroughly, and incubated at room temperature (RT) for 3 min. Subsequently, 4 mL of 2 % sodium carbonate solution was added, and the mixture was incubated in the dark for 1 h at RT. Absorbance was measured at 765 nm using a spectrophotometer, with a reagent blank used as the control. All measurement were performed in triplicate, and results were expressed as gallic acid equivalents (GAE).

2.2.3. Total flavonoid content (TFC)

The total flavonoid content of the flour extracts was determined using a modified colorimetric method (Chang et al., 2002). A 0.5 mL aliquot of extract (1 mg/mL) was mixed with 1.5 mL of 95 % ethanol, 0.1 mL of 10 % aluminum chloride hexahydrate, 0.1 mL of 1 M potassium acetate, and 2.8 mL deionized water. The mixture was incubated in the dark at 25 °C for 60 min. Absorbance was then recorded at 415 nm using a spectrophotometer, with a blank solution as reference. Quercetin (Sigma-Aldrich, USA) was used as the standard, and results were expressed as quercetin equivalents (QE) per gram of sample. All analyses were conducted in triplicate.

2.3. Structural characteristics

2.3.1. Surface morphology by field emission scanning electron microscopy (FE-SEM)

The HG-R, HG-G, and HG-C samples were mounted onto stubs using double-sided adhesive tape, following the method of Medhe et al. (2023). A thin platinum coating was applied using a Polaron SC515 Sputter Coater (VG Microtech, UK). The coated samples were then examined using a FE-SEM (Hitachi, Japan) operated at accelerating voltages ranging from 1 to 7 kV.

2.3.2. Fourier transform infrared (FTIR) spectroscopy analysis

The spectral properties of flour samples were analyzed using an FTIR spectrometer (Thermo Scientific, USA) (Kamble et al., 2022; Medhe et al., 2022). Samples were prepared by mixing flour with potassium bromide (KBr) in a 1:100 (w/w) ratio, finely grinding the mixture, and compressing it into pellets. Spectra were recorded in the 800–4000 cm⁻¹ range with a resolution of 4 cm⁻¹. Each sample was scanned 64 times, and the results were averaged to enhance precision. All measurements were performed in triplicate to ensure consistency and reliability.

2.4. Functional properties

2.4.1. Gelation capacity

Gelation capacity was evaluated using a modified method described by Ajibola et al. (2016). Flour solutions (2–30 % w/v) were prepared in distilled water, heated at 95 °C for 1 h, then cooled at 10 °C for 14 h. Gelation was visually assessed by inverting the test tubes to observe droplet separation. The minimum concentration at which no separation occurred was recorded as the lowest concentration.

2.4.2. Foaming properties

The foaming capacity was determined using a slightly modified method of Siddiq et al. (2010). Briefly, 2 g of flour was mixed with 100 mL of distilled water and homogenized using an IKA stirrer (RW20, Germany) at 4470 × g for 2 min. The foaming capacity was calculated using the following formula:

$$\text{Foaming capacity (\%)} = ((\text{Final volume} - \text{Initial volume}) / \text{Initial volume}) \times 100$$

The foam stability was assessed by measuring the volume of foam retained after 8 h at 25 °C, expressed as a percentage of the initial foam volume.

2.4.3. Emulsifying properties

Emulsifying properties were assessed following a modified method described by Neto et al. (2001). A mixture of 50 g flour, 5 mL distilled water, and 5 mL of soybean oil, was homogenized and centrifuged at 1100 × g for 5 min. The emulsifying capacity was calculated as:

$$\text{Emulsifying capacity (\%)} = (\text{Height of emulsified layer} / \text{Total height}) \times 100$$

To assess stability, the emulsions were heated at 80 °C for 30 min and centrifuged again under the same conditions. The stability was determined using:

$$\text{Emulsion stability (\%)} = (\text{Post-heating emulsified layer height} / \text{Pre-heating emulsified layer height}) \times 100.$$

2.4.4. Pasting properties

Pasting behavior was evaluated using a Rapid Visco Analyser (RVA-4, Australia) with 29 g flour dispersions. The test involved increasing the temperature from 50 °C to 95 °C at a rate of 6 °C/min, holding at 95 °C for 5 min, then cooling back to 50 °C. A 1-min stabilization period was applied at both the beginning and end. Parameters including pasting temperature, peak viscosity, trough, final viscosity, breakdown, and setback were recorded (Nasrin et al., 2015).

2.4.5. Thermal properties

Thermal characteristics were analyzed using a differential scanning calorimeter (DSC-1 STAR System, Mettler-Toledo, Switzerland). Approximately 20 mg of each sample was sealed in aluminum pans, and equilibrated at RT for 24 h. The temperature was increased from 20 °C to 110 °C at a 10 °C/min. An empty sealed pan was served as a reference. The onset temperature (T_o), peak temperature (T_p), and enthalpy change (ΔH) were recorded in triplicate, and presented as thermograms (Henshaw et al., 2003).

2.4.6. Rheological properties

The rheological behavior of the flour dispersions (10 % w/v) was evaluated using a rotational rheometer (HAAKE MARS 40) equipped with a 50 mm parallel plate system. The gap was set to 1 mm, with 2 % strain and 1 Hz frequency. The temperature was ramped from 40 °C to 95 °C, then cooled to 25 °C at 1 °C/min. After a 5-min equilibrium at 25 °C, a frequency sweep (0.1 to 25 Hz) was conducted to assess viscoelastic properties.

2.5. Antioxidant activity

2.5.1. Radical scavenging activity using DPPH assay

The antioxidant activity of flour extracts was evaluated using the 1,1-diphenyl-2-picryl-hydrayl (DPPH) free radical scavenging assay (Chen et al., 2013). Extract concentrations ranging from 2000 to 125 µg/mL were mixed 0.1 mM DPPH in 95 % ethanol and incubated in complete darkness for 30 min. The absorbance was measured at 517 nm using a spectrophotometer. A blank containing 95 % ethanol and DPPH-only control were used for calibration. All measurements were performed in triplicate. The percentage of DPPH radical inhibition was calculated using the following formula:

$$\text{DPPH \% inhibition} = ((\text{Control absorbance} - \text{Sample absorbance}) / \text{Control absorbance}) \times 100$$

2.5.2. Ferric reducing-antioxidant power (FRAP) assay

The ferric reducing-antioxidant power (FRAP) of the flour extracts was determined according to the method of Benzie and Strain (1996). A total of 2 mL of freshly prepared FRAP reagent was mixed with 0.2 mL of the diluted extract. The FRAP reagent was prepared by combining 0.3 M acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, and 20 mM FeCl₃·6H₂O in a ratio of 10:1:1. The mixture was incubated at RT for 10 min, and the absorbance was measured at 593 nm. The antioxidant activity was quantified using a FeSO₄·7H₂O calibration curve and expressed as mg Fe²⁺ equivalents per gram of extract.

2.5.3. Reducing power assay

The reducing power of the flour extracts was assessed using a modified protocol (Yildirim et al., 2001). Extracts at concentrations ranging from 2 to 0.125 mg/mL were mixed with 0.2 M phosphate buffer (pH 6.6) and potassium ferricyanide, followed by incubation at 50 °C for 30 min. The reaction was cooled to RT, after which 10 % trichloroacetic acid was added. The mixture was then centrifuged at 3000 × g for 10 min. The supernatant was diluted with distilled water, mixed with 1 g/L ferric chloride solution, and the absorbance was measured at 700 nm to determine the reducing power.

2.6. Antidiabetic properties: inhibition of α-amylase and α-glucosidase enzymatic activity

The inhibitory potential of flour extracts against α-amylase was evaluated using a modified method (Worthington, 1993). Extracts (2–8 µg/mL) were mixed with 500 µL of 0.02 M sodium phosphate buffer (pH 6.9, 0.006 M NaCl) containing 0.5 mg/mL porcine pancreatic α-amylase (EC 3.2.1.1) and incubated at 25 °C for 10 min. Subsequently, 500 µL of a 1 % starch solution was added, the mixture was incubated for an additional 10 min at 25 °C. The reaction was terminated by adding 1 mL of dinitrosalicylic acid reagent, followed by heating in boiling water for 5 min, and then cooling to RT. The mixture was diluted with 10 mL of distilled water, and the absorbance was measured at 540 nm using a UV-visible spectrophotometer. The percentage inhibition of α-amylase was calculated.

The α-glucosidase inhibition activity of flour extracts was assessed using a modified method (Apostolidis et al., 2007). Extracts (2–8 µg/mL) were combined with 100 µL of α-glucosidase (1.0 U/mL) in 0.1 M phosphate buffer (pH 6.9), and incubated at 25 °C for 10 min. The reaction was initiated by adding 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside in the same buffer, followed by incubation for 5 min at 25 °C. Absorbance was then measured at 405 nm, and the inhibitory activity was calculated accordingly.

2.7. Statistical analysis

All experiments were performed in triplicate, and the results are expressed as mean values ± standard error (SE). Statistical analyses were conducted using SPSS software, version 29 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to determine significant differences among treatment means, followed by Tukey's post hoc test for multiple comparisons. Differences were considered statistically significant at $p < 0.05$.

Table 1

Proximate composition (g/100 g) and phenolic content of horse gram (*Macrotyloma uniflorum*) in raw, germinated, and cooked flours.

	HG-R	HG-G	HG-C
Proximate composition (g/100 g)			
Crude Protein	13.25 ± 0.48 ^b	23.48 ± 0.35 ^a	19.19 ± 0.36 ^a
Crude Lipid	0.50 ± 0.00 ^a	0.40 ± 0.00 ^a	0.40 ± 0.00 ^a
Crude Fiber	8.10 ± 0.10 ^a	9.10 ± 0.20 ^b	8.50 ± 0.20 ^{ab}
Moisture	3.00 ± 0.10 ^a	3.10 ± 0.0 ^a	3.10 ± 0.2 ^a
Ash	3.10 ± 0.00 ^b	3.00 ± 0.00 ^b	2.20 ± 0.10 ^a
Carbohydrate *	72.05 ± 0.34 ^a	60.92 ± 0.53 ^b	66.61 ± 0.21 ^c
Phenolic content			
TPC (mg GAE/g)	34.10 ± 0.20 ^b	57.30 ± 0.10 ^c	30.60 ± 0.10 ^a
TFC (mg QE/g)	14.30 ± 0.50 ^b	15.50 ± 0.10 ^c	12.20 ± 0.30 ^a

* The difference was utilized to calculate carbohydrate values.

Values are presented as mean ± SE ($n = 3$). Means within a row with different superscript letters (a, b, c) differ significantly (Tukey's test, $p < 0.05$).

3. Results and discussion

3.1. Chemical composition

3.1.1. Proximate composition

The proximate composition of HG-R, HG-G, and HG-C flours is summarized in Table 1. The protein content was significantly higher ($p < 0.05$) in HG-G (23.48 g/100 g) compared to HG-C (19.19 g/100 g) and HG-R (13.25 g/100 g). These results align with previous studies that reported an increase in protein content following germination in various legumes: green gram, cowpea, lentil, and chickpea increased from 27.7 g, 25.2 g, 26.5 g, and 22.1 g to 29.1 g, 27.2 g, 28.5 g, and 24.2 g, respectively (Ghavidel and Prakash, 2007); mung bean seeds increased from 23.13 % to 25.77 % (Wintersohle et al., 2024); stink beans from 31.40 % to 35.61 % (Medhe et al., 2023); and nitta beans from 17.27 % to 37.34 % (Medhe et al., 2022). Germination enhances protein hydrolysis and synthesis due to enzymatic activity in the testa and embryonic axis (Atudorei et al., 2021; Xu et al., 2017). This activity, regulated by plant hormones, activates functional enzymes such as amylases, lipases, and proteases, which support tissue development and increase the water-soluble protein content (do Nascimento et al., 2022). The elevated protein content in HG-C (19.19 g/100 g) relative to HG-R (13.25 g/100 g) is likely due to the thermal processing, which may deactivate protein-inhibiting compounds and induce protein denaturation. This denaturation unfolds globulin structures, thereby enhancing protein bioavailability. These observations are consistent with previous findings (Atudorei et al., 2021; Piecyk et al., 2012).

The lipid and moisture contents showed no significant differences ($p > 0.05$) among HG-R (0.50 g/100 g, 3.00 g/100 g), HG-G (0.40 g/100 g, 3.10 g/100 g), and HG-C (0.40 g/100 g, 3.10 g/100 g). While some studies have reported reductions in lipid content during germination, others found no notable changes, highlighting the complexity and variability of the biochemical processes involved (Nemzer and Al-Taher, 2023).

Crude fiber content was substantially elevated ($p < 0.05$) in HG-G (9.10 g/100 g) than in HG-C (8.50 g/100 g) and HG-R (8.10 g/100 g). These findings are in agreement with previous studies showed increased crude fiber content in sprouted grains and legumes, such as quinoa (from 4.06 % to 4.58 %) (Bhatthal et al., 2017), kidney beans (by 121.07 %) (Srenuva et al., 2023), and moth beans (from 6 % to 7 %) (Medhe et al., 2019). The observed increase in crude fiber can be attributed to the formation of new cells and the accumulation of cell wall components such as lignin, hemicellulose, and cellulose during germination (Nkhata et al., 2018).

Ash content was significantly lower ($p < 0.05$) in HG-C (2.20 g/100 g) than in HG-R (3.10 g/100 g) and HG-G (3.00 g/100 g). A similar trend was reported in hydrothermally treated, germinated and raw stink bean, with ash content of 4.03 g/100 g, 6.14 g/100 g and 6.26 g/100 g, respectively (Medhe et al., 2023). The reduced ash content in HG-C likely results from the leaching of macro- and micronutrients during soaking and cooking (Medhe et al., 2019), as well as mineral loss into the cooking water (Piecyk et al., 2012; Wang et al., 2008).

Carbohydrate content was significantly lower ($p < 0.05$) in HG-G (60.92 g/100 g) and HG-C (66.61 g/100 g) than in HG-R (72.05 g/100 g), indicating notable variation across treatments. These results are consistent with previous findings in mung bean, where carbohydrate content decreased from 62.3 g/100 g to 61.7 g/100 g after germination (Mubarak, 2005). Similarly, stink beans and nitta beans also showed a significant reduction ($p < 0.05$) in carbohydrate content after germination and cooking (Medhe et al., 2022, 2023). This reduction is likely due to the metabolic conversion of carbohydrates to meet the energy demands of germination (Atudorei et al., 2021). During germination, hydrolytic enzymes break down complex carbohydrates into monosaccharides, which serves as energy sources for sprouting process (Mubarak, 2005).

3.1.2. Total phenolic and flavonoid content

The TPC of HG-G (57.30 mg GAE/g) was significantly higher ($p < 0.05$) than that of HG-R (34.10 mg GAE/g) and HG-C (30.60 mg GAE/g) flours (Table 1). This increase is primarily attributed to the activation of endogenous enzymes during germination, which hydrolyze complex phenolic compounds into simpler, more extractable forms. Additionally, germination stimulates the de novo synthesis of phenolic compounds as part of the plant's defense response to sprouting stress (Khang et al., 2016). In contrast, thermal processing during cooking leads to the degradation of phenolic compounds, resulting in a reduced TPC values (Singh et al., 2020). Similar TPC enhancements have been reported in germinated horse gram (Pal et al., 2016) and nitta bean flours (Medhe et al., 2022). A comparable trend was observed in stink bean flours, where raw, germinated and hydrothermally samples showed TPC values of 14.30 mg GAE/g, 15.50 mg GAE/g and 12.20 mg GAE/g, respectively (Medhe et al., 2023). These findings highlight the critical role of enzymatic activity during germination in enriching bioactive compounds in legumes, thereby enhancing their nutritional and functional properties.

The TFC of HG-G flour (15.50 mg QE/g) was also significantly higher than that of HG-R (14.30 mg QE/g) and HG-C (12.20 mg QE/g) flours (Table 1). This improvement is primarily due to enzymatic activation during germination, which facilitates the biosynthesis of flavonoids and other phenolic compounds (Pal et al., 2016). Moreover, germination reduces antinutritional factors such as tannins and phytic acid, which otherwise bind flavonoids and limit their extractability and bioavailability (Pagar et al., 2021). These results are consistent with findings in germinated black soybeans (Huang et al., 2024) and common beans (Zhang et al., 2022).

3.2. Structural characteristics

3.2.1. Surface morphology

The microstructural characteristics of HG-R, HG-G, and HG-C flours were examined using SEM, as shown in Fig. 1A–F. Distinct morphological differences were observed among the treatments, highlighting the effects of germination and cooking on surface structure.

HG-R (Fig. 1A and B) exhibited a compact, irregular granular morphology with relatively smooth surfaces and tightly packed particles, indicating low porosity. This structural integrity reflects the native, undisturbed arrangement of macromolecules such as starch, proteins, and lipids. The higher magnification in Fig. 1B further illustrates the intact and dense granular packing, typical of untreated legume seeds (Medhe et al., 2023).

In contrast, HG-G (Fig. 1C and D) displayed significant

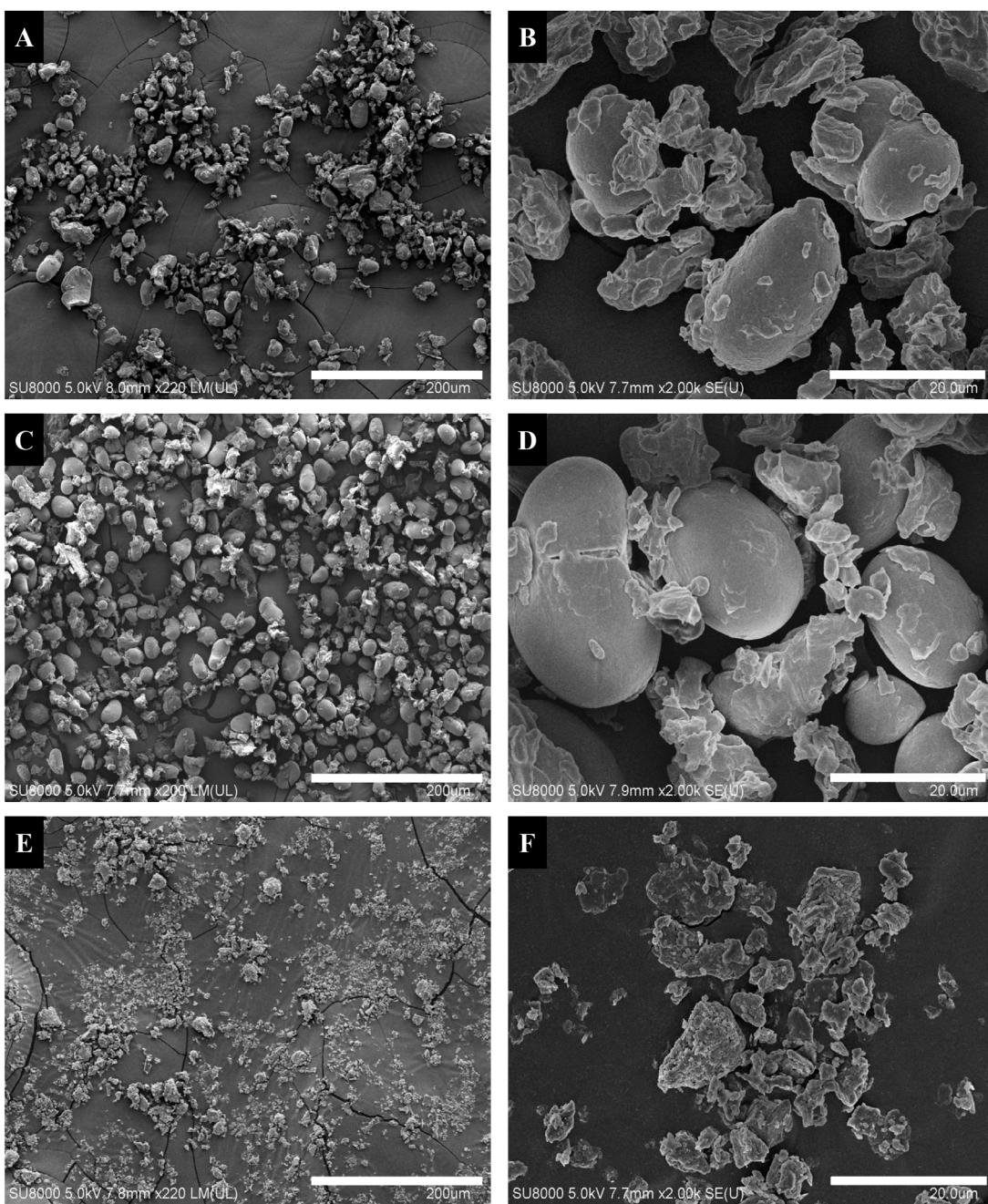


Fig. 1. Morphology of (A-B) raw (HG-R), (C-D) germinated (HG-G), and (E-F) cooked (HG-C) horse gram (HG) flours. Scale bars = 200 and 20 μm .

disaggregation of particles, increased porosity, and rougher surface textures. Fig. 1C shows loosened structures and pore formation, likely resulting from enzymatic hydrolysis during germination. Enzymes such as α -amylase and proteases degrade starch and protein matrices, generating low-molecular-weight fragments that increase surface roughness. At higher magnification (Fig. 1D), the increased porosity and fragmentation are more evident, confirming extensive microstructural breakdown. These observations align with findings in germinated legumes such as moth beans (Medhe et al., 2019), nitta beans (Medhe et al., 2022), and stink beans (Medhe et al., 2023), where enzymatic modification significantly alter surface architecture (Atudorei et al., 2021; Khang et al., 2016). Similar trends have been reported in other legumes, including beans, lentils, soybeans, chickpeas, and lupines (Atudorei et al., 2021). The smaller, more dispersed granules observed in HG-G further support the degradation of macromolecular aggregates

typically induced by germination.

HG-C (Fig. 1E and F) revealed additional morphological changes. Fig. 1E displays partial swelling, surface cracking, and particle aggregation, attributed to heat-induced gelatinization and protein denaturation. At higher magnification (Fig. 1F), disrupted surface layers and uneven textures become evident, indicating structural breakdown and moisture uptake resulting from thermal processing. The observed coalescence of partially gelatinized starch granules is consistent with findings in other thermally treated legumes, including moth beans (Medhe et al., 2019), nitta beans (Medhe et al., 2022), and stink beans (Medhe et al., 2023), where cooking leads to deformation and increased porosity through thermal modification of cell wall components (Guo et al., 2018; Villetti et al., 2002). Collectively, these SEM findings demonstrate that both germination and cooking significantly alter the surface morphology of horse gram flour. These structural modifications likely contribute to

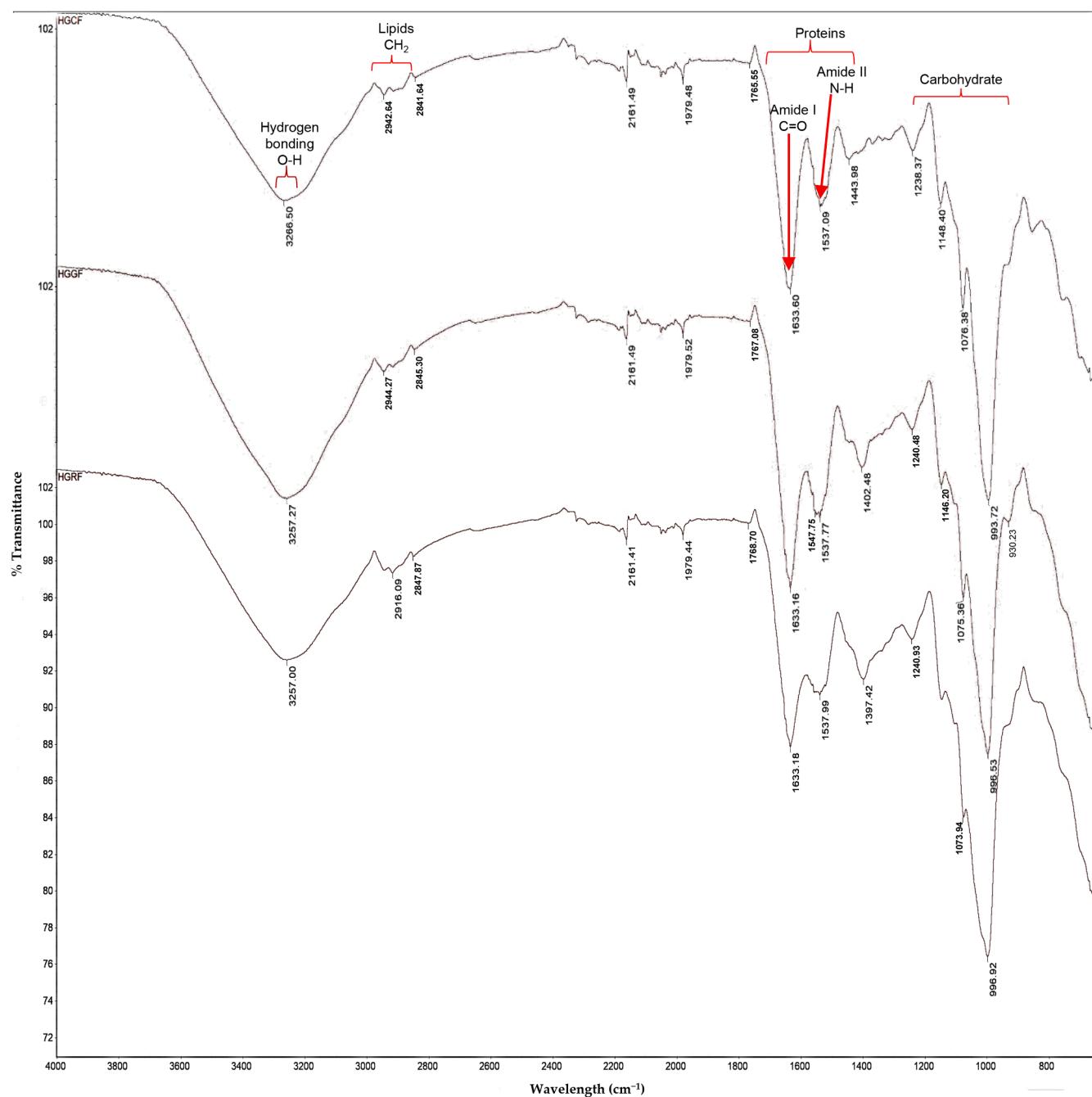


Fig. 2. FTIR spectra of raw (HG-R), germinated (HG-G), and cooked (HG-C) horse gram (HG) flours.

the functional and nutritional enhancements observed in other parts of this study.

3.2.2. FTIR analysis

A FTIR spectroscopy was employed to investigate the structural and compositional changes in HG-R, HG-G, and HG-C flours. Spectra were recorded in the range of 800–4000 cm⁻¹ to analyze molecular alterations associated with proteins, carbohydrates, and lipids induced by germination and cooking. Fig. 2 presents the FTIR spectra of the three flour types, highlighting differences in their functional group regions. A prominent absorption band was observed between 3100 and 3700 cm⁻¹, corresponding to the stretching vibrations of hydroxyl (OH) groups present in all samples. Among them, HG-G exhibited the higher absorbance at 3257 cm⁻¹. This increase is attributed to enhanced hydrogen bonding due to enzymatic degradation of carbohydrates and proteins

during germination, leading to the release of free OH groups. Similar findings have been reported in other germinated legumes and stink beans, where improved hydration and bonding capacity were observed (Khang et al., 2016; Medhe et al., 2023). Additional peaks in the 3050–2800 cm⁻¹ region were associated with asymmetric and symmetric CH stretching vibrations, characteristic of triglycerides (lipid molecules). These results are consistent with earlier studies on germinated and cooked flours of stink beans and nitta beans (Medhe et al., 2022, 2023).

Protein-associated bands, including amide I ($C = O$) and amide II ($N—H$), were observed within the 1750–1550 cm^{-1} region (Xu et al., 2017). The amide I band is primarily linked to carbonyl ($C = O$) stretching in peptide bonds (Ghumman et al., 2016), while the amide II band reflects N—H bending and C—N stretching vibrations (Atudorei et al., 2021). The increased intensities of these bands in HG-G indicate

elevated protein content and structural modification. Enzymatic activity during germination likely promotes protein hydrolysis and synthesis, exposing more C = O and N—H groups, thereby enhancing protein digestibility and bioavailability (Handa et al., 2017; Moktan and Ojha, 2016). In contrast, HG-C showed a reduced Amide II band intensity, indicating heat-induced protein denaturation and aggregation that disrupts hydrogen bonding and alters secondary structures (Kaur and Singh, 2007; Singh et al., 2003).

Bands between 1200 and 900 cm⁻¹ confirmed the presence of carbohydrates in all flour types (El Darra et al., 2017). A decrease in peak intensities at 1238, 1148, and 1076 cm⁻¹ was observed in HG-G and HG-C compared to HG-R. This reduction is attributed to enzymatic hydrolysis during germination, in which α -amylase and β -amylase break down complex carbohydrates such as starch into simpler sugars for seedling development (Muñoz-Llanes et al., 2023). Additionally, heat treatment contributes to the degradation of polysaccharide structures via depolymerization cleavage of glycosidic bonds cleavage, and solubilization of carbohydrates, further reducing their measurable carbohydrate content (Guo et al., 2018; Villette et al., 2002).

3.3. Functional properties

3.3.1. Gelation capacity

The complete gelation concentration for HG-R was 22 g/100 mL, significantly higher than that of HG-G (18 g/100 mL) and HG-C (16 g/100 mL) flour (Table 2). These results are consistent with previous studies on germinated and cooked moth beans, which reported partial and complete gelation concentrations of 24 and 26 g/100 mL for raw flour, and 12 and 14 g/100 mL for germinated flour, respectively (Medhe et al., 2019). Similar findings have been observed in germinated non-conventional legumes (Benítez et al., 2013). This enhancement is likely due to the activity of amylase enzymes produced during germination, which interact with starch components and improve gelation behavior. Variations in gelation among the different treatments can be attributed to differences in the proportions of proteins, carbohydrates, and fats, highlighting the importance of molecular interactions in determining their functional properties (Chinma et al., 2009).

3.3.2. Emulsion and foaming properties

The emulsifying and foaming properties of HG-R, HG-G and HG-C flours are presented in Table 2. HG-C showed the lowest emulsion capacity (3.30 mL/100 mL) and showed no emulsion stability compared to HG-R (42.91 and 58.81 mL/100 mL) and HG-G (27.82 and 60.9 mL/100 mL). A similar reduction in emulsion stability was reported in soaked,

Table 2

Functional properties, including gelation, emulsification, foaming capacity, and pasting behavior of horse gram (*Macrotyloma uniflorum*) in raw, germinated, and cooked flours.

	HGR	HGG	HGC
Gelation capacity (g/100 mL)	22	18	16
Emulsion properties			
Capacity (mL/100 mL)	42.91 ± 0.70 ^c	27.82 ± 1.90 ^b	3.3 ± 0.90 ^a
Stability (mL/100 mL)	58.81 ± 5.60 ^b	60 ± 9.6 ^b	00 ^a
Foaming properties			
Capacity (mL/100 mL)	86.96 ± 1.80 ^c	56.04 ± 6.60 ^b	12.15 ± 4.10 ^a
Stability (mL/100 mL)	67.41 ± 5.30 ^c	27.31 ± 0.30 ^b	1.12 ± 0.20 ^a
Pasting properties			
Pasting Temperature (°C)	81.10 ± 0.84	82.22 ± 0.64	–
Pasting times (min)	4.710 ± 0.02 ^a	4.510 ± 0.08 ^a	4.75 ± 0.04 ^a
Peak Viscosity (cP)	95.44 ± 2.02 ^c	72.42 ± 0.47 ^b	13.39 ± 0.13 ^a
Trough viscosity (cP)	73.19 ± 1.54 ^c	48.19 ± 0.18 ^b	12.19 ± 0.46 ^a
Breakdown viscosity (cP)	22.25 ± 0.84 ^b	24.22 ± 0.62 ^b	1.19 ± 0.36 ^a
Final viscosity (cP)	89.56 ± 1.93 ^c	58.69 ± 0.43 ^b	14.00 ± 0.50 ^a
Set back viscosity (cP)	16.36 ± 0.42 ^c	10.50 ± 0.30 ^b	1.81 ± 0.18 ^a

Values are presented as mean ± SE ($n = 3$). Means within a row with different superscript letters (a, b, c) differ significantly (Tukey's test, $p < 0.05$).

cooked, and dehydrated chickpeas (4.10 %) as compared to raw chickpeas (12.30 %) (Aguilera et al., 2011a). Comparable trends have been observed in cooked moth beans (Medhe et al., 2019), hydrothermally cooked nitta beans (Medhe et al., 2022) and stink beans (Medhe et al., 2023). The reduced emulsion properties in HG-C are likely due to heat-induced protein denaturation (Setia et al., 2019). Germination also caused a decline in emulsion capacity, possibly due to changes in protein concentration, as observed in cowpea, dolichos, jack bean, and Mucuna (Karaca et al., 2011). Alterations in the balance of hydrophobic and hydrophilic protein domains may impair their ability to form stabilizing films around oil droplets (Medhe et al., 2019).

The foaming capacity and stability of HG-C (12.15 and 1.12 mL/100 mL) were significantly lower ($p < 0.05$) than those of HG-R (86.96 and 67.41 mL/100 mL) and HG-G (56.04 and 27.31 mL/100 mL). This results aligns with studies showing reduced foaming capacity in boiled yellow pea flour (Ferawati et al., 2019) and cooked cannellini beans (Aguilera et al., 2011b). Heat denaturation likely disrupts protein structure, reducing its ability to stabilize air-water interfaces. Additionally, cooking diminishes protein solubility and alters surface-active properties, impairing foam formation (Medhe et al., 2022; Setia et al., 2019).

3.3.3. Pasting properties

The pasting temperatures of HG-R and HG-G were 81.10 °C and 82.22 °C, respectively (Table 2), consistent with those reported in cooked carioca beans (80.7–84.1 °C) (Correia Bento et al., 2022) and yellow peas (79.3 °C) (Waduge et al., 2017). The elevated temperatures are attributed to non-starch components such as proteins, oligosaccharides, and cellulose that limit water availability during starch gelatinization (Romero and Zhang, 2019). A high amylose content also contributes by promoting molecular alignment and strengthening starch granules (Lin and Fernández-Fraguas, 2020).

Peak viscosity was significantly lower ($p < 0.05$) in HG-C (13.39 cP) compared to HG-R (95.44 cP) and HG-G (72.42 cP). The reduction in HG-C is likely due to starch gelatinization disrupting granule structure and reducing water-holding capacity (Medhe et al., 2023). Germination also lowered peak viscosity, likely due to enzymatic starch hydrolysis, as reported for other legumes (Acevedo et al., 2017; Medhe et al., 2019). Although amylose-to-amylopectin ratios can influence pasting behavior (Kaushal et al., 2012), these were not measured in this study, so the observed effects are attributed primarily to starch modification.

HG-R exhibited the highest trough viscosity (73.19 cP) compared to HG-G (48.19 cP) and HG-C (12.19 cP), which may be due to enhanced amylose leaching, amylose-lipid complex formation, and granule swelling (Liu et al., 1997). The low breakdown viscosity in HG-C indicates higher paste stability (Zhang et al., 2019). Additionally, HG-R showed higher final and setback viscosities, suggesting a greater tendency for retrogradation (Demiate et al., 2016; Li et al., 2016). Variability in pasting properties across samples can be attributed to differences in starch structure, crystallinity, and non-starch components (Frohlich et al., 2021; Lin and Fernández-Fraguas, 2020; Romero and Zhang, 2019).

3.3.4. Thermal properties

The onset temperature for gelation in HG-R flour (63.01 °C) was significantly higher ($p < 0.05$) than that observed in HG-G (58.42 °C) and HG-C (58.63 °C) (Fig. 3), which can be attributed to the more complex structural composition of HG-R. Similarly, HG-R exhibited a higher peak transition temperature (68.73 °C) compared to HG-G (66.65 °C) and HG-C (66.50 °C), indicating greater ionic strength and structural integrity (Sánchez-Arteaga et al., 2015). These results suggest that while gelatinization begins at comparable temperatures in HG-G and HG-C, the peak temperature is influenced by differences in amino acid profiles and the extent of protein denaturation (Sharma et al., 2015).

The conclusion temperature was also higher in HG-R (74.11 °C) and HG-G (73.79 °C) compared to HG-C (73.33 °C). The reduced thermal

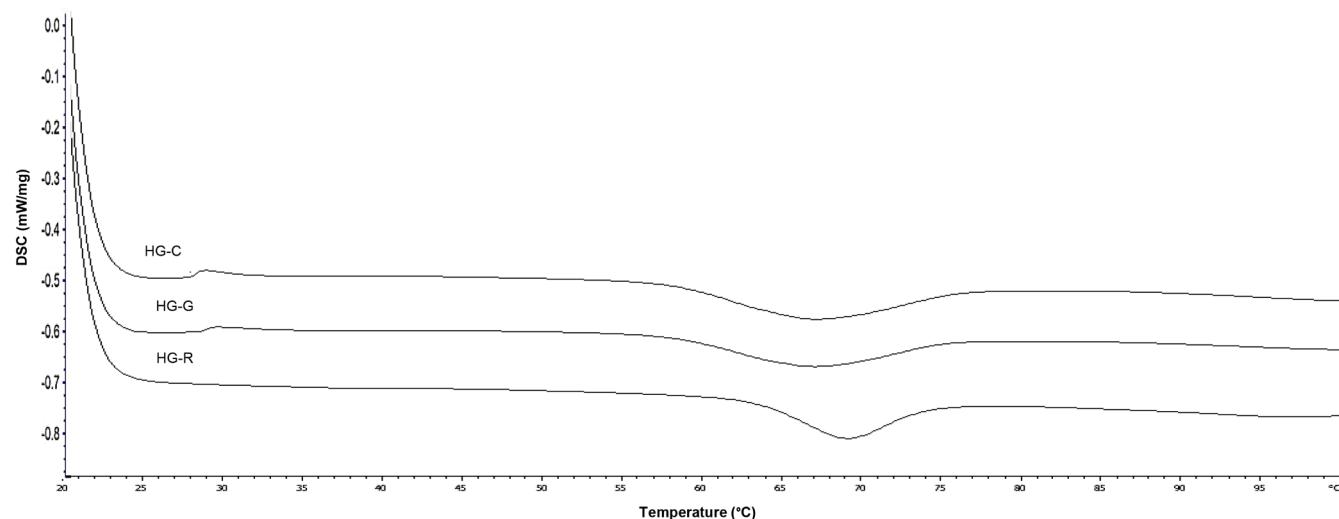


Fig. 3. Thermal properties of raw (HG-R), germinated (HG-G), and cooked (HG-C) horse gram (HG) flours.

properties observed in HG-G may result from fat loss during germination, whereas the lower conclusion temperature in HG-C likely reflects thermal degradation of starch–protein and starch–lipid interactions (Bekele and Admassu, 2023; Li et al., 2020).

Notably, HG-R showed lower enthalpy values relative to HG-G and HG-C, indicating that less energy was required to disrupt its native starch structure. In contrast, the higher enthalpy values in HG-G and HG-C may be attributed to processing-induced structural rearrangements. In HG-G, enzymatic activity during germination likely promoted the removal of degraded fragments and enriched the starch with more ordered double-helical structures. Similarly, cooking may have caused partial gelatinization and retrogradation, resulting in reformed crystalline regions that require more energy to break (Bekele and Admassu, 2023; Li et al., 2020).

3.3.5. Rheological properties

Raw materials such as flour dough typically exhibit shear rate-dependent viscosity behavior (Dogan and Kokini, 2006). Evaluating viscosity across varying shear rates is essential for understanding how material's respond under specific processing conditions (Peressini et al., 2008). For example, viscosity at low shear rates reflects storage stability and leveling behavior, while medium shear rates relate to pumping and spreading. High shear rates are relevant to applications like spraying and rubbing. Rheological curves plotted across these ranges can help characterize whether a material behaves more like solid (yield stress) or liquid (zero-shear viscosity) (Cristiano et al., 2019).

In this study, the shear rate was increased from 1 to 300 s⁻¹ for HG-R, HG-G, and HG-C flours, and the resulting apparent viscosity was recorded (Fig. 4). All samples showed a decreased viscosity with increasing shear rate, demonstrating typical shear-thinning behavior. These findings are consistent with prior studies on wheat flour (Cristiano

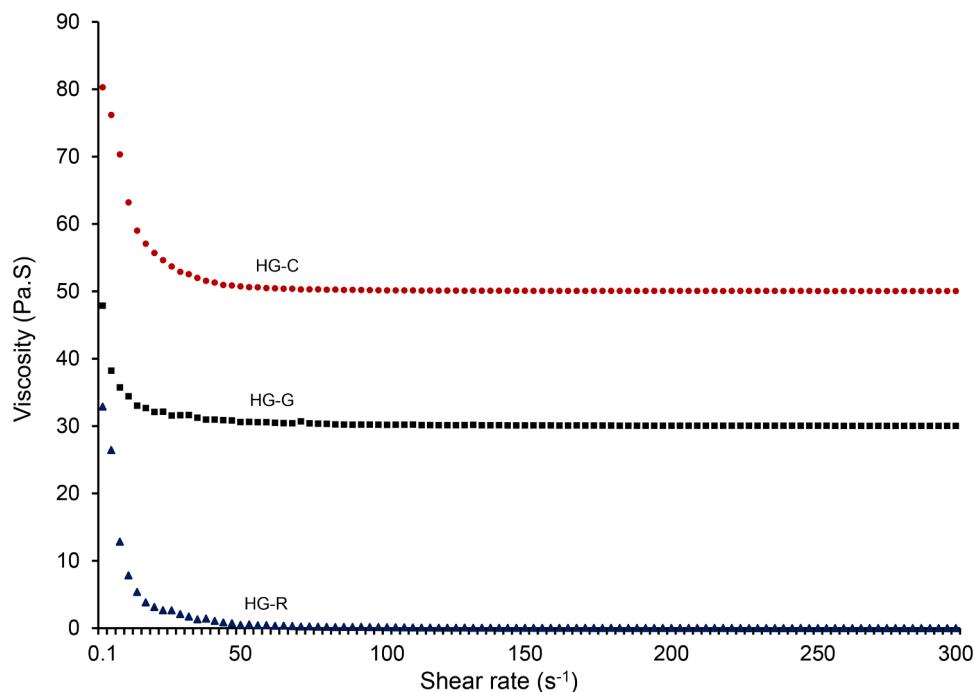


Fig. 4. Flow curves of raw (HG-R), germinated (HG-G), and cooked (HG-C) horse gram (HG) flours using rotational rheometer at a shear rate of 0.1–300 s⁻¹ and a constant temperature of 25 °C.

et al., 2019), chestnut flour (Moreira et al., 2010), and brown rice flour (Wang et al., 2020). Processed flours (HG-G and HG-C) exhibited higher viscosity across the full shear rate range compared to HG-R. Similar trends have been observed in starch-lipid systems subjected to thermal treatment, where increased viscosity results from partial gelation, frictional resistance, water loss, and Maillard browning reactions (Alvarez-Ramirez et al., 2018; Avila Ruiz et al., 2016).

The impact of germination duration was also evident: longer germination time associated with lower apparent viscosity. HG-G showed an intermediate viscosity—lower than HG-C but higher than the HG-R. Wang et al. (2020) similarly found that brown rice germinated for 9 h had lower viscosity than that germinated for 5 h, suggesting that enzymatic activity during germination influences shear behavior. This shear-thinning characteristics is desirable for processing techniques like extrusion, filling, and modeling, where lower viscosity under shear helps reduce energy input.

3.4. Antioxidant activity

Antioxidant activity of horse gram flour was significantly influenced ($p < 0.05$) by germination and cooking treatments (Table 3). The higher DPPH radial scavenging activity was observed in HG-G (86.40 %), followed by HG-R (81.70 %) and HG-C (71.10 %) flours (Table 3). A similar trend has been reported in chickpea, soybean, lentil, mung bean, and kidney bean, where germination enhanced DPPH activity compared to raw forms (Mamilla and Mishra, 2017). This improvement is attributed to the activation of hydrolytic enzymes, such as amylases and proteases, which generate intact phenolics compounds during germination (Medhe et al., 2023).

Additionally, the FRAP values were significantly higher ($p < 0.05$) in HG-G (0.36 µg/mL) compared to HG-R (0.30 µg/mL) and HG-C (0.13 µg/mL), consistent with previous findings in germinated black beans (Hannachi et al., 2025). The elevated antioxidant capacity is likely due to increased biosynthesis of phenolic compounds that protect the hypocotyl from oxidative stress during sprouting (Guzmán-Ortiz et al., 2017).

The reducing power was also significantly enhanced ($p < 0.05$) in HG-G (421.40 µg AA/mL), followed by HG-R (392.20 µg AA/mL) and HG-C (351.10 µg AA/mL), a trend consistent with that observed in germinated lentils (Fouad and Rehab, 2015; Świeca and Gawlik-Dziki, 2015). The increased metal-chelating capacity in germinated samples may be associated with reduced phytic acid content, which enhances the mineral bioavailability (Sharma and Sahni, 2021).

Overall, the antioxidant activity in germinated horse gram is positively correlated with its TPC and TFC, confirming previous observations in lentils and other legumes where antioxidant capacity is closely linked to polyphenol content (Fouad and Rehab, 2015; Medhe et al., 2022, 2023; Świeca and Gawlik-Dziki, 2015).

3.5. Antidiabetic properties

Managing postprandial plasma glucose levels is crucial in the early stages of diabetes treatment (Kim et al., 2005). One effective strategy involves inhibiting carbohydrate-digesting enzymes, particularly α -amylase and α -glucosidase (Kim et al., 2005). In the present study, both HG-G (29.80 µg/mL for α -amylase and 70.20 µg/mL for α -glucosidase) and HG-C (18.30 µg/mL and 68.20 µg/mL, respectively) flours exhibited significantly higher ($p < 0.05$) inhibitory activity compared to the HG-R (14.60 µg/mL and 62.80 µg/mL) (Table 3). These findings are consistent with previous reports on germinated lentils (Casarin et al., 2021; de Souza Rocha et al., 2015), cooked rice (Adedayo et al., 2018), and cooked common beans (Ombra et al., 2018), which also showed enhanced enzyme inhibition.

The increased antidiabetic potential of germinated and cooked horse gram is likely due to elevated levels of TPC and TFC. These phytochemicals possess known antioxidant and enzyme-inhibiting properties and may interact with digestive enzymes to suppress their activity (Medhe et al., 2023; Tan et al., 2017). Germination reduces anti-nutritional factors such as phytic acid and tannins (Ayet et al., 1997), which can otherwise interfere with enzyme binding, thereby enhancing the functional activity of bioactive compounds.

Although the current *in vitro* results are promising, they may not fully replicate the complex physiological environment of living organisms. Therefore, future studies should include *in vivo* models to validate the antidiabetic potential and assess the bioavailability and metabolic fate of the active compounds in processed horse gram.

4. Conclusion

Germination significantly increased protein, fiber, phenolic, and flavonoid contents, along with enhanced antioxidant and antidiabetic activities. In contrast, cooking improved carbohydrate availability but resulted in reduced protein levels and antioxidant potential due to heat-induced degradation. Both processing methods altered the bioactive compound profile, highlighting their potential to enhance the overall nutritional quality of horse gram flour. These findings support the use of processed horse gram as a functional ingredient in nutraceuticals and health-focused diets.

Nonetheless, this study has several limitations. The antidiabetic activity was assessed solely through *in vitro* assays, which may not fully represent *in vivo* physiological responses. Future studies should incorporate animal models or clinical trials to validate the efficacy and bioavailability of the bioactive compounds. Moreover, the specific bioactive compounds responsible for enzyme inhibition were not isolated or structurally characterized. Further research is warranted to investigate additional processing techniques—such as fermentation or enzymatic hydrolysis—and to identify and clarify the mechanisms of key bioactive constituents. These efforts will be essential for fully unlock the therapeutic potential of horse gram.

Ethical statement

This study does not involve any research with human participants or animals. Therefore, ethical approval was not required. All experimental procedures were conducted in accordance with institutional guidelines and regulations.

CRediT authorship contribution statement

Seema Vijay Medhe: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Manoj Tukaram Kamble:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation. **Anil K. Anal:** Writing –

Table 3
Antioxidant activity, and antidiabetic activity of horse gram (*Macrotyloma uniflorum*) in raw, germinated, and cooked flours.

	HGR	HGG	HGC
Antioxidant activity			
DPPH (%)	81.70 ± 0.10 ^b	86.40 ± 0.20 ^c	71.10 ± 0.10 ^a
FRAP (µg/mL)	0.30 ± 0.01 ^b	0.36 ^c ± 0.00	0.13 ^a ± 0.00
Reducing Power (µg AA/mL)	392.20 ± 5.50 ^b	421.40 ± 0.80 ^c	351.10 ± 9.90 ^a
Antidiabetic properties			
α -amylase (µg/mL)	14.60 ± 2.33 ^a	29.80 ± 2.29 ^b	18.30 ± 0.43 ^c
α -glucosidase (µg/mL)	62.80 ± 0.40 ^a	70.20 ± 0.20 ^b	68.20 ± 0.30 ^b

Values are presented as mean ± SE ($n = 3$). Means within a row with different superscript letters (a, b, c) differ significantly (Tukey's test, $p < 0.05$).

review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Muhammad Umar:** Writing – review & editing, Validation, Software, Formal analysis. **Kim D. Thompson:** Writing – review & editing, Validation, Software, Formal analysis. **Balasaheb Ramdas Chavan:** Writing – review & editing, Validation, Software, Formal analysis. **Aurawan Kringkasemsee Kettawan:** Writing – review & editing, Validation, Software, Formal analysis. **Parunya Thiyajai:** Writing – review & editing, Validation, Software, Formal analysis. **Aikkarakch Kettawan:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis. **Nopadon Pirarat:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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