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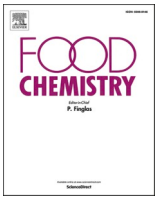

Thermo-mechanical pre-treatments modulate the structure, protein and starch digestibility, and thermal and pasting properties of sorghum flour

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ABSTRACT

This study examined the effects of five thermal-mechanical pre-treatments, including popping, roasting, parboiling, pressure cooking, and extrusion, on the structure, digestibility, and thermal and pasting properties of sorghum flour. The results indicated that dry-heat treatments disrupted the starch-protein matrix, which caused granule swelling, fusion and partial gelatinisation. Protein digestibility was highest after extrusion (76.2 %) and lowest in pressure-cooked sorghum flour (42.6 %). Wet-heat treatments, including parboiling and pressure cooking, consistently increased thermal stability, as evidenced by higher onset and peak temperature (To, Tp) and enthalpy changes (ΔH), while roasting resulted in the lowest ΔH, consistent with partial gelatinisation. FTIR analysis revealed protein unfolding, particularly in dry heat-treated samples, and SEM imaging showed changes in the morphological microstructure, confirming granule swelling and formation of an amorphous matrix. These findings underscore the potential of these treatments to enhance the properties of sorghum flour and broaden its use in food products.

1. Introduction

Sorghum (*Sorghum bicolor L Moench*) is a cereal from the grass family *Poaceae*, and is ranked fifth most important cereal crop globally (in terms of production) following rice, wheat, maize and barley (USDA, 2024). Its high cultivation is predominantly attributed to its resilience against high temperature, drought, mycotoxins and fungi (Zheng et al., 2024). It is a stable food in Africa, Asia, and Latin America, nourishing approximately 750 million people (Taylor & Taylor, 2011). Sorghum is gaining popularity in the food industry due to its environmental sustainability, adaptability to shifting dietary trends in emerging economies, and rising demand for convenient, nutrient-dense, and gluten-free products (Alavi et al., 2019; Hegde & Singh, 2023).

Sorghum is nutritionally rich, providing approximately 36.05 g of carbohydrates, 5.3 g of protein, 3.4 g of fibre, 1.5 g of lipids and 6.2 g of moisture per 50 g (Haytowitz et al., 2020). Sorghum starch is predominantly composed of resistant and slowly digestible starch (Shah et al., 2024). Its protein content ranges from 6 % to 18 % (*w*/w) and is classified into water-soluble albumins, salt-soluble globulins, alcohol-soluble kafirins (alcohol and reducing agent-soluble), and alkaline-soluble glutelins (Belton et al., 2006; Taylor & Taylor, 2018; Xiao et al., 2017). Kafirin, a prolamin protein, is the main protein fraction of sorghum and constitutes approximately 70 % (w/w) of total protein, while non-prolamins comprise approximately 30 % (w/w) (Shah et al., 2021). In terms of its lipid profile, sorghum is predominantly unsaturated, with polyunsaturated fatty acids being the most abundant (Verbruggen et al., 1998; Xiong et al., 2019). The major fatty acids in sorghum include oleic, palmitic, linoleic, linolenic, and stearic acid (Stefoska-Needham et al., 2015). Furthermore, sorghum provides essential micronutrients, including iron, zinc, magnesium, phosphorus, and B-complex vitamins, although their bioavailability may be reduced by anti-nutritional factors like phytates and tannins.

Despite sorghum's favourable nutritional profile, its use in food products is restricted due to some intrinsic structural factors that reduce its digestibility and functionality (Taylor & Taylor, 2018). A high proportion of resistant and slowly digestible starch is a characteristic feature of sorghum flour, in which starch is stabilised by tight complexations with the prolamin protein kafirin and phenolic compounds,

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such as tannins (Duodu et al., 2003). Kafirin is made of α-, β-, and γ-fractions, and γ-kafirin has the capacity to form disulfide-linked aggregates that encase starch granules, which limit its enzyme access (Xiao et al., 2017). Kafirins are water-insoluble and exhibit strong hydrophobic interactions with starch and lipid components, thereby hindering starch gelatinisation and reducing protein digestibility (Xiao et al., 2017). Additionally, tannins and phytates form insoluble complexes with proteins and divalent cations (e.g., Ca^{2+}, Zn^{2+}, Fe^{2+}), which further reduce the nutrient bioavailability (Duodu et al., 2003). Structurally, sorghum starch granules exhibit higher crystallinity and tightly packed amylopectin double helices, which contribute to their high resistance to thermal and enzymatic treatments (Haytowitz et al., 2020). The tight embedding of sorghum starch within its protein matrix, along with its intermolecular interactions with polyphenols, presents a significant challenge for developing sorghum-based food formulations with desirable functional properties and digestibility.

Recent thermal-mechanical treatments have shown promise for overcoming these intrinsic restrictions by modifying the structural, thermal and physicochemical properties of grains. These treatments combine heat, moisture, and mechanical shear to disrupt starch crystallinity, denature proteins, and enhance enzymatic accessibility (Mapengo & Emmambux, 2020). Studies on maize grains, whose prolamin protein, zein, closely resembles sorghum kafirin (in terms of primary structure), showed that thermal-mechanical processing can alter grain components (Gu et al., 2024; Luo et al., 2022; Mapengo & Emmambux, 2020). These transformations in the flour include partial gelatinisation of starch (Zhang et al., 2022) and protein denaturation (Sun et al., 2016), leading to enhanced enzymatic accessibility (digestibility) and reduced hydrophobicity. As a result, the thermally and mechanically processed flour exhibits improved texture, water absorption, and swelling capacity, while protein denaturation weakens the starch-protein network.

Similar treatments have been reported for sorghum, though research is still limited compared to maize. Recent studies have explored the use of heat-moisture, infrared, and microwave treatments to enhance the nutritional and functional properties of sorghum. For example, Semwal and Meera (2025) treated sorghum with infrared irradiation (IR; 1.1–1.2 μm wavelength, 0.26 kWm^{-2}), microwave heating (2450 MHz frequency and power of 1.2 kW for 1 to 6 min), and pressure cooking (101.325 kPa atmospheric pressure for 15, 30 and 45 min). Apparently, IR treatment increased starch digestibility by reducing the crystallinity, while pressure cooking improved pasting stability (Semwal & Meera, 2025). In the study by Vu et al. (2017), heat-moisture treatment increased the resistant starch content from 5.6 % to 22.1 % (*w*/w) in sorghum flour. The rise was primarily due to amylose-lipid complex formation and heat-induced protein structural transformations (Vu et al., 2017). While wet and dry treatments can enhance the properties of flours, more research is needed to extend beyond individual methods or isolated functions, particularly in sorghum, where studies on how these treatments affect flour structure and functionality are limited.

This study compared the effects of various thermal-mechanical pretreatments on the physical structure, chemical composition, and digestibility and functional properties of sorghum flour. Five treatments, including microwave popping, roasting, parboiling, pressure cooking, and extrusion, were applied to white sorghum grain flour. Their impacts on pasting and thermal properties, starch and protein digestibility, and the molecular and morphological structure were investigated. The findings could offer new insights into the process-structure-function relationships of pre-treated sorghum flour, enhancing its potential for sustainable food systems.

2. Materials and methods

2.1. Materials

Five kilograms of whole white sorghum grain (*Sorghum bicolor L Moench* 'Liberty') were sourced from Pacific Seeds Pty Ltd. (Queensland, Australia). Liberty cultivar is commercially grown in Australia. It is a food-grade hybrid, known for its high starch content, white pericarp, and uniform grain size. The grains were dried, vacuum-packed, and stored at 4 °C before further use.

The grains were then processed for pre-treatments using methods as follows: (a) microwave popping; (b) salt roasting; (c) parboiling; (d) pressure cooking; and (e) extrusion. These treatments were selected such that a spectrum of thermal-mechanical inputs, ranging from dry-heat treatments (microwave, popping and roasting) to wet-heat methods (parboiling and pressure cooking), and a high-shear thermal-mechanical process (extrusion). This gradient enabled a systematic evaluation of how varying processing intensities influence properties of sorghum flour. The raw unprocessed sorghum grains were served as the control throughout the study.

The processed sorghum grains and control were milled into fine powders using a grinding mill (Cemotec 1090 sample mill, Foss Tecator AB, Mulgrave, VIC, Australia), followed by high-speed blending (ZM 200 blender, Retsch Gmbh & Co, Haan, Germany) for homogeneity across milling batches. The blended samples were passed through a 200-μm sieve, resulting in an 85 % (*w*/w) recovery. The remaining 15 % (w/w) was re-blended and re-sieved, and combined with the initial 85 %, yielding a total recovery of ~95 %, while ~5 % was discarded. The final sieved fractions had an average particle size of 200 μm, measured using a particle size analyser (Master Sizer 2000, Malvern Instruments Ltd., Malvern, UK). All reagents were of analytical grade.

2.2. Pre-gelatinisation of sorghum flour

2.2.1. Microwave popping

Microwave popping of sorghum was performed as described by de Morais Cardoso et al. (2014). A 100 g batch of sorghum grains, with an initial moisture content of 10.66 ± 0.29 % (*w*/w), was divided into 20 g portions and microwaved (Panasonic NN-6455a, Newark, NJ, USA) at 900 W (2450 MHz) for 3 min. The grains were spread evenly on a microwave-safe dish to ensure uniform heating. After popping, the popped grains were manually separated from the unpopped ones, cooled to ambient temperature, and stored in airtight containers for later use.

2.2.2. Salt roasting

Sorghum grains with an initial moisture content 10.66 ± 0.29 % (w/w) were salt roasted in a preheated pan over medium heat for 5 min. The dry-heat process evaporated surface moisture, giving the samples a golden-brown appearance. Following roasting, the grains were cooled, visually inspected, and those with a uniform golden-brown colour were selected for further use, while the rest were discarded. Milling and blending followed the method in Section 2.1.

2.2.3. Parboiling and pressure cooking

Approximately 200 g of sorghum grains were soaked in water (1,1 grain-to-water ratio, w/w) for 72 h at ambient temperature. The soaked grains were divided into two portions (100 g each) for parboiling and pressure cooking. In the parboiling process, a total of 100 g of soaked grains were placed in boiling water (90–95 °C) for 7 min. For pressure cooking, the remaining 100 g of soaked grains were cooked in a 6 L pressure cooker (ISA S.p.A., Pordenone, Italy) at ~110 kPa for 7 min. After processing, the grains were drained through muslin cloth to remove excess water, then dried in a hot-air oven (Model 854, Memmert GmbH, Schwabach, Germany) at 50 °C for 16 h. The dried samples were ground into fine sorghum flour as described in Section 2.1.

2.2.4. Extrusion processing

Extrusion cooking was performed using a twin-screw extruder (MPF 19:25, APV Baker Ltd., Peterborough, England) at a constant screw speed of 250 rpm and a flour feed rate of 2.5 kg/h. The barrel temperature was maintained at 100 °C. The extruder's heating zones 1 and 2

were controlled to ensure uniform cooking. After extrusion, the pellets were dried overnight in a hot-air oven at 60 °C, then ground and blended as described in Section 2.1. The feed rate was modified based on preliminary trials and the literature of Kumar (2017), with screw speed maintained at 250 rpm and a feed rate of 14 kg/h.

2.3. Proximate analysis

Proximate analysis, including moisture, crude protein, crude fat, ash, crude and dietary fibre and carbohydrates, was analysed following the Association of Official Analytical Chemists (AOAC) Method (AOAC International, 2005). Crude protein content was determined by the Kjeldahl method, where total nitrogen was measured by distillation and titration, and a conversion factor of 6.25 was used to convert percent nitrogen to protein. The moisture content was determined by drying the sample in a forced-air oven at 105 °C to constant weight. Ash content was determined by incinerating the samples at 550 °C until a constant weight was achieved. Crude fat was extracted using Soxhlet extraction with petroleum ether, and crude fibre was measured by acid and alkali digestion. Carbohydrate content was calculated by difference, subtracting the sum of the moisture, protein, fat, ash, and fibre from the total weight. Dietary fibre was quantified using the AOAC 985.29 method, determining the crude fibre content in foods.

2.4. Pasting properties of sorghum flour

The pasting properties of sorghum flours were analysed using a rapid visco analyser (RVA Tecmaster, Newport Scientific Pty, Ltd., New South Wales, Australia). Three grams of sorghum flour were placed in an aluminium RVA sample canister, to which 25 mL of distilled water was added to achieve a total sample weight of 28.0 g. The pasting properties of the samples were determined at 50 °C, with stirring at 160 rpm, using a 13-min RVA temperature profile, following a similar procedure of 160 rpm for 20 min (Alves Cayres et al., 2021). All measurements were performed in duplicates ($n = 2$).

2.5. Starch content and starch digestibility

The starch content of sorghum flours was examined using the Total Starch Assay Kit (AA/AG) (Neogen Corporation, Lansing, MI, USA), containing α-amylase and amyloglucosidase, following the manufacturer's procedure. The procedure is recognised by AACC Method 76–13.01, AOAC Method 996.11, and ICC Standard Method No. 168. A 100 mg sample was weighed into test tubes, wetted with 0.2 mL of 80 % (*v*/v) aqueous ethanol, and mixed with dimethyl sulfoxide using a vortex mixer, followed by a second vortex mixing. The samples were incubated at 95 °C for 6 min, with manual stirring at 2- and 4-min intervals. Following this, 4 mL of 0.2 M sodium acetate buffer (pH 2) and 0.1 mL of amyloglucosidase (Sigma A-7420 from Aspergillus Niger, 30–60 units/mg, Saint Louis, USA) were added, and then the mixture was vortexed. The samples were incubated at 50 °C for 30 min. After mixing thoroughly, the samples were centrifuged at 3000 ×*g* for 10 min. A 1 mL aliquot from each sample was transferred into new test tubes in duplicate. Then, 9 mL of distilled water was added to each duplicate, and the tubes were shaken. A 0.1 mL aliquot from each diluted solution was then transferred to new test tubes, followed by the addition of 3.0 mL of glucose oxidase-peroxidase 4-aminoantipyrine reagent to each tube, including the glucose controls and reagent blanks. The tubes were incubated at 50 °C for 20 min. Absorbance was measured at 510 nm against the reagent blank, and the total starch content (on a dry weight basis) was calculated as follows:

$$\text{Starch} = (\Delta A \times F \times 1000 \times 1/1000 \times 100/W \times 162/180) = \Delta A \times F/W \times 90$$

In the above calculation, ΔA represents the absorbance measured against the blank. F is given by 100 (μg of glucose) divided by the absorbance of 100 μg of glucose. The volume correction factor is 1000, accounting for the 0.1 mL sample taken from the 100 mL solution. The term 1/1000 is used for conversion from micrograms to milligrams. To express starch as a percentage of flour weight, the factor 100/W is applied, where W is the weight of the flour sample in milligrams (on an as-is basis), and 162/180 is an adjustment to convert free glucose to anhydroglucose, as it occurs in starch. Starch digestibility was measured using the procedure described by Sopade and Gidley (2009).

2.6. In vitro protein digestibility

The in vitro protein digestibility was determined using a modified pepsin-pancreatin digestion method (Villarino et al., 2015). Approximately 50 mg of sorghum flour samples were incubated in a water bath at 37 °C with 0.75 mg pepsin (2500 units/mg activity) in 7.5 mL of 0.1 N HCl for 3 h. The mixtures were neutralised by adding 3.75 mL of 0.2 mol/m^3 sodium hydroxide, followed by neutralisation with 2 g of pancreatin in 2.75 mL of pH 8.0 phosphate buffer. The samples were incubated at 37 °C for 24 h to mimic small intestinal digestion. After incubation, 5 mL of the digesta was mixed with 25 mL of 10 % (*v*/v) trichloroacetic acid to precipitate undigested protein. The sample was centrifuged at 1000 *g* for 30 min at ambient temperature. Nitrogen content in the supernatant was determined using the Kjeldahl digestion and distillation method, and the in-vitro protein digestion of protein was calculated as:

$$\text{In}-\text{vitro protein digestion} = \frac{\textit{total nitrogen}\left(\frac{g}{ml}\right) - \textit{nitrogen in the supernatant}\left(\frac{g}{ml}\right)}{\textit{total nitrogen}\left(\frac{g}{ml}\right)} \times 100$$

2.7. Attenuated total reflection - Fourier transform infrared spectroscopy (ATR-FTIR)

The secondary structure of processed and unprocessed sorghum flour samples was investigated with attenuated total reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR; Thermo Scientific, Nicolet 50 ABX, Australia) following the method described by Shah et al. (2021). A single-bounce diamond ATR crystal was coupled with the instrument. A total of 64 co-added scans were collected over the spectral range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1}. The background spectra were recorded from the pure crystal to minimise beam current degradation. The spectra were analysed using OPUS software (V 7.0, Bruker Corporation, Ettlingen, Germany). The complete FTIR spectra for all the samples were obtained, followed by vector normalisation and baseline correction.

2.8. Thermal properties of sorghum flour

A differential scanning calorimeter (DSC; Model DSC25, TA

Instruments – Waters LLC, New Castle, DE, USA) was used for the thermal analysis of sorghum flour. Approximately 3 mg of the sample was weighed and placed in a zero-aluminium pan, with three times its volume of water was added (3,9 sample-to-water ratio). An identical empty pan was employed as a reference. The samples were kept at ambient temperature overnight. Heating was carried out in a nitrogen atmosphere (50 mL/min) from 25 °C to 100 °C at 5 °C/min. The device was calibrated using phase transitions of 99 + % adamantane (−65.54 °C), 18.2 MΩ.cm water (0.010 °C), 99.999 % indium (156.5985 °C), and 99.99 + % tin (231.93 °C). The cell constant was determined using the heat of fusion of 99.999 % indium (28.47 J/g) (Shah et al., 2016). Thermograms were analysed and corrected using the TRIOS software (TA Instruments, New Castle, DE, USA). Thermal transitions were assessed by the onset temperature (To), peak temperature (Tp), and the enthalpy change (ΔH), expressed in J/g, of sorghum flour.

2.9. Morphological properties of sorghum flour

The surface morphology of processed sorghum flour and control was investigated using secondary electron (SE) imaging on a dual-beam field-emission scanning electron microscope (Vega 3 VP-SEM, Tescan Vega, Czech Republic), following the method described by Shah et al. (2021). Samples were stored in a desiccator, mounted on an aluminium stub with carbon tape, and coated with a 6 nm layer using a sputter coater (208 HR, Cressington, Watford, UK). An accelerating voltage of 10 kV and a working distance of approximately 20 mm were applied.

2.10. Statistical analysis

Statistical analysis was performed using one-way ANOVA followed by Tukey's Honest Significant Difference (HSD) test, with statistical significance at $p < 0.05$. Rapid Visco Analyser (RVA) analysis was performed in duplicate, while all other measurements were conducted in triplicate. Statistical analysis was performed using SPSS v23 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Proximate analysis

Table 1 represents the proximate composition of sorghum flour samples. The results for moisture content and starch content of processed sorghum flours differed significantly from the control, ranging from 10.66 ± 0.29 g/100 g to 6.50 ± 0.48 g/100 g and from 67.23 ± 2.5 g/100 g to 47.70 ± 1.1 g/100 g, respectively. The protein content increased significantly from 7.89 ± 0.07 g/100 g to 11.49 ± 0.19 g/100 g in extruded flour, suggesting that the thermal-mechanical treatments improved protein accessibility and apparent concentration through moisture loss and structural modification. There was no significant difference ($p < 0.05$) in fat (between 3.07 ± 0.03 g/100 g and 3.46 ± 0.24 g/100 g), carbohydrate content (between 73.33 ± 1.36 g/100 g and 75.98 ± 0.40 g/100 g), and ash (between 1.36 ± 0.01 g/100 g and 2.28 ± 0.71 g/100 g) between processed sorghum flours and the control. The results for proximate composition are consistent with those reported by Palavecino et al. (2016), except for the quantity of dietary fibre.

The dietary fibre content ranged between 2.12 ± 0.09 g/100 g and 2.98 ± 0.06 g/100 g, which is lower than typical values reported for the whole grain sorghum (5 to 8 g/100 g). The Liberty cultivar used in this study is a white-pericarp sorghum with a thin bran layer, resulting in less dietary fibre than pigmented varieties (Pontieri et al., 2022). Additionally, the analytical method employed in this study was AOAC 985.29 (2005), which only quantifies insoluble dietary fibre, therefore, yields lower content than those that quantify total dietary fibre, such as AOAC 991.43 or AOAC 2009.01 (Phillips et al., 2019). Given these concerns, the impact of processing on dietary fibre was not observed, as previously reported. For example, heat-moisture treatment can increase resistant starch while the remaining proximate analysis remains the same as that of unprocessed sorghum (Vu et al., 2017). Specifically, sorghum flour treated at 20 % moisture and 100 °C for 4 h showed a significant increase in resistant starch, rising from 5.6 % in native sorghum flour to 22.1 % in the treated samples. This increase was attributed to the enhancement in the amylose-lipid complex formation and heat-induced structural changes in the protein fraction (Vu et al., 2017). This suggests that, based on Vu et al.'s findings, we may observe higher dietary fibre content in pre-gelatinised sorghum flours when analysed using a different method.

3.2. Effect of pre-treatments on pasting property of sorghum flour samples

The pasting properties of the sorghum flour via microwave popping, parboiling, sand roasting, pressure cooking, and extrusion are presented in Table 2 and Supplementary Fig. 1. The unprocessed sorghum flour exhibited the highest pasting properties: peak viscosity of 1785.5 ± 36.5 cP, holding viscosity of 1439 ± 28.99 cP, final viscosity of 4306 ± 60.81 cP, and pasting temperature of 84.25 ± 0.07 °C ($n = 3$; mean [M] ± standard deviation [SD]). The results differ slightly from those reported for unprocessed sorghum flour (Sharanagat et al., 2019), likely due to variations in the sorghum cultivars used.

Proximate composition, particularly starch content, can vary considerably among sorghum varieties and directly influence their functional properties, including pasting properties. The white sorghum cultivar Liberty used in this study is characterised by a higher starch content, whereas Haryana Jowar 513, used by Sharanagat et al. (2019), contains relatively less starch and is a creamy-white, tannin-free sorghum variety. The higher starch content in Liberty likely contributes to a greater swelling capacity and, consequently, a higher peak viscosity (1785.5 cP ± 36.5), consistent with previous studies. Moreover, the high starch-to-protein ratio of Liberty may also enhance greater peak and final viscosities (Table 2), as less protein restricts starch swelling (Cai et al., 2021; Li et al., 2016; Taylor & Taylor, 2011).

All pre-treatments significantly reduced the viscosity of sorghum flours (Table 2 and Supplementary Fig. 1), indicating that each had an effect on the starch molecular structure and functionality. The reduction in peak viscosity followed the order: roasting (624 ± 44.54 cP) < microwave popping (664 ± 15.56 cP) < pressure-cooking (757 ± 57.98 cP) < parboiling (829 ± 16.2 cP) < extrusion (853 ± 20.51 cP), with all

Table 1
Proximate composition of pre-gelatinised and raw sorghum flour.

Samples	Moisture	Fat	Ash	Protein	Dietary fibre	Starch content	Carbohydrate
Raw sorghum flour	10.66 ± 0.29^{a}	3.07 ± 0.03^{a}	2.25 ± 0.04^{a}	7.89 ± 0.07^{d}	2.80 ± 0.01^{a}	67.23 ± 2.5^{a}	73.33 ± 1.36^{a}
Popped sorghum flour	7.35 ± 0.34^{b}	3.46 ± 0.24^{a}	2.28 ± 0.71^{a}	8.81 ± 0.08^{cd}	2.12 ± 0.09^{a}	52.08 ± 2.9^{c}	75.98 ± 0.4^{a}
Roasted sorghum flour	6.93 ± 0.58^{b}	3.40 ± 0.05^{a}	1.92 ± 0.06^{a}	9.43 ± 0.03^{bc}	2.36 ± 0.71^{a}	51.79 ± 2.5^{c}	75.95 ± 3.61^{a}
Extruded sorghum flour	6.50 ± 0.48^{b}	3.39 ± 0.29^{a}	1.36 ± 0.01^{a}	11.49 ± 0.19^{a}	2.20 ± 0.02^{a}	59.21 ± 2.9^{b}	75.04 ± 1.44^{a}
Par-boiled sorghum flour	6.70 ± 0.45^{b}	3.36 ± 0.19^{a}	1.78 ± 0.02^{a}	10.38 ± 0.55^{ab}	2.98 ± 0.06^{a}	47.71 ± 1.1^{c}	74.80 ± 0.84^{a}
Pressure-cooked sorghum flour	7.33 ± 0.04^{b}	3.39 ± 0.15^{a}	1.66 ± 0.32^{a}	10.70 ± 0.52^{ab}	2.39 ± 0.09^{a}	48.61 ± 2.1^{c}	74.51 ± 2.26^{a}

Values are expressed as mean ± standard deviation ($n = 3$). Different superscript letters (a–d) within the same column indicate significant differences ($p < 0.05$). All parameters are expressed.

Table 2
Pasting properties of unprocessed and processed sorghum flours.

Samples	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown viscosity (cP)	Final viscosity (cP)	Setback viscosity (cP)	Peak time (S)	Pasting temperature (°C)
Unprocessed	1785.5 ± 36.5^{a}	1439 ± 28.99^{a}	346 ± 7.07^{b}	4306 ± 60.81^{a}	2866.5 ± 31.1^{a}	5.5 ± 0.04^{b}	84.25 ± 0.07^{b}
Extrusion	853.5 ± 20.51^{b}	469.5 ± 6.36^{e}	384 ± 14.4^{a}	836.5 ± 9.19^{d}	367 ± 2.82^{d}	4.72 ± 0.07^{c}	78.52 ± 0.03^{c}
Pressure cooked	757 ± 57.98b^{c}	727 ± 59.39^{bc}	30 ± 1.41^{c}	1300 ± 118.9^{c}	573 ± 59.39^{c}	6.93 ± 0.05^{a}	87.9 ± 0.5^{a}
Parboiled	829.5 ± 16.2^{b}	813 ± 15.55^{b}	16.5 ± 0.70^{c}	1857 ± 19.79^{b}	1044 ± 4.24^{b}	6.75 ± 0.04^{a}	89.13 ± 0.03^{a}
Popping	664 ± 15.56^{c}	626 ± 14.14^{cd}	38 ± 1.41^{c}	831.5 ± 30.41^{d}	205.5 ± 16.26^{e}	6.97 ± 0.01^{a}	88.27 ± 0.1^{a}
Roasting	624.5 ± 44.54^{c}	584 ± 49.66^{de}	40.5 ± 2.12^{c}	885.5 ± 86.97^{d}	301.5 ± 40.31^{de}	6.97 ± 0.07^{a}	90.37 ± 1.73^{a}

At $P < 0.05$, values along the columns with distinct superscripts (a, b, c and d) are considered statistically significant.

pre-treatment methods resulting in lower viscosities compared to the control (1785.5 ± 36.5 cP) (Table 1). Several mechanisms have been proposed to explain the reduction in viscosity: (a) heat and mechanical shear may cause starch swelling and gelatinisation, with heat leading to partial degradation, reducing gelling ability and lowering peak and final viscosities (Mathobo et al., 2021); (b) thermal-mechanical stress may strengthen protein-starch cross-links, restricting starch swelling (Ezeogu et al., 2005; Scott & Awika, 2023); (c) thermal treatment may denature sorghum protein, hindering starch hydration (Batariuc et al., 2021); (d) extrusion may cause granular degradation and structural breakdown, leading to amylose fragmentation and reduced retrogradation upon cooling (Sandrin et al., 2018). In short, viscosity reduction results from the disruption of starch molecular structure and protein interactions, limiting starch swelling and water-binding capacity after processing. A similar effect is observed in microwave-parboiled sorghum, where rapid microwave heating alters the lamellar structure of starch, generating molecular vibrations that cause gelatinisation and reduced viscosity (Dhanya et al., 2024).

3.3. *Effect of pre-treatments on in vitro protein digestibility of sorghum flour samples*

The in vitro protein digestibility of sorghum flours is presented in Fig. 1. Control, the unprocessed sorghum, exhibited lower protein digestibility compared to several pre-gelatinised sorghum variants, including microwave-popped, roasted, and extruded sorghum. These findings are consistent with previous studies that proteins in unprocessed sorghum had low digestibility (Baah et al., 2024). The low protein digestibility of unprocessed sorghum is primarily due to its high kafirin content, which accounts for approximately 70 % (*w*/w) of total sorghum protein. Protein digestibility is influenced by both endogenous factors, such as high disulfide cross-linking (Hamaker et al., 1986) and racemisation and isopeptide formation (Liardon & Hurrell, 1983), and exogenous factors, including interactions with non-protein compounds, such as polyphenols (e.g., tannins) (Hahn et al., 1984), phytic acid (Elkhalil et al., 2001), cell wall components (Glennie, 1984), and starch (Seckinger & Wolf, 1973).

Protein digestibility of sorghum flours varied significantly between treatments ($p < 0.05$). The highest digestibility was observed in extruded flour (76.2 %), followed by microwave-popped flour (66.1 %) and sand-roasted samples (65.7 %). In contrast, parboiled (45.3 %) and pressure-cooked (42.6 %) sorghum flour exhibited lower protein digestibility. These results align with previous studies, which suggest that heat-moisture or wet treatments, such as parboiling and pressure cooking, may induce protein aggregation, complex reformation, and strong protein-starch cross-links, all of which hinder enzymatic hydrolysis and reduce protein digestibility (Duodu et al., 2003; Hamaker et al., 1986; Nunes et al., 2004).

These differences between wet and dry heat treatments are primarily due to their distinct effects on protein structure (specifically prolamin-non-prolamin interactions) and protein-starch interactions. In wet-heat treatments, such as parboiling, sorghum components are exposed to high temperature and moisture, promoting extensive protein unfolding and aggregation. This resulted in large and insoluble protein aggregates that are poorly accessible to proteolytic enzymes (Duodu et al., 2003; Emmambux & Taylor, 2009). Additionally, during parboiling, simultaneous starch gelatinisation may entrap denatured proteins within a protein-starch matrix, and subsequent starch retrogradation further reinforces the structure, restricting enzyme accessibility (Wong et al., 2009). The Maillard-type crosslinking between protein and reducing sugars occurs more readily under moist conditions, further reducing digestibility (Lund & Ray, 2017).

In contrast, dry-heat treatments (i.e., extrusion, microwave popping, and sand roasting) induce partial denaturation without extensive aggregation. The low-moisture environment limits disulfide exchange and protein-starch cross-linking, resulting in a more open microstructure and greater accessibility of peptide bonds to digestive enzymes (Duodu et al., 2001; Duodu et al., 2003; Rooney & Pflugfelder, 1986).

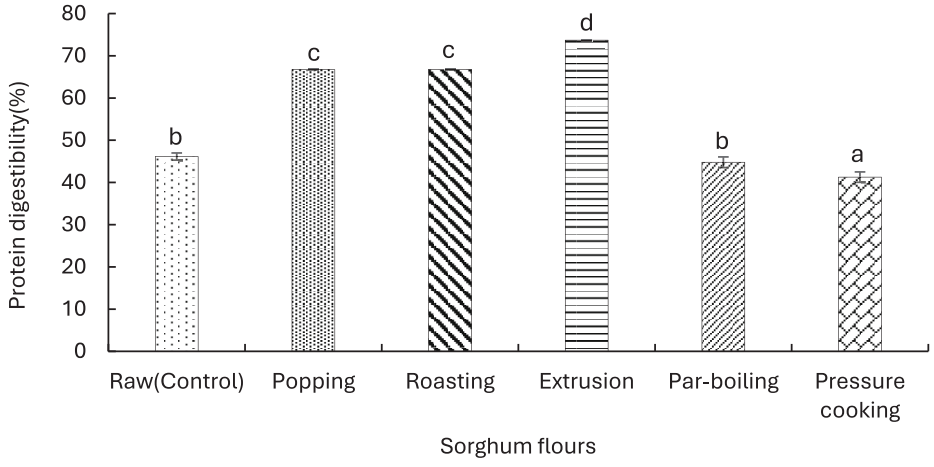

Fig. 1. In vitro protein digestibility of sorghum flour samples. A similar superscript represents no significant difference ($P < 0.05$).

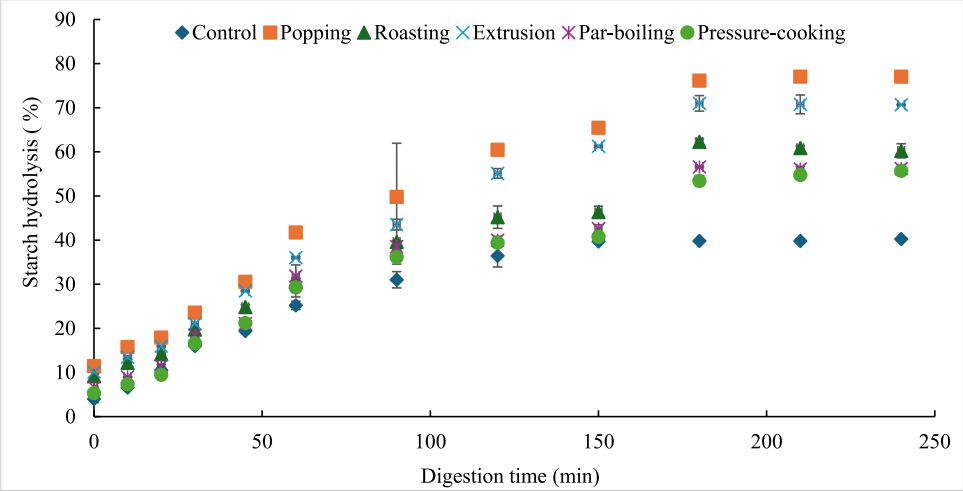

Fig. 2. Starch digestibility of processed and unprocessed sorghum flour samples.

Furthermore, high-heat treatments of sorghum may reduce the anti-nutritional factors, particularly tannins and phytic acid, which, otherwise, could form insoluble complexes with proteins, inhibiting digestion (Duodu et al., 2003). Similar improvements in protein digestibility have been reported for extrusion cooking (Bhattarai et al., 2025; Wang et al., 2020) and popping (Duodu et al., 2002). Conversely, Oria et al. (1995) reported that wet cooking of sorghum flour (200 mg flour heated with 5 mL at 100 °C for 5 min) significantly reduced pepsin digestibility, as measured by the Digestibility Determination Test (DDT), from 69.2 to 43.6 %.

3.4. *Effect of pre-treatments on in vitro starch digestibility of sorghum flour samples*

Fig. 2 shows the digestion profile of the processed sorghum flours and the control. Unprocessed sorghum (control) exhibited the lowest starch digestibility (Fig. 2), while the thermal-mechanical processed sorghum flours had increased digestibility. Among the treatments, microwave popping resulted in the highest digestibility throughout the hydrolysis period (Supplementary Table 1), followed by extrusion > roasting > pressure cooking > parboiling. The low digestibility of unprocessed sorghum may be due to a compact starch-kafirin protein matrix (hydrophobic protein), limiting enzyme accessibility (Xiao et al., 2017). In contrast, the increased digestibility in processed sorghum likely results from enhanced substrate permeability, due to disruption of starch-kafirin complexes during processing. These findings are consistent with previous reports (Wong et al., 2009). The extrusion-induced changes to the starch granules and their impact on digestibility have been recently reviewed by Bhattarai et al. (2025).

During the hydrolysis period, all sorghum flours showed increased hydrolysis up to 170 min, followed by a plateau until 249 min. This suggests rapid hydrolysis of readily available starch fractions, such as

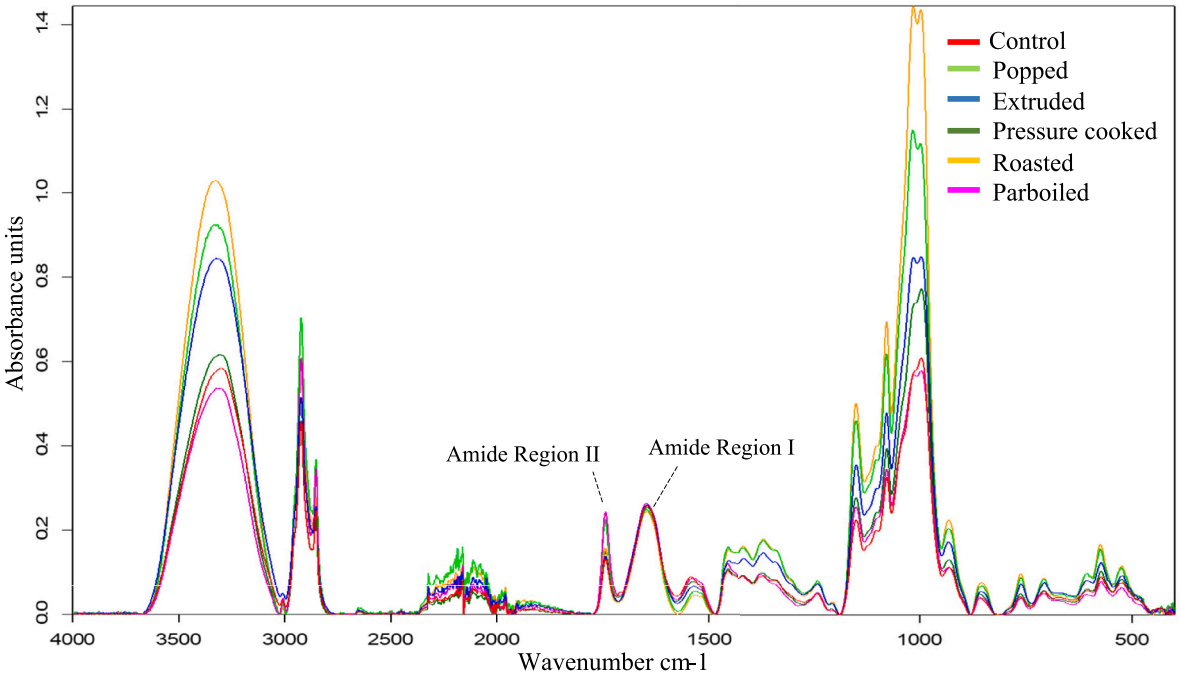

Fig. 3. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopic characterisation of the secondary structure of processed and unprocessed sorghum flour.

amorphous and partially gelatinised starch, in the early digestion phase. As the hydrolysis progressed, the remaining starch became more resistant, likely due to strong interactions with the kafirin protein matrix and other non-starch compounds, such as phytates and tannins (Gu et al., 2024; Hamaker et al., 1986). The processing employed in this study was a pre-gelatinisation treatment, which involved the transition of starch granular structure into gelatinised molecular form. At the granular level, the crystalline structure, primarily A-type polymorphism, restricts water retention and makes it resistant to enzymatic hydrolysis (Haziman et al., 2025; Ma & Boye, 2018). The increased digestibility, promoted by the processing treatments, suggests disruption of this crystalline structure, leading to granule swelling, loss of crystallinity, and partial gelatinisation, which enhances enzymatic hydrolysis. These findings align with previous studies on physical modifications of sorghum starch (Uzizerimana et al., 2021), where changes in crystalline structure and granule morphology were observed. However, excessive heat treatment, under suitable moisture conditions, can cause starch retrogradation, resulting in resistant starch and reduced digestibility. For example, infrared thermal treatment increased resistant and slowly digestible starch, along with water absorption capacity and thermal stability, but also resulted in higher crystallinity (Semwal & Meera, 2021).

3.5. *Effect of pre-treatments on the secondary structure of sorghum flour samples*

The representative FTIR spectrum of processed and unprocessed sorghum flour is shown in Fig. 3. It exhibits characteristic absorption banding corresponding to its major biochemical constituents, including carbohydrates, proteins, and lipids. Sorghum flour samples exhibited a broad absorption peak at 3600–3200 cm^{-1}, attributed to stretching vibrations of OH groups, indicating the presence of hydrogen bonds, primarily from polysaccharides and water. A peak around 2900 to 2850 cm^{-1} corresponds to C—H stretching vibrations of methyl and methylene groups in lipids and carbohydrates (Ezeogu et al., 2008; Semwal & Meera, 2025; Xiao et al., 2015).

Prominent peaks at around 1200 cm^{-1} to 900 cm^{-1}, known as the fingerprint region for carbohydrates, displayed strong absorption banding near 1047 cm^{-1} and 1022 cm^{-1}, corresponding to C—O stretching vibrations of starch, specifically related to the glycoside bonds in the polysaccharide structure. (Castro-Campos et al., 2021; Duodu et al., 2001; Jafari et al., 2017; Lin et al., 2021). A slight shift in this carbohydrate fingerprint region, ranging from 1100 to 1000 cm^{-1}, was seen upon pre-treatments, in particular, banding centred at ~1047 cm^{-1} and ~ 1022 cm^{-1}, indicating glycosidic linkages of starch and a decrease in the starch crystallinity (loss of ordered structure) upon thermal-mechanical treatment (Semwal & Meera, 2025). Such absorbance peaks indicate that the processing treatments might have disrupted the native crystalline structure of starch granules (Castro-Campos et al., 2021). This might have led to an overall increase in amorphous content, further enhancing accessibility to hydroxyl groups (-OH) and glycosidic (C-O-C) functional groups (Jafari et al., 2017). This spectral arrangement confirmed the thermal-mechanical processing of sorghum (where starch granules usually swell and lose birefringence), as well as altered molecular interactions and vibrational characteristics. Such findings are consistent with the literature, which analysed waxy maize starch using FTIR, showing that during starch gelatinisation, an increase in the band at 1022 cm^{-1} indicated a loss of ordered structures (Wilson et al., 1987). Additionally, popping the sorghum flour was also found to

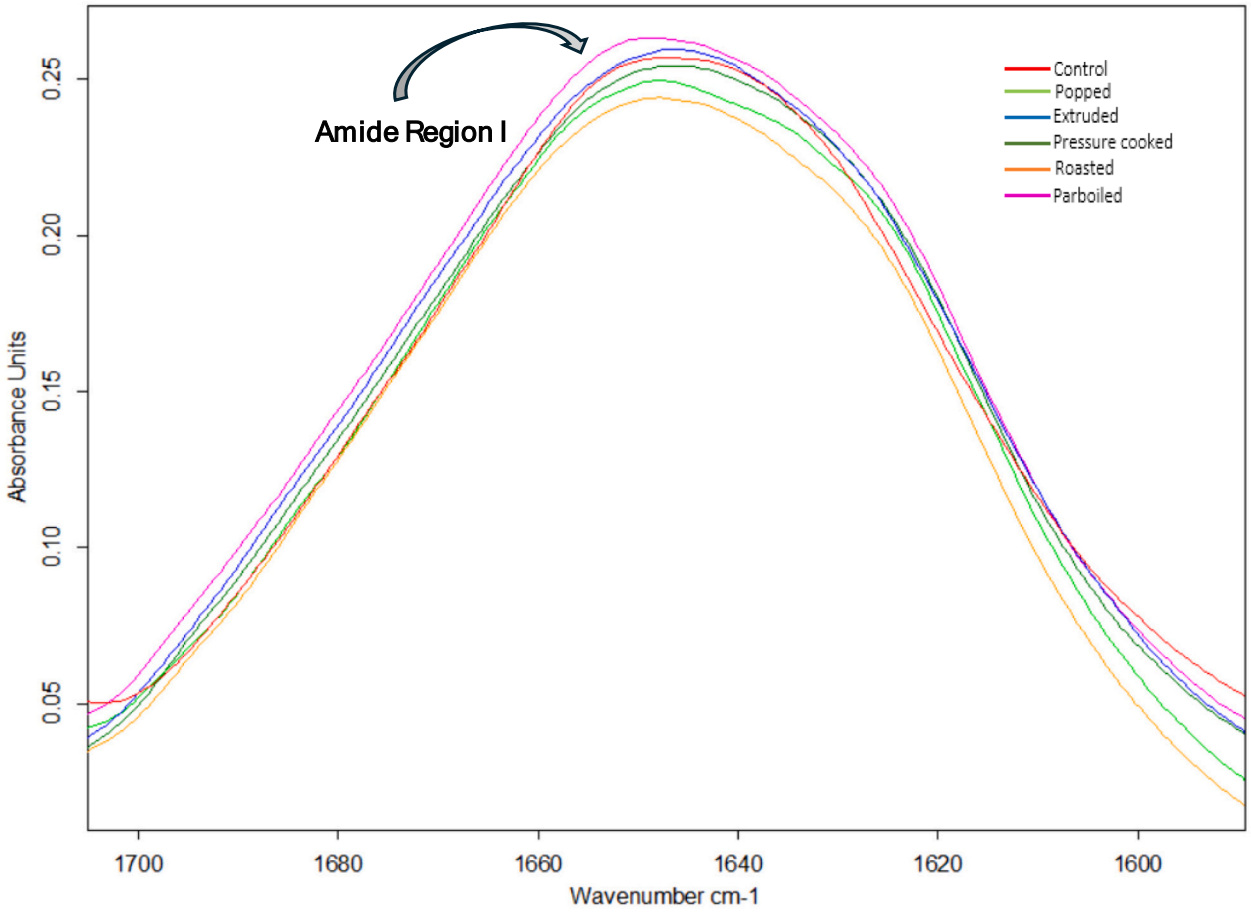

Fig. 4. Amide *I* region of attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy of processed and unprocessed sorghum flour.

alter the secondary structure of sorghum (Castro-Campos et al., 2021). The authors reported higher-order changes in popping sorghum flour, particularly in the short-range structure.

The peak at ~1650 cm^{-1} is representative of amide I (C═O stretching), and the peak at ~1540 cm^{-1} represents amide II (N—H bending and C—H stretching) bands. The amide I is sensitive to protein secondary structure due to two main factors: (1) distinct secondary structures are stabilised by specific hydrogen-bonding patterns involving the carbonyl group in the amide linkage, and (2) hydrogen bonding influences the C═O vibrational frequency (Elliott & Ambrose, 1950; Jackson & Mantsch, 1995; Surewicz et al., 1993; Susi & Byler, 1983). Fig. 4 represents the magnified amide I region of the baseline-corrected and vector-normalised spectra. The broad absorption peak at ~1650 cm^{-1} was found in raw and pre-treated sorghum flour samples, which is mainly associated with α-helical and random-coil conformations, with minor contributions from β-structures (β-sheets and β-turns).

Upon pre-treatments, clear spectral differences were observed. The wet heat treatments, such as parboiling and pressure cooking, exhibited a shift in the amide I peak from 1650 cm^{-1} to ~1630 cm^{-1} with reduced peak intensity. Previous literature related such shifts to the increased β-sheet formation and stronger intermolecular hydrogen bonding. This, in turn, reflects protein aggregation and a more ordered structure (Shah et al., 2021). Duodu et al. (2001) reported an increase in the intensity of the amide I band, centred around ~1635 cm^{-1}, following wet cooking. Our findings also align with those of Gao et al. (2005), who reported that sorghum protein showed enhanced β-sheet aggregation upon high-heat treatment. In another study, cooked sorghum protein was evaluated for secondary structure, and the authors found alterations in its secondary structure upon cooking, particularly shifts in the intensities of amide bands (Ezeogu et al., 2008). In contrast, dry heat treatments (extrusion, popping, and roasting) resulted in minor changes, with only a slight reduction in peak intensity. This suggests that partial unfolding of α-helices and a slight increase in random-coil structures enhanced molecular flexibility and reduced protein aggregation.

The wet heat-treated sorghum flour (parboiling and pressure cooking) showed lower digestibility (Section 3.3) compared to that subjected to dry heat treatment. FTIR findings support it, as the formulation of aggregated β-sheet structures under wet-heat conditions likely limits enzyme accessibility and reduces proteolytic degradation efficiency. In dry-heat treatments, partial unfolding and reduced aggregation are consistent with the in vitro protein digestibility results, which showed higher digestibility. This is because partial unfolding might render the sorghum protein more open and flexible (see SEM Fig. 6), thereby exposing additional peptide bonds to digestive enzymes and facilitating hydrolysis.

While the amide I band alone does not allow precise determination of protein secondary structure, it is widely used to monitor relative changes in protein conformation. Numerous studies, including those for sorghum protein, have successfully used this approach to evaluate changes in the secondary structure under various processing treatments (Gao et al., 2005; Jackson & Mantsch, 1995; Kong & Yu, 2007; Miller et al., 2013; Shah et al., 2021; Tidy et al., 2017). The amide I region, therefore, provides robust qualitative evidence of conformational modification. Although detailed quantitative deconvolution of the amide I band using Gaussian fitting algorithms is beyond the scope of this study, the comparative spectral approach effectively captured

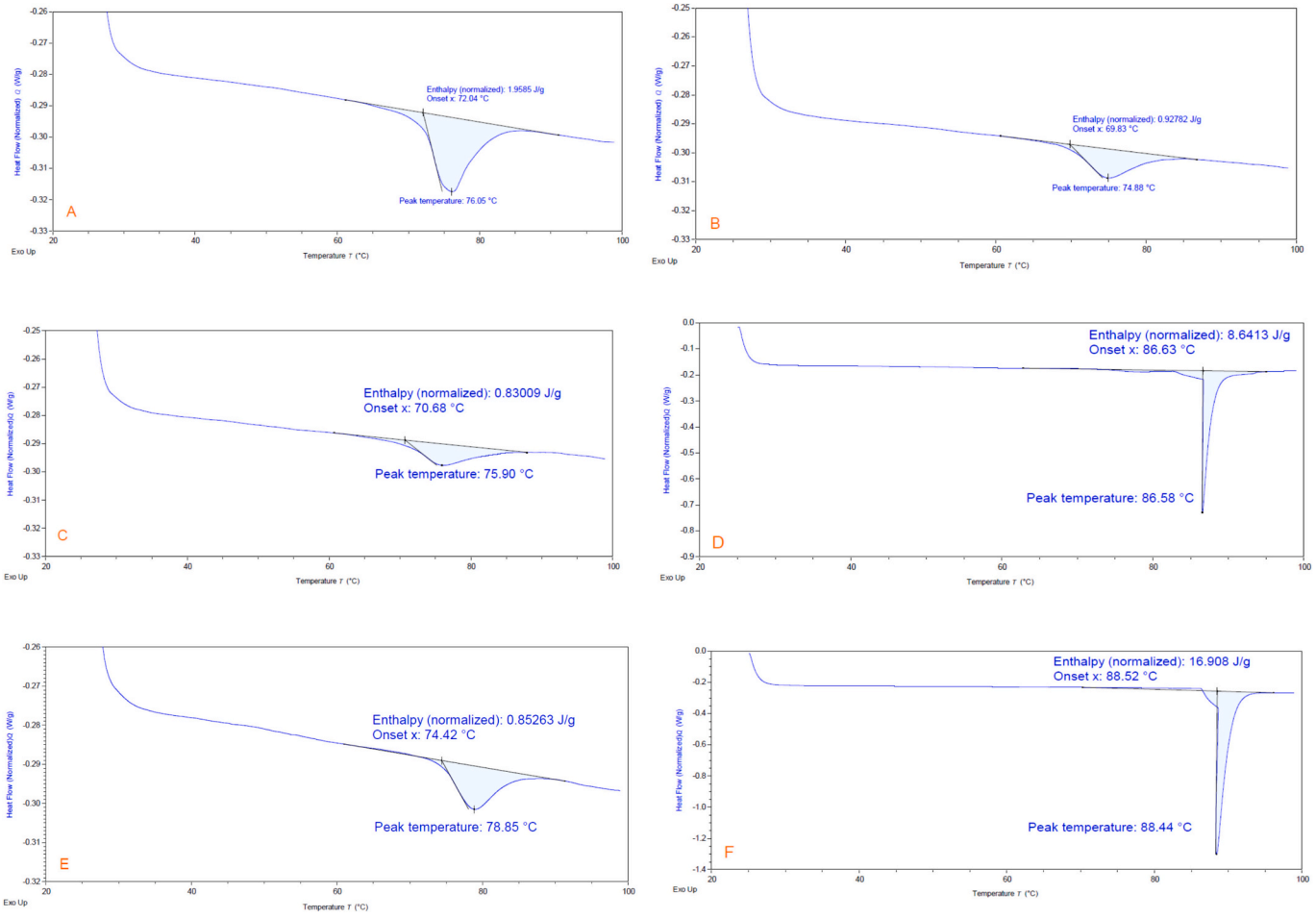

Fig. 5. DSC thermograms of sorghum flour. (A) Unprocessed sorghum flour (control); (B) popped sorghum flour; (C) roasted sorghum flour; (D) pressure-cooked sorghum flour; (E) parboiled sorghum flour; and (F) extruded sorghum flour.

relative structural shifts across pre-treatments.

3.6. Effect of pre-treatments on thermal properties of sorghum flour samples

DSC data, including onset temperature (To), peak temperature (Tp), and the enthalpy change (ΔH), are shown in Fig. 5. The three wet-heat processing methods (i.e., pressure cooking, extrusion, and parboiling) resulted in higher values of To, Tp, and ΔH than the control, while the two dry-heat processing methods (i.e., roasting and popping) had reduced values of the three parameters. As shown in the FTIR results (Section 3.5), wet-heat treatments induced protein aggregation and β-sheet formation, whereas dry heat treatment led to partial unfolding and an increase in random-coil content. The β-sheet aggregates strengthened protein-starch interactions through hydrogen bonding between starch hydroxyl groups and protein amide groups, and hydrophobic associations between unfolded protein nonpolar regions (Scott & Awika, 2023). As a result, a compact network was formed, which restricts water diffusion and granule swelling; consequently, greater thermal energy (higher ΔH and Tp) is required to disrupt the stabilised matrix. Marston et al. (2016) and Sharanagat et al. (2024) also reported that reducing water absorption increases the energy required for structural modification. These findings support the results of extrusion and pressure-cooking processing, aligning with previous studies that showed heat-moisture treatment increased the enthalpy change or gelatinisation temperature (Sun et al., 2014).

Unlike the wet-heat processing, dry-heat treatments likely led to more flexible, less aggregated protein conformations, weakening interactions and promoting starch gelatinisation at lower temperatures, resulting in lower ΔH values. Furthermore, dry heat may cause partial gelatinisation, thermal degradation, and a reduction in molecular order, which contribute to the need for little energy to dissociate the starch structure (Batariuc et al., 2023). This was supported by the low values of the thermal parameters for the roasting and popping treatments in this study.

3.7. Effect of pre-treatments on the morphological structure of sorghum flour samples

Morphological imaging of processed sorghum flour showed significant changes compared with the control (Fig. 6). Unprocessed sorghum primarily exhibited polygonal and oval starch granules alongside spherical, compactly folded proteins, which are consistent with the report of native sorghum morphology (Mahasukhonthachat et al., 2010). After the pre-gelatinised treatments, the micrographs showed swelling, ruptures, and granule fusion, forming an amorphous matrix that suggests damage to the granular structure as well as partial gelatinisation.

The microstructural features observed in the SEM images seem to align with the formation of a protein-starch network. Among the five treatments, parboiling and pressure cooking generated smoother and more compact structures, which may be due to the interactions of unfolded protein molecules and gelatinised starch through hydrophobic and disulphide bonds. Such an interaction may contribute to the reduced starch digestibility due to the restriction of starch granular swelling (Emmambux & Taylor, 2009; Wong et al., 2009). In contrast, popping and roasting lacked such smooth and gel-like features, while some individual starch granules are visible in the SEM images. Having less protein-starch complexation may offer a greater starch surface area for enzymatic hydrolysis and a higher starch digestibility (Jafari et al., 2017; Nathakattur Saravanabavan et al., 2013). Extruded sorghum flours show irregular and partially damaged particles with a gel-like feature, which suggests that extrusion may induce amylose leaching and elevated amorphous regions (Jafari et al., 2017; Zhang et al., 2016).

4. Conclusion

Thermal-mechanical treatments, including parboiling, roasting, extrusion, and pressure cooking, altered the structure and impacted the physicochemical and thermal properties, which in turn modulated the digestibility and functionality of sorghum flour. Dry-heat treatments, including microwave popping, roasting, and extrusion, effectively

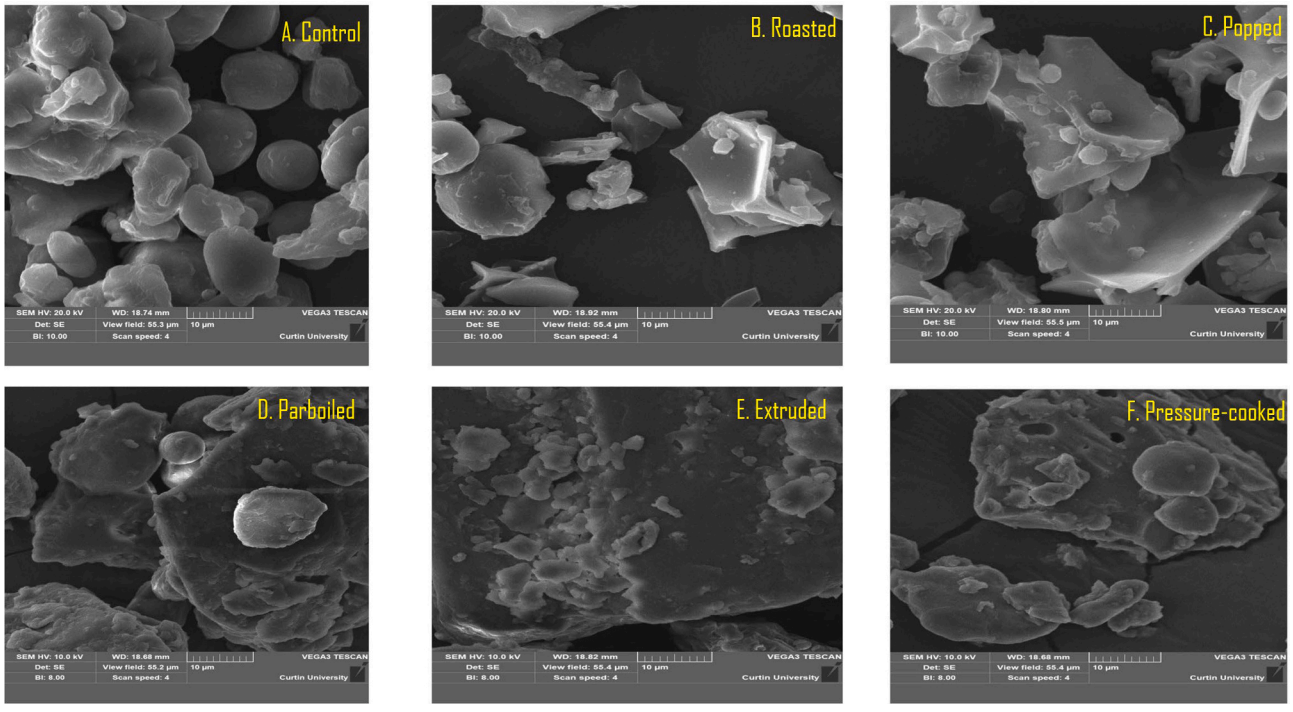

Fig. 6. Field emission-scanning electron micrograph (FE-SEM) of sorghum flours (A) un-processed sorghum flour (control); (B) roasted sorghum flour; (C) popped sorghum flour; (D) parboiled sorghum flour; (E) extruded sorghum flour; and (F) pressure-cooked sorghum flour.

disrupted the starch-protein network, enhancing protein and starch digestibility and reducing paste viscosities (roasting ≈ popping > extrusion). In contrast, wet-heat treatments, including parboiling and pressure cooking, promoted protein-starch aggregation, resulting in lower protein digestibility but enhancing thermal stability. Extrusion, due to its pronounced effects on microstructural reorganisation, exhibited excellent thermal stability and the highest protein digestibility. Parboiling and pressure cooking also offer excellent thermal stability, accompanied by lower starch digestibility. Roasting and popping are preferred for applications requiring reduced viscosity. These findings underline the critical relationship between sorghum structure and its functional properties, offering valuable insights for optimising processing conditions to achieve desired nutritional and technological outcomes.

CRediT authorship contribution statement

Sumi Shah: Writing – original draft, Methodology, Investigation, Formal analysis. **Umar Shah:** Writing – review & editing, Data curation. **Adil Gani:** Writing – review & editing. **Amy Hui-Mei Lin:** Writing – review & editing. **Darren Greetham:** Writing – review & editing. **Rewati R. Bhattarai:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2025.147496.

Data availability

Data will be made available on request.

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