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Effect of Enzymatic Extrusion Treatment on Pregelatinized Sorghum Flour

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Abstract. *Sorghum flour has gained considerable attention as a gluten-free cereal with high nutritional value; however, its functional properties require improvement for industrial applications. This study aimed to investigate the effects of combined extrusion and enzymatic treatment on the morphological characteristics, thermal properties, and carbohydrate composition of pregelatinized sorghum flour. Three flour samples were prepared: native sorghum flour (PS1), extrusion-treated flour (PS2), and extrusion-enzymatic-treated flour with α-amylase (PS3). Morphological analysis was performed using polarized light microscopy and scanning electron microscopy. Thermal properties were assessed by differential scanning calorimetry (DSC), and carbohydrate composition was determined by high-performance liquid chromatography (HPLC). Results showed progressive loss of the Maltese cross pattern in PS2 and PS3, indicating disruption of crystalline structure, along with granule aggregation caused by amylose leaching. DSC analysis revealed that PS2 exhibited the most significant reduction in gelatinization temperature (T_o: 77.84 °C) and enthalpy (ΔH: 2.60 J/g) compared to native sorghum starch (T_o: 82.38 °C; ΔH: 7.52 J/g), representing a 57% decrease in ΔH. In contrast, PS3 showed no detectable endothermic peak (n.d.), indicating complete loss of residual crystalline structure. HPLC analysis confirmed extensive starch hydrolysis in PS3, with glucose concentration increasing more than 15-fold (77.31 mg/mL) compared to PS1, along with elevated maltose (12.05 mg/mL) and fructose (8.13 mg/mL) concentrations. These findings demonstrate that extrusion-enzymatic treatment effectively modifies sorghum flour structure, enhancing sugar release and altering thermal characteristics, thereby indicating improved functional properties for applications in instant foods, bakery products, and fermentation processes.*

Keywords: *birefringence; carbohydrate profile; enzymatic extrusion; pregelatinization sorghum flour; thermal properties.*

Type of the Paper: Regular Article.

1. Introduction

Sorghum (*Sorghum bicolor* L. Moench) has gained considerable attention as a promising alternative cereal crop, particularly for individuals with celiac disease and gluten-related disorders owing its naturally gluten-free properties [1,2]. Beyond its nutritional benefits, including high protein content, dietary fiber, and bioactive compounds such as polyphenols, sorghum presents opportunities for developing functional food ingredients through various modification techniques [3,4]. The conversion of native sorghum starch into pregelatinized flour through controlled processing offers advantages including improved digestibility, enhanced functional properties, and modified sugar release patterns, which are crucial for formulating specialized food products and

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instant applications [5].

Physical and enzymatic modifications have been extensively employed to produce pre-gelatinized flours with tailored characteristics. Extrusion processing, recognized as a versatile high-temperature short-time (HTST) technology, induces significant structural changes in starch granules through the combined effects of heat, moisture, and mechanical shear forces [6,7]. When coupled with enzymatic treatment, the extrusion-enzymatic process offers enhanced control over starch modification, leading to products with tailored functional properties [8,9]. A recent study by Usman et al. [10] demonstrated that extrusion-enzymatic treatment at varying feed moisture levels (20-35% db) and α-amylase concentrations (0.1-1.5% db) significantly altered the physicochemical, thermal, and pasting properties of sorghum flour, with observable changes in swelling power, solubility, and viscosity profiles. However, the study did not comprehensively investigate the sugar formation patterns and their relationship with structural characteristics of the modified flour.

The degree of starch gelatinization and subsequent sugar formation is fundamentally related to the disruption of crystalline structure within starch granules. Polarized light microscopy serves as a powerful tool for assessing structural changes in starch, as the birefringence patterns and characteristic Maltese cross reflect the integrity of crystalline regions [11,12]. The progressive loss of birefringence during gelatinization indicates molecular reorganization and increased amorphous content, which correlates with enhanced enzyme accessibility and hydrolysis potential [13,14]. Complementary microscopic techniques, such as scanning electron microscopy (SEM), reveal changes in surface morphology and granule aggregation patterns that result from starch leaching and molecular interactions during processing [15].

Thermal properties, assessed using differential scanning calorimetry (DSC), provide quantitative information about the degree of starch modification including crystallinity of material. The gelatinization temperature range and associated enthalpy change reflect the energy required to disrupt the crystalline within the starch granule [16]. Extrusion processing significantly reduces both the gelatinization temperature and enthalpy, with the extent of reduction depending on processing conditions such as barrel temperature, screw speed, and feed moisture content [17]. Studies have demonstrated that higher processing intensity (e.g., higher barrel temperature, higher screw speed, or lower feed moisture) leads to greater structural disruption, manifested as lower residual gelatinization enthalpy and narrower gelatinization temperature ranges [18,19]. Understanding the thermal behavior of modified flours is essential for predicting their performance during subsequent food manufacturing operations.

The sugar composition resulting from starch hydrolysis plays a crucial role in determining the functional applications and nutritional properties of modified flours. Different sugar profiles—

characterized by the relative proportions of glucose, maltose, and higher maltooligosaccharides—affect sweetness, hygroscopicity, fermentability, and glycemic response [20,21]. Understanding the relationship between microstructure and sugar content is essential for rational design and optimization of modified sorghum flour production. While previous research has documented the effects of extrusion-enzymatic treatment on general physicochemical properties [10], the mechanistic link between granular morphology, crystallinity disruption, and sugar formation patterns requires elucidation. Such knowledge would enable precise control over modification processes to achieve target sugar profiles for specific applications, ranging from low-glycemic index products to rapidly fermentable ingredients for bioprocessing [22,23].

Therefore, this study aimed to investigate the morphological characteristics, thermal properties, and carbohydrate composition of pre-gelatinized sorghum flour produced through extrusion-enzymatic treatment. Specific objectives included characterizing morphological changes in starch granules using polarized light microscopy and scanning electron microscopy to asses structural disruption and granule integrity; evaluating thermal properties through differential scanning calorimetry to quantify the degree of gelatinization and remaining crystallinity; and determining individual sugar composition and total carbohydrate content using high-performance liquid chromatography to establish the extent of enzymatic hydrolysis. This integrated approach addresses the current knowledge gap by linking structural disruption to sugar release patterns, thereby providing a scientific basis for developing modified sorghum flours with predictable functional properties for food applications.

2. Materials and Methods

2.1. Materials

Three types of sorghum flour were prepared using the Sorghum Kawaii variety grain supplied by the Cereal Crops Research Institute, Indonesian Agency for Agricultural Research and Development (IAARD), Indonesia: native flour (PS1), extrusion-treated flour (PS2), and extrusion-enzymatic treated flour (PS3). The preparation followed the method of Usman et al. [10] with the following modification: sorghum flour was prepared by dehulling followed by dry milling and sieving using a 200-mesh sieve. The dry sorghum flour was extruded using a twin-screw extruder (the HAAKE^{TM} Rheomex CTW 100 OS, Thermo Fisher, US) at 100 °C across all heating zone without a die, under the following conditions: feed rate of 1kg/h and srew speed was increased to 100 rpm. PS1 served as the control (no extrusion, no α-amylase addition). PS2 was extruded with 30% feed moisture (dry basis, db). PS3 was extruded with 30% feed moisture (db) and 1% α-amylase (enzyme/substrate ratio: 0.72 KNU-S/g). The extrudates were cooled and dried using convection oven (50 °C for 6 h), then stored in sealed plastic bags at room temperature (25 ± 2 °C).

2.2. Birefringence observation

The loss of starch granule birefringence was observed at 400× magnification using an Olympus BX61 light microscope (Olympus Optical Corporation, Japan) equipped with a polarized filter, following the method described by Muñoz et al. [24] with slight modification. Starch granule suspensions were prepared prior to observation by suspending 1 mg each flour in 1 mL of distilled water followed by vortex mixing for 30 s at 2,500 rpm. The suspensions were equilibrated for 2 h at room temperature (25 ± 2 °C). Several drops of each suspension were then deposited onto a concave slide and covered with coverslip. Each spesimen was observed under normal and polarized light. For each sample, at least 100 starch granules were observed across three randomly selected fields. Images were captured using a digital camera attached to the microscope. The loss of birefringence was qualitatively assessed based on the disappearance or weakening of the Maltese cross pattern. Observations were performed in duplicate for each flour sample.

2.3. Carbohydrate content analysis

Carbohydrate content was analyzed using high-performance liquid chromatography (HPLC) (UltiMate 3000, Thermo Fisher Scientific, Germany) equipped with a refractive index detector (RID) (Vanquish, Thermo Fisher Scientific, Germany). Data acquisition and processing were performed using Chromeleon software (Thermo Fisher Scientific, Germany). HPLC separation was carried out using a Dionex CarboPac PA10 analytical column (4 × 250 mm) equipped with a CarboPac PA10 guard column (4 × 50 mm). The mobile phase (specify composition, e.g., '100 mM NaOH') was filtered under a vacuum through a 0.2 µm membrane filter and degassed by sonication for 15 min prior to use. Prior to injection, all samples were filtered through a 0.45 µm syringe filter (4 mm diameter). The volume of the injected sample was 20 µL. Standard solutions were prepared by dissolving 1.00 g of a carbohydrate standard mix (containing mono-, di-, and trisaccharides, e.g., glucose, fructose, sucrose, and maltose) in ultrapure water using separate 100.00 mL volumetric flasks. Both stock and diluted standard solutions were stored in glass volumetric flasks protected from light at 4 °C until analysis. The sensitivity, repeatability, and reproducibility of the HPLC-RID method were determined according to the International Conference on Harmonization (ICH) Q2(R1) guidelines. Observations were performed in duplicate for each flour sample

2.4. Thermal properties

Thermal properties were measured by placing 3.0 mg of each sample into an aluminum DSC pan and adding distilled water at a sample-to-water ratio of 1:3 (w/w). The pan was hermetically sealed and then analyzed using a differential scanning calorimeter (DSC; Lab SYS-DSC 8500, model N5340501, Perkin Elmer, Norwalk, CT, USA), following the method described by Sun et

al. [25]. Samples were heated from 25 °C to 120 °C at a heating rate of 10 °C/min under a nitrogen atmosphere (flow rate: 20 mL/min). An empty aluminum pan was used as a reference. The onset temperature (To), peak temperature (Tp), conclusion temperature (Tc), and gelatinization enthalpy (ΔH) were recorded. ΔH was expressed in joules per gram (J/g) of dry sample.

2.5. Data analysis

The Morphological properties (birefringence patterns) were analyzed qualitatively by visual observation. Thermal properties and sugar content were analyzed quantitatively. Carbohydrate composition data were statistically analyzed using one-way analysis of variance (ANOVA) with a significance level of $\alpha = 0.05$. When significant differences were detected, Tukey's honestly significant difference (HSD) post hoc test was applied for multiple comparisons. All statistical analyses were performed using RStudio software (version 2026.01.0, RStudio, PBC, Boston, MA, USA). Prior to ANOVA, normality of residuals was assessed using the Shapiro–Wilk test, and homogeneity of variances was verified using Levene's test. Both assumptions were met ($p > 0.05$). All statistical analyses were performed using R software (version 4.3.0; R Core Team, Vienna, Austria) via the RStudio integrated development environment (version 2026.01.0; RStudio, PBC, Boston, MA, USA). All quantitative results are presented as mean ± standard deviation (SD) from triplicate measurements. Birefringence observations were performed in duplicate for each sample, with at least 100 starch granules examined per replicate, as described in Section 2.2.

3. Results and Discussion

3.1 Effect on morphological characteristics

Combined extrusion and enzymatic treatment significantly altered the morphological properties of sorghum flour, including loss of birefringence, granule aggregation, and structural disruption. Changes in the Maltese cross pattern of starch granules are shown in Fig. 1. Surface morphology and granule aggregation were examined using scanning electron microscopy (SEM) (Fig. 2). As shown in Fig. 1, the Maltese cross pattern (indicated by bright birefringence) was clearly visible in PS1 but progressively weakened in PS2 and PS3. This loss of birefringence is attributed to starch swelling and subsequent disruption of the crystalline structure during extrusion. When starch granules are exposed to water and temperatures above the gelatinization temperature, they absorb water and swell, leading to the loss of molecular order within the crystalline regions [26]. Notably, the Maltese cross pattern did not completely disappear in PS2 or PS3, indicating that the extrusion and enzymatic treatments resulted in only partial disruption of the crystalline structure under the conditions applied.

The differences in birefringence between PS2 and PS3 reflect distinct mechanisms and degrees of crystalline disruption. In PS2, extrusion with limited moisture content (30% db) induced

partial gelatinization, leaving some intact crystalline regions and observable Maltese cross patterns [27,28]. In contrast, PS3 underwent synergistic damage: extrusion thermally and mechanically opened the granule structure, after which α-amylase hydrolyzed glycosidic bonds in the exposed amorphous regions, further disrupting the remaining crystalline regularity and nearly eliminating the Maltese cross pattern [29–32].

SEM micrographs (Fig. 2) revealed that most starch granules were polygonal or irregularly spherical with angular surfaces, which is typical for sorghum starch. In PS2 and PS3, granule aggregation was observed, with some granules adhering to one another. In Fig. 2, surface morphology shows smooth, intact granules in PS1, whereas PS2 and PS3 exhibit rough surfaces and inter-granule adhesion (indicated by white arrows). This adhesion is attributed to amylose leaching during extrusion. As amylose is released from swollen granules, it forms a continuous amorphous matrix that binds adjacent granules together upon cooling [33]. In PS3, despite partial hydrolysis by α-amylase, sufficient amylose remained to facilitate granule aggregation.

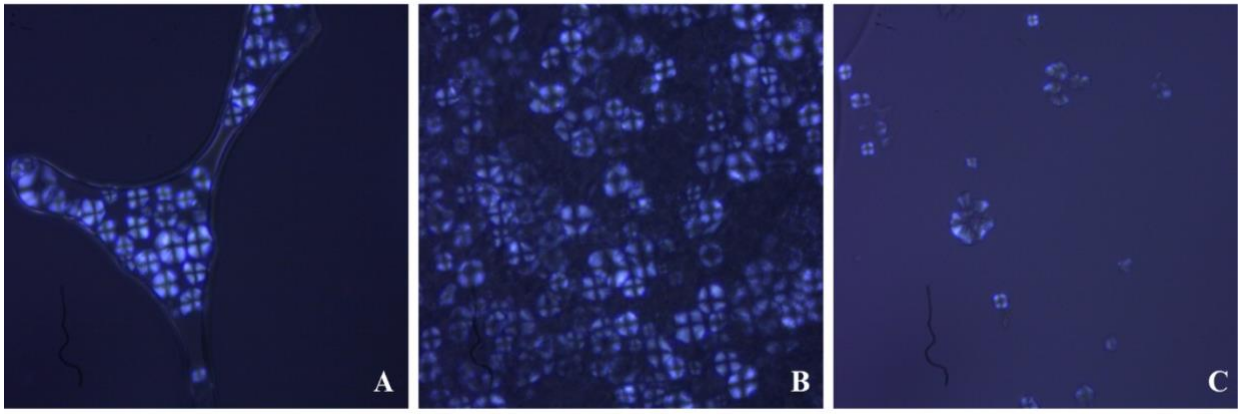

Fig. 1. Birefringence of starch on pre-gelatinized sorghum flour (A: native sorghum flour (PS1); B: extrusion only (PS2); and C: extrusion-enzymatic treatment (PS3)).

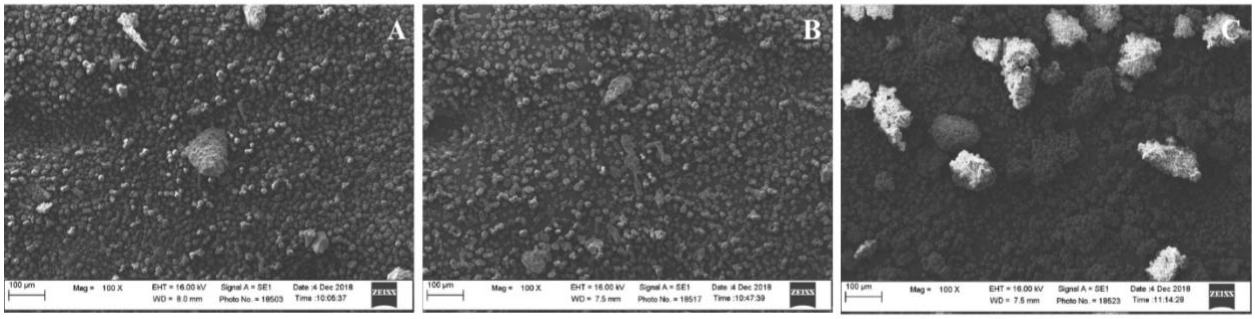

Fig. 2. Morphology of starch on pre-gelatinized sorghum flour flour (A: native sorghum flour (PS1); B: extrusion only (PS2); and C: extrusion-enzymatic treatment (PS3)).

3.2 Effect on thermal properties

Differential scanning calorimetry (DSC) analysis revealed a progressive decrease in gelatinization temperatures (onset, peak, and conclusion) from native flour (PS1) to extrusion-treated flour (PS2). As shown in Table 1, native sorghum starch and PS1 exhibited distinct

endothermic peaks corresponding to gelatinization. PS2 also showed an endothermic peak, albeit with reduced thermal parameters. In contrast, no endothermic peak was detected for PS3 (n.d.), indicating complete loss of residual crystalline structure.

Compared to native sorghum starch, both PS1 (native flour) and PS2 (extrusion-treated flour) exhibited lower To, Tp, Tc, and ΔH values (Table 1). The reduction from native sorghum starch (To = 82.38 °C; ΔH = 7.52 J/g) to PS1 (To = 79.38 °C; ΔH = 6.11 J/g) is attributed to the mechanical stress and localized heating during dry milling and sieving, which may have induced partial starch gelatinization prior to extrusion. The effect of extrusion alone is evident when comparing PS1 and PS2. Extrusion reduced the onset temperature (To) from 79.38 °C (PS1) to 77.84 °C (PS2) and decreased gelatinization enthalpy (ΔH) from 6.11 J/g to 2.60 J/g—a reduction of approximately 57%. This substantial decrease in ΔH confirms that extrusion induced a high degree of starch gelatinization, leaving fewer intact crystallites [27].

The most distinct result was observed in PS3 (extrusion combined with α-amylase treatment), where no endothermic peak was detected (n.d.). This absence indicates that virtually no residual crystalline structure remained prior to DSC analysis. This outcome likely results from the synergistic effect of thermal-mechanical disruption (extrusion) and enzymatic hydrolysis (α-amylase), which together destroyed the starch crystalline order [29-32]. The complete absence of an endothermic peak in PS3, compared to the measurable residual enthalpy in PS2 (2.60 J/g), demonstrates that enzymatic hydrolysis dramatically enhanced the disruption of crystalline structures.

The DSC findings are consistent with the morphological observations (Section 3.1). The progressive loss of the Maltese cross pattern under polarized light (Fig. 1) and the granule aggregation observed by SEM (Fig. 2) correspond to the decreasing gelatinization enthalpy (ΔH) from PS1 (6.11 J/g) to PS2 (2.60 J/g) and the complete absence of an endotherm in PS3 (n.d.). Previous studies have shown that higher feed moisture content during extrusion promotes greater starch gelatinization, resulting in lower residual gelatinization enthalpy (ΔH) and a narrower gelatinization temperature range (Tc-To) [17].

The onset temperature (To), peak temperature (Tp), and conclusion temperature (Tc) represent the beginning, maximum rate, and completion of starch crystallite melting, respectively, while the enthalpy value (ΔH) reflects the total energy required to disrupt the crystalline order within starch granules [31,32].

Previous studies have shown that increasing feed moisture content during extrusion reduces both the gelatinization temperature range (Tc-To) and enthalpy (ΔH) [17]. Compared to native sorghum starch (pure starch), both PS1 (native flour) and PS2 (extrusion-treated flour) exhibited lower To, Tp, Tc, and ΔH values (Table 1). This decline indicates that the flour preparation process

(dehulling, milling, and sieving) partially disrupted the crystalline structure, with extrusion causing further reduction. As noted by Chakraborty [31], physical and thermal modification of starch commonly result in reduced gelatinization temperature and enthalpy compared to native starch, reflecting a decrease in the quantity and quality of remaining crystallites. The enthalpy value (ΔH) is particularly informative as it directly reflects the degree of residual crystallinity: a lower ΔH value indicates a greater extent of prior gelatinization [34].

Table1. Thermal properties of pregelatinized sorghum flour with different treatments.

Sample	To (°C)	Tp (°C)	Tc (°C)	Tc-To (°C)	ΔH (J/g)
Native sorghum starch (pure starch)	82.38	85.52	91.89	9.51	7.52
PS1 (native flour)	79.38	82.87	88.78	9.40	6.11
PS2 (extrusion-treated flour)	77.84	82.01	84.05	6.21	2.60
PS3 (extrusion-enzymatic-treated flour)	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*

*n.d., not detected.

The effect of extrusion alone is evident when comparing PS1 and PS2. PS2 exhibited lower To, Tp, Tc, and ΔH values than PS1, indicating a higher degree of gelatinization achieved through extrusion. This is consistent with findings that extrusion—combining heat, mechanical shear, and pressure simultaneously—effectively disrupts starch granules even under limited moisture conditions, leaving fewer intact crystallites detectable by subsequent DSC analysis [27].

The most distinct result was observed in PS3 (extrusion combined with α-amylase treatment), where no endothermic peak was detected (n.d.). This absence indicates that no residual crystalline structure remained prior to DSC measurement [32]. This outcome reflects the synergistic effect of extrusion and enzymatic hydrolysis: extrusion partially disrupted the granule structure and exposed the amorphous regions, after which α-amylase preferentially hydrolyzed the α-1,4 glycosidic bonds in both the amorphous and semi-crystalline lamellae [29,30]. The combined action of thermal-mechanical and enzymatic treatments thus resulted in near-complete destruction of the crystalline order, consistent with the near-total disappearance of the Maltese cross pattern observed under polarized light microscopy in PS3 [28].

The DSC findings are consistent with the morphological observations (Section 3.1). The progressive loss of the Maltese cross pattern under polarized light (Fig. 1) and the granule aggregation observed by SEM (Fig. 2) correspond to the decreasing gelatinization enthalpy (ΔH) from PS1 (6.11 J/g) to PS2 (2.60 J/g) and the complete absence of an endotherm in PS3 (n.d.). While not measured in this study, such structural changes are known to influence swelling power and solubility, which affect food texture [35,36].

3.3 Effect on carbohydrate composition

The carbohydrate composition of pregelatinized sorghum flour was quantified using high-performance liquid chromatography (HPLC). The results are presented in Table 2.

The results of the analysis showed significant changes in the carbohydrate profile due to

extrusion and enzymatic treatment. In PS1 (native sorghum flour), only fructose and glucose were detected as free monosaccharides, with relatively low concentrations. Sucrose and maltose were not detected, which may be attributed to thermal hydrolysis during milling or to concentrations below the HPLC detection limit. Extrusion treatment alone (PS2) reduced fructose content to 0.746±0.327 mg/mL, indicating thermal degradation or Maillard reactions during the extrusion process at high temperatures [37]. However, small amounts of sucrose (0.203±0.288 mg/mL) and maltose (0.024±0.034 mg/mL) began to be detected. The appearance of maltose is notable because maltose is a starch hydrolysis product consisting of two glucose units indicating that extrusion with high temperature and shear force caused partial hydrolysis of starch chains into oligosaccharides and disaccharides [38].

Table 2. Carbohydrate content of pregelatinized sorghum flour.

Sample	Fructose (mg/mL)	Glucose (mg/mL)	Sucrose (mg/mL)	Maltose (mg/mL)
PS1	3.871±1.837^{ab}	2.503±0.00^{a}	n.d	n.d
PS2	0.746±0.327^{ac}	8.149±0.955^{a}	0.203±0.288	0.024±0.034
PS3	8.127±2.291^{bc}	77.310±2.782^{b}	n.d	12.050±4.393

Note: n.d. = *not detected*.

The most dramatic change was observed in PS3 sample (extrusion-enzymatic treatment), where the glucose concentration increased dramatically to 77.310±2.782 mg/mL, representing a more than 15-fold increase compared to PS1 and PS2. This increase resulted from α-amylase activity, which randomly hydrolyzes α-1,4-glycosidic bonds in starch chains, producing dextrins, maltose, and ultimately glucose [39,40].

Maltose content also increased significantly in PS3 to 12.050±4.393 mg/mL. Maltose is a typical intermediate product of starch hydrolysis by α-amylase. The ratio of maltose to glucose can provide information about the degree of starch hydrolysis [41]. In PS3, the relatively low maltose:glucose ratio (approximately 1:6) indicates extensive hydrolysis, whereby most of the maltose formed was further hydrolyzed into glucose, suggesting extended enzyme activity or the possible presence of α-glucosidase activity in the enzyme preparation.

The increase in fructose concentration in PS3 (8.127±2.291 mg/mL) compared to PS2 (0.746±0.327 mg/mL) may be attributed to several factors, such as (1) release of fructose from sucrose complexes or fructooligosaccharides present in sorghum; (2) residual invertase activity in the commercial enzyme preparation; or (3) partial isomerization of glucose to fructose under alkaline conditions, though this mechanism is less common under neutral pH [42]. Increased fructose concentration can contribute to the sweetness and hygroscopic properties of modified starch.

The detection of sucrose only in PS2, but not in PS1 or PS3, suggests that extrusion alone may promote sucrose formation or release, whereas enzymatic treatment in PS3 likely hydrolyzed any sucrose present into glucose and fructose [43]. These changes in carbohydrate composition

have important implications for the functional properties and applications of pregelatinized sorghum flour. Increased simple sugar content (glucose, fructose, maltose) increases sweetness, decreases water activity, increases water absorption, and affects browning reactions during processing [44]. From a nutritional perspective, increasing simple sugars can raise a product's glycemic index, which should be considered in product formulation for consumers with diabetes or those concerned about metabolic health [45]. However, for applications such as fermentation or bakery products that require fermented sugars, the high sugar profile of PS3 is advantageous.

While maltose itself is not a prebiotic, longer-chain maltooligosaccharides (not quantified in this study) have been reported to exhibit prebiotic potential [46]. Additionally, a more complex sugar profile can provide better sensory characteristics, with a more rounded sweetness profile compared to single monosaccharides. Maltose, with a sweetness level of approximately 30–40% of sucrose, provides mild sweetness, while combinations with glucose and fructose create desirable flavor complexity in many food applications [47].

The HPLC results corroborated the relationship between structural changes (observed by microscopy and DSC) and molecular composition. The loss of the Maltese cross pattern and the decrease in gelatinization enthalpy in PS2 and PS3 were associated with increased starch hydrolysis products, indicating that disruption of the crystalline structure facilitated enzyme accessibility and accelerated starch degradation [22]. A comprehensive understanding of this structure-function relationship is essential for optimizing processes and designing products with desired characteristics.

4. Conclusions

The combined extrusion and enzymatic treatment of sorghum flour resulted in significant changes in morphology, thermal properties, and carbohydrate composition. Morphological characterization using polarized light microscopy and SEM showed progressive weakening of the Maltese cross pattern (partial in PS2, near-complete in PS3) and adhesion of starch granules, indicating disruption of the crystalline structure. Thermal analysis by DSC revealed a progressive decrease in gelatinization temperature and enthalpy from PS1 to PS2, with PS2 exhibiting a 57% reduction in ΔH (2.60 J/g) compared to PS1 (6.11 J/g). PS3 showed no detectable endothermic peak (n.d.), indicating complete loss of residual crystalline structure. Further enzymatic treatment (PS3) caused dramatic changes in the carbohydrate profile, with a significant increase in glucose (77.31 mg/mL) and maltose (12.05 mg/mL), resulting from enzymatic hydrolysis of starch. Key quantitative findings included a more than 15-fold increase in glucose concentration compared to PS1 and PS2.

The integration of these three characterization aspects provides a comprehensive understanding of the modification mechanism of sorghum flour and may inform the development of modified sorghum flour for applications in instant foods, bakery products, and fermentation processes. These findings provide a scientific basis for optimizing the production of modified sorghum flour with predictable and controllable characteristics, including sugar profile and degree of gelatinization, tailored to specific application requirements.

While this study focused on morphological, thermal, and compositional changes, further research is needed to evaluate the functional properties (e.g., swelling power, solubility, pasting behavior) and potential prebiotic effects of the modified flours.

Abbreviations

DSC	Differential Scanning Calorimeter
HPLC-RID	High Performance Liquid Chromatography-Refractive Index Detector
ND	Not Detected
SP	sorghum pre-gel (sample code)
SEM	Scanning Electron Microscopy

Data Availability Statement

Supporting data on this study will be shared (limited) upon reader request and consideration.

CRediT Authorship Contribution Statement

Sukmawati Usman: Conceptualization, Writing-Original Draft, Methodology, Formal Analysis, Data Curation. **Endang Yuli Purwani**: Methodology, Investigation, Data Curation, Writing-Review, Supervision, Resources.

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.

Declaration of Use of AI in the Writing Process

During the preparation of this manuscript, the author(s) used the following AI-assisted technologies to enhance the writing and research process: 1) SciSpace: used for literature discovery, synthesizing existing research, and preliminary of relevant papers to ensure a comprehensive literature review; 2) Bohrium AI: employed to assist in structuring the initial draft and optimizing the logical flow of the scientific arguments; and 3) DeepL: used for language translation and refining the linguistic accuracy of the text to ensure clarity and professional academic tone. The author(s) reviewed and edited the content as needed and take full responsibility for the final content of the publication. All AI-generated suggestions were critically evaluated for accuracy and aligned with the empirical data presented in this study.

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