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Enhancing physicochemical and functional properties of jack bean (*Canavalia ensiformis*) starch with heat-moisture treatment

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Abstract

Heat-moisture treatment (HMT) represents a standard procedure used to adjust the properties of jack bean starch (JBS) and enhance its functionality in food applications. Therefore, this study aimed to analyze the influence of HMT on the JBS's functional and physicochemical properties. Native starch was subjected to HMT with varying moisture contents (15, 20, and 25%) and durations (1, 2, and 3 h) at a constant temperature of 110°C. The influence of HMT on the physicochemical properties, pasting properties, crystalline pattern, crystallinity, and morphology of starch granules was evaluated. Color analysis revealed a decline in lightness (L*) and greenness (a*), accompanied by an increase in yellowness (b*) as the heating time increased. Swelling power and solubility decreased with increasing moisture content. Meanwhile, amylose content increased with prolonged heating compared to that of native starch. Elevated moisture content and extended heating duration increased pasting temperature and peak time but simultaneously reduced breakdown viscosity. X-ray diffraction (XRD) exhibited that HMT maintained the A-type crystalline pattern of the native starch. Nevertheless, the crystallinity showed a complex trend, increasing at 25% moisture after one hour of heating (from 42.95 to 43.19%). FTIR spectra confirmed that HMT did not change the molecular structure of the starch, even though higher moisture content led to surface damage and granule cracking. HMT effectively modified jack bean starch, offering potential for improved functionality in food applications.

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Jack Bean Starch, Heat-moisture Treatment, Physicochemical Properties, Functional Properties.

1. Introduction

Starch is a renewable plant-derived biopolymer, recognized for its widespread availability, affordability, consistent reproducibility, superb biocompatibility, and biodegradability (1). This polymer is broadly utilized in diverse sectors, such as cosmetics, pharmaceuticals, and food (1–3). Native starch shows limited viscosity stability and thermal resistance during food processing at high temperatures and shear forces; therefore, starch materials with functional properties that can be improved through controlled thermal modification without changing their chemical structure are needed (4).

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The jack bean (*Canavalia ensiformis*) is an underused member of the Leguminosae family, comprising over 12,000 species in approximately 480 genera (5,6). It is extensively distributed throughout Africa, Latin America, Asia, the West Indies, India, and Indonesia (6). In Indonesia, jack bean cultivation yields approximately 5 tons annually from 1,590 ha of land (6,7). The plant is rich in proteins (22–35%), carbohydrates (45–65%), and dietary fiber (4–17%) (8,9), making it a viable candidate for starch extraction. Jack bean starch (JBS) contains relatively high levels of amylose and residual protein, which influence gelatinization and structural reorganization during heating. Yet, its functional response to physical modification for food applications has been less extensively studied than that of other legume starches, such as chickpea, lentil, and mung bean starch (6,10).

The commercial use of native starch is restricted because of its limited thermal stability, insufficient shear resistance, and large granule size (3). These limitations include low solubility, poor dispersibility, and poor tolerance to different processing conditions (11). These issues can be addressed using physical, chemical, enzymatic, and biotechnological methods for starch modification, owing to the widespread presence of hydroxyl groups and simple glycosidic bonds (12). Modification of native starch improves its functional properties, thereby expanding its applications as a polymeric material for water treatment, antimicrobial films, drug delivery, biodegradable film fillers, and particles for stabilizing emulsions (13,14).

Heat-moisture treatment (HMT) represents an important physical modification method for starch, providing benefits such as eco-friendliness and flexibility (15). HMT is a physical modification technique for starch, involving heating for a specific duration (15 min–16 h) to a temperature above the glass transition point while maintaining a moisture content below 35% (16). In this context, starch molecules undergo partial breakdown and chain rearrangement, leading to enhanced granule functional properties, including reduced swelling power, increased gelatinization temperature, and more stable paste viscosity (3). This method induces changes in the amorphous region and the crystallinity of starch without compromising granule integrity, thereby affecting the fine structure, crystallinity, paste properties, thermal stability, digestibility, and application characteristics (17–19).

HMT has been shown to increase starch crystallinity by compacting amylopectin into a double helical structure (20). Its effectiveness is influenced by various parameters, including the starch source, composition, morphology, and amylose content (21). HMT increases starch chain interactions, thereby breaking the structure and separating the crystal from the double helix (18). This method enhances the shear resistance and thermal resilience of starch (22). Research has demonstrated that HMT affects the physicochemical properties, digestibility, and structure of potatoes, corn, sorghum, and rice (15,23).

There is limited data on how HMT affects jack bean starch (JBS), and no comprehensive study has examined its physicochemical properties or potential use in the food industry. HMT improved the texture, stability, and digestibility of starch without altering its fundamental structure (10,24). Ridawati and Alsuhendra found that post-HMT moisture changes enhanced starch behavior; however, the benefits decreased at excessive moisture levels, suggesting an ideal level (25). HMT also improves starch gelatinization and pasting effects for food applications, such as baking and thickening (26). Previous studies have indicated that starches with high amylose content are more responsive to hydrothermal treatment. In contrast, moisture content and heating duration play key roles in controlling molecular mobility, amylose rearrangement, and interactions with endogenous proteins during HMT (22,27). Accordingly, this study hypothesizes that varying moisture content (15%, 20%, and 25%) and

heating time interact with the amylose–protein matrix of jack bean starch, leading to distinct molecular reorganizations and corresponding changes in physicochemical and functional properties (19,28). Hence, this study examined the effect of HMT on the functional and physicochemical properties of JBS to understand its structure-function relationship and optimize its use. HMT is a feasible technique to improve the stability and functionality of JBS in food applications.

2. Materials and Methods

Jack bean (*Canavalia ensiformis*) was obtained from Yayasan Gita Pertiwi and produced by farmers in Wonogiri, Central Java, Indonesia. In this study, distilled water was used to modify starch with HMT and other analytical-grade reagents.

2.1. Isolation of Native Starch

Jack beans were immersed in water having 0.45% Na-metabisulfite for 9 h to lower HCN levels and inhibit browning. Jack bean skin was removed by hand, and native starch was produced using the protocol established by Ariyantoro *et al*. (7). The peeled skin was blended for 2 min until smooth in distilled water at a 1:3 ratio. The solution was then filtered through a cloth. The remaining dregs were combined with distilled water at a 3:1 ratio and filtered. The filtrate was allowed to settle for 2 h. The starch precipitate was removed and rinsed five times with distilled water, with 1 h of precipitation between each wash. Wet starch was parched for 6 h at 50°C using a cabinet dryer, pulverized with a blender, and sifted through a 100-mesh sieve.

2.2. Heat-Moisture Treatment (HMT)

HMT was performed on the native JBS using the protocol reported by Ariyantoro et al. (7), with some modifications. Native starch (30 g) was weighed carefully and placed in a glass jar. The moisture content was set to 15%, 20%, and 25% by introducing a suitable quantity of distilled water. The sealed jars were incubated at room temperature for 24 h to equilibrate. The jar was subsequently heated in an oven set to 110°C for 1, 2, and 3 h. The combination of moisture content and heating duration was based on a study by Dai *et al*. (29). A temperature of 110°C was applied for heating, following the results of Ariyantoro *et al*. (10). After heating, the samples were placed in a cabinet dryer for 5 h at 40°C, milled, and sieved through a 100-mesh sieve to obtain HMT-modified JBS.

2.3. Color Analysis

The color parameters of the starch samples, including b* (yellowness-blueness), L* (lightness), and a* (redness-greenness), were evaluated using a Minolta color spectrophotometer (CR-400/410, Minolta) at room temperature. The analytical equipment was standardized using a Minolta standard white reflection plate before measurements. The L* value indicates brightness, scaled from 1 (black) to 100 (white); the a* value represents positive (red) or negative (green) color; and the b* value indicates the presence of yellow (positive) or blue (negative) color. The whiteness index (WI) was calculated using equation (27).

$$\mathrm{WI} = 100 - [(100 - \mathrm{L})^2 + \mathrm{a}^2 + \mathrm{b}^2]^{1/2} \quad (1)$$

2.4. Physicochemical Properties Analysis

The amylose content, swelling power, and solubility were analyzed to describe the physicochemical properties of the HMT-modified and native JBS. Solubility and swelling power were analyzed using the methods described by Kittipongpatana & Kittipongpatana (30). Starch (0.1 g) was measured, placed into a test tube, and combined with 10 mL of distilled water by vortexing for 1 min. The sample was heated in a water bath at 95°C for 30 min, then cooled to room temperature. Centrifugation was performed for 15 min at 3000 rpm to remove the supernatant. A 5 mL aliquot of the supernatant was dried at 110°C to achieve a constant weight. The solubility was computed as the percentage ratio of the weight of dried solids in the supernatant to the initial dry weight of the sample. The swelling power was calculated as the percentage of wet sediment weight relative to the initial dry sample weight. The amylose content was estimated using the procedure reported by Munjal *et al*. (6).

2.5. Pasting Properties Analysis

The pasting properties of the HMT-modified and native JBS were assessed using a Rapid Visco Analyzer (RVA-4500; Perten Instruments, Waltham, MA, USA). Samples (3.5 g, dry basis) were mixed with 25 mL of distilled water in an RVA tube and heated. RVA analysis obtained values for trough viscosity (TV), peak viscosity (PV), breakdown viscosity (BD), final viscosity (FV), setback viscosity (SB), and pasting temperature (31).

2.6. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Fourier-transform infrared spectroscopy was performed on the samples using a spectrophotometer (Nicolet iS-10, Thermo Scientific, Madison, USA). A dried starch sample without the KBr mixture was placed directly on the diamond sensor of the instrument to obtain three spectral readings at a resolution of 4 cm^{-1}. The recorded FTIR spectra covered wavenumbers ranging from 400 cm^{-1} to 4000 cm^{-1}. The peaks at specific wavenumbers indicated the presence of specific functional groups. The spectra were examined using OMNIC (Thermo Fisher's OMNIC software) and Origin 2024, as described by Kumar *et al*. (22).

2.7. X-ray Diffraction Analysis

The crystalline patterns of the starch samples were characterized using XRD (X-ray diffraction), as described by Dai et al. (32). The diffraction angle (2θ) of the scanned area ranged from 10° to 40°. Spectrum analysis software was used to determine the relative crystallinity of the samples by plotting the peak baselines and the diffraction pattern areas. Additionally, the degree of relative crystallinity (RC) was calculated based on the ratio between the crystal and total curve area, utilizing the equation below:

$$\mathrm{RC}\ (\%) = \left(\frac{\mathrm{Ac}}{\mathrm{At}}\right) \mathrm{x}\ 100 \qquad (2)$$

Where Ac is the crystalline peak area, and At is the total curve area.

2.8. Granule Morphology Analysis

The surface morphology of the starch granules was observed using scanning electron microscopy (SEM) (JSM-6510LA, JEOL Ltd., Japan) at a voltage of 15–200 kV and magnifications ranging from 200x to 3000x. The starch was individually attached to the pieces using double-sided sticky tape, and the starch samples were covered with gold (33).

2.9. Statistical Analysis

The measurements are reported as the mean ± standard deviation. Statistical analysis was performed using one-way ANOVA followed by Duncan's multiple-range test to determine significant differences, while Pearson's correlation analysis was used to assess correlations among parameters. Analyses were conducted using SPSS version 26.0 (IBM Corporation, Armonk, NY, USA) at $p < 0.05$, and figures were prepared using Origin 2024.

3. Results and Discussion

3.1. Color

Color parameters of the HMT-modified and native JBS were L*, a*, and b*, respectively (Table 1). HMT modification significantly decreased the L* and WI values of starch. The decrease in brightness and whiteness index values was caused by the heating process, during which interactions occur between reducing sugars and the amino groups of proteins (34,35). This reaction, known as the Maillard reaction, produces the dark pigment melanoidin. Melanoidin results from the interactions between the carbonyl groups of reducing sugars and amines from amino acids, particularly lysine (10). Under HMT conditions, elevated temperature and extended heating promote melanoidin formation even with limited moisture, while starch granules remain relatively color stable (36).

The native and HMT-modified JBS had negative a* values, indicating a greenish color, which decreased with increasing HMT time. The length of HMT heating increased the b* value, indicating a tendency toward a yellowish color. According to Ariyantoro *et al.*, the higher yellowness was due to greater melanoidin formation in the HMT-modified JBS than in the native variant (37). Temperature and time are key factors in the Maillard response, as a longer HMT time significantly affects the a* and b* values (35).

Table 1. Color properties of native and HMT-modified JBS.

Samples	L*	a*	b*	WI
Native	93.96 ± 0.02^{f}	-5.52 ± 0.01^{e}	11.65 ± 0.01^{a}	85.76 ± 0.00^{j}
HMT15_1	93.81 ± 0.03^{g}	-5.30 ± 0.01^{d}	11.97 ± 0.01^{d}	85.52 ± 0.02^{h}
HMT15_2	93.39 ± 0.02^{d}	-5.18 ± 0.01^{c}	12.90 ± 0.02^{g}	84.61 ± 0.01^{d}
HMT15_3	92.99 ± 0.01^{c}	-4.98 ± 0.01^{a}	13.94 ± 0.01^{j}	83.62 ± 0.01^{b}
HMT20_1	93.83 ± 0.03^{gh}	-5.56 ± 0.01^{f}	11.93 ± 0.01^{c}	85.47 ± 0.02^{g}
HMT20_2	93.65 ± 0.01^{e}	-5.51 ± 0.01^{e}	12.55 ± 0.01^{f}	84.90 ± 0.01^{e}
HMT20_3	92.95 ± 0.02^{b}	-5.32 ± 0.01^{d}	13.33 ± 0.01^{h}	84.01 ± 0.01^{c}
HMT25_1	93.86 ± 0.01^{h}	-5.81 ± 0.03^{h}	11.70 ± 0.01^{b}	85.57 ± 0.01^{i}
HMT25_2	93.69 ± 0.02^{f}	-5.61 ± 0.01^{g}	12.27 ± 0.02^{e}	85.10 ± 0.01^{f}
HMT25_3	91.37 ± 0.02^{a}	-5.02 ± 0.01^{b}	13.85 ± 0.01^{i}	82.93 ± 0.01^{a}

** Data are summarized as mean ± SD. a-j: Mean values marked with the same letter in one column are not significantly different ($p < 0.05$). HMT15_1, HMT15_2, HMT15_3, HMT20_1, HMT20_2, HMT20_3, HMT25_1, HMT25_2, and HMT25_3 are HMT-modified JBS samples that were treated with moisture contents of 15% for 1 h, 15% for 2 h, 15% for 3 h, 20% for 1 h, 20% for 2 h, 20% for 3 h, 25% for 1 h, 25% for 2 h, and 25% for 3 h.*

3.2. Swelling Power and Solubility

Table 2 shows the effect of HMT modification on the solubility, amylose content, and swelling power of starch granules. The swelling power, solubility, and amylose content ranged from 13.03 to 16.49 g/g, 6.08 to 10.67%, and 44.40% to 52.98%, respectively. The solubility and swelling power of native JBS exceeded those of the HMT-modified variants. The modification altered the arrangement of the starch particles and strengthened the bonds between amylose-amylose and amylose-amylopectin chains. The solubility and swelling power of the starch chains in the amorphous and crystalline domains indicate the magnitude of these interactions (38). The reduction in swelling power and HMT promotes stronger intermolecular associations, including amylose–amylose aggregation and amylose–lipid inclusion complexes, which limit water penetration and granule expansion (39,40).

The starch's swelling power was used to assess water absorption in the granules. This parameter is closely related to the capacity of starch molecules to retain water through hydrogen bonding. As shown in Table 2, the swelling power of the HMT-modified JBS was lower than that of the native variant. The native starch exhibited the highest swelling power (16.49 g/g), which was significantly different from that of the HMT-modified variant. HMT reduced the swelling power, particularly at moisture contents of 15% and 20%. Furthermore, HMT25_1 exhibited relatively higher swelling power than the other HMT treatments. Structural alterations in starch granules due to HMT include increased cross-linking or altered crystallinity, and reduced swelling power (41,42). The rearrangement of amylopectin double helices and increased crystalline perfection after HMT reduces the availability of hydroxyl groups for hydration, resulting in restricted water uptake and lower swelling power (43).

Table 2. Physicochemical properties of HMT-modified and native JBS.

Samples	Swelling Power (g/g)	Solubility (%)	Amylose (%)
Native	16.49 ± 0.85^{c}	10.67 ± 0.30^{f}	44.40 ± 0.87^{a}
HMT15_1	14.03 ± 0.95^{ab}	7.40 ± 0.40^{cd}	51.22 ± 0.08^{de}
HMT15_2	13.03 ± 0.38^{a}	6.47 ± 0.35^{ab}	52.98 ± 0.08^{g}
HMT15_3	13.69 ± 0.55^{a}	6.08 ± 0.41^{a}	51.54 ± 0.11^{ef}
HMT20_1	13.16 ± 0.64^{a}	6.81 ± 0.59^{abc}	47.01 ± 0.40^{b}
HMT20_2	13.64 ± 0.41^{a}	7.59 ± 0.51^{cd}	50.85 ± 0.16^{c}
HMT20_3	13.62 ± 0.39^{a}	7.30 ± 0.31^{bcd}	52.01 ± 0.20^{f}
HMT25_1	14.90 ± 0.33^{b}	8.57 ± 0.90^{bcd}	48.10 ± 0.16^{b}
HMT25_2	13.55 ± 0.31^{a}	7.75 ± 0.19^{d}	51.37 ± 0.43^{def}
HMT25_3	13.72 ± 0.41^{a}	9.79 ± 0.45^{e}	51.61 ± 0.28^{ef}

** Data are summarized as mean ± SD. a-f: Mean values marked with the same letter in one column are not significantly different ($p < 0.05$). HMT15_1, HMT15_2, HMT15_3, HMT20_1, HMT20_2, HMT20_3, HMT25_1, HMT25_2, and HMT25_3 are HMT-modified JBS samples that were treated with moisture contents of 15% for 1 h, 15% for 2 h, 15% for 3 h, 20% for 1 h, 20% for 2 h, 20% for 3 h, 25% for 1 h, 25% for 2 h, and 25% for 3 h.*

Modification of JBS using HMT reduced the swelling power as the moisture content increased (10). The HMT process changed the arrangement of starch particles and strengthened the bonds between amylose and amylose–amylopectin. Therefore, amylose-amylopectin molecules have fewer hydroxyl groups for hydration and diffusion (44). The decreased swelling power after HMT treatment was caused by several factors, including the

reorganization and re-linking of starch chains, which restricted the ability to absorb water, and changes in crystallite perfection and starch granule strength. Crystallite formation increased granule stability and reduced the degree of granule swelling (38).

The solubility of the HMT-modified JBS exceeded that of the native version (Table 2). Native starch exhibited the highest solubility (10.67%), which was significantly different from that of all the HMT treatments. The HMT15_2 and HMT15_3 samples exhibited the lowest solubilities. The strengthening of internal hydrogen bonds in starch granules by HMT decreases solubility, thereby reducing starch's ability to dissolve in water (45). This decrease in solubility is also associated with the formation of amylose–lipid complexes and tighter molecular packing after HMT, which limits starch chain leaching into the aqueous phase and stabilizes the granular structure (39,46).

Pearson correlation analysis showed a strong positive correlation between moisture content and solubility ($r = 0.793$, $p < 0.05$), indicating that higher moisture content during heat–moisture treatment (HMT) enhanced molecular mobility and promoted partial starch solubilization, although the overall solubility remained lower than that of native starch due to HMT-induced structural stabilization (19). Swelling power exhibited a moderate positive correlation with solubility ($r = 0.485$), reflecting concurrent granule hydration and limited molecular leaching, constrained by increased structural order (47).

3.3. Amylose Content

Starches with high and low amylose contents have diverse structures and physicochemical characteristics (48). Table 2 describes the amylose contents of HMT-modified and native JBS. HMT increased the amylose content of the JBS. Native starch had the lowest amylose content (44.40%), which was significantly different from that of the HMT-modified JBS. This increase was most evident in the HMT treatments with moisture contents of 15% and 20%. This was caused by the structural modifications of amylose and amylopectin during HMT, which affected the measurement methods (6). Research has shown that the hydrothermal processes lead to the breaking and formation of amylopectin and amylose chains (33).

The amylose content of legume starch ranges from 24% to 88%. This result was consistent with Munjal *et al.* (6), where the amylose contents of native JBS from India and Indonesia were 47.78% and 29.31%, respectively (7). According to Su *et al.* (2024), partial starch gelatinization at high temperatures causes the starch structure to disintegrate, thereby releasing amylose (41). In this context, HMT-modified JBS exhibited a greater amylose content than the native variant. HMT induces side chains in amylopectin molecules, forming short chains, such as amylose (49). However, no substantial difference was observed in the amylose content of the samples with a moisture content of 25%. Previous findings have indicated that HMT induces high pressure and heat, encouraging the migration of water molecules into starch particles and breaking of hydrogen bonds (41). The decrease in the solubility of HMT-modified starch was attributed to a decline in granule stability, which led to the unraveling of potential double helices within the crystal structure of native starch granules (38). The solubility of the HMT-modified starch was reduced because the bonds were stronger (42). This change resulted from increased interaction between amylopectin and amylose molecules.

3.4. Pasting Properties

RVA was used to investigate the pasting properties of both the HMT-modified and native JBS. Pasting properties included TV (Trough Viscosity), PV (Peak Viscosity), BD (Breakdown Viscosity), SB (Setback Viscosity), and FV (Final Viscosity) (50). BD shows the difference between TV and PV, and indicates how starch granules withstand heat and shear stress (43). The discrepancy between TV and FV was demonstrated by SB, which reported the magnitude of starch reshaping and potential for gel formation after cooling (51). Additionally, the number of straight chains, the granule size, and the crystalline structure of the starch significantly affected the characteristics of the starch paste (52).

Table 3 lists the properties of HMT-modified and native JBS pastes. HMT modification altered the properties of the HMT-modified JBS paste. HMT modification with 15% moisture content increased TV, PV, SB, and FV values. Furthermore, BD, PV, TV, FV, and SB decreased with increasing moisture content. An increase in swelling power enhanced the viscosity (31), but the reported results showed the opposite effect. Other factors, such as protein and lipid content, amylose release, and friction between swollen granules, also affect thickening characteristics.

Table 3. Pasting properties of HMT-modified and native JBS.

Samples	*Peak viscosity* (PV)	*Trough viscosity* (TV)	*Breakdown viscosity* (BD)	*Final viscosity* (FV)	*Setback viscosity* (SB)	*Peak time* (TP)	*Pasting temperature* (PT)
	cP	cP	cP	cP	cP	min	°C
Native	2860 ± 26^{bc}	1755 ± 69^{a}	1105 ± 43^{d}	3785 ± 113^{a}	2030 ± 44^{ab}	8.50 ± 0.04^{a}	81.8 ± 0.3^{a}
HMT15_1	2977 ± 41^{c}	2385 ± 57^{bc}	592 ± 16^{c}	5844 ± 301^{cc}	3459 ± 245^{c}	8.78 ± 0.16^{ab}	82.4 ± 0.5^{ab}
HMT15_2	3045 ± 37^{c}	2533 ± 81^{cd}	513 ± 45^{bc}	6028 ± 366^{d}	3495 ± 284^{c}	8.94 ± 0.09^{bc}	82.8 ± 0.5^{ab}
HMT15_3	3141 ± 6^{c}	2691 ± 1^{d}	450 ± 7^{abc}	6222 ± 134^{d}	3531 ± 132^{c}	9.04 ± 0.05^{bc}	83.1 ± 0.0^{ab}
HMT20_1	2640 ± 2^{ab}	2303 ± 116^{b}	354 ± 89^{abc}	5030 ± 212^{bc}	2727 ± 96^{b}	9.23 ± 0.14^{cd}	84.4 ± 0.4^{bc}
HMT20_2	2636 ± 94^{ab}	2269 ± 47^{b}	367 ± 47^{abc}	4952 ± 122^{b}	2684 ± 169^{ab}	9.03 ± 0.14^{bc}	84.1 ± 0.0^{b}
HMT20_3	2586 ± 93^{ab}	2335 ± 185^{b}	251 ± 92^{a}	4665 ± 908^{at}	2330 ± 723^{ab}	9.67 ± 0.19^{e}	86.2 ± 2.3^{cd}
HMT25_1	2533 ± 34^{a}	2244 ± 146^{b}	339 ± 41^{ab}	4408 ± 370^{at}	2164 ± 224^{ab}	9.47 ± 0.09^{de}	88.0 ± 0.2^{d}
HMT25_2	2501 ± 267^{a}	2215 ± 61^{b}	286 ± 206^{ab}	4185 ± 292^{at}	1970 ± 231^{a}	9.67 ± 0.09^{e}	88.0 ± 0.7^{d}
HMT25_3	2498 ± 254^{a}	2256 ± 72^{b}	242 ± 18^{a}	4592 ± 273^{at}	2336 ± 201^{ab}	10.00 ± 0.28^{f}	88.1 ± 0.0^{d}

** Data are presented as mean ± SD. a-f: Mean values sharing the same letter within a column are not significantly different ($p < 0.05$). HMT15_1, HMT15_2, HMT15_3, HMT20_1, HMT20_2, HMT20_3, HMT25_1, HMT25_2, and HMT25_3 are HMT-modified JBS samples that were treated with moisture contents of 15% for 1 h, 15% for 2 h, 15% for 3 h, 20% for 1 h, 20% for 2 h, 20% for 3 h, 25% for 1 h, 25% for 2 h, and 25% for 3 h.*

HMT modification decreased the PV of starch. Samples HMT25_1, HMT25_2, and HMT25_3 had the lowest PV, which were noticeably different from those of native and HMT-modified JBS with 15% and 20% water content. Proteins present on the surface of the starch granules led to an initial increase in viscosity. The first increase in thickness occurred because proteins on the surface of the starch granules formed bonds with starch molecules, preventing liquids from escaping and maintaining thickness. Protein removal increased amylose exudation and viscosity. Exposure to high temperatures during treatment causes protein denaturation and reduces its ability to bind amylose molecules. This increased amylose release and the formation of more viscous mixtures (31). A decrease in PV suggests diminished capacity of starch to swell and form a thick paste. HMT alters the starch granule

structure, leading to increased crosslinking between starch molecules. TV increased after HMT, but only a few treatments showed significant differences. This increase was attributed to the enhanced stability of the starch paste after HMT.

In the current study, HMT significantly decreased BD levels. Native JBS had the highest BD value and was the least stable paste after heating and stirring. Sample HMT25_3 exhibited the lowest BD, indicating the starch granules' susceptibility to mechanical and thermal shear. SB indicates the tendency of starch pastes to be retrograde. Upon heating, starch granules with limited expansion are less prone to disruption by heat and mechanical shear, resulting in a decreased BD. Upon cooling, less soluble starch resulted in lower SB values (22). The lower BD of HMT-modified JBS indicates its high stability at high temperatures and mechanical stress. Rearrangement of starch chains or granules strengthens the link between amylopectin and amylose side chains (53). The reduced BD indicates that HMT-modified JBS starch has improved resistance to shear stress and heat, as reflected in restricted granule swelling and strengthened internal molecular interactions. This enhanced stability, attributed to starch chain restructuring and increased crystalline order, suggests its suitability for high-temperature food applications requiring viscosity stability, such as extruded products, sauces, and ready-to-eat foods (43,54).

The starch content increased at the pasting temperature (PT) during heating and was related to paste formation. The higher PT observed in HMT25_3 (88.1°C) than in native starch (81.8°C) indicated an increase in gelatinization temperature. HMT enhanced the PT of JBS by improving crystal perfection through reorientation of starch granule molecules. This was caused by the increased strength of intragranular bonding forces, as starch requires more thermal energy to disrupt its structure and form a paste. Higher heating temperatures are required for paste formation and structural breakdown due to increased crosslinking and reinforcement of starch granules (53,55).

The high moisture level in the HMT increased the pasting temperature. HMT-modified JBS had a higher PT because processing or treatment improved crystal perfection, likely due to the reorientation of granule molecules or chains. Higher heating temperatures are required to break down the structure and form a paste due to the enhanced forces and cross-linking within starch granules.

Pearson correlation analysis showed that moisture content was the main factor affecting the pasting properties of HMT-modified JBS, with strong negative correlations with viscosity parameters and a positive correlation with pasting temperature. This indicates that higher moisture enhances molecular rearrangement and granule rigidity, limiting viscosity development and increasing resistance to gelatinization, while heating time has a minor effect. These results are consistent with previous reports on HMT-induced structural reinforcement and restricted granule disintegration in starches (27).

3.5. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR effectively characterizes the molecular arrangement of starch, particularly its double helical structure. The broad absorption band in the range of 3800–3000 cm^{-1} is attributed to the stretching vibrations of the starch hydroxyl groups. The peak strength increased following HMT, indicating that the treatment strengthened the connections between the double helices in starch by forming more hydrogen bonds (3,56).

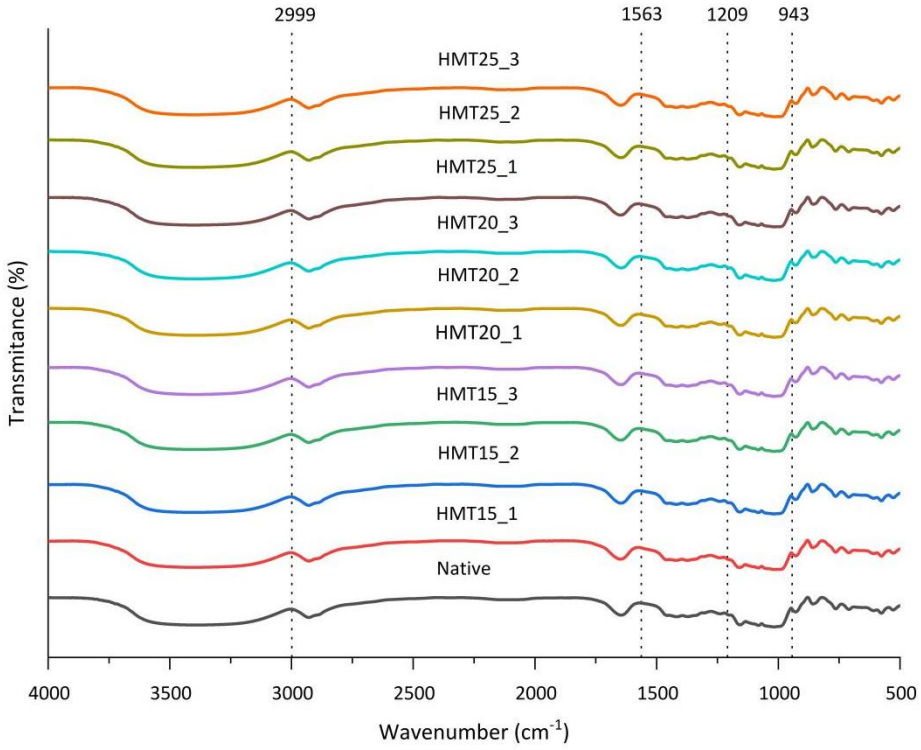

Figure 1. FTIR spectroscopy of HMT-modified and native JBS. HMT15_1, HMT15_2, HMT15_3, HMT20_1, HMT20_2, HMT20_3, HMT25_1, HMT25_2, and HMT25_3 are HMT-modified JBS samples that were treated with moisture contents of 15% for 1 h, 15% for 2 h, 15% for 3 h, 20% for 1 h, 20% for 2 h, 20% for 3 h, 25% for 1 h, 25% for 2 h, and 25% for 3 h.

The impact of HMT on crystallinity and starch properties was determined using FTIR based on functional groups. Figure 1 displays the FTIR spectra of the HMT-modified and native JBS. The spectra of the experimental samples showed no new absorption peaks, suggesting that no substituents were present on the polymer chains. Therefore, HMT did not alter the molecular structure of starch. The prominent sharp absorption peaks of HMT-modified and native JBS were detected at 2999, 1563, 1209, and 943 cm^{-1}.

The starch skeleton mainly consists of -CH-, -CH_2-, C-OH, and -OH groups (35,57). The absorbance bands at 929, 1022, 1047, and 1080 cm^{-1} were ascribed to the interaction between amylose and water, indicating C-OH and CH_2. Meanwhile, 1157 cm^{-1} corresponded to C-C and C-O stretching (14), and the contribution of C-OH groups (58). The peak at 2999 cm^{-1} indicated C-H stretching, 1563 cm^{-1} represented O-H bending in bound water, 1209 cm^{-1} indicated C-O vibrations, and 943 cm^{-1} was associated with the characteristic pattern of the glycosidic ring structure.

3.6. X-ray Diffraction Pattern and Relative Crystallinity

Figure 2 presents the XRD patterns and RC for both HMT-modified and native JBS. The HMT-modified and native JBS displayed an A-type crystal structure, as indicated by peaks at 15°, 17°, 18°, and 23° in the diffraction angle (2θ) (22,59). The classification of JBS as A-type crystalline starch is consistent with previous findings that reported typical A-type diffraction peaks at similar 2θ values (60). Although legume starches are generally classified as C-type,

these results confirm that JBS exhibits A-type crystallinity. These peaks indicate that the crystalline structure of starch remained unchanged even after HMT, consistent with previous research on oat and buckwheat starches from different cultivars (18). Therefore, HMT modification did not change the crystalline polymorphic form (59).

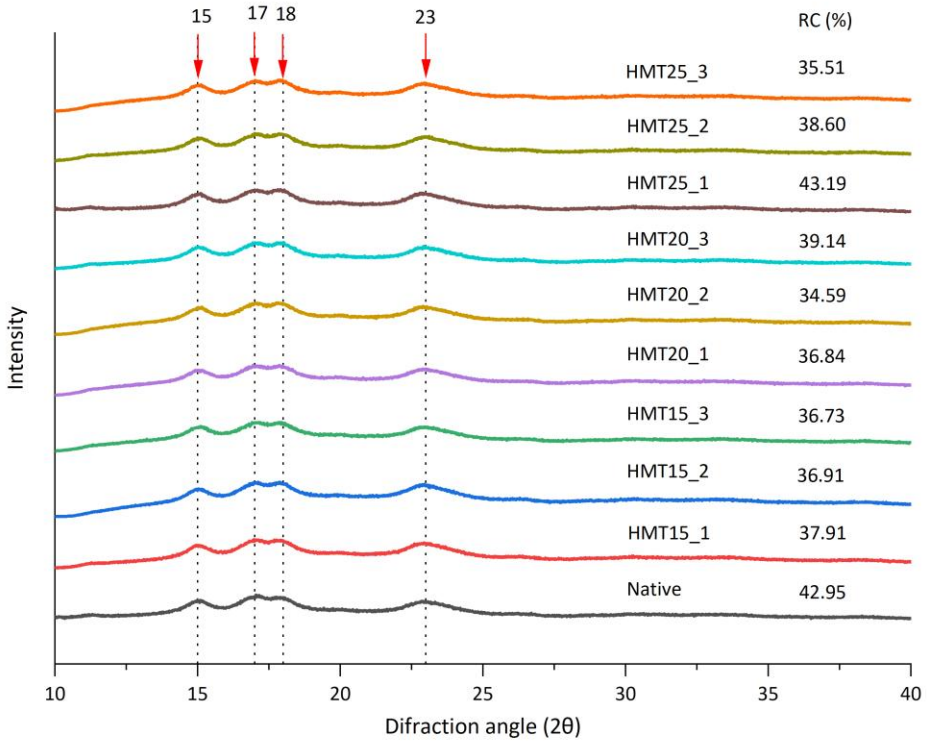

Figure 2. XRD pattern of HMT-modified and native JBS. HMT15_1, HMT15_2, HMT15_3, HMT20_1, HMT20_2, HMT20_3, HMT25_1, HMT25_2, and HMT25_3 are HMT-modified JBS samples that were treated with moisture contents of 15% for 1 h, 15% for 2 h, 15% for 3 h, 20% for 1 h, 20% for 2 h, 20% for 3 h, 25% for 1 h, 25% for 2 h, and 25% for 3 h.

The relative crystallinity of starch was assessed by examining diffraction peaks in X-ray patterns and distinguishing between amorphous and crystalline regions. This variable is influenced by several factors, including crystal size, branched amylose content, length of branched amylose chains, orientation of double helices within the crystal region, and overall compactness (50). Figure 2 shows that the relative crystallinity decreased after HMT modification but increased after treatment with 25% moisture and 1 h of heating. This decline indicated that the hydrogen bonds broke down as the HMT treatment time increased (61).

HMT modification changed the unstable structures of the starch granules by enhancing the helical structure and microcrystalline organization in the presence of a specific amount of moisture (62). The degree of crystallinity of HMT-modified JBS is related to changes in the crystalline fraction of starch, water loss, and the movement of double helices. This disturbs starch crystallinity and shifts the crystal structure from a semi-crystalline state to a less organized or partially melted state (63). At the molecular level, HMT enhances the mobility of starch chains and induces partial reorientation of amylopectin double helices. Under limited moisture conditions, elevated temperatures promote helix slippage and

structural rearrangement, resulting in reduced long-range crystalline order without altering the polymorphic type (39,40). Changes in the diffraction patterns caused by HMT resulted from dehydration, which created two double helices in the central channel. This process breaks down starch crystallites and shifts the crystal orientation (38).

3.7. Granule Morphology

Figure 3 shows the granule morphology of HMT-modified and native JBS, as visualized by SEM. The native starch granules were predominantly oval and had smooth surfaces, indicating minimal structural disruption during extraction. HMT at 15% and 20% moisture contents did not markedly alter granule morphology, although surface roughness was observed, suggesting initial granule destabilization. In contrast, treatment with 25% moisture induced pronounced morphological changes, including surface roughness, cracks, and hollow formations at the granule center. These alterations were attributed to molecular rearrangements in amylose and amylopectin during HMT. Consistent with previous reports, elevated moisture levels during HMT promoted partial gelatinization and granule agglomeration (44,64).

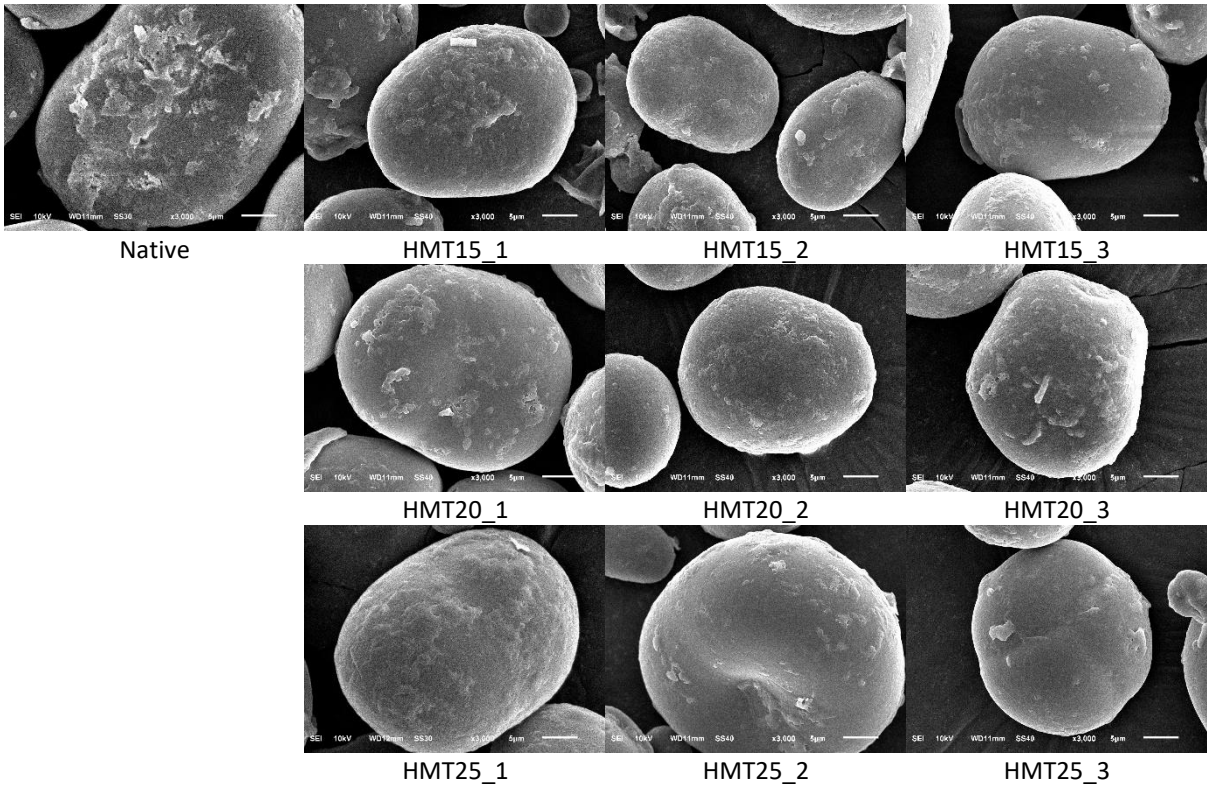

Figure 3. SEM images comparing native and HMT-modified JBS. HMT15_1, HMT15_2, HMT15_3, HMT20_1, HMT20_2, HMT20_3, HMT25_1, HMT25_2, and HMT25_3 are HMT-modified JBS samples that were treated with moisture contents of 15% for 1 h, 15% for 2 h, 15% for 3 h, 20% for 1 h, 20% for 2 h, 20% for 3 h, 25% for 1 h, 25% for 2 h, and 25% for 3 h.

According to Liu *et al.* (2021), increasing the moisture content during HMT tends to produce starch granules with rougher, more irregular surfaces, often characterized by small indentations (64). When moisture levels approach the upper threshold, internal molecular

restructuring accelerates, leading to more pronounced morphological changes. The SEM analysis conducted in this study supports these findings, demonstrating that HMT substantially changed the surface structure of JBS granules. These structural modifications are likely to influence the starch's physical and functional properties. The extent of these changes is determined by processing conditions and the inherent properties of starch, highlighting the need to select suitable HMT conditions to achieve the targeted functionality for specific food applications (34,64).

These microstructural modifications were reflected in the RVA pasting behavior of JBS. The presence of surface cracks and hollow granules at 25% moisture content indicates partial gelatinization and strengthened intragranular interactions, which limit granule swelling during heating, thereby reducing PV and BD. During cooling, HMT-induced molecular rearrangements enhanced paste stability, resulting in a higher FV than that of native starch. This confirms that microstructural reorganization under high-moisture HMT plays a key role in governing the paste stability and shear resistance of JBS (43,54).

4. Conclusions

HMT greatly affected the JBS's functional and physicochemical properties. HMT altered the color, resulting in reduced L* and a* values and increased b* with longer heating times. The HMT-modified JBS exhibited low swelling power and solubility. The amylose content increased with increasing heating time after HMT. The gelatinization temperature and pasting time increased, and the breakdown viscosity decreased as the moisture content and heating time increased. The high moisture content of the jack beans did not affect the XRD pattern but did influence the degree of starch crystallinity. Although the HMT-modified starch granules retained their initial morphology, an increase in water content led to surface roughness, cracks, and hollow centers within the granules. HMT helped the starch molecules rearrange into a long-chain, double-helix crystal structure. This process increased the gelatinization temperature, stability of the starch at high temperatures, and crystallinity. Among the evaluated treatments, HMT at 25% moisture content and 110°C for 1 h resulted in the most balanced functional and physicochemical properties, as indicated by increased crystallinity, higher pasting temperature, reduced swelling power, and lower breakdown viscosity. These characteristics demonstrate improved starch stability during heating, suggesting that this modified starch is suitable for food systems subjected to prolonged thermal processing, such as canned and retorted products.

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Author Contributions

B.C.A. Conceptualization, methodology, investigation, data curation, visualization, writing–original draft, writing–reviewing, and editing; C.A. methodology, investigation, validation, supervision, writing, reviewing, and editing; M.Z.Z. methodology, investigation, validation, supervision, writing-reviewing, and editing; D.P. conceptualization, methodology, investigation, validation, writing-reviewing, and editing, supervision, and funding acquisition.

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Not applicable.

Data Availability Statement

The available data are presented in this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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