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The effect of heat-moisture treatment on the molecular changes of starch and protein in high- and low-amylose rice flours and its physicochemical properties

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Abstract

Heat-moisture treatment (HMT) could alter the starch and protein structures of rice flour resulting in the change of physicochemical properties. The aim of the study was to investigate the effect of HMT (moisture content of 20%, at 110°C for 14 h) on the molecular changes of starch and protein, pasting properties, and textural characteristics of high-(PTT80 and KTH17) and low-(KDML105) amylose rice flours. The protein and fat contents of all HMT-modified rice flours (HPTT, HKTH and HKDML) were altered slightly and the resistant starch content of high-amylose rice flour increased. In addition, after HMT, the total relative crystallinity (RC) of all rice flour increased. For high-amylose rice flour, the RC of V-type complexes increased after HMT. The HMT-modified rice flours with high-amylose content (HPTT and HKTH) displayed starch molecular rearrangement. The HPTT exhibited increased short-range ordered structures; conversely, the HKDML, low-amylose content, had decreased short-range ordered structures. Furthermore, the FTIR results showed that the secondary protein structure was altered, β-turn and random coil to β-sheet structures, after HMT. The gelatinization temperatures of HPTT and HKTH were higher than their native rice flour. All HMT-modified rice flours have a higher pasting temperature, lower peak viscosity, and lower breakdown viscosity compared to their native rice flour. Additionally, after HMT, the high-amylose rice flour gel (both HPTT and HKTH) had greater hardness but lower cohesiveness than the low-amylose rice flour gel (HKDML). This study suggested that HMT not only affects the starch structural changes but the protein structure as well and their change is responsible for their pasting and textural properties. The HMT-modified rice flour will be utilized in gluten-free and other functional products.

Keywords: Rice flour; Amylose content; Heat-moisture treatment; Secondary protein structure; Resistant starch

1. Introduction

In the present, rice flour has been used as a major alternative flour to replace wheat flour in gluten-free products (Demirkesen and Ozkaya, 2022; El Khoury *et al.*, 2018) due to its lack of gluten and hypo-allergenicity (Park and Kim, 2023). However, there are disadvantages of rice flour properties, for example, poor tolerance and stability during process and storage (Schafranski *et al.*, 2021). Therefore, the modification (chemical and physical) of rice flour has increased in demand. Flours from different rice varieties differ in gelatinization and viscoelastic properties due to their structural differences, such as amylose and amylopectin and other components (lipid and protein) which might lead to the change of physicochemical properties after modification.

Heat-moisture treatment (HMT) is a physical modification, which is gaining industrial interest exclusively nowadays due to its safe and absence of chemical waste. HMT-modified starch by heating starch with low moisture (< 35% w/w) at a temperature above glass transition but below the gelatinization of starch for a certain time. HMT could induce the molecular rearrangement of starch molecules and the formation of amylose-lipid complexes (ALCs) (Asare *et al.*, 2021; Gunaratne and Hoover, 2002; Hoover, 2010). After modification, starch molecules have higher gelatinization temperature, lower granular swelling and higher thermal stability (Zavareze *et al.*, 2010). Furthermore, the HMT modification could also increase the resistant starch content (RS) (Noro *et al.*, 2018). In addition, the pasting temperature of high amylose rice flour was greatly higher than that of low amylose rice flour after HMT modification (Kunyanee and Luangsakul, 2022).

Besides starch, the structural properties of protein in flour could be altered during HMT especially the secondary structures (Lv *et al.*, 2022), which are α-helix, β-sheet, β-turn, and random coil. During HMT, the hydrophobic region of protein could be exposed (Seguchi *et al.*, 2004) by heat and led to the increase of starch-protein interaction (Scott and Awika, 2023; Seguchi *et al.*, 2004; Yuan *et al.*, 2018). Moreover, Khamthong and Lumdubwong (2012) has also showed that the HMT modification induced β-turn conformation in rice proteins within normal and waxy rice flours. Although, there were studies carried out on the effect of HMT modification on rice flours with varying amylose content (Puncha-Arnon and Uttapap, 2013; Ruiiz *et al.*, 2018), the research frequently focused on the change of starch. There is still limited information on the molecular changes of both starch and protein and their effect on the pasting properties when modified rice flour with different varieties and amylose contents by HMT. The objective of this study was to investigate the effect of HMT on the molecular changes of starch and protein, pasting properties, and textural characteristics of high- and low-amylose rice flours.

2. Materials and Methods

Pathum Thani 80 or RD31 (PTT80, high amylose content) and Khao Dawk Mali 105 (KDML105, low amylose content) rice grains were received from the Rice Department, Bangkok (Thailand). Khao Tah Haeng 17 (KTH17, high amylose content) was obtained from a local market.

2.1 Rice flour preparation

Polished rice grains were wet milled by a colloid mill. Then, the rice slurry was centrifuged to remove extra water before drying at 45°C until it reached the moisture content of 10-12%. Then, rice flour was ground using a rotor mill and passed through a 100-mesh sieve. Rice flour samples were then packed in polyethylene bags, sealed, and kept at -18°C.

2.2 Heat-moisture-treated rice flour preparation

Rice flour (50 g, dry basis) was weighed in an aluminum foil pouch and then slowly added distilled water to rice flour to get 20% moisture content before sealed. The sample was then equilibrated at 4°C for 24 h before heated at 110°C for 14 h. The treated samples were subsequently dried at 45°C until 10–12% moisture content was obtained. Later, the samples were ground and passed through a 100-mesh sieve (150 μm) (Chen *et al.,* 2017; Asare *et al.,* 2021; Khunae *et al.,* 2007).

2.3 Chemical composition and resistant starch content

The moisture and lipid contents of the native and HMT rice flour were determined according to the Official Standard Method AACC (2000) (AACC method 44-15.02) and AOAC (1990) (AOAC method 922.06), respectively. The total nitrogen amount was evaluated according to the Official Standard Method AOAC (1990) and the protein content was calculated by 5.95 as the conversion factor (AOAC method 920.87). The amylose content was analyzed by following (Juliano, 1971) and expressed as the proportion by weight of amylose (g/100 g dry basis). Potato amylose, type II was used for the standard curve. Resistant starch (RS) content of native and HMT rice flour was quantified using the Megazyme Resistant Starch Assay Kit (K-RSTAR, Megazyme Bray, Co. Wicklow, Ireland) by following Official Standard Method AOAC (1990) (AOAC method 2002.02).

2.4 Chemical structure

The chemical structure of native and HMT rice flours was obtained using a Fourier Transform Infrared Spectrophotometer (FTIR) (Bruker Tensor 27, Germany) with an Attenuated Total Reflectance (ATR) accessory. Each measurement consisted of 64 scans in the range 400 to 4000 cm^{-1} at a resolution of 4 cm^{-1}. The deconvolution data was used Analyzer software (OriginPro, 2018). The measurement was done in two replications.

2.5 Crystalline structure

The crystalline structure of native and HMT rice flours was determined using an X-ray diffractometer (D8 ADVANCE, Bruker, USA). The samples were scanned from 5° to 35° (2θ) at 0.02° step size and 0.75s of time per step. The measurement was done in two replications. The relative crystallinity (RC) was determined using MDI JADE 6 software. RC (%) was the percentage of crystallization area and total area (crystallization area + amorphous area) ratio.

2.6 Thermal properties

Thermal properties of native and HMT rice flour were measured using a Differential Scanning Calorimeter (DSC, DSC 1, STARe system Mettler Toledo, USA). The sample (10.0 mg, dry basis) was weighed accurately into a stainless-steel pan and distilled water was added at a ratio of 3:1 (water: sample). The pan was sealed and equilibrated overnight at room temperature. Then, the samples in sealed pans were heated from 30 to 140°C at a heating rate of 10°C/min and then cooled at 10°C/min to 30°C. Samples were then reheated at 10°C/min to their same corresponding temperature. The onset temperature (T_o), endothermic peak (T_p), conclusion temperature (T_c) and enthalpy change (ΔH) were calculated by Pyris software (Version 12, USA).

2.7 Pasting properties

The pasting properties of native and HMT rice flour were determined following AACC method 61-02 (AACC, 2000) using Rapid Visco Analyser (RVA) (model 3D, Newport Scientific, Warriewood, Australia). The sample was weighed (3 g dry basis) directly into the aluminum RVA sample canister, and 25 mL distilled water was added and mixed thoroughly.

A programmed heating and cooling cycle were used where the samples were held at 50°C for 2 min, heated to 95°C in 7.5 min, held at 95°C for 5 min before cooling to 50°C in 7.5 min, and holding at 50°C for 5 min. Pasting temperature, peak, trough, breakdown, final, and setback viscosity were recorded. All samples were done in two replications.

2.8 Texture profile analysis of rice flour gel

The 20% w/w rice flour gel was prepared as follows. The rice flour (native or HMT) and distilled water were weighed. Then, the mixture was mixed at 25°C for 10 min and then heated to 60°C. Consequently, the pastes were poured onto the aluminum trays and steamed for 20 min before cooling at 25°C for 3 h.

Texture profile analysis (TPA) was used to determine textural properties of native and HMT rice flour gel by a Texture Analyser (TA.XT.plusC, Stable Micro Systems, Surrey, UK) equipped with a load cell of 5 kg. Gel was cut to the size of 10 × 10 × 12 mm and the texture measurement was performed with a 36 mm diameter cylindrical probe (P/36R) and the deformation level was at 40% strain. The hardness, springiness, cohesiveness, gumminess, and resilience were measured. The test was done in two replications.

2.9 Statistical analysis

The measurements were reported as the means ± standard deviations (SD). The experimental data were analyzed using analysis of variance with the SPSS V.26 statistical software package (SPSS (Thailand) Co., Ltd; Bangkok, Thailand). Significant differences ($P<0.05$) were tested using Duncan's multiple range test.

3. Results and Discussion

3.1 Chemical composition and resistant starch content

The chemical compositions (moisture, protein, fat, amylose and resistant contents) of native and HMT rice flour with different varieties are shown in Table 1. When compared between high (PTT80, KTH17) and low (KDML105) amylose groups, the results showed that the protein and fat of high amylose group were significantly higher than those of low amylose group. After HMT, the protein content of KDML105 was increased (P<0.05), while that of PTT80 was decreased (P<0.05). However, the protein content of KTH17 was not significantly different after HMT (P≥0.05). In Table 1, the results showed that the fat contents of HPTT, HKTH and HKDML were not significantly different (P≥0.05) compared to their native rice flours. After HMT modification, the protein and fat content were altered slightly, which might be due to the starch, protein and lipid molecular chains binding (Xiang *et al.,* 2023).

Table 1 The amylose content, chemical composition, and resistant starch content of native and HMT rice flour.

Treatment	Amylose content (% dry basis)	Chemical composition (%)			Resistant starch content (% dry basis)
		Moisture (% dry basis)	Protein (% dry basis)	Fat (% dry basis)	
PTT80	33.51 ± 0.23^{a}	8.89 ± 0.02^{d}	7.78 ± 0.01^{a}	0.37 ± 0.04^{abc}	3.46 ± 0.13^{b}
KTH17	30.99 ± 0.42^{b}	8.02 ± 0.06^{e}	7.58 ± 0.02^{b}	0.40 ± 0.02^{ab}	0.61 ± 0.00^{d}
KDML105	18.41 ± 0.10^{c}	7.98 ± 0.05^{e}	6.32 ± 0.03^{e}	0.25 ± 0.05^{d}	0.06 ± 0.01^{e}
HPTT		11.56 ± 0.03^{a}	7.26 ± 0.02^{c}	0.29 ± 0.04^{cd}	9.17 ± 0.15^{a}
HKTH		11.08 ± 0.08^{b}	7.56 ± 0.03^{b}	0.43 ± 0.01^{a}	1.42 ± 0.00^{c}
HKDML		10.86 ± 0.19^{c}	6.49 ± 0.04^{d}	0.33 ± 0.03^{bcd}	0.09 ± 0.00^{e}

Note: All data were reported as mean and standard deviations (n=2). Values with different superscripts in the same column are significantly different (P<0.05).
PTT80: Pathum Thani 80, KTH17: Khao Tah Haeng 17, KDML105: Khao Dawk Mali 105
HPTT: HMT of PTT80, HKTH: HMT of KTH17, HKDML: HMT of KDML105

As shown in Table 1, the resistant starch content (RS) of rice flour with different varieties was significantly difference regarding the amylose content (P<0.05). Furthermore, the results also exhibited that after HMT modification, the RS content of rice flour with high amylose content was increased (Table1); while that of rice flour with low amylose content was not significantly difference. The results agree with previous study that the RS content of rice starches was positively correlated with amylose content (Tian and Sun, 2020). Moreover, the protein and lipid in flour may also affect the RS content by interacting with starch and preventing the hydrolysis of enzymes (Li and Hu, 2023).

After HMT modification, the RS content has increased drastically in the high-amylose group compared to the low-amylose rice flour. The RS content of HPTT and HKTH were higher than that of native flour 2.65 and 2.32 times (P<0.05), respectively. This result indicated that the HMT process might promote the interaction among starch, protein and lipid on the starch granules' surfaces of high-amylose rice flour greater than low-amylose rice flour (Xiang *et al.,* 2023). In addition, the protein or lipid and starch interactions and ALCs (V-type complexes) may restrict the enzyme digestion and led to the decrease of starch digestibility and increase the RS content (Lu *et al.*, 2019; Ye *et al.*, 2018). Therefore, the factors that affect the RS content in native and HMT rice flour are the amylose content, and protein and fat content (Li and Hu, 2023; Tian and Sun, 2020).

3.2 Crystalline and short-range molecular ordered structure

The XRD patterns and relative crystallinity (RC) of native and HMT rice flours are shown in Table 2 and Fig 1. All samples (both native and HMT rice flours) have shown a strong reflection peak at 15.0° and 23.0° and an unresolved doublet at 2θ of 17.1° and 17.9°, which exhibited an A-type crystalline pattern consistent with the previous studies (Chen *et al.*, 2017; Khunae *et al.*, 2007). Furthermore, there are strong reflection peaks at 13.0° and 20.0°, which referred to starch-lipid complexes and this exhibited the V-type crystalline pattern (Raza *et al.*, 2022; Xiang *et al.*, 2023; Yang *et al.*, 2022). In addition, the previous study reported that the formation of ALCs during HMT was primarily responsible for the significant increase in peak intensity around 20° (Yang *et al.*, 2022). From the XRD diffractogram, the result confirms that the crystalline pattern of rice flour remained A-type after HMT modification, which agreed with other studies (Kaur and Singh, 2019; Khunae *et al.*, 2007). Typically, heat moisture treated starch is done under low moisture content (<35 %) at the temperature around 84-120°C for a period of 15 min to 16 h (Gunaratne and Hoover, 2002). The arrangement of starch chains within the amorphous and crystalline domains is altered during HMT modification without disrupting the starch's crystalline structure (Hoover, 2010; Kumar *et al.*, 2023).

Table 2 Relative crystallinity (RC), degree of double helix (DD) and degree of order (DO) of native and HMT rice flour.

Treatment	Relative crystallinity (RC) (%)			DD (995/1022)	DO (1045/1022)
	A-Type	V-Type	Total (A+V-Types)		
PTT80	21.49 ± 0.06^{d}	0.89 ± 0.12^{c}	22.38 ± 0.17^{d}	1.74±0.15^{b}	0.18±0.02^{b}
KTH17	19.53 ± 0.02^{e}	1.08 ± 0.06^{bc}	20.61 ± 0.07^{e}	1.72±0.16^{b}	0.19±0.01^{ab}
KDML105	22.52 ± 0.11^{c}	1.13 ± 0.22^{bc}	23.65 ± 0.11^{c}	1.95±0.04^{ab}	0.24±0.01^{a}
HPTT	25.16 ± 0.45^{a}	1.50 ± 0.16^{ab}	26.66 ± 0.61^{a}	2.18±0.25^{a}	0.24±0.04^{a}
HKTH	22.29 ± 0.35^{c}	1.69 ± 0.27^{a}	23.98 ± 0.63^{c}	1.97±0.03^{ab}	0.21±0.01^{ab}
HKDML	24.25 ± 0.39^{b}	1.44 ± 0.02^{ab}	25.69 ± 0.38^{b}	1.79±0.00^{b}	0.19±0.00^{b}

Note: All data were reported as mean and standard deviations (n = 2). Values with different superscripts in the same column are significantly different (P<0.05).
PTT80: Pathum Thani 80, KTH17: Khao Tah Haeng 17, KDML105: Khao Dawk Mali 105
HPTT: HMT of PTT80, HKTH: HMT of KTH17, HKDML: HMT of KDML105

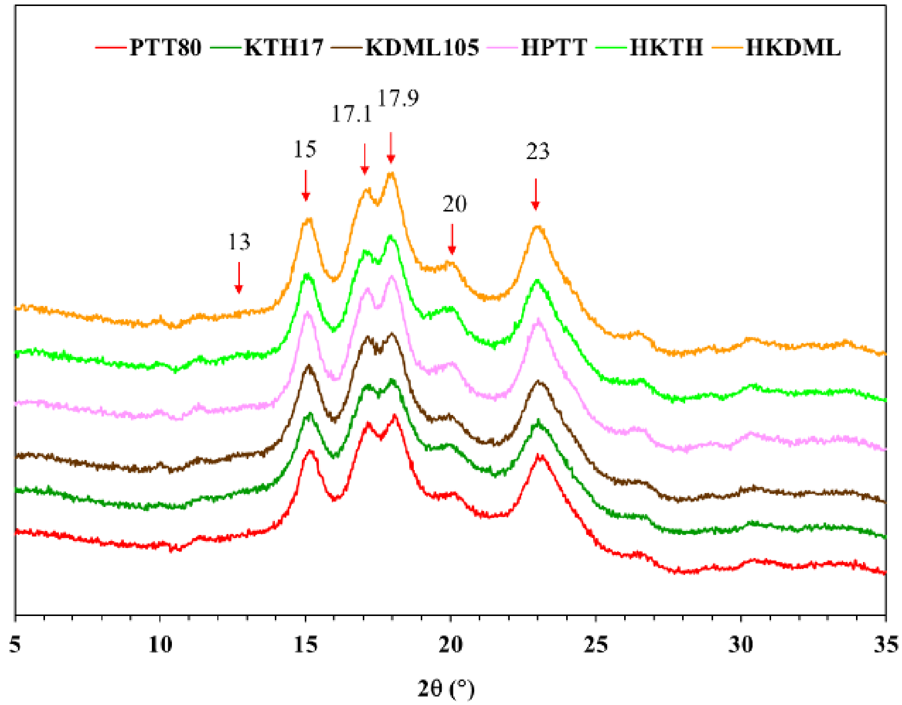

Fig 1 X-ray diffraction patterns for native and HMT rice flour
PTT80: Pathum Thani 80, KTH17: Khao Tah Haeng 17, KDML105: Khao Dawk Mali 105, HPTT: HMT of PTT80, HKTH: HMT of KTH17, HKDML: HMT of KDML105

The RC was calculated using the ratio of crystallization area and total area (both crystallization and amorphous area) as shown in Table 2. The results showed that the total RC of KDML105 was higher than that of PTT80 and KTH17, respectively ($P<0.05$). After HMT, the total RC of rice flour with different varieties increased because there might be the rearrangement of starch molecules like amylose-amylose or amylose-amylopectin side chain interaction and also the formation of a complex between amylose and endogenous lipid (Asare *et al.*, 2021). In addition, this might be attributed to the binding of starch, protein, and lipids molecular chains (Xiang *et al.*, 2023). The RC of A-type of high- and low-amylose rice flour also increased after HMT modification (Table 2), suggesting that HMT could induce the formation of double helices. The thermal energy and moisture during HMT cause double helices to shift within the crystallites and to adopt a more closely packed and ordered crystalline structure. This would explain the observed increase in X-ray intensities, especially in A-type starches, in which the adjacent double helices within crystallites are mainly linked by direct hydrogen bonding (Hoover, 2010). The RC of V-type of high-amylose rice flour also increased after modification, which could imply that HMT could induce the formation of ALCs (Wang *et al.*, 2018).

The FTIR spectra of native and HMT rice flour with high- and low-amylose content are shown in Fig 2. The results showed the absorption peaks of both native and HMT with different rice varieties at about 1149–1150 and 1078–1079 cm^{-1} attributed to coupling of C-O, C-C, and O-H bond stretching, bending and asymmetric stretching of the C-O-C glycosidic bridge (1150 and 1080 cm^{-1}) (Ma and Boye, 2018). In addition, they showed similar spectra at about 995, 1022 and 1045 cm^{-1}, ascribed to molecular order, amorphous or disorder phase and crystalline phase of starch molecules, respectively (Ma and Boye, 2018). In this study, the ratios of 995/1022 and 1045/1022 cm^{-1} were calculated as shown in Table 2. The ratio of 995/1022 cm^{-1} correlates to the internal changes in the degree of double helix (DD), while the ratio of 1045/1022 cm^{-1} represents the short-range molecular order related to the degree of order (DO) (Yang *et al.,* 2021). The degree of order is the amount of ordered crystalline region to the starch amorphous region or the degree of starch short-range ordered structure (Warren *et al.,* 2016). The greater the value of the ratio of 1045/1022 cm^{-1}, the higher the ordered degree. Previous research indicated that the increase in crystalline structure was associated with the rise in the 1045/1022 cm^{-1} ratio following HMT (Sudlapa and Suwannaporn, 2023). The DD and DO of KTH17 rice flours were not different ($P \geq 0.05$) after HMT. The increase in DD and DO of PTT80 rice flour after HMT might be due to the amylose-amylose and amylose-amylopectin side-chain interaction (Gunaratne and Hoover, 2002; Hoover, 2010). However, the DO of KDML105 rice flour decreased after HMT ($P<0.05$). The decrease in DO of HKDML might be due to the alteration of the amylopectin in crystalline region after HMT (Wang et al., 2021).

3.3 Secondary protein structure

The FTIR spectra of HMT rice flour provided a strong peak at about 1639–1641 cm^{-1} that shifted from the absorption peak of the native rice flour at about 1644–1645 cm^{-1} (Fig 2). The FTIR spectra range of 1600–1700 cm^{-1} represented amide I, which related to the secondary structure of the protein: 1610–1640 cm^{-1} and 1670–1690 cm^{-1} related to β-sheet and 1640–1650, 1650–1658 and 1660–1700 cm^{-1} were assigned for random coil, α-helix and β-turn respectively (Shevkani et al., 2019). Therefore, the shift of these spectra after HMT could relate to the change in the secondary structures of protein. The amide band intensities in Table 3 are proportionate to the fraction of each secondary structure, as determined by the peak fitting calculations (Pelton and McLean, 2000). There were reports that the β-sheet structure has exposed more hydrophobic residues and facilitated the binding to starch molecules (Scott and Awika, 2023; Seguchi *et al.,* 2004). The results showed that the HMT could alter the secondary protein structure of high- and low-amylose rice flour by changing from β-turn and random coil to β-sheet structures (Table 3). It was suggested that the HMT modification provided the reduction of β-turn and random coil structures and the formation of β-sheet structures in high- and low-amylose rice flour, thus, implied the formation of protein and starch interactions.

Table 3 Relative content of the secondary structure of proteins in native and HMT-modified rice flour with different amylose contents.

Treatments	Peak area (%)			
	α-Helix [ns]	β-Sheet	β-Turn	Random coil
PTT80	18.57±0.43	48.87±0.33[c]	24.43±0.13[b]	8.14±0.23[a]
KTH17	18.86±0.23	48.44±0.06[c]	25.43±0.19[a]	7.26±0.48[ab]
KDML105	19.55±0.57	49.68±0.28[b]	24.23±0.08[b]	6.54±0.38[bc]
HPTT	18.80±0.17	53.15±0.44[a]	22.51±0.42[c]	5.55±0.15[c]
HKTH	18.83±0.42	52.89±0.03[a]	22.89±0.15[c]	5.40±0.61[c]
HKDML	18.81±0.36	52.98±0.09[a]	22.48±0.14[c]	5.72±0.31[c]

Note: Data are expressed as means of two replications. Values with different superscripts in the same column are significantly different ($P<0.05$), ns: not significant ($P\geq0.05$).
PTT80: Pathum Thani 80, KTH17: Khao Tah Haeng 17, KDML105: Khao Dawk Mali 105
HPTT: HMT of PTT80, HKTH: HMT of KTH17, HKDML: HMT of KDML105

The FTIR spectra of HMT and native rice flour of all rice varieties exhibited the peak at about 2928–2930 and 3285–3303 cm^{-1} (Fig 2), which attributed to O-H groups and CH_2 deformations and O-H-stretching vibration (bound hydroxyl groups) (Ma and Boye, 2018). The HKTH has shifted to a higher wavenumber from 3289 to 3298 cm^{-1} compared to KTH17, possibly because of the breakage of inter- and intramolecular hydrogen bonds in the main chains of starch as well as the formation of hydrogen bonds between starch and protein or lipid. In addition, the wavenumber of HPTT decreased, which might be due to the amylose-amylose and amylose-amylopectin interaction. This result was consistent with the increase in double helix order and crystalline structures of HPTT. However, the wavenumber of HKDML remained unchanged, indicating that the double helix order of HKDML was not altered (Table 2). From these results, the HMT provided the different conversion of β-turn and random coil to β-sheet structure as well as hydrogen bonds formation of high-and low-amylose rice flour. These may induce the different ability of the protein to bind to starch and lipids. This suggests that HMT could promote interactions between amylose-amylose, starch-lipid and starch-protein in PTT80, while promoting interactions between starch-protein and starch-lipid in KTH17. This might be due to the difference of the starch fine structure and storage protein composition and structures of PTT80 and KTH17. The most significant factor in starch-protein interactions affecting physical properties is amylose, followed by amylopectin and protein (Ma *et al.*, 2024).

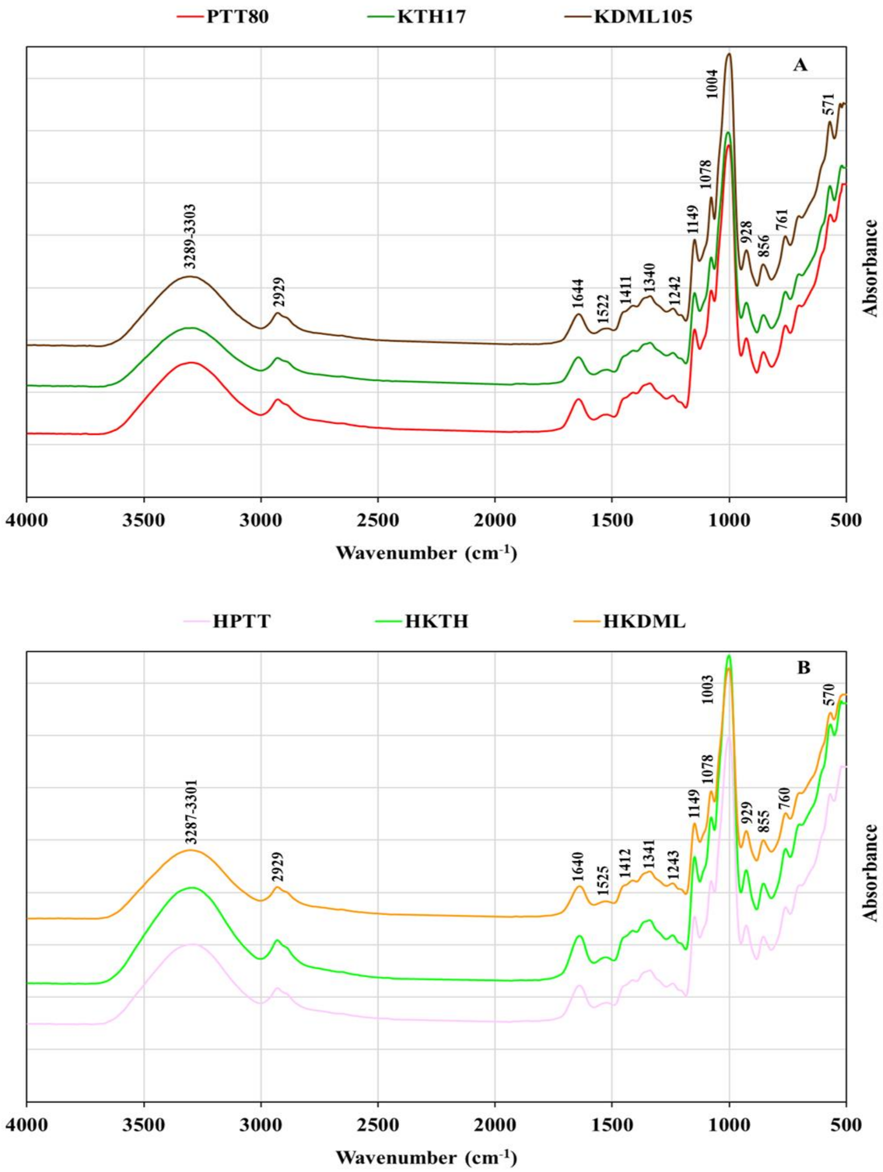

Fig 2 FTIR spectra of (A) native and (B) HMT rice flour
PTT80: Pathum Thani 80, KTH17: Khao Tah Haeng 17, KDML105: Khao Dawk Mali 105, HPTT: HMT of PTT80, HKTH: HMT of KTH17, HKDML: HMT of KDML105

3.4 Thermal properties

The thermal properties of native and HMT rice flour with different amylose contents are summarized in Table 4 and Table 5. The native and HMT rice flour exhibited the peak of the 1st heating that was attributed to the gelatinization temperature range (Table 4). They also showed the peak of the 2nd heating (after cooling) that attributed to the ALCs Type I dissociation temperature range (Table 5). The onset (T_o), peak (T_p), and conclusion (T_c) temperatures of PTT80 was higher than those of KTH17 and KDML105 ($P < 0.05$) (Table 4). The gelatinization temperature range (T_c–T_o) of PTT80 was lower than those of KTH17 and KDML105 ($P<0.05$). The gelatinization enthalpy (ΔH) of all rice flour were not significantly different ($P \geq 0.05$) (Table 4).

Table 4 Thermal properties of native and HMT rice flour (1st heating (Peak I)).

Treatment	1st heating (Peak I)				
	T_o (°C)	T_p (°C)	T_c (°C)	T_c- T_o (°C)	ΔH (J/g) ns
PTT80	75.66 ± 0.00a	80.84 ± 0.23b	88.10 ± 0.04b	12.44 ± 0.04c	7.65 ± 0.08
KTH17	64.59 ± 1.38c	72.17 ± 1.18c	82.79 ± 0.38c	18.21 ± 1.00b	6.43 ± 1.10
KDML105	65.37 ± 1.03c	73.17 ± 0.71c	82.06 ± 1.15c	16.69 ± 0.11b	7.43 ± 0.10
HPTT	77.39 ± 0.43a	86.17 ± 2.83a	94.29 ± 0.07a	16.91 ± 0.36b	7.38 ± 0.11
HKTH	68.38 ± 0.69b	78.84 ± 3.06b	91.82 ± 2.16a	23.44 ± 1.46a	6.48 ± 1.74
HKDML	69.72 ± 0.86b	78.34 ± 1.18b	87.34 ± 0.90b	17.62 ± 0.04b	7.61 ± 0.49

Note: Data are expressed as means of two replications. Values with different superscripts in the same column are significantly different (P<0.05), ns: not significant (P≥0.05).
T_o, T_p, T_c, and ΔH are onset temperature, peak temperature, conclusion temperature, and enthalpy change, respectively.
PTT80: Pathum Thani 80, KTH17: Khao Tah Haeng 17, KDML105: Khao Dawk Mali 105
HPTT: HMT of PTT80, HKTH: HMT of KTH17, HKDML: HMT of KDML105

Table 5 Thermal properties of native and HMT rice flour (2nd heating (Peak I)).

Treatment	2nd heating (Peak I)			
	T_o (°C) ns	T_p (°C) ns	T_c (°C) ns	ΔH (J/g) ns
PTT80	95.72 ± 1.13	103.34 ± 0.47	108.69 ± 0.63	0.53 ± 0.16
KTH17	94.84 ± 0.21	103.00 ± 0.47	108.65 ± 0.88	0.61 ± 0.11
KDML105	95.96 ± 0.09	103.59 ± 0.83	108.89 ± 0.84	0.49 ± 0.10
HPTT	96.15 ± 0.93	104.17 ± 0.71	109.14 ± 1.25	0.44 ± 0.12
HKTH	95.52 ± 0.00	103.67 ± 0.47	108.96 ± 1.13	0.58 ± 0.21
HKDML	96.42 ± 1.32	104.17 ± 0.71	109.40 ± 1.62	0.53 ± 0.21

Data are expressed as means of two replications. Values with different superscripts in the same column are significantly different (P<0.05), ns: not significant (P≥0.05).
T_o, T_p, T_c, and ΔH are onset temperature, peak temperature, conclusion temperature, and enthalpy change, respectively.
PTT80: Pathum Thani 80, KTH17: Khao Tah Haeng 17, KDML105: Khao Dawk Mali 105
HPTT: HMT of PTT80, HKTH: HMT of KTH17, HKDML: HMT of KDML105

As shown in Table 4, the T_o, T_p, and T_c of HPTT, HKTH and HKDML were higher than those of native rice flour (P<0.05). The gelatinization temperatures (T_c–T_o) of HPTT and HKTH were higher than their native rice flour (P<0.05). The HKTH provided the highest T_c–T_o of all HMT rice flour. However, the T_c–T_o of KDML105 was not significantly different (P≥0.05) after HMT. In addition, the gelatinization enthalpy (ΔH) of all HMT rice flours was not significantly different (P ≥ 0.05). After undergoing HMT, there was an increase in T_o, T_p, and T_c compared to the untreated flour. This might be due to changes in the structure of the starch granules, which involved interactions between amylose and lipids, proteins, and amylose, leading to a reduction in the mobility of the amorphous regions. Consequently, higher temperatures are needed for swelling and crystalline regions disruption in HMT-modified starches, which increases T_o, T_p, and T_c (Hoover, 2010; Ruiiz *et al.*, 2018; Xiang *et al.*, 2023; Zavareze and Dias, 2011). There are studies reported that during the HMT modification, the amylopectin chain mobility was restricted due to the interaction between amylose molecules in amorphous region and the amylopectin side chain in the crystalline region and this resulted in the increase of the transition temperature of melting (Hoover, 2010; Ruiiz *et al.*, 2018).

There were no significant differences (P≥0.05) in the gelatinization enthalpy of all rice flour after HMT; however, the gelatinization temperature range (T_c–T_o) was increased after HMT. This might be because the disruption of the crystalline region occurred simultaneously with the formation of starch, protein, and lipid interaction, which is agreeable with the total RC, DD and DO results in Table 2 as well as the FTIR spectra result (Table 3 and Fig 2). However, the ALCs dissociation temperature and enthalpy of HMT rice flour with different amylose contents did not show extensive change compared to those of native rice flour (Table 5), unlike the X-ray diffractograms (Fig 1 and Table 2) that showed ALCs peak and RC of V-types content with significant change except HKDML. This could imply that the ALCs that formed during HMT might not be reformed after being melted during the first scan (Tufvesson *et al.*, 2003).

3.5 Pasting properties

The pasting properties of native and HMT of high- and low-amylose rice flour are shown in Table 6. The pasting temperatures of PTT80 and KTH17 were not significantly different (P≥0.05) but higher than that of KDML105. The peak viscosity of KDML105 rice flour was found to be the highest, followed by that of PTT80 and KTH17 rice flour. In Table 6, the results show that the breakdown viscosity of all native rice flours is in the following order: KDML105 > PTT80 > KTH17. The setback viscosity of high-amylose rice flours was not significantly different (P≥0.05), but higher than that of KDML105. From the pasting profiles, the results show that the amylose content could affect the pasting temperature, peak, breakdown, final and setback viscosity (Tufvesson *et al.*, 2003). There was research showed that the amylose could restricted the starch granules swelling (higher pasting temperature and lower peak viscosity), which could led to higher granule strength (lower breakdown) (Vamadevan and Bertoft, 2020). However, during starch gelatinization, there were amylose molecules leaching from the granules and these molecules could re-associate during cooling, showing a higher final viscosity (Liu *et al.*, 2019). For this reason, the PTT80 and KTH17 that have high amylose content have higher final viscosity compared to KDML105 (low amylose content). In addition, their setback viscosity is also higher and this could lead to a higher retrogradation rate (Puncha-Arnon and Uttapap, 2013). However, not only amylose content but other chemical compositions (protein and lipid) and the fine structure could affect the pasting properties as well (Tester and Morrison, 1990).

Table 6 The pasting properties of native and HMT rice flours.

Treatment	Pasting Temperature (°C)	Peak Viscosity (cP)	Trough Viscosity (cP)	Breakdown Viscosity (cP)	Final Viscosity (cP)	Setback* (cP)
PTT80	77.95±0.49[b]	5260.0±214.96[b]	2246.0±145.66[cd]	3014.0±69.30[b]	4908.0±230.52[b]	2662.0±84.85[b]
KTH17	78.88±0.81[b]	4149.5±154.86[c]	2017.5±82.73[d]	2132.0±72.12[c]	4512.5±205.77[bc]	2495.0±123.04[b]
KDML105	72.28±0.04[d]	7321.5±279.31[a]	2651.5±21.92[b]	4670.0±301.23[a]	3884.5±2.12[cd]	1233.0±19.80[c]
HPTT	82.70±0.64[a]	3197.0±14.14[d]	2443.5±94.05[bc]	753.5±79.90[e]	5692.5±36.06[a]	3249.0±57.98[a]
HKTH	80.93±1.45[a]	2490.5±143.54[e]	1995.5±190.21[d]	495.0±46.67[e]	3578.5±562.15[d]	1583.0±371.94[c]
HKDML	75.93±0.04[c]	4980.5±300.52[b]	3491.5±212.84[a]	1489.0±87.68[d]	5809.0±271.53[a]	2317.5±58.69[b]

Note: All data were reported as mean and standard deviations (n=2). Values with different superscripts in the same column are significantly different (P<0.05).
*Setback (Setback from Trough).
PTT80: Pathum Thani 80, KTH17: Khao Tah Haeng 17, KDML105: Khao Dawk Mali 105
HPTT: HMT of PTT80, HKTH: HMT of KTH17, HKDML: HMT of KDML105

After HMT modification, all rice flour samples have higher pasting temperature (P<0.05) compared to native rice flour. In addition, the results indicated that all rice flours had lower peak and breakdown viscosity after HMT. Additionally, the final and setback viscosity of HKTH decreased, except for those of HPTT and HKDML. These parameters indicated that the HMT rice flour promoted the strong interactions between starch molecules by strengthening intra-granular binding forces contributed to the resistance to deformation of starch granules. In addition, high-amylose rice flour treated with HMT increased the abundance of ALCs or V-type complexes (Table 2), both inside and on the surface of the starch granules. Additionally, the denaturation of rice protein (Table 3) strengthens the adherence of starch granules, forming a more rigid network on the starch surface and a stronger protein-starch matrix. These changes resulted in the increased granule rigidity, reduced water penetration and granular swelling, and increased temperature requirements for gelatinization (higher pasting temperature and lower peak and breakdown viscosity) (Becker *et al.*, 2001; Hoover, 2010; Kunyanee and Luangsakul, 2022). These changes could imply that the starch granules may resist to starch digestion (Balet *et al.*, 2019; Chen *et al.*, 2017; Silva et al., 2017). In addition, the final and setback viscosity of HPTT and HKDML increased, while these values decreased for HKTH. The decrease in the final and setback viscosity of HKTH may be due to the presence of ALCs (V-type complexes by XRD; Table 2) of starch granules, preventing amylose leaching as well as restricting the reassociation of starch molecules (Becker et al., 2001; Kunyanee and Luangsakul, 2022). Furthermore, the β-turn level in HKTH decreased more significantly than in HPTT and HKDML. This resulted in the formation of hydrogen bonds between starch, protein, and lipid in starch granules (as mentioned in section 3.3). These bonds contribute to the hydrophobicity of the granules' surface, giving them greater rigidity and resistance to quick heating by altering their swelling behavior as well as reducing amylose leaching from starch granules (Mathobo *et al.*, 2021). The starch granule's hydrophobicity was caused by changes to the surface protein of the starch granule (Seguchi *et al.*, 1998). During heating with shearing in RVA, gelatinization occurs and leads to the formation of ALCs and starch-protein interactions, which can impede starch reassociation (Scott and Awika, 2023). Consequently, these interactions may lower the final and setback viscosity of HKTH. In addition, the increase in the final and setback viscosity of HPTT might be due to the formation of rigid non-fragmented swollen granules and starch granule ghost (Zhang *et al.*, 2014). HPTT led to an increase in double helices, ordered structures, V-type complexes, total RC, and starch-protein interaction after undergoing HMT (as shown in Table 2 and 3). When HPTT is heated with shearing in RVA, it can cause an increase in the rigidity of starch granules and a decrease in their swelling, which leads to the formation of rigid non-fragmented swollen granules and starch ghosts. This, in turn, results in an increase in final and setback viscosity. The viscosity of starch paste can increase due to the presence of starch ghosts (Li and Wei, 2020). However, the increase in the final and setback viscosity of HKDML might be due to the fact that HKDML105 did not increase the degree of double helix and ALCs during HMT, as mentioned earlier. This resulted in an increase in the amylose leaching from starch granules during the heating process with shearing (RVA). Moreover, the formation of starch-protein interaction (β-turn and random coil conversion in Table 3) may provide the rigid non-fragmented swollen granules during the heating process with shearing (RVA). These increase in the final and setback viscosity.

The HMT modification of high- and low-amylose rice flour induces differences in hydrogen bond formation as well as β-turn and random coil conversion. These alter the pasting properties, caused by interactions between starch, protein, and lipid. HMT-modified high-amylose rice flour exhibits a high conversion of β-turn to β-sheet level, which leads to starch-protein and/or starch-protein-lipid interactions. Additionally, there is a high conversion of random coil to β-sheet level, which leads to the formation of starch granule ghosts. These differences alter the final and setback viscosity of HMT-modified high amylose rice flour. Furthermore, the final and setback viscosity of HPTT and HKTH exhibited an inverse trend, possibly due to the influence of the starch fine structure and the amylose chain length distributions.

3.6 Texture profile analysis

Texture profile analysis of native and HMT of high- and low-amylose rice flour are shown in Table 7. The hardness and springiness of PTT80 rice flour gel were higher than those of KTH17 and KDML105 rice flour gel ($P<0.05$), respectively. The amylose content in starch can affect its gelatinization and retrogradation properties. High amylose starches promote starch reassociation, while low amylose starches result in a more open structure that disintegrates more easily in water (Biduski *et al.*, 2018). Rice flour with high amylose content contributes to greater hardness, and elasticity in rice flour gels. Due to amylose promotes the production of crystalline, short-range ordered, and compact network structures, which can increase the strength and elasticity of gels (Kunyanee and Luangsakul, 2022; Dun *et al.*, 2021; Tian and Sun, 2020).

Table 7 Texture profile analysis of native and HMT rice flour gel

Treatment	Hardness (N)	Springiness	Cohesiveness	Gumminess (N)	Resilience
PTT80	4.22 ± 0.45^{b}	0.91 ± 0.01^{a}	0.69 ± 0.02^{d}	2.90 ± 0.33^{b}	0.47 ± 0.02^{c}
KTH17	3.08 ± 0.42^{c}	0.32 ± 0.02^{c}	0.79 ± 0.03^{b}	2.44 ± 0.31^{c}	0.59 ± 0.02^{a}
KDML105	0.90 ± 0.13^{e}	0.22 ± 0.03^{e}	0.83 ± 0.03^{a}	0.75 ± 0.13^{d}	0.61 ± 0.04^{a}
HPTT	5.98 ± 1.12^{a}	0.79 ± 0.07^{b}	0.59 ± 0.03^{f}	3.57 ± 0.83^{a}	0.37 ± 0.02^{d}
HKTH	3.95 ± 0.70^{b}	0.27 ± 0.02^{d}	0.66 ± 0.03^{e}	2.61 ± 0.48^{bc}	0.46 ± 0.04^{c}
HKDML	1.36 ± 0.14^{d}	0.22 ± 0.03^{e}	0.77 ± 0.03^{c}	1.04 ± 0.13^{d}	0.51 ± 0.03^{b}

All data were reported as mean and standard deviations (n=2). Values with different superscripts in the same column are significantly different ($P<0.05$).
PTT80: Pathum Thani 80, KTH17: Khao Tah Haeng 17, KDML105: Khao Dawk Mali 105
HPTT: HMT of PTT80, HKTH: HMT of KTH17, HKDML: HMT of KDML105

After modification with HMT, the rice flour gels had higher hardness, but lower cohesiveness compared to their native rice flour gels ($P<0.05$). Gelatinized starches usually consist of a combination of solubilized polymers (mostly leached amylose and amylopectin molecules) and starch remnant or ghost (Zhang *et al.*, 2014; Derek *et al.*, 1992). The presence of swollen starch granules or remnants with well-maintained integrity (starch ghost) is crucial for the development of a strong gel during the cooking of native, granular starch (Wang *et al.*, 2022). Additionally, amylose molecules diffusing out from granules could associate with other molecules and further enhance the firmness of the gel network (Wang *et al.*, 2022). From the previous results (Table 2), it showed that heat-moisture treatment modification could promote starch-starch, starch-protein and starch-lipid interaction in starch granules causing the increase of granule strength. Consequently, these phenomena led to the increase of pasting temperature and the decrease of peak and breakdown viscosity (Table 6). Due to the increase of granule strength as mentioned above, therefore during the heating to develop gel under low shear condition; therefore, there was rigid non-fragmented swollen granules and/or remnants (starch ghost) left in starch gel network, resulting in increased hardness and decreased cohesiveness of gel (Kunyanee and Luangsakul, 2022; Wang *et al.*, 2022; Biduski *et al.*, 2018).

Furthermore, the hardness of gel was also positively correlated to its β-sheet content (Zhou *et al.*, 2017; Ye *et al.*, 2023). Due to the conversion of α-helix to β-sheet structures, more hydrophobic groups of protein exposed and it could facilitate the interaction between starch and protein molecules (Ye *et al.*, 2023). This study observed that HPTT and HKTH rice flours provided different conversions of β-turn and random coil to β-sheet levels (Table 3). This change may lead to increased starch granule rigidity and reduced swelling, in which granules remain intact during gelatinization resulting in greater hardness and reduced cohesiveness of the gel (Biduski *et al.*, 2018).

4. Conclusion

Heat-moisture treatment modification had a significant impact on high-amylose rice flour, particularly when it had a high protein content. After modification, the RS content of high-amylose rice flour increased significantly. Furthermore, the short-range ordered structure and V-type complexes in high-amylose rice flour could also be promoted by the HMT modification. Moreover, HMT also caused different changes in the secondary protein structure (β-turn and random coil) and hydrogen bond formation between high- and low amylose rice flour leading to the alteration of gel hardness. The HMT also could promote the higher gel hardness of rice flour gel. The amylose and protein content in rice flour, along with the variety of rice, played a critical role in the changes in properties associated with HMT. This new information will be useful when the HMT rice flour will be used as an alternative flour in gluten free product or other functional products.

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