Effects of Heat Moisture Treatment on Physicochemical Properties and Starch Digestibility of Rice Flours Differing in Amylose Content

Elvira Ruiz[1], Khongsak Srikaeo[2,*] and Lucía Segovia de la Revilla[1]

Abstract

Rice flours from the varieties which differ in amylose content, Chai Nat 1 and KDML 105, were modified by heat moisture treatment (HMT). HMT condition was 25 g/100g moisture content and heating at 110ºC or 120ºC for 5 h. Both HMT treated rice flours were investigated for their morphology and physicochemical properties including starch digestibility. It was found that HMT induced the changes of HMT treated samples when compared to the native flours. Starch granules as observed by SEM images were more aggregated. Gelatinization temperatures as examined by a DSC were shifted to higher temperatures and a broader range. Inconsistent results were found for swelling and solubility. This could be the influence from other components, rather than starch, in the flours. In terms of digestibility, high amylose rice (Chai Nat 1) exhibited lower estimated glycaemic index (GI) than KDML 105. Within the same variety, HMT at the condition used in this study did not significantly affect starch digestion rate and GI values.

Keywords: Rice flour, Heat moisture treatment, Physicochemical property, Starch digestion, Glycaemic index

1. Introduction

Rice is a cereal grain that is the most widely consumed staple food for a large part of the world's human population, especially in Asia. Most of the rice is consumed as intact kernels, but lately a significant raise in the rice flour production has been observed due to its employment in various food products such as noodles, and novel products as gluten free based foods.

Commercial rice flour is produced either by dry or wet milling of broken rice, whereas rice starch is generally obtained by the alkaline steeping method with multi-stage purification. Usually, the flours contain almost the same major components as rice kernels (Puncha-arnon and Uttapap, 2013). High purity starch with low surface protein-lipid contamination is desired for industry use. Due to complex purification process, rice starch is expensive (Lumdubwong and Seib, 2000) and that limits the utilization in many products. Rice flours are widely used instead.

[1] Faculty of Pharmacy, University of Granada, 18071, Granada, Spain.

[2] Faculty of Food and Agricultural Technology, Pibulsongkram Rajabhat University, Muang, Phitsanulok, 65000, Thailand

*Corresponding author(s), e-mail: khongsak@live.psru.ac.th

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Rice flour in its native form, does not always have the appropriate physical and chemical properties for certain types of processing. However, modification can improve the flour properties and increase their uses in various industries. They are often modified by physical, chemical, and enzymatic processes. Recently, there has been interest in physical modification due to its simplicity and lack of environmental impact.

Heat moisture treatment (HMT) is a hydrothermal treatment technique that heats starch granules at low levels of moisture (<35% slurry in water) and a relatively high temperature (80–140°C) above its glass transition temperature but below the temperature when gelatinization occurs (Jacobs and Delcour, 1998; Hoover, 2010). It is considered to be natural and safe compared to chemical modification. HMT-mediated changes of starch properties are often found in products with starch in intact granular form. HMT decreases starch solubility, swelling power, amylose leaching and peak viscosity, and increases the pasting temperature (Sui *et al*., 2011; Varatharajan *et al*., 2011). The effects of HMT on various aspects of rice starch properties have been reported by several researchers (Hormdok and Noomborn, 2007; Khunae *et al*., 2007; Shih *et al*., 2007; Zavareze *et al*., 2010), whereas relatively little work has been done on properties of rice flour (Cham and Suwannaporn, 2010; Lorlowhakarn and Naivikul, 2006; Puncha-arnon and Uttapap, 2013). However, rice flour is widely used, more than rice starch. Other components, rather than starch, in rice flours could affect the properties of HMT treated rice flours. For example, it has been reported that protein components account for the differences in thermal and pasting properties of rice starch and rice flour (Hamaker and Griffin, 1993; Zhu *et al*., 2010). Therefore, this study aimed to determine the effects of HMT on physicochemical properties and starch digestibility of rice flours. Two rice varieties differing in amylose contents were selected.

2. Materials and methods

2.1 Rice flour preparation and HMT process

Two rice varieties were obtained from Bureau of Rice Research and Development of Thailand, Chai Nat 1 (amylose 26–27%) and KDML 105 (amylose 12–17%). The paddy were aged for 4 months and milled as polished rice. The milled rice grains were steeped in water for 4 h to soften the kernels and then ground by a double-disk stone mill (Super Masscolloider, MKPB6-2, Masuko Sangyo Co., Ltd., Japan) with water at a ratio of water to rice of 2:1 (w/w). The rice slurry was poured into a thick cloth bag and centrifuged using a basket centrifuge (locally made in Thailand) for 10 min to remove the excess water. The rice cake was then dried in a tray dryer at 45±5°C to uniform moisture content (less than 12%). The rice flour samples were ground by a hammer mill and passed through a 100-mesh sieve and packed in

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polyethylene bags, sealed and stored at 4°C for further modification and determinations. The HMT of the rice flours was carried out using the method published elsewhere (Lorlowhakarn and Naivikul, 2006). Briefly, the moisture content of the native rice flours was adjusted to 25 g/100g wet basis by mixing thoroughly with distilled water and equilibrated at 4°C for 24 h. The native rice flours (50 g dry basis) were weighed into aluminum pouches, sealed and then placed in a tray dryer. The temperature conditions were 110°C and 120°C, both for 5 h. Afterwards, HMT treated rice flours were taken out of the pouches and spread on aluminum trays, then dried in a hot-air drying oven at 45±5°C to uniform moisture content (less than 12%). The HMT treated rice flours were then milled, sieved through a 100-mesh screen and kept in sealed polyethylene bags and stored at 4°C.

2.2 Scanning Electron Microscope (SEM)

Starch granule morphology was examined by a SEM (Mini-SEM model SNE-3200M) at 1,000x magnification. All the samples were coated with gold prior to examination.

2.3 Differential Scanning Calorimetry (DSC)

The thermal properties as determined by a DSC were conducted using the Mettler Toledo (DSC 1) equipped with a refrigerated cooler. Flour samples, 6 mg, were weighed into the aluminum pan (capacity 120 μL) and water was added in a proportion of 1:2 (w/w). The pan was sealed and allowed to stand for 24 h at 4°C. Nitrogen was used as a purging gas. The software used for the analysis of the resulting thermograms was Star^{e} software (Mettler Toledo). An empty pan was used as a reference. The scanning temperature range and heating rate were 25–100°C and 5°C/min, respectively. The transition temperatures reported were the onset temperature (To), peak temperature (Tp), and conclusion temperature (Tc). The enthalpy change on gelatinization (ΔH) was estimated by integrating the area between the thermograms and a baseline under the peak. It was expressed in terms of J/g of dry starch. All the samples were conducted in triplicate.

2.4 Swelling power and solubility

The solubility and swelling power were obtained using the method from Schoch (1964) with slight modifications. Samples (0.5 g) were dispersed in 15 mL distilled water. The suspensions were heated to 50, 70, 80, 90°C in a water-bath with periodic mixing over a 30 min period. The cooked paste samples were centrifuged at 2,200 rpm for 15 min. The supernatants were taken and placed in pre-weighed aluminum can before drying at 105°C to gain constant weight. The dried supernatants were weighed as soon as the samples reached room temperature. After the supernatants were removed the swollen sediment samples were weighed. The solubility and swelling power were then calculated using Equations (1) and (2):

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$$\text{Solubility (\%)} = \frac{\text{Weight of soluble matter in supernatant (g)}}{\text{Weight of sample (g dry basis)}} \times 100 \quad (1)$$

$$\text{Swelling power (\%)} = \frac{\text{Weight of swollen matter (g)}}{\text{Weight of sample (g dry basis)} \times (100\text{–Solubility})} \times 100 \quad (2)$$

2.5 Total starch and in vitro starch digestion and modeling of starch digestograms

Total starch content was determined enzymatically using the Megazyme assay kit (Megazyme International, Ireland), following the approved AACC method 76.13 (AACC, 2009).

Time-course starch digestion was determined using a rapid in vitro digestibility assay based on glucometry (Sopade and Gidley, 2009; Mahasukhonthachat et al., 2010). About 0.5 g of ground sample was weighted and mixed with distilled water (1:1.5 w/w) and boiled at 100°C for 20 min to obtain the gelatinized samples. To avoid the effect of retrogradation, immediately after cooking, the samples were treated with artificial saliva containing porcine α-amylase (Sigma A3176 Type VI-B) before pepsin (Sigma P6887; pH 2.0) was added and incubated at 37°C for 30 min in reciprocating water bath. The digesta was neutralized with NaOH before adjusting the pH to 6 (sodium acetate buffer) prior to the addition of pancreatin (Sigma P1750) and AMG (Novozymes AMG 300 L). The mixture was incubated for 2 h, during which the glucose concentration in the digesta was measured with Accu-Check® Performa® glucometer at specific periods (0, 10, 20, 30, 45, 60, 90 and 120 min). Digested starch per 100 g dry starch (DS) was calculated as in Equation (3).

$$DS = \frac{0.9 \times G_G \times 180 \times V}{W \times S[100 - M]} \quad (3)$$

where G_G = glucometer reading (mM/L), V = volume of digesta (mL), 180 = molecular weight of glucose, W = weight of sample (g), S = starch content of sample (g/100 g sample), M= moisture content of a sample (g/100 g sample), and 0.9 = stoichiometric constant for starch from glucose contents.

The digestogram (digested starch at a specific time period) of each sample was modeled using a modified first-order kinetic model, Equation (4), as described before (Mahasukhonthachat *et al*., 2010).

$$D_t = D_0 + D_{\infty-0} (1 - \exp[-Kt]) \quad (4)$$

where D_t (g/100 g dry starch) is the digested starch at time t, D_0 is the digested starch at time t= 0, D_∞ is the digestion at infinite time (D_0 + $D_{\infty-0}$), and K is the rate constant (min^{-1}).

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The Microsoft Excel Solver® was used to compute the parameters of the model by minimising the sum of squares of residuals (SUMSQ) and constraining $D_\infty \leq 100$ g per 100 g dry starch, and $D_0 \geq 0$g per 100 g dry starch. In addition to the coefficient of determination (r^2), the predictive ability of the models was assessed with the mean relative deviation modulus (MRDM) as described elsewhere (Mahasukhonthachat *et al.*, 2010).

In order to calculate the estimated glycaemic index (GI) of the samples, the areas under the digestograms (AUC_{exp}) were computed with Equation (5):

$$AUC_{exp} = \left[D_{\infty t} + \frac{D_{\infty-0}}{K} \exp(-Kt) \right]_{t_1}^{t_2} \quad (5)$$

The hydrolysis index (HI) of each sample was calculated by dividing the area under its digestogram by the area under the digestogram of a fresh white bread (Goñi *et al.*, 1997) which was calculated to be about 23,000 min g/100 g dry starch. Using the parameters of the modified first-order kinetic model for both the samples and fresh white bread, estimated GIs of the samples were also calculated, and the average GI (GI_{AVG}) for each sample was defined as Equation (6):

$$\text{Estimated GIa}_{AVG} = \frac{[(39.51 + 0.803H_{45}) + (39.51 + 0.803HI)]}{2} \quad (6)$$

2.6 Statistical analysis

Experimental data were analyzed using one-way analysis of variance (ANOVA) and expressed as mean values ± standard deviations. Tukey's method was conducted to examine significant differences among experimental mean values with a confidence level of 95%. Minitab® version 16 was used.

3. Results and discussion

3.1 Morphology and physicochemical properties

Scanning electron micrographs of the native and HMT treated rice flours are shown in Figure 1. Native rice starch granules show angular and polyhedral shapes. Generally, it has been reported that HMT did not alter the size or shape of starch granules from various sources (Zavareze and Dias, 2011). However, in this study, HMT affected the format and degree of agglomeration of the starch granules (Figure 1 b, c and e, f). They lost their granule integrity presenting flatter and smother surface than the native ones. The granules were more aggregated. These caused by the swollen or partial gelatinized granules (Lorlowhakarn and Naivikul, 2006; Hormdok and Noomborm, 2007; Anderson and Guraya, 2006). This effect was

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pronounced in lower amylose rice (KDML 105) when compared to higher amylose sample (Chai Nat 1) (Figure 1 c, f). In rice starch, it has been reported that the HMT (25% moisture) of the high- and medium-amylose rice starch granules slightly affected the format and degree of agglomeration, making the granules more aggregated and the surface of the granules more irregular, as compared with the native starches (Zavareze *et al*., 2010). Starch granule morphology has been reported to affect the composition, gelatinization and pasting properties, enzyme susceptibility, crystallinity, swelling and solubility (Lindeboom *et al*., 2004).

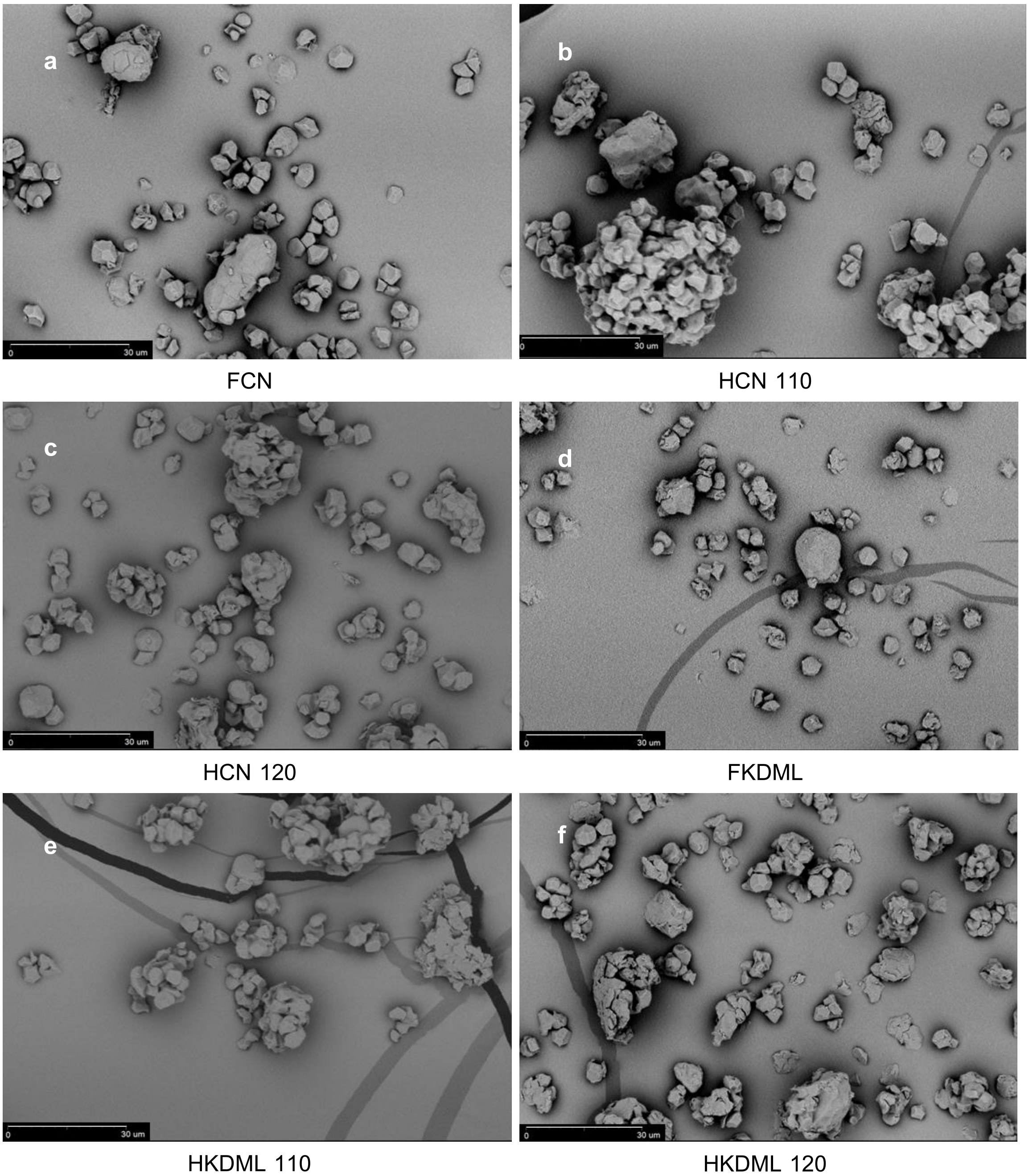

Figure 1 SEM images of the rice flour samples. FCN = native Chai Nat 1 flour, HCN 110 = HMT treated Chainat 1 at 110ºC, HCN 120 = HMT treated Chainat 1 at 120ºC, FKDML = native KDML 105 flour, HKDML 110 = HMT treated KDML 105 at 110ºC, HKDML 120 = HMT treated KDML 105 at 120ºC.

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For thermal properties, DSC results showing To, Tp and Tf of the native and HMT rice flour samples are provided in Table 1.

Table 1 DSC parameters of the rice flour samples

Samples	To (ºC)	Tp (ºC)	Tf (ºC)	△H (J/g dry sample)
High amylose				
FCN	73.0±3.0^{b}	77.2±2.0^{b}	81.3±2.1^{b}	1.20±0.9^{a}
HCN 110	80.5±2.3^{a}	83.1±2.8^{a}	85.6±3.5^{ab}	0.50±0.3^{b}
HCN 120	77.8±3.9^{ab}	84.3±1.2^{a}	88.1±1.3^{a}	0.60±0.3^{b}
Medium amylose				
FKDML	61.5±1.2^{a}	67.5±2.0^{b}	72.0±2.1^{b}	0.70±0.3^{ns}
HKDML 110	68.3±1.5^{a}	73.9±1.5^{a}	78.7±1.4^{a}	0.50±0.2
HKDML 120	64.7±5.1^{a}	73.7±1.6^{a}	77.5±0.7^{a}	0.35±0.1

Note: FCN = native Chai Nat 1 flour, HCN 110 = HMT treated Chainat 1 at 110ºC, HCN 120 = HMT treated Chainat 1 at 120ºC, FKDML = native KDML 105 flour, HKDML 110 = HMT treated KDML 105 at 110ºC, HKDML 120 = HMT treated KDML 105 at 120ºC.

Values are means ± standard deviations.

For each parameter (column for each variety), values with the same letters are not significantly different (p>0.05).

HMT affected the thermal properties of the samples as it induced the changes of gelatinization temperatures. Generally, the endotherms of the HMT treated samples were shifted to a higher temperature and a broader range when compared to native flours. The result was obvious for Tp values, as so they were all statistically different (p≤0.05). It should be noted that the temperatures used in this study (110ºC and 120ºC) did not change gelatinization temperatures of the treated samples, providing similar To, Tp, and Tc for both HMT treated samples at both temperatures. Comparing among both samples, the changes were found to be in similar trend. The increased To, Tp, and Tc have been attributed to structural changes within the starch granules, which involve amylose–amylose and amylose–lipid interactions (Hoove and Vasanthan, 1993; Adebowale *et al*., 2009). The process of gelatinization which involves the melting of crystalline regions and double helices, is determined by the hydration and swelling of the amorphous regions of starch granules. When the amorphous region swells, it imparts a stress on the crystalline regions and polymer chains are stripped from the surface of starch crystallites. After HMT, the amylose–amylose and amylose–lipid interactions reduce the mobility of the amorphous region. As a result, HMT treated starches require a higher temperature in order for swelling and disruption of the

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crystalline regions to occur, leading to the increased To, Tp, and Tc (Zavareze and Dias, 2011). In terms of △H, although the results were not statistically different in KDML 105 samples, but HMT treated samples tended to provide less △H than the native samples (significantly different in Chai Nat 1 samples). Most published literatures suggested the reduction of the enthalpies due to HMT though some found no changes. The decrease of △H due to HMT was reported to be a result of the disruption of double helices present in the crystalline and non-crystalline regions of the granules (Gunaratne and Hoover, 2002). It might also be attributed to the transformation of the intercrystalline part into an amorphous phase, and thus the crystalline regions could melt more easily (lower energy) (Lim *et al*., 2001). However, Hormdok and Noomborm (2007) argued that the reduction in △H could be due to the partial gelatinization of amylose and amylopectin molecules that are less stable during heating.

In terms of swelling power and solubility, the results are shown in Figure 2. The swelling power of rice starch and flour was reported to be about 2–14 g/g and increased as the temperature increased (Yu *et al*., 2012). In this study, the swelling power for all samples slightly increased as the temperature increased. However, comparing between the native and HMT treated samples at the same temperature, the results were found to be inconsistent. Some samples were found to be increased; some showed the decrease and also no change (see Figure 2). Most of the published literatures suggested the reduction of swelling power in HMT treated starches (Hoover, 2010). The reduction has been attributed to increased crystallinity, reduced hydration (Waduge *et al*., 2006), increased interactions between amylose and amylopectin molecules, strengthened intramolecular bonds (Jacobs *et al*., 1995), the formation of amylose–lipid complexes (Waduge *et al*., 2006) and changes in the arrangements of the crystalline regions of starch (Hoove and Vasanthan, 1993). The reduced swelling power of HMT treated samples could impede the increased swelling power of the rice samples when temperature increased from 60–90°C and therefore only slight increase was observed over the temperature range in this study. In addition, the inconsistent results found in this study might relate to the interferences from other components in the flour samples. Yu *et al*. (2012) reported that the swelling power of rice flours was lower than starches as caused by the other compositions e.g. proteins and lipids in rice flours or channels in rice flour granules.

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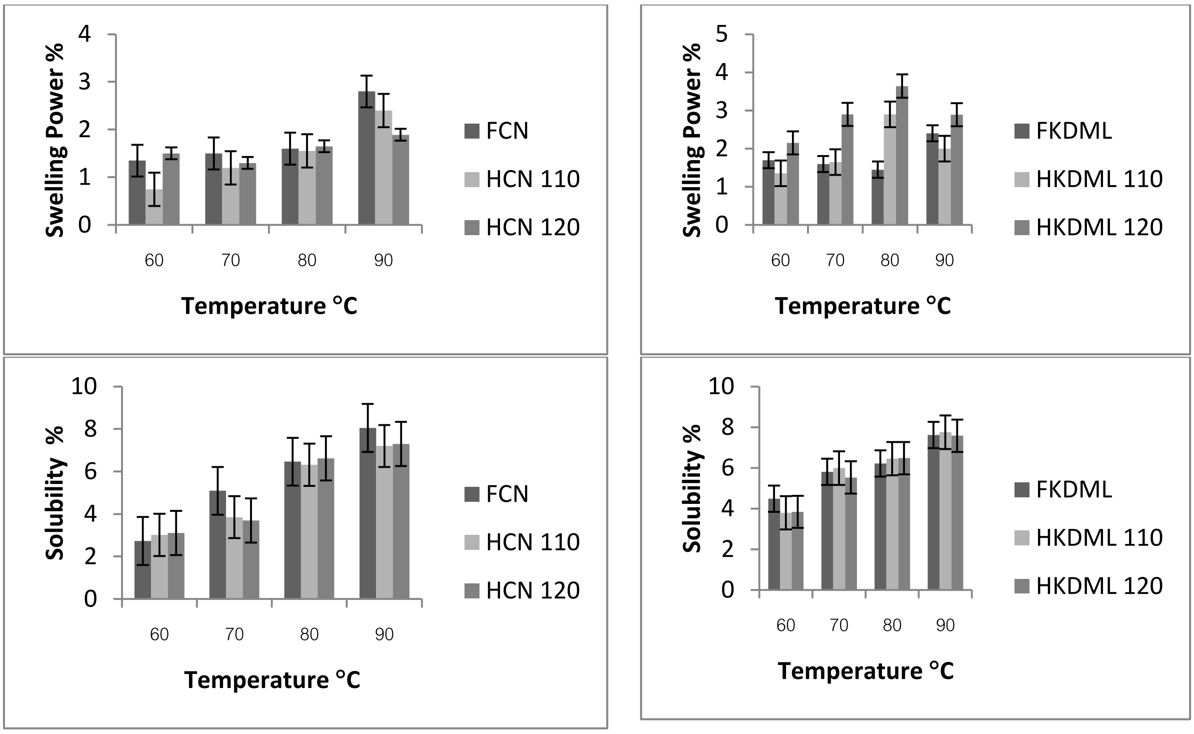

Figure 2 Swelling power and solubility of the rice flour samples

FCN = native Chai Nat 1 flour, HCN 110 = HMT treated Chainat 1 at 110ºC, HCN 120 = HMT treated Chainat 1 at 120ºC, FKDML = native KDML 105 flour, HKDML 110 = HMT treated KDML 105 at 110ºC, HKDML 120 = HMT treated KDML 105 at 120ºC.

For solubility, in native form, the solubility of rice flour samples increased significantly with temperatures. Several researchers have reported the reduction of solubility in HMT treated starches (Adebowale and Lawal, 2002; Olayinka *et al*., 2008). However, the others reported no changes (Hormdok and Noomborm, 2007). Starch solubility results from leaching of amylose, which dissociates and diffuses out of granules during swelling. This leaching represents a transition from order to disorder within the starch granules that occurs when starch is heated with water (Tester and Morrison, 1990; Zavareze and Dias, 2011). In this study, inconsistent results for all the samples were obtained. At 60°C, for Chai Nat1, the HMT treated samples exhibited higher solubility than the native form, on the contrary at 70°C and 90°C. Medium-amylose sample (KDML 105) at 60°C seemed to be less soluble than the HMT treated samples, while from 80°C to 90°C the solubility increased homogeneously for all the samples as the temperature increased. The highest swelling power and solubility for all the samples in this study was obtained at 90°C, when most of the granules were gelatinized or swollen.

3.2 Starch digestibility

Total starch and digestibility model parameters are shown in Table 2. In addition, starch digestograms are shown in Figure 3. The samples contained total starch in the range of 84–91 g/100 g dry sample. The content found in this study is slightly lower than usually

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reported in rice flour samples. This might be due to other components such as protein and ash of the samples used in this study. It was found that the modified first-order kinetic model showed suitable in describing the digestograms (r^2 = 0.90–0.99; MRDM = 8–15%; SUMSQ = 67–148).

Table 2 Model parameters, hydrolysis index (HI) and estimated GI (average) of the rice flour samples

Samples	Total starch (g/100g dry sample)	K x 10^{-3} (min^{-1})	HI	GI_{AVG}
High amylose				
FCN	88.09 ± 2.54^{a}	2.41 ± 0.50^{c}	50.54 ± 5.35^{c}	63.13 ± 2.45^{b}
HCN 110	85.56 ± 7.42^{b}	4.25 ± 0.91^{a}	63.53 ± 2.66^{ab}	69.27 ± 1.48^{a}
HCN 120	86.00 ± 5.62^{b}	3.68 ± 0.36^{b}	64.38 ± 2.19^{a}	69.66 ± 0.99^{a}
Medium amylose				
FKDML	84.16 ± 1.72^{b}	6.17 ± 0.34^{ab}	88.02 ± 2.95^{c}	81.55 ± 1.53^{b}
HKDML 110	90.72 ± 0.50^{a}	6.70 ± 0.92^{a}	92.26 ± 7.74^{ab}	83.79 ± 4.06^{a}
HKDML 120	89.92 ± 3.20^{a}	6.94 ± 0.32^{a}	94.44 ± 2.51^{a}	84.92 ± 1.33^{a}

Note: FCN = native Chai Nat 1 flour, HCN 110 = HMT treated Chainat 1 at 110ºC, HCN 120 = HMT treated Chainat 1 at 120ºC, FKDML = native KDML 105 flour, HKDML 110 = HMT treated KDML 105 at 110ºC, HKDML 120 = HMT treated KDML 105 at 120ºC.

Values are means ± standard deviations.

For each parameter (column for each variety), values with the same letters are not significantly different (p>0.05).

In general, Chai Nat 1 group gave lower estimated GI values than KDML 105 group. HMT treated samples exhibited higher GI values than the native flours. This trend was observed in K and HI as well. Low GI diets have been recommended as a healthy diet. Rice is generally known to have a relatively high GI compared to other starchy foods. The GI values of rice have been reported to be varied. It is likely that much of the variation in the GI of rice is due to differences in the proportion of starch present as amylose e.g. amylose/amylopectin ratio. Amylose content of rice may vary from 0–60 g/100g depending on their varieties. High amylose rice was found to exhibit lower GI value than low amylose rice due to its less enzymatic susceptibility (Hu *et al*., 2004). This inherent characteristic was also found in HMT treated rice flours as Chai Nat 1 (high amylose) was found to exhibit lower GI values than KDML 105 (medium amylose) (see Table 2).

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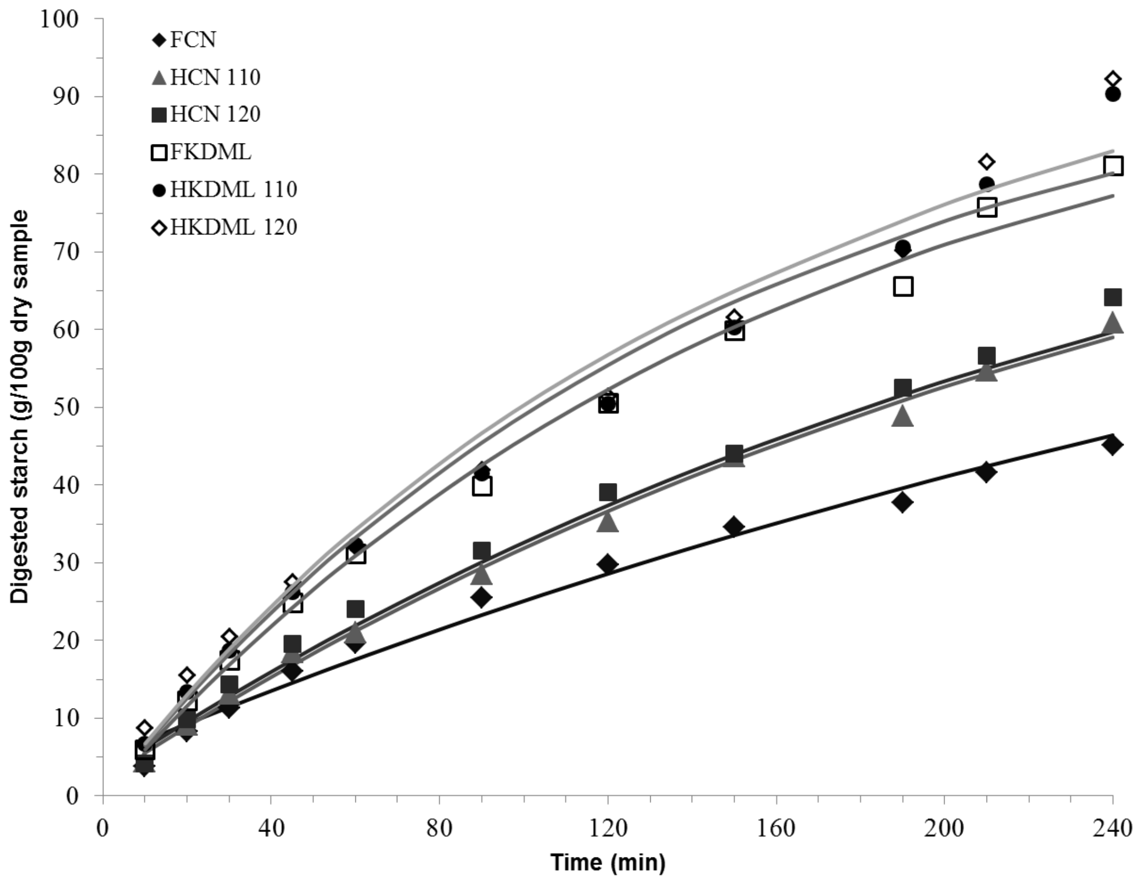

Figure 3 Starch digestograms of the rice flour samples

FCN = native Chai Nat 1 flour, HCN 110 = HMT treated Chainat 1 at 110ºC, HCN 120 = HMT treated Chainat 1 at 120ºC, FKDML = native KDML 105 flour, HKDML 110 = HMT treated KDML 105 at 110ºC, HKDML 120 = HMT treated KDML 105 at 120ºC.

The changes of starch digestibility by HMT were reported to probably occur in the amorphous regions of the starch granules, which are more accessible to hydrolysis. These amorphous areas are more rapidly degraded by α-amylases than the crystalline areas. Moreover, HMT specifically promotes crystalline disruption and the dissociation of double helical structures in the amorphous region, which can facilitate the attack of α-amylase within the granules. The structural rearrangement of starch caused by HMT facilitates enzymatic accessibility to the amorphous areas (Gunaratne and Hoover, 2002; Kweon *et al*., 2000; Franco *et al*., 1995).

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4. Conclusion

HMT is a physical modification of starches that has been widely used due to its safety and chemical free. The properties of HMT treated rice flours with different in amylose content (high and medium amylose content) were investigated. HMT condition in this study (25 g/100 g moisture content and 110–120°C for 5 h) was found to alter the properties of rice flours, both high and medium amylose samples). It induced the changes in physicochemical properties and starch granule morphology of the rice flour samples. In addition, HMT at the condition used in this study did not significantly affect starch digestion rate and glycaemic response of the modified rice flour samples.

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