Physicochemical properties and digestibility of hydrothermally treated waxy rice starch

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ABSTRACT

Waxy rice starch was subjected to annealing (ANN) and heat-moisture treatment (HMT). These starches were also treated by a combination of ANN and HMT. The impact of single and dual modifications (ANN–HMT and HMT–ANN) on the molecular weight (M_w), crystalline structure, thermal properties, and the digestibility were investigated. The relative crystallinity and short-range order on the granule surface increased on ANN, whereas decreased on HMT. All treated starches showed lower M_w than that of the native starch. Gelatinization onset temperature, peak temperature and conclusion temperature increased for both single and dual treatments. Increased slowly digestible starch content was found on HMT and ANN–HMT. However, resistant starch levels decreased in all treated starches as compared with native starch. The results would imply that hydrothermal treatment induced structural changes in waxy rice starch significantly affected its digestibility.

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1. Introduction

Starch is the principal carbohydrate in cereal grains and an important source of nourishment for humans. From a nutritional point of view, starch is generally classified as rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) based on the rate and extent of its digestibility (Englyst, Kingman, & Cummings, 1992). RDS causes an increase in blood glucose levels immediately after ingestion and SDS is digested completely in the small intestine but this process is slow. RS is not digested in the small intestine but fermented in the large bowel into short-chain fatty acids (Cummings, Beatty, Kingman, Bingham, & Englyst, 1996). SDS offers the advantage of a slow increase of postprandial blood glucose level and sustains blood glucose levels over time compared to RDS with its drastic fluctuation. SDS might be helpful in controlling and preventing hyperglycemia related diseases. Consequently, starch ingredients with high levels of SDS and RS can improve the nutritional function of foods.

Hydrothermal treatments, including annealing (ANN) and heat-moisture treatment (HMT), are physical modifications that change the physicochemical properties of starch without destroying its granule structure (Zavareze, Storck, de Castro, Schirmer, & Dias, 2010). Both ANN and HMT are involved in the starch to moisture ratio, with temperature and heating time needed to be controlled. Annealing occurs under an excess of water (>40%) and low temperature (below the gelatinization temperature), while the HMT is carried out under restricted moisture content (10–30%) and higher temperatures (90–120 °C) (Maache-Rezzoug, Zarguili, Loisel, Queveau, & Buléon, 2008).

A substantial amount of studies have been focused on the effect of ANN and HMT on the physicochemical properties and digestibility of various starches (Dias, Da Rosa Zavareze, Spier, de Castro, & Gutkoski, 2010; Jacobs, Eerlingen, Spaepen, Grobet, & Delcour, 1997; Lee, Kim, Choi, & Moon, 2012; Singh, Chang, Lin, Singh, & Singh, 2011; Varatharajan et al., 2011). The above studies have shown that ANN and HMT result in structural changes within the amorphous and crystalline regions to different extents, which in turn influence granular swelling, amylose leaching, pasting properties, gelatinization parameters, molecular structure, crystalline structure, and susceptibility towards enzymes and acids. However, only a few studies have reported the effect of the combination of ANN and HMT on starch structure and properties (Chung, Hoover, & Liu, 2009; Chung, Liu, & Hoover, 2010; Stute, 1992). These studies showed the effect of combinative hydrothermal treatments on the crystalline structure, thermal properties,

nutritional fractions of various starches and led to either an increase or decrease in SDS and RS content. However, data is still scarce on the effect of dual hydrothermal treatments on starch. Further studies are still essential on the combinative effect of ANN and HMT on starch molecule structure and properties.

Rice starch is used as an additive in various foods, industrial products, desserts, bakery products and as a fat mimetic in foods such as ice cream, yoghurt and salad dressings. Because of its wide-ranging food and industrial applications, waxy rice starch has been extensively studied. To our knowledge, no further report was found using a combination of ANN and HMT treatment on the physicochemical properties of waxy rice starch. Thus, the objective of this study was to investigate to what extent changes to molecular weight, crystalline structure, gelatinization properties and nutritional fractions of waxy rice starch on ANN, HMT, HMT–ANN and ANN–HMT are influenced by amylopectin structure.

2. Materials and methods

2.1. Materials

Waxy rice starch (0% amylose) was obtained from Jiangsu Baobao Group (Nantong, China). Porcine pancreas α-amylase (EC3.2.1.1, 16 U/mg) type-B and amyloglucosidase (EC 3.2.1.3, 300 U/ml) from *Aspergillus niger* were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Megazyme glucose assay kit (GOPOD method) was bought from Megazyme International Ireland Ltd. (Wicklow, Ireland). Other chemicals and solvents were all of analytical grade.

2.2. Hydrothermal treatment

For annealing treatment (ANN), native waxy rice starch (50 g dry basis) slurries (80% moisture) were incubated at 50 °C in a water bath with shaking for 24 h. After the incubation period, samples were centrifuged (6000 rpm) for 10 min and the supernatant was decanted. The annealed starches were washed once with deionized water and air dried at 40 °C overnight. With regard to heat-moisture treatment (HMT), native waxy rice starch (50 g dry basis) samples were weighed and the moisture content was adjusted to 25% by adding appropriate amount of distilled water. Specimens were weighed into polytetrafluoroethylene containers and sealed, then packed into stainless steel reaction still. The containers were kept for 12 h at 4 °C, and then placed in a forced air oven at 110 °C for 8 h. Afterwards the containers were opened, and the starch samples were air-dried to uniform moisture content (about 12%). For dual modification, annealed starch samples were subjected to heat-moisture treatment (ANN–HMT) and heat-moisture treated starch samples were subjected to annealing (HMT–ANN) as referred to by Stute (1992) and Chung et al. (2009). Finally, all starch samples were dried at 40 °C overnight then gently ground by a pestle and mortar to pass through a 100-mesh sieve.

2.3. Swelling power and solubility

Swelling power of starch samples was determined in duplicate by adopting the method of Tester and Morrison (1990). Swelling power is the ratio in weight of the wet sediment to the initial weight of dry starch. The solubility of starch was measured according to the method of Schoch (1964) with modifications. The solubility is the ratio of the dried supernatant weight to the initial weight of dry starch. Experimental data are the means of duplicates.

2.4. ATR-FIR analysis

ATR-FTIR analysis of starches was obtained with an FT-IR spectrometer (TENSOR27, BRUCK, Germany) equipped with a deuterated triglycine sulphate (DTGS) detector using an attenuated total reflectance (ATR) mode. For each spectrum, 16 scans were recorded at a resolution of 4 cm^{-1} at room temperature. Spectra were baseline-corrected and then deconvoluted over the range of 1200–800 cm^{-1}. A half- width of 22 cm^{-1} and a resolution enhancement factor of 2.2 were used. The amplitudes of absorbance for each spectrum at 1022 and 1047 cm^{-1} were noted and the ratio of amplitudes of absorbance at 1047 cm^{-1} and at 1022 cm^{-1} was calculated per sample to estimate the degree of order of starch at the surface (Sevenou, Hill, Farhat, & Mitchell, 2002).

2.5. X-ray diffraction and relative crystallinity

X-ray diffraction analysis was performed with an X-ray diffractometer (D8 ADVANCE, Bruker, Germany) operated at 40 kV and 40 mA producing Cu Kα radiation of 1.5418 Å wavelength, scanning through the 2θ range from 3° to 35° at a rate of 2°/min. The moisture of a specimen was regulated to about 15% by storage in a sealed desiccator over water at 25 °C. Relative crystallinity was calculated by the ratio of the crystalline area to the total diffractogram area (Nara & Komiya, 1983).

2.6. High-performance size-exclusion chromatography (HPSEC) and multi-angle laser-light scattering (MALLS) with refractive index (RI) detector

Starch sample (12.5 mg) was stirred in 25 ml of dimethyl sulfoxide (DMSO) contain 50 mM LiBr and heated in a boiling water bath for 30 min. After that, the liquid system was stirred for 24 h at room temperature. The solutions were then filtered through a nylon filter (0.22 μm type membrane, Millipore, USA) before injection into the MALLS system (Wyatt Technology, Santa Babara, CA, USA) consisting of a pump (P2000, Spectra System, San Hose, CA, USA), an injector valve with a 1 ml loop, SEC column (P8514-806, Showa Denko, Tokyo, Japan), a MALLS (Dawn DSP-F, Wyatt Technology, Santa Barbara, CA, USA) fitted with an argon laser (488 nm), and an Optilab 903 RI detector (Wyatt Technology, Santa Barbara, CA, USA). The sample (1 ml) was injected into the system and ran at a flow rate of 0.3 ml/min. The mobile phase was DMSO and degassed under vacuum. The column oven temperature was controlled at 40 °C. The molecular weights were calculated using ASTRA 6.1 software program (Wyatt Technology).

2.7. Differential scanning calorimetry

The thermal transitions of starches were investigated with the use of a differential scanning calorimetry (DSC 8000, Perkin Elmer Inc., Norwalk, USA). A starch sample (3 mg) was weighed in a DSC pan and the excess water was added to obtain a starch/water ratio of 3:7. The pans were then sealed, equilibrated for 4 h at room temperature, then heated from 30 to 130 °C at the rate of 10 °C/min. Gelatinization onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), gelatinization range (ΔT) and enthalpy values (ΔH) were measured to characterise the thermal properties of starch.

2.8. In vitro digestibility

Starch nutrition fractions were analysed according to the method of (Englyst et al., 1992) with minor modifications. Enzyme solution containing porcine pancreas α-amylase and amyloglucosidase was

prepared immediately before use. Starch (200 mg) was dispersed in 15 ml of sodium acetate buffer (0.2 mol/l, pH 5.2) by vortexing. Then six glass balls and 10 ml mixtures of porcine pancreatic α-amylase (290 U/ml) and amyloglucosidase (15 U/ml) were added. Enzyme digestion was carried out at 37 °C and 0.5 ml aliquots of hydrolysed solution was withdrawn at different time intervals and mixed with 4 ml of absolute ethanol to denature the enzymes. The glucose in the supernatant gained from centrifugation (4000 rpm, 10 min) was measured with the Megazyme glucose assay kit (GOPOD method).

The rapidly digestible starch (RDS) content was measured as the amount of glucose released in 20 min of incubation. The slowly digestible starch (SDS) fraction was defined as the fraction digested between 20 and 120 min of hydrolysis. The starch not hydrolysed within 120 min was designated resistant starch (RS) content.

2.9. Statistical analysis

Mean values and standard deviations were reported. Duncan's least significant test was used to compare means at the 5% significance level. All the statistical analyses were conducted using the SPSS for Windows 12.0 software (SPSS, Chicago, IL, USA).

3. Results and discussion

3.1. Swelling power and solubility

The swelling power and solubility of native and treated starches are presented in Fig. 1. The swelling power and solubility of all starches increased as a result of increasing the treatment temperature, due to the starch gelatinization.

In all starches, swelling power decreased upon treatment with ANN, HMT, ANN–HMT and HMT–ANN (Fig. 1A). The results showed that the decrease in swelling power on ANN is influenced to a large extent by the interplay between the extent of crystalline perfection and on the extent of interaction involving AMP–AMP chains. Both crystalline perfection and interactions involving amylopectin chains would decrease hydration of the amorphous regions, thereby decreasing granular swelling (Jayakody, Hoover, Liu, & Donner, 2009). Many researchers have shown a reduction in granular swelling on ANN (Jayakody et al., 2009; Lan et al., 2008; Waduge, Hoover, Vasanthan, Gao, & Li, 2006). The reduction in swelling power following HMT has been attributed to internal rearrangement of the starch granules, which causes further interaction amongst the starch functional groups (Hoover & Manuel, 1996), making it form more ordered double helical amylopectin side chain clusters. Several authors have observed a reduction in the swelling power on HMT (Chung et al., 2009; Gunaratne & Hoover, 2002; Olayinka, Adebowale, & Olu-Owolabi, 2008). The decrease in swelling power was further enhanced (Fig. 1A) when HMT followed ANN (ANN–HMT) and ANN followed HMT (HMT–ANN). We suspects that the higher extent of swelling power reduction in the HMT–ANN may have been influenced by its larger decrease in relative crystallinity as compared with HMT (Fig. 2B). The results indicate that when ANN starch is subjected to HMT (ANN–HMT), both perfect crystallites as well as the imperfect crystallites would disrupt leading to further decreases in swelling power.

There was a significant reduction in the solubility of the treated starches as compared to the native starch (Fig. 1B). The reduction in the solubility of the annealed starch reflects a strengthening of the bonds; as the interactions between amylopectin molecules increased, they were prevented from leaching out of the granules. Decreased solubility on annealing has also been observed in rice starches with various amylose contents (Dias et al., 2010). Waduge et al. (2006) and Lan et al. (2008) studied cassava, barley
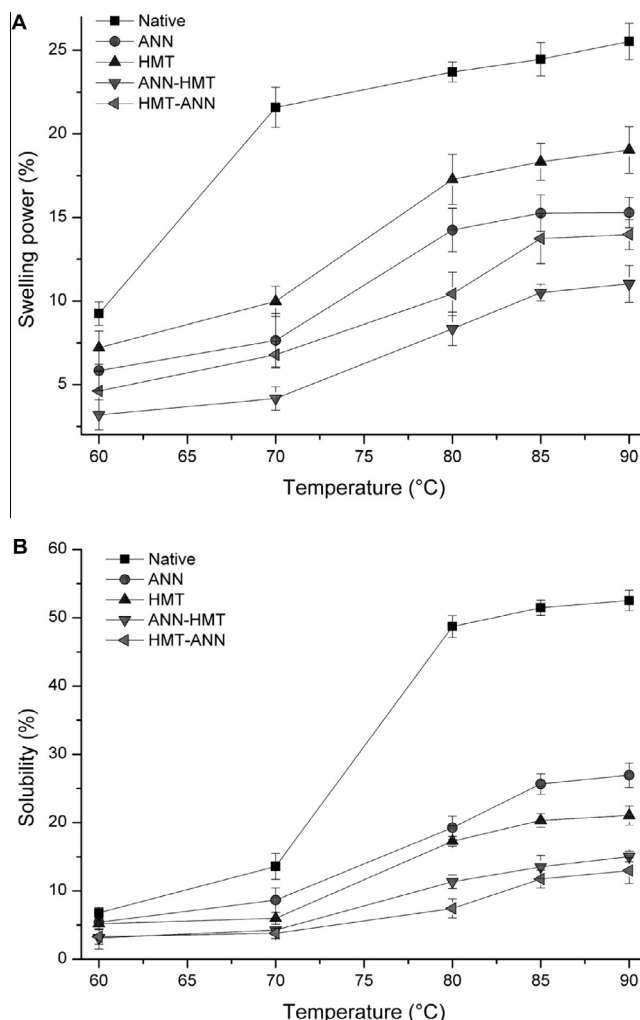

Fig. 1. Swelling power and solubility of native and treated starches. Experimental data are the means of duplicates.

and wheat starches, respectively, also found that annealing treatment reduced the swelling power and solubility of the starches. The highest solubility for all of the starches was obtained at 90 °C, where most of the granules were gelatinized or swollen (Fig. 1B). The decrease in the solubility of HMT starch also suggests that there was a strengthening of the bonds, with an increase in the interactions amongst amylopectin and amylopectin molecules, impeding them from leaching out of the granules. Combined treatment of the ANN–HMT and HMT–ANN showed lower solubility than the single treatment, indicating that there was a strengthening of the bonds. The decrease in solubility was further enhanced when ANN followed HMT (HMT–ANN).

3.2. ATR-FTIR spectra

The deconvoluted FT-IR spectra of native and treated starches are presented in Fig. 2A. The modified waxy rice starches showed similar deconvoluted FT-IR spectra to native starch. FT-IR has been suggested to be sensitive to changes in structure on a molecular level (short-range order). Furthermore, the FT-IR technique yields information on the structural organisation of starch chains near the granule surface, since the IR beam penetrates only to a depth of 2 μm into the granule (Sevenou et al., 2002; van Soest, Tournois, de Wit, & Vliegenthart, 1995). The IR bands at 1047 and 1022 cm^{-1} have been revealed to be associated with ordered
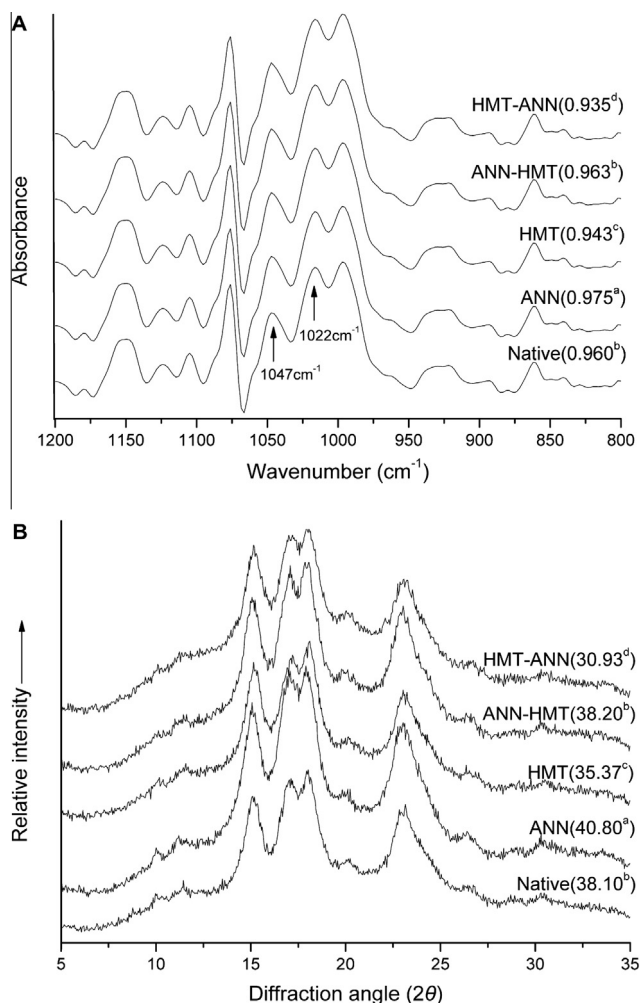
Fig. 2. ATR-FTIR spectra and X-ray diffraction patterns of native and treated starches: (A) ATR-FTIR spectra, value in a bracket is the ratio of 1047 cm^{-1}/1022 cm^{-1}; and (B) X-ray diffraction patterns, value in a bracket is relative crystallinity (%). Values followed by the different superscripts amongst treatments within each starch are significantly different ($P < 0.05$). Experimental data are the means of duplicates.

starch and amorphous structures of starch, respectively (Capron, Robert, Colonna, Brogly, & Planchot, 2007). The ratio of the heights of bands at 1047 and 1022 cm^{-1} expresses the amount of ordered starch to amorphous starch. Amongst the native and modified starches, the 1047/1022 cm^{-1} ratio followed the order: ANN > ANN–HMT > native starch > HMT > HMT–ANN.

Fig. 2A shows that the 1047 cm^{-1}/1022 cm^{-1} ratio increased on ANN, but decreased on HMT. The increased ratio suggests that the moderate thermal energy and the high moisture content prevailing during ANN cause more efficient packing of double helices within the crystalline lamella (Chung et al., 2009). The decrease in the ratio on HMT could be attributed to dissociation and unraveling of double helices forming the crystalline array. This is consistent with the reported variation tendency of the single and dual hydro-thermal treatment of normal corn starch (Chung et al., 2009). The decrease in the above ratio is more pronounced in HMT than in ANN–HMT starch, since in the former, the increased ratio in ANN and the decreased ratio in ANN–HMT reflect disruption of those crystallites that were perfected on ANN. The decrease in the above ratio is more pronounced in HMT–ANN than in HMT starch, reflecting crystallites that were disrupted on HMT and could not be further improved to perfection. The result was consistent with the

result of relative crystallinity from the XRD pattern. van Soest et al. (1995) observed that the band at 1022 cm^{-1} increases with decreasing crystallinity, whereas the band at 1047 cm^{-1} increases with increasing crystallinity on potato starch.

3.3. X-ray diffraction and relative crystallinity

The X-ray diffraction patterns and relative crystallinities (RC) of native and treated starches are presented in Fig. 2B. The relative crystallinity calculated from X-ray diffraction patterns ranged from 30.93% to 40.80% (Fig. 2B). The native waxy rice starch displayed the typical A-type diffraction pattern with peaks at 15°, 17°, 18° and 23° (2θ), which is the common characteristic of the A-type starch in most rice starches. The XRD traces of all treated starches remained unchanged.

Starch crystallinity has been shown to be influenced by: (1) amylopectin content, (2) average amylopectin chain length, (3) orientation of the double helices (within the crystallites) to the X-ray beam, and (4) crystallite size (Lan et al., 2008; Tester & Morrison, 1990). ANN is a thermal treatment that induces a rearrangement of starch molecules resulting in an increase in crystal perfection and/or an increase in crystallite size (Genkina, Wasserman, Noda, Tester, & Yuryev, 2004; Tester, Debon, & Karkalas, 1998). Increased relative crystallinities and X-ray intensities at 15°, 17° and 18° (2θ) in ANN was noted as compared with the native starch. The X-ray intensities have been shown to increase slightly in potato, lentil, oat, wheat (Hoover & Vasanthan, 1994a) and barley (Waduge et al., 2006) starches on annealing. It could conclude that increased crystallinity in ANN to native starch is resulted from an increase in crystal perfection.

Decrease in RC on HMT could be attributed to disruption of amylopectin crystallites, which was evidenced by a decrease in gelatinization enthalpy (Table 2). This is because that gelatinization enthalpy reflects the overall crystallinity (quality and amount of starch crystallites) of amylopectin (Tester & Morrison, 1990). Excessive heat or moisture content during HMT has been reported to reduce crystallinity (Lee et al., 2012; Vermeylen, Goderis, & Delcour, 2006), and HMT of tuber starches reduced X-ray diffraction intensities (Gunaratne & Hoover, 2002; Vermeylen et al., 2006). The reduced intensities and crystallinities observed in this study indicated that the crystalline regions of the starch granules were disrupted by HMT. Double-helical movement during HMT may disrupt and change the orientation of crystalline structures in starch granules (Gunaratne & Hoover, 2002).

The RC of ANN–HMT starch was much lower than that in ANN starch. This suggests that HMT of ANN starch have disrupted crystallites that were perfected on ANN. On the other hand, the RC was lower in HMT–ANN than HMT, since double helices disrupted on HMT could not become better aligned and/or could not form larger crystals on subsequent ANN. Meanwhile, the RC was lower in HMT–ANN than in ANN–HMT starch, which was in agreement with trend of gelatinization enthalpy (Table 2). However, studies examining the dual HMT–ANN treatment of normal corn starch and pulse starches have yielded contradictory observations. A reduction in RC was lower in HMT–ANN than in ANN–HMT, which was reported by Chung et al. (2009) and Chung et al. (2010). The discrepancy in the RC of dual HMT–ANN modified starches might be caused by the inherent properties of each type of starch, as well as the conditions used for starch modification.

3.4. Weight-average molar mass, gyration radius and molecular density

The weight-average molar mass (M_w), gyration radius (R_z) and molecular density (ρ) from the native and treated starches are shown in Table 1.

Table 1
Weight-average molar mass (M_w), radius of gyration (R_z), and density (ρ) of starches before and after hydrothermal treatment.

Starch sample	$M_w \times 10^7$ (g/mol)	R_z (nm)	ρ (g/mol/nm³)	$M_n \times 10^7$ (g/mol)	M_w/M_n
Native	10.38 ± 1.16[a]	337.35 ± 3.27[a]	2.83 ± 1.11[b]	8.33 ± 1.43[a]	1.25 ± 0.08[ab]
ANN	9.60 ± 0.34[a]	342.15 ± 3.32[a]	2.40 ± 0.02[b]	7.37 ± 0.26[a]	1.30 ± 0.01[ab]
HMT	1.79 ± 0.87[c]	149.40 ± 2.59[d]	5.17 ± 0.08[a]	1.52 ± 0.82[c]	1.20 ± 0.08[b]
ANN–HMT	4.59 ± 0.84[bc]	296.20 ± 0.99[c]	1.77 ± 0.34[b]	3.36 ± 0.49[bc]	1.36 ± 0.05[ab]
HMT–ANN	7.50 ± 0.56[ab]	322.40 ± 8.63[b]	2.24 ± 0.01[b]	5.10 ± 0.56[ab]	1.48 ± 0.07[a]

M_w, weight-average molar mass; R_z, z-average radius of gyration; ρ, density (M_w/R_z^3); M_n, number-average molar mass.
Values followed by the different superscripts in the same column amongst treatments within each starch are significantly different ($P < 0.05$).
Experimental data are the means of duplicates.

Table 2
Gelatinization parameters of native and modified starches.

Starch sample	T_o/°C	T_p/°C	T_c/°C	ΔT/°C	ΔH/J g^{-1}
Native	70.2 ± 0.0[e]	75.0 ± 0.0[e]	82.0 ± 0.0[c]	11.8 ± 0.1[ab]	12.4 ± 0.3[a]
ANN	73.6 ± 0.0[d]	77.6 ± 0.0[d]	83.5 ± 1.1[c]	9.9 ± 1.1[bc]	13.2 ± 0.5[a]
HMT	79.2 ± 0.1[a]	84.6 ± 0.0[a]	92.5 ± 0.2[a]	13.3 ± 0.2[a]	8.9 ± 1.1[b]
ANN–HMT	77.8 ± 0.1[c]	82.1 ± 0.0[c]	86.7 ± 0.2[b]	8.9 ± 0.3[c]	8.0 ± 0.9[b]
HMT–ANN	78.6 ± 0.1[b]	82.2 ± 0.0[b]	90.5 ± 0.3[a]	11.9 ± 0.4[ab]	6.4 ± 1.0[b]

T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔT, gelatinization temperature range; ΔH, gelatinization enthalpy.
Values followed by the different superscripts in the same column amongst treatments within each starch are significantly different ($P < 0.05$).
Experimental data are the means of duplicates.

The M_w of native waxy rice starch was larger than those of treated counterparts. A larger M_n observed for the native waxy rice starch may be the result of its large proportion of short branch-chains (Rocha, Felizardo, Jane, & Franco, 2012; Yoo & Jane, 2002). This indicated that native waxy rice starch was composed of highly polymerised amylopectin. The M_w of native waxy rice starch obtained in our study was similar to that reported in Shin, Choi, Park, and Moon (2010). M_w of native starch was approximately tenfold larger than that of HMT starch. The lower M_w of the treated starches may be a result of the degradation of some amylopectin molecules.

The radius of gyration (R_z) is related to the theoretical probability of finding the molecule at a given distance from the centre. The changes in R_z and dispersed molecular density ($\rho = M_w/R_z^3$) of starches after the hydrothermal treatment were significantly different depending on the conditions of treatment. The dispersed molecular density of waxy rice starch decreased the most after the ANN–HMT treatment. Highly branched, compact starch has been found to show greater dispersed molecular density compared with less branched starch (Yoo & Jane, 2002). ANN starch had the greatest R_z amongst the starches studied. The larger proportion of long branch-chains of amylopectin of the waxy rice starch may result in higher R_z, since it is dependent on the volume occupied by the molecule in solution (Millard, Dintzis, Willett, & Klavons, 1997). These results indicated that the amylopectin molecules of the native and treated starches were significantly different in their conformation in solution.

The ratio of the weight-average molar mass to the number-average molar mass (M_w/M_n) is known as the polydispersity index (PDI), which is related to the variety of molecular shapes. The PDI approaches 1.0 (the lower limit) for special polymers with very narrow mass distributions, but it is normally greater than 1.0 for typical polymers (Lowry, 1963). The PDI of native and treated starches followed the order: HMT–ANN > ANN–HMT > ANN > Native > HMT. The higher value of the PDI indicates the wider of the molecular weight distribution. When the value of the PDI is close to 1.0, the molecular weight of the polymer tends to be homogeneous (Zhang, Zeng, Wang, Zeng, & Zheng, 2014). The fact that the M_w/M_n ratio of HMT were close to 1 confirmed that starch on HMT was degraded during the process of heat-moisture treatment, formed molecular chains with a low degree of polymerisation, and provided a number of chain ends able to easily form double

helical structures. Since the capacity of amylopectin molecules to move relative to each other was enhanced, they were thus more likely to form a stable double helix structure facilitating an increase in the overall orderly level of the starch molecules. The PDI of the HMT–ANN starch was higher than those of the other starch samples. This suggested that the molecular weight distribution of the HMT–ANN starch was more heterogeneous, and includes various chains as compared with the other starch samples.

3.5. Gelatinization parameters

ANN increased T_o, T_p, T_c and ΔH but decreased ΔT. HMT increased T_o, T_p, T_c and ΔT, but decreased ΔH (Table 2). Increased T_o, T_p and T_c on ANN and HMT have been partly attributed to interaction between amylose–amylose (AM–AM) and/or amylose–amylopectin chains (AM–AMP) and/or amylopectin–amylopectin chains (AMP–AMP), and to the formation of additional complexes between amylose and starch lipids (Hoover & Vasanthan, 1994a, 1994b; Lan et al., 2008; Waduge et al., 2006). The lower ΔT values after ANN and ANN–HMT were caused by decreased crystallite heterogeneity within the granules. The decreased ΔH values following the HMT of waxy rice starch reflect the loss of double helices and some crystallites. Decreased ΔH following HMT has also been reported in normal corn starch (Chung et al., 2009), pea, lentil and navy bean starches (Chung et al., 2010) and waxy potato starch (Lee et al., 2012). Lopez-Rubio, Flanagan, Gilbert, and Gidley (2008) recently suggested that ΔH is due to the melting of imperfect amylopectin-based crystals, with potential contributions from crystal-packing and helix melting enthalpies. The ΔH decrease on HMT suggests that the high temperature prevailing during HMT may have increased the mobility of double helices (forming the crystalline structure) leading to a disruption of some of the hydrogen bonds linking adjacent double helices. This seems plausible since the RC (Fig. 2B), M_w (Table 1) and the IR ratio between 1047 cm^{-1} and 1022 cm^{-1} (Fig. 2A) were decreased after HMT.

As discussed earlier, X-ray diffraction (Fig. 2B) showed no evidence of amylose–lipid interactions during ANN and HMT. Consequently, the increase in T_o, T_p and T_c on ANN could be attributed solely to crystalline perfection (Hoover & Vasanthan, 1994a; Lan et al., 2008; Waduge et al., 2006), whereas the above increase on HMT suggests that crystallites disrupted on HMT may have

aggregated to form larger crystallites. The decrease in relative crystallinity on HMT (Fig. 2B) suggests that these large crystals are probably not arranged in a crystalline array. The decrease in ΔT on ANN could be attributed to crystalline perfection, whereas the increase in ΔT on HMT suggests melting of crystallites of different stabilities. Consequently, the number of double helices that unravel and melt during gelatinization of HMT starch would be lower than its native counterpart.

Increase in T_o, T_p and T_c was observed in ANN–HMT starch (Table 2). Furthermore, the extent of this increase was higher than in the ANN starch for T_o, T_p and T_c. However, ΔT and ΔH of ANN–HMT starch were narrower and lower, respectively, than in the ANN starch. In addition, amongst the native and modified starches, ANN–HMT starch showed the lowest ΔT (Table 2). This suggests that HMT of ANN starch decreased crystallite heterogeneity within the granules.

In HMT–ANN starch, the increase in T_o, T_p and T_c was lower than in HMT starch (Table 2). This indicates that crystallites disrupted on HMT could not become better aligned on ANN. In addition, amongst the native and modified starches, HMT–ANN starch showed the lowest gelatinization enthalpy (Table 2), crystallinity (Fig. 2B) and ratio of 1047 and 1022 cm^{-1} (Fig. 2A). This result indicates that ANN of HMT starch may promote disruption of some of the crystallites when compared to HMT starch.

In conclusion, the dually modified starches (ANN–HMT and HMT–ANN) displayed different gelatinization parameters (Table 2). HMT–ANN had higher gelatinization temperatures but lower enthalpy than that of ANN–HMT. This suggests that the crystalline structure of dually modified starch could be influenced by the treatment sequence.

3.6. In vitro digestibility

Table 3 shows the amounts of rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) in native and treated waxy rice starches. ANN increased RDS levels but decreased SDS and RS levels. However, HMT decreased RDS and RS levels but increased SDS level. ANN–HMT led to an increase in RDS and SDS, but a decrease in RS level. HMT–ANN increased RDS, and decreased SDS and RS levels.

It is difficult to acquire a consensus on the effect of ANN and HMT from previous researches on in vitro starch digestibility due to distinctions in enzyme source and concentration, time of hydrolysis, conditions of annealing, time and temperature of HMT and starch source or species (Chung et al., 2009). ANN has been suggested either to increase or decrease enzyme susceptibility. Decreased digestibility on ANN has been attributed to the interplay of the following factors: (1) interaction between AM–AM and/or AM–AMP chains, (2) crystalline perfection and (3) amylose–lipid complex formation (Hoover & Vasanthan, 1994a; Jacobs, Eerlingen, Rouseu, Colonna, & Delcour, 1998). O'Brien and Wang (2008) found that an increase in digestibility of annealed starch

has been attributed to an increase in granule porosity. This suggests that the increase in RDS and the decreases in SDS and RS levels could be attributed to an increase in granule porosity (facilitates the entry of the enzyme into the granule interior) which probably plays a more important impact of crystalline perfection on digestibility. The effect of HMT on enzyme digestibility has been shown to be influenced by: (1) starch source and/or species, (2) moisture content during HMT, (3) temperature and duration of HMT, (4) amylose–lipid interactions and (5) AM–AM, AM–AMP and/or AMP–AMP interaction (Franco, Ciacco, & Tavares, 1995; Gunaratne & Hoover, 2002; Hoover & Vasanthan, 1994b).

As discussed above, the increase or decrease in RDS levels on ANN reflects the interplay between crystalline perfection (decreases digestibility) and increase in granule porosity (increases digestibility). Further increases in the RDS and SDS levels and decreases in RS levels in ANN–HMT starch are indicative of disruption of those crystallites that were perfected on ANN. Whereas the decrease in the SDS and RS levels and increases in RDS level in HMT–ANN starch might result from the disrupted crystallites that could not be perfected any more, which is consistent with the crystallinity data (Fig. 2B).

The increase in RDS and decreases in SDS and RS levels on HMT–ANN indicate that crystallite disruption on HMT, as evidenced by decreased crystallinity (Fig. 2B) and gelatinization enthalpy (Table 2), may have exposed the α-(1 → 4) glycosidic and/or α-(1 → 6) glycosidic that were previously buried within the starch crystallites, and thus readily accessible to enzyme attack in starch granules. The changes in HMT–ANN starch in vitro digestibility observed in our study were consistent with the results of previous studies examining HMT–ANN effects on normal corn starch (Chung et al., 2009).

4. Conclusions

Hydrothermally treated starches possessed differences in physicochemical properties and in vitro digestibility. Native waxy rice starch had the highest value for M_w, whereas HMT starch had the lowest value for M_w. Both single and dual hydrothermal treatment did not change the XRD pattern, but relative crystallinity was found increased in ANN and decreased in HMT. The gelatinization temperature and enthalpy of treated starches increased as compared to that of native starch. The maximum SDS content was observed when starch exposed to the HMT. Both single and dual hydrothermal treatment changed the conformation of starch molecules. These findings suggest that the single and dual hydrothermal treatment can regulate and control molecule structure of starch with various digestibility. The results of this study would help food processors to tailor the properties of hydrothermally treated waxy rice starch. Additional studies are required to determine the hydrolysis index (HI) and expected glycemic index (eGI) of the modified starch products.

Table 3
The amount of RDS, SDS and RS of native and modified starches.

Starch sample	RDS (%)	SDS (%)	RS (%)
Native	32.4 ± 1.4[c]	45.5 ± 2.0[bc]	22.1 ± 1.7[a]
ANN	38.7 ± 0.3[b]	43.6 ± 1.3[bc]	17.7 ± 1.2[ab]
HMT	27.3 ± 0.8[d]	54.6 ± 1.8[a]	18.1 ± 1.4[ab]
ANN–HMT	34.2 ± 1.7[bc]	49.3 ± 1.6[ab]	16.5 ± 0.4[b]
HMT–ANN	45.8 ± 1.3[a]	40.5 ± 0.6[c]	13.7 ± 1.6[b]

RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch.
Values followed by the different superscripts in the same column amongst treatments within each starch are significantly different ($P < 0.05$).
Experimental data are the means of duplicates.

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