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The impact of heat-moisture treatment on the molecular structure and physicochemical properties of normal and waxy potato starches

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ABSTRACT

Normal and waxy potato starches were heat-moisture treated (HMT) at 100 °C for 16 h at a moisture content of 24%. Starch chain interactions, X-ray pattern and intensities, crystallite orientation, molecular order and polymorphic composition changed on HMT. However, birefringence intensity and amylopectin chain length remained unaltered in both starches on HMT. The above structural changes on HMT increased gelatinization temperatures widened the gelatinization temperature range and increased thermal stability in both starches. However, HMT decreased granular swelling, extent of amylose leaching, iodine complexing ability, peak viscosity, extent of set-back, enthalpy of gelatinization, acid susceptibility and the steepness of the adsorption isotherm in both normal and waxy starches. The extent of changes to starch structure and properties on HMT was different in normal and waxy starches due to differences in their amylose content and amylopectin chain mobility.

1. Introduction

Starch has been widely used in a wide variety of products, either as a food ingredient or as an industrial material. Total annual world production of starch is approximately 60 million MT. Native starches usually do not meet industrial needs in which starch should be able to withstand low acidity, high temperatures and high shear forces. Consequently, starches have been modified physically (heat-moisture treatment, annealing, pre-gelatinization, high pressure treatment) and chemically (cross-linking, substitution, acid hydrolysis, oxidation) in order to extend the range of starch applications in the food and non-food sectors. Heat-moisture treatment has been used to a limited extent in the food industry to impart shear and thermal stability. During HMT, starch granules at low moisture levels [<35% water (w/w)] are heated at temperatures in the range 84–120 °C for a periods ranging from 15 min to 16 h (Hoover, in press). HMT has been shown to modify the structural arrangement of starch chains within the amorphous and crystalline domains of the starch granule, resulting in changes to granular swelling, crystallinity, amylose leaching, gelatinization parameters, retrogradation, thermal stability and pasting properties (Hoover, in press). Most HMT studies have been on normal starches. The impact of HMT on structural changes within the granule interior of starches varying in amylose content has been reported only in cereal (maize and rice) starches (Hoover & Manuel, 1996; Kweon, Haynes, Slade, & Levine, 2000; Zavareze, Storck, Castro, Schirmer, & Dias, 2010). Unlike in cereal starches, amylose has been shown to be co-crystallized with amylopectin (Hoover & Vasanthan, 1994; Saibene, Zobel, Thompson, & Seetharaman, 2008; Zobel, 1988) in normal potato starch. Therefore, it is hypothesized that amylose may have a significant impact on the extent to which double helical chains forming the crystalline lamellae of potato amylopectin move and rearrange during HMT. A survey of the literature also revealed that there is a dearth of information on changes to lamellar organization, molecular order, amylopectin chain length distribution and retrogradation on HMT. Furthermore, conflicting information exists on the impact of HMT on acid and enzyme hydrolysis. Thus, the objective of this study was to use different physical probes to determine changes to molecular structure (crystallinity, molecular order, amylopectin chain length distribution) of normal (33.5%) and waxy (3.4% amylose) potato starches on HMT and their impact on physicochemical properties.

2. Materials and methods

2.1. Materials

Normal potato starch was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Waxy potato starch (Eliane 100) was

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a gift from Louise Lynch (Chemroy, Canada Inc, Laval, Quebec, Canada). All chemicals and solvents were of ACS certified grade.

2.2. Chemical composition of starch

Quantitative estimation of moisture, ash, nitrogen, was performed by the standard AACC methods (2000). Starch lipids were determined by the procedures outlined in an earlier publication (Vasanthan & Hoover, 1992). Amylose content was determined by a colorimetric method (Hoover & Ratnayake, 2004). In order to correct for over estimation of apparent and total amylose content, amylose was calculated from a standard curve using mixtures of pure potato amylose and amylopectin (over the range 0–100% amylose). Total phosphorus content was determined by the method of Morrison (1964).

2.3. Heat-moisture treatment (HMT)

Starch samples were equilibrated to a water activity of 0.97 in a desiccator using a saturated salt solution of K_2SO_4. Following equilibration the moisture content (24%) of the samples was determined by the standard AACC (2000) method. The starch samples were then placed in a forced air oven at 100 °C for 16 h. The HMT starches were subsequently air dried to uniform moisture content (~11%).

2.4. Amylopectin chain length distribution

Isoamylase debranching of whole starch accompanied by high performance anion exchange chromatography with pulsed amperometric detection was used to determine the average chain length ($\overline{CL}$) and the branch chain length distribution of the native and HMT starches (Liu, Gu, Donner, Tetlow, & Emes, 2007).

2.5. Iodine binding of native and HMT starches

The ability of iodine to complex starch chains before and after HMT was determined by equilibrating starch samples at room temperature in a desiccator (25 cm diameter and 2.0 cm deep) containing saturated K_2SO_4 (300 ml) solution. Samples were weighed and equilibrated to 0.97 water activity (a_w). Sodium azide (1%, w/w) was added to the K_2SO_4 saturated salt solution to prevent microbial growth. To determine iodine binding, a thin layer of the equilibrated starch sample (0.2 g) was spread in a plastic dish and exposed for 24 h at room temperature to iodine vapour generated from 2 g of iodine crystals placed in the desiccator containing the starch samples. The desiccator was covered with aluminum foil to avoid light exposure.

2.6. Reflectance spectra of native and HMT normal and waxy starches before and after iodine staining

The reflectance spectra of the above starches were determined using the Kubetta and Munk equation:

$$\frac{K}{S} = \frac{(1-R)^2}{2R}$$

where K is an absorption coefficient, S is a scattering coefficient, and R is the reflectance expressed as fraction between 0 and 1. The K/S value of the starches (represents the absorption corrected to the light scattering) was measured over a wavelength range from 400 to 700 nm (CM 3500-d spectrophotometer, Konica Minolta, Mahwah, NJ) at 10 nm intervals.

2.7. Wide angle X-ray diffraction

Native (normal and waxy) and HMT (normal and waxy) starches for X-ray diffraction measurements were kept in a desiccator over saturated solutions K_2SO_4 (a_w – 0.97), at 25 °C for 14 days. The hydrated native (moisture content 25%) and HMT (moisture content 23%) starches were then packed tightly into a round aluminum holder. X-ray diffractograms of native and HMT starches were obtained with a Rigaku Ultima IV X-ray diffractometer with operating conditions of target voltage 40 kV; current 44 mA; scanning range 3–35°; scan speed 1.00°/min; step time 0.95; divergence slit width 0.5°; scatter slit width 0.5°; sampling width 0.03° and receiving slit width 0.3 mm.

2.8. Amylose leaching (AML)

Starches (20 mg/db) in water (10 ml) was heated at 50–90 °C in volume calibrated sealed tubes for 30 min (tubes were shaken by hand every 5 min to suspend the starch slurry). The tubes were then cooled to room temperature and centrifuged at 2000 × *g* for 10 min. The supernatant liquid (1 ml) was withdrawn and amylose content determined as described by Hoover and Ratnayake (2004). AML was expressed as percentage of amylose leached per 100 g of dry starch. Three replicate samples were used in the determination.

2.9. Swelling factor (SF)

The SF of the native and HMT starches when heated to 50–90 °C in excess water (5 ml) was determined according to the method of Tester and Morrison (1990). Starch samples (50 mg db) were weighed into screw cap tubes, 5 ml water were added and heated in a shaking water bath at appropriate temperature for 30 min. The tubes were then cooled rapidly to 20 °C in an ice water bath and 0.5 ml of Blue Dextran (Pharmacia MW 2×10^6, 5 mg/ml) was added and the contents mixed by inverting the closed tubes several times. The tubes were centrifuged at 1500 × *g* for 10 min and the absorbance of the supernatant was measured at 620 nm. The absorbance of the reference tube that contained no starch was also measured. Three replicate samples were used in the determination. The swelling factor was reported as the ratio of the volume of swollen starch granule to the volume of the dry starch.

2.10. Sorption isotherm

Native and HMT starches (2.0 g) were equilibrated at 25 °C in desiccators containing 300 ml of different saturated salt solutions [LiCl (a_w – 0.11), CH_3COOK (a_w – 0.23), $MgCl_2$ (a_w – 0.33), $COCl_2$ (a_w – 0.67), $SrCl_2$ (a_w – 0.71), NaCl (a_w – 0.75), KCl (a_w – 0.86), K_2SO_4 (a_w – 0.97)] until there was no discernible weight change. Each desiccator held one set of triplicate samples. Following equilibration, the moisture content of the samples was measured according to the standard AACC (2000) method.

2.11. Birefringence

Birefringence of native and HMT starch granules was observed under polarized light with a binocular microscope (DME, Leica, Canada, Mississauga, ON, Canada) equipped with a real time viewing (Micropublisher 5.0, Qimaging, Surrey, BC, Canada). The images were recorded at the same magnification (600×) for all starch samples (1% starch suspension).

2.12. Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectra of native and HMT starches were recorded on a Digilab FTS 7000 spectrometer (Digilab USA, Randolph, MA, USA)

equipped with a thermoelectrically cooled deuterated triglycine sulphate (DIGS) detector using an attenuated total reflectance (ATR) accessory at a resolution of 4 cm^{-1} by 128 scans. Spectra were base line-corrected and then deconvoluted by drawing a straight line between 1200 and 800 cm^{-1} (using Win-IR Pro software). A half-band width of 15 cm^{-1} and a resolution enhancement factor of 1.5 with Bessel apodization were employed. Intensity measurements were performed on the deconvoluted spectra by recording the peak height of the absorbance bands from the base line.

2.13. Differential scanning calorimetry (DSC)

Gelatinization parameters of native and HMT starches were measured using a Mettler Toledo differential scanning calorimeter (DSC1/700/630/GC200) equipped with a thermal analysis data station and data recording software (STAR@ SW 9.20). Water (11 μl) was added with a microsyringe to starch (3.0 mg db) in the DSC pans, which were then sealed, reweighed and allowed to stand overnight at room temperature before DSC analysis. The scanning temperature range and the heating rates were 30–110 °C and 10 °C/min, respectively. In all measurements, the thermogram was recorded with an empty aluminum pan as a reference. During the scans, the space surrounding the sample chamber was flushed with dry nitrogen to avoid condensation. The transition temperatures reported are the onset (T_o), peak (T_p) and conclusion (T_c). The enthalpy of gelatinization (ΔH) was estimated by integrating the area between the thermogram and a base line under the peak and was expressed in terms of Joules per gram of dry starch; three replicates per sample were analyzed.

2.14. Pasting properties

A Rapid Visco Analyzer RVA-4 (Newport Scientific Pty. Ltd., Warriewood, NSW, Australia) was employed to measure the pasting properties of native and HMT starches (5% db, 25 g total weight). The samples were equilibrated at 50 °C for 1 min, heated at 6 °C/min at 95 °C, held at 95 °C for 5 min, cooled at 6 °C/min at 50 °C, and held at 50 °C for 2 min. The speed was 960 rpm for the first 10 s, then 160 rpm for the remainder of the experiment.

2.15. Acid hydrolysis

Native and HMT starches were hydrolysed in triplicate with 2.2 M HCl (1 g db, starch/40 ml) at 35 °C in a water bath (New Brunswick Scientific, G76D, Edison, NJ, USA) for periods ranging from 0 to 32 days. The starch slurries were vortexed daily to resuspend the deposited starch granules. Aliquots taken at specific time intervals were neutralized with 2.2 M NaOH and centrifuged (2000 rpm/10 min). The amount of total reducing sugar in the supernatant was determined by the method of Bruner (1964).

3. Results and discussion

3.1. Chemical composition

The data on the chemical compositions are presented in Table 1. The purity of the starches was judged on the basis of low nitrogen (0.13% [normal potato], 0.14% [waxy potato]) and low ash (0.22% [normal potato], 0.21% [waxy potato]) levels. There was no significant difference between the starches with respect to total phosphorus (0.07–0.08%), free lipids (0.02%) and bound lipid (0.05–0.08%) contents (Table 1). However, the starches differed significantly with respect to total amylose content (33.5% [normal potato], 3.4% [waxy potato]). The total phosphorus content which is mainly in the form of phosphate monoesters (McPherson & Jane, 1999) was in agreement with those reported in the literature (Gunaratne & Hoover, 2002; McPherson & Jane, 1999).

3.2. Amylopectin branch chain length distribution

The chain length distribution and the average chain length ($\overline{CL}$) of native normal and waxy potato starches were nearly similar (Table 2), and were in the range reported by Svegmark et al. (2002). However, McPherson and Jane (1999) reported slightly lower values for DP 6 to 36 for normal potato (DP 6–12, [13.1%], DP 13–24 [44.4%] DP 25–36 [14.0%]) and waxy potato (DP 6–12, [14.8%], DP 13–24 [48.4%], DP 25–36 [14.4%]), but higher values for DP > 37 (normal potato, [28.5%], waxy potato [22.4%]) and $\overline{CL}$ (normal potato [28.5%], waxy potato [25.8%]). In both starches, amylopectin chain length distribution remained unchanged on HMT. This suggests that amylopectin crystallites were not degraded under the HMT conditions used in this study. However, Lu, Chen, and Lii (1996) and Vermeylen, Goderis, and Delcour (2006) have reported crystalline disruption in normal potato starch but at temperatures exceeding 120 °C.

3.3. X-ray diffraction

Native normal (Fig. 1a) and waxy (Fig. 1c) potato starches exhibited the typical B-type X-ray pattern with reflection intensities centered at 5.5°, 17.1° and 22–24° 2θ. However, in waxy potato starch, the characteristic B-type reflection centered at 5.5° 2θ was of a lower intensity, and the doublet centered at 22–24° 2θ was less well resolved than in normal potato starch. This suggests the presence of B and A-type unit cells in waxy potato starch. Both starches also exhibited a peak centered at 20° 2θ (Fig. 1a, c), which generally reflects V-type crystallinity resulting from interactions of granular monoacyl lipids with single amylose helices (Morrison, Law, & Snape, 1993). However, the presence of only trace quantities of bound lipids (0.08%) in both normal and waxy potato starches (Table 1) suggests that the 20° 2θ peak probably represents single helices of linear starch chains arranged in a crystalline array, rather than V-type lipid-amylose complexes. Lopez-Rubio, Flanagan, Gilbert, and Gidley (2008) and Saibene et al. (2008) have also postulated that the 20° 2θ peak observed in native wheat, rice, barley, maize and potato starches represents single left handed helices. Heat-moisture treatment (HMT) changed the X-ray pattern (B to A + B) of both normal (Fig. 1b) and waxy (Fig. 1d) potato starches. The extent of this polymorphic transformation was most pronounced in the latter (Fig. 1d). The presence of more A-type unit cells in HMT waxy potato starch was reflected by the greater reduction of the peak intensities centered at 5.5° 2θ (Fig. 1d) and 22–24° 2θ, and by the wider broadening of the 17° 2θ peak (Fig. 1d). Gunaratne and Hoover (2002) have attributed the change in X-ray pattern on HMT of potato starches (B → A + B) as being due to dehydration of the 36 water molecules in the central channel of the B-type unit cell and to the movement of a pair of double helices into the central channel (that was originally occupied by the vapourized water molecules). The decrease in intensity of the 5.5° 2θ peak seen in both starches (normal < waxy) on HMT (Fig. 1b, d) could be attributed to crystallite reorientation and/or loss of larger order packing of these crystallites, rather than to disruption of double helices forming the crystalline array. This is based on the observation that the AMP chain length distribution of both starches remained unchanged on HMT (Table 2). The extent of the above intensity decrease is lower in normal potato starch (Fig. 1b) due to co-crystallization of amylose with amylopectin (Hoover & Vasanthan, 1994; Saibene et al., 2008; Zobel, 1988). This seems plausible, since in normal potato starch, the thermal energy and moisture content during HMT may have increased chain mobility, facilitating additional and/or stronger interactions between amy-

Table 1
Chemical composition (%) of normal and waxy potato starches[a].

Potato starches	Moisture (%)	Ash (%)	Nitrogen (%)	Phosphorus (%)	Amylose content (%)		Lipid (%)	
					Apparent[b]	Total[c]	CM[d]	PW[e]
Normal potato	17.01 ± 0.04[p]	0.22 ± 0.01[p]	0.13 ± 0.01[p]	0.08 ± 0.01[p]	32.3 ± 0.2[p]	33.5 ± 0.3[p]	0.02 ± 0.01[p]	0.08 ± 0.03[p]
Waxy potato	15.20 ± 0.04[q]	0.21 ± 0.01[p]	0.14 ± 0.03[p]	0.07 ± 0.01[p]	3.4 ± 0.5[q]	3.4 ± 0.3[q]	0.02 ± 0.01[p]	0.05 ± 0.02[p]

[a] All data reported on dry basis and represents the mean of three determinations. Means of normal and waxy starches with different superscripts within the same column are significantly different ($P < 0.05$).
[b] Determined by iodine binding without removal of free and bound lipids.
[c] Determined by iodine binding after removal of free and bound lipids.
[d] Lipid extracted from the starch by chloroform–methanol (CM) 2:1(v/v) at 25 °C (mainly free lipids).
[e] Lipid extracted by hot 1-propanol–water (PW) 3:1(v/v) from the residue left over CM extraction (mainly bound lipids).

lose and amylopectin chains. These interactions may have hindered movement of double helices into the central channel of the B-type unit cell. This would then account for the lower proportion of A-type unit cells formed on HMT of normal potato starch. The complete disappearance of the 20° 2θ peak in both normal (Fig. 1b) and waxy (Fig. 1d) potato starches on HMT, indicates reorientation of single helices of amylose and/or amylopectin (that were arranged in a crystalline array within the native starches) and/or to interactions between these helices. Saibene et al. (2008) have shown by using iodine vapour, the existence of polymer mobility in granular starch even at moisture contents as low as 5.0%. Thus, it was hypothesized, that starch chain movement during HMT could be demonstrated by the use of iodine vapour. Iodination of both native normal (Fig. 1e) and waxy (Fig. 1f) starches reduced the intensities (normal > waxy) of the diffraction peaks centered at 5.5°, 17°, 23° and 24° 2θ. In native normal potato starch, the difference between the intensities of the peaks centered at 17° 2θ and 20° 2θ (1092 CPS) decreased to 518 CPS on iodination (Fig. 1e). Whereas, in native waxy potato starch the corresponding values were 1145 CPS and 818 CPS, respectively. This is indicative of iodine complexation with single helical structures that are arranged in a crystalline array in both native (normal and waxy) starches. Saibene et al. (2008) have shown by studies on both normal corn and potato starches, that at high water activity levels (0.97), iodine is able to penetrate the granule and form a complex with lipid free amylose and long linear branches of AMP. This complex formation reduces X-ray intensities due to absorption of the scattered radiation by the heavy iodine atoms. This would then partly explain the greater reduction in X-ray intensities exhibited by normal potato starch (due to the presence of amylose) on iodination. Complex formation between single helices and iodine should have theoretically increased the intensity of the peak centered at 20° 2θ. The absence of such an increase (Fig. 1e, f) in both starches on iodination, suggests that the overall intensity reduction of all peaks due to absorption of scattered radiation by iodine atoms negates this increase. It is likely that co-crystallization of amylose with amylopectin may have been also a factor influencing the decrease in peak intensities in normal potato starch. Complexation of iodine with amylose chains not involved in co-crystallization could lead to a mechanical stress in the crystalline packing resulting in some of the B-type crystallites being oriented in a non-crystalline array. This seems plausible, since the extent of decrease in the intensities of all peaks on iodination were more pronounced in normal (Fig. 1e) than in waxy (Fig. 1f) potato starches. Iodination also decreased the peak intensities of HMT (Fig. 1g, h) starches (normal > waxy). However, the extent of this reduction was much less than that observed for the native starches (Fig. 1e, f). This suggests single helical reorientation and/or interaction among single helices on HMT may have hindered amylose-iodine complexation.

3.4. Amylose leaching (AML) and swelling factor (SF)

The AML and SF of normal and waxy potato starches at different temperatures are presented in Fig. 2a and b, respectively. In both native normal and waxy potato starches, AML increased with increase in temperature and was detectable only at temperatures exceeding 70 °C (Fig. 2a). In the native starches, the SF of normal potato (Fig. 2b) progressively increased with increase in temperature. The extent of this increase being more pronounced in the temperature range 60–70 °C (Fig. 2b). However, in waxy potato, SF increased only until 70 °C, and then decreased thereafter (Fig. 2b). The SF in the temperature ranges 60–70 °C and 80–90 °C followed the order waxy > normal and normal > waxy, respectively (Fig. 2b). The SF of starches has been shown to the influenced by amylopectin content and structure and starch–lipid complexes (Chung, Liu, & Hoover, 2009; Gomand, Lamberts, Visser, & Delcour, 2010). Since there was no difference in amylopectin structure and bound lipid content between the starches (Table 2), the higher SF of waxy potato (Table 1) in the temperature range 60–70 °C reflects its higher amylopectin content (confers granule rigidity). Whereas, the lower SF of waxy potato starch in the temperature range (Fig. 2b) 80–90 °C, suggests that the higher granular swelling at lower temperatures may have rendered the granules very fragile and thus susceptible to partial disintegration at higher temperatures. In both starches, AML (Fig. 2a) decreased on HMT. The decrease in AML can be attributed to amylose–amylose (AM–AM) and/or

Table 2
Amylopectin chain length distribution of native and heat-moisture treated potato starches[a].

Starch source	Treatment	Distribution (%)[b]				CL[c]
		DP 6–12	DP 13–24	DP 25–36	DP 37–50	
Normal potato	Native	17.67 ± 0.93[p]	49.99 ± 1.35[p]	16.93 ± 0.17[p]	15.41 ± 0.59[p]	22.41 ± 0.07[p]
	HMT[d]	17.95 ± 0.04[p]	49.51 ± 1.52[p]	16.62 ± 0.63[p]	15.92 ± 0.84[p]	22.54 ± 0.32[p]
Waxy potato	Native	19.47 ± 0.96[p]	48.77 ± 0.51[p]	15.83 ± 0.44[p]	15.93 ± 0.01[p]	22.28 ± 0.13[p]
	HMT[d]	19.89 ± 0.88[p]	48.66 ± 0.05[p]	15.29 ± 0.62[p]	16.16 ± 0.21[p]	22.24 ± 0.21[p]

Means within the same column with different superscripts for native starch and its HMT counterpart are significantly different ($P < 0.05$).
[a] All data reported on dry basis.
[b] DP_n: indicates degree of polymerization. Total relative area was used to calculate the percent distribution.
[c] Average chain length (CL) calculated by $\sum(DP_n \times \text{peak area})/\sum(\text{peak area}_n)$.
[d] Heat-moisture treated (100 °C, 24% moisture, 16 h).

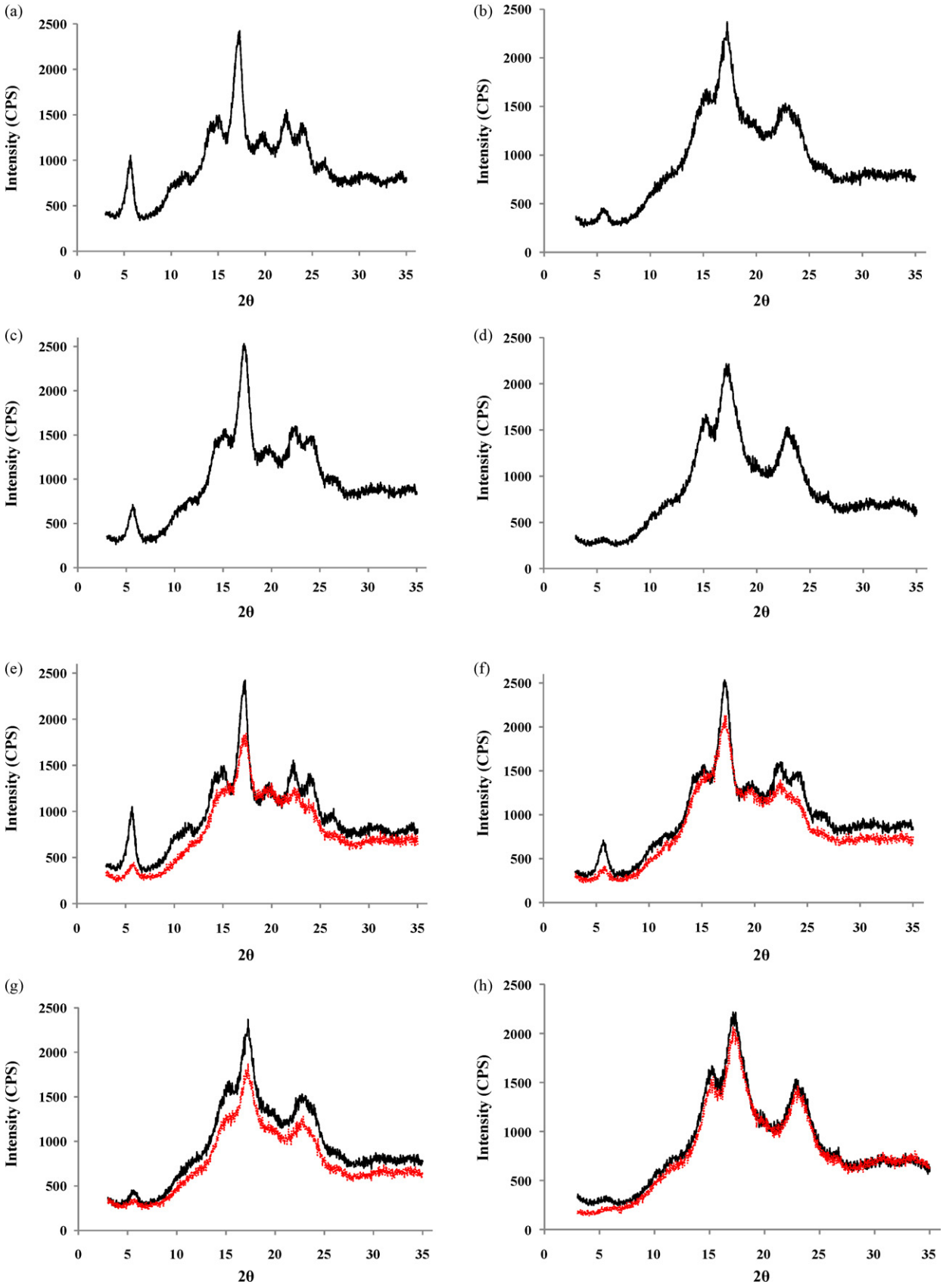

Fig. 1. X-ray patterns of native and HMT normal and waxy potato starches before (—) and after (- - -) iodine treatment. (a) Native normal potato starch. (b) HMT normal potato starch. (c) Native waxy potato starch. (d) HMT waxy potato starch. (e) Native and iodinated normal potato starch. (f) Native and iodinated waxy potato starch. (g) HMT and iodinated HMT normal potato starch. (h) HMT and Iodinated HMT waxy potato starch.

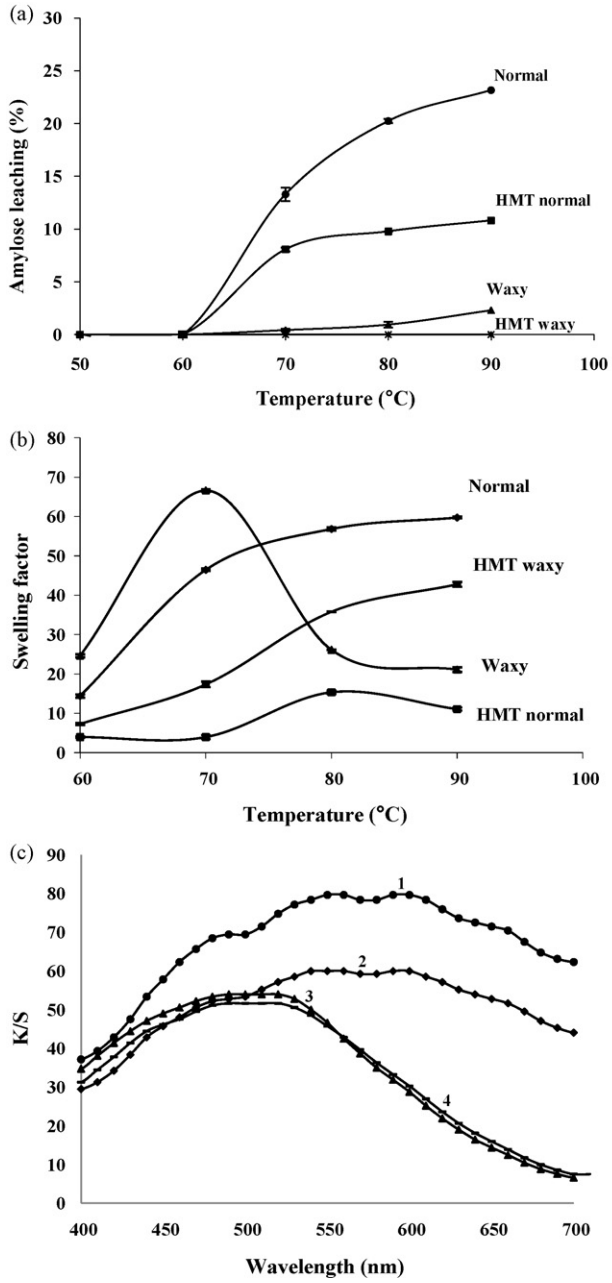

Fig. 2. (a) Amylose leaching of native and HMT normal and waxy potato starches in the range 50–90 °C. (b) Swelling factor of native and HMT normal and waxy potato starches in the range 50–90 °C. (c) Reflectance spectra of native normal potato (1), HMT normal potato (2), native waxy potato (3) and HMT waxy potato (4) starches.

amylose–amylopectin (AM–AMP) interactions. This seems plausible, since the intensity of the absorption spectrum of iodinated HMT starches (Fig. 2c) were much lower (normal > waxy) than their native counterparts (AM–AM and/or AM–AMP interactions would reduce the ability of amylose chains to form a single helical complex with iodine). At all temperatures, the SF of HMT normal potato starch was lower than its native counterpart (Fig. 2b). However, the SF of HMT waxy potato starch was lower than its native counterpart in the temperature range 60–70 °C, but higher in the temperature range 80–90 °C (Fig. 2b). Reduction in AML and SF on HMT has also been reported in cereals, pulses and tuber starches (Chung et al., 2009; Hoover, Hughes, Chung, & Liu, 2010; Jayakody, Hoover, Liu, & Donner, 2007). The reduction in SF on HMT could be attributed to AM–AM, AM–AMP and AMP–AMP interactions (reduces the number of free hydroxyl groups available for interaction with water molecules) and to the change in polymorphic form (B → A + B). The reduction in SF due to the B → A + B transformation could be explained by considering the unit cell structure. The A-polymorph has been shown to crystallize in a face centered monoclinic unit cell corresponding to 12 glucose residues located in two left handed helices that contain 4 water molecules between the helices whereas in the B-polymorph, 12 glucose residues crystallizes in a hexagonal packing unit cell with a more open structure in which two left handed, parallel-stranded, double helices are arranged in parallel containing 36 water molecules (Gidley & Bociek, 1985). Since the double helices of the A-polymorph are more compactly packed than those of the B-polymorph, the polymorphic transformation on HMT could decrease the ingress of water into the amorphous and crystalline domains of the granule during SF measurements. This is indicative that the decrease in SF (in the range 60–90 °C) exhibited by HMT normal potato starch is influenced by the B to A + B transformation and starch chain interactions (AM–AM, AMP–AM, AMP–AMP). Whereas, in HMT waxy potato, the decrease in SF (in the range 60–80 °C) reflects mainly AMP–AMP interactions and the B to A + B transformation. The difference in SF between native waxy and HMT waxy potato starches at temperatures beyond 80 °C (HMT waxy > native waxy) could be attributed to loss of granule integrity in native waxy starch at temperatures beyond 70 °C (Fig. 2b).

3.5. Moisture sorption isotherm

The moisture sorption isotherms of native and HMT normal and waxy potato starches equilibrated above saturated salt solutions at 25 °C are presented in Fig. 3. Both native and HMT starches (normal and waxy) exhibited a sigmoidal shape (Fig. 3). However, the sigmoidal shape of native (normal and waxy) starches were steeper than their HMT counterparts. This suggests that starch chain interactions and polymorphic transformation that occurs on HMT, decreases the rate and extent of water uptake. In both normal (Fig. 3a) and waxy (Fig. 3b) starches, the decrease in the rate and extent of water uptake on HMT was more pronounced at water activity levels (a_w) beyond 0.7 (Fig. 3). This is not surprising, since at a_w in the range 0.7–0.97, the granules are more swollen, and crystalline stability is much weaker (due to greater swelling of the amorphous regions which destabilizes the crystallites) than at $a_w < 0.7$. Consequently, the impact of HMT in strengthening granule structure becomes more evident at $a_w > 0.7$.

3.6. Birefringence

Native normal and waxy potato starches exhibited the characteristic birefringence patterns under polarized light (figures not shown). Birefringence indicates that amylose and amylopectin chains are arranged radially within the granule at right angles to the surface with their single reducing end group towards the hilum. The intensity of birefringence of both normal and waxy starches at the granule periphery and granule center remained unchanged on HMT. This suggests that the radial arrangement of the chain axis of the polysaccharide was not influenced by crystallite orientation or starch chain interactions on HMT. Kweon et al. (2000) also reported an unchanged birefringence pattern on HMT of corn starch (110 °C, 16 h, 18% mc). However, disappearance of birefringence at the granule center on HMT (100 °C and 120 °C, 2 h, 30% mc) has been reported by Chung et al. (2009) for pea, lentil and corn starches, Vermeylen et al. (2006) for normal potato starch (100 °C, 120 °C, 130 °C, 2 h, 20 and 26% mc) and by Kawabata et al. (1994) for normal potato starch (110 °C, 30 min, 16% mc).

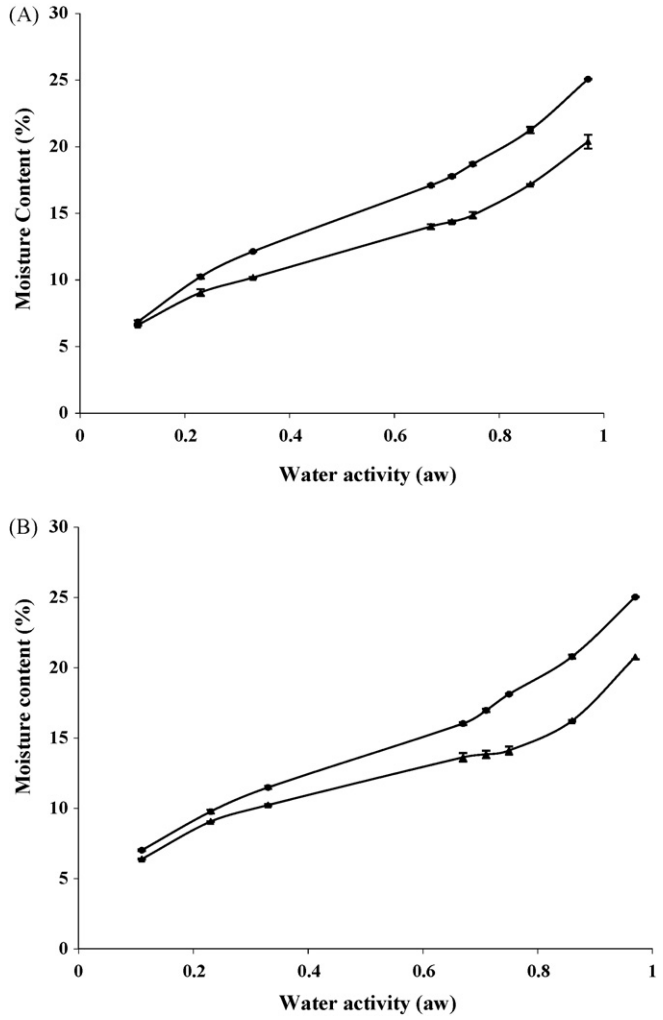

Fig. 3. Moisture sorption isotherms of: (a) native normal (●) and HMT normal (▲) potato starches, (b) native waxy (●) and HMT waxy (▲) potato starches.

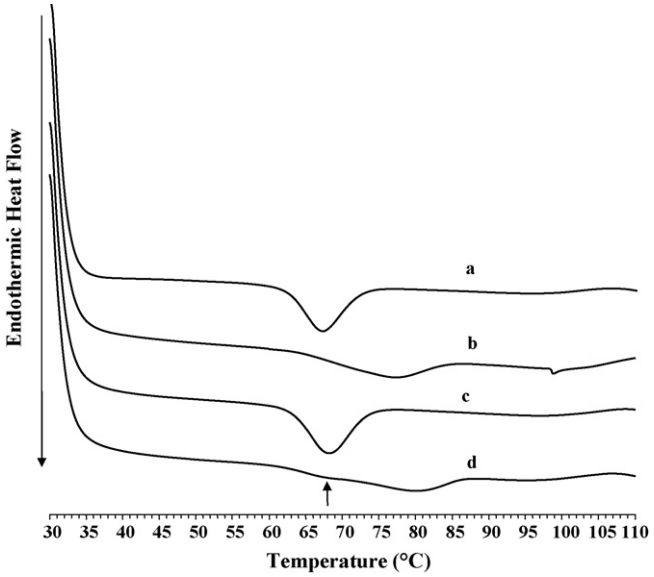

Fig. 4. DSC thermograms of: (a) native normal (b) HMT normal, (c) native waxy, (d) HMT waxy (arrow points to the minor endothermic peak) potato starches.

3.7. Gelatinization parameters

The gelatinization transition temperatures [(T_o, onset), (T_p, midpoint), (T_c, conclusion)], the gelatinization temperature range ($T_c - T_o$) and the enthalpy (ΔH) of gelatinization of normal and waxy potato starches are presented in Table 3 and Fig. 4. HMT increased T_p and T_c, widened $T_c - T_o$ and decreased ΔH in both starches (Table 3 and Fig. 4). However, there was no significant increase in T_o in both starches on HMT. Similar changes on HMT have been reported (Eerlingen, Jacobs, Van Win, & Delcour, 1996; Gunaratne & Hoover, 2002; Hoover & Vasanthan, 1994) for normal potato starch. Furthermore, both native and HMT normal potato starches (Fig. 4a, b) exhibited only a single endothermic peak. Whereas, waxy potato starch behaved differently in exhibiting a single endothermic peak in its native form (Fig. 4c), and a minor endothermic peak preceding the main melting peak of the crystallites on HMT (Fig. 4d). In HMT normal potato starch, the increase in T_o, T_p and T_c represents melting of: (a) B-type crystallites present in the native granules, (b) A-type crystallites formed on HMT and (c) crystallites resulting from interactions between AM–AM, AM–AMP and AMP–AMP chains on HMT. Whereas, in HMT waxy potato starch, the above increases represents melting of: (a) B-type and some A-type crystallites that were present in the native granules, (b) A-type crystallites formed on HMT and (c) crystallites resulting from AMP–AMP interactions. Kweon et al. (2000) and Hoover and Manuel (1996) have shown that the increase in T_o, T_p and T_c on HMT in normal and waxy maize starches (the X-ray patterns remains unchanged on HMT) is lower in the latter due to the absence of AM–AM and AM–AMP interactions. Thus, if this is true of all waxy starches, the nearly similar increase in T_o, T_p and T_c exhibited by both normal and waxy potato starches on HMT (Table 3) most probably reflects the formation of a larger number of A-type crystallites that are formed on HMT of waxy potato starch. The presence of only a single endotherm on HMT of normal potato starch, suggests that crystallites formed on HMT may have been nearly of the same size and stability. Whereas, the presence

Table 3
Gelatinization parameters of native and heat-moisture treated potato starches as determined by differential scanning calorimetry.

Starch source	Treatment	Gelatinization transition parameters[a]				
		T_o (°C)[b]	T_p (°C)[b]	T_c (°C)[b]	$(T_c - T_o)$ (°C)[c]	ΔH (J/g)[d]
Normal potato	Native	62.0 ± 0.3^p	67.0 ± 0.2^p	73.3 ± 0.2^p	11.1 ± 0.4^p	13.2 ± 0.1^p
	HMT[e]	63.9 ± 0.3^p	76.8 ± 0.2^q	85.4 ± 0.2^q	21.6 ± 0.4^q	9.9 ± 0.5^q
Waxy potato	Native	62.5 ± 0.0^p	67.8 ± 0.2^p	74.0 ± 0.1^p	11.5 ± 0.3^p	14.5 ± 0.3^p
	HMT[e]	61.8 ± 0.2^p	78.3 ± 0.1^q	86.5 ± 0.0^q	24.6 ± 0.0^q	12.5 ± 0.2^q

[a] All data reported on dry basis. Means within each column with different superscripts for native potato starch and its heat-moisture treated counterparts are significantly different ($P < 0.05$) by Tukey's HSD test.
[b] T_o, T_p, T_c represent the onset, peak and conclusion temperature, respectively.
[c] $(T_c - T_o)$ represents the gelatinization temperature range.
[d] Gelatinization enthalpy of starch expressed in J/g of dry starch.
[e] Heat-moisture treated (100 °C, 24% moisture, 16 h).

Table 4
Short-range molecular order of native and heat-moisture treated potato starches measured by attenuated total reflectance–Fourier-transform infrared spectroscopy (ATR–FT-IR).

Starch source	Treatment	R (1048/1016 cm^{-1})[a]	R (995/1016 cm^{-1})[a]
Normal potato	Native	1.146 ± 0.011[p]	1.177 ± 0.011[p]
	HMT[b]	1.010 ± 0.014[q]	1.165 ± 0.011[q]
Waxy potato	Native	1.167 ± 0.011[p]	1.198 ± 0.011[p]
	HMT[b]	1.028 ± 0.012[q]	1.167 ± 0.004[q]

Means within each column with different superscripts for the native potato starch and its heat-moisture treated counterparts are significantly different ($P < 0.05$) by Tukey's HSD test.

[a] Intensity ratio.

[b] Heat-moisture treated (100 °C, 24% moisture, 16 h).

of two endotherms, in HMT waxy potato starch (Fig. 4d), suggests that crystallites formed on HMT may have been more heterogeneous (differences in crystalline thickness and stability) than the crystallites in HMT normal potato starch. Consequently, the two endotherms probably represent melting of crystallites of widely differing thickness and stability. This explanation seems logical, since Kozlov, Blennow, Krivandin, and Yuryev (2007) have postulated that the presence of two distinct peaks in granule bound starch synthase suppressed potato starch (12.3% amylose) represent the melting of crystallites with different thermostability. The difference in the extent of decrease in ΔH (normal > waxy) on HMT is indicative that the ability of double helices of AMP to dissociate, unravel and melt during gelatinization is hindered due to interactions between AM–AMP and AMP–AMP chains in normal potato starch, and between AMP–AMP chains in waxy potato starch.

3.8. Fourier transform infrared spectroscopy (FT-IR)

The FT-IR spectroscopy of native and HMT (normal and waxy) potato starches are presented in Table 4. The FT-IR spectra have been used for investigating changes to starch structure on a short-range molecular level. Short-range order refers to double helical order, as opposed to long range order related to the packing of double helices (Cooke & Gidley, 1992; Sevenou, Hill, Farhat, & Mitchell, 2002). Sevenou et al. (2002) showed by X-ray and FT-IR studies on native and acid hydrolysed wheat and potato starches, that FT-IR is not able to differentiate between the A and B polymorphs and thus the long range packing. The above authors showed that FT-IR differences between starch varieties is only related to variations in the ratio of the amounts of ordered to unordered fractions within the starch granule. The FT-IR spectrum of starch has been attributed to three main vibrational modes with maximum absorbance at 995, 1022 and 1047 cm^{-1}. The FT-IR absorbance at 1045 cm^{-1} is related to crystallinity, because this band increases with crystallinity. Whereas, the band at 1022 cm^{-1} has been attributed with vibrational modes within the amorphous domains of starch granules [since this band has been observed to decrease with increase in crystallinity (Capron, Robert, Colonna, Brogly, & Planchot, 2007)]. The band at 995 cm^{-1} which is mainly due to COH bending vibrations, is especially sensitive to the water content and these vibrations involve water–starch interaction, for example, hydrogen bonding, which influences the COH bending modes. The band at 995 cm^{-1} represents hydrated crystalline domains (Capron et al., 2007). Both the ratio between the bands at 1047/1022 cm^{-1} and 995/1022 cm^{-1} have been used to quantify the degree of order in starches (Lopez-Rubio et al., 2008; Sevenou et al., 2002; Van Soest, Tournois, de Wit, Johannes, & Vliegenthart, 1995). Lopez-Rubio et al. (2008) have shown by studies on enzyme digested extruded high amylose maize starches, that the ratio of the absorbances at 995/1022 cm^{-1} is a better indicator of changes in helical order than the 1047/1022 cm^{-1} ratio.

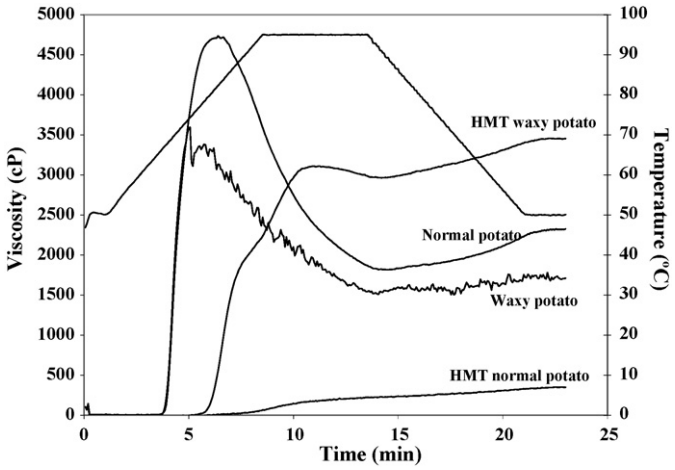

Fig. 5. RVA profiles of native and HMT normal and waxy potato starches.

The results (Table 4) showed that in both normal and waxy potato starch the 1047/1022 cm^{-1} ratio decreased nearly to the same extent on HMT (normal [1.146–1.010], waxy [1.167–1.028]). Whereas, the decrease in the 995/1022 cm^{-1} ratio on HMT was 1.177–1.165 (normal) and 1.198 and 1.167 (waxy). This suggests that the 995/1022 cm^{-1} ratio is more sensitive than the 1047/1022 cm^{-1} ratio in determining changes to the alignment of double helices at short-range order. The A-type unit cells have been shown to contain less water than B-type unit cells (Saibene et al., 2008). Furthermore, as shown earlier (Fig. 1), more A-type unit cells are formed in waxy (Fig. 1f) than in normal (Fig. 1b) potato starch on HMT. Consequently, the loss of water during the polymorphic transformation (B → A + B) that occurs on HMT would be greater in waxy than in normal potato starch. Van Soest et al. (1995) have postulated that removal of water would decrease molecular order. This would then explain the decrease in the 995/1022 cm^{-1} ratio being greater in waxy than in normal potato starch (Table 4).

3.9. Pasting characteristics

The pasting properties of native and HMT normal and waxy potato starches measured using a Rapid Visco Analyzer (RVA) are presented in Fig. 5. All RVA parameters were higher in native normal potato starch. Studies have shown that peak viscosities are influenced by amylose content, extent of amylose leaching, granular swelling, friction between swollen granules, phosphate monoester content and/or the proportion of long amylopectin branch chains (Jane et al., 1999; Jayakody et al., 2007). As shown in Tables 1 and 2, there was no significant difference in phosphate monoester content and in the proportion of long amylopectin chains (DP 37–50), between native normal and waxy potato starches. Therefore, the difference in peak viscosity (normal > waxy) between the two native starches could be attributed to AML (normal > waxy) (Fig. 2a) negating the effect of granular swelling (waxy > normal) (Fig. 2b) on peak viscosity. McPherson and Jane (1999) also showed that normal potato starch displays a larger peak viscosity than waxy potato starch. This was attributed to AM–AMP interactions within native normal potato starch which confers granule rigidity, thereby enabling granules to swell and to attain a higher peak viscosity than waxy potato starch. Higher granule fragility (resulting from higher granular swelling) may have been responsible for the extent of viscosity breakdown being higher in normal potato starch (Fig. 3). The higher set-back of native normal potato starch reflects more extensive amylose leaching (Fig. 2a). In both starches, HMT decreased peak viscosity (normal > waxy), breakdown viscosity (normal > waxy)

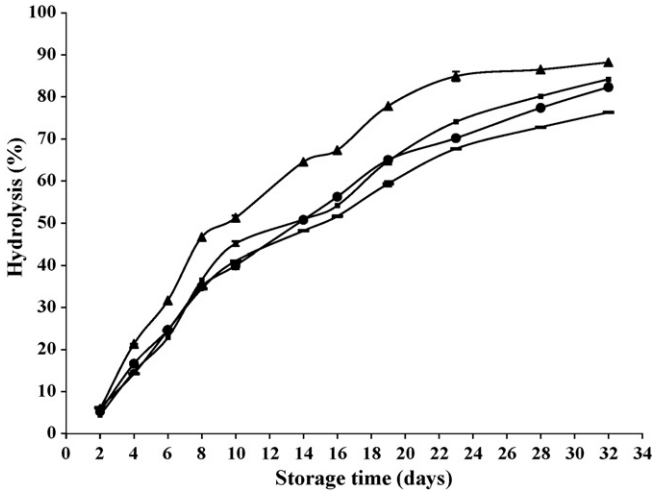

Fig. 6. Time course of acid hydrolysis (%) of native normal (■) HMT normal (−) native waxy (▲) and HMT waxy potato (●) starches.

and increased the pasting temperature (normal > waxy). However, set-back decreased in normal potato starch, but increased in waxy potato starch on HMT (Fig. 3). The reduction in peak viscosity on HMT reflects decreased AML (Fig. 2a) and SF (Fig. 2b) as a result of enhanced interaction between AM–AM, AM–AMP and AMP–AMP chains. Reduction of peak viscosity is lower in waxy potato starch, since contribution from amylose to the above interactions is negligible. The above interactions are also responsible for the increase in pasting temperature on HMT being higher in normal potato starch. Jayakody et al. (2007) have shown that both amylose gelation and presence of rigid swollen granules embedded within the leached amylose network influence the extent of set-back. SF (Fig. 2b) and AML (Fig. 2a) data showed that HMT decreased the SF and AML of normal potato starch, but increased SF of waxy potato starch. This suggests that the increased set-back on HMT of waxy potato starch reflects an increase in the proportion of swollen intact granules (provides increased resistance to shearing during the cooling cycle in the RVA).

3.10. Acid hydrolysis

Acid hydrolysis of native and HMT normal and waxy potato starches are presented in Fig. 6. The results showed that native waxy potato was hydrolysed to a greater extent than native normal potato starch throughout the time course of hydrolysis. A similar result was reported by Bertoft (2004). In this study (Fig. 6), the difference in hydrolysis between the native starches was more pronounced between the 8th and 23rd day. It has been suggested that heterogeneous acid hydrolysis preferably attacks the more amorphous regions of the granule, whether they be at the surface or interior. In contrast, crystalline regions are less accessible to attack by hydrated protons (H_3O^+), and are attacked only after a period of 10–12 days (Hoover, 2000). The large difference in hydrolysis between native normal and waxy potato starches between the 8th and 23rd day, suggests that crystallites of native waxy potato starch are more accessible to penetration by H_3O^+. Surface gelatinization studies on native normal potato starch granules (Jane & Shen, 1993) have shown that amylose content is greater at the periphery than at the core. The above authors postulated that the increased concentration of amylose at the granule periphery enhance their interaction with amylopectin. This suggests, that the difference in hydrolysis between the native (waxy > normal) starches (Fig. 6) may reflect AM–AMP interactions (predominant in native normal potato starch) which renders the α (1–4) and α (1–6) linkages less accessible to hydrolysis by H_3O^+. Jenkins and Donald (1995) have shown by small angle X-ray scattering studies on normal, waxy and high amylose barley, maize and pea starches that increasing the amylose content has the effect of increasing the size of the crystalline portion of the amylopectin cluster. This was based on the observation that the crystalline lamella electron density decreases with increasing amylose content. The above authors proposed two mechanisms to explain the disrupting effect of amylose on packing of amylopectin chains within the crystalline lamellae: (1) co-crystallization between amylose and amylopectin and (2) penetration of amylose into the amorphous region of the cluster (regions where branch points are located). This suggests that the difference in susceptibility of native potato starches (waxy > normal) towards acid hydrolysis could also reflect the larger size of the crystalline portion in native potato starch. Acid hydrolysis decreased (waxy > normal) on HMT. The influence of HMT on acid hydrolysis has been shown to vary widely among starch sources. In cereal starches, susceptibility towards acid hydrolysis after HMT decreases marginally in wheat (Hoover & Vasanthan, 1994) and maize (Hoover & Manuel, 1996) starches, whereas tuber starches (potato, cassava, taro) show increased hydrolysis (1–13%) during the first 5 days of hydrolysis on HMT, thereafter they are hydrolysed to lesser extent (2–15%) than their native counterparts (Gunaratne & Hoover, 2002; Hoover & Vasanthan, 1994). In this study, the decrease in acid hydrolysis on HMT could be explained on the basis of change in crystalline polymorphism (B → A + B) and starch chain interactions (AM–AM, AMP–AM, AMP–AMP). As discussed earlier, the unit cells of A-type starches are more compactly packed than those of B-type starches. This suggests that closely packed A-type AMP chains could hinder the accessibility of H_3O^+ towards the α (1–4) and α (1–6) glycosidic linkages. Jane, Wong, and McPherson (1997) have shown that in B-type starches, the α (1–6) branch points are mainly clustered in the amorphous regions whereas in A-type starches, the α (1–6) branch points are located both within crystalline and amorphous lamellae. This suggests that α (1–6) branch points inside the crystalline lamella of A-type starches would be protected and hence resistant to acid hydrolysis. Thus, the higher proportion of A-type unit cells formed on HMT in waxy potato starch (Fig. 1f) may have been partly responsible for the extent of decrease in acid hydrolysis on HMT being higher than in HMT normal potato starch (Fig. 6). It has been shown (Hoover, 2000) that a conformational transformation (chair → half chair) is required for efficient protonation of glycosidic oxygen. Interactions (AM–AM, AM–AMP, AMP–AMP) between starch chains on HMT would reduce chain flexibility, thereby hindering the above conformational change. This would also then explain the decrease in hydrolysis on HMT. On this basis, the decrease in hydrolysis on HMT should have been higher in normal potato starch due to interaction involving amylose chains. This leads to the conclusion that the extent of decrease in acid susceptibility on HMT is mainly influenced by the extent of polymorphic transformation (waxy > normal).

4. Conclusion

The results showed that differences in amylose content between the starches (normal > waxy) and the close association of amylose with amylopectin chains in normal potato starch were the main causative factors influencing starch chain interactions and the extent of the polymorphic phase transition (waxy > normal) of the B to A-type crystal lattice on HMT. These changes in turn influenced the extent of decrease on AML, SF, iodine complexing ability, peak viscosity, set-back and acid susceptibility, and the extent to which gelatinization transition temperatures and the gelatinization temperature range increased on HMT. Studies are underway to study

the effect of HMT on lamellar organization, enzyme susceptibility and retrogradation.

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