Hydrothermal Treatment of Water Yam Starch in a Non-granular State: Slowly Digestible Starch Content and Structural Characteristics

Khanh Son Trinh, Chang Joo Lee, Seung Jun Choi, and Tae Wha Moon

Abstract: Gelatinized water yam starch was subjected to hydrothermal treatment (25, 30, and 35% moisture content for 1, 8, 16, and 24 h at 100 °C) and characterized by X-ray diffractometry, solid-state ^{13}C cross-polarization and magic-angle spinning nuclear magnetic resonance, differential scanning calorimetry, and digestibility analysis. The slowly digestible starch (SDS) content of the starch treated at 30% moisture content for 24 h reached 49.1%, 31.9% higher than that of the control starch. The B-type pattern of native starch was re-crystallized to the A-type by hydrothermal treatment. The SDS content showed negative correlations with T_o, T_p, T_c, and T_r, but showed a positive correlation with melting enthalpy. Furthermore, SDS was positively correlated with hydrothermal reaction time, moisture content, relative crystallinity, and the double-helix proportion. The structural changes in hydrothermally treated water yam starches resulted in the enhancement of SDS.

Keywords: differential scanning calorimetry, hydrothermal treatment, re-crystallization, slowly digestible starch, water yam starch

Practical Application: The hydrothermally treated water yam starch could be used as a food ingredient for slow-energy supply or dietary fiber.

Introduction

Starch is the main source of metabolic energy and carbohydrate in foods. Based on the rate and extent of digestion, starches are generally classified into (i) rapidly digestible starch (RDS), the fraction digested within the first 20 min in the mouth and intestine; (ii) slowly digestible starch (SDS), the portion digested from 20 to 120 min in the small intestine; and (iii) resistant starch (RS), the remaining fraction that cannot be further digested in the small intestine but is fermented mainly in the colon (Englyst and others 1992). RDS usually leads to a rapid increase in blood glucose due to the rapid conversion to glucose in the early stage of the digestive process. Because of the unique characteristics of SDS, products rich in SDS can prevent a rapid increment in blood glucose and provide a sustained supply of glucose. Consequently, these products may reduce postprandial blood glucose levels and lower postprandial insulinemia. Thus, SDS could affect physical and menta performance, satiety, and diabetes management (Lehmann and Robin 2007).

Heat-moisture treatment (HMT) is a physical technique that involves heating starch at a temperature above its gelatinization point for a certain period of time with insufficient moisture (<35%) to cause gelatinization (Eliasson and Gudmundsson 2006). Zhang and others (2010) reported that microwave HMT of *Canna edulis* Ker starch in a granular state increased both the SDS and RS contents from 11.7 and 27.7% to 13.6 and 55.5%, respectively. He and others (2008) investigated the effect of further HMT of esterified waxy-maize starch on SDS formation and reported an increase from 28 to 42.8%, although the RDS level did not significantly change after treatment. However, hydrothermal treatment does not always have a positive effect on the SDS content. Chung and others (2009) reported that the SDS content in HMT corn, pea, and lentil starches decreased, whereas the RS content increased. Few studies have been carried out to determine the impact of HMT without destroying granular structure on SDS formation. As most starchy foods are cooked before consumption, the gelatinization of starch is a critical phenomenon in the food industry. Native or nearly native starch is not used widely in the food industry due to its poor functional properties. Therefore, the changes in the structural characteristics and digestibility after hydrothermal treatment of gelatinized starch may provide very useful information for the food industry.

The purpose of the current study was to determine the impact of hydrothermal treatment of gelatinized water yam starch on SDS formation and the relationship between its digestibility and structural characteristics. Water yam was chosen for this study because it is grown widely in many subtropical and tropical countries and is used as a dietary staple due to its high starch content, which is 71 to 85% of dry matter (Huang and others 2006).

Materials and Methods

Materials

Tubers of water yam (*Dioscorea alata*) grown at a farm in the Mekong Delta region of Vietnam were harvested, rinsed, sliced,

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doi: 10.1111/j.1750-3841.2012.02703.x

Journal of Food Science A Publication of the Institute of Food Technologists

and dried at 40 °C for 3 d in an air-drying oven to reach approximately 11% moisture content. Pancreatin (P7545, activity 8×USP/g) and isoamylase (15284, activity ≥3,000,000 units (U)/mg protein) were obtained from Sigma–Aldrich (St. Louis, Mo., U.S.A.) and amyloglucosidase (AMG 300L, activity 300 AGU/mL) from Novozymes (Bagsvaerd, Denmark). All chemicals used were of analytical reagent grade.

Starch isolation

Water yam starch was isolated by the method of Hoover and Hadziyev (1981), with slight modification. Dried slices were soaked in 0.2% NaOH overnight at ambient temperature and then ground in a laboratory blender. Ground tubers were passed through a 150-μm sieve. The filtrate was allowed to stand at 4 °C overnight, and the supernatant was decanted. The residue was rinsed with 0.2% NaOH and again allowed to stand at 4 °C. This step was repeated every 8 h for a week. Finally, the starch residue was suspended in distilled water, adjusted to pH 7.0 with 1 N HCl. The residue was collected, washed with distilled water, and dried in a forced-air oven overnight at 40 °C. The dried starch was finely ground with a mortar and pestle to pass through a 150-μm sieve for further experiments.

The proximate analysis of isolated water yam starch, carried out by Approved Methods (08–17, 30–25, and 46–10) of the AACC (2000), showed dry-weight percentages of 0.09, 0.12, 0.05, and 99.74% for crude ash, crude fat, crude protein, and nitrogen-free extract, respectively. The amylose content was 18.6%, determined by the Megazyme amylose/amylopectin assay procedure outlined by the manufacturer, using the commercial kit (Megazyme Ireland International, Ltd., Bray, Ireland).

Hydrothermal treatment

To observe the effect of various storage time during hydrothermal treatment and to use as a reference for structural analysis, gelatinized (control) and amorphous starches were prepared by autoclaving native starch (40 and 5% suspension, respectively; dry basis) at 121 °C for 30 min, followed by drying in an air-drying oven at 40 °C for 24 h. The initial moisture content, measured according to Approved Method 44–15 of the AACC (2000), for gelatinized starch was around 11%. For hydrothermal treatment, gelatinized starch was weighed into a glass container, and the moisture content was adjusted to 25, 30, or 35% by adding appropriate amount of water and mixed gently with a spatula. The glass container was sealed and allowed to stand at room temperature for 24 h to reach equilibrium. Then, starch samples were stored at 100 °C for 1, 8, 16, and 24 h in an air-drying oven. The samples were dried in an air-drying oven at 40 °C for 24 h to reach a final moisture content of around 11%. All samples were ground and passed through a 150-μm sieve.

Side-chain-length distribution

The side-chain-length distribution of water yam starch was analyzed by high-performance anion-exchange chromatography using pulsed amperomeric detection (HPAEC-PAD; Dionex, Sunnyvale, Calif., U.S.A.) and a CarboPac PA1 anion-exchange column (250 × 4 mm; Dionex). Starch (15 mg) was dissolved in 90% DMSO (3 mL) and boiled for 15 min with vortexing. Ethanol (15 mL) was added, and centrifuged at 10,000 × g for 10 min. After removing the supernatant, distilled water (1.5 mL) was added and boiled for 30 min. Sodium acetate buffer (50 mM, pH 4.3) was also added during boiling of the sample. For hydrolysis of α-1,6-glycosidic linkages, 30 μL isoamylase (1000 U, Sigma–Aldrich) was used and incubated at 45 °C for 2 h. Enzyme reaction was finished by boiling for 10 min. Debranched sample was filtered through a 0.45-μm membrane filter and analyzed with the following gradients of elution: linear gradients from 0 to 20% for 0 to 5 min, from 20 to 45% for 6 to 30 min, from 45 to 55% for 31 to 60 min, from 56 to 60% for 61 to 80 min, from 81 to 100% for 96 to 100 min.

X-ray diffractometry (XRD) and degree of relative crystallinity (DRC)

XRD was determined using a powder X-ray diffractometer (Model D5005, Bruker, Karlsruhe, Germany). The operating conditions were 40 kV and 40 mA with Cu-Kα radiation of 0.15406 nm (Nickel filter; time constant, 4 s). Each scan was performed from 3 to 30° (2θ). DRC was calculated using the equation DRC = Ac/ (Ac + Aa), where Ac is the area of crystalline portion and Aa is the area of amorphous portion, according to the method of Nara and Komiya (1983) with peak-fitting software (Origin-version 7.5, OriginLab, Northampton, Mass., U.S.A.).

Differential scanning calorimetry (DSC)

The thermal properties of starch samples were determined using a differential scanning calorimeter (Diamond DSC, Perkin-Elmer, Waltham, Mass., U.S.A.). Water (40 μL) was added to a sample (10 mg) in an aluminum DSC pan, which was then sealed, and allowed to stand for 4 h at room temperature to reach equilibrium. The sample pan was heated from 30 to 130 °C at 5 °C/min with an empty pan as reference. Endothermic transition parameters including onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) of melting, melting temperature range ($T_r = T_c - T_o$), and melting enthalpy (ΔH) were determined with the Pyris software (Perkin–Elmer).

Solid-state ^{13}C cross-polarization and magic-angle spinning (CP/MAS) nuclear magnetic resonance (NMR) spectroscopy

The quantification of the ordered and non-ordered structures was performed by comparing spectra of each sample and amorphous starches (Gidley and Bociek 1985). Data were obtained at a ^{13}C frequency of 400 MHz on a Bruker DSX-400 spectrometer (Brucker Instrument, Billerica, Mass., U.S.A.), equipped with CP/MAS accessories. The samples were spun at 5 kHz and room temperature. The acquisition time was 35 ms, time domain points 2.2 K and line broadening 10 Hz. The samples were packed in a 4-mm diameter rotors and spectral width was 3.1 kHz. Spectra were obtained using the high-field resonance and referenced to adamantine (29.5 ppm). The data processing and calculation of integrated peak areas were performed using the MestRe-C package software (Mestrelab Research, Santiago de Compostela, Spain).

Starch digestibility

Starch digestibility was determined according to the method of Brumovsky and Thompson (2001), with slight modification. Pancreatin (2 g, Sigma–Aldrich) was dissolved in distilled water (24 mL) and stirred for 10 min. It was centrifuged at 1500× g for 10 min, and then 20 mL of supernatant was mixed with 3.6 mL of distilled water, and 0.4 mL of amyloglucosidase (AMG 300L, Novozymes). This enzyme solution was stored in a 37 °C water bath for at least 10 min. Each starch sample (30 mg) was placed in a 2-mL microtube with a glass bead. After adding 0.75 mL of sodium acetate buffer (pH 5.2), the tube was stored in

a shaking incubator (37 °C, 10 min, 240 rpm). Then, 0.75 mL of the prepared enzyme solution was added to the tube, and the tube was shaken continuously. The reaction was stopped after 10 or 240 min by boiling for 10 min. The glucose present in the supernatant obtained by centrifugation (5000× *g*, 5 min) was measured using a GOD-POD kit (BCS, Anyang, Korea).

Starch fractions were classified based on the rate of hydrolysis. Rapidly digestible starch (RDS) and slowly digestible starch (SDS) were measured by the glucose concentration after enzyme reaction for 10 and 240 min, respectively. Resistant starch (RS) constituted the fraction undigested after 240 min.

Statistical analysis

All experiments were performed in triplicate, and the mean value and the standard deviation are reported. Analysis of variance (ANOVA) was conducted, and the mean separations were analyzed by Duncan's multiple-range test ($P < 0.05$). The Pearson correlation analysis (bivariate correlations algorithms, $P < 0.01$ and $P < 0.05$) was performed to summarize the relationships among characteristics of hydrothermally treated starches. All statistical analyses were conducted using SPSS software (Ver. 17.0, SPSS, Chicago, Ill., U.S.A.).

Results and Discussion

In our preliminary study (data not shown), native water yam starch in a granular state was subjected to HMT (25, 30, 35, and 40% moisture content at 100 °C for 1 to 24 h). However, the HMT starches showed no significant difference in *in vitro* digestibility compared with the native starch, except for a slight increase in RDS and a decrease in RS in the starch treated at 40% moisture content for 24 h. Light and polarized-light micrographs revealed no changes in granular structure, aside from the starch treated at 40% moisture content for 24 h, which exhibited some cracks on granules. Obviously, HMT had no effect on the morphological characteristics and digestibility of granular starch. This was the main reason for applying hydrothermal treatment to the gelatinized starch in this study.

Side-chain-length distribution

The side-chain-length distribution of the control or the hydrothermally treated starches was very similar to that of the native starch. Therefore, the side-chain-length distribution of only native water yam starch is represented in Figure 1. The native starch showed a bell-shaped distribution with the highest peak at DP 14. According to the previous classification (Hanashiro and others 1996), the side chains of amylopectin could be categorized into four fractions: fa, DP 6–12; fb_1, DP 13–24; fb_2, DP 25–36; and fb_3, DP > 37. A cluster model for amylopectin composed of a number of A-chains without branching and B-chains carrying A- or other B-chains is generally accepted (Hizukuri 1986). Water yam starch showed an average chain length of 20.5, and the largest proportion of fraction fb_1 (61.3%) and very short-chain fraction (DP ≤ 5) was extremely low (1.5%). The proportions of fa, fb_1, fb_2, and fb_3 for water yam starch were 14.1, 61.3, 17.6, and 5.5, respectively, which are different from those of a previous report (18, 56, 15, and 11, respectively). Besides, these differences of fa, fb_1, fb_2, and fb_3 proportions were observed in potato starch (18, 48, 15, and 18; respectively) and in rice starch (27, 52, 12, and 9; respectively) (Hanashiro and others 1996). These results showed good agreement with the highest proportion of fb_1 and the lowest of fb_3; however, the ratio of fa to fb_1, fb_{1-2}, and fb_{1-3} in this study was less than that reported previously (Hanashiro and others 1996).

XRD pattern and DRC

The XRD patterns of starch samples are presented in Figure 2. The diffractograms of the amorphous and control samples were very similar to each other, not showing any peaks, mostly due to considerable amorphous regions. The native starch displayed the typical characteristics of B-type structure (distinct peaks at 5.7 and 17.1° and small peaks at 14.9, 22.2, and 24.1°). Hydrothermally treated starches (Figure 2B and C) showed strong diffraction at 2θ of about 15.3 and 23° and an unresolved doublet at 17.0 and 18.1°, which was close to the A-type crystalline pattern (Bogracheva and others 2001). The re-association of gelatinized starch molecules to an ordered structure during drying or cooling of gelatinized starch is referred to as retrogradation or re-crystallization. It generally occurs during the storage of gelatinized starch with 45 to 50% moisture content at temperatures between glass transition temperature, the critical temperature at which an amorphous material changes its behavior from being glassy (hard and brittle) to being rubbery (elastic and flexible), and room temperature (Eliasson and Gudmundsson 2006). This retrogradation is affected greatly by the storage temperature. The hydrothermal treatment conditions applied in this study, especially the temperature, were not ideal for re-crystallization, even though re-crystallization could occur to some extent during hydrothermal treatment. In general, retrogradation is a three-step procedure (Levine and Slade 1989): (i) nucleation, formation of nuclei; (ii) propagation, growth of crystals from the nuclei; and (iii) maturation, crystal perfection. The nucleation rate is almost zero at the melting temperature of the crystal, and the propagation rate is maximal at the melting temperature. The melting temperature for amylose crystals is about 150 °C (Ring and others 1987). The temperature (100 °C) used for hydrothermal treatment in this study was positioned between the glass transition and melting temperatures. Therefore, during the hydrothermal treatment, nucleation was limited, and propagation was favored. Previous studies have demonstrated that crystallization tends to favor the more stable A-type at a higher crystallization (Gidley 1987; Gidley and Bulpin 1987). Presumably, the hydrothermal treatment of gelatinized starch induced the formation of A-type crystalline structure, clearly distinct from the

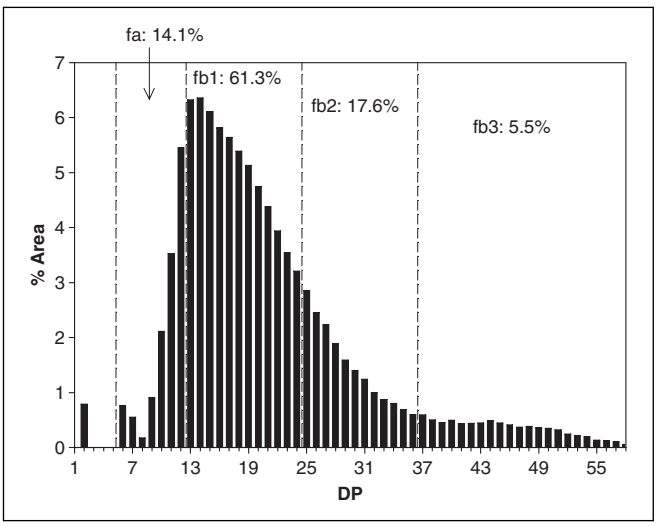

Figure 1–Side-chain-length distribution of water yam starch.

crystalline structure of the native starch. The DRC data for the starch samples are given in Table 1. Much higher DRC values were obtained for hydrothermally treated starches than for the control, but they were less than that of native starch. The DRC showed a positive correlation with moisture content ($r = 0.868$, $P < 0.01$) and a weak correlation with reaction time ($r = 0.429$, $P < 0.05$). This indicated that both the moisture content and reaction time of hydrothermal treatment positively affected the DRC

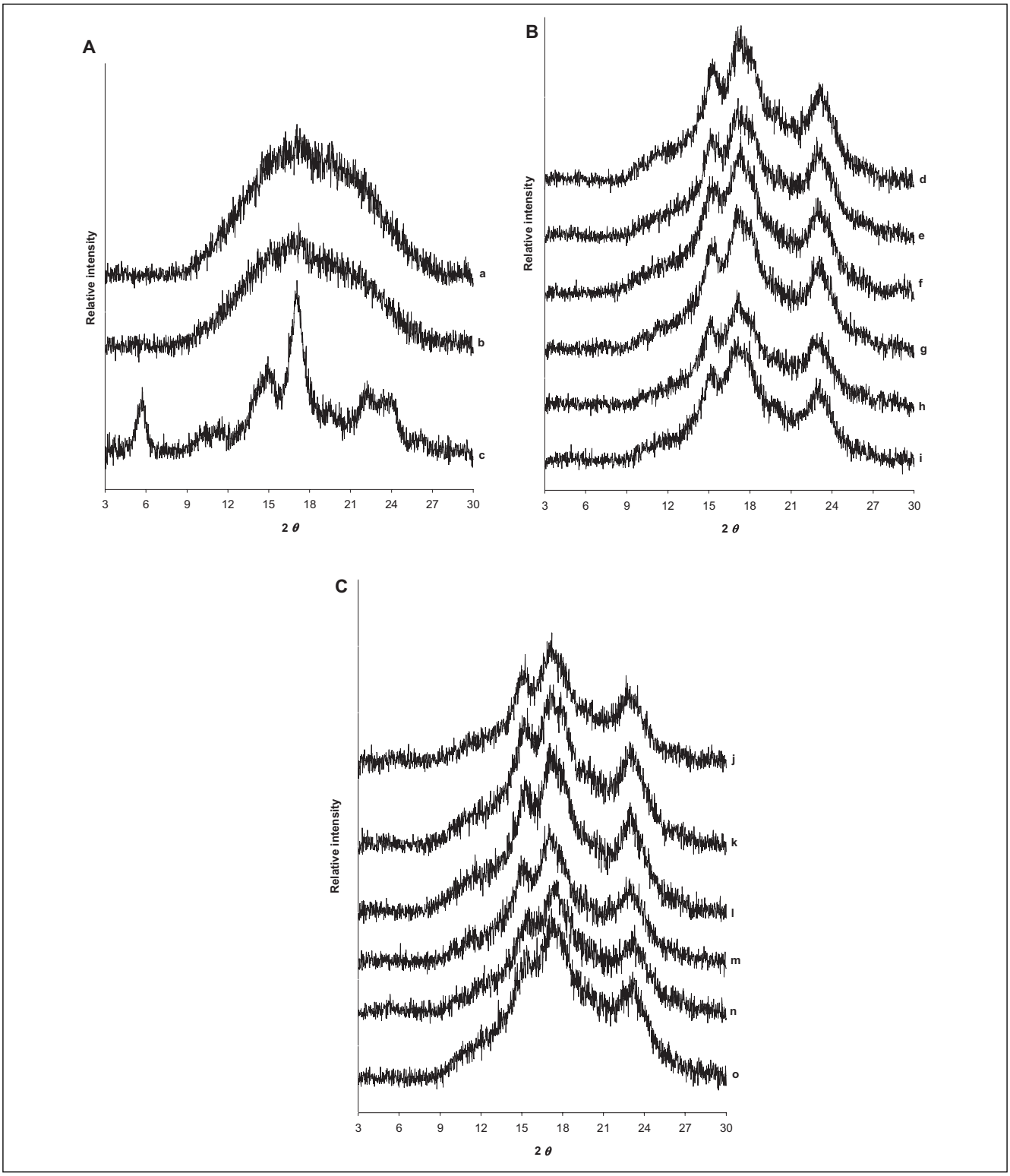

Figure 2–X-ray diffraction patterns of (a) amorphous (prepared by autoclaving 5% suspension of native starch at 121 °C for 30 min, followed by drying in an air-drying oven at 40 °C for 24 h), (b) control (gelatinized starch prepared by autoclaving 40% suspension of native starch at 121 °C for 30 min, followed by drying in an air-drying oven at 40 °C for 24 h), (c) native, (d) 25% 1 h, (e) 30% 1 h, (f) 35% 1 h, (g) 25% 8h, (h) 30% 8h, (i) 35% 8 h, (j) 25% 16 h, (k) 30% 16 h, (l) 35% 16 h, (m) 25% 24 h, (n) 30% 24 h, and (o) 35% 24 h starches.

of hydrothermally treated starches and that the moisture content had a greater impact in hydrothermal treatment. Furthermore, although the Pearson's correlation coefficients of RDS-DRC and SDS-DRC were −0.720 and 0.622, respectively, there was no association between RS and DRC.

Thermal properties

The endothermic transition parameters (T_o, T_p, T_c, T_r, and ΔH) for native and hydrothermally treated starches are presented in Table 2. Higher scanning temperature range (up to 190 °C, data not shown) was examined, but no further endothermic transition parameters were observed. Native starch showed a significantly sharper endothermic peak when compared with the control and hydrothermally treated starches (Figure 3). No obvious retrogradation peaks were detected in the control and 1-h-treated starches, regardless of the moisture content. It is known that no melting endotherm of amylose gel is obtained in the temperature interval of 10 to 130 °C (Eliasson and Gudmundsson 2006). There was no envidence of this endotherm of 1-h-treated starches within the temperature range examined in this study. However, according to the XRD observations, the DRC values for 1-h-treated starches ranged from 22.0 to 31.8%, indicating that they had a crystalline structure to some extent. This implied that the results of DRC could be attributed to the crystalline structure formed with amylose molecules at the early stage of hydrothermal treatment and that the 1-h-treated starches had an insignificant extent of crystalline regions formed with amylopectin molecules. When the treatment time was longer than 8 h, retrogradation peaks, caused by the melting of re-crystallized amylopectin molecules, were detected. It has been suggested that the side chains in amylopectin with less than DP 15 do not take part in the re-crystalllization (Ring and others 1987). Therefore, a large proportion (about 80%, Figure 1) of side chains with more than DP 15 presumably promoted the re-crystallization of amylopectin, and the 8 h treatment time might be long enough for amylopectin molecules to re-crystallize under the hydrothermal treatment conditions applied. These peaks became broader and shallower and showed lower ΔH compared with the native starch. Under the conditions of hydrothermal treatment, T_o, T_p, and T_c tended to increase with increasing moisture content, regardless of the treatment time (Table 2). The endothermic peaks at high temperature (from 79.9 to 91.5 °C) observed for hydrothermally treated starches with 30 and 35% moisture contents (Figure 3B and C) suggested that more stable crystallites might be present in hydrothermally treated starches than in the native starch (T_p 79.8 °C). This result, observed by XRD, was consistent with a change in the crystalline structure from B- to A-type, a more stable polymorph. A decrease in the ΔH of all hydrothermally treated starches could be due mainly to a decrease in the amount of crystallites compared with the native starch. The broader T_r after hydrothermal treatment indicated that crystallites having high heterogeneity, meaning various sizes and degrees of perfection, developed by re-crystallization during hydrothermal treatment (Vasanthan and Bhatty 1996).

In our study, T_o, T_p, T_c, and ΔH were positively correlated with moisture content (Table 3). In contrast, Hoover and Vasanthan (1994) reported a negative correlation between ΔH of yam starch and the moisture level during HMT. This discrepancy between our result and that of Hoover and Vasanthan (1994) could be due to the gelatinization of water yam starch used in our study, along with the different variety.

Table 1–Degree of relative crystallinity (DRC) and the proportion of ordered structure of hydrothermally-treated starches[a].

Starch samples		DRC (%)	Crystal pattern	Ordered[b] structure[b]
Amorphous[c]		13.4 ± 0.4^{i}	-	30.0^{i}
Control[d]		16.3 ± 0.5^{i}	-	30.7^{i}
Native		49.5 ± 0.6^{a}	B	47.3^{a}
Hydrothermally treated starches				
Moisture content	Reaction time			
25%	1 h	22.0 ± 0.4^{h}	A	38.7^{h}
30%	1 h	28.3 ± 0.6^{ef}	A	39.1^{gh}
35%	1 h	31.8 ± 0.1^{b}	A	41.2^{d}
25%	8 h	26.3 ± 0.1^{fg}	A	40.9^{d}
30%	8 h	25.5 ± 0.0^{g}	A	39.5^{fg}
35%	8 h	30.2 ± 0.1^{c}	A	39.8^{ef}
25%	16 h	28.9 ± 0.2^{cde}	A	41.5^{cd}
30%	16 h	29.6 ± 0.4^{cd}	A	42.4^{bc}
35%	16 h	29.5 ± 0.1^{cd}	A	42.0^{cd}
25%	24 h	27.6 ± 0.7^{ef}	A	42.3^{b}
30%	24 h	29.3 ± 1.0^{cd}	A	40.3^{e}
35%	24 h	28.6 ± 0.1^{cde}	A	42.2^{b}

[a]The values with different superscripts in a same column are significantly different ($P < 0.05$).
[b]Mean ($n = 2$).
[c]Amorphous starch was prepared by autoclaving 5% suspension of native starch at 121 °C for 30 min, followed by drying in an air-drying oven at 40 °C for 24 h.
[d]Gelatinized starch prepared by autoclaving 40% suspension of native starch at 121 °C for 30 min, followed by drying in an air-drying oven at 40 °C for 24 h.

Table 2–Thermal properties of native, control, and hydrothermally treated starches[a].

Starch samples		T_o[b] (°C)	T_p (°C)	T_c (°C)	T_r (°C)	ΔH (J/g)
Native		76.3 ± 0.1^{b}	79.8 ± 0.2^{d}	82.5 ± 0.1^{e}	4.1 ± 3.6^{c}	19.9 ± 0.4^{a}
Control[c]				ND		
Hydrothermally-treated starches						
Moisture content	Reaction time					
25%	1 h			ND		
30%	1 h			ND		
35%	1 h			ND		
25%	8 h	75.2 ± 0.6^{bc}	80.0 ± 1.0^{d}	88.0 ± 1.2^{d}	9.6 ± 1.7^{bc}	2.9 ± 0.1^{b}
30%	8 h	78.2 ± 2.5^{bc}	84.9 ± 0.0^{b}	92.9 ± 0.8^{c}	13.1 ± 2.3^{bc}	4.2 ± 0.1^{b}
35%	8 h	80.1 ± 0.5^{b}	90.4 ± 0.0^{ab}	99.5 ± 1.1^{a}	19.0 ± 2.0^{a}	4.1 ± 0.1^{b}
25%	16 h	72.5 ± 3.0^{c}	79.9 ± 0.5^{d}	86.0 ± 0.6^{d}	15.0 ± 2.0^{abc}	3.6 ± 0.8^{b}
30%	16 h	74.9 ± 2.8^{bc}	84.7 ± 2.3^{c}	91.6 ± 0.9^{c}	16.7 ± 1.9^{ab}	6.0 ± 0.6^{b}
35%	16 h	79.8 ± 1.4^{b}	89.1 ± 0.1^{b}	95.8 ± 0.2^{b}	16.0 ± 1.7^{ab}	7.2 ± 2.1^{b}
25%	24 h	73.9 ± 1.9^{bc}	81.4 ± 0.6^{d}	87.7 ± 0.7^{d}	13.8 ± 2.4^{abc}	5.1 ± 0.7^{b}
30%	24 h	77.8 ± 2.0^{bc}	85.7 ± 0.1^{c}	91.7 ± 0.3^{c}	14.0 ± 2.3^{abc}	6.0 ± 0.9^{b}
35%	24 h	86.2 ± 3.1^{a}	91.5 ± 0.3^{a}	96.3 ± 0.2^{b}	10.1 ± 2.9^{bc}	3.8 ± 0.4^{b}

[a]The values with different superscripts in a same column are significantly different ($P < 0.05$).
[b]T_o = Onset temperature; T_p = Peak temperature; T_c = Conclusion temperature; T_r = Gelatinization temperature range; ΔH = Melting enthalpy; ND = Not detected.
[c]Gelatinized starch prepared by autoclaving 40% suspension of native starch at 121 °C for 30 min, followed by drying in an air-drying oven at 40 °C for 24 h.

On the other hand, no correlation was observed between the hydrothermal reaction time and endothermic transition parameters, except for ΔH. The hydrothermal reaction time positively correlated with ΔH ($r = 0.768$, $P < 0.01$), which reflects the melting of imperfect amylopectin-based crystals, with potential contributions from both crystal-packing and helix-melting enthalpies (Lopez-Rubio and others 2008).

^{13}C CP/MAS NMR spectroscopy

NMR spectra of the starch samples are shown in Figure 4. The resonances around 60.5 ppm were assigned to C-6, and the wide signal around 71–73 ppm was collectively associated with C-2, C-3, and C-5 sites. The resonances at 81 ppm and 98–102 ppm were associated with C-4 and C-1 sites, respectively. The native starch was resolved into a doublet in the range between 98.5 and

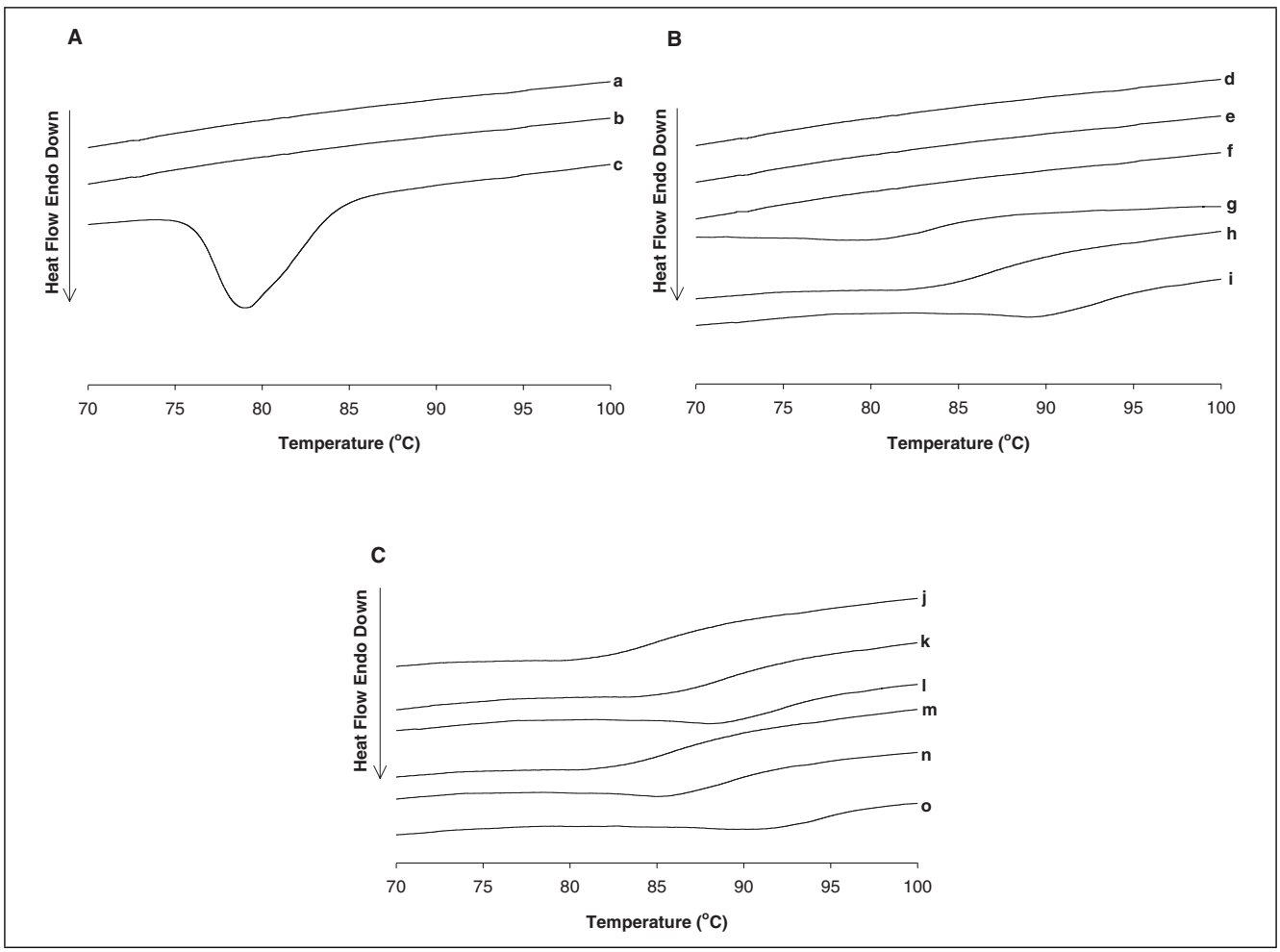

Figure 3–Differential scanning calorimetric thermograms of (a) amorphous (prepared by autoclaving 5% suspension of native starch at 121 °C for 30 min, followed by drying in an air-drying oven at 40 °C for 24 h), (b) control (gelatinized starch prepared by autoclaving 40% suspension of native starch at 121 °C for 30 min, followed by drying in an air-drying oven at 40 °C for 24 h), (c) native, (d) 25% 1 h, (e) 30% 1 h, (f) 35% 1 h, (g) 25% 8 h, (h) 30% 8 h, (i) 35% 8 h, (j) 25% 16 h, (k) 30% 16 h, (l) 35% 16 h, (m) 25% 24 h, (n) 30% 24 h, and (o) 35% 24 h starches.

Table 3–Matrix of Pearson's correlation coefficient (*r*) of hydrothermally-treated starches.

	MC[a]	RT	RDS	SDS	RS	T_o	T_p	T_c	T_r	ΔH	DRC	Ord
MC	1.000	0.296	−0.552**	0.422*	0.485**	0.792**	0.975**	0.957**	0.223	0.362*	0.868**	0.770**
RT		1.000	−0.791**	0.758**	0.269	0.130	0.090	−0.144	−0.373	0.768**	0.429*	0.595**
RDS			1.000	−0.944**	−0.378*	0.192	0.315	0.516*	0.508*	−0.707**	−0.720**	−0.770**
SDS				1.000	0.05	−0.511*	−0.674**	−0.775**	−0.455*	0.595**	0.622**	0.747**
RS					1.000	0.573**	0.685**	0.611**	0.125	0.456**	0.384	0.196
T_o						1.000	0.836**	0.730**	−0.368	−0.002	0.110	−0.062
T_p							1.000	0.949**	0.153	0.273	0.428	−0.103
T_c								1.000	0.367	0.209	0.434	−0.245
T_r									1.000	0.287	0.495*	−0.279
ΔH										1.000	0.430*	0.538**
DRC											1.000	0.852**
Ord												1.000

[a]MC = Treatment moisture content; RT = Treatment time; RDS = Rapidly digestible starch; SDS = Slowly digestible starch; T_o = Onset temperature; T_p = Peak temperature; T_c = Conclusion temperature; T_r = Gelatinization temperature range; ΔH = Melting enthalpy; DRC = Degree of relative crystallinity; Ord = Proportion of ordered structure.
* Correlation is significant ($P < 0.05$).
** Correlation is highly significant ($P < 0.01$).

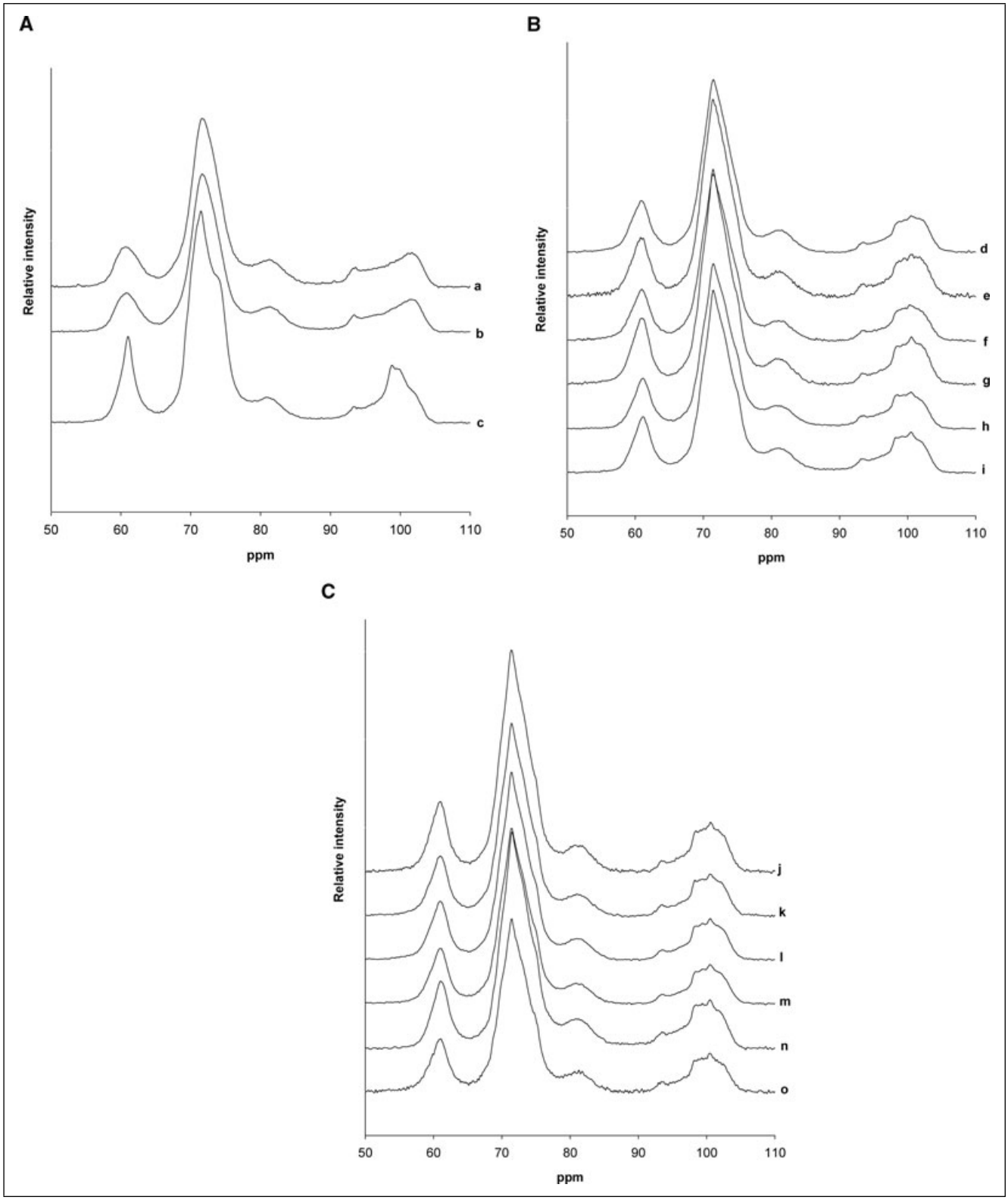

Figure 4—^{13}C CP/MAS NMR spectra of (a) amorphous (prepared by autoclaving 5% suspension of native starch at 121 °C for 30 min, followed by drying in an air-drying oven at 40 °C for 24 h), (b) control (gelatinized starch prepared by autoclaving 40% suspension of native starch at 121 °C for 30 min, followed by drying in an air-drying oven at 40 °C for 24 h), (c) native, (d) 25% 1 h, (e) 30% 1 h, (f) 35% 1 h, (g) 25% 8 h, (h) 30% 8 h, (i) 35% 8 h, (j) 25% 16 h, (k) 30% 16 h, (l) 35% 16 h, (m) 25% 24 h, (n) 30% 24 h, and (o) 35% 24 h starches.

99.5 ppm, the characteristic peaks for B-type crystalline structure. However, C-1 resonances of hydrothermally treated starches appeared as unclear triplets, which indicated that these starches had typical A-type crystalline structure (Bogracheva and others 2001).

The degree of crystalline structure corresponded well with DRC data from XRD. However, the values for the ordered (double-helical) structures, obtained by NMR, in the hydrothermally treated starches were approximately 10% higher than those obtained by XRD. This could be due to the irregular packing of the crystalline structure and the significant extent of non-crystalline double helices (Cooke and Gidley 1992). Both the moisture content and reaction time had positive correlations with the proportion of ordered structures (Tables 1 and 3). Additionally, the moisture content exerted a stronger influence on the development of ordered structures during hydrothermal treatment. Besides, the proportion of ordered structures was positively correlated with SDS content and negatively correlated with the RDS content of the hydrothermally treated starches. Similarly, DRC showed a positive correlation with SDS content, such as the proportion of the ordered structures, indicating that the ordered structures developed during hydrothermal treatment may influence the proportion of SDS and RS fractions in hydrothermally treated starches. It is well known that the resistance to amylolysis can be increased by both the ordered structures and crystalline regions (Cooke and Gidley 1992; Wei and others 2010).

Starch digestibility

The *in vitro* digestibility of starch samples is listed in Table 4. Native starch had a high-RS content, in agreement with the resistant property of granular starch having B-type crystalline structure observed by Hoover and Vasanthan (1994). The control starch had the highest RDS content (>57%). Because the autoclaving destroyed the semi-crystalline and crystalline structures of native starch granules, a loss of RS content and increase in RDS content could occur. The SDS content was elevated significantly by hydrothermal treatment and reached its highest point when treated at 30% moisture content for 24 h compared with the control starch. When starch is retrograded, amylose molecules begin to re-associate as double helices and then form highly ordered and stable-crystalline structures, which can later form an enzyme-resistant fraction when re-associated with amylopectin (Eliasson and Gudmundsson 2006). However, the hydrothermal treatment conditions in this study were not ideal for retrogradation, so the harsh conditions of hydrothermal treatment could possibly prevent crystallites from becoming highly ordered and bigger. This observation is consistent with the DRC data and the proportion of ordered structure. For the native starch, DRC was very similar to the proportion of ordered structure, indicating that most of the double helices were present in the crystalline structure. However, there was a significant difference between DRC and the proportion of ordered structure for the hydrothermally treated starches. This suggested that a significant amount of double helices could not take part in the crystalline structure during the hydrothermal treatment and that they remained as a semi-crystalline structure, which is related to the high-SDS content, after hydrothermal treatment.

Table 4–Relative amounts of RDS, SDS, and RS of hydrothermally treated starches[a,b].

Starch samples		RDS (%)	SDS (%)	RS (%)
Native		0.1 ± 0.1^{g}	4.9 ± 0.9^{i}	95.0 ± 0.8^{a}
Control[c]		57.7 ± 1.8^{a}	17.2 ± 1.7^{h}	25.1 ± 1.4^{c}
Hydrothermally-treated starches				
Moisture content	Reaction time			
25%	1 h	50.1 ± 1.1^{a}	22.3 ± 1.1^{g}	27.7 ± 1.1^{bc}
30%	1 h	38.9 ± 0.5^{bc}	33.8 ± 1.0^{ef}	27.3 ± 1.6^{bc}
35%	1 h	41.8 ± 1.7^{b}	31.7 ± 1.0^{f}	26.4 ± 1.8^{bcd}
25%	8 h	31.7 ± 0.1^{de}	43.5 ± 0.7^{bcd}	24.9 ± 0.6^{cd}
30%	8 h	33.7 ± 1.5^{d}	39.0 ± 1.7^{de}	27.4 ± 0.2^{bc}
35%	8 h	38.3 ± 1.6^{c}	34.9 ± 1.1^{ef}	26.9 ± 1.5^{bc}
25%	16 h	29.2 ± 0.1^{ef}	46.9 ± 0.1^{bc}	24.0 ± 0.0^{cd}
30%	16 h	32.2 ± 1.6^{de}	39.2 ± 0.1^{de}	28.6 ± 1.7^{bc}
35%	16 h	33.4 ± 0.0^{de}	38.7 ± 0.0^{de}	27.8 ± 0.0^{bc}
25%	24 h	29.4 ± 1.7^{ef}	48.4 ± 0.9^{ab}	22.2 ± 0.9^{d}
30%	24 h	23.2 ± 0.8^{g}	49.1 ± 0.7^{a}	27.7 ± 0.1^{bc}
35%	24 h	27.5 ± 0.6^{f}	42.1 ± 0.7^{cd}	30.4 ± 1.2^{b}

[a]The values with different superscripts in a same column are significantly different ($P < 0.05$).
[b]RDS, SDS, and RS denote rapidly digestible starch, slowly digestible starch, and resistant starch, respectively.
[c]Gelatinized starch prepared by autoclaving 40% suspension of native starch at 121 °C for 30 min, followed by drying in an air-drying oven at 40 °C for 24 h.

The RDS content showed negative correlations with the moisture content and reaction time, whereas the RS content had a positive relationship with moisture content (Table 3). The SDS content showed positive correlations with the reaction time ($P < 0.01$) and moisture content ($P < 0.05$). Thus, the impact of reaction time on SDS formation was greater than that of moisture content, but the opposite behavior was observed for RS.

The SDS content showed negative correlations with thermal parameters (T_o, T_p, T_c, and T_r), while the RS content showed positive correlations with these parameters. Whereas the SDS and RS contents displayed positive correlations with ΔH, the RDS content displayed a negative correlation with ΔH. Hoover and Manuel (1996) reported that a marginal decrease in α-amylase hydrolysis after HMT of maize starch granules is probably due to increased lipid binding and/or an increased association of starch chains in the amorphous regions. However, in our study, no evidence of amylose-lipid complex formation during hydrothermal treatment was observed. The DSC thermograms showed no endothermic peaks at temperatures ranging from 100 to 120 °C, the general melting temperatures of amylose-lipid complexes at high- or intermediate-water contents (Eliasson and Gudmundsson 2006). Besides, there were no distinguishable peaks that might represent amylose-lipid complexes in X-ray diffraction patterns.

Therefore, the decrease in starch digestion after hydrothermal treatment was due mainly to the increased association of starch chains in the amorphous regions, indicating that the description given by Hoover and Manuel (1996) could not fit the hydrothermally treated starches prepared with gelatinized starch.

Conclusion

Under the hydrothermal treatment conditions used in this study, the structural properties changed with the re-crystallization of gelatinized water yam starch, resulting in a conversion from RDS to SDS. Moreover, the hydrothermal treatment caused an alteration of the B-type crystalline structure of the native starch to A-type and increased the DRC values and the proportion of ordered structures as compared with the gelatinized starch. The reaction time showed a greater impact than moisture content on SDS enhancement. The relationship between structure and digestibility reflected the heterogeneous semi-crystalline structure of the SDS fraction, suggesting various sizes and degrees of perfection.

Acknowledgments

This work was supported by the Dept. of Agricultural Biotechnology and Center for Agricultural Biomaterials, and by a grant from the GSFS and BK21 Scholarship Program, Seoul National Univ.

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