Recrystallization of starches by hydrothermal treatment: digestibility, structural, and physicochemical properties

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Abstract Gelatinized starches were recrystallized under hydrothermal treatment and their properties were characterized by X-ray diffractometry, solid-state ¹³C cross-polarization and magic-angle spinning nuclear magnetic resonance, differential scanning calorimetry, gel-permeation chromatography, high-performance anion-exchange chromatography using pulsed amperometric detection, high-performance size-exclusion chromatography with attached multiangle laser light scattering and refractive index detectors, and digestibility analysis. Amylopectin molecules of hylon (V, VII) and water yam starch contained long side-chains with high proportion of fb₁ and fb₂. Under hydrothermal treatment, the double helix proportion and relative crystallinity significantly increased and reached maxima of water yam (48.7 and 28.2 %, respectively). Except water yam starch, X-ray diffraction pattern of all starches exhibited the evidence of type 2 amylose-lipid complex. Besides, under DSC measurement, potato and hylon starches showed the endotherm of amylose-amylose interaction. The hydrothermal treatment caused the recrystallization resulting in the decrease of RDS, especially in case of hylon and water yam starch. HTT water yam contained highest SDS (48.3 %) and HTT hylon VII contained highest RS (44.5 %). The relationship between structure and digestibility was observed, in which, high amylose content and specific structures of amylopectin molecule

were necessary for the production of RS and/or SDS of hydrothermally treated starches.

Keywords Hydrothermal treatment · Recrystallization · Resistant starch · Slowly digestible starch · Water yam starch

Introduction

Starch is the major component of many food plants and is the main carbohydrate source of energy for human body. Starch digestibility in human small intestine can vary from rapid digestion and to indigestibility (Lehmann and Robin 2007). Slowly digestible starch (SDS) cause the stabilization of postprandial blood glucose level and sustained this level overtime compared to rapid digestible starch (RDS). High SDS gives the advantage of physical and mental performance, satiety, cardiovascular disease and diabetes management (Jenkins et al. 2004). Resistant starch not only positive effect on hypocholesterolemia such as lower plasma cholesterol and triglycerides (Sajilata et al. 2006) but also the inhibition of fat accumulation (Higgins et al. 2005). Rich-RS foods not only have beneficial effects on diabetes and impaired glucose tolerance, but also can apply in the treatment of obesity and weight management. The potential benefits of RS in ameliorating the symptoms of inflammatory bowel diseases such as ulcerative colitis were reported in previous studies (Nugent 2005).

There are some factors effect on SDS content such as crystallite structure, the packing of amorphous phase and amylose content... (Colonna et al. 1990; Park and Rollings 1994). High RDS and SDS contents were observed in shorter double helices and interior crystallites in A-type starches (Jane et al. 1997). The side-chain length of amylopectin molecules affected on digestibility (Srichuwong et al. 2005). Amylose content

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correlated with RS formation (Berry 1986). Besides, the aggregation of amylose helices in a crystalline B-type structure over a particular region of the chain (about 24 glucose units) resulted in the formation of RS (Eerlingen et al. 1993).

A previous study (Park et al. 2009) reported that the recrystallization of waxy maize starch cause the increase of both SDS and RS (around 50 and 40 %, respectively) at a cycle of 4 °C for 2 d and 30 °C for 2 d. According to maize mutant starches, another paper (Zhang et al. 2008) stated that the recrystallization of HLCS (highest long-chain starch) caused the increase of both SDS and RS (~45 % of each) compared to that of HSCS (highest short-chain starch) (< 10 %). The side-chain length of amylopectin was associated to the digestibility: DP 8–12 and DP 16–26 were positively and negatively correlated with the digestion, respectively (Srichuwong et al. 2005).

Because of the cooking process before consumption of most starchy foods, the gelatinization of starch is a key factor in the food industry. Native or nearly native starch contains poor functional properties so they are not used widely in food industry. Correspondingly, the change in structure and digestibility under hydrothermal treatment of gelatinized starch may provide very useful information for the food industry.

The purpose of the current study was formation of recrystallized SDS and RS under hydrothermal treatment, characterized the impact of this recrystallization on their structure changes, and investigated the relationship between their digestibility and structural characteristics. Six different starches were chosen for this study because they exhibited different starch sources (cereal, tuber), amylose content...etc.

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, and dried at 40 °C for 3 d in a forced-air oven to reach approximately 11 % moisture content. Rice (Sigma-Aldrich, Co., Belgium), potato (KMC Ingredients, Brande, Denmark), corn (Samyang Corporation, Seoul, Korea), hylonTMV and hylonTMVII (National Starch Food Innovation, National Starch & Chemical Ltd., Seoul, Korea) starches are commercial products. The crude fat measurement of rice, corn, potato, hylon V, and hylon VII, carried out by Approved Methods 30–25 of the AACC (American Association of Cereal Chemists, 2000), showed dry weight percentages 0.17, 0.17, 0.18, 0.22, and 0.28 %; respectively.

Pancreatin (P7545, activity 8 × USP/g) and isoamylase (15, 284, activity ≥3,000,000 units/mg protein) were obtained from Sigma-Aldrich (St. Louis, Mo., U.S.A.) and amyloglucosidase (AMG 300 L, 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 a previous paper (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 (American Association of Cereal Chemists, 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.

Hydrothermal treatment

Autoclaved starch (5 % suspension, dry basis) was dried in a forced-air oven at 40 °C for 24 h to reach the moisture content of around 11 %. To prepare hydrothermally-treated (HTT) starch, autoclaved starch was weighed into a glass container and the moisture content was adjusted to 25 % with distilled water. The glass container was sealed and allowed to stand at room temperature for 24 h for equilibrium. Starch samples were stored at 100 °C for 24 h in a forced-air oven. Then, the samples were dried in a forced-air 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.

Amylose content

Amylose content (AM) was measured by using Amylose/Amylopectin Assay Kit of Megazyme (Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland).

Amylose isolation and average degree of polymerization (DP_n) of isolated amylose

The isolation of amylose from native starches was carried out following a previous study (Jane and Chen 1992). The average degree of polymerization (DP_n) of isolated amylose was

determined by dividing total carbohydrate content by its reducing residue (Chung and Liu 2009). Total carbohydrate content was analyzed by the phenol-sulfuric acid method (Dubois et al. 1956). The reducing residue was measured by dinitrosalicylic acid (DNS) method (Miller 1972).

Amylopectin side-chain length distribution

Starch (15 mg) was solubilised in 90 % DMSO (3 mL) and heated for 15 min with mixing every 2 min. Ethanol (15 mL) was added and then centrifuge at $10,000 \times g$ for 10 min. This step was done twice. The residue was collected and dry at room temperature. Distilled water (1.5 mL) was added and then heated for 10 min with mixing. Sodium acetate buffer (1.5 mL, 0.05 M, pH 4.3) was added and then heated for further 5 min. Starch was debranched by 30 μ L Isoamylase (Megazyme, E-ISAMYL, diluted 10 times) at 45 °C, 30 rpm for 2 h and boiling for 10 min to stop reaction. A debranched sample was filtered through a 0.45 μ m filter (Khanh Son Trinh et al. 2012).

The analysis was performed using HPAEC-PAD on CarboPac PA-1 anion exchange column (4 mm $\times$ 250 mm, Dionex, Sunnyval, CA, USA). 150 mM NaOH was used to equilibrate the column and 600 mM Sodium acetate in 150 mM NaOH was used to elute sample with flow rate of 1 mL/min. The gradients of sodium acetate used were as follows: linear gradients from 0 to 20 % for 0–5 min, from 20 to 45 % for 6–30 min, from 45 to 55 % for 31–60 min, from 56 to 60 % for 61–80 min, from 60 to 65 % for 81–90 min, from 66 to 80 % for 91–95 min, and from 81 to 100 % for 96–100 min. The mixture of malto-oligosaccharides (DP 1–7, Sigma Chemical) was used as standard (Khanh Son Trinh et al. 2012).

Macromolecular characterization of amylopectin

The macromolecular characterization of amylopectin molecules were done using HPSEC-MALLS-RI system (PU-2086 Plus, Jasco, Tokyo, Japan), with 200 μ L injector loop, a model degasser (NO-OX Vaccum Station, Alltech Corporate, Deerfield, IL, USA), a differential refractive index detector (OptiLab, Wyatt Technology, Santa Barbara, CA, USA). The temperature of the columns was maintained at 50.0 °C using a column heater module (Waters, USA) and a temperature control module (Waters, USA). To separate amylopectin from amylose, a Shodex OH Pak SB-G guard column and a SB-804 and a SB-806 analytical column (Showa Denko, Tokyo, Japan) were used (Stevenson et al. 2007). The mobile phase was 100 mM NaNO₃ solution containing 0.02 % sodium azide that had been filtered through 0.22 μ m filter (Milipore, USA) and degassed by a vacuum pump for at least 2 h before using. The flow rate was 0.4 mL/min and the experiment data was collected by DAWN DSP/OptiLab system

processed by ASTRA software (Version 4.90.07, Wyatt Technology) (Sang Ick Shin et al. 2009).

Starch (25 mg) was dispersed in 5.0 mL of dimethyl sulfoxide (DMSO). The suspension was continuously shaken while heating in boiling water for 15 min. Ethanol (25 mL) was added, mixed well, and this suspension was centrifuged at $10,000 \times g$ for 10 min; this step was repeated twice. The starch pellet was dry at room temperature by a hair-dryer and then dissolved in the mobile phase buffer (5.0 mL) and mixed for 15 min in boiling water. The hot sample solution was filtered through a 5.0 μ m nylon membrane filter and then injected into a HPSEC system.

Molecular weight distribution

Starch molecular weight distribution profiles were determined by using a gel-permeation chromatography (GPC) column (2.0 cm i.d. $\times$ 52 cm) packed with Sepharose CL-2B gel (Pharmacia Inc., Piscataway, NJ), mainly following the method of previous study (Jane and Chen 1992). Starch (0.5 g) was suspended with 95 % dimethyl sulfoxide (DMSO) (45 mL). The suspension was mechanically stirred while heating in a boiling water bath for 1 h and then stirred for 24 h at 25 °C to prepare a starch solution. An aliquot (2 mL) of the starch solution was mixed with absolute ethyl alcohol (8 mL) to precipitate the starch, followed by centrifugation ($10,000 \times g$, 10 min). The precipitated starch was dissolved in distilled water (10 mL) with glucose (1.5 mg, as a marker) and stirred for 30 min, and the solution was filtered through a 5.0 μ m nylon membrane filter to remove the insoluble residues. The supernatant (3.0 mL) then was injected into the column. The column was run in the ascending mode. A solution made of distilled water containing 25 mM NaCl and 1 mM NaOH was used as an eluent at a flow rate of 26.5 mL/h. Fractions of 84 drops (2230 μ L) per cup were collected. One mL of each fraction was used to determine the total carbohydrate (CHO) following a previous procedure (Dubois et al. 1956). Blue value (BV) measurement was carried out following modified-previous method (Morrison and Laignelet 1983), in which, 0.2 mL of each fraction in 0.2 mL 0.01 N I₂/KI (1.27 g/I₂L + 3 g/I KI) and 0.6 mL distilled water. This mixture stood in dark place for 30 min (25 °C) and the absorbance at $\lambda = 635$ nm was observed using a spectrophotometer (Jasco V-530, Tokyo, Japan). The ratio of blue value and total carbohydrate (BV/CHO) at peak of amylopectin fractions were calculated to compare the side-chain length of different amylopectin molecules.

Thermal properties

The thermal properties of starch samples were determined using a differential scanning calorimeter (Diamond DSC, Perkin-Elmer, Waltham, Mass., U.S.A.). Distilled water

(40 μ L) was added to a sample (10 mg) in an aluminum Perkin-Elmer DSC pan (0319–1525), which was then sealed by an O-ring (0319–1535) and a cover (0319–1526), reweighed and allowed to stand for 24 h at room temperature to attain an even distribution of water. The range of scanning temperature was 30 to 185 °C. Endothermic transition parameters included onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) of gelatinization, gelatinization temperature range ($T_r = T_c - T_o$), and enthalpy of recrystallization (or enthalpy of gelatinization, in case of native starch) (ΔH) were determined with the Pyris software (Khanh Son Trinh et al. 2012).

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-K α radiation of 0.15406 nm (Nickel filter; time constant, 4 s). Each scan was performed from 3 to 30° (2θ) (Khanh Son Trinh et al. 2012). DRC was calculated using the method of Nara (Nara and Komiya 1983) with peak-fitting software (Origin-version 7.5, OriginLab, Northampton, Mass., U.S.A.). DRC was the ratio of $A_c/(A_c + A_a)$. In this, A_c was area of crystalline portion and A_a was area of amorphous portion. The dDRC was the difference between DRC of hydrothermally treated starches and that of autoclaved starches.

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

A Bruker DSX-400 (Bruker Instrument Inc., Billerica, MA, USA) equipped with CP-MAS accessories was used for ^{13}C CP/MAS NMR analysis. Cross-polarization (CP), magic angle sample spinning (MAS), and high power decoupling conditions was used to observe NMR spectra (single scan). The acquisition time was 35 ms, time domain points, 2.2 k, and line broadening 10 Hz. The samples were spun at a rate of 5 kHz at room temperature in a 4-mm rotor with a spectral width of 3.1 kHz. Spectra were referenced to the high-field resonance of adamantane (29.5 ppm) (Sang Ick Shin et al. 2009). The the ordered (double-helical) to amorphous ratio was obtained by comparison between spectra of sample and amorphous starches (Gidley and Bociek 1985). The MestRe-C package software (Mestrelab Research, Santiago de Compostela, Spain) was used for the data processing and calculation of integrated peak areas were performed using. The dOrd was the difference between Ord (proportion of ordered structure) of hydrothermally treated starches and that of autoclaved starches.

Starch digestibility

Starch digestibility was determined according to the slightly modified method of Brumovsky and Thompson (2001). Pancreatin (2 g, P-7545, activity $8 \times \text{USP/g}$, Sigma-Aldrich, St. Louis, Mo., U.S.A) was dissolved in distilled water (24 mL) and stirred for 10 min. It was centrifuged at $1500 \times g$ 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 300 L, activity 300 AGU/mL, Novozymes, Bagsvaerd, Denmark). 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 ($5000 \times g$, 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 content after enzyme reaction for 10 and 240 min, respectively. Resistant starch (RS) constituted the fraction undigested after 240 min. dRDS, dSDS, and dRS were the differences of RDS, SDS, RS content of hydrothermally treated starches and autoclaved starches.

Statistical analysis

All experiments were performed in at least duplicate, 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 (Rodgers and Nicewander 1988) analysis (bivariate correlations algorithms, $p < 0.01$ and $p < 0.05$) were performed to characterize the relationships among characteristics of samples. All statistical analyses were conducted using SPSS software (Ver. 17.0, SPSS, Chicago, Ill., U.S.A.).

Results and discussion

Amylopectin side-chain length distribution

Side-chain length distribution of amylopectin molecules were presented on Table 1. According to the previous classification (Hanashiro et al. 1996), the side chains of amylopectin could be categorized into four fractions as follows: fa, DP 6–12; fb₁, DP 13–24; fb₂, DP 25–36; and fb₃, DP > 37. The branch-chain

Table 1 Amylopectin side-chain length distribution of starches¹

Sample	DP ² ≤ 5	fa DP 6–12	fb ₁ DP 13–24	fb ₂ DP 25–36	fb ₃ DP ≥ 37	AveDP ³	fa/fb ₁	fa/fb ₁₋₂	fa/fb ₁₋₃
Rice	3.0 ± 0.8 ^c	33.5 ± 0.2 ^a	46.8 ± 0.5 ^{cd}	10.2 ± 0.3 ^d	6.6 ± 0.2 ^a	17.3 ± 0.1 ^c	0.72 ± 0.00 ^a	0.59 ± 0.00 ^a	0.53 ± 0.00 ^a
Corn	4.5 ± 0.7 ^b	31.8 ± 0.3 ^a	45.9 ± 0.4 ^d	12.5 ± 0.2 ^c	5.3 ± 0.0 ^a	17.2 ± 0.1 ^c	0.69 ± 0.00 ^a	0.54 ± 0.01 ^{ab}	0.50 ± 0.01 ^a
Potato	8.0 ± 0.3 ^a	28.6 ± 0.7 ^b	46.2 ± 0.1 ^d	10.4 ± 0.1 ^d	6.8 ± 0.7 ^a	17.2 ± 0.3 ^c	0.62 ± 0.02 ^b	0.50 ± 0.01 ^b	0.45 ± 0.02 ^b
Hylon V	7.7 ± 0.1 ^a	22.6 ± 0.9 ^c	48.1 ± 0.5 ^c	14.0 ± 1.0 ^b	7.5 ± 0.8 ^a	18.4 ± 0.3 ^b	0.47 ± 0.07 ^d	0.37 ± 0.06 ^d	0.33 ± 0.05 ^c
Hylon VII	5.1 ± 0.3 ^b	25.5 ± 0.2 ^{bc}	50.1 ± 0.2 ^b	14.1 ± 0.3 ^b	5.3 ± 0.3 ^a	18.1 ± 1.1 ^b	0.51 ± 0.00 ^c	0.40 ± 0.01 ^c	0.37 ± 0.00 ^c
Water yam	1.5 ± 0.4 ^d	14.1 ± 0.3 ^d	61.3 ± 0.4 ^a	17.6 ± 0.1 ^a	5.5 ± 1.0 ^a	20.5 ± 0.2 ^a	0.23 ± 0.01 ^c	0.18 ± 0.01 ^c	0.17 ± 0.01 ^d

¹ The values with different superscripts in a column are significantly different ($p < 0.05$)

² The degree of polymerization

³ The average of amylopectin side-chain length

length distribution of water yam amylopectin showed a quite difference from other amylopectins. Although all starches showed the highest proportion of fb₁, their values were below 50 %, except for amylopectin of water yam (61.3 %). In amylopectin side-chain length distribution of starches, water yam showed the second-highest proportion of fb₂ but the other samples showed those of fa. Potato and Hylon (V and VII) contained the highest ratio of DP ≤ 5 (7.7–8.1 %) and this ratio of water yam was the lowest (1.5 %). The highest proportions of fb₁ and fb₂ in water yam amylopectin could be a possible reason of the highest AveDP among the tested starch samples. The values of AveDP were water yam > hylon V > hylon VII > rice > corn ≈ potato. Theoretically, the chains with DP < 15 were too short to take part in the recrystallization (Robin et al. 1974) or the minimum chain-length required for crystallization was 10, although oligomers as short as maltohexaose could co-crystallize in the presence of longer chains (Gidley and Bulpin 1987).

Macromolecular characteristics of amylopectin

Macromolecular characteristics of amylopectin were shown in Table 2. Amylopectin is very large molecular weight, large polydispersity and susceptibility to shear degradation thus amylopectin molecular weight measurement is difficult, and results vary with the analytical technique and method used to disperse the sample. M_w of amylopectins were determined by using HPSEC-MALLS-RI system vary between 10^7 – 10^9 g/mol (Jay-lin Jane 2009). Potato and water yam exhibited the high value of M_w (the weight-average molecular weight) (5.024 and 3.127×10^7 g/mol, respectively) while two hylon starches had lowest M_w (0.117 and 0.134×10^7 g/mol). Theoretically, M_w of normal starch is smaller than that of the waxy starch counterpart. This difference is caused by carbon flux partitioning between amylopectin and amylose molecules. Some normal starch amylopectins have M_w value are substantially higher than their M_n (number-average molecular weight), which indicate the presence of small molecules (Jay-

lin Jane 2009). This behavior was observed in our current study.

A previous paper (Han and Lim 2004) stated the positive correlation between the polydispersity and amylose content, which linearly raised the amylopectin polydispersity. This correlation of $AM-M_w/M_n$ in our study was 0.944^{**} (Table 6). So, our current study has an agreement with the previous one. Actually, amylopectin chains distribute in narrower molecular weight range than that of amylose does, hence polydispersity of amylose is usually greater than that of amylopectin. The amylose presence may effect the structure information of amylopectin during measurement. Some of amylose chains, which are in proximity of amylopectin structure, reside with amylopectin as contaminants (Han and Lim 2004). This statement explains the high polydispersity of hylon V and hylon VII (4.64 and 4.48, respectively), which higher than that of the other starches. The other starches showed the M_w/M_n value in the range of 1.14–1.53 as nearly similar a previous result (Han and Lim 2004). Potato and water yam exhibited lowest value of M_w/M_n (1.14 and 1.20, respectively).

The degree of molecular compactness relates to the inverse of SV_g (the specific volume for gyration). The SV_g of a macromolecule exhibits in a solution is significantly reduced by branching (Whistle and BeMiller 1997). This value gives the mass-based information on the density and degree of branching from its theoretical gyration volume per unit of molar mass. Generally, SV_g of amylose is always higher than that of amylopectin exhibiting the substantial effects of branching on molecular compactness. In our current study, this statement was observed in highly positive correlation (0.929^{**} , Table 6) of $AM-SV_g$. SV_g value in Table 2 reflected the descending degree of branching, the density and the compactness as potato > water yam > rice > corn > hylon V > hylon VII. The R_g (theoretical probability of finding the molecule at a given distance from the center) has no basis in mass (You and Lim 2000). Base on the R_g values in Table 2, the descending size of amylopectin molecules were potato > water yam > corn > rice > hylon VII > hylon V.

Table 2 Macromolecular characteristics of amylose and amylopectin molecules¹

Samples	Amylose		Amylopectin			
	AM ²	DPn	M _w	M _w /M _n	R _g	SV _g
rice	19.9 ± 0.8 ^d	844 ± 13 ^f	2.019 ± 0.026 ^c	1.39 ± 0.02 ^d	123.3 ± 5.1 ^d	0.234 ± 0.025 ^d
corn	21.3 ± 0.8 ^c	1364 ± 12 ^b	1.576 ± 0.112 ^d	1.53 ± 0.10 ^c	131.9 ± 7.0 ^c	0.368 ± 0.033 ^c
potato	14.8 ± 0.8 ^c	871 ± 18 ^e	5.024 ± 0.028 ^a	1.14 ± 0.00 ^f	150.1 ± 1.2 ^a	0.170 ± 0.004 ^d
hylon V	49.3 ± 0.4 ^b	1803 ± 13 ^a	0.134 ± 0.009 ^e	4.64 ± 0.09 ^a	73.5 ± 2.7 ^f	0.748 ± 0.060 ^b
hylon VII	70.3 ± 0.7 ^a	1251 ± 8 ^c	0.117 ± 0.003 ^e	4.48 ± 0.06 ^b	105.6 ± 0.4 ^e	2.612 ± 0.087 ^a
water yam	18.6 ± 0.7 ^d	931 ± 17 ^d	3.127 ± 0.025 ^b	1.20 ± 0.00 ^e	142.1 ± 2.9 ^b	0.232 ± 0.014 ^d

¹ The values (average ± standard deviation) with different superscripts in a same column are significantly different ($p < 0.05$)

² AM: amylose content; DPn: average degree of polymerization of isolated amylose; M_w: the weight-average molecular weight ($\times 10^7$ g/mol) (calculated by Astra software); M_w/M_n: the polydispersity; R_g: the radius of gyration (nm) (calculated by Astra software); SV_g: the specific volume for gyration (cm^3/g) ($=2.522 \times R_g^3/M_w$)

Macromolecular characteristics of amylose

The amylose content and macromolecular characteristics of amylose molecules were displayed in Table 2. The ordered amylose contents were hylon VII > hylon V > corn > rice > yam > potato and the ordered DPn values were hylon V > corn > hylon VII > water yam > potato > rice. In a previous paper (Chung and Liu 2009), the authors reported the longer potato amylose (DPn = 4915) could restrict amylose chains association resulting in lower endothermic transition temperatures and enthalpy of recrystallization. On the other hand, other amylose molecules ranged between 850 and 1410 did not exhibit any relationships between DPn of amylose and amylose association. In our current study, DPn of amylose ranged from 844 to 1803, DPn value moderately correlated with endothermic transition temperatures (data not shown), indicated the perfection of crystalline structure, but did not correlated with Ord, dOrd, DRC and dDRC. These ones explained the behavior of the first endothermic transition temperatures mainly related to the reassociation of amylopectin and DPn of amylose did not display relationship with reassociation of our samples.

Molecular weight distribution

The elution profiles of six starches presented in Fig. 1. Because of large molecular weight of amylopectin, it was located at the first peak and was excluded from the gel and appeared at the void volume. The second peak, evident in a great blue value response, is considered to be amylose. The peak at fraction 45 is glucose, added to mark the end of the elution. Intermediate material (IM), which eluted between the first peak and the second peak, was detected either as a small hump or, more likely, by an elevated baseline between these peaks. This material could not clear separated between these peaks (Wang et al. 1993). There was not clear baseline separation between the first and the second peaks, observed in

GPC profile of corn, potato and water yam starches, so the amount of IM in these starches were little. But an elevated baseline was observed in GPC profile of hylons starches (Fig. 1) indicating the presence of IM in these samples.

Base on total carbohydrate pattern, the second peak of hylon V and hylon VII were exceptionally broader and larger area than that of the other starched indicating high amylose content and broader molecular weight distribution. Results showed that ratio of blue value to total carbohydrate (BV/CHO) of amylopectin of rice, corn, potato (0.06–0.10) were lower than that of the others. These ratios of hylon (V, VII) were around 0.18 and the highest ratio was around 0.24 of water yam. These results suggested that the ordered larger proportions of longer branched chains of amylopectin molecules were yam > hylon V > hylon VII > potato > corn > rice (Franco et al. 2002). The trend of these result agreed with the result of amylopectin side-chains length above reflecting in the positive correlation (0.884**, Table 6) between AveDP and BV/CHO values.

Thermal properties

Thermal properties of autoclaved and hydrothermally treated starches were shown in Table 3. There are no any endothermic peaks were observed in autoclaved starches. However, according to the XRD observations (Table 4), the DRC value of autoclaved starches ranged from 8.5 to 13.2 % indicating that they had a crystallite structure to some extent. These implied that the results of DRC could be attributed to the crystalline structure formed with amylose molecules after autoclaving and autoclaved starches had an insignificant extent of crystalline regions formed with amylopectin molecules.

Most hydrothermally treated starches showed two endothermic peaks while HTT water yam showed the first endotherm. Under hydrothermal treatment, the first endotherm, caused by the melting of recrystallized amylopectin

Fig. 1 Sepharose CL-2B gel permeation chromatograms of starches. The values, above the first peaks, are BV/CHO of amylopectin fractions and those with different superscripts are significantly different ($p < 0.05$)

Table 3 Thermal properties of autoclaved and hydrothermally-treated starches¹

Sample	First endotherm					Second endotherm				
	T_o (°C) ²	T_p (°C)	T_c (°C)	T_r (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	T_r (°C)	ΔH (J/g)
Autoclaved starches										
Rice	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Corn	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Potato	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hylon V	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hylon VII	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Water yam	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hydrothermally- treated starches										
Rice	52.5 ± 0.1 ^c	68.1 ± 0.0 ^c	73.0 ± 0.1 ^c	20.5 ± 0.2 ^b	6.6 ± 0.3 ^a	96.0 ± 0.0 ^d	97.4 ± 0.0 ^d	105.0 ± 0.0 ^c	9.0 ± 0.0 ^c	2.5 ± 0.0 ^{bc}
Corn	63.0 ± 0.0 ^f	70.3 ± 0.0 ^d	79.5 ± 0.1 ^c	16.5 ± 0.1 ^d	5.3 ± 0.2 ^b	92.2 ± 0.0 ^e	97.2 ± 0.0 ^e	105.7 ± 0.0 ^d	13.5 ± 0.1 ^b	2.2 ± 0.1 ^c
Potato	47.9 ± 0.0 ^d	72.4 ± 0.0 ^c	78.9 ± 0.4 ^d	31.0 ± 0.4 ^a	6.7 ± 0.2 ^a	139.2 ± 0.0 ^c	143.2 ± 0.0 ^c	156.4 ± 0.0 ^b	17.2 ± 0.1 ^a	2.7 ± 0.1 ^b
Hylon V	88.0 ± 0.1 ^a	95.1 ± 0.0 ^a	105.2 ± 0.3 ^a	17.2 ± 0.2 ^c	6.1 ± 0.2 ^a	140.7 ± 0.1 ^b	146.6 ± 0.0 ^b	157.8 ± 0.1 ^a	17.1 ± 0.1 ^a	1.5 ± 0.1 ^d
Hylon VII	87.9 ± 0.1 ^b	95.1 ± 0.0 ^a	105.4 ± 0.2 ^a	17.6 ± 0.2 ^c	4.9 ± 0.2 ^b	142.9 ± 0.5 ^a	151.5 ± 0.0 ^a	156.3 ± 0.1 ^c	13.4 ± 0.4 ^b	3.5 ± 0.3 ^a
Water yam	74.9 ± 0.2 ^c	81.3 ± 0.0 ^b	88.6 ± 0.2 ^b	13.7 ± 0.3 ^c	5.4 ± 0.6 ^b	ND	ND	ND	ND	ND

¹ The values with different superscripts in a column are significantly different ($p < 0.05$)

² T_o , T_p , T_c , and T_r indicate the temperature of the onset, peak, conclusion of gelatinization, and the gelatinization temperature range, respectively; ΔH indicates the enthalpy of gelatinization

ND: Not detected

Table 4 Degree of relative crystallinity (DRC) and the proportion of ordered structure of hydrothermally treated starches¹

Starch samples	DRC ² (%)	dDRC (%)	Crystal pattern	Ord	dOrd
Autoclaved starches ³					
Aut rice	9.3 ± 0.1 ^b			31.7 ± 0.1 ^{bc}	
Aut corn	9.3 ± 0.5 ^b			34.2 ± 0.1 ^{abc}	
Aut potato	7.8 ± 0.2 ^d			33.0 ± 0.5 ^c	
Aut hylon V	8.5 ± 0.4 ^c			36.6 ± 0.2 ^a	
Aut hylon VII	8.5 ± 0.4 ^c			36.7 ± 0.0 ^a	
Aut water yam	13.2 ± 0.1 ^a			35.9 ± 0.2 ^{ab}	
Hydrothermally treated starches					
HTT rice	15.0 ± 0.6 ^f	5.7 ± 0.4 ^c	V _A	37.2 ± 0.2 ^e	5.5 ^d
HTT corn	19.1 ± 0.1 ^d	9.8 ± 0.6 ^d	V _A	39.8 ± 0.3 ^d	5.6 ^c
HTT potato	17.3 ± 0.1 ^e	9.5 ± 0.0 ^d	V _A	37.1 ± 0.2 ^f	4.1 ^f
HTT hylon V	22.4 ± 0.2 ^c	13.9 ± 0.5 ^c	V _A	42.8 ± 0.1 ^b	6.2 ^b
HTT hylon VII	25.6 ± 0.3 ^b	17.1 ± 0.7 ^a	V _A	41.9 ± 0.2 ^c	5.2 ^e
HTT water yam	28.2 ± 0.4 ^a	15.0 ± 0.4 ^b	A	48.7 ± 0.1 ^a	12.8 ^a

¹ The values (average ± standard deviation) with different superscripts in a same column are significantly different ($p < 0.05$)

² DRC: the degree of relative crystallinity; dDRC: the difference between DRC of hydrothermally treated starch and that of autoclaved starch; Ord: the ordered proportion; dOrd: the difference between Ord of hydrothermally treated starches and that of autoclaved starches

³ Autoclaved and hydrothermally treated starches were separately statistical analyzed

molecules, were detected. It has been reported that the side chain in amylopectin is less than DP 15 do not take part in the recrystallization (Ring et al. 1987). Correspondingly, a large proportion of side chains with more than DP 15 (Table 1), especially fractions of fb₁ and fb₂, presumably promoted the recrystallization of amylopectin. Recrystallized amylopectin molecules have rather low melting temperatures (T_p in the range of 55–70 °C) depending on the chain length of amylopectin and treatment condition (Eerlingen and Delcour 1995). In the first endotherm, HTT hylon (V, VII) exhibited the highest T_p (95.1 °C); the lowest T_p (68.1 °C) was belonged to HTT rice. Obviously, the long-chain double-helical crystallites, which were mainly derived from long side-chain amylopectin molecules of water yam starch or from high amylose content of hylon starches, had higher gelatinization temperatures of the first endotherm compared to other starches (Jiang et al. 2010; Eerlingen and Delcour 1995). The high endothermic peak temperatures suggested heat-stable or more perfect crystallites might be present in hydrothermally treated starches. HTT potato showed the broadest T_r (31.0 °C); whereas, HTT water yam displayed the narrowest T_r (13.7 °C). The broader T_r indicated the crystallites contained high heterogeneity, meaning various sizes and degrees of perfection, developed by recrystallization during hydrothermal treatment (Vasanthan and Bhatti 1996).

Differential scanning calorimetry (DSC) thermograms of hydrothermally treated starches (except HTT water yam) show an additional thermal transition peak, the second endotherm, at a higher temperature than the starch gelatinization

peak. Theoretically, depending on the amount and nature of lipid in samples, monoglycerides of short chain fatty acids, such as capric and lauric acids, complex with amylose directly to form the type-2 semicrystalline structure, this is a polycrystalline aggregate with well-developed long-range order displaying a V-type X-ray pattern. The lamellar crystalline of type-2 amylose-lipid complex melts at 100–125 °C (Jay-lin Jane 2009; Eliasson and Gudmundsson 2006). The similarity was found in Table 3. In which, hydrothermally rice and corn exhibited the second endothermic transition temperatures of type-2 amylose-lipid complex at around 97 °C that is slightly lower than previous report (Jay-lin Jane 2009) but higher than those of another published paper (Biliaderis and Galloway 1989). Biliaderis and Galloway stated that the transition-peak temperature, particularly of type-1 amylose-lipid complex, increase with increasing chain-length of ligand molecule. When amylose leached out of the starch samples during gelatinization, the lipids form complexes with the exuded amylose. At 95 °C in system with excess or intermediate water contents, many amylose-lipid complexes begin to melt. The presence of lipid caused a decreased melting endotherm of recrystallized amylopectin, as well as an increased size of the endotherm associated with the amylose-lipid complex transition (Eliasson and Gudmundsson 2006). The unclear crystalline amylose-lipid complex of potato, which was observed in X-ray pattern (Fig. 1), may be so little or weak to be detected by DSC measurement. Furthermore, the amylose-lipid complex of hylon (V, VII) starches (observed in Fig. 1) may be mixed in their first endotherm (Table 3) or may be so

little or weak to be detected by DSC measurement. Thus, the first endothermic enthalpy of these starches was contributed by not only the melting of imperfect amylopectin-based crystals, with potential contributions from both crystal-packing and helix-melting enthalpies (Lopez-Rubio et al. 2008), but also the melting of amylose-lipid complexes. Generally, the recrystallization was effected by amylose-lipid complex. The cocrystallization of amylose and amylopectin is possible to some extent and amylose-lipid complex eliminated the participation of amylose in the recrystallization process. Furthermore, amylopectin-lipid complex, which is contributed by outer branches of the amylopectin, could retard the recrystallization (Eliasson and Gudmundsson 2006).

Furthermore, the endothermic peaks of amylose crystal, which has the melting temperature around 150 °C, were found in hydrothermally treated potato, hylon (V, VII) (Ring et al. 1987).

¹³C CP/MAS NMR spectroscopy

The ¹³C CP/MAS NMR spectra and proportion of ordered (double-helical) structure of autoclaved and modified starches were exhibited in Fig. 2 and Table 4. The resonances around 60–67 ppm was assigned to C-6 and the wide signal around 70–79 ppm was collectively associated with C-2, C-3, and C-5 sites. The peak resonances at 82–86 ppm and 100–107 ppm were associated with C-4 and C-1 sites, respectively (Gidley and Bociek 1985; Bogracheva et al. 2001). Particularly, striking differences of hydrothermally treated starches were observed in the C-1 region, where peak intensity was shifted left to low field compared to their autoclaved starches. These differences reflected the increase of ordered (double helix) regions of modified starches under hydrothermal treatment (Gidley and Bociek 1985). The proportion of ordered structure of hydrothermally treated starches are significantly higher (dOrd from 4.1 to 6.2) than that of their autoclaved starches. Especially, the strong reassociation was showed by the highest Ord and dOrd values of water yam starch (48.7 and 12.8, respectively). The Ord value corresponded well with DRC value from XRD (0.920**, Table 6). The highly positive correlations (Table 6) between NMR results (Ord, dOrd) and amylopectin structures (fb₁, fb₂, aveDP and BV/CHO) suggested the positive effect of amylopectin structure on the reassociation under hydrothermal treatment of non-granular starches.

XRD pattern and DRC

X-ray diffraction patterns and their DRC of autoclaved and hydrothermally treated starches were showed in Fig. 1 and Table 4. All autoclaved starches did not showed any peaks reflecting their mainly amorphous structure. This statement was supported by the results from DSC scan without any

endothermic peaks of autoclaved starches were detected. All hydrothermally treated starches displayed A-type pattern with an unresolved doublet at 17.0 and 18.1 (Bogracheva et al. 2001) and two peaks at 2θ of about 15.3 and 23°. The peak around 20°, indicating the present of amylose-lipid complex (Zobel et al. 1967; Zobel 1988), was found at the X-ray diffraction pattern of hydrothermally treated (except water yam) starches. Hydrothermally treated water yam starch was A-type crystallite structure and others showed V_A-type crystal pattern. The reassociation of gelatinized starch molecules to an ordered structure during drying or cooling of gelatinized starch is referred to as retrogradation or recrystallization. Generally, the recrystallization occurred during storage of gelatinized starches with 45–50 % moisture content at temperature between glass transition temperature (indicates a change in the mobility of the molecular structure of materials, T_g = −5.0 °C) and room temperature (Eliasson and Gudmundsson 2006; Kaletunç and Breslauer 2003) resulted in the increase of DRC of hydrothermally treated starches. Previous studies reported the reassociation tends to favor the more stable A-type at a higher temperature (Gidley 1987; Gidley and Bulpin 1987). Presumably, the hydrothermal treatment of gelatinized starch induced the formation of A or V_A-type crystalline structure.

Actually, the values for ordered structures, obtained by NMR, in both autoclaved and hydrothermally starches were approximately 16.3 to 28.2 % higher than those obtain by XRD. These could be due to the significant extent of non-crystalline double helices and the irregular packing of crystalline structure (Cooke and Gidley 1992). Except autoclaved water yam starch, which had highest DRC (13.2 %), the others had DRC from 7.8–9.3 %. Under hydrothermal treatment, DRC of all starches significantly was increased. The highest DRC was HTT water yam (28.2 %) and the lowest DRC was rice (15.0 %). The difference of DRC (dDRC) of rice, corn and potato starches are fewer than that of hylon (V, VII) and water yam starches. The highest dDRC was hylon VII (17.1 %) while the lowest dDRC was rice (5.7 %). They suggested specifically structural characteristics of hylons and water yam starch affect their formation of crystalline structure under hydrothermal treatment.

In general, retrogradation is a three-step procedure (Levine and Slade 1989): (i) nucleation, formation of nuclei; and (ii) propagation, growth of crystals from the nuclei; (iii) maturation, crystal perfection. Even though recrystallization could occur to some extent during hydrothermal treatment, the high temperature (100 °C) applied in our hydrothermal treatment were not ideal for recrystallization. At the melting temperature of the crystal there is no nucleation, and the propagation rate is maximal at the melting temperature. The temperature used in this study was positioned between the glass transition and melting temperatures of amylose crystals (around 150 °C) (Ring et al. 1987). Therefore, during the hydrothermal

Fig. 2 ^{13}C CP/MAS NMR spectra of autoclaved and hydrothermally treated starches

treatment, the propagation was favored, and the nucleation was limited. Normally, in starch granule, amylose molecules are present in amorphous material and amylopectin molecules are in crystalline structure. Especially in case of water yam and hylons starches, the increasing in Ord and DRC of hydrothermally treated starches reflected that amylose molecules interacted with amylopectin molecules to form the double-helical crystalline structure during the treatment (Jiang et al. 2010). Furthermore, when starch contain high amount of amylose, the cocrystallization can be promoted (Eliasson and Gudmundsson 2006).

Table 6 displayed the positive correlations between fb_1 -DRC, fb_2 -DRC, BV/CHO-DRC, BV/CHO-dDRC ($>0.891^{**}$) and the moderate correlations of fb_1 -dDRC, fb_2 -dDRC and aveDP-dDRC (0.547^* to 0.619^*). These reflected that under dual steps (autoclaving and hydrothermal) treatment, both fb_1 and fb_2 fractions and amylopectin side-chains length well effect on the accumulated crystalline. But these

fractions moderately effected on the increase of these regions under hydrothermal treatment only.

Starch digestibility

In case of autoclaved starches, hylon (V, VII) and water yam exhibited high concentration of RS than that of the other starches. Under hydrothermal treatment, these starches gathered higher SDS + RS proportion than that of the other starches, especially SDS reflected by high dSDS values (Table 5). Theoretically, the initial stages of starch gelation are dominated by amylose. In a further stage reassociation of the amylopectin molecules, a slow process continuing over a period of several days or weeks, occurs (Eerlingen and Delcour 1995). When amylopectin concentration above 10 %, amylopectin gels, which is partially crystalline structure, can be obtained. The short branched chains (DP 14–20), which were observed in higher proportion of fb_1 fraction of

Table 5 Digestibility of starches¹

Sample	RDS ²	SDS	RS	dRDS	dSDS	dRS
Autoclaved starches						
Rice	76.7 ± 0.5 ^c	5.5 ± 0.4 ^c	17.8 ± 0.8 ^c			
Corn	77.8 ± 0.4 ^b	7.6 ± 0.4 ^b	14.6 ± 0.2 ^c			
Potato	82.3 ± 0.4 ^a	5.4 ± 0.2 ^c	12.3 ± 0.3 ^f			
Hylon V	63.9 ± 0.4 ^d	7.0 ± 0.7 ^b	29.1 ± 0.8 ^b			
Hylon VII	57.0 ± 0.5 ^c	5.5 ± 0.7 ^c	37.4 ± 0.2 ^a			
Water yam	63.1 ± 0.7 ^d	20.7 ± 0.3 ^a	16.2 ± 1.0 ^d			
Hydrothermally-treated starches						
Rice	64.5 ± 0.4 ^a	5.2 ± 0.9 ^f	30.3 ± 0.7 ^c	−12.2 ± 0.6 ^c	−0.2 ± 1.0 ^c	12.5 ± 1.1 ^b
Corn	59.6 ± 0.5 ^b	11.4 ± 0.3 ^d	29.0 ± 0.9 ^d	−18.2 ± 0.6 ^d	3.8 ± 0.5 ^d	14.4 ± 0.9 ^a
Potato	64.6 ± 0.3 ^a	9.8 ± 0.9 ^e	25.6 ± 0.7 ^e	−17.7 ± 0.5 ^d	4.4 ± 0.4 ^d	13.3 ± 0.8 ^{ab}
Hylon V	27.7 ± 0.4 ^d	32.7 ± 0.9 ^b	39.6 ± 0.6 ^b	−36.2 ± 0.6 ^a	25.7 ± 1.1 ^b	10.5 ± 1.0 ^c
Hylon VII	25.7 ± 0.5 ^c	29.8 ± 0.4 ^c	44.5 ± 0.4 ^a	−31.3 ± 0.7 ^c	24.3 ± 0.8 ^c	7.1 ± 0.4 ^d
Water yam	29.4 ± 1.2 ^c	48.3 ± 0.6 ^a	22.3 ± 0.6 ^f	−33.7 ± 1.4 ^b	27.6 ± 0.7 ^a	6.1 ± 1.2 ^d

¹ The values (average ± standard deviation) with different superscripts in a column are significantly different ($p < 0.05$), and autoclaved and hydrothermally-treated starches were separately statistical analyzed

² RDS: rapidly digestible starch; SDS: slowly digestible starch; RS: resistant starch; dRDS, dSDS, and dRS: the difference between RDS, SDS, and RS of hydrothermally-treated starch and autoclaved starch, respectively

both hylon (V, VII) and water yam starches (Table 1), reassociated to form a network of amylopectin molecules. Because of limitation of the chains, this reassociation results in less perfect crystals or in the increasing of double-helix structure (Lehmann and Robin 2007). While amylopectin is the major component of SDS, an optimum molecular structure of amylopectin was required for SDS formation (Zhang and Hamaker 2009). The length of amylopectin side-chains correlates with digestibility. The high ratio of DP 8–12 and DP 16–26 resulted in high and low hydrolysis (Srichuwong et al. 2005). This observation can be seen in high proportion of fa fraction of rice, corn and potato resulted in high value of their RDS or high proportion of fb₁ + fb₂ fractions of hylon (V, II) and water yam starches resulted in high SDS content. Furthermore, hylon (V, II) and water yam displayed longer amylopectin side-chains length (AveDP). This property of amylopectin molecules lead to form longer and more stable helices, which are further stabilized by H-bonds, distributed over the entire crystalline materials and caused low digestibility (Lehmann and Robin 2007). The positive correlations between SDS-AveDP, dSDS_AveDP, SDS_BV/CHO and dSDS_BV/CHO (0.921**, 0.787**, 0.989** and 0.965**, respectively) were agree with above statement and reflected the well relation between longer amylopectin side-chains length and SDS formation under hydrothermal treatment. In Table 6, the fractions of fb₁ and fb₂ positive correlated with SDS and dSDS exhibiting the agreement with previous statement (Jiang et al. 2010). This structure of amylopectin was result in higher SDS content of not only autoclaved water yam

but also hydrothermally treated water yam starch and hylon (V, VII).

Amylose is mainly related with RS formed through retrogradation during food storage (Sajilata et al. 2006). In our current study (Table 6), amylose content positive correlation with RS and dSDS ($r = 0.947^{**}$ and 0.600^{**} , respectively) reflected that amylose is not only the major component of RS, accumulated under the combination of autoclaving and hydrothermal treatment, but also one of the component of hydrothermally treated SDS of hylons and water yam starches. After autoclaving, during cooling/drying, amylose molecules were quickly recrystallized as the linear structure facilitates cross-linkages be means of hydrogen bonds (Sajilata et al. 2006). This recrystallization of amylose was identified as the main mechanism for the formation of RS (Berry 1986; Bjorck et al. 1990).

Actually, the long-chain double-helical crystallites, which can be seen in hydrothermally treated hylon V and water yam starch, and amylose-amylose complexes, especially in hydrothermally treated hylon (V, VII), formed block structures to protect the bulk of the amylopectin and amylose molecules from enzymatic hydrolysis (Jiang et al. 2010). This mechanism supported for the lower RDS, higher SDS + RS of hydrothermally treated hylon (V, VII) and water yam than that of other starches. Furthermore, the chain length of amylose molecule (DP_n) showed the moderately positive correlation ($r = 0.631^{**}$) with RS content of HTT starches (Table 6). Amylose molecules had DP_n in range of 100–610 were reported to increase the yield of RS from 23 to 28 % (Eerlingen

Table 6 Matrix of Pearson's correlation coefficient (r) between structural properties and digestibility of hydrothermally treated starches

	M_w	R_g	M_w/M_n	SV_g	fa	fb ₁	fb ₂	aveDP	AM	DPn	Ord	dOrd	DRC	dDRC	BV/CHO
RDS	0.572*	0.567*	-0.718**	-0.594**	0.710**	-0.592**	-0.670**	-0.710**	-0.717**	-0.503*	-0.800**	-0.484*	-0.900**	-0.924**	-0.913**
SDS					-0.925**	0.848**	0.880**	0.921**			0.967**	0.790**	0.952**	0.838**	0.989**
RS	-0.835**	-0.814**	0.936**	0.841**					0.947**	0.631**					
dRDS		-0.536*	0.661**		-0.785**	0.583*	0.725**	0.727**	0.600**	0.567*	0.815**	0.502*	0.877**	0.902**	0.941**
dSDS			0.618**	0.476*	-0.815**	0.674**	0.742**	0.787**	0.600**		0.856**	0.573*	0.924**	0.917**	0.965**
dRS				-0.496*	0.745**	-0.791**	-0.653**	-0.798**	-0.480*		-0.801**	-0.654**	-0.875**	-0.784**	-0.848**
M_w	1	0.849**	-0.822**	-0.639**					-0.796**	-0.739**					
R_g		1	-0.892**						-0.734**	-0.831**					
M_w/M_n			1	0.762**					0.944**	0.768**				0.635**	
SV_g				1					0.929**					0.675**	
fa					1						-0.918**	-0.846**	-0.821**	-0.650**	-0.914**
fb ₁						1					0.903**	0.946**	0.803**	0.547*	0.801**
fb ₂							1				0.946**	0.889**	0.806**	0.591**	0.847**
aveDP								1			0.947**	0.922**	0.836**	0.619**	0.884**
AM									1	0.580*				0.699**	
DPn										1					
Ord											1	0.897**	0.920**	0.732**	0.936**
dOrd												1	0.697**		0.717**
DRC													1	0.926**	0.963**
dDRC														1	0.891**
BV/CHO															1

*Correlation is significant ($p < 0.05$)**Correlation is highly significant ($p < 0.01$)

et al. 1993). In our current study, although amylose molecules had DPn around 844–1803 which out off the range above but the relationship between DPn of amylose and RS formation exhibited the agreement with the previous report. As mention above, amylose-amylopectin association could slightly attributed to the perfection of crystalline structure result in the increase of RS content.

Interestingly, the relationship between digestibility and macromolecular characteristics of amylopectin were observed in Table 6, in which, SDS did not show any correlations. However, the negative correlation of RS- M_w and RS- R_g (-0.835^{**} and -0.814^{**} , respectively) and the positive correlation of RS- M_w/M_n and RS-SV_g (0.936^{**} and 0.841 , respectively) suggested that the specifically macromolecular characteristics of amylopectin molecular highly effect on the formation of RS, in which, lower molecular weight, smaller size, lower degree of molecular compactness and lower branching structure lead to formation of RS₃ under our treatment.

Base on results of X-ray and ^{13}C NMR measurements (Table 4, Fig. 2 and Fig. 3), all autoclaved starches contained proportion of ordered (double-helical) structure from 31.7 to 36.7 displayed in SDS and RS of 5.5–20.7 % and 12.3–

Fig. 3 X-ray diffraction patterns of autoclaved and hydrothermally-treated starches

37.4 %, respectively. Evidently, SDS and RS of autoclaved starches mainly are in double-helical regions. In case of rice, corn and potato starches, their dDRC slightly increased (5.7–9.8 %, Table 4) under hydrothermally treatment. In the opposite, dDRC of hydrothermally treated hylon (V, VII) and water yam significantly increased (13.9–17.1 %, Table 4). Furthermore, dOrd of all HTT starches around 4.1–6.2 %, except water yam starch (12.8 %, Table 4). These behaviors indicated that both ordered and crystalline structure were increase in water yam starch under hydrothermal treatment. It was reported that in nucleation step, lower T_p value and SDS formation were favored under recrystallization at low temperature while propagation and in maturation steps, higher T_p value, A-type polymorphs, and RS formation were favored under recrystallization at high temperature (Guraya et al. 2001). In our current study, there are agreements of X-ray patterns and melting temperature values with that previous report but the behavior of digestibility changes are more complicated than those of Guraya's study. Under hydrothermal treatment, rice, corn, and potato slightly increased RS fraction whereas, hylon (V, VII) and water yam increased SDS fraction. It is well known that the resistance to amylolysis can be increased by both the ordered structures and crystalline regions. In Table 5, rice, corn, potato and hylon V showed higher dRS but hylon (V, VII) and water yam showed higher dSDS than that of the others starches. Not only positive correlations of dSDS-(Ord, dOrd, DRC, and dDRC) but also the negative correlations of dRS-(Ord, dOrd, DRC, and dDRC) indicated that the reassociation mainly favor SDS formation than RS formation under hydrothermal treatment. The harsh condition of hydrothermal treatment, especially the temperature, was not ideal for the retrogradation leading to the possibly prevention of crystallites from becoming highly ordered and bigger. This suggested that a significant amount of double-helical regions could not take part in the crystalline structure during hydrothermal treatment and these regions remained as a semi-crystalline structure, which related to the high SDS level.

Conclusion

Under hydrothermal treatment, the optimum molecular structure of amylopectin with long side-chain length, especially high proportion of both fb₁ and fb₂, is necessary for non-granular SDS formation. On the opposite, rice, corn and potato starches, which have low amylose content, short branched chains, were impossible for low digestible starch formation under hydrothermal treatment. While, the major factors effect on the formation of RS type 3 under hydrothermally treatment was not only the high concentration and the length of amylose but also the amylopectin molecules which have lower molecular weight, smaller size, broader molecular weight range and

lower degree of molecular compactness and lower branching structure resulted in high RS and low RDS concentrations under hydrothermal treatment. Finally, both amylose content and specific structure of amylopectin molecules resulted in different digestible starch fractions under hydrothermal treatment.

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