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Preparation of starches with low glycaemic response using amylosucrase and their physicochemical properties

Hee Jung Shin[a], Seung Jun Choi[a], Cheon Seok Park[b], Tae Wha Moon[a,*]

[a] Department of Agricultural Biotechnology, Center for Agricultural Biomaterials, and Research Institute for Agriculture and Life Sciences, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul 151-921, Republic of Korea
[b] Department of Food Science and Biotechnology, Kyunghee University, Yongin, Gyeonggi-do 446-701, Republic of Korea

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ABSTRACT

Enzymatic modification by amylosucrase (AS) from *Neisseria polysaccharea* was used on waxy rice, normal rice, waxy corn, normal corn, waxy potato, and normal potato starches to prepare the starches with a high content of slowly digestible starch (SDS) fraction. The AS-treated starches contained similar amounts of resistant starch, decreased rapidly digestible starch, and increased SDS compared to controls. After the AS treatment, the SDS contents increased by approximately 25% in waxy starches but about 8% in normal starches. The yields of the insoluble fraction of waxy starches were about 15% higher than those of normal starches. The branch chain length was increased by DP 13–19. The AS-treated starches showed a B-type X-ray diffraction pattern, 5–9 J/g for retrogradation enthalpy, 88–89 °C for peak temperature, and much larger moduli for their gels compared with raw starches.

1. Introduction

Starch is a storage polysaccharide in plant organs such as grains, tubers, and roots and is the most important source of human nutrition. For nutritional purposes, starch is generally classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) depending on the rate and extent of digestion (Englyst, Kingman, & Cummings, 1992). SDS is completely but slowly digested in the small intestine. RS is known as the starch fraction that escapes digestion in the small intestine. It reaches the large intestine, where it can be more or less fermented by the intestinal microflora.

The physiological advantages of SDS and RS have been reviewed (Lehmann & Robin, 2007; Sajilata, Singhal, & Kulkarni, 2006). Briefly, the principal health effect of SDS is a slow increase of postprandial blood glucose level. This can have implications for physical and mental performance, satiety, and diabetes management. RS has beneficial health functions including hypoglycaemic and hypocholesterolaemic effects, inhibition of fat accumulation, and absorption of minerals. Furthermore, both SDS and RS have low glycaemic index (GI) values. Consumption of low-GI diets is linked with higher high density lipoprotein (HDL)–cholesterol concentrations and reduced risk of developing diabetes and cardiovascular disease (Jenkins et al., 2002).

Heat, chemical, and enzymatic treatments can lead to higher SDS and RS contents in starch (Brumovsky & Thompson, 2001; Eerlingen, Crombez, & Delcour, 1993; Guraya, James, & Champagne, 2001a,b; Wolf, Bauer, & Fahey, 1999). Recently, many researchers have preferred to use enzymatic treatment because enzymatic modification is safer and healthier for humans and the environment than are other modification methods. Additional advantages of enzymatic treatment are more specific reactions, higher yield, fewer by-products, and less purification requirement (Kennedy & Dziedzic, 1995).

Most enzyme-treated starches have been studied using hydrolysis enzymes. Han et al. (2006) investigated the possibility of preparing SDS and RS concentrates from maize starch. They prepared a novel starch having 35% reduced GI using partial α-amylase treatment. Guraya et al. (2001a,b) reported the production of SDS from debranched rice starch by pullulanase with heating and cooling. High enzyme concentration and less debranching time decreased the amount of SDS, whereas longer times accelerated the production of RS.

In the present study, as a different approach for enzymatic modification, amylosucrase from *Neisseria polysaccharea* was used to prepare SDS. Amylosucrase catalyses a transglycosylation reaction to produce $(1 \rightarrow 4)$-α-glucans using sucrose as a substrate while releasing fructose in the reaction medium (Büttcher, Welsh, Willmitzer, & Kossmann, 1997). In particular, in the presence of an acceptor such as a glucosyl unit, amylosucrase catalyses the elongation of some external chains at their non-reducing end (Potocki de Montalk et al., 2000). Starch was used as an acceptor of amy-

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* Corresponding author. Tel.: +82 2 880 4854; fax: +82 2 873 5095.
E-mail address: twmoon@snu.ac.kr (T.W. Moon).

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losucrase in our study. Rolland-Sabaté, Colonna, Potocki-Véronèse, Monsan, and Planchot (2004) reported that high RS contents were obtained after α-glucans were modified with amylosucrase and explained that this result arose from the elongation of linear chains. Judging from their result, the reaction with amylosucrase could enhance SDS and RS contents of some starches. Therefore, the objectives of the present study were to enhance SDS and RS contents of several starches by amylosucrase treatment and to elucidate the relationship between the physicochemical characteristics and the digestibility of amylosucrase-treated starch.

2. Materials and methods

2.1. Materials

Waxy corn starch, corn starch (Genex Corp., Inchon, Korea), waxy rice starch (Samlip, Siheung, Korea), rice starch (Sigma, St. Louis, MO, USA), waxy potato starch (AVEBE, Veendam, Netherland), and potato starch (KMC, Brande, Denmark) were used. The apparent amylose contents were 0, 25.1, 0, 18.0, 7.5, and 29.3% for waxy corn, normal corn, waxy rice, normal rice, waxy potato and normal potato starches, respectively. Pancreatin (P-7545, activity 8× USP/g) was obtained from Sigma and amyloglucosidase (AMG 300L, activity 300 AGU/mL) from Novozymes (Bagsvaerd, Denmark). Amylosucrase (AS) from *N. polysaccharea* was provided by Food Microbiology and Bioengineering Laboratory of Kyunghee University.

2.2. Enzyme assay of AS activity

Amylosucrase was purified following the method of Jung et al. (2009). Enzyme activity was analyzed according to the method of van der Veen et al. (2004) with slight modification. It was measured under standard conditions (0.1 mL of 4% sucrose, 0.1 mL of 1% glycogen, 0.05 mL of diluted enzyme, and 0.25 mL of 100 mM sodium citrate buffer (pH 6.0)), and reacted for 10 min. The released fructose was quantified using the dinitrosalicylic acid method (Miller, 1959). One unit (U) of AS was defined as the amount of enzyme that catalyzes the consumption of 1 μmol of sucrose per min in the assay condition.

2.3. Preparation of AS-treated starches

Starches (2%, w/w) and sucrose (100 mM) were suspended in 100 mM sodium citrate buffer (pH 6.0). The suspension was boiled for 10 min to increase enzyme accessibility. After the solution was cooled to 30 °C, AS (40,000 U) was added, and incubated at 30 °C for 40 h. The total volume of solution with the enzyme was 30 mL. At the end of the reaction, soluble fraction and insoluble fraction were separated by centrifugation (10,000 × g, 10 min). The soluble fraction was washed off three times with distilled water and then once with 70% ethanol. The pellet was freeze-dried and ground to pass through a 100-mesh sieve.

Control and cooked starches were prepared to compare the properties with AS-modified starches. For control starches, starches without enzymatic modification were prepared under the same thermal regimes as the method of AS modification. Cooked starches were prepared by boiling 2% (w/w) raw starch suspension for 10 min. Both samples were also freeze-dried, ground, and sieved.

2.4. Determination of starch fractions

Starch fractions were determined according to the method of Englyst et al. (1992) with some modifications for analysis of essentially protein-free starch samples. Pancreatin (2 g) was added to 24 mL of distilled water and stirred for 10 min. This suspension was centrifuged at 1500 × g for 10 min. The supernatant (20 mL) was transferred to a beaker containing 0.4 mL of amyloglucosidase and 3.6 mL of distilled water.

For the determination of starch fractions, each sample (30 mg) was placed into a 2 mL-microtube containing 0.75 mL of 0.1 M sodium acetate buffer (pH 5.2) and a glass ball. The microtubes were equilibrated in a shaking incubator with a stroke speed of 240 rpm/min at 37 °C for 10 min. Then, 0.75 mL of the prepared enzyme solution was added to each microtube. The microtubes were removed each time at 10, 20, 60, 120, and 240 min and boiled in a cooker to stop the reaction. The glucose in the supernatant gained from centrifugation (5000 × g, 5 min) was measured with the GOD-POD kit (BCS Co., Anyang, Korea).

To measure the content of released glucose, a 0.1 mL diluted supernatant was added to a 2 mL-microtube containing 1.5 mL of glucose oxidase and peroxidase reagent. The microtube was incubated in a water bath at 37 °C for 20 min. The absorbance of the sample at 505 nm was then read.

The RDS content was measured as the amount of glucose released after 10 min of digestion. The SDS fraction was defined as digested fraction between 10 and 240 min of digestion. The unhydrolysed fraction after 240 min of digestion was measured as RS content.

2.5. Analysis of soluble fraction after AS treatment

Sugar concentration of the soluble fraction after enzyme reaction was determined by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC–PAD) on CarboPac^{TM} PA-1 anion-exchange column (4 mm × 250 mm, Dionex, Sunnyvale, CA, USA). The soluble fraction (0.1–0.15 mL) filtered through a 0.45-μm membrane filter (Millipore, Bedford, MA, USA) was injected and eluted with a gradient of 600 mM sodium acetate (0–5 min, increasing from 0 to 20%; 6–30 min, increasing from 20 to 45%; 31–60 min, 45 to 55%; 61–80 min, 56 to 60%; 81–90 min, 61 to 65%; 91–95 min, 66 to 80%, 96–100 min, 81 to 100%) in 150 mM NaOH with a flow rate of 1 mL/min.

2.6. Determination of branched chain distribution by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC–PAD)

The branched chain distributions of starches before and after AS treatment were determined by debranching the starch with isoamylase that specifically hydrolyses α-1,6-glycosidic linkages. Starch (10 mg) was solubilized in 90% DMSO (0.5 mL) and boiled for 15 min. Distilled water (0.5 mL) was added to the solution. The solution (0.5 mL) was mixed with 0.49 mL of 0.2 M sodium acetate buffer (pH 3.5) and 0.01 mL of isoamylase (Sigma). The mixture was incubated at 40 °C for 16 h and the reaction was stopped by boiling for 10 min. After centrifugation at 10,000 × g for 10 min, the supernatant was filtered using a 0.45-μm membrane filter. Debranched samples were analyzed using HPAEC–PAD on a CarboPac^{TM} PA-100 anion-exchange column (4 mm × 250 mm, Dionex). Filtered sample (0.1–0.15 mL) was injected and the same acetate gradient system was applied as previously described. DP values from 1 to 7 were designated using a mixture of maltooligosaccharides (DP 1–7, Sigma).

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

A 25-mg sample was dispersed in 5 mL of DMSO and heated for 15 min with intermittent vortexing. The solution was mixed with 25 mL of ethanol to precipitate starch. Ethanol-precipitated starch was separated by centrifugation at 10,000 × *g* for 10 min. The starch pellet was redissolved in 5 mL of 100 mM $NaNO_3$ and boiled in a cooker for 15 min. The hot sample solution was filtered through a 5.0-μm membrane filter (Millipore) and injected into a HPSEC–MALLS–RI system. The final concentration of the starch solution filtrate was determined by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Pebers, & Smith, 1956).

The HPSEC–MALLS–RI system consisted of a model PU-2080 Plus pump (JASCO, Tokyo, Japan), with a 200-μL injector loop, a degasser (NO-OX Vacuum Station, Alltech, Deerfield, IL, USA), a differential refractive index detector (Opti-Lab, Wyatt Technology, Santa Barbara, CA, USA), and Shodex OH-Pak 804 and 806 columns (Showa Denko, Tokyo, Japan). The mobile phase used for HPSEC was aqueous $NaNO_3$ (100 mM) solution containing 0.02% sodium azide that had been filtered through 0.22-μm filters (Millipore) and degassed before use. The flow rate was 0.4 mL/min and the experimental data collected from the DAWN DSP/OptiLab system were processed with ASTRA software (Version 4.09.07, Wyatt Technology).

2.8. X-ray diffractometry and relative crystallinity

X-ray diffraction analysis was performed with an X-ray diffractometer (D5005, Bruker, Karlsruhe, Germany) operating at 40 kV and 40 mA with Cu Kα radiation of 0.154 nm (nickel filter). The sample was scanned through 2θ range from 3° to 30°. The relative crystallinity was calculated from the following equation according to the method of Nara and Komiya (1983) using Origin 5.0 (Microbial, Northampton, MA, USA).

$$\text{relative crystallinity}\,(\%) = \left[\frac{A_c}{A_a + A_c}\right] \times 100$$

A_a: area of amorphous region;
A_c: area of crystalline region.

2.9. Differential scanning calorimetry (DSC)

Thermal properties of samples were investigated using a differential scanning calorimeter (Diamond DSC, Perkin-Elmer, Waltham, MA, USA). Indium was used as a calibration standard. A 3-mg sample was placed in a Seiko high-pressure stainless steel pan, and 15 mg of distilled water was added. The sample pan was sealed and kept at room temperature overnight. An empty pan was used as a reference. The sample pan was heated from 30 to 130 °C at 5 °C/min.

2.10. Measurement of rheological properties

The rheological properties of starch gel were measured using an oscillatory rheometer (Rheostress 1, Thermo HAAKE, Karlsruhe, Germany) with a cone-plate system (35 mm diameter, cone angle 1°). Starch suspensions (2, 4, 6%, w/w) were autoclaved at 121 °C for 15 min. Each autoclaved sample was placed between the cone and plate in a rheometer and then cooled to 25 °C for 1 h to form starch gel. After expelled materials were trimmed off, the sample at the edge of the system was covered by thin layer of silicone oil to prevent drying. A solvent trap was also used to minimize the water loss during the measurement. The linear viscoelastic region was determined by means of a strain sweep measurement. The frequency sweep measurement was carried out in a frequency range of 0.1–10 Hz at 25 °C.

3. Results and discussion

3.1. Yield and composition after AS treatment

The yield of the insoluble fraction and composition of the soluble fraction after the enzyme reaction were determined. After 40 h of the enzyme reaction, HPAEC–PAD detected only sucrose and fructose in the soluble fraction of all starches, suggesting that only starch chain elongation was involved. Residual sucrose concentration depended on the type of starch tested. For waxy corn, waxy rice, and waxy potato starches, the sucrose concentrations remaining after reaction were approximately 3.4, 2.5, and 2.3 mM, respectively, indicating that almost all of the initially added sucrose (100 mM) was consumed for the enzyme reaction for waxy starches. For normal starches, however, residual sucrose represented about 7 mM. The rate of sucrose consumption of waxy starches was also higher than that of normal starches. Almost all the sucrose in waxy starch samples was consumed at 15 h after the enzyme treatment, whereas most of the sucrose in normal starch samples was consumed after 40 h from the start of the enzyme treatment. Amylosucrase forms glucose by sucrose hydrolysis, releasing fructose. Consumption of sucrose increased along with production of fructose. Therefore, the fructose concentration of waxy starches after the reaction was higher than that of normal starches. Waxy starch consumed more sucrose than did normal starch during the AS treatment because waxy starch has more non-reducing ends than does normal starch.

The percentage of yield was determined as the weight percentage of the freeze-dried insoluble fraction after the reaction per weight of raw starch and sucrose before the reaction. The yields of the insoluble fraction of waxy starches, ranging from 66.7 to 69.1%, were around 15% higher than those of normal starches (data not shown).

The AS/raw values were determined using the weight ratio of freeze-dried insoluble fraction after the reaction to raw starch before the reaction. For waxy starches, the AS/raw values were approximately 2, indicating that starch was doubled after the AS treatment. However, the weights of normal starches after the AS treatment were approximately 1.5 times those before the AS treatment (data not shown).

As mentioned above, waxy starch has more non-reducing ends than does normal starch due to its high amylopectin content. Therefore, the elongation reaction occurred to a greater extent on waxy starch, leading to a higher yield, fructose production, and sucrose consumption compared with results for normal starch.

3.2. In vitro starch digestibility

Fig. 1 displays the enzymatic digestion profiles of cooked, control, and AS-modified starches. The digestion rates of the AS-modified starches were slower than those of cooked and control starches. Cooked and control starches at 10 min were digested over 70 and 50%, respectively. At 240 min, the digestion values of cooked and control starches were similar to those at 10 min. In contrast, the AS-modified waxy rice starch at 10, 20, 60, 120, and 240 min showed digestibilities of 21.2, 26.8, 33.0, 43.7, and 50.2%, respectively. However, the AS-modified normal rice starches at 240 min were digested 41.4, 44.5, 46.0, 50.0, and 53.8%, respectively. A similar pattern was also observed for waxy and normal potato starches. The digestion rates of the AS-modified waxy starches were slower than those for the AS-modified normal starches. Thus, amylosucrase treatment improved the slow digestion property of starch. In particular, the effect on waxy starches was greater than that on normal starches. Because the branched structure possessing a significant amount of short chains had a tendency toward insufficient retrogradation and formation of imperfect crystalline structure

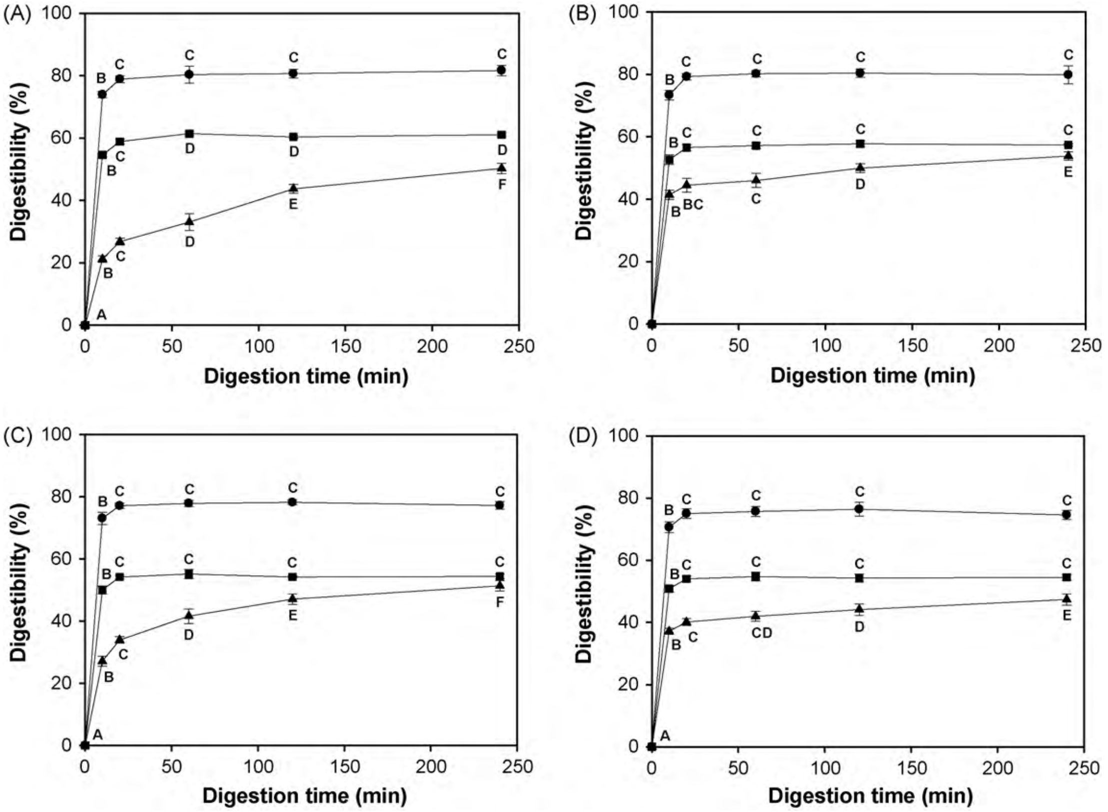

Fig. 1. Hydrolysis patterns of cooked, control, and AS-modified starches. (A) Waxy rice starch; (B) normal rice starch; (C) waxy potato starch; (D) normal potato starch; (●) cooked starch; (■) control starch; (▲) AS-modified starch.

(Würsch & Gumy, 1994), elongated amylopectin made a larger contribution to increasing SDS content than to increasing RS content.

3.3. Determination of starch fractions

The content changes in the starch fractions of cooked, control, and AS-modified starches are presented in Table 1. The RDS contents of all cooked starches were more than 70%, showing high digestibility. Because the cooking process destroys the semicrystalline structure of raw starch granules, cooking starches results in the loss of SDS and an increase of RDS (Cousin, Cuzon, & Guillaume, 1996). Control starches showed less RDS (about 50%) and more RS contents (about 40%) compared to cooked starches. The reduced digestibility of control samples compared with cooked samples is explained by retrogradation under the same conditions of AS treatment (30 °C, 40 h). Gelatinised starch is recrystallised when it is stored at ambient to refrigerated temperatures with appropriate moisture content. This change is known as retrogradation. Guraya et al. (2001b) suggested that the net rate of crystallisation has a maximum at the temperature of $T = 1/2(T_g + T_m)$ (T_g: glass transition temperature, T_m: melting temperature), usually close to room temperature. Therefore, the reaction temperature of 30 °C and long reaction time (40 h) could induce rearrangement of chains in gelatinised starch, causing a low digestibility. Retrograded starch could result in a reduction of GI value due to the increased resistance to digestive enzymes (Eerlingen et al., 1993).

The AS-modified starches produced similar amounts of RS but decreased RDS and increased SDS contents compared with control starches. In the presence of starch as an acceptor, AS catalyses the elongation of amylopectin and amylose chains at their non-reducing ends (Rolland-Sabaté et al., 2004). Therefore, the AS-modified starches seemed to have longer branched chain length than did raw starches. Jane et al. (1999) reported that starch with long branched chain tends to show a high degree of retrogradation. In addition, a high degree of retrogradation for Indica rice starch was attributed to the extra-long chain branches in the amylopectin (Lu, Chen, & Lii, 1997). When the extent of retrogradation increases, the susceptibility to digestive enzyme decreases (Eerlingen et al., 1993). Thus, the AS-modified starches showed higher SDS contents compared to control starches.

In particular, the SDS contents of the AS-modified waxy starches were 19.8–25.0% higher than those of the control starches, whereas

Table 1
Contents of RDS, SDS, and RS of cooked, control, and AS-modified starches.

Starch	Cooked starch			Control starch			AS-modified starch		
	RDS (%)	SDS (%)	RS (%)	RDS (%)	SDS (%)	RS (%)	RDS (%)	SDS (%)	RS (%)
Waxy corn	74.0 ± 2.5^a	7.5 ± 0.4^a	18.5 ± 2.8^c	53.4 ± 1.3^a	5.2 ± 1.5^a	41.4 ± 0.3^d	24.8 ± 1.3^d	30.2 ± 1.0^a	45.0 ± 0.9^d
Normal corn	72.4 ± 0.7^a	7.1 ± 0.5^{ab}	20.5 ± 0.4^{bc}	49.6 ± 0.1^c	3.8 ± 0.6^a	46.7 ± 0.6^a	43.2 ± 1.0^a	12.7 ± 0.8^c	44.2 ± 1.5^d
Waxy rice	73.9 ± 2.1^a	7.8 ± 1.7^a	18.4 ± 0.9^c	54.6 ± 0.7^a	4.9 ± 1.2^a	40.5 ± 0.6^d	21.2 ± 1.0^e	29.1 ± 1.2^a	49.8 ± 1.7^b
Normal rice	73.4 ± 1.6^a	6.5 ± 1.8^{bc}	20.1 ± 2.9^{bc}	52.7 ± 1.6^{ab}	4.7 ± 1.2^a	42.6 ± 0.4^c	41.4 ± 1.5^a	12.4 ± 0.6^c	46.2 ± 1.3^{cd}
Waxy potato	73.1 ± 2.0^a	4.1 ± 2.9^{bc}	22.8 ± 1.3^{ab}	50.0 ± 1.2^c	4.4 ± 1.4^a	45.6 ± 0.8^{ab}	27.1 ± 1.6^c	24.2 ± 0.8^b	48.7 ± 1.7^{bc}
Normal potato	70.6 ± 1.8^a	3.9 ± 1.2^c	25.4 ± 1.5^a	50.9 ± 1.1^{bc}	3.7 ± 1.4^a	45.5 ± 0.9^b	37.2 ± 0.9^b	10.2 ± 2.0^d	52.6 ± 1.8^a

The values with different superscripts in each row are significantly different ($P < 0.05$) by Duncan's multiple range test.

Table 2
Branched chain distributions of starches before and after AS treatments.

Starch		Relative area (%)				Highest detectable DP
		DP6–12	DP13–24	DP25–36	≥DP37	
Waxy rice	Raw	38.0	50.6	8.9	2.5	52
	AS-modified	1.2	32.2	43.3	23.3	71
Normal rice	Raw	33.7	52.9	10.0	3.5	54
	AS-modified	3.6	38.6	37.9	19.9	70
Waxy potato	Raw	27.1	55.9	11.5	5.5	59
	AS-modified	1.9	28.4	47.5	22.1	73
Normal potato	Raw	24.6	55.5	13.4	6.5	55
	AS-modified	1.9	32.7	45.3	20.0	68

their RDS contents were lower. The highest SDS content was obtained for the AS-modified waxy corn starch (30.2%). On the other hand, the SDS contents of normal starches were increased by around 8% after the AS treatment. Therefore, the increment of SDS content was significantly different between waxy and normal starches after the AS treatment, although no difference in the proportions of starch fractions was observed between starch sources. Due to high amylopectin content, waxy starch has more non-reducing ends than normal starch has. Therefore, as compared with normal starch, the enzyme reaction on waxy starch could be carried out to a greater extent, resulting in a higher SDS content.

3.4. Branch chain length distribution determined by HPAEC–PAD

The branch chain length distribution of amylopectin affects starch gelatinisation temperature, gelatinisation enthalpy change, retrogradation, and pasting properties (Jane et al., 1999). The chain length distributions of debranched raw and AS-modified starches, determined by HPAEC–PAD, are summarized by the sum of their relative area in Table 2. The branch chain length distribution was determined as a percentage of the total peak area, and variation in the detector response with degree of polymerization (DP) was disregarded (Hanashiro, Abe, & Hizukuri, 1996). Grouping of DP numbers followed that of Hanashiro et al. (1996), who categorized branched chains into the following four fractions: DP 6–12, 13–24, 25–36, and ≥37, corresponding to A, B_1, B_2, and B_3 and longer chains. However, this classification is no longer valid after the AS treatment. Rice amylopectin had larger proportions of fraction A and smaller proportions of fraction B_3 and longer chains, whereas potato amylopectin had a relatively smaller proportion of fraction A and a larger proportion of chains longer than B_1. These results show a clear relationship between A- and B-type crystalline polymorphs. The A type had a relatively abundant fraction A, but the B type had less. Raw starches had larger proportions (24.6–38.0%) of short chains (DP 6–12) and smaller proportions (2.5–6.5%) of long chains (≥DP 37) than did the AS-modified starches, which had 1.2–3.6 and 19.9–23.3% short and long chains, respectively. The fractions making up the largest proportions were DP 13–24 for raw starches and DP 25–36 for AS-modified starches. The longest chain lengths were found for DP 52–59 for raw starches and DP 68–73 for AS-modified starches. The branched chain length was increased by DP 13–19 after the AS treatment.

Using partial α-amylase treatment, Han et al. (2006) developed a novel maize starch having slowly digestible and resistant character compared to raw maize starch. They reported that the proportion of short chain was reduced in the partially α-amylase-treated starch having higher SDS and RS contents than raw starch and that the proportion of long chain progressively increased in the partially α-amylase-treated starch. The significant amount of short chains in amylopectin molecules could interrupt the formation of crystallites, perfectly resistant to digestion, during retrogradation. Amylopectin molecules having small amounts of short chain and large amounts of long chain could preferentially form the relatively perfect crystallites, resistant to starch-hydrolysing enzymes. In our study, AS treatment of starches increased the branched chain length of amylopectin. The decreased proportion of short chains and increased proportion of long chains in amylopectin molecules could allow the formation of crystallite having the property of slow digestion. Although the RS contents of the AS-modified starches were slightly higher than those of control starches, with the exception of normal corn starch, the RS contents of the AS-modified starches were similar to each other. In our study, the elongation of branched chains in amylopectin molecules and the retrogradation occurred at the same time. Amylose, a very long linear chain, could be rapidly retrograded rather than elongated during the AS treatment. Therefore, the similar RS contents between the AS-modified starches and control starches resulted from the retrogradation of amylose molecules. In short, the increase in SDS contents after the AS treatment could be related to the elongation of external chains in amylopectin molecules.

3.5. Molecular weight determined by a HPSEC–MALLS–RI system

Table 3 shows the weight-average molar mass (M_w) and radius of gyration (R_z) of starches determined using a HPSEC–MALLS–RI system. The M_w and R_z of starches obtained in our study were

Table 3
Weight-average molar mass (M_w), radius of gyration (R_z), and density (ρ) of starches before and after AS treatment.

Starch		M_w ($\times 10^7$, g/mol)	R_z (nm)	ρ (g/mol/nm^3)
Waxy rice	Raw	10.89 ± 0.85^{a}	182 ± 12^{de}	18.18 ± 1.62^{a}
	AS-modified	$20.54 \pm .2.56^{a}$	260 ± 13^{a}	11.75 ± 1.54^{c}
Normal rice	Raw	5.24 ± 0.49^{c}	214 ± 12^{bc}	5.32 ± 0.55^{e}
	AS-modified	10.21 ± 0.97^{a}	230 ± 13^{b}	8.34 ± 0.87^{d}
Waxy potato	Raw	5.17 ± 0.36^{c}	164 ± 11^{e}	11.82 ± 0.99^{c}
	AS-modified	10.43 ± 0.89^{a}	209 ± 12^{bc}	11.44 ± 1.08^{c}
Normal potato	Raw	2.53 ± 0.13^{d}	120 ± 9^{f}	14.66 ± 1.00^{d}
	AS-modified	4.86 ± 0.38^{c}	201 ± 10^{cd}	5.96 ± 0.52^{e}

The values with different superscripts in each row are significantly different ($P < 0.05$) by Duncan's multiple range test.

Table 4
Gelatinisation parameters of raw, control, and AS-modified starches.

Starch		T_o (°C)	T_p (°C)	T_c (°C)	$T_c - T_o$ (°C)	ΔH (J/g)
Waxy rice	Raw	58.3 ± 0.1^f	64.9 ± 0.2^b	76.0 ± 0.4^d	17.6 ± 0.3^e	11.6 ± 0.2^b
	AS-modified	75.6 ± 0.5^b	88.7 ± 0.1^a	100.1 ± 0.4^c	24.4 ± 0.8^c	7.3 ± 0.5^d
	Control	ND				
Normal rice	Raw	55.8 ± 0.7^g	62.6 ± 0.1^c	76.5 ± 0.1^d	20.7 ± 0.7^d	9.8 ± 0.2^c
	AS-modified	73.7 ± 0.8^c	88.8 ± 0.2^a	101.8 ± 1.0^b	28.1 ± 0.9^b	5.3 ± 0.3^e
	Control	ND				
Waxy potato	Raw	61.0 ± 0.2^e	65.7 ± 0.2^b	70.2 ± 0.2^e	9.1 ± 0.4^f	19.2 ± 1.5^a
	AS-modified	70.8 ± 0.4^d	88.8 ± 0.2^a	104.2 ± 0.5^a	33.4 ± 0.4^a	9.0 ± 0.5^c
	Control	ND				
Normal potato	Raw	56.1 ± 0.4^g	59.9 ± 0.1^d	64.3 ± 0.3^f	8.2 ± 0.7^f	18.4 ± 0.7^a
	AS-modified	80.4 ± 0.6^a	89.3 ± 1.3^a	102.0 ± 0.1^b	21.6 ± 0.5^d	7.6 ± 0.5^d
	Control	ND				

The values with different superscripts in each row are significantly different ($P < 0.05$) by Duncan's multiple range test.

smaller than those reported in other studies (Fisher, Rodriguez, & Chau, 1996; Yoo & Jane, 2002). The difference between our results and the reported results was possibly due to the different methods used for the preparation of starch dispersion, which could affect molecular weight determination (Millard, Dintzis, Willett, & Klavons, 1997). We did not divide the RI chromatograms into two fractions (i.e., the earlier fraction and the later fraction, representing amylopectin and amylose, respectively) because there were no clear criteria for dividing into amylopectin and amylose after the AS treatment. The M_w of waxy starches were larger than those of normal starch counterparts. A possible reason for our observation was that the amylopectins of raw waxy starches had larger M_w than those of raw normal counterparts as reported by Yoo and Jane (2002). After the AS treatment, the M_w of the AS-modified starches were approximately twice as large as those of raw starches. However, the changes in R_z and dispersed molecular density ($\rho = M_w/R_z^3$) of starches after the AS treatment were significantly different depending on the starch origin. Highly branched, compact starch has been found to show greater dispersed molecular density compared with less branched starch (Yoo & Jane, 2002). A possible reason for these results involves differences in the branched chain lengths and branching patterns of A- and B-type starches. Amylopectins of B-type starches make up longer branched chains and have larger proportions of long B chains and fewer branches than do those of A-type starches (Hizukuri, Abe, & Hanashiro, 2006; Jane, Wong, & McPherson, 1997; Jane et al., 1999; Yoo & Jane, 2002). The dispersed molar density of waxy rice starch decreased after the AS treatment, but that of waxy potato starch did not change. In addition, the dispersed molar density of normal rice starch increased, whereas that of normal potato starch decreased after the AS treatment. These results suggested that type of starch was not an important factor of the change in dispersed molar density after the AS treatment. To further understand the relationship between the change in dispersed molar density and the AS treatment, changes in dispersed molar density should be monitored following AS treatment of starches having the same type of X-ray pattern but different branched chain length and branching pattern.

3.6. *Thermal properties determined by DSC*

When starches are heated in a DSC, a melting endotherm of crystallised amylopectin is shown on the DSC thermogram. The thermal properties of the raw, control, and AS-modified starches are represented in Table 4. Raw starches showed typical DSC peaks for starch gelatinisation. Waxy starches exhibited significantly higher peak gelatinisation temperatures than normal starches did, in agreement with the findings of Yasui, Matsuki, Sasaki, and Yamamori (1996). However, the control and AS-modified starches displayed the curves observed for retrogradation. Endotherm peaks were not observed for control starches, suggesting an insignificant extent of retrogradation. In contrast, endotherm peaks were shown in the AS-modified starches. The retrogradation was accelerated by the amylopectin with longer average chain length (Kalichevsky, Orford, & Ring, 1990). Previous researchers have reported retrogradation enthalpy values for starches of below 2 J/g and T_p of approximately 50 °C under similar conditions (Kim, Kim, & Shin, 1997; Kohyama, Matsuki, Yasui, & Sasaki, 2004). However, the AS-modified starches showed values of 5–9 J/g for retrogradation enthalpy and 88–89 °C for T_p, higher than those reported for retrograded unmodified starches. This result suggests that the crystallites of the AS-modified starches in this study were more stable than those of the control and other retrograded starches.

Some starches including waxy rice, sweet rice, and waxy amaranth show low retrogradation rates. However, not all waxy starches have low retrogradation rates. Jane et al. (1999) reported that waxy maize displayed a retrogradation rate similar to that of normal maize starch. They also reported that *du* waxy maize and *ae* waxy maize, having very long branched chain length, exhibited the highest retrogradation rate among waxy rice, sweet rice, waxy amaranth, waxy maize, wheat, barley, mung bean, and other starches in their study. In general, amylose crystallites are more stable than amylopectin crystallites (Zobel, 1988), and the T_p of amylopectin crystallites is lower than that of amylose crystallites (Karim, Norziah, & Seow, 2000). During retrogradation, the behaviour of amylopectin with very long branched chains in the AS-modified starches was similar to that of long linear amylose. However, the ordered structures consisting of elongated branched chains in amylopectin of the AS-modified starches were of less perfect crystallites compared to those consisting of very long linear amylose. The retrograded RS product, prepared from amylomaize containing about 70% amylose, is very resistant to enzyme digestion (Gruchala & Pomeranz, 1993). Therefore, increased SDS contents of the AS-modified starches could be due to less perfect crystallites consisting of elongated branched chains in amylopectin. These results suggest that elongated branched chains of amylopectin could be more effective than short branched chains for formation of denser crystalline structure.

3.7. *X-ray diffraction pattern and relative crystallinity*

The X-ray diffraction patterns of raw and AS-modified starches are shown in Fig. 2. Raw normal and waxy rice starches displayed a typical A-type pattern, with diffraction peaks corresponding to Bragg angles of 15°, 17°, 18°, and 23°, as reported by Hizukuri et al. (2006). AS treatment of rice starches resulted in change in

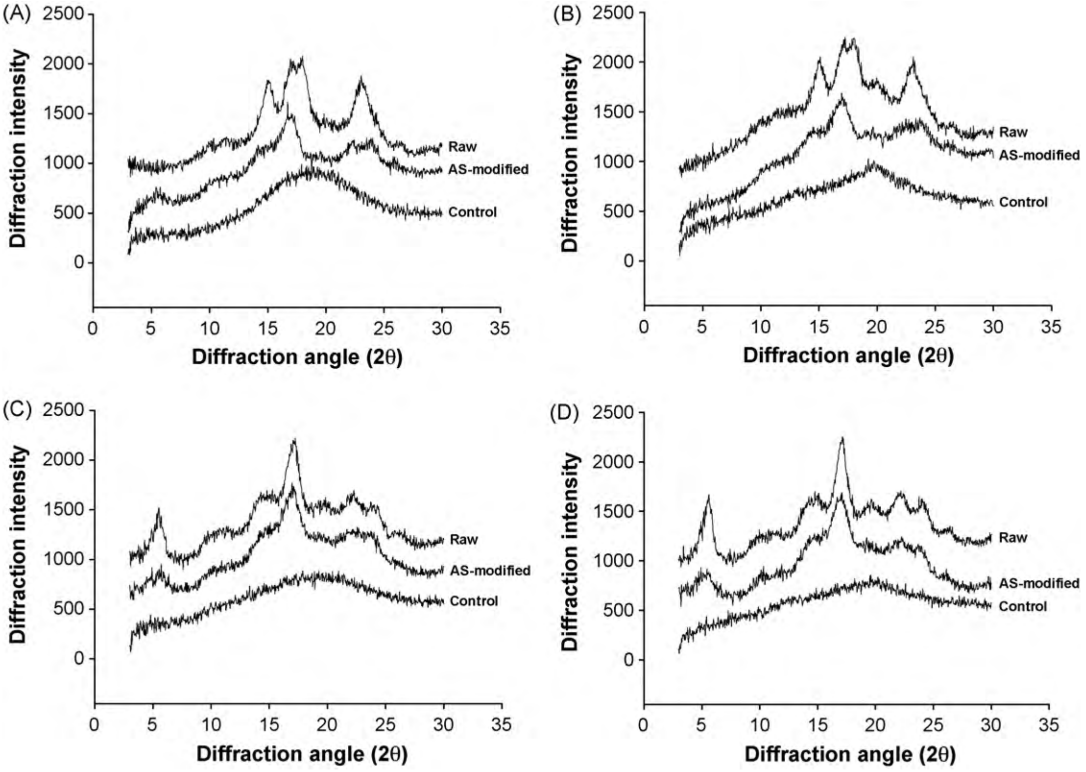

Fig. 2. X-ray diffractograms of AS-modified starches. (A) Waxy rice starch; (B) normal rice starch; (C) waxy potato starch; (D) normal potato starch.

their crystalline structure. The recorded diagrams of AS-treated rice starches were characteristic of B-type crystalline structure with main peaks emerging at 5.5°, 15°, 17°, 22°, and 24°, even though their peak intensities were slightly weak. However, no peaks were detected in control rice starches. The X-ray diffraction traces of raw normal and waxy potato starches showed a typical B-type pattern. The peaks of AS-modified potato starches were similar to that of raw starch, but their peak intensities were lower than those of raw starch. Control potato starches, like control rice starches, also had no peaks in X-ray diffractograms. These results suggest that control starches contained amorphous regions to a significant extent, which was reflected as background on their X-ray diffractograms. Leeman, Karlsson, Eliasson, and Björck (2006) have reported that retrograded starches give a B-type X-ray diffraction pattern. In addition, B-type crystals were found in retrograded cereal starch that showed an A-type structure before gelatinisation (Eerlingen et al., 1993; Kim et al., 1997). In this experiment, B-type crystalline structure in the AS-modified starches could be induced not only by the AS reaction but also by retrogradation during enzyme reaction performed at temperature of 30 °C. This suggests that at 30 °C the entanglement and formation of ordered structural crystallites among elongated branched chains of amylopectin and elongated amylose in the AS-modified starches could occur more easily compared to in control starches.

Table 5 gives the relative crystallinities of starches. Typically, natural starch granules range in degree of crystallinity from about 15 to 45% (Zobel, 1988), similar to the values obtained in this study. Relative crystallinities of raw waxy starches were higher than those of normal starches. Cheetham and Tao (1998) reported that the amylopectin content had a positive correlation with the degree of crystallinity. It has been proposed that amylose disrupts the crystalline packing of amylopectin (Jenkins & Donald, 1995). In the case of control starches, the crystalline structure of raw starches disappeared, and relative crystallinities became low because of gelatinisation before the enzyme reaction. Relative crystallinities of the AS-modified starches were higher than those of control starches, but similar to those of raw starches. As described above, elongated chains of the AS-modified starches decreased the amorphous region and increased the crystalline region. The higher crystallinities of the AS-modified starches could imply that the crystalline structures were more densely packed than in control starches.

3.8. Rheological properties

The mechanical spectra of raw and AS-modified starches at different concentrations after gelation in a frequency range of 0.1–10 Hz at 25 °C are presented in Fig. 3. The value of storage (G') and loss (G'') moduli increased with starch concentration. In regard of starch gelation, a linear relationship between the G' and concentration has been reported (Evans & Haisman, 1979). At the same concentration of starch, the moduli for AS-modified starches were always much larger than those for raw starches, indicating the increase in gel strength after the AS treatment. One possible reason for this result was that the elongated branched chains of amylopectin by AS behave like linear chains of amylose (Rolland-Sabaté et al., 2004). Jane and Chen (1992) reported that long branched chain amylopectin had a strong tendency to gel. All raw starches showed solid-like behaviour (G' larger than G'')

Table 5
Relative crystallinities of raw, control, and AS-modified starches.

Starch	Raw starch (%)	Control starch (%)	AS-modified starch (%)
Waxy rice	48.1 ± 0.7^{b}	10.7 ± 0.1^{d}	47.5 ± 0.3^{a}
Normal rice	40.7 ± 0.2^{d}	18.6 ± 0.3^{b}	39.1 ± 0.2^{d}
Waxy potato	49.1 ± 0.4^{a}	11.4 ± 0.5^{c}	44.4 ± 0.2^{b}
Normal potato	46.0 ± 0.3^{c}	19.6 ± 0.2^{a}	40.0 ± 0.2^{c}

The values with different superscripts in each row are significantly different ($P < 0.05$) by Duncan's multiple range test.

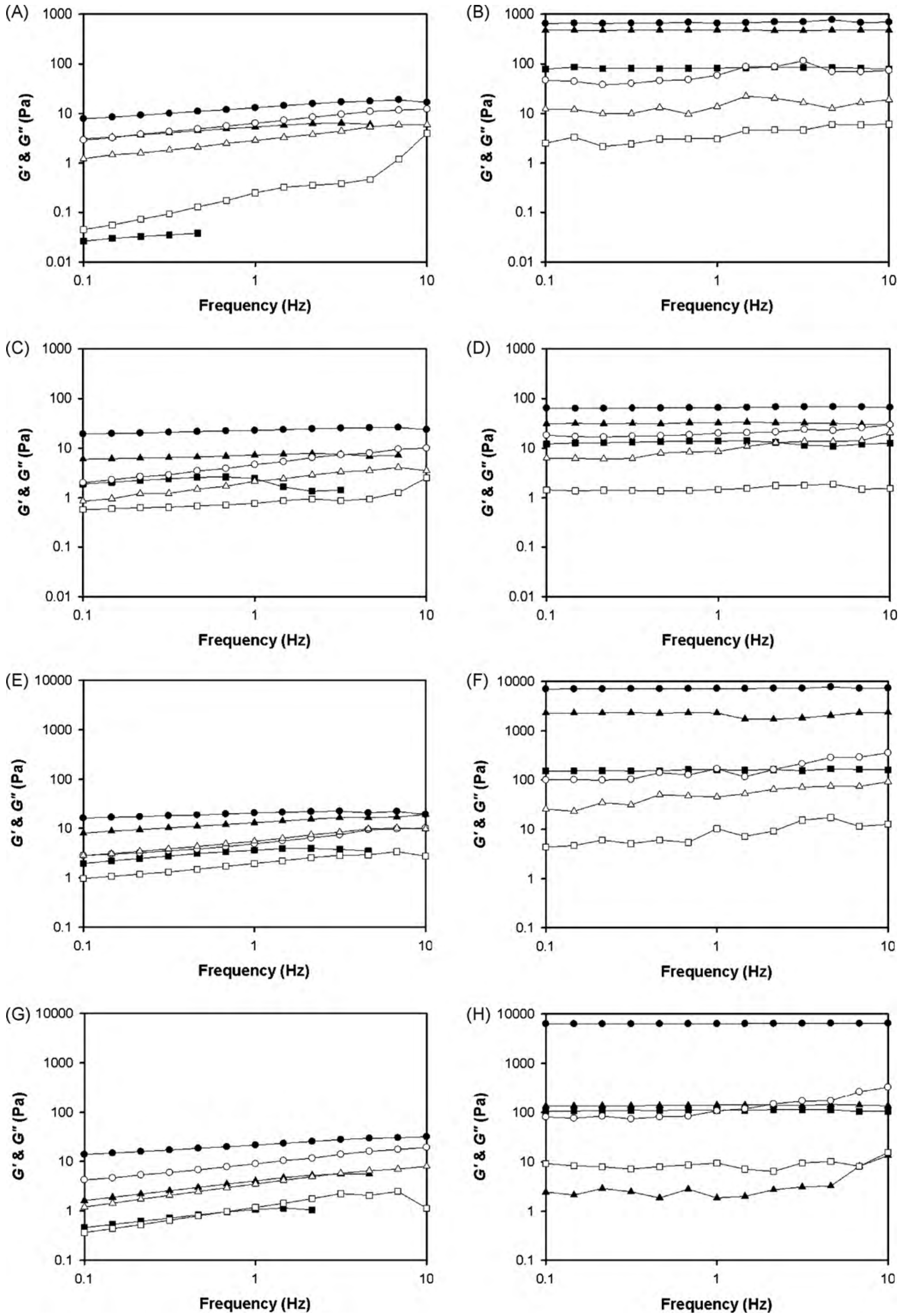

Fig. 3. Frequency dependence curves for rice and potato starch gels. (A) Raw waxy rice starch; (B) AS-modified waxy rice starch; (C) raw normal rice starch; (D) AS-modified normal rice starch; (E) raw waxy potato starch; (F) AS-modified waxy potato starch; (G) raw normal potato starch; (H) AS-modified normal potato starch. (●) 6% starch gel; (▲) 4% starch gel; (■) 2% starch gel. Filled symbols, G'; open symbols, G''.

at concentration of 6% and only a slight frequency dependence in the entire frequency range. On the other hand, the AS-modified starches showed solid-like behaviour at concentrations of 2, 4, and 6%. The G' values for 6% raw starches were equivalent to those for 2% AS-modified starches. In other words, 6% for raw starches and 2% for the AS-modified starches were the thresholds above which solid-like gelation occurred. The elongated branched chains of amylopectin by AS treatment had a strong tendency to interact and entangle between chains because the elongated branched chains of amylopectin had many possible contact points.

4. Conclusion

Amylosucrase treatment on several starches led to an increase in SDS fraction. The decreased proportion of short chains and increased proportion of long chains due to the elongation of external chains in amylopectin molecules could allow the formation of crystallites having the property of slow digestion. The mechanical spectra of starch pastes showed that the G' values for 6% raw starches were equivalent to those for 2% AS-modified starches, indicating the gelation of AS-modified starches at much lower concentrations compared with raw starches. The results of this study indicate that a novel starch having a high SDS content and gel-forming ability at low concentration could be prepared by AS treatment of starch. From a practical viewpoint, further systematic study is required to assess the thermal stability of AS-modified starches with low glycaemic response.

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