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The structural characteristics of amylosucrase-treated waxy corn starch and relationship between its *in vitro* digestibility

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Abstract The glucotransferase amylosucrase (AS) influences the structural properties of starch, but its precise effects are unclear. The structural characteristics and *in vitro* digestibility of waxy corn starch modified by AS from *Neisseria polysaccharea* were examined. AS-treated starch exhibited a higher slowly digestible starch (SDS) fraction, the weak B-type polymorph, lower relative crystallinity, and lower double helix content than those of native starches based on X-ray diffractometry, solid-state ^{13}C CP/MAS NMR, and FT-IR. AS-treated starches exhibited increased proportions of degree of polymerization (DP) 25–36 and DP≥37 chains. Higher SDS and resistant (RS) fractions, higher proportions of DP 25–36 and DP≥37 chains, more double helices, higher relative crystallinity, and less difference between double helix and relative crystallinity were observed for starch treated with 460 U than with 230 U of AS. AS re-built the double-helical and rearranged crystalline structure of gelatinized starch and consequently influenced the SDS and RS fractions.

Keywords: amylosucrase, modified starch, crystalline structure, double helix, *in vitro* digestibility

Introduction

Starch is one of the main components of various food products and is the main energy source for humans. From a nutritional point of view, starch is classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (1). SDS is digested slowly throughout the small intestine, thereby stabilizing and sustaining blood glucose concentrations and providing a prolonged release of glucose with a low glycemic index. SDS has several health benefits, e.g., preventing diabetes and cardiovascular disease and improving mental performance. Therefore, it is rapidly gaining attention as a new functional food component (2). SDS preparation by chemical, physical, or enzymatic treatment or multiple modifications of various starch sources has been reported (3-5). Enzymatic modification of the molecular structure of starch occurs by hydrolysis (α-amylase), debranching (isoamylase and pullulanase), elongation of amylose and branch chains of amylopectin (amylosucrase, AS), and dual enzyme reactions (i.e., branching enzyme with β-amylase) (3,6-9).

The molecular structure of amylopectin, and not amylose, is related to the formation of SDS (2,7,10); in particular, the chain length of amylopectin is correlated with SDS content. Lehmann and Robin (2) reported positive and negative correlations of degree of polymerization (DP) 8–12 and DP 16–26 chains with SDS content, respectively, whereas Zhang *et al*. (10) reported that DP 31–69 and DP 5–30 chains are positively and negatively correlated with SDS content, respectively.

Therefore, the length of amylopectin is a critical factor determining the SDS content, despite the results did not coincide in previous studies. Accordingly, the use of AS to modify the amylopectin branch chain length distribution has received attention. AS produces (1→4)-α-glucans by catalyzing the transglycosylation reaction using sucrose as the sole substrate, and it elongates the non-reducing end of amylopectin and amylose by up to 13–19 glucose units (11). This decreases DP<12 and increases DP>24 in amylopectin chains (3). A higher proportion of extended branched chains in amylopectin is associated with more stable double helices, further stabilized by hydrogen bonds, distributed over the entire crystalline region, resulting in decreased digestibility and an increased SDS content (2,3). In addition, AS-treated starch shows a higher RS content than that of control starch (12-14).

Various techniques are used to determine starch structural properties. X-ray diffraction (XRD) can be used to monitor the crystalline structure and relative amounts of crystalline and amorphous phases in starch in long-range order. However, this technique is limited owing to the inability to detect an irregularly packed structure (15,16). High-resolution ^{13}C cross-polarization/magic-angle spinning (^{13}C CP/MAS) NMR can be used to identify long-range structural organization at the molecular level in starches

with two polysaccharide structures: a non-ordered structure, known as an amorphous structure (single-chain), and an ordered structure, known as a crystalline structure (double helix) (15). Moreover, Fourier transform infrared (FT-IR) spectroscopy of starch is sensitive to changes in structure at the molecular level, which are related to starch chain conformation, helicity, crystallinity, and retrogradation in short-range order (17,18). Changes in the polymorphism and crystallinity of AS-treated starch have been measured by XRD (3,13,19). A combination of NMR and FT-IR analyses is expected to provide important information about the secondary structures and molecular order of AS-treated starches.

Therefore, the aim of this study was to qualitatively and quantitatively characterize starch structure using various techniques and to elucidate the effects of AS treatment on starch properties and digestibility.

Materials and Methods

Experimental materials Waxy corn starch was obtained from MSC Co., Ltd. (Yangsan, Korea). Sucrose, porcine pancreatin (P7545, EC 232-468-9, activity 8× USP (The United States Pharmacopeia)/g of pancreatin) and amyloglucosidase from *Aspergillus niger* (AMG A7095, EC 3.2.1.3, activity ≥260 U/mL of aqueous solution) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Isoamylase (EC 3.2.1.68) was purchased from Megazyme (Wicklow, Ireland). All chemicals were of analytical grade.

Amylosucrase from *Neisseria polysaccharea* The gene encoding amylosucrase (AS, EC 2.4.1.4) from *Neisseria polysaccharea* was cloned and expressed in *Escherichia coli*. The amylosucrase from *N. polysaccharea* was purified by affinity chromatography using nickel-nitrilotriacetic acid (Ni-NTA; Qiagen, Hombrechtikon, Switzerland). AS activity was determined as previously described by Jung *et al.* (20). One unit of AS corresponds to the amount of enzyme that catalyzes the production of 1 μmol fructose per minute.

Preparation of amylosucrase-treated starch A starch suspension (2%, w/w) was prepared by mixing starch with 100 mM sodium citrate buffer (pH 7.5), and 100 mM sucrose was added as a substrate, followed by boiling for 30 min. After cooling to 30°C, AS (230 U and 460 U) was added to the suspension and incubated in a water bath at 30°C for 20 h. The reaction was terminated by adding three volumes of ethanol. The AS-treated starch was precipitated by centrifugation at 10,000×*g* for 10 min, and the supernatant was removed. The precipitate was washed three times with distilled water by centrifugation at 10,000×*g* for 10 min to remove the soluble fraction. The pellet was freeze-dried, ground, and sieved with a 100-μm screen. Control starch was prepared according to the same procedure, except AS was not added. Cooked starches were prepared by boiling the 2% (w/w) raw waxy corn starch suspension for 15 min. Both samples were freeze-dried, ground, and sieved following the methods used to prepare AS-modified starches.

Determination of starch digestibility The starch fraction was measured based on the methods of Brumovsky and Thompson (21), as modified by Shin *et al.* (3), with a slight additional modification. Pancreatin (1 g) was added to distilled water (12 mL) and stirred well for 10 min. This dispersion was precipitated by centrifugation at 1,500×*g* for 10 min. The supernatant (10 mL) and amyloglucosidase (0.2 mL) were mixed with distilled water (1.8 mL) in a beaker. For the determination of the starch fraction, the starch sample (30 mg) was dispersed in a microtube (2 mL) with sodium acetate buffer (0.75 mL, 0.1 mM, pH 5.2) and a glass bead. The microtubes were equilibrated in a shaking incubator (200 rpm, 37°C) for 10 min. Then, the prepared enzyme solution (0.75 mL) was added to each microtube. The microtubes were removed at 10 and 240 min. The reaction was terminated by boiling for 10 min. To obtain glucose produced by the hydrolysis of starch, the microtube was centrifuged at 10,000×*g* for 10 min. The glucose content of the hydrolysates after starch digestion was measured using a GOD-POD kit (Embiel, Gunpo, Korea). The RDS fraction was defined as the amount of glucose released after 10 min of digestion. The SDS fraction was defined as the amount digested between 10 and 240 min of hydrolysis. The RS fraction was defined as the unhydrolyzed fraction after 240 min of digestion.

X-ray diffraction patterns and relative crystallinity An XRD analysis was conducted using an X-ray diffractometer (D8 ADVANCE with DAVINCI; Bruker, Karlsruhe, Germany) with a LYNXEYE XE detector (Bruker) at 40 kV and 40 mA with CuK_{α} radiation with wavelength 2. The starch sample was scanned at a diffraction angle (2θ) ranging from 3° to 40° and a step time of 1 s. The relative crystallinity (*RC*) was calculated from the following equation according to the method of Nara and Komiya (22) using TOPAS software (Bruker):

$$RC(\%) = \frac{A_c}{A_a + A_c}$$

where A_a is the area of the amorphous region and A_c is the area of the crystalline region.

Fourier transform infrared spectroscopy Fourier transform infrared (FT-IR) spectra of starch granules were obtained using a Nicolet 6700 (Thermo Scientific, Madison, WI, USA) with an attenuated-total-reflectance (ATR) single-reflectance cell with a ZnSe/diamond crystal. For each spectrum, 256 scans were recorded over the range of 1,200–800 cm^{-1} at a resolution of 4 cm^{-1}. The unresolved FT-IR spectra were analyzed by two methods. In the first method, all spectra were baseline corrected and then deconvoluted using OMNIC software (version 9.2; Thermo Scientific). A half-bandwidth of 15 cm^{-1} and an enhancement factor of 1.5 with triangular apodization were employed. In the second method, Gaussian curves were fitted to all spectra to resolve the crystalline and amorphous

peaks using Origin (version 8; OriginLab, Northampton, MA, USA). Intensity was measured for the deconvoluted spectra or Gaussian peaks by recording the height of the absorbance bands from the baseline.

Solid-state ^{13}C CP/MAS NMR High-resolution solid-state ^{13}C CP/MAS NMR experiments were conducted using an AVANCE 400 (Bruker) equipped with CP/MAS accessories at a frequency of 100 MHz in high-power decoupling conditions. Approximately 200 mg of starch was packed and a rotor of 4 mm in diameter was used. The samples were spun at a rate of 5 kHz with a spectral width of 3.1 kHz, acquisition time of 35 ms, and 2.2 k time domain points at room temperature. At least 4,000 scans were accumulated for each spectrum. The data processing and resonance peak spectrum integration were performed using TOPSPIN 1.3 software (Bruker BioSpin, Silberstreifen, Germany). The double helix to amorphous ratio was obtained as described by Gidley (15).

Determination of branch chain length distribution The amylopectin branch chain-length distribution was determined by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD), as previously described (3).

Statistical analysis The data are reported as the means of triplicate measurements with standard deviations. Analysis of variance (ANOVA) was performed, and differences in means were analyzed using Duncan's multiple range tests (p<0.05). The correlation between nutritional starch fractions and measured structural properties of starch were analyzed by Pearson correlation coefficients using SAS (version 9.3; SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Determination of nutritional fractions in starch The RDS, SDS, and RS proportions in native waxy corn starch, gelatinized starch, control starch, and AS-treated starches are shown in Table 1. The RDS, SDS, and RS fractions in native waxy corn starch were 34.9, 54.8, and 10.3%, respectively. Control starch was rapidly digested by hydrolytic enzymes, and had 85.3% RDS, 5.3% SDS, and 9.4% RS. After AS modification, the SDS fraction in starch increased by 30.3% and 42.7% for 230 U and 460 U, respectively. The results were in good agreement with previous reports indicating that the SDS fraction increases in response to AS (3,19). Further, the RS content for starch treated with 460 U of AS was significantly different from that of other samples (p<0.05). Similar results were obtained by Seo (14), who found that the RS content increased as the AS quantity increased, and Kim *et al.* (12), who observed a high RS fraction in AS-modified waxy adlay starch, both *in vitro* and *in vivo*. Ryu *et al.* (13) also determined that the insoluble RS content of AS-modified waxy corn starches was up to 22.3% greater than that of native starch counterparts. The SDS and RS contents were inversely proportional to the RDS content in AS-treated starches.

Table 1. Nutritional fractions of AS-treated waxy corn starches

Starch	RDS (%)	SDS (%)	RS (%)
Native	34.9±0.6[3)]	54.8±0.9	10.3±0.7
Gelatinized	90.9±1.8[a4)]	5.7±1.7[c]	3.4±1.2[a]
Control[1)]	85.3±0.9[a]	5.3±1.8[c]	9.4±1.5[b]
230 U-AS[2)]	60.5±1.5[b]	30.3±0.3[b]	9.2±1.5[b]
460 U-AS[2)]	24.6±0.6[c]	42.7±1.6[a]	32.7±2.3[c]

[1)]Control of AS-treated starches
[2)]230U, 460U-AS: amount of amylosucrase
[3)]Data are expressed as mean values and standard deviation.
[4)]Values with different superscript letters in each column are significantly different (p<0.05) by Duncan's multiple range test.

The original crystalline structure of the AS-modified starches prepared in this study was completely disrupted by the 30-min gelatinization step. Subsequently, the starch crystalline structure might be rebuilt and rearranged via intermolecular double helix formation by elongated AP with AS addition and retrogradation during 20 h of incubation (13,19). The hydrogen bonding between AP determines the crystalline structure quantity, thereby affecting the SDS and RS contents (19,23). Accordingly, XRD, FT-IR, and NMR were used to obtain the crystallinity and double helix amount in order to elucidate starch structural changes after treatment with 230 U and 460 U of AS.

X-ray diffraction patterns and relative crystallinity XRD patterns were evaluated to determine starch crystallinity and long-range molecular order. Native waxy corn starch displayed a typical A-type pattern, with major diffraction peaks at 15°, 17°, 18°, and 23° (2θ), as reported in the literature (24). Control starch did not exhibit any peaks, indicating that the starch lost crystallinity and comprised amorphous regions. After AS treatment, the diffraction peaks occurred at 5.7°, 15°, 17°, 22°, and 24°. Reflection intensity peaks (5.7°, 22°, and 24°) were characteristic of the typical B polymorph, even when they were very weakly detected (Fig. 1A). Therefore, the A-type pattern of waxy corn starch was altered to the B-polymorph by enzymatic modification. The relative crystallinities were calculated for native starch (42.9%), control starch (10.5%), 230 U AS-treated starch (24.2%), and 460 U AS-treated starch (28.0%) based on the diffraction intensities (Table 2). The relative crystallinity of 460 U AS-treated starch was not significantly different from that of 230 U AS-treated starch, but was lower than that of native starch.

For AS modification, the starch sample was perfectly gelatinized by cooking for 30 min, which completely disrupted the original crystalline structure (gelatinized starch). Subsequently, AS catalyzed the transglycosylation and elongation of amylopectin external chains, disrupted the crystalline structure, and regenerated and reordered the crystalline structure (230 U and 460 U AS-treated starch). Elongated long chains of amylopectin and retrograded AS-modified starch subjected to enzymatic reaction at 30°C for 20 h formed the B-type

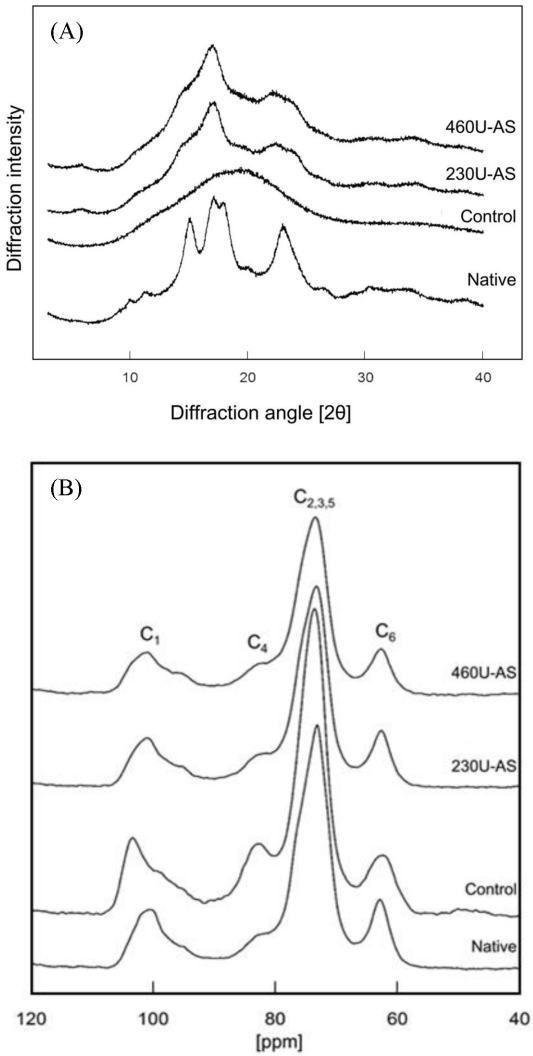

Fig. 1. X-ray diffraction patterns (A) and ^{13}C CP/NMR spectra (B) of native, AS-control, 230 U, and 460 U AS-treated waxy corn starches. Native, Native waxy corn starch; Control, AS control starch; 230U-AS, 230 U of AS-treated starch; 460U-AS, 460 U of AS-treated starch

polymorph of starch because longer amylopectin chains and retrograded starch favor the formation of the B-type crystallite (24). A- and B-type polymorphs differ in the packing of their double helices and water content; the B-type crystallite is known to be less susceptible to digestive enzymes than the A-type polymorph (25). Therefore, the X-ray pattern explained, in part, the slower digestion rate of AS-modified starch than non-modified starch, along with the B-type crystalline structure.

Solid-state ^{13}C CP/MAS nuclear magnetic resonance Solid-state ^{13}C CP/MAS NMR spectroscopy for long-range ordering is another method to determine starch crystallinity based on estimates of double helices and amorphous sites. Based primarily on chemical shifts and the line shape of the difference in C-1 and C-4 sites involved in glycosidic linkages sensitive to polysaccharide conformation, C-1 and C-4 sites were assigned to double helical and amorphous (single-chain) sites, respectively (Fig. 1B). CP/MAS resonance peaks in the regions of 80–87 and 103–104 ppm are specific of amorphous sites, whereas the signal at 99–102 ppm is characteristic to double helices (15,26). The integration of these peaks can be used to obtain the percentages of double helices and amorphous sites in starch, as shown in Table 2.

The ratio of double helix to amorphous sites for waxy corn starch was 42.9:57.1, consistent with a previous report (15). Control starch showed a 25.6% double helix content. In AS-treated waxy corn starches, the crystalline region was lower (99–102 ppm) and the resonance peak assigned to the amorphous region was higher (80–87 ppm and 103–104 ppm) comparing the properties of AS-treated starches with those of control starch. The double helix to amorphous ratio was 37.8:62.2 for 230 U and 36.2:63.8 for AS-460 U treated starches. Crystallinity and amorphous regions did not differ significantly with respect to the AS amount (i.e., 230 U vs. 460 U).

The degree of crystallinity based on the ^{13}C CP/MAS NMR analysis was similar to the results of the XRD study, but the crystallinity of AS-treated starch was lower than that of native starch. When comparing ^{13}C CP/MAS NMR and XRD data, however, the percentage of double helices was higher than the crystallinity percentage, indicating that not all double helices participated in the crystalline structure in the control and AS-treated starches.

The differences between double helix content and XRD crystallinity for control starch and starch treated with 230 U and 460 U of AS were 15.3, 13.6, and 8.2, respectively. These results indicated that control and AS-treated starches showed misalignment or less unwinding of the double helices due to the disruption of a few of the

Table 2. Crystallinity and molecular order of AS-treated waxy corn starches

	XRD	FTIR	^{13}C CP/MAS NMR		Crystallinity (XRD)-Double Helix (NMR)
	Crystallinity (%)	1047/1022	Double Helix (%)	Amorphous (%)	
Native	42.9±0.1[3)a4)]	0.550±0.003[a]	42.9±0.1[a]	57.1±0.0[c]	N/A
Control[1)]	10.3±0.1[c]	0.514±0.002[c]	25.6±0.0[d]	74.4±0.1[a]	15.3
230 U-AS[2)]	24.2±0.0[b]	0.531±0.001[b]	37.8±0.0[b]	62.2±0.1[b]	13.6
460 U-AS[2)]	28.0±0.1[b]	0.533±0.001[b]	36.2±0.1[bc]	63.8±0.0[b]	8.2

[1)]Control of AS-treated starches
[2)]230U, 460U-AS: amount of amylosucrase
[3)]Data are expressed as mean values and standard deviation.
[4)]Values with different superscript letters in each column are significantly different (p<0.05) by Duncan's multiple range test.

hydrogen bonds linking adjacent double helices, consequently resulting in the formation of imperfect double helices and an irregularly packed crystalline structure (15,27). Control and 230 U AS-treated starches contained more double helices not involved in the crystalline structure, resulting in more imperfect crystallinity and an irregularly packed structure. This probably explains the RDS, SDS, and RS contents shown in Table 1. High quantities of amorphous sites might be related to the high RDS content of control starch, and the larger difference between double helix content and XRD crystallinity was related to imperfect crystallinity and an irregularly packed structure that is easy to access for enzyme hydrolysis, explaining a high SDS content. In contrast, 460 U AS-treated starch is more perfect and regular than that treated with 230 U of AS, resulting in a higher RS content.

A similar phenomenon has been observed in previous studies (15,28). The two different techniques provided complementary information about the molecular structure of AS-treated starch. XRD detects the regularly repeating double helices formed by the amylopectin side chains, but not in irregularly packed molecular structures. In contrast, ^{13}C CP/MAS NMR provides information on the molecular organization at shorter scales than those probed by XRD, and is thus typically able to analyze a sample as a composite of ordered double helices and the amorphous component (15,27).

Fourier transform infrared spectroscopy The FT-IR spectrum is used to investigate changes in starch structure owing to sensitivity to changes in starch chain conformation, water content, crystallinity, and retrogradation at a short-range molecular level (17,18). The crystalline state was identified by the development of a band at 1047 cm^{-1} and amorphous starch was characterized by an absorbance band around 1022 cm^{-1}, which might be assigned to the C-O-H bending and CH_2-related modes; therefore, the ratio of 1047/1022 was used to resolve the proportion of ordered crystalline to amorphous domains in starches (18).

First, resolution enhancement was performed by deconvolution of FT-IR spectra. The bands at 1,022 and 1,047 cm^{-1} were distinct band

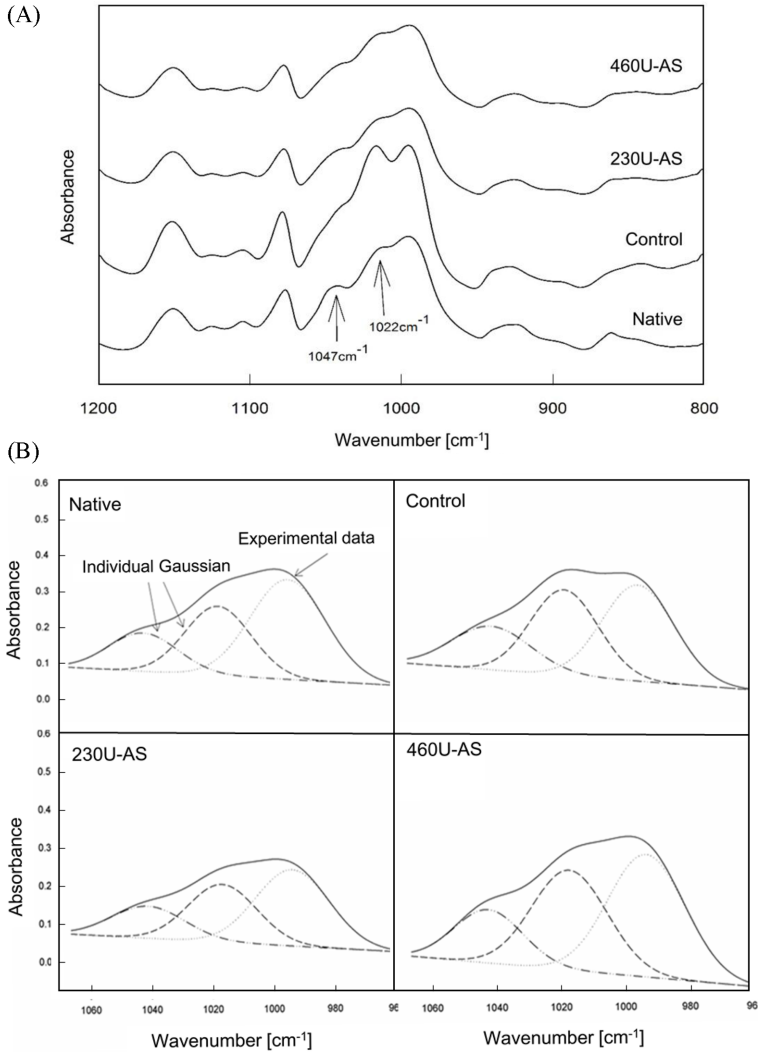

Fig. 2. Deconvoluted spectra of 1,200–800 cm^{-1} (A) and Gaussian-fitted FT-IR spectra of 965–1,070 cm^{-1} to obtain individual peaks (B) of native, AS-control, 230 U, and 460 U AS-treated waxy corn starches.

Table 3. The side chain length distributions of AS-treated waxy corn starches

	Relative peak area (%)			
	DP 6–12	DP 13–24	DP 25–36	≥DP 37
Native	22.4±0.0	51.6±0.2	18.4±0.3	7.1±0.2
Control[1)]	20.9±0.8[3)a4)]	49.1±0.7[a]	17.0±0.7[c]	8.7±0.1[c]
230 U-AS[2)]	7.1±0.4[b]	43.5±0.4[b]	33.9±0.6[b]	14.8±0.5[b]
460 U-AS[2)]	5.7±0.5[b c]	36.3±1.1[c]	39.2±1.1[a]	18.4±0.7[a]

[1)]Control of AS-treated starches
[2)]230U, 460U-AS: amount of amylosucrase
[3)]Data are expressed as mean values and standard deviation.
[4)]Values with different superscript letters in each column are significantly different (p<0.05) by Duncan's multiple range test.

Table 4. Pearson correlation coefficient for parameters describing the structure, gelatinization, and pasting properties of rice starches

	RDS	SDS	RS
Crystallinity (XRD)	0.682*[1)]	-0.588	-0.312
Double helix (NMR)	-0.728*	0.638	0.376
Amorphous (NMR)	0.731*	-0.643	-0.379
1047/1022 (FT-IR)	0.727*	-0.631	-0.370
DP 6–12	0.855**	-0.786*	-0.563
DP 13–24	0.998***	-0.979***	-0.892**
DP 25–36	-0.917***	0.862**	0.666*
≥DP 37	-0.968***	0.928***	0.771*

[1)]*, **, and *** indicate significance at the 0.05, 0.01, and 0.001 levels, respectively.

for native starch, and an intense broad band at 1,022 cm^{-1} was detected for AS control starch, as expected (Fig. 2A). However, the resolution of the peak at 1,047 cm^{-1} for control starch and AS-treated starches was limited and did not provide an individual peak. Therefore, the Gaussian curve fitting method was used as an alternative method. The Gaussian curve fitting at 965–1,070 cm^{-1} revealed three individual peaks at approximately 1,047, 1,022, and 995 cm^{-1}, as shown in Fig. 2B. The estimated ratios of 1,047/1,022 for native, control, and 230 U and 460 U AS-treated starches are presented in Table 2. FT-IR data also showed the most highly ordered structure in native waxy corn starch, followed by 460 U and then 230 U AS-treated starches, while control starch showed the least ordered structure. The ratio of 1,047/1,022 was not significantly different between 230 U and 460 U AS-treated starches. The quantitative short-range molecular order in starches obtained by FT-IR spectroscopy was in agreement with the results of XRD and ^{13}C CP/MAS NMR measurement revealing the long-range molecular ordering.

Branched chain-length distribution The relative percentages of the peak area of the DP of the branch chain length for native, control, and AS-treated starches are presented in Table 3. Amylopectin side chains were classified as A chains (DP 6–12), B_1 chains (DP 13–24), B_2 chains (DP 25–36), or B_3 chains (DP≥37) (29) according to their chain length, location in AP, and whether the chains carried other chains. However, A chains of native starch gain properties of B_1 chains via elongation after AS treatment. It is not accurate to classify these as B_1 chains because they did not carry other chains. Therefore, this classification may not apply to AS-modified starches.

After treatment with AS (230 U or 460 U), the proportion of short DP 6–12 and DP 13–24 chains decreased, and the proportion of DP 25–36 and DP≥37 chains increased considerably in amylopectin molecules. The largest fraction of native starch was observed DP 13–24 (51.6%), but DP 25–36 was the largest fraction in starch treated with 460 U of AS (39.2%). Therefore, the DP occupying the maximum peak area gradually shifted to the right after AS modification compared with those of native and control starchs (Table 3).

AS catalyzes the elongation of external branch chains of amylopectin via 13–19 glucose units. As a result of enzymatic modification, the proportion of DP 25–36 and DP≥37 chains increased, whereas the DP 6–12 and DP 13–24 fractions decreased. Assuming that 6 glucose units constitute 1 turn of the helix, the shortest chains (DP 6–12) cannot form stable double helix and thus are likely to be easily attacked by hydrolytic enzymes (30). In addition, DP 6–12 chains might disrupt the formation of an ordered crystalline structure and negatively affect the perfection of the amylopectin crystalline structure. An increase in the proportion of DP 6–12 has been found to decrease the resistance of starch to hydrolysis (31). Because DP 25–36 and DP≥37 chains form intra and intercluster connections, they are important determinants of the semicrystalline structure of starch granules (30). Through enzymatic modification, the elongation of external branch chains of amylopectin favors the formation of double helices and intercluster connections. This hinders enzyme access and decreases the rate of enzymatic digestion (3). In addition, long branched chains of starch favor retrogradation, reducing susceptibility to digestive enzymes (32). These changes might explain the increased SDS and decreased RDS fractions of AS-treated starches in the present study.

Starch nutritional fractions in relation to structural properties Pearson correlation coefficients for the relationship between the starch fraction (RDS, SDS, and RS) and molecular structural properties of AS-treated starches are presented in Table 4. The correlation coefficients were used to assess molecular structural properties for various nutritional fractions of starch. As expected, the SDS fraction was highly positively correlated with double helix (0.638), DP 25–36 (0.862, p<0.01), and DP≥37 (0.928, p<0.001) of amylopectin branch chain length, but were negatively correlated with amorphous amount, 1,047/1,022 (-0.631), DP 6–12 (−0.786, p<0.05), and DP 13–24 (−0.979, p<0.001) of amylopectin branch chain length. The RS fraction showed positive correlations with DP 25–36 (0.666, p<0.05) and DP≥37 (0.771, p<0.05) and a negatively correlation with DP 13–24 (−0.892, p<0.01) of amylopectin branch chain length. The side chain length distribution was more highly correlated with starch nutritional fractions in AS-treated starches. In particular, DP>25 was positively correlated with SDS and RS and negatively correlated with the RDS content in this study. Side chains of AP are very important

for the formation of double helices and further comprise the crystalline region, determined by NMR, XRD and, FT-IR. Therefore, the correlation coefficient were greater and more highly significant for the side change lengths of AP than for other parameters. In the present study, SDS and RS content was positively correlated with DP>25 and DP≥37; however, these results are not comparable and not consistent with those of previous studies (2,10) because AS elongates does not belong to the classical classification, as mentioned in the discussion of branch chain length distribution; the proportion of DP 25–36 and DP≥37 chains was not significantly correlated with the starch nutritional fraction in native rice starches with differing amylose content (33).

The elongation of the side chain of amylopectin by AS trans-glycosylation provides the longer branch chain and retrogradation of AS-treated starches changed the A type to the B type. NMR data revealed that elongated AP external chains formed double helices and induced rearrangement, but a greater fraction of the double-helices did not participate in the crystalline structure. The rearranged crystalline structure in starch treated with AS-230 U contributed to the SDS fraction, and the crystalline structure for 460 U may be stronger with more numerous chains, leading to digestion resistance (RS).

The results of this study showed that side chain length distribution was a critical factor determining the SDS and RS content. The current findings thus improve our understanding of the structural properties of the reduced digestibility fraction of starch, including the SDS and RS contents, which have a low glycemic index in response to AS modification.

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