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Branch chain elongation by amylosucrase: Production of waxy corn starch with a slow digestion property

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ABSTRACT

Starches with high slowly digestible starch (SDS) contents were prepared by treating completely gelatinized waxy corn starch with amylosucrase. The structural properties of the prepared starches were then investigated. The content of SDS increased by up to 38.7% after amylosucrase modification, and the portion of chains with degree of polymerisation (DP) 25–36 increased, while the portion of chains with DP $\leqslant$ 12 decreased. Amylosucrase-modified starches showed a weak B-type crystalline structure. A slight increase in the degree of relative crystallinity was observed with increased reaction time. The thermal properties, including melting temperature and enthalpy, of the amylosucrase-modified starches were higher than for the control starch. Although the amylosucrase-modified starches showed varying structural properties according to reaction time (1–45 h), their digestibilities did not change much after 6 h. By controlling the reaction time of the amylosucrase treatment, a tailored starchy food containing the desired amount of SDS can be produced.

1. Introduction

Starch is the major energy source for humans and is related to human health. The release and absorption of glucose generated by the hydrolysis of starch is related to blood glucose level, which is linked directly with health. Starch has been classified into three categories, rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992). RDS is digested rapidly in the mouth and the small intestine, is found mainly in cooked food, and can be used as an urgent energy source. SDS, which can aid in maintaining energy supply and blood glucose level, is digested completely but slowly in the small intestine. RS resists digestive enzymes, is fermented in the colon, and thus affects intestinal health (Englyst, Vinoy, Englyst, & Lang, 2003). In addition, SDS has many beneficial physiological effects and can be helpful in reducing the severity of many common chronic diseases such as obesity, diabetes, and cardiovascular disease. The glycemic index (GI) is defined as the increasing region in the blood glucose response curve after ingesting a certain amount of carbohydrates in a sample food compared with the same amount of available carbohydrate in a reference food such as glucose or white bread. Foods containing high amounts of SDS and therefore a medium or low GI help to reduce the glycemic load, whereas rapidly digestible food displays a high GI (Ells, Seal, Kettlitz, Bal, & Mathers, 2005; Englyst et al., 2003). Intake of SDS could result in a beneficial metabolic response and protection from diabetes. A meal with a high content of SDS allows for a relatively low postprandial glycemic response to carbohydrates and moderates insulin demand in type 2 diabetes (Ells et al., 2005). Also, since SDS is able to keep a postprandial insulin at a stable and low level, SDS is expected to have a high satiety effect (Lehmann & Robin, 2007).

Research regarding the production, structure, mechanism, and physiological effects of RS has been plentiful, but SDS has yet to be clearly elucidated. Several articles regarding the formation and structure of SDS by enzymatic treatment have been published (Ao et al., 2007; Casarrubias-Castillo, Hamaker, Rodriguez-Ambriz, & Bello-Pérez, 2012; Shin, Choi, Park, & Moon, 2010; Shin et al., 2004). Han et al. (2006) produced a modified maize starch via an α-amylase treatment; the starch maintained its SDS and RS content after cooking and had a low GI. It has also been established that using a high concentration and short reaction time while debranching by pullulanase is an effective strategy for the formation of SDS, and that fast cooling and storage of debranched starches increase the SDS content (Miao, Jiang, & Zhang, 2009). Shin et al. (2004) established optimal conditions for waxy sorghum starch which allow for maximum SDS content; debranching by isoamylase treatment for 8 h followed by storage at 1 °C for 3 days.

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The researchers reported that SDS consists of mainly imperfect crystalline regions containing small portions of double helices as well as amorphous regions.

Amylosucrase (AS), a glucosyltransferase from *Neiserria polysaccharea*, produces an insoluble α-1,4-linked glucan polymer by consuming sucrose and releasing fructose. This reaction does not require α-D-glucosyl-nucleotide-diphosphate like ADP- or UDP-glucose, but rather uses the energy generated by splitting sucrose in order to synthesise the glucan polymer. Moreover, sucrose as substrate is relatively inexpensive, plentiful, and eco-friendly. Because of these capabilities, many investigators have been interested in the synthesis of amylose by AS. When glycogen is used as the acceptor, elongation of the glucosyl units occurs at the non-reducing ends of the external chains, resulting in the precipitation of modified glycogen (Potocki de Montalk et al., 2000; Rolland-Sabaté, Colonna, Potocki-Véronèse, Monsan, & Planchot, 2004).

A previous study reported that amylose content was significantly correlated with RS content but not with SDS content (Zhang, Ao, & Hamaker, 2008). On the other hand, the fine structure of amylopectin is related to the formation of SDS (Ao et al., 2007; Zhang, Sofyan, & Hamaker, 2008; Zhang, Ao et al., 2008). Although SDS does not have a uniform molecular structure, amylopectin with a high proportion of either short chains or long chains tends to produce a high amount of SDS, showing a parabolic relationship between SDS content and the weight ratio of amylopectin short chains to long chains. SDS with a low ratio of short chains to long chains is a physical entity with long branch chains facilitating association among molecules, whereas SDS with more short chains is a chemical entity inherent in the special structure with high branching density and short chains of amylopectin (Zhang, Ao et al., 2008). Shin et al. (2010) reported very similar findings that the chain elongation of amylopectin and/or amylose by amylosucrase elevated the content of SDS in waxy and non-waxy starches. After the amylosucrase treatment, the proportion of long chains increased, resulting in a decrease in the weight ratio of amylopectin short chains to long chains. It could be a concrete evidence for the production of a relatively high amount of SDS by the amylosucrase treatment on starch. However, the amylosucrase treatment was carried out on all starch samples for 40 h, thus they could not monitor the changes in the branch chain length distribution and the weight ratio of amylopectin short chains to long chains during the amylosucrase reaction. Also, the relationship between the digestion property and the weight ratio of amylopectin short chains to long chains could not be determined during the amylosucrase reaction. Therefore, in this study, we prepared slowly digestible waxy corn starch by the AS treatment and investigated the effect of AS reaction time on its branch chain length distribution. Further, we examined the relationship between the proportion of long chains and their length in AS-treated waxy corn starch and its slow digestion property.

2. Materials and methods

2.1. Materials

Waxy corn starch was obtained from Samyang Genex Corporation (Incheon, Korea). Amylosucrase (AS, 230 U/mL) from *N. polysaccharea* was provided by the Food Microbiology and Bioengineering Laboratory of Kyunghee University. One unit (U) of amylosucrase was defined as the amount of enzyme catalysing the release of 1 μM fructose per min by consumption of sucrose (Potocki de Montalk et al., 2000). All other chemicals were of analytical reagent grade.

2.2. Preparation of AS-modified starches

Waxy corn starch (2%, w/w) was suspended in 100 mM sodium citrate buffer (pH 7.0) and made 100 mM in sucrose added as substrate. The starch suspension was boiled for 30 min and then cooled to 30 °C. Amylosucrase (230 U/mL) was added to the suspension and incubated at 30 °C for 1, 3, 6, 9, 15, and 45 h. Threefold ethanol was added to terminate the reaction, then the AS-modified starch was precipitated by centrifugation at 7000*g* for 10 min. The supernatant was retrieved to measure the content of released fructose during the AS treatment. The precipitate was washed three times with distilled water by centrifugation at 7000*g* for 10 min. The pellet was freeze-dried, pulverized and passed through a 100-mesh sieve. The control was prepared using the same method used for AS-modified starches except without the addition of enzyme. Cooked starch was prepared by boiling starch suspension for 15 min.

2.3. Determination of starch fractions based on digestibility

Starch fractions related to digestibility were determined by the method of Brumovsky and Thompson (2001), although with slight modifications. Pancreatin (2 g, P-7545, activity 8 × USP/g, Sigma–Aldrich, St. Louis, MO, USA) was dissolved in distilled water (24 mL), stirred for 10 min, and centrifuged at 1500*g* for 10 min. The supernatant (20 mL) was mixed with amyloglucosidase (0.4 mL, AMG 300L, activity 300 AGU/mL, Novozymes, Bagsvaerd, Denmark) and distilled water (3.6 mL). This mixture was incubated at 37 °C for 10 min to obtain the enzyme solution, which was newly prepared every time prior to the experiment.

A sample (30 mg) and a glass bead were placed in a 2 mL-microtube, and 100 mM sodium acetate buffer (0.75 mL, pH 5.2) was added. This mixture was then kept in a shaking incubator at 37 °C for 10 min with a stroke speed of 240 rpm. Then, the prepared enzyme solution (0.75 mL) was added to each microtube at regular intervals. The microtubes were then kept in a shaking incubator at 37 °C and tested at 10 and 240 min. To terminate the enzyme reaction, each microtube was boiled for 10 min. The glucose released by the hydrolysis of the starch samples was measured using a GOD-POD kit (BCS, Anyang, Korea) following centrifugation at 5000*g* for 5 min.

Starch fractions were determined according to the degree of hydrolysis. RDS was measured as the amount of glucose released after the reaction was allowed to progress for 10 min. SDS was the fraction digested between 10 and 140 min. The undigested fraction that remained after 240 min was measured as RS.

2.4. Analysis of the soluble fraction after amylosucrase treatment

Following the AS reaction, the remaining sucrose and fructose that were released were retrieved from the supernatant after the first centrifugation (5000*g*, 15 min) to determine the composition of the soluble fraction. Ethanol was evaporated using a SpeedVac Concentrator (Savant AES 1010, GMI, Ramsey, MN, USA) for 4 h. The fraction obtained was redissolved in distilled water. All of the samples were filtered through a 0.45-μm membrane filter and analysed using HPAEC on a CarboPac PA-1 anion exchange column (250 × 4 mm; Dionex, Sunnyvale, CA, USA) with a pulsed amperometric detector (PAD, Dionex). The analysis was performed using 150 mM sodium hydroxide for column equilibration and 600 mM sodium acetate in 150 mM sodium hydroxide for sample elution with a flow rate of 1 mL/min. The linear gradients for the latter were from 0% to 15% for 0 to 10 min and from 15% to 100% for 10 to 15 min.

2.5. Determination of branched chain length distribution

Sample (15 mg) was dissolved in 90% DMSO (3 mL) and boiled for 15 min with vortexing. Ethanol (15 mL) was added, and then the mixture was centrifuged twice at 10,000*g* for 10 min. After removing the supernatant, distilled water (1.5 mL) and sodium acetate buffer (1.5 mL, 50 mM, pH 4.3) were added, and then the sample mixture was boiled. Isoamylase (30 μL, 1000 U, Sigma–Aldrich) was added to the mixture and incubated at 45 °C for 2 h. The enzyme reaction was finished by boiling for 10 min. Debranched samples were filtered through a 0.45-μm membrane filter and analysed under the following conditions: linear gradients from 0% to 20% for 0 to 5 min, from 20% to 45% for 6 to 30 min, from 45% to 55% for 31 to 60 min, from 56% to 60% for 61 to 80 min, from 61% to 65% for 81 to 90 min, from 66% to 80% for 91 to 95 min, and from 81% to 100% for 96 to 100 min. A mixture of maltooligosaccharides (DP 1–7, Sigma Chemical) was used as a standard to determine the degree of polymerisation (DP) of the resulting samples.

2.6. Determination of X-ray diffraction pattern

X-ray diffraction (XRD) analysis was performed using a Powder X-ray diffractometer (D5005, Bruker, Karlsruhe, Germany) operating at 40 kV and 40 mA with Cu Kα radiation of 0.154 nm (nickel filter; time constant, 4 s). The sample was scanned through 2θ range from 3° to 30°.

Relative crystallinity was calculated according to the following equation (Nara & Komiya, 1983) using Origin 5.0 (MicroCal, Northampton, MA, USA):

$$\text{Relative crystallinity } (\%) = [A_c/(A_a + A_c)] \times 100$$

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

2.7. Determination of thermal properties

The thermal properties of raw, control, and AS-modified starches were determined using a differential scanning calorimeter (Diamond DSC, Perkin–Elmer, Waltham, MA, USA). Samples (15 mg) were placed in an aluminium pan (Seiko, Tokyo, Japan), and distilled water (45 mg) was added. The samples were then sealed and equilibrated at room temperature for at least 4 h. DSC scan was performed from 30 to 130 °C at a rate of 5 °C/min. An empty pan was used as a reference.

2.8. Statistical analysis

All experiments were performed in triplicate, and mean values are reported with standard deviations. Analysis of variance (ANOVA) was performed, and the mean separation was analysed by Duncan's multiple range test ($p < 0.05$). All statistical analyses were conducted using SPSS for Windows 12.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Yield and soluble fraction composition

The yield of the insoluble fraction and the composition of the soluble fraction after AS modification are shown in Table 1. The percentage of yield was calculated by dividing the weight of the freeze-dried insoluble fraction after the reaction by the weight of raw starch.

Table 1
Yield and soluble fraction composition.

Reaction time (h)	Yield (%)	Soluble fraction composition	
		Sucrose (mM)	Fructose (mM)
1	64.8 ± 2.9^{c}	88.5 ± 3.2^{d}	6.0 ± 0.8^{e}
3	85.0 ± 2.8^{b}	85.4 ± 3.2^{cd}	17.9 ± 0.9^{d}
6	106.1 ± 1.6^{a}	78.7 ± 5.0^{b}	24.7 ± 2.7^{c}
9	109.2 ± 2.1^{a}	79.5 ± 3.7^{bc}	29.7 ± 1.8^{b}
15	110.7 ± 2.3^{a}	76.6 ± 0.8^{ba}	29.7 ± 0.6^{b}
45	106.6 ± 4.1^{a}	71.4 ± 3.7^{a}	33.5 ± 2.7^{a}

The values having different superscripts in the same column are significantly different ($p < 0.05$) by Duncan's multiple range test.

After AS modification, the yield gradually increased with reaction time. When AS modification was conducted for 1 and 3 h, 64.8% and 85.0%, respectively, insoluble starch fractions were obtained. This result suggests that the raw starch originally contained soluble compounds such as oligomers and small-sized amylopectin molecules. When the reaction was allowed to progress for up to 3 h, the soluble molecules were not elongated enough to be insoluble. After the reaction progressed for over 6 h, the yield of the insoluble fractions was over 100%, suggesting that the elongation reaction extensively occurred on amylopectin and soluble molecules; consequently, the elongated molecules became insoluble.

For AS modification, sucrose, the sole substrate, had to be added. Once the reaction began, the consumption of sucrose and the release of fructose both occurred. These processes decreased and increased respectively with reaction time. However, sucrose remained between 71.4 and 88.5 mM over the entire AS modification reaction, indicating that the added AS could not use all of the substrate during the enzyme reaction. Table 1 shows that the rates of sucrose consumption and fructose release were not the same at all time intervals during the reaction. The rate of sucrose consumption was high during the early phases of the reaction and decreased as the reaction time increased. Neither reduced AS activity or lack of substrate could be the reason for the change in the reaction rate during the reaction. Our previous experiments indicated that AS maintained almost 100% of its initial activity during a 45 h reaction (data not shown). Also, as described above, after a 45 h reaction, sucrose still remained at 71% of its initial concentration. One of the hypotheses for the action of amylosucrase on glycogen is the elongation of all external chains from the non-reducing ends, with DP increasing from 12 to 18 glucosyl units (Potocki de Montalk, Remaud-Simeon, Willemot, Planchot, & Monsan, 1999). Therefore, chain elongation by attaching 12–18 glucosyl units occurred over the course of the entire reaction, but the attachment of glucose extensively occurred during the early phase of the reaction.

3.2. Branched chain length distribution

The branched chain length distributions of debranched AS-modified starches are presented in Fig. 1 and Table 2. Hanashiro, Abe, and Hizukuri (1996) categorised branched chains into four fractions: A, B_1, B_2, and B_3 chains having chain lengths of DP 6–12, 13–24, 25–36, and $\geqslant$37, respectively, based on their length, the location in amylopectin, and whether the chains were carrying other chains. However, this classification may no longer be valid after AS modification.

With increasing reaction time, the DP of branched chains representing the largest proportion became greater (Fig. 1). The proportion of short chains (DP $\leqslant$ 12) decreased due to the elongation by AS. The fraction making up the largest proportion was DP 13–24, and the proportion of very long chains (DP $\geqslant$ 37) did not change significantly. The proportion of DP 25–36 increased gradually with reaction time. These changes in chain-length distribution seemed

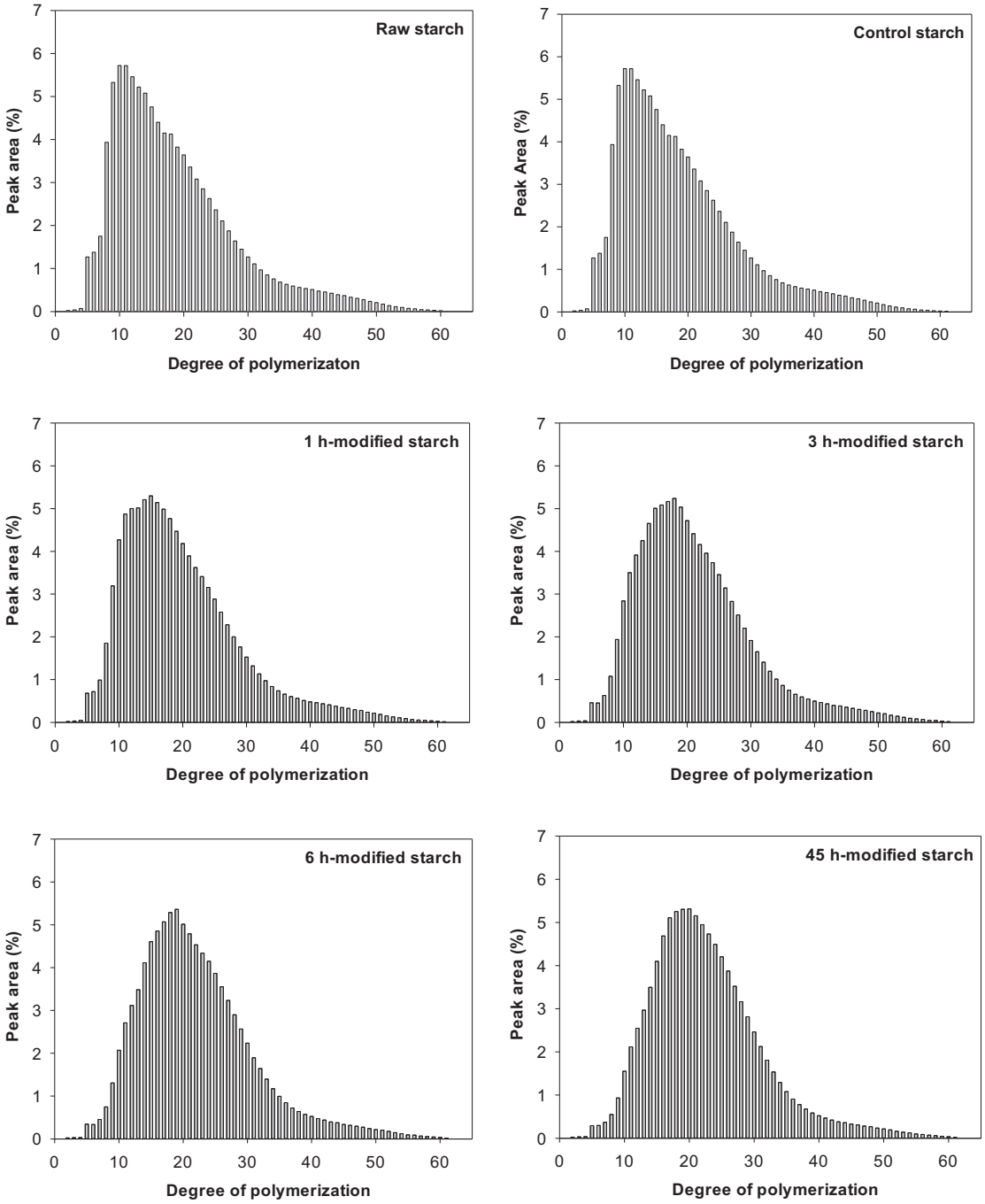

Fig. 1. Branched chain-length distributions of AS-modified starches.

Table 2
Branched chain length distributions of AS-modified waxy corn starches.

Sample	Relative peak area (%)				
	DP ⩽ 5	DP 6–12	DP 13–24	DP 25–36	DP ⩾ 37
Raw	1.4 ± 0.1[a]	29.3 ± 0.6[a]	47.1 ± 0.1[c]	15.7 ± 0.4[f]	6.5 ± 0.4[a]
Reaction time (h)					
1	0.8 ± 0.1[b]	20.9 ± 0.1[b]	53.2 ± 1.0[b]	19.0 ± 0.5[e]	6.5 ± 0.3[a]
3	0.5 ± 0.0[c]	14.4 ± 0.1[c]	55.4 ± 1.1[a]	23.0 ± 0.5[d]	6.7 ± 0.4[a]
6	0.4 ± 0.0[cd]	10.7 ± 0.0[d]	55.6 ± 0.3[a]	26.3 ± 0.1[c]	7.0 ± 0.1[a]
9	0.5 ± 0.1[cd]	10.0 ± 0.5[e]	55.6 ± 1.1[a]	27.2 ± 0.2[b]	6.8 ± 0.3[a]
15	0.4 ± 0.0[d]	9.4 ± 0.0[e]	55.8 ± 0.0[a]	27.5 ± 0.1[b]	6.9 ± 0.1[a]
45	0.4 ± 0.0[d]	8.4 ± 0.1[f]	55.6 ± 0.4[a]	28.8 ± 0.3[a]	6.9 ± 0.3[a]

The values having different superscripts in the same column are significantly different ($p < 0.05$) by Duncan's multiple range test.

insignificant after the AS reaction proceeded for 6 h. As described above, the results of branched chain-length distribution suggest that the elongation of branched chains occurred extensively during the early phases of the AS reaction (within 6 h from the start of the reaction), and that elongation continued to occur after 6 h, though with a lower rate than during the early phases.

As described above, the AS reaction is known to lead to the elongation of external chains by the attachment of 18 glucosyl units at non-reducing ends (Potocki de Montalk et al., 1999). It is anticipated that the proportion of short chains decreases, since short chains are located outside of the cluster structure as A-chains and short B-chains, and these short chains are readily accessed by AS. Waxy cereal starches are relatively abundant and include about 70–80% A-chains (DP 6–24) (Chung, Han, Yoo, Seib, & Lim, 2008). Therefore, it is suggested that AS preferentially reacts at the external chains and extends them up to DP 25–36. The extended branched chains by amylosucrase modification favored the formation of double helices which hinder enzyme access. The increased proportion of long chains and the decreased proportion of short chains could lead to the formation of more perfect crystallites resulting in the resistance to starch-digestive enzymes (Shin et al., 2010). Therefore, the elongation of branched chains in amylopectin could reduce accessibility of digestive enzymes and it might be related to the increase of both SDS and RS levels.

3.3. XRD pattern and relative crystallinity

XRD patterns and relative crystallinities of the raw, control, and AS-modified starches are presented in Fig. 2. The waxy corn starch used in this study displayed a typical A-type XRD pattern, with diffraction peaks at Bragg angles (2θ) of 15°, 17°, 18.1°, and 23.3°, as reported by Hizukuri et al. (2006). No peak was detected with the control starch, indicating that the control starch contained amorphous regions to a significant extent. The AS-modified starches displayed an XRD pattern similar to B-type with peaks at 5°, 17°, and 24°, but the peak intensities were very weak. Although slightly increased with increasing AS reaction time, the peak intensities remained weak. Retrograded cereal starches normally give a B-type XRD pattern, although they show an A-type pattern before gelatinization (Leeman, Karlsson, Eliasson, & Björck, 2006). The B-type crystalline structure is less sensitive to digestive enzymes than the A-type crystalline structure (Zhang, Venkatachalam, & Hamaker, 2006). Rolland-Sabaté et al. (2004) reported that AS-modified amylopectin mainly consists of RS3 and shows a B-type XRD pattern. The AS-modified starches prepared in this study exhibited a very weak B-type crystallinity and were digested slowly, although they were not resistant to digestive enzymes. For AS modification, samples were perfectly gelatinized by cooking for 30 min. Consequently, the original crystalline structure was completely disrupted, suggesting that the disrupted crystalline structure might be entangled and reordered to a B-type crystalline structure. The B-type crystalline structure in the AS-modified starches was produced not only by the AS reaction, but also by retrogradation during the AS reaction at 30 °C. The formation of ordered structural crystallites and entanglement occurred more easily among the elongated branched chains of amylopectin in the AS-modified starches than in the control starch.

Raw starch had a relative crystallinity of 44.5%, in good agreement with the report of Lopez-Rubio, Flanagan, Gilbert, and Gidley (2008). The AS-modified starches had higher relative crystallinities (24.0–29.7%) compared with the control starch (Table 3). The gelatinization that occurred before the enzyme reaction was a main factor in the low relative crystallinity of the control starch. The difference in relative crystallinity might be caused by the interaction of such factors as crystal size, amount of crystalline region, origin of the double helices within the crystalline domains, and extent of interaction between double helices (Miao et al., 2009). Double helices formed by the elongated chains of AS-modified starch could contribute to the formation of crystalline structure.

3.4. Thermal properties

Thermal properties of raw, control, and AS-modified starches are described in Table 3. Raw waxy corn starch showed a typical endothermic peak for starch gelatinization with a range from 66.1 to 78.4 °C and a melting enthalpy (ΔH) of 15.1 J/g. However, only very weak endothermic peaks were observed for the control starch, indicating an insignificant extent of retrogradation. Peak temperature (T_p), melting temperature range ($T_r = T_c - T_o$), and ΔH increased with increasing AS modification time. T_p increased with the reaction time of AS modification, in agreement with a previous report stating that T_p depends on chain length in the B-type crystalline polymorph of starch (Moates, Noel, Parker, & Ring, 1997). T_r of retrograded starch is much broader than that of raw starch (Ottenhof, Hill, & Farhat, 2005), corresponding with our result. A previous study has reported retrogradation enthalpy values for waxy corn starches of about 10 J/g (Ottenhof et al., 2005), lower than those of the AS-modified starches in our experiment. This result suggests that the crystallites of the AS-modified starches were more stable than those of the control starch, and that the stability of crystallites increased as AS-modification time increased.

Although there was little difference among the AS-modified starches, T_r and ΔH increased with increasing AS reaction time. The broad melting endotherm indicated a double helical structure, consisting of chains with varying DPs (Zhang, Sofyan et al., 2008). The difference in melting temperature range could be due to the presence of crystallites, each possessing slightly different crystal strength (Vasanthan & Bhatty, 1996). In general, amylose crystallites are more stable than amylopectin crystallites, and the T_p of amylopectin crystallites is lower than that of amylose crystallites (Karim, Norziah, & Seow, 2000). During retrogradation, the elongated chains of amylopectin in the AS-modified starch behaved like long linear amylose chains. Therefore, all AS-modified starches had higher T_p than the control starch. However, the elongated chains of amylopectin in the AS-modified starch were reordered to less perfect crystallites as compared with the crystallites consisting of very long linear amylose. If the elongated chains were rearranged into a highly ordered structure, RS content had to increase dramatically with increasing AS reaction time. However, SDS content, not RS content, increased gradually as the reaction time of AS increased (Fig. 3). Therefore, the increased SDS content of the AS-modified starches could be due to less perfect crystallites consisting of elongated branched chains of amylopectin.

3.5. Starch fractions of AS-modified starches

The *in vitro* digestibilities of AS-modified starches are represented in Fig. 3. The contents of RDS, SDS, and RS in raw waxy corn starch were 35.2%, 55.5%, and 9.3%, respectively. Raw waxy corn starch showed a relatively high SDS content, which is in agreement with the slow digestion property of granular starch with the A type crystalline structure (Zhang, Ao, & Hamaker, 2006). In AS-modified starches, RDS decreased and SDS increased as the reaction time increased from 1 to 6 h, reaching a plateau after 6 h. During the 45 h AS treatment, the contents of RDS and SDS remained relatively steady at 49.0–56.4% and 35.7–38.7%, respectively. Although the RS content slightly changed over the entire enzyme reaction, the extent of the change was not significant. The content of SDS was in inverse proportion to that of RDS (Fig. 3). Shin et al. (2010) reported that the AS modification of gelatinized waxy corn, rice, and potato starches led to increased SDS contents, similar to our observation. In general, retrograded starch is inherently more resistant to enzymatic

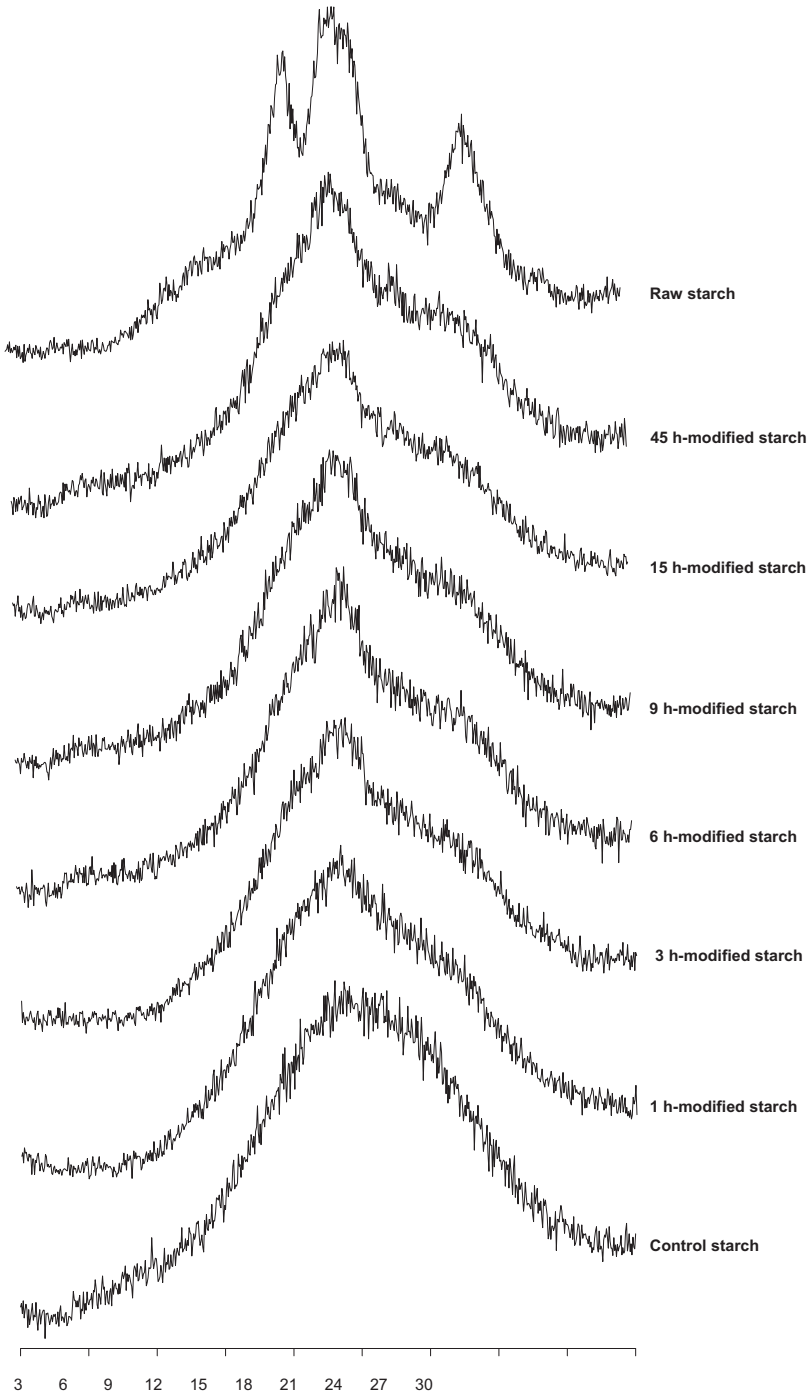

Fig. 2. X-ray diffraction patterns of AS-modified starches.

hydrolysis and the crystalline structure formed by retrogradation could reduce the hydrolytic enzyme susceptibility (Fredriksson et al., 2000). Therefore, it is suggested that the degree of retrogradation and/or the perfectness and rigidity of crystalline structure built during retrogradation could be one of the key factors affecting the resistant level of starch to hydrolytic enzymes. The previous findings hypothesised follows: (i) branched chains with lengths shorter than DP 15 are resistant to recrystallization during retrogradation (Shi & Seib, 1992); (ii) DP 10 is the minimum chain length required for recrystallization during retrogradation (Robin, Mercier, Charbonniere, & Guilbot, 1974); (iii) if a considerable amount of longer chains (DP > 10) is present, the short chains can behave as co-crystals (Gidley & Bulpin, 1987); and (iv) starches with long branched chains tend to show a high degree of retrogradation (Jane et al., 1999). Considering these hypotheses, the high SDS level of AS-modified starch could be due to the easy retrogradation of elongated chains. Through the easier access of AS to the external chains than the internal ones of amylopectin (Potocki de Montalk et al., 2000), the external chains of amylopectin can be elongated enough to take part in the recrystallization, probably longer than DP 10 (Table 2). Those chains that are elongated enough form a more perfect crystalline structure compared with the control starch. However, the crystalline structures in the AS-modified starches formed during retrogradation were not very compact because of the lack of significant changes in the

Table 3
Thermal properties and relative crystallinities of AS-modified starches.

Sample	T_o (°C)	T_p (°C)	T_c (°C)	T_r (°C)	ΔH (J/g)	Relative crystallinity (%)
Raw	66.1 ± 0.3	72.2 ± 0.2	78.4 ± 0.3	12.2 ± 0.2	15.1 ± 0.5	44.5 ± 3.3[a]
Control	50.3 ± 0.4[b]	60.3 ± 1.3[f]	80.4 ± 1.1[e]	30.1 ± 0.9[d]	1.0 ± 0.2[f]	21.8 ± 1.1[d]
Reaction time (h)						
1	52.5 ± 0.3[a]	61.4 ± 0.2[e]	72.0 ± 0.7[d]	19.5 ± 0.9[e]	6.4 ± 0.2[e]	24.0 ± 1.4[d]
3	49.8 ± 0.3[c]	66.1 ± 0.3[d]	85.0 ± 0.9[c]	35.3 ± 1.2[c]	12.1 ± 0.2[d]	27.0 ± 0.7[c]
6	49.4 ± 04[c]	74.3 ± 0.1[c]	91.0 ± 0.3[b]	41.6 ± 0.1[b]	14.5 ± 0.1[b]	29.4 ± 0.7[bc]
9	49.2 ± 0.5[c]	75.9 ± 0.0[b]	91.5 ± 0.4[b]	42.3 ± 0.8[b]	13.7 ± 0.6[c]	29.7 ± 1.7[b]
15	49.4 ± 0.5[c]	75.9 ± 0.3[b]	91.8 ± 0.0[b]	42.4 ± 0.5[b]	14.0 ± 0.4[bc]	26.9 ± 0.5[c]
45	50.4 ± 0.2[b]	80.3 ± 0.6[a]	98.5 ± 0.6[a]	48.0 ± 0.7[a]	15.4 ± 0.8[a]	28.2 ± 1.1[bc]

The values having different superscripts in the same column are significantly different ($p < 0.05$) by Duncan's multiple range test.

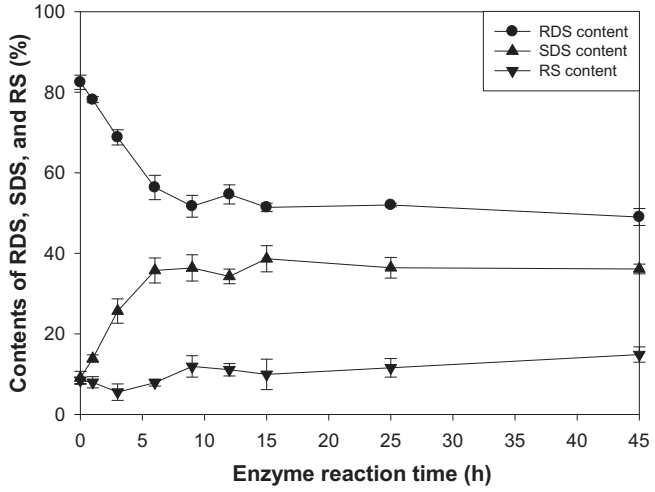

Fig. 3. Effect of AS reaction time on the RDS, SDS, and RS contents of AS-modified starches.

amount of RS with the enzyme reaction (Fig. 3). The thermal properties of AS-modified starches in Table 3 showed a concrete evidence for the formation of a more perfect crystalline structure. As AS reaction time increased, melting temperature and enthalpy gradually increased, meaning the increment of the formation of double-helical structures between elongated chains, a main mechanism of retrogradation (Gidley et al., 1995). However, it looked like that although the double-helical structure was formed to the relatively high extent, all of them did not participate to build the highly ordered crystalline structure. As shown in Table 3, the findings that the change in relative crystallinity was not significant as much as those of the melting temperature and enthalpy suggest that the AS-modified starch could contain a greater amount of the double-helical structure but that the ordered structure built with the double-helical structure was not compact and rigid than initially expected. The rearranged crystalline structures in the AS-modified starch contributed to slow digestion but not to digestion resistance. Thus, the structure of AS-modified starches relatively loosened through perfect gelatinization was rearranged, which might cause a change from RDS to SDS in the digestion property. As described above, AS mainly elongated the external chains of amylopectin and does not easily react with the internal chains of amylopectin because of steric hindrance. This result suggests that the crystalline structures in the AS-modified starch are the result of the formation of intermolecular double helices among amylopectins with elongated external chains. The degree of mutual binding by hydrogen bonds between amylopectins is responsible for the amount of crystalline structure (Imberty, Chanzy, Perez, Buleon, & Tran, 1988). When these bonds are strong and numerous, the chains associate as crystalline structures, resulting in high SDS and/or RS content (Ao et al., 2007). However, the internal structures of AS-modified starch were not significantly different from the control. This is a plausible explanation for the insignificant change in RS content of the AS-modified starches with the varying reaction times.

4. Conclusions

Amylosucrase treatment on waxy corn starch led to an increase in SDS content. The elongation of the outer branch chains of amylopectin reduced starch digestion rate. The increase in the proportion of long branch chains, resulting in the reduction of weight ratio of short chains to long chains, could promote the crystalline structures, leading to the slow digestion property during enzyme reaction. Our findings could be useful to describe and explain the structural characteristics of SDS and the mechanism of SDS formation. However, for clearer understanding of the mechanism of SDS formation by amylosucrase, the modification should be carried out with starches having amylose/amylopectin ratios, polymorphisms, and branched chain distributions different from those of the waxy corn starch used in this study.

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