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Impact of amylosucrase modification on the structural and physicochemical properties of native and acid-thinned waxy corn starch

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ABSTRACT

Recombinant amylosucrase from *Neisseria polysaccharea* was utilized to modify native and acid-thinned starches. The molecular structures and physicochemical properties of modified starches were investigated. Acid-thinned starch displayed much lower viscosity after gelatinization than did the native starch. However, the enzyme exhibited similar catalytic efficiency for both forms of starch. The modified starches had higher proportions of long (DP > 33) and intermediate chains (DP 13-33), and X-ray diffraction showed a B-type crystalline structure for all modified starches. With increasing reaction time, the relative crystallinity and endothermic enthalpy of the modified starches gradually decreased, whereas the melting peak temperatures and resistant starch contents increased. Slight differences were observed in thermal parameters, relative crystallinity, and branch chain length distribution between the modified native and acid-thinned starches. Moreover, the digestibility of the modified starches was not affected by acid hydrolysis pretreatment, but was affected by the percentage of intermediate and long chains.

1. Introduction

Starch, one of the major ingredients in processed foods, is an important energy source for humans. In plants, it is found in the chloroplasts of green leaves and the amyloplasts of seeds, roots, and tubers (Ellis et al., 1998). Based on the rate and extent of digestion, starch is nutritionally divided into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992). Among these, RS is defined as the sum of the starch fractions that escape digestion in the small intestine of healthy humans. In addition, RS has been subdivided into four types: physically protected starch (RS_1), ungelatinized resistant starch granules (RS_2), retrograded starch (RS_3), and chemically-modified starches (RS_4) (Brown, Mcnaught, & Moloney, 1995; Englyst et al., 1992). Much work has been done to investigate the physiological advantages of RS in past decades (Nugent, 2005). It appears to possess considerable benefits in preventing colon cancer in humans. Higgins et al., 2004 reported that the intake of RS in a meal could reduce fat accumulation in the long term. Moreover, compared to digestible starch, RS facilitates the absorption of minerals in the intestines. Among the four types of RS, the most attention is currently focused on the preparation of RS_3.

Amylosucrase (EC 2.4.1.4) is a remarkable glucosyltransferase from glycoside-hydrolase family 13 (Hehre, 1949; Potocki De Montalk, Remaud-Simeon, Willemot, Planchot, & Monsan, 1999). To date, the recombinant amylosucrase (NpAS) from *Neisseria ploysaccharea* has been extensively investigated among the known amylosucrases. It has the specific capacity to synthesize linear α-glucan from sucrose, releasing fructose. In contrast with other polymerases involved in α-glucan synthesis, NpAS does not require expensive α-D-glucosyl-nucleoside-diphosphate (e.g. ADP- or UDP-glucose) as a glucosyl donor (Wang, Kim, Kim, Park, & Yoo, 2011). Furthermore, with the appearance of polymers with (1 → 4)-α- or (1 → 4)-α- and (1 → 6)-α- linkages as acceptors, it elongates the non-reducing ends of some external chains of the polymers randomly (Rolland-Sabaté, Colonna, Potocki-Véronèse, Monsan, & Planchot, 2004). Afterward, the elongated polymers have a highly resistant starch content. Rolland-Sabaté et al. (2004) suggested that the appearance of polymers with a high RS content is probably due to the formation of retrograde starch (RS_3) after enzymatic modification. In order to obtain efficient production of RS_3, Ryu

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et al. (2010) utilized the unique catalytic properties of NpAS to modify corn starches. Kim et al. (2013) also conducted an NpAS treatment reaction with a series of rice and barley starches as acceptors. In their studies, the RS contents were significantly enhanced for modified starches relative to native starches. However, a low starch concentration was commonly used in the reaction. Ryu et al. (2010) found that solutions containing more than 3% waxy corn starch could not be used in their study because the high viscosity of the starch solution made the enzymatic reaction difficult to handle.

It is known that, after diluted acid hydrolysis, the starch has a low viscosity with slight loss in weight (Sandhu, Singh, & Lim, 2007; Wang, Truong, & Wang, 2003). To the best of our knowledge, there has been little work done to study NpAS-modified acid-thinned starches. The aim of this study was to investigate the differences in structural and physicochemical properties, especially RS contents, between NpAS-treated native and acid-thinned waxy corn starches.

2. Materials and methods

2.1. Materials

Waxy corn starch (WCS) was obtained from Ingredion Inc. (Westchester, IL, USA). Sucrose and fructose were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Mo, USA). Isoamylase was purchased from Megazyme (Wicklow, Ireland). Other chemicals and reagents were all analytical grade.

2.2. Preparation of NpAS

The synthesis of the *N. polysaccharea* amylosucrase gene (GenBank: AJ011781.1) was carried out by Sangon Biotech (Shanghai, China). The company provided us with the gene cloned into the pRSET-B vector. The constructed plasmid was then transformed into *E. coli* BL21, and subsequently induced by isopropyl-β-D-thiogalactoside to express amylosucrase protein. NpAS was purified as previously described (Jung et al., 2009). Enzyme activity was measured using the method proposed by Ryu et al. (2010). Enzyme assays were carried out at 35 °C using 0.10 M sucrose as the substrate and 1 mg/mL WCS as the acceptor, with a reaction time of 10 min. The reaction medium was 50 mM Tris-HCl buffer (pH 7.0). One unit (U) of NpAS activity was defined as the amount of NpAS that catalyzes the release of 1 μmol of fructose per minute under the assay conditions. The amount of fructose released during the reaction was quantified by the dinitrosalicylic acid method (DNS) (Sumner & Howell, 1934).

2.3. Preparation of acid-thinned waxy corn starch

Acid-thinned waxy corn starch (AWCS) was prepared following the method of Sandhu et al. (2007) with slight modifications. A starch slurry (40%, w/w) was prepared by mixing WCS (40 g, dry-weight basis) with 0.2 M aqueous hydrochloric acid (HCl) to a final weight of 100 g. The slurry was incubated in a water bath at 50 °C, and allowed to react for 8 h. Thereafter, the slurry was washed three times with distilled water, and the insoluble fraction was separated by centrifugation (5000*g*, 10 min). Then, AWCS was freeze-dried and ground so that it passed through a 200-mesh sieve.

2.4. Starch modification by NpAS-treatment

The starch samples (3.0 g, dry-weight basis) were suspended in 50 mM Tris-HCl buffer (pH 7.0) and gelatinized in a boiling water bath for 45 min in order to increase enzyme accessibility. After cooling to room temperature, the sucrose (0.03 mol) and enzyme (1.61 mg of purified NpAS, 100 U) were then added, and the total volume of the reaction mixture was 100 mL. The enzymatic reactions were conducted at 35 °C, and each reaction mixture was stirred using a mechanical overhead stirrer (RW 20, IKA, Germany) at a speed of 200 rpm. Samples of WCS and AWCS that were elongated to different extents were prepared by allowing the reaction to proceed for 0.5, 3, and 6 h. At the end of the reaction, the reaction mixtures were boiled for 10 min to inactivate the enzyme, cooled to room temperature, and stored in a refrigerator at 4 °C overnight. The supernatant was collected for further study after centrifugation (12,000*g*, 20 min), whereas the insoluble fraction was washed five times with distilled water, then freeze-dried and ground so that it passed through a 200-mesh sieve.

2.5. Determination of the pasting properties of WCS and AWCS

Pasting characteristics of WCS and AWCS were determined using a Rapid Visco Analyzer (RVA 4500, Perten, Stockholm, Sweden). The starch slurries (9.2 g/100 g, 28 g total weight) were held at 50 °C for 1 min, heated to 95 °C at a rate of 12 °C/min, and held at 95 °C for 2.5 min, then cooled to 50 °C at a rate of 12 °C/min, and held at 50 °C for 1.4 min.

2.6. Chromatographic analysis of soluble fractions

The soluble fractions were analyzed by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD), using a previously described method (Wang et al., 2012).

2.7. Analysis of branch chain length distribution by HPSEC

The branch chain length distributions of starch samples were determined by high-performance size exclusion chromatography (HPSEC), using a previously described process with modifications (Zhou, Wang, Yoo, & Lim, 2011). To avoid linear chain precipitation during isoamylase debranching, the concentration of the starting starch solution was decreased to 0.5 mg/mL, and the temperature of the debranching reaction was increased to 50 °C.

2.8. Scanning electronic microscopy (SEM)

The morphology of the starch samples was characterized by SEM (Quanta200, FEI, Netherlands). The dried samples were mounted on aluminum stubs using double-sided sticky tape, and then coated with a thick film of gold (10 nm) under vacuum. The digital images of the samples were obtained at an accelerating voltage of 5 kV.

2.9. X-ray diffraction (XRD)

An X-ray powder diffractometer (D8, Bruker, Germany), equipped with Cu Kα radiation, was used to determine the crystallinity of the starch samples. Diffractograms of the samples were obtained at 40 kV and 300 mA. Before analysis, the moisture content of the starches was equilibrated in a hermetic chamber with 100% relative humidity. Then, the samples were placed on a plate and scanned at a rate of 2°/min from 2θ 4.5° to 40° at room temperature. Relative crystallinity (X_c) was calculated by using the following equation (Nara & Komiya, 1983):

$$X_C(\%) = \left(\frac{A_C}{A_C + A_a}\right) \times 100$$

where A_c is the crystalline area and A_a is the amorphous area of the X-ray diffraction profiles.

2.10. Thermal analysis

The thermal characteristics of the starches were analyzed using a differential scanning calorimeter (X-DSC7000, Seiko instruments Inc., Chiba, Japan). The samples (3 mg, dry-weight basis) were weighed into aluminum DSC pans (Seiko instruments), and mixed with distilled water (7 μL). Then, the pans were hermetically sealed and stored in a 4 °C refrigerator overnight to equilibrate the moisture content. The prepared pans were scanned by heating from 20 °C to 130 °C at a rate of 5 °C/min. An empty pan was used as a reference.

2.11. Determination of resistant starch content

The RS contents were quantified by AACC method 32-40.01. The well-ground samples (100 mg, dry-weight basis) were digested using a mixture of pancreatic α-amylase (Megazyme, 10 U/mL) and amyloglucosidase (Megazyme, 3 U/mL) at 37 °C for 16 h. Afterward, the insoluble fractions were dissolved in 2 M KOH, and incubated with amyloglucosidase (0.1 mL, 3300 U/mL) for 0.5 h. The released glucose was determined using a glucose oxidase assay kit (Megazyme).

2.12. Isolation of resistant starch

At the end of 16 h-digestion, the RS and soluble fractions were separated by centrifugation (6000*g*, 15 min). Carefully decanted the supernatants, the RS was re-suspended in 8 mL of 50% (v/v) ethanol with vigorous stirring, and then the mixture was centrifuged at 6000*g* for 15 min. Repeated the above wash steps, and RS was subsequently freeze-dried. The molecular structure of RS was characterized by HPSEC using a method as Section 2.7 described.

2.13. Statistical analysis

All numerical results are averages of at least two independent replicates, and values are represented as the mean ± standard deviation. Statistical significance analysis was performed by using SPSS (version 21.0, SPSS Inc., Chicago, IL, USA) and applying Duncan's multiple range test ($p < 0.05$).

3. Results and discussion

3.1. Pasting properties of WCS and AWCS

The pasting profiles of WCS and AWCS, measured by RVA, are presented in Fig. 1. A significant difference in the viscosity curves was observed for WCS and AWCS. The viscosity of WCS increased from 17 to over 3500 cP during the RVA measurement, and the peak and final viscosities were 3814 and 2112 cP, respectively. After acid hydrolysis, the viscosity of AWCS was very low (17 cP) and it remained nearly constant during RVA measurements. Similar observations were reported by Sandhu et al. (2007). According to Jane et al. (1999), the pasting properties of starch are affected not only by the amylose and lipid contents, but also by the branch chain length distribution of the amylopectin. In this study, the WCS mainly consisted of amylopectin, and thus the significant decrease in the values of the pasting viscosity of AWCS was possibly due to hydrolysis of the glycosidic bonds of the amylopectin in the amorphous region.

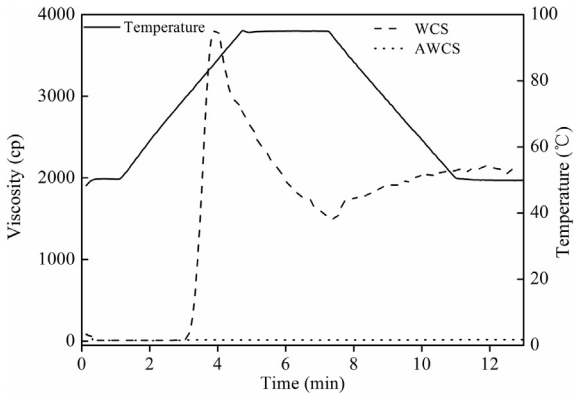

Fig. 1. Pasting profiles of waxy corn starch and acid-thinned waxy corn starch.

3.2. Branch chain elongation by NpAS

In the presence of an acceptor, NpAS is more efficient in catalyzing branch chain elongation than synthesizing insoluble linear α-1, 4-linked glucan from sucrose. For NpAS modifications of WCS and AWCS, the compositions of the soluble fractions in the reaction mixtures were determined in order to assess the influence of different acceptors on the enzymatic reaction. After reaction, no other oligosaccharides were detected in the reaction mixtures, except fructose and sucrose (data not shown). This indicated that only branch chain elongation was occurring, in which the D-glucose residue from sucrose was exclusively incorporated into the acceptor molecules. In addition, the amount of sucrose consumed at different reaction times was quantified, and it seemed to be independent of the acceptors used and dependent only on the reaction time (Table 1). After acid hydrolysis, AWCS has an extremely low viscosity compared to native starch. Thus, the reaction was expected to be more efficient when AWCS was used as the acceptor, due to the superior mobility of the reaction substrates and enzyme molecules. However, no significant difference in sucrose consumption was observed between the two acceptors. Indeed, Potocki de Montalk, Remaud-Simeon, Willemot, and Monsan (2000) suggested the presence of a non-catalytic binding site in the NpAS molecule, and that the branch chains of glycogen could strongly bind to this site. Following this line of thought, in this study, as soon as the enzyme was added to the reaction mixtures, NpAS would instantly combine with the branch chains of the starches, inducing the formation of the branch chain-NpAS complex. This would also explain why the catalytic efficiency of NpAS was not significantly affected by the decrease in viscosity of the reaction mixture, which resulted from acid hydrolysis of the starches.

3.3. Branch chain length distributions of the starches

Because of the limited resolution of HPAEC, the branch chain length distributions of WCS, AWCS, and NpAS-modified starches were analyzed by HPSEC after isoamylase debranching, and the obtained RI signals were normalized for easy comparison (Fig. 2). Based on the classification of Kim et al. (2013) and Miao, Jiang, Zhang, Jin, and Mu (2011), the branch chain length of amylopectin, expressed as the degree of polymerization (DP), was divided into three categories: Fr I (DP > 33), Fr II (DP 13–33), and Fr III (DP < 13), and their proportions were obtained from the sum of the relative peak areas and are listed in Table 1. As shown in Fig. 2A, WCS and AWCS synchronously displayed a major peak at DP 14, indicating an extremely similar composition of short chains. An exception was that parts of the long chains (DP 33–76), which

Table 1
Quantification of residual sucrose in the reaction mixtures and branch chain fractions of waxy corn starch, acid-thinned waxy corn starch, and amylosucrase-modified starches. The enzymatic reactions were carried out at 35 °C for 0.5 h, 3 h, and 6 h with amylosucrase activity of 1000 U/L. The acceptor and sucrose concentrations were 3.0% (W/V) and 0.30 M, respectively.

Acceptor	Reaction time (h)	Sucrose concentration (mM)	Relative percentage areas (%)		
			Fr I (DP > 33)	Fr II (DP 13–33)	Fr III (DP < 13)
Waxy corn starch	–	300	26.1 ± 0.6^{a}	53.7 ± 0.3^{bc}	20.2 ± 0.5^{e}
	0.5	259.8 ± 0.7^{d}	29.1 ± 0.6^{b}	63.3 ± 0.7^{f}	7.6 ± 0.6^{c}
	3	210.4 ± 0.8^{c}	42.6 ± 0.7^{d}	55.8 ± 1.0^{cd}	1.6 ± 0.4^{a}
	6	163.7 ± 2.5^{a}	50.4 ± 1.2^{f}	48.3 ± 0.4^{a}	1.3 ± 0.3^{a}
Acid-thinned waxy corn starch	–	300	25.0 ± 0.9^{a}	53.3 ± 0.8^{b}	21.7 ± 0.3^{e}
	0.5	257.6 ± 0.5^{d}	30.4 ± 1.2^{b}	59.5 ± 0.7^{e}	10.1 ± 0.6^{d}
	3	205.0 ± 0.3^{b}	39.0 ± 0.7^{c}	57.7 ± 0.8^{de}	3.4 ± 0.5^{b}
	6	165.9 ± 0.1^{a}	46.1 ± 0.5^{e}	52.2 ± 1.3^{b}	1.7 ± 0.5^{ab}

Values with different superscripts letters in each column are significantly different ($P < 0.05$).

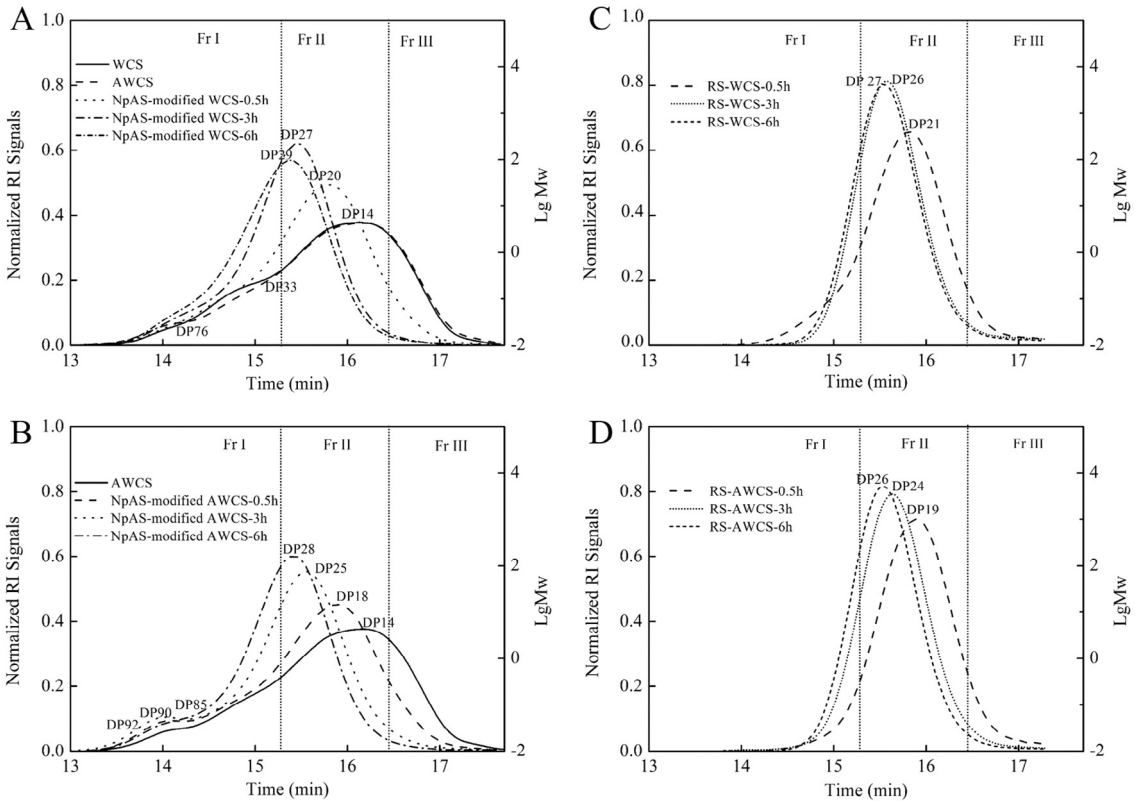

Fig. 2. Normalized high-performance size exclusion chromatography profiles of debranched waxy corn starch, acid-thinned waxy corn starch, amylosucrase-modified starches and resistant starches: (A) waxy corn starch, acid-thinned waxy corn starch, and amylosucrase-modified waxy corn starches; (B) acid-thinned waxy corn starch and amylosucrase-modified acid-thinned waxy corn starches; (C) Resistant starches isolated from amylosucrase-modified waxy corn starches; (D) Resistant starches isolated from amylosucrase-modified acid-thinned waxy corn starches.

were defined as B_2 and B_3 chains (chains extending into 2 and 3 clusters) (Hizukuri, 1986), were slightly decreased after acid hydrolysis. Native starch granules consist of alternating amorphous regions and crystalline layers (Perera, Lu, Sell, & Jane, 2001). In contrast to the crystalline layers, the amorphous regions are easily accessible to acid hydrolysis, and thus the long chains that extended through the amorphous regions would be readily hydrolyzed rather than the short chains.

For all NpAS-modified starches, a considerable shift in the major peak towards higher DP was observed. After enzymatic modification for 0.5 h, 3 h, and 6 h, the peak maxima for the corresponding NpAS-modified WCSs (Fig. 2A) were DP 20, 27, and 29, respectively, whereas those of NpAS-modified AWCSs (Fig. 2B) were DP 18, 25, and 28, respectively. These results suggest that NpAS specifically elongated the chains of acceptor molecules, irrespective of whether WCS or AWCS was employed. However, NpAS-modified AWCSs exhibited subordinate peaks at DP 85–92 (Fig. 2B), which differed from NpAS-modified WCSs. This observation could be attributed to the destruction of the molecular structure of amylopectin by acid hydrolysis. The cluster model of amylopectin, proposed by Hizukuri (1986), has shown that the connections between the clusters are linked by the long inner chains (such as B_2, B_3, and B_4 chains). As for the modification of native WCS, the non-reducing ends of the long inner chains would have been less accessible to NpAS, because of the high viscosity of the reaction mixture and the hindering effect of the short chains of the clusters. The acid hydrolysis occurred preferentially at the amorphous regions as previously described, and a number of clusters would be hydrolyzed from the amylopectin molecule. Therefore, some of the long inner chains would be exposed to the exterior of the

amylopectin molecules. As a result of the decreased viscosity, these long chains of AWCS would also possess better mobility than would the long chains of WCS. Hence, the presence of subordinate peaks was probably due to the better accessibility of NpAS to the long inner chains, which would facilitate branch chain elongation and induce the production of long chains (DP 85–92).

3.4. Morphology

The morphology of WCS, AWCS, and NpAS-modified starches was characterized by SEM. For easy comparison, WCS and AWCS were observed at a magnification of 1200×, and all of the NpAS-modified starches were observed at a magnification of 600×. As shown in Fig. 3A, WCS exhibited spherical or polyhedral shaped granules with diameters ranging from 5 μm to 20 μm. Similar observations were made for AWCS, and the granular structure of the starches did not show signs of any visible destruction (Fig. 3E). This might be the result of the low level of hydrolysis. These results were consistent with an earlier report (Sandhu et al., 2007). As a result of NpAS modification, the granular structures of WCS and AWCS were completely disrupted. The images of the NpAS-modified starches obtained after freeze-drying and subsequent grinding are shown in Fig. 3B–D and F–H. All NpAS-modified starches displayed similar irregularly shaped particles with densely packed structures, and the surfaces of the particles seemed to be slightly roughened, similar to the morphology of retrograde starches. Kim et al. (2013) reported similar findings. Moreover, it has been suggested that amylopectin, with its elongated branched chains, exhibits similar behaviors to long linear amylose (Shin, Choi, Park, & Moon, 2010). In this study, after the starches were modified by NpAS, it is possible that the elongated branch chains of amylopectin assumed amylose-like properties, and thus the elongated starches retrograded and aggregated afterwards, leading to the formation of the densely packed particles.

3.5. X-ray diffraction

X-ray diffraction profiles of the starch samples are shown in Fig. 4. As reported by Zobel (1988), the crystal structures of starches are classified into A-, B-, and C-type. In this study, both WCS and AWCS exhibited typical A-type crystallites with reflection

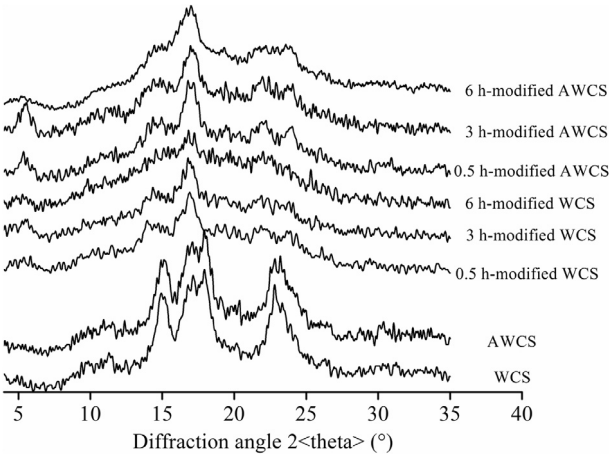

Fig. 4. X-ray diffraction profiles of waxy corn starch, acid-thinned waxy corn starch, and amylosucrase-modified starches.

angles (2θ) of 15°, 17°, 18°, and 23°, indicating that the acid treatment did not change the crystal structure. For all of the NpAS-modified starches, the X-ray diffraction profiles showed a weak peak at 2θ of 5.5° and a stronger peak at 2θ of 17°, indicating the crystal structure of NpAS-modified starches was a characteristic B-type, consistent with that reported in the literature (Shin et al., 2010). In general, the humidity, temperature, and especially the branch chain length of amylopectin affect the crystal structure of the starches to a significant extent (Gidley, Bulpin, Gidley, & Bulpin, 1987). In the present study, control starch samples should ideally be prepared with the same protocol but without incubation with NpAS. Nevertheless, preparations of control starch samples all failed since they were difficult to retrograde under the same protocol, i.e. 3% starch concentration, gelatinized, incubated at 35 °C, boiled for 10 min, and stored at 4 °C overnight. Thus, it was impossible to separate the control starches from the starch sucrose mixtures by centrifugation. This behavior was probably due to the high proportions of short chains (Fr III) in WCS and AWCS (corresponding to 20.2% and 21.7%), which hindered the retrogradation of the starches. For the preparation of modified starches, the branch chain

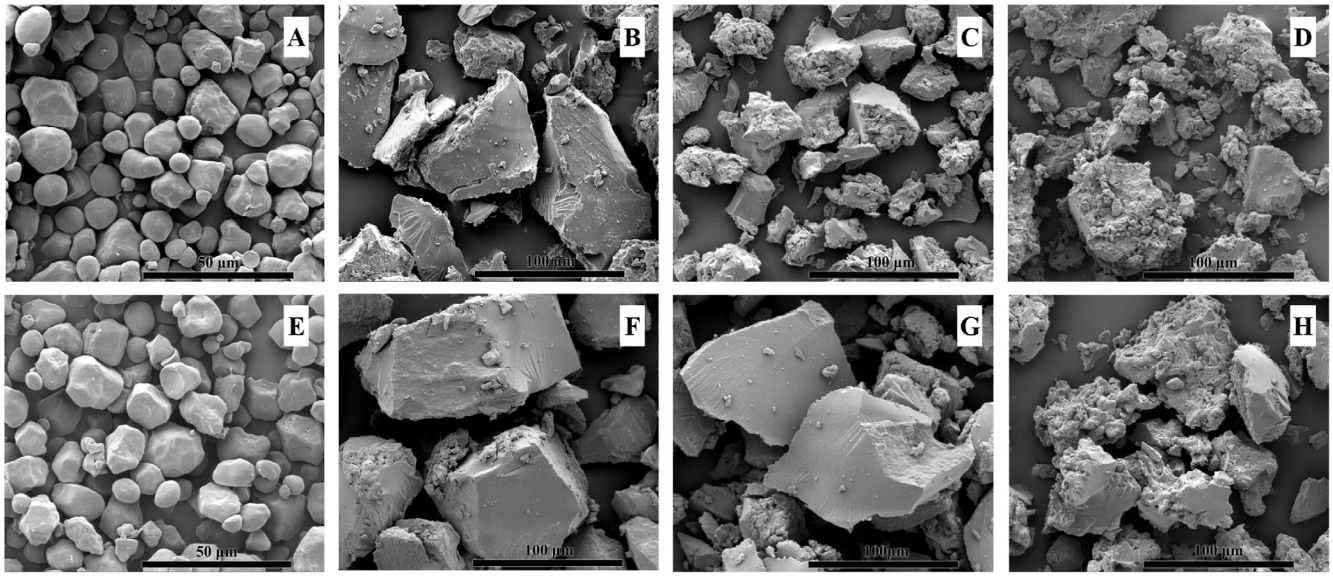

Fig. 3. Scanning electron micrographs of initial waxy corn starch (A), waxy corn starch modified with amylosucrase for 0.5 h (B), 3 h (C), and 6 h (D), initial acid-thinned waxy corn starch (E) and acid-thinned waxy corn starch modified with amylosucrase for 0.5 h (F), 3 h (G), and 6 h (H).

elongation decreased the proportion of short chains significantly, and the reaction mixtures were subsequently stored in the refrigerator after reaction. Such conditions are well known to favor the formation of a B-type crystalline structure. The calculated relative crystallinities (X_c) of the starch samples are listed in Table 2. X_c of WCS was 41.71%. After acid treatment, the X_c of AWCS increased to 45.50%, which could be attributed to the preferential hydrolysis of the amorphous regions as described above. However, after 0.5 h and 6 h of enzymatic reactions, the X_c values of NpAS-modified WCSs and AWCSs decreased from 34.22% to 23.45% and 35.03% to 28.91%, respectively. As pointed out by Miao, Jiang, and Zhang (2012), the shorter chains tend to yield precipitates, whereas the longer chains tend to gel. For NpAS-modified starches, the elongated branch chains might induce gelation, which could decrease the mobility of branch chains. Therefore, it would be difficult for longer branch chains to associate as an orderly crystalline structure might cause a decrease in crystallites when the reaction time increases.

3.6. Thermal properties

The thermal properties of WCS, AWCS, and NpAS-modified starches are summarized in Table 2. WCS displayed a sharp endothermic peak (curve not shown) ranging from 64.8 °C to 78.9 °C with an endothermic enthalpy (ΔH) of 15.6 J/g. After acid treatment, the onset melting temperature (T_o), peak melting temperature (T_p), and conclusion temperature (T_c) of AWCS were slightly decreased, whereas there was almost no difference in the value of ΔH. According to Donovan, 1979, when native starch granules were heated in excess water, the crystallites were responsible for the endothermic peak of starches. During gelatinization, the crystallites were destabilized and melted cooperatively, because of the tensions exerted by the adjacent amorphous domains, which were fully hydrated and swollen. As a result of the destruction of the amorphous region, it was proposed that the decreases of T_o, T_p, and T_c were presumably due to better hydration and swelling of AWCS. For NpAS-modified starches, remarkable increases in T_o, T_p, and T_c were observed with prolonged reaction time, indicating that the thermal characteristics of the modified starches were elevated. NpAS-modified starches with elongated branch chains might favor the formation of long-range double helices during retrogradation, and thus exhibit higher thermostability. As to the value of ΔH, it primarily represents the loss of double helical order rather than the loss of crystallinity (Cooke & Gidley, 1992). Interestingly, the values of ΔH for NpAS-modified starches gradually decreased as the reaction time increased. This implies that the modified starches with higher degrees of elongation possessed fewer double helices.

3.7. Resistant starch content and characterization of isolated resistant starch

The RS contents of WCS, AWCS, and NpAS-modified starches are summarized in Table 2. WCS had an RS content of 1.2%, in agreement with previous studies (Cai, Shi, Rong, & Hsiao, 2010; Shi, Chen, Yu, & Gao, 2013) reporting that the native cereal starches with A-type crystallites contain low levels of RS. As for AWCS, the RS content was slightly decreased to 0.7%, indicating that the mild acid hydrolysis did not change the starch digestibility significantly. After 0.5 h of enzymatic reaction, the contents of RS in NpAS-modified WCS and AWCS were 19.1% and 19.7%, respectively, and the values of RS gradually increased as the reaction progressed. This observation revealed that the branch chain elongation by NpAS dramatically enhanced the contents of RS in the modified acceptors, in accordance with the findings of Rolland-Sabaté et al., 2004), who speculated that the high RS content in modified acceptors could be attributed to the elongation of branch chains and the formation of B-type crystallites. After 16 h of digestion, the RS components were isolated from the modified starches. Fig. 2 shows the branch chain length distributions obtained for the RS samples. The peak maxima for isolated RS samples differed between 20 and 27 glucosyl units, and the RS mostly consisted of intermediate branch chains. Moreover, the peak values of RS were similar to those of the corresponding modified starches. In this study, the long branch chains of modified starches were extensively hydrolyzed during the enzymatic digestion. Thus, the intermediate chains of RS might be hydrolysis products of the long branch chains of modified starches and, as another possibility, the intermediate branch chains of modified starches might be less efficiently hydrolyzed by enzymatic degradation. Nevertheless, at the same reaction time, no significant difference in RS content was observed between NpAS-modified WCS and NpAS-modified AWCS. Of further importance was that the RS isolated from NpAS-modified WCS and NpAS-modified AWCS had similar compositions of intermediate and long branch chains. Overall, the results suggest that the fraction of intermediate and long branch chains might be a crucial factor, which could affect the RS contents in the modified starches.

Compared to the amorphous region of starch, it is well known that the crystalline region is more resistant to enzymatic hydrolysis. However, regardless of the acceptor, the RS contents in modified starches were inversely correlated to the relative crystallinity (Table 2) in the present study. Generally, the rate of starch enzymatic hydrolysis is mainly controlled by porcine pancreatic α-amylase (Zhang, Ao, & Hamaker, 2006). Due to its limited space, the binding site of α-amylase could not accommodate large and stiff fragments (such as double helices) (Casset, Imberty, Haser, Payan, & Perez, 1995), and thus it seems possible that the digestibility of modified starches was affected not only by the

Table 2
Thermal properties, relative crystallinity, and resistant starch content of waxy corn starch, acid-thinned waxy corn starch, and amylosucrase-modified starches.[a,b]

Acceptor	Reaction time (h)	T_o (°C)	T_P (°C)	T_C (°C)	ΔH (J/g)	X_c (%)	RS (%)
Waxy corn starch	–	64.8 ± 0.4^{b}	70.0 ± 0.6^{a}	78.9 ± 0.3^{b}	15.6 ± 0.1^{f}	41.71	1.2 ± 0.1^{a}
	0.5	68.8 ± 2.1^{c}	80.3 ± 2.3^{c}	90.2 ± 1.0^{d}	10.5 ± 0.3^{d}	34.22	19.1 ± 0.1^{b}
	3	77.3 ± 1.6^{d}	90.2 ± 1.1^{d}	102.6 ± 1.1^{f}	8.2 ± 0.3^{b}	26.76	38.7 ± 0.4^{d}
	6	84.0 ± 0.6^{f}	102.5 ± 1.4^{e}	108.2 ± 0.5^{h}	7.3 ± 0.1^{a}	23.45	48.1 ± 0.2^{e}
Acid-thinned waxy corn starch	–	60.6 ± 0.7^{a}	68.4 ± 0.6^{a}	73.7 ± 0.4^{a}	16.2 ± 0.0^{g}	45.50	0.7 ± 0.1^{a}
	0.5	65.2 ± 1.3^{b}	75.3 ± 1.8^{b}	84.5 ± 0.7^{c}	11.1 ± 0.2^{e}	35.03	19.7 ± 0.3^{c}
	3	75.9 ± 0.8^{d}	80.4 ± 1.1^{c}	94.8 ± 1.0^{e}	9.3 ± 0.1^{c}	30.65	38.2 ± 0.5^{d}
	6	80.4 ± 1.6^{e}	90.0 ± 0.8^{d}	104.6 ± 0.6^{g}	7.6 ± 0.1^{a}	28.91	49.1 ± 0.4^{g}

[a] T_o, T_P, T_C, ΔH represent the onset melting temperature, peak melting temperature, concluding temperature, and endothermic enthalpy, respectively; X_c represents the relative crystallinity; RS represents the resistant starch content.
[b] Values with different superscript letters in each column are significantly different ($P < 0.05$).

relative crystallinity, but also by the structure of the double helices. As shown by HPSEC and DSC data, the modified starches with longer chains tended to generate larger size double helices (helices with more turns). These double helices with more turns would be less susceptible to α-amylase hydrolysis. This would explain why, as the reaction time progressed, the contents of RS in the modified starches gradually increased.

4. Conclusions

NpAS modification of AWC was investigated for the first time. Under the experimental conditions, the viscosity of the reaction mixture did not affect the catalytic efficiency of NpAS significantly in the presence of acceptor, because of the non-catalytic binding site of the NpAS molecule. The enzymatic reaction decreased the proportion of short chains significantly, and the modified starches formed B-type crystalline structures preferentially. Furthermore, branch chain elongation dramatically enhanced the RS contents of modified acceptors, and the values of RS gradually increased as the reaction progressed. The digestibility of modified starches was affected not only by the relative crystallinity, but also by the structures of the double helices in the crystalline region. The results of this study also suggest that the fraction of intermediate and long branch chains might be a crucial factor, which could affect the RS contents in the modified starches.

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References

Brown, I. L., Mcnaught, K. J., & Moloney, E. (1995). Hi-Maize™: New directions in starch technology and nutrition. *Food Australia, 47*(6).

Cai, L., Shi, Y. C., Rong, L., & Hsiao, B. S. (2010). Debranching and crystallization of waxy maize starch in relation to enzyme digestibility. *Carbohydrate Polymers, 81*(2), 385–393.

Casset, F., Imberty, A., Haser, R., Payan, F., & Perez, S. (1995). Molecular modelling of the interaction between the catalytic site of pig pancreatic α-amylase and amylose fragments. *European Journal of Biochemistry, 232*(1), 284–293.

Cooke, D., & Gidley, M. J. (1992). Loss of crystalline and molecular order during starch gelatinisation: Origin of the enthalpic transition. *Carbohydrate Research, 227*(92), 103–112.

Potocki De Montalk, G., Remaud-Simeon, M., Willemot, R. M., Planchot, V., & Monsan (1999). Sequence analysis of the gene encoding amylosucrase from Neisseria polysaccharea and characterization of the recombinant enzyme. *Journal of Bacteriology, 181*(2), 375–381.

Donovan, J. W. (1979). Phase transitions of the starch–water system. *Biopolymers, 18*(2), 263–275.

Ellis, R. P., Cochrane, M. P., Dale, M. F. B., Duffus, C. M., Lynn, A., Morrison, I. M., et al. (1998). Starch production and industrial use. *Journal of the Science of Food & Agriculture, 77*(3), 289–311.

Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition* (Suppl. 2(Suppl. 2)), S33–S50.

Gidley, M. J., Bulpin, P. V., Gidley, M. J., & Bulpin, P. V. (1987). Crystallisation of malto-oligosaccharides as models of the crystalline forms of starch: Minimum chain-length requirement for the formation of double helices. *Carbohydrate Research, 161*(2), 291–300.

Hehre, E. J. (1949). Synthesis of a polysaccharide of the starch-glycogen class from sucrose by a cell free, bacterial enzyme system. *Journal of Biological Chemistry, 177*(1), 267–279.

Higgins, J. A., Higbee, D. R., Donahoo, W. T., Brown, I. L., Bell, M. L., & Bessesen, D. H. (2004). Resistant starch consumption promotes lipid oxidation. *Nutrition & Metabolism, 1:8*(1), 8.

Hizukuri, S. (1986). Polymodal distribution of the chain lengths of amylopectins, and its significance. *Carbohydrate Research, 147*(2), 342–347.

Jane, J., Chen, Y. Y., Lee, L. F., Mcpherson, A. E., Wong, K. S., Radosavljevic, M., et al. (1999). Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chemistry, 76*(5), 629–637.

Jung, J.-H., Seo, D.-H., Ha, S.-J., Song, M.-C., Cha, J., Yoo, S.-H., et al. (2009). Enzymatic synthesis of salicin glycosides through transglycosylation catalyzed by amylosucrases from Deinococcus geothermalis and Neisseria polysaccharea. *Carbohydrate Research, 344*(13), 1612–1619.

Kim, B. S., Kim, H. S., Hong, J. S., Huber, K. C., Shim, J. H., & Yoo, S. H. (2013). Effects of amylosucrase treatment on molecular structure and digestion resistance of pre-gelatinised rice and barley starches. *Food Chemistry, 138*(2–3), 966–975.

Miao, M., Jiang, B., & Zhang, T. (2012). Effect of pullulanase debranching and recrystallization on structure and digestibility of waxy maize starch. *Carbohydrate Polymers, 112*(8), 2009–2017.

Miao, M., Jiang, B., Zhang, T., Jin, Z., & Mu, W. (2011). Impact of mild acid hydrolysis on structure and digestion properties of waxy maize starch. *Food Chemistry, 126*(2), 506–513.

Nara, S., & Komiya, T. (1983). Studies on the relationship between water-satured state and crystallinity by the diffraction method for moistened potato starch. *Starch – Stärke, 35*(12), 407–410.

Nugent, A. P. (2005). Health properties of resistant starch. *Nutrition Bulletin, 30*(1), 27–54.

Perera, C., Lu, Z., Sell, J., & Jane, J. (2001). Comparison of Physicochemical Properties and Structures of Sugary-2 Cornstarch with Normal and Waxy Cultivars 1. *Cereal Chemistry, 78*(3), 249–256.

Potocki de Montalk, G., Remaud-Simeon, M., Willemot, R.-M., & Monsan, P. (2000). Characterisation of the activator effect of glycogen on amylosucrase from *Neisseria polysaccharea*. *FEMS Microbiology Letters, 186*(1), 103–108.

Rolland-Sabaté, A., Colonna, P., Potocki-Véronèse, G., Monsan, P., & Planchot, V. (2004). Elongation and insolubilisation of α-glucans by the action of Neisseria polysaccharea amylosucrase. *Journal of Cereal Science, 40*(1), 17–30.

Ryu, J.-H., Lee, B.-H., Seo, D.-H., Baik, M.-Y., Park, C.-S., Wang, R., et al. (2010). Production and characterization of digestion-resistant starch by the reaction of Neisseria polysaccharea amylosucrase. *Starch – Stärke, 62*(5), 221–228.

Sandhu, K. S., Singh, N., & Lim, S. T. (2007). A comparison of native and acid thinned normal and waxy corn starches: Physicochemical, thermal, morphological and pasting properties. *LWT – Food Science and Technology, 40*(9), 1527–1536.

Shi, M., Chen, Y., Yu, S., & Gao, Q. (2013). Preparation and properties of RS III from waxy maize starch with pullulanase. *Food Hydrocolloids, 33*(1), 19–25.

Shin, H. J., Choi, S. J., Park, C. S., & Moon, T. W. (2010). Preparation of starches with low glycaemic response using amylosucrase and their physicochemical properties. *Carbohydrate Polymers, 82*(2), 489–497.

Sumner, J. B., & Howell, S. F. (1934). A method for determination of saccharase activity. *Journal of Biological Chemistry, 108*, 51–54.

Wang, R., Bae, J. S., Kim, J. H., Kim, B. S., Yoon, S. H., Park, C. S., et al. (2012). Development of an efficient bioprocess for turanose production by sucrose isomerisation reaction of amylosucrase. *Food Chemistry, 132*(2), 773–779.

Wang, R., Kim, J.-H., Kim, B.-S., Park, C.-S., & Yoo, S.-H. (2011). Preparation and characterization of non-covalently immobilized amylosucrase using a pH-dependent autoprecipitating carrier. *Bioresource Technology, 102*(10), 6370–6374.

Wang, Y. J., Truong, V. D., & Wang, L. (2003). Structures and rheological properties of corn starch as affected by acid hydrolysis. *Carbohydrate Polymers, 52*(3), 327–333.

Zhang, G., Ao, Z., & Hamaker, B. R. (2006). Slow digestion property of native cereal starches. *Biomacromolecules, 7*(11), 3252–3258.

Zhou, X., Wang, R., Yoo, S. H., & Lim, S. T. (2011). Water effect on the interaction between amylose and amylopectin during retrogradation. *Carbohydrate Polymers, 86*(4), 1671–1674.

Zobel, H. F. (1988). Starch crystal transformations and their industrial importance. *Starch – Stärke, 40*(1), 1–7.