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Debranching and crystallization of waxy maize starch in relation to enzyme digestibility

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ABSTRACT

Molecular and crystal structures as well as morphology during debranching and crystallization of waxy maize starch at a high solid content (25%, w/w) were investigated, and the results were related to the digestibility of debranched products. The starch was cooked at 115–120 °C for 10 min, cooled to 50 °C and debranched by isoamylase. After 1 h of debranching, wormlike objects with 5–10 nm width and ca. 30 nm length were observed by transmission electron microscopy. Further release of linear chains and crystallization led to assembly of semi-crystalline structures in the form of nano-particles and subsequent growth of nano-particles into large aggregates. After 24 h at 50 °C, a debranched starch product with an A-type X-ray diffraction pattern, a high melting temperature (90–140 °C), and high resistant starch content (71.4%) was obtained. Small-angle X-ray scattering results indicated that all debranched products were surface fractal in a dry state (4% moisture) but had a mass fractal structure when hydrated (e.g. 45% moisture).

1. Introduction

Starch, a semi-crystalline polymer, is biosynthesized as granules in higher plants and generally consists of two types of α-D-glucose polymers: amylose and amylopectin. Amylose is a mixture of lightly branched and linear molecules with a molecular weight (MW) of approximately 1×10^5–1×10^6 g/mol, whereas amylopectin is a much larger molecule with a MW of 1×10^7–1×10^9 g/mol and a highly branched structure consisting of about 95% α-1,4- and 5% α-1,6-linkages (Hizukuri, Abe, & Hanashiro, 2006). Waxy starch is comprised of essentially 100% amylopectin.

The branching points, or α-1,6-linkages, can be cleaved by debranching enzymes such as isoamylase and pullulanase (Hizukuri et al., 2006; Manners, 1989). For an amylose-containing starch, debranching enzymes cleaves branching points in both amylose and amylopectin and produce a mixture of long and short linear chains; for a waxy starch, debranching releases short linear side chains from amylopectin (Shi, Capitani, Trzasko, & Jeffcoat, 1998).

A number of patents have applied debranching techniques to produce linear starch chains of low MWs (Kurimoto & Sugimoto, 1975; Kurimoto & Yoshida, 1974; Sugimoto, Hirao, & Yoshida, 1973) that function as resistant starch (RS) (Chiu, Henley, & Altieri, 1994; Gross & Haralampu, 1999; Haralampu & Gross, 1998; Kettlitz, Coppin, Roper, & Bornet, 2000; Shi, Cui, Birkett, & Thatcher, 2006), slowly digestible starch (SDS) (Shi, Cui, Birkett, & Thatcher, 2005a; Shi, Cui, Birkett, & Thatcher, 2005b), opacifying agent (Chiu, 1992; Chiu & Henley, 1993), fat replacer (Chiu, 1990; Chiu & Mason, 1998; Harris & Little, 1995; Stanley, Harris, Little, & Schanefelt, 1995), tabletting excipient for controlled release (Arends-Scholte et al., 2000; Besemer & Lerk, 1996; Besemer & Lugt, 1997; Dumoulin & Carriere, 1999), and tablet binder (Chiu & Kasica, 1995; Shi, Cui, & Chakrabarti, 2003). In addition, enzyme-debranched starch products are used in cosmetics (Rouse, Valles, Martino, & Chiu, 1996), extruded products (Lehmann, Jacobasch, & Schmiedl, 2002; Zallie, Altieri, Chiu, & Henley, 1996), and many other foods. The properties of debranched products are dependent on a number of factors, including amylose content and MW distribution of starting starch, degree of debranching, type of debranching enzyme used, and crystallization conditions such as solids content, temperature, time, and type and intensity of mixing. Debranching an amylose-containing starch produces a mixture of short and long chains. Those linear chains have a broad MW distribution, which inhibits forming products with a high degree of crystallinity. In contrast, short linear chains released from a waxy starch have a relatively narrow MW distribution and may be crystallized to produce products with a high degree of crystallinity (Shi et al., 2006). However, products debranched from high-amylose starches (Chiu et al., 1994) generally have better thermal stability or a higher melting temperature than those from debranched waxy starches. Compared with com-

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pletely linear molecules, partially debranched starch products have a different gelling rate, gel strength, degree of crystallinity, rate of crystal aggregation, particle size, and enzyme digestibility.

The type of enzyme used in the debranching process is also important. Isoamylase and pullulanase, the two commonly used debranching enzymes, do not act the same on starch (Hizukuri et al., 2006; Manners & Matheson, 1981; Yokobaya et al., 1973). Compared with pullulanase, isoamylase has superior activity for debranching amylopectin (Hizukuri et al., 2006). Pullulanase hydrolyzes amylopectin slowly by exo-wise action and produces A-chains at the initial stages, whereas isoamylase hydrolyzes both inner and outer branching linkages (Manners & Matheson, 1981; Yokobaya et al., 1973). In many patents (e.g. Chiu & Henley, 1993; Chiu & Kasica, 1995; Chiu & Mason, 1998; Chiu et al., 1994; Kettlitz et al., 2000), a debranching technique is combined with physical treatment, such as annealing, to prepare products with various degrees of crystallinity and differing functional properties. However, the detailed structure–function relationships are not discussed in the patent literature.

Nutritionally, starches are classified into rapidly digestible starch (RDS), SDS, and RS on the basis of their digestion rate (Englyst, Kingman, & Cummings, 1992). A high ratio of the sum of SDS and RS to RDS in a starchy food indicates a low glycemic index (Englyst, Englyst, Hudson, Cole, & Cummings, 1999). Because of the great interest in digestibility of starch in relation to carbohydrate nutrition, many researchers have applied debranching techniques to prepare RS (Berry, 1986; Gonzalez-Soto, Agama-Acevedo, Solorza-Feria, Rendon-Villalobos, & Bello-Perez, 2004; Gonzalez-Soto, Mora-Escobedo, Hernandez-Sanchez, Sanchez-Rivera, & Bello-Perez, 2007; Lehmann, Jacobasch, & Schmiedl, 2002; Leong, Karim, & Norziah, 2007; Onyango & Mutungi, 2008) and SDS (Guraya, James & Champagne, 2001a; Guraya, James, & Champagne, 2001b; Shin et al., 2004). However, the detailed structural changes that occur during debranching of starch are not well understood. Pohu, Planchot, Putaux, Colonna, & Buleon (2004) investigated the crystallization during debranching of maltodextrin derived from tapioca starch at 25% (w/v) by isoamylase. They found that a loose B-type network containing linear and branched chains of high MW was formed mainly during the initial stage of debranching, whereas aggregates of A-type crystals consisting of short linear chains were produced during the late stage of debranching. The resulting RS product appeared as a thick, dense precipitate (Pohu, Putaux, Planchot, Colonna, & Buleon, 2004). However, the detailed ultrastructure of the RS product after hydrolysis by α-amylase was difficult to define because the morphology was too complex and the transmission electron microscopic images were of poor quality. Instead, the authors used two model substrates, waxy maize starch nano-crystals obtained by acid hydrolysis and A-type amylose crystals prepared from a low degree of polymerization (DP) amylose, to explain the origin of the limited α-amylolysis of the RS product, and they suggested that the RS aggregates resulted from the epitaxial growth of elementary crystalline A-type platelets (Pohu, Putaux, et al., 2004).

In this study, instead of using soluble maltodextrin, we investigated structural changes during debranching and crystallization of waxy maize starch at high concentration. Multiple analytical techniques, including gel permeation chromatography (GPC), scanning electron microscopy (SEM), transmission electron microscopy (TEM), differential scanning calorimetry (DSC), synchrotron wide-angle X-ray diffraction (WAXD) and small-angle X-ray scattering (SAXS) techniques, were used to determine the molecular and crystal structures as well as morphology during debranching and crystallization of waxy maize starch at a high solid content (25%, w/w). The fractal concept (Suzuki, Chiba, & Yano, 1997) was used to analyze the SAXS results. Based on "self-similarity", this fractal concept uses a non-integer dimension called the fractal dimension and provides quantitative evaluation of irregular structures of polymers (Daoud & Martin, 1989). Suzuki et al. (1997) applied the fractal concept to interpret the small-angel X-ray scattering from maize and potato starches during gelatinization, swelling and retrogradation and concluded that the scattering from native starches with low moisture contents are due to the surface fractal structure of the starches obeying a power law with an exponent of ca. −4 but the physical arrangement of gelatinized starch molecules is a "mass fractal", i.e. a self-similar structure, in nature.

Compared to soluble maltodextrin, there are challenges and advantages in using waxy maize starch as a starting material for debranching and crystallization. Waxy maize starch granules have to be cooked or gelatinized prior to debranching, but the step of converting starch to maltodextrin is omitted. The debranching kinetics and chain-length distribution of waxy maize starch are expected to be different from those of maltodextrin. The specific objectives of the current work were to (1) investigate the debranching and crystallization mechanism of waxy maize starch and (2) determine morphology, structure, and physicochemical properties of debranched products and their impacts on digestibility.

2. Materials and methods

2.1. Materials

Waxy maize starch was obtained from National Starch LLC (Bridgewater, NJ, USA), and isoamylase (EC 3.2.1.68) from Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). The enzyme activity was 1.41×10^6 IAU/g, and 1 IAU was defined as the amount of isoamylase that increased reducing-power absorbance of the reaction mixture by 0.008 in 30 min under the conditions of the isoamylase assay (FAO JECFA Monographs, 2007). All chemicals were reagent-grade.

2.2. Debranching of starch

Waxy maize starch (150 g, dry basis) was mixed with water to give a 25 wt.% solids content. The slurry was adjusted to pH 4.0 by adding 0.5N HCl, cooked at 115–120 °C in a Parr reactor (Parr Instrument, Moline, IL, USA) for 10 min, and cooled to 50 °C. The debranching reaction was started by adding 0.5 wt.% isoamylase based on the dry weight of starch. The mixture was kept at 50 °C with stirring. At 1, 2, 4, 8, 16, and 24 h intervals after adding enzyme, sample slurries (about 40 mL) were taken, immediately frozen in a dry ice acetone bath, freeze-dried, and saved for analysis. The precipitates after 24 h of crystallization were filtered, washed with water, and dried in an oven at 40 °C overnight.

In separate experiments, after the starch was debranched and crystallized at 50 °C for 24 h, the digestion mixture was cooled to 25 °C and held for another 24 h to further increase the yield of crystallized product.

To determine the yield of crystallized product, an aliquot (1.0 mL) of starch slurry was taken and centrifuged ($13{,}226 \times g$) for 10 min. The carbohydrate concentration in the supernatant was determined with a portable refractometer (Fisher Scientific Inc., Pittsburgh, PA, USA). The blank reading was determined by the same procedure on an uncooked starch slurry mixed with isoamylase. The level of precipitation of carbohydrate was calculated by difference. Each measurement was done in duplicate.

2.3. GPC

Each starch sample (4 mg) was mixed with dimethyl sulfoxide (DMSO) (4 mL) and stirred in a boiling water bath for 24 h. The sample was filtered through a 2 μm filter and then injected by an autosampler into a PL-GPC 220 system (Polymer Laboratories Inc.,

Amherst, MA, USA) with three Phenogel columns (00H-0642-K0; 00H-0644-K0; 00H-0646-K0; Phenomenex Inc., Torrance, CA, USA), one guard column (03B-0290-K0, Phenomenex Inc., Torrance, CA, USA), and a differential refractive index detector. The eluenting solvent was DMSO containing with 0.5 mM $NaNO_3$, and the flow rate was 0.8 mL/min. The column oven temperature was controlled at 80 °C. Standard dextrans (American Polymer Standards Co., Mentor, OH, USA) with different MWs were used for MW calibration.

2.4. SEM

The isolated crystalline samples were coated with gold-palladium by using a sputter coater (Denton Vacuum, LLC, Moorestown, NJ) and viewed at 1000× resolution with a scanning electron microscope (S-3500N, Hitachi Science Systems, Ltd., Japan) operating at an accelerating voltage of 20 kV.

2.5. TEM

A drop of starch suspension was deposited on a 200 mesh copper Formvar/carbon-coated support film grid. Excessive liquid was wiped with filter paper. A drop of 2% uranyl acetate negative stain was added, and the suspension film was left to dry. The samples were observed and images were recorded with a Philips CM100 transmission electron microscope (FEI Company, Hillsboro, Oregon, USA) with 64,000× magnification.

2.6. DSC

A 25 wt.% starch suspension in water was prepared and sealed in a DSC pan and analyzed with a TA Q5000 instrument (TA Instruments, New Castle, DE, USA). An empty pan was used as a reference. Samples were heated from 10 °C to 160 °C at 10 °C/min. The onset (T_o), peak (T_p), and conclusion (T_c) temperatures and enthalpy (ΔH) were calculated from the DSC endotherm (TA Instruments, New Castle, DE, USA). Experiments were conducted in duplicate.

2.7. Synchrotron X-ray scattering and diffraction measurements

WAXD and SAXS experiments were carried out at the Advanced Polymers Beamline (X27C) in the National Synchrotron Light Source, Brookhaven National Laboratory, in Upton, NY. The details of the experimental setup at the X27C beamline have been reported elsewhere (Chen et al., 2006, 2007; Chu & Hsiao, 2001). The wavelength used was 0.1371 nm. The sample-to-detector distance was 155.6 mm for WAXD and 2018.5 mm for SAXS, respectively. A 2D MAR-CCD (MAR USA, Inc.) X-ray detector was used for data collection. In addition to native waxy maize starch (ca. 11% moisture) and freeze-dried debranched products (ca. 4% moisture), samples were mixed (hydrated) with water to form starch pastes (45% moisture) and examined by the WAXD and SAXS. The SAXS data were analyzed on the basis of the fractal concept (Suzuki et al., 1997).

2.8. In vitro digestion method

The in vitro starch digestion profile was determined by a modified Englyst procedure (Englyst et al., 1992; Sang & Seib, 2006). Samples (~0.6 g) were mixed with guar gum (50 mg) and pepsin (50 mg) in 0.05 M hydrochloric acid (10 mL). Then, sodium acetate solution (0.25 M, 10 mL) and 30 glass beads (~8.4 g) were added. Guar gum (50 mg) in 0.1 M sodium acetate buffer (pH 5.2, 20 mL) and glucose standard solution (20 mL) were used as the blank and standard, respectively. After the enzyme (pancreatin and amyloglucosidase) solution (5 mL) was added, the mixture was shaken in a water bath (Models 25 and 50, Precision, Winchester, VA) at 37 °C and 90 strokes/min. At 20 and 120 min intervals, a 250 μL aliquot of the mixture was taken and added into 66.6% ethanol solution (10 mL). After centrifugation, 100 μL of supernatant was taken, and glucose content was determined by a D-glucose assay procedure with a Megazyme glucose assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland). Percentages of RDS, SDS, and RS were calculated by (% digestible starch at 20 min), (% digestible starch at 120 min – % digestible starch at 20 min) and (100% – % digestible starch at 120 min), respectively.

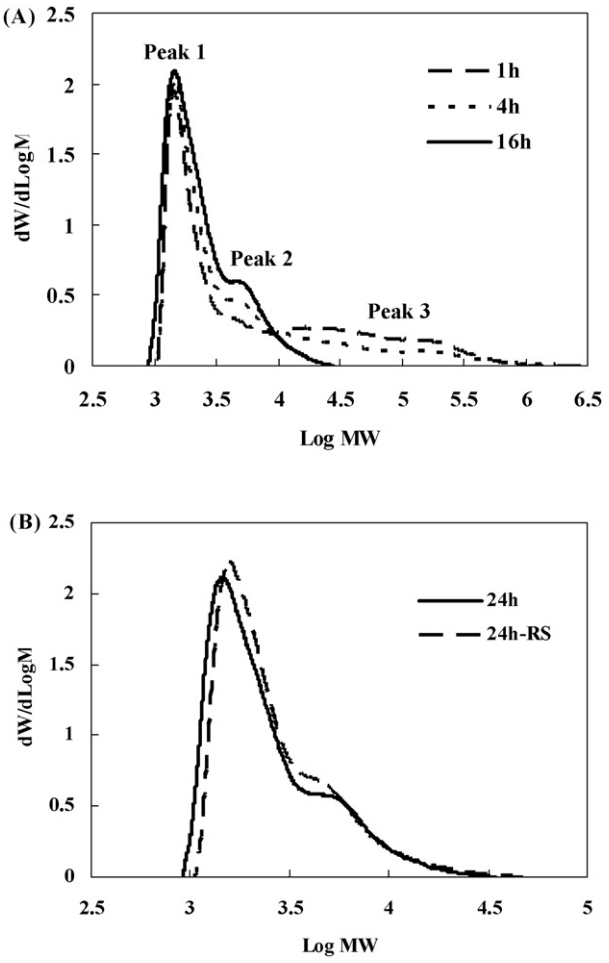

Fig. 1. Molecular weight distribution of (A) waxy maize starch (25% solids) debranched and crystallized at 50 °C at different times, and (B) waxy maize starch debranched and crystallized at 50 °C for 24 h and its corresponding isolated resistant starch.

2.9. Isolation of resistant starch

At 120 min into the *in vitro* digestion method, the entire digest of ~25 mL was mixed with ethanol (100 mL). The sediment was collected by filtration and washed three times with distilled water. The residue was dried under room condition and was called isolated resistant starch.

3. Results

3.1. Degree of debranching and MW distribution

The MW distribution of waxy maize starch at different debranching times is shown in Fig. 1. For samples debranched at 1, 2, 4 and 8 h, 3 peaks were observed. Peaks 1 and 2 represent the unit chains released by debranching whereas Peak 3, ranged from about 1.3×10^4 g/mol (DP 80) to 1×10^6 g/mol (DP 6000), corre-

Table 1
Percentage of debranched starch at different debranching times.

Time (h)	1	2	4	8	16	24
Percentage of debranching (%)	68 ± 2	75 ± 2	80 ± 2	89 ± 2	100	100

sponds to branched molecules. Dividing the sum of areas of Peaks 1 and 2 by the total area of the 3 peaks gave the percentage of debranched starch at different debranching times (Table 1). From 1 to 24 h, Peaks 1 and 2, predominantly short linear chains (DP about 6–80), increased while Peak 3 decreased and disappeared at 16 h. Those short linear chains had similar molecular characteristics to amylose and were mainly linked by α-1,4 linkage. Debranching of the starch started very rapidly after the isoamylase was added. Two-thirds of the debranching was achieved during the first hour. No changes were detected in the MW distribution curve between 16 and 24 h samples, indicating that the waxy maize starch was totally debranched within 16 h (Table 1).

3.2. Crystallization and precipitation

Crystallization occurred at the same time as the release of short linear chains (short-chain amylose, DP about 6–80) from amylopectin during debranching. The percentages of starch precipitate as a function of debranching time are shown in Fig. 2A. The amount of starch precipitate was initially low at 0–4 h, but increased significantly at 4–24 h. The precipitate accounted for about two-thirds of the weight of the initial starch after 24 h. If the temperature of the digestion mixture was cooled to 25 °C and held for another 24 h, the yield of precipitate increased to more than 90% (Fig. 2A). The remarkable changes from the starch polymer solution at the beginning of debranching to the cloudy slurry after 24 h of crystallization at 50 °C are depicted in Fig. 2B. The aggregation and growth of crystallized short-chain amylose produced particles (Fig. 2C) that could be recovered by filtration with a high yield.

The effects of incubation time on starch yield can be explained by the conformational changes of linear short-chain amylose during crystallization. On the molecular level, amylose chains in the aqueous solution are known to behave as random coils (Ring, Lanson, & Morris, 1985), along with some single helical structure, which could associate together and form double helices. The further association of double helices results in crystallites and aggregates of crystals. Therefore, coil-helix structure and formation of double helices from short linaer chains appeared to predominate in the first 4 h, whereas crystallization and aggregation took place in the following stage.

3.3. Morphology

The morphology of waxy maize starch at different debranching times was analyzed by TEM. Fig. 3A shows the morphology of waxy maize starch debranched at the first hour. The sample appeared to consist of wormlike objects with 5–10 nm width and 30–80 nm length. Aggregates (50–100 nm diameter) composed of semi-crystalline units were detected in the following hour (Fig. 3B). After 4 h of debranching, particles with 5–30 nm diameter were evident, and double helices were probably aggregated (Fig. 3C). Larger particles or a long-piece appearance of aggregates were observed after 8 h (Fig. 3D–F). Therefore, the morphology of the short-chain amylose (DP about 6–80) during crystallization had three steps: (1) association of starch chains into double helixes and forming clustering, wormlike structure, (2) rearrangement of semi-crystalline units into nano-particles, and (3) growth of particles into large aggregates.

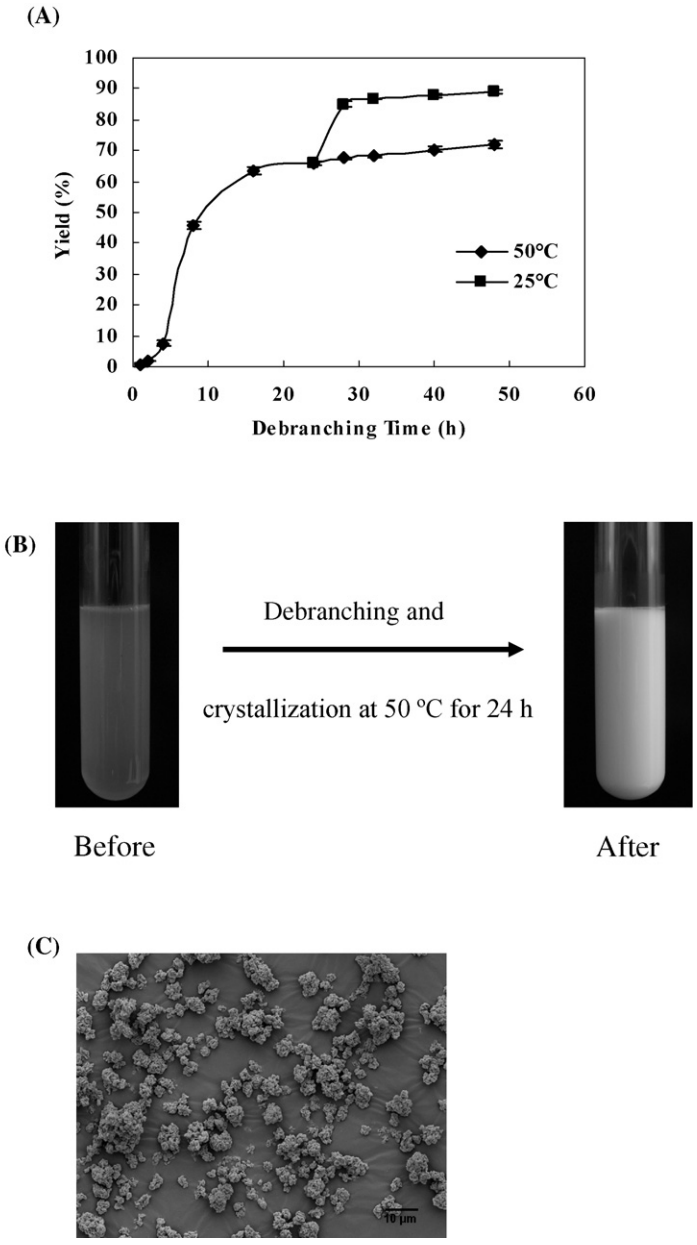

Fig. 2. (A) Yield of starch precipitate based on the weight of waxy maize starch at different debranching times; –◆– crystallization at 50 °C; –■– crystallization at 25 °C after the debranched starch was crystallized at 50 °C for 24 h; (B) Starch polymer solution at the beginning of debranching and cloudy slurry after 24 h of crystallization at 50 °C; and (C) SEM images of waxy maize starch debranched and crystallized at 50 °C for 24 h recovered by filtration and drying in an oven at 40 °C.

3.4. Thermal properties

The thermal properties of native waxy maize starch and the freeze-dried digests of waxy maize starch at different debranching times are shown in Table 2. Native starch was characterized by a sharp endothermic peak at about 65–95 °C with an enthalpy of 18.9 J/g. After debranching for 1, 2, and 4 h, a broad endothermic peak ranging from about 40 to 100 °C with enthalpy of 18.5, 18.3, and 20.6 J/g (Table 2), respectively, was observed (curves not shown), indicating that the materials solidifying at the early stage of debranching had weak crystalline structure and a large variation in crystal size and perfection.

Two endothermic peaks, one ranging from about 43 to 90 °C and the other ranging from about 99 to 138 °C, were observed in the sample debranched for 8 h. After 16 h, only one endothermic peak

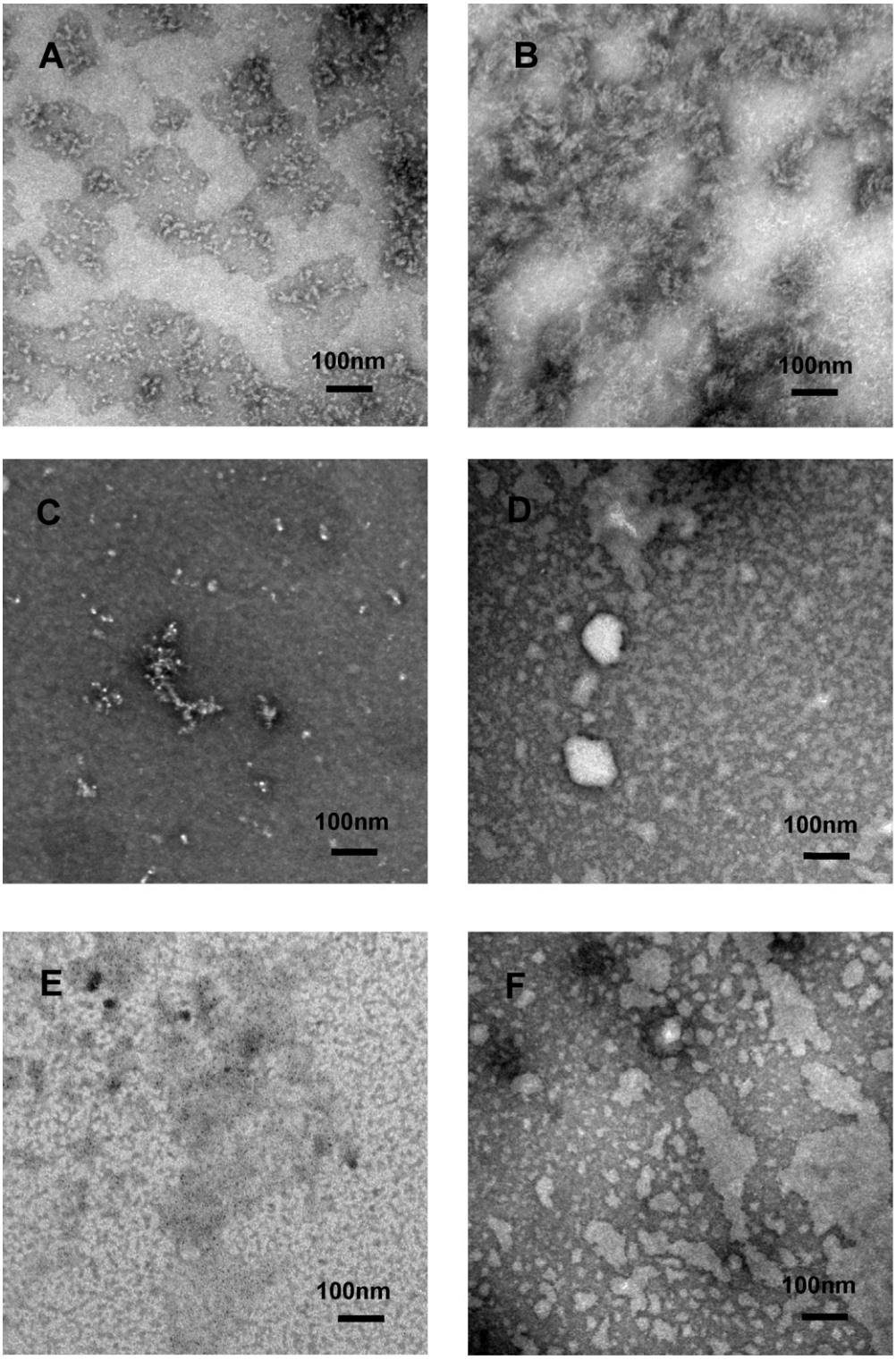

Fig. 3. Transmission electron microscopic images of waxy maize starch (25% solids) debranched at different times at 50 °C: (A) 1, (B) 2, (C) 4, (D) 8, (E) 16, and (F) 24 h.

with melting temperature of about 115 °C and enthalpy of about 20 J/g was detected. Moates, Noel, Parker, and Ring (1997) reported that the dissolution temperature of short-chain amylose crystallites increased form 57–119 °C with an increasing chain length in the range of 12–55 glucose units. In the present study, from 1 to 24 h, a high percentage of short chains (Peak 1 in Fig. 1A) released first and associated into weak crystals, and then more long chains (Peak 2 in Fig. 1A) released with increasing debranching time and formed into crystals with high melting temperature. In addition, the long incubation time (24 h) at 50 °C also facilitated the growth of crystals that required a high temperature to melt.

3.5. Synchrotron SAXS results

Fig. 4A and B shows SAXS patterns of native waxy maize starch and freeze-dried digests of waxy maize starch at different

Table 2
Thermal properties of native waxy maize starch and waxy maize starch debranched at different times as determined by differential scanning calorimetry.

Samples	Peak 1				Peak 2			
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH(J/g)
Native	64.3 ± 0.3	73.5 ± 0.1	94.6 ± 0.1	18.9 ± 0.1	–	–	–	–
1 h	42.3 ± 0.1	73.4 ± 1.9	86.9 ± 0.6	18.5 ± 0.2	–	–	–	–
2 h	44.4 ± 1.2	69.6 ± 1.1	90.1 ± 0.4	18.3 ± 0.3	–	–	–	–
4 h	42.7 ± 0.1	73.6 ± 0.3	99.5 ± 0.5	20.6 ± 0.1	–	–	–	–
8 h	43.3 ± 0.1	68.5 ± 3.1	89.7 ± 2.2	10.9 ± 0.1	99.3 ± 2.1	114.4 ± 1.4	137.8 ± 0.4	8.3 ± 0.0
16 h	–	–	–	–	92.9 ± 0.5	113.6 ± 0.9	138.7 ± 0.4	19.2 ± 0.4
24 h	–	–	–	–	92.3 ± 0.3	114.0 ± 0.2	141.8 ± 0.5	20 ± 0.2

debranching times. As reported by Donald, Kato, Perry, and Waigh (2001); Donald, Perry, and Waigh (2001), the 9 nm lamellar peak of native waxy maize starch was absent in a dry state (Fig. 4A) but appeared after hydration (Fig. 4B). The presence of water molecules is thought to solvate the amorphous region containing branching points, which enables a decoupling between the main backbone of the amylopectin molecules and the side chains. The decoupling allows the side chains to be reconciled into order. However, no lamellar peaks were observed for debranched samples in dry or hydrated states in this study. This could be due to the lack of cluster structure of debranched samples. The released starch chains could be crystallized but lacked regularity between crystalline and amorphous regions.

The log–log plots of SAXS data of native waxy maize starch and freeze-dried digests of waxy maize starch debranched at different times are shown in Fig. 4C and D. According to Suzuki et al. (1997), the SAXS curves could be interpreted on the basis of fractals with the scattering power law: $I \sim q^{\alpha}$, where I is the scattering intensity and q is the scattering vector. The exponent α, which can range from -1 to -4, relates to the fractal characteristics of the scattering objectives. For $-4 < \alpha < -3$, the scattering source is classified as a surface fractal. The surface fractal dimension $D_s = 6 + \alpha$. For $-3 < \alpha < -1$, the scattering source is classified as a mass fractal. The mass fractal dimension $D_m = -\alpha$.

In Fig. 4C, the slope α of the straight lines in all curves was -4.0, which is a well-known behavior described by Porod's law (Porod, 1951). The α value of samples after 1, 4 and 16 h of debranching was also -4.0 (curves not shown). These results suggest that native starch and debranched samples were "surface fractal" in a dry state and that scattering was reflected from the surface or interface. The surface fractal dimension (D_s) was 2.0 when $\alpha = -4.0$, indicating that the surface of native starch and debranched samples was smooth (Suzuki et al., 1997).

In contrast, all debranched samples when hydrated, had slope α of around -2.0 (Fig. 4D), suggesting that hydrated starch aggregates were mass fractal and had self-similar structure in nature. When water molecules were present, they replaced the air around the starch molecules. Because the density of water was much closer to that of the starch than that of air, the scattering reflected the inner structure of starch rather than the surface structure. The mass fractal dimension (D_m) was 2 for all hydrated, debranched samples. Using small-angle neutron scattering, Vallera, Cruz, Ring, and Boue (1994) reported that amylose gels and sols had D_m of 2.6 and 2.0, respectively. It is interesting that the D_m of our hydrated debranched starches, which was 2.0, was close to that of amylose sols. The D_m value of our hydrated debranched starches was similar to that of gelatinized corn and potato starch, which was estimated to be 2.1 and 1.9, respectively (Suzuki et al., 1997).

In contrast to the hydrated debranched products, the hydrated native starch obeyed the power law at q value ranged from 0.06 to 0.2 nm^{-1} with an exponent of $\alpha = -3.7$, which could still be interpreted as surface fractal structure. The surface of starch granules was too rigid and remained smooth in a hydrated state (Suzuki et al., 1997). The inner structure of starch granules was masked by the surface scattering.

3.6. Synchrotron WAXD results

Fig. 5 shows WAXD patterns of native waxy maize starch and waxy maize starch at different debranching times. In the dry state, the diffraction pattern of waxy maize starch debranched at 1, 2, and 4 h was unclear. An A-type crystalline structure was detected for native waxy maize starch and 16 and 24 h samples. In the hydrated state, a typical B-type structure with a peak around 5° (2θ) was observed for samples debranched for 1, 2, and 4 h. It seems that B-type crystallization was preferred during the first 8 h of incubation, followed by A-type crystallization between 16 and 24 h. This dual-stage crystallization phenomenon was also noted during the crystallization of debranched maltodextrin (Pohu, Planchot, et al., 2004).

Using line-broadening analysis (Cairns, Bogracheva, Ring, Hadley, & Morris, 1997), we estimated the size of crystallites in the native waxy maize starch and products debranched at different time (Table 3). At 1, 2, 4 h of debranching, the size of the crystallites was small (<5 nm in dry state), but dramatically increased to ~10 nm in dry state (ca. 4% moisture) and ~12 nm in hydrated state (45% moisture) after 8 h of debranching.

3.7. In vitro digestion profile

Table 4 shows the *in vitro* digestion profiles of native waxy maize starch, waxy maize starch at different debranching times, and isolated crystalline materials. Native waxy maize starch had a very low RS content (<5%). After debranching, RS content increased with time. The rate of increase in RS content was slow from 0 to 4 h but significantly increased from 4 to 24 h. After 24 h at 50 °C, a product with 71.4% RS content was formed. The results confirmed the precipitate yield data (Fig. 2A). The long incubation time resulted in thick, dense crystallites, which were characterized as resistant

Table 3
Average size of polymorph crystallites of native waxy maize starch and waxy maize starch debranched at different times as determined by wide angle X-ray diffraction.

Samples	Size (nm)	
	Powder (ca. 4% moisture)	Hydrated (45% moisture)
Native starch[a]	8.1	9.4
Debranched		
1 h	3.8	9.7
2 h	4.0	8.8
4 h	4.8	8.5
8 h	10.3	12.4
16 h	9.5	14.8
24 h	9.8	14.5

[a] The moisture of native waxy maize starch powder was about 11%.

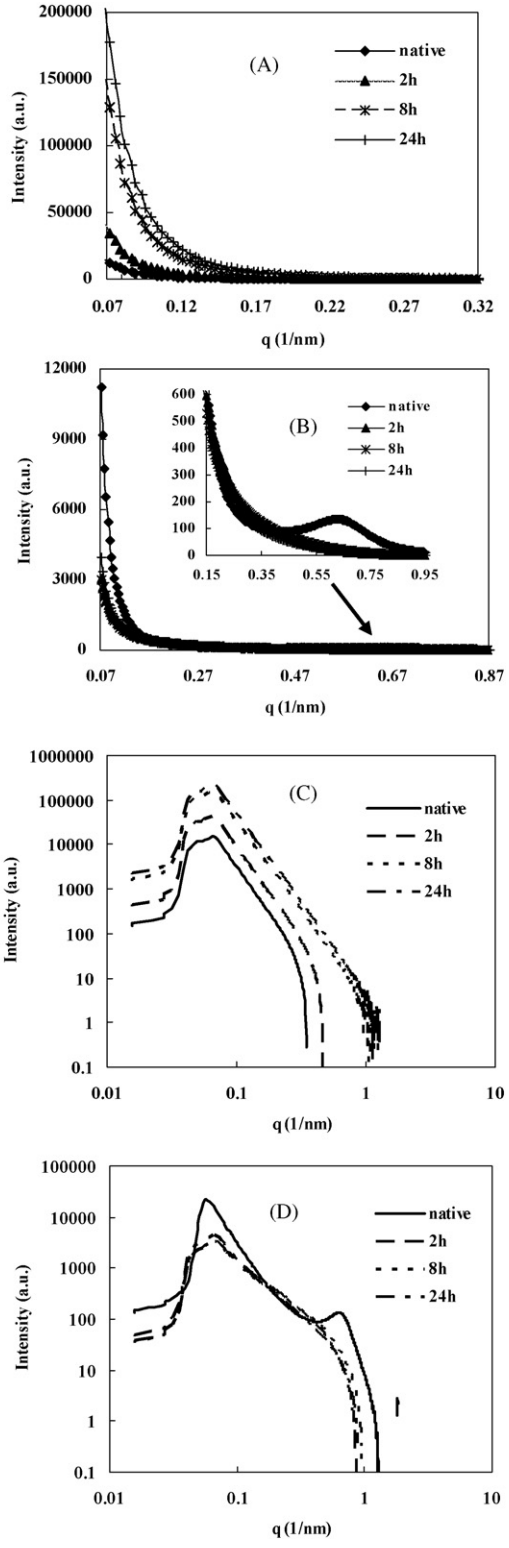

Fig. 4. Small angle X-ray scattering of native waxy maize starch and freeze-dried digests of waxy maize starch debranched and crystallized at 50 °C at different times with (A) 4% moisture (except that native starch was 11% moisture), (B) 45% moisture; (C) log–log plots, 4% moisture (except that native starch was 11% moisture) and (D) log–log plots, 45% moisture.

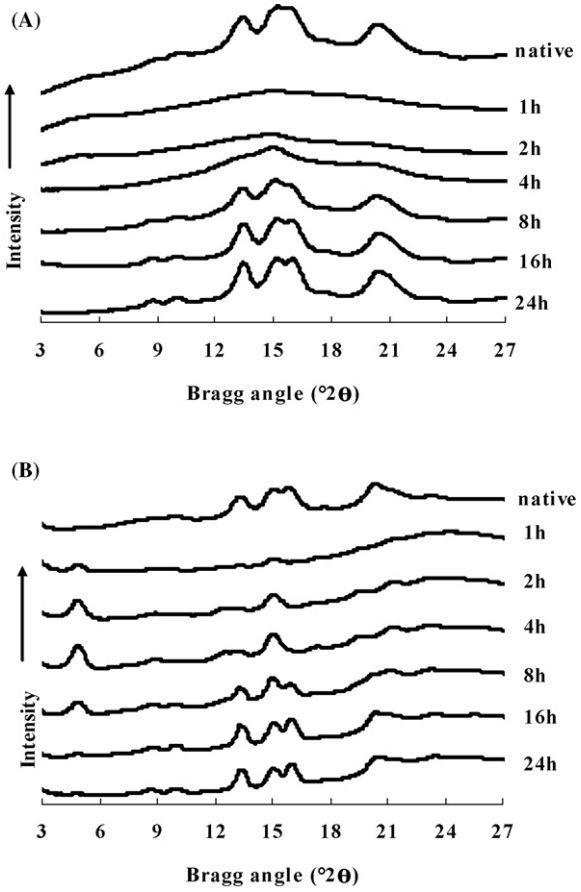

Fig. 5. Wide-angle X-ray diffraction of native waxy maize starch and freeze-dried digests of waxy maize starch debranched and crystallized at 50 °C at different times with (A) 4% moisture (except that native starch was 11% moisture) and (B) 45% moisture.

starch. After 24 h of crystallization, the isolated crystalline materials had a RS content of 86.9% (Table 4).

It is interesting to note that the changes of RDS and SDS content were not linear with debranching time. The SDS content decreased in the first hour of debranching but increased from 1 to 4 h. During the first 4 h of the reaction, the crystallized particles were small in size and weak in crystalline structure (Table 3 and Fig. 5). Alpha-amylase could digest these weak crystalline materials, but at a slow rate. After 4 h of reaction, aggregation and crystallization increased and the crystallized particles became thick and dense. Alpha-amylase has limited access to double helices in the crystalline phase, so resistant starch was formed. Thus, RS formation

Table 4
Levels of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) content in native waxy maize starch, debranched waxy maize starch produced at different times at 50 °C and isolated crystalline materials.

Samples	RDS (%)	SDS (%)	RS (%)
Native starch	29 ± 0.4	66.7 ± 0.4	4.3 ± 0.8
Debranched			
1 h	59.8 ± 0.3	32.9 ± 1.4	7.3 ± 1.7
2 h	58.5 ± 1.7	31.5 ± 0.5	10 ± 1.2
4 h	43.5 ± 0.3	38.9 ± 0.6	17.6 ± 0.9
8 h	33.9 ± 1.1	23.3 ± 0.3	42.8 ± 1.4
16 h	25.1 ± 0.4	8.3 ± 0.9	66.6 ± 0.5
24 h	22.5 ± 0.3	6.1 ± 0.1	71.4 ± 0.4
Isolated crystalline materials[a]	4.9 ± 0	8.2 ± 0.8	86.9 ± 0.8

[a] Isolated crystalline materials were obtained by filtering debranched waxy maize starch product after 24 h of crystallization and drying at 40 °C in an oven over night.

could be characterized as the aggregation and arrangement of double helices from short linear chains in a crystalline structure. The differences in enzyme digestion behaviors among debranched waxy maize starches suggest that the debranching technique combined with controlled crystallization could be used to design the structure of starches with different digestibility.

3.8. Molecular origin of RS

After *in vitro* digestion, the MW distribution of waxy maize starch debranched at 24 h was slightly shifted (Fig. 1B), suggesting that linear chains with a lower molecular weight is more susceptible to the enzyme attack. The low MW linear chains (mostly DP <10) was too short to form the stable double helices (Gidley & Bulpin, 1987); thus, it failed to aggregate and was easily digestible. Lopez-Rubio, Flanagan, Shrestha, Gidley, and Gilbert (2008) reported that the average characteristic dimension of RS crystals was ~5 nm, which corresponds to 2.2 helix turns, with ~13 glucose per helix. For waxy maize starch, the CL distribution was peaked at DP 16 as determined by high-performance anion-exchange chromatograph (Cai & Shi, 2010). The size of the crystallites in the final crystallized product was ~14.5 nm (Table 3). The nature of the RS product in this work was attributed to its dense crystalline structure and compact morphology. Pohu, Putaux, et al. (2004) suggested an epitaxial growth of elementary crystalline A-type platelets for the origin of the α-amylase resistance of debranched maltodextrin. The accessibility of double helices to α-amylase was strongly limited by aggregation.

In this study, the RS product produced from the debranching of waxy maize starch had a relative low DP which was assembled into a highly crystalline structure. In contrast, high amylose starch contains long linear molecules. Lopez-Rubio et al. (2008) noted a significant increase in molecular order and crystallinity when extruded high amylose starches were digested by α-amylase and glucoamylase, suggesting that during the digestion process, amylose chains were rearranged into enzyme-resistant structures of higher crystallinity. In that case, two competing factors, the kinetics of enzyme hydrolysis and the kinetics of amylose retrogradation, determine the resistance to enzyme digestion of a specific processed starch (Lopez-Rubio et al., 2008).

4. Discussion

There are two challenges when waxy maize starch is debranched at a high solids content (25%, w/w). First, the slurry may become very viscous during gelatinization. In this study, a pressure cooker coupled with mechanical stirring was used to totally gelatinize the starch and perform the debranching reaction. Second, amylose can either precipitate or form a gel (network). When the amylose concentration is higher than 1.5%, amylose gels can be formed (Gidley, 1989). Therefore, we needed to use conditions that led to crystallization instead of gelation. Increasing the mobility of starch chains at 50 °C helped achieve that goal. With continuous stirring, the resulting products could be recovered by filtration, which made them practical for large-scale production.

Depending on CL, concentration, temperature and solvent used, amylose can be crystallized from solution to form gels, aggregates, precipitates, spherulites or lamellar crystals with A or B allomorphic type (Buleon, Veronese, & Putaux, 2007). Combining debranching of starch with controlled crystallization provides a practical way of producing A- and B-type crystalline starches with different morphology. In the present work, the crystallization behaviors of debranched waxy maize starch were investigated in a concentrated solution (25%, w/w). After crystallization at 50 °C, debranched starch with an A-type X-ray diffraction pattern and high RS content (71.4%) was obtained. In contrast, when a low starch concentration (5%, w/w) was used, debranching of waxy maize starch at 50 °C followed by crystallization at 25 °C resulted in crystalline short-chain amylose with a B-type X-ray pattern (Cai & Shi, 2010). Our results were in agreement with previous findings on debranched glycogen (Gidley & Bulpin, 1987) and amylodextrin (Hizukuri, 1961) that A-type polymorph is favored by high concentration and high temperature. However, the exact solid concentration and crystallization temperature needed to produce A- or B-type polymorph were different for different materials. For instance, A-type crystals were produced from debranched glycogen by crystallization at 30 °C with 50% solids (Gidley & Bulpin, 1987). The average CL of debranched glycogen was 11.2 (Gidley & Bulpin, 1987), much shorter than the debranched waxy maize starch (average CL 24.1) (Cai & Shi, 2010). Indeed, CL is of primary importance in determining the type of polymorph formed during crystallization (Gidley & Bulpin, 1987; Hizukuri, Kaneko, & Takeda, 1983; Pfannemuller, 1987). Moreover, the yield of crystalline short-chain amylose increases as the CL increases (Cai & Shi, 2010). The yield of A-type crystalline debranched glycogen is 52% after crystallization at 30 °C for 10–14 days (Gidley & Bulpin, 1987). In comparison, in this study, the yield of crystalline short-chain amylose was greater than 90% after debranching and crystallization of waxy maize starch at 50 °C for 24 h followed by precipitation at 25 °C for 6 h (Fig. 2A). The remarkable high yield and possible recovery by filtration make the large production of short-chain amylose from debranched waxy maize starch feasible.

5. Conclusions

The simultaneous debranching of waxy maize starch and solidification of debranched products at a high solid content were investigated for the first time by combined analytical techniques. Short-chain amylose crystallized upon release from amylopectin during debranching. A RS product with an A-type crystalline structure, high melting temperature (90–140 °C), and high RS content (71.4%) was obtained. The yield of the crystallized product was ca. 90% after the starch was completely debranched at 50 °C and further precipitated at 25 °C. Combining debranching techniques with controlled crystallization may be used to design the structure of starch with targeted digestibility. Further research is needed to determine health impact of starches with different digestibility.

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