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Preparation, structure, and digestibility of crystalline A- and B-type aggregates from debranched waxy starches

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ABSTRACT

Highly crystalline A- and B-type aggregates were prepared from short linear α-1,4 glucans generated from completely debranched waxy maize and waxy potato starches by manipulating the chain length and crystallization conditions including starch solids concentration and crystallization temperature. The A-type crystalline products were more resistant to enzyme digestion than the B-type crystalline products, and the digestibility of the A- and B-type allomorphs was not correlated with the size of the aggregates formed. Annealing increased the peak melting temperature of the B-type crystallites, making it similar to that of the A-type crystallites, but did not improve the enzyme resistance of the B-type crystalline products. The possible reason for these results was due to the compact morphology as well as the denser packing pattern of double helices in A-type crystallites. Our observations counter the fact that most B-type native starches are more enzyme-resistant than A-type native starches. Crystalline type per se does not seem to be the key factor that controls the digestibility of native starch granules; the resistance of native starches with a B-type X-ray diffraction pattern is probably attributed to the other structural features in starch granules.

1. Introduction

Starch, a natural biopolymer, is the most important source of food energy. Interest is growing in understanding the relationships between digestion of starch and its effects on human health (Lehmann & Robin, 2007; Zhang & Hamaker, 2009). Fundamental knowledge of the relationship between starch structure and digestion is needed for the development of ingredients and products with health benefits (Tester, Karkalas, & Qi, 2004; Tester, Qi, & Karkalas, 2006).

The susceptibility of starch granules to enzymes and the extent of enzymatic hydrolysis are controlled by many factors such as botanical origins of starch, enzyme sources, enzyme and substrate concentration, hydrolysis temperature, and time as well as the presence of other components (Bird, Lopez-Rubio, Shrestha, & Gidley, 2009; Tester et al., 2006). Within starch granules, the feature of granular morphology (Brewer, Cai, & Shi, 2012; Colonna, Leloup, & Buleon, 1992; Fanon, Hauber, & BeMiller, 1992; Noda et al., 2005; Shrestha et al., 2012; Tester et al., 2006), arrangement of crystalline and amorphous regions in the granule (Blazek & Gilbert, 2010; Jiang & Liu, 2002; Planchot, Colonna, Gallant, & Bouchet, 1995; Tester et al., 2006), size of blocket that contains both amorphous and crystalline lamella (Gallant, Bouchet, & Baldwin, 1997), fine structure of amylose and amylopectin (Jane, Wong, & McPherson, 1997; Jiang & Liu, 2002; Srichuwong, Sunnarti, Mishima, Isono, & Hisamatsu, 2005; Syahariza, Sar, Hasjim, Tizzotti, & Gilbert, 2013), crystal perfection and interrelations, and crystalline types have been suggested to have large effects on the rate and extent of enzymatic hydrolysis (Buleon, Colonna, Planchot, & Ball, 1998). The exact underlying mechanism of enzymatic digestion of starch granule is complicated because these structural features are often interconnected (Bird et al., 2009).

In general, native starches with a B-type X-ray diffraction pattern (e.g., potato and high-amylose starches) are more resistant to enzyme hydrolysis than starches with an A-type crystalline structure (e.g., waxy and normal cereal starches) (Dreher, Dreher, & Berry, 1984; Gallant et al., 1997; Gallant, Guilbot, & Mercier, 1972; Jane et al., 1997; McCleary & Monaghan, 2002; Planchot et al., 1995; Srichuwong et al., 2005a; Srichuwong, Sunnarti et al., 2005). However, the relative enzyme resistance of A- and B-type allomorphs within C-type starches is not consistent in the literature (Li, Gao, Wang, Jiang, & Huang, 2011; Man et al., 2012; Qin et al., 2011; Xia et al., 2012). Two studies showed that B-type allomorph of C-type starches was more enzyme resistant than A-type allomorph in high-amylose rice starch (Man et al., 2012; Qin et al., 2011), whereas other researchers suggested that B-type allomorph was preferentially hydrolyzed by enzymes than A-type allomorph in the C-type *Pueraria lobata* (Willd.) Ohwi and *Pueraria thomsonii* Benth. starches (Xia et al., 2012) and Chinese yam starches (Li, Gao, Wang et al. 2011; Li, Gao, Jiang, Wang, Guo, & Huang, 2011).

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Whether the enzyme resistance of native starch is due to its crystalline type or the other structural features of starch granules is unclear. According to Gallant et al. (1997), the enzyme resistance of potato and high-amylose starches (both B-type structures) may be linked to their large blocklet size. These starch granules appear to have a thick peripheral layer of large stacked blocklets comprising the crystalline and amorphous lamellae of the amylopectin that are organized into larger, more or less spherical structures; however, as pointed out by Gallant at al. (1997), wrinkled pea starch has a smaller blocklet size than smooth pea but is more resistant to enzyme digestion (Gallant, Bouchet, Buleon, & Perez, 1992), indicating that other factors besides blocklet size determine the resistance to α-amylase. The presence of pores and channels in A-type starches has been attributed to their high enzyme digestibility (Blazek & Gilbert, 2010; Zhang, Venkatachalam, & Hamaker, 2006). In studying three maize starches with different amylose contents, Shrestha et al. (2012) concluded that the major factor controlling enzyme susceptibility is granule architecture, particularly the features of surface pores. Short length scales (e.g., amylopectin branch lengths, helix form, crystallinity or lamellar organization) play a secondary role. However, they suggested that the presence of B-type crystallites within the granule may be connected with the absence of the extensive pores and channels in B-type starches.

Studies that compare enzyme digestibility of pure A- and B-type crystals are limited. Williamson et al. (1992) and Planchot, Colonna, & Buleon (1997) prepared A- and B-type spherulite crystals and found that B-type spherulites were more resistant to α-amylase than A-type spherulites. The crystals prepared by these authors were from acid hydrolyzed (referred as lintnerized) potato starch and presumably contained a mixture of linear and branched molecules because potato starch comprises amylose and amylopectin. In those studies, the B-type spherucrystals were crystallized in water whereas the A-type spherucrystals were produced in a water–ethanol mixture. The extensive acid hydrolysis removes amorphous regions in starch, but leads to high starch loss during filtration. In addition, organic solvent (a mixture of water and ethanol) was used to produce the A-type spherucrystals. As a result, the process is not cost effective or commercially viable.

In our previous work (Cai & Shi, 2010; Cai, Shi, Rong, & Hsiao, 2010), we prepared highly crystalline A- and B-type products from short linear chains (short-chain amylose, SCA) generated from completely debranched waxy starches in an aqueous environment. In this study, systematic experiments were designed to determine how crystallization conditions and starch sources affected the formation of A- and B-type allomorphs in aqueous systems. Our overall goal was to compare the structure and enzyme digestibility of highly pure A- and B-type starch crystals and to understand the roles of crystalline types in starch digestibility. The specific objectives were to (1) prepare highly crystalline materials with A- and B-type allomorphs by manipulating the processing conditions such as starch solids concentration, crystallization temperature, and chain length (starch sources), (2) characterize the properties and digestibility of the resulting materials, and (3) relate the starch allomorphs to enzyme digestion.

2. Materials and methods

2.1. Materials

Waxy maize starch was obtained from National Starch LLC (Bridgewater, NJ, USA), waxy potato starch was provided by Penford Food Ingredients Company (Centennial, CO, USA), and isoamylase (EC 3.2.1.68) was obtained from Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). The enzyme activity was 1.41×10^6 IAU/g, where 1 IAU of activity 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.

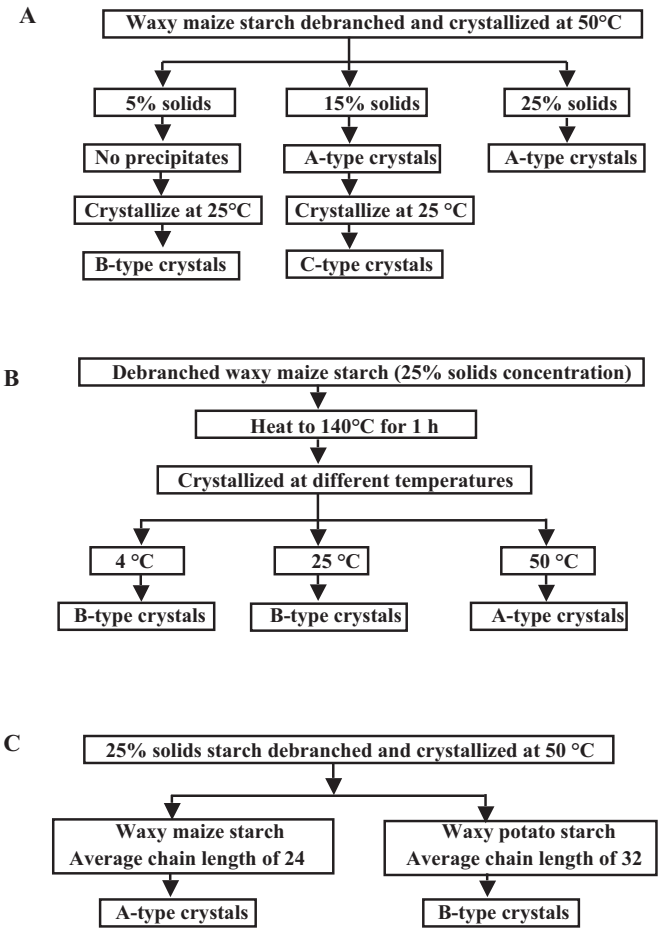

Fig. 1. Production of A- and B-type crystalline products from short-chain amylose by changing (A) starch solids, (B) crystallization temperature, and (C) chain length.

2.2. Manipulation of A- and B-type allomorphs from SCA

Three approaches were used to produce A- and B- type starch allomorphs from SCA (Fig. 1). The first approach was to use different solids concentrations of starch as described in our previous studies with minor modifications (Cai & Shi, 2010; Cai et al., 2010). Three different starch levels (5%, 15%, and 25%) were used. To debranch starch, waxy maize starch was first mixed with acetic acid buffer (0.01 M, pH 4.0) in a pressure bottle and heated in a boiling water bath with stirring for 30 min followed by heating at 120 °C in an oven for 30 min to ensure that the starch was fully gelatinized. After the mixture was cooled to 50 °C, the debranching reaction was started by adding 1% isoamylase based on the dry weight of starch. For the 25% solids sample, the precipitates were filtered after 24 h of debranching reaction. Because none or fewer crystalline materials were obtained after being reacted at 50 °C for 24 h for 5% and 15% solids samples, the mixtures were then cooled to 25 °C and held for another 24 h in order to obtain enough crystalline materials. The precipitates were filtered, washed with water, dried in an oven at 40 °C overnight, and ground by a mortar and pestle. For the first approach, the crystalline products were formed *in situ* during the debranching of waxy maize starch.

The second approach to favor A- or B-type crystalline product was to manipulate crystallization temperatures. Waxy maize starch (25% solids concentration) was debranched for 24 h as described

above, then the mixture was heated at 140 °C in an oven for 1 h to completely melt the crystals. The clear solution (amorphous debranched waxy maize starch) was held at 4, 25, or 50 °C for 24 h to induce crystallization. The precipitates were filtered, washed with water, dried in an oven at 40 °C overnight, and ground by a pestle and mortar. For the second approach, the A- and B-type aggregates were obtained from amorphous debranched waxy maize starch.

The third approach to prepare A- or B-type crystalline products was to use SCA with different chain lengths (CL). Waxy potato starch (25% solids concentration), which has longer unit chains than waxy maize starch (Cai & Shi, 2010), was debranched for 24 h as described above, then the precipitates were filtered, washed with water, dried in an oven at 40 °C overnight, and ground with a mortar and pestle. The yield of all crystallized product was determined as previously described (Cai et al., 2010). For the third approach, A- and B-crystalline products were gained *in situ* during the debranching of starch.

To compare the results in the literature, the SCA from debranched waxy maize starch was used to prepare A- and B-type crystals by the method of Planchot et al. (1997). Briefly, amorphous SCA was obtained by heating 10% SCA aqueous solution at 140 °C in an oven for 1 h, followed by cooling to and equilibrating at 95 °C. B-type crystalline products were obtained by cooling the aqueous SCA solution from 95 °C to 25 °C at a rate of ca. 5 °C/h, whereas A-type crystalline materials were prepared by adding the same volume of warm ethanol to SCA solution at 78 °C during cooling. All the starch materials were proved to be completely debranched and contained short linear chains (Cai & Shi, 2010; Cai et al., 2010).

2.3. Annealing of starch crystals

Immediately after debranching for 24 h, waxy potato starch slurry (25% solids) was annealed at 100 °C in a water bath for 4 h with continuous stirring. The mixture was filtered, dried at 40 °C in an oven overnight, and saved for further digestion analysis.

2.4. Particle size determination

Crystalline samples of SCA were suspended in aqueous 1% sodium azide, and after sonication, several drops of the suspension were added into a Beckman Coulter LS 13 320 Laser Diffraction Size Analyzer (Beckman Coulter, Inc. Brea, CA, USA) equipped with the universal liquid module. The average particle size was recorded. Each sample was measured twice.

2.5. Gel permeation chromatography (GPC)

The starch samples were examined by GPC as previously described (Cai & Shi, 2010; Cai et al., 2010). Dextrans with different molecular weight (MW) were used to calibrate the columns. An equivalent molecular size to dextran standards was reported.

2.6. Scanning electron microscopy (SEM)

The samples were coated with gold-palladium using a sputter coater (Denton Vacuum, LLC, Moorestown, NJ, USA) and viewed at 1000× and 4000× magnification with a scanning electron microscope (S-3500N, Hitachi Science Systems, Ltd., Japan) operating at an accelerating voltage of 20 kV.

2.7. Wide-angle X-ray diffraction

X-ray diffraction was conducted with a Philips X-ray diffractometer with Cu-Ka radiation at 35 kV and 20 mA, a theta-compensating slit, and a diffracted beam monochromator. The moisture of all samples was adjusted to about 15% in a sealed container that contained water at the bottom at room temperature (ca. 25 °C) before analysis. The diffractograms were recorded between 2 and 35 ° (2θ). The relative crystallinity of samples was estimated by the ratio of the peak areas to the total diffractogram area (Komiya & Nara, 1986).

Table 1
Yields, average particle size and relative crystallinity of short-chain amylose products prepared by three different approaches (mean ± standard deviation).

a. Effect of solids concentration

Solids (%)	5	15	25
Yield (%)	0.0[a]	32.0 ± 0.4[a]	65.8 ± 0.8[a]
	59.2 ± 1.4[b]	77.4 ± 0.5[b]	89.8 ± 0.3[b]
Average particle size (μm)	9.0 ± 0.4[b]	7.4 ± 0.3[b]	4.7 ± 0.1[a]
Relative crystallinity (%)	87.2 ± 0.6[b]	85.3 ± 1.5[b]	86.9 ± 1.3[a]

b. Effect of crystallization temperature[c]

Crystallization temperature (°C)	4	25	50
Yield (%)	90.4 ± 1.1	88.0 ± 0.8	72.4 ± 0.6
Average particle size (μm)	13.5 ± 0.5	10.1 ± 0.4	8.9 ± 0.3
Relative crystallinity (%)	84.3 ± 0.9	84.3 ± 1.5	87.4 ± 2.4

c. Effect of chain length[d]

Average chain length (Glucose units)	Waxy maize starch	Waxy potato starch
	24	32
Yield (%)	65.8 ± 0.8	72.8 ± 1.4
Average particle size (μm)	4.7 ± 0.1	15.9 ± 0.1
Relative crystallinity (%)	86.9 ± 1.3	83.2 ± 1.0[e]

[a] Waxy maize starch was debranched at 50 °C for 24 h.
[b] Waxy maize starch was debranched at 50 °C for 24 h, followed by holding at 25 °C for another 24 h.
[c] Waxy maize starch (25% solids) was debranched at 50 °C for 24 h, then heated to 140 °C for 1 h and crystallized at different temperatures.
[d] Starch (25% solids) was debranched and crystallized at 50 °C for 24 h.
[e] After being annealed at 100 °C in a water bath for 4 h with continuous stirring and filtration, the crystallinity of debranched waxy potato starch was 79.8 ± 1.3%.

2.8. Differential scanning calorimetry (DSC)

Starch suspension (25% solids) was sealed in a DSC pan and equilibrated overnight at 25 °C with the intention to completely hydrate the samples. Each sample was heated from 10 °C to 160 °C at 10 °C/min by using a DSC (TA Q200 instrument, New Castle, DE, USA). An empty pan was used as a reference. The onset (T_o), peak (T_p), and conclusion (T_c) temperatures and enthalpy change (ΔH) were calculated from the DSC thermogram.

2.9. In vitro digestion method

The *in vitro* starch digestion profile was determined by a modified Englyst procedure (Englyst, Kingman, & Cummings, 1992) as previously described (Sang & Seib, 2006).

3. Results

3.1. Formation of A- and B-type allomorphs

Wide-angle X-ray diffraction patterns and relative crystallinity of SCA prepared from different approaches are shown in Fig. 2 and Table 1, respectively. All samples displayed a diffraction pattern with sharp peaks that were associated with a crystalline structure of 83–87% crystallinity. However, no lamellar peaks were observed for debranched waxy starches by small-angle X-ray scattering (Cai et al., 2010; Cai & Shi 2013). After starch granules were cooked and destroyed, short linear chains from debranching of waxy starches were readily crystallized but lacked regularity between crystalline and amorphous regions. A crystalline product with A-type allomorph was obtained by debranching and crystallization of 25% solids waxy maize starch at 50 °C for 24 h (Fig. 2A). Debranching 5% solids waxy maize starch and crystallization at 50 °C for

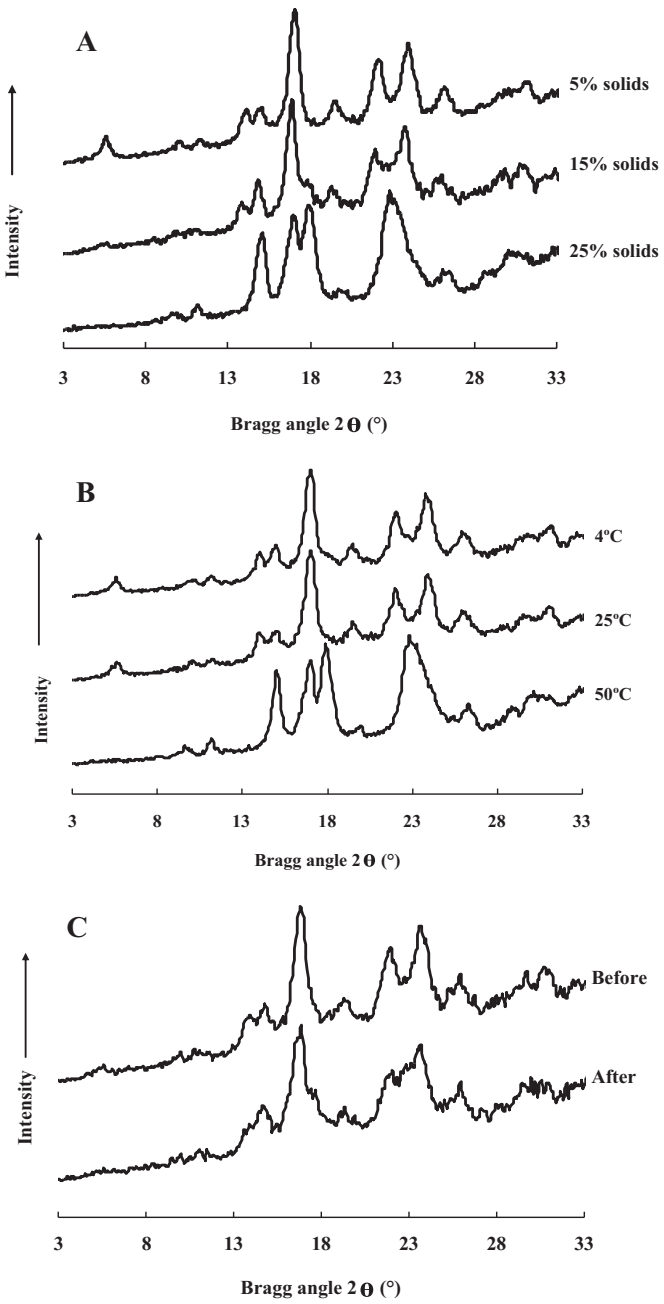

Fig. 2. Wide-angle X-ray diffraction of short-chain amylose prepared from debranched waxy maize starch with different (A) solids concentration, (B) crystallization temperatures, and (C) debranched waxy potato starch before and after annealing.

24 h followed by holding at 25 °C for another 24 h resulted in a B-type allomorph. A mixture of A- and B-type starch crystallites was observed for 15% solids sample (Fig. 2A). At the same starch concentration (25% solids), an amorphous sample of SCA was transformed into A-type starch crystals at 50 °C, whereas B-type crystalline materials were formed at 4 and 25 °C (Fig. 2B). It should be noted that although A-type crystalline aggregates were produced in both Method A and Method B (Fig. 1), they were not formed in the same way. In Method A, the crystalline products were formed *in situ* during the debranching of waxy maize starch, whereas in Methods B, the A- and B-type aggregates were obtained from amorphous debranched waxy maize starch. Therefore, even though the solid content (25%) and the crystallization temperature (50 °C) were the same in both approaches, the products could have different digestibility because in Method B, prior to crystallization, SCA was melt to an amorphous state after debranching was complete. In contrast with waxy maize starch (Fig. 2A), 25% solids waxy potato starch debranched and crystallized at 50 °C showed the B-type X-ray diffraction pattern (Fig. 2C). Our results agree with the general findings that higher solids and temperature and shorter chain length favor the formation of A-type crystallites, and the reverse conditions induce B-type crystallization (Buleon, Veronese, & Putaux, 2007; Cai & Shi, 2010; Cai et al., 2010; Gidley & Bulpin, 1987; Helbert, Chanzy, Planchot, Buleon, & Colonna, 1993; Pfannemuller, 1987; Planchot et al., 1997; Ring, Miles, Morris, Turner, & Colonna, 1987; Whittam, Noel, & Ring, 1990; Williamson et al., 1992).

However, the exact conditions to produce highly crystalline A- or B-type products were different depending on the starting materials. For SCA prepared from extensive acid hydrolysis of native starches, the B-type crystalline structure was obtained by cooling the aqueous solution directly at a slow rate, whereas the A-type allomorph was obtained by addition of water–ethanol mixture during cooling (Helbert et al., 1993; Planchot et al., 1997; Ring et al., 1987; Whittam et al., 1990; Williamson et al., 1992). Pohu et al., 2004a studied debranching of maltodextrins at 25% solids concentration by isoamylase and found that a B-type network was produced during the first 12 h of debranching, followed by formation of A-type lamellar crystals in subsequent reaction. Similar results were observed during debranching of 25% solids concentration of waxy maize starch (Cai et al., 2010). CL also affects on the crystallization of pure malto-oligomers in aqueous solution (Gidley & Bulpin, 1987; Pfannemuller, 1987). Malto-oligomers of degree of polymerization (DP) 10–12 gave an A-type X-ray diffraction pattern, chains of DP13 and above showed a B-type pattern, and chains shorter than DP10 did not crystallize. According to Gidely & Bulpin (1987), the A-type crystals could be produced from 50% solids debranched glycogen by crystallization at 30 °C, and B-type structure could be obtained by crystallization of 30% or 40% aqueous solution at 15 °C.

The yields of A- and B-type crystalline products are listed in Table 1. Debranching 5% solids waxy maize starch at 50 °C for 24 h gave no precipitate, but 59.2% crystalline material was obtained after holding the mixture at 25 °C for another 24 h. Debranching 15 and 25% solids waxy maize starch at 50 °C for 24 h yielded 33% and 65.8% precipitates, respectively, and 77.4% and 89.8% products, respectively, after holding at 25 °C for another 24 h. Cooling debranched waxy maize starch that has been preheated to 140 °C for 1 h to 4, 25, and 50 °C for 24 h generated 90.4, 88.0, and 72.4% starch crystals, respectively. The yield of waxy potato starch debranched at 50 °C for 24 h was 72.8%. The results showed that yield increased as solids concentration and chain length increased and crystallization temperature decreased. The high yields of crystalline SCA indicated their great potential for industry applications.

3.2. MW distribution of A- and B-type allomorphs

The MW relative to dextran standards was obtained in this study. The relative MW distributions of A- and B-type crystalline products are presented in Fig. 3. A bimodal distribution with a low molecular weight peak and a high molecular weight peak was observed for all samples. Crystalline product prepared from 25% solids had a higher proportion of low molecular weight fraction than those from 5% solids (Fig. 3A). Molecules with small molecular weight seem favored to crystallize at higher solids concentration. At the same solids concentration, the distribution pattern of SCA crystallized at 4, 25, and 50 °C was essentially the same (Fig. 3B). Compared with samples prepared from debranched waxy maize starch, those generated from debranched waxy potato starch had a higher percentage of large molecular weight molecules (Fig. 3C).

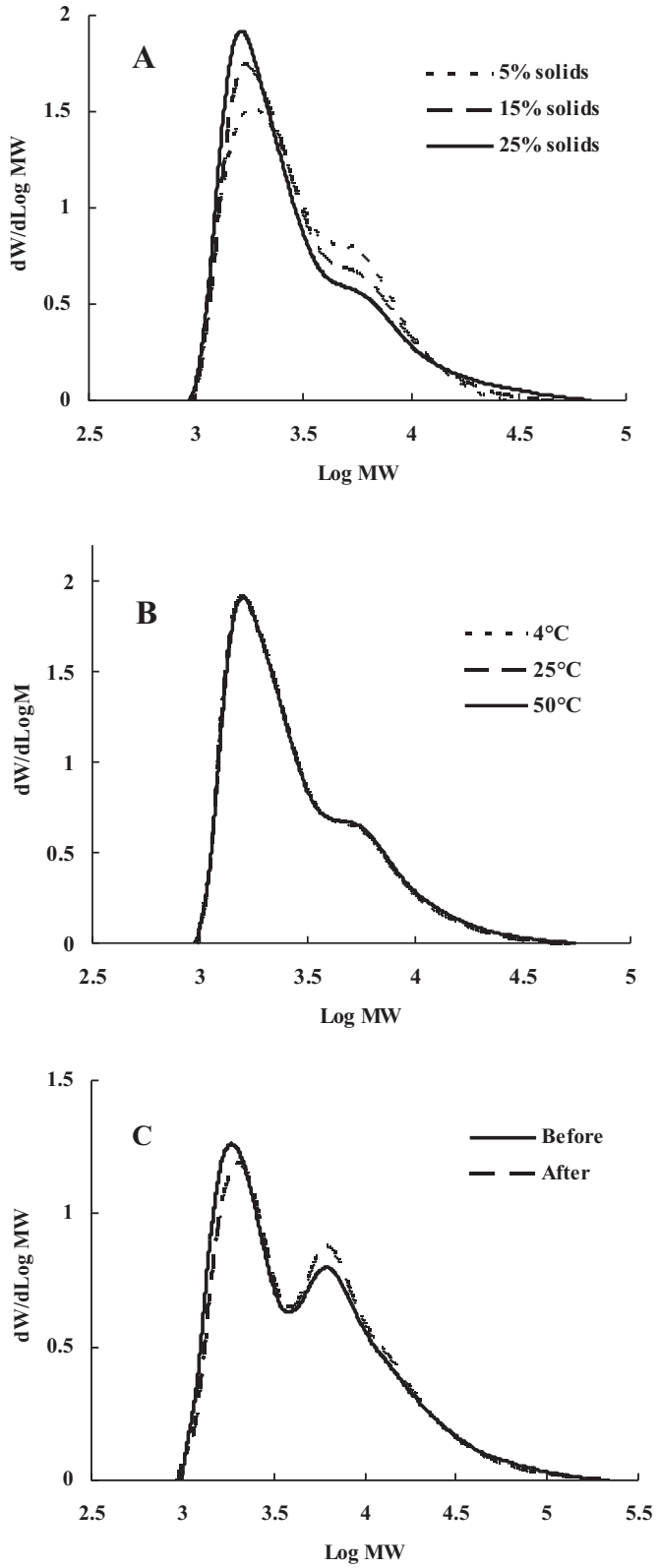

Fig. 3. Molecular weight distribution of short-chain amylose prepared from debranched waxy maize starch with different (A) solids concentration, (B) crystallization temperatures, and (C) debranched waxy potato starch before and after annealing.

The average CL of waxy maize and waxy potato starch was 24.1 and 32.1 glucose units, respectively (Cai & Shi, 2010).

3.3. Morphology and average particle size of crystalline A- and B-type aggregates

All A- and B-type crystalline products contained aggregates and particles with a coarse surface and irregular shape (Fig. 4). Starch granules were cooked and completely disrupted before debranching and crystallization. The new solid crystalline products formed reflected a composite structure and were aggregates of smaller crystals (Fig. 4). A wide distribution of particle sizes was revealed in the products, which contained small isolated particles (<1 μm) along with large pieces of particle clusters (>10 μm) in the same sample. The average particle sizes of SCA prepared from debranched waxy maize starch at 5, 15 and 25% solids were 9, 7.4 and 4.7 μm respectively (Table 1). The average dimension of SCA particles produced from debranched waxy maize starch at 4, 25, and 50 °C were 13.5, 10.1 and 8.9 μm, respectively (Table 1). The B-type crystalline aggregates prepared from amylose synthesized *in vitro* by amylosucrase (Potocki-Veronese et al., 2005) partly resemble the products shown in this study.

3.4. Thermal properties of A- and B-type crystalline products

The thermal properties of A- and B-type crystalline products are given in Fig. 5 and Table 2. The B-type crystalline product obtained from 5% solids showed an endotherm with a melting temperature ranging from 67 to 104 °C and an enthalpy of 15.9 J/g, whereas the A-type crystalline product displayed a melting peak ranging from 98 to 140 °C with an enthalpy of 20.5 J/g. For samples prepared from 15% solids, two endotherms with a low melting peak and a high melting peak were observed, reflecting the presence of a mixture of A- and B-type structures (Fig. 5A and Table 2a). Under the same concentration of 25% solids, the A-type structure prepared at a higher temperature had a higher melting temperature than the B-type allomorph formed at lower temperatures (Fig. 5B and Table 2b).

The melting temperature of recrystallized SCA in excess water with the A-type structure was higher than that of its B-type counterpart as noted in this study (Table 2a and b and Fig. 5) and previous work (Cai & Shi, 2010; Cai et al., 2010; Planchot et al., 1997; Whittam et al., 1990; Williamson et al., 1992). With the same B-type allomorph, crystallites generated from debranched waxy potato starch displayed a higher peak melting temperature than those generated from debranched waxy maize starch (Fig. 5C and Table 2c). This phenomenon could be explained by stronger double helices formed from debranched waxy potato starch with longer unit chains than that in debranched waxy maize starch (Cai & Shi, 2010).

Due to the concern that the lower thermal stability of B-type structure may result in high susceptibility to the digestive enzymes, which is discussed in the next section, the debranched waxy potato starch was annealed to achieve a higher melting peak centered around 120 °C, similar to that of A-type structure (Fig. 5). This annealed sample retained predominant B-type X-ray diffraction pattern (Fig. 2C) and similar crystallinity (Table 1) and morphology (Fig. 4C). It was thus used as a B-type sample with improved thermal stability to compare the digestibility with that of A-type crystals.

3.5. In vitro digestibility of A- and B-type crystalline products

The rate and extent of hydrolysis of crystalline A- and B-type aggregates by a mixture of α-amylase and glucoamylase are shown in Fig. 6. The A-type crystalline product prepared from 25% solids displayed lower digestibility than the mixture of crystalline

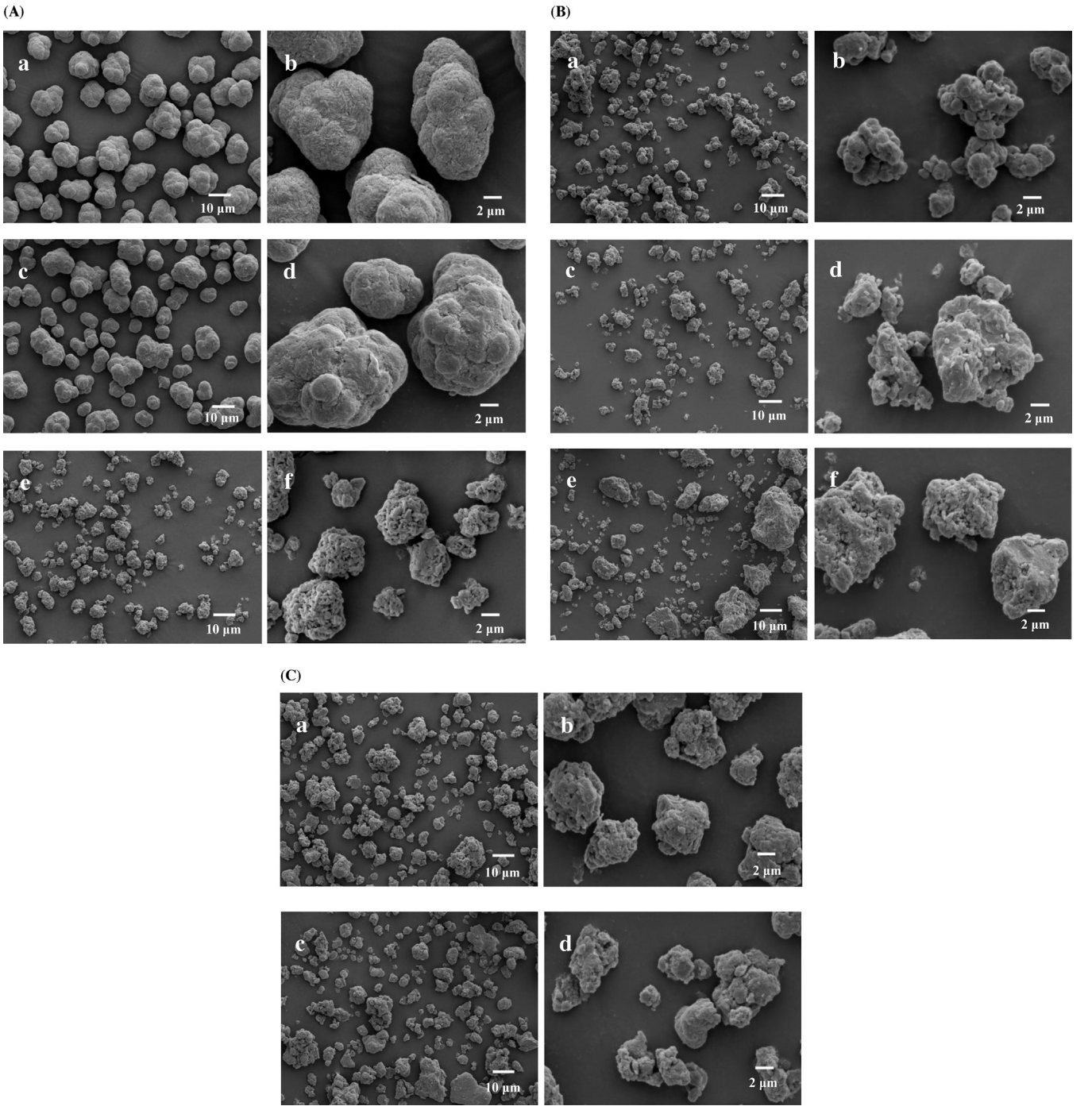

Fig. 4. Scanning electron microscopic images of short-chain amylose prepared from debranched waxy maize starch with (A) different starch solids concentration: a and b, 5% solids; c and d, 15% solids; e and f, 25% solids; (B) different crystallization temperatures : a and b, 4 °C; c and d, 25 °C; e and f, 50 °C; and (C) debranched waxy potato starch: a and b, before annealing; c and d, after annealing.

products and the B-type crystalline materials prepared at 15% and 5% solids, respectively (Fig. 6A). The A-type crystalline product obtained from debranching of 25% waxy maize starch at 50 °C for 24 h gave 16.6% digestion after 3 h incubation. In contrast, the B-type crystals obtained by debranching 5% waxy maize starch at 50 °C for 24 h followed by holding at 25 °C for another 24 h gave 38.9% digestion after 3 h incubation. Similarly, A-type product crystallized at 50 °C from the amorphous debranched waxy maize starch had a lower hydrolysis extent (28.0% digested after 3 h hydrolysis) than B-type crystalline product formed at 4 and 25 °C (41.7% and 44.5% hydrolysis after 3 h digestion, respectively, Fig. 6B). With the same preparation approach, the B-type crystalline product formed from debranched waxy potato starch with larger CL was less resistant to enzyme digestion (27.6% hydrolyzed after 3 h digestion) than the A-type crystalline product prepared from debranched waxy maize starch (16.6% digestibility after 3 h hydrolysis, Fig. 6C). Our results demonstrated that with similar crystallinity (Table 1 and Fig. 2), A-type allomorph prepared from the same approach was more resistant to the enzyme digestion than their B-type counterparts. The digestibility of the A- and B-type products (Fig. 6) did not seem to correlate with the size of the aggregates formed (Table 1). For instance, the highly crystalline A-type

Table 2
Thermal properties of short-chain amylose prepared at different conditions as determined by differential scanning calorimetry.

a. Debranched waxy maize starch with different solids concentration[a]

Solid (%)	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)
5	67.4 ± 0.5	87.0 ± 0.1	104 ± 0.6	15.9 ± 0.1	–	–	–	–
15	76.6 ± 0.1	95.9 ± 0.0	110.4 ± 0.8	12.7 ± 0.5	115.5 ± 0.2	127.3 ± 0.5	140.4 ± 0.4	3.15 ± 0.1
25					97.6 ± 0.8	117.9 ± 0.8	139.5 ± 0.9	20.5 ± 1.2

b. Debranched waxy maize starch with different crystallization temperature[b]

Crystallization temperature (°C)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
4	63.8 ± 0.4	92.1 ± 0.4	108.2 ± 0.6	17.2 ± 0.2
25	68.3 ± 0.9	89.4 ± 1.2	108.2 ± 0.4	18.7 ± 0.1
50	99.0 ± 1.3	115.9 ± 1.1	140.3 ± 0.3	19.5 ± 0.7

c. Debranched waxy potato starch before and after annealing[c]

Debranched waxy potato starch	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
Before annealing	83.8 ± 0.7	104.3 ± 0.1	123.4 ± 0.8	20.0 ± 0.3
After annealing	83.5 ± 0.6	119.6 ± 0.6	143.0 ± 0.4	16.8 ± 0.8

[a] Mean ± standard deviation values are reported.
[b] Mean ± standard deviation values are reported.
[c] Mean ± standard deviation values are reported.

product crystallized from 25% solids at 50 °C had a small average particle size of 4.7 μm (Table 1) and a large surface area, yet was more resistant to enzyme digestion (Fig. 6). However, we should point out that the particle size, morphology, and surface characteristics of crystalline aggregates would be affected by grinding method. Further study is needed to investigate how the digestion of crystalline products is affected by large scale, commercial grinding devices.

The B-type crystalline products had a lower melting temperature in excess water than A-type crystals (Fig. 5 and Table 2). The high digestibility of B-type crystalline products was probably due to their low thermal stability and imperfect crystals. To improve the thermal stability, we annealed B-type crystalline products. Debranched waxy maize starch with a B-type structure (5% solids debranched at 50 °C for 24 h and held at 25 °C for another 24 h) was annealed at 60 °C and 80 °C, respectively, to increase its thermal stability; however, the peak melting temperature of the sample was increased only slightly from 87 °C to 90.6 °C after annealing at 60 °C for 8 h. Upon annealing at 80 °C, the sample displayed a peak melting temperature of 98.0 °C, but with a significantly decreased enthalpy of 4.1 J/g. Debranched waxy maize starch with a B-type allomorph was concluded to have a weak crystalline structure that was difficult to strengthen by annealing.

Thus, the crystallized B-type allomorph with a relatively high melting temperature of 104.3 °C obtained from debranched waxy potato starch was used as the starting material. After annealing, the B-type crystals had a similar peak melting temperature compared with the A-type crystals (119.6 °C vs. 117.9 °C, Table 2a and c). However, the B-type crystalline product exhibited an increase in the extent of hydrolysis rather than a decrease (Fig. 6D). We noticed that the enthalpy was slightly decreased after annealing (Table 2c), suggesting that some weak crystallites were melt during annealing, and therefore prone to enzyme hydrolysis. In addition, there was slight change in molecular size for annealed product (Fig. 3C), probably because some weak crystallites (mostly from short chains) were melt, solubilized and removed during filtration.

Our enzyme digestion results conflict with the observations on the digestibility of A- and B-type crystals in the literature. Planchot et al. (1997) prepared A- and B-type starch crystals from lintnerized potato starch and found that the A-type structure was more susceptible to α-amylase hydrolysis. Williamson et al. (1992) reported that the B-type crystalline starch was more resistant to α-amylase, β-amylase, and glucoamylase 1. Notably, those authors prepared A- and B-type starch crystals using mild acid-treated potato starch. Their crystalline solids contained α-1,6 branch points and a mixture of linear and branched polymers. The B-type starch crystals were prepared by cooling the solution of acid-treated starch at a slow rate, whereas the A-type crystals were crystallized from an ethanol–water mixture. The A-type spherocrystals grown by the mixture of ethanol and water were reported to be fragile and that was used to explain their easy susceptibility to α-amylase digestion (Helbert et al., 1993). To understand and explain the differences between this work and previous studies, SCA generated from debranched waxy maize starch was used in this study as a starting material to prepare A- and B-type crystals by the same procedures described in the literature (Planchot et al., 1997). Once again, the A-type crystals from debranched waxy maize starch were still more enzyme-resistant than their corresponding B-type crystals. The A-type structure gave only 22.9% hydrolysis in 3 h compared with 33.4% for the B-type structure (Fig. 6E). The different starting materials used to prepare the model products may have led to the opposite digestion results.

In the case of native starches, most A-type allomorphs are found to have higher enzyme digestibility than B-type allomorphs (Dreher et al., 1984; Gallant et al., 1997; Gallant et al., 1972; Jane et al., 1997; McCleary & Monaghan, 2002; Planchot et al., 1995; Srichuwong, Isono et al., 2005; Srichuwong, Sunnarti et al., 2005), but the aggregates of the A-type starch crystals were found to be more enzyme-resistant than that of the B-type crystals in this study. Thus, the crystalline type per se does not seem to be the key factor that affects the digestibility of native starch granules. The resistance of native starches with the B-type X-ray diffraction pattern is probably attributed to the other structural features of starch granules. Shrestha et al. (2012) studied the effects of partial enzyme digestion of three maize starches differing in amylose content and found that granule architecture is the major factor controlling enzyme susceptibility of native starch. Brewer, Cai & Shi (2012) investigated the actions of α-amylase and amyloglucosidase on four maize starches with different amylose contents, and similarly proposed that the extent of enzyme digestion is largely controlled by the granule architecture and diffusion of the enzymes within densely packed starch granules. Low degree of crystallinity does not result

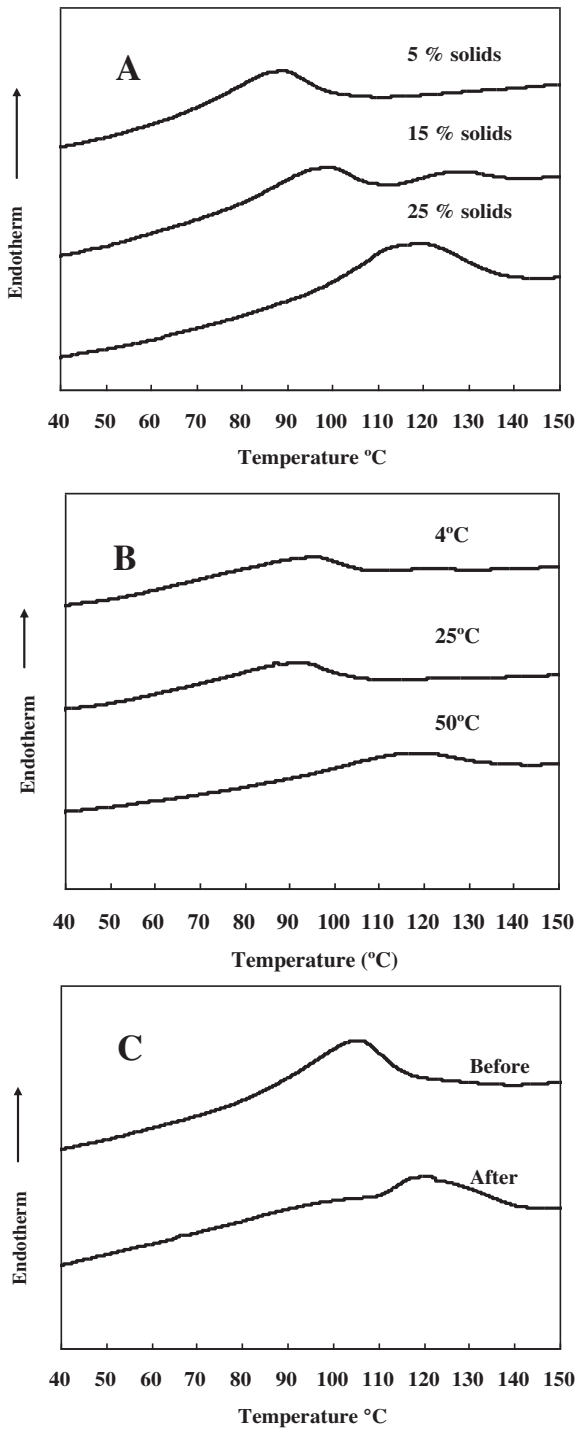

Fig. 5. Thermal properties of short-chain amylose prepared from debranched waxy maize starch with different (A) solids concentration, (B) crystallization temperatures, and (C) debranched waxy potato starch before and after annealing.

in high digestibility. Compared to waxy and normal maize starch, high-amylose maize starch has a lower degree of crystallinity but is more resistant to enzyme digestion (Brewer et al., 2012; Shrestha et al., 2012)

4. Discussion

The digestibility of starch crystals depends on multiple factors such as crystalline types, morphology, chain length, ultrastructure of crystals, crystal defects etc. (Buleon et al., 1998; Gallant et al., 1997; Jane et al., 1997; Jiang & Liu, 2002; Planchot et al., 1995; Srichuwong, Sunnarti et al., 2005). In this study, using the same linear short chains from the debranched waxy maize starch as the starting material, we were able to produce highly crystalline A- and B-type allomorphs through specific crystallization conditions (Fig. 1). Those highly crystalline products appeared to be aggregates of small crystals (Fig. 4). The digestibility of those aggregates is largely controlled by the accessibility of the enzymes. We found that the crystalline products with A-type allomorph were more resistant to enzyme digestion than their B-type counterparts (Fig. 6). Although our work was the first to show the direct comparison of the digestibility of A- and B-type allomorphs with A-type allomorph being less digestible, high resistance of crystalline A-type aggregates from debranched maltodextrin was previously reported by Pohu, Planchot et al. (2004). They found the morphology of the A-type aggregates was too complex and images of poor quality to understand the mechanism (Pohu, Putaux, Planchot, Colonna, & Buleon, 2004). Consequently, they investigated the hydrolysis of model A-type crystalline systems comprised of short linear chains and found that A-type nanocrystals prepared from acid hydrolyzed potato starch (DP15) were not degraded homogeneously. Alpha-amylase hydrolyzed the nanocrystals on the side of the double helices, not their ends at the surface of lamellar crystals. Based on these results, they attributed the enzymatic resistance of the A-type aggregates from debranched maltodextrin to the dense and compact morphology resulted from the epitaxial growth of elementary crystalline A-type platelets. The double helices were organized parallel to the long axis of the crystal. The hydrolysis by α-amylase was limited because of the reduced accessibility to double helices by aggregation. The mechanism proposed by Pohu, Putaux et al. (2004) can be applied to explain the lower digestibility of the crystalline A-type aggregates in this study.

On a molecular level, A- and B-type allomorphs differ in the packing pattern of double helices (monoclinic and hexagonal geometry, respectively) and the number of water molecules (8 and 36, respectively) in the crystal unit cell (Imberty, Chanzy, Perez, Buleon, & Tran, 1988; Imberty & Perez, 1988; Popov et al., 2009; Takahashi, Kumano, & Nishikawa, 2004). The A-type allomorph has a denser and tighter structure. The crystallized SCA with an A-type structure seemed to be stronger than the B-type crystallites as reflected in the DSC data (Fig. 5, Table 2) observed in this study and previous work (Cai & Shi, 2010; Cai et al., 2010; Planchot et al., 1997; Whittam et al., 1990; Williamson et al., 1992). In addition, we noted that it was difficult to improve the quality of B-type crystallites generated from the SCA by annealing. The aggregates of B-type crystallites from the SCA were probably not as compact as A-type aggregates, consisted of a large number of individual tiny crystallites, and organized into the structures with many defects, and thus were prone to enzyme hydrolysis as compared to that of A-type crystallites.

In another study (Cai & Shi, 2013), we heated the SCA after debranching to 180 °C followed by cooling and crystallization and obtained well-developed spherulites. In consistent with the present study, the spherulites crystallized at 50 °C had an A-type diffraction pattern, a higher melting temperature, and a lower digestibility compared with the spherulites crystallized at low temperatures (4 and 25 °C) which had a B-type starch X-ray diffraction pattern, a lower melting temperature, and a higher digestibility.

In this study, the alpha-amylase used is from pancreatin. It would be interesting to investigate the digestibility of the highly crystalline A- and B-type products by a new type of alpha-amylase that was very aggressive to raw native starch in future (Tawil, Vikso-Nielsen, Rolland-Sabate, Colonna, & Buleon, 2011; Tawil, Vikso-Nielsen, Rolland-Sabate, Colonna, & Buleon, 2012).

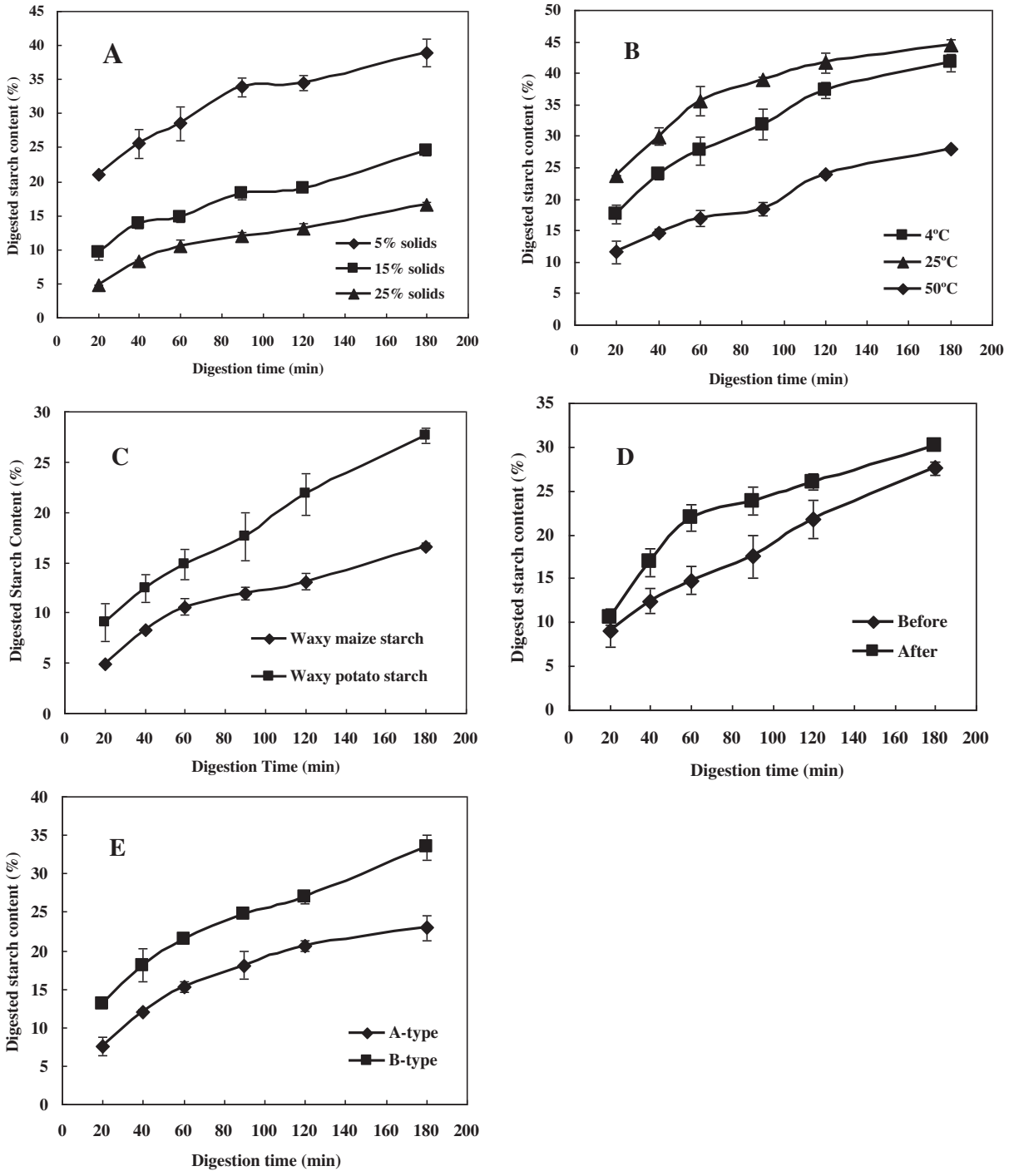

Fig. 6. In vitro digestion profile of short-chain amylose prepared from (A) debranched waxy maize starch with different solids concentration, (B) debranched waxy maize starch with different crystallization temperatures, (C) 25% solids debranched waxy maize and waxy potato starches, (D) debranched waxy potato starch before and after annealing, and (E) the methods by Planchot et al. (1997).

5. Conclusions

The present study highlights the potential use of debranching technique combined with controlled crystallization to design starch products with different allomorphs and digestibility. The A-type crystalline SCA could be formed at high concentration, high temperature, and short CL, whereas the B-type allomorph was produced at low concentration, low temperature, and long CL. The A-type crystalline product prepared from the same approach had a higher melting temperature and was more resistant to enzyme digestion than its B-type counterpart. The higher enzyme resistance of the aggregates of A-type crystallites was probably due to the dense and compact morphology with reduced accessibility of double helices to the enzymes. In striking contrast to the fact that most A-type native starches are less enzyme-resistant, we have showed that it is possible to have highly crystalline A-type products that are more enzyme-resistant. The A- and B-crystalline type per se does not seem to be the key factor that affects the digestibility of native starch granules. The resistance of native starch with the B-type X-ray diffraction pattern is probably attributable to the surface features and other organizational structures of starch granules.

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