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Self-Assembly of Short Linear Chains to A- and B-Type Starch Spherulites and Their Enzymatic Digestibility

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ABSTRACT: A novel process combining enzymatic debranching, melting, and crystallization was developed to produce spherulites from short linear α-1,4-linked glucans (short-chain amylose, SCA) with controlled enzyme digestibility. SCA was obtained by completely debranching waxy maize starch at 50 °C and 25% solids in 0.01 M sodium acetate buffer. The mixture was then heated to 180 °C followed by cooling and crystallization to form well-developed spherulites. Multiple analytical techniques including light microscopy, scanning electron microscopy, differential scanning calorimetry, wide-angle X-ray diffraction, and synchrotron small-angle X-ray scattering (SAXS) covered over 5 orders of length scale and were applied to study the morphology and structure of the spherulites. Spherulites crystallized at low temperatures (4 and 25 °C) had a large size (5−10 μm), a B-type starch X-ray diffraction pattern, a lower melting temperature (70−110 °C), and a higher digestibility (Englyst method) compared to the spherulites crystallized at 50 °C, which had a small size (1−5 μm), an A-type diffraction pattern, a higher melting temperature (100−140 °C), and a lower digestibility. Intact spherulites along with small fragments were observed after digestion with a mixture of α-amyase and amyloglucosidase, indicating that digestion was not homogeneous and preferentially occurred in weak spherulites. A second exposure of the undigested residues to the amylases showed a similar digestive pattern as with the parent spherulites, suggesting that the spherulites were hydrolyzed by enzymes at essentially a constant digestion rate between 20 min and 3 h.

KEYWORDS: *short-chain amylose, waxy maize starch, crystallization, spherulites, digestibility*

INTRODUCTION

Spherulites are important structural features found in many polymers crystallized from a melt.[1,2] Starch-based spherulites may be obtained by heating a starch suspension to produce a solution state and then cooling without disturbance.[3−7] The overall morphology of spherulites is dependent on starch source, amylose content, and crystallization conditions, such as heating temperature, concentration of starting material, cooling rate, and crystallization temperature.[5,7] High-amylose starches form spherical structure (spherulite) with birefringence more readily than normal and waxy starches.[3−5] Spherulites were obtained over a wide range of cooling rates (1−250 °C/min), provided that the amylose solution (10−20%, w/w) had been preheated to >170 °C.[8] Spherulitic crystals display dimensions and structural characteristics consistent with the hilum and core region of native starch granules, and they have been proposed as a model for starch granule initiation in vivo.[9] However, in those studies,[8,9] amylose was isolated from granular starch by an aqueous leaching process, and the spherulites were prepared in small amount in a differential scanning calorimetry (DSC) pan. The small sample size was insufficient for conducting digestion study.[8,9]

Spherutilic crystallization has been documented[10−14] starting from a solution of amylodextrin produced from potato starch. Amylodextrin (lintnerized starch) is prepared by subjecting starch granule to acid hydrolysis below the gelatinization temperature. Helbert et al.[10] prepared spherulites by mixing ethanol with hot aqueous solutions of low molecular weight amylose followed by slow cooling to 4 °C. The precipitates had a diameter on the order of 10 μm and exhibited an A-type X-ray diffraction pattern. In contrast, spherulites with a B-type polymorph and diameters of 10−15 μm were produced by direct cooling to 2 °C of a 5−20% w/w aqueous solution of amylodextrin from potato starch.[11] Because A- and B-amylose spherulites mimic both granular morphology and the crystalline types of native starches, they have been used as model systems to study the enzymatic hydrolysis of starch crystallites.[12,13] In those studies, the amylodextrins used to prepare spherulites were obtained by extensive acid hydrolysis of native starches with hydrochloric acid. Significant loss of starch occurred during acid treatment because washing was needed to remove water-soluble fractions and recover acid-resistant products. The product presumably still contained α-1,6-branch linkages.[12,13]

We used a "single medium" approach to prepare spherulites in this study. Completely linear short-chain amylose (SCA, average DP about 24) was produced by enzymatic debranching of waxy maize starch in sodium acetate buffer. The SCA was not isolated but instead was crystallized directly into spherulites in high yield. We investigated the conditions and mechanism of self-assembly of SCA molecules into spherulites and also measured their digestibility with a mixture of α-amylase and amyloglucosidase (Englyst method). Our specific goals were to (1) investigate the necessary aqueous dissolution temperature for SCA to form spherulites upon cooling; (2) study the effects of crystallization temperature on morphology, crystalline structure, and digestibility of the resulting products; and (3)

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explore the mechanism of forming spherulites and their digestion patterns.

MATERIALS AND METHODS

Waxy maize starch was obtained from National Starch LLC (Bridgewater, NJ, USA), and isoamylase (EC 3.2.1.68) was obtained from Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). The enzyme activity of isoamylase was 1.41×10^6 isoamylase activity units (IAU)/g as determined[15] by incubating the enzyme with soluble waxy maize starch as a substrate in the presence of iodine for 30 min under the assay conditions (pH 3.5, 40.0 ± 0.1 °C). One IAU was defined as the amount of isoamylase that increased absorbance of the reaction mixture by 0.008 in 30 min.[15] All chemicals were of reagent grade.

Methods. *Debranching of Starch and Formation of Spherulites.* Waxy maize starch (25 g, dry basis) was slurried in acetate buffer (75 mL, 0.01 M, pH 4.0) in a pressure bottle (Ace Glass Inc., Vineland, NJ, USA). The slurry was heated in a boiling water bath with continuous stirring for 30 min and then held at 120 °C in an oven for 30 min. After the mixture was cooled to 50 °C, 1 wt % isoamylase based on the dry weight of starch was added, and the mixture was stirred at 50 °C for 24 h. The debranched starch molecules were confirmed to be linear.[16] Three portions (15 mL each) of the mixture were sealed in pressure tubes (Ace Glass Inc.) followed by heating in an oven at 180 °C for 20 min. The individual tubes were stored at 4, 25, or 50 °C for 24 h. The precipitate in a tube was filtered, washed with water, dried at 40 °C in an oven overnight, and gently ground by a mortar and pestle. To determine the optimum heating temperature required to form spherulites, two other portions of the mixture (15 mL each) were sealed and heated to 170 and 190 °C for 20 min, respectively, and then crystallized at 4 °C for 24 h.

To determine the yield of spherulites, a mixture containing the crystals was centrifuged (13226*g*) for 10 min, and an aliquot (1.0 mL) of the supernatant was analyzed for the soluble carbohydrate with a portable refractometer (Fisher Scientific Inc., Pittsburgh, PA, USA). The blank reading was determined according to the same procedure on uncooked starch slurry that contained isoamylase. The level of precipitation of carbohydrate was calculated by the difference between refractive index measurement on a sample and the blank. Each measurement was done in duplicate.

Gel Permeation Chromatography (GPC). The molecular size distribution of chains in spherulites was examined by GPC as previously described.[16,17] Starch sample (4 mg) was stirred with dimethyl sulfoxide (DMSO) (4 mL) in a boiling water bath for 24 h, then filtered through a 2 μm filter, and 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.), and a differential refractive index detector. The eluenting solvent was DMSO containing 0.5 mM $NaNO_3$, and the flow rate was 0.8 mL/min. The column oven temperature was controlled at 80 °C. The molecular size was relative to dextran standards (American Polymer Standards Co., Mentor, OH, USA).

Light Microscopy. A drop of sample suspension (1% in water) was deposited on a microscope slide and covered with a coverslip. The sample was observed by an Olympus BX51TF microscope (Olympus Optical Co. Ltd., Shinjuku-ku, Tokyo, Japan). The images in both normal light and polarized light backgrounds were captured using a SPOT 18.2 Color Mosaic camera (Diagnostic Instruments Inc., Sterling Heights, MI, USA).

Scanning Electron Microscopy (SEM). The spherulite 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.

Wide-Angle X-ray Diffraction. The crystalline structure of spherulites was studied by wide-angle X-ray diffraction. The experiment was conducted with a Philips X-ray diffractometer with Cu Kα radiation at 35 kV and 20 mA, a θ-compensating slit, and a diffracted beam monochromator. The moisture of a sample was adjusted to about 15% by storage in a sealed desiccator over water at 25 °C. The diffractograms were recorded between 2 and 35° (2θ).

Synchrotron Small-Angle X-ray Scattering (SAXS). SAXS experiments were carried out at the Advanced Polymers Beamline (X27C) in the National Synchrotron Light Source, Brookhaven National Laboratory, in Upton, NY, USA. The wavelength used was 0.1371 nm, and the sample-to-detector distance was 1782.97 mm. A 2D MAR-CCD (Rayonix, LLC, formerly MAR USA, Inc., Evanston, IL, USA) X-ray detector was used for data collection. Both samples were examined at an "as is" moisture level and at ∼50%. The hydrated sample was mixed with an equal weight of water and the mixture equilibrated at 25 °C.

DSC. Spherulite suspension in water (25% solids) was prepared and sealed in a DSC pan and analyzed by DSC (TA Q200 instrument, New Castle, DE, USA). Each sample was heated from 10 to 160 °C at 10 °C/min. An empty pan was used as a reference. The onset (T_o), peak (T_p), and conclusion (T_c) temperatures and enthalpy (ΔH) were calculated from the DSC thermogram.

In Vitro Digestion. An in vitro digestion of spherulites was conducted according to a modified[18,19] Englyst procedure. After 3 h at 37 °C, the digestion was stopped by adding 200 mL of ethanol. The solids were recovered by filtration and dried in an oven at 40 °C overnight. The in vitro digestion test was repeated once more on the recovered solids.

RESULTS

Molecular Size Distribution and Yield. Figure 1 shows the size distribution of SCA molecules that comprise the spherulites, which was determined by GPC and dextran standards. A bimodal distribution with low- and high-molecular sizes was observed for the spherulites, which was anticipated on the basis of data for debranched waxy maize starch. Spherulites that were crystallized at different temperatures had almost identical molecular size distributions. However, spherulites obtained at 4 °C had a slightly larger proportion of the low-molecular size fraction as compared to those produced at 25 and 50 °C. This observation suggests that short linear chains associate more at low temperature. The associated molecules crystallized and precipitated from solution.

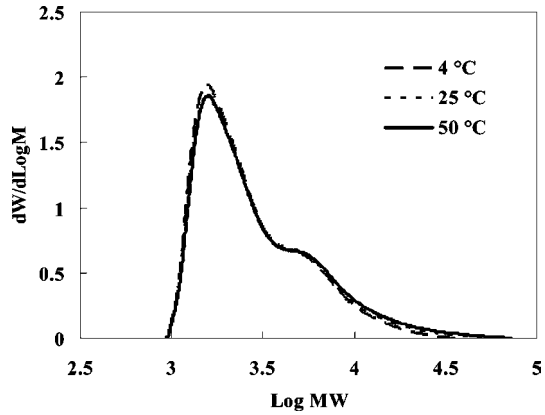

Figure 1. Molecular size distribution of short-chain amylose spherulites produced by heating debranched waxy maize starch (25% w/w) in ∼0.06% sodium acetate, pH 4, to 180 °C and crystallizing at different temperatures.

The recovery of SCA spherulites from the 25% SCA solution increased from 50 to 88% as the crystallization temperature decreased from 50 to 4 °C (Table 1). A similar increase in yield

Table 1. Yield of Short-Chain Amylose Spherulites Produced by Heating Debranched Waxy Maize Starch (25% w/w) to 180 °C and Crystallizing at Different Temperatures[a]

	crystallization temperature		
	4 °C	25 °C	50 °C
yield (%)	87.6 ± 2.1	72 ± 1.4	50 ± 1.6

[a]Mean ± standard deviation values are reported.

was observed after debranching of a 25% solids concentration of waxy maize starch at 50 °C followed by immediate cooling to form aggregates of small crystals.[17] The yield of the aggregate increased to about 90% upon crystallization of the debranched waxy maize starch at 25 °C for 6 h, as opposed to 65% yield by crystallization at 50 °C.[17] Large-scale production of spherulites seems feasible because of their high yield and recovery by conventional filtration.

Morphology. Microscopic images of SCA spherulites under both normal and polarized light backgrounds are shown in Figure 2. For materials crystallized at 4 and 25 °C, birefringence and a Maltese cross were observed under polarized light, confirming the formation of spherulites. The size of the spherulites ranged from 5 to 10 μm. Increasing the crystallization temperature to 50 °C resulted in less-well-

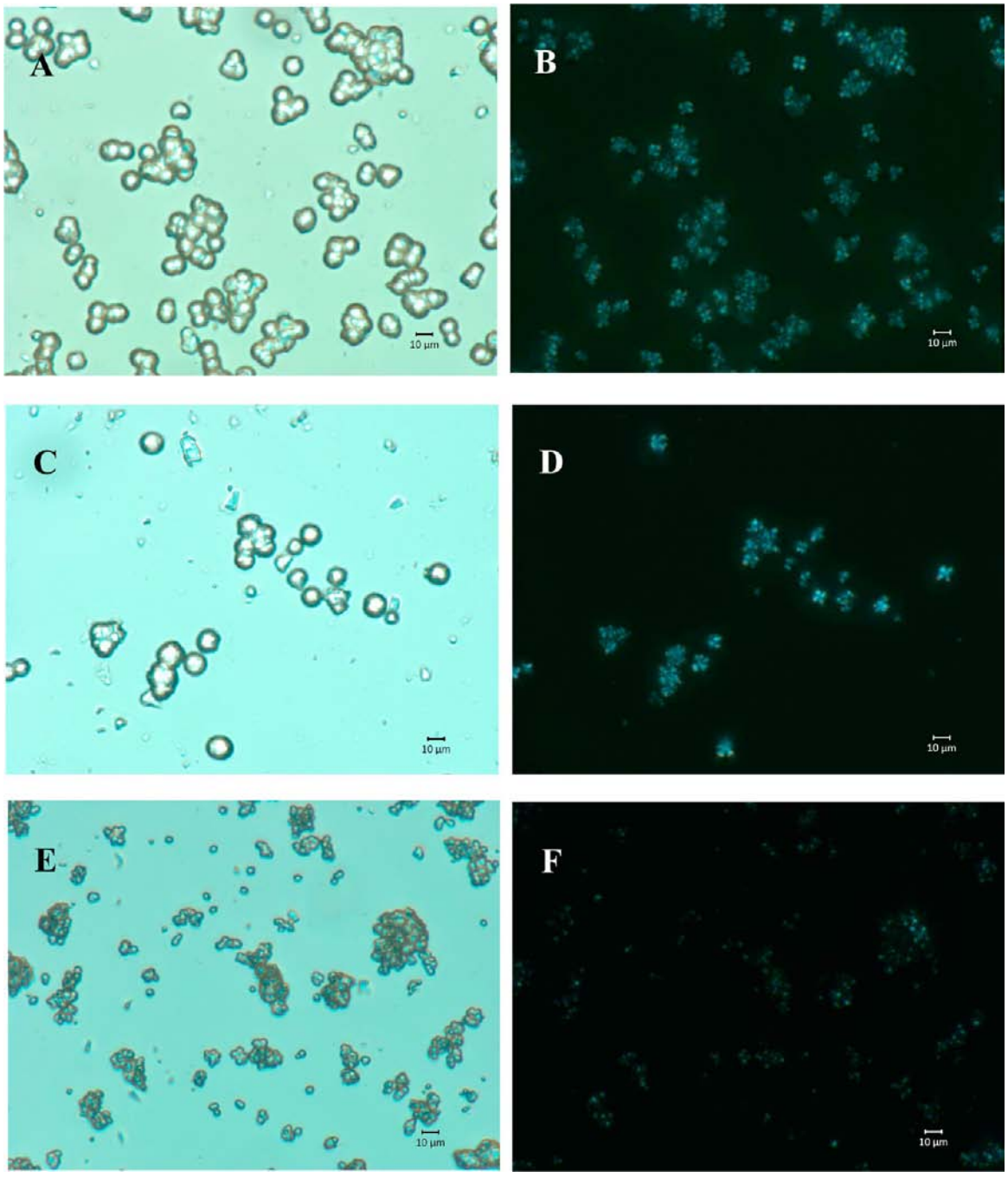

Figure 2. Microscopic images of short-chain amylose spherulites produced by heating debranched waxy maize starch (25% w/w) to 180 °C and crystallizing at different temperatures: (A, B) 4 °C; (C, D) 25 °C; (E, F) 50 °C. All scale bars represent 10 μm.

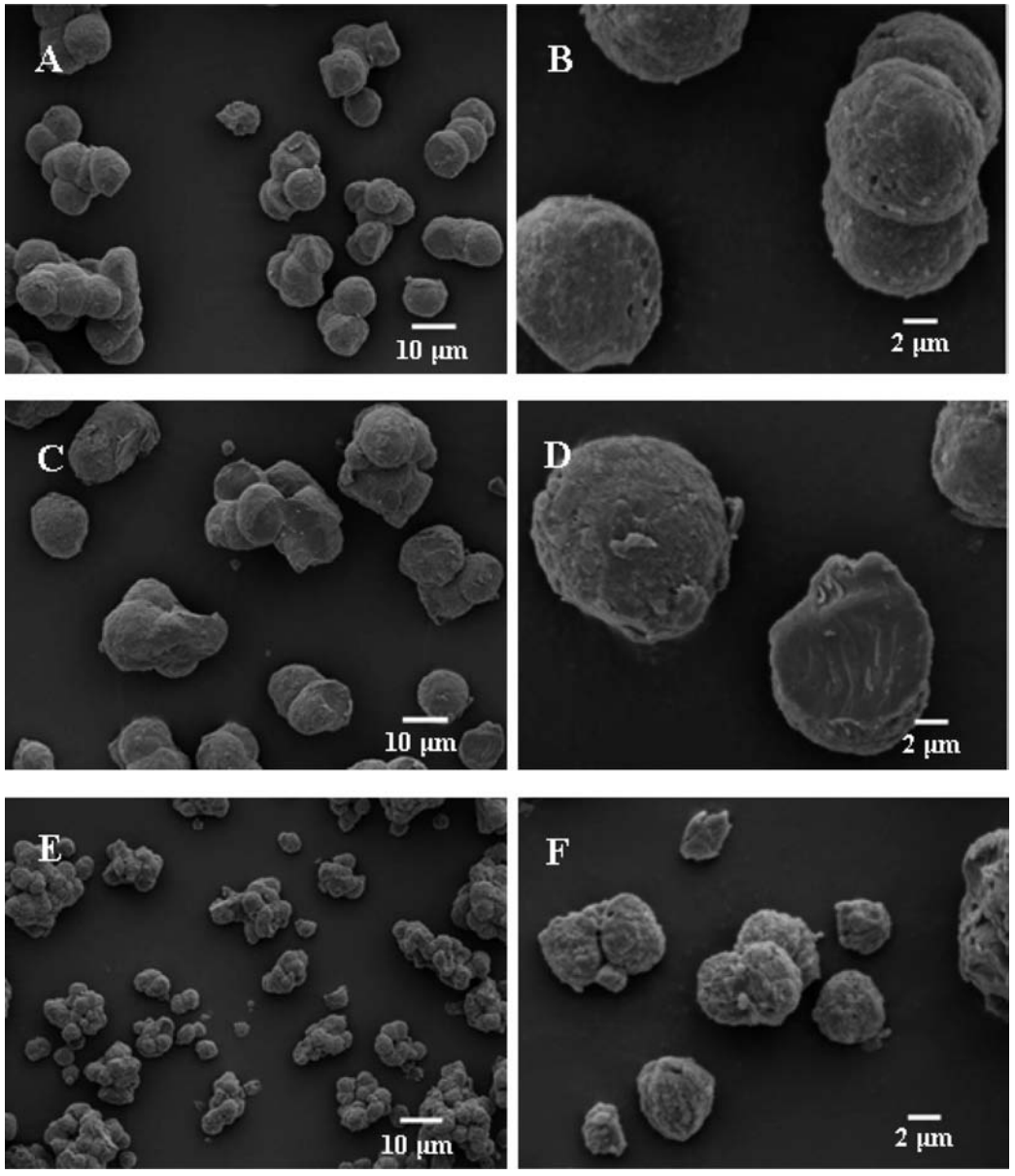

Figure 3. Scanning electron microscopic images of short-chain amylose spherulites produced by heating debranched waxy maize starch (25% w/w) to 180 °C and crystallizing at different temperatures: (A, B) 4 °C; (C, D) 25 °C; (E, F) 50 °C.

developed spherulites with a reduced particle size (ca. 1−5 μm) and weak birefringence and Maltese cross pattern. Some small spherulites appeared to be fused together (Figure 2), but during the light microscopy experiments, movement of those individual small spherulites was observed. The apparent aggregation of those small spherulites suggests that there was surface adhesion between those spherulites, particularly after water was evaporated.

To study the optimum heating temperature for forming spherulites, the solution of SCA was heated at 170 and 190 °C for 20 min, respectively, and crystallized at 4 °C for 24 h. At both heating temperatures (170 and 190 °C), the spherulites were not well developed. At 170 °C, small-size particles of ca. 1−5 μm were obtained with weak birefringence, whereas at 190 °C large particles of ∼10 μm formed, which were birefringent but without the Maltese cross pattern. Thus, the optimum temperature to preheat SCA at 25% solids for spherulite preparation seems to be 180 °C, which is the same as that found for the long-chain amylose.[8,9]

Figure 3 shows the SEM images of SCA spherulites produced at different crystallization temperatures. A similar morphology and size (ca. 5−10 μm) were found for spherulites formed at 4 and 25 °C, but spherulites crystallized at 50 °C had a smaller size (ca. 1−5 μm). The shape of spherulites prepared in this study was similar to the one reported previously,[11] but different morphologies of spherulites can be obtained depending on the starting material, concentration, solvent, and preparation method.[20] A-type spherulites consisting of a radial assembly of thin elongated single crystals and B-type spherulites with a smoother surface and two symmetrical "eyes" (uncrystallized holes)[21] were reported and used as models to study starch digestibility.[12,20] In this study, both A- and B-type spherulites showed the same spherical shape with a similar surface smoothness. The minimal morphology difference between

these A- and B-type spherulites appears to provide an improved model to compare their enzymatic digestibility.

Characterization of Crystalline Structure. The wide-angle X-ray diffraction patterns of spherulites are shown in Figure 4. Spherulites formed at 4 and 25 °C exhibited B-type

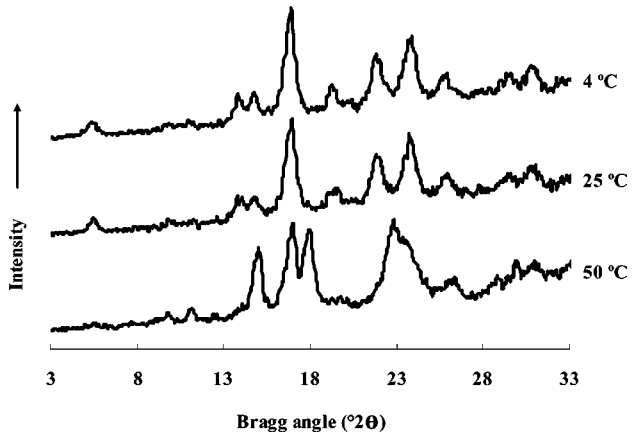

Figure 4. Wide-angle X-ray diffraction of short-chain amylose spherulites produced by heating debranched waxy maize starch (25% w/w) to 180 °C and crystallizing at different temperatures.

crystallinity, whereas the A-type allomorph was formed at 50 °C. The phenomena followed the general rule in SCA crystallization that high temperature favors the formation of A-type crystallites, whereas the reverse condition induces B-type crystallization.[14,16,17,20,22]

According to Gidley,[23] the A-type crystalline allomorph of starch is the thermodynamic product, whereas the B-type allomorph results from a kinetic event. The melting temperatures of SCA spherulites are largely dependent on their allomorphic form and water content.[14] The thermal properties of spherulites prepared at three different crystallization temperatures are shown in Figure 5 and Table 2. In excess water, an endothermic peak centered around 90 °C was observed for spherulites obtained at 4 and 25 °C, whereas the endotherm of spherulites formed at 50 °C shifted to a higher peak melting temperature of 120 °C, indicating that differences

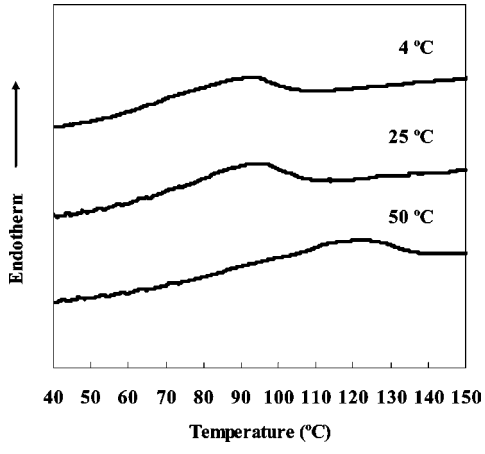

Figure 5. Thermal properties of short-chain amylose spherulites produced by heating debranched waxy maize starch (25% w/w) to 180 °C and crystallizing at different temperatures as determined by differential scanning calorimetry.

in crystallization temperatures can lead to different melting temperatures. In our case, the A-type structure prepared at 50 °C was more thermally stable with a melting temperature about 30 °C higher than the B-type structure obtained at 4 and 25 °C (Figure 5 and Table 2). These results were in agreement with the work conducted by Whittam et al.,[14] who reported that the A-type SCA spherulites melted at temperatures about 20 °C higher than the B-type spherulites with a water content of >40% (w/w).

The peak melting temperature of the B-type SCA spherulites, which had been prepared from lintnerized potato starch, was 70−80 °C in excess water conditions,[11,13,14] whereas the peak melting temperature was ca. 90 °C for the A-type spherulites.[13,14] Interestingly, for the same crystalline type, spherulites produced in this study had 20−30 °C higher melting temperatures in excess water than those in the literature.[13,14]

It is interesting to note that in this study the enthalpy values were similar among the three spherulites prepared at three different crystallization temperatures, even though those spherulites had different thermal stabilities as noted by their melting temperatures (Table 2). The enthalpy value reflects the degree of crystallinity of starch,[24] and in this study those spherulites had a similar relative degree of crystallinity and were estimated to be 90−94% (Figure 4).

It should be noted that spherulites prepared from long-chain amylose (isolated from native starch) revealed only the B-type X-ray diffraction pattern and that they showed a higher melting temperature than the B-type SCA spherulites.[3,8] According to Nordmark and Ziegler,[3] amylose purified from high-amylose maize starch could form spherulites with an endotherm ranging from ca. 90−140 °C and a peak melting temperature of 125−130 °C. Spherulitical materials obtained from preheated maize amylose over a wide range of cooling rates showed a melting temperature ranging from 100 to 140 °C, and the endotherm did not change significantly in response to a change in cooling rate.[8] Apparently, the double helices present in spherulites of long-chain amylose are more resistant to disassociation upon heating in excess water. In model studies, the melting temperature of amylose crystals in water at a volume fraction of 0.8 increased from 57 to 119 °C as the chain length increased from 12 to 55 residues. The extrapolated dissolution temperature for high molecular amylose in water was 147 °C.[25]

The SAXS curves of spherulites are presented in Figure 6. No lamellar peak was observed for all spherulite samples with a low moisture content (ca. 5%). For hydrated spherulite samples at ∼50% water content, a weak and broad peak was detected for spherulites formed at 4 °C, whereas this peak was basically invisible in spherulites formed at 25 and 50 °C, suggesting that no regular alternating crystalline and amorphous structure was developed in SCA spherulites, especially at the two higher crystallization temperatures. These results suggest that even though spherulites produced in this study were birefringent and had a Maltese cross, the organization of the molecules was different from those in native starch. For hydrated native starch granules, a narrow 9 nm lamellar peak is observed by SAXS, which is attributed to a repeating structure of alternating amorphous and crystalline regions.[26] It is possible that the chain length of SCA is too short and irregular in length to form partially crystalline particles with repeating distance between highly packed double helices and poorly packed irregular zones. Thus, no long length scale-order beyond the crystal packing of double helices was observed by SAXS, as is the case for the

Table 2. Thermal Properties of Short-Chain Amylose Spherulites Produced by Heating Debranched Waxy Maize Starch (25% w/w) to 180 °C and Crystallizing at Different Temperatures As Determined by Differential Scanning Calorimetry[a]

crystallization temperature (°C)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH(J/g)
4	64.8 ± 1.2	91.4 ± 2.3	108.5 ± 0.7	20.9 ± 0.1
25	73.5 ± 0.32	91.8 ± 1.9	109.1 ± 0.9	21.0 ± 0.4
50	99.9 ± 1.1	117.5 ± 1.9	139.1 ± 0.5	20.5 ± 0.5

[a]Waxy maize starch (25% solids) was debranched and heated to 180 °C and cooled to different crystallization temperatures.

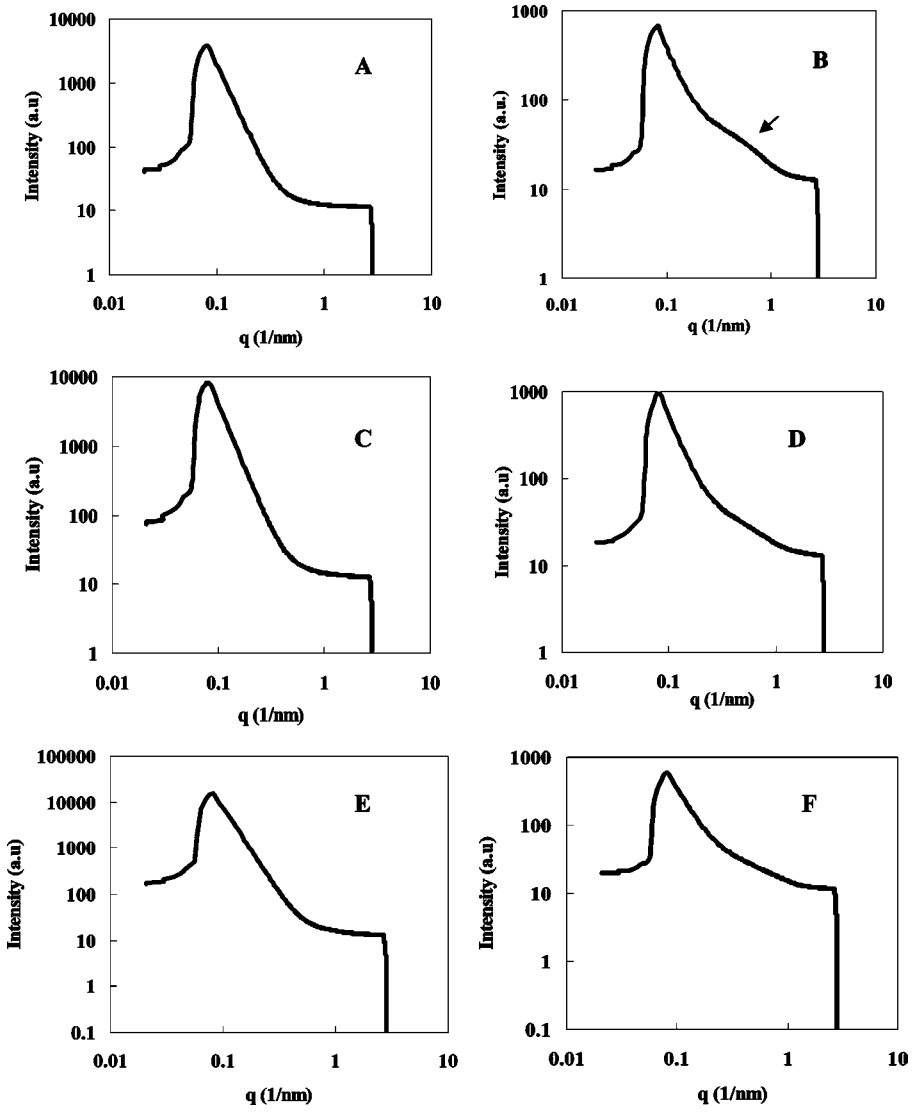

Figure 6. Synchrotron small-angle X-ray scattering of short-chain amylose spherulites produced by heating debranched waxy maize starch (25% w/w) to 180 °C and crystallizing at different temperatures: (A) 4 °C, as is; (B) 4 °C, hydrated; (C) 25 °C, as is; (D) 25 °C, hydrated; (E) 50 °C, as is; (F) 50 °C, hydrated.

native starch. However, at the low crystalline temperature of 4 °C, a weak broad peak ranging from ca. 0.3 to 1.2 nm^{-1} was observed (Figure 6), suggesting that some SCA molecules transversed double-helical crystals to form regions of low density.

It is worth comparing the SAXS results in this study with those on acid-hydrolyzed starches. After amorphous regions in starches had been extensively removed by acid, the intensity of the SAXS lamellar peak was reduced.[27,28] This reduction in SAXS peak seems to be consistent with the SAXS results in this study. The interconnected crystalline and amorphous regions were no longer in place after the waxy maize starch granules were cooked and debranched. Instead, the short linear chains that were released became well aligned during the crystallization and they formed double helices and strong crystallites.

In Vitro Digestion Profile. The spherulites prepared at 50 °C, which had an A-type X-ray diffraction pattern (Figure 4), showed the lowest digestibility among the three spherulites crystallized at different temperatures (Figure 7A) even though the spherulites crystallized at 50 °C had the smallest particle size (Figures 2 and 3). Only 15.4% of the spherulites were digested after 3 h. The spherulites formed at 4 and 25 °C gave a B-type X-ray pattern (Figure 4) with a larger particle size (Figures 2 and 3), and they were digested in 3 h to extents of

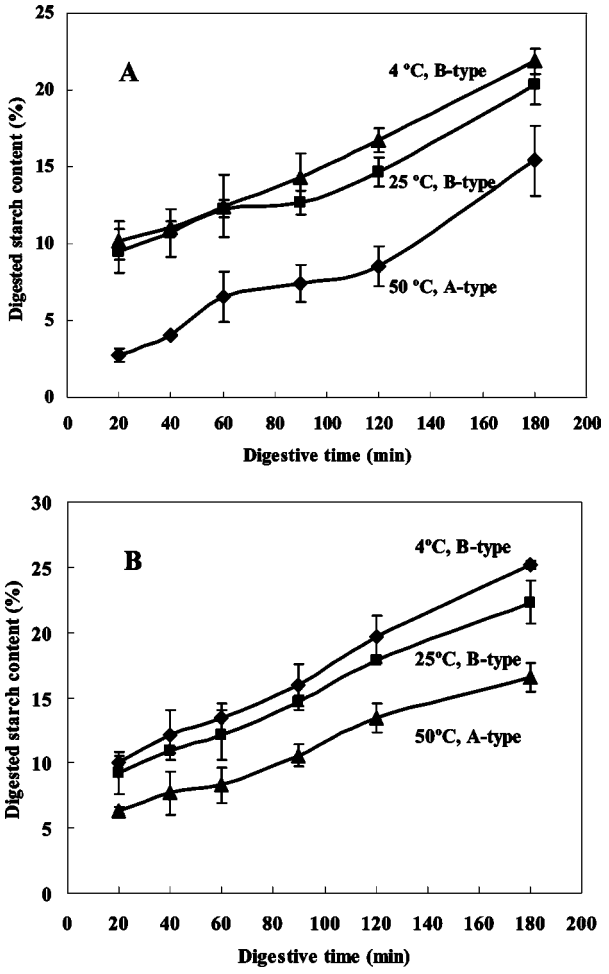

Figure 7. In vitro digestion (Englyst procedure[17,18]) profile of (A) short-chain amylose spherulites produced by heating debranched waxy maize starch (25% w/w) to 180 °C and crystallizing at different temperatures and (B) their digestive residues.

20.4 and 21.9%, respectively. One remarkable feature of the digestion curve of the spherulites formed at 4 °C was that the rate was essentially linear as a function of digestion time between 20 min and 3 h (Figure 7A). The digestion test was repeated once more on the recovered undigested solids. Digestion profiles of the undigested residues showed a similarly linear digestive pattern as the parent spherulites (Figure 7B), demonstrating that the spherulites were digested at essentially a constant rate between 20 min and 3 h. The residues from the first digestion of spherulites that had been formed at 50 °C were again more enzyme resistant than those that had formed at 4 and 25 °C. A slight increase in digestion was observed for all of the digestive residues after 3 h of the second digestion (Figure 7B) as compared to their parent counterparts (Figure 7A), which may be attributed to an altered morphology or structure in the residues after the first digestion.

To further understand the digestion mechanism, we used light microscopy to examine the residues of spherulites after the first digestion step (Figure 8). The residues still contained intact spherulites with a clear Maltese cross along with small pieces of fragmented spherulites. These results suggest that enzyme hydrolysis was not homogeneous and that some spherulites were completely hydrolyzed, whereas other spherulites remained birefregient. In general, the particle size appeared to be smaller after digestion, particularly for the B-type spherulites (Figures 2 and 8). SEM data (Figure 9) showed a rough surface structure for all of the digestion residues of spherulites, indicating that surface erosion rather than the endocorrosion had occurred during starch digestion. It is known that the enzymatic degradation of native starch granules is not homogeneous. The enzymes enlarge holes occurring as weak points on a granule's surface. Those holes lead to channels inside the granules, which are the path for enzymes to access the less resistant regions near the hilum in a granule.[29] In contrast, no holes or pores were observed in this study during digestion of the spherulites by the mixtures of α-amylase and glucoamylse.

It is worth noting that the rate of enzyme hydrolysis or the digestion curve of starch is dependent on the level of enzyme activity.[30] The linear rate of digestion observed in this study (Figure 7A) seems to suggest that the enzyme hydrolysis proceeded one spherulite at the time or had a mode of "single spherulite attack" as opposed to "multi spherulite attack". Microscopic data (Figures 2 and 8) suggested that the enzyme hydrolysis was not homogeneous. It would be interesting to determine how the rate of digestion of the spherulites is affected by different levels of enzyme activities in the future work.

DISCUSSION

Formation of Spherulites from SCA. Amylose is known to be the effective starch component that induces the formation of spherulites.[3−5,9] Short chains with branch points obtained after acid hydrolysis of starch were also used to produce spherulites.[11−13] In this study, short, complete linear chains obtained from debranched waxy maize starch were used to prepare spherulites for the first time. A schematic drawing of the transitions of SCA from starch granules to SCA spherulites is presented in Figure 10. Native starch granules are proposed to contain an amorphous background interrupted by semi-crystalline growth rings that are composed of regularly repeating crystalline and amorphous lamella.[26] After being cooked in water at a high temperature, the crystalline structure of the starch granule was destroyed, allowing branch points to be cleaved by isoamylase and producing completely linear chains.

Upon debranching 25% waxy maize starch with isoamylse at 50 °C, the liquid reaction mixture appeared opaque. However, the mixture was converted to a clear solution after it was heated to 140 °C. When the solution was cooled from this temperature (or the temperature range of 140−180 °C), aggregates of particles with irregular shapes were observed. Those materials showed weak birefringence and a sharp wide-angle X-ray diffraction pattern, confirming the formation of double helices and a crystalline structure. However, the Maltese cross was not observed in these particles under polarized light, suggesting that the double helices were not radially orientated. Interestingly, when the solution of SCA was cooled from 180 °C, a weak lamellar structure was formed as evidenced by the broad peak detected in SAXS curves of the hydrated spherulites (Figure 6).

According to Zieger et al.,[9] the production of spherulite crystals of amylose from aqueous solution depends on the relative rates of phase separation and polymer association (crystallization). It is only when phase separation is induced well before the nucleation of crystallization that the formation of spherulites occurs. At the temperature of 180 °C, the molecules in our SCA solution shifted in conformation from

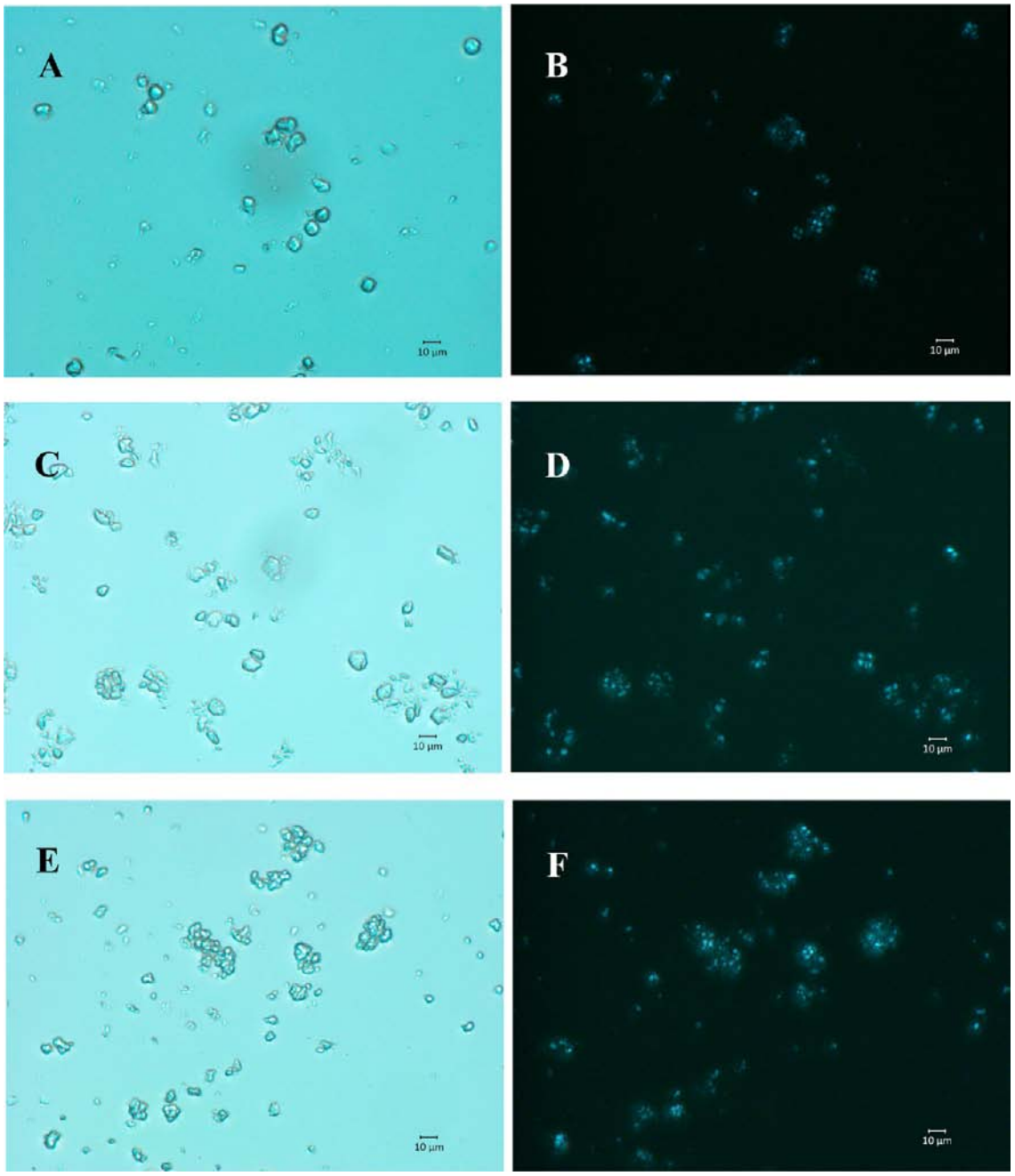

Figure 8. Microscopic images of digestive residues from short-chain amylose spherulites produced by heating debranched waxy maize starch (25% w/w) to 180 °C and crystallizing at different temperatures: (A, B) 4 °C; (C, D) 25 °C; (E, F) 50 °C. All scale bars represent 10 μm.

helix to coil state, and the solution separated into polymer-poor and polymer-rich phases at ~140−180 °C. Upon further cooling, the double helices formed in the polymer-rich phase became self-aligned into crystalline arrangement, which grew into spherulites that precipitated from the liquid medium. Thus, spherulite formation from SCA appeared to involve a conformational transition from coils to double helices, alignment of double helices into bundles that crystallized with limited amorphous loose ends, and radial orientation of the bundles into spherulites (Figure 10). Spherulites can be classified by their fiber density as described in a 2012 review.[31] The spherulites formed from SCA in this study appear to be compact and do not contain free space between individual crystallites. We postulate that the spherulites from SCA grew radially from the nucleation site and formed via central multidirectional growth.

Digestibility of A- and B-Type Spherulites. Given their well-defined morphology and high crystallinity, A- and B-amylose spherocrystals were used as model substrate to study the mode of enzymatic digestibility of starch crystallites.[12,13] Our results show that it was possible to produce spherulites with the A-type crystallinity that were more resistant to enzymatic hydrolysis than the B-type (Figure 7). However, this observation differs from the results presented by Planchot et al.[12] and Williamson et al.[13] Those authors prepared A- and B-type spherulite crystals from potato amylodextrins and reported that the B-type spherulites were more resistant to α-amylase digestion than the A-type. However, their A- and B-spherulites

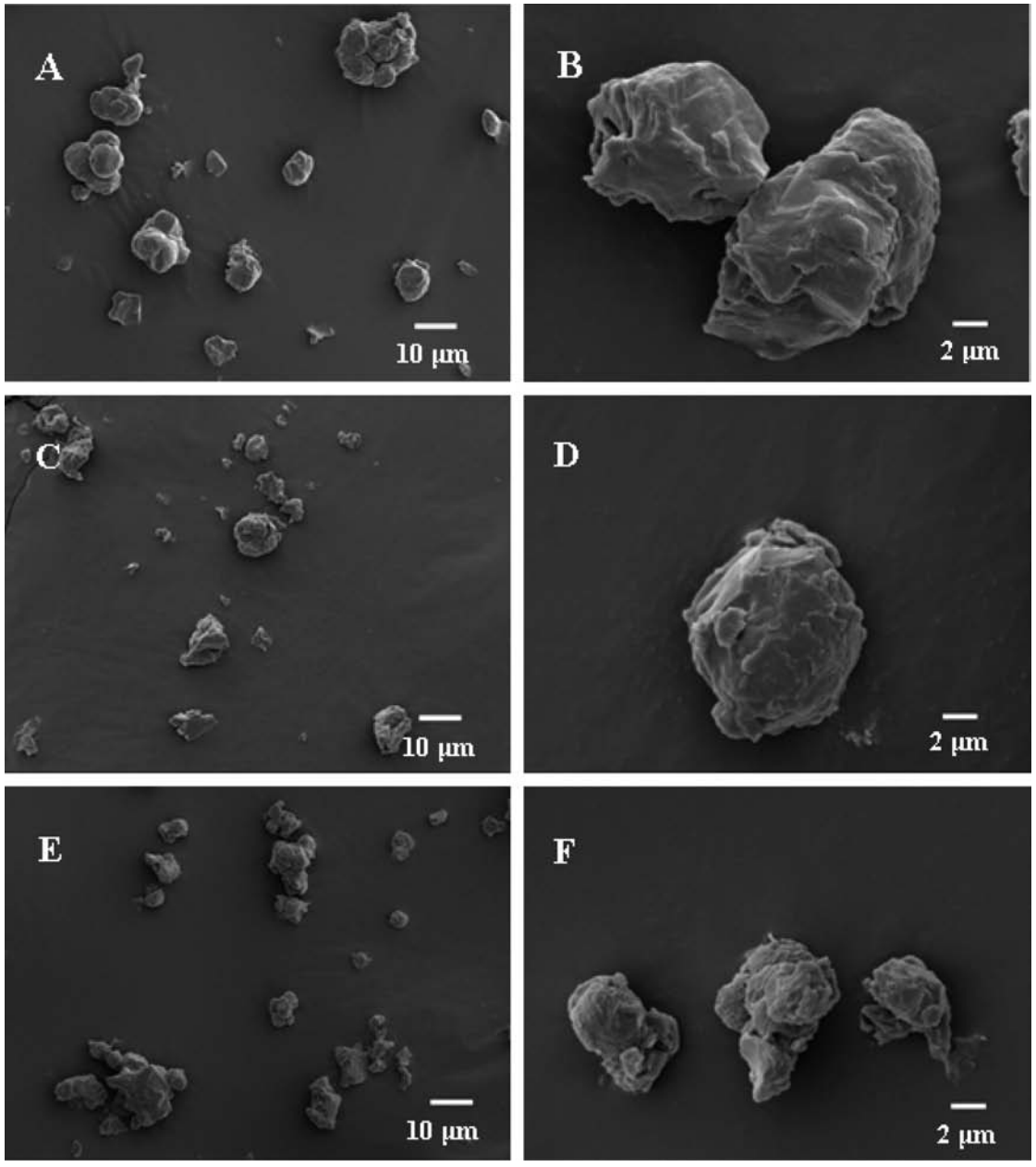

Figure 9. Scanning electron microscopic images of digestive residues from short-chain amylose spherulites produced by heating debranched waxy maize starch (25% w/w) to 180 °C and crystallizing at different temperatures: (A, B) 4 °C; (C, D) 25 °C; (E, F) 50 °C.

contained branched molecules, and as pointed out by the authors, the presence of α-1,6- linkages and the ratio of linear and branched chains may have affected amylase susceptibility. In the present work, the A- and B- spherulites were composed of only linear chains, and both types had similar molecular size distribution (Figure 1). In addition, Planchot et al.[12] noted that the difference in A- or B-type crystallinity could not account for the digestibility behavior of all the amylodextrins they examined. Other factors such as morphology, crystal defects, and the interrelation of the crystallites should be considered. It is possible that the radial organization of molecules in their A-type spherulite[12,20] was responsible for its higher degree of enzyme hydrolysis, compared to the tangential organization of the molecules in their B-type spherulite. The A- and B-type spherulites in the present study displayed a radial orientation of molecules (birefringence with Maltese cross) and similar surface smoothness (Figures 2 and 3), although the Maltese cross was not well observed in A-type spherulites due to their small particle size.

In this study, the spherulite size was not correlated with the digestibility. The A-type spherulite had a smaller particle size (larger surface area) than the two other B-type spherulites (Figure 3), but the A-type was still more enzyme-resistant (Figure 7). Moreover, the A-type spherulite had the highest melting temperature (Table 2; Figure 5), indicating it contained strong crystallites, that is, tighter packing of its double helices.

The spherulitic morphology has been proposed to closely resemble the in vivo initiation of native granular structure during the early stage of biosynthesis.[9] In this study, the B-type spherulite was more susceptible to α-amylase/amyloglucosidase hydrolysis than the A-type spherulite (Figure 7). This

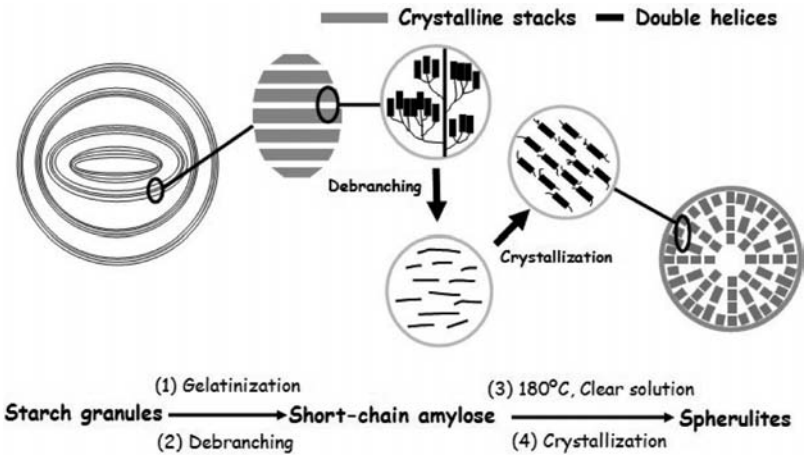

Figure 10. Schematic drawing of transition from short linear chains generated from debranching of waxy maize starch into spherulites.

observation is in striking contrast to the fact that native starch granules with a B-type X-ray pattern are more amylase-resistant than those with an A-type X-ray pattern.[32−39] Recent work has indicated that crystalline type per se does not appear to be the rate-limiting factor in the digestibility of starch granules.[40,41] Instead, granule architecture and structural barriers that limit diffusion of enzymes within a granule are purported to be the major factors controlling enzyme susceptibility of native starch.[40,41] A dense and compact morphology resulting from the epitaxial growth of elementary crystalline A-type platelets was used[42] to explain the highly enzyme-resistant aggregates of A-type crystals generated from the debranched maltodextrin.[43] The packing pattern of double helices in the A-type crystal structure is known to be denser than in its B-type counterpart.[44−47] The denser structure in the A-type spherulites most likely inhibited access of the enzymes to starch molecules and led to their lower digestibility compared to the B-type.

In conclusion, a novel high-yield process combining enzymatic debranching, melting, and crystallization was developed to convert waxy maize starch at high solids (~25%) to spherulites with a high melting temperature and a controlled enzyme digestibility. When the spherulites were digested by a mixture of α-amylase and amyloglucosidase, solids were lost at essentially a constant rate up to ~25%. The A-type spherulites had a smaller particle size (1−5 μm) but were still more resistant to enzyme digestion than the B-type spherulites (5−10 μm). Our findings suggested that size or surface area is not a determining factor controlling the digestibility of spherulites. As reflected in their greater thermal stability, the organization of A-type crystallites seemed to be more compact and stronger than that of B-type crystallites and therefore was more resistant to enzyme digestion.

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ABBREVIATIONS USED

DSC, differential scanning calorimeter; SCA, short-chain amylose; GPC, gel permeation chromatography; SEM, scanning electron microscopy; SAXS, synchrotron small-angle X-ray scattering

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