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Spherulitic self-assembly of debranched starch from aqueous solution and its effect on enzyme digestibility

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ABSTRACT

Debranched waxy rice starch (DWRS) and debranched normal rice starch (DNRS) were crystallized into spherulites from aqueous solutions. DNRS, which contained extra-long chains, yielded small sintered-like spherulites with rough surface, which exhibit positive birefringence and, therefore, had radial orientations. Large spherulites with smooth surfaces, negative birefringence and tangential orientation were formed with DWRS which essentially contained short linear chains. Both spherulites showed a B-type crystalline structure with an exceptionally high crystallinity and a similar melting behavior. However, DWRS spherulites had higher resistance to enzymatic hydrolysis than DNRS spherulites. The higher enzyme resistance of DWRS spherulites was attributed to their tangential organization and their smoother and denser surface as observed by light microscopy and scanning electron microscopy. The impact of the different structural features of the two types of spherulites is discussed in relation to their resistance to enzymatic hydrolysis.

1. Introduction

Spherulites are spherical semi-crystalline structures which exhibit a specific birefringence under the form of 'Maltese Cross' when observed with a polarized light microscopy. This birefringence arises from a preferred orientation of anisotropic crystallites (Bassett, 2003). The sign of the birefringence is given by the difference between the radial and tangential refractive indices which can be positive or negative (Murayama, 2002). It is directly related to the orientation of the macromolecules. In spherulites, the macromolecules are preferentially oriented in the radial direction and in the tangential direction for positive and negative spherulites, respectively (Yoshioka, Fujimura, Manabe, Yokota, & Tsuji, 2007).

Recently, two different ways were reported for the preparation of spherulites from amylose and native starches. The first, which involved inclusion complexes of amylose with small ligands, led to V-type amylose spherulites with a single helical conformation (Conde-Petit, Handschin, Heinemann, & Escher, 2007; Heinemann, Escher, & Conde-Petit, 2003). This spherulitic crystallization occurred during the slow cooling of amylose-ligand mixtures after heating at 140 °C (Fanta, Felker, Shogren, & Salch, 2008) and the process was termed 'high temperature retrogradation' (Davies, Miller, & Procter, 1980). The second comprised heating of aqueous starch dispersions above 170 °C, named as "clearing temperature", followed by rapid cooling (Creek, Ziegler, & Runt, 2006; Nordmark & Ziegler, 2002; Ziegler, Creek, & Runt, 2005). These spherulites were mainly composed of amylose and lightly branched starch polymers, and showed a weak B-type crystallinity and a radial orientation which was consistent with the core region of native granules (Nordmark & Ziegler, 2002; Ziegler et al., 2005). Thus, this mode of spherulitic crystallization was proposed as a model for *in* vivo starch granule initiation (Creek et al., 2006). A wide range of cooling rate (1–250 °C min^{-1}) can be used (Nordmark & Ziegler, 2002). The spherulites formation depended on the starch sources, the most perfect spherulites were obtained from high amylose starch while spherulites never formed from waxy starch (Singh, Lelane, Stewart, & Singh, 2010; Ziegler, Nordmark, & Woodling,

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2003). Similarly, Ma, Floros, and Ziegler (2011) reported that the spherulitic crystallization of starch was favored by a higher ratio of linear to branched molecules.

An alternative method using crystallization of concentrated amylose short-chain solutions into A and B-type spherulites was also reported (Cai & Shi, 2013; Helbert, Chanzy, Planchot, Buleon, & Colonna, 1993; Ring, Miles, Morris, Turner, & Colonna, 1987). A-type spherulites were prepared by ethanolic precipitation (Ring et al., 1987), showing radial organization and a weak cohesion between long crystalline elements, were more susceptible to α-amylase hydrolysis (Helbert et al., 1993; Planchot, Colonna, & Buleon, 1997a). B-type spherulites obtained by cooling at a low temperature (Ring et al., 1987) exhibited spherical shapes with smooth surfaces and were more resistant to enzymatic hydrolysis (Planchot et al., 1997a). On the contrary, Cai and Shi (2013) prepared A- and B-type spherulites from short linear chains crystallized at different temperatures and found that A-type spherulites were more compact and more resistant to enzyme digestion than B-type spherulites. This dependence on the crystalline type does not seem to be the determining factor for the behavior of enzyme digestion. However, starch spherulites have been explained to consider the differences in morphology and enzyme susceptibility. The spherulites morphology and degradation pattern could be implemented for application on materials for controlled or targeted release systems (Bhosale & Ziegler, 2010; Shi & Cai, 2014).

There have been limited works describing about the spherulitic self-assembly of highly polydisperse material as debranched starch. This study is focused on the impact of molecular weight distribution on the formation of spherulitic particles by using debranched waxy rice starch (DWRS) and debranched normal rice starch (DNRS), containing only short chains and a wider range of chain length, respectively. The structural features and the digestibility of resulting spherulites are described and some mechanisms for the formation of such spherulites are debated. This fundamental finding could be applied for designing novel starch for food and pharmaceutical industry.

2. Materials and methods

2.1. Materials

Waxy (RD6 variety) and normal rice grains (Chai-Nat 1 variety, 30% amylose) were obtained from the Bureau of Rice Research and Development, Thailand. Rice starches were extracted according to the method of Ju, Hettiarachchy, and Rath (2001). Isoamylase (EC 3.2.1.68, E-ISAMY) and a resistant starch assay kit were purchased from Megazyme (Megazyme International Ireland Ltd., Bray, Ireland). The isoamylase activity was 1.2×10^3 isoamylase activity units (IAU) mL^{-1} at 50 °C and pH 4.5.

2.2. Debranching

Starch (10 g, dry basis, db) was mixed with 90 mL sodium acetate buffer (0.05 M, pH 4.5) in a screw top glass bottle. The slurry was gelatinized and subsequently debranched using isoamylase (5 IAU g^{-1} of dry starch) at 50 °C for 24 h, according to the method of Kiatponglarp, Tongta, Rolland-Sabaté, and Buléon (2015). After 24 h debranching time, the debranched starch was rapidly frozen using liquid nitrogen and then kept at −40 °C overnight. The sample was freeze-dried and used for spherulites preparation. To confirm that the starch was completely debranched, the debranched starch was analyzed for β-amylolysis limit using β-amylase following the method of Mutungi, Onyango, Jaros, Henle, & Rohm (2009).

2.3. Molecular characterization

The molecular size distribution of the debranched starches was examined by high performance size exclusion chromatography coupled with multi-angle laser light scattering and differential refractometric detection (HPSEC-MALLS-DRI) as previously described (Kiatponglarp et al., 2015). The debranched starches (50 mg) were solubilized in 1 M KOH (500 μL) at 4 °C for 2 days and subsequently diluted with 4.5 mL of deionized (DI) water. The resulting solution was neutralized, then filtered through a 0.45 μm filter and immediately injected into the HPSEC-MALLS-DRI setup. The SEC columns used were three HemaBio (1000, 100 and 40) (250 mm × 8 mm) coupled to a HemaBio guard column (Sopares, Gentilly, France). Their temperature was maintained at 40 °C. The two-online detectors were a Dawn® Heleos™ MALLS instrument fitted with a K5 flow cell and a GaAs laser (λ = 658 nm) from Wyatt Technology Corporation (Santa Barbara, CA, USA) and an RID-10A refractometer from Shimadzu (Kyoto, Japan). The eluent was 0.1 N KOH with the flow rate of 0.4 mL min^{-1}.

2.4. Starch spherulites preparation

Approximately 15 mg of debranched waxy rice starch (DWRS) or debranched normal rice starch (DNRS) were weighted in high-volume (60 μL) stainless steel differential scanning calorimetry (DSC) pan and 35 μL of DI water was added to obtain about 30% (w/w) solid concentration. The sample pan was hermetically sealed and allowed to equilibrate overnight at room temperature. The sample was heated in a TA Q100 DSC (TA Instruments, New Castle, DE, USA) from 25 to 170 °C at a rate of 5 °C min^{-1}, and immediately cooled to 10 °C at rates of 25 °C $min^{-1}$for DWRS or 10 °C $min^{-1}$for DNRS. The sample was stored overnight at room temperature before analysis.

2.5. Thermal analysis

After storing overnight, the sample was heated in a TA Q100 DSC from 10 to 200 °C at 3 °C min^{-1}. The DSC was calibrated with indium, and a 35 μL of DI water was used as a reference. The data were analyzed using TA Advantage Software v5.5.3 (TA Instruments–Waters LLC, New Castle, DE, USA). The measurement was conducted in duplicate.

2.6. Light microscopy (LM)

Immediately after opening the sample pans, approximately 5 mg of the sample was taken and dispersed in a drop of DI water on a microscopy slide, and covered with a cover slip. The sample was observed using an Olympus BX51 microscope (Olympus, Deutschland GmbH, Hamburg, Germany) equipped with a polarizing filter and 530 nm compensating filter. The optical and polarized images were recorded using a digital CCD camera (SONY, XCD-SX90CR, Tokyo, Japan). The signs of birefringence of the spherulites were determined by means of a primary filter (λ-plate) located diagonally between cross polars. In this way the first and third quarters of the sight are blue and the second and fourth are yellow when the spherulites are positive, while a reversed arrangement of the quarters are observed for negative spherulites (Murayama, 2002; Yoshioka et al., 2007).

In parallel, approximately 5 mg of each sample was stained with a KI/I_2 solution. Twenty optical images with 10 × magnifications were captured at different positions using a digital CCD camera. Twenty global images were used to analyze the size distribution of particles based on the image analysis using mathematical morphology. All color images were converted into monochromatic