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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.,