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Molecular structure and enzymatic hydrolysis properties of starches from high-amylose maize inbred lines and their hybrids

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ABSTRACT

High-amylose maize starch has health benefits and special industrial uses. In this study, starches were isolated from normal maize and high-amylose inbred and hybrid maizes. Their molecular structure and enzymatic hydrolysis properties were investigated and analyzed. The high-amylose hybrid maize starch contained lower amylose, intermediate component, amylopectin long branch-chains, and amylopectin average chain length, and higher amylopectin short branch-chains than did high-amylose inbred maize starch. High-amylose maize starch was more resistant to α-amylase and amyloglucocidase hydrolysis and had a significantly lower hydrolysis rate coefficient than normal maize starch did. The native, gelatinized and retrograded starches of the high-amylose hybrid maize had significantly higher rapidly digestible starch and lower resistant starch than those of the high-amylose inbred maize. The retrogradation of gelatinized starch markedly increased the resistance of high-amylose starch to *in vitro* digestion. The high contents of amylose and intermediate component and the long branch-chains of amylopectin increased the resistance of maize starch to enzymatic hydrolysis and *in vitro* digestion.

1. Introduction

Starch consists of two main components: amylose and amylopectin. Many studies have reported that high-amylose starch also contains an intermediate component that has branched structures with branch-chains longer than amylopectin but molecular weights smaller than amylopectin and similar to amylose (Jiang, Campbell, Blanco, & Jane, 2010; Jiang et al., 2015; Li, Jiang, Campbell, Blanco, & Jane, 2008). Sepharose CL-2B gel-permeation chromatography (GPC) is generally used to investigate the molecular weight distribution (amylopectin, amylose, and intermediate component) of native starch, especially for high-amylose starch (Alves, Polesi, Aguiar, & Sarmento, 2014; Jiang et al., 2010, 2015; Kubo et al., 2010; Li et al., 2008; Marti, Pagani, & Seetharaman, 2011; Pinto et al., 2015; Yoo et al., 2009; Zhang, Zhao, & Xiong, 2010; Zhu et al., 2015).

Amylose content has significant effects on structural and functional properties of starch. Starches with high-amylose content have a high content of resistant starch, a portion of starch that cannot be hydrolysed in the upper gastrointestinal tract and functions as a substrate for bacterial fermentation in the large intestine for digestion (Carciofi et al., 2012; Englyst, Kingman, & Cummings, 1992; Man et al., 2012; Regina et al., 2006; Slade et al., 2012; Zhu et al., 2012). High-amylose cereal starch is presently of interest because it can lower glycaemic and insulin responses and reduce the risk for developing type II diabetes, obesity, and cardiovascular disease through its resistant starch (Nugent, 2005). In addition, high-amylose starch is also potentially of special value for industrial utilization. Therefore, many high-amylose cereal varieties have been developed via mutation or transgenic breeding approaches (Carciofi et al., 2012; Regina et al., 2006; Slade et al., 2012; Zhu et al., 2012).

Abbreviations: AAG, *Aspergillus niger* amyloglucosidase; GPC, gel-permeation chromatography; HPAEC, high-performance anion-exchange chromatography; PPA, porcine pancreatic α-amylase; RDS, rapidly digestible starch; RS, resistant starch; SDS, slowly digestible starch.

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Maize is an important food and feed crop because its seeds provide large amounts of starch. Maize starch (especially amylose) is also an important industrial raw material (Guan et al., 2011; Jiang et al., 2013). High-amylose maize has health benefits through its high resistant starch content (Jiang et al., 2010). Many high-amylose maize inbred lines have been developed (Guan et al., 2011; Jiang et al., 2010; Li et al., 2008). For the practical and economical value of hybrid vigour and grain yield, hybrid maize is widely grown by farmers, and relatively few maize inbred lines are grown. To the best of our knowledge, high-amylose hybrid maize starch is seldom reported in the literature. A high-amylose maize mutant was demonstrated in our previous study (Li et al., 2014). We had utilized this mutant to develop some high-amylose maize inbred lines and their hybrids through crossing, backcrossing, and self-crossing methods (Lin et al., 2016b). Maize starch granules become smaller in size and more spherical, oval, or elongated in shape with increasing amylose content. Hybrid maize starch with a C_B-type crystallinity has lower amylose content than inbred maize starch with a B-type crystallinity (Lin et al., 2016b). However, the molecular structure and functional properties of starches from high-amylose maize inbred lines and their hybrids are unclear, which impedes their utilization.

Starch hydrolysis is very important in the applications of starch in food and non-food industries. Enzymatic hydrolysis of starch is involved in many biological and industrial processes (Tawil, Viksø-Nielsen, Rolland-Sabaté, Colonna, & Buléon, 2011). Enzyme molecules affect starch granules in two ways: enzymes erode the outer surface of the granule and cause the occurrence of characteristic fissures and pits (exocorrosion), and enzymes create channels leading to the granule centre, which weakens granule integrity and consequently leads to its breakdown (endocorrosion) (Li, Gao, Wang, Jiang, & Huang, 2011; Li, Vasanthan, Hoover, & Rossnagel, 2004). The α-amylase is an endoamylase that cleaves the α-1,4 glycosidic bonds of amylose or the amylopectin chain at internal positions (endo) to yield products (oligosaccharides with varying lengths and branched oligosaccharides called limit dextrins) with an α-configuration. The amyloglucosidase is an exoamylase and catalyses the hydrolysis of both α-1,4 and α-1,6 glycosidic bonds at the branching point to release β-D-glucose residues of the polymer substrate (Li et al., 2004, 2011). The enzymatic hydrolysis properties of starch are generally determined using α-amylase and amyloglucosidase in many studies. In most mammals, starch digestion and glucose absorption occur mostly in the small intestine. The *in vitro* digestion of starch is generally carried out in an enzyme solution (pH 6.0) containing pancreatic α-amylase and amyloglucosidase at 37 °C to simulate and investigate the digestion of starch in the small intestine (Englyst, Kingman, Hudson, & Cummings, 1996; McCleary, McNally, & Rossiter, 2002; Witt, Gidley, & Gilbert, 2010).

As a follow-up study on our previous paper (Lin et al., 2016b), we have further investigated and analyzed the molecular structure and enzymatic hydrolysis properties of starches from normal maize and high-amylose inbred and hybrid maizes in this study. We aimed to compare the molecular structure and enzymatic hydrolysis properties of starches from high-amylose maize inbred lines and their hybrids and reveal the relationship between molecular structure and enzymatic hydrolysis properties of starch. This study could provide important information for utilizing starches from high-amylose maize hybrids in food and non-food industries.

2. Materials and methods

2.1. Plant materials

One normal maize cultivar, Xianyu 335, four high-amylose maize inbreds (Zae28, Zae35, Zae49, and Zae50), and two high-amylose maize hybrids (Zae35 × Zae28 and Zae49 × Zae50) were used in this study (Lin et al., 2016b). Plants were grown in the experimental field of Northwest A&F University, Yangling, China. Mature seeds were used to isolate starch.

2.2. Starch isolation

Starch was isolated from maize mature seeds following the method of Lin et al. (2016b).

2.3. Molecular weight distribution of native starch

The molecular weight distribution of native starch was determined using GPC on Sepharose CL-2B following the method of Li et al. (2008) with some modifications. Native starch (18 mg) was defatted and dispersed using 1.8 mL of 90% DMSO in a ThermoMixer with continuous shaking (500 rpm) at 95 °C for 1 h and 25 °C for overnight. The sample was centrifuged at 4000 g for 10 min, and the supernatant (1.5 mL) was mixed with 6 mL of absolute ethanol to precipitate the starch. The precipitation was washed with absolute ethanol and then dispersed using 5 mL of warm deionized water and incubated in boiling water for 30 min. The sample was cooled to room temperature and then centrifuged at 4000 g for 10 min. The supernatant was filtered by using a 5 μm filter, and 2 mL of supernatant was then injected into a GPC column (1.6 cm ID × 50 cm) packed with Sepharose CL-2B (Sigma CL2B300). The column was eluted using an eluent containing 25 mM NaCl and 1 mM NaOH at a flow rate of 0.5 mL/min in a descending mode. Fractions of 1.5 mL each were collected. The aliquots (1 mL) of the fractions were used for determination of carbohydrate by using an anthrone-H_2SO_4 method. The iodine absorption spectrum was checked as follows: a 0.4 mL aliquot of the fractions was added to 1.56 mL deionized water and 0.04 mL iodine solution (0.2% I_2 and 2% KI, w/v). The solution was mixed and allowed to stand at room temperature for 10 min to react. Finally, the reaction mixture was scanned from 400 to 900 nm using a spectrophotometer (Ultrospec 6300 pro, Amersham Biosciences). The iodine absorbance value was the extinction at 630 nm under the above experimental conditions. The λ_{max} was the wavelength (in nm) at which the extinction was the highest over the range of wavelengths.

2.4. Chain length distribution of amylopectin

The starch was deproteinized with protease and sodium bisulfite and debranched with isoamylase following the method of Lin et al. (2016a). The debranching treatment was terminated by adding NaOH and heating at 80 °C. The sample was centrifuged at 10000 g for 10 min and then filtered (0.22 μm nylon filter) and injected into a high-performance anion-exchange chromatography (HPAEC) system with a pulse amperometric detector (PAD) system. The HPAEC system consisted of a Dionex ICS-5000 (Thermo Fisher Scientific, USA), a DC detector/chromatography module, a CarboPac PA200 guard column (3 × 50 mm), a CarboPac PA200 analytical column (3 × 250 mm), and an AS-AP autosampler. The separation was achieved using a gradient eluent with eluent A (150 mM NaOH) and eluent B (500 mM sodium acetate in 150 mM NaOH) at a flow rate of 0.5 mL/min. The gradient of eluent B was 35% from 0 to 2 min, increased from 35% to 60% for 15 min and from 60% to 80% for 13 min, maintained 80% for 10 min, and finally reduced from 80% to 30% for 0.2 min. Maltohexaose was used as a standard. The chain length distribution was characterized as a percentage of the total peak area.