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Thermal and rheological properties of granular waxy maize mutant starches after isoamylase modification

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ABSTRACT

This work investigated the changes in the thermal and rheological property of two waxy maize mutant starches (Hsyn73 *wxwx* and Hsyn73 *duwx*) after they were treated with isoamylase in the granular state for various periods. The hydrolysis degrees reached in 168 h were 4.0% and 9.5% for the *wxwx* and the *duwx*, respectively. With increasing degree of hydrolysis, both *wxwx* and *duwx* generally showed an increase in gelatinization and retrogradation temperatures and retrogradation enthalpy. The weight average molar mass (M_w), *z*-averaged radius of gyration (R_z), and pasting viscosity of both starches decreased when isoamylase hydrolysis progressed, however, the *wxwx* showed a greater decrease in M_w and R_z than the *duwx* at the same hydrolysis degree. Both *wxwx* and *duwx*, native or isoamylase-treated, displayed a similar pasting viscosity during holding and cooling when their amylopectin molecules had a similar R_z. The study indicates the importance of R_z in determining the pasting properties of waxy maize starch.

1. Introduction

Starch is used widely in processed foods as a thickening, texturizing, binding or stabilizing agent because of its swelling and viscosity development. It is known that amylose and lipids inhibit swelling of granular starch, whereas amylopectin contributes to swelling (Tester & Morrison, 1990a). Jane and Chen (1992) reported synergistic effects on paste viscosity when amyloses and amylopectins were mixed, with the long branch-chain amylopectin and the intermediate molecular size amylose producing the greatest effect. Jane et al. (1999) further proposed that the very long branch-chains of amylopectin were responsible for holding the integrity of starch granules during heating and shearing and the decrease in peak viscosity and breakdown.

Waxy (*wx*) and *wx*-containing genotypes, such as double- or triple mutants, consist of essentially 100% of amylopectin, and the presence of extra mutant gene(s) creates additional modifications in amylopectin structure. Waxy mutant starches serve as good models to study amylopectin structure–function relationship because of little influence from amylose and lipids. Yuan, Thompson, and Boyer (1993) studied three waxy maize mutant starches (*wx*, *du wx*, and *ae wx*) from two inbred lines and found that higher gelatinization temperature and enthalpy were associated with a higher proportion of longer chains (degree of polymerization (DP) > 30). Shi and Seib (1995) reported that both amorphous and crystalline regions were altered in four waxy maize mutant starches (*wx*, *du wx*, *ae wx*, and *ae du wx*), and the onset melting temperature and heat uptake for both gelatinization and retrogradation increased with increasing proportion of amylopectin long chains (DP > 16).

Starch granules are resistant to degradation by amylases and their susceptibility to amylases varies with sources of starch granules and amylases. Alpha-amylase and glucoamylase are more efficient than β-amylase in degrading starch, and more susceptible starch granules possess pores or a sponge-like structure to facilitate amylase attacks (Leach & Schoch, 1961; Sarikaya, Higasa, Adachi, & Mikami, 2000). Kimura and Robyt (1996) demonstrated that isoamylase from *Pseudomonas amyloderamosa* was also capable of hydrolyzing the α-(1 → 6) linkages in native starch granules to a limited extent, depending on the starch type from 3.6% for tapioca to 11.9% for amylomaize-7, at 37 °C after 32 h. Nevertheless, there was no correlation between the starch types that were susceptible to glucoamylase degradation and those that were susceptible to isoamylase degradation.

This objective of this work was to investigate the thermal and rheological properties of two waxy maize mutant starches, *wxwx* and *duwx*, after hydrolysis by isoamylase to varying degrees in the granular state. We (Mendez-Montealvo, Wang, & Campbell, 2010) recently reported that β-amylase hydrolyzed Hsyn73 *duwx* to a greater extent than Hsyn73 *wxwx*, and a greater proportion of amylopectin long B chains may be responsible for a high viscosity profile. This work attempted to alter the branching degree in waxy

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maize mutant starches in the granular state to better understand how amylopectin structural characteristics affect the thermal and rheological properties of granular waxy maize starches.

2. Materials and methods

2.1. Materials

Two synthetic populations developed by Dr. David Glover at Purdue University were derived from a common synthetic variety known as Hsyn73 developed by combining a number of public inbreeds of the stiff-stalk heterotic group having a normal endosperm type. The genetic materials were grown in 2004 at the Truman State University Research Farm near Kirksville, Missouri, as described in Mendez-Montealvo et al. (2010). Starch was isolated according to the method of Eckhoff et al. (1996), and the damaged starch content was determined by following AACC Method 76-31 (2000). Isoamylase (EC 3.2.1.68, 59,000 U/mL) was purchased from Hayashibara Biochemical Laboratories (Okayama, Japan) and used without further treatment.

2.2. Methods

2.2.1. Hydrolysis by isoamylase

A 6% (w/v) starch slurry in 0.1 M acetate buffer (pH 3.5) was incubated at 45 °C and 100 rpm in a reciprocating shaker (Boekel Scientific, Feasterville, PA). Hydrolysis was initiated by the addition of 96 μL isoamylase (2360 U/g starch), and aliquots (9 mL) were taken periodically over a 168-h time period. The aliquot was centrifuged at 2000 × *g* for 10 min, and the supernatant was determined for soluble sugars using the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The recovered starch was washed with 80% ethanol, centrifuged at 2000 × *g* for 10 min, washed and centrifuged again, dried at room temperature, powdered with mortar and pestle, and stored at room temperature. At least two samples were prepared for each hydrolysis degree.

$$\text{Degree of hydrolysis (\%)} = 100 \times \frac{\text{Soluble sugar produced by enzyme hydrolysis}}{\text{Starch dry weight}}$$

2.2.2. Molecular size and chain-length distribution of debranched amylopectin

Starch was debranched according to the method of Mendez-Montealvo et al. (2010). The relative proportions of debranched amylopectin fractions were calculated from the area of their corresponding peaks using high-performance size-exclusion chromatography with refractive index detection (HPSEC–RI) following the method of Kasemsuwan, Jane, Schnable, Stinard, & Robertson (1995).

2.2.3. Structural characteristics of amylopectin

Starch (20 mg) was mixed with 4 mL of 90% dimethyl sulfoxide in a screw-cap test tube, and the mixture was stirred gently at room temperature for 16 h. One milliliter aliquot was precipitated with 10 mL ethanol, allowed to stand for 30 min, and centrifuged at 800 × *g* for 10 min. The supernatant was discarded, and the precipitate was re-dispersed in 6 mL of deionized water and then autoclaved at 121 °C for 15 min. After cooling, the sample was filtered through a 5.0-μm membrane filter (mixed cellulose esters, SMWP, Millipore, Bedford, MA) prior to injection into a HPSEC–MALLS–RI system (HPSEC with multi-angle laser light scattering and refractive index detection) as described in Patindol, Gu, & Wang (2009). The coefficients of photodiode were standardized using a pullulan standard of 22,500 M_w (Showa Denko K.K., Tokyo, Japan) as reference. The values reported were averages of three measurements.

2.2.4. Thermal properties

Thermal properties were assessed by a Pyris-1 differential scanning calorimeter (DSC) (Perkin Elmer Co., Norwalk, CT). The instrument was calibrated with indium and an empty pan was used as reference. Starch (~4.0 mg, dry basis) was weighed into an aluminum DSC pan and then moistened with 8 μL of deionized water using a micro-syringe. The pan was hermetically sealed and allowed to stand for 1 h prior to analysis. The sample was scanned from 25 to 120 °C at a heating rate of 10 °C/min. The onset (T_o), peak (T_p) and conclusion (T_c) gelatinization temperature and enthalpy (ΔH) were computed. Gelatinized samples were stored at 4 °C for 7 days, and then the samples were equilibrated at room temperature for 1 h prior to re-scanning using the same conditions described previously to obtain retrogradation T_o, T_p and T_c and ΔH. Retrogradation degree was the percentage of retrogradation enthalpy over gelatinization enthalpy of the same sample.

2.2.5. Rheological properties

The pasting profile of starch dispersion (5% w/v, dry base) was measured by a rotational test in a AR2000 Rheometer (TA Instruments, New Castle, DE) using parallel plates (sandblasted plate) with a diameter of 40 mm and a gap of 1000 μm at a heating or cooling rate of 2.0 °C/min and a shear rate of 50 1/s. The parallel plates were covered with mineral oil to avoid water evaporation during the test. The rheometer was programmed for running time sweeps of a cycle of heating from 25 to 90 °C, holding at 90 °C for 10 min, cooling from 90 °C to 25 °C, and maintaining at 25 °C for 5 min.

3. Results and discussions

3.1. Hydrolysis profile by isoamylase

The damaged starch contents in native *wxwx* and *duwx* were 2.3% and 3.5%, respectively, on a dry basis. The hydrolysis profiles of the two starch mutants over a 168-h period are shown in Fig. 1. An initial rapid hydrolysis followed by a steady increase over a 168-h period was observed for both starches. The rate of hydrolysis was greater for the *duwx*, and the hydrolysis degrees at 168 h were approximately 9.5% and 4.0% for the *duwx* and *wxwx*, respectively.

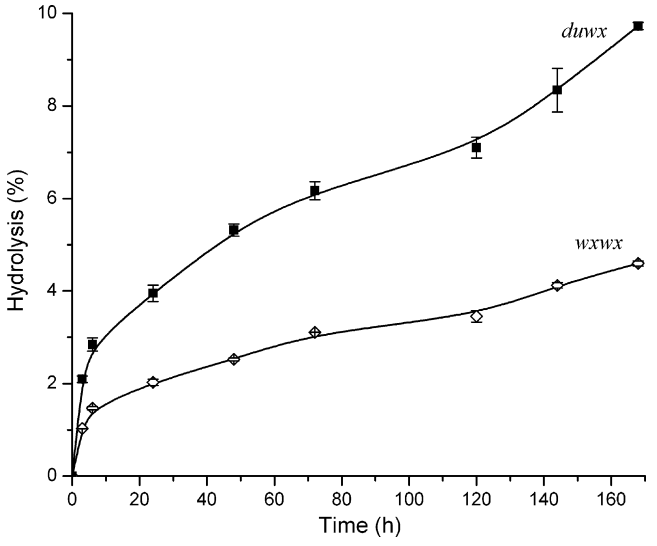

Fig. 1. Percent hydrolysis of *wxwx* (◊) and *duwx* (■) by isoamylase over a 168-h period.