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Thermal and rheological properties of granular waxy maize mutant starches after β-amylase modification

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ABSTRACT

The objective of this work was to investigate the impact of β-amylolysis on the physicochemical properties of two granular waxy maize mutant starches. The β-amylolysis progressed rapidly at the beginning and then gradually leveled at approximately 5% for *wxwx* and 8.5% for *duwx*. With increasing β-amylolysis, both starches showed a decrease in the proportion of long B chains, average chain length, molar mass, and *z*-averaged radius of gyration, but an increase in the proportion of short B and A chains, and chains with 2–5 glucose units. The onset gelatinization temperature and pasting viscosity of hydrolyzed samples did not decrease until reaching the hydrolysis degree of 5% for the *wxwx* and 8.5% for the *duwx*. Moreover, both *wxwx* and *duwx* retained their respective characteristics in molecular size distribution and rheological properties during β-amylolysis. The rheological properties of waxy maize starch were more affected by the crystalline structure and the proportion of long chains in amylopectin.

1. Introduction

Starch is the major component of many staple foods and also one of the most versatile ingredients in the food industry. The texture and quality of starch-containing foods is mainly controlled by the state of starch granular disorganization (Greenwood, 1979), such as gelatinization and swelling. When starch is heated in excess water, starch granule swells and increases in volume and at the same time part of the components becomes soluble, giving rise to a suspension of swollen particles dispersed in a macromolecular continuous phase. Swelling contributes to important functional properties of starch such as water-holding and thickening.

Starch swelling is considered a property of amylopectin, and amylose acts as a diluent (Tester & Morrison, 1990). Sasaki and Matsuki (1998) found that larger proportions of amylopectin long chains (degree of polymerization, DP ≥ 35) contributed to increased starch swelling from studying 12 wheat samples. Jane et al. (1999) reported that the very long branch chains in amylopectin behaved similar to amylose and restricted starch swelling. Han and Hamaker (2001) found a negative relationship between proportion of long chains in amylopectin and paste breakdown. Vandeputte, Derycke, Geeroms, and Delcour (2003) found that amylopectin branch chains of DP 12–22 decreased swelling power, but there were no significant correlations between amylopectin chain-length distribution and peak, breakdown, setback and final viscosities. The inconsistent results in the literature are attributed to the complex nature of structural features in starches.

Maize is genetically the most accessible and most characterized among the higher plants. Mutants that have effects on carbohydrate composition typically show differences in the percentage of amylose content, the structures of amylose and amylopectin, the degree of branching, the chain length, and the amount of the intermediate component (Shannon & Garwood, 1984). Twelve recessive genes have been identified to alter endosperm carbohydrate deposition (Watson, 2003). The waxy gene encodes granule-bound starch synthase I (GBSSI), which is responsible for amylose synthesis. Mutants showing very low GBSSI activity produce starch with almost 100% amylopectin. The dull1 (*du1*) mutants have reduced activity in the endosperm of starch synthase II (SSII) and starch branching enzyme IIa (SBEIIa) (Boyer & Preiss, 1981). The starch from *du1* mutant kernels has slightly or greatly elevated amylose content and higher degree of branching compared with normal kernels (Inouchi, Glover, & Fuwa, 1987; Wang, White, & Pollak, 1993; Wang, White, Pollak, & Jane, 1993). The amylopectin of dull waxy (*duwx*) mutant was reported to have a decreased proportion of long B chains and an increased proportion of short B chains (Fuwa et al., 1987). Shi and Seib (1995) reported that *duwx* starch had a reduced percentage of short chains particularly DP 6–9, a larger percentage of DP 12–16 chains, and a lower percentage of DP 17–30 chains.

The objective of this study was to utilize the known difference in amylopectin structure of the two waxy corn mutant starches (*wxwx* and *duwx*) to study the structure impact on thermal and rheological

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properties of granular waxy maize starch. Because of the absence of amylose in the waxy maize mutants and the hydrolysis conducted at the granular state, it is possible to use the results from this study to obtain a better understanding how amylopectin structural characteristics affect the thermal and rheological properties of granular waxy maize starches.

2. Materials and methods

2.1. Materials

Genetic material was grown in 2004 at the Truman State University Research Farm near Kirksville, Missouri. Two synthetic populations developed by Dr. David Glover at Purdue were derived from a common synthetic variety known as Hsyn73 developed by combing a number of public inbreds of the stiff-stalk heterotic group having a normal endosperm type. The synthetic was backcross converted to both a homozygous *wxwx* synthetic (Hsyn73 *wxwx*) and a separate homozygous *dudu wxwx* synthetic (Hsyn73 *wxwx dudu*). During conversion individuals test crossed onto genetic stocks of *wxwx* and *duduwx* to confirm the presence of mutant alleles. Both synthetics were grown in a similar breeding nursery and plants chain crossed to maintain heterosis, population uniformity while maintaining the genetic breadth of the synthetic. 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). Beta-amylase (*B. cereus*, 20,000 U/mL) was purchased from Megazyme (Wicklow, Ireland) with less than 0.0001% α-amylase. Isoamylase (EC 3.2.1.68, 59,000 U/mL) was purchased from Hayashibara Biochemical Laboratories (Okayama, Japan). Both enzymes were used without further treatment.

2.2. Methods

2.2.1. Hydrolysis by β-amylase

Starch slurry (10%, w/v) in 50 mM acetate buffer (pH 4.8) was incubated in a reciprocating shaker (Boekel Scientific, Feasterville, PA) at 40 °C and 100 rpm. Hydrolysis was initiated by the addition of β-amylase (500 U/g starch) to the slurry, and the amount of enzyme was chosen to obtain controllable hydrolysis for both starches within 7 days. Aliquots (9 mL) were taken periodically over 168 h. 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 unhydrolyzed 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 β-amylase-treated starch samples were prepared for each sampling time:

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

2.2.2. Fine structure of debranched amylopectin

Debranched amylopectin was prepared by boiling 10 mg starch sample in 3.2 mL deionized water in a test tube with stirring for 30 min. After cooling to room temperature, 0.4 mL of 0.1 M acetate buffer (pH 3.5) and 5 μL of isoamylase were added, and the mixture was incubated in the water bath shaker at 45 °C and 150 rpm for 2 h. Isoamylase activity was arrested by adding 0.21 mL of 0.2 M NaOH and heating the tube in a boiling water for 15 min. After cooling, the sample was filtered through a 0.45-μm membrane (NYL w/GMF, Whatman, Clifton, NJ) prior to injection into HPLC systems.

The relative proportions of debranched amylopectin fractions were calculated from the area of their corresponding peaks by using high-performance size-exclusion chromatography with refractive index detection (HPSEC-RI) following the method of Kasemsuwan, Jane, Schnable, Stinard, and Robertson (1995) with modifications (Patindol, Gu, & Wang, 2009).

The chain-length distribution of debranched amylopectin was characterized by high-performance anion-exchange chromatography equipped with pulsed-amperometric detection (HPAEC-PAD) according to the method of Kasemsuwan et al. (1995). The HPAEC-PAD system (Dionex ICS-3000, Sunnyville, CA) consisted of the components of AS40 autosampler, single pump, detector/chromatography module (DC), 4 × 50-mm CarboPac PA1 guard column and 4 × 250-mm CarboPac PA1 analytical column.

2.2.3. Molecular size and 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 millilitre 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 et al. (2009). The coefficients of photodiode were standardized using pullulan standard of 22,500 Mw (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 mg, db) 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, equilibrated at room temperature for 1 h, and then re-scanned using the same conditions described previously to obtain retrogradation T_o, T_p and T_c and ΔH.

2.2.5. Rheological properties: rotational test

The pasting profile of starch dispersion (1.3 mL, 5%, w/v, db) was measured by running rotational test in an 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 °C/min and a shear rate of 50 s^{-1}. The parallel plates were covered with mineral oil to avoid water evaporation during the test. The rheometer was programmed for running time sweeps of cycle of heating from 25 to 90 °C, holding at 90 °C for 10 min, cooling from 90 to 25 °C, and maintaining at 25 °C for 5 min.

3. Results and discussions

3.1. Beta-amylolysis profile

The damaged starch contents in native *wxwx* and *duwx* were 2.3 and 3.5%, respectively, on a dry weight basis. The β-amylolysis profiles of both waxy maize mutant starches over a 168-h period are shown in Fig. 1. Both starches underwent rapid hydrolysis at the beginning, and then gradually reached a plateau of approximately 5 and 8.5% for the *wxwx* and *duwx*, respectively, after 48 h of hydrolysis. The β-amylolysis proceeded rapidly at the first few hours, which

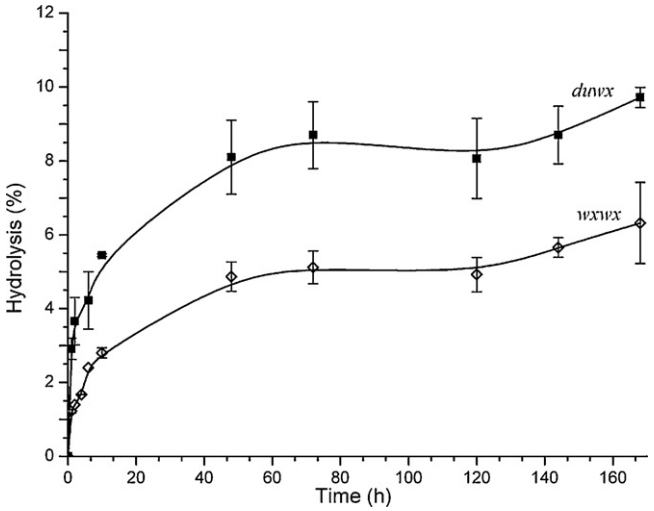

Fig. 1. Percent hydrolysis of *wxwx* (◊) and *duwx* (■) by β-amylase over 168 h.

was suspected to result from the presence of damaged starch. Moreover, the *duwx* was consistently hydrolyzed to a greater degree than was the *wxwx*, and their differences in the hydrolysis degree could not be solely explained by their different damaged starch contents. The results indicate differences in the organization of amylopectin molecules between the two waxy maize mutant starches, and the differences affected the hydrolysis by β-amylase.

It is known that granular starch is very resistant to enzymatic attack when compared to gelatinized starch, and the efficiency by which amylolytic enzymes attack granular starch varies widely. 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 enzyme attacks (Leach & Schoch, 1961; Sarikaya, Higasa, Adachi, & Mikami, 2000). Although β-amylase has not been as extensively studied as α-amylase in terms of factors affecting its rate and profile of hydrolyzing granular starch, β-amylase, similar to α-amylase, is capable of penetrating granular starch and hydrolyzing the amorphous regions prior to crystalline regions (Bertoft, Manelius, & Zhu, 1993a; Bertoft, Manelius, & Zhu, 1993b; Gallant, Bouchet, & Perez, 1992). Herbert, Schulein, and Henrissat (1996) described the concept of centrifugal (hydrolysis of regions and layers) and centripetal (hydrolysis from surface to core) hydrolysis of granular maize starch by α-amylase. Sarikaya et al. (2000) observed slight centripetal hydrolysis of granular maize starch by β-amylase with no deep holes. In their study, β-amylase from *B. cereus* solubilized approximately 1.5% of granular maize starch compared with 16% solubilization by α-amylase from *B. amyloliquefaciens* at 35 °C for 24 h.

Table 1
Fine structure of debranched *wxwx* and *duwx* as resolved by HPSEC-RI[a] after hydrolysis by β-amylase to various degrees.[b]

Hydrolysis (%)	Fr. I[c] (%)	Fr. II[c] (%)	Fr. II/Fr. I
wxwx			
0	20.7 ± 0.4	79.3 ± 0.4	3.8 ± 0.1
1.5	20.7 ± 0.1	79.3 ± 0.1	3.8 ± 0.0
3.5	19.2 ± 0.5	80.8 ± 0.5	4.2 ± 0.1
5.0	19.4 ± 0.2	80.6 ± 0.2	4.2 ± 0.1
duwx			
0	16.3 ± 0.2	83.7 ± 0.2	5.1 ± 0.1
3.5	16.2 ± 0.2	83.8 ± 0.2	5.2 ± 0.1
5.5	15.7 ± 0.1	84.3 ± 0.1	5.4 ± 0.1
8.5	15.8 ± 0.1	84.2 ± 0.1	5.3 ± 0.0

[a] High-performance size-exclusion chromatography with refractive index detection.
[b] Mean of duplicate measurements ± standard deviation.
[c] Fr. I, amylopectin long B chains; Fr. II, amylopectin A and B short chains.

3.2. Fine structure of debranched amylopectin

Table 1 lists the relative proportions of Fraction I (Fr. I) and Fraction II (Fr. II) from native and β-amylase-treated *wxwx* and *duwx* as eluted by the HPSEC-RI system. Fr. I included amylopectin long B chains, and Fr. II consisted of amylopectin A and short B chains (Akai, Yokobayashi, Misaki, & Harada, 1971). The ratio of Fr. II to Fr. I may be used as an index of the extent of amylopectin branching, the greater the ratio, the higher the degree of branching (Biliaderis, Grant, & Vose, 1981). There were significant differences in the distribution of amylopectin chains between the two waxy maize mutant starches. The *duwx* had a larger proportion of Fr. II, consequently a greater ratio of Fr. II/Fr. I, i.e. higher degree of branching, than the *wxwx*, which agreed with results by Fuwa et al. (1987) and Yuan, Thompson, and Boyer (1993). The higher degree of branching indicates the presence of more short chains, subsequently more non-reducing groups in the *duwx*. Kim, Kong, Kim, and Lee (2008) proposed that the number of contact points between substrate and enzyme molecules determined the initial stage of amylolysis. We propose that the larger amount of non-reducing groups in the *duwx* facilitated β-amylolysis, thus the *duwx* was hydrolyzed to a greater extent than was the *wxwx*. During the course of β-amylolysis, both *duwx* and *wxwx* showed a slight increase in the proportion of Fr. II as a result of chain shortening.

The chain-length distributions of native and β-amylase-treated *wxwx* and *duwx* are summarized in Table 2. Amylopectin branch chains were grouped into four chain types; namely, A, B1, B2, and B3+ chains corresponding to their chain length DP 6–12, 13–24, 25–36, and >36, respectively, according to Hanashiro, Abe, and

Table 2
Amylopectin chain-length distribution of *wxwx* and *duwx* after hydrolysis by β-amylase to varying degrees.[a]

Hydrolysis (%)	Average chain length	Percent distribution (%)				
		DP[b] 2–5	DP 6–12 (A chains)	DP 13–24 (B1 chains)	DP 25–36 (B2 chains)	DP 37+ (B3+ chains)
wxwx						
0	20.8 ± 0.0	0	22.3 ± 0.1	49.8 ± 0.1	17.1 ± 0.1	10.4 ± 0.1
1.5	20.3 ± 0.0	2.1 ± 0.1	22.5 ± 0.0	49.2 ± 0.0	16.2 ± 0.0	10.0 ± 0.1
3.5	20.3 ± 0.0	2.1 ± 0.1	22.5 ± 0.0	49.1 ± 0.1	16.3 ± 0.0	10.0 ± 0.0
5.0	20.1 ± 0.0	3.3 ± 0.3	22.4 ± 0.0	48.3 ± 0.3	16.1 ± 0.1	9.9 ± 0.1
duwx						
0	20.4 ± 0.0	0	21.5 ± 0.2	51.0 ± 0.1	18.8 ± 0.0	8.0 ± 0.1
3.5	19.5 ± 0.0	4.5 ± 0.0	21.6 ± 0.1	48.9 ± 0.0	17.6 ± 0.1	7.4 ± 0.1
5.5	19.7 ± 0.2	2.9 ± 0.1	22.6 ± 0.4	49.6 ± 0.4	17.6 ± 0.4	7.5 ± 0.2
8.5	19.5 ± 0.1	3.9 ± 0.3	22.4 ± 0.0	48.9 ± 0.1	17.3 ± 0.2	7.6 ± 0.0

[a] Mean of duplicate measurements ± standard deviation.
[b] Degree of polymerization as expressed as glucose unit.

Table 3
Molecular size and structural characteristics of *wxwx* and *duwx* after hydrolysis by β-amylase to varying degrees.[a]

Hydrolysis (%)	Mn/Mw[b]	Mw (E+08) (g/mol)	R_z (nm)	ν_z
wxwx				
0	2.22 ± 0.10	3.66 ± 0.15	324 ± 6	0.36 ± 0.00
1.5	2.14 ± 0.11	3.50 ± 0.18	306 ± 5	0.36 ± 0.01
3.5	2.21 ± 0.16	3.41 ± 0.18	312 ± 5	0.36 ± 0.01
5.0	2.04 ± 0.06	3.12 ± 0.09	303 ± 2	0.37 ± 0.00
duwx				
0	2.11 ± 0.12	3.64 ± 0.30	305 ± 2	0.37 ± 0.01
3.5	2.01 ± 0.04	3.75 ± 0.49	301 ± 3	0.38 ± 0.01
5.5	2.08 ± 0.03	3.14 ± 0.18	296 ± 1	0.38 ± 0.00
8.5	2.02 ± 0.05	3.22 ± 0.28	292 ± 4	0.38 ± 0.00

[a] Mean of three measurements ± standard deviation.
[b] Mw, weight-average molar mass; Mn, number-average molar mass; R_z, *z*-averaged radius of gyration; Mn/Mw, polydispersity; ν_z, slope of Mw versus R_z.

Hizukuri (1996). The native *wxwx* was composed of smaller proportions of chains with DP 13–36 but larger proportions of chains with DP 6–12 and >36, consequently a slightly higher average chain length when compared with the *duwx* at the native form. When β-amylolysis progressed, a group of chains with DP 2–5 appeared, the proportions of chains with DP >12 steadily decreased, and the average chain length also gradually decreased for both starches. The group of chains with DP 2–5 that not originally present in native starch was a result of chain shortening from β-amylolysis. Although the chain distribution changed during β-amylolysis, both *wxwx* and *duwx* still displayed different chain-length distribution patterns after various degrees of β-amylolysis.

3.3. Molecular size and structural characteristics of amylopectin

The molecular size and structural characteristics of native and β-amylase-treated samples as analyzed by HPSEC-MALLS-RI are summarized in Table 3. The percent dissolution reached for the samples prepared were approximately 82.0–97.0%, similar to those reported by Rolland-Sabaté, Colonna, Mendez-Montealvo, and Planchot (2007). The molecular size and structural characteristics govern the physicochemical properties such as viscosity, gelatinization and retrogradation behavior, and pasting properties of starch (Jane & Chen, 1992; Jane et al., 1999). Both untreated *wxwx* and *duwx* had similar polydispersity (Mn/Mw) and molar mass (Mw), but the *duwx* had a smaller *z*-averaged radius of gyration (R_z), indicating a slightly more branched structure of the *duwx*, agreeing with HPSEC results (Table 1). The Mw and R_z of *wxwx* were similar to those reported by Buléon, Colonna, Planchot, and Ball (1998), Mua and Jackson (1997), Morrison and Karkalas (1990), and Rolland-Sabaté et al. (2007). With increasing β-amylolysis, there was no change in Mn/Mw and ν_z, but a slight decrease in R_z and Mw for both starches.

The dependency of the molar mass with radius of gyration can be described using the power law relationship (Eq. (1)) (Hanselmann, Burchard, Ehrat, & Widmer, 1996):

$$R_{zi} = K_z M_i^{\nu_z} \quad (1)$$

where M_i and R_i are the molar mass and the radius of gyration of component *i* of a series of particles of the same architecture but with different molar masses (Roger, Tran, Lesec, & Colonna, 1996). When plotting R_z versus molar mass, structural information may be obtained by the exponent ν_z value from Eq. (1). The values of ν_z are affected by polymer shape, the temperature, and the interaction of polymer and dissolvent, with $\nu_z = 0.33$ for a sphere, $\nu_z = 0.5$–0.6 for a random coil, and $\nu_z = 1$ for a rod (Hanselmann et al., 1996). The values of the native and hydrolyzed *wxwx* and *duwx* were close to 0.33, indicating that both amylopectin molecules, when solubilized, were present in the spherical shape and still maintained this shape after the various degrees of chains shortening from β-amylolysis in the granular state.

3.4. Thermal properties

The gelatinization and retrogradation properties of native and β-amylase-treated *wxwx* and *duwx* as measured by DSC are presented in Table 4. For gelatinization, the *wxwx* had a higher T_o and T_p, a smaller gelatinization range (T_c–T_o), and a slightly larger ΔH when compared with the *duwx*. The gelatinization temperature of *wxwx* and *duwx* seems to be influenced by the inbred line. Yuan et al. (1993) reported that *wxwx* had a higher T_o than *duwx* in Ia5125 line, but the opposite was observed in W64A line. There were no changes in T_p, T_c, and gelatinization range during the initial stages of hydrolysis until 5% hydrolysis for the *wxwx* and 8.5% for the *duwx*. The results suggest that there was a greater proportion of amylopectin chains present in the amorphous regions and accessible to β-amylase in the *duwx* than in the *wxwx*, which agrees with the previous finding that the *duwx* was consistently hydrolyzed to a greater degree than the *wxwx* (Fig. 1). The greater proportion of chains present in the amorphous regions of the *duwx* could also explain its lower gelatinization temperature at the native state when compared with the *wxwx*, even though the *wxwx* had a greater proportion of A chains of DP 6–12 than the *duwx*. The ΔH of the *wxwx* slightly decreased after 3.5% hydrolysis, but that of the *duwx* remained relatively unchanged during the β-amylolysis. Although amylopectin chains were gradually shortened during β-amylolysis, the order structure was mostly unaffected during the hydrolysis as shown by the little change in ΔH.

Table 4
Gelatinization and retrogradation temperatures and enthalpies of *wxwx* and *duwx* after hydrolysis by β-amylase to varying degrees.[a]

Hydrolysis (%)	Gelatinization					Retrogradation					
	T_o[b] (°C)	T_p (°C)	T_c (°C)	T_c–T_o (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	T_c–T_o (°C)	ΔH (J/g)	Retrogradation degree (%)
wxwx											
0	67.9 ± 0.1	72.2 ± 0.1	78.2 ± 0.4	10.2 ± 0.4	13.7 ± 0.3	42.7 ± 0.4	53.0 ± 0.4	63.5 ± 0.8	20.9 ± 0.4	7.6 ± 0.5	53.1 ± 1.8
1.5	68.3 ± 0.2	72.5 ± 0.2	78.2 ± 0.0	9.9 ± 0.2	13.3 ± 0.2	41.9 ± 0.2	53.5 ± 0.1	63.5 ± 0.2	21.6 ± 0.2	7.9 ± 0.4	61.3 ± 1.3
3.5	68.1 ± 0.1	72.5 ± 0.2	78.1 ± 0.1	10.0 ± 0.0	11.7 ± 0.4	44.2 ± 0.2	54.4 ± 0.2	63.5 ± 0.0	19.5 ± 0.1	7.3 ± 0.0	63.6 ± 1.6
5.0	66.3 ± 0.2	72.4 ± 0.2	79.0 ± 0.5	12.7 ± 0.4	12.2 ± 0.2	44.9 ± 0.4	54.8 ± 0.1	64.0 ± 0.1	19.1 ± 0.3	7.9 ± 0.4	64.5 ± 3.5
duwx											
0	66.0 ± 0.4	71.3 ± 0.5	77.8 ± 1.4	11.8 ± 1.0	12.7 ± 0.5	42.1 ± 0.1	52.5 ± 0.3	63.1 ± 0.7	21.0 ± 0.6	8.5 ± 0.4	70.2 ± 2.4
3.5	66.3 ± 0.2	71.4 ± 0.4	78.1 ± 0.3	11.8 ± 0.1	12.0 ± 0.2	42.5 ± 0.6	53.1 ± 0.3	63.1 ± 0.2	20.6 ± 0.5	8.8 ± 0.2	73.2 ± 0.9
5.5	66.7 ± 0.7	72.1 ± 0.1	77.4 ± 0.4	10.8 ± 0.6	11.5 ± 0.1	43.7 ± 0.3	53.7 ± 0.4	63.3 ± 0.3	19.5 ± 0.1	9.2 ± 0.1	80.2 ± 1.1
8.5	64.5 ± 0.6	71.6 ± 0.3	79.9 ± 0.3	15.4 ± 0.8	12.3 ± 0.1	45.7 ± 0.5	54.3 ± 0.4	63.5 ± 0.2	17.7 ± 0.3	8.2 ± 0.1	64.0 ± 0.2

[a] Mean of three measurements ± standard deviation.
[b] T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; T_c–T_o, temperature range; ΔH, enthalpy; retrogradation degree, retrogradation ΔH/gelatinization ΔH.

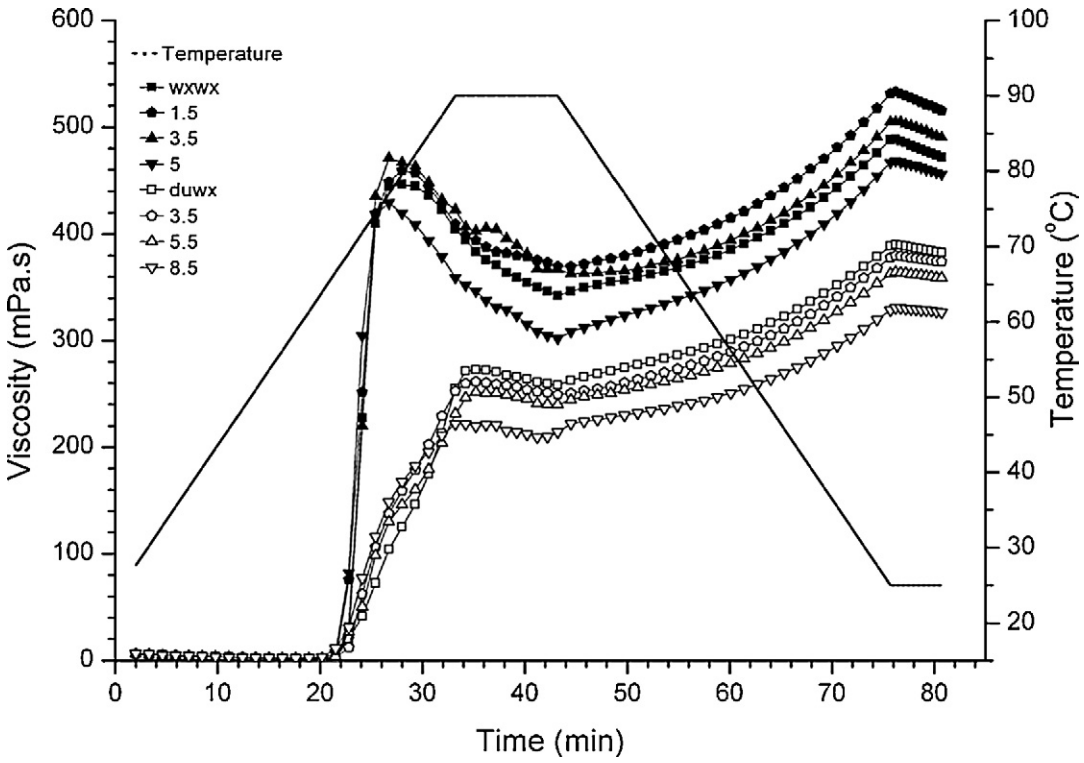

Fig. 2. Pasting profiles of *wxwx* (filled symbols), and *duwx* (open symbols) after hydrolysis by β-amylase to various degrees. The numbers indicates the percent degree of hydrolysis.

For retrogradation properties, the *duwx* generally had a slightly higher ΔH and retrogradation degree than the *wxwx* for both native and hydrolyzed samples, except the 8.5% hydrolysis. The retrogradation degree is the ratio of retrogradation enthalpy to gelatinization enthalpy for the same sample. Yuan et al. (1993) reported 83–90% of retrogradation degree in the *duwx* and 47–73% retrogradation degree in the *wxwx* for 30% starch concentration and stored for 7 days. Shi and Seib (1995) also reported a similar trend of the *duwx* having a greater retrogradation enthalpy than the *wxwx*. The greater retrogradation degree of the *duwx* was attributed to its larger proportion of chains with DP 13–36, which was similar to the findings by Shi and Seib (1995). Both starches showed an increase in retrogradation T_o and a decrease in retrogradation range (T_c–T_o) at 3.5% hydrolysis for the *wxwx* and at 5.5% hydrolysis for the *duwx*. The decrease in amylopectin chain length had little impact on retrogradation enthalpy, and the increase in retrogradation degree of hydrolyzed samples was mostly due to their decrease in the gelatinization enthalpy.

3.5. Rheological properties

The pasting profiles of *wxwx* and *duwx* as measured by a dynamic rheometer using rotational tests are displayed in Fig. 2. The *wxwx* had higher peak, greater breakdown and higher final viscosity values than the *duwx*. Although the two waxy mutant starches shared a similar Mw (Table 3), the *wxwx* had a greater proportion of long B chains (Fr. I, Table 1), a less branching structure, and a slightly large R_z value than the *duwx*, which might contribute its significantly higher viscosity. The greater proportion of long B chains and longer average chain length in the *wxwx* might provide a stronger association of the amylopectin molecules with each other to better preserve granule integrity, thus producing higher overall viscosity profiles (Asaoka, Okuno, Sugimoto, & Fuwa, 1985; Asaoka, Okuno, Sugimoto, Kawakami, & Fuwa, 1984; Sasaki & Matsuki, 1998; Tester & Karkalas, 2001). The slower swelling of the *duwx* was speculated to be caused by its larger proportion of chains with DP 13–36, which constitute the crystalline structure of the granular starch and consequently delay the onset of swelling. Furthermore, the more branched structure and the slightly smaller R_z might be responsible for the lower overall viscosity profile of the *duwx*.

When β-amylolysis progressed, both starches did not show evident decreases in pasting viscosity until 5% hydrolysis for the *wxwx* and 8.5% hydrolysis for the *duwx*. Moreover, the overall pasting profiles of the *wxwx* were still higher than those of the *duwx*. In addition, the changes in pasting profile coincided with the changes in onset gelatinization temperature where no changes were noted until 5% hydrolysis for the *wxwx* and 8.5% for the *duwx*. It is noted that although the *wxwx* at 5% hydrolysis had a lower Mw than was the native *duwx*, the *wxwx* at 5% hydrolysis still had a higher viscosity profile than did the native *duwx*. Tester and Morrison (1990) hypothesized that crystallites within the amylopectin molecule determine the onset of swelling and gelatinization, but maximum swelling factors may relate to the molar mass and shape of the whole amylopectin molecule. These results support their hypothesis and further suggest that amylopectin Mw is not the primary determinant for the viscosity development in waxy maize starch. The proportion of long chains and R_z may play more important roles in determining the viscosity profile of waxy maize starch.

4. Conclusions

Although both *wxwx* and *duwx* are composed of only amylopectin, they show district thermal and rheological properties at both their native states and after β-amylolysis because of their differences in structural characteristics. During the course of β-amylolysis, the *duwx* was hydrolyzed consistently to a greater extent than was the *wxwx*, which was attributed to a greater proportion of amylopectin chains present in the amorphous regions in the *duwx*. While both waxy mutant starches showed steady decreases in molecular sizes, chain length, Mw, and R_z during

β-amylolysis, their onset gelatinization temperature and pasting viscosity did not significantly decrease until 5% hydrolysis for the *wxwx* and 8.5% for the *duwx*. The results suggest that a greater proportion of amylopectin long B chains would allow for more interactions among molecules, thus providing a stronger network structure and consequently a high viscosity profile. The amylopectin Mw was not as important in determining the pasting properties of waxy maize starch.

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