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

Guadalupe Mendez-Montealvo [a], Ya-Jane Wang [a,*], Mark Campbell [b]

[a] Department of Food Science, University of Arkansas, Fayetteville, AR, USA
[b] Department of Agricultural Science, Truman State University, Kirksville, MO, USA

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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.
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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

* Corresponding author at: Department of Food Science, 2650 N. Young Avenue, University of Arkansas, Fayetteville, AR 72704, USA. Tel.: +1 479 575 3871; fax: +1 479 575 6936.
E-mail address: yjwang@uark.edu (Y.-J. Wang).

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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.

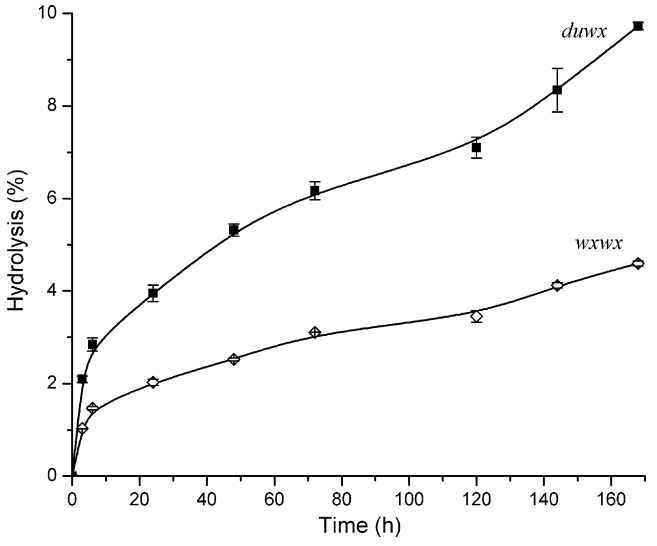

Fig. 1. Percent hydrolysis of *wxwx* (◊) and *duwx* (■) by isoamylase over a 168-h period.

Table 1
Fine structure of debranched *wxwx* and *duwx* as resolved by HPSEC–RI[a] after hydrolysis by isoamylase to varying degrees.[b]

Hydrolysis (%)	Fr. I[c] (%)	Fr. II[c] (%)	Fr. II/Fr. I
wxwx			
0	20.7 ± 0.5	79.3 ± 0.5	3.8 ± 0.1
1.0	20.9 ± 0.1	79.1 ± 0.1	3.8 ± 0.0
2.0	19.8 ± 0.1	80.2 ± 0.1	4.1 ± 0.0
3.0	21.3 ± 0.3	78.7 ± 0.3	3.7 ± 0.1
4.0	20.0 ± 0.0	80.0 ± 0.0	4.0 ± 0.0
duwx			
0	16.3 ± 0.2	83.7 ± 0.2	5.1 ± 0.1
2.0	15.9 ± 0.1	84.1 ± 0.1	5.3 ± 0.0
4.0	16.2 ± 0.2	83.8 ± 0.2	5.2 ± 0.1
6.5	16.1 ± 0.1	83.9 ± 0.1	5.2 ± 0.1
9.5	16.0 ± 0.0	84.0 ± 0.0	5.2 ± 0.0

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

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

Hydrolysis (%)	M_w/M_n[b]	M_w ($E+08$) (g/mol)	R_z (nm)	ν_z
wxwx				
0	2.22 ± 0.10	3.66 ± 0.15	324 ± 6	0.36 ± 0.00
1.0	2.10 ± 0.10	3.04 ± 0.36	303 ± 1	0.37 ± 0.01
2.0	2.16 ± 0.04	2.75 ± 0.08	307 ± 2	0.38 ± 0.00
3.0	2.15 ± 0.00	3.15 ± 0.04	307 ± 0	0.37 ± 0.01
4.0	2.17 ± 0.07	2.46 ± 0.10	296 ± 3	0.38 ± 0.01
duwx				
0	2.11 ± 0.11	3.64 ± 0.30	305 ± 2	0.37 ± 0.01
2.0	2.02 ± 0.08	3.45 ± 0.19	293 ± 5	0.38 ± 0.01
4.0	2.12 ± 0.01	3.54 ± 0.16	296 ± 4	0.38 ± 0.00
6.5	1.92 ± 0.17	2.87 ± 0.23	282 ± 1	0.40 ± 0.00
9.5	1.96 ± 0.18	2.58 ± 0.22	275 ± 5	0.38 ± 0.01

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

The initial more rapid hydrolysis was attributed to the presence of damaged starch, which facilitated the penetration of isoamylase into starch granules. However, the greater hydrolysis (2–5.5%) of the *duwx* could not be solely ascribed to its higher damaged starch content. A similar trend was also observed when both starches were subjected to hydrolysis by β-amylase with the *duwx* showing a greater β-amylolysis degree (Mendez-Montealvo et al., 2010). Unlike β-amylase hydrolysis that leveled off after approximately 72 h under the conditions studied (Mendez-Montealvo et al., 2010), the hydrolysis by isoamylase in the present study showed a continuous increase for both waxy maize starches.

Isoamylase is an endo-acting enzyme and requires the binding of two starch chains to hydrolyze the α-(1 → 6) branch linkages. Although being a relative large molecule (M_r = 86,000 Da), isoamylase does penetrate into starch granules and hydrolyze branch chains to significant extents (Kimura & Robyt, 1996). The greater extent of hydrolysis of the *duwx* suggests that the *duwx* has more branching points and/or has more branching points located in the amorphous regions than the *wxwx*. Therefore, the structural differences between the two starches were responsible for their differences in the enzyme accessibility and hydrolysis kinetics.

3.2. Fine structure and chain-length distributions of debranched amylopectin

The fine structure and chain-length distributions of both isoamylase-treated waxy maize mutant starches are listed in Tables 1 and 2, respectively. The differences in structural characteristics of both native waxy maize mutants have been discussed in our previous work (Mendez-Montealvo et al., 2010). The isoamylase hydrolysis did not change any of these distributions, which reflected the purity of isoamylase used in this study and confirmed that the hydrolysis results (Fig. 1) were mainly from isoamylase. The *duwx* had a greater proportion of Fr. II (amylopectin A and short B chains), a higher ratio of Fr. II/Fr. I, i.e. higher degree of branching, and greater proportions of chains with DP (degree of polymerization) 13–36 when compared with the *wxwx*. The higher degree of branching implies that more α-1–6 linkages were present in the *duwx* for isoamylase to attack, thus supporting previous hydrolysis results that the *duwx* was hydrolyzed to a greater extent than the *wxwx*. In addition, no change in the ratio of Fr. II/Fr. I after the hydrolysis by isoamylase for both starches suggests that the branching points of different chains were evenly distributed in the amorphous regions.

3.3. Molecular size and structural characteristics of amylopectin

The molecular size and structural characteristics of native and isoamylase-treated samples as analyzed by HPSEC–MALLS–RI are summarized in Table 3. The percent dissolution achieved for the samples prepared was approximately 82.0–95.0%, similar to those reported by Rolland-Sabaté, Colonna, Mendez-Montealvo, and Planchot (2007). It is known that 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 native *wxwx* and *duwx* shared a similar polydispersity (M_w/M_n) and molar mass (M_w), but the *duwx*

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

Hydrolysis (%)	Average chain length	Percent distribution (%)			
		DP[b] 6–12 (A chains)	DP 13–24 (B1 chains)	DP 25–36 (B2 chains)	DP 37+ (B3+ chains)
wxwx					
0	20.9 ± 0.0	22.4 ± 0.1	50.0 ± 0.1	17.2 ± 0.1	10.4 ± 0.1
1.0	21.1 ± 0.1	21.9 ± 0.1	49.7 ± 0.2	17.6 ± 0.2	10.8 ± 0.2
2.0	21.1 ± 0.1	21.9 ± 0.1	49.6 ± 0.2	17.6 ± 0.1	10.9 ± 0.2
3.0	20.9 ± 0.2	21.9 ± 0.0	50.4 ± 0.5	17.4 ± 0.0	10.2 ± 0.6
4.0	20.8 ± 0.1	22.8 ± 0.2	49.8 ± 0.1	17.1 ± 0.3	10.3 ± 0.0
duwx					
0	20.5 ± 0.0	21.6 ± 0.1	51.4 ± 0.0	19.0 ± 0.1	8.0 ± 0.1
2.0	20.5 ± 0.0	21.5 ± 0.0	51.5 ± 0.1	19.0 ± 0.1	8.0 ± 0.1
4.0	20.5 ± 0.0	21.5 ± 0.1	51.6 ± 0.1	19.0 ± 0.1	8.0 ± 0.1
6.5	20.5 ± 0.0	21.7 ± 0.0	51.6 ± 0.2	18.8 ± 0.0	7.9 ± 0.2
9.5	20.5 ± 0.1	21.6 ± 0.1	51.5 ± 0.3	18.8 ± 0.2	8.1 ± 0.2

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

Table 4
Gelatinization and retrogradation properties of *wxwx* and *duwx* after hydrolysis by isoamylase 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	67.8 ± 0.2	71.9 ± 0.2	77.8 ± 0.2	10.0 ± 0.1	13.3 ± 0.3	41.0 ± 0.3	52.7 ± 0.4	61.9 ± 0.1	20.9 ± 0.3	7.0 ± 0.2	52.2 ± 2.2
1.0	68.4 ± 0.1	72.3 ± 0.2	77.6 ± 0.3	9.3 ± 0.3	12.9 ± 0.5	41.8 ± 0.2	53.5 ± 0.2	62.5 ± 0.5	20.7 ± 0.6	7.9 ± 0.4	61.0 ± 2.4
2.0	68.7 ± 0.2	72.5 ± 0.2	77.8 ± 0.4	9.1 ± 0.3	13.4 ± 0.7	42.8 ± 0.4	54.0 ± 0.2	62.9 ± 0.1	20.1 ± 0.3	8.1 ± 0.2	60.6 ± 2.2
3.0	68.6 ± 0.2	72.8 ± 0.2	78.7 ± 0.5	10.1 ± 0.4	13.7 ± 0.8	43.4 ± 0.4	54.1 ± 0.0	63.4 ± 0.4	20.0 ± 0.1	9.0 ± 0.1	65.8 ± 2.8
4.0	66.8 ± 0.3	72.6 ± 0.3	79.2 ± 0.3	12.4 ± 0.5	13.8 ± 0.7	44.0 ± 0.2	54.3 ± 0.0	63.5 ± 0.3	19.5 ± 0.5	9.0 ± 0.4	65.5 ± 1.4
duwx											
0	66.3 ± 0.3	71.1 ± 0.2	77.8 ± 0.2	11.5 ± 0.2	12.2 ± 0.5	40.7 ± 0.7	50.3 ± 0.2	61.8 ± 0.3	21.1 ± 0.7	8.9 ± 0.1	72.7 ± 2.5
2.0	66.6 ± 0.4	71.4 ± 0.3	78.0 ± 0.3	11.4 ± 0.1	12.5 ± 0.4	41.3 ± 0.2	51.9 ± 0.5	62.7 ± 0.1	21.4 ± 0.2	10.4 ± 0.2	82.8 ± 1.0
4.0	67.3 ± 0.2	71.7 ± 0.1	77.7 ± 0.2	10.5 ± 0.0	12.5 ± 0.1	42.5 ± 0.1	52.6 ± 0.3	62.5 ± 0.1	20.0 ± 0.1	10.2 ± 0.1	81.5 ± 0.9
6.5	68.0 ± 0.2	72.5 ± 0.1	78.3 ± 0.2	10.3 ± 0.4	12.2 ± 0.1	44.1 ± 0.4	54.1 ± 0.0	63.5 ± 0.1	19.4 ± 0.5	10.2 ± 0.3	83.6 ± 1.8
9.5	67.5 ± 0.0	72.5 ± 0.3	79.5 ± 0.4	11.9 ± 0.4	12.1 ± 0.4	45.1 ± 0.0	54.4 ± 0.3	64.0 ± 0.3	18.9 ± 0.3	10.5 ± 0.1	86.9 ± 2.4

[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 (%) = 100 × (retrogradation ΔH/gelatinization ΔH).

had a smaller *z*-averaged radius of gyration (R_z), thus a slightly more branched structure, agreeing with HPSEC results (Table 1). The M_w and R_z of both *duwx* and *wxwx* progressively decreased with increasing hydrolysis by isoamylase, whereas their M_w/M_n and ν_z remained relatively unchanged. The ν_z values of the native and isoamylase-treated *wxwx* and *duwx* were close to 0.33, indicating that both amylopectin molecules, when solubilized, were present in the spherical shape (Hanselmann, Burchard, Ehrat, & Widmer, 1996) and still maintained this shape after the various degrees of debranching from isoamylase in the granular state.

When the decrease in M_w and R_z of isoamylase-treated waxy starches relative to their respective native one were compared at the same hydrolysis degree, the decreases were significantly greater for the *wxwx* than for the *duwx*. For example, at 4% hydrolysis, the M_w and R_z of the *wxwx* significantly decreased from 3.66×10^8 g/mol and 324 nm at its native state to 2.46×10^8 g/mol and 296 nm, respectively, whereas the *duwx* showed no change in M_w and only a slight decrease in R_z. It is suspected that because of its more branching structure, the *duwx* was capable of accommodating a greater degree of debranching without changing its M_w and R_z. Nevertheless, the M_w and R_z of the *duwx* significantly decreased when the hydrolysis reached and exceeded 6.5%.

3.4. Thermal properties

Table 4 lists the gelatinization and retrogradation properties of native and isoamylase-treated *wxwx* and *duwx*. For gelatinization, the *wxwx* had a higher T_o, T_p, and ΔH, and a smaller gelatinization range ($T_c - T_o$) than the *duwx*. With increasing hydrolysis by isoamylase, both starches generally showed an increase in T_o, T_p, and T_c, but little change in ΔH. A significant increase in T_o was noted for the *wxwx* at 1% hydrolysis, and for the *duwx* at 4% hydrolysis. A similar trend was also noted in the changes of structural characteristics (Table 3), where the *wxwx* showed a significant decrease in M_w and R_z at 1% hydrolysis, but the *duwx* showed no change in M_w until 6.5% hydrolysis and no change in R_z until 2% hydrolysis. Again, the more branching nature of the *duwx* probably allowed for a greater degree of debranching without affecting its gelatinization temperature. In contrast, our previous study (Mendez-Montealvo et al., 2010) found that the T_o and T_p of both *wxwx* and *duwx* generally remained unchanged or decreased after β-amylase hydrolysis. These results demonstrate that hydrolysis in the amorphous lamellae affects starch gelatinization temperature, but the changes are affected by the type of hydrolysis. At low degrees of hydrolysis, shortening amylopectin chains as in β-amylase hydrolyzed samples seemed to have no impact or slightly decreased their T_o and T_p, whereas debranching as in isoamylase hydrolyzed samples slightly increased their T_o and T_p. The shorter branches as in β-amylase hydrolysis probably help destabilize the crystalline structure to result in lower gelatinization temperature, whereas the less branching structure as in isoamylase hydrolysis probably is a more stable structure.

For retrogradation properties, both starches showed a significant increase in T_o, T_p, T_c, ΔH and retrogradation degree with increasing hydrolysis by isoamylase. With increasing hydrolysis by isoamylase, both starches became less branched, which promoted the re-association of amylopectin chains and consequent formation of crystalline structures after the starch was gelatinized. The retrogradation enthalpy values of debranched *wxwx* and *duwx* was generally greater than those of β-amylase-treated ones as reported in our previous study (Mendez-Montealvo et al., 2010).

3.5. Rheological properties

The pasting profiles obtained by a dynamic rheometer using rotational tests of native and isoamylase-debranched *wxwx* and *duwx* are displayed in Fig. 2. The *wxwx* had higher peak, greater breakdown and higher final viscosity values than the *duwx*. Although the two mutant starches shared a similar M_w (Table 3), the *wxwx* had a greater proportion of long B chains (Fr. I, Table 1), a lower degree of branching, and a slightly large R_z value compared

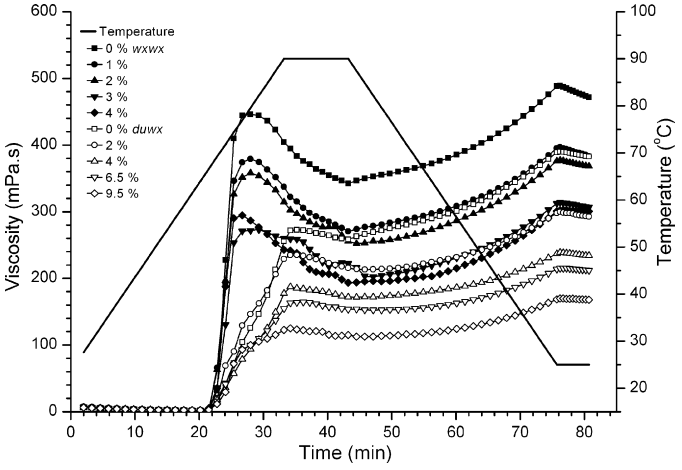

Fig. 2. Pasting profiles of *wxwx* (filled symbols) and *duwx* (open symbols) after hydrolysis by isoamylase to varying degrees. The number indicates the percent degree of hydrolysis.

with the *duwx*, which might contribute its significantly higher viscosity.

When hydrolysis by isoamylase progressed, both starches showed significant decreases in pasting viscosity. During the holding and cooling stages, the pasting profiles of the *wxwx* at 1% and 2% hydrolysis were similar to that of native *duwx*, and the pasting profiles of the *wxwx* at 3% and 4% hydrolysis were similar to that of the *duwx* at 2% hydrolysis. We noted that the *wxwx* at 1–2% hydrolysis had a lower M_w but a similar R_z when compared with native *duwx*, and the *wxwx* at 3–4% hydrolysis also had a lower M_w but a similar R_z when compared with the *duwx* at 2% hydrolysis. Therefore, the present study supports our previous findings (Mendez-Montealvo et al., 2010) that amylopectin M_w is not the primary determinant for the viscosity profile, and R_z may play a more important role in determining the viscosity profile of waxy maize starch. Although R_z value is derived from individual amylopectin molecules, and the pasting viscosity is obtained from granular starch, it is proposed that amylopectin molecules with larger R_z would be able to have stronger interactions with other amylopectin molecules, thus resulting in a higher viscosity profile. It was proposed that the formation of a semi-permeable membrane-like surface structure during gelatinization and swelling is a result of molecule entanglement after gelatinization (Wang, Kuo, Wang, & Patindol, 2007). Therefore, stronger interactions among amylopectin molecules allow for higher viscosity profiles for waxy corn starch.

Tester and Morrison (1990b) reported that crystallites within the amylopectin molecule determined the onset of swelling and gelatinization, but maximum swelling factors may relate to the molar mass and shape of the whole amylopectin molecule. The present results suggest that amylopectin shape, as represented by R_z, was the primary determinant for the extent of viscosity development in waxy maize starch. The branching degree of amylopectin had a strong impact on R_z and consequent pasting viscosity of waxy starch. The importance of branching degree is also supported by a greater extent of decrease in pasting viscosity of debranched waxy starch than β-amylase-treated one at the same degree of hydrolysis (Mendez-Montealvo et al., 2010).

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 isoamylase hydrolysis. The *duwx* was consistently hydrolyzed by isoamylase to a greater extent than was the *wxwx*, which was attributed to a higher degree of branching present in the amorphous lamellae in the *duwx*. Isoamylase-treated *wxwx* and *duwx* generally displayed slightly higher gelatinization temperatures, retrogradation enthalpy and retrogradation degree than their respective native ones. The amylopectin branching structure in waxy starches helped to destabilize the crystalline structure during gelatinization and hinder reassociation upon cooling. Therefore, when amylopectin became less branched the gelatinization temperature and the retrogradation tended to increase. The M_w, R_z, and pasting viscosity of both waxy starches steadily decreased with increasing hydrolysis by isoamylase. When the *wxwx* and *duwx* had a similar R_z, regardless of the degree of hydrolysis, they displayed similar pasting profiles during holding and cooling stages. These results suggest that the pasting viscosity of waxy maize starch is primarily governed by amylopectin R_z than by M_w and/or long B chains among amylopectin structural characteristics. Any modification resulting in significant changes in R_z would have a strong impact on the pasting properties of waxy starch.

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