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Genotype diversity in structure of amylopectin of waxy rice and its influence on gelatinization properties

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ABSTRACT

A set of 13 waxy rice genotypes prepared by chemically-induced mutation of non-waxy rice variety TNG67 and 7 waxy rice varieties widely planted in Taiwan were screened for various structural and gelatinization properties of starches. Wide variation on physicochemical properties and molecular structure of amylopectin for the 20 waxy rice starches were obtained and relationship between gelatinization properties and molecular structure of starch were discussed. More attributes on swelling and gelatinization thermal properties, comparing to pasting attributes, showed significant correlation with molecular structure parameters. The swelling and gelatinization thermal properties of waxy rice starch did not show significant correlation with molecular size of amylopectin, while significant correlations were found between the swelling or gelatinization thermal properties and chain length of amylopectin. Results suggest that the swelling and pasting of waxy starch is essentially dominated by granule architecture and is dependent on the interactions among amylopectin chains.

1. Introduction

Starch is the main component of cereal crops such as rice, wheat, corn, sorghum and barley. The cooking quality of the cereal grains is largely determined by physicochemical properties of starch. Eating and cooking quality of rice has been related to the amylose content (Juliano, Onate, & Del Mundo, 1965) and the fine structure of amylopectin (Ong & Blanshard, 1995).

Tester and Morrison (1990a) indicated that starch swelling is considered a property of amylopectin and amylose acts as a diluent. Jane et al. (1999) investigated 13 non-waxy starches from different botanical sources to illustrate the effect of amylopectin branch chain length on gelatinization and pasting properties of starch. They reported that the very long branch-chains in amylopectin behaved similar to amylose and restricted starch swelling. Study on 12 non-waxy wheat starch samples found that larger proportions of amylopectin long chains (degree of polymerization, DP ≥ 35) contributed to the swelling of starch (Sasaki & Matsuki, 1998). Han and Hamaker (2001) selected 10 rice starches with a fairly narrow range of amylose content (15.1–17.9%) to study a possible relationship between amylopectin fine structure and RVA paste viscosity parameters. They found a negative relationship between proportion of long chains in amylopectin and paste breakdown. Result from waxy and non-waxy rice starches showed that amylopectin branch chains of DP 12–22 led to decrease swelling power, but no significant correlations between amylopectin chain-length distribution and peak, breakdown, setback and final viscosities were found. Higher swelling power for waxy rice starches than normal rice starches at the temperature range between 55 and 85 °C were observed (Vandeputte, Derycke, Geeroms, & Delcour, 2003a). The authors also indicated that proportion of short amylopectin chains (DP 6–9) had positive impact in raising swelling between 55 and 85 °C for normal and waxy rice starches. Cai and Shi (2010) reported that debranched waxy potato starch had a longer average chain length than both debranched waxy wheat and waxy maize starches. Rolland-Sabaté et al. (2012) concluded that waxy cassava starch exhibited similar molecular weight and gyration radius of amylopectin to waxy maize starch, while waxy potato starch showed lower molecular size and longer average chain length comparing to those of waxy cassava and waxy maize starches. However, both waxy maize and waxy potato starches exhibited higher gelatinization onset and peak temperatures than those of waxy cassava starch. Ji, Ao, Han, Jane, and BeMiller (2004) proposed that the differences in thermal properties among waxy maize starch granules could be attributed to the structure of amylopectin. Waxy maize starch granules gelatinized at high temperature (>74 °C) had lower concentration of A chain (DP 6–12) than that of starch granules gelatinized at relative lower temperature (<74 °C). It was

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found that the results on literatures that mention above were not consistent, and this could be attributed to the influencing factors, such as granule size, crystalline structure, presence of amylose and amylose content, of starch granules, which are too complicated to tell the effect of one factor from another.

Qi, Tester, Snape, and Ansell (2003) indicated that a way to tease out the role of amylopectin on gelatinization characteristics starch granules is to utilize waxy starches. However, rare result could be found in literatures due to lack of source of waxy starch, especially from the same species. Thus, most literatures are focus on non-waxy starches, waxy and non-waxy starches or starches from different botanical sources systems. Waxy rice starches contain little amylose, lipid and phosphorylation, and provide simplistic system for probing the molecular basis of starch gelatinization properties than other starches (Qi et al., 2003). To the best of our knowledge, literature review indicates that a few studies exist on investigation of relationship among pasting, thermal properties and molecular weights of amylopectin for waxy rice starches. Previous studies (Qi et al., 2003; Tester & Morrison, 1990b; Vandeputte et al., 2003a; Vandeputte, Derycke, Geeroms, & Delcour, 2003b; Wang & Wang, 2002), involving small number of waxy rice genotypes, were focused on relationship between chain lengths or DP of amylopectin and other attributes of waxy rice starches. The present investigation involved starches from large number of waxy rice genotypes including 13 mutants and 7 widely grown named varieties. The starches were almost pure amylopectin as no measurable iodine affinity was detected hence no influence of amylose–lipid complexes could be observed.

Rice variety Tainung 67 (TNG67) has been widely planted in Taiwan. Generally, TNG67 rice starch exhibited amylose content around 20%. A mutation pool of TNG67 mutants was established by treating with NaN_3 (Jeng, Wang, Tseng, Chen, & Sung, 2003), and the responses of starch biosynthesizing enzymes to grain growth had been investigated (Jeng, Wang, Tseng, & Sung, 2007). The aim of this study was focused at assessing the difference in physicochemical properties between mutant genotypes, NaN_3-induced from variety TNG67, and named varieties by measuring various physicochemical, thermal and pasting properties of starches. The viability of starches isolated from the 13 mutant genotypes for food industry utilization was studied by comparison with starches from the named varieties. Furthermore, Pearson correlation was applied to understand the relationship between the molecular structures of amylopectin and various characteristics of starch cooking behavior.

2. Materials and methods

2.1. Materials

Seven waxy rice genotypes namely BW, ESW, TCSW1, TCW70, TKW1, TKW5 and TSW2 were widely planted varieties in Taiwan. The other 13 waxy rice accessions, WR1–WR13, were NaN_3-induced mutants of the named variety TNG67. The waxy rice genotypes were obtained from Agricultural Research Institute of Taiwan, Wufong, Taichung, Taiwan. The 7 named genotypes were planted in different areas of Taiwan, and the 13 mutants were grown and harvested at Wufong under the same planting conditions. All reagents used were of analytical grade.

2.2. Methods

2.2.1. Isolation of starch

Starch isolation was done following a modified alkali steeping method proposed by Lu, Duh, Lin, and Chang (2008). Rice kernels were steeped and milled with 0.1% NaOH solution, and the slurry was diluted and stood for separating into three layers. The top and bottom impurity layers exhibited yellow color, and the middle layer was starch layer. The middle starch layer was siphoned out, while the top and bottom impurity layers were collected to repeat the processes of diluting, standing, and siphoning until the starch layer was clear. The siphoned starch layers were collected and centrifuged at 10,000 × *g* in a continuous phase centrifuge (T1A, Sharples, Warminster, PA, USA). The precipitate was suspended and neutralized with 0.1% HCl. The neutralized slurry was repeatedly washed and centrifuged by distilled water until the absence of NaCl (detecting by 1% $AgNO_3$) was found in supernatant. The precipitate was re-suspended in 95% ethanol and oven-dried at 40 °C for 24 h. Protein contents of starches isolated from the 20 rice genotypes were all below 0.2%. The starches were almost pure amylopectin as no measurable iodine affinity was detected hence no influence of amylose–lipid complexes could be observed.

2.2.2. Physicochemical properties

Size distribution of starch granule was determined by using a laser light scattering based particle size analyzer (Mastersizer Micro, Malvern Instruments, UK). Blue value and maximum absorbance (λ_{max}) of starches were determined according to the method proposed by Morrison and Laignelet (1983). Swelling factor (SF) of starches at temperatures of 55, 57.5, 60 and 62.5 °C was determined by the method as outlined in Tester and Morrison (1990b).

2.2.3. Chain length and β-amylolysis

Total carbohydrate and reducing terminal residues were determined by the phenol–sulfuric acid method and the modified Park–Johnson method, respectively (Mizukami, Takeda, & Hizukuri, 1999). The number-average chain length (CL) was calculated by dividing total carbohydrates by the reducing residues. β-Amylolysis limit was conducted and calculated as described by Suzuki, Hizukuri, and Takeda (1981). The external (ECL) and internal chain lengths (ICL) were calculated according to the following equations:

$$\mathrm{ECL} = \mathrm{CL} \times \beta\text{-amylolysis}\,(\%) + 2$$

$$\mathrm{ICL} = \mathrm{CL} - \mathrm{ECL} - 1$$

2.2.4. Molecular weight distribution

Weight percentage and weight-average degree of polymerization (DP_w) of starch were measured according to the procedures reported by Chang, Lin, and Chang (2006) using a high-performance size exclusion chromatography (HPSEC) system. The system consisted of an isocratic pump (G1310A series, Hewlett Packard, Wilmington, DE), a multi-angle laser light scattering (MALLS, model Dawn DSP, Wyatt Tech., Santa Barbara, CA) and a refractive index (RI) detectors (HP1047A). The columns used were PWH (guard column), G5000PW and G4000PW (TSK-Gel, Tosoh, Tokyo, Japan) columns connected in series and kept at 70 °C. The mobile phase was 100 mM $NaNO_3$ containing 0.02% NaN_3 and injected at flow rate of 0.5 ml/min.

2.2.5. Gelatinization thermal properties

Thermal properties of starch during heating were determined by using of a differential scanning calorimeter (DSC, model 2910, TA Instruments, Surrey, England). Starch sample (about 2.5 mg, db.) was weighed in the sample pan, mixed with distilled water (about 5.0 mg), and sealed. The sample pans were heated from 30 to 120 °C at a rate of 10 °C/min. Gelatinization temperatures of onset (T_o), peak (T_p), conclusion (T_c) and range ($R = T_c - T_o$), as well as, gelatinization enthalpy change (ΔH) were quantified.

2.2.6. Pasting properties

Pasting properties of starch were determined with Rapid Visco-Analyzer (RVA 3D+, Newport Scientific, Australia). Each starch suspension (8%, w/w, 28 g total weight) was equilibrated at 35 °C for 1 min, heated to 95 °C at a rate of 6 °C/min, maintained at 95 °C for 4 min, then cooled to 35 °C at a rate of 6 °C/min, and maintained at 35 °C for 5 min. Paddle speed was set at 960 rpm for the first 10 s and then 160 rpm for the rest of the analysis. Pasting parameters determined were peak viscosity (PV), hot paste viscosity (HPV), setback (SB), final viscosity (FV), breakdown (BD), pasting temperature (PT), breakdown ratio (BDr) and setback ratio (SBr). BDr (%) and SBr (%) are defined as the ratios of BD to PV and SB to HPV, respectively.

2.2.7. Statistical analysis

The data reported are averages of triplicate observations. Statistical analysis was conducted with Statistical Analysis System (SAS Institute, Cary, NC, USA) to perform Pearson correlation analysis between various parameters.

3. Results and discussion

3.1. Physicochemical properties

The 20 waxy rice starches in the present study showed granule size in the range from 2.57 μm (WR10) to 3.74 μm (TSW2) (Table 1). On an average, granule size of starches from the 7 named varieties (3.27 μm) was higher than that of starches from the 13 mutant genotypes (2.95 μm).

Waxy rice starches displayed blue value between 0.134 and 0.205 (Table 1), which corresponding to the undetectable of iodine affinity of the waxy rice starch samples. The maximum blue value was observed for variety BW and the minimum for mutant genotype WR12. Blue value for the waxy rice starches in the present study could be contributed by longer branch chains of amylopectin as confirmed from λ_{max} values exhibited by them as well as from significant correlation between the two parameters ($r^2 = 0.998$; $p < 0.005$).

The maximum starch-iodine absorbance (λ_{max}) for the 20 waxy rice starches ranged between 506 and 521 nm. The highest λ_{max} was shown by starch from variety BW and the lowest by mutant genotype WR12. On an average, the λ_{max} of starches from the 7 named varieties was observed at 516 nm whereas λ_{max} of starches from the 13 mutant genotypes was recorded at 512 nm. The λ_{max} of starch provides an idea about degree of polymerization and average chain length of amylose and amylopectin (Banks, Greenwood, & Khan, 1971).

Due to unable to separate waxy rice starch granules from suspension after heating at 65 °C or higher temperature, swelling factor (SF) of waxy rice starches was examined at 55–62.5 °C at intervals of 2.5 °C (Table 1). SF of starches determined at 55, 57.5, 60 and 62.5 °C ranged from 0.2 to 4.3, 2.0 to 8.0, 4.6 to 32.4 and 8.2 to 50.0, respectively (Table 1). SF of the 20 waxy starches increased with increasing temperature, especially for temperature at 60 and 62.5 °C. The difference in swelling factor between waxy rice starches measured at 60 and 62.5 °C were more profound than that measured at temperature below 60 °C. Among the 13 mutant genotype starches, WR1, WR9 and WR11 showed maximum extent of SF. These 3 mutant waxy rice starches exhibited obviously higher SF than that of starch from named varieties measured at 62.5 °C. This may be attributed to the difference in amylopectin chain structure, and reveals that the chemically-induced mutation could be used to enhance the diversity in molecular structure and characteristics of waxy rice starch.

3.2. Molecular structure of waxy rice starches

Waxy rice starches showed β-amylolysis limits between 47.9 and 54.4% (Table 2). The highest value of β-amylolysis limit was shown by variety TCSW1 and the lowest by mutant genotype WR2. β-Amylolysis limit was 54% for waxy rice amylopectin (Betroft, Zhu, Andtfolk, & Jungner, 1999), 56–58% for wheat amylopectin (Shibanuma, Takeda, & Hizukuri, 1996), 53–54% for barley amylopectin (Yoshimoto, Takenouchi, & Takeda, 2002) and 57% for amylopectin from lily starch (Takeda, Takeda, & Hizukuri, 1983). On an average, mutants showed lower value of β-amylolysis limit than that of named varieties, however, mutant genotype WR11 had higher β-amylolysis limit than other varieties except TSCW1.

The average chain length (CL) of starches varied between 13.0 and 15.1 (Table 2). The highest value of CL was shown by varieties

Table 1
Granule size, blue value, λ_{max} and swelling factor parameters of waxy rice starches.

Genotype	Granule size (μm)	Blue value	λ_{max} (nm)	Swelling factor			
				SF_{55} [a]	$SF_{57.5}$	SF_{60}	$SF_{62.5}$
BW	3.61	0.205	521	1.9	2.4	6.1	8.2
ESW	3.21	0.185	516	2.3	2.2	7.6	23.9
TCSW1	3.15	0.181	516	2.9	3.6	7.4	24.8
TCW70	3.01	0.194	519	1.9	2.0	5.4	14.4
TKW1	2.68	0.168	514	3.8	6.9	24.5	37.7
TKW5	3.49	0.164	512	4.2	6.0	18.5	38.9
TSW2	3.74	0.191	518	2.6	2.2	4.6	16.3
WR1	2.85	0.151	509	3.4	7.8	26.6	45.3
WR2	3.38	0.176	516	0.2	3.2	7.7	30.9
WR3	2.80	0.168	514	1.6	2.4	6.7	25.8
WR4	2.75	0.164	516	1.3	4.4	13.8	31.9
WR5	3.44	0.165	516	0.4	2.9	7.8	28.9
WR6	2.67	0.162	513	1.8	2.9	6.5	28.9
WR7	3.30	0.159	513	1.8	2.8	8.9	32.2
WR8	3.02	0.157	516	1.0	4.1	11.4	32.8
WR9	2.89	0.148	511	3.8	6.7	26.0	50.0
WR10	2.57	0.140	510	4.3	5.6	21.5	41.0
WR11	2.90	0.142	508	3.2	4.3	24.1	44.9
WR12	2.64	0.134	506	2.8	8.0	32.4	41.5
WR13	3.10	0.146	511	1.4	4.2	15.0	30.7
SD [b]	<0.01	<0.004	<1	<0.7	<0.8	<0.7	<0.8

[a] Swelling factor (SF) at 55, 57.5, 60 and 62.5 °C.
[b] Standard deviation.

Table 2
Molecular characteristics of waxy rice starches.

Genotype	β-Amylolysis (%)	CL[a]	ECL[a]	ICL[a]	DP_w[b] ($\times 10^3$)	Molecular weight distribution[b]			
						F1%	$F1DP_w$ ($\times 10^3$)	F2%	$F2DP_w$
BW	50.2	14.9	9.5	4.4	727	92.6	784	7.4	1666
ESW	52.4	14.4	9.5	3.9	689	94.3	731	5.7	1746
TCSW1	54.4	15.0	10.2	3.8	798	93.8	851	6.2	1870
TCW70	49.5	15.1	9.5	4.6	908	96.0	946	4.0	1506
TKW1	51.7	14.6	9.5	4.1	911	96.7	942	3.3	2302
TKW5	50.5	15.1	9.6	4.5	700	94.0	745	6.0	1809
TSW2	51.7	14.7	9.6	4.1	642	93.6	686	6.4	1368
WR1	49.4	14.4	9.1	4.3	745	97.2	767	2.8	2315
WR2	47.9	14.9	9.2	4.9	928	93.4	994	6.6	1988
WR3	48.4	14.0	8.8	4.2	643	92.7	693	7.3	806
WR4	48.6	14.6	9.1	4.5	721	94.5	763	5.5	1976
WR5	49.6	13.6	8.7	3.9	773	95.5	809	4.5	1740
WR6	52.4	14.1	9.4	3.7	715	96.7	740	3.3	813
WR7	48.9	14.2	8.9	4.3	757	94.7	799	5.3	1445
WR8	50.9	13.0	8.6	3.4	793	93.9	844	6.1	1588
WR9	51.4	14.7	9.6	4.1	683	94.0	727	6.0	2006
WR10	51.4	14.7	9.6	4.1	571	95.2	600	4.8	1628
WR11	52.8	14.2	9.5	3.7	822	95.2	863	4.8	1500
WR12	52.2	14.5	9.6	3.9	859	96.4	891	3.6	1436
WR13	49.7	14.0	9.0	4.0	667	95.9	695	4.1	1795
SD[c]	<1.4	<0.3	<0.3	<0.3	<58	<0.5	<60	<0.5	<74

[a] CL, ECL and ICL stand for average chain length, exterior chain length and interior chain length of starch, respectively.
[b] DP_w, $F1DP_w$ and $F2DP_w$ stand for weight-average degree of polymerization of F1 + F2, F1 and F2 fractions, while F1% and F2% stand for the weight percentage of F1 and F2 fractions, respectively.
[c] Standard deviation.

TCW70 and TKW5, and the lowest by mutant genotype WR8. In this study, λ_{max} showed significant positive relationships with both CL (r^2 = 0.810, $p < 0.01$) and blue value (r^2 = 0.656, $p < 0.05$). No significant correlation between β-amylolysis limit and CL was found, this implies that the β-amylolysis limit of starch depends not only the length of starch chains, but also the arrangement of starch chains or distribution of branch point. Results showed that difference in chain arrangement or branch point distribution between waxy rice starches was applied in this study. External chain length (ECL) of amylopectin from 20 waxy rice starches varied from 8.6 to 10.2 and internal chain length (ICL) from 3.4 to 4.9. Betroft et al. (1999) reported ECL and ICL of 11 and 4.8, respectively, for waxy rice amylopectin. Average values of ECL and ICL of named varieties (9.6 and 4.2, respectively) were slightly higher than the corresponding value of mutants (9.2 and 4.1, respectively).

Average DP_w of the 20 waxy rice starches ranged from 571×10^3 to 928×10^3 anhydro glucose unit (AGU), with a mean value of 752×10^3 AGU (Table 2). Mutant genotype WR2 and WR10 showed the highest and the lowest DP_w, respectively. Generally, HPSEC profile of starch showed bimodal distribution. The first fraction (F1) with lower retention time mainly corresponds to amylopectin, and the second fraction (F2) corresponds to the amylose or low molecular weight molecules (Lin & Chang, 2006). Due to undetectable iodine affinity of waxy rice starches in this study, F2 can be defined as fraction of amylopectin with low molecular weight. DP_w of fractions F1 and F2 for the 20 waxy rice starches ranged from 600×10^3 to 994×10^3 and 806–2315 AGU, respectively. While, the average proportions of fractions F1 and F2 for the 20 waxy rice starches were observed to be 94.8 and 5.2%, respectively. Thus, the maximum contribution to DP_w of starches came from fraction F1. DP_w for fractions F1 and F2 of the 7 named varieties ranged from 686×10^3 (TSW2) to 946×10^3 (TCW70) and 1368 (TSW2) to 2302 (TKW1) AGU, respectively. DP_w for fractions F1 and F2 of starches from the 13 waxy rice mutants varied from 891×10^3 (WR12) to 600×10^3 (WR10) and 806 (WR3) to 2315 (WR1) AGU, respectively. In short, on an average DP_w of fractions F1 and F2 of the 7 named varieties were higher than those of starches from the 13 mutant genotypes. However, the proportions of F1 and F2 chains were comparable between starches of mutant genotypes and named varieties.

3.3. Gelatinization thermal properties

T_o, T_p and T_c of starches ranged from 57.1 to 64.1 °C, 64.6 to 70.3 °C and 80.6 to 86.4 °C, respectively, for the 20 waxy rice starches (Table 3), while R varied between 19.1 (TSW2) and 24.8 °C (WR4). It was observed that the average T_o, T_p and T_c of mutants (59.7, 66.4 and 82.8 °C, respectively) were obviously lower than the corresponding value of named varieties (62.0,

Table 3
Gelatinization thermal properties[a] of waxy rice starches.

Genotype	T_o (°C)	T_p (°C)	T_c (°C)	R (°C)	ΔH (J/g)
BW	64.1	70.2	83.9	19.8	15.4
ESW	63.0	67.8	83.1	20.1	15.0
TCSW1	62.0	68.0	86.4	24.4	14.9
TCW70	63.5	70.3	86.4	22.9	16.5
TKW1	59.1	66.9	82.1	23.1	15.7
TKW5	59.1	65.6	80.6	21.4	15.3
TSW2	63.5	69.6	82.6	19.1	15.7
WR1	58.3	65.9	81.5	23.1	14.6
WR2	61.1	66.5	82.4	21.3	15.4
WR3	61.8	68.2	84.0	22.1	17.2
WR4	58.8	66.9	83.6	24.8	16.2
WR5	61.0	66.4	82.5	21.6	16.3
WR6	61.9	67.5	85.2	23.3	17.1
WR7	61.1	67.2	82.7	21.5	16.6
WR8	60.0	66.0	83.5	23.5	16.0
WR9	58.1	64.6	81.1	23.0	15.1
WR10	59.0	66.5	83.5	24.5	15.7
WR11	58.7	65.9	82.3	23.5	15.4
WR12	57.4	65.4	81.5	24.0	15.5
WR13	59.2	66.4	82.7	23.6	15.3
SD[b]	<0.2	<0.3	<1.1	<1.0	<0.5

[a] T_o, T_p, T_c, R and ΔH stand for the onset, peak, conclusion and range of gelatinization temperature, as well as enthalpy change of gelatinization, respectively.
[b] Standard deviation.

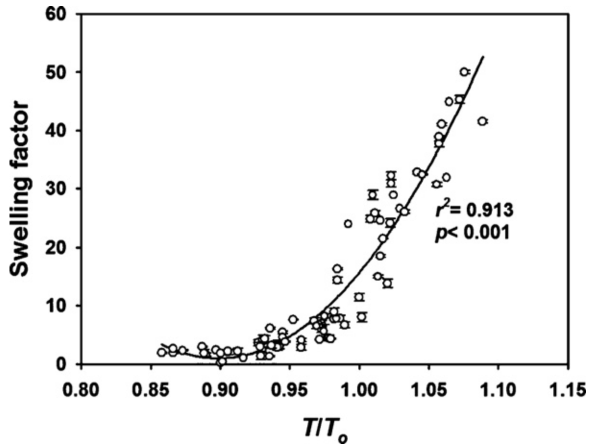

Fig. 1. Correlation between swelling factor and T/T_o value of waxy rice starches studied. T and T_o stand for the temperature of swelling factor determination and gelatinization onset temperature determined by DSC, respectively.

68.3 and 83.6 °C, respectively). ΔH ranged between 14.6 (WR1) and 17.2 J/g (WR3) for the 20 waxy rice starches. Cooke and Gidley (1992) proposed ΔH as an indirect measurement of molecular order rather than loss of crystallinity. Gelatinization and swelling properties have been observed to depend on the molecular structure of amylopectin (perfection and ordering of amylopectin crystallites, length of external chains of amylopectin, extent of branching, molecular weight and polydisperisty), starch composition (amylose/amylopectin ratio, lipid complexed amylose chains and granule architecture, that is crystalline to amorphous ratio) (Tester, 1997). Due to lack of amylose for waxy rice starches studied in this report, the differences in gelatinization thermal properties and molecular properties among the 20 waxy rice starches will be useful for elucidating the contribution of molecular structure of amylopectin on gelatinization thermal or swelling properties of waxy starch by correlation analysis.

For the 20 waxy rice starches, the increase in SF was maximum between 57.5 and 60 °C for the starches with T_o below or near 60 °C (Tables 1 and 3). Furthermore, it is interesting that a quadratic correlation (r^2 = 0.913, p < 0.001) was found between SF and T/T_o (ratio of temperature used for SF determination and gelatinization onset temperature of starch) in spite of rice genotype (Fig. 1). SF of waxy rice starch with T/T_o lower than 0.95 was less than 10, then SF rapidly increased with increasing value of T/T_o. Tester (1997) indicated the gelatinization and swelling properties have been observed to depend on numerous factors as previously described. However, swelling of the 20 waxy rice starches investigated in this study strongly depends on the ratio between temperature used for SF determination and gelatinization onset temperature. This dependence can be attributed to the lack of amylose of waxy starches, which simplifies the swelling of starch granules despite the discrepancy in molecular structure of the 20 rice starches. Moreover, T/T_o is an useful index for predicting swelling extent of waxy starch granules heated in different temperatures.

3.4. Pasting properties

Pasting properties of the 20 waxy rice starches are shown in Table 4. Pasting temperature (PT) was measured between 65.5 (WR9) and 71.2 °C (ESW) for the 20 waxy rice starches, and peak viscosity (PV) for starches varied between 1535 (WR12) and 2079 cP (WR2). Vandeputte et al. (2003b) reported PV of 10 waxy rice starches at 10% suspensions between 3360 and 3720 cP. Vandeputte et al. (2003a) observed that amylose did not contribute to swelling of rice starches in the temperature range of 55–85 °C. These authors, however, showed lower PV for waxy rice starches and attributed it to low rigidity of waxy rice starch granules. Amylopectin has been reported to contribute to swelling and pasting of starch granules while amylose and lipids impede the swelling (Tester & Morrison, 1990b). However, Han and Hamaker (2001) did not find any significant relationship between amylopectin architecture and PV of starches.

Hot paste viscosity (HPV) for the 20 waxy rice starches varied between 583 (BW) and 769 cP (WR4). Breakdown (BD), a measure of thermal stability of starch granules, was observed to range from 922 (WR6) to 1351 cP (WR2). Breakdown ratio (BDr), the ratio of BD and PV, was calculated to range between 59.4 (TCW70) and 67.2% (TKW5). BD depends on granule characteristics like size, shape and swelling extent. Han and Hamaker (2001) observed negative and positive correlations of BD with extra long (DP > 100) and short amylopectin chains (average DP ~ 17), respectively. These authors postulated that involvement of extra long amylopectin chains in more than one cluster reduces their dispersion due to entanglement with other amylopectin molecules. The other reason cited by them was that increase in viscosity was affected with increase in gyration radius of amylopectin molecules due to higher proportion of long amylopectin chains.

Setback (SB) is related to retrogradation of starches. SB was recorded between 585 (BW) and 687 cP (TSW2) and setback ratio (SBr), the ratio of SB and HPV, was ranged from 87.8 to 112.1%. Due to the absence of gel network formed by amylose, waxy starches exhibited low SB values. Since rice starches studied showed undetectable amylose content, SB can be attributed to the re-association tendency of amylopectin chains during cooling, especially for the long chains of amylopectin. Final viscosity (FV) depends on leaching extent of amylose and granule architecture. The 20 waxy rice starches showed FV varying between 1168 (BW) and 1443 cP (WR4). Yoshimoto et al. (2002) showed lower SB and FV for waxy barley starch than normal barley starch and attributed this to higher swelling and granule fragility of waxy barley starches. Vandeputte et al. (2003b) showed that SB was not correlated with the low amylose contents of waxy rice starches. In absence of amylose, FV may depend on amylopectin architecture and granule fragility.

3.5. Correlations

Correlations between physicochemical properties and parameters of molecular structure (Table 5) showed that SF determined at 60 °C negatively correlated with λ_{max}, BV, granule size, ICL, proportion of low molecular weight amylopectin (F2%), and positively correlated with proportion of high molecular weight amylopectin (F1%). Negative relationship on λ_{max}, BV, granule size, ECL and ICL with SF determined at 62.5 °C were also obtained. For SF determined at 60 or 62.5 °C, it can be read as swelling of waxy rice starch granules at initial stage of gelatinization, because of T/T_o of waxy rice starches at this temperature range were higher than 0.93 (see Table 3 and Fig. 1). Correlation results showed that the swelling of initial gelatinization stage of waxy starches is affected by chain length, because of high negatively correlation on λ_{max} and BV. While correlation result on chain length parameters showed that the swelling of waxy starch granules is not dependent on the average chain length (CL), but dependent on the chain length of constituted chains. Negative relationship on SF with ECL and ICL were observed, especially for ICL. Generally, longer ICL referred to relatively high entanglement of amylopectin molecules as compared to that of shorter ICL. Correlation results of SF showed that more amylopectin entanglement (longer ICL) causes less swelling of waxy starch granules during initial gelatinization stage.

Correlations between gelatinization thermal parameters and parameters of molecular structure showed positively correlations

Table 4
Pasting properties[a] of waxy rice starches.

Genotype	PT (°C)	PV (cP)	HPV (cP)	BD (cP)	BDr (%)	FV (cP)	SB (cP)	SBr (%)
BW	68.7	1655	583	1072	64.8	1168	585	100.5
ESW	71.2	1772	644	1129	63.7	1255	611	95.0
TCSW1	68.4	1795	682	1113	62.0	1315	633	92.8
TCW70	69.8	1640	666	974	59.4	1336	670	100.5
TKW1	67.0	1624	638	986	60.7	1263	625	98.0
TKW5	66.4	1849	607	1242	67.2	1267	660	108.9
TSW2	69.4	1661	613	1049	63.1	1299	687	112.1
WR1	66.1	1678	640	1038	61.9	1263	623	97.5
WR2	67.2	2079	728	1351	65.0	1391	663	91.1
WR3	68.4	1724	695	1030	59.7	1308	614	88.5
WR4	67.6	1954	769	1185	60.7	1443	674	87.8
WR5	67.7	1996	694	1302	65.2	1339	645	92.9
WR6	68.5	1540	618	922	59.9	1229	611	98.8
WR7	67.3	1841	666	1175	63.8	1313	647	97.3
WR8	67.3	1993	748	1245	62.5	1374	626	83.7
WR9	65.5	1945	648	1297	66.7	1253	605	93.3
WR10	67.2	1582	629	953	60.2	1237	607	96.5
WR11	66.5	1692	664	1028	60.7	1305	641	96.6
WR12	66.1	1535	606	928	60.5	1212	606	99.9
WR13	66.9	1819	673	1146	63.0	1308	635	94.3
SD[b]	<0.5	<33	<24	<29	<1.4	<43	<40	<6.0

[a] PT, PV, HPV, BD, BDr, FV, SB and SBr stand for pasting temperature, peak viscosity, hot paste viscosity, breakdown (PV – HPV), break down ratio (BD/PV × 100%), final viscosity, setback (FV – HPV), and setback ratio (SB/HPV × 100%), respectively.
[b] Standard deviation.

between T_o, as well as T_p, and λ_{max}, BV, ECL and ICL. Positively relationship on T_c with λ_{max} and BV were also obtained. Similarly, no significant correlation between gelatinization temperatures and CL was observed. Positively correlation on ECL and ICL reveals that high entanglement of amylopectin make waxy starch granules with high thermal stability. Moreover, no significant correlations between gelatinization temperatures and parameters of molecular size distribution were found. Correlations on temperature range of gelatinization (R) showed reverse result as correlations on T_o. R was also positively correlated with F1%, and negatively correlated with F2%. R has been recognized an index of homogeneous of order structure for starch granule, high value of R reveals less homogeneous on order structure of starch granules. Correlations for R and parameters of molecular structure indicated that high homogeneous order structure could be present in waxy starch granules with larger granule size, higher entanglement of amylopectin (lower ICL), or higher proportion of low molecular weight amylopectin. Only negatively correlation with molecular size of low molecular weight amylopectin was observed for ΔH of gelatinization ($p < 0.01$).

PT showed significant relationship with λ_{max} and BV (Table 5). PV and shear stability are dependent on amylose content (Jane et al., 1999) and the proportion of amylopectin branch chains of DP 13–24 and DP > 37 (Han & Hamaker, 2001; Stevenson, Domoto, & Jane, 2006). A significant correlation between granule size and BD, as well as BDr, was also observed, and FV also showed relationship with β-amylolysis (Table 5). Vandeputte et al. (2003b) did not find any relationship between amylopectin chain length distribution and pasting parameters with exception of pasting temperature for normal and waxy rice starches. Pasting properties of waxy rice starches can be affected by a number of factors like proportion of long and short chains of amylopectin and interactions among starch chains. PT also indicates the swelling capacity of starch granules. However we find no relationship of PT with chain lengths of amylopectin although PT was significantly related to T_p ($r^2 = 0.654$, $p < 0.05$). Thus PT can also be related to crystallite quality.

The correlation between physicochemical properties and molecular structure amylopectin of waxy rice starches indicates that the effects of molecular structure on physicochemical properties of waxy rice starches were more profound on swelling and gelatinization thermal properties than pasting properties. Although SBr was positively correlated with CL, no significant relationship on PV, HPV or SB with molecular parameters was found. Generally, gelatinization and pasting of starch are influenced by granule characteristic (granule size and architecture), amylose

Table 5
Pearson correlations between physicochemical parameters and molecular structure parameters of waxy rice starches.

Parameters	SF_{60}	$SF_{62.5}$	T_o	T_p	T_c	R	ΔH	PT	PV	HPV	BD	BDr	FV	SB	SBr
λ_{max}	**−0.829****[a]	**−0.860****	**0.831****	**0.769****	**0.530***[b]	**−0.516***	–[c]	**0.705****	–	–	–	–	–	–	–
BV	**−0.760****	**−0.879****	**0.893****	**0.854****	**0.489***	**−0.642***	–	**0.768****	–	–	–	–	–	–	–
Granule size	**−0.575***	**−0.569***	**0.592***	–	–	**−0.814****	–	–	–	–	**0.497***	**0.686****	–	–	–
β-Amylolysis	–	–	–	–	–	–	–	–	–	–	–	–	**−0.445***	–	–
CL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	**0.509***
ECL	–	**−0.605***	**0.626***	**0.603***	–	–	–	**0.667***	–	–	–	–	–	–	–
ICL	**−0.559***	**−0.609***	**0.538***	**0.543***	–	**−0.541***	–	–	–	–	–	–	–	–	–
Average DP_w	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
F1%	**0.491***	–	–	–	–	**0.471***	–	–	–	–	**−0.463***	**−0.444***	–	–	–
$F1DP_w$	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
F2%	**−0.491***	–	–	–	–	**−0.471***	–	–	–	–	**0.463***	**0.444***	–	–	–
$F2DP_w$	–	–	–	–	–	–	**−0.716****	–	–	–	–	–	–	–	–

[a] Significant at $p < 0.01$.
[b] Significant at $p < 0.05$.
[c] $p > 0.05$.

content, and leaching of amylose during gelatinization. In this study, waxy rice starches have similar granule size and very low amylose content, consequently the discrepancy in molecular structure of amylopectin of the waxy rice starches could refer to the different architectures of starch granules. In other words, the pasting of waxy starch is essentially dominated by granule architecture. Moreover, the swelling and gelatinization thermal properties of waxy rice starch did not show significant correlation with molecular size of amylopectin, but significant correlations were found between the swelling or gelatinization thermal properties and chain length of amylopectin. This implies that the gelatinization, swelling of granules or disorder of order structure, of waxy starch granules depend on the interactions among amylopectin chains.

4. Conclusions

Heterogeneity in amylopectin molecular structure between the starches of mutant genotypes and named waxy rice varieties was reflected in molecular weight and chain lengths despite the botanical source being the same for all. Granule architecture of waxy starch essentially dominates the variations in swelling, pasting and thermal attributes which are dependent on the interactions among amylopectin chains. Mutation led to change in amylopectin molecular structure as well as inter and intra-molecular bonding. It has been proposed that NaN_3-induced mutation affected not only the GBSS locus, but also other loci related to starch biosynthesis (Jeng et al., 2003, 2007). Rice mutants will be helpful for enhancing application possibility of starch, and is worth to further investigation on the relation between genetic expression of mutant and physicochemical properties of starch.

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