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Molecular weight, chain profile of rice amylopectin and starch pasting properties

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ABSTRACT

Differences in fine structure, average molecular size of amylopectin (AP) as well as clarity of the AP solution from indica waxy rice and high amylose (HAM) rice were examined. Despite similar amylose content (AM), rice starches displayed different pasting properties. Waxy APs had higher values of both number–average and weight–average molecular weight ($\overline{M_n}$ and $\overline{M_w}$) but lower values of intrinsic viscosity [η], compared to HAM APs. HAM APs had higher values of average chain length ($\overline{CL}$), average external chain length ($\overline{ECL}$), and a proportion of DP $\geq$ 37. Statistical correlations of mol proportions of debranched AP, branching parameters, and molecular weight of AP were calculated. The study showed that starch pasting properties and clarity of AP solutions were influenced by molecular weight and branching characteristics of AP.

1. Introduction

Amylopectin (AP) fine structure and its influences on starch functional properties are topics of interest and have been extensively investigated in the past decades (Han & Hamaker, 2001; Jane & Chen, 1992; Jane et al., 1999; Patindol, Gu, & Wang, 2009). Chain length (CL) distribution, branching characteristics, and intramolecular organization of the macromolecules are the principal subjects of AP chain profile that have been explored (Bertoft, Piyachomkwan, Chatakanonda, & Sriroth, 2008; Hanashiro, Abe, & Hizukuri, 1996; Hizukuri, 1986; Jane & Chen, 1992; Jane et al., 1999; Vandeputte, Derycke, Geeroms, & Delcour, 2003). Using different enzymes based on their selectivity to hydrolyze AP molecules is a major approach used in the studies.

Compared to chain profile investigations, studies related to the molecular weight of AP are rather scarce (Chung, Han, Yoo, Seib, & Lim, 2008; Ma et al., 2007; Wang & Wang, 2002; Yoo & Jane, 2002; Zhong, Yokoyama, Wang, & Shoemaker, 2006). Unlike the particular means and specific parameters used in the study of chain profiles, there are several ways to determine the average molecular weight (MW) of polymers. Number–average molecular weight ($\overline{M_n}$) represents MW of the most prevalent polymer molecules and small polymer molecules generally have a major influence on the value. Weight–average molecular weight ($\overline{M_w}$) emphasizes large molecules more than small molecules whereas $\overline{M_v}$ indicates the molecular volumes of polymers in a solution.

Numerous studies (Jane et al., 1999; Shi & Seib, 1995; Vandeputte et al., 2003; Yuan, Thompson, & Boyer, 1993) have emphasized the influence of AP chain profiles on starch functional properties. In regard to pasting properties, debranched medium-amylose (AM) rice starch with a higher amount of the B_3 fraction ($\overline{DP}37-60$) displayed higher peak viscosity, hot paste viscosity, and breakdown viscosity than did others (Patindol & Wang, 2002). Paste breakdown of low-AM rice starches was negatively and positively correlated with long-chain ($\overline{DP}_n > 100$) and short-chain ($\overline{DP}_n \sim 17$) fractions of debranched starch, respectively (Han & Hamaker, 2001). In contrast, the findings about the significance of average molecular weight of AP on functional properties of starch are somewhat contradictory. $\overline{M}_n$ of AP was reported to be positively correlated with peak viscosity of wheat starch (Shibanuma, Takeda, & Hizukuri, 1996), swelling power of waxy rice (Wang & Wang, 2002), and induction time of retrogradation of normal rice starches (Lai, Lu, & Lii, 2000). $\overline{M}_w$ was negatively correlated with peak viscosity and setback of normal rice starches (Patindol et al., 2009). Mufumbo et al. (2011) reported a negative correlation between $\overline{M}_w$ and setback but not between $\overline{M}_w$ and peak viscosity of cassava

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starches. Lin et al. (2013) reported no correlations between $\overline{M_w}$ and pasting properties of Taiwanese waxy rice starches.

Besides the inconclusive results of the effects of MW on starch functional properties, few studies have simultaneously covered both AP chain profile and its average molecular weight (Lu, Chen, & Lii, 1997; Takeda, Hizukuri, & Juliano, 1987; Vilaplana & Gilbert, 2010; Wang & Wang, 2002). Additionally, to the best of our knowledge, no studies have been conducted on the various kinds of average MW and the chain profile of the same AP sample. It is worthwhile to elucidate disagreement in the effects of MW on starch functional properties due to measurements of different kinds of average MW performed on AP from various starches. Thus, the objectives of the present study were to investigate the different kinds of average MW, the chain profile, solution clarity of identical AP samples as well as their starch pasting properties. Any indications of relationships between the AP chain profile and average MW as well as effects of the structural parameters on starch pasting properties and clarity of the AP solutions were also examined. Thai rice starches were used as the model for this study.

2. Materials and methods

2.1. Materials

Three waxy rice varieties – RD6, Hang Yee 71 (HY71) and Sew Mae Jan (SMJ) and three high amylose (HAM) rice varieties – Prajeenburi 1 (PJ1), Chainart 1 (CN1), and Suphanburi 1 (SP1) – were obtained from the Rice Research Center (Thailand). β-Amylase from sweet potato was purchased from Sigma–Aldrich (St. Louis, MO), and isoamylase was obtained from Hayashibara (Okayama, Japan). Analytical-grade chemicals were used in the study unless otherwise noted.

2.2. General methods

Moisture, protein, lipid and ash contents of the starches were determined using AACC methods (AACC methods 08-01, 30-10, 44-15A and 46-11A). The conversion factor of N × 5.95 was applied to convert nitrogen to crude protein content. Total amylose (AM) content of starch was measured by the method of Chrastil (1987). Pasting properties of all starches were determined by a rapid visco analyzer (Newport Scientific, Narrabeen, Australia) (Lumdubwong & Seib, 2000). The clarity of AP solution (1%, w/w) was measured by the method of Craig, Maningat, Seib, and Hoseney (1989). Swelling power and water solubility (%) of all starches (1% w/w) were determined by the method of Holm, Björck, Asp, Sjöberg, and Lundquist (1985).

2.3. Preparation of AP samples

All starches were isolated using the alkaline steeping method (Lumdubwong & Seib, 2000). All waxy AP samples in this study were used in the form of non-granular (NG) starch. Waxy AP solutions were prepared by the method of Yoo & Jane (2002) but the solutions were stirred for 48 h instead of 24 h. The starch solutions were then precipitated with absolute ethanol, followed by centrifugation at 3000 × *g* for 10 min and the supernatant was discarded. After washing three times with ethanol, the NG starches were dried in a vacuum oven.

For HAM AP samples, the starch was first prepared in the form of NG starch in the same manner as waxy samples. The NG starch was then re-dissolved in distilled water and boiled for 1 h. The HAM AP was further extracted from the NG starch solution using the alcohol precipitation method of Takeda et al. (1987) and the precipitated AP samples were dried in a vacuum oven at ambient temperature. The purity of HAM AP samples was verified using high-performance size-exclusion chromatography (HPSEC). For further analyses, all powdered AP samples were re-dissolved in deionized water and boiled for 1 h prior to uses.

2.4. Number–average molecular weight measurement

Number–average molecular weight measurement ($\overline{M_n}$) was obtained by multiplying the number–average degree of polymerization ($\overline{DP_n}$) by 162. $\overline{DP_n}$ was measured using the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and a modified Park–Johnson procedure (Takeda et al., 1987).

2.5. Weight–average molecular weight measurement

Weight–average molecular weight ($\overline{M_w}$) of AP was measured by both a laser light scattering (LLS) instrument and by high-performance size-exclusion chromatography with multi-angle laser light scattering coupled with refractive index detection (HPSEC–MALLS–RI).

For static LLS, AP was dissolved in 90% DMSO at a concentration of 0.2 mg/ml. The solution was simultaneously gently stirred and heated for 1 h in a boiling water bath, and diluted with 90% DMSO to five concentrations ranging from 0.004 to 0.012 mg/ml. All solutions were filtered with a 5 μm PTFE filter before LLS measurement. The scattered light intensities were measured from 5 to 18 angles by a MALLS instrument with a He–Ne laser source (λ = 690 nm) and a K-5 flow cell.

The AP solution (0.2 mg/ml) using water as dispersing medium was prepared in the same manner as with 90% DMSO, and was injected into a HPSEC–MALLS–RI system consisting of: a 515 HPLC pump (Waters, Milford, MA) equipped with an injection valve (200 μl sample loop, model 7725i; Rheodyne); a MALLS detector (DAWN® EOS; Wyatt Technology, Santa Barbara, CA) with a He–Ne laser source (λ = 690 nm) and K-5 flow cell; and a refractive index detector (Optilab rEX; Wyatt Technology). The SEC columns were composed of a guard column (OHpak SB-G; Shodex, Tokyo, Japan) and two analytical columns (OHpak SB-804HQ and SB-806HQ; Shodex). The columns were maintained at 55 °C and the temperature of the RI detector was maintained at 30 °C. The mobile phase was deionized water (18.2 MΩ) and the flow rate was 0.6 ml/min.

A dextran standard ($\overline{M_w} = 2.50 \times 10^4$, 0.4 mg/ml) was used for normalization of the multi-angle photodiode detector used in MALLS and LLS. Astra software was used for data acquisition and analysis. The curve fitting method and calculation of $\overline{M_w}$ were based on the second-order Berry method (Yoo & Jane, 2002; Zhong et al., 2006).

2.6. Intrinsic viscosity measurement

Waxy starches and NG HAM AP samples were dispersed in different solvents, 90% DMSO and water, at a concentration of 3×10^{-3} g/ml. When dispersed in 90% DMSO, the samples were heated in a boiling water bath for 15 min and stirred overnight at room temperature. When dispersed in water, the samples were gently stirred and boiled for 1 h. The final concentrations of the solutions were 0.5–3.0 × 10^{-3} g/ml. Intrinsic viscosity measurements were obtained using a Cannon-Ubbelohde glass capillary viscometer (Cannon Instrument Co., State College, PA) immersed in a water bath at 25 ± 1 °C for 90% DMSO and at 40 ± 1 °C for water. The intrinsic viscosity [η] was calculated from the *y*-axis intercept of the Huggins plot of η_{sp}/c against *c* and the Kramer plot of $\ln \eta_{rel}/c$ against *c*.

Table 1
Chemical compositions of waxy and high amylose starch from different cultivars.

Cultivars	Moisture content (%)	Protein content (%)	Fat content (%)	Amylose content (%)	Ash content (%)
HY71	10.9^{bc}	0.02^{a}	0.06^{a}	1.6^{a}	0.06^{a}
RD6	11.1^{d}	0.02^{a}	0.05^{a}	1.0^{a}	0.06^{a}
SMJ	10.8^{ab}	0.02^{a}	0.07^{a}	1.0^{a}	0.08^{a}
PJ1	11.0^{cd}	0.33^{c}	0.05^{a}	31.6^{b}	0.13^{bc}
CN1	10.7^{a}	0.32^{c}	0.06^{a}	32.8^{b}	0.14^{c}
SP1	10.7^{a}	0.29^{b}	0.06^{a}	32.4^{b}	0.11^{b}

Means in the same column with different letters are significantly different ($P < 0.05$).

2.7. Analysis of average chain length and chain profile of APs

The AP samples were debranched using isoamylase by the method of Jane & Chen (1992). The average chain length distribution of debranched AP was determined by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC–PAD), using the method of Hanashiro et al. (1996). The average chain length ($\overline{CL}$) was obtained by calculating the mol ratio of total carbohydrate to reducing ends of debranched AP. The average external chain length ($\overline{ECL}$) and the average internal chain length ($\overline{ICL}$) were calculated as shown: $\overline{ECL} = (\overline{CL} \times \%\beta) + 2$; $\overline{ICL} = \overline{CL} - \overline{ECL} - 1$.

2.8. Percent of β-amylolysis of AP

Percent of β-amylolysis of AP samples was determined using the method of Zhu and Bertoft (1997).

2.9. Statistical analyses

Each AP structural measurement, starch chemical compositions, pasting properties, and solution clarity were done at least in duplicate. Analysis of variance (ANOVA), Duncan's multiple comparison, and Pearson's correlation were performed using SPSS 11.0 software.

3. Results and discussion

3.1. Average molecular weight of rice AP

The fat content of waxy and HAM indica rice starch samples was low and not statistically different. The amylose content of waxy and HAM starches was ~1.2% and 32.3%, respectively. In addition, the HAM starch samples had higher protein and ash content than the waxy samples (Table 1).

The number–average degree of polymerization ($\overline{DP_n}$) of Thai indica waxy rice APs ranged from 6228 to 8262 anhydroglucose unit (AGU) (Table 2), which are ~2–3 times greater than the $\overline{DP_n}$ of japonica and Taiwanese indica waxy rice APs reported by Wang and Wang (2002). However, the values were similar to those of Taiwanese waxy indica AP reported by Lu et al. (1997). The $\overline{DP_n}$ values of Thai indica HAM rice APs ranged from 2653 to 3069 AGU and were similar to those of Taiwanese HAM indica AP (Lu et al., 1997). The $\overline{DP_n}$ value of PJ1, however, was significantly higher than others. The order of $\overline{M_n}$ was found to be RD6 > HY71 > SMJ > PJ1 > CN1 ~ SP1. It appears that the prevalent molecules of waxy APs were larger than those of HAM APs since the $\overline{M_n}$ values of HAM APs were ~2–3 times less than those of waxy APs.

It is known that HPSEC separation induce degradation of AP molecules due to chain scission (Cave, Seabrook, Gidley, & Gilbert, 2009). Thus, the $\overline{M_w}$ values in the study obtained from the HPSEC–MALLS method are relative and comparable among samples determined by the same method. The weight–average molecular weight ($\overline{M_w}$) of the waxy samples, determined by HPSEC–MALLS using water as the mobile phase, ranged from 6.13 to 8.20 × 10^8 g/mol. Since $\overline{M_w}$ measurement emphasizes the mass of molecules, RD6 and HY71 possibly possess larger molecules than SMJ. The polydispersity index (PI) suggested that SMJ had the narrowest size distribution of molecular mass. The values of $\overline{M_w}$ and the average radius of gyration ($\overline{R_g}$) in our study were ~7–8 times and ~2.2 times less, respectively, than values previously presented (Yoo & Jane, 2002). Fewer aggregates of AP due to higher AP dilution used, probably is the cause of the discrepancy in the $\overline{M_w}$ values (Zhong et al., 2006).

No studies have been done on $\overline{M_w}$ measurement of AP from rice containing ≥28% AM which is HAM rice according to rice classification of Juliano (1998). The values of $\overline{M_w}$ of HAM APs ranged from 3.04 to 4.34 × 10^8 g/mol, which were ~1.4–2.7 times less than those of waxy APs. The average size of large molecules of PJ1 and CN1 was similar but larger than that of SP1. The high PI of HAM APs suggested that the discrepancy in average molecular size of prevalent and large molecules of the samples was larger than that of waxy APs. Compared to the large difference in $\overline{M_w}$ of HAM and waxy APs, the $\overline{R_g}$ values of HAM APs were only ~0.96–1.25 times less than those of waxy APs. Thus, it is likely that waxy AP had more compact conformation than HAM APs when using water as a solvent.

When AP was dispersed in 90% DMSO and its $\overline{M_w}$ was determined using batch mode static MALLS, the order of AP molecular sizes remained the same, but the $\overline{M_w}$ and the $\overline{R_g}$ values were reduced to 5.92–8.80 × 10^7 g/mol and to 135–187 nm, respectively. The ratio of $\overline{M_w}$ of waxy to HAM APs was ~1.25, confirming that the average size of large waxy AP molecules was actually larger than that of HAM APs. The ratio of $\overline{R_g}$ of the waxy to HAM APs is ~1.1, suggesting that the dispersing ability in 90% DMSO solvent of both waxy and HAM APs were rather similar. Several studies have suggested that DMSO and 90% DMSO are the "optimal" solvents for starch molecule dispersion, in a thermodynamic sense, compared

Table 2
Average molecular weight and intrinsic viscosity of rice amylopectin from different cultivars.

Cultivars	Reducing end analysis		Water by HPSEC-MALLS-RI			90% DMSO by Batch MALLS		[η] (ml/g)	
	$\overline{DP_n}$ (AGU)	$\overline{M_n}$ (×10^6 g/mol)	$\overline{M_w}$ (×10^8 g/mol)	$\overline{R_g}$ (nm)	PI (×10^2)	$\overline{M_w}$ (×10^7 g/mol)	$\overline{R_g}$ (nm)	Water	90% DMSO
HY71	7430^{b}	1.20^{b}	7.99^{a}	393.8^{a}	6.64	7.75^{ab}	178.2^{a}	107.4^{b}	124.3^{c}
RD6	8262^{a}	1.34^{a}	8.20^{a}	390.2^{a}	6.12	8.80^{a}	187.2^{b}	106.7^{b}	123.5^{c}
SMJ	6228^{c}	1.01^{c}	6.13^{b}	330.2^{bc}	6.07	7.24^{bc}	164.0^{c}	95.4^{c}	114.3d
PJ1	3069^{d}	0.50^{d}	4.34^{c}	335.9^{bc}	8.72	6.24^{c}	157.6^{c}	116.8^{a}	147.0^{a}
CN1	2785^{e}	0.45^{e}	4.21^{c}	345.0^{b}	9.33	6.76^{bc}	146.2^{d}	115.1^{a}	143.3^{a}
SP1	2653^{e}	0.43^{e}	3.04^{d}	314.0^{c}	7.07	5.92^{c}	134.8^{e}	115.2^{a}	139.2^{b}

Means in the same column with different letters are significantly different ($P < 0.05$).

Table 3
Branch chain profiles of rice amylopectin from different cultivars.

Structural properties	Rice cultivars					
	HY71	RD6	SMJ	PJ1	CN1	SP1
$\overline{NC}$	429^{b}	490^{a}	373^{c}	153^{d}	137de	133^{e}
% β-Amylolysis	54.2^{d}	55.9^{c}	55.6^{c}	61.2^{a}	61.8^{a}	59.8^{b}
$\overline{CL}$ (AGU)	17.30^{b}	16.88^{bc}	16.70^{c}	20.03^{a}	20.47^{a}	20.07^{a}
$\overline{ECL}$ (AGU)	11.38^{c}	11.43^{c}	11.30^{c}	14.23^{ab}	14.63^{a}	14.00^{b}
$\overline{ICL}$ (AGU)	4.92^{a}	4.44^{b}	4.40^{b}	4.77^{a}	4.80^{a}	5.07^{a}
Mol fraction (%) (DP 6 to DP 58)						
f_a (%) (DP 6–12)	44.88^{a}	45.17^{a}	44.94^{a}	44.19^{b}	35.51^{c}	35.35^{c}
f_{b1} (%) (DP 13–24)	47.57^{d}	47.35^{d}	47.99^{c}	48.34^{b}	56.72^{a}	56.73^{a}
f_{b2} (%) (DP 25–36)	6.28^{ab}	6.20^{ab}	5.86^{c}	6.11^{bc}	6.29^{ab}	6.46^{a}
f_{b3} (%) (DP ≥ 37)	1.27^{c}	1.28^{c}	1.21^{d}	1.36^{b}	1.48^{a}	1.46^{a}

Means in the same row with different letters are significantly different ($P < 0.05$).

to water (Jackson, 1991; Ma et al., 2007; Millard, Wolf, Dintzis, & Willett, 1999). The ratios of $\overline{M_w}$ and $\overline{R_g}$ of waxy AP dispersed in water to that in 90% DMSO are ~8–10 and ~2.0–2.2 whereas the ratios of HAM AP are ~5–7 and 2.1–2.4, respectively. Therefore, AP molecular aggregations likely occurred in water and the aggregation was more pronounced for waxy AP, compared to HAM AP. Each aggregate may contain a greater number of molecules, and with more compact conformation, compared to those in 90% DMSO (Ma et al., 2007). The supposition was supported by the study of Callaghan and Lelievre (1985) that AP aggregated in water and its shape was more spherical than AP in DMSO. Lee, You, Kweon, Chung, & Lim (2014) also reported that AP chains inclined to aggregate by the excess amount of water.

The intrinsic viscosity [η], an index of $\overline{M_v}$, of APs when 90% DMSO was used as a solvent was higher than that of APs dissolved in water (Table 2), confirming that higher hydrodynamic volume of the polymers (better polymer dispersion) occurred in 90% DMSO. Not only differences in the [η] values were found between waxy and HAM APs, but also were found among APs from rice starch containing similar AM content. HAM APs displayed higher [η] than waxy APs, suggesting that the shape effect was likely stronger than the size effect within the range of $\overline{M_n}$ and $\overline{M_w}$ values of APs reported in this study. HAM APs might have smaller size but more elongated than those of waxy APs which is probably due to the presence of extra long chain in HAM APs (Lu et al., 1997). It was found that SMJ and SP1, the smallest waxy and HAM APs, had the least [η] among the waxy and HAM samples, respectively.

3.2. Branching characteristics of Thai rice APs

The values for the number of chains ($\overline{NC}$) of Thai waxy and HAM rice APs were high (~373–490) and low (~133–153) respectively (Table 3). PJ1 had rather high branching, compared to other HAM samples. Although the $\overline{NC}$ was somewhat proportional to the $\overline{M_n}$, the values of $\overline{CL}$ of debranched APs were different. HAM APs had higher $\overline{CL}$ than waxy APs. The $\overline{CL}$ of RD6 and HY71 was slightly greater, compared with SMJ whereas the $\overline{CL}$ of all HAM APs was similar. The external chains of HAM APs were not only longer than those of waxy APs ($\overline{ECL}$~14.3 *vs* 11.3 AGU), but their length was slightly different, depending on rice varieties. The lower values of $\overline{ICL}$ of RD6 and SMJ than others as shown in Table 3 indicated that branch points of the APs were closer together, i.e., that the molecule is more compact.

When based on % area, the chain length distribution of waxy APs and PJ1 was bimodal but that of the other two HAM APs was unimodal (Fig. 1). When based on % mol fraction, the chain length distribution of all APs was unimodal. The highest peak was DP 11 (HY71, RD6, SMJ, PJ1), and DP 12 (CN, SP1) respectively (Fig. 2). When the CL distribution of debranched AP was classified using the method of Hanashiro et al. (1996) – i.e., f_a chains (DP 6–12), f_{b1} chains (DP 13–24), f_{b2} chains (DP 25–36) and f_{b3} chains (DP ≥ 37) – all waxy samples had a higher proportion of the shortest chain (f_a chains) than did HAM samples (~45% *vs* 35%), except PJ1 whose f_a fraction is very closed to that of waxy samples. Due to % f_{b1} fraction, it appears that CN1 and SP1 contained the highest proportion of a mixture of longer A-chains and the short B-chains while HY71 and RD6 had the least. CN1 and SP1 also had the highest proportions of the longer chains (f_{b2} and f_{b3} chains), whereas SMJ had the least fraction (Table 3).

3.3. Pasting properties and paste clarity of Thai rice starches

All starches contained similar fat content (Table 1). Thus, any differences in pasting properties among the indica waxy and HAM rice starches (Table 4) were unlikely due to their lipid content. SMJ had the lowest peak viscosity (PV), trough or the highest hot paste viscosity (HV), and the lowest breakdown (BD) among the waxy starches. It also had lower swelling power (SP) and solubility (%SS) than did other waxy samples, indicating that SMJ had less granule swelling, less granule fragility, and less granule fragments than others (Han & Hamaker, 2001). Among HAM starches, SP1 displayed the lowest PV, HV, and final viscosity (FV). The starch did not only have a low pasting temperature and rather low SP, but also had high solubility. The data implied that SP1 not only had less granule swelling, but also had lower granule rigidity, compared to other HAM starches. Differences in setback (SB) viscosity of the HAM starches were also found, implying that other factors besides AM affect the property.

One would expect that waxy starches should have higher peak viscosity (PV) than HAM starches since swelling property of starch granules primarily depends on AP content and AM behaves as both a diluent and a swelling inhibitor, particularly in the presence of lipid (Tester & Morrison, 1990). However, SMJ had lower PV than CN1 although it had lower AM but higher SP (~1% *vs* 32%, 28.9 g/g *vs* 19.6 g/g). Noosuk, Hill, Farhat, Mitchell, & Pradipasene (2005) reported that it is possible that starches which had less granule swelling can provide higher viscosity than starches which had higher swollen granules at higher concentrations. Depending on starch concentrations, the extent of granule swelling and granule rigidity when the swollen granules close pack, govern the starch viscosity. This should be the case in the study since the starch concentration used in the RVA measurement was about 11%. Both starches (SMJ and CN1) displayed similar BD, but CN1 as expected had higher FV.

The clarity of AP solution was measured in the study rather than starch paste clarity in order to eliminating effects of granule rigidity, granule remnants, and starch soluble, which possibly interfere clarity measurement. Thus, any differences in clarity of AP solutions

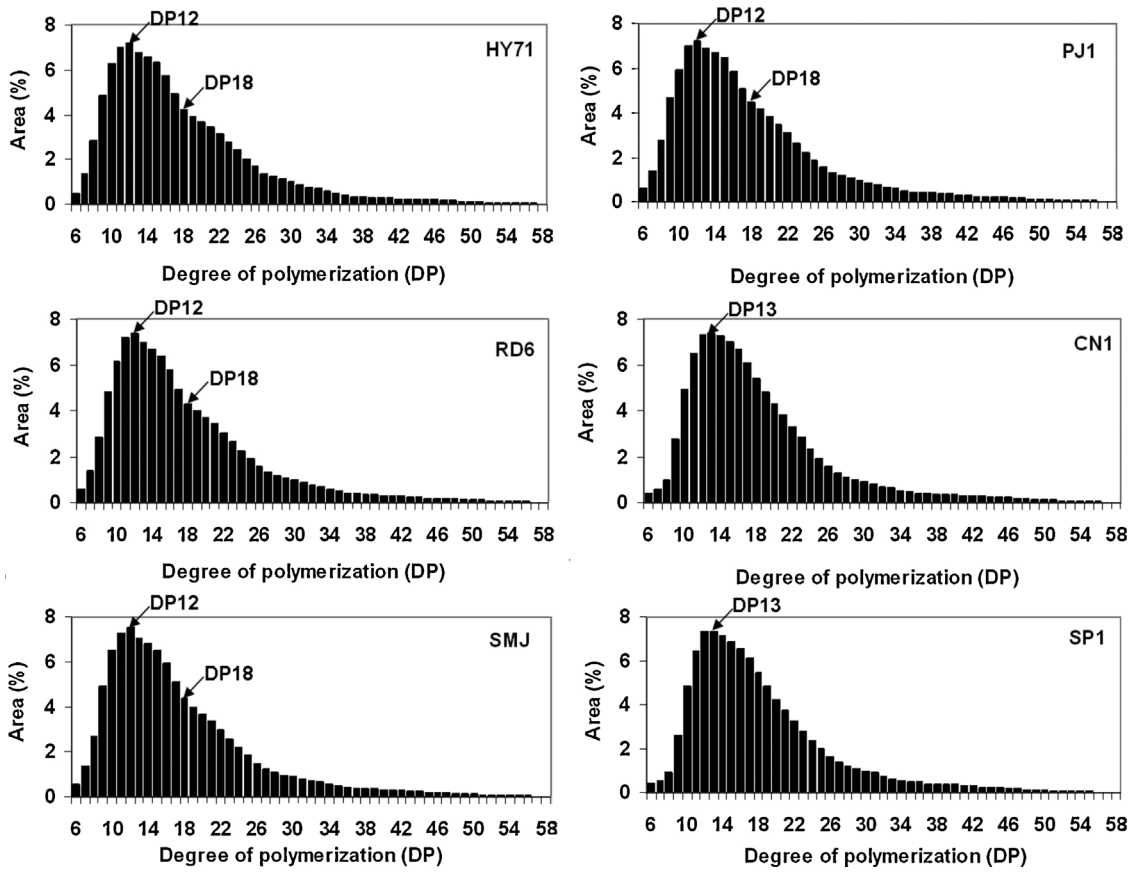

Fig. 1. Chain length distribution of rice amylopectin by peak areas.

found in the study should be due to differences in AP molecular characteristics. The clarity of indica rice AP solutions ranged from 36%T (light transmittance) to 55%T. SMJ and PJ1 had the lowest value among waxy and HAM rice APs (Table 4). Waxy APs, except SMJ, had higher %T than HAM APs.

3.4. Possible relationships between molecular size and chain profile of rice APs

A strong correlation between $\overline{M_n}$ and $\overline{M_w}$ ($r = 0.96$) (Table 5) indicated that when the prevalent AP molecules were of increased molecular sizes, the large AP molecules also were of increased molecular sizes, or vice versa. The negative correlations of molecular size and branching characteristics ($\overline{CL}$ and $\overline{ECL}$) implied that larger AP molecules tended to had shorter average chain length and external chain length. In addition, correlations between molecular size and shape of prevalent molecules were stronger than those of large molecules ($r = -0.94$ *vs* $r = -0.84$). It seems that branch chain length and external chain length mutually increased ($r = 0.99$), and both parameters ($\overline{CL}$ and $\overline{ECL}$) were more positively correlated with [η] of APs than did molecular size within the range reported in the study.

Positive and negative correlations between the proportions of unit chains (mol basis) and molecular size of AP molecules were shown in Table 5. The range of unit chains from DP 6 to DP 58 was used in all calculations and considered as 100% of the chains. The positive correlation of the proportion of very short chains (f_a, DP 6–12) and the negative correlations of f_{b1} (DP 13–24) and f_{b3} (DP ≥ 37) and molecular size ($\overline{M_n}$ and $\overline{M_w}$) suggested that larger AP molecules contained a higher proportion of very short chains but lower proportions of DP 13–24, and DP ≥ 37. Additionally, it appears that APs containing higher proportions of those fractions displayed high average chain length and external chain length.

Table 4
Pasting properties of rice starches from different cultivars.[A]

Starches	PT (°C)	Viscosity (cP)					SP	SS (%)	T (%)
		PV	HV	BD	FV	SB			
HY71	67.4^{c}	4533^{a}	2103^{d}	2429^{b}	2468^{d}	365^{d}	32.18^{a}	10.48^{d}	55.15^{a}
RD6	66.2^{c}	4530^{a}	1989^{e}	2540^{a}	2401^{d}	412^{d}	32.60^{a}	10.40^{d}	52.95^{a}
SMJ	68.8^{bc}	3781^{c}	2312^{c}	1469^{c}	2424^{d}	111^{e}	28.96^{b}	5.88^{e}	41.30^{b}
PJ1	72.6^{b}	3557^{d}	2714^{a}	842^{e}	5793^{a}	3078^{a}	16.15^{e}	15.22^{c}	36.75^{c}
CN1	78.7^{a}	3878^{b}	2416^{b}	1462^{c}	5089^{b}	2673^{b}	19.65^{c}	17.34^{b}	41.60^{b}
SP1	72.5^{b}	3193^{e}	2246^{c}	946^{d}	3957^{c}	1711^{c}	17.36^{d}	21.00^{a}	39.35^{bc}

Means in the same column with different letters are significantly different ($P < 0.05$).

[A] PT, pasting temperature; PV, peak viscosity; HV, hot paste viscosity; BD, breakdown viscosity (PV–HV); FV, final viscosity; SB, setback viscosity (FV–HV); SP, swelling power; SS, soluble starch; T, transmittance.

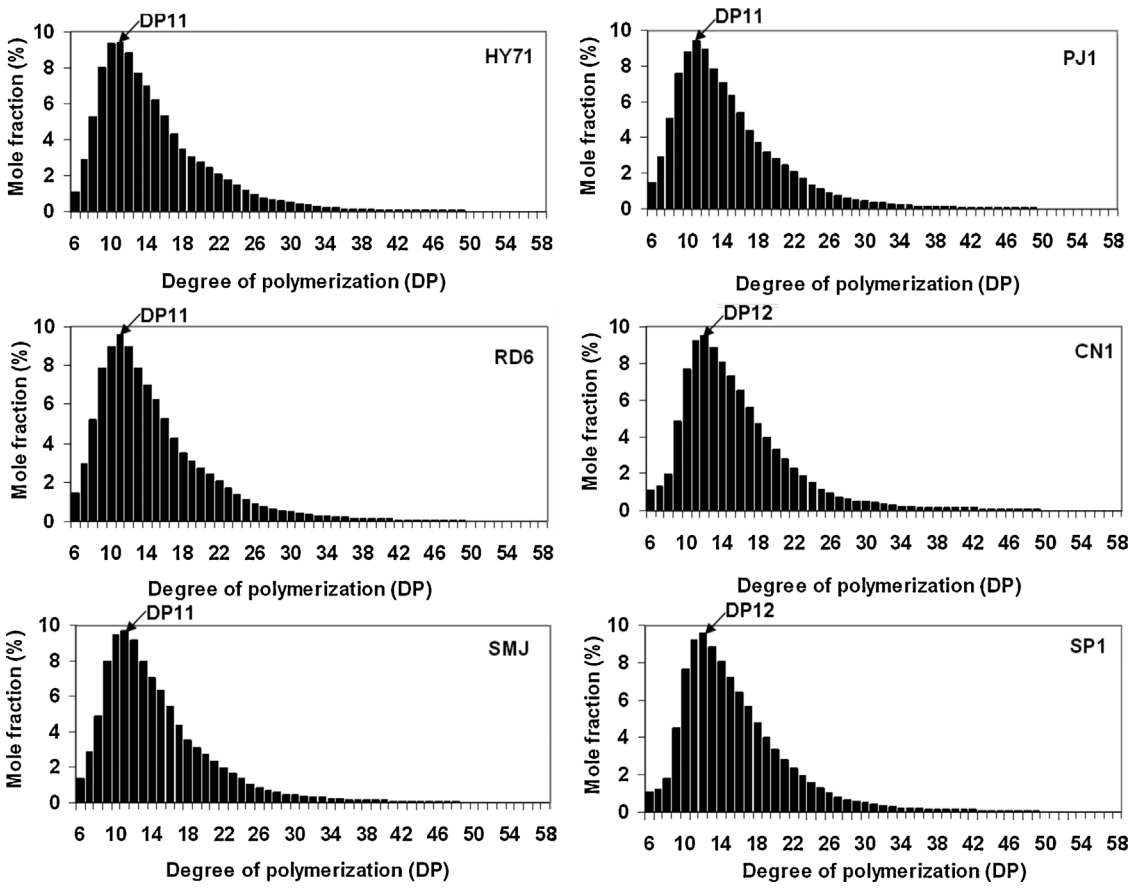

Fig. 2. Chain length distribution of rice amylopectin by mol fraction.

3.5. *Possible relationships between molecular parameters of rice APs and pasting properties of rice starches*

The starch peak viscosity (PV) mutually increased not only with an increase of molecular size of AP ($\overline{M_n}$ and $\overline{M_w}$) ($r = 0.86$, $r = 0.92$), but also with increases of the proportion of very short chains (DP 6–12) ($r \sim 0.58$) (Table 6). On the contrary, the PV decreased when $\overline{CL}$, $\overline{ECL}$, as well as the proportions of f_{b1} (DP 13–24) and f_{b3} (DP ≥ 37) increased ($r \sim -0.52$ to -0.60) or vice versa. Based on their correlation coefficient values, molecular size seemingly had a stronger relationship with starch PV than did the branching characteristics and proportions of chain distribution. The negative values of r between $\overline{M_n}$, $\overline{M_w}$ ($r = -0.73$, $r = -0.63$) and hot paste viscosity (HV) implied that starch containing larger AP molecules, especially the prevalent molecules, had lower resistance to shear. Additionally, $\overline{CL}$ and $\overline{ECL}$ mutually increased with HV ($r \sim 0.65$–0.68). The longer external chains probably facilitated molecular entanglement with other molecules, resulting in maintaining gelatinized starch granule structure and thus, providing higher shear resistance (Han & Hamaker, 2001). Similar findings of the correlations between AP molecular parameters and breakdown (BD) which is the difference between PV and HV as those of the molecular parameters and PV were reported. Higher proportions of the f_{b1} and f_{b3} seemingly decreased BD whereas higher proportions of the very short chains (f_a) increased it. The strong correlations of $\overline{M_n}$, $\overline{M_w}$, $\overline{ECL}$, the proportion of f_{b3} (DP > 37), FV and SB (Table 6) suggested that starch containing smaller AP molecules which carried longer external chains and contained a higher proportion of long chains tended

Table 5
Pearson's correlation between average molecular weight and branching characteristics of rice amylopectin.

Parameters	$\overline{M_n}$	$\overline{M_w}$	$[\eta]$	$\overline{CL}$	$\overline{ECL}$	$\overline{ICL}$	$\%\beta$
$\overline{M_n}$	1.00						
$\overline{M_w}$	0.96**	1.00					
$[\eta]$	−0.68*	−0.56	1.00				
$\overline{CL}$	−0.94**	−0.84**	0.88**	1.00			
$\overline{ECL}$	−0.94**	−0.85**	0.86**	0.99**	1.00		
$\overline{ICL}$	−0.50*	−0.42	0.62*	0.62**	0.50*	1.00	
%β-Amylolysis	−0.90*	−0.83**	0.80**	0.92**	0.97**	0.27	1.00
Mol fraction (%) (DP 6 to DP 58)							
f_a (%) (DP 6–12)	0.76**	0.75**	−0.58*	−0.77**	−0.75**	−0.55*	−0.68**
f_{b1} (%) (DP 13–24)	−0.76**	−0.76**	0.56	0.76**	0.74**	0.53*	0.68**
f_{b2} (%) (DP 25–36)	−0.26	−0.23	0.67*	0.46	0.40	0.61**	0.29
f_{b3} (%) (DP ≥ 37)	−0.81**	−0.72**	0.82**	0.91**	0.89**	0.60**	0.82**

* Correlation is significant at the 0.05 level ($P < 0.05$).
** Correlation is significant at the 0.01 level ($P < 0.01$).

Table 6
Correlations between amylopectin structure and rice starch properties.[A]

Parameters	RVA parameters						SP	SS (%)	T (%)
	PT	PV	HV	BD	FV	SB			
$\overline{M_n}$	−0.81**	0.86**	−0.73**	0.89**	−0.86**	−0.86**	0.97**	−0.77**	0.84**
$\overline{M_w}$	−0.70*	0.92**	−0.63*	0.90**	−0.75**	−0.75**	0.93**	−0.73**	0.85**
$[\eta]$	0.57*	−0.36	0.42	−0.42	0.80**	0.85**	−0.76**	0.87**	−0.32
$\overline{CL}$	0.82**	−0.67*	0.65*	−0.73**	0.90**	0.92**	−0.94**	0.87**	−0.66*
$\overline{ECL}$	0.82**	−0.68*	0.68*	−0.74**	0.92**	0.94**	−0.94**	0.84**	−0.70*
$\overline{ICL}$	0.42	−0.33	0.18	−0.31	0.36	0.39	−0.47*	0.68**	−0.14
%β-Amylolysis	0.79**	−0.66*	0.70*	−0.74*	0.92**	0.93**	−0.92**	0.75**	−0.74*
Mol fraction (%) (DP 6 to DP 58)									
f_a (%) (DP 6–12)	−0.74*	0.58*	−0.16	0.49*	−0.48*	−0.53*	0.66**	−0.83**	0.45
f_{b1} (%) (DP 13–24)	0.75*	−0.60*	0.17	−0.50*	0.48*	0.52*	−0.66**	0.82**	−0.47
f_{b2} (%) (DP 25–36)	0.29	−0.09	−0.11	−0.02	0.19	0.25	−0.27	0.71**	0.09
f_{b3} (%) (DP ≥ 37)	0.77*	−0.52*	0.33	−0.50*	0.70*	0.75*	−0.78**	0.90**	−0.43

* Correlation is significant at the 0.05 level ($P < 0.05$).
** Correlation is significant at the 0.01 level ($P < 0.01$).
[A] PT, pasting temperature; PV, peak viscosity; HV, hot paste viscosity; BD, breakdown viscosity (PV–HV); FV, final viscosity; SB, setback viscosity (FV–HV); SP, swelling power; SS, soluble starch; T, transmittance.

to have a higher final viscosity (FV). The shape of those AP molecules probably was rather closer to AM molecules which easily associate between themselves and significantly contribute a network formation, resulting in increases of FV and SB during cooling (Noosuk et al., 2005; Park, Kim, Kim, & Lim, 2009). The directions of the statistical correlations of molecular shape and size of AP, SP, and soluble starch (SS) also appeared to support the explanation of significance of size and shape of AP molecules and starch pasting properties.

The percentage of light transmittance of 1% NG APs was positively correlated with the size of AP molecules ($\overline{M_n}$ and $\overline{M_w}$), but negatively correlated with $\overline{CL}$ and $\overline{ECL}$ ($r \sim 0.8$, $r \sim -0.6$ to -0.7). It appeared that large molecules with shorter external chains increased light transmittance of the solution. Craig et al. (1989) reasoned that collapsed conformation of the starch molecules occur during starch dissolution, which results in formation of ordered junction zones. Any junction zones which are larger than the wavelength of the illumination decreased paste clarity since the light scattered from the surface of those junction zones when returned to the observer causes light transmittance reduction. Probably, both high branching and short chain length of AP large molecules were the keys which deter intermolecular hydrogen bonding, double helical formation, helical aggregation, which are the causes of junction zone. Consequently, smaller sizes of junction zones were formed and the solution was rather clear.

4. Conclusions

The $\overline{M_n}$ and $\overline{M_w}$ of HAM APs were ~33–50% and ~71–91% of those of waxy APs. The HAM APs containing higher $\overline{CL}$, $\overline{ECL}$, and a proportion of DP ≥ 37 displayed $[\eta] \sim 12$–28% higher than waxy APs. Besides PJ1, HAM APs had the mol proportion of DP 6–12 ~ 10 percentage point less, but had the proportion of DP 13–24 ~ 9 percentage point higher, compared with waxy APs. Different starch pasting properties were found not only between waxy and HAM starches, but also found within starches containing similar AM content. The waxy AP solutions from HY71 and RD 6 yielded the highest value of % light transmittance whereas the HAM AP solution from PJ1 yielded the lowest value. It appeared that larger AP molecules had shorter average chain length and external chain length, and AP with higher proportions of f_{b1} (DP 13–24), and f_{b3} (DP ≥ 37) had high $\overline{CL}$ and $\overline{ECL}$. It was found that when molecular size of AP increased, PV of starch increased but HV decreased. In contrast, smaller size of AP with higher $\overline{ECL}$ and a higher proportion of DP > 37 had higher FV of starch. The light transmittance of AP solution increased when molecular size of AP increased or vice versa.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.02.081.

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