Structures of Four Waxy Rice Starches in Relation to Thermal, Pasting, and Textural Properties

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ABSTRACT

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Waxy rice starches from three japonica cultivars (Taichung Waxy 1 [TCW1], Taichung Waxy 70 [TCW70], Tachimemochi) and one indica cultivar (Tainung Sen Waxy 2 [TNSW2]) were characterized for chemical and physicochemical properties. The amylopectin structures were different for the four waxy rice starches in terms of degree of polymerization (DP), average chain length (CL), exterior chain lengths (ECL), and distribution of chains, indicating the existence of varietal differences. The order of swelling power was TCW1 > TCW70 > TNSW2 > Tachimemochi; the order of water solubility index was TCW70 > TNSW2 > Tachimemochi > TCW1. The low water solubility index of TCW1 might be ascribed to a high DP. All starches shared similar gelatinization temperatures and enthalpies but showed distinct retrogradation patterns.

TNSW2 showed the highest retrogradation rate, followed by TCW2, Tachimemochi, and TCW70. TCW70 exhibited the highest overall pasting viscosity, followed by TNSW2, TCW1, and Tachimemochi. The hardness of waxy rice starch pastes from a Brabender amyloviscograph increased rapidly after storage at 5°C for one day and remained the same or slightly increased after seven days of storage, whereas the opposite trend was observed for adhesiveness. The lower degree of retrogradation of TCW70 was probably a result of a larger amount of A chain and a shorter ECL. The changes in hardness correlated with the amount of A and B1 chains. The texture attributes of waxy rice starch pastes were significantly affected by amylopectin retrogradation during storage.

Waxy rice (*Oryza sativa* L.), also called glutinous or sweet rice, is characterized by its opaque appearance and very low amylose content. Waxy rice can be classified into two subspecies, indica and japonica, and there are many cultivars in each subspecies. In Asia, indica and japonica waxy rices are used for different rice products. Indica waxy rice is used in rice tamale and rice pudding for its tenderness, while japonica waxy rice is used for sweetened rice cake due to its stickiness (Villareal et al 1993). Earlier studies have shown that waxy rices with high gelatinization temperature (GT) had a harder texture than did the low-GT rices (Antonio and Juliano 1974). The intermediate-GT waxy rice starch had a higher gel viscosity than did the low-GT starch (Perdon and Juliano 1975).

Because the amylose content in waxy rice starch is usually <2%, the amylopectin structure plays a determinant role in the physicochemical properties of this starch, including the average degree of polymerization (DP), average chain length (CL), degree of branching, and chain-length distribution profile. Shi and Seib (1992) reported that the retrogradation of waxy starches was directly proportional to the mole fraction of unit chains with DP 14–25 and inversely proportional to the mole fraction of unit chains with DP 6–9. The DP of amylopectin negatively correlated with the hardness of stale cooked rice for low-GT waxy rices (Villareal et al 1993). The amylopectin content affected the swelling behavior of cereal starches (Tester and Morrison 1990; Morrison et al 1993) and the integrity of swollen starch granules determined the rheological properties of a starch paste or gel (Tsai et al 1997). Although varietal differences in amylopectin structure of waxy rice starches and the relation to physicochemical properties have been reported (Perdon and Juliano 1975; Juliano and Villareal 1987; Villareal et al 1993; Lu et al 1997; Tsai et al 1997; Villareal et al 1997), there is limited information on the contribution of starch structure to the different pasting and textural properties of waxy rice starches. Because many cultivars of waxy rice with different functionalities are commercially available and waxy rice starch consists of essentially 100% amylopectin, they serve as a good model to study the structure-functionality relationship of amylopectin. This study was initiated to provide additional information to better understand the relationship of amylopectin structure to thermal, pasting, and textural properties of four waxy rice starches.

MATERIALS AND METHODS

Starches

Samples of four cultivars of milled waxy rice were obtained from the Taiwan Agricultural Research Institute, Taiwan, in 2000. There were three japonica cultivars (Taichung Waxy 1 [TCW1], Taichung Waxy 70 [TCW70], Tachimemochi) and one indica cultivar (Tainung Sen Waxy 2 [TNSW2]).

Starch was isolated from milled rice by following a modified alkaline steeping method (Yang et al 1984). Dried starch was defatted with water-saturated-1-butanol (WSB) by shaking the suspension (5 g of starch in 25 mL of WSB) on a rotary shaker for 24 hr at room temperature and then centrifugation (15 min at 3,000 rpm). Defatted starch was dried in a convection oven at 40°C overnight. The apparent amylose content was determined by measuring the iodine affinity of defatted starch according to Schoch (1964).

Structural Characterization

The carbohydrate distributions of native and debranched maltodextrins were evaluated by using high performance size-exclusion chromatography (HPSEC) and high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). A Waters HPSEC system equipped with 515 HPLC pump with an injector of 100 μL sample loop, an in-line degasser, and a 2410 refractive index detector maintained at 40°C was used in this study. The separation of both native and debranched starches was accomplished by a series of Shodex OHpak columns, including a guard column, KB-802 and KB-804 columns, and columns were maintained at 55°C with a column heater. The mobile phase was $0.1M$ $NaNO_3$ and 0.2% NaN_3 at a rate of 0.7 mL/min. Dextran standards, ranging from 5,200 to 872,300 of weight average molecular-weight (M_w) (PSS Polymer Standards Service-USA) and α 1-4 linked sugars with degree of polymerization (DP) 1 to 7 (Sigma Chemical Co.) were used to construct the regression line for molecular weight (MW) determination.

The debranched maltodextrins were prepared by following the method of Kasemsuwan et al (1995), except that 6 mg of starch was dissolved in 3.2 mL of distilled water. The branch chain-length distribution of debranched starch was further elucidated with a Dionex DX-500 HPLC system (Sunnyvale, CA) by following the method of Kasemsuwan et al (1995). Sugars with DP 1–7 were used to identify the chromatographic peaks. The assignment for the chromatographic peaks with DP > 7 was based on the assumption that each successive peak represented a saccharide that was 1 DP longer than that of the previous peak. A mixed bed exchange resin

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(IONAC NM-60, J.T. Baker) was added to the debranched solutions for 1 min to eliminate the interference from the buffer before HPSEC and HPAEC-PAD analyses. The branch chain-length distribution was calculated based on the relative peak areas up to DP 63.

The β-amylolysis limit was determined by hydrolyzing the starch samples (9.0 mg) with β-amylase (150 U) at 30°C in 50 mM acetate buffer (pH 4.8) for 180 min. Maltose produced was determined by the methods of Somogyi (1952) and Nelson (1944). The average degree of polymerization of amylopectin was calculated from the reducing value equivalent to glucose and total carbohydrate measured by the anthrone-H_2SO_4 method (Koehler 1952). The average number of chains per molecule was calculated as DP/average chain length.

Physicochemical Properties

Swelling power (SP) and water solubility index (WSI) were measured by suspending a 1.7% starch suspension (0.5 g of starch in 30 mL of water) into a centrifuge tube with cap and heating the tube from 55 to 95°C at 10°C intervals and the sample was kept at that temperature for 30 min. The heated sample was cooled rapidly to room temperature in an ice water bath and centrifuged at 8,000 rpm for 20 min. SP was determined by measuring the sedimented paste weight and WSI by the solid content of the supernatant (Holm et al 1985).

Thermal properties, including gelatinization and retrogradation, were assessed by a Perkin-Elmer Pyris-1 differential scanning calorimeter (DSC, Perkin-Elmer Co., Norwalk, CT) following the method of Wang et al (1992). Gelatinized samples were stored at 5°C for 7, 14, 21 and 28 days and then subsequently rescanned to determine the retrogradation enthalpy with the same conditions used in the gelatinization test. Degree of retrogradation was calculated as the ratio of gelatinization enthalpy to retrogradation enthalpy. Rescanning was done after allowing the samples to equilibrate for 1 hr at room temperature. Triplicate measurements were performed on each cultivar per storage period.

Pasting properties of rice starch were characterized according to Approved Method 61-01 (AACC 2000) using a Brabender Viskograph-E (C.W. Brabender Instruments, South Hackensack, NJ) equipped with a 700-cmg cartridge operated at a speed of 75 rpm. Starch (45 g, 10% db) was mixed with water to a final weight of 450 g and the slurry was heated from 50 to 95°C at a rate of 1.5°C/min, held at 95°C for 20 min and cooled down to 50°C at a rate of 1.5°/min. The gelatinized starch pastes prepared by the Brabender Viskograph-E were used to measure textural properties

with a texture analyzer (TA-XT2i, Texture Technologies Corp., Scarsdale, NY) after 0, 1, and 7 days of storage at 5°C. The gel was compressed at a speed of pretest 2.0 mm/sec, test 0.5 mm/sec, and posttest 2.0 mm/sec, with 15-g trigger force to a distance of 1.0 mm with an aluminum plate probe (5.08 cm dia.) under the measure force in compression test mode. The peak force of the first penetration was reported as hardness and the negative force area after the first penetration was reported as adhesiveness (Bourne 1978). Triplicate measurements were performed on each starch sample.

Statistical Analysis

All analyses were done in duplicate unless otherwise indicated. Experimental data were analyzed by using the general linear models procedure (SAS Software Institute, Cary, NC 1999) to identify differences among data. All significant differences were reported at the 95% confidence interval unless otherwise indicated.
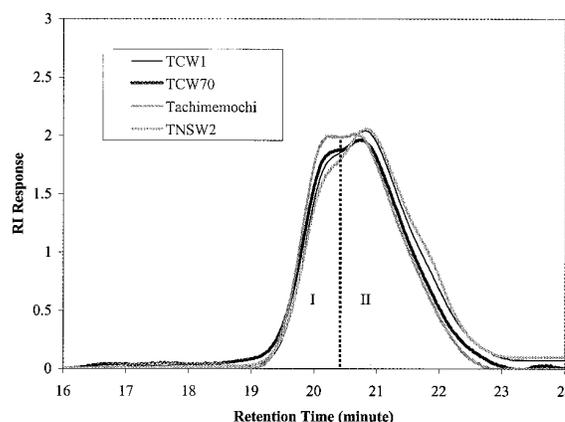

Fig. 1. Carbohydrate profiles of isoamylase-debranched waxy rice starches by HPLC. Fraction I: long B chains. Fraction II: A and short B chains.

Fig. 2. Swelling power (**A**) and water solubility index (**B**) of waxy rice starches.

TABLE I
Chain-Length Distribution of Waxy Rice Starches[a]

| Sample | Mole Fraction (%) | | | | |
	A (DP 5-12)	B1 (DP 13-24)	B2 (DP 25-36)	B3+ (DP 37-63)	A/B1-3+
TCW1	46.6	41.9	7.3	4.2	0.87
TCW70	46.9	42.2	6.9	4.0	0.88
Tachimemochi	47.1	41.2	7.3	4.3	0.89
TNSW2	44.7	43.0	7.8	4.5	0.81

[a] DP = degree of polymerization.

TABLE II
Structural Parameters of Waxy Rice Starches[a]

Sample	β-Amylolysis Limit (%)	DP	CL	NC	ECL	ICL
TCW1	69.2	3,218	20.2	159.3	16.0	3.2
TCW70	44.9	2,740	20.0	137.0	11.0	8.0
Tachimemochi	43.2	2,721	20.4	133.4	10.8	8.6
TNSW2	59.8	2,736	20.7	132.2	14.4	5.3

[a] DP = degree of polymerization; CL = average chain length in glucose units; NC = average number of chain (DP/CL); Exterior chain length in glucose units, ECL = (CL × β-amylolysis limit) + 2; ICL = interior chain length in glucose units (CL − ECL) − 1.
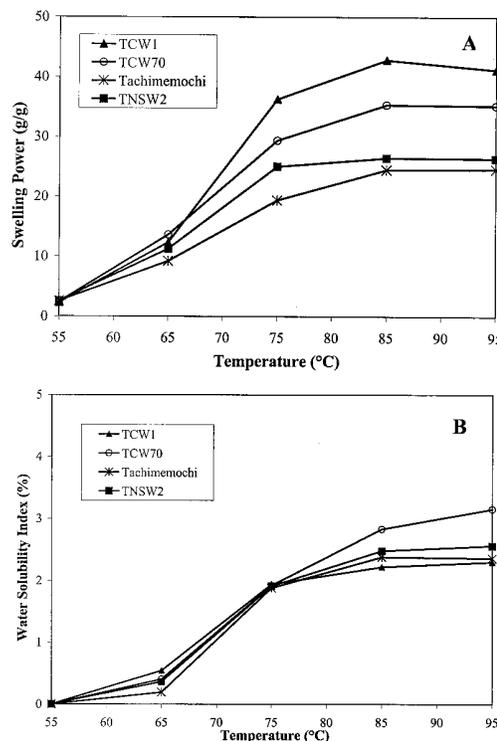
RESULTS AND DISCUSSION

Structural Characterization

All waxy rice starches had iodine affinity values close to 0, indicating that little apparent amylose was present in these samples. Therefore, the influence of amylose on the physicochemical properties of waxy rice starches could be neglected in this study. Fig. 1 presents the carbohydrate profiles of isoamylase-debranched waxy rice starches. All cultivars exhibited similar profiles with two fractions: fraction I (Fr. I) consisted of HMW molecules including long B chains, and fraction II (Fr. II) consisted of LMW molecules including A and short B chains. TCW1 and TNSW2 consisted of a significantly larger proportion of Fr. II (68%) than did TCW70 and Tachimemochi (62 and 61%, respectively). The mole fractions of amylopectin calculated from HPAEC-PAD results are grouped into chain types and the corresponding DP according to Hanashiro et al (1996) and given in Table I. TNSW2, an indica cultivar, had a significantly lower percentage of A chain (44.7%) and a higher percentage of B1 chain (43.0%). TCW70 consisted of a larger amount of A+B1 chains (89.1%), whereas TNSW2 contained a smaller amount of A+B1 chains (87.7%). Consequently, TNSW2

had the lowest A/B ratio (0.81), while the three japonica cultivars shared a similar ratio (0.87–0.89).

Table II summarizes the structural parameters of four waxy rice starches. TCW1 had the highest β-amylolysis limit, followed by TNSW2, TCW70, and Tachimemochi. The β-amylolysis limit values correlated with the proportion of Fr. II (Fig. 1), with TCW1 having the highest β-amylolysis limit and the largest Fr. II. TCW1 also had the largest DP (3,218) while the other three had a similar DP. TNSW2 had the longest average chain length (20.7) and TCW70 had the shortest CL (20.0). The differences in the average number of chains of amylopectin reflected differences in DP. TNSW2, an indica cultivar, did not contain fewer chains than the japonica cultivar as found by Lu et al (1997). Although TCW1, TCW70, and Tachimemochi were from japonica subspecies and shared a similar A/B ratio, they showed very different exterior (ECL) and interior (ICL) chain lengths besides their differences in DP. Both TCW70 and Tachimemochi had a shorter ECL and a longer ICL, corresponding to their low β-amylolysis limit, whereas TCW1 and TNSW2 had a longer ECL and a shorter ICL. TCW1 had the largest DP and also the longest ECL. These results clearly indicate that the fine structures of amylopectins from different waxy rice starches were different in addition to differences in mean molecular size and molecular weight distribution proposed by Juliano and Villareal (1987).

Swelling Power and Water Solubility Index

The changes in swelling power (SP) and water solubility index (WSI) of waxy rice starches during heating are shown in Fig. 2A and 2B, respectively. All starches exhibited a moderate swelling during 55–65°C and 75–85°C, little change between 85–95°C, and a rapid swelling from 65 to 75°C, where the gelatinization occurred. No two-stage swelling was noted in this study as observed by Tsai et al (1997), possibly due to different experimental conditions or sample variation. Similarly, all cultivars also experienced a rapid rise in WSI from 65–75°C and a slow increase in other temperature ranges.

TABLE III
Thermal Properties of Waxy Rice Starches[a]

Sample	Onset Temp. (°C)	Peak Temp. (°C)	Enthalpy (J/g)
TCW1	64.9a	69.8ab	14.5a
TCW70	63.3b	69.2b	14.5a
Tachimemochi	64.7a	70.3a	14.6a
TNSW2	64.3a	69.6ab	14.5a

[a] Mean value of triplicates in the same column and same starch with different letters are significantly different ($P < 0.05$).
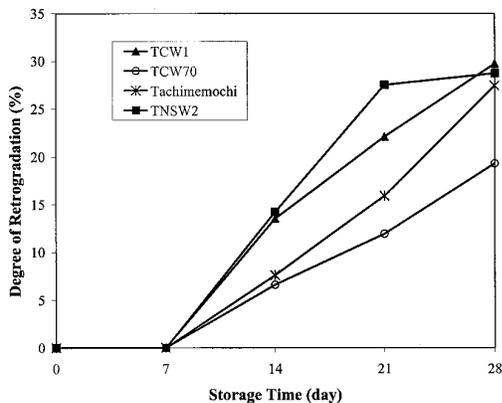

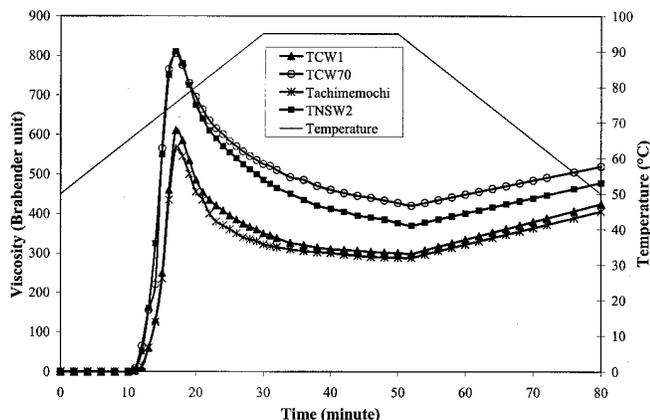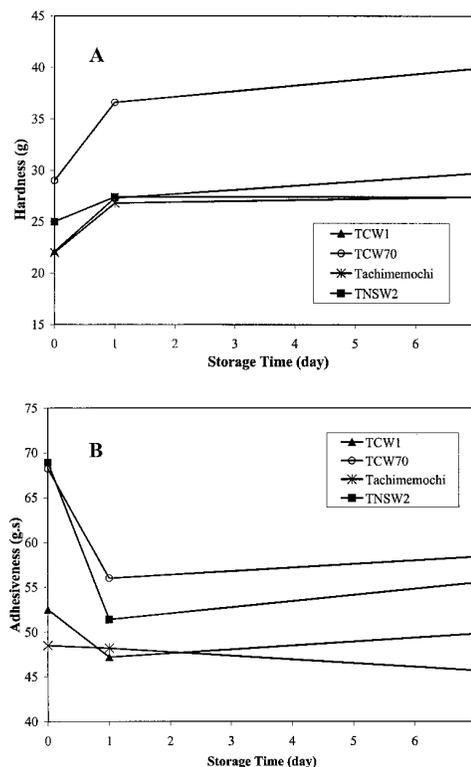**Fig. 3.** Degree of retrogradation of waxy rice starches during storage at 5°C.

Fig. 4. Brabender amylographs (10% starch, db) of waxy rice starches.

Fig. 5. Hardness (**A**) and adhesiveness (**B**) of waxy rice starch pastes during storage at 5°C.

TCW1 exhibited the highest degree of swelling, followed by TCW70, TNSW2, and Tachimemochi. Because the swelling behavior of cereal starch is primarily related to amylopectin (Tester and Morrison 1990) and the rigidity of starch granule was inversely proportional to the SP (Lii et al 1996), the high SP suggests less rigid granular structure of TCW1 and TCW70. The order of WSI was similar to that of SP except TCW1 had the lowest WSI. Unlike the others, the WSI of TCW70 increased continuously at >75°C and reached a WSI of 3.15% at 95°C. The lowest WSI of TCW1 may be related to the largest DP, which was also observed by Juliano and Villareal (1987). The large DP molecule might have less tendency to leach out during heating. The high WSI of TCW70 might result from a higher degree of swelling and a lower DP.

Thermal Properties

Table III lists the gelatinization temperatures and enthalpies of four waxy rice starches measured by DSC. The samples were comparable in onset temperature, peak temperature, and enthalpy except TCW70 had significantly lower onset and peak temperatures. Although TNSW2 consisted of a smaller amount of A+B1 chains, which constituted the crystalline regions of starch granule, similar energy was consumed by TNSW2 as by the others. No retrogradation was noted for gelatinized samples stored at 5°C until after seven days of storage and the order of retrogradation was TNSW2 > TCW1 > Tachimemochi > TCW70 (Fig. 3) during the course of storage. No correlation was found between A chains and peak temperature-enthalpy as suggested by Noda et al (1998, 2001). The greater retrogradation of TNSW2 can be explained by a higher proportion of B1 chain and a longer CL, which agrees with previous results of Shi and Seib (1992) and Suzuki et al (1985), respectively. It is possible that B1 chains with DP 18–21 of amylopectin representing the full length of the crystalline region (Jane et al 1999) and longer branches tended to form helix. The smaller retrogradation of TCW70 and Tachimemochi can be attributed to higher proportions of A chain and a shorter ECL. Although TCW1 had a similar amount of A chain as TCW70 and Tachimemochi, the ECL of TCW70 was significantly longer, which might contribute to greater retrogradation similar to that of TNSW2. The present results indicate the importance of ECL in affecting starch retrogradation and suggest that retrogradation of amylopectin might be controlled by a combined effect of the amount of A chain, B1 chain, CL, and ECL.

Pasting Properties

The Brabender amylograms of four waxy rice starches are presented in Fig. 4. They exhibited a similar pasting temperature but TCW70 and TNSW2 showed significantly higher peak, hot, and cool paste viscosities than did TCW1 or Tachimemochi. TCW70 and TNSW2 also had a significantly larger breakdown and setback but a slightly smaller gel consistency compared with TCW1 and Tachimemochi. The order of Brabender viscosity followed a trend similar to the SP, except that TCW1 had a low viscosity similar to that of Tachimemochi. It is possible that TCW1 had a weaker granular integrity as suggested by high SP (Tsai et al 1997) and was prone to fragmentation from shear exerted by Brabender amylograph. Svegmark and Hermansson (1990, 1991) also observed a major effect from shearing than from heating when studying the viscoelastic behavior of potato starch dispersions and stated that shear significantly changed the viscoelastic behavior of starch pastes. The high peak viscosity of TCW70 and TNSW2 could be a result of a higher SP and a higher concentration of hot-water soluble as evidenced by high WSI.

Textural Properties

Although no real gels formed, the hardness of starch pastes increased rapidly during the first day of storage and then remained unchanged or slightly increased, whereas the opposite trend was observed for adhesiveness (Fig. 5A and 5B). As hardness increased, adhesiveness decreased during storage. TCW70 displayed a significant increase in hardness and a significant decrease in adhesiveness during storage, indicating that the retrogradation of amylopectin would have a great impact on the texture attributes. Both TCW70, an japonica cultivar, and TNSW2, an indica cultivar, exhibited similar adhesiveness, supporting the work by Villareal et al (1993) that japonica waxy rices were not necessarily more sticky than indica waxy rices.

When comparing structural parameters with textural properties, the increase in hardness positively correlated with the amount of A+B1 chain ($r = 0.99$, $P = 0.08$). The results suggest that the recrystallization of A and B1 chain contributed to the hardness of waxy rice starch paste during storage, which agrees with Villareal et al (1997) that retrogradation of amylopectin was enhanced by the A+B1 fraction, located in one cluster of the starch granule (Hizukuri 1986). As discussed earlier, retrogradation of waxy rice starch as measured by DSC was affected by the amount of A chain, B1 chain, CL, and ECL. In this study, gelatinization by DSC described the order-disorder transition of starch granule heated in excess of water (Donovan 1979), while pasting by Brabender amylograph was related to the swelling of granules and the solubilization of starch molecules (Svegmark and Hermansson 1990). Because starch granules were swollen but not disrupted during gelatinization (Abd Karim et al 2000), starch molecules might still retain their inherent molecular arrangement; thus A chain and ECL might be a more important factor in impeding retrogradation. On the other hand, as the shear of Brabender amylograph disrupted the inherent molecular arrangement to form the solubilized molecules and starch fragments, both A and B1 chains might assist in the recrystallization process and a large amount of A+B1 chains would contribute to more recrystallized domains and consequently a firmer texture.

As granular structure and composition were suggested as the major factors affecting the rheological properties of rice starch pastes and gels (Lii et al 1996), the present results provide additional information that the DP, ECL, and the amounts of A and B1 chains might be the important structural parameters determining the physicochemical properties of waxy rice starches.

CONCLUSIONS

The present study demonstrated that the fine structures of amylopectins were different for different waxy rice cultivars and their differences in structure determined their different physicochemical properties. The amylopectin retrogradation as measured by DSC was likely not controlled by the same mechanism as that measured by the change in hardness of starch paste from Brabender amylograph because of the involvement of shear in Brabender amylograph. Shear disrupted the starch granular structure and released more water-soluble components compared with only swollen but undisrupted granule in DSC measurement. Therefore, the retrogradation of waxy rice starch might be reduced by the presence of a large amount of A chain and a short ECL, whereas the increase in hardness of waxy rice starch paste might be favored by a large amount of A+B1 chains. The large DP of amylopectin might contribute to a lower WSI despite of its high SP.

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