

# Properties of Starches from Several Low-Amylose Rice Cultivars

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## ABSTRACT

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The starch properties of five low-amylose rice cultivars, Yawarakomachi, Soft 158, Hanabusa, Aya, and Snow Pearl, were compared with those of two normal amylose rice cultivars, Nipponbare and Hinohikari. There were no large differences in the distributions of the amylopectin chain length determined by high-performance anion-exchange chromatography, and the starch gelatinization properties determined by differential scanning calorimetry, between normal and low-amylose rice

cultivars. Results obtained using rapid viscosity analysis indicated that low-amylose rice starches had lower peak viscosity, breakdown, and setback values than normal amylose rice starches. Starch granules from low-amylose rice cultivars had a higher susceptibility to glucoamylase than those from normal amylose rice cultivars. The results of this study showed some differences between normal and low-amylose rice starches in pasting properties and enzymatic digestibility.

Because starch is the largest component of rice endosperm, the texture of cooked rice is largely governed by the starch properties. Therefore, the development of new cultivars that possess unique starch properties is one objective of rice breeding. Starch is composed of essentially linear amylose and highly branched amylopectin. Normal starch generally consists of 15–25% amylose and 75–85% amylopectin. It is well established that the amylose-amylopectin ratio is a major factor influencing the physicochemical properties of starch. Amylose has the ability to form a firm gel, whereas amylopectin displays low syneresis and high resistance to retrogradation. Several mutant genes of rice have affected the amylose content of starch. Starch from a waxy mutant is composed of only amylopectin and no amylose. The endosperm from a dull mutant of rice contains starch with an intermediate amylose content between waxy (0%) and normal amylose (15–25%) starches (Okuno et al 1983). A dull mutant of rice is defined as a representative low-amylose cultivar. The boiled rice of a low-amylose cultivar is stickier and glossier than that of a normal amylose cultivar. A promising use for low-amylose rice cultivars is for blending. For example, the eating quality of cooked rice can be improved by blending a low-amylose rice cultivar with some normal amylose cultivars. In Japan, low-amylose rice cultivars have been developed in the past decade (Uehara et al 1995; Tanno et al 1997; Higashi et al 1999; Okamoto et al 2001).

The properties of starches from low-amylose rice cultivars have been studied by several research groups (Okuno et al 1983; Asaoka et al 1989, 1993, 1994a,b; Kuno et al 2000). Low-amylose rice cultivars exhibited higher peak viscosity and breakdown and lower setback (Kuno et al 2000). Furthermore, concerning the structure of amylopectin, no conspicuous differences in the amylopectin chain length were found between normal and low-amylose (dull-mutant) rice cultivars (Okuno et al 1983; Asaoka et al 1989, 1993, 1994a); however, Kuno et al (2000) concluded that the ratios of the shorter to longer chains of low-amylose rice cultivars were slightly smaller than those of normal amylose rice cultivars. However, not much is known about the characteristics of starches from several low-amylose rice cultivars recently released in Japan (Kuno et al 2000).

Accordingly, the present report focuses on the differences in the starch properties of normal and low-amylose rice cultivars to determine the effects of a marked reduction in amylose content on the properties of the endosperm starch of rice. Five Japanese low-amylose

rice cultivars, Yawarakomachi, Soft 158, Hanabusa, Aya, and Snow Pearl, and two Japanese normal amylose cultivars, Nipponbare and Hinohikari, were used in this study.

## MATERIALS AND METHODS

### Rice Samples

Two Japanese normal amylose rice cultivars, Nipponbare and Hinohikari, and a Japanese low-amylose rice cultivar, Yawarakomachi, were grown at the National Agricultural Research Center for the Kyushu Okinawa Region (formerly, the Kyushu National Agricultural Experiment Station) at Chikugo, Fukuoka (Kyushu region) during the 1998 and 1999 growing seasons. Five Japanese low-amylose rice cultivars, Yawarakomachi, Soft 158, Hanabusa, Aya, and Snow Pearl, were grown at Fukuoka Agricultural Research Center at Chikushino, Fukuoka (Kyushu region) during the 1999 growing season. Nipponbare, Hinohikari, Yawarakomachi, and Soft 158 are suitable for the Kyushu region in southwestern Japan, whereas Hanabusa and Aya are suitable cultivars for the Hokkaido region in the northernmost part of Japan and Snow Pearl for the Tohoku region in the second northernmost part of Japan.

The rice seeds were hulled and milled into flour. The rice flours (50 g) were steeped in 0.2% sodium hydroxide (400 mL) at 4°C overnight to remove the protein. The supernatant was discarded, and the alkaline extraction was repeated twice. The residue was successively washed with distilled water and passed through a 62- $\mu$ m sieve. The filtrate was allowed to stand until the starch granules precipitated. The starch granules were then washed with distilled water and ethanol several times and dried at room temperature.

### Analytical Methods

The blue value (BV) at 680 nm was recorded on mixtures (25 mL) containing 1 mg of starch, 2 mg of iodine, and 20 mg of potassium iodine according to a modified method (Noda et al 1992a), eliminating the step of defatting of starch. The amylose content was calculated according to the equation proposed by Asaoka et al (1994a), who determined the properties of 51 rice starches: Amylose content =  $99.1 \times (\text{blue value}) + 5.0$ .

The enzymatic digestibility of the raw starch by crystalline glucoamylase of *Rhizopus niveus* was evaluated for 4 hr at 40°C with a substrate concentration of 2% (Noda et al 1992b).

Each starch was digested by crystalline isoamylase from *Pseudomonas amyloclavata*, as described earlier (Noda et al 1995a). The linear maltosaccharides produced after enzyme digestion were analyzed by high-performance anion-exchange chromatography (BioLC, Dionex Co., Sunnyvale, CA) equipped with pulsed amperometric detection (HPAEC-PAD) and a CarboPac PA1 Column (4  $\times$  250 mm) (Koizumi et al 1991). The area of each peak of linear chains up to DP 30 was determined using a Hitachi D-2500 Chromatointegrator.

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Differential scanning calorimetry (DSC) measurements were conducted at a heating rate of 10°C/min over the temperature range 30–130°C (DSC-7, Perkin-Elmer Co., Norwalk, CT) equipped with a 1020 TA workstation (Noda et al 2001b).

Measurements were made at 10% starch suspension using the Rapid Visco Analyser (RVA-3D) (Newport Scientific Pty. Ltd., Australia), as described previously (Noda et al 2000). The viscosity was measured in RVA units.

### Statistical Analysis

Determinations of amylose content and amylase digestibility were performed in triplicate. Other tests were done in duplicate. Each value is the mean of triplicate or duplicate measurements. Correlation coefficients between two parameters of structural properties and eight parameters of other physicochemical properties in 11 rice starches were calculated. Calculations were done using Microsoft Excel 5.0 for Macintosh.

## RESULTS AND DISCUSSION

### Amylose Content

The absorbance at 680 nm (blue value) and the amylose content are presented in Table I. The blue values (BV) were 0.200–0.224 and 0.086–0.148 for normal and low-amylose rice cultivars, respectively. Normal amylose cultivars definitely showed higher amylose

contents calculated from the BV (14.8–17.2%) than low-amylose cultivars (3.5–9.7%). Hinohikari, a normal amylose cultivar that was released to improve the eating quality of cooked rice had a lower amylose content (14.8–15.3%) than the cultivar formally known as Nipponbare (17.1–17.2%). Among the low-amylose cultivars, Yawarakomachi and Soft 158, which are suitable cultivars for the Kyushu region, exhibited a higher amylose content (7.4–9.7%). In contrast, the amylose content was lower for Hanabusa (3.9%), Aya (3.5%), and Snow Pearl (4.4%), which are adaptable to the normal season culture of northern Japan. These results were in good agreement with the data of Kuno et al (2000), who reported that the amylose contents were 9.6 and 3.3% for Soft 158 and Snow Pearl, respectively. In contrast, compared to our values, obviously higher values of amylose contents were obtained for Aya (9–16%) (Asaoka et al 1994a; Tanno et al 1997) and Snow Pearl (7–9%) (Higashi et al 1999) when they were grown under chillier conditions than those of the Kyushu region. Previous studies confirmed that the amylose content decreases as the environmental temperature increases during grain filling in rice (Asaoka et al 1984, 1985, 1989; Tanno et al 1997; Higashi et al 1999). In the case of low-amylose rice samples, dull mutants (Asaoka et al 1989), Aya (Tanno et al 1997), and Snow Pearl (Higashi et al 1999), the amylose content was lowered by 3–10% when the environ-

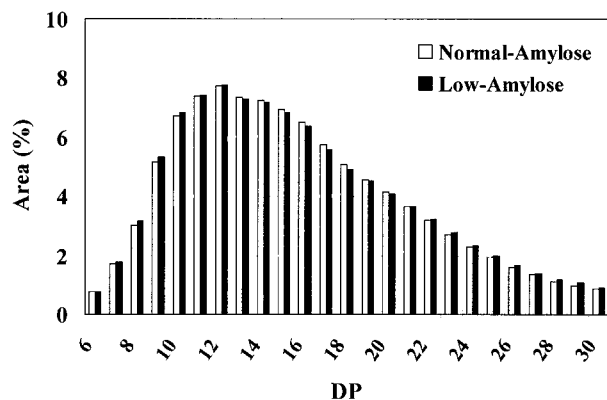


Fig. 1. Average values of chain length distributions (DP 6–30) of amylopectins from normal ( $n = 4$ ) and low-amylose ( $n = 7$ ) rice cultivars.

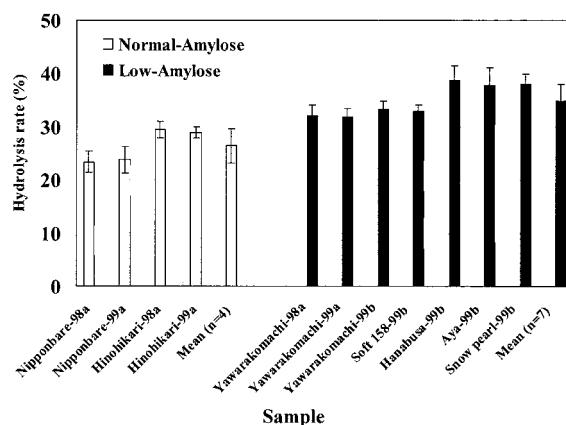


Fig. 2. Hydrolysis rate of starch granules from normal and low-amylose rice cultivars using crystalline glucoamylase. Values are means  $\pm$  SD of three determinations. See Table I for sample codes.

TABLE I  
Amylose Content, Amylopectin Chain Length, Gelatinization Properties by Differential Scanning Calorimetry (DSC) in Normal and Low-Amylose Rice Cultivars

Sample <sup>a</sup>	Blue Value <sup>b,c</sup>	Amylose <sup>c</sup> (%)	Amylopectin Chain Length <sup>d,e</sup>	DSC Characteristics <sup>f</sup> ( $n = 2$ )		
				$T_o$ (°C)	$T_p$ (°C)	$\Delta H$ (J/g)
Normal amylose						
Nipponbare 98a	0.224 $\pm$ 0.002	17.2 $\pm$ 0.2	0.328	62.2	67.0	15.4
Nipponbare 99a	0.223 $\pm$ 0.003	17.1 $\pm$ 0.3	0.321	62.2	67.9	15.0
Hinohikari 98a	0.200 $\pm$ 0.003	14.8 $\pm$ 0.3	0.331	58.9	65.4	14.8
Hinohikari 99a	0.205 $\pm$ 0.002	15.3 $\pm$ 0.2	0.327	59.2	65.9	14.5
Mean $\pm$ SD ( $n = 4$ )	0.213 $\pm$ 0.012	16.1 $\pm$ 1.2	0.327 $\pm$ 0.004	60.6 $\pm$ 1.8	66.6 $\pm$ 1.1	14.9 $\pm$ 0.4
Low amylose						
Yawarakomachi 98a	0.126 $\pm$ 0.001	7.4 $\pm$ 0.1	0.328	61.1	67.8	16.5
Yawarakomachi 99a	0.125 $\pm$ 0.003	7.4 $\pm$ 0.3	0.332	60.8	67.4	15.6
Yawarakomachi 99b	0.127 $\pm$ 0.002	7.6 $\pm$ 0.1	0.327	61.2	68.0	14.8
Soft 158 99b	0.148 $\pm$ 0.001	9.7 $\pm$ 0.1	0.333	60.0	65.7	14.7
Hanabusa 99b	0.090 $\pm$ 0.001	3.9 $\pm$ 0.1	0.327	60.3	67.8	15.1
Aya-99b	0.086 $\pm$ 0.001	3.5 $\pm$ 0.1	0.329	59.6	66.0	15.4
Snow Pearl 99b	0.094 $\pm$ 0.000	4.4 $\pm$ 0.0	0.336	58.3	65.9	15.0
Mean $\pm$ SD ( $n = 7$ )	0.114 $\pm$ 0.024	6.3 $\pm$ 2.3	0.330 $\pm$ 0.003	60.2 $\pm$ 1.0	66.9 $\pm$ 1.0	15.3 $\pm$ 0.6

<sup>a</sup> Harvested in 1998 or 1999; a, grown at National Agricultural Research Center for Kyushu Okinawa Region; b, grown at Fukuoka Agricultural Research Center.

<sup>b</sup> Absorbance at 680 nm.

<sup>c</sup> Values are means of three determinations.

<sup>d</sup> Peak area ratio of DP 6–12 to DP 6–30.

<sup>e</sup> Values are means of two determinations.

<sup>f</sup> Gelatinization onset, peak, and enthalpy values, respectively.

mental temperature during the 20 days after heading increased by 4–6°C. From these results, the lowering of the amylose content for Hanabusa, Aya, and Snow Pearl in this study appeared to be caused by the elevation of the environmental temperature during grain filling.

### Amylopectin Structure

To compare the structural differences in amylopectin molecules between normal and low-amylose rice cultivars, the distributions of the amylopectin chain length were measured by the HPAEC-PAD method. We used the rice starch samples debranched with *Pseudomonas amyloideramosa* isoamylase for HPAEC-PAD analysis. As shown in Fig. 1, the average values of the chain length distributions of amylopectin from low-amylose rice cultivars were similar to those from normal amylose rice cultivars. All amylopectins examined in this study showed a peak at DP 12. This observation was consistent with the results of Umemoto et al (1999), who analyzed several rice amylopectins using HPAEC-PAD. The peak area ratio of DP 6–12 to DP 6–30 was used as an indicator of the distribution of the amylopectin chain length. As shown in Table I, the ratios obtained for normal and low-amylose starches were 0.321–0.331 and 0.327–0.336, respectively. Thus, the present study showed that there were no remarkable differences in the amylopectin structure between normal and low-amylose starches. Similar results were obtained by Okuno et al (1983) and Asaoka et al (1989, 1993, 1994a) using gel filtration chromatography. Asaoka et al (1993) also demonstrated that higher environmental temperatures in the earlier developmental stages of seeds resulted in a lower ratio of the shorter to longer chains in dull mutants of rice. Kuno et al (2000) concluded that starches from low-amylose cultivars had a lower ratio of the shorter to longer chains in amylopectin molecules than those from normal amylose cultivars, although the differences between the ratios of normal and low-amylose cultivars were not so large.

### Thermal Properties

The gelatinization properties of normal and low-amylose rice starches measured by DSC are presented in Table I. DSC parameters recorded were onset ( $T_o$ ), peak ( $T_p$ ), gelatinization temperatures, and gelatinization enthalpy ( $\Delta H$ ). The  $T_o$ ,  $T_p$ , and  $\Delta H$  of normal amylose starches were 58.9–62.2°C, 65.4–67.9°C, and 14.5–15.4 J/g, respectively, while those of low-amylose starches were 58.3–61.2°C, 65.7–68.0°C, and 14.7–16.5 J/g, respectively. Therefore, the DSC parameters of low-amylose starches were not significantly different from those of normal amylose starches. The results of the present study were in good agreement with those of Asaoka et al (1993), who showed that the dull mutant did not have significant effects on the DSC characteristics in rice.

Lower values of gelatinization temperatures,  $T_o$  (56°C) and  $T_p$  (64°C), were obtained for Aya when it was cultivated in the Hokkaido region, which is much colder than the Kyushu region (Asaoka et al 1994b). Besides the amylose content and amylopectin structure, the DSC characteristics also were sensitive to environmental temperature during grain filling in the dull mutant of rice (Asaoka et al 1989). The higher environmental temperature was associated with a higher gelatinization temperature. Overall, presumably due to the higher environmental temperature during grain filling, comparatively higher values of  $T_o$  (59.6°C) and  $T_p$  (66.0°C) for Aya were obtained in the present study.

### Pasting Properties

Significant differences in starch pasting properties, as analyzed by RVA, between normal and low-amylose rice cultivars are summarized in Table II. Low-amylose cultivars tended to exhibit lower peak viscosity (205–357 RVU) than normal amylose cultivars (345–438 RVU). Similarly, the breakdown was generally lower for low-amylose cultivars (115–268 RVU) than for normal amylose cultivars (254–338 RVU). As expected, distinctly lower values of setback, which is the recovery of the viscosity by cooling after heating starch suspension, were obtained from low-amylose cultivars (44–73 RVU) than from normal amylose cultivars (113–132 RVU). Low-amylose starches were likely to have lower pasting temperatures (66.9–70.3°C) than normal amylose starches (70.0–71.0°C). The values of peak viscosity and breakdown were somewhat higher for starches from the Nipponbare, Hinohikari, and Yawarakomachi cultivated in 1998 than for those cultivated in 1999. Among the low-amylose rice cultivars, Soft 158 displayed the highest value of peak viscosity (357RVU), followed by Yawarakomachi, Aya, Snow Pearl, and Hanabusa. The orders of breakdown and setback were similar to those of peak viscosity. The pasting temperature varied in the following order: Yawarakomachi  $\approx$  Hanabusa > Soft 158  $\approx$  Aya > Snow Pearl. Kuno et al

TABLE III  
Correlation Coefficients for Rice Starches ( $n = 11$ )

	Amylose Content	Peak Area Ratio of DP 6–12
$T_o$	0.364	-0.667 <sup>a</sup>
$T_p$	-0.088	-0.601*
$\Delta H$	-0.290	-0.023
Peak viscosity	0.877**	-0.166
Breakdown	0.880**	-0.174
Setback	0.986**	-0.453
Pasting temperature	0.721**	-0.791**
Digestibility by amylase	-0.949**	0.508

<sup>a</sup> \*\* and \* =  $P < 0.01$  and  $0.05$ , respectively.

TABLE II  
RVA Pasting Properties in Normal and Low-Amylose Rice Cultivars<sup>a</sup>

Sample <sup>b</sup>	Peak Viscosity (RVU)	Breakdown (RVU)	Setback (RVU)	Pasting Temp. (°C)
Normal amylose				
Nipponbare 98a	381	281	132	70.7
Nipponbare 99a	345	254	130	71.0
Hinohikari 98a	438	338	124	70.0
Hinohikari 99a	365	268	113	70.4
Mean $\pm$ SD ( $n = 4$ )	382 $\pm$ 40	285 $\pm$ 37	124 $\pm$ 8	70.5 $\pm$ 0.4
Low amylose				
Yawarakomachi 98a	296	201	68	70.3
Yawarakomachi 99a	255	162	66	69.5
Yawarakomachi 99b	235	153	60	69.4
Soft 158 99b	357	268	73	68.6
Hanabusa 99b	205	115	45	69.5
Aya 99b	231	136	50	68.7
Snow Pearl 99b	208	118	44	66.9
Mean $\pm$ SD ( $n = 7$ )	255 $\pm$ 54	165 $\pm$ 54	58 $\pm$ 12	69.0 $\pm$ 1.1

<sup>a</sup> Values are means of two determinations.

<sup>b</sup> Harvested in 1998 or 1999; a, grown at National Agricultural Research Center for Kyushu Okinawa Region; b, grown at Fukuoka Agricultural Research Center.

(2000) reported that the values of peak viscosity and breakdown were higher and those of setback were lower for low-amylose cultivars than for normal amylose cultivars. Our results of peak viscosity and breakdown were not in good agreement with their data.

### Digestibility by Amylase

The digestibility of raw starches by crystalline glucoamylase of *Rhizopus niveus* is presented in Fig. 2. The hydrolysis rates of starches from low-amylose cultivars were higher than those from normal amylose cultivars. Among lower amylose cultivars, starches from Hanabusa, Aya, and Snow Pearl, which showed extremely lower amylose content, were digested faster than those from Yawarakoamchi and Soft 158. No starch examined in this investigation was digested <20% after 4 hr of reaction. Distinctly lower hydrolysis rates (1.97–7.34%) were observed in 30 sweetpotato starches under the same condition for enzymatic reaction (Noda et al 1993). These results were in good agreement with the data of Fuwa et al (1977), who revealed that the relative order of hydrolysis by amylase is potato < sweetpotato < maize < rice. Asaoka et al (1994) showed that starches from low-amylose rice cultivars such as Aya were digested faster than those from normal amylose rice cultivars. However, in another report by Asaoka et al (1993), similar hydrolysis rates of raw starch by amylase were obtained between normal and low-amylose rice cultivars, which was not the case of our data.

### Correlation Analysis

Correlation coefficients were calculated to examine the relationships between structural properties and other physicochemical properties in 11 kinds of rice starches examined in this study (Table III). The branch chain length of amylopectin is related to the crystalline structure of starch granules (Hizukuri 1986; Gidley and Bulpin 1987; Hanashiro et al 1996). Our previous investigations have confirmed negative correlations of the amount of extremely short chains of amylopectin to gelatinization temperature and gelatinization enthalpy within the same botanical origin for sweetpotato (Noda et al 1998, 2001a, 2002), buckwheat (Noda et al 1998), and wheat (Noda et al 2001b) starches. According to Gidley and Bulpin (1987), the presence of shorter chains with DP<10 decreases the stability of the double helix in amylopectin molecules, and this may lower the gelatinization temperature and gelatinization enthalpy. Our present study indicated that the amount of amylopectin short chains with DP 6–12 correlated negatively with gelatinization temperatures,  $T_o$  ( $r = -0.667$ ) and  $T_p$  ( $r = -0.601$ ). Unexpectedly, it did not correlate with  $\Delta H$  ( $r = -0.023$ ). In this study, the amylose content was not correlated with all DSC parameters,  $T_o$  ( $r = 0.363$ ),  $T_p$  ( $r = -0.088$ ), and  $\Delta H$  ( $r = -0.290$ ), which agrees with our previous results (Noda et al 1993, 1998, 2002). Currently, amylose content appears to play a critical role in determining starch pasting properties by Brabender amylograph or RVA, as amylose suppresses starch swelling. Lower amylose content was associated with higher peak viscosity (Collado and Corke 1997; Zeng et al 1997). Contrary to this, amylose-free starches from wheat (Sasaki et al 2000; Yamamori and Quynh 2000), potato (Kortstee et al 1998), and sweetpotato (Noda et al 2002) did not exhibit higher peak viscosity than amylose-containing starches. From the present data, a positive, rather than a negative, correlation was found between the amylose content and peak viscosity ( $r = 0.877$ ). These results suggest that a marked decrease in amylose content does not increase peak viscosity. In low-amylose starch, the decrease in the amount of leached-out amylose results in lower viscosity during cooling. As expected, a highly positive correlation was obtained between amylose content and setback ( $r = 0.986$ ) in this study. Several studies were undertaken to clarify the relationship between amylose content and digestibility by amylase, using starch samples with high ranges of amylose content (Juliano and Perez 1990; Asaoka et al 1994; Noda et al 2002). Negative correlation coefficients were obtained

between amylose content and digestibility by amylase in these studies. Also, in our present study, amylose content was negatively correlated with digestibility by crystalline glucoamylase ( $r = -0.949$ ). These results lead to the conclusion that amylose in starch granules is resistant to digestion by amylase.

### CONCLUSIONS

In the present study, we examined the differences between normal and low-amylose rice cultivars in the physicochemical properties of starches. The chain length distribution profiles of amylopectin of low-amylose rice cultivars were similar to those of normal amylose rice cultivars. DSC data also showed no remarkable differences in starch gelatinization properties between normal and low-amylose rice cultivars. The digestibility of raw starch by glucoamylase was higher for low-amylose rice cultivars than for normal amylose rice cultivars. Our experimental results would provide useful information for the food industry and other industries making use of low-amylose rice cultivars.

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