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Starch Properties of Mutant Rice High in Resistant Starch

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As the staple food of over half the world's population, hot cooked rice high in resistant starch (RS) is of particular interest, which will have greater impact in the dietary prevention of diabetes and hyperlipidemia. A mutant rice high in RS in hot cooked rice, described as RS111, was comparatively studied with the wild type and common rice. Despite obviously low RS content in the raw milled rice, the RS content in the hot cooked rice of mutant RS111 was significantly higher than that of the wild type and common rice and, correspondingly, in vitro starch hydrolysis by porcine pancreatic α-amylase tends to be incomplete with low hydrolysis extent for the cooked mutant rice high in RS. Obvious differences in physicochemical properties, starch granule morphology, pasting properties, thermal properties, and X-ray diffraction pattern were observed among the mutant RS111, wild type, and common indica rice. The high-RS mutant was characterized by significantly higher apparent amylose content and crude lipid content, higher percentage of oval-shaped granules and bigger oval size, reduced paste viscosity, and low onset temperature, peak temperature, final temperature, enthalpy of gelatinization, and crystallinity.

KEYWORDS: **Rice; resistant starch; starch hydrolysis; physicochemical properties; morphology; starch paste properties; thermal properties; X-ray diffraction**

INTRODUCTION

Cereals constitute the "starchy staples" in the human diet and as such are the primary source of dietary carbohydrate (*1*, *2*). In the traditional view, starch is thought to be completely digested; it is now recognized that a portion resistant to digestion by human enzymes in the small intestine will pass into the large bowel, where they may or may not be fermented by gut bacteria (*3*, *4*). In fact, starch contains a large digestible portion (digestible starch, DS) and a small nondigestible portion called enzymatic resistant starch (RS). RS is defined as "the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals" (*5*, *6*).

RS slowly absorbed in the small intestine results in decreased postprandial glucose and insulin responses. This behavior has significant implications for use of RS in food formulations to help persons with diabetes normalize glucose pressure. There is evidence that slowly digested and absorbed carbohydrates are favorable for the dietary management of metabolic disorders such as diabetes and hyperlipidemia (*7*, *8*). The starch fraction including RS fermented by microflora will produce short-chain fatty acids such as acetic, propionic, and butytic acid that are greatly helpful in preventing colonic diseases (*9*, *10*).

Rice is the most important cereal crop and the staple food of over half the world's population. As the primary dietary source of carbohydrates in these populations, rice plays an important role in meeting energy requirements and nutrient intake. Cooked rice is readily digested because it contains a higher percentage of DS and a lower percentage of RS (*11*). The content of RS in rice cooked according to traditional domestic methods is always below 3% (*12*−*17*). In view of the current concept of nutrition, rice with a higher content of DS and a lower content of RS is not the fittest food for health. RS from both rice and corn was found to significantly lower plasma total lipid and cholesterol concentrations compared to the diabetic control (*18*).

The development and incorporation of RS-rich ingredients into common food products is a challenge for the food industry (*19*). Multiple approaches have been explored to produce high amounts of RS, and most of them focus on physically modifying starch structure through the processing technology (*17*, *20*, *21*). RS levels and formation were influenced by the ratio of amylose to amylopectin in rice and in pea (*13*, *15*, *22*, *23*). Commercial attempts to produce high RS content were achieved in high-amylose corn. A high-amylose and -RS barley cultivar was proved to have potential health benefits through reduction of plasma cholesterol and production of increased large-bowel short-chain fatty acids (SCFA) (*24*). In the context of treatment of non-insulin-dependent diabetes, breeding for rice high in RS is of particular interest, as it will be easily to incorporated into

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the dietary-prevention strategy. To address this problem, a rice mutant described RS111 with high RS in hot cooked rice was induced by γ-ray irradiation (*25*). Starch properties of this mutant were comparatively studied with the wild type and common rice, in order to provide information for food technologists and plant breeders to improve RS in rice.

MATERIALS AND METHODS

Materials. The mutant rice RS111 high in RS when cooked, induced from hybrid rice restorer R7954 (*25*), was selected for the current study, together with the wild type R7954 and common indica rice cultivar ZHONG9B. The wild type R7954 and common rice ZHONG9B are the leading commercial indica-type hybrid rice restorer and maintainer (*26*, *27*). Mutant RS111 was crossed with the wild type R7954, intermediate apparent amylose content (AAC) rice cultivar II-32B (AAC = 21.3%), and low AAC rice cultivar Yixiang B (AAC = 12.7%), respectively, to understand the mechanism of RS enrichments. The F_1 plants were bagged and self-pollinated to obtain F_2 progenies. The contents of RS and AAC were investigated in three populations of hybrid F_1 and F_2 individual plants.

Sample Preparation. The rough rice was dehulled in a Satake dehuller (Satake Co.), and the resulting brown rice was milled in a Satake miller (Satake Co.). The milled rice was ground in a Udy Cyclone Mill with a 100-mesh sieve for AAC, ash content (AC), DS, gel consistency (GC), crude lipid content (LC), protein content (PC), and total starch (TS) analysis. Milled rice (100 mg) was cooked according to the traditionally domestic method using a rice cooker with varying ratios of water to rice ranging from 1.0 to 2.1. The hot cooked rice was sampled for RS determination after the rice cooker automatically shifted to warm-holding status at 50 °C. Starch was separated from rice flour by the alkaline extraction method described by Verwimp et al. (*28*). Briefly, 2 g of rice flour was suspended in 10 mL of 0.25% NaOH solution, stirred at room temperature for 1 h, and then centrifuged at 3000*g* for 10 min. The sediment was washed with distilled water, stirred, and centrifuged several times and then suspended and neutralized with 0.2 M HCl. After removal of the upper grayish layer, the white starch was dispersed in distilled water and passed through a 30-μm mesh sieve using a stirring rod and a vacuum air pump. The washings were centrifuged and sediments collected. After oven-drying at 40 °C for 24 h, the isolated starch was gently ground through a 100-mesh sieve and stored in a sealed plastic bag for later use.

Physiochemical Properties and Total, Digestible, and Resistant Starch Determination. AAC was estimated by using the simplified assay with three repeats (*29*). The standard samples with four levels of AAC (1.2, 11.2, 16.8, and 26.8%) were provided by the China National Rice Research Institute (CNRRI). AC was determined according to the China National Standard methods of GB/T5009.10-2003. GC was determined using the procedure outlined by Cagampang et al. (*30*). LC was determined according to the method described by the AACC (*31*). PC was measured by the micro-Kjeldahl method with three repeats (*29*). To comparatively verify RS111 high in RS and eliminate the disparity of RS values caused by different determination methods or probably incomplete starch hydrolysis in the RS assay, RS was determined according to three common methods detailed by Englyst et al. (*32*), Göni et al. (*33*), and the Megazyme RS Kit (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). TS was determined according to the method described by Garcia-Alonso et al. (*34*). DS was calculated on the basis of the difference between TS and RS determined by Göni et al. (*33*).

In Vitro Starch Digestion. In vitro starch digestion was determined according to the methods of Goñi et al. (*14*) and Holm et al. (*35*). Starch hydrolysis was initiated by the addition of 5 mL of Tris−maleate buffer containing 3.0 IU of Megazyme porcine pancreatic α-amylase (Megazyme International Ireland Ltd.). The reaction mixture was incubated in a shaking water bath at 37 °C with moderate agitation. Samples (0.5 mL) were taken from each flask every 30 min from 0 to 3 h, and the α-amylase was inactivated by adding 0.5 mL of 1.2 M acetate. The total reducing sugar content was determined by using the 3,5-dinitrosalicylic acid (DNS) reagent. Maltose was used to make the standard curve. The extent of hydrolysis was calculated as the proportion (percent; maltose equivalent) of starch degraded to maltose in total starch. Each sample and treatment were analyzed in triplicate.

Starch Paste Properties. Paste viscosity of rice flour was determined by a Rapid Visco Analyzer (RVA, model-3D, Newport Scientific Inc.) according to the protocol of the AACC (*31*), and its analysis was subjected to Thermo Cycle for Windows (TCW) software. The major parameters of the RVA profile are peak viscosity (PKV), hot paste viscosity (HPV), cool paste viscosity (CPV), breakdown viscosity (BDV, PKV minus HPV), and setback viscosity (SBV, CPV minus PKV).

Thermal Properties. The thermal properties of starch isolated from rice cultivars were determined using a differential scanning calorimeter (DSC) (Q100 T, TA Inc., Newcastle, DE) and calculated with the Universal Analysis program, version 3.8B. Five-milligram samples were placed in an aluminum cup, and 20 μL of distilled water was added. The cup was hermetically sealed and then heated from 30 to 110 °C at a rate of 10 °C/min. The major parameters of the DSC profile were described as onset temperature (T_O), peak temperature (T_P), enthalpy of gelatinization (ΔH_{gel}), and final temperature (T_C).

Scanning Electron Microscopy. Starch powers were homogeneously stuck on double-adhesive tap fixed on a metallic stub, then treated in an IB-5 ion coater (Eiko Co.) for 30 min under argon atmosphere, coated with the Pt ion, and visualized with a scanning electron microscope (XL30ESEM, Philips Co.) at 20 kV. Micrographs of starch sample were taken at 2000× magnification, and the size of starch granules was measured in the Adobe Photoshop Elements 2.0.

X-ray Diffraction Pattern. The X-ray pattern of the starch was measured with Cu Kα radiation ($\lambda = 0.154$ nm) using a diffractometer (D/max 2550PC, Rigku Inc.) and calculated using the Universal Analysis program, version 3.8B. The diffraction was operated at 30 mA and 40 kV. The region of the two-theta angle (2θ) was scanned over the range from 3.0 to 70.0° with a 0.05° step size and a count time of 2 s. The photographs were drawn in the SigmaPlot software (SYSTAT).

Statistical Methods. The data obtained were subjected to a one-way analysis of variance followed by Duncan's multiple-range tests.

RESULTS AND DISCUSSION

As the staple food of over half the world's population, rice plays an important role in providing a dietary source of carbohydrate and nutrient intake. Generally, rice has a high content of DS and a low content of RS, and the content of RS in rice cooked according to traditionally domestic methods is always below 3%. Despite being high in amylose, the mutant RS111 has a soft texture and improved eating quality.

Resistant Starch Content in Mutant RS111. The RS values were variable when determined by three methods; however, the distinct differences in RS contents were consistent among the three cultivars tested (**Table 1**). The RS values in both the raw rice and the hot cooked rice were all highest when determined according to the method of Englyst et al. (*32*) and lowest when determined by using the Megazyme RS Kit. In the raw milled rice of mutant RS111, the RS contents were significantly lower compared to the wild type and common rice. However, significant RS enrichments in mutant RS111 were found in the hot cooked rice, whereas the RS contents were obviously reduced in the cooked rice of wild type and common rice. The amounts of RS in the hot cooked rice of three rice cultivars tested were all increased with increasing ratios of water to rice. Different cooking requirements were observed; the optimal cooking ratio of water to rice was 1.8 for the high-RS mutant, whereas for the wild type and common rice the optimal ratio was 1.5. As determined by Göni et al. (*14*), the RS contents in mutant RS111 were increased from 5.47 to 7.56%, whereas that of the wild type R7954 and common rice ZHONG9B ranged from 2.27 to 2.88% and from 1.72 to 2.62%, respectively (**Figure 1**). In the *Australian Guide to Healthy Eating*, it has

Table 1. Major Nutritional Components and Physicochemical Properties in Mutant RS111, Wild Type R7954, and Common Indica Rice ZHONG9B on a Dry Weight Basis[a]

			RS in raw milled rice (%)			RS in hot cooked rice[b] (%)							
rice	TS (%)	DS (%)	Englyst	Göni	Megazyme	Englyst	Göni	Megazyme	AAC (%)	LC (%)	AC (%)	PC (%)	GC (mm)
RS111 (mutant)	75.2a	67.6a	3.23a	2.27a	1.49a	8.17a	7.56a	3.5a	32.8a	1.24a	0.65a	12.0a	51a
R7954 (wild type)	75.4a	72.3a	13.2b	11.2b	5.83b	3.09b	2.88b	0.7b	24.0b	0.91b	0.75b	11.3a	33b
ZHONG9B (common rice)	72.3b	69.7a	11.6b	9.82b	5.14b	2.79b	2.62b	0.6b	23.9b	0.62b	0.52b	12.9a	34b

[a] TS, total starch; RS, resistant content; DS, digestible starch; AAC, apparent amylose content; LC, crude lipid content; AC, ash content; PC, protein content; GC, gel consistency. Different letters following the means indicate significant differences at $p = 0.05$ compared to the high-RS mutant RS111. [b] RS contents in hot cooked rice were determined by Englyst et al. (*32*), Göni et al. (*33*), and Megazyme RS Kit when cooked with the ratio of water to rice of 2.1 to 1.0.

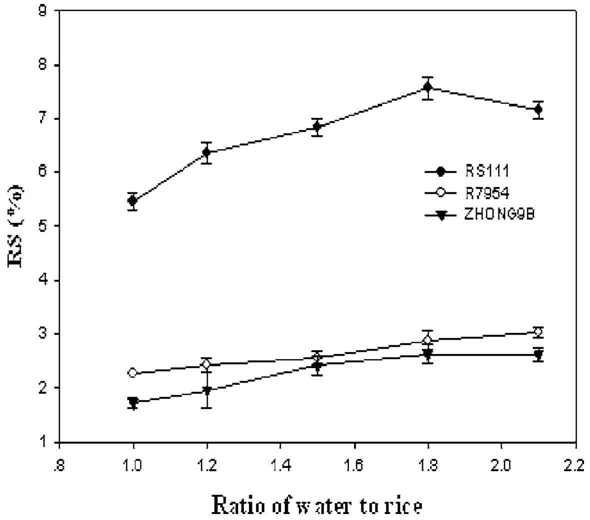

Figure 1. RS contents determined by Göni method in the hot cooked rice of mutant RS111, wild type R7954, and common indica rice ZHONG9B when cooked with ratios of water to rice from 1.5 to 2.1.

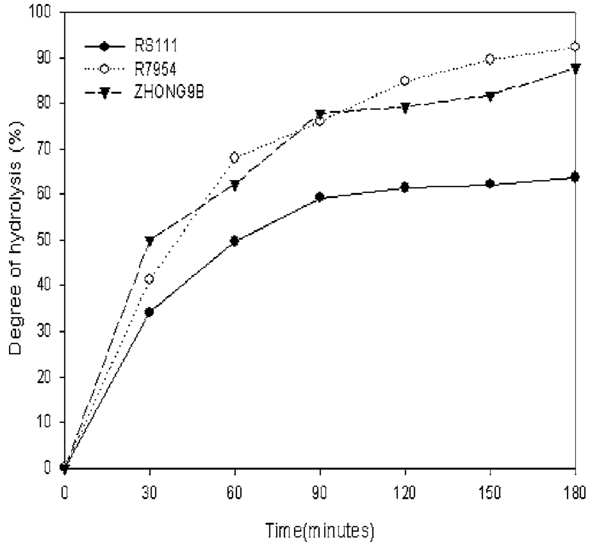

Figure 2. In vitro hydrolysis of crack of the hot cooked rice of mutant RS111, wild type R7954, and common indica rice ZHONG9B.

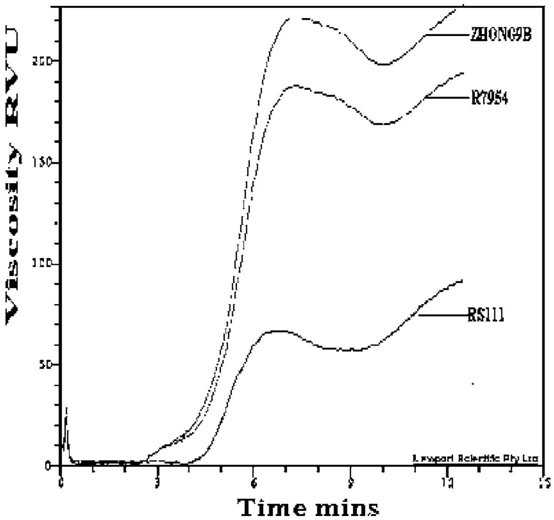

Figure 3. Pasting viscosity of rice flour from mutant RS111, wild type R7954, and common indica rice ZHONG9B.

been estimated that resistant starch intake in Australia is ~5−7 g/person/day. Approximately 20 g a day is recommended to obtain the beneficial health effects of RS. Therefore, eating 250−300 g of cooked RS111 per day is enough to basically meet the RS requirement for a normal person. Clinical observation on non-insulin-dependent diabetes was very positive when RS111 was eaten daily as a staple food (data not shown).

In Vitro Starch Hydrolysis. Corresponding to the RS results, the rate and extent of hydrolysis were different among cracked grains of the hot cooked rice (**Figure 2**). The high-RS mutant was highly resistant to hydrolysis, and hydrolysis was complete in a low degree of 59.3% after 90 min, while the wild type R7954 and common rice ZHONG9B hydrolysis still have not reached equilibrium after 90 min, but >76% of the starches have already been hydrolyzed. At the final time of 180 min, only 63.6% of RS111 starch had been hydrolyzed, whereas 92.3 and 87.7% of the wild type and common rice starches had been hydrolyzed. Therefore, although absolute high RS content in mutant RS111 was still very low, the slowly digested starch in RS111 would also play an important role in the dietary management of metabolic disorders such as diabetes and hyperlipidemia.

Physicochemical Properties, Starch Pasting Viscosity, and Major Nutritional Components. Significantly higher content of AAC was detected in the mutant RS111, compared to the wild type and common rice (**Table 1**). This result was consistent with previous studies. The high-amylose rice cultivars were found to be relatively high in RS in the previous papers of Miller et al. (*36*), Frei et al. (*13*), and Hu et al. (*15*). Because of its compact linear structure (*37*), the high-amylose starch is more resistant than amylopectin to starch digestion and more prone to form amylose−lipid complexes, a property that can be utilized in the formulation of foods with high RS content. Closely related to amylose increase, the LC was obviously increased in mutant RS111. Interestingly, accompanied by the enhanced LC, a specific good aroma was detected during the cooking process of the mutant RS111, which concurred with a previous report that the fatty acids will affect the flavor of rice (*38*). To verify RS111 as being high in RS and not a typical high-amylose rice,

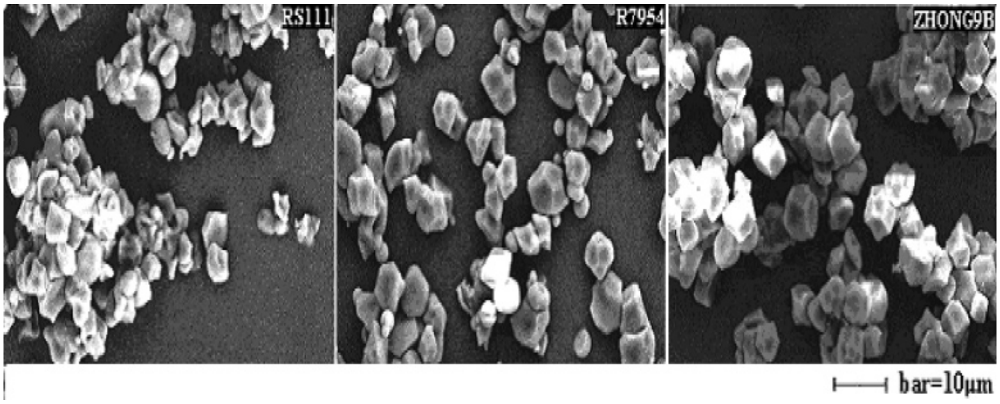

Figure 4. Scanning electron micrographs of starch granules from mutant RS111, wild type R7954, and common indica rice ZHONG9B (×2000).

Table 2. Starch Granule Characters of Mutant RS111, Wild Type R7954, and Common Indica Rice ZHONG9B[a]

rice	no. of granules observed	shape	range (μm)	av granule size (μm)	variation coefficient (%)	rate of oval (%)	av oval granule size (μm)
RS111 (mutant)	606	polygonal, angular, and oval	1.40–6.34	3.60a	27.01a	19.76a	2.55a
R7954 (wild type)	506	polygonal, angular, and oval	1.23–6.83	3.55a	38.18b	9.37b	2.13b
ZHONG9B (common rice)	427	polygonal, angular, and oval	1.08–7.49	4.29b	24.12b	3.51b	2.09b

[a] Different letters following the means indicate significant differences at $p = 0.05$ compared to the high-RS mutant RS111.

observations on the RS contents of hybrid F_1 and F_2 individual plants derived from RS111 crossed to the wild type, intermediate-amylose rice II-32B, and low-amylose rice Yixiang B indicated that the high-RS mutation in RS111 was a recessive gene controlled character and was not inherited with the high amylose in three populations (data not shown). This fact suggested that the high RS in mutant RS111 might be caused both by the increased amylose starch and by amylose−lipid complex.

The wild type R7954 and common rice had a hard texture and low GC characteristics. Despite the significantly high amylose and RS, an obviously improved GC was observed in the mutant RS111. Three major RVA parameters, PKV, HPV and CPV, were significantly reduced in the mutant RS111, compared to the wild type and common rice (**Figure 3**). This result concurred with our previous study, in which early indica rice cultivar ZF210 high in RS was characterized by a significantly reduced starch paste viscosity (*15*). This suggested that the improved GC character might be caused by the reduced starch paste viscosity.

Starch Granule Morphology. The starches isolated from the high-RS mutant, the wild type, and common rice had the same white appearance when dried at 40 °C in an oven for 24 h according to the common method. However, if dried at 50 °C, the isolated starch of the high-RS mutant would be gelatinized with a transparent appearance, but no changes were observed in the starches from the wild type and common rice, reflecting the fact that the high-RS starch has a lower gelatinization temperature. Scanning electron micrographs of starch granules are shown in **Figure 4**. Significant variations in the shape and size of starch granules were observed between the high-RS mutant and common rice (**Figure 5**). In agreement with the results of Singh et al. (*39*), the majority of starch granules from three rice cultivars were of pentagonal and angular shape. However, an obviously higher percentage of oval shape and a bigger average oval size were observed in the high-RS mutant, and common rice ZHONG9B was characterized by an obviously higher percentage of pentagonal shape (**Table 2** and **Figure 4**). As in previous studies (*40*, *41*), the mutant RS111 high in RS may be partly due to the granule size and the differences in the surface/volume ratio.

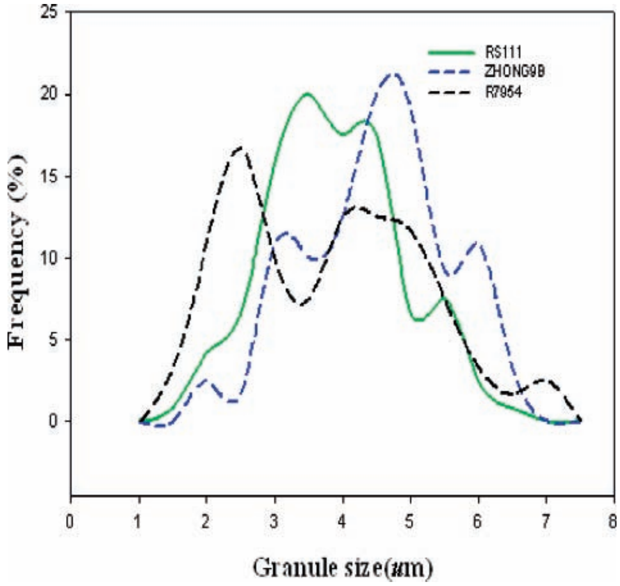

Figure 5. Distribution of starch granule size from mutant RS111, wild type R7954, and common indica rice ZHONG9B.

Thermal Properties, X-ray Pattern, and Crystallinity. Gelatinization temperatures such as T_O, T_P, and T_C and the ΔH_{gel} of starches of the mutant RS111 were significantly different from those of the wild type R7954 and common rice ZHONG9B, which showed significantly lower T_O, T_P, T_C, and ΔH_{gel} values (**Table 3** and **Figure 6**). Corresponding with the scanning electron micrographs, X-ray diffraction of starch granules in pentagonal and angular shape from three rice cultivars exhibited a typical A-type pattern, which has peaks at $2T = 15.10$, 16.98, 18.05, and 22.90 °C and an additional peak at $2T = 19.94$ °C, respectively (**Figure 6**). According to the study of Shamai et al. (*42*), a peak at $2T = 19.9$ °C is characteristic to V-type polymorph. The extent of crystallinity seemed to be closely related with the gelatinization temperatures. The crystallinity

Table 3. DSC and X-ray Diffraction Parameters of Starch from Mutant RS111, Wild Type R7954, and Common Indica Rice ZHONG9B

rice	T_O (°C)	T_P (°C)	T_C (°C)	ΔH_{gel} (J/g)	crystallinity (%)
RS111 (mutant)	60.42a	64.64a	72.80a	3.485a	28.13a
R7954 (wild type)	68.24b	72.02b	79.65b	7.124b	35.65b
ZHONG9B (common rice)	72.38b	77.60b	85.18b	9.358b	40.09b

[a] T_O, T_P, T_C, and ΔH_{gel} represent onset temperature, peak temperature, final temperature, and enthalpy of gelatinization, respectively. Different letters following the means indicate significant differences at $p = 0.05$ compared to the high-RS mutant RS111.

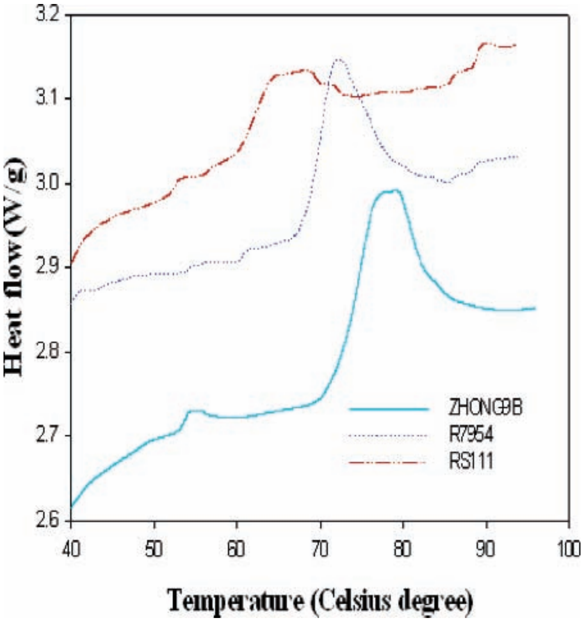

Figure 6. DSC curves of starch from mutant RS111, wild type R7954, and common indica rice ZHONG9B.

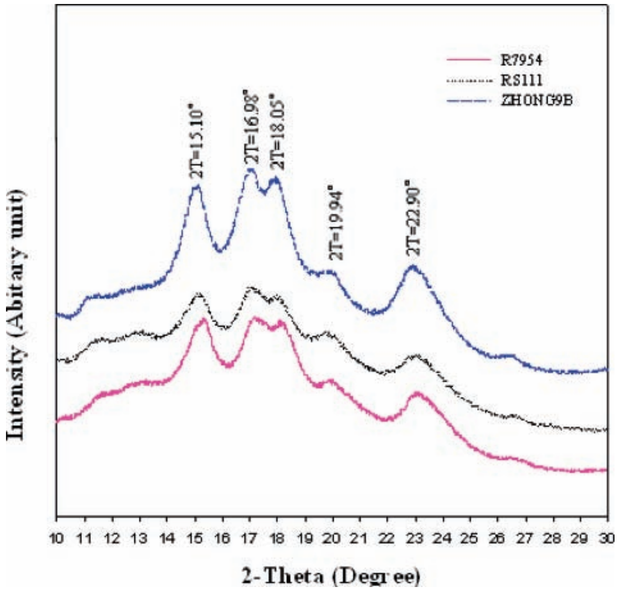

Figure 7. X-ray diffraction pattern of starch isolated from mutant RS111, wild type R7954, and common indica rice ZHONG9B.

in the mutant was obviously lower than that of the wild type and common indica rice (**Table 3** and **Figure 7**). From the study of Barichello et al. (*43*), high transition temperatures mainly result from a high degree of crystallinity that provides structural stability and makes the granule more resistant toward gelatinization. Tester (*44*) has postulated that the extent of crystalline perfection is reflected in the gelatinization temperatures, and amylopectin plays a major role in crystallinity. The current result was consistent with the previous findings (*45*); the lower transition temperature for starch in mutant RS111 may result from significantly higher amylose and the presence of the higher lipids.

Conclusions. In addition to processing methods, the inherent characteristics of rice play the key important role in the formation of RS in hot cooked rice. Both the absolutely high RS and the slowly digested starch character in mutant RS111 will play an important role in the dietary management of metabolic disorders such as diabetes and hyperlipidemia. The high-RS rice was significantly different from the wild type and common rice in different aspects of starch properties such as high amylose, starch granule shape and size, reduced starch paste viscosity, low gelatinization temperature, and crystallinity. The high-RS rice was accompanied with high amylose in this and previous studies; however, this does not imply that the high-RS rice must have high amylose and poor eating quality. Mutants high in RS have been identified from the low-RS and glutinous rice and wheat cultivars in subsequent studies. The current study provides useful information for the food technologist to identify distinct rice cultivars for the production of high-RS food and for the plant breeder to develop rice high in RS.

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