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ARTICLE

Slow Digestion Properties of Rice Different in Resistant Starch

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The hydrolysis of starch is a key factor for controlling the glycemic index (GI). Slow digestion properties of starch lead to slower glucose release and lower glycemic response. Food with high resistant starch (RS) possesses great value for controlling the GI. To elucidate the factors that play a role in slow digestibility, seven rice mutants different in RS contents were selected for comparative studies. The degree of hydrolysis showed highly significant correlation with RS, apparent amylose content (AAC), lipid content (LC), and other starch physiochemical properties in all these materials with different RS contents. The rate of in vitro digestible starch correlated positively with RS, whereas digestibility was affected mostly by lipid content for those mutants with similar RS. Starch−lipid complexes and short chains with degrees of polymerization (DP) of 8−12 strongly influenced starch digestion. The integrity of aggregated starch and the number of round starch granules might influence the digestibility of starch directly.

KEYWORDS: Slow digestion; rice; resistant starch; starch structure

INTRODUCTION

The glycemic index (GI), a scale that ranks carbohydrate-rich foods by how much they raise blood glucose levels compared to glucose or white bread, is a useful measurement for managing the blood glucose of those affected by diabetes. It can be used to scientifically describe the effects of carbohydrate-rich food on postprandial glucose in the blood. Dietary carbohydrates provide half the energy for bodily functions. The Dietary Guidelines for Americans recommend that 55−60% of daily calories should come from carbohydrate foods. Intake of large amounts of high-GI food can be detrimental to health and lead to obesity, diabetes, and cardiovascular disease (*1*). Dietary foods with lower GI are suggested to be healthier, and low-GI diets appear to improve overall blood glucose control in people with diabetes (*2*).

Starch is the most important dietary carbohydrate. It can be classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) according to in vitro digestion (*3*). The rate of starch digestion is directly related to the glycemic and insulin responses, and the GI of food products is positively correlated with the amount of RDS (*4*). SDS and RS will lead to slower glucose release and lower glycemic response. Resistant starch (RS), as the indigestible starch portion in the stomach and digested in the small intestine, can provide functional control of the GI, help body weight management, and prevent heart disease, cancer, diabetes, and cardiovascular disease (*5*). It has attracted great interest in the context of type II diabetes treatment.

Rice is a staple food source throughout the world, especially in Asia, and contains about 76−78% starch. It is a relatively high-GI food compared to other starchy foods (*6*). In cooked rice, there is a high percentage of RDS and a low percentage of RS. The content of RS in hot cooked rice is generally under 3% (*7*). The rate of starch digestion was affected by both enzyme activities and starch properties. RS has a clear impact on the rate of starch digestion. Yang et al. (*8*) reported a rice mutant with high RS content (major RS3, about 7%) which clearly showed a slow rate of starch digestion (*9*). In previous studies, we reported the starch properties and effects of amylopectin on RS (*9*−*11*). Frei et al. (*12*) showed that rice with high amylose content tended to be hydrolyzed more slowly. Fässler et al. (*13*) reported that in two in vitro digestion models, the digestibilities of RS3 were similar, whereas that of RS2 was not the same. Stevnebø et al. (*14*) demonstrated that the amylose level and the amylose lipid complex are the limiting factors for starch degradation in barley cultivars. To develop methods to predict these possible food effects in vivo, Englyst et al. (*15*) and Goñi et al. (*16*) carried out studies on the in vitro digestion of starchy foods.

Although several studies have demonstrated that starch is in vitro digested at various rates, very little information is available on the digestibility of stable food with different RS contents. In the present study, we selected series of mutants with various RS contents to evaluate the in vitro starch hydrolysis profile, to elucidate the mechanism of slow digestibility of rice with different RS contents, and to provide a better choice for high-RS rice consumed as the staple food in regulating glucose control for diabetic patients.

MATERIALS AND METHODS

Plant Materials. Seven rice mutants from the same genetic background were selected in this study (**Table 1**). All of these mutants were

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Table 1. Major Nutritional Components and Starch−Iodine Bonding Properties in High-RS Rice Mutants

material	TS[a] (%)	RS[a] (%)	AAC[a] (%)	proteins[a] (%)	lipids[a] (%)	BV[a] (680 nm)	λ_{max} (nm)	$OD_{620}-OD_{680}$
RS14	74.84 ± 0.72 Ab	3.28 ± 0.01 Aa	25.8 ± 0.6Aa	11.97 ± 0.00 EFf	1.02 ± 0.00 Cc	0.294 ± 0.005 a	589	0.0685
RS30	79.95 ± 0.52 Bc	3.97 ± 0.17 Ab	25.9 ± 0.7 Aa	11.91 ± 0.00 DEe	0.79 ± 0.01 Aa	0.297 ± 0.003 a	592	0.0665
RS12	84.56 ± 0.78 De	5.19 ± 0.27 Bc	27.3 ± 0.7 ABb	11.65 ± 0.00 Cc	1.06 ± 0.01 Dd	0.310 ± 0.001 ab	595	0.071
RS25	74.92 ± 0.63 Ab	5.20 ± 0.16 Bc	25.8 ± 0.2Aa	11.84 ± 0.00 Dd	0.83 ± 0.01 Bb	0.297 ± 0.004 a	592	0.0665
RS13	73.10 ± 0.10 Aa	5.87 ± 0.24Bd	28.1 ± 0.6 Bb	11.99 ± 0.02 Ff	1.04 ± 0.02 CDd	0.319 ± 0.001 bc	598	0.0685
RS9	82.47 ± 0.45 Cd	6.02 ± 0.01 Bd	30.5 ± 0.2 Cc	11.69 ± 0.04 Cc	1.23 ± 0.00 Ee	0.337 ± 0.015 c	595	0.0725
RS4	78.77 ± 0.34 Bc	11.65 ± 0.31 Ce	40.4 ± 0.2 Dd	9.77 ± 0.01 Aa	2.01 ± 0.00 Ff	0.438 ± 0.009 d	602	0.0895
R^b	−0.413	−0.894**	−0.911**	0.858*	−0.924**	−0.910**	−0.900**	−0.918*
	(−0.537)	(−0.624)	(−0.75)	(0.570)	(−0.784)	(−0.765)	(−0.758)	(−0.848*)

[a] TS, total starch; RS, resistant content; AAC, apparent amylose content; BV, blue value. The same capital letter indicates no significant differences at the 0.01 level; the same lower case letter indicates no significant differences at the 0.05 level. [b] The correlation between HD (the value at 30 min) in all materials, and the value in blank means the correlation between HD in all materials except RS4: *, significant correlation at the 0.05 level; **, significant correlation at the 0.01 level.

obtained from RS111, which was derived from R7954 (*8*) by irradiation with 300 Gy of cobalt γ rays. All mutants had agronomic characters similar to those of R7954 but were different in resistant starch content.

Sample Preparation. All samples were grown and harvested at the same time in the summer of 2007 in Hangzhou, Zhejiang, China. Paddy rice was dehulled in a Satake dehuller (Satake Co., Hiroshima, Japan), ground in an Udy Cyclone Mill (Fort Collins, CO), passed through a 100 mesh sieve, and stored in a dryer.

Physiochemical Properties and Total Starch and Resistant Starch Measurements. Apparent amylose content (AAC) was determined by the simplified assay (*17*); 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). Iodine-binding samples were scanned at wavelengths ranging from 400 to 700 nm and the values at maximum wavelength (λ_{max}), blue value (BV, absorbance at $\lambda = 680$ nm, A_{680}), and A_{620} (the λ at which AAC was determined) were recorded with a UNICAM UV300 spectrophotometer (Thermo Electron Co., Waltham, MA). Lipid content (LC) was determined according to the method described by the AACC (*18*), and protein content (PC) was measured by the micro-Kjeldahl method (*19*). Total starch was determined according to the method described by García-Alonso et al. (*20*). RS was measured according to the method of Shu et al. (*11*) with rice flour, and the ratio of water/rice is 5. All analyses were repeated at least twice.

Enzymatic Starch Hydrolysis. Starch hydrolysis kinetics was measured using the method of Shu et al. (*10*). The degree of hydrolysis (HD) was indicated as the percentage of digested starch to total starch; it reflected the rate of starch digestion and can be regarded as an index of starch digestion: the lower the HD, the slower the starch digestion. The procedure was also used to prepare prehydrolyzed starch samples by stopping the hydrolyzing reaction at 0, 30, 60, and 120 min intervals using an equal volume of 95% ethanol and then centrifugation at 5000 rpm for 10 min; the residues were washed twice with distilled water and freeze-dried (Virtis freeze-dryer). Native starch was isolated from rice flour by treatment with 600 UI pepsin (Amersco, Solon, OH) for 1 h at 37 °C, and residues obtained by centrifugation (5000 rpm, 10 min) were washed twice with distilled water and freeze-dried. All treated samples were passed through a100 mesh sieve and stored for use.

Amylopectin Chain Length Profile. The amylopectin chain length distribution profile was analyzed by high-performance anion exchange chromatography with pulsed amperometric detection according to the method of Nakamura et al. (*21*).

Scanning Electronic Microscopy (SEM). Starches and samples with different degrees of hydrolysis prepared as above were homogenously stuck on double-adhesive tape fixed on a metallic stud, coated with Pt ions under argon atmosphere for 30 min (IB-5 ion coater, Eiko Co.), and then visualized with a scanning electron microscope (XL30ESEM, Philips Co.) at 20 kV.

Differential Scanning Calorimetry (DSC). A differential scanning calorimeter (DSC 200, NETZSCH, Germany) was used to examine the thermal properties of starch samples. Rice flour (2.5 mg) was mixed with 5 μL of distilled water and hermetically sealed in aluminum pans. After equilibrating for 12 h at room temperature, samples were scanned at a heating rate of 10 °C/min from 30 to 110 °C, cooled at a rate of 3 °C/min, and then stored for 24 h at room temperature and resubmitted to DSC.

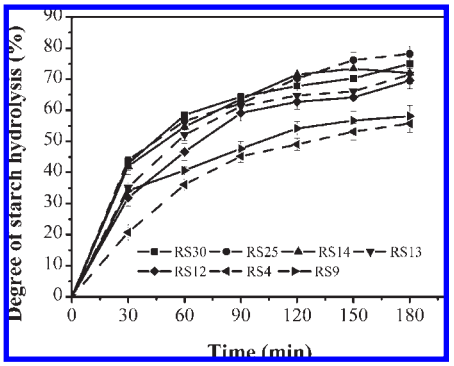

Figure 1. In vitro digestion curve for different rice cultivars (3U amylase).

The major parameters of the DSC profile were described as onset temperature (T_o), peak temperature (T_p), final temperature (T_c), and the change of enthalpy during gelatinization (ΔH).

X-ray Diffraction. A diffractometer (D/max 2550PC, Rigaku Inc., Tokyo, Japan) was used to examine the X-ray diffraction patterns and crystalline property of rice samples. The diffractometer was operated at 300 mA and 40 kV to generate copper K radiation of $\lambda = 0.154$ nm. Samples were scanned from 2θ 40° to 60° with a step size of 0.05° and a count time of 2 s. The crystalline degree was calculated with Jade 5.0.

Statistical Analysis. All data analysis was performed with SPSS program version 15.0. Multiple-comparison analysis among all materials was conducted by Duncan's tests; correlations between the degree of hydrolysis (HD) and other parameters were also evaluated. HD and other variables were subjected to factor analysis following the FACTOR procedure using principal component analysis (PCA).

RESULTS AND DISCUSSION

Resistant Starch and Starch Digestion Properties. Seven mutants with the same genetic background were selected for this study. The contents of RS are given in **Table 1**. RS values ranged from 3.3 to 11.7%. There were no significant differences between RS12 and RS25, RS9, and RS13 ($P > 0.05$), whereas there were significant differences among the others ($P < 0.05$). The materials with different RS contents showed different HD values. The higher the RS was, the lower the HD. RS4, with the highest RS content, showed the lowest HD among all of these materials. Only 55% of the starch was hydrolyzed after 3 h (**Figure 1**), whereas nearly 80% of starch was digested for RS25. However, for materials with similar RS contents such as RS9 and RS13, the HD also showed some differences (**Figure 1**).

To investigate the differences of HD among all of these materials and study the whole digestion dynamics, a PCA was conducted. The differences of all materials in the whole digestion process are shown in **Figure 2A**. RS4 showed the highest resistance to enzyme, whereas RS25, RS14, and RS30 showed similar

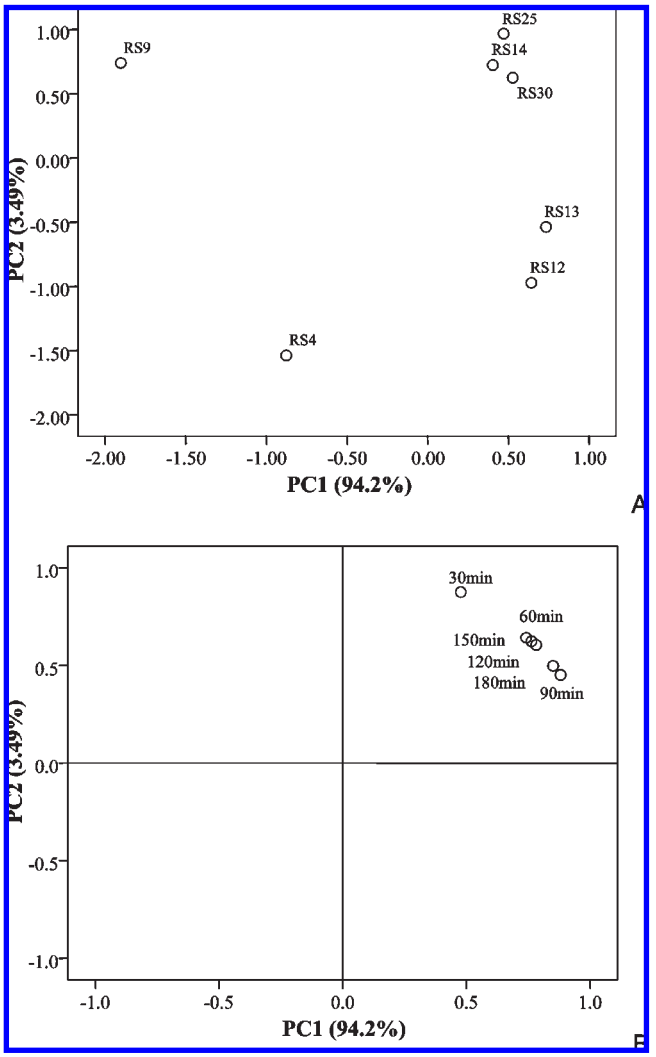

Figure 2. Score plot (**A**) and loading plot (**B**) of in vitro digestion rate in seven rice samples by PCA. Only the first two main factors were released; PC1 explained the majority variances among all variables.

HD values. According to the loading plots of the first two factors, the HD at 30 min clusters alone could explain the second variance among all of these mutants (**Figure 2B**), which was then selected as the parameter for correlation analysis and overview PCA.

The HD shows good correlation with RS except RS4, for which there was no significant correlation between RS and HD ($R = -0.624$). RS9, RS13, RS12, and RS25, which had no significant differences in RS content, showed significant differences in HD (**Figures 1** and **2A**). For these four materials, lipid content associated significantly with HD (**Table 1**). The lipid/starch complexes may influence digestion by reducing the contact between starch and enzyme or reducing swelling of the starch granule (*22*) and is consistent with the previous results (*9*).

Starch Physiochemical Properties and Starch Digestion Property. *AAC and Amylose−Lipid Complex*. It can be seen from **Table 1** and **Figure 1** that the higher the ACC content was, the lower the HD. There are some studies about the influence of the amylose content on starch digestibility (*7*, *12*). Although amylose content was not the only factor determining starch digestibility, HD is significantly correlated with AAC content (*12*). In barley, the degree of starch hydrolysis was higher in normal-amylose cultivars than in high-amylose cultivars (*14*). That might be due to the easier formation of the amylose−lipid complex in higher amylose materials or the easier retrogradation of amylose than of

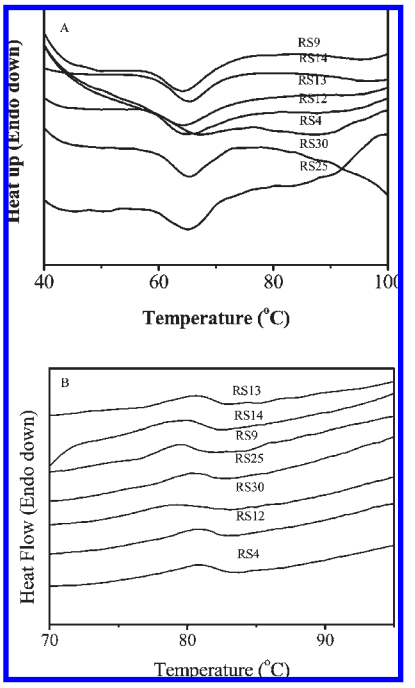

Figure 3. DSC (**A**) and immediate cooling curve (**B**) of seven rice varieties with different RS contents. The exothermic peaks in the cooling curve reflect the amylose−lipid complex formation during the cooling process after gelatinization.

Table 2. Parameters of DSC and Starch Crystallinity of All Rice Materials

material	T_o (°C)	T_p (°C)	T_c (°C)	ΔH_1[a] (J/mg)	ΔH_2[a] (J/mg)	crystalline (%)
RS13	58.0	64.2	73.6	4.20	−0.71	29.9
RS14	58.9	65.4	72.9	5.14	−0.73	35.8
RS9	58.7	64.2	72.4	4.70	−0.72	34.7
RS25	59.5	65.1	70.8	5.27	−0.74	32.9
RS30	59.9	65.4	71.9	4.79	−0.85	38.9
RS12	58.5	64.4	71.8	4.30	−0.98	29.6
RS4	60.3	67.3	72.8	3.99	−1.02	39.6

[a] T_o, onset temperature; T_p, peak temperature; T_c, final temperature; ΔH_1, endothermic during heating; ΔH_2, exothermic during cooling.

amylopectin after cooking. Fässler et al. (*13*) found that the materials with higher amounts of nonstarch compounds may compete for water binding and thus reduce the starch swelling and slow the in vitro digestion.

In all mutants studied, lipids and proteins showed significant correlation to the RS content ($r = 0.927$ and -0.783, respectively) and also affected the HD greatly. With the decreased RS, the degree of starch hydrolysis increased (**Table 1**). That might be because starch granules may be wrapped by proteins during dretrogradation and be released in the digestion process. Mangala et al. (*23*) observed that the percent recovery of RS increased 2−3-fold after defatting and autoclaving.

In all of these rice mutants, there is no significant difference in the gelatinization properties (**Figure 3A** and **Table 2**). Panlasigui et al. (*24*) reported that the degree of gelatinization showed a considerable correlation with the rate of in vitro starch hydrolysis with α-amylase The similar gelatinization properties suggest that the influence of gelatinization on starch hydrolysis of all these mutants might be the same.

The endothermic ΔH_1 values are higher and correlated negatively with the lipid and AAC contents in those high-lipid-containing samples (**Tables 1** and **2**). The absolute value of exothermic ΔH_2 increased with the increase in degree of complex

formation, which showed a positive correlation with the lipid and AAC contents (**Tables 1** and **2**). With DSC analysis, there is an additional endothermic peak at about 90 °C in the first heating run of RS4 (**Figure 3A**). Amylose−lipid complexes were formed during gelatinization and can be evidenced in the cooling DSC curve (**Figure 3B**). During the gelatinization, part of the free lipids may form a helical inclusion complex with the amylose molecules. The complex formation is an exothermic process and results in a decrease in the observed endothermic gelatinization enthalpy (*25*). The complex can significantly slow the in vitro starch digestion, although it might restrict the retrogradation of amylose after cooking (*26*, *27*). It is possible that the amylose portion of starch formed complexes with lipids during retrogradation, so that it is less susceptible to enzymatic attack, which might result in increased RS content as measured according to ref *26* and slowing of HD. Holm et al. (*28*) reported that the formation of amylose−lipid complexes showed a slower in vitro digestion and may be responsible for the modulated digestibility. Kwaśniewska-Karolak et al. (*29*) found the susceptibility of amylose−lipid complexes to degradation by α-amylase did not depend on its amount but probably on its structure.

Starch Polymerization and Chain Length. The λ_{max} of starch was reported to be related to degree of polymerization and average chain length of amylose and amylopectin and showed a significant relationship with RS. The longer amylose chains in nonwaxy rice starch would prevent the ordered aggregation of double helices and result in the formation of a cross-linked network during cooling, which make it easier to be digested (*30*). Srichuwong et al. (*31*) found that A-type starches were more sensitively digested than B- and C-type starches. Eerlingen et al. (*32*) concluded that the longer the chains were, the more the RS obtained among those materials with similar amylose when the DP was 100−610. The high-amylose wheat starches also had higher blue values (BV) but lower λ_{max} than the normal wheat starch (*33*). The starch of RS4 has the maximum wavelength of absorbance of the starch−iodine complex and the lowest in vitro digest rate, which suggests its average DP was the highest.

BV reflected the apparent amylose content and the affinity of starch for iodine. Amylopectin having long chains showed high affinity for iodine. $OD_{620}-OD_{680}$ reflected the amylopectin portions in AAC. Starch of RS4 had the strongest affinity for iodine, as measured by BV at 680 nm (0.44), and also had the largest $OD_{620}-OD_{680}$ and λ_{max}, which indicated much longer amylopectin existed in RS4, and this might explain partly its higher amylose contents. Higher amylopectin content in waxy starches or higher amount of branching favors slow digestion after debranching (*30*). BV and $OD_{620}-OD_{680}$ showed strong correlation to HD, which indicated that the structure of amylopectin might play a role in HD.

Digestion Properties of Amylopectin and Starch. The chain length profiles of five mutants are shown in **Figure 4A**. The short chains ($8 \leq DP \leq 12$) showed positive correlation to the HD for materials with similar RS but different HD (**Figure 4B**). The same trends were also observed among mutants with different RS and HD (**Figure 4C**). Hydrolysis of starch granules in A-type starches by α-amylase was positively correlated with the proportions of unit chains with DP8−11 and negatively correlated with the proportions of unit chains with 18−23 (*31*). Ring et al. (*34*) reported that the retrogradation of starch was mainly caused by the recrystallinization of short amylopectin chains such as DP15−18 and retrograded starch was the major RS in cooked rice. Ao et al. (*35*) found that amylopectin with a higher proportion of short chains, especially short A chains ($DP < 13$), due to its higher branch density and short chain length, was more easily retrograded, thereby reducing enzyme susceptibility, which made

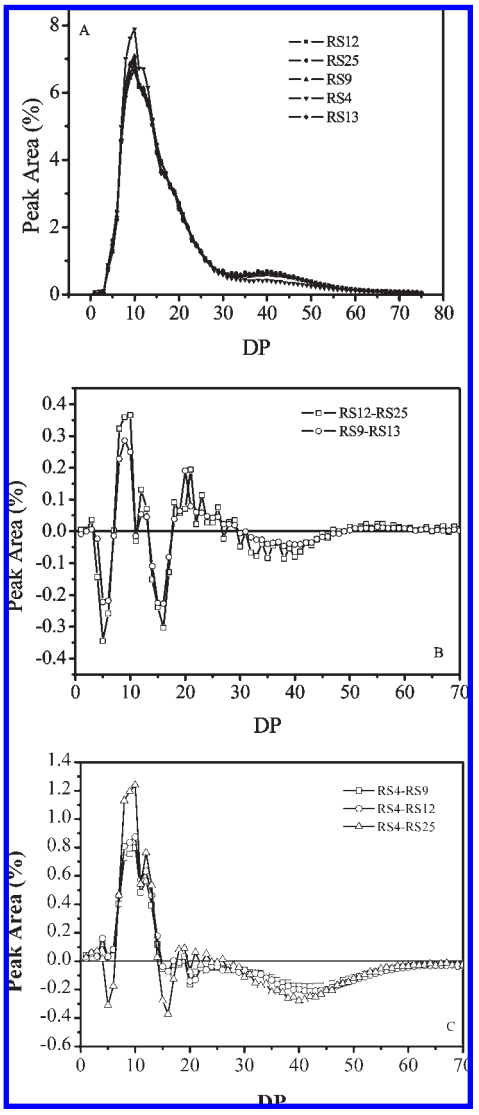

Figure 4. Chain length profiles of five mutants and differences among them.

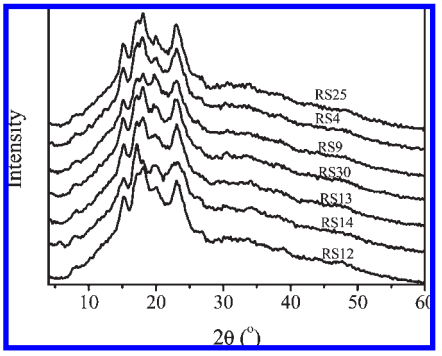

Figure 5. X-ray diffraction profile of all rice materials.

it more difficult for the amylolytic enzymes to digest. Amylopectin is the starch molecule associated with SDS (*36*); amylopectin with a higher amount of either short chains or long chains was more slowly digestible. Maize mutants high in long chains represent a physical entity that produces a slow digestion property, and mutants high in short chains represent a chemical entity of slow digestion property (*37*).

The more short chains there were, the lower the HD. This is different from those studies showing that slowly digestible starch

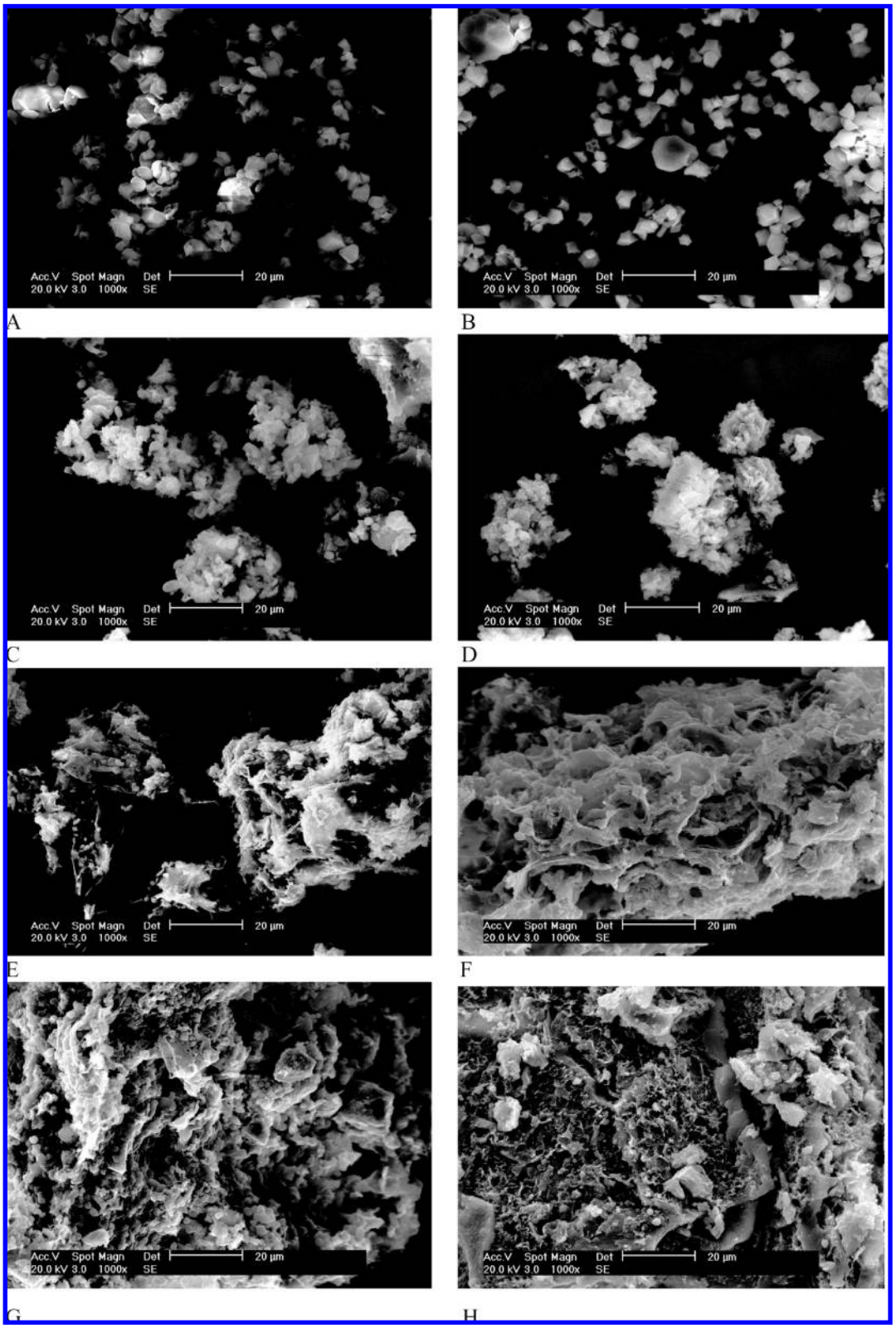

Figure 6. SEM images of native rice starch isolated by protease (**A**, **B**) and cooked rice starch digested for 0 (control, **C**, **D**), 30 (**E**, **F**), and 120 min (**G**, **H**). Images **A**, **C**, **E**, and **G** show starch granules observed after different pretreatments of RS12, and images **B**, **D**, **F**, and **H** are of RS25.

was negatively correlated with chains of DP < 13 in 12 rice cultivars with a narrow range of amylose contents (around 16%) (*38*). A negative correlation between RS and DP6−12 indicated that the granule resistance decreased with an increase in the proportion of short chains in the starch granules (*39*). The observed differences might be due to variations in the materials used.

Starch Crystallinity. Although the materials showed different short-chain fractions, their starch crystallinity had no correlation with the hydrolysis rate (**Table 2**). All materials showed an X-ray diffraction profile characteristic of A-type starch, whereas sharp reflection peaks at angles of 20° 2θ were observed, which is a typical amylose−lipid complex diffraction peak (**Figure 5**). There

is no significant correlation between the fraction of short chains and the degree of crystallinity, suggesting not all $A+B_1$ (fraction short chain) chains participate in the crystalline lamella in an ordered crystalline structure (*40*). Amylose−lipid complexes in barley starches, lentil, oat, and wheat were all shown to have a slower digestibility in vitro (*30*).

In native rice starches, the chains of DP6−9 were negatively correlated with crystallinity (*41*). A higher proportion of the short-chain amylopectin (SAC) fraction results in less perfect crystallites; a high amount of short A chains of DP6−11 suggests an accumulation of dangling chains or defective crystallites that favor a high initial hydrolysis rate (*42*). The high proportion of SDS in cereal starches was related to their A-type crystalline structure with a lower degree of perfection as indicated by a higher amount of shortest A chains with a DP of 5−10. The A-type crystalline structure determines the slow digestion property of native cereal starches (*39*). However, Zhang et al. (*43*) showed that amorphous and crystalline regions are evenly digested and starch was digested evenly inside-out and side-by-side. Although the short amylopectin chains defect the crystallites, they are densely packed and arranged tightly to the crystalline regions, which inhibit a favored hydrolysis. For wheat starches, it was shown that the rate of acidic hydrolysis increased with the increasing defectiveness of the crystalline lamellae, irrespective of differences in structural organization of the amorphous lamellae (*44*).

Starch Granule Structure. Two typical materials with similar RS contents but different hydrolysis rates, RS12 and RS25, were selected for starch granule investigation during the process of in vitro digestion with 3U amylase. Starch granules isolated by protease were mostly of pentagonal and angular shape, whereas in RS12, a higher percentage of oval shape was observed (**Figure 6A**,**B**). After gelatinization, the starch aggregated and formed gels during cooling; only a few round starch granules can be seen (**Figure 6C**,**D**). Yang et al. (*9*) observed that rice mutants with high RS have a much higher proportion of round starch granules. The higher RS content may be party due to the granule size and the differences in the surface/volume ratio. Retrogradation starch will enhance the resistance to enzyme hydrolysis and nonstarch polysaccharides, and amylose−lipid complexes reduce starch digestibility considerably (*45*).

During the course of in vitro digestion, the integrated starch gels of RS12 and RS25 were broken to different extents. For RS25, there were only skeletons of the aggregated starch left, and pores were enlarged after 30 min of digestion, whereas for RS12, there were still well-aggregated starch gels and also small round starch granules could be seen (**Figure 6E**,**F**). With progressive hydrolysis, the integrated starch gels in RS25 showed many fewer residues than in RS12, compared with the residues observed in RS25, and there were still some round starch granules with smooth surfaces that remained on the surface of the integrated starch gels in RS12 even after 120 min of digestion. For native cereal starch, the hydrolysis began with enlargement of the surface pores and channels with concurrent hydrolysis from the helical region toward the outside of the granules (*46*). This so-called "inside-out" digestion pattern fits only starches with pores and channels, whereas for starches without pores, such as B-type starches, an "exo-pitting" starch hydrolysis pattern is observed. For the integrated starch gels after cooking, these two hydrolysis patterns might exist concomitantly. The round starch granules and crystalline aggregated starch after cooking might explain for the different hydrolyses of starch in rice materials with different RS contents.

Principal Component Analysis—Overview of the Materials. PCA was used to visualize the variation of all of these materials

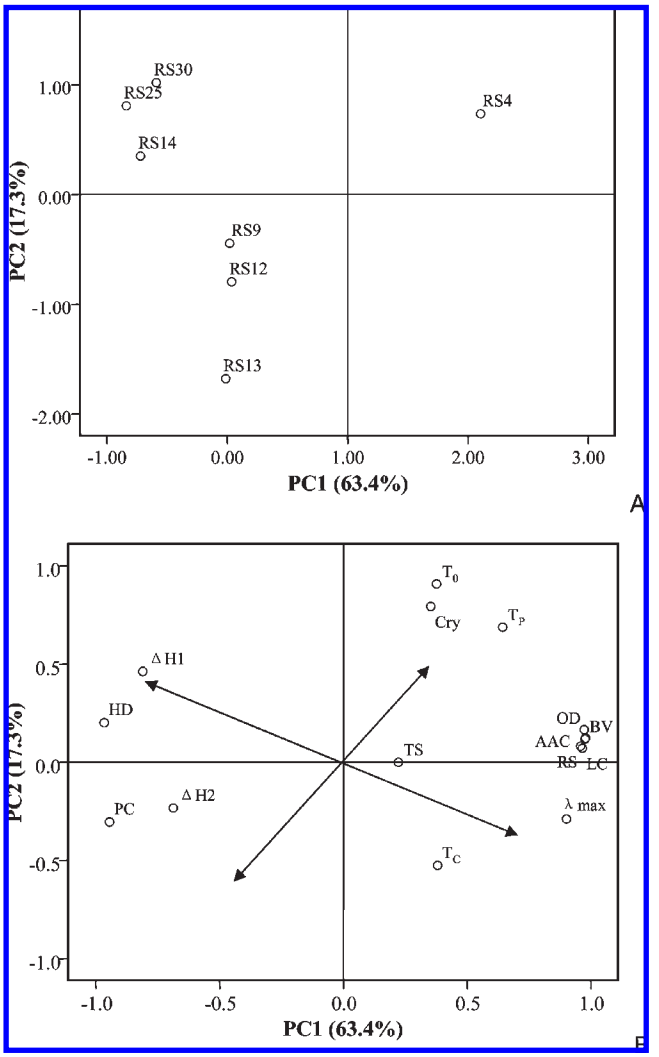

Figure 7. Score plot (**A**) and loading plot (**B**) of principal components 1 and 2 (PC1 and PC2), describing the variation in all of the parameters of the seven rice samples. Arrows indicate main trends in correlations between the different measured variables in the studied materials.

in starch properties such as AAC, gelatinization and retrogradation, and in vitro digestion. With this statistical method, a large number of variables is reduced to a few orthogonal variables, which describe the greatest covariance in the data analyzed. The PCA plots provide an overview of the similarities and differences between different rice samples as well as the interrelationships between the measured properties. The first and second PCs describing 63.4 and 17.3% of the variance, respectively, provided an overview of the samples. The distance between the locations of any two samples on the score plot is directly proportional to the degree of difference or similarity between them (**Figure 7A**); RS4 departed greatly from other materials, indicating that the differences between RS4 and other materials are mainly caused by PC1. RS14, RS25, and RS30 clustered together and differed from the left three samples, RS13, RS9, and RS12, mainly in PC2. The variances showed little difference with the PCA of in vitro hydrolysis (**Figure 2A**).

The loading plot of the two first PCs provided information regarding the correlations between measured properties, which described 80.7% of the variance in all variables (**Figure 7B**). Variables found close to each other in pairs or groups indicate a positive correlation. The variables of the gelatinization enthalpy (ΔH_1 and ΔH_2) and the protein content (PC) all showed positive

correlation to the degree of hydrolysis (HD). Variables found in orthogonal directions, as indicated by the arrows in **Figure 7B**, varied independently of each other, whereas those in the opposite directions are negatively correlated. HD showed highly significant correlations with RS, AAC, LC and other starch physiochemical properties in all of these materials with different RS contents.

In conclusion, starch hydrolysis is affected by many factors. For rice materials with different RS contents, the RS showed significant positive correlation with the degree of starch hydrolysis, whereas for those rice materials with similar RS contents, the content of lipids and starch−lipid complex was shown to be a key factor for starch hydrolysis. In addition, starch structure played a great role in starch hydrolysis; short chains of DP8−12 correlated negatively with the digestibility of starch, and the integrity of aggregated starch and the numbers of round granules observed after cooking contributed greatly to slow starch digestibility.

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LITERATURE CITED

(1) Ludwig, D. Dietary glycemic index and obesity. *J. Nutr.* **2000**, *130* 2S, 280−283.

(2) Brand-Miller, J.; Hayne, S.; Petocz, P.; Colagiuri, S. Low-glycemic index diets in the management of diabetes. *Diabetes Care* **2003**, *26*, 2261–2267.

(3) Englyst, H.; Hudson, G. The classification and measurement of dietary carbohydrates. *Food Chem.* **1996**, *57*, 15–21.

(4) Englyst, K.; Englyst, H.; Cumming, J. Rapidly available glucose in foods: an vitro measurement that reflects the glycemic resoponse. *Am. J. Clin. Nutr.* **1999**, *69*, 448–454.

(5) Nugent, A. Health properties of resistant starch. *Nutr. Bull.* **2005**, *30*, 27–54.

(6) Tetens, I.; Biswas, S.; Glits, L.; Kabir, K.; Thilsted, S.; Choudhury, N. Physiochemical characteristics as indicators of starch availability from milled rice. *J. Cereal Sci.* **1997**, *26*, 355–361.

(7) Hu, P.; Zhao, H.; Duan, Z.; Zhang, L.; Wu, D. Starch digestibility and estimated glycemic score of different types of rice differing in amylose content. *J. Cereal Sci.* **2004**, *20*, 1–7.

(8) Yang, C.; Li, C.; Shu, X.; Zhang, Z.; Zhang, L.; Zhao, H.; Ma, C.; Wu, D. Starch properties of rice mutant enriched with resistant starch. *Chinese J. Rice Sci.* **2005**, *19*, 516–520.

(9) Yang, C.; Shu, X.; Zhang, L.; Wang, X.; Zhao, H.; Ma, C.; Wu, D. Starch properties of mutant rice high in resistant starch. *J. Agric. Food Chem.* **2006**, *54*, 523–528.

(10) Shu, X.; Jiao, G.; Fitzgerald, M.; Yang, C.; Wu, D. Starch structure and digestibility of rice high in resistant starch. *Starch/Staerke* **2006**, *58*, 411–417.

(11) Shu, X.; Jia, L.; Gao, J.; Nakamura, K.; Wu, D. The influences of chain length of amylopectin on resistant starch in rice (*Oryza sativa* L.). *Starch/Staerke* **2007**, *59*, 504–509.

(12) Frei, M.; Siddhuraju, P.; Becher, K. Studies on the in vitro starch digestibility and the glycemic index of six different indigenous rice cultivars from the Philippines. *Food Chem.* **2003**, *83*, 395–402.

(13) Fässler, C.; Arrigoni, E.; Venema, K.; Hafner, V.; Brouns, F.; Amado, R. Digestiblity of resistant starch containing preparations using two in vitro models. *Eur. J. Nutr.* **2006**, *45*, 445–453.

(14) Stevnebø, A.; Sahlstőm, S.; Svihus, B. Starch structure and degree of starch hydrolysis of small and large starch granules from barley varieties with varying amylose content. *Anim. Feed Sci. Technol.* **2006**, *130*, 23–38.

(15) Englyst, H.; Kingman, S.; Hudson, G.; Cummings, J. Measurement ofresistant starch in vitro and in vivo. *Br. J. Nutr.* **1996**, *75*, 749–755.

(16) Goñi, I.; Garcia-Alonso, A.; Saura-Calixto, F. A starch hydrolysis procedure to estimate glycemic index. *Nutr. Res.* (*N.Y.*) **1997**, *17*, 427–437.

(17) Mei, S.; Jia, L.; Gao, J.; Yang, C.; Shu, X.; Wu, D. A simplified method for the determination of amylose content in rice. *Acta Agric. Nucl. Sin.* **2007**, *21*, 246–248.

(18) American Association of Cereal Chemists. *Approved Methods of the AACC*, 9th ed.; AACC: St. Paul, MN, 1995; Method 08-10.

(19) Juliano, B.; Pascual, C. Quality characteristics of milled rice grown in different countries. *IRRI Res. Pap. Ser.* **1980**, No. *48*, 25.

(20) García-Alonso, A.; Jiménez-Escrig, A.; Martí′n-Carrón, N.; Bravo, L.; Saura-Calixto, F. Assessment of some parameters involved in the gelatinization and retrogration of starch. *Food Chem.* **1999**, *66*, 181–187.

(21) Nakamura, Y.; Kubo, A.; Shimamune, T.; Matsuda, T.; Harada, K.; Satoh, H. Correlation between activities of starch debranching enzyme and α-polyglucan structure in endosperm of sugary-1 mutants of rice. *Plant J.* **1997**, *12*, 143–153.

(22) Svihus, B.; Uhlen, A.; Harstad, O. Effect of starch granule structure, associated components and processing on nutritive value of cereal stach: a review. *Anim. Feed Sci. Technol.* **2005**, *122*, 303–320.

(23) Mangala, S.; Udayasankar, K.; Tharanathan, R. Resistant starch from processed cereals: the influence of amylopectin and non-carbohydrate constituents in its formation. *Food Chem.* **1999**, *64*, 391–396.

(24) Panlasigui, L.; Thompson, L.; Juliano, B.; Perez, C.; Yiu, S.; Greenberg, G. Rice varieties with similar amylose content differ in starch digestibility and glycemic response in humans. *Am. J. Clin. Nutr.* **1991**, *54*, 871–877.

(25) Eliasson, A.; Krog, N. Physical properties of amylose−monoglyceride complex. *J. Cereal. Sci.* **1985**, *3*, 239–248.

(26) Goñi, I.; Carcía-Diz, L.; Mañas, E.; Saura-Calixto, F. Analysis of resistant starch: a method for foods and food products. *Food Chem.* **1996**, *56*, 445–449.

(27) Guraya, H.; Kadan, R; Champagne, E. Effect of rice starch−lipid complexes on in vitro digestibility, complexing index, and viscosity. *Cereal Chem.* **1997**, *74*, 561–565.

(28) Holm, J.; Björck, I.; Ostrowska, S.; Eliasson, A.; Asp, N.; Larsson, K.; Lundquist, I. Digestibility of amylose−lipid complexes in vitro and in vivo. *Starch/Staerke* **1983**, *35*, 294–297.

(29) Kwaśniewska-Karolak, I.; Nebesny, E.; Rosicka-Kacamarek, J. Characterization of amylose−lipid complexes derived from different wheat varieties and their susceptibility to enzymatic hydrolysis. *Food Sci. Technol. Int.* **2008**, *14*, 29–37.

(30) Lehmann, U.; Robin, F. Slowly digestible starch—its structure and health implications: a review. *Trends Food Sci. Technol.* **2007**, *18*, 346–355.

(31) Srichuwong, S.; Sunarti, T.; Mishima, T.; Isono, N.; Hisamatsu, M. Starches from different botanical sources I: contribution of amylopectin fine structure to thermal properties and enzyme digestibility. *Carbohydr. Polym.* **2005**, *60*, 529–538.

(32) Eerlingen, R.; Deceunick, M.; Delcour, J. Enzyme-resistant starch. II. Influence of amylose chain on resistant starch formation. *Cereal Chem.* **1993**, *70*, 345–350.

(33) Hung, P.; Maeda, T.; Miskelly, D.; Tsumori, R.; Morita, N. Physicochemical characteristics and fine structure of high-amylose wheat starches isolated from Australian wheat cultivars. *Carbohydr. Polym.* **2008**, *71*, 656–663.

(34) Ring, S.; Colonna, P.; I'Anson, K.; Kalichevsky, M.; Miles, M.; Morris, V.; Orford, P. Gelation and crystallization of amylopectin. *Carbohydr. Res. 2987*, *162*, 277−293

(35) Ao, Z.; Simsek, S.; Zhang, G.; Venkatachalam, M.; Reuhs, B.; Hamker, B. Starch with a slow digestion property produced by altering branch density, chain length and crystalline structure. *J. Agric. Food Chem.* **2007**, *55*, 4540–4547.

(36) Zhang, G.; Ao, Z.; Hamaker, B. Nutritional property of endosperm starches from maize mutants: a parabolic relationship between slowly digestible starch content and amylopectin fine structure. *J. Agric. Food Chem.* **2008**, *56*, 4686–4694.

(37) Zhang, G.; Sofyan, M.; Hamaker, B. Slowly digestible state of starch: mechanism of slow digestion property of gelatinied maize starch. *J. Agric. Food Chem.* **2008**, *56*, 4695–4702.

(38) Benmoussa, M.; Moldenhauer, K.; Hamaker, B. Rice amylopectin fine structure variability affects starch digestion properties. *J. Agric. Food Chem.* **2007**, *55*, 1475–1479.
(39) Charles, A.; Chang, Y.; Ko, W.; Sriroth, K.; Huang, T. Influence of amylopectin structure and amylose content on the gelling properties of five cultivars of cassava starches. *J. Agric. Food Chem.* **2005**, *53*, 2717–2725.
(40) Zhang, G.; Venkatachalam, M.; Hamaker, B. Structural basis for the slow digestion property of native cereal starches. *Biomacromolecules* **2006**, *7*, 3259–3266.
(41) Vandeputte, G.; Vermeylen, R.; Geeroms, J.; Delcour, J.; Rice starches, I. Structural aspects provide insight into crystallinity characteristics and gelatinisation behaviour of granular starch. *J. Cereal Sci.* **2003**, *38*, 43–52.
(42) Gerard, C.; Colonna, P.; Buleon, A.; Planchot, V. Amylolysis of maize mutant starches. *J. Sci. Food Agric.* **2001**, *81*, 1281–1287.
(43) Zhang, G.; Ao, Z.; Hamaker, B. Slow digestion property of native cereal starches. *Biomacromolecules* **2006**, *7*, 3252–3258.
(44) Kozlov, S.; Noda, T.; Bertoft, E.; Yuryev, V. Structure of starches extracted form near isogenic wheat lines. *J. Therm. Anal. Calorim.* **2006**, *86*, 291–301.
(45) Edwards, C. The physiological effects of dietary fibre. In *Dietary Fiber in Health and Disease*; Kristchevsky, D., Bonfield, C., Eds.; Eagan Press: St. Paul, MN, 1990; pp 58−71.
(46) Gallant, D.; Bouchet, B.; Buléon, A.; Pérez, S. Physical characteristics of starch granules and susceptibility to enzymatic degradation. *Eur. J. Clin. Nutr.* **1992**, *46*, S3–S6.

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