



Digestibility and physicochemical properties of rice (*Oryza sativa* L.) flours and starches differing in amylose content

Li-Jia Zhu^{a,b}, Qiao-Quan Liu^{a,*}, Jeff D. Wilson^c, Ming-Hong Gu^a, Yong-Cheng Shi^{b,**}

^a Key Laboratory of Plant Functional Genomics of the Ministry of Education, Jiangsu Key Laboratory of Crop Genetics and Physiology, Yangzhou University, Yangzhou 225009, PR China

^b Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506, USA

^c USDA-ARS Center for Grain and Animal Health, Manhattan, KS 66502, USA

ARTICLE INFO

Article history:

Received 11 May 2011

Received in revised form 30 June 2011

Accepted 9 July 2011

Available online 19 July 2011

Keywords:

Rice

Starch digestibility

Amylose

Resistant starch

ABSTRACT

Digestibility of starches in four rice samples with amylose content (AC) from 1.7 to 55.4%, including a newly developed high-amylose rice, was investigated. An *in vitro* enzymatic starch digestion method and an AOAC method were applied to correlate rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS), and total dietary fiber (TDF) content with the AC in the samples. SDS content decreased and RS and TDF content increased with the increase in AC. The low-amylose rice (AC=16.1%) had starch granules with weak crystalline structure and was lower in RS and TDF content even though it had a higher AC compared to waxy rice. The digestibility of the starches was not correlated with granule size or degree of crystallinity. The newly developed high-amylose rice starch exhibited a predominant B-type X-ray diffraction pattern, a great proportion of long chains in amylopectin, high gelatinization temperature, and semi-compound starch granules which are attributed to its increased resistance to enzyme digestion.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Starch is classified as rapidly digestible starch (RDS), slowly digestible starch (SDS), or resistant starch (RS) (Englyst, Kingman & Cummings, 1992). RS is defined as the portion of dietary starch that is not digested in the small intestine of a healthy human (Asp, 1992). When ingested, RS causes less starch to be converted to glucose, which has potential impact on diabetes and energy intake; provides fermentable carbohydrates for colonic bacteria; and produces short chain fatty acids that have direct health benefits to the colon and may play other physiological roles (Bird, Lopez-Rubio, Shrestha & Gidley, 2009). RDS content has been positively correlated to the glycemic index (GI) of starchy foods (Englyst, Englyst, Hudson, Cole & Cummings, 1999; Englyst, Vinoy, Englyst & Lang, 2003). The potential health benefits associated with SDS have been reviewed by Zhang and Hamaker (2009).

Many factors, including surface organization (e.g. pores), granular architecture, starch composition, type of crystal polymorph, granular size, and the presence of compound granules, affect the rate and extent of digestion of starch granules (Bird et al., 2009). The exact underlying mechanism of relative resistance of starch granules is complicated because those factors are often interconnected. In general, however, RS content of granular starches is positively correlated with the level of amylose (Benmoussa, Moldenhauer & Hamaker, 2007; Cone and Wolters, 1990; Evans and Thompson, 2004; Li, Vasanthan, Hoover & Rossnagel, 2004; Rendleman, 2000; Riley et al., 2004; Sang, Bean, Seib, Pedersen & Shi, 2008; Themeier, Hollman, Neese & Lindhauer, 2005; Vasanthan and Bhatti, 1996), although exceptions in pea starches are notable in that pea starches with medium amylose contents have high RS contents (Themeier et al., 2005).

Maize starches with amylose content from 0 to 90% are available (Shi, Capitani, Trzasko & Jeffcoat, 1998) and a strong correlation has been reported between amylose and RS content (Morita, Ito, Brown, Ando & Kiriyama, 2007). Great efforts have been made to develop other crops containing high-amylose starch because of its unique functional and nutritional properties (Richardson, Jeffcoat & Shi, 2000). High-amylose potato (Schwall et al., 2000), barley (Morell et al., 2003), and wheat (Regina et al., 2006) have been developed. Rice is the second-highest-produced grain in the world after maize and is a staple food in Asia. We have been developing high-amylose rice since 2000 (Chen, 2003). The objectives of this study were to

Abbreviations: AACC, American Association of Cereal Chemistry; AC, amylose content; AOAC, Association of Official Analytical Chemists; DMSO, dimethyl sulfoxide; DSC, differential scanning calorimeter; GBSS, granular bound starch synthetase; GPC, gel permeation chromatography; RDS, rapidly digestible starch; RS, resistant starch; SDS, slowly digestible starch; SEM, scanning electron microscope; TDF, total dietary fiber.

* Corresponding author. Tel.: +86 514 8799 6648; fax: +86 514 8799 6817.

** Corresponding author. Tel.: +1 785 532 6771; fax: +1 785 532 7010.

E-mail addresses: qqliu@yzu.edu.cn (Q.-Q. Liu), ycshi@ksu.edu (Y.-C. Shi).

determine the digestibility of rice starches with a wide range of amylose contents, including a newly developed rice with greater than 50% amylose content, and to investigate factors affecting their digestibility.

2. Materials and methods

2.1. Rice samples

The mature grains of four *indica* rice cultivars/lines with different amylose contents were grown from May to October in 2007 at the experimental farm of Agricultural College, Yangzhou University, Yangzhou, China. The cultivars 'Yang-fu-nuo', 'Yang-dao 6', and 'Te-qing' contained very low (waxy rice), low, and intermediate contents of amylose, respectively, and the high-amylose rice (HAR) was a transgenic rice line generated from the cultivar 'Te-qing' (Zhu, 2009). After the mature seeds were harvested and air-dried, seeds were dehusked in an electrical dehusker (Model SDL-A, Rice Product Quality Supervision and Inspection Center of Ministry of Agriculture, China) and polished by a grain polisher (Model Kett, Tokyo, Japan). The polished rice was ground by a mill (FOSS 1093 Cyclotec Sample Mill, Höganäs, Sweden) with a 0.5 mm screen.

2.2. Starch isolation

Rice starches were isolated from polished rice by an alkaline protease method (Lumdubwong and Seib, 2000) with slight modifications (Zhu, Liu, Sang, Gu & Shi, 2010).

2.3. Composition analysis

Amylose content was determined by a modified Con A method developed by Yun and Matheson (1990), and total starch was determined by the AACC Method 76-13 (AACC International, 2000) each with an assay kit from Megazyme International Ltd. (Wicklow, Ireland). The moisture content was measured according to the AACC Air Oven Method 44-19 (AACC International, 2000) at 135 °C for 2 h.

Crude protein content was measured by nitrogen combustion with a nitrogen determinator (LECO FP-528, St. Joseph, MI) according to AOAC method 990.03 (AOAC International, 1999). Nitrogen (N) values were converted to protein content by $N \times 5.95$. Crude ash was determined by AOAC method 942.05 (AOAC International, 1999).

All the tests were done in duplicate.

2.4. Starch digestion profile

RDS, SDS and RS were determined by a modified procedure of Englyst method (Sang and Seib, 2006).

2.5. Total dietary fiber

Total dietary fiber (TDF) was determined by the AOAC Method 991.43 (AOAC International, 2003) using a TDF assay kit from Megazyme International Ltd. (Wicklow, Ireland).

2.6. Thermal properties

The thermal transition temperatures of gelatinization and retrogradation of rice starch were measured by a differential scanning calorimeter (DSC) (DSC Q100, TA Instruments, New Castle DE) as previously described by Zhu et al. (2010). The solid content was 33.3%. Gelatinization was determined by heating starch in a DSC pan from 10 °C to 140 °C at a heating rate of 10 °C/min. The onset (T_o), peak (T_p), and conclusion (T_c) of gelatinization temperatures

and the enthalpy of gelatinization (ΔH) were determined. For retrogradation, gelatinized samples were stored at 4 °C for 1 week and then rescanned with the same conditions used in the gelatinization test.

2.7. Debranching of starch

Purified waxy rice and low-amylose rice starches were debranched by isoamylase (EC3.2.1.68, Hayashibara Biochemical Laboratories, Inc., Okayama, Japan) as follows. Each starch (20 mg) was added to 10 ml 0.01 N acetate buffer (pH 4.2) in a 12-ml glass vial with a microstir bar. The vial was placed in a boiling water bath for 1 h. After the solution was cooled to 25 °C, isoamylase (50 μ l) was added. The vial was placed in a water bath at 50 °C overnight, cooled to 25 °C, frozen in a dry ice/acetone bath, and freeze-dried. Purified intermediate-amylose rice and high-amylose rice starches were debranched by isoamylase as follows. Starch (30 mg) was added to 3 ml dimethyl sulfoxide (DMSO) in a 30 ml glass vial with a microstir bar. The vial was placed in a boiling water bath for 20 min with constant stirring. After the mixture was cooled to 25 °C, 10 ml sodium acetate buffer (50 mM) and isoamylase (3 μ l) was added. The vial was placed in a 50 °C water bath for 16 h, then 117 ml of ethanol (ethanol:solution=9:1, v/v) was added to precipitate the debranched starch and the mixture was held at 4 °C overnight. The mixture was centrifuged at 2200 \times g for 10 min, and the supernatant was discarded. The sediment was frozen in a dry ice/acetone bath, and freeze-dried.

2.8. Gel permeation chromatography (GPC)

Molecular weight distribution of starch was determined by GPC (PL-GPC 220, Polymer Laboratories Varian, Inc. Amherst, MA) as previously described (Zhu et al., 2010).

For GPC analysis, 4 mg (dry basis) of each debranched starch was dissolved in 4 ml DMSO solution in a boiling water bath for 24 h with constant stirring. Each starch solution was filtered through a 2.0- μ m filter, and the filtrate was injected into a GPC system by an autosampler.

2.9. X-ray diffraction analysis

The crystalline structure of the starches was analyzed by using a Philips X-ray diffractometer (MAC Science Co. MO3XHF22, Tokyo, Japan) at 35 kV and 20 mA Cu-K α radiation. Diffractograms were obtained from 2° to 35° (2θ) at a scan rate of 2°/min. In addition to as is samples, starches were hydrated with water and examined at 20 and 40% moisture content.

2.10. Particle size analysis

Particle size distributions of rice flours and starches were measured using a Beckman Coulter LS 13 320 Laser Diffraction Size Analyzer (Beckman Coulter, Inc. Brea, CA, USA). Starch samples were suspended in 1% sodium azide–water solution, sonicated for 30 s and then introduced into the universal liquid module until the required obscuration of 8–12% was achieved. The suspension was sonicated again for 30 s to assure the suspension had not agglomerated. Approximately 2 g of the flour samples were analyzed dry, in the tornado module. At least 2 replicates were done on each sample.

2.11. Scanning electron microscope (SEM)

The native starch samples with their residue after digestion were coated with gold palladium using a sputter coater (Denton Vacuum, LLC, Moorestown, NJ) and viewed at 1000 \times and

4000× resolution, respectively, with a scanning electron microscope (S-3500N, Hitachi Science Systems, Ltd., Japan) operating at an accelerating voltage of 20 kV.

2.12. Statistical analysis

For characterization of the samples, at least two replicate measurements were performed, unless otherwise specified. All data were reported as the mean ± standard deviation (mean ± SD). The results were analyzed by using analysis of variance (ANOVA) (SPSS version 13.0, SPSS Inc., Chicago, IL), and the Student's *t* test was used to examine the differences. Results with a corresponding probability value of $p < 0.05$ were considered to be statistically significant.

3. Results and discussion

3.1. Composition of flours and starches

Table 1 shows the composition of flours and starches from the four rice samples. The amylose content as determined by a ConA method in endosperm starches of the four rice samples were 1.7, 16.1, 22.5 and 55.4%, respectively. Protein content in intermediate-amylose rice and high-amylose rice starches was higher than that in waxy rice and low-amylose rice, presumably because amylose was synthesized by granular bound starch synthetase (GBSS), and with increasing amylose content, GBSS, which was bound to the starch granule and difficult to remove, increased. High-amylose maize starch tends to have higher protein content than normal maize starch (Morita et al., 2007). Ash content was increased with increase in amylose content. In flours, high-amylose rice contained a low total starch content. Intermediate-amylose rice and waxy rice had higher protein content than low-amylose rice and high-amylose rice in flours.

3.2. Structural characterization of starch

The molecular weight distributions of isoamylase-debranched starches from four rice cultivars were examined by GPC (Fig. 1). Because waxy rice was ~100% amylopectin, only two well resolved fractions were observed when the starch was separated by GPC. The other three rice starches, low-amylose rice, intermediate-amylose rice, and high-amylose rice, contained both amylose and amylopectin, and thus three well resolved fractions were observed. The first two fractions, fraction I (F1) and fraction II (F2), were unit chains from amylopectin (Fig. 1). F1 consists of high molecular weight molecules including long B chains; F2 consists of low molecular weight molecules including A and short B chains. The third fraction represented amylose in starch.

GPC analysis indicated that amylopectin chains in high-amylose rice and intermediate-amylose rice were longer than that in waxy rice and low-amylose rice (Fig. 1). High-amylose rice appeared

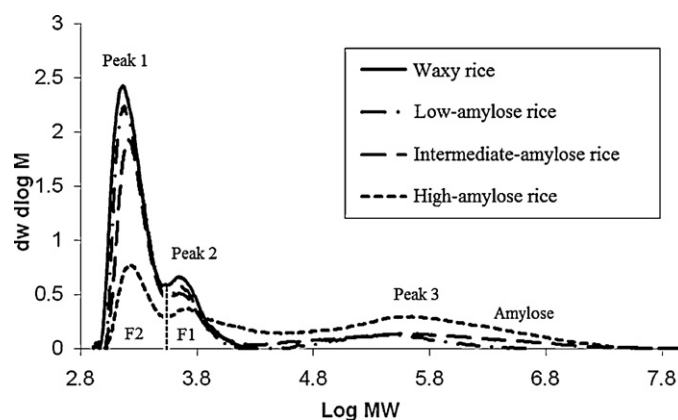


Fig. 1. Gel-permeation chromatograms of isoamylase-debranched rice starches.

to contain the highest proportion of long chains in amylopectin among the four rice starches. The same trend was observed in maize starches (Shi et al., 1998). High-amylose rice and intermediate-amylose rice also had longer amylose chains compared to low-amylose rice. It is interesting to note the longer amylose chains in high-amylose rice. In contrast, amylose chains in high-amylose maize starches were shorter than that in normal maize starch (Hizukuri, Abe & Hanashiro, 2006; Shi et al., 1998).

3.3. Gelatinization and retrogradation

The four samples exhibited broad endotherms in gelatinization and retrogradation (Fig. 2). Waxy rice starch had the highest gelatinization onset temperature (Table 2). The high-amylose rice starch had the highest T_c (106.0°C). In agreement with maize starches with different amylose contents (Shi et al., 1998), the endotherm of high-amylose rice starch was the broadest among all samples, followed by intermediate-amylose rice, waxy rice, and low-amylose rice starch. The low-amylose rice starch had the lowest gelatinization temperature.

Retrogradation endotherms were observed at 50.4–69.6°C for waxy rice, 43.4–66.8°C for low-amylose rice, 41.8–68.5°C for intermediate-amylose rice, and 43.2–75.3°C for high-amylose rice (Table 2). Waxy rice still had the highest T_0 and T_p , and high-amylose rice had the highest T_c and broadest endotherm. Melting of amylose-lipid complex also was observed in retrograded low-amylose rice, intermediate-amylose rice, and high-amylose rice starches (Fig. 2B).

3.4. X-ray diffraction patterns

Fig. 3 shows the X-ray diffraction patterns of the four rice starches. The intensity of the 20° peak, representing amylose-lipid complex, was increased along with amylose content. Waxy rice,

Table 1
Composition of the four rice flours and starches.^a

| Sample | Waxy rice | Low-amylose rice | Intermediate-amylose rice | High-amylose rice |
|-------------------|--------------|------------------|---------------------------|-------------------|
| <i>Starch</i> | | | | |
| Amylose (%) | 1.7 ± 0.3 | 16.1 ± 0.7 | 22.5 ± 0.3 | 55.4 ± 0.4 |
| Crude protein (%) | 0.38 ± 0.00 | 0.25 ± 0.00 | 0.61 ± 0.00 | 0.83 ± 0.00 |
| Ash (%) | 0.02 ± 0.01 | 0.11 ± 0.00 | 0.14 ± 0.01 | 0.24 ± 0.02 |
| Moisture (%) | 8.8 ± 0.0 | 11.9 ± 0.2 | 11.8 ± 0.1 | 12.9 ± 0.0 |
| <i>Flour</i> | | | | |
| Total starch (%) | 81.5 ± 0.0 | 80.7 ± 0.3 | 83.5 ± 0.8 | 77.3 ± 0.3 |
| Crude protein (%) | 10.66 ± 0.03 | 9.24 ± 0.02 | 11.39 ± 0.00 | 9.37 ± 0.01 |
| Ash (%) | 0.55 ± 0.01 | 0.56 ± 0.00 | 0.97 ± 0.00 | 0.49 ± 0.00 |
| Moisture (%) | 11.0 ± 0.0 | 11.1 ± 0.0 | 9.8 ± 0.1 | 9.5 ± 0.1 |

^a All data are means ± standard deviations, $n = 2$.

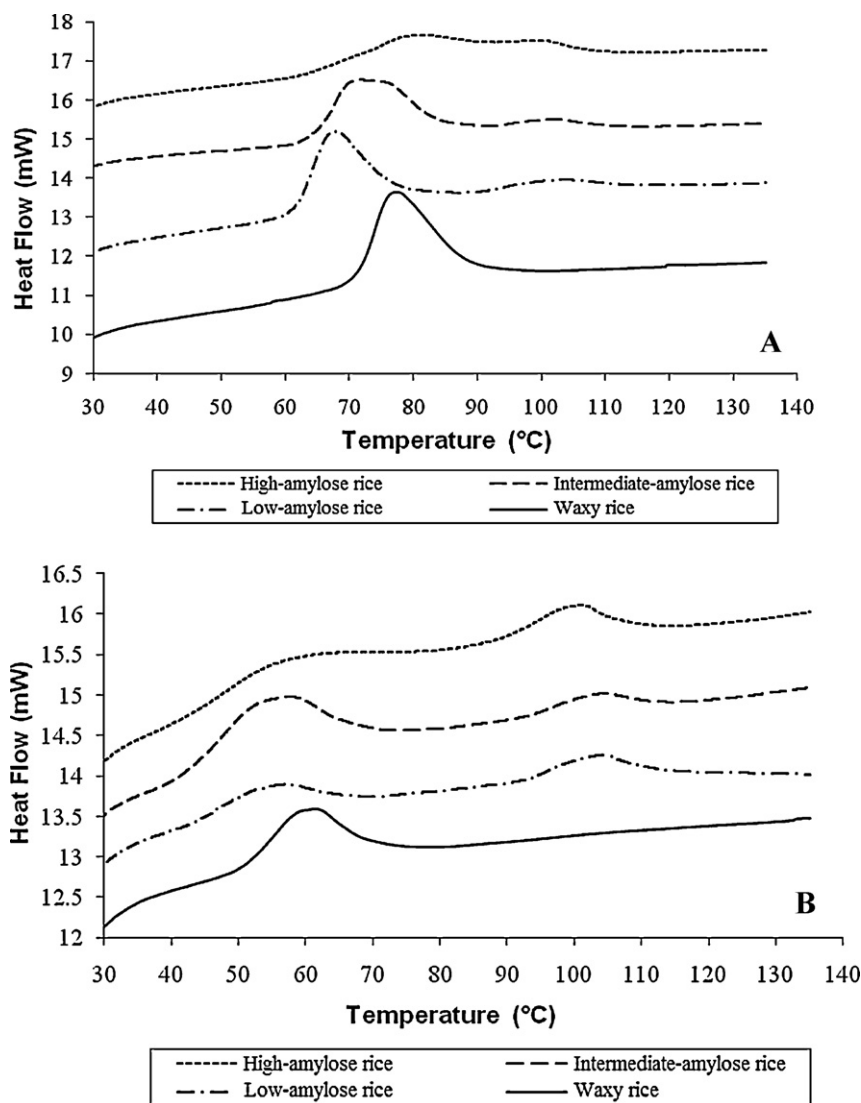


Fig. 2. The gelatinization (A) and retrogradation (B) of four rice starches at 33.3% starch solid as determined by differential scanning calorimetry.

low-amylose rice, and intermediate-amylose rice starches had an A-type X-ray diffraction pattern with the diffractions at 15° , a doublet at 17° and 18° , and 23° (2θ). For the high-amylose rice starch at 20% moisture content, instead of an unresolved doublet at 17° and 18° 2θ , only one peak at around 17° 2θ was observed. In addition,

a weak peak at 5° 2θ and a broad peak between 21° and 25° 2θ were noted. These features (Wei et al., 2010) suggest that the high-amylose rice starch had a C-type X-ray diffraction pattern at approximately 20% moisture content. However, when the high-amylose starch was hydrated to 40% moisture content, a relatively

Table 2
Gelatinization and retrogradation properties of four rice starches as determined by differential scanning calorimetry.

| Sample | Temperature ($^\circ\text{C}$) | | | Enthalpy ^a (J/g) ΔH |
|----------------------------------|----------------------------------|----------------|-----------------|---|
| | T_o | T_p | T_c | |
| <i>Gelatinization</i> | | | | |
| Waxy rice starch ^b | 70.9 ± 0.3 | 77.0 ± 0.1 | 89.8 ± 0.5 | 18.4 ± 0.2 |
| Low-amylose rice starch | 61.6 ± 0.0 | 67.5 ± 0.0 | 78.4 ± 0.1 | 14.9 ± 0.1 |
| Intermediate-amylose rice starch | 65.0 ± 0.2 | 70.9 ± 0.1 | 85.0 ± 0.6 | 15.6 ± 1.3 |
| High-amylose rice starch | 65.2 ± 0.6 | 79.9 ± 0.2 | 106.0 ± 0.0 | 15.8 ± 0.7^c |
| <i>Retrogradation</i> | | | | |
| Waxy rice starch | 50.4 ± 0.0 | 58.4 ± 1.9 | 69.6 ± 2.4 | 7.1 ± 1.7 |
| Low-amylose rice starch | 43.4 ± 0.0 | 55.5 ± 0.1 | 66.8 ± 0.1 | 3.5 ± 0.2 |
| Intermediate-amylose rice starch | 41.8 ± 0.3 | 54.5 ± 0.0 | 68.5 ± 0.4 | 9.5 ± 0.3 |
| High-amylose rice starch | 43.2 ± 0.7 | 56.7 ± 0.1 | 75.3 ± 0.3 | 4.6 ± 0.3 |

^a Enthalpy values are based on dry weight of starch. Data are means \pm standard deviation. Starch solid was 33.3%.

^b From Zhu et al. (2010).

^c Since the gelatinization peak in the high-amylose rice was merged with the peak of amylose-lipid complex, the enthalpy in high-amylose rice showed in this table was the summation of the gelatinization peak and the melting of amylose-lipid complex peak.

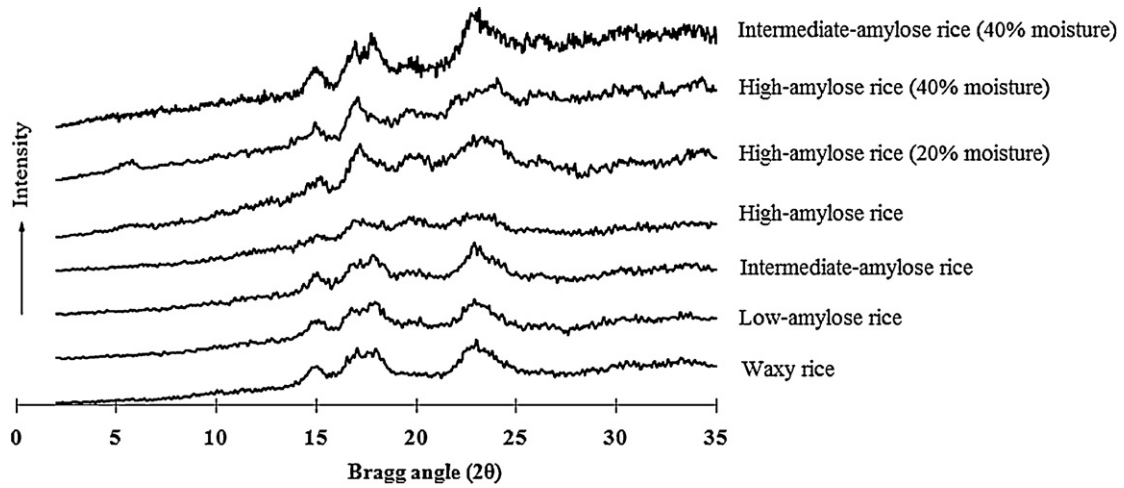


Fig. 3. X-ray diffraction patterns of four rice starches. (All starches had as-is moisture content of about 12%, except for additional runs of high-amylose rice starch at 20% and 40% water content, and intermediate-amylose rice starch at 40% water content.)

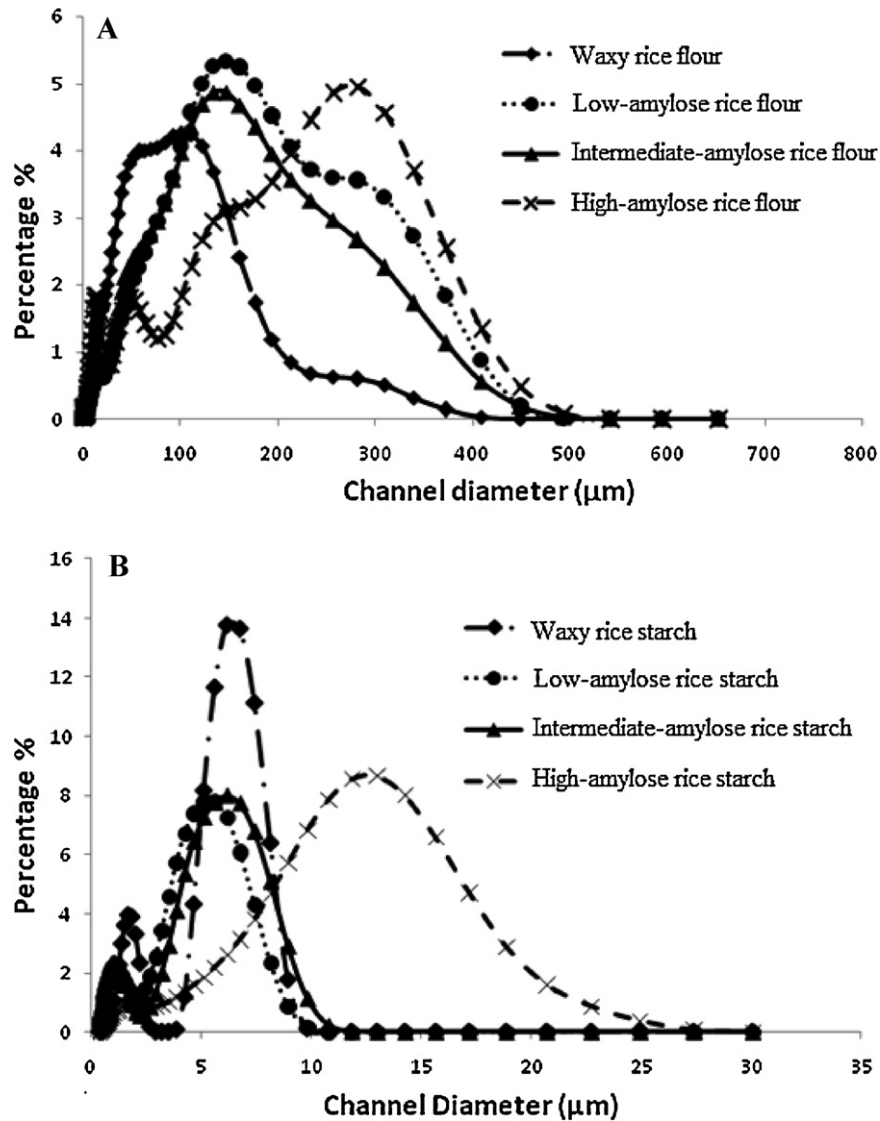


Fig. 4. Particle size of rice flours (A) and starches (B) with different amylose contents.

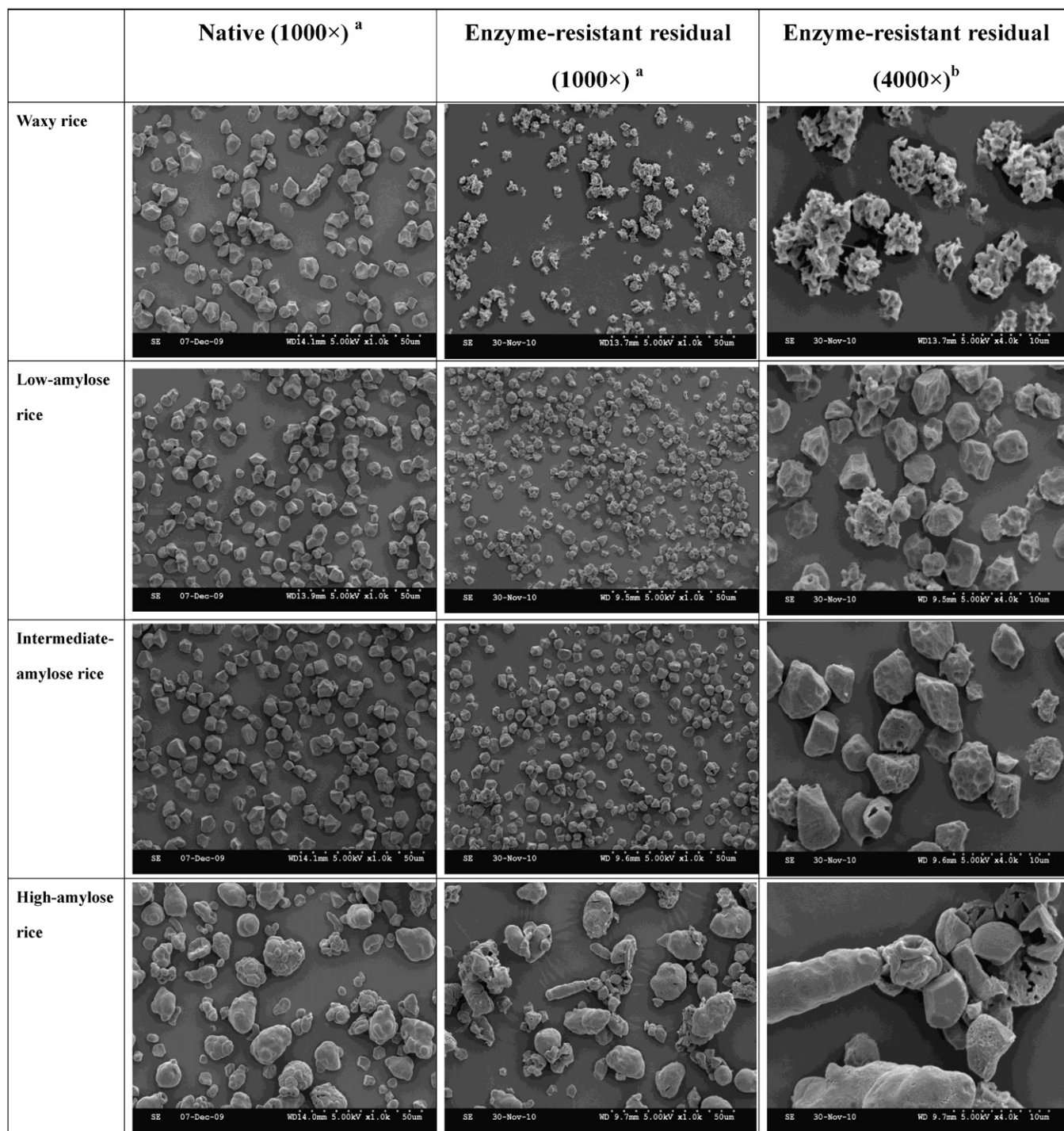


Fig. 5. Scanning electron microscopy (SEM) micrographs of native and enzyme-resistant residues.

Table 3
Rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS), and total dietary fiber content (TDF) of the isolated starches and flours from four rice samples.^a

| Sample | RDS ^b | | SDS ^b | | RS ^b | | TDF ^c | |
|---------------------------|------------------|--------------|------------------|---------------|-----------------|--------------|------------------|--------------|
| | Starch | Flour | Starch | Flour | Starch | Flour | Starch | Flour |
| Waxy rice | 23.1 ± 0.2 b | 35.4 ± 1.1 b | 64.2 ± 2.0 d | 59.77 ± 0.9 d | 12.76 ± 1.7 a | 4.9 ± 0.2 b | 1.1 ± 0.0 a | 5.1 ± 0.2 a |
| Low-amylose rice | 31.9 ± 0.4 c | 46.9 ± 0.0 d | 57.5 ± 1.1 c | 55.75 ± 0.7 c | 10.32 ± 1.2 a | 0.0 ± 0.7 a | 0.9 ± 0.2 a | 6.3 ± 0.0 b |
| Intermediate-amylose rice | 21.2 ± 0.3 a | 33.2 ± 0.1 a | 45.5 ± 0.7 b | 48.50 ± 1.2 b | 33.26 ± 0.4 b | 18.3 ± 1.3 c | 1.7 ± 0.1 b | 6.8 ± 0.1 c |
| High-amylose rice | 23.1 ± 0.2 b | 39.0 ± 0.4 c | 21.6 ± 0.0 a | 27.68 ± 0.9 a | 55.34 ± 0.2 c | 33.4 ± 0.5 d | 9.3 ± 0.4 c | 15.2 ± 0.3 d |

^a All values were reported on dry basis of starch; Data are means ± standard deviation. Means not sharing a common letter in a column are significantly different at $p \leq 0.05$.

^b Measured by the Englyst method (Sang and Seib, 2006).

^c Measured by AOAC method 991.43 using an assay kit from Megazyme International Ltd. (Wicklow, Ireland).

stronger peak at $5^\circ 2\theta$ was observed and a doublet between 21° and $25^\circ 2\theta$ became apparent, suggesting a predominant B-type crystalline structure. The crystallinity of the B-type starch is known to depend strongly on hydration and increase with water content (Buléon, Bizot, Delage & Multon, 1982; Buléon, Bizot, Delage & Pontoire, 1987; Buléon, Véronèse & Putaux, 2007). The A-type crystalline pattern of the three rice starches did not change when the moisture content was increased from 20 to 40%. As an example, the X-ray diffraction pattern of the intermediate-amylose rice starch is shown in Fig. 3. In maize, the crystalline type of starch is reported to change from A to B via C with an increase in amylose content, and the transition occurs at about 40% amylose (Cheetham and Tao, 1998). Maize starches with greater than 50% have a B-type crystalline pattern (Cheetham and Tao, 1998; Shi et al., 1998).

3.5. Particle size distribution of rice flours and starches

The particle size distributions of rice flours (A) and starches (B) are shown in Fig. 4. The flour particle size was the greatest in high-amylose rice, followed by low-amylose rice, intermediate-amylose rice, and waxy rice. In isolated starches, high-amylose rice starch still showed the biggest granule size, followed by waxy rice, intermediate-amylose rice, and low-amylose rice starches. Mean

value of granule size diameter has been suggested to be a factor that affects starch digestibility (Morita et al., 2007; Tester, Qi & Karkalas, 2006); however, in this study, no correlation between granule size and digestibility was found (Figs. 4 and 5 and Table 3). Morita et al. (2007) reported that in maize starches, granule size decreases as amylose content increases. We did not find this correlation in the four rice starches examined. The newly developed high-amylose rice had semi-compound starch granules built from individual granules (Wei et al., 2010). Compound granules in rice seem to have complicated the relationship between granule size and amylose content or digestibility of starch. In addition to granule size, other factors such as pores on starch (Zhang, Ao & Hamaker, 2006) and molecular structure (Zhang, Venkatachalam & Hamaker, 2006) may affect the digestibility of starch.

3.6. SEM image

SEM micrographs of four rice starch granules before and after digestion are shown in Fig. 5. In agreement with the particle size distribution results (Fig. 4), high-amylose rice with compound starch granules had the biggest granule size, followed by waxy rice, intermediate-amylose rice, and low-amylose rice (Fig. 5). Waxy rice starch after digestion showed a coarse honeycomb-like net-

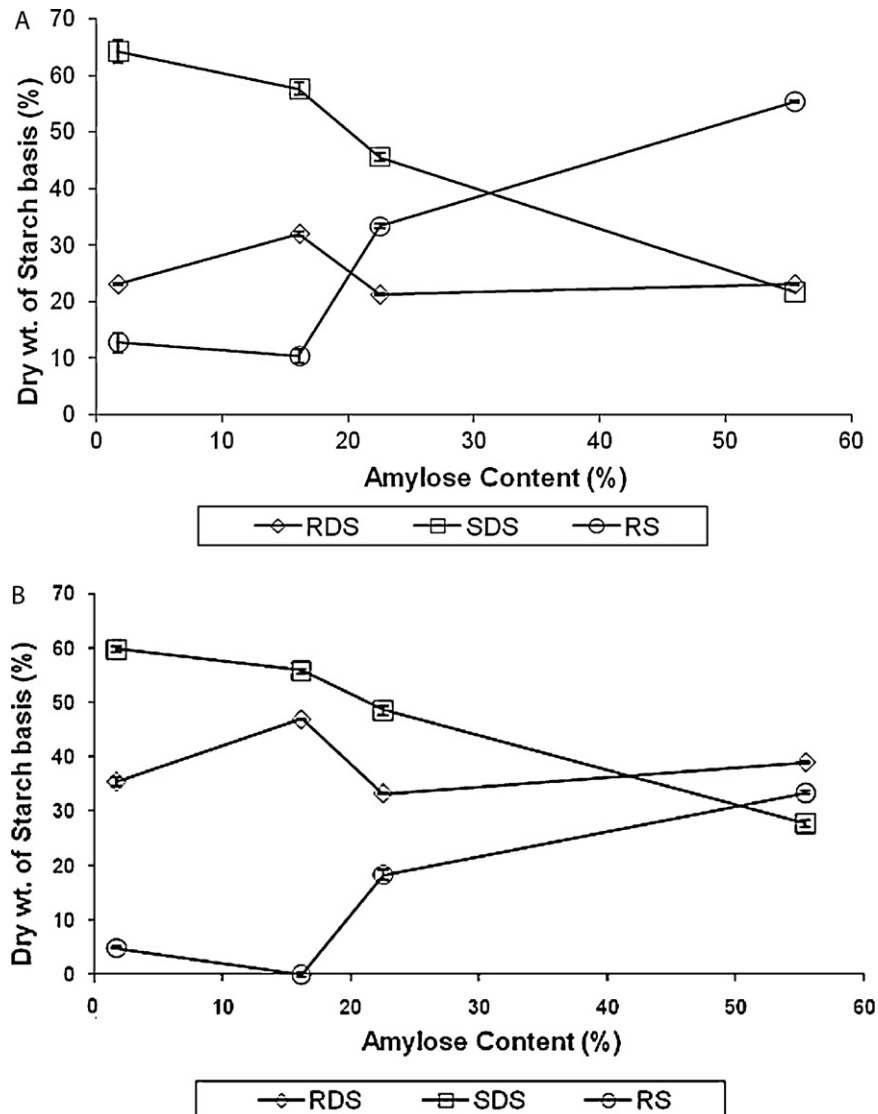


Fig. 6. The digestibility of starches (A) and flours (B) with increasing amylose content.

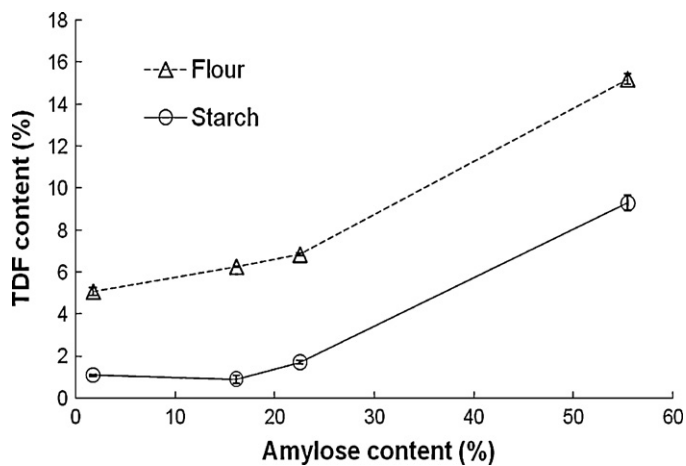


Fig. 7. Relationship between amylose content and total dietary fiber (TDF) content in rice flours and starches.

work structure as observed by SEM. After digestion the size of intermediate-amylose rice and low-amylose rice starch granules was smaller than that of native starches. Some of the high-amylose rice starch granules showed cracks and were broken into pieces after digestion, but most compound granules retained a smooth surface and were resistant to enzyme attack.

3.7. Starch digestibility

A positive correlation was evident between RS and amylose content (Fig. 6, $R=0.93$ in starch, $R=0.91$ in flour). SDS decreased as amylose content increased and the correlation between SDS and amylose content was high (Fig. 6, $R=0.99$ in starch, $R=0.99$ in flour). Almost no correlation occurred between RDS and amylose content (Fig. 6, $R=0.33$ in starch, $R=0.18$ in flour). A similar trend was observed for the TDF results in starches (Table 3 and Fig. 7). TDF from waxy rice, low-amylose rice, intermediate-amylose rice, and high-amylose rice starches were 1.1, 0.9, 1.7 and 15.2%, respectively. The same trends also were observed in rice flours, although levels of RDS, SDS, RS, and TDF differed between flour and starch.

With the increasing levels of amylose, RS content increased, except for low-amylose rice. Notably, waxy rice has higher RS content than low-amylose rice (Table 3). Compared to waxy rice starch, low-amylose rice starch had a lower gelatinization temperature, lower enthalpy value (Table 2), and lower crystallinity (Fig. 3), indicating that the low-amylose rice starch granules were weak and consequently more prone to α -amylase digestion. The results observed above indicate that other factors aside from amylose content have an impact on starch digestibility. This was consistent with previous studies on rice (Eggum, Juliano, Perez & Acedo, 1993; Hu, Zhao, Duan, Zhang & Wu, 2004) and maize (Morita et al., 2007).

Compared to low-amylose rice and waxy rice starches, the intermediate-amylose rice starch had a higher proportion of amylose and also longer chains in amylopectin, which have the ability to form more stable double helices and stronger crystallites to reduce enzyme susceptibility (Fig. 1). As a result, the intermediate-amylose rice starch had higher RS and TDF than the low-amylose and waxy rice starches. Based on the peak area, the four rice starches had the relative degree of crystallinity in the order of waxy rice > intermediate amylose rice > low amylose rice > high amylose rice (Fig. 3). The high-amylose rice had a lower degree of crystallinity than other rice starches (Fig. 3), yet had a higher RS content (obtained after digestion at 37 °C) and TDF content (obtained after digestion at 95–100 °C) (Table 3), reflecting the important role of amorphous regions in controlling digestibility of starch (Htoon et al., 2009). Amylose molecules in the amorphous regions may

initially be hydrolyzed by amylases but hydrolyzed molecules may associate and become resistant to enzyme digestion. Furthermore, the high-amylose rice had semi-compound starch granules consisting of individual granules, reducing the capacity for amylase to bind to granule surface and restrict hydrolysis.

4. Conclusion

In conclusion, amylose content was positively correlated with RS and TDF content in rice flours, and starches ranged from 1.7 to 55% amylose content. However, no correlation between granule size and digestibility of starch or amylose content was found in the rice starches. Degree of crystallinity and digestibility also were not correlated. Compound granules in rice play an important role in controlling digestion of starch. The newly developed high-amylose rice had a predominate B-type crystalline structure and semi-compound starch granules, which reduce amylase binding and digestion.

Acknowledgments

This study was supported by Natural Science Foundation (Grant Nos. 30828021 and 31071383), the National Key Project for Basic Research (2011CB100202) of China, and the Priority Academic Program Development from Jiangsu Government (grant no. 2011CB100202). L.J. Zhu thanks the Department of Grain Science and Industry at Kansas State University for the support and opportunity to work as a visiting student. We thank Dr. Sajid Alavi for the use of DSC equipment.

This is Contribution no. 11-244-J from the Kansas Agricultural Experiment Station, Manhattan, KS.

References

- AACC International (2000). Approved Methods of the American Association of Cereal Chemists, 10th Ed. Method 44-19 and 76-13. The Association: St. Paul, MN.
- AOAC International (1999). Official Methods of Analysis, 16th Ed. 5th revision. Methods 942.05 and 990.03. The Association: Gaithersburg, MD.
- AOAC International (2003). Official Methods of Analysis, 17th Ed. 2nd revision. Method 991.43. The Association: Gaithersburg, MD.
- Asp, N. G. (1992). Resistant starch—proceedings from the second plenary meeting of EURESTA: European FLAIR concerted action, 11 on physiological implications of the consumption of resistant starch in man. Preface. *European Journal of Clinical Nutrition*, 46, S1.
- Benmoussa, M., Moldenhauer, K. A. K., & Hamaker, B. R. (2007). Rice amylopectin fine structure variability affects digestion properties. *Journal of Agriculture and Food Chemistry*, 55, 1475–1479.
- Bird, A. R., Lopez-Rubio, A., Shrestha, A. K., & Gidley, M. J. (2009). Resistant starch in vitro and in vivo: Factors determining yield, structure, and physiological relevance. In S. Kasapis, I. T. Norton, & J. B. Ubbink (Eds.), *Modern biopolymer science*. San Diego, CA: Academic Press, pp. 449–510.
- Buléon, A., Bizot, H., Delage, M. M., & Multon, J.-L. (1982). Evolution of crystallinity and specific-gravity of potato starch versus water – adsorption and desorption. *Starch/Starke*, 34, 361–366.
- Buléon, A., Bizot, H., Delage, M. M., & Pontoire, B. (1987). Comparison of X-ray diffraction and sorption properties of hydrolyzed starches. *Carbohydrate Polymers*, 7, 461–482.
- Buléon, A., Véronèse, G., & Putaux, J.-L. (2007). Self-association and crystallization of amylose. *Australian Journal of Chemistry*, 60, 706–718.
- Cheetham, N. W. H., & Tao, L. P. (1998). Variation in crystalline type with amylose content in maize starch granules: An X-ray powder diffraction study. *Carbohydrate Polymers*, 36, 277–284.
- Chen, X. H. (2003). Introduction of antisense Sbe genes into rice to increase its amylose content. Yangzhou University, Yangzhou, Jiangsu, China. (Ph.D Dissertation).
- Cone, J. W., & Wolters, M. G. E. (1990). Some properties and degradability of isolated starch granules. *Starch-Starke*, 42, 298–301.
- Eggum, B. O., Juliano, B. O., Perez, C. M., & Acedo, E. F. (1993). The resistant starch, undigestible energy and undigestible protein contents of raw and cooked milled rice. *Journal of Cereal Science*, 18, 159–170.
- Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition*, 46, S33–S50.

- Englyst, K. N., Englyst, H. N., Hudson, G. J., Cole, T. J., & Cummings, J. H. (1999). Rapidly available glucose in foods: An in vitro measurement that reflects the glycemic response. *American Journal of Clinical Nutrition*, 69, 448–454.
- Englyst, K. N., Vinoy, S., Englyst, H. N., & Lang, V. (2003). Glycaemic index of cereal products explained by their content of rapidly and slowly available glucose. *British Journal of Nutrition*, 89, 329–339.
- Evans, A., & Thompson, D. B. (2004). Resistance to alpha-amylase digestion in four native high-amylose maize starches. *Cereal Chemistry*, 81, 31–37.
- Htoon, A., Shrestha, A. K., Flanagan, B. M., Lopez-Rubio, A., Bird, A. R., Gilbert, E. P., et al. (2009). Effects of processing high amylose maize starches under controlled conditions on structural organization and amylase digestibility. *Carbohydrate Polymer*, 75, 236–245.
- Hu, P. S., Zhao, H. J., Duan, Z. Y., Zhang, L. L., & Wu, D. X. (2004). Starch digestibility and the estimated glycemic score of different types of rice differing amylose contents. *Journal of Cereal Science*, 40, 231–237.
- Hizukuri, S., Abe, J. I., & Hanashiro, I. (2006). Starch: Analytical aspects. In A.-C. Eliasson (Ed.), *Carbohydrates in food* (2nd ed., pp. 305–390). Boca Raton: CRC Press.
- Li, J. H., Vasanthan, T., Hoover, R., & Rossnagel, B. G. (2004). Starch from hull-less barley: V In-vitro susceptibility of waxy, normal, and high-amylose starches towards hydrolysis by alpha-amylases and amyloglucosidase. *Food Chemistry*, 84, 621–632.
- Lumdubwong, N., & Seib, P. A. (2000). Rice starch isolation by alkaline protease digestion of wet-milled rice flour. *Journal of Cereal Science*, 31(1), 63–74.
- Morell, M. K., Kosar-Hashemi, B., Cmiel, M., Samuel, M. S., Chandler, P., Rahman, S., et al. (2003). Barley sex6 mutants lack starch synthase IIa activity and contain a starch with novel properties. *Plant Journal*, 34, 173–185.
- Morita, T., Ito, Y., Brown, I. L., Ando, R., & Kiriyama, S. (2007). In vitro and in vivo digestibility of native maize starch granules varying in amylose contents. *Journal of AOAC International*, 90(6), 1628–1634.
- Regina, A., Bird, A., Topping, D., Bowden, S., Freeman, J., Barsby, T., et al. (2006). High-amylose wheat generated by RNA interference improves indices of large-bowel health in rats. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 3546–3551.
- Rendleman, J. A. (2000). Hydrolytic action of alpha-amylase on high-amylose starch of low molecular mass. *Biotechnology and Applied Biochemistry*, 31, 171–178.
- Richardson, P. H., Jeffcoat, R., & Shi, Y. C. (2000). High-amylose starches: From biosynthesis to their use as food ingredients. *Material Research Society Bulletin*, 25, 20–24.
- Riley, C. K., Wheatley, A. O., Hassan, I., Ahmad, M. H., Morrison, E. S., & Asemota, H. N. (2004). In vitro digestibility of raw starches extracted from five yam (*Dioscorea spp.*) species grown in Jamaica. *Starch–Starke*, 56, 69–73.
- Sang, Y., & Seib, P. A. (2006). Resistant starches from amylose mutants of corn by simultaneous heat-moisture treatment and phosphorylation. *Carbohydrate Polymers*, 63, 167–175.
- Sang, Y., Bean, S., Seib, P. A., Pedersen, J., & Shi, Y. C. (2008). Structure and functional properties of sorghum starches differing in amylose content. *Journal of Agricultural and Food Chemistry*, 56, 6680–6685.
- Schwall, G. P., Safford, R., Westcott, R. J., Jeffcoat, R., Tayal, A., Shi, Y.-C., et al. (2000). A Production of very-high-amylose potato starch by inhibition of SBE A and B. *Nature Biotechnology*, 18(5), 551–554.
- Shi, Y. C., Capitani, T., Trzasko, P. T., & Jeffcoat, R. (1998). Molecular structure of a low-amylopectin starch and other high-amylose maize starches. *Journal of Cereal Science*, 27, 289–299.
- Tester, R. F., Qi, X., & Karkalas, J. (2006). Hydrolysis of native starches with amylases. *Animal Feed Science and Technology*, 130, 39–54.
- Thiemeier, H., Hollman, J., Neese, U., & Lindhauer, M. G. (2005). Structural and morphological factors influencing the quantification of resistant starch II in starches of different botanical origin. *Carbohydrate Polymers*, 61, 72–79.
- Vasanthan, T., & Bhatta, R. S. (1996). Physicochemical properties of small- and large-granule starches of waxy, regular, and high-amylose barleys. *Cereal Chemistry*, 73, 199–207.
- Wei, C., Qin, F., Zhou, W., Chen, Y., Xu, B., Wang, Y., et al. (2010). Formation of semi-compound C-type starch granule in high-amylose rice developed by anti-sense RNA inhibition of starch-branching enzyme. *Journal of Agriculture of Food Chemistry*, 58(20), 11097–11104.
- Yun, S. H., & Matheson, N. K. (1990). Estimation of amylose content of starches after precipitation of amylopectin by concanavalin A. *Starch/Starke*, 42, 302–305.
- Zhang, G., Ao, Z., & Hamaker, B. R. (2006). Slow digestion property of native cereal starches. *Biomacromolecules*, 7, 3252–3258.
- Zhang, G., Venkatachalam, M., & Hamaker, B. R. (2006). Structural basis for the slow digestion property of native cereal starch. *Biomacromolecules*, 7, 3259–3266.
- Zhang, G., & Hamaker, B. R. (2009). Slowly digestible starch: Concept, mechanism, and proposed extended glycemic index. *Critical Reviews in Food Science and Nutrition*, 49, 852–867.
- Zhu, L. J. (2009). Studies on starch structure and functional properties of high-amylose transgenic rice and different waxy rice varieties. Yangzhou University, Yangzhou, Jiangsu, China (Ph.D Dissertation).
- Zhu, L. J., Liu, Q. Q., Sang, Y., Gu, M. H., & Shi, Y. C. (2010). Underlying reasons for waxy rice flours having different pasting properties. *Food Chemistry*, 120, 94–100.