JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Physicochemical Properties of A- and B-Starch Granules Isolated from Hard Red and Soft Red Winter Wheat

Wenhao Li, Yulin Shan, Xinlong Xiao, Qingui Luo, Jianmei Zheng, Shaohui Ouyang, and Guoquan Zhang*

College of Food Science and Engineering, Northwest A&F University, Yangling 712100, China

ABSTRACT: Large A- and small B-starch granules separated from hard red and soft red winter wheat grains were investigated for their morphological, structural, and physicochemical properties. A-granules displayed a disk or lenticular shape, and B-granules showed a spherical or polygonal shape according to SEM. XRD analysis showed that both A- and B-granules had A-type crystallinity. A-granules contained a higher amount of amylose and a lower protein content and amylopectin/amylose ratio than B-granules. A-type granules exhibited a higher hydrolysis extent and swelling power and a lower iodine affinity than did B-granules. A-granules showed a higher peak, trough, breakdown and final viscosity, and gelatinization enthalpy than did B-granules, while B-granules exhibited a higher gelatinization temperature. The study demonstrated that the A- and B-granules separated from both hard red and soft red winter wheat grains exhibited a similar structure and very different physicochemical properties.

KEYWORDS: *starch, swelling power, pasting, gelatinization, amylose, amylopectin*

■ INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops in the world and is one of the staple foods consumed by humans. Starch is the most abundant constituent in wheat endosperm, making up 60−65% of the dry weight in the mature wheat grain and playing a significant role in the quality of the end-use product.[1] It is one of the primary sources of calories in the human diet and may be used as a thickener, gelling agent, stabilizer, and fat replacer in numerous food industries. Starch is mainly composed of two types of glucose polymers, amylose, which is an almost linear α-1,4-glucan molecule that comprises about 25−30% wheat starch, and amylopectin, which is highly branched and comprises 70−75% wheat starch.[1,2] Specifically, it is widely accepted that starch from wheat has a bimodal granule size distribution, which may be classified as type-A and type-B according to their shape and size. A-type starch granules are disk-like or lenticular in shape with an average diameter from 10 to 48 μm, while the B-type starch granules are less than 10 μm in size and spherical or polygonal in shape.[3,4]

Wheat A-type and B-type starch granules have been reported to differ in chemical composition such as amylose, amylopectin, lipid, and protein content as well as in functional properties such as gelatinization and pasting properties, swelling, crystallinity, enzyme susceptibility, and baking properties.[2,5] Early studies showed that the B-granules have higher contents of lipids than the A-granules, while A-granules have higher amylose contents than B-granules.[6] The A- and B-granules are developed in the endosperm during the different development period of grain. A-granules start to synthesize at about 4−5 days after anthesis, with granule growth and development continuing until the end of the endosperm cell division phase, while the formation of B-granules occurs 10−14 days after anthesis and they continue to enlarge until 21 days postanthesis.[1,6] The different sizes and shapes also result in the two starch granule types being used differently in food and nonfood applications. In wheat, B-type starches have a negative effect on flour processing and bread-making quality, but have a positive affected on pasta making.[7] Wheat A-granule starch can be used in carbonless copy paper, and wheat B-granule starch is a good material for plastic film filler.[6]

The granule size and shape have been related to the molecular architecture of amylopectin and its molecular arrangement within the granule. The ratio of long branch chain to short branch chain affects the shapes of the amylopectin molecules, which affects their packing and further affects the morphology and size of the starch granule.[2] Studies have revealed that the physicochemical properties and the size distribution of starch granules in wheat are both genetically and environmentally controlled.[8] Variety is a key determinant factor that affects wheat plant development and starch accumulation. Wheat cultivars can be classified according to hardness of the grain as hard wheat and soft wheat. The grain hardness of wheat, reflecting the character of endosperm texture, is one of the most important determinants of milling, baking, and end-use quality. Soft wheat varieties have less protein content and mill easier than the hard wheat, and soft wheat flour is preferred for biscuits, French bread, breakfast foods, etc., while hard wheat flour offers a very high quality for bread making. Several studies have been conducted on physicochemical functional differences between A- and B-granules. In this study, the characteristics of starches and separated A- and B-granules from hard red winter wheat and soft red winter wheat varieties with regard to morphological, structural, physicochemical, thermal,

Received: February 28, 2013
Revised: June 1, 2013
Accepted: June 11, 2013
Published: June 11, 2013

Table 1. Diameters and Purities of Wheat Starch and A- and B-Separated Granule Starch[a]

variety	sample	$d_{4,3}$ (μm)[b]	$d_{3,2}$ (μm)[b]	A-granule (%)	B-granule (%)
Xinong 9718	unfractionated	16.81 ± 0.28 b	6.35 ± 0.12 a	75.43 ± 0.81 b	24.57 ± 0.81 b
	A-granule	19.08 ± 0.34 a	8.98 ± 0.21 a	92.32 ± 0.16 a	7.68 ± 0.16 c
	B-granule	7.60 ± 0.11 b	3.83 ± 0.09 c	13.29 ± 0.12 c	86.71 ± 0.12 a
Shannong 138	unfractionated	18.39 ± 0.44 b	6.709 ± 0.23 b	74.95 ± 0.13 b	25.05 ± 0.13 b
	A-granule	21.57 ± 0.31 a	9.79 ± 0.28 a	91.01 ± 0.02 a	8.99 ± 0.02 c
	B-granule	8.58 ± 0.20 c	4.52 ± 0.14 c	12.54 ± 0.26 c	87.46 ± 0.26 a

[a]All values are means of triplicate determinations ± SD. Means within columns with different letters for the same wheat varieties are significantly different ($p < 0.05$). [b]$d_{4,3}$ is the volume mean diameter; $d_{3,2}$ is the area mean diameter.

granule size distribution, and pasting properties were investigated.

MATERIALS AND METHODS

Materials. The wheat (*Triticum aestivum* L.) variety Xinong 9718 (hard red winter wheat) and Shannong 138 (soft red winter wheat) were used in this study. They were provided as grains by the College of Agriculture, Northwest A&F University, Yangling, China, from the 2010 harvest. The wheat grain was milled by using a laboratory mill (mill type Perten 3100, Swedish Perten Company, Sweden) to obtain a whole-wheat meal. All the chemicals and reagents used were of analytical grade.

Isolation of Starch. The wheat starch granules were isolated from the flours following the method described by Singh et al. (2010).[2]

Separation of the A- and B-Granule Starch. The A- and B-granules were separated by the method described by Zeng et al. (2011).[9]

Determination of Granule Size Distribution. Starch granule size distribution was analyzed by using a laser light-scattering particle size analyzer (Mastersizer-2000, Malvern Instruments Ltd., Malvern, UK). A ~0.1 g starch sample was dissolved in 100 mL of distilled water and mixed using a magnetic stirrer for 30 min at room temperature before measurement. The focal length of 100 mm and obscuration level of 20% were maintained during measurements on the analyzer.

Determination of Starch Composition. Total starch, amylose, and amylopectin contents were determined using the colorimetric method reported by Jarvis and Walker (1993).[10] The protein content in starch was measured according to AACC Methods 46−30 (AACC 2000).[11] The results were reported on a dry basis.

Light Microscopy. The starch sample was suspended in a glycerol solution (glycerol/H_2O_2, v/v) and was observed using a polarizing light microscope (DMBA400, Motic China Group Co., Ltd., Guangzhou, China) with a 40× objective.

Scanning Electron Microscopy (SEM). A starch sample was mounted on an SEM stub with double-sided adhesive tape and coated with gold. Scanning electron micrographs were taken using a scanning electron microscope (JSM-6360LV, JEOL, Japan).

Enzymatic Hydrolysis. The hydrolysis properties of starches were analyzed for resistance to porcine pancreatic α-amylase hydrolysis following procedures modified from Englyst et al.(1992).[12] The 120 mg samples were mixed with 30 mL of water with continuous mixing by using a magnetic stirrer and separated into 5 mL aliquots. To each starch suspension were added 4 mL of sodium acetate buffer (0.1 M, pH 5.2) and 4 mL of α-amylase (Sigma). The suspensions were incubated at 37 °C and 120 rpm in a water bath shaker. The samples were withdrawn for analysis of reducing sugar after 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 h of hydrolysis using the 3,5-dinitrosalicylic acid method described by Maache-Rezzoug et al. (2009).[13] The hydrolysis properties of starch samples were expressed as the percent of hydrolyzable starch. Samples were studied in triplicate, and controls without enzymes were subjected to the same experimental conditions.

Determination of Swelling Power and Blue Value. The swelling powers of starch samples were determined following the modified method of Leach, McCowen, and Schoch (1959).[14] The starch suspension was stirred at 90 °C for 30 min, cooled, and centrifuged at 3000*g* for 15 min. The values for swelling power were calculated in grams per gram, and the solubility index was calculated as a percent at 90 °C. The blue values of starches were determined according to the procedure of Morrison and Laignelet (1983)[15] with a slight modification. Starch (40 mg) was dispersed in 10 mL of 10%-urea-containing dimethyl sulfoxide solution. The mixture was kept at room temperature for 10 min followed by heating in a boiling water bath for 20 min with shaking to completely dissolve all starch granules. After cooling to room temperature, an aliquot (1 mL) of dispersion was weighed into a 100 mL volumetric flask. About 95 mL of distilled water and 2 mL of 0.2% iodine solution were added and then filled up to 100 mL and mixed immediately. The mixture was left standing for 20 min at room temperature, and the blue value of samples was measured as the absorbance at 640 nm with a spectrophotometer (UV-1700, Shimadzu, Japan).

Determination of Pasting Properties. Pasting properties of starches were determined using a Rapid Visco-Analyzer (RVA) model Master (Newport Scientific, Pty Ltd., Australia). Deionized water (25.0 g) was added to starch (2.0 g, db) in the RVA canister to obtain a total constant sample weight of 27.0 g. The slurry was then manually homogenized using the plastic paddle to avoid lump formation before the RVA run. The starch slurry was heated from 50 °C to 95 °C at a rate of 12 °C/min, maintained at 95 °C for 2.5 min, and then cooled to 50 °C at the same rate.

Determination of Thermal Properties. Thermal properties were measured by a differential scanning calorimeter (DSC-Q2000, TA, New Castle, DE, USA). Starch (3 mg) was directly measured into the aluminum DSC pan, distilled water (12 μL) was added with a microsyringe, and an empty pan was used as reference for all measurements. The scanning temperature and the heating rates were 30−120 °C and 10 °C/min, respectively.

Statistical Analysis. All the experiments were performed at least in triplicate, and experimental data also were analyzed using analysis of variance (ANOVA) and expressed as mean value ± standard deviation. Duncan's multiple range test was conducted to assess significant differences among experimental mean values ($p < 0.05$). All statistical computations and analyses were conducted using SPSS version 13.0 for Windows.

RESULTS AND DISCUSSION

Granule Size Distributions of Wheat Starch and Isolated A- and B-Granules. The mean particle diameter of volume and surface area of unfractionated wheat starch and A- and B-granules are presented in Table 1. The granule size distributions of both kinds of unfractionated wheat starch granules exhibited bimodal size distributions (Figure 1A and B), with a mean particle size of 16.81 and 18.39 μm, respectively (Table 1). The results of particle size distribution in total starch fractions in our experiment showed a significantly ($p < 0.05$) higher proportion of small B-granules in wheat starch compared to A-granules (Table 1). A-type granules exhibited average diameters ranging from 18.39 to 19.08 μm, whereas B-type granules possessed average diameters of 7.60 to 8.58 μm (Table 1). These values were in agreement with the result obtained by Kim and Huber (2010),[16] who reported

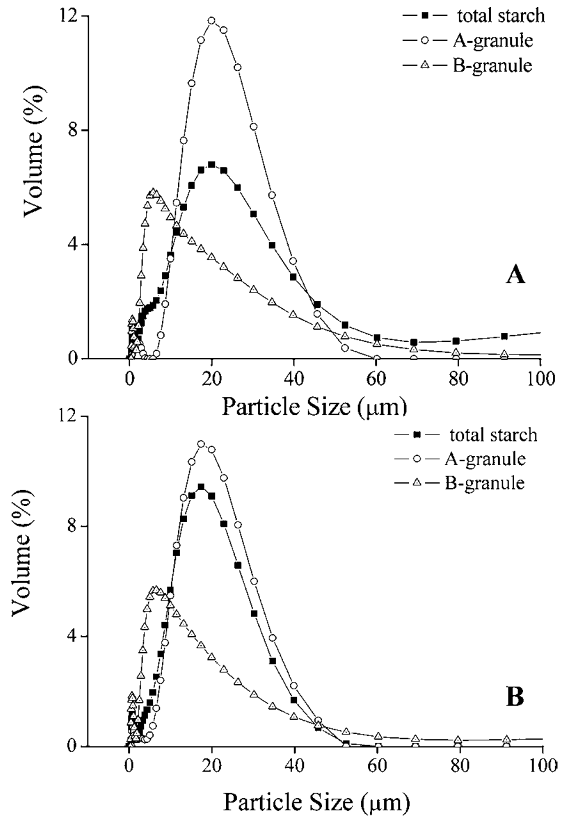

Figure 1. Particle size distribution of starches separated from hard red (A: Xinong 9718) and soft red winter wheat (B: Shannong 138) and their isolated A- and B-granules at different times.

mean granule diameter ranges of 20.9 to 21.9 μm and 6.2 to 6.3 μm for A- and B-type granules of waxy and normal soft wheat starch. Fraction purities of isolated A-type and B-type granules of hard red and soft red winter wheat starch were 91.0 to 92.32% and 86.71 to 87.46%, respectively, which suggest that there was existing contamination between A- and B-type starch granule fractions.

Scanning Electron Microscope Photograph and Microscopy Observations. The scanning electron micrographs of the unfractionated wheat starches and A- and B-granules isolated from two starch varieties are shown in Figure 2A–F. The SEM of both kinds of unfractionated wheat starch granules also showed a clearly bimodal pattern and the large starch granules surrounded by many small starch granules (Figure 2A and D). The large A-granules appeared to be smooth and displayed a disk or lenticular shape with diameters ranging from 10 to 40 μm (Figure 2B and E). The isolated B-granules showed an irregular, spherical or polygonal shape with diameters of about 2 to 5 μm, and a proportion of B-granules was associated with small amounts of proteins (Figure 2C and F). In addition, SEM observations revealed that A-type starch granules were contaminated to a very minor degree with B-type starch granules, and B-granules were also contaminated by some disc-shaped A-granules, which coincided with the particle size distribution analysis. According to the report, the A-type granule is formed 4 days after flowering and may continue to grow throughout the grain filling period, while the B-type granules initiated about 12–14 days after flowering and increase in size up to 10 μm, which leads to the different size and shape of A- and B-type starch granules.[17] The differences in granule size and shape might influence the physicochemical and functional properties of these wheat starches.

Figure 2a–f shows the birefringence micrographs of the unfractionated and A- and B-starch granules isolated from two wheat varieties. All starch granules exhibited a typical "Maltese cross" under polarized light. It is seen that there is no observable difference for both unfractionated wheat starch granules (Figure 2a and d). However, the B-starch (Figure 2c and f) granules displayed a relative weak and obscure "Maltese cross" compared to A-starch granules (Figure 2b and e). The phenomenon of birefringence under polarized light is known due to the radial orientation of crystallites, which reveal the crystalline organization of the granules.[18] As a result, the weak "Maltese cross" of B-starch granules may be attributed to their lower order of crystalline organization within the granules.

Composition of Wheat Starch and Isolated A- and B-Granules. The composition of unfractionated wheat starch and their A- and B-granules, including total starch, amylose, amylopectin, and protein content, and ratio of amylopectin/amylose were analyzed, and the results are presented in Table 2. It is rather difficult to obtain pure starch from plant tissues, because of other constituents such as protein, lipid, fiber, minerals, etc. For all samples, the values of starch purity (expressed on dry basis) were higher than 97.0%, indicating their high purity. Herein, the values of starch purity (expressed on dry basis) of all samples were higher than 97.0%, indicating their high purity. The protein content was highest in B-granules and lowest in A-granules for two wheat varieties.

The difference of amylose contents among unfractionated starches was not significant ($p < 0.05$) (Table 2). The amylose content of the A-granules (25.26% and 25.30% for *cv.* Xinong 9718 and Shannong 138, respectively) was significantly ($p < 0.05$) higher than that of unfractionated starch (22.25% and 23.79%, respectively) and B-granules (19.19% and 21.58%) for both varieties. These results are consistent with previous reports on wheat, triticale, and barley starch[6] and on normal and partially waxy wheat starch.[19] Amylose constitutes the amorphous part in starch granules, and a higher amylose content in starch granules may result in a lesser percentage of crystallinity of starch.[6] As a result, the B-granule starch may have a lower percentage of crystallinity than the A-granule.

However, the amylopectin content and amylopectin/amylose ratio of the A-granules showed the opposite situation. The values of amylopectin content and amylopectin/amylose ratio of A-granules were significantly ($p < 0.05$) lower than unfractionated starch and B-granules for both varieties, while B-granules had the highest amylopectin content and amylopectin/amylose value (Table 2). The amylopectin content and amylopectin/amylose ratio of starch granules have been related to the starch crystalline structure. In the present study, approximately more than 98% of the chemical composition of unfractionated starches and A- or B-type granules was amylose and amylopectin, and more than 70% of their chemical composition was amylopectin. So the content and structure of amylopectin play a critical role in the physicochemical characteristics and functional properties of starch granules.

X-ray Diffraction Analysis. The X-ray diffraction patterns of unfractionated starches and A- and B-granules are presented in Figure 3. Table 3 shows the intensity of the main peaks corresponding to the X-ray diffractograms and the relative crystallinity of unfractionated and A- and B-granules of both wheat starch varieties. The unfractionated starches and A- and

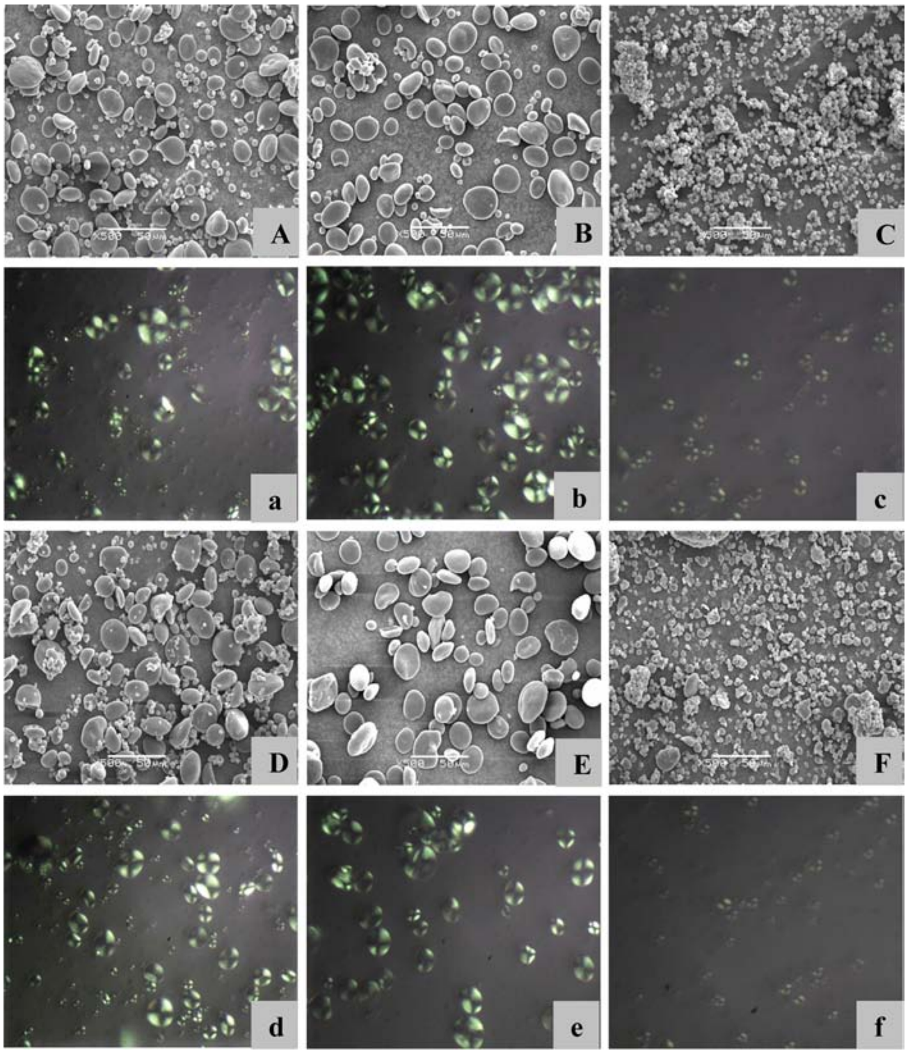

Figure 2. Scanning electron micrographs and polarized light micrographs of hard red (A, a: Xinong 9718) and soft red winter wheat (D, d: Shannong 138) and their isolated A- (B, b and E, e, respectively) and B-granules (C, c and F, f, respectively) (scale bar = 50 μm).

Table 2. Starch Purity and Composition of Unfractionated Wheat Starch and Isolated A- and B-Granules[a]

variety	sample	starch purity (%)	protein (%)	amylose (%)	amylopectin (%)	amylopectin/amylose
Xinong 9718	unfractionated	98.78 ± 0.79 a	0.25 ± 0.01 a	22.25 ± 0.26 b	76.53 ± 0.48 b	3.44 ± 0.11 b
	A-granule	99.43 ± 0.98 a	0.16 ± 0.02 b	25.26 ± 0.17 a	74.17 ± 0.24 c	2.94 ± 0.06 c
	B-granule	97.85 ± 1.02 b	0.23 ± 0.04 a	19.19 ± 0.19 c	78.66 ± 0.24 a	4.10 ± 0.09 a
Shannong 138	unfractionated	98.02 ± 0.88 a	0.33 ± 0.03 a	23.79 ± 0.01 b	74.23 ± 0.50 b	3.16 ± 0.01 b
	A-granule	98.12 ± 0.92 a	0.18 ± 0.02 b	25.30 ± 0.22 a	72.82 ± 0.06 c	2.88 ± 0.03 c
	B-granule	97.29 ± 0.75 b	0.28 ± 0.02 a	21.58 ± 0.21 c	75.71 ± 0.53 a	3.36 ± 0.01 a

[a]All values are means of triplicate determinations ± SD. Means within columns for the same varieties with different letters are significantly different ($p < 0.05$).

B-granules of each wheat variety all showed similar X-ray diffraction patterns, with the strongest diffraction peak at around 15°, 17°, 18°, 20°, and 23° (Figure 3A andB), which are typical of the A-type crystallinity. The starches showed differences in the peak intensity values and relative crystallinity. The A-granule of Xinong 9718 had higher peak intensities than its unfractionated starch and B-granules, while the A-granule of Shannong 138 had the lowest intensities (Table 3). This could be due to the difference in protein, amylose, and amylopectin content between both starch varieties.

The degree of crystallinity is calculated on the basis of the total area and amorphous area of X-ray diffractograms, and A-granules of both wheat varieties showed the highest relative crystallinity value, consistent with the result of birefringence pattern analysis. A significant positive correlation ($r = 0.951$, $p < 0.05$) between amylose content and relative crystallinity and a negative correlation ($r = -0.863$, $p < 0.05$) between amylopectin content and relative crystallinity were found. Our results are in agreement with the results obtained by Chiotelli and LeMeste (2002),[20] while opposite of the results found by Xie et al. (2009),[17] who reported a positive correlation between crystallinity and amylose content ($r = 0.981$) in wheat starch. This may be due to the difference in wheat starch varieties. Generally, amylose in the starch granules is amorphous, and

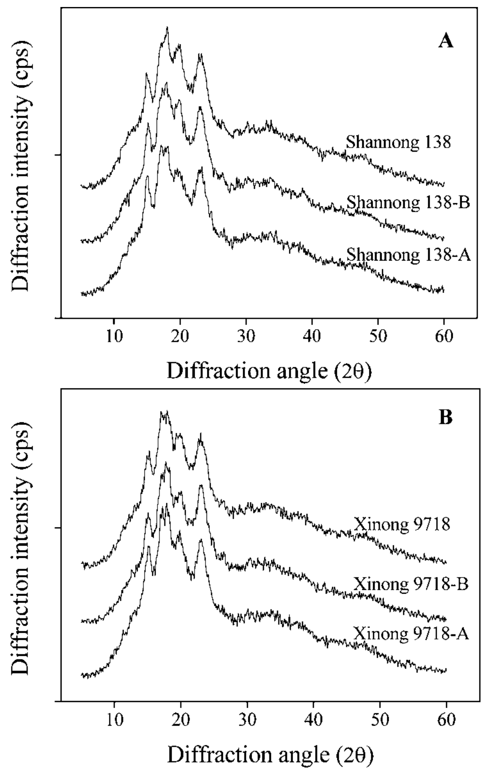

Figure 3. X-ray diffraction (XRD) patterns of starches separated from hard red (A: Xinong 9718) and soft red winter wheat (B: Shannong 138) and their isolated A- and B-granules.

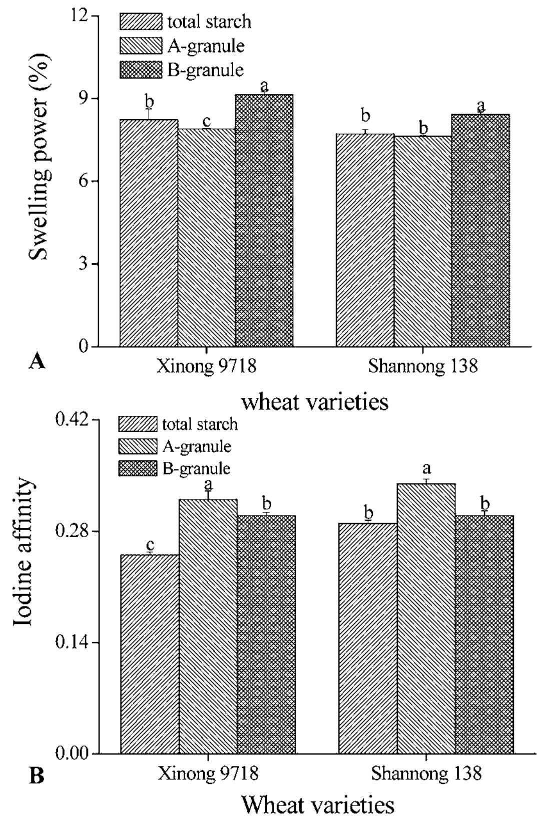

Figure 4. Swelling power (A) and iodine affinity (B) of starches separated from hard red and soft red winter wheat and their isolated A- and B-granules. Bars bearing the same letter within a particular cultivar are not significantly different ($p < 0.05$).

high amylose content may lead to a weak crystallinity of starch. As a result, the relationship between crystallinity and amylose in wheat starch granules needs to be further investigated.

Swelling Power and Iodine Affinity. The swelling power and iodine affinity of unfractionated starches and A- and B-granules are shown in Figure 4. The swelling power and iodine affinity of different starch samples differed significantly ($p < 0.05$). The A-starch granules of both wheat varieties exhibited the lowest swelling power and highest iodine affinity, whereas the B-granules of both wheat varieties showed the highest swelling power and the unfractionated starch granules gave the lowest iodine affinity (Figure 4A and B). During heating of starch granules in excess water, the molecular structure is broken and the water molecules are bound to the free hydroxyl groups of amylose and amylopectin by hydrogen bonding, which causes an increase in granule swelling. The swelling power of cereal starches has been reported to be related to amylopectin, amylose, and lipid content.[21] Swelling power can be used to assess the extent of interaction between starch chains within the amorphous and crystalline domains of the starch granule, and starch with a higher crystallinity would show a lower swelling power.[2,18] This conclusion was in agreement with our results; the swelling powers of wheat starch have been increased with a decrease in the degree of crystallinity. In addition, the higher swelling power value of B-type wheat granules may also be associated with their lower amylose content. The higher amylose contents of the A-granule wheat starches have been illustrated by the higher blue values than those of the B-type granules.

Hydrolysis Properties of the A- and B-Granule Wheat Starches. The results of enzymatic hydrolysis of unfractionated and large A- and small B-wheat starch granules at various times are presented in Figure 5. Between 0.5 and 1.5 h, the B-granules of both wheat starch varieties exhibited a greater hydrolysis extent than the A-granules, but after 0.5, 1.0, and 1.5 h the A-granules were digested more extensively than the

Table 3. Intensity of the X-ray Diffractogram Main Peaks and Relative Crystallinity of Wheat Starch and A,B-Granules[a]

		peak intensity at 2θ values (cps)					
variety	sample	15°	17°	18°	20°	23°	degree of crystallinity (%)
Xinong 9718	unfractionated	1850.00	2316.67	2366.67	2033.00	1941.67	33.74 ± 0.63 a
	A-granule	2141.67	2533.33	2566.00	2191.67	2148.33	34.47 ± 0.70 a
	B-granule	1758.33	2258.33	2550.00	2066.67	2141.67	31.05 ± 0.58 b
Shannong 138	unfractionated	1808.33	2550.00	2691.67	2175.00	2183.00	32.78 ± 0.44 b
	A-granule	1866.67	2275.00	2350.00	2025.00	2075.00	34.82 ± 0.59 a
	B-granule	1900.00	2416.67	2558.33	2233.33	2200.00	30.98 ± 0.41 c

[a]Means ± SD within columns with different letters for the same variety are significantly different ($p < 0.05$).

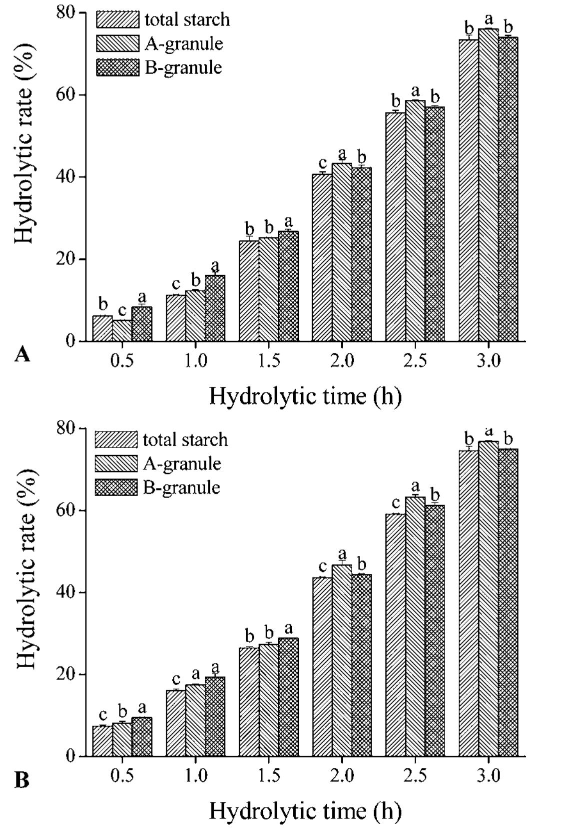

Figure 5. Hydrolysis of starches separated from hard red (A: Xinong 9718) and soft red winter wheat (B: Shannong 138) and their isolated A- and B-granules at different times. Bars bearing the same letter within a particular time are not significantly different ($p < 0.05$).

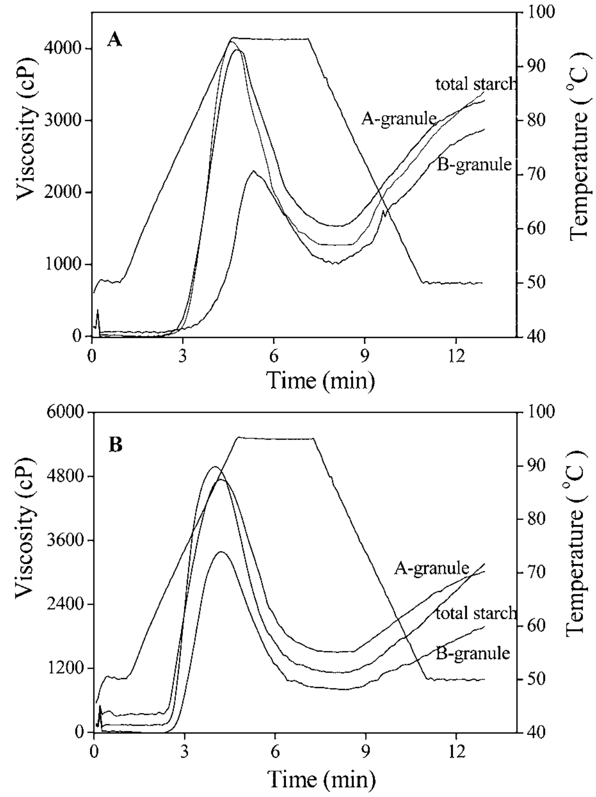

Figure 6. Pasting viscosity profiles of starches separated from hard red (A: Xinong 9718) and soft red winter wheat (B: Shannong 138) and their isolated A- and B-granules.

corresponding B-granules (Figure 5A and B). The A-type granules had a higher hydrolysis extent than did the B-type granules. The rate and extent of enzymatic hydrolysis of starch granules could be influenced by many factors that can affect access of the enzyme to its substrate and the release of reaction products.[22] These factors include granule size, granule integrity, relative surface area, crystallinity, porosity of the granules, and amylose to amylopectin ratio.[22] The higher hydrolysis rate of B-type granules at the initial stage may be attributed to their higher surface-to-volume ratio than that of A-type starch granules; larger A-granules will be less susceptible than small B-granules to α-amylase hydrolysis, due to their smaller surface area. The A-granules of both wheat varieties had higher amylose content than their B-granule counterparts (Table 2), and higher amylose content could lead to formation of a relatively larger amorphous area in starch granules, which enables α-amylase easy access to the glucosidic bonds. In addition, the hydrolysis rate of B-granules may be influenced by their higher protein content (Table 2), since the proteins can inhibit the action of α-amylase on starch.

Pasting Properties of the A- and B-Granule Wheat Starches. Pasting profile of A-granule starch, B-granule starch and unfractionated starches of wheat are presented in Figure 6, and the pasting parameters are summarized in Table 4. The unfractionated starches of both wheat varieties had the highest peak viscosity, breakdown viscosity, and setback viscosity. A-granules showed the highest trough viscosity and final viscosity, while B-granules exhibited the highest pasting temperature and peak time (Table 4). For Shannong 138, A-granules showed a significantly ($p < 0.05$) higher peak, trough, breakdown, final, and setback viscosities than the B-granule starch, while the B-granule starch had significantly ($p < 0.05$) higher pasting temperature than the A-granule starch counterparts. However, the setback viscosity of B-granules for Xinong 9718 was significantly ($p < 0.05$) higher than that of A-granules, and the difference of peak time between A- and B-granules is not significant ($p < 0.05$) (Table 4). These results agreed with those reported by Sahlström et al. (2003)[5] and Ao and Jane (2007).[6] Variations in pasting characteristics for hard red and soft red winter wheat starch may be attributed to their different protein and amylose content, as shown in Table 2.

During the RVA pasting test, starch granules are heated in excess water, and with an increase in temperature, the starch granules start to imbibe water. The pasting temperature indicates the temperature at which the viscosity begins to increase during the heating process. The high pasting temperatures of starch indicated a higher resistance to swelling and rupture.[18] Meanwhile, starch molecules begin to leach out into the solution, and the viscosity of the suspension increases. The maximum viscosity attained during heating is peak viscosity, which indicates the water-holding capacity of starch in terms of the resistance of swollen granules to shear and the swelling performance of granules. The higher peak viscosity of unfractionated starch granules may be due to the presence of granules with a wide size distribution range, leading to different swelling patterns.[23] In addition, the higher peak viscosity of A-granules compared to B-granules may be attributed to their larger granule size, which would possess a loose packing ability and occupy a relatively larger volume compared to B-granules at a similar concentration.[2]

dx.doi.org/10.1021/jf400943h | J. Agric. Food Chem. 2013, 61, 6477−6484

Table 4. Pasting Properties of Unfractionated Wheat Starch and Isolated A and B Granules[a]

variety	sample	PV (cP)	TV (cP)	BD (cP)	FV (cP)	SB (cP)	PT (min)	GT (°C)
Xinong 9718	unfractionated	4102 ± 73 a	1400 ± 19 b	2702 ± 32 a	3276 ± 27 a	1876 ± 14 a	4.60 ± 0.02 c	70.9 ± 0.0 b
	A-granule	3981 ± 54 b	1530 ± 27 a	2451 ± 46 b	3291 ± 25 a	1761 ± 21 b	4.73 ± 0.0 b	69.3 ± 0.1 b
	B-granule	2302 ± 60 c	1005 ± 31 c	1297 ± 17 c	2895 ± 22 b	1890 ± 7 a	5.33 ± 0.03 a	75.1 ± 0.0 a
Shannong 138	unfractionated	4983 ± 47 a	1119 ± 9 b	3864 ± 65 a	3197 ± 36 a	2078 ± 17 a	4.00 ± 0.00 b	65.5 ± 0.3 b
	A-granule	4748 ± 41 b	1504 ± 15 a	3244 ± 48 b	3206 ± 45 a	1522 ± 11 b	4.20 ± 0.01 a	54.4 ± 0.0 c
	B-granule	3385 ± 48 c	803 ± 9 c	2582 ± 33 c	2008 ± 31 b	1205 ± 23 c	4.20 ± 0.00 a	67.1 ± 0.0 a

[a]All values are means of triplicate determinations ± SD. Means within columns with different letters for the same wheat varieties are significantly different ($p < 0.05$). [b]PV, peak viscosity; TV, trough viscosity; BD, breakdown; FV, final viscosity; SB, setback; GT, pasting temperature; PT, peak time.

Table 5. Thermal Properties of Unfractionated Wheat Starch and Isolated A- and B-Granules[a]

variety	sample	T_o (°C)	T_p (°C)	T_c (°C)	ΔT_r (°C)	ΔH (J/g)
Xinong 9718	unfractionated	56.40 ± 0.17 b	60.84 ± 0.31 b	66.72 ± 0.38 b	10.31 ± 0.03 a	5.85 ± 0.03 b
	A-granule	56.52 ± 0.24 b	60.91 ± 0.41 b	66.10 ± 0.46 b	10.26 ± 0.07 a	6.86 ± 0.00 a
	B-granule	58.73 ± 0.19 a	62.41 ± 0.32 a	68.84 ± 0.29 a	10.80 ± 0.01 a	4.46 ± 0.01 c
Shannong 138	unfractionated	56.32 ± 0.41 b	61.49 ± 0.47 b	67.50 ± 0.75 a	11.41 ± 0.08 a	6.06 ± 0.04 b
	A-granule	56.60 ± 0.39 b	61.05 ± 0.34 b	66.48 ± 0.38 a	9.58 ± 0.01 c	6.78 ± 0.02 a
	B-granule	58.03 ± 0.26 a	62.75 ± 0.31 a	67.34 ± 0.31 a	10.62 ± 0.01 b	5.87 ± 0.00 c

[a]All values are means of triplicate determinations ± SD. Means within columns for the same starch variety with different letters are significantly different ($p < 0.05$). T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔT_r, gelatinization temperature range ($\Delta T_r = T_c - T_o$); ΔH, enthalpy of gelatinization.

During the holding period at a constant high temperature of about 95 °C, a breakdown in viscosity to a holding strength or trough takes place (Figure 6A and B). The breakdown in viscosity is caused by rupture of the swollen granules. Breakdown viscosity is the difference between the peak viscosity and the trough viscosity, which was used to examine the fragility of the granules and stability of the starch paste during shearing at high temperatures. Thus, the higher breakdown viscosity value of A-granules revealed their higher stability compared to B-starch granules. The final viscosity indicates the stability of the cooled−cooked paste under low shear. The setback viscosity (the difference between the peak viscosity and the final viscosity) is the viscosity increase resulting from the rearrangement of amylose molecules that have leached from swollen starch during cooling, which is generally used as a measure of the gelling ability or retrogradation tendency of starch.[24] The different setback of A- and B-starch granules could be attributed to their different amylose content.

Thermal Properties of the A- and B-Granule Wheat Starches. The gelatinization properties of the unfractionated wheat starches and A- and B-granules isolated from two starches were measured by differential scanning calorimetry, and the results are presented in Table 5. The B-type starch granules exhibited higher onset gelatinization (T_o), peak (T_p), and conclusion (T_c) temperatures than unfractionated and A-granules, and no significant differences ($p < 0.05$) were found between the T_o, T_p, and T_c of the unfractionated and A-granule starches. The results are in agreement with the results presented by Ao and Jane (2007)[6] and Zeng et al. (2011).[9] The gelatinization temperature reflects the heat stability of the crystalline structure, which depends on the particle size distribution, where small granules usually have lower gelatinization temperature values than large granules.[17] However, the present results do not agree with this theory. The A-granules showed the highest gelatinization enthalpy (ΔH) compared with the unfractionated and B-starch granules. The results are in accordance with research of Zeng et al. (2011).[9] However, Xie et al. (2008),[17] Chiotelli and LeMeste (2002),[20] and Wong and Lelievre (1982)[25] reported a completely opposite result. The gelatinization enthalpy (ΔH) has been related to the degree of crystallinity. Therefore, the result is consistent with the observation that A-granules of both wheat varieties showed a higher relative crystallinity value and required more energy for gelatinization than the unfractionated and B-starch granules.

The starches isolated from both hard red and soft red winter wheat grain seeds exhibited a clearly bimodal size distribution. The large disk shape or lenticular A-type and small, spherical B-type starch showed significantly different chemical compositions, including total starch, amylose, amylopectin, and protein content, and ratio of amylopectin/amylose. The physicochemical properties of the A-type wheat starch granules were also significantly different from those of the B-type granules. The A-type granules had a higher iodine affinity value, enzymatic hydrolysis extent, and transition enthalpy and lower gelatinization temperature than did the B-type granules. A-granules showed the highest trough viscosity and final viscosity, while B-granules exhibited the highest pasting temperature and peak time. Both the A- and B-type granules exhibited typical A-type crystallinity, while A-granules showed a higher crystallinity degree than B-type granules. The physicochemical property difference between A- and B-type granules could be due to the difference in protein, amylose, and amylopectin content and their different arrangements. The present study demonstrated the compositions, physicochemical properties, and structures of A- and B-type granules individually, so as to better understand the relationship among properties, structure, and functionality of starch.

■ AUTHOR INFORMATION

Corresponding Author

*Fax: +86 02987092171. E-mail: zhanggq98@nwsuaf.edu.cn.

Funding

This project was financially supported by the Scientific and Technological Innovation Foundation of Northwest A&F University (ZD2012019) and the Modern Wheat Technological System Foundation of China (MATS).

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Ni, Y.; Wang, Z.; Yin, Y.; Li, W.; Yan, S.; Cai, T. Starch granule size distribution in wheat grain in relation to phosphorus fertilization. *J. Agric. Sci.* **2012**, *150*, 45−52.

(2) Singh, S.; Singh, N.; Isono, N.; Noda, T. Relationship of granule size distribution and amylopectin structure with pasting, thermal, and retrogradation properties in wheat starch. *J. Agric. Food Chem.* **2010**, *58*, 1180−1188.

(3) Vermeylen, R.; Goderis, B.; Reynaers, H.; Delcour, J. A. Gelatinisation related structural aspects of small and large wheat starch granules. *Carbohydr. Polym.* **2005**, *62*, 170−181.

(4) Dai, Z. M. Starch granule size distribution in grains at different positions on the spike of wheat (*Triticum aestivum* L.). *Starch/Stärke* **2009**, *61*, 582−589.

(5) Sahlström, S.; Bævre, A. B.; Bråthen, E. Impact of starch properties on hearth bread characteristics. II. Purified A- and B-granule fractions. *J. Cereal Sci.* **2003**, *37*, 285−293.

(6) Ao, Z. H.; Jane, J. L. Characterization and modeling of the A- and B-granule starches of wheat, triticale, and barley. *Carbohydr. Polym.* **2007**, *67*, 46−55.

(7) Park, S. H.; Wilson, J. D.; Seabourn, B. W. Starch granule size distribution of hard red winter and hard red spring wheat: its effects on mixing and breadmaking quality. *J. Cereal Sci.* **2009**, *49*, 98−105.

(8) Peña, R. J.; Trethowan, R.; Pfeiffer, W. H.; van Ginkel, M. Quality (end use) improvement in wheat: compositional, genetic, and environmental factors. *J. Crop Prod.* **2002**, *5*, 1−37.

(9) Zeng, J.; Li, G. L.; Gao, H. Y.; Ruv, Z. G. Comparison of A and B starch granules from three wheat varieties. *Molecules* **2011**, *16*, 10570−10591.

(10) Jarvis, C. E.; Walker, J. R. L. Simultaneous, rapid, spectrophotometric determination of total starch, amylose and amylopectin. *J. Sci. Food Agric.* **1993**, *63*, 53−57.

(11) American Association of Cereal Chemists. *Method 44-15A, 46-12, 61-03, and 76-21 in Approved Methods of the AACC*, 10th ed.; AACC: St. Paul, MN, 2000.

(12) Englyst, H. N.; Kingman, S. M.; Cummings, J. H. Classification and measurement of nutritionally important starch fractions. *Eur. J. Clin. Nutr.* **1992**, *46*, 33−50.

(13) Maache-Rezzoug, Z.; Maugard, T.; Zarguili, I.; Bezzine, E.; Marzouki, M. N. E.; Loisel, C. Effect of instantaneous controlled pressure drop (DIC) on physicochemical properties of wheat, waxy and standard maize starches. *J. Cereal Sci.* **2009**, *49*, 346−353.

(14) Leach, H. W.; McCowen, L. D.; Schoch, T. J. Structure of the starch granule, swelling and solubility patterns of various patterns of various starches. *Cereal Chem.* **1959**, *36*, 534−544.

(15) Morrison, W. R.; Laignelet, B. An improved colorimetric procedure for determining apparent and total amylose in cereal and other starches. *J. Cereal Sci.* **1983**, *1*, 9−20.

(16) Kim, H. S.; Huber, K. C. Physicochemical properties and amylopectin fine structures of A- and B-type granules of waxy and normal soft wheat starch. *J. Cereal Sci.* **2010**, *51*, 256−264.

(17) Xie, X. J.; Cui, S. W.; Li, W.; Tsao, R. Isolation and characterization of wheat bran starch. *Food Res. Int.* **2008**, *41*, 882−887.

(18) Li, W. H.; Shu, C.; Zhang, P. L.; Shen, Q. Properties of starch separated from ten mung bean varieties and seeds processing characteristics. *Food Bioprocess Technol.* **2011**, *4*, 814−821.

(19) Bertolini, A. C.; Souza, E.; Nelson, J. E.; Huber, K. C. Composition and reactivity of A- and B-type starch granules of normal, partial waxy, and waxy wheat. *Cereal Chem.* **2003**, *80*, 544−549.

(20) Chiotelli, E.; LeMeste, M. Effect of small and large wheat starch granules on thermo-mechanical behavior of starch. *Cereal Chem.* **2002**, *79*, 286−293.

(21) Morrision, W. R.; Tester, R. F.; Snape, C. E.; Law, R.; Gidley, M. J. Swelling and gelatinization of cereal starches. 4. Some effects of lipidcomplexed amylose and free amylose in waxy and normal barley starches. *Cereal Chem.* **1993**, *70*, 385−391.

(22) Blazek, J.; Copeland, L. Amylolysis of wheat starches. II. Degradation patterns of native starch granules with varying functional properties. *J. Cereal Sci.* **2010**, *52*, 295−302.

(23) Lovedeep, K.; Jaspreet, S.; Owen, J. M.; Harmit, S. Physicochemical, rheological, and structural properties of fractionated potato starches. *J. Food Eng.* **2007**, *82*, 383−394.

(24) Karim, A. A.; Norziah, M. H.; Seow, C. C. Methods for the study of starch retrogradation. *Food Chem.* **2000**, *71*, 9−36.

(25) Wong, R. B.; Lelievre, J. Comparison of crystallinities of wheat starches with different swelling capacities. *Starch/Stärke* **1982**, *34*, 159−161.