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RESEARCH ARTICLE

Characterization of A- and B-type starch granules in Chinese wheat cultivars

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Abstract

Starch is the major component of wheat flour and serves as a multifunctional ingredient in food industry. The objective of the present study was to investigate starch granule size distribution of Chinese wheat cultivars, and to compare structure and functionality of starches in four leading cultivars Zhongmai 175, CA12092, Lunxuan 987, and Zhongyou 206. A wide variation in volume percentages of A- and B-type starch granules among genotypes was observed. Volume percentages of A- and B-type granules had ranges of 68.4–88.9% and 9.7–27.9% in the first cropping seasons, 74.1–90.1% and 7.2–25.3% in the second. Wheat cultivars with higher volume percentages of A- and B-type granules could serve as parents in breeding program for selecting high and low amylose wheat cultivars, respectively. In comparison with the B-type starch granules, the A-type granules starch showed difference in three aspects: (1) higher amount of ordered short-range structure and a lower relative crystallinity, (2) higher gelatinization onset (T_o) temperatures and enthalpies (ΔH), and lower gelatinization conclusion temperatures (T_c), (3) greater peak, though, and final viscosity, and lower breakdown viscosity and pasting temperature. It provides important information for breeders to develop potentially useful cultivars with particular functional properties of their starches suited to specific applications.

Keywords: bread wheat, A- and B-type starch granules, short-range molecular order, relative crystallinity, gelatinization and pasting properties

1. Introduction

Starch is the major component of wheat flour and serves as a multifunctional ingredient in food industry. Starch occurs as granules in the endosperm of wheat grain. The granule shape, size and its hierarchical structure are important determinants of starch functionality (Lindeboom *et al*. 2004; Park *et al*. 2004). Wheat starch consists of two distinct forms of granules: A- and B-type granules. The A-type starch granules are disk-like or lenticular in shape with a

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diameter of >10 μm, while the B-type starch granules are less than 10 μm in diameter and spherical or polygonal in shape (Vermeylen *et al*. 2005; Ao and Jane 2007; Kim and Huber 2008; Wang *et al*. 2014). In wheat, A-type granules contribute to more than 70% total weight of the starch (Bechtel *et al*. 1990; Peng *et al*. 1999; Shinde *et al*. 2003), whereas B-type granules comprise up to 90% of granules in number (Raeker *et al*. 1998).

The proportions of B- and A-starch granules, by weight, volume and number, differ among genotypes (Raeker *et al*. 1998; Li *et al*. 2001). A wide range of variation (17–50%) for B-starch granule volume was observed in bread wheat, suggesting possibilities of genetic manipulation of granule size distribution (Stoddard 1999). The B-granules occupied volumes in a range of 28.5–56.2% for hard red winter and hard red spring wheat (Park *et al*. 2009). The volume percentages of A- and B-type starch granules were 52.7–65.5% and 34.5–47.3% in seven Chinese wheat cultivars (Dai *et al*. 2009). In several studies, environmental stress such as temperature (Liu *et al*. 2011), water deficit (Dai *et al*. 2009; Zhang *et al*. 2010), nutrient supplementation (Ni *et al*. 2012; Li *et al*. 2013), and light intensity (Li *et al*. 2010) significantly changed starch granule size distribution and amylose content in wheat.

Wheat starch A- and B-type granules differ in composition, chain length distribution of amylopectin, relative crystallinity, microstructure (e.g., surface pores, channels, cavities), and they have been summarized in details (Soulaka and Morrison 1985; Fortuna *et al*. 2000; Chiotelli and Le Meste 2002; Bertolini *et al*. 2003; Shinde *et al*. 2003; Van Hung and Morita 2005; Geera *et al*. 2006; Kim and Huber 2008; Kim 2009; Salman *et al*. 2009). The differences in these structural characteristics lead to variations in swelling, gelatinization, retrogradation and pasting properties of the two types of starch granules (Eliasson and Kaelsson 1983; Fortuna *et al*. 2000; Chiotelli and Le Meste 2002; Shinde *et al*. 2003; Geera *et al*. 2006; Soh *et al*. 2006; Kim 2009). A-type granules have higher gelatinization enthalpy, amylose content, pasting parameters such as peak, trough, breakdown, final and setback viscosities, and lower gelatinization onset and peak temperatures, whereas B-type granules have higher lipid-complexed amylose content and swelling power, broader gelatinization ranges, and lower gelatinization enthalpy (Sahlström *et al*. 2003; Geera *et al*. 2006; Soh *et al*. 2006; Kim and Huber 2010a; Yin *et al*. 2012). Shinde *et al*. (2003) and Soh *et al*. (2006) observed that peak and final viscosities of wheat starch reduced with increasing proportion of B-type granules. Thus, the proportion of A- and B-type granules impacts wheat starch structural characteristics and functional properties. However, there are yet inconsistent reports on amylopectin chain-length distribution, relative crystallinity, and microstructure of A- and B-type wheat starch granules (Vermeylen *et al*. 2005; Liu *et al*. 2007; Salman *et al*. 2009). Much of this inconsistency is likely attributable to the different genotypes used in previous reports. In addition, there are very little information available on amylopectin chain-length distribution, relative crystallinity and microstructure of A- and B-type starch granules in Chinese cultivars.

The objective of the present study was to investigate starch granule size distribution in Chinese wheat cultivars, and to assess A- and B-type starch granule characteristics of four leading cultivars in morphology, amylose content, chain length distribution of amylopectin, short-range molecular order, relative crystallinity, and gelatinization and pasting properties. The results of this study will help breeders to develop potentially useful cultivars with particular functional properties of their starches suited to specific applications.

2. Materials and methods

2.1. Experimental materials

A total of 345 Chinese leading cultivars and advanced lines, including 66 from the Northern China Plain zone, 251 from the Yellow and Huai Valley zone, 12 from the middle and low Yangtze Valley zone, 3 from the southwestern China zone, and 13 introductions from other countries were grown in Anyang, Henan Province in two seasons, including 245 and 208 genotypes in 2010–2011 and 2011–2012 cropping seasons, respectively. Only 108 genotypes were grown in two consecutive cropping seasons. They were used to determine the distribution of A- and B-type starch granules. Among them, four leading cultivars Zhongmai 175, CA12092, Lunxuan 987, and Zhongyou 206 collected from the same field in 2012–2013 season in Beijing were used to analyze morphology, amylose content, chain length distribution of amylopectin, short-range molecular order, relative crystallinity, and gelatinization and pasting properties of the A- and B-type starch granules. Zhongmai 175, Lunxuan 987 and Zhongyou 206 have been released in the Northern China Plain zone, and CA12092 is an advanced line currently including in the regional yield trials. Zhongmai 175 is characterized by high yielding potential and broad adaptation, soft kernel, excellent noodle, and steamed bread qualities, and is currently a leading cultivar and also serves as a check cultivar in the regional yield trials. Zhongyou 206 possessed hard kernel, strong gluten and excellent bread-making quality. CA12092 and Lunxuan 987 showed high yielding potential and broad adaptation, hard kernel, but poor gluten quality, and averaged qualities for noodles and steamed bread.

2.2. Flour milling

Grain hardness was measured on 300-kernel sample with a

Perten Single Kernel Characterization System (SKCS) 4100 (Perten Instruments, Springfield, IL, USA). The tested samples were tempered overnight to 14.5 and 16.5% moisture for soft and hard wheat, respectively. 200 g grain samples from each genotype were milled using a Brabender Quadrumat Junior mill (Brabender Inc., Duisberg, Germany). Grain samples of Zhongmai 175 and CA12092 were tempered overnight to 14.5% moisture, and those of Lunxuan 987 and Zhongyou 206 were also tempered overnight to 16.5% moisture and milled using the same mill as mentioned above.

2.3. Starch isolation

Starch was extracted according to the methods of Liu *et al*. (2007) and Park *et al*. (2005) with minor modifications, the tailings were centrifuged twice and all the starch portions were combined. To separate gluten from starch, dough was made by mixing 6 g of flour with 4 g of distilled water, stood for 10 min, and then washed with 60 mL of water. The gluten was washed twice with 20 mL of water to ensure the complete separation of all the starch. The combined starch suspensions were filtered using a nylon cloth (75 µm openings). The resulting starch filtrate was centrifuged at 2 500×g for 15 min, and the supernatant was discarded. The precipitate was divided into two portions and the upper gray-colored tailings were transferred to another tube. Water was added into the lower light-colored portions and slurries were centrifuged again. These steps were repeated until there were no gray-colored tailings on top of the starch. The collected tailings from each repeat were re-suspended and centrifuged twice. Then, the top layer was discarded as described above. The resulting starch from the above steps was combined and freeze-dried. The dried starch granules were ground lightly with a mortar and pestle and passed a 100-mesh sieve.

2.4. Fractionation of A- and B-type starch granules

Large A- and small B-type starch granules were separated from prime starch by repeated suspensions in six cycles (Park *et al*. 2005). The sediment comprised mainly A-type starch granules and the supernatant contained mainly B-type granules. The sediment and supernatant were centrifuged for 15 min (4 000×g), and the A- and B- starch granule factions were collected, respectively. The fractions were frozen, lyophilized, and ground with a household coffee mill to pass through a 149-µm mesh sieve.

2.5. Granule size distribution

The proportion of A- and B-type granules in wheat starch was determined using a Sympatec Helos/Rodos laser diffraction particle size analyzer (Sympatec GmbH, Clausthal-Zellerfeld, Germany), and the data were calculated as the volume percentage (%) occupied by starch granules. Granules with size of <10.0 µm and between 10.1–35.0 µm in diameter were classified as B- and A-type starch granules, respectively (Peng *et al*. 1999). Granules with diameters >35.0 µm were considered to be impurities or compound granules. Each sample was measured twice, and the differences between two measurements of B-type granule contents were less than 0.5%.

2.6. Granule morphology

Starch samples were fixed onto the surface of double-sided, carbon-coated adhesive tape attached to an aluminium stub. The mounted starch samples were coated with gold prior to imaging in a scanning electron microscope (SU1510, Hitachi High-technologies Corporation, Japan). The accelerating voltage was 10.0 kV.

2.7. Starch crystallinity

Starch crystallinity was measured using a Panalytical X' Pert Pro X-ray diffractometer (PANalytical, Holand) with a Co-K_α source (λ=0.1789 nm) operating at 45 kV and 35 mA. The detailed operating conditions and sample treatment before measurement were described elsewhere (Wang *et al*. 2009). The relative crystallinity was quantitatively estimated as a ratio of the crystalline area to the total area between 4–40°(2θ) using the Origin software (ver. 7.5, Microcal Inc., Northampton, MA, USA).

2.8. Chain length distribution of amylopectin

Chain length distribution of amylopectin was analyzed using a high-performance anion-exchange chromatography equipped with a pulsed amperometric detector (HPAEC-PAD) (Dionex Corporation, Sunnyvale, CA) according to the method of Liu *et al*. (2007). 18 mg starch was dispersed in 6 mL of sodium acetate buffer (pH 3.5) by stirring in a boiling water bath for 20 min. After cooling, isoamylase solution (10 µL) was added. The sample was incubated at 37°C with slow stirring for 16 h. The enzyme was inactivated by boiling the samples for 15 min. The sample was filtered (0.45 µm nylon syringe filter) and injected into the HPAEC-PAD System.

The HPAEC-PAD System consisted of a Dionex DX2500 equipped with an ED50 electrochemical detector with a gold working electrode, GP50 gradient pump, LC30 chromatography oven, and AS40 automated sampler (Dionex Corporation, Sunnyvale, CA, USA). The standard quintuple potential waveform was employed, with the following periods and pulse potentials: $T1$=0.20 s, $E1$=0.1 V; $T2$=0.40 s, $E2$=0.1 V; $T3$=0.41 s, $E3$=–2 V; $T4$=0.43 s, $E4$=0.6 V; $T5$=0.44 s, *E*5=−0.1 V. Data were collected using Chromeleon software, ver. 8.00 (Dionex Corporation, Sunnyvale, CA, USA). The mobile phase was prepared in deionized water with helium sparging and contained eluent A (100 mmol L^{-1} NaOH) and eluent B (50 mmol L^{-1} sodium acetate in 100 mmol L^{-1} NaOH). Flow rate was 1.0 mL min^{-1}. Linear components were separated on a Dionex CarboPac™ PA100 column with gradient elution (0–5 min, 40% A; 15–50 min, 70% A; 15–50 min, 70% A+30% B) at a column temperature of 26°C and a flow rate of 1 mL min^{-1}. A CarboPac™ PA100 guard column was installed in front of the analytical column.

2.9. Fourier transform infrared spectroscopy (FT-IR)

The FT-IR spectra of wheat starch samples were obtained using a Tensor 27 FT-IR spectrometer (Bruker, Germany) equipped with a DLATGS detector. The sample preparation and operation conditions were described elsewhere (Wang *et al*. 2014). The ratios of absorbance at 1 045 cm^{-1}/1 022 cm^{-1} were used to characterize the short-range ordered structure of starch.

2.10. Amylose content

0.1 mg of starch sample was dissolved in 1 mL of 95% ethanol (v/v) and 9 mL of NaOH solution (1 N) and placed overnight in a refrigerator at 4°C. Distilled water was added to make up 100 mL in a volumetric flask containing the dissolved starch sample. The amount of amylose was determined using an automated chemistry analyzer (FS3100, OI Analytical, USA) according to the operator's manual. Rice amylose and amylopectin from the China National Rice Research Institute, Chinese Academy of Agricultural Sciences were used to establish a calibration curve using a set of starches with amylose concentrations of 1.5, 10.4, 16.2, 19.3, and 22.5%. An additional rice starch with 26.5% amylose was used as the control sample.

2.11. Thermal analysis

Thermal transition analysis of starch samples was made using a differential scanning calorimeter (DSC 200 F3, NETZSCH, Germany) equipped with a thermal analysis data station and data recording software, according to the method of Wang and Copeland (2012). About 3 mg of starch granules were weighed into 40 µL aluminum pans. Distilled water was added to the starch with a microsyringe to obtain a starch/water ratio of 1:3 in the DSC pans. Care was taken to ensure that the starch samples were completely immersed in the water by gentle shaking before the pans were sealed, reweighed and left overnight at room temperature before analysis. An empty pan was used as a reference. The pans were heated from 30 to 115°C at a scanning rate of 10°C min^{-1}. The instrument was calibrated using indium as a standard. The onset (T_o), peak (T_p) and conclusion (T_c) temperatures and the enthalpy change (ΔH) were determined through data recording software.

2.12. Pasting properties

The pasting profiles were analyzed using a newport scientific rapid visco analyser 3 (RVA-3) (Newport Scientific, Australia). Starch slurries containing 8% (w/w) starch (dry weight) in a total weight of 28 g were held at 50°C for 1 min before heating at a rate of 6°C min^{-1} to 95°C, holding at 95°C for 5 min, and then cooling at a rate of 6°C min^{-1} to 50°C and held at 50°C for 2 min. The speed of the mixing paddle was 960 r min for the first 10 s, then 160 r min^{-1} for the remainder of the experiment. Peak viscosity (PV), viscosity at trough (also known as minimum viscosity, MV) and final viscosity (FV) were recorded, and breakdown (BD, which is PV minus MV) and setback (SB, which is FV minus MV) were calculated using the Thermocline software provided with the instrument.

2.13. Statistical analysis

All testings were conducted at least twice and the results were reported as the mean value and standard deviations excluding the X-ray diffraction, chain length distribution of amylopectin and FT-IR measurements. Mean, standard deviation, variable coefficient and Duncan's test ($P<0.05$) were conducted using the SPSS 17.0 Statistical Software Program (SPSS Inc. Chicago, IL, USA).

3. Results

3.1. Starch granule size distribution of Chinese cultivars

Significant differences in granule size distribution for A- and B-type granules were observed in the tested cultivars (Table 1). The volume percentages of A- and B-type granules had a range of 68.4–88.9% and 9.7–27.9% in 2010–2011 cropping season, 74.1–90.1% and 7.2–25.3% in 2011–2012 cropping season. This suggested that improvement for starch quality could be achieved through breeding given the wide variation for starch granule distribution present in Chinese wheat cultivars. The A-starch granules in most cultivars exhibited the volume percentage of 74.0–82.0% and 78.0–86.0%, while the B-type starch granules in most cultivars showed the volume percentage of 14.0–20.0% and 12.0–18.0% in 2010–2011 and 2011–2012 cropping seasons, respectively (Fig. 1). This indicated that cropping season had impacts on starch granule distribution in Chinese cultivars.

Table 1 Mean, range, standard deviation (SD), and coefficient of variance (CV%) of the volume percentages of A- and B-type starch granules in the tested cultivars

Season	No. of cultivars	Starch granule[1)]	Mean	Range	SD	CV (%)
2010–2011	245	A	79.0	68.4–88.9	3.46	4.4
		B	18.9	9.7–27.9	3.13	16.5
2011–2012	208	A	82.6	74.1–90.1	3.02	3.7
		B	16.4	7.2–25.3	3.21	19.6

[1)] A, the volume percentages of A-type starch granules (%); B, the volume percentages of B-type starch granules (%).

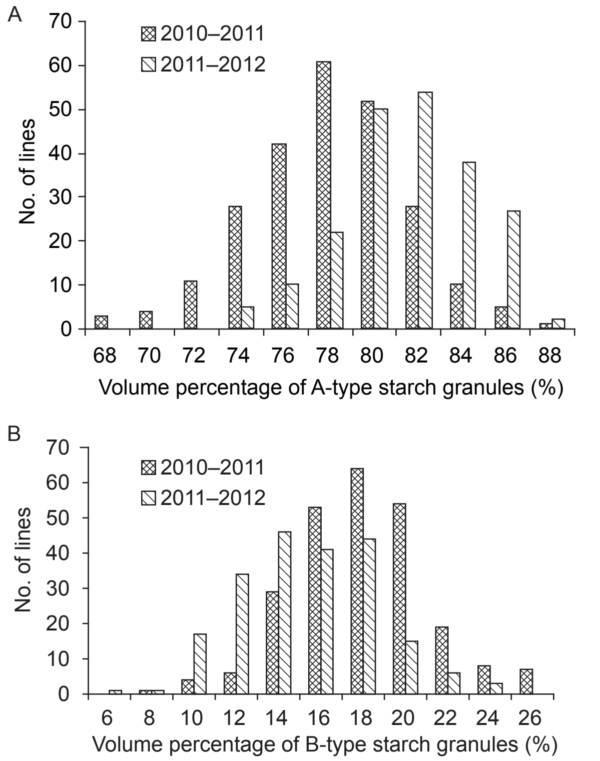

Fig. 1 The frequency distribution of percentage volumes of A- and B-type starch granules in the tested cultivars. The horizontal axes indicate the percentage volumes of A- or B-type starch granules (%).

Twenty-two cultivars with higher volume percentage of A-type granules and lower volume percentage of B-type granules were selected. Other 22 cultivars also showed higher volume percentage of B-type granules and lower volume percentage of A-type granules. Name of these cultivars are listed in Table 2. Among them, Wheatear, Zhou 9811-1, Ruzhou 0319, and Chuanmai 42-white with higher volume percentage of A-type granules and lower volume percentage of B-type granules, and 07CA266 and Bainong 64 with higher volume percentage of B-type granules and lower volume percentage of A-type granules were found in two consecutive cropping seasons. They could serve as crossing parents for selecting high amylose cultivars in breeding programs due to A-type granules with more amylose relative to B-type granules (Liu *et al*. 2007), which increase the resistant starch in wheat suitable for improving public health (Rahman *et al*. 2007). Meanwhile 07CA266 and Bainong 64 could also become crossing parents for selecting low amylose wheat cultivars that can positively contribute to noodle quality improvement. For the above other cultivars only grown in single cropping season, further investigation will be needed to confirm starch granule distribution of them.

3.2. Characterization of A- and B-type starch granules in four cultivars

Granule morphology The granular morphologies of A- and B-type granules separated from starch of Zhongmai 175, CA12092, Lunxuan 987, and Zhongyou 206 were similar, as observed from the representative images of Zhongmai 175 in Fig. 2. A-type starch granules displayed a disk-like shape with diameter in the range of 10–30 µm. In contrast, B-type granules displayed a spherical shape with diameter less than 10 µm, and granules with diameter of about 5 µm were predominant. The morphology of the starch was in agreement with previous reports (Jane *et al*. 1994; Song and Jane 2000; Yoo and Jane 2002; Wang *et al*. 2014).

FT-IR spectroscopy Similar FT-IR patterns were also observed for unfractionated, A- and B-type starch granules from four cultivars, hence only FT-IR spectra of Zhongmai 175 is presented (Fig. 3-A). The deconvoluted FT-IR spectrum in the range of 800–1 200 cm^{-1} of the B-type starch granules differed greatly from that of unfractionated and A-type starch granules (Fig. 3-B). The 1 047 cm^{-1}/1 022 cm^{-1} ratios of unfractionated starch, A- and B-type starch granules are presented in Table 2. Interestingly, A-type large granules showed the highest 1 047 cm^{-1}/1 022 cm^{-1} ratios, whereas B-type small granules presented the lowest values. This indicated that A-type large granules had a larger amount of ordered short-range structure than B-type small granules.

3.3. Branch chain-length distribution of amylopectin

The amylopectin chain length distribution of the starches was classified into four categories: short chains with degree of polymerization (DP) 6–12, medium length chains with DP 13–24, long chains with DP 25–36, and very long chains with DP>36 (Table 3). Amylopectin of unfractionated, A- and

Table 2 Cultivars with higher volume percentage of A- and B-type granules in two consecutive cropping seasons

Cropping season	Cultivars[1)]	A	B	Cultivars[2)]	A[3)]	B[4)]
2010–2011	Wheatear	88.9	9.7	Yumai 34	71.7	27.9
	Zhoumai 26	87.4	10.7	07CA266	72.4	27.2
	Fumai 8	87.1	11.8	Xin 9408	73.3	26.7
	Zhi4001	86.8	10.8	BAY24	72.8	26.7
	Zhou9811-1	86.5	11.8	Jagger	73.1	26.5
	U07-6308	86.0	12.9	AAY08	71.8	26.1
	04Zhong70	85.4	13.3	CA9507-dwarf	72.9	26.0
	Zhou 18	85.2	13.3	08CA137	68.6	25.3
	Yubao 2	85.1	12.5	10CS4801	74.8	24.8
	Ruzhou 0319	84.8	13.8	Xin 05-1241	74.8	24.7
	Chuanmai 42-white	84.7	13.5	Bainong 64	68.4	24.3
2011–2012	Zhouheimai 1	90.1	7.2	Tainong 2413	74.1	25.3
	Wheatear	90.0	8.4	Luomai 05123	74.6	24.8
	Brula	87.9	11.3	Luyuan 502	75.2	24.3
	Ruzhou 0319	87.9	10.3	Shixin 733	76.8	22.6
	09CA86	87.5	10.6	Kenong 2011	76.9	22.6
	Luomai 6082	87.2	11.8	Pubing 3228	76.1	22.6
	Luomai 24	87.2	10.6	Zimai 12	76.1	22.5
	Chuanmai 42-white	87.1	11.0	Shixin 828	77.3	22.3
	Yan 4110	87.1	11.8	Tainong 9862	77.2	22.2
	Zhou 24	87.1	11.2	07CA266	77.8	21.6
	Zhou 9811-1	86.7	11.5	Bainong 64	77.7	21.2

[1)] Cultivars with higher volume percentage of A-type granules and lower volume percentage of B-type granules.
[2)] Cultivars with higher volume percentage of B-type granules and lower volume percentage of A-type granules.
[3)] A, the volume percentages of A-type starch granules (%).
[4)] B, the volume percentages of B-type starch granules (%).

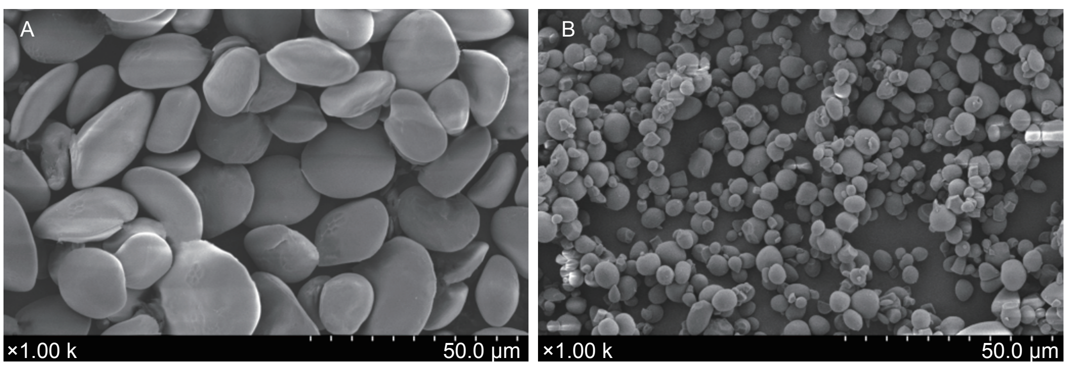

Fig. 2 Scanning electron micrographs of A-type (left) and B-type (right) granules in Zhongmai 175. Scale bar=5.0 μm.

B-type starch granules contained a higher proportion of medium chains with DP 13–24 (47.1–65.8%) and short chains with DP 6–12 (16.3–36.5%), and a smaller proportion of long chains with DP 25–36 (11.1–17.9%) and very long chains with DP>36 (0–1.9%). Differences in chain length distribution of amylopectin of A- and B-type granules separated from four cultivars were observed. The A-type granules of Zhongmai 175 and Zhongyou 206 contained less branch chains of DP 6–12 and more branch chains of DP 13–24 than did the B-type granules, in general agreement with the results from Ao and Jane (2007) and Salman *et al*. (2009). However, the A-type granules of CA12092 and Lunxuan 987 contained more branch chains of DP 6–12 and less branch chains of DP 13–24 than did the B-type granules, consistent with the report by Vermeylen *et al*. (2005). In contrast to B-type granules, the A-type granules of four cultivars had lower branch chains of DP 25–36 and DP>36. These data indicate that amylopectin molecules of the A- and B-type granules have distinct fine structures, and they are likely genetically controlled during their biosynthesis (Peng *et al*. 2000).

3.4. X-ray crystallinity

Unfractionated, A- and B-type starch granules of four cul-

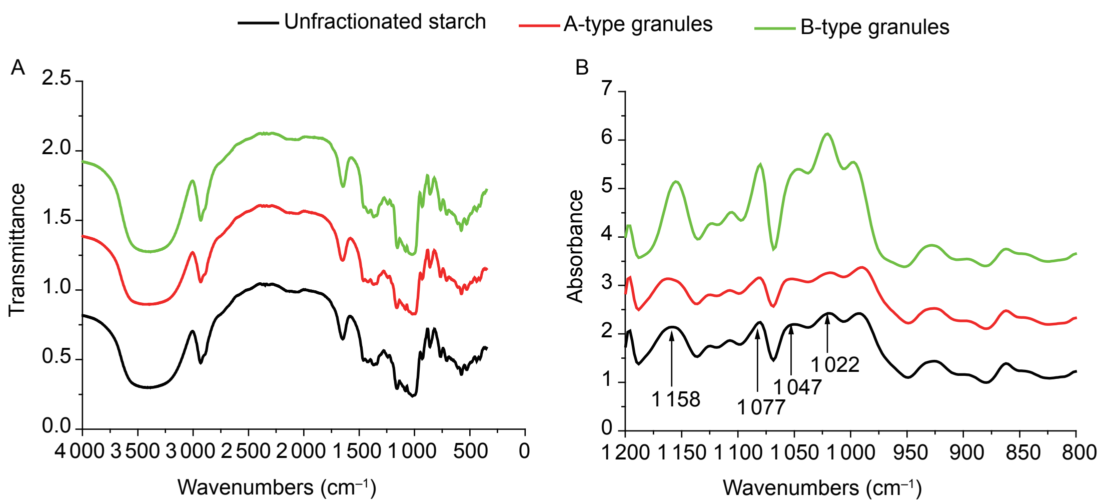

Fig. 3 Fourier transform infrared spectroscopy (FT-IR) average spectra (A) and the subtraction spectra (B) of unfractionated starch, A- and B-starch granules in Zhongmai 175.

Table 3 Ratio between 1 047 and 1 022 cm−1 of fourier transform infrared spectroscopy (FT-IR) spectra, relative crystallinity and branch chain length distribution for unfractionated starch, A- and B-starch granules of four wheat cultivars

Cultivar	Starch source	1 047 cm−1/ 1 022 cm−1	Relative crystallinity (%)	Distribution (%)[1)]			
				DP 6–12	DP 13–24	DP 25–36	DP>36
Zhongmai 175	Unfractionated starch	0.86 a	25.6 b	25.0 a	59.2 b	14.4 c	1.5 c
	A-granules	0.90 a	24.7 c	23.3 c	60.4 a	14.6 b	1.7 b
	B-granules	0.74 b	27.3 a	24.7 b	58.4 c	15.1 a	1.8 a
CA12092	Unfractionated starch	0.88 a	25.1 b	28.3 a	58.7 c	12.0 c	1.0 c
	A-granules	0.89 a	23.6 c	26.7 b	59.4 b	12.8 b	1.1 b
	B-granules	0.79 b	26.7 a	16.3 c	65.8 a	16.1 a	1.9 a
Lunxuan 987	Unfractionated starch	0.88 a	24.8 b	34.7 a	53.3 c	11.1 b	0.9 b
	A-granules	0.90 a	23.7 c	31.8 b	56.4 b	10.9 c	0.9 b
	B-granules	0.79 b	25.4 a	20.2 c	63.8 a	14.6 a	1.5 a
Zhongyou 206	Unfractionated starch	0.89 a	24.6 b	20.8 c	61.4 a	17.9 a	0 b
	A-granules	0.90 a	23.4 c	26.8 b	59.4 b	13.1 c	0 b
	B-granules	0.80 b	25.8 a	36.5 a	47.1 c	16.4 b	0.7 a

[1)] DP, degree of polymerization

Values followed by the same letter in the same column are not significantly different (*P*<0.05). The same as below.

tivars presented similar XRD patterns, as observed from the representative patters of Zhongmai 175 (Fig. 4). Four characteristic diffraction peaks were noted at 2θ 15.5, 17.4, 18.7, and 23° (Fig. 4), indicating the presence of A-type crystalline polymorphs in wheat starches. The relative crystallinity of B-type starch granules was the highest, while it was the lowest for A-type starch granules (Table 3). The result indicated that B-type starch granules had a larger amount of crystallites than A-type starch granules, consistent with Ao and Jane (2007).

3.5. Thermal properties

DSC data showed that the gelatinization temperatures of

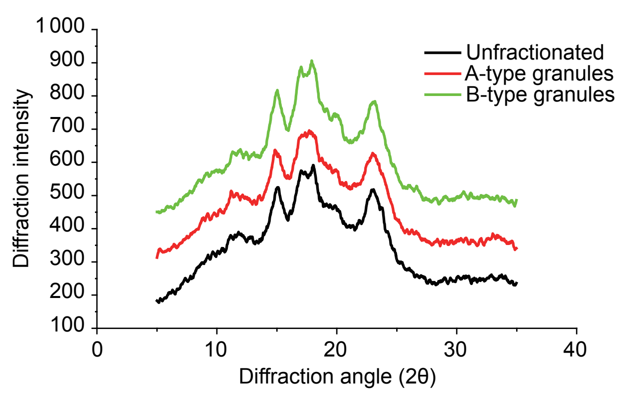

Fig. 4 X-ray diffraction patterns of unfractionated starch, A- and B-starch granules in Zhangmai 175.

unfractionated, A- and B-type starch granules of four wheat cultivars varied from 54.2 to 69.3°C, and the gelatinization enthalpy changes were in the range of 8.1–11.1 (Table 4). No significant difference was found for gelatinization parameters changes of the A- and B-type starch granules among four cultivars. A-type starch granules relative to B-type starch granules exhibited higher gelatinization onset (T_o), temperature and enthalpy changes (ΔH), but lower gelatinization conclusion temperatures (T_c).

3.6. Pasting properties

Amylose content and pasting parameters of unfractionated, A- and B-type starch granules from four wheat cultivars are shown in Table 5. A-type starch granules had a higher amylose content (31.4–33.3%) than B-type starch granules (29.9–32.5%), in agreement with reports by Soulaka and Morrison (1985), Peng *et al*. (1999) and Shinde *et al*. (2003). A-type starch granules exhibited greater peak, trough, and final viscosities, and lower breakdown viscosity and pasting temperature than B-type starch granules. This was consistent with previous reports (Franco *et al*. 2002; Shinde *et al*. 2003; Ao and Jane 2007), suggesting greater swelling power of A-type starch granules.

4. Discussion

4.1. Starch granules size distribution

Previous studies indicated that starch granule size distribution was mainly affected by environmental factors such as growing season temperature, rainfall patterns and humidity, growth locations, and sustained or episodic environmental stresses (Raeker *et al*. 1998; Dai *et al*. 2009; Beckles *et al*. 2014), generally consistent with our observations that some differences were found in the volume percentage of A- and B-type starch granules of most cultivars grown at the same location among two cropping seasons. Moreover, a wide variation in volume percentages of A- and B-type starch granules among cultivars grown in the same environment was observed in the present study, indicating genotype can play a major role in determining the variation in granule size

Table 4 Thermal properties of unfractionated starch, A- and B-starch granules in four wheat cultivars[1)]

Cultivar	Starch	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J g^{-1})
Zhongmai 175	Unfractionated starch	57.4 a	62.1 a	67.7 a	9.0 a
	A-granules	56.5 a	61.5 b	66.6 b	9.2 a
	B-granules	54.5 b	61.0 c	68.6 a	8.1 b
CA12092	Unfractionated starch	56.7 a	62.4 a	68.4 b	10.8 a
	A-granules	56.6 a	62.0 b	67.5 c	11.1 a
	B-granules	54.3 b	62.3 a	70.1 a	9.2 b
Lunxuan 987	Unfractionated starch	56.5 a	62.6 a	68.9 b	10.5 a
	A-granules	56.7 a	62.1 a	68.6 b	10.6 a
	B-granules	54.2 b	61.6 b	70.4 a	9.6 b
Zhongyou 206	Unfractionated starch	57.0 a	63.1 a	69.3 b	10.4 a
	A-granules	56.5 a	62.6 b	68.6 c	10.3 b
	B-granules	55.3 b	62.1 c	69.9 a	10.2 b

[1)] T_o, T_p and T_c, onset, peak and complete temperature, respectively; ΔH, enthalpy change.

Table 5 Amylose and rapid visco analyzer (RVA) parameters of unfractionated starch, A- and B-starch granules in four wheat cultivars

Cultivar	Sample	Amylose (%)	Peak viscosity (cP)	Trough (cP)	Breakdown (cP)	Final viscosity (cP)	Pasting temperature (°C)
Zhongmai 175	Unfractionated starch	29.7 b	1 848 b	1 326 b	523 b	1 929 b	92.5 a
	A-granules	32.5 a	2 454 a	2 043 a	412 b	2 497 a	88.4 c
	B-granules	30.2 b	1 232 c	430 c	802 a	1 275 c	90.6 b
CA12092	Unfractionated starch	31.8 a	2 002 b	1 326 b	588 a	2 201 b	87.1 b
	A-granules	31.9 a	2 230 a	2 043 a	275 c	2 358 a	87.2 b
	B-granules	30.4 b	1 534 c	430 c	442 b	1 688 c	95.4 a
Lunxuan 987	Unfractionated starch	32.3 b	2 045 b	1 739 b	306 a	2 295 b	88.1 b
	A-granules	33.3 a	2 370 a	2 134 a	236 b	2 609 a	84.4 c
	B-granules	32.5 ab	860 c	560 c	300 a	988 c	95.2 a
Zhongyou 206	Unfractionated starch	31.2 a	2 306 b	1 813 b	493 b	2 567 b	89.7 a
	A-granules	31.4 a	2 643 a	2 333 a	310 c	2 857 a	84.9 c
	B-granules	29.9 b	1 398 c	754 c	645 a	1548 c	85.6 b

distribution of wheat starch, in agreement with previous reports (Dengate and Meredith 1984; Stoddard 2000, 2003).

4.2. FT-IR spectroscopy, X-ray crystallinity and branch chain-length distribution of amylopectin

The FT-IR spectrum of starch is sensitive to changes in structure of a short-range molecular level (double helices). The absorbance bands at 1 022 and 1 047 cm^{-1} are characteristics of amorphous and ordered structures in starch (Van Soest *et al*. 1995). Thus, the ratio of 1 047 cm^{-1}/1 022 cm^{-1} can be used to characterize the short-range molecular order of double helices in starches (Van Soest *et al*. 1995; Capron *et al*. 2007).

In the present study, A-type starch granules presented a higher ratio of 1 047 cm^{-1}/1 022 cm^{-1} than B-type starch granules, indicating the presence of a larger amount of ordered short-range double helices in A-type starch granules. This was in general agreement with the chain length distribution of amylopectin in Zhongmai 175 and Zhongyou 206, showing that A-type starch granules have a higher proportion of intermediate chains (DP 13–24). The length of amylopectin chains acceptable for the formation of double helices is in the range of 10–25 glucosyl residues, preferentially 12–18 (Genkina *et al*. 2007). For A-type starch granules, higher proportion of intermediate chains would result in the formation of more double helices, causing a higher IR ratio of 1 047 cm^{-1}/1 022 cm^{-1} compared with B-type starch granules. However, inconsistent results were observed for starches from CA 12092 and Lunxuan 987. These inconsistencies observed between molecular order and chain length distribution of starch granules suggested that the molecular order of starch granules is not determined solely by starch chains that may form double helices. B-type starch granules presented higher relative crystallinity compared with A-type starch granules, in agreement with reports from Ao and Jane (2007) and Sahal and Jackson (1996). Generally, the higher IR ratio of 1 047 cm^{-1}/1 022 cm^{-1}, the higher relative crystallinity starch granules have. In this study, A-type starch granules with higher IR ratio of 1 047 cm^{-1}/1 022 cm^{-1} yet presented lower relative crystallinity. The different result might be due to the impact of amylose molecules on the conformation of amylopectin double helices. Kozlov *et al*. (2007) found that an increase in amylose content is accompanied by accumulation of amylose tie-chains in amylopectin clusters forming defects in crystalline lamellae. Disordered ends of amylopectin double helices not participating in the formation of crystals are also proposed to be contributing factors for defects of the crystalline regions and for greater disorder in the packing of the lamellar structure (Koroteeva *et al*. 2007a, b). A-type starch granules have higher amylose content, which would result in the formation of more defects of the crystalline regions, thus leading to lower relative crystallinity. Our results also corroborated the fact that starch crystallites are formed by the long-range regular array of double helices, and that double helices content are higher than relative crystallinity.

4.3. Thermal and pasting properties

DSC measures the heat required for the melting of double helices or starch crystallites following granule swelling during gelatinization (Wang and Copeland 2012; Wang and Copeland 2013). DSC gelatinization parameters are influenced by the molecular structure of amylopectin, amylose/amylopectin ratio, crystalline/amorphous ratio, or a combination thereof (Noda *et al*. 1998). The enthalpy change primarily reflects the loss of molecular order (double helices) rather than the melting of starch crystallites (Cooke and Gidley 1992). A-type starch granules presented higher onset temperature and enthalpy change than did B-type starch granules. The higher enthalpy change of A-type starch granules could be attributed to the higher amount of ordered short-range molecular structure. Our results were in agreement with Ao and Jane (2007). However, Liu *et al*. (2007) reported that A-type starch granules had lower gelatinization temperature, and Ghiasi *et al*. (1982) found that A- and B-type starch granules had similar gelatinization temperature regimes. Thus, further investigation is needed to confirm the association between starch granules and gelatinization property.

A-type starch granules had greater peak, trough and final viscosities, and lower breakdown viscosity and pasting temperature than B-type starch granules, in agreement with reports by Sahlstrom *et al*. (2003), Ao and Jane (2007) and Kim and Huber (2010b). Pasting properties of starch are affected by starch granule size, amylose and lipid content, and amylopectin structure. Amylopectin is primarily responsible for granule swelling, whereas amylose and lipid restrict the swelling (Tester and Morrison 1990). The B-type starch granules had more lipids than the A-type starch granules, and lipids can form helical complexes with amylose, which restricted granule swelling (Ao and Jane 2007). Thus, the B-type starch granules developed lower peak viscosity at a higher pasting temperature.

5. Conclusion

A wide variation in volume percentages of A- and B-type starch granules was observed in Chinese wheat cultivars. Volume percentage of A- and B-type granules had ranges of 68.4–88.9% and 9.7–27.9% in 2010–2011, 74.1–90.1% and 7.2–25.3% in 2011–2012. Wheatear, Zhou 9811-1, Ruzhou 0319 and Chuanmai 42-white with higher volume percentage of A-type granules and lower volume percentage of B-type granules, and 07CA266 and Bainong 64 with higher volume percentage of B-type granules and lower volume percentage of A-type granules could serve as parents in breeding program for selecting high and low amylose wheat cultivars, respectively. The unfractionated, A- and B-type starch granules in four leading cultivars presented significant differences in granule morphology, IR ratio of 1 047 cm^{-1}/1 022 cm^{-1}, amylopectin chain length distribution, relative crystallinity, gelatinization, and pasting properties. Some differences were also observed for amylopectin chain length distribution of A- and B-type granules separated from starches of four tested cultivars. The A-type granules starch had a higher amount of ordered short-range structure, but a lower relative crystallinity as compared with the B-type starch granules. The A-type granules starch displayed higher gelatinization onset (T_o) temperatures and enthalpies (ΔH), and lower gelatinization conclusion temperatures (T_c) than the B-type starch granules. The A-type starch granules compared to the B-type granules starch had greater peak, though, and final viscosity, and lower breakdown viscosity and pasting temperature.

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References

Ao Z, Jane J L. 2007. Characterization and modeling of the A- and B-granule starches of wheat, triticale, and barley. *Carbohydrate Polymers*, **67**, 46–55.

Bechtel D B, Zayas I, Kaleikau L, Pomeranz Y. 1990. Size-distribution of wheat starch granules during endosperm development. *Cereal Chemistry*, **67**, 59–63.

Beckles D M, Thitisaksakul M. 2014. How environmental stress affects starch composition and functionality in cereal endosperm. *Starch/Stärke*, **66**, 58–71.

Bertolini A C, Souza E, Nelson J E, Huber K C. 2003. Composition and reactivity of A- and B-type starch granules of normal, partial waxy, and waxy wheat. *Cereal Chemistry*, **80**, 544–549.

Capron I, Robert P, Colonna P, Brogly M, Planchot V. 2007. Starch in rubbery and glassy states by FTIR spectroscopy. *Carbohydrate Polymers*, **68**, 249–259.

Chiotelli E, Le Meste M. 2002. Effect of B- and A-wheat starch granules on thermomechanical behavior of starch. *Cereal Chemistry*, **79**, 286–293.

Cooke D, Gidley M J. 1992. Loss of crystalline and molecular order during starch gelatinization: Origin of the enthalpic transition. *Carbohydrate Research*, **227**, 103–112.

Dai Z M, Yin Y P, Wang Z L. 2009. Starch granule size distribution from seven wheat cultivars under different water regimes. *Cereal Chemistry*, **86**, 82–87.

Dengate H, Meredith P. 1984. Variation in size distribution of starch granules from wheat grain. *Journal of Cereal Science*, **2**, 83–90.

Eliasson A C, Karlsson R. 1983. Gelatinization properties of different size classes of wheat starch granules measured with differential scanning calorimetry. *Starch/Stärke*, **35**, 130–133.

Fortuna T, Januszewska R, Juszczak L, Kielski A, Palasinski M. 2000. The influence of starch pore characteristics on pasting behaviour. *International Journal of Food Science and Technology*, **35**, 285–291.

Franco C M L, Wong K S, Yoo S H, Jane J L. 2002. Structural and functional characteristics of selected soft wheat starches. *Cereal Chemistry*, **79**, 243–248.

Geera B P, Nelson J E, Souza E, and Huber K C. 2006. Composition and properties of A- and B-type starch granules of wild-type, partial waxy, and waxy soft wheat. *Cereal Chemistry*, **83**, 551–557.

Ghiasi K, Hoseney R C, Varriano-Marston E. 1982. Gelatinization of wheat starch III. Comparison by differential scanning calorimetry and light microscopy. *Cereal Chemistry*, **59**, 258–262.

Genkina N K, Wikman J, Bertoft E, Yuryev V P. 2007. Effects of structural imperfection on gelatinization characteristics of amylopectin starches with A- and B-type crystallinity. *Biomacromolecules*, **8**, 2329–2335.

Jane J, Kasemsuwan T, Leas S, Zobel H, Robyt J F. 1994. Anthlolgy of starch granule morphology by scanning electron microscopy. *Starch/Stärke*, **46**, 121–129.

Kim H S. 2009. Wheat starch A- and B-type granule microstructure and reactivity. Ph D thesis, University of Idaho, USA.

Kim H S, Huber K C. 2008. Channels within soft wheat starch A- and B-type granules. *Journal of Cereal Science*, **48**, 159–172.

Kim H S, Huber K C. 2010a. Impact of A/B-type granule ratio on reactivity, swelling, gelatinization, and pasting properties of modified wheat starch. Part I: Hydroxypropylation. *Carbohydrate Polymers*, **80**, 94–104.

Kim H S, Huber K C. 2010b. Physicochemical properties and amylopectin fine structures of A- and B-type granules of waxy and normal soft wheat starch. *Journal of Cereal Science*, **51**, 256–264.

Koroteeva D A, Kiseleva V I, Krivandin A V, Shatalova O V, Blaszczak W, Bertoft E. 2007a. Structural and thermodynamic properties of rice starches with different genetic background. Part 2. Defectiveness of different supramolecular structures in starch granules. *International Journal of Biological Macromolecules*, **41**, 534–547.

Koroteeva D A, Kiseleva V I, Sriroth K, Piyachomkwan K, Bertoft E, Yuryev P V. 2007b. Structural and thermodynamic properties of rice starches with different genetic background. Part 1. Differentiation of amylopectin and amylose defects. *International Journal of Biological Macromolecules*, **41**, 391–403.

Kozlov S S, Krivandin A V, Shatalova O V, Noda T, Bertoft E, Fornal J. 2007. Structure of starches extracted from near-isogenic wheat lines. Part II. Molecular organization of amylopectin clusters. *Journal of Thermal Analysis and Calorimetry*, **87**, 575–584.

Li J H, Vasanthan T, Rossnagel B, Hoover R. 2001. Starch from hull-less barley: I. Granule morphology, composition and amylopectin structure. *Food Chemistry*, **74**, 395–405.

Li W, Yan S, Yin Y, Wang Z. 2010. Starch granules size distribution in wheat grain in relation to shading after anthesis. *Journal of Agricultural Science*, **148**, 183–189.

Li W H, Shan Y L, Xiao X L, Zheng J M. 2013. Effect of nitrogen and sulfur fertilization on accumulation characteristics and physicochemical properties of A- and B-wheat starch. *Journal of Agricultural and Food Chemistry*, **61**, 2418–2425.

Lindeboom N, Chang P R, Tyler R T. 2004. Analytical, biochemical and physicochemical aspects of starch granule size with emphasis on B- granule starches: A review. *Starch/Stärke*, **56**, 89–99.

Liu P, Guo W, Jiang Z, Pu H. 2011. Effects of high temperature after anthesis on starch granules in grains of wheat (*Triticum aestivum* L.). *Journal of Agricultural Science*, **149**, 159–169.

Liu Q, Gu Z, Donner E, Tetlow I, Emes M. 2007. Investigation of digestibility *in vitro* and physicochemical properties of A- and B-type starch from soft and hard wheat flour. *Cereal Chemistry*, **84**, 15–21.

Ni Y, Wang Z, Yin Y, Li W. 2012. Starch granules size distribution in wheat grain in relation to phosphorus fertilization. *Journal of Agricultural Science*, **150**, 45–52.

Noda T, Takahata Y, Sato T, Suda I, Morishita T, Ishiguro K, Yamakawa O. 1998. Relationships between chain length distribution of amylopectin and gelatinization properties within the same botanical origin for sweet potato and buckwheat. *Carbohydrate Polymers*, **37**, 153–158.

Park S H, Chung O K, Seib P A. 2005. Effects of varying weight ratios of A- and B- wheat starch granules on experimental straight-dough bread. *Cereal Chemistry*, **82**, 166–172.

Park S H, Wilson J D, Chung O K, Seib P A. 2004. Size distribution and properties of wheat starch granules in relation to crumb grain score of pup-loaf bread. *Cereal Chemistry*, **81**, 699–704.

Park S H, Wilson J D, Seabourn B W. 2009. Starch granule size distribution of hard red winter and hard red spring wheat: Its effects on mixing and breadmaking quality. *Journal of Cereal Science*, **49**, 98–105.

Peng M, Gao M, Abdel-Aal E S M, Hucl P, Chibbar R N. 1999. Separation and characterization of A- and B-type starch granules in wheat endosperm. *Cereal Chemistry*, **76**, 375–379.

Peng M, Gao M, Baga M, Hucl P, Chibbar R N. 2000. Starch-branching enzymes preferentially associated with A-type starch granules in wheat endosperm. *Plant Physiology*, **124**, 265–272.

Raeker M O, Gaines C S, Finney P L, Donelson T. 1998. Granule size distributions and chemical composition of starches from 12 soft wheat cultivars. *Cereal Chemistry*, **75**, 721–728.

Rahman S, Bird A, Regina A, Li Z Y, Ral J P, McMaugh S, Topping D, Morell M. 2007. Resistant starch in cereal: Exploiting genetic engineering and genetic variation. *Journal of Cereal Science*, **46**, 251–260.

Sahal D, Jackson D S. 1996. Structural and chemical properties of native corn starch granules. *Starch/Stärke*, **48**, 249–255.

Sahlström S, Bævre A B, Bråthen E. 2003. Impact of starch properties on health bread characteristics. II. Purified A- and B-granule fractions. *Journal of Cereal Science*, **37**, 285–293.

Salman H, Blazek J, Lopez-Rubio A, Gilbert E P, Hanley T, Copeland L. 2009. Structure-function relationships in A and B granules from wheat starches of similar amylose content. *Carbohydrate Polymers*, **75**, 420–427.

Shinde S V, Nelson J E, Huber K C. 2003. Soft wheat starch pasting behavior in relation to A- and B-type granule content and composition. *Cereal Chemistry*, **80**, 91–98.

Van Soest J J G, Tournois H, De Wit D, Vliegenthart J F G. 1995. Shot-range structure in (partially) crystalline potato starch determined with attenuated total reflectance Fourier-transform IR spectroscopy. *Carbohydrate Research*, **279**, 201–214.

Soh H N, Sissons M J, Turner M A. 2006. Effect of starch granule size distribution and elevated amylose content on durum dough rheology and spaghetti cooking quality. *Cereal Chemistry*, **83**, 513–519.

Song Y, Jane J. 2000. Characterization of barley starches of waxy, normal and high amyloase varieties. *Carbohydrate Polymers*, **41**, 365–377.

Soulaka A B, Morrison W R. 1985. The amylose and lipid contents, dimensions, and gelatinization characteristics of some wheat starches and their A- and B-granule fractions. *Journal of the Science of Food and Agriculture*, **36**, 709–718.

Stoddard F L.1999. Survey of starch particle-size distribution in wheat and related species. *Cereal Chemistry*, **76**, 145–149.

Stoddard F L. 2000. Genetics of wheat starch B-granule content. *Euphytica*, **112**, 23–31.

Stoddard F L. 2003. Genetics of starch granule size distribution in tetraploid and hexaploid wheats. *Australian Journal of Agricultural Research*, **54**, 637–648.

Tester R, Morrison W R. 1990. Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose and lipids. *Cereal Chemistry*, **67**, 551–557.

Van H P, Morita N. 2005. Physicochemical properties of hydroxypropylated and cross-linked starches from A-type and B-type wheat starch granules. *Carbohydrate Polymers*, **59**, 239–264.

Vermeylen R, Goderis B, Reynaers H, Delcour J A. 2005. Gelatinization related structural aspects of B- and A- wheat starch granules. *Carbohydrate Polymers*, **62**, 170–181.

Wang S, Copeland L. 2012. Effect of alkali treatment on structure and function of pea starch gramles. *Food Chemistry*, **135**, 1635–1642.

Wang S, Copeland L. 2013. Molecular disassembly of starch granules during gelatinization and its effect on starch digestibility: A review. *Food & Function*, **4**, 1564–1580.

Wang S, Yu J, Zhu Q, Yu J, Jin F. 2009. Granular structure and allomorph position in C-type Chinese yam starch granule revealed by SEM, 13C CP/MAS NMR and XRD. *Food Hydrocolloids*, **23**, 426–433.

Wang S J, Luo H, Zhang J, Zhang Y, He Z H, Wang S. 2014. Alkali-induced changes in functional properties and *in vitro* digestibility of wheat starch: The role of surface proteins and lipids. *Journal of Agricultural and Food Chemistry*, **62**, 3636–3643.

Yin Y A, Qi J C, Li W H, Cao L P, Wang Z B. 2012. Formation and developmental characteristics of A- and B-type starch granules in wheat endosperm. *Journal of Integrative Agriculture*, **11**, 73–81.

Yoo S H, Jane J. 2002. Structural and physical characteristics of waxy and other wheat starches. *Carbohydrate Polymers*, **49**, 297–305.

Zhang T, Wang Z, Yin Y, Cai R. 2010. Starch content and granule size distribution in grains of wheat in relation to post-anthesis water deficits. *Journal of Agronomy and Crop Science*, **196**, 1–8.

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