Carbohydrate Polymers 75 (2009) 420–427

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Structure–function relationships in A and B granules from wheat starches of similar amylose content

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ARTICLE INFO

Article history:
Received 30 March 2008
Received in revised form 31 July 2008
Accepted 1 August 2008
Available online 12 August 2008

Keywords:
Wheat starch
A granules
B granules
SAXS
XRD
Swelling power
Starch functionality
In vitro digestibility of starch

ABSTRACT

Five wheat (*Triticum aestivum* L.) starches, from the varieties Sunco, Sunsoft, SM1118, and SM1028, with similar amylose content, and a waxy wheat were separated into large (A) and small (B) granules. The unfractionated starches, and isolated A and B granules, were characterized structurally and evaluated for their functional properties. The amylopectin chain length distribution revealed that A granules had a lower proportion of short chains with degree of polymerization (DP) 6-12 and a higher proportion of chains with DP 25-36 than B granules. X-ray diffraction (XRD) patterns showed predominantly A-type crystallinity for all of the starches. No differences in the crystallinity were found between unfractionated, A and B granules. Small-angle X-ray scattering (SAXS) patterns of the starches at 55% hydration showed that the lamellar repeat distance in A granules was larger than that of B granules for all the starches examined. However, the lamellar distances of both A and B granules from the waxy wheat were smaller than those of Sunco, Sunsoft, SM1118 and SM1028 starches. The swelling power of the B granules was greater than that of A granules from all five starches. The kinetics of digestion of A and B granules with α-amylase in vitro were complex, with B granules initially digested to a greater extent than A granules. After 4 h of incubation, A granules showed greater digestibility than B granules, except in the case of waxy starch where unfractionated and fractionated granules had similar in vitro digestibility. Correlations between structural and functional parameters were more significant for the isolated A and B granules than for the unfractionated starches. This study demonstrates that A and B granules differ in structure and functionality, and that some correlations between these properties could be masked in unfractionated starches with bimodal granule size distribution.

1. Introduction

Starches from wheat (*Triticum aestivum* L.), barley, rye and triticale have a bimodal granule size distribution (Peng, Gao, Abdel-Aal, Hucl, & Chibbar, 1999; Soulaka & Morrison, 1985; Stoddard, 1999). Specifically for wheat, there is one population of small spherical granules ranging in size from approximately 1–10 μm, which are referred to as B granules, and another population of larger lenticular-shaped granules ranging from about 15 to 40 μm, known as A granules. The A and B granules are considered to differ according to the time of biosynthesis during grain filling. Synthesis of A granules begins four days after anthesis, with granule growth and development continuing over the next 20 days. Initiation of B granule synthesis occurs 10 days after anthesis, with significant granule growth beginning 20 days after anthesis (Bechtel, Zayas, Kaleikau, & Pomeranz, 1990; Parker, 1985; Shinde, Nelson, & Huber, 2003). The temporal variation in the biosynthesis is considered to affect the size of the granules, and result in differences in the molecular organisation of the amylose and amylopectin fractions (Tester, 1997).

The size, shape and size distribution of granules are important distinguishing morphological features of starch from different botanical sources. Granule size has been reported to influence the functional and baking properties (Chiotelli & Le Meste, 2002; Liu, Gu, Donner, Tetlow, & Emes, 2007; Park, Wilson, Chung, & Seib, 2004; Sahlstrom, Baevre, & Brathen, 2003) and the pasting behaviour of starch (Ao & Jane, 2007; Shinde et al., 2003). However, variation in functional properties of starch is also likely to be due to the internal structure of the granules.

Granule size is related to the molecular architecture of amylopectin and its molecular arrangement within the granule (Geera, Nelson, Souza, & Huber, 2006; Jane, 2006; Raeker, Gaines, Finney, & Donelson, 1998). Amylopectin with its multiple branched chains of (1–4)-α-glucans interlinked by (1–6)-α-linkages is usually the major component of starch, with the essentially unbranched amylose making up the minor fraction. The current accepted model for the molecular structure of the starch granule consists of repeating

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doi:10.1016/j.carbpol.2008.08.001

amorphous and semicrystalline layers termed growth rings (Jenkins & Donald, 1995; Manners, 1989). The amorphous growth rings contain amylopectin and amylose macromolecules in a relatively disordered conformation, whereas the semicrystalline growth rings consist of amylopectin clusters that contain alternating crystalline and amorphous regions of approximately 9–11 nm thickness in a lamellar arrangement (Kozlov, Noda, Bertoft, & Yuryev, 2006; Qi et al., 2004). External chains of amylopectin with more than 10 glucose units are predominantly found in the crystalline region of these clusters and are organized into a double helical form that crystallizes into either A or B polymorphs, or a mixture of both which is sometimes referred to as a C-polymorph (Buleon, Colonna, Planchot, & Ball, 1998; Vermeylen, Goderis, Reynaers, & Delcour, 2005). The longer amylopectin chains are considered to pass from the crystalline region into the amorphous region of the lamellae (Qi et al., 2004). Amylose is considered to be present in both the amorphous and crystalline regions (Jane, 2006; Kozlov et al., 2006). Shorter branch chains of amylopectin may disrupt the order of the granule structure, resulting in lobed or compound starch granules and small granules. The ratio of long branch chains to short branch chains affects the shape of the amylopectin molecules, which affects their packing and, in turn, the morphology and size of the starch granule (Jane, 2007).

Several studies have reported structural and functional differences between A and B granules (Ao & Jane, 2007; Liu et al., 2007; Peng et al., 1999; Shinde et al., 2003; Tang, Ando, Watanabe, Takeda, & Mitsunaga, 2001; Vermeylen et al., 2005). In this study, structural and functional properties of unfractionated starches and separated A and B granules of four wheat starches of similar amylose content but from varied genetic backgrounds are compared. A waxy wheat was also studied to contrast with the other four starches. The structure and functionality of starches with wider range of amylose content has been described in Blazek et al. (submitted for publication).

2. Materials and methods

Five wheat (*Triticum aestivum L.)* varieties were used in this study. Two of the varieties, Sunco (a prime hard wheat) and Sunsoft (a soft wheat) are commercial varieties obtained from Australian Grain Technologies, Narrabri, Australia. Varieties SM1118 and SM1028 were obtained from the Value Added Wheat Cooperative Research Centre Ltd. breeding program. The waxy wheat was provided by George Weston Technologies, Australia. All of the varieties were provided as grains.

Starch granules were isolated from 500 g of grain from each variety according to the method of Matheson and Welsh (1988) as follows. Grains were soaked for 24 h in 0.2 M ammonium hydroxide solution (2.5 L) and the softened grains blended for 30 s with fresh 0.2 M ammonium hydroxide solution. The slurry was poured through two layers of muslin and extraction of the residue was repeated. The combined filtrates were allowed to settle overnight and the supernatant decanted. The sedimented starch was washed by successively resuspending and decanting in distilled water, 0.2 M acetic acid, distilled water and ethanol, and the washed preparation dried in vacuum. Isolated starch (5 g) was fractionated into A and B granules using Percoll density gradient centrifugation, according to the method of Peng et al. (1999). For SM1028 and waxy varieties, isolated B granules were suspended in distilled water and filtered through size 11 μm mesh to further increase the enrichment.

Starch granules were examined using a Philips XL30 scanning electron microscope. The granules were mounted on an aluminium stub using double-sided adhesive tape. The stubs were coated with gold and the starch was viewed at an accelerating voltage of 10 kV.

Particle size distribution of wet suspensions of the unfractionated starches and A and B granules was measured by laser scattering on triplicate samples using a Malvern Mastersizer S (Malvern Instruments Ltd., UK) according to the instructions supplied with the instrument.

Starch damage was estimated using the Megazyme Starch Damage Assay Kit (Megazyme International Ireland Ltd.). Amylose content was estimated in two ways, using the Megazyme Amylose Amylopectin (AM/AP) Assay Kit and the iodine binding method according to Chrastil (1987).

Starch swelling power (SSP) was determined by measuring the weight of water taken up by 40 mg of starch at 92.5 °C during a defined gelatinisation process according to the method of Konik-Rose et al. (2001).

The digestibility of the starch samples was estimated by incubating 20 mg of isolated starch granules in a shaking water bath at 37 °C in 5 ml sodium acetate buffer (100 mmol, pH 4.5) containing a mixture of porcine pancreatic α-amylase (Sigma; 14 U) and amyloglucosidase (Megazyme; 0.33 U). An aliquot of 0.1 ml of the incubation mixture was collected at 30 min, 1, 2, 4, 6 and 24 h and heated in a boiling water bath for a few minutes. Glucose content was measured after incubating this aliquot with 3 ml of Megazyme glucose oxidase peroxidase reagent (GOPOD) for 20 min at 50 °C. Absorbance was read at 510 nm and converted to the amount of glucose released by comparison with a standard curve. The extent of hydrolysis was calculated as the amount of glucose released from total amount of glucose equivalents in the starch sample.

Amylopectin chain length distribution was determined in the laboratories of CSIRO Plant Industry, Canberra by fluorophore-assisted carbohydrate electrophoresis, using the Beckman P/ACE System 5010, as described by Morell, Samuel, and O'Shea (1998) and O'Shea et al. (1998).

X-ray diffraction (XRD) patterns of the starch samples were obtained using a Shimadzu S6000 diffractometer operating at 40 V and 30 mA (Cu Kα radiation of 0.154 nm). Starches were equilibrated above saturated potassium chloride at room temperature before analysis. The intensity was measured from 5 to 35 °C as a function of 2θ and at a scanning speed of 0.5°/min and a step size of 0.05°.

Small angle X-ray scattering (SAXS) measurements were performed on a Bruker Nanostar SAXS camera, with pin-hole collimation for point focus geometry. The instrument source is a copper rotating anode (0.1 mm filament) operating at 50 kV and 24 mA, fitted with cross coupled Göbel mirrors, resulting in CuK α radiation wavelength 1.54 Å. The SAXS camera is fitted with a Hi-star 2D detector (effective pixel size 100 μm). The sample to detector distance was chosen to be 700 mm, which provided a q-range from 0.02 to 0.3 $Å^{-1}$, where q is the scattering vector defined as $q = (4\pi\sin\theta)/\lambda$, in which λ is the wavelength and 2θ is the scattering angle. Starch samples, at 55% hydration, were presented in 2 mm glass capillaries. The optics and sample chamber were under vacuum to minimize air scatter. Scattering files were background subtracted, normalized to sample transmission and then averaged radially using macros written in the Igor software package (Wavemetrics, Lake Oswego, Oregon, USA). SAXS curves were plotted as a function of relative intensity, I, versus q.

Each analysis was performed using separate duplicate samples unless stated otherwise. Statistical analysis was performed using GenStat7 software. Pearson's correlation coefficients (r) were calculated between pairs of measured parameters for the unfractionated starch samples and the A and B granules. A statistically significant relationship between the two variables is indicated for a P-value of less than 0.05. An r value $\geqslant 0.950$ for the unfractionated starches ($n = 4$) and $r \geqslant 0.707$ for A and B granules ($n = 8$), was statistically significant at a level of $P < 0.05$. The waxy wheat

unfractionated starch and A and B granules were excluded from statistical analysis so as not to artificially affect the correlations.

3. Results

Granules from the unfractionated wheat starches from all varieties studied showed a high degree of intactness as indicated by scanning electron microscopy (Fig. 1). Assays for starch damage gave low values (0.3–0.5%) for all of the unfractionated starches. From the particle size distribution based on the distribution of the diameters of equivalent spheres, the ratio of A–B granules in the unfractionated starches ranged from 33:67 for Sunsoft to 45:55 for the waxy starch (Table 1). Enriched preparations of A and B granules were obtained by centrifuging starch granules through Percoll density gradients. The preparations of A granules were cross-contaminated with 8–10% of B granules, whereas the B granule preparations contained between 17% and 34% of A granules (Table 1). The mode of the size distribution of B granules of the SM1028 and waxy starches was greater (7.7 and 12.2 μm, respectively) than the mode of 6.6 μm for the other B granule preparations (Table 1).

The amylose content of Sunco, Sunsoft, SM1118 and SM1028 starches was between 23.7% and 27.9% and was not significantly different between these starches as measured using the Megazyme assay kit and the iodine binding method. Waxy wheat had amylose content of 4.8% measured by the Megazyme method and 1.2% according to the iodine binding method. No significant differences in amylose content were found between the respective pairs of A and B granule populations of any of the starches using either the iodine binding or the Megazyme method (Table 2).

The amylopectin chain length distribution of the starches was classified into four categories: short (DP 6-12), medium (DP 13-24), long (DP 25-36) and very long chains (DP > 36), as shown in Table 2. The overall pattern of chain length distributions was similar amongst the starches, with SM1028 having the highest percentage of the short chains (42.6%) and the lowest percentage of long and very long chains (8.2% and 1.6%, respectively). Sunsoft had the lowest proportion of short chains (39.2%) and the highest of very long chains (4.5%), as shown in Table 2.

Except for the waxy starch, the proportion of chains with DP 6-12 was consistently lower in A granules than B granules, whereas medium and long chains were more abundant in A than B granules in all the starch samples (Table 2). There was no obvious pattern in the distribution of very long chains between A and B granules. Waxy starch A granules had a lower percentage of short chains, similar medium chains and more long chains compared to B granules (Table 2).

The water uptake of Sunco, Sunsoft, SM1118 and SM1028 starches as measured by the swelling power test ranged between 7.6 and 8.4, whereas the swelling power of unfractionated waxy starch was 15.3 (Table 2). The swelling power of B granules was greater than that of the corresponding A granules in all of the varieties studied.

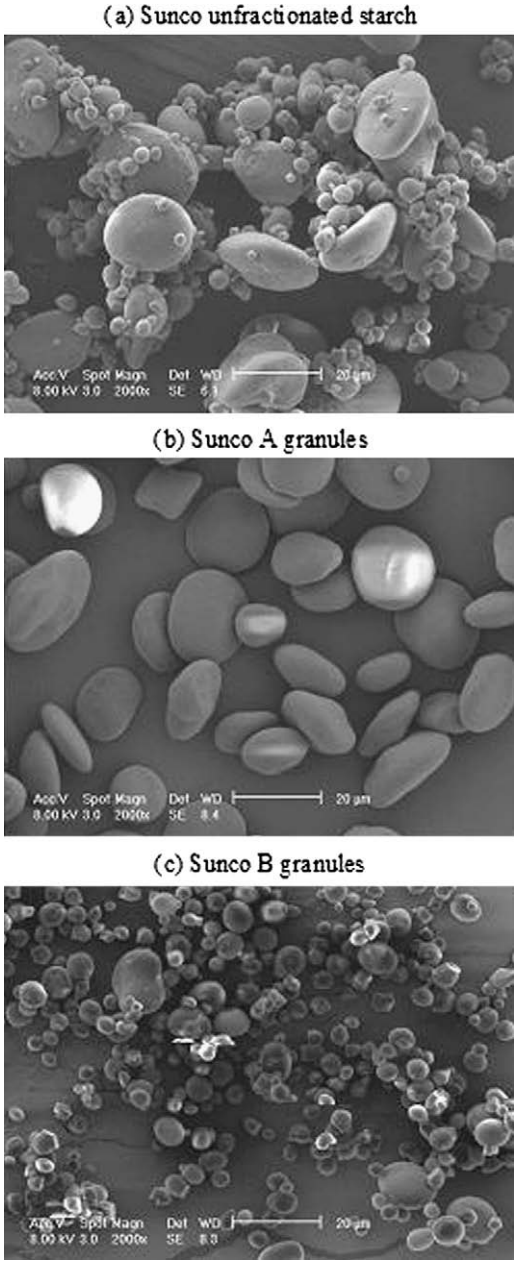

Fig. 1. Scanning electron microscope images of unfractionated Sunco starch (a) and isolated A (b) and B granules (c).

3.1. Digestibility of the starches

The extent of starch hydrolysis at various times of digestion when the different samples were incubated with a mixture of α-amylase and amyloglucosidase are shown in Table 2. The B granules of Sunco, Sunsoft, SM1118 and SM1028 were digested to a greater extent than the A granules initially, but after 4, 6 and 24 h the A granules were digested more extensively than the corresponding B granules (Table 2). Waxy starch was more susceptible to hydrolysis than the other varieties studied and, except for the greater initial digestibility of B granules, no significant differences were observed between the extent of hydrolysis, initially and after 24 h, of the isolated waxy A and B granules and the unfractionated starch.

As discussed subsequently, few significant correlations were found between structural and functional parameters for the unfractionated starches, in contrast to the separated A and B preparations. Hence, the swelling and digestibility characteristics of unfractionated granules may not necessarily be intermediate between those of A and B granules.

3.2. Crystallinity and nanostructural characteristics

The unfractionated starches and A and B granules all gave XRD patterns at 2θ that were consistent with A-type crystal packing, with the first peak around 15°, the second peak near 17°, and the third main reflection around 23°. In general, the XRD intensities of the unfractionated starches, and the corresponding A granules

Table 1
Particle size distribution and efficiency of separation for A and B starch granules

Wheat	Unfractionated starch		A granules			B granules		
Variety	A (%)	B (%)	A (%)	B (%)	Mode (μm)	A (%)	B (%)	Mode (μm)
Sunco	36.7	63.3	89.8	10.2	22.5	17.4	82.8	6.6
Sunsoft	32.5	67.5	89.6	10.4	22.5	19.6	80.4	6.6
SM1118	38.5	61.5	90.3	9.7	22.5	18.8	81.2	6.6
SM1028	34.6	65.4	92.0	8.0	26.2	30.3	69.7	7.7
Waxy	45.3	54.7	92.0	8.0	22.5	33.5	66.5	12.2

The distributions are on a number basis and are expressed in terms of the diameters of equivalent spheres. The mode is the diameter in μm that occurred most often. The values are the average of triplicate measurements.

Table 2
Amylose content, amylopectin chain length distribution, SAXS characteristics, swelling power and starch hydrolysis after 4 and 24 h of incubation with α-amylase and amyloglucosidase of wheat starches and their A and B granules

Starch	Amylose content		Amylopectin chain length distribution				SAXS		Swelling	% of starch hydrolysed					
	IB	Megz	DP6-12	DP 13–24	DP 25–36	DP > 36	q_{max}	I_{max}	SSP	0.5 h	1 h	2 h	4 h	6 h	24 h
Sunco	24.8±2.3	24.8±0.7	40.5	48.5	8.4	2.6	0.061	103.5	7.6 ± 1.0	1.1 ± 0.7	2.8 ± 0.7	6.4 ± 2.0	11.0 ± 1.1	16.5 ± 1.2	48.2 ± 0.6
Sunco-A	25.5 ± 0.3	29.6 ± 3.3	39.9	48.3	8.8	3	0.06	125.9	8.2 ± 0.2	1.2 ± 0.3	3.1 ± 0.9	7.0 ± 0.8	14.2 ± 2.6	20.5 ± 2.8	59.3 ± 0.5
Sunco-B	24.7 ± 2.4	28.0 ± 2.3	43.3	46	7.9	2.8	0.065	158.4	10.0 ± 0.9	2.3 ± 0.8	4.2 ± 0.6	7.7 ± 0.8	12.6 ± 1.7	16.9 ± 1.7	42.8 ± 4.2
Sunsoft	23.7 ± 3.1	25.2 ± 1.9	39.2	47.7	8.6	4.5	0.062	142.5	7.9 ± 1.4	1.6 ± 0.5	3.7 ± 0.4	8.0 ± 0.5	15.7 ± 1.4	22.5 ± 0.4	59.1 ± 0.6
Sunsoft-A	21.6 ± 2.5	27.0 ± 3.8	40.4	49.4	8.5	1.7	0.055	120.4	8.9 ± 0.5	1.3 ± 0.5	3.5 ± 0.8	7.7 ± 0.5	15.3 ± 1.4	22.2 ± 1.3	63.2 ± 3.5
Sunsoft-B	21.0 ± 3.4	26.5 ± 1.3	43.1	46.6	7.5	2.8	0.062	154.1	9.9 ± 0.5	2.4 ± 0.4	4.7 ± 0.2	8.1 ± 0.2	13.3 ± 1.3	17.6 ± 0.9	48.1 ± 2.5
SM1118	27.1 ± 0.6	26.5 ± 0.4	41.3	48.3	8.6	1.8	0.061	129.4	8.3 ± 0.1	2.2 ± 0.4	4.7 ± 0.4	9.0 ± 0.5	15.8 ± 0.3	21.6 ± 0.5	60.0 ± 1.9
SM1118-A	24.0 ± 2.0	29.4 ± 3.8	40.6	47.9	9.2	2.3	0.059	141.4	7.4 ± 0.1	1.5 ± 0.6	3.80.2	7.8 ± 0.7	13.6 ± 1.7	18.9 ± 0.6	46.9 ± 4.8
SM1118-B	24.6 ± 3.4	30.2 ± 0.9	42.7	47.1	8.5	1.8	0.062	125	8.5 ± 0.4	2.4 ± 0.6	4.6 ± 0.1	7.5 ± 0.2	12.3 ± 1.5	15.6 ± 1.7	37.3 ± 11.6
SM1028	26.0 ± 0.8	27.9 ± 1.4	42.6	47.6	8.2	1.6	0.061	141.1	8.4 ± 0.1	2.2 ± 0.3	4.8 ± 0.1	9.3 ± 1.1	18.3 ± 2.1	27.0 ± 3.4	60.6 ± 4.1
SM1028-A	25.5 ± 1.3	30.0 ± 1.9	41.3	48.6	8.6	1.5	0.059	125.5	9.0 ± 0.4	1.8 ± 0.4	4.7 ± 2.3	9.5 ± 3.2	17.4 ± 1.4	26.1 ± 0.9	64.8 ± 13.2
SM1028-B	22.5 ± 4.8	30.1 ± 0.8	43.3	47.2	7.8	1.6	0.061	140.2	10.5 ± 0.6	2.4 ± 0.1	4.5 ± 0.1	8.03 ± 0.8	13.5 ± 1.3	18.9 ± 1.3	46.6 ± 6.6
Waxy	1.2 ± 0.4	4.8 ± 0.4	40.2	49.3	8.7	1.9	0.058	339	15.3 ± 1.6	2.6 ± 0.91	6.1 ± 0.9	11.4 ± 1.8	21.9 ± 1.9	29.0 ± 2.9	66.3 ± 4.2
Waxy-A	1.2 ± 0.6	6.7 ± 0.8	38	50.2	9.8	2	0.058	310.2	13.5 ± 5.3	2.6 ± 0.23	5.7 ± 0.7	11.5 ± 1.7	22.2 ± 3.2	32.5 ± 3.5	71.8 ± 2.5
Waxy-B	0.8 ± 0.6	4.2 ± 0.5	39.3	50.3	8.6	1.9	0.06	319.3	20.8 ± 3. 7	3.9 ± 0.87	7.4 ± 0.2	13.3 ± 0.8	22.6 ± 3.4	32.0 ± 0.9	72.4 ± 2.2

and B granules were almost identical for each variety, as indicated by the representative XRD patterns shown in Fig. 2. The waxy starch gave sharper XRD patterns than the other starches, consistent with its higher level of crystallinity (Fig. 2).

SAXS patterns at 55% hydration showed well-defined peaks at a value of q of about 0.06 $Å^{-1}$ (Table 2). The SAXS peak intensity of waxy wheat was higher than the intensities observed for Sunco, Sunsoft, SM1118, and SM1028, which were all similar. Fig. 3 shows the difference in SAXS patterns between the unfractionated starch and A and B granules for Sunco and waxy starches.

The parameters of the SAXS peak for the A and B granules were obtained by fitting the scattering profile to a Lorentzian function, which describes the SAXS peak, plus a Power Law function, accounting for the small angle scattering where A is a prefactor and δ is the Power Law exponent:

$$I(q) = I_{max}[1 + (2(q - q_{max})/\Delta q)^2]^{-1} + Aq^{-\delta}$$

SAXS peaks were characterized by the following set of parameters: intensity at peak maximum I_{max}, position of the peak q_{max} and width of the peak Δq, which are positive and adjustable parameters (Yuryev et al., 2004). The Bragg spacing d, representing the average interlamellar distance, was calculated according to the Bragg equation ($d = 2\pi/q_{max}$). For the unfractionated starches, the SAXS patterns were fitted with two Lorentzian peaks, accounting for the mixed population of A and B granules, together with a third peak accounting for the second order reflection present in the curves (Fig. 4), plus the power-law function describing the underlying small angle scattering. An average peak position (q_{max}) for the lamellar repeat was calculated taking into account the ratio of the area of the first two peaks obtained from the fitting of the curves. An example of the curve fitting analysis is shown in Fig. 4. The calculated peak position of the starches showed a range between 0.058 $Å^{-1}$ for waxy and 0.062 $Å^{-1}$ for Sunsoft starch. Sunco, SM1118, and SM1028 gave a similar q_{max} value of 0.061 $Å^{-1}$. The corresponding lamellar repeat distances (d) were, therefore, in the range of 10 (Sunsoft) to 11 nm (waxy starch) with Sunco, SM1118 and SM1028 having intermediate values. The intensity at peak maximum (I_{max}) ranged between 104 relative units (RU) for Sunco and 339 RU for waxy starch.

The SAXS profiles for the fractionated granule preparations showed that the peak position was shifted to lower q values for A granules compared to B granules for all the varieties studied (Table 2). The Bragg spacing d, representing the lamellar distance, ranged from 10.2 to 11.0 nm for A granules and from 10.0 to 10.3 for B granules, indicating larger lamellar spacing in A granules compared to B granules in all the starches examined. Lamellar distances of both A and B granules of the waxy starch were lower than the respective values for A and B granules of the other starches. The intensities at peak maximum (I_{max}) of A and B granules of Sunco, Sunsoft, SM1028 and SM1118 starches were in the range between 120.4 and 158.4 RU (Table 2).

The analysis of the correlation between the various parameters determined for the unfractionated starches showed that the only strong correlation was between swelling power and digestibility at 0.5 and 1 h ($r = 0.992$ and 0.996, respectively). No other significant correlations at ($P < 0.05$) were found.

Once the A and B granules were studied separately, an increased number of significant correlations were found between the tested parameters (Table 3). Significant correlations were noted between amylopectin chain length and SAXS parameters. For instance, the proportion of short amylopectin chains correlated positively with peak positions ($r = 0.894$), while medium and long amylopectin

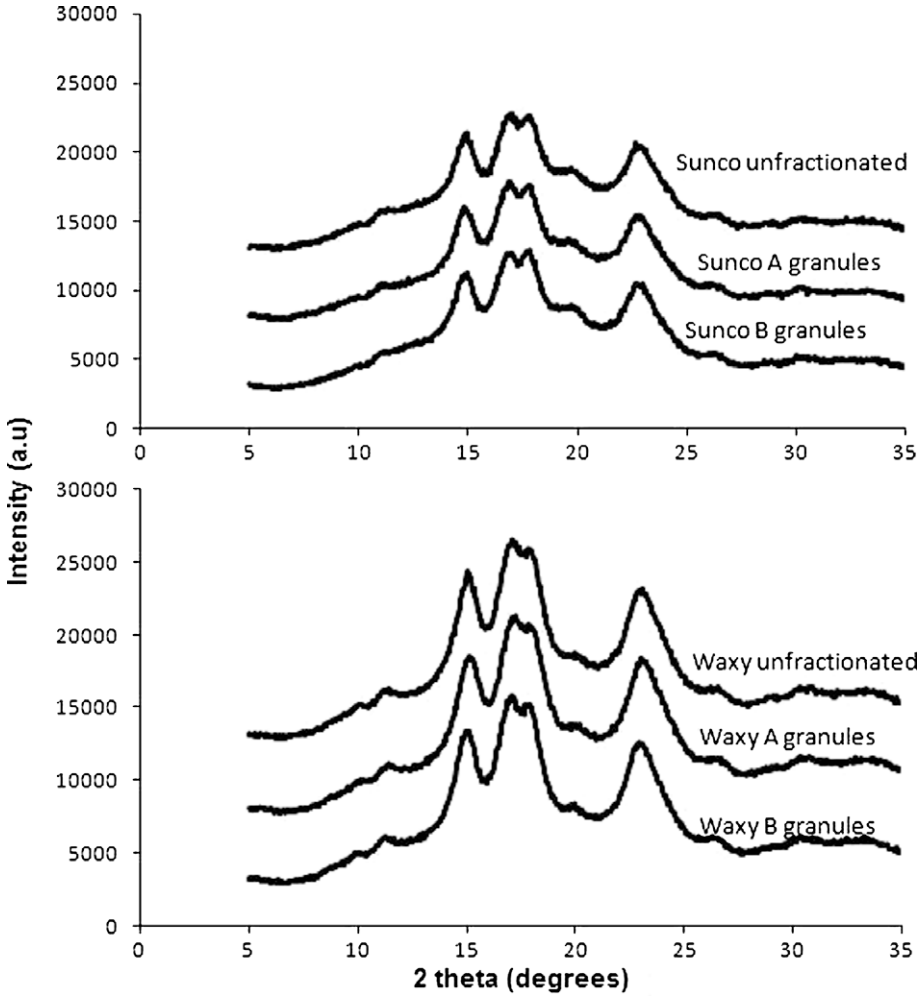

Fig. 2. XRD patterns of unfractionated starch, A and B granules of Sunco and waxy varieties. Data have been offset for clarity.

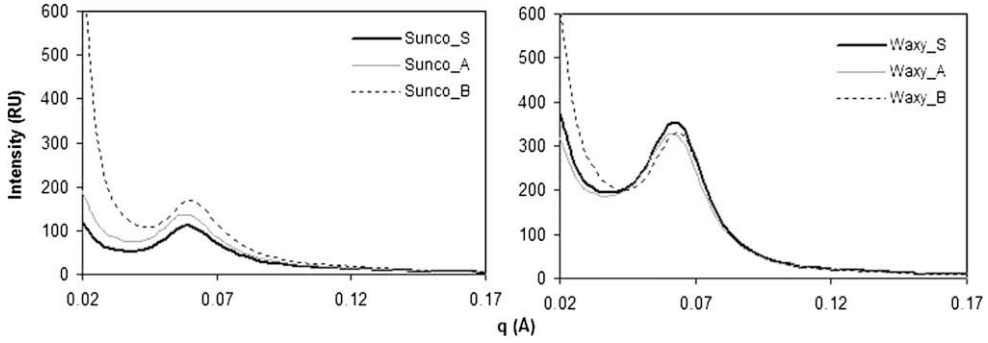

Fig. 3. SAXS patterns of Sunco and waxy unfractionated starch (—), A granules (—) and B granules (- - -).

chain lengths correlated negatively with peak position ($r = -0.823$, -0.799, respectively). The medium amylopectin chain length also correlated negatively with peak intensity ($r = -0.781$). Significant correlations were also recorded between functionality and structural parameters. Swelling power correlated positively with short amylopectin chains ($r = 0.778$) and negatively with long amylopectin chains ($r = -0.935$). Extent of digestion correlated positively with short amylopectin chains ($r = 0.982$), negatively with medium and long amylopectin chains ($r = -0.822$, -0.754, respectively) after 0.5 h, positively with short amylopectin chains ($r = 0.787$) after 1 h and positively with medium length amylopectin chains ($r = 0.751$, 0.772, and 0.824) after 4, 6 and 24 h, respectively. Digestibility after 0.5 h also correlated positively with SAXS peak position ($r = 0.909$).

4. Discussion

Chemical, structural and functional properties of the unfractionated starches and the corresponding isolated A and B granules from four wheat varieties with similar amylose content and a waxy variety were investigated in this study. Differences were noted in

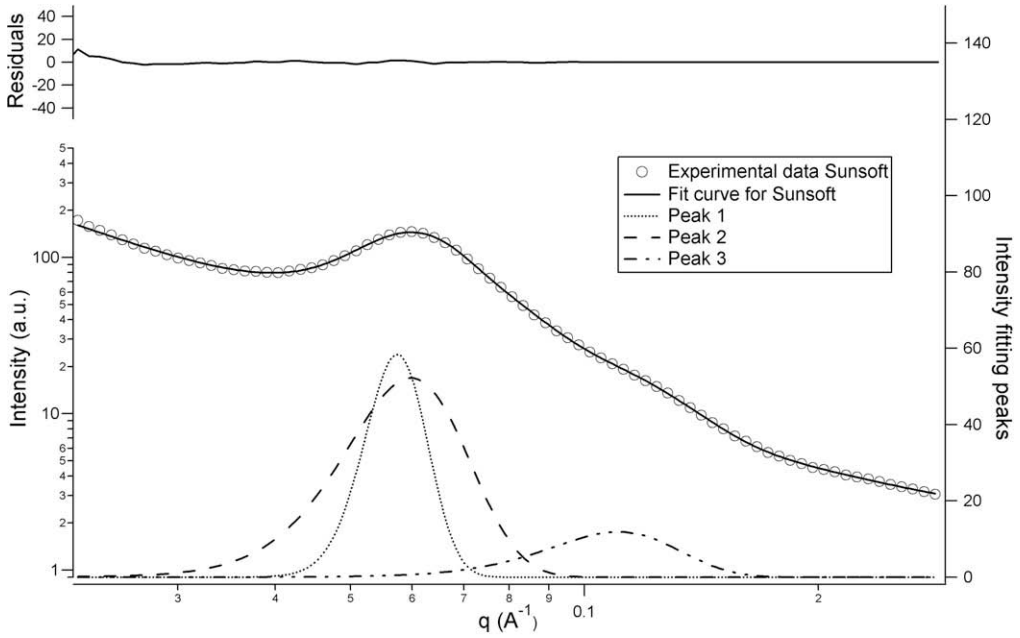

Fig. 4. SAXS patterns of Sunsoft starch fitted with the three peak model.

Table 3
Correlation matrix between structural (amylose content, amylopectin chain lengths, q_{max} and I_{max} from SAXS) and functional (swelling power and digestibility) properties of A and B granules of Sunco, Sunsoft, SM1118 and SM1028 starches (Pearson correlation coefficients)

		Amylose content		Amylopectin chain length distribution				SAXS		Swelling
		IB	Megazyme	DP6-12	DP 13–24	DP 25–36	DP > 36	q_{max}	I_{max}	SSP
Amylose content	IB	1								
	Megazyme	0.7	1							
Amylopectin chain length distribution	DP6-12	−0.27	−0.089	1						
	DP 13-24	0.037	0.09	−0.846	1					
	DP25-36	0.551	0.462	−0.825	0.63	1				
	DP>36	0.079	−0.385	−0.027	−0.443	−0.129	1			
SAXS	q max	−0.123	−0.021	**0.894**	**−0.823**	**−0.799**	0.168	1		
	I max	−0.102	−0.339	0.514	**−0.781**	−0.474	0.643	0.386	1	
Swelling	SSP	−0.424	−0.286	**0.778**	−0.503	**−0.935**	−0.069	0.692	0.4	1
% of starch hydrolysed	0.5 h	−0.217	0.006	**0.982**	**−0.822**	**−0.754**	−0.084	**0.909**	0.412	0.685
	1 h	−0.147	0.09	**0.787**	−0.515	−0.557	−0.371	0.681	0.154	0.526
	2 h	0.049	0.102	0.146	0.151	−0.109	−0.531	−0.006	−0.109	0.234
	4 h	0.14	0.06	−0.522	**0.751**	0.298	−0.449	−0.535	−0.505	−0.125
	6 h	0.185	0.107	−0.571	**0.772**	0.341	−0.419	−0.585	−0.483	−0.141
	24 h	0.045	−0.125	−0.696	**0.824**	0.301	−0.187	−0.627	−0.472	−0.163

* Numbers in bold indicate values are significant at $\alpha = 0.05$.
Values greater than 0.707 indicate a significant correlation at $\alpha = 0.05$.

amylopectin chain length distributions, swelling power and susceptibility to enzymic hydrolysis between the A and the B granules of each variety. However unlike in other studies (Peng et al., 1999), no differences were found in amylose content between the respective pairs of A and B granules, which may have been related to the varieties used, or due to limitations of the Megazyme and iodine binding analytical methods.

XRD patterns of all of the tested starches displayed typical A-type crystal patterns. The intensity of the diffraction peaks indicated similarity in the degree of crystallinity for the unfractionated starches except for waxy starch, which had the highest crystallinity. A-type patterns have been previously reported for native wheat starches with low or medium amylose contents (Atwell, 2001). Granule crystallinity is considered to be mainly associated with the amylopectin component (Bayer, Cagiao, & Balta Calleja, 2006).

Using SAXS it was found that the nanostructure of A and B granules vary consistently across varieties. Lower q_{max} values were observed in the A granules compared to B granules indicating thicker lamellae and larger repeat distances. Thicker lamellae in A granules may be related to higher percentages of medium (DP 13–24) and long (DP 25–36) amylopectin chains compared to B granules, consistent with the strong negative correlation between q_{max} and the percentage of amylopectin of these chain lengths. However, there are likely to be additional factors to explain the differences in lamellar thickness. Although the same trends were observed for waxy wheat, there were no significant differences in the percentage of medium length amylopectin chains between the A and B granules for this variety. Furthermore, there was no correlation between I_{max} and the amylose content of the starches analysed in this study, which may be due to the narrow range of amylose content in the starches investigated here.

Starch functionality as measured by swelling power and enzymic hydrolysis showed consistent differences between the A and B granules. Swelling power is a measure of water uptake which is related primarily to the quantity of amylopectin molecules (Sterling, 1978; Tester & Morrison, 1990) and the size of the granules (Wong & Lelievre, 1982). In the present study, the significant positive correlation between swelling power and the short amylopectin chains is consistent with the destabilising effect of these chains on the lamellar structure. However, the inverse relationship between swelling power and long-chain population could indicate the formation of a stronger crystalline network by long chains within the large starch granules as reported by Tang et al. (2001).

As the starches in this study were subjected to enzymic hydrolysis using the same conditions, differences in the rate and extent of hydrolysis between unfractionated starches and A and B granules can be considered to be mainly due to the physicochemical and structural properties of the starch. All of the starches had low levels of damage (0.3–0.5%) and this is unlikely to be a cause of differences in the initial rate of enzymic digestion or swelling power. In the initial stages, enzymic hydrolysis is dependent mainly on contact between the enzyme and the surface of starch granules and, thus, the surface area of the granules would be important in determining the initial attack on the granules by the enzyme. The larger relative surface area of small granules is consistent with their greater initial digestibility by enzymes compared to the large granules. Similar findings were reported by Kong, Kim, Kim, and Kim (2003), who compared the rate of digestion of monodisperse maize (large granules) and rice starches (small granules). Moreover, we found a strong positive correlation between digestibility in the first hour and the proportion of short amylopectin chains, and a strong negative correlation between the digestibility at 0.5 h and the amount of medium length amylopectin chains. These correlations are consistent with the proposal that the surface of the granules is mainly composed of short amylopectin chains and that the B granules had more short-branch chains than A granules (Ao & Jane, 2007).

In the first stages of digestion, the rate of hydrolysis was primarily determined by the surface characteristics of the granules, with the smaller B granules having greater specific surface area and being digested at a faster rate. After 4 h of digestion, the rate of hydrolysis seemed to be influenced more by volume and internal structure of the granules. It was noted in Table 2 that the extent of hydrolysis of the unfractionated starches did not always fall between the corresponding values for the separated A and B granules. The hydrolysis kinetics of the starches will be complex and comparison between samples is unlikely to be simple because of the unequal numbers of granules in the starch samples, the differences between the A and B granules in specific area, volume and structure, the lack of a clear transition between initial and later stages of digestion, and the possibility that the enzyme acts preferentially on particular granules. Experiments with purified preparations of A and B granules mixed in known proportions may help address some of these uncertainties.

With prolonged exposure to the hydrolytic enzymes, A granules were digested to a greater extent. A positive correlation was observed between medium length chains of amylopectin and digestibility, which indicates that the internal organisation of the granules is different from that of the surface. The greater digestibility of the waxy starch compared with the other starches investigated, and the similar extent of hydrolysis of waxy A and B granules are likely to be related to the absence of amylose. Initially, water absorption by starch granules, which occurs in the amorphous regions (Jenkins & Donald, 1998), may be less constrained in waxy starch granules than in granules that contain amylose. Our results are consistent with amylose being present both in the amorphous and crystalline regions. The improved contrast with increasing amylose content observed by SAXS is consistent with the amylose being aligned with the amylopectin clusters. On the other hand, the presence of amylose resulting in lower crystallinity is indicative of amylose generally being in a disordered state within the granule and/or amylose disrupting the crystal order in the amylopectin cluster.

After separating wheat starches into their A and B granular fractions, not only consistent differences between their lamellar repeat distances were observed, but increased number of significant correlations between the nanostructure and the digestibility were also found in comparison with the unfractionated wheat starch granules. The positive correlation between q_{max} and digestibility within the first hour of enzymatic treatment seems to indicate that an initial faster hydrolysis is associated with decreased lamellar repeat distances. The lack of the correlation between I_{max} and digestibility may arise from the similarity in the amylose content in the studied starches although we cannot exclude subtle transmission differences due to variations in packing density of the powdered samples.

5. Conclusions

The proportion of A and B granules can influence the properties of wheat starch. The A and B granules in starches from four wheat varieties of similar amylose content differed in their structural and functional properties, and significant correlations were found between a number of these characteristics of the isolated A and B granules. Our results demonstrate the value of studying A and B granules separately, so as to better understand the relationship between structure and functionality of starch.

Acknowledgments

H.S. was the recipient of a scholarship from Value Added Wheat CRC. Megazyme assay kits were provided to this project by Dr. Barry McCleary, Megazyme International Ireland Ltd. We thank Dr. Oscar Larroque for the chain length analysis of the amylopectin and Allied Mills for the use of the Particle Size Analyzer.

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