

Composition and Properties of A- and B-type Starch Granules of Wild-Type, Partial Waxy, and Waxy Soft Wheat

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ABSTRACT

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Starch A- and B-type granules were isolated from soft wheat (*Triticum aestivum* L.) genotypes representing the four granule bound starch synthase I (GBSSI) classes, and characterized according to composition and properties. While total (TAM) and apparent (AAM) amylose contents of both granule fractions decreased as starch waxy character increased, the A-type granules possessed higher TAM and AAM contents than B-type granules for a given genotype. From wild-type to waxy, a general transition was observed from B- to A-type starch granule fractions with higher levels of lipid-complexed amylose (LAM) and phospholipid. Within a genotype, A-type (relative to B-type) granules possessed higher gelati-

nization enthalpies, while B-type granules exhibited higher gelatinization peak and completion temperatures (broader gelatinization ranges) than A-type granules. Normal (wild-type) and waxy A- and B-type starch granule pasting rates were affected by starch granule lipids; the granule type within a genotype with the lowest LAM and phospholipid levels generally exhibited the shortest time to pasting. For normal and waxy starches, A-type granules exhibited higher pasting viscosities than B-type granules throughout the pasting profile. Thus, the A:B-type granule ratio is important to understanding overall wheat starch composition and behavior.

Mature wheat (*Triticum aestivum* L.) endosperm contains at least two distinct starch granule populations, commonly referred to as A- and B-type granules (Evers 1971). A third population of starch granules, the C-type, was reported by Bechtel et al (1990). For practical purposes, C-type granules are most often considered a subpopulation of the B-type granule fraction, as they represent only a minor portion ($\approx 3\%$) of the total endosperm starch by weight (Bechtel et al 1990). For the two predominant wheat starch granule populations, A-type granules are larger in size ($>10 \mu\text{m}$), lenticular in shape, and initiated early in the grain filling period as compared with B-type starch granules, which are smaller in size ($<10 \mu\text{m}$), more spherical in shape, and initiated during the later stages of grain filling (Evers et al 1974; Bechtel et al 1990; Peng et al 1999). By number, B-type (including C-type) granules comprise up to 99% of granules within the wheat endosperm (Raeker et al 1998), whereas A-type granules constitute the majority of the starch (50–87%) by weight (Dengate and Meredith 1984; Soulaka and Morrison 1985; Bechtel et al 1990; Raeker et al 1998; Peng et al 1999; Shinde et al 2003; Geera et al 2005a). Aside from morphological differences, significant effort has been expended to characterize and define wheat starch A- and B-type granule characteristics and properties. The two starch granule fractions differ in regard to granule composition (Kulp 1973; Soulaka and Morrison 1985; Morrison 1988; Raeker et al 1998; Lu et al 1999; Peng et al 1999; Shinde et al 2003), molecular structure (Jane et al 2003), granule swelling (Wong and Lelievre 1982; Tester and Morrison 1990; Hayashi et al 2005; Van Hung and Morita 2005), gelatinization properties (Bathgate and Palmer 1972; Kulp 1973; Wong and Lelievre 1982; Eliasson and Karlson 1983; Peng et al 1999), pasting/rheological behavior (Fortuna et al 2000; Shinde et al 2003), and reactivity to modifying agents (Bertolini et al 2003; Stapley and BeMiller 2003; Van Hung and Morita 2005). Based on differences in characteristics and properties, the proportion of A- to B-type starch granules in wheat flour has an effect on the quality of traditional wheat-based products such as breads (Sahlström et al 1998; Park et al 2004, 2005) and noodles (Black et al 2000).

The more recent description of partial waxy and waxy wheat lines, which possess reduced levels of amylose, has introduced further compositional and structural variability among genotypes (Hayakawa et al 1997; Sasaki et al 2002; Yoo and Jane 2002). To date, the majority of studies characterizing wheat starch A- and B-type granules have focused solely on wild-type genotypes. Though it is anticipated that starch characteristics of A- and B-type granules within partial waxy and waxy wheat lines differ, the specific contribution of A- and B-type granules to overall starch composition and behavior has not been established for wheat lines with varying degrees of waxy character. As the ratio of A- to B-type starch granules has greatly affected the pasting properties of both wheat flour (Geera et al 2005a,b) and starch (Shinde et al 2003), a knowledge of A- and B-type granule composition and properties is paramount to comprehending wheat starch behavior.

The objective of this study was to investigate the relative compositions, gelatinization behaviors, and pasting properties of individual A- and B-type starch granule fractions isolated from wild-type, partial waxy, and waxy soft wheat lines to enhance fundamental understanding of wheat starch behavior.

MATERIALS AND METHODS

Grain Source and Milling

Soft wheat genotypes varied according to granule bound starch synthase I (GBSSI) class and consisted of wild-type (Jubilee and Whitebird), one gene null partial waxy (Alturas and Penawawa: *Wx-B1a*), two gene null partial waxy (IDO563 and IDO565: *Wx-A1a*, *Wx-B1a*), and waxy (Leona and A98054S-Gwxy-1: *Wx-A1a*, *Wx-B1a*, *Wx-D1a*) cultivars. All genotypes were derived from common parentage (with coefficients of parentage similar to half sibs or closer, except for Penawawa) and were grown at two diverse Idaho locations (Aberdeen/irrigated and Tetonia/rain-fed) over two successive crop years (2000/2001) to ensure that material utilized in experiments was truly representative of each respective line and reflected environmental fluctuation. However, for A98054S-Gwxy-1 and Leona (waxy lines), wheat from the 2000 crop year at Tetonia was not available for study. Thus, a total of 30 genotype/crop year/growing location combinations were utilized in the study. For each genotype/crop year/growing location combination, grain was milled to straight-grade flour, and native starch (defined as the population of starch granules present in straight-grade flour) was isolated from milled flours as described in a companion article (Geera et al 2005a).

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Fractionation of A- and B-Type Starch Granule Populations

For each genotype/crop year/growing location combination, isolated native starch was fractionated into respective A- and B-type granule populations. A combination of centrifugation and micro-screening techniques was used, as illustrated in Fig. 1. Native starch (2.5 g, db) was suspended in 20 mL of aqueous sucrose solution (80%, w/v) by vortexing thoroughly within a centrifuge tube (28.8 × 106.7 mm, Nalgene Corp., Rochester, NY), after which the solution level within the tube was marked on the outer test tube wall. The starch-sucrose suspension was centrifuged (350 × g, 8 min), after which ≈13 mL of supernatant containing B-type starch granules was gently pipetted from the tube and set aside (supernatant 1). Fresh sucrose solution was again added to the tube to the prescribed level, after which the tube was vortexed and recentrifuged (350 × g, 9 min). The supernatant (≈13 mL) containing B-type starch granules was pipetted from the tube and set aside (supernatant 2). This process was repeated three more times with accompanying centrifugations (350 × g, 2.5 min) to generate supernatants 3, 4, and 5 (containing mixtures of A- and B-type granules) and a final pellet of A-type granules. For each repeat of the process, fresh sucrose solution was added before centrifugation to compensate for the supernatant that had been removed and set aside. Also, for the fifth (last) centrifugation step of the series, the entire supernatant (supernatant 5) was carefully collected and set aside, rather than just 13 mL.

Supernatants 3, 4, and 5 (Fig. 1), which all contained mixtures of A- and B-type starch granules, were combined and centrifuged (2,500 × g, 20 min) to recover the starch material. The combined starch material (<1 g) was washed extensively 3× with 30 mL of deionized water, resuspended in deionized water (50 mL), and passed onto a 10-μm precision sieve (ATM Corp., Milwaukee, WI) enhanced with mechanical vibration to augment flow to fractionate both A-type (overs) and B-type (throughs) starch granules. Additional deionized water was added during the filtration step as necessary to facilitate the process.

Supernatants 1 and 2 and the sieve throughs obtained from filtration were combined and recovered by centrifugation (2,500 × g, 20 min) to yield the B-type starch granule fraction. The final pellet left after the five initial centrifugation steps and the sieve overs from the filtration step were similarly combined and collected to yield the A-type starch granule fraction (Fig. 1). Each purified A- and B-type starch granule fraction was washed extensively 3× with 200 mL of deionized water, resuspended in 50 mL

of absolute ethanol, recovered on a Büchner funnel, and allowed to air-dry. Though the procedure was described here on the basis of a single tube, up to four tubes were processed simultaneously, allowing 10 g (db) of native starch to be fractionated at a time.

The fractionation procedure described above was used to obtain purified A- and B-type starch granule fractions for both wild-type and one gene null partial waxy genotypes.

Adjustments were made to optimize the process for two gene null partial waxy and waxy genotypes. The number of initial centrifugation steps used to recover B-type granules was increased from two to three for these genotypes with accompanying centrifugation times of 5, 6, and 6 min for steps 1, 2, and 3, respectively.

Measurement of Starch Granule Size Distribution

Granule size distributions of native starches representing each genotype/crop year/growing location combination and the respective purified A- and B-type starch granule fractions were determined using an Accusizer model 780 with SW 788 Windows software (Particle Sizing Systems, Santa Barbara, CA) as previously described (Geera et al 2005a). A 10-μm cutoff was used to differentiate A- and B-type starch granule populations.

Determination of Amylose

Apparent (AAM), total (TAM), and lipid-complexed (LAM) amylose contents of A- and B-type starch granule fractions were determined using the colorimetric method of Morrison and Laignelet (1983). The AAM content was measured before the removal of starch lipids, while TAM was determined on defatted starch. The difference between TAM and AAM provided a measure of LAM.

Gelatinization Properties by DSC

Gelatinization properties of genotype A- and B-type starch granule fractions were analyzed on a Pyris-1 differential scanning calorimeter (Perkin Elmer, Norwalk, CT). Starch (10 mg, db) was weighed directly into a stainless steel pan followed by addition of deionized water (20 μL). Pans were sealed, allowed to equilibrate overnight at ambient temperature before analysis, and scanned at a rate of 10°C/min over a temperature range of 20–180°C. A pan containing a 1:2 ratio of Al₂O₃ to water was used as a reference. DSC measurements recorded were onset (*T*_o), peak (*T*_p), and completion (*T*_c) gelatinization temperatures, as well as gelatinization enthalpy (ΔH).

Evaluation of Starch Pasting Properties

Pasting properties of A- and B-type starch granule fractions (wild-type and waxy genotypes only) were determined using the Rapid Visco Analyser (RVA) (Newport Scientific, NSW, Australia) as described by Geera et al (2005a) for the analysis of native wheat starch. Pasting analyses were restricted to A- and B-type starch granule fractions of wild-type and waxy genotypes due to limiting amounts of fractionated starch material.

Miscellaneous Analyses

Moisture contents of A- and B-type starch granule fractions were determined according to AOAC Method 925.09 (AOAC 1990). Phosphorous contents of A- and B-type starch granule fractions were determined using inductively-coupled plasma-atomic emission spectroscopy (ICP-AES) according to the method of Anderson (1996) to approximate starch phospholipid contents.

Experimental Design

Fractionation of native starch into its respective A- and B-type starch granule populations was conducted one time (four tubes) for each genotype/crop year/growing location combination. For calculation of genotype mean A- and B-type granule fraction yields and purities after fractionation, each crop year/growing location combination was considered a replicate within a genotype (total of four replicates/genotype). After calculating fraction yields and

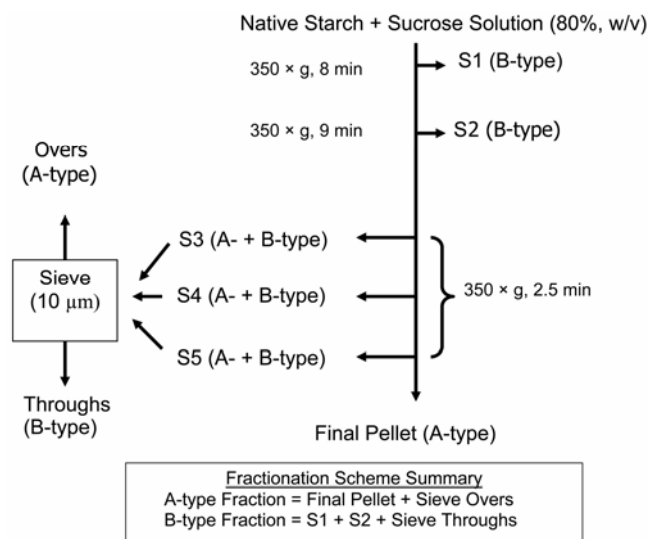


Fig. 1. Outline for fractionation of native starch into A- and B-type starch granule populations through a combination of centrifugation and micro-sieving techniques.

recoveries, the respective A- and B-type starch granule populations of each genotype were bulked across all crop year/growing location combinations to generate single sources of A- and B-type starch granules for each genotype. The bulked genotype A- and B-type starch granule fractions were the sources of starch for all further analyses. Analysis of variance (ANOVA) was used to test for significant differences between A- and B-type starch granule characteristics and properties within a genotype; LSD tests ($P < 0.05$) were used to statistically differentiate mean values for the various starch analyses. All statistical computations were conducted using SAS v. 8.2 for windows (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Yields and Purities of Genotype A- and B-Type Starch Granule Fractions

Genotype native starch A- and B-type granule contents were assessed through particle size analysis to provide a reference for calculating A- and B-type starch granule recoveries after fractionation. Mean A- and B-type granule contents of isolated native starches by genotype had ranges of 61.6–76.8 g/100 g of starch (db) and 23.2–38.40 g/100 g of starch (db), respectively (Table I). A more detailed discussion of genotype native starch A- and B-type granule contents has been provided elsewhere (Geera et al 2005a). The primary discussion here will focus on characteristics and properties of the isolated A- and B-type starch granule fractions from the genotypes in this study.

Mean yields of fractionated A-type starch granules from native starches for all genotypes had a range of 61.7–75.5% (by weight, db), while those of fractionated B-type starch granules had a range of 15.2–28.5% (by weight, db) (Table I). Combined mean yields for A- and B-type starch granule fractions for all genotypes accounted for 88.4–92.4% of the original native starch subjected to fractionation, and did not differ statistically among genotypes. As the combined yields of A- and B-type starch granule fractions fell short of the amounts of original native starch utilized for fractionation, a proportion of the native starch (7.6–11.6%, depending on genotype) was unrecovered in the fractionation process. Fraction yields for B-type starch granules fell well shy of the established B-type granule contents of the respective native starches (Table I). In contrast, there was little difference between the established genotype A-type granule contents of the native starches and the respective genotype A-type granule yields obtained by

fractionation. These observations suggest that predominantly B-type granules were lost during the fractionation process. Previous researchers have also expressed an inability to recover the entire B-type starch granule fraction (Soulaka and Morrison 1985; Peng et al 1999; Shinde et al 2003). Nevertheless, the combined recoveries of A- and B-type granules in our study approached 90% of the original native starch used for fractionation.

The relative purities of genotype A- and B-type starch granule fractions (wb), which were assessed by particle size analysis, had ranges of 92.7–94.6% and 88.7–91.4%, respectively (Table I). The A- and B-type granule fractions did not differ statistically in regard to fraction purity. Starch granule fraction purities in this study were comparable to those of most previous reports (Bertolini et al 2003; Shinde et al 2003), but fell short of those reported by Peng et al (1999), though no mention of overall starch recovery after fractionation was provided in the latter report. Emphasis in our study was not given merely to obtaining purified A- and B-type fractions, but also to recovering a maximal amount of starch through the fractionation process in the form of isolated A- and B-type starch granule fractions. Thus, isolated A- and B-type starch granule fraction yields in our study represented a balance between starch recovery and fraction purity.

Amylose Characteristics of A- and B-Type Starch Granule Fractions

Starch total (TAM), apparent (AAM), and lipid-complexed (LAM) amylose contents, as well as phosphorus levels (as an approximation of granule phospholipids) within isolated A- and B-type starch granule fractions generally decreased over the range of wild-type to waxy (Table II). Genotype A- and B-type starch granule fractions exhibited trends in starch amylose and lipid contents characteristic of those reported for native (unfractionated) wheat starches spanning the range from wild-type to waxy (Kim et al 2003; Mangalika et al 2003; Geera et al 2005a).

A majority of previous studies have reported that A-type granules of wild-type wheat possess higher TAM and AAM contents compared with those of B-type granules (Peng et al 1999; Shinde et al 2003). Results of our study extend this finding also to partial waxy and waxy genotypes. The A-type granule fractions exclusively exhibited higher TAM and AAM contents than the respective B-type granule fractions of the same genotype (Table II). For A-type starch granule fractions, reductions in TAM and AAM contents occurred very gradually over the range of wild-type to

TABLE I
Mean Yields and Purities of Genotype A- and B-Type Granule Fractions Isolated from Native Wheat Starch^a

Genotype	A-Type Granules			B-Type Granules			Combined A- and B-Type Granule Fraction Yield ^c
	Native Starch ^b	Fraction Yield ^c	Purity ^d	Native Starch ^b	Fraction Yield ^c	Purity ^d	
Wild-type							
Whitebird	75.6 ± 2.8ab	74.2 ± 3.3ab	94.6 ± 1.1a	24.4 ± 2.8cd	16.1 ± 1.6cd	90.2 ± 1.9a	90.3 ± 2.6a
Jubilee	75.3 ± 1.8ab	73.4 ± 3.2ab	94.1 ± 1.8a	24.7 ± 1.8cd	15.2 ± 2.2de	89.4 ± 4.1a	88.6 ± 4.0a
One Gene Null							
Alturas	71.7 ± 3.1c	69.3 ± 3.1c	93.2 ± 2.5a	28.3 ± 3.1b	19.1 ± 2.6bc	89.1 ± 2.6a	88.4 ± 2.3a
Penawawa	76.8 ± 1.5a	75.5 ± 2.5a	94.4 ± 2.6a	23.2 ± 1.5d	12.2 ± 1.7e	90.2 ± 2.1a	87.7 ± 3.5a
Two Gene Null							
IDO563	72.4 ± 2.1c	71.0 ± 2.1bc	93.3 ± 2.6a	27.6 ± 2.1b	21.4 ± 4.9b	89.0 ± 2.4a	92.4 ± 3.2a
IDO565	73.5 ± 2.1bc	71.2 ± 1.8bc	93.6 ± 2.9a	26.5 ± 2.3bc	17.6 ± 2.4b–d	88.7 ± 3.0a	88.6 ± 2.7a
Waxy							
Leona	63.6 ± 1.5d	63.1 ± 2.0d	92.7 ± 1.8a	36.4 ± 1.5a	26.3 ± 1.5a	90.6 ± 1.2a	89.4 ± 1.6a
A98054S-Gwxy-1	61.6 ± 2.3d	61.7 ± 2.1d	93.0 ± 1.4a	38.4 ± 2.3a	28.5 ± 2.2a	91.4 ± 1.4a	90.2 ± 2.3a
P value	<0.0001	<0.0001	0.9264	<0.0001	<0.0001	0.8679	0.4419

^a Genotype mean values calculated across four crop year/growing location combinations. Values within a column followed by the same letter are not significantly different ($P < 0.05$).

^b A- or B-type granule content by weight of original native starch (g/100 g of native starch) determined by particle size analysis (reprinted from Geera et al [2005]).

^c Yield of fraction (A- or B-type) starch granules obtained from fractionation of native starch (g/100 g of native starch).

^d A- or B-type granule content of isolated starch fraction by weight (g/100 g of starch) determined by particle size analysis; based on a 10- μ m cutoff for differentiation of A- and B-type granules.

^e Combined A- and B-type starch granule fraction yields after fractionation from native starch (g/100 g of native starch).

two gene null genotypes, followed by a drastic reduction for waxy genotypes (Table II). For B-type starch granule fractions, the reduction in TAM and AAM contents occurred in a more step-wise fashion from wild-type to two gene null genotypes, followed by the anticipated drastic reduction for waxy genotypes. Thus, the number-fold difference in TAM or AAM observed between genotype A-type and B-type starch granule fractions (ratio of the A-type:B-type TAM or AAM content) appeared to increase over the range of wild-type to waxy.

Genotype LAM and phosphorous contents of both A-type and B-type starch granule fractions generally decreased as the degree of waxy character increased. According to past reports, B-type granules (wild-type wheat) traditionally possess higher phospholipid contents than A-type granules (Peng et al 1999; Shinde et al 2003). However, in this study, a transition from B-type starch granule fractions in wild-type genotypes to A-type starch granule fractions in waxy genotypes with higher levels of LAM and phosphorus was generally observed (Table II). This transition has not been previously reported and will be discussed further in relation to starch fraction pasting behaviors.

Gelatinization Properties of A- and B-Type Starch Granule Fractions

Gelatinization properties of A- and B-type starch granule fractions for genotypes are depicted in Table III. Starch gelatinization onset temperatures (T_o) for both A- and B-type granule fractions did not exhibit any obvious pattern across genotypes. For both granule types, gelatinization peak (T_p) and completion (T_c) temperatures generally increased with a decrease in amylose content over the range of wild-type to waxy. Gelatinization enthalpy (ΔH), which is a measure of the overall crystallinity (i.e., indicative of both the quality and quantity of starch crystals) (Tester and Morrison 1990), also increased for both granule fractions with a decrease in amylose content (from wild-type to waxy). Previous studies have reported higher gelatinization enthalpies for waxy wheat starches compared with wild-type wheat starches (Fujita et al 1998; Sasaki et al 2000; Yoo and Jane 2002; Abdel-Aal et al 2002; Mangalika et al 2003). Both A- and B-type fractions exhibited similar trends across genotypes. In the absence of amylose-rich amorphous regions, more energy is required to melt the crystalline regions (Krueger et al 1987). This phenomenon likely

TABLE II
Genotype A- and B-Type Starch Granule Fraction Mean Amylose Characteristics and Phosphorus Levels^a

Starch Fraction ^b	Wild-Type Genotype		One Gene Null Genotype		Two Gene Null Genotype		Waxy Genotype	
	Jubilee	Whitebird	Alturas	Penawawa	IDO563	IDO565	Leona	A98480-Gwxy-1
TAM								
A-type	25.5 ± 0.7a	25.5 ± 0.4a	24.6 ± 0.4a	24.3 ± 1.0a	21.8 ± 0.5a	19.4 ± 0.2a	3.4 ± 0.8a	3.5 ± 0.2a
B-type	21.0 ± 0.4b	20.9 ± 0.9b	17.6 ± 0.3b	16.4 ± 0.4b	12.3 ± 0.3b	11.9 ± 0.1b	1.3 ± 0.3b	0.6 ± 0.2b
P value	0.0006	0.0011	<0.0001	0.0003	<0.0001	<0.0001	0.0119	<0.0001
AAM								
A-type	20.6 ± 0.4a	20.8 ± 0.4a	20.0 ± 0.3a	18.9 ± 0.6a	17.4 ± 0.3a	15.0 ± 0.4a	2.3 ± 0.2a	2.7 ± 0.3a
B-type	14.8 ± 0.7b	14.9 ± 0.8b	12.7 ± 0.7b	12.4 ± 0.5b	8.7 ± 0.6b	8.7 ± 0.1b	0.6 ± 0.2b	0.5 ± 0.1b
P value	0.0002	0.0003	<0.0001	<0.0001	<0.0001	<0.0001	0.0004	0.0001
LAM								
A-type	4.9 ± 0.7a	4.7 ± 0.4b	4.6 ± 0.1a	5.1 ± 0.7a	4.5 ± 0.3a	4.3 ± 0.3a	1.2 ± 0.8a	0.8 ± 0.1a
B-type	6.2 ± 1.1a	6.1 ± 0.4a	5.0 ± 0.4a	4.1 ± 0.1a	3.6 ± 0.3b	3.2 ± 0.1b	0.6 ± 0.2a	0.1 ± 0.2b
P value	0.1564	0.0093	0.2122	0.0548	0.0362	0.0031	0.3081	0.0045
Phosphorous content ^c								
A-type	475 ± 7b	480 ± 0b	440 ± 0b	500 ± 14a	465 ± 7a	450 ± 0a	130 ± 0a	100 ± 0a
B-type	590 ± 0a	570 ± 14a	570 ± 0a	535 ± 7a	405 ± 7b	455 ± 20a	82 ± 0b	44 ± 1b
P value	0.0019	0.0121	<0.0001	0.0887	0.0136	0.7706	<0.0001	<0.0001

^a Mean values of at least two measurements; A- and B-type values for a particular characteristic within a genotype followed by the same letter are not significantly different ($P < 0.05$).

^b TAM, total amylose (g/100 g of starch); AAM, apparent amylose (g/100 g of starch); LAM, lipid-complexed amylose (g/100 g of starch).

^c Approximation of relative starch phospholipid content (ppm).

TABLE III
Genotype A- and B-Type Starch Granule Fraction Mean Gelatinization Properties^a

Properties ^b	Wild-Type Genotype		One Gene Null Genotype		Two Gene Null Genotype		Waxy Genotype	
	Jubilee	Whitebird	Alturas	Penawawa	IDO563	IDO565	Leona	A98480-Gwxy-1
T_o								
A-type	57.0 ± 0.1a	57.6 ± 0.2a	59.9 ± 0.1a	57.9 ± 0.0a	58.2 ± 0.0a	57.7 ± 0.3a	57.0 ± 0.0a	57.3 ± 0.1a
B-type	57.5 ± 0.2a	57.4 ± 0.0a	58.5 ± 0.0b	57.8 ± 0.1a	58.1 ± 0.1a	57.8 ± 0.2a	55.8 ± 0.2b	56.4 ± 0.3a
P value	0.1430	0.3350	0.0016	0.1794	0.3125	0.7746	0.0114	0.0722
T_p								
A-type	60.9 ± 0.2b	61.7 ± 0.2b	63.7 ± 0.0b	61.5 ± 0.0b	62.8 ± 0.1b	63.1 ± 0.0b	64.7 ± 0.0b	65.5 ± 0.1b
B-type	63.5 ± 0.2a	62.7 ± 0.2a	65.3 ± 0.1a	64.2 ± 0.0a	66.7 ± 0.5a	66.2 ± 0.2a	65.9 ± 0.1a	66.5 ± 0.2a
P value	0.0059	0.0315	0.0028	<0.0001	0.0076	0.0030	0.0045	0.0300
T_c								
A-type	66.3 ± 0.6b	67.6 ± 0.1b	68.9 ± 0.1b	67.2 ± 0.1b	70.0 ± 0.2b	70.3 ± 0.0b	75.5 ± 0.0b	74.6 ± 0.1b
B-type	72.5 ± 0.3a	70.4 ± 0.0a	74.4 ± 0.2a	73.6 ± 0.2a	76.0 ± 0.5a	77.7 ± 0.5a	77.8 ± 0.4a	78.5 ± 0.2a
P value	0.0051	0.0013	0.0008	0.0008	0.0032	0.0019	0.0151	0.0023
ΔH								
A-type	11.3 ± 0.0a	11.5 ± 0.3a	12.1 ± 0.1a	12.6 ± 0.1a	13.1 ± 0.1a	13.3 ± 0.0a	15.4 ± 0.1a	15.1 ± 0.1a
B-type	9.8 ± 0.0b	10.1 ± 0.1b	11.5 ± 0.2a	11.2 ± 0.1b	11.9 ± 0.5a	11.4 ± 0.2b	12.9 ± 0.2b	13.6 ± 0.0b
P value	0.0003	0.0294	0.0582	0.0058	0.0770	0.0045	0.0044	0.0012

^a Mean values of at least two measurements. A- and B-type values within a genotype followed by the same letter are not significantly different for a particular gelatinization characteristic ($P < 0.05$).

^b Gelatinization onset, peak, and completion temperatures (°C) (T_o , T_p , T_c , respectively); gelatinization enthalpy (ΔH , J/g).

explained the trend toward higher gelatinization enthalpies for A- and B-type starch granule fractions with increasing waxy character (Table III).

Irrespective of the GBSSI class, B-type granules generally exhibited the highest starch gelatinization peak and completion temperatures, while A-type granules consistently exhibited the highest gelatinization enthalpies. These results are consistent with previous reports for normal wheat starch A- and B-type starch granules (Peng et al 1999; Chiotelli and Le Meste 2002). In our study, B-type granules also exhibited broader gelatinization temperature ranges ($T_c - T_o$) irrespective of genotype. Within a genotype, lower gelatinization enthalpies and greater gelatinization ranges were observed for B-type (relative to A-type) granule fractions in spite of the fact that they possessed relatively lower TAM and AAM contents. For normal wheat starch, Chiotelli and Le Meste (2002) suggested that B-type granules possessed a lower percentage of crystals or less stable crystals compared with A-type granules. Another factor could be the presence of more short chains (A and B1) and fewer long chains for amylopectin of B-type (relative to A-type) starch granules, as observed for normal wheat starch (Jane et al 2003). Both phenomena warrant further investigation in explanation of partial waxy and waxy wheat starch A- and B-type granule gelatinization behavior.

Pasting Properties of A- and B-Type Starch Granule Fractions

Mean pasting attributes for A- and B-type granules of wild-type (Whitebird and Jubilee) and waxy (Leona, A98054S-Gwxy-1) genotypes are shown in Table IV. Examples of pasting profiles for wild-type (Whitebird) and waxy (Leona) genotype A- and B-type starch granule fractions are shown in Figs. 2 and 3, respectively. Waxy genotype A- and B-type starch granule fractions exhibited higher peak and breakdown viscosities, respectively, compared with wild-type genotype A- and B-type starch granule fractions (Table IV). In contrast, the respective A- and B-type starch granule fractions of wild-type genotypes exhibited higher trough, final, and setback viscosities relative to those of waxy genotypes. Differences in the pasting properties of waxy and wild-type genotype starch granule fractions (A- and B-type) appeared to be attributable to the obvious differences in TAM, AAM, and LAM contents, following trends previously reported for isolated native starches of wild-type and waxy wheat (Kim et al 2003; Geera et al 2005a).

Though B-type starch granule fractions of all genotypes possessed lower TAM and AAM levels compared with the respective A-type starch granule fractions (Table II), they exhibited relatively lower pasting viscosities along virtually all points of the pasting profile (Figs. 2 and 3). Within a genotype, A-type starch granule fractions exhibited higher peak, trough, final, breakdown, and setback viscosities relative to B-type starch granule fractions (Table IV), similar to pasting patterns observed for normal wheat

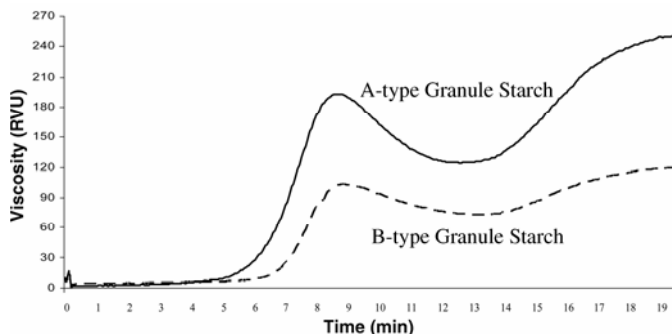


Fig. 2. Pasting profiles of A- and B-type starch granule fractions for Whitebird (wild-type).

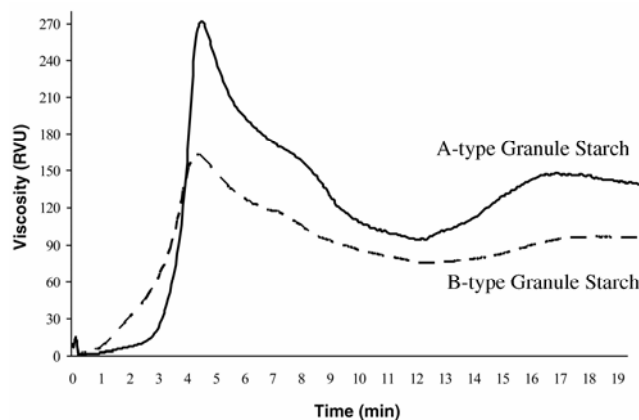


Fig. 3. Pasting profiles of A- and B-type starch granule fractions for Leona (waxy).

TABLE IV
Wild-Type and Waxy Genotype A- and B-Type Starch Granule Fraction Mean Pasting Properties^a

Genotype and Starch Fraction	Viscosity (RVU)					Time to Pasting (min)
	Peak	Breakdown	Trough	Final	Setback	
Wildtype						
Jubilee						
A-type	145.4 ± 0.0a	63.9 ± 0.9a	81.4 ± 0.9a	165.5 ± 1.8a	84.0 ± 1.0a	6.2 ± 0.0a
B-type	113.4 ± 1.3b	35.7 ± 0.0b	77.7 ± 1.3a	120.9 ± 1.1b	43.2 ± 0.2b	7.2 ± 0.1b
P value	0.0008	0.0005	0.0847	0.0011	0.0003	0.0023
Whitebird						
A-type	193.1 ± 0.6a	68.5 ± 0.7a	124.5 ± 0.1a	250.2 ± 2.0a	125.6 ± 2.2a	6.3 ± 0.0a
B-type	103.9 ± 0.1b	30.6 ± 0.1b	73.3 ± 0.3b	119.9 ± 0.0b	46.6 ± 0.3b	7.4 ± 0.0b
P value	<0.0001	0.0002	<0.0001	0.0001	0.0004	<0.0001
Waxy						
Leona						
A-type	273.8 ± 3.0a	179.2 ± 2.0a	94.5 ± 0.9a	139.2 ± 0.6a	44.5 ± 0.5a	3.1 ± 0.7a
B-type	163.9 ± 2.9b	88.1 ± 2.6b	75.5 ± 0.6b	96.0 ± 0.4b	20.4 ± 0.2b	2.0 ± 0.0b
P value	0.0007	0.0007	0.0017	0.0002	0.0002	0.0019
A98480-Gwxy-1						
A-type	239.2 ± 0.6a	159.6 ± 0.1a	79.3 ± 0.8a	128.5 ± 1.6a	49.2 ± 0.8a	3.3 ± 0.0a
B-type	178.0 ± 3.2b	101.6 ± 1.9b	76.4 ± 1.3a	98.5 ± 0.3b	22.1 ± 1.0b	2.1 ± 0.1b
P value	0.0014	0.0005	0.1155	0.0015	0.0012	0.0019

^a Mean values of at least two measurements. Mean values for A- and B-type starch fraction pasting attributes within a genotype followed by the same letter are not significantly different ($P < 0.05$).

starch A- and B-type granules (Sahlström et al 2003; Shinde et al 2003). Thus, differences in amylose characteristics, which have explained the primary pasting differences between waxy and normal starches (Kim et al 2003; Mangalika et al 2003), did not provide a reasonable explanation for pasting differences between the A- and B-type granules within a genotype.

Phospholipids influence the swelling behavior of starch by inhibiting the movement of water into granules, resulting in reduced starch granule swelling, amylose leaching, and peak paste viscosity (Tester and Morrison 1990). It has been suggested that the lower pasting viscosities of B-type starch granules, compared with those of A-type starch granules, might be the result of the relatively higher phospholipid and LAM contents. Our findings support the premise that phospholipids influence the rate of starch swelling and pasting. The time to pasting (defined as the run time at which starch viscosity increased 30 RVU units above baseline) for wild-type genotype B-type starch granule fractions was delayed compared with that of A-type starch granule fractions (Table IV, Fig. 2). In this scenario, it is significant that normal B-type granules possessed higher levels of LAM and phospholipid relative to the A-type counterparts (Table II). This observation supports the finding of Gaines et al (2000), who reported that a high phospholipid content was associated with a delayed starch pasting time and temperature. For waxy genotypes of this study, the pattern was reversed, as a shorter time to pasting was observed for B-type relative to A-type starch granule fractions (Table IV, Fig. 3). This reversal in time to pasting for the two granule fractions of waxy (relative to wild-type) genotypes occurred in concert with a similar transition in fraction phospholipid contents. For waxy starches, A-type granules generally possessed relatively higher phospholipid or LAM contents compared with B-type starch granules (Table II). Within a genotype (waxy or wild-type), the starch fraction with the lowest levels of LAM or phospholipid exhibited the shortest time to pasting (Table IV, Figs. 2 and 3). Thus, LAM and phospholipid levels appeared to influence the rate of starch fraction swelling and pasting.

Though waxy B-type starch granules possessed slightly lower TAM, AAM, LAM, and phospholipid contents relative to waxy A-type starch granules, attributes that would tend to favor waxy B-type over A-type granules for higher pasting viscosities (Table II), they nevertheless displayed comparatively lower pasting viscosities at almost all points along the pasting curve (Fig. 3). Despite the compositional differences noted between the two granule types within both wild-type and waxy genotypes (Table II), a similar magnitude difference in pasting viscosity was consistently maintained between the two starch granule types of a genotype (Figs. 2 and 3). Basic differences in starch chemical composition such as amylose content, phospholipid content, etc., studied here did not solely explain the observed magnitude pasting differences between A- and B-type starch granule fractions within a genotype. This observation supports the previous assertion that granule size itself might explain in part the pasting property differences of A- and B-type granule fractions (Shinde et al 2003). At equal concentrations (weight basis), suspensions of swollen A-type granules would be expected to occupy a greater volume fraction (Wong and Lelievre 1981, 1982) within a starch paste (compared with swollen B-type granules) and contribute more significantly to viscosity development. The potential role of granule type and size, which could also be related to starch molecular structure, has not yet been fully elucidated for wheat starch A- and B-type granule fractions. In any event, the relative proportion of A- and B-type granules within a starch has the potential to greatly influence wheat starch composition and properties.

SUMMARY AND CONCLUSIONS

Genotype native starches of varying *Wx* gene dosage were fractionated into respective A- and B-type granule populations. Amy-

lose characteristics, gelatinization behavior, and pasting properties of A- and B-type starch granule fractions varied significantly among genotypes and also between the two granule types within a genotype.

Amylose (TAM, AAM, and LAM) and phospholipid contents for both starch granule fractions generally decreased over the range of wild-type to waxy with the gradual loss of functional *Wx* genes. Trends in amylose characteristics for genotype A- and B-type granules were primarily a function of GBSSI genes. Within all genotypes, A-type starch granules possessed higher TAM and AAM contents than B-type starch granules. From wild-type to waxy, a general transition from B- to A-type starch granule fractions with higher amounts of LAM and phospholipid was observed. Gelatinization peak and completion temperatures as well as gelatinization enthalpies gradually increased over the range of wild-type to waxy concurrent with decreasing TAM and AAM contents for both A- and B-type starch granule fractions. Within a genotype, B-type granules consistently exhibited higher starch gelatinization peak and completion temperatures, while A-type granules consistently exhibited higher gelatinization enthalpies.

Pasting properties of wild-type and waxy A- and B-type granule fractions exhibited similar trends, but different magnitude viscosities at virtually all points of the pasting curve and mirrored pasting profile patterns typifying normal and waxy wheat starches. In comparing like granule fractions of normal and waxy starches, the genotype A- or B-type granule fraction with the lowest TAM and AAM contents (waxy) displayed the highest peak and breakdown viscosities, while that with the highest TAM and AAM contents (wild-type) exhibited the highest trough, setback, and final viscosities. Pasting rates of normal and waxy A- and B-type granules within a genotype appeared to coincide with starch phospholipid contents. For a given genotype, the starch granule fraction with the least amount of LAM and phospholipids exhibited the shortest time to pasting. Phospholipid and LAM contents appeared to influence the rate more significantly than the magnitude of starch fraction pasting viscosity within a genotype. The A-type starch granule fractions consistently displayed higher pasting viscosities at virtually all points of the pasting curve relative to those of B-type starch granule fractions for both wild-type and waxy genotypes. Compositional differences alone did not explain the relative magnitude differences in the pasting viscosities between the two granule types, suggesting a potential role of granule size itself. Regardless of GBSSI class, the relative ratio of A- to B-type granules within a wheat starch can be expected to influence starch swelling, gelatinization, and pasting properties and will likely affect wheat end use characteristics and product quality.

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