

# Separation and Characterization of A- and B-Type Starch Granules in Wheat Endosperm

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## ABSTRACT

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Mature wheat (*Triticum aestivum* L.) endosperm contains two types of starch granules: large A-type and small B-type. Two methods, microsieving or centrifugal sedimentation through aqueous solutions of sucrose, maltose, or Percoll were used to separate A- and B-type starch granules. Microsieving could not completely separate the two types of starch granules, while centrifuging through maltose and sucrose solutions gave a homogenous population for B-type starch granules only. Centrifuging through two Percoll solutions (70 and 100%, v/v) produced purified populations of both the A- and B-type starch granules. Analysis of starch granule size distribution in the purified A- and B-type granule populations and in the whole-starch granule population obtained directly from wheat endosperm con-

firmed that the purified A- and B-type starch granule populations represented their counterparts in mature wheat endosperm. Centrifugations through two Percoll solutions were used to purify A- and B-type starch granule populations from six wheat cultivars. The amylose concentrations and gelatinization properties of these populations were analyzed. All of the A-type starch granules contained higher amylose concentrations and had higher gelatinization enthalpies than did B-type starch granules. Although A- and B-type starch granules started to gelatinize at a similar temperature, B-type starch granules had higher gelatinization peak and completion temperatures than did A-type starch granules.

Wheat grain is one of the major cereals consumed by human beings. Starch, the predominant component of the wheat grain, constitutes two-thirds to three-quarters of the dry weight of a wheat kernel, depending on the cultivar (Hucl and Chibbar 1996). At maturity, wheat endosperm contains two types of starch granules: large A- and small B-type (Evers 1971). A-type starch granules are disk-like or lenticular in shape with an average diameter of 10–35  $\mu\text{m}$  and contribute >70% of the total weight and  $\approx$ 3% of the total granule number of endosperm starch. On the other hand, B-type starch granules are roughly spherical or polygonal in shape, ranging from 1 to 10  $\mu\text{m}$  in diameter. These account for >90% of the total granule number but <30% of the total weight of starch in wheat endosperm (Evers 1973, Morrison and Gadan 1987).

Wheat A- and B-type starch granules are reported to have significantly different chemical compositions and functional properties such as amylose, amylopectin, lipid, and protein concentrations; pasting characteristics; and baking properties (Seib 1994, Maningat and Seib 1997). These differences result in the two starch granule types being utilized differently, both in food and nonfood uses. For example, starch with predominantly B-type starch granules can be used as a fat substitute (Lim et al 1992), while starch with a high percentage of A-type starch granules has applications in the manufacture of biodegradable plastic film and carbonless copy paper (Nachtergaele and Van Nuffel 1989). Thus, wheat cultivars with predominantly B- or A-type starch granules would be very useful to the food and nonfood industries, respectively. To develop these wheat cultivars, the biochemical characterization and ontogeny of A- and B-type starch granules during wheat kernel development must be understood. For this purpose, it is essential to develop methods that will efficiently separate the two types of wheat starch granules.

To separate wheat A- and B-type starch granules, several procedures, including microsieving (Evers 1973), sedimentation in a water column (Morrison and Gadan 1987), and elutriation (MacGregor 1979) have been used. The separated starch granules were evaluated for chemical composition and functional properties. However, these procedures usually could not completely separate A- and B-type starch granules. Thus, the results from the subsequent charac-

terization of A- and B-type starch granules should be used with caution. The objectives of the present study were: 1) to develop an efficient procedure to completely separate wheat A- and B-type starch granules, 2) to determine whether the separated A- and B-type starch granule populations could represent their counterparts in mature wheat endosperm, 3) to measure the amylose concentrations in A- and B-type starch granules, and 4) to study the gelatinization properties of A- and B-type starch granules.

## MATERIALS AND METHODS

### Starch Granule Isolation

Wheat starch granules were isolated from mature seeds of *Triticum aestivum* cv. CDC Teal. Wheat grains (5.7 g) were steeped in 40 mL of double-distilled water (DDW) at 4°C for 16 hr. The softened seeds were degermed and ground in an autoclaved mortar and pestle. The slurry was filtered through four layers of cheesecloth to remove endosperm cell debris and centrifuged at  $3,500 \times g$  for 5 min. The yellow gel-like layer on top of the packed white starch granule pellet was carefully removed. The starch granule pellet was then suspended in 5 mL of DDW, overlaid on 30 mL of 80% (w/v) cesium chloride, and centrifuged at  $3,500 \times g$  for 5 min. The supernatant and cesium chloride with debris were discarded. The starch granule pellet, referred to as the whole-starch granule population, was washed twice with 30 mL of wash buffer (62.5 mM tris-(hydroxymethyl)aminomethane-HCl, pH 6.8; 10 mM sodium ethylenediaminetetraacetate; and 4% SDS), four times with DDW, and once with acetone. Then it was air-dried and stored at  $-20^\circ\text{C}$ .

### Separation of A-Type and B-Type Starch Granules

The wheat starch preparation containing both A- and B-type granules was separated into the two types of granules.

**Microsieving.** Wheat starch (0.5 g) was suspended in 200 mL of DDW. The starch suspension was filtered through two layers of nylon screen with a mesh diameter of 10  $\mu\text{m}$ . The starch fraction retained by the screen was the A-type starch granule population, and that from the filtrate comprised the B-type starch granule population.

**Centrifugation through maltose (80%, w/v).** A 5-mL starch suspension in DDW (0.1 g/mL) was laid on the top of 10 mL of 80% (w/v) maltose in a 15-mL glass tube and centrifuged at  $10 \times g$  for 10 min. The supernatant which contained B-type starch granules was removed to another tube. The pellet was washed twice in DDW, suspended in 5 mL of DDW, and centrifuged four times in fresh 80% maltose solution. The starch pellet constituted the A-type starch granule population. The supernatants were pooled and centrifuged

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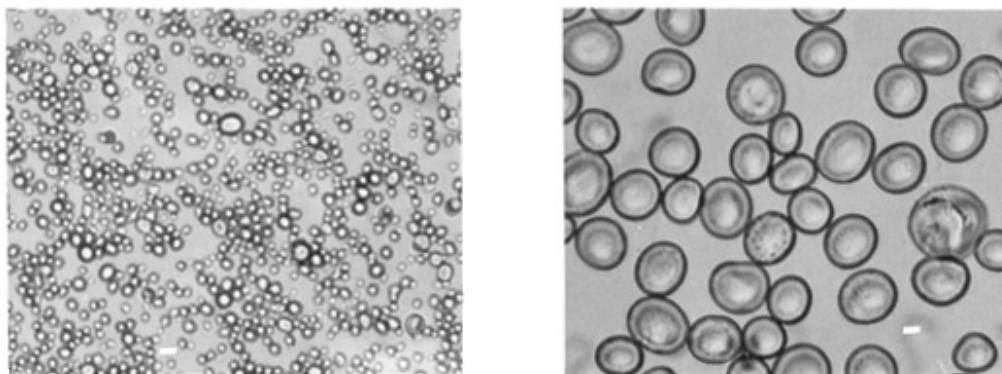
at  $3,500 \times g$  for 5 min, and the resulting starch pellet comprised the B-type starch granule population. Finally, A- and B-type starch granules were washed three times in DDW and once in acetone and then air-dried.

*Centrifugation through sucrose (80%, w/v).* The separation procedure was the same as for maltose centrifugation except that maltose was replaced by 80% (w/v) sucrose.

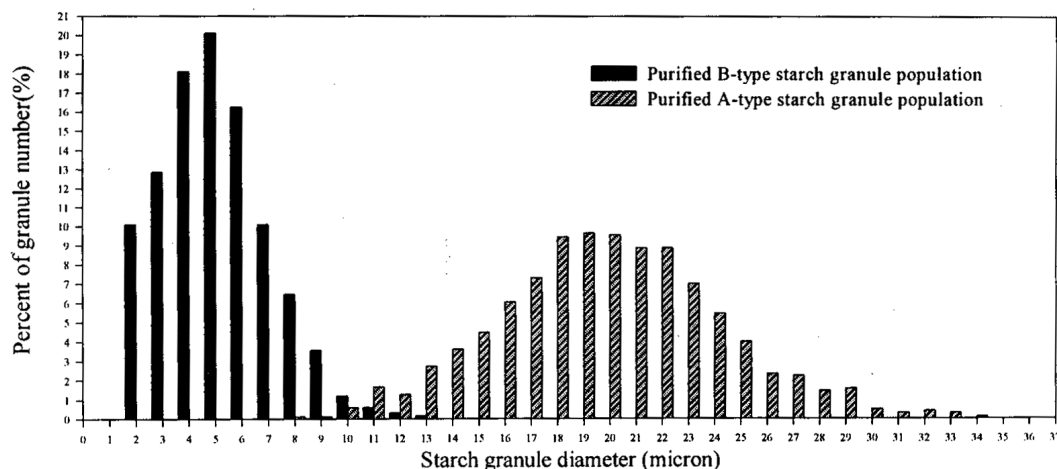
*Centrifugations through Percoll (70 and 100%, v/v).* A 5-mL starch suspension (0.1 g/mL) was laid on the top of 10 mL of 70% (v/v) Percoll solution (Pharmacia Biotech, Quebec) in a 15-mL

centrifugation tube and centrifuged at  $10 \times g$  for 10 min. The supernatant, containing B-type starch granules, was removed to another tube. The pellet was washed twice in DDW, suspended in 5 mL of DDW, and centrifuged three times in 70% (v/v) Percoll solution at  $10 \times g$  for 10 min. The pellet, containing predominantly A-type starch granules, was washed in DDW, suspended in 5 mL of DDW, and purified to a homogeneous A-type starch granule population by three cycles of Percoll (100%) centrifugation ( $10 \times g$ , 10 min in each cycle). Starch granules obtained from the pooled supernatants were considered B-type starch granules. A- and B-type

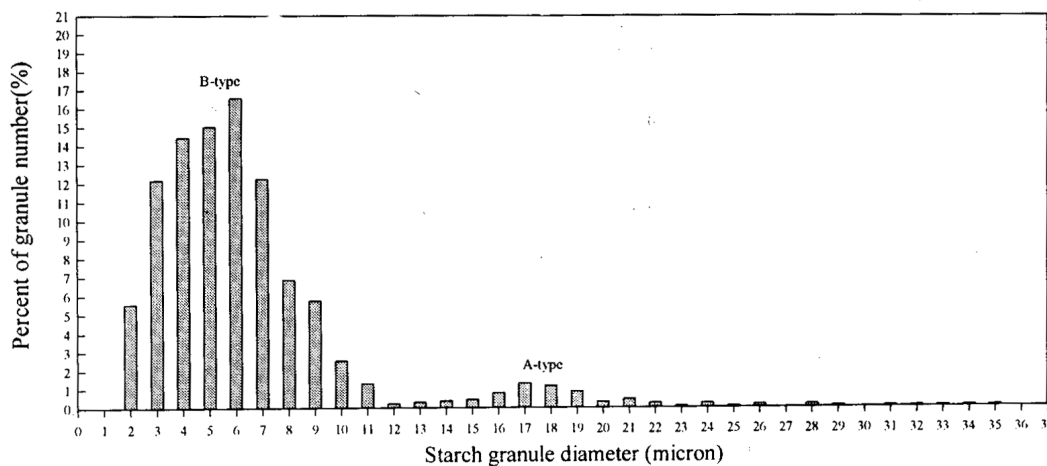
**A**



**B**



**C**



**Fig. 1.** Separation of A- and B-type starch granules. **A**, purified large A-type starch granules (right) and small B-type starch granules (left) (scale bar = 10  $\mu$ m). **B**, size-distribution curve of purified B- and A-type starch granule populations; >55% of the B-type starch granules were 4–6  $\mu$ m in diameter. In the purified A-type starch granule population, >60% were 16–22  $\mu$ m in diameter. **C**, size-distribution curve of the whole-starch granule population. Two peaks appeared: first peak (4–6  $\mu$ m) represented B-type starch granules, second peak (16–19  $\mu$ m) represented A-type starch granules.

starch granules were washed and dried as described above for maltose centrifugation.

### Starch Granule Size Analysis

The sizes of starch granules from the purified A- and B-type starch granule populations and from the whole-starch granule population (directly from wheat endosperm) were analyzed. Starch granules were suspended in 90% (v/v) ethanol. A drop of the starch suspension was spread on a microscope slide and air-dried. The slide was then placed on the stage of a light microscope (Leitz Laborluc K and D, Wetzlar, Germany). Images of starch granules were analyzed using an image analyzer equipped with image acquisition and processing software (BioQuant System IV, Image Technology, New York). More than 1,000 starch granules from each of the three starch granule populations (the purified A- and B-types and the whole-starch granule population) were analyzed. Starch granules were grouped according to diameter (Fig. 1B and C), and the number of starch granules in each group was counted. Plotting the relative number of starch granules against granule diameters produced a starch granule size-distribution curve.

### Determination of Amylose Concentration and Starch Gelatinization Properties

Starch granules were isolated from the mature endosperm of five hexaploid wheat cultivars (*T. aestivum* cvs. CDC Teal, McKenzie, AC Karma, AC Crystal, and Fielder) and one tetraploid durum wheat (*T. turgidum* cv. Plenty). A- and B-type starch granules were separated using two Percoll solution centrifugations.

The total starch concentration of A- and B-type starch granules was analyzed using the  $\alpha$ -amylase/amyloglucosidase procedure (Megazyme total starch analysis kit, Megazyme, Wicklow, Ireland) (McCleary et al 1994). A 100-mg starch sample (A- or B-type starch granules) was wetted with 0.2 mL of ethanol and completely dissolved by partial hydrolysis with thermostable  $\alpha$ -amylase. Dextrins were then quantitatively hydrolyzed to glucose by amyloglucosidase. The amount of glucose was determined, and the starch concentration was calculated on an as-is moisture basis as described by McCleary et al (1994).

Amylose concentration in A- and B-type starch granules was analyzed as described by Gibson et al (1997). A 25-mg starch sample was dissolved in 1 mL of dimethyl sulfoxide solution by boiling for 15 min. Ethanol was added to precipitate starch and to remove lipid. The starch pellet was redissolved in dimethyl sulfoxide. An aliquot (0.5 mL) was used to determine the total starch content. In another aliquot (1.0 mL), the amylopectin was precipitated with concanavalin A. The quantity of amylose remaining in the solution was determined after quantitative hydrolysis to glucose by amyloglucosidase/ $\alpha$ -amylase and was expressed as a proportion (%) of the total starch.

Gelatinization properties of A- and B-type starch granules were determined by differential scanning calorimeter (Mettler TA3000) as described by Abdel-Aal et al (1997). A starch sample (4–5 mg) was loaded into a differential scanning calorimeter pan, and the requisite amount of water was added to give a volume ratio of 3:1 between water and starch. The sample was then heated at 10°C/min and the transition temperatures recorded from a plot of heat flow versus temperature (30–100°C). The onset ( $T_o$ ), peak ( $T_p$ ), and completion ( $T_c$ ) temperatures were taken from the curve and expressed in °C. The enthalpy of gelatinization ( $\Delta H$ , J/g) was calculated from measurement of the curve area using indium as the reference standard.

## RESULTS AND DISCUSSION

### Separation of A-Type and B-Type Starch Granule Populations

Wheat A- and B-type starch granules have significantly different sizes and rates of sedimentation in a water column (Seib 1994), by which the two types of starch granules may be separated. In this

study, microsieving separated A- and B-type starch granules according to size, and centrifugation through maltose, sucrose, and Percoll separated the two types of starch granules on the basis of distinct rates of sedimentation in these solutions. Table I shows that microsieving could not completely separate A- and B-type starch granules, while centrifugation through maltose or sucrose solution produced a purified B-type starch granule population but could not give a homogeneous A-type starch granule population. However, centrifugation twice through Percoll (70 and 100%, v/v) completely separated the two types of starch granules (Fig. 1A).

In microsieving, many small B-type starch granules were attached to the surface of large A-type starch granules and were retained in the A-type starch granule population. Some large A-type starch granules passed through the nylon screen and, hence, were present with B-type starch granules in the filtrate. Eliasson and Karlsson (1983) found that the shape of starch granules also affected passage through microsieves. In this study, after microsieving, the A-type starch granule population contained  $\approx$ 67% B-type starch granules, and the B-type starch granule population had  $>$ 20% A-type starch granules (Table I).

Sedimentation in a water column and elutriation separated A- and B-type starch granules based on the difference in sedimentation rates. Because the density of water is low, the difference between sedimentation rates of A- and B-type starch granules in water is not large enough to separate the two types of granules. Therefore, neither sedimentation in a water column nor elutriation could completely separate A- and B-type starch granules (MacGregor 1979, Eliasson and Karlsson 1983). Eliasson and Karlsson (1983) reported that the A-type starch granule population obtained from sedimentation in a water column contained  $\approx$  20% (by number) B-type starch granules, while the B-type starch granule population contained  $\approx$ 30% (by weight) A-type starch granules.

We attempted to separate A- and B-type starch granules based on different sedimentation rates in maltose, sucrose, and Percoll solutions. Because maltose, sucrose, and Percoll solutions have higher density than water, they may make the sedimentation rate difference between A- and B-type starch granules large enough to separate the two types of starch granules. Thus, in the present study, centrifugation through a single concentration of maltose or sucrose or through two concentrations of Percoll were employed. The B-type starch granule populations separated by these three methods contained 100% B-type starch granules (Table I, Fig. 1A), while only centrifugation through two Percoll concentrations yielded a homogeneous A-type starch granule population (Table I, Fig. 1A). A-type starch granule populations from maltose or sucrose centrifugation contained 8–10% B-type starch granules (Table I). This is most likely due to the highly viscous solutions of 80% (w/v) maltose and sucrose, which made some B-type starch granules stick to the surface of A-type starch granules after centrifugation. Percoll is not sticky even at 100% (v/v) concentration. Thus, the separation

TABLE I  
Efficiency of Different Methods in Separating A- and B-Type Starch Granule Populations

Method	B-Type Granule Population (%)		A-Type Granule Population (%)	
	A-Type	B-Type	A-Type	B-Type
Microsieving	21.68 <sup>a</sup>	78.32	32.36	67.64
Maltose <sup>b</sup>	0	100	91.98	8.02
Sucrose <sup>c</sup>	0	100	89.56	10.44
Percoll <sup>d</sup>	0	100	100	0

<sup>a</sup> Diameter of starch granules determined with a micrometer under a light microscope. A-type starch granules  $>$  10  $\mu$ m. B-type starch granules  $<$  10  $\mu$ m.  $\approx$ 300 starch granules analyzed for each method.

<sup>b</sup> Centrifugation through maltose (80%, w/v).

<sup>c</sup> Centrifugation through sucrose (80%, w/v).

<sup>d</sup> Centrifugation through two Percoll (70 and 100%, v/v) concentrations.

of A- and B-type starch granules by centrifugation through two Percoll concentrations is complete and is not affected by the shape and size of the starch granules.

### Purified A- and B-Type Starch Granules and Their Counterparts in Mature Wheat Endosperm

Although centrifugation through two Percoll concentrations produced purified A- and B-type starch granules, some starch granules (a mixture of A- and B-types) remained in the Percoll phase even after six cycles of centrifugation through two Percoll concentrations. This gave rise to the question of whether the purified A- and B-type starch granules actually represented their counterparts in the whole-starch granule population. Analysis and comparison of the starch granule size distributions in the whole-starch granule population and the purified A- and B-type starch granule populations were used to address this question.

Starch granule sizes are usually measured by electrozone, image analysis, or laser-based time of transition methods (Maningat et al 1996). Harrigan (1997) found that using image analysis to determine starch granule size could yield accurate and reproducible data. Thus, in this study, image analysis was used to determine wheat starch granule size distribution. Plotting the relative number of starch granules against granule diameters showed that the whole-starch granule population had a typical bimodal granule size distribution (Fig. 1C). The first peak was at 4–6  $\mu\text{m}$  and represented B-type starch granules. The second peak was at 16–19  $\mu\text{m}$  and represented A-type starch granules. If >50% of the granules in the purified B-type starch granule population are 4–6  $\mu\text{m}$  in diameter and the diameters of most starch granules in the purified A-type starch granule population are in the range of the second peak, then the purified B- and A-type starch granule populations can represent their counterparts in a mature wheat endosperm. Figure 1B showed that in the purified B-type starch granule population,  $\approx$ 55% of the starch granules were 4–6  $\mu\text{m}$  in diameter, and in the purified A-type starch granule population, >60% of the starch granules were 16–22  $\mu\text{m}$  in diameter. As expected, most starch granules retained in the Percoll phase were mixtures of A- and B-type starch granules, and they ranged from 7 to 11  $\mu\text{m}$  in diameter (data not shown). These starch granules accounted for the differences between A- and B-type starch granule peaks in the starch granule size-distribution curve of the whole-starch granule population (Fig. 1C).

### Amylose Concentration and Gelatinization Properties of Wheat A- and B-Type Starch Granules

The characteristics of intact A- and B-type starch granules in mature wheat endosperm could be estimated by analysis of the chemical composition and gelatinization properties of the purified A- and B-type starch granules. In this study, amylose concentration, gelatinization temperatures, and enthalpy were used to characterize A- and B-type starch granules.

Purified A- and B-type starch granules were obtained from six wheat cultivars. The data in Table II show that A- and B-type starch granules had similar total starch concentrations but significantly different amylose concentrations. A-type starch granules contained 30–36% amylose, while B-type starch granules contained 24–27% amylose. This observation was consistent with those previously reported (Seib 1994). However, in this study, the range of difference (3–10%) in amylose concentrations between A- and B-type starch granules was much larger than those published previously (2–3%). This is most likely due to the increased purity of the A- and B-type starch granule populations. In previous studies (Eliasson and Karlsson 1983, Soulaka and Morrison 1985), A- and B-type starch granules were usually separated by sedimentation in a water column; therefore, the purity was lower than in the present study. The second reason may be that, in the current study, amylose was determined using the procedure described by Gibson et al (1997), which is a more precise method than other commonly used methods such as the measurement of the iodine binding capacity of amylose or the separation of native amylose and amylopectin by size-exclusion chromatography (Gibson et al 1997).

The gelatinization properties of wheat A- and B-type starch granules are usually measured by differential scanning calorimetry. It has been reported that A- and B-type starch granules have different gelatinization temperature regimes (Eliasson and Karlsson 1983, Soulaka and Morrison 1985). Compared with A-type starch granules, B-type granules started gelatinization at a lower  $T_0$  but had higher  $T_p$  and  $T_c$  (Seib 1994). In this study, B- and A-type starch granules started gelatinization at a similar temperature, while the  $T_p$  and  $T_c$  of B-type starch granules were higher by 1–2°C than those of A-type starch granules (Table II). This is consistent with the observations of Eliasson and Karlsson (1983) and Soulaka and Morrison (1985), but differed from the results of Ghiasi et al (1982), who reported that A- and B-type starch granules had similar gelatinization temperature regimes.

There are conflicting reports about the gelatinization enthalpy of wheat A- and B-type starch granules. Eliasson and Karlsson (1983) and Soulaka and Morrison (1985) reported that A-type starch granules had higher gelatinization enthalpy than B-type starch granules, while Stevens and Elton (1971) found the gelatinization enthalpies of wheat starch granules of different size classes to be similar. In contrast, Wootton and Bamunuarachchi (1979) reported that A-type starch granules had a lower gelatinization enthalpy than did B-type starch granules. From the measurements in this study, it was evident that the gelatinization enthalpy of A-type starch granules (10.0–12.2 J/g) was higher than that of B-type starch granules (8.0–10.0 J/g). This was consistent with the results of Eliasson and Karlsson (1983) and Soulaka and Morrison (1985). Imprecise measurements of the gelatinization enthalpy of A- and B-type starch granules were suggested as the cause of the conflicting reports (Soulaka and Morrison 1985).

TABLE II  
Amylose Concentrations and Gelatinization Properties of Purified Wheat A- and B-Type Starch Granules from Six Wheat Cultivars

Cultivar	Type	Amylose (%)	Starch (%)	Gelatinization by DSC <sup>a</sup>			
				$T_0$ (°C)	$T_p$ (°C)	$T_c$ (°C)	$\Delta H$ (J/g)
CDC Teal	A	35.54 $\pm$ 2.86 <sup>b</sup>	92.08 $\pm$ 2.17	56.2	62.2	69.4	11.2
	B	26.36 $\pm$ 2.02	91.93 $\pm$ 1.85	55.4	63.4	71.6	9.2
McKenzie	A	34.93 $\pm$ 1.62	90.41 $\pm$ 2.41	52.2	60.2	70.3	10.2
	B	25.15 $\pm$ 0.46	90.50 $\pm$ 1.46	53.8	61.3	70.8	8.0
AC Karma	A	30.94 $\pm$ 0.56	91.29 $\pm$ 2.17	53.0	60.7	70.8	12.2
	B	27.16 $\pm$ 2.37	90.98 $\pm$ 1.85	54.4	62.4	73.2	9.0
AC Crystal	A	34.09 $\pm$ 2.94	91.32 $\pm$ 1.87	54.2	61.3	71.0	10.4
	B	26.75 $\pm$ 1.04	91.80 $\pm$ 1.59	55.0	62.3	72.6	8.3
Fielder	A	29.89 $\pm$ 2.00	90.75 $\pm$ 2.36	52.8	60.6	68.0	11.4
	B	24.55 $\pm$ 1.78	92.26 $\pm$ 2.18	53.4	61.8	68.4	10.0
Plenty	A	35.42 $\pm$ 2.75	91.13 $\pm$ 1.66	52.8	58.0	63.8	10.0
	B	27.37 $\pm$ 1.32	91.64 $\pm$ 1.52	52.2	59.6	69.6	8.8

<sup>a</sup> Differential scanning calorimetry.

<sup>b</sup> Mean  $\pm$  standard error. Values calculated from three repetitions.

## CONCLUSIONS

Starch granules from mature wheat endosperm had a typical bimodal granule size distribution. The first peak represented small, B-type starch granules, and the second represented large, A-type granules. Centrifugation through two Percoll concentrations completely separated A- and B-type starch granules. The purified A- and B-type starch granules were confirmed to represent their counterparts in mature wheat endosperm. Thus, the characteristics of intact A- and B-type starch granules in mature wheat endosperm could be estimated by analysis of the chemical composition and gelatinization properties of the purified A- or B-type starch granules. Our experimental results demonstrated that A- and B-type starch granules have significantly different chemical compositions and gelatinization properties, indicating that they may have distinct applications in food and nonfood industries.

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