



Starch granule size distribution of hard red winter and hard red spring wheat: Its effects on mixing and breadmaking quality

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ABSTRACT

Starch was isolated from 98 hard red winter (HRW) wheat and 99 hard red spring (HRS) wheats. Granule size/volume distributions of the isolated starches were analyzed using a laser diffraction particle size analyzer. There were significant differences in the size distribution between HRW and HRS wheats. The B-granules (<10 μm in diameter) occupied volumes in the range 28.5–49.1% (mean, 39.9%) for HRW wheat, while HRS wheat B-granules occupied volumes in the range 37.1–56.2% (mean, 47.3%). The mean granule sizes of the distribution peaks less than 10 μm in diameter also showed a significant difference (HRW, 4.32 vs. HRS, 4.49 μm), but the mean sizes of the distribution peaks larger than 10 μm were not significantly different (21.54 vs. 21.47 μm). Numerous wheat and flour quality traits also showed significant correlation to starch granule size distributions. Most notably, protein content was inversely correlated with parameters of B-granules. Crumb grain score appeared to be affected by starch granule size distribution, showing significant inverse correlations with B-granules. Furthermore, the linear correlations were improved when the ratio of B-granules to protein content was used, and the polynomial relation was applied. There also appeared to be an optimum range of B-granules for different protein content flour to produce bread with better crumb grain.

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1. Introduction

Starch is an important part of wheat endosperm, not only because starch accounts for 65–73% of dry flour mass when milling extraction is <80% (Pomeranz, 1988), but also because wheat starch has unique properties in breadmaking that are not replaceable by other starches from corn, potato, and cassava (Hoseney et al., 1971; Sahlstrom et al., 1998; Sollars and Rubenthaler, 1971). Wheat starch granules have been reported to have bimodal size distribution (Dengate and Meredith, 1984; Evers and Lindley, 1977; Karlsson et al., 1983; Morrison and Scott, 1986; Simmonds and O'Brien, 1981; Stoddard, 1999), but trimodal size distributions have also been reported (Bechtel et al., 1990; Raeker et al., 1998). The large A-granules (generally larger than 10 μm in diameter) are formed first in developing endosperm, whereas the small B-granules (smaller than 10 μm in diameter) are formed late in kernel development (Dengate and Meredith, 1984; Karlsson et al., 1983; Sandstedt, 1961). Bechtel et al. (1990) reported the formation of very small C-type granules (less than 5 μm) that were initiated very late in grain filling. Different size starch granules have different physical, chemical, and functional properties (Chiotelli and Le

Meste, 2002; Dronzek et al., 1972; Eliasson, 1989; Kulp, 1973; Lineback, 1984; Meredith, 1981; Morrison and Gadan, 1987; Nikuni, 1978; Park et al., 2004; Peng et al., 1999; Soulaka and Morrison, 1985a). In addition, there has been considerable debate on the role of B-granules in breadmaking, which was discussed in a previous report by Park et al. (2005).

Starch granule size distribution is an important factor that affects the quality of many final products. A wide variation (17–50%) of B-starch granule volume was found from a survey of hexaploid wheats, suggesting prospects of genetic manipulation of granule size distribution (Stoddard, 1999). However, there has been limited research conducted to find relationships between starch granule size distribution and final product quality. Also, there are no reports comparing the starch granule size distribution of HRW and HRS. Therefore, the objective of this study was to investigate starch granule size distribution of HRW and HRS, and determine its relationship to wheat, flour, dough mixing, and breadmaking properties.

2. Experimental

2.1. Materials

A total of 197 wheat samples, including 98 hard red winter and 99 hard red spring harvested in 2002 and 2003, were provided by

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Nomenclature

A-granules	larger than 10 μm in diameter
B-granules	smaller than 10 μm in diameter
HRS	hard red spring
HRW	hard red winter
r	simple correlation coefficient

R^2	coefficient of determinant
*	$P < 0.01$
**	$P < 0.001$
***	$P < 0.0001$
SEM	standard error of the mean
SKCS	single kernel characterization system

the Federal Grain Inspection Service (FGIS) Technical Center, (Kansas City, MO), Grain Inspection, Packers, and Stockyards Administration (GIPSA), U.S. Department of Agriculture. Detailed information about the samples has been previously reported (Maghirang et al., 2006).

2.2. Starch isolation and determination of granule size distribution

Starch was isolated by enzymatic digestion using pepsin A (P7012, Sigma, St. Louis, MO), hemicellulase 90 (90,000 U/g activity, Amano Enzyme U.S.A., Lombard, IL), and cleaned further by a detergent mix (5% SDS, 5% Triton X-100, 5% Tween 40, and 5% Triton X-15) (Bechtel and Wilson, 2000).

The size distribution of isolated starch granules was measured using a single wavelength Beckman Coulter LS 13 320 Particle Size Analyzer (Beckman Coulter, Miami, FL) with the Universal Liquid Module for liquid-based measurements. Each starch sample was slurried with 1.0 mL of water and vortexed before analysis. The standard refractive indices used were 1.31 for water and 1.52 for starch, which is within the sample concentration range of the instrument's specifications. Volumes of all starch granules were calculated on the assumption that all granules were spherical in shape.

2.3. Evaluation of wheat and flour properties

The following properties of wheat were analyzed: test weight (lb/bu) by American Association of Cereal Chemists (AACC, 2000) Approved Method 55-10; protein content using near-infrared reflectance (AACC Approved Method 39-25); single kernel hardness (AACC Approved Method 55-31), weight, and size using the SKCS 4100 (Perten, Springfield, IL); and ash content (AACC Approved Method 08-01). Wheat was milled using a Brabender Quadrumat Sr. experimental mill (AACC Approved Method 26-10A). Flour protein and ash content were measured using AACC Approved Method 39-11 and 08-01, respectively. Flour color (L^* , a^* , and b^*) was determined using a colorimeter (CR-300, Minolta, Osaka, Japan). Mixing characteristics of flour were evaluated using Mixograph (AACC Approved Method 54-40A) and Farinograph (AACC Approved Method 54-21). A modified optimized straight-dough breadmaking method (AACC Approved Method 10-10B) was used for evaluation of experimental breadmaking properties of flours. The detailed baking method and description of crumb grain score were reported previously (Park et al., 2004). All tests were conducted with at least 2 duplicates.

2.4. Statistical analysis

A complete randomized experimental design was used. The difference between starch granule size and volume distributions of HRW and HRS was analyzed using the General Linear Models procedure of the Statistical Analysis System (SAS Institute, Cary, NC). Statistical abbreviations were simple correlation coefficient (r), coefficient of determinant (R^2), $P < 0.01$ (*), $P < 0.001$ (**), $P < 0.0001$ (***), and standard error of the mean (SEM).

3. Results and discussion

3.1. Starch granule size distributions of HRW and HRS

Fig. 1 shows a typical bimodal size distribution of wheat starch granules, plotting proportions by volume % of granules in equal diameter intervals against diameter. Point 1 and 3 represent the most frequent differential volume (%) of the small and large granules, and Point 2 is differential volume (%) between small and large granules that sets apart small and large starch granules. The respective integrating areas (%) (A, B, and C) represent proportion of volume distribution (%) split by each point.

Starch granule size distributions of HRW ($n = 98$) and HRS ($n = 99$) showed significant differences in mean values of different aspects of granule size distribution including differential volume, diameter, and volume distribution (Table 1). Table 1 shows that HRW has less B-granules as indicated by lower differential volume compared to HRS wheat (2.25% vs. 2.80%, respectively). Differential volumes of HRW and HRS wheats at Point 2, however, were not significantly different. At Point 3, differential volume for HRW wheat was higher (6.55%) than that of HRS (6.03%), and is due to the fact that differential volume (%) represents proportionality. HRW and HRS wheats have similar starch granule size distribution range from less than 1 μm to about 40 μm , thus, a lower % in one proportion results in higher % proportion elsewhere. Fig. 2 shows typical starch granule size distribution curves of HRW and HRS wheats, demonstrating that HRS wheat had higher and lower differential volumes at Point 1 and 3, respectively, compared with HRW wheat. It should be noted that even though these curves (differential volume) were obtained from calculations using real numbers of starch granules at a specific size, the differential volume represents the proportion of starch granule size distribution in the kernel and not the actual volume.

The mean values of starch granule diameter at each point, compared to differential volume, represent actual size. HRW had significantly smaller size of B-granules (4.32 μm) compared with

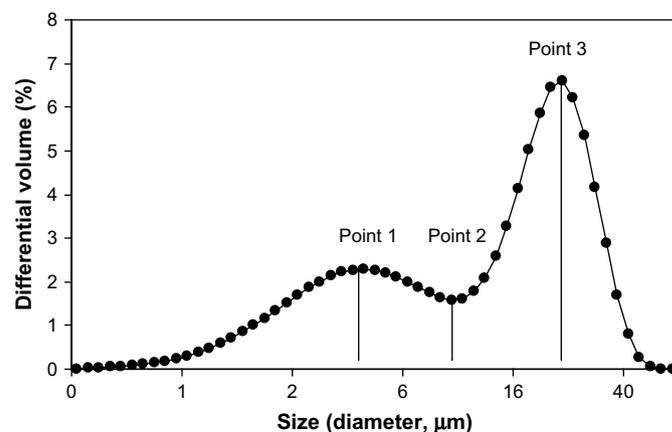


Fig. 1. Starch granule size distribution indicating high and low points (Point 1, 2, and 3) of differential volume and their volume percents (Area A, B, C).

Table 1
Starch granule size distribution in differential volume (%), diameter (μm), and volume percent distribution (%) of HRW and HRS^a

Size Distribution ^b	HRW				HRS			
	Mean	Minimum	Maximum	SEM ^c	Mean	Minimum	Maximum	SEM ^c
<i>Differential volume (%)</i>								
Point 1	2.25***	1.30	3.15	0.025	2.80***	1.91	4.12	0.029
Point 2	1.53	0.91	1.96	0.015	1.56	0.73	2.14	0.022
Point 3	6.55***	5.17	7.91	0.029	6.03***	4.74	7.20	0.034
<i>Diameter (μm)</i>								
Point 1	4.32***	3.52	5.11	0.030	4.49***	3.86	5.11	0.029
Point 2	8.72***	5.61	10.78	0.063	9.46***	8.15	10.78	0.043
Point 3	21.49	18.86	24.95	0.087	21.46	18.86	24.95	0.088
<i>Volume distribution (%)</i>								
A	23.53***	14.94	30.87	0.245	29.35***	20.06	38.24	0.254
A + B	38.01***	12.57	49.07	0.402	46.88***	34.03	57.81	0.308
A + B + C	75.78***	57.59	89.00	0.300	80.05***	73.34	87.30	0.180

^a *** = Mean values of HRW and HRS in the same row are significantly different at $P = 0.0001$.

^b Explanation of specific points and area are given in Fig. 1.

^c SEM = standard error mean.

HRS (4.49 μm) at Point 1 (Table 1). The observation that HRW wheat had a smaller size of B-granules compared with HRS wheat was confirmed by the fact that the threshold diameters at Point 2 were 8.72 μm for HRW wheat and 9.46 μm for HRS wheat. The mean diameter of A-granules, however, was not significantly different at Point 3 (21.49 and 21.46 μm , respectively).

The HRW and HRS wheats showed a wide range of volume distributions in terms of A- and B-granules. The volume distribution of HRW wheat B-granules ranged from 12.57% to 49.07%, while HRS wheat B-granules ranged from 34.03% to 57.81% (Table 1, Fig. 1). The mean volume distributions of HRW wheat B-granules were significantly lower (23.53% and 38.01% for area A and A + B, respectively) than that of HRS (29.35% and 46.88% for area A and A + B, respectively). It is obvious that HRW wheat had smaller B-granules in size and number.

The volume distributions of subdivided ranges of starch granule size distribution are shown in Table 2. This data confirms that HRW wheat has significantly smaller proportions of B-granules (less than 10 μm) compared with HRS wheat in our study.

Specific volume ranges for A- and B-granules derived from HRW and HRS wheats have not been reported in the literature, and most

previous reports on the proportion of B-granules in total starch were on a weight basis (Brocklehurst and Evers, 1977; D'Appolonia and Gilles, 1971; Dengate and Meredith, 1984; Evers, 1973; Evers and Lindley, 1977; Hughes and Briarty, 1976; Meredith, 1981; Park et al., 2004; Soulaka and Morrison, 1985a). Our data, however, can still be compared with previous reports because the difference in density of A- and B-starch granules is negligible (Dengate et al., 1979; Meredith, 1981). Our volume data on the range of B-granules was higher than most previous reports. D'Appolonia and Gilles (1971) reported less than 10% B-granules by weight in total starch from 12 HRS flours, whereas others reported 30% B-granules (Evers, 1973; Evers and Lindley, 1977; Hughes and Briarty, 1976). Park et al. (2004) observed 15.7–27.0% B-granules from 12 HRW wheat flours, and Soulaka and Morrison (1985a) found 13–35%. Stoddard (1999) reported a similar range of B-granule volume (%), showing 17–50% of B-granules using laser diffraction sizing methods from 130 Australian hexaploid wheat cultivars and Landraces from Asia. Bechtel et al. (1990) found 48% of B- and C-type granules (less than 15.9 μm) from mature HRW wheat, but this discrimination was larger than the commonly used 10- μm range. These different observations on the proportion of B-granules in total starch were most likely due to different cultivars, growing conditions, and starch extraction and analysis methods.

The smaller size and number of B-granules in HRW wheat compared with HRS wheat could be a characteristic of wheat class, or it could be caused by higher temperature during the grain-filling period. Dengate and Meredith (1984) found that drought affected granule size distribution (weight %), decreasing B-granules (4–10 μm) and increasing smaller A-granules (10–20 μm). The volume % of B-granules decreased when growth temperature was increased from 15 °C to 40 °C during grain-filling period (Shi et al., 1994). High temperature also seemed to be associated with a reduction in the number of B-granules (Bhullar and Jenner, 1985). Barley, which also has a bimodal starch granule size distribution, showed a similar low ratio in number of B-granules induced by high temperature (MacLeod and Duffus, 1988; Tester et al., 1991). These environmental effects could cause a difference in the grain-filling pattern between A- and B-granules. The A-granule starts to form in the amyloplast at about 4–5 days after anthesis (Bechtel et al., 1990; Parker, 1985), and continues to increase in size until reaching a maximum diameter of 25–50 μm at physiological maturity (Bechtel et al., 1990; Dengate and Meredith, 1984; Simmonds and O'Brien, 1981). The final number of A-amyloplasts, however, is achieved much earlier at approximately seven days post-anthesis when cell division ceases (Briarty et al., 1979). On the other hand,

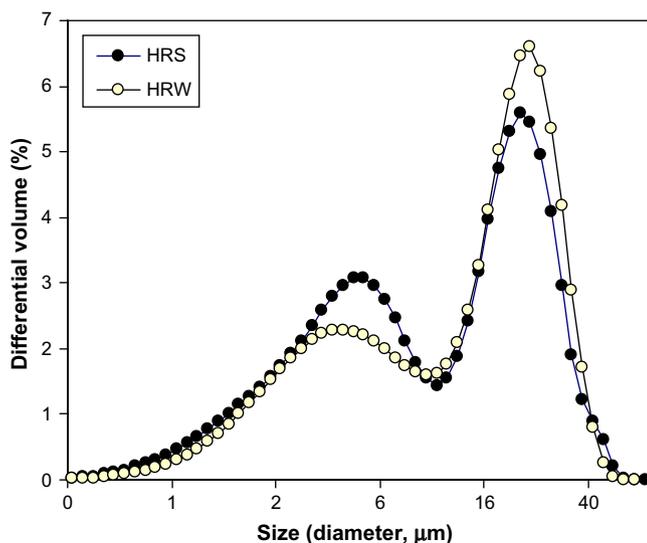


Fig. 2. Starch granule size distribution of HRW (GIPSA identification number: 03027680) and HRS (GIPSA identification number: 03036243).

Table 2
Volume percent distribution (%) on different ranges of granule size of HRW and HRS^a

Range (μm)	HRW				HRS			
	Mean	Minimum	Maximum	SEM ^b	Mean	Minimum	Maximum	SEM ^b
<2	6.6***	3.9	8.2	0.06	8.2***	6.1	10.1	0.05
2–5	18.5***	12.0	23.1	0.16	22.1***	16.5	26.8	0.13
5–10	14.7***	11.8	18.5	0.10	17.1***	13.7	22.1	0.11
10–20	26.8***	19.5	36.7	0.20	24.6***	17.7	29.9	0.18
20–30	23.7***	18.6	27.7	0.12	21.6***	16.3	26.1	0.13
>30	9.6***	2.2	22.9	0.24	6.5***	3.3	13.3	0.13
<5	25.2***	16.4	30.6	0.20	30.2***	23.2	36.6	0.17
<10	39.9***	28.5	49.1	0.28	47.3***	37.1	56.2	0.25

^a *** = Mean values of HRW and HRS in the same row are significantly different at $P < 0.0001$.

^b SEM = standard error mean.

B-granules are initiated at 10–12 days after anthesis and continue to enlarge until 21 days post-anthesis, and up to approximately 9 μm by maturity (35 days after anthesis) (Bechtel et al., 1990). Thus, considering the nature of the growth pattern of B-granules that initiate and enlarge toward the end of the grain-filling period, a high temperature event could cause a decrease in starch synthase activity and reduce the duration of grain filling, resulting in smaller size and number of B-granules in the endosperm. The U.S. Wheat Associates' harvest report of 2002 stated that 2002 HRW wheat production was the lowest since 1970 because of persistent drought conditions throughout the Great Plains region (U.S. Wheat Associate, 2002). In this study, however, we could not verify that drought conditions or high temperatures caused smaller size and number of B-granules in HRW wheat due to lack of detailed weather information concerning growing conditions for tested wheats, and no HRW wheats were grown in the HRS wheat growing region or vice versa. In addition, there is no previous comprehensive study on this subject because various U.S. wheat varieties adapt to diverse growing regions and respond differently to the environment (Altenbach et al., 2003).

It must also be pointed out that our volume data, which was generated by laser diffraction analysis, was calculated based on the assumption that all starch granules were spherical in shape. Starch granules larger than 5 μm in diameter are typically oblate spheroids in shape (Bechtel et al., 1990; Bechtel and Wilson, 2000). Therefore, differential volume of granules over 5 μm in diameter in this study could be over-estimated. In particular, the volume of small granules between 5 and 10 μm could be over-estimated because the number of B-granules composes over 90% of total number of starch granules (Evers and Lindley, 1977; Morrison and Gadan, 1987; Park et al., 2004). Wilson et al. (2006) found that volume data obtained from laser diffraction analysis needed a correction factor for better correlation with data from digital image analysis.

3.2. Relationships with wheat and flour properties

Starch granule size distribution showed many significant correlations with wheat and flour properties. HRS wheat, in particular, was highly correlated when compared with HRW wheat (Table 3). Since we do not know the cause of this difference, our discussion at this point will focus primarily on HRS wheat.

Protein content in HRS wheat showed negative correlations with differential volume at Point 1 ($r = -0.72^{***}$), but displayed positive relationships at Point 2 ($r = 0.48^{***}$) and 3 ($r = 0.62^{***}$). HRW showed similar relationships, but there was no significant correlation at Point 3. Starch granule size (diameter) at Points 1 and 2 was negatively correlated with HRS wheat protein content ($r = -0.52^{***}$ and $r = -0.67^{***}$, respectively), although there was no significant correlation at Point 3. In addition, area range A and A + B, representing volume proportion of B-granules, showed negative correlations with wheat protein content. All relationships

between wheat protein content and starch granule size distribution indicated that size and number of B-granules decrease when protein content increases, or vice versa. Altenbach et al. (2003) reported that in HRS wheat exposed to high temperature regimes (37/17 or 37/27 °C day/night), onset and cessation of starch accumulation occurred earlier and overall accumulation period was shortened. They, however, observed slight changes in the timing and duration of protein accumulation during the temperature regime. In addition, previous studies reported that high temperature stress during maturation significantly reduced kernel weight as much as 85% (Stone and Nicolas, 1994; Tashiro and Wardlaw, 1990). Therefore, it is highly likely for individual kernels to have high protein content on a percentage basis when they were exposed to high temperature, and as described earlier, high temperature affects the starch granule synthase, thus decreasing the number and size of B-granules. This may be an explanation as to why wheat protein content (%) showed inverse correlations to B-granules, including differential volume at Point 1 ($r = -0.72^{***}$), diameter at Points 1 and 2 ($r = -0.52^{***}$ and -0.67^{***} , respectively), and area range of A and A + B ($r = -0.68^{***}$ and -0.66^{***} , respectively).

On the other hand, test weight was positively correlated with B-granule parameters, such as differential volume at Point 1 ($r = 0.72^{***}$), diameter at Points 1 and 2 ($r = 0.45^{***}$ and 0.64^{***} , respectively), and area range A and A + B ($r = 0.70^{***}$ and 0.71^{***} , respectively). Test weight generally increased when kernel weight increased (Ohm et al., 1998), and kernel weight and dimensions are decreased by high temperature during grain filling (Gibson et al., 1998; Gibson and Paulsen, 1999; Tashiro and Wardlaw, 1990). Since wheat kernels exposed to high temperature tend to have a low proportion of B-granules, this could explain the positive correlation between test weight and volume of B-granules. The proportion of large kernels and single kernel weight and size showed similar relationships with parameters of starch granule size distribution because of the reasons we mentioned earlier (Table 3). Therefore, our data confirms previous reports and links the separate observations with this large data set. Flour protein showed similar relationships as wheat protein, as expected. Flour brightness (L), showed positive correlation with parameters of B-granules, possibly due to the inverse correlation between flour brightness and flour protein content ($r = -0.56^{***}$, data not shown). In other words, flour color gets darker as protein content increases, and as explained previously, wheat kernels with high protein content tend to have a smaller ratio of B-granules.

3.3. Relationships with mixing properties

The relationship between mixing properties and starch granule size distribution showed similar trends to the relationship between protein content and starch granule size distribution (Table 4). In HRS wheat, differential volume at Point 1 ($r = -0.67^{***}$), diameter

Table 3
Correlations between starch granule size distribution of HRW and HRS and wheat and flour properties

	Differential volume (%)						Diameter (μm)						Area range (%)					
	Point 1		Point 2		Point 3		Point 1		Point 2		Point 3		A	A + B	A + B + C			
<i>HRW</i>																		
Wheat protein (%)	-0.39	***	0.40	***					-0.49	***	-0.47	***						
Test weight (bu/lb)					-0.36	**			0.32	*								
<i>Kernel size distribution (%)</i>																		
Large kernel	0.56	***	-0.32	*	-0.40	***	0.33	**	0.61	***	0.28	*	0.40	***	0.41	***		
Medium kernel	-0.56	***	0.33	**	0.39	***	-0.31	*	-0.60	***	-0.28	*	-0.39	***	-0.41	***		
Small kernel	-0.28	*					-0.28	*	-0.36	**			-0.27	*				
<i>Single kernel character</i>																		
Weight (mg)	0.40	***			-0.38	***			0.48	***	0.34	**			0.29	*		
Size (mm)	0.49	***			-0.45	***	0.30	*	0.53	***	0.30	*	0.33	**	0.36	**		
Hardness	-0.29	*			0.30	*			-0.31	*								
Flour protein (%)	-0.35	**	0.41	***					-0.44	***	-0.45	***						
Flour color: L	0.32	*			-0.41	***			0.32	*								
<i>HRS</i>																		
Wheat protein (%)	-0.72	***	0.48	***	0.62	***	-0.52	***	-0.67	***		-0.68	***	-0.66	***	-0.30	*	
Test weight (bu/lb)	0.72	***	-0.41	***	-0.67	***	0.45	***	0.64	***		0.70	***	0.71	***	0.26	*	
<i>Kernel size distribution (%)</i>																		
Large kernel	0.68	***	-0.50	***	-0.60	***	0.53	***	0.70	***	0.30	*	0.67	***	0.63	***	0.29	*
Medium kernel	-0.68	***	0.50	***	0.60	***	-0.53	***	-0.70	***	-0.30	*	-0.67	***	-0.64	***	-0.29	*
Small kernel	-0.29	*	0.28	*	0.29	*			-0.28	*								
<i>Single kernel character</i>																		
Weight (mg)	0.59	***	-0.49	***	-0.53	***	0.56	***	0.48	***	0.27	*	0.59	***	0.45	***		
Size (mm)	0.63	***	-0.44	***	-0.58	***	0.57	***	0.54	***			0.63	***	0.53	***		
Hardness							-0.29	*			-0.27	*						
Flour protein (%)	-0.72	***	0.51	***	0.59	***	-0.54	***	-0.70	***	-0.29	*	-0.68	***	-0.64	***	-0.28	*
Flour color: L	0.53	***	-0.33	**	-0.50	***	0.44	***	0.52	***			0.54	***	0.50	***	0.31	*

*, **, and *** = significant correlations at $P < 0.01$, $P < 0.001$, and $P < 0.0001$, respectively.

at Points 1 and 2 ($r = -0.53^{***}$, -0.65^{***} , respectively), and area range A and A + B ($r = -0.64^{***}$, -0.59^{***} , respectively) showed significant inverse correlations with mixing absorption. This observation is most likely due to mixing absorption being generally positively correlated to protein content (Ohm and Chung, 1999; Park et al., 2006). The B-granules were reported to have higher

swelling power (intragranular plus entrained intergranular water), which was due to higher water absorption capability, and was triggered only when temperature was much higher than normal mixing temperature, above 90 °C (Kulp, 1973; Park et al., 2004; Seib, 1994; Wong and Lelievre, 1982). Haraszi et al. (2004) observed a decrease in water absorption when protein content decreased

Table 4
Correlations between starch granule size distribution and mixing properties

	Differential volume (%)						Diameter (μm)						Area range (%)					
	Point 1		Point 2		Point 3		Point 1		Point 2		Point 3		A	A + B	A + B + C			
<i>HRW</i>																		
<i>Mixograph</i>																		
Absorption	-0.30	*	0.42	***					-0.40	***	-0.42	***						
Mix time																		
Mix tolerance							0.26	*										
<i>Farinograph</i>																		
Absorption			0.35	**					-0.30	*	-0.36	**						
Development time	-0.32	*	0.32	*	0.28	*			-0.31	*	-0.39	***						
Stability																		
Tolerance	0.34	**																
Breakdown	-0.29	*	0.36	**							-0.34	**						
<i>HRS</i>																		
<i>Mixograph</i>																		
Absorption	-0.67	***	0.47	***	0.56	***	-0.53	***	-0.65	***	-0.30	*	-0.64	***	-0.59	***	-0.27	*
Mix time	-0.59	***	0.29	*	0.53	***	-0.36	**	-0.45	***			-0.58	***	-0.57	***	-0.35	**
Mix tolerance	-0.55	***	0.35	**	0.47	***	-0.41	***	-0.39	***			-0.55	***	-0.49	***	-0.32	*
<i>Farinograph</i>																		
Absorption	-0.29	*							-0.38	***			-0.29	*	-0.35	**		
Development time	-0.59	***	0.33	**	0.49	***	-0.37	**	-0.54	***			-0.59	***	-0.61	***	-0.31	*
Stability	-0.48	***	0.38	***	0.41	***	-0.46	***	-0.48	***			-0.48	***	-0.40	***		
Tolerance	0.33	**							0.39	***	0.34	**	0.27	*	0.29	**		
Breakdown	-0.62	***	0.37	**	0.52	***	-0.43	***	-0.60	***			-0.61	***	-0.61	***	-0.29	*

*, **, and *** = significant correlations at $P < 0.01$, $P < 0.001$, and $P < 0.0001$, respectively.

with starch addition. Also, mixing absorption is highly influenced by protein subclass, 50% 1-propanol insoluble polymeric protein content in flour (Park et al., 2006). HRW wheat showed similar trends, but with rather weak and insignificant relationships.

Mixograph mixing time in HRS wheat showed negative correlations with B-granules, whereas in HRW wheat, a positive correlation was observed between mix time and differential volume at Point 1 (Table 4). Park et al. (2004) found that mix time did not show significant correlations to protein content with 49 HRW wheat flours due to contrasting effects among protein subclass on mix time. However, they suggested that mix time would be shorter as protein content increases because they also found that 50% 1-propanol insoluble polymeric protein based on total protein was positively correlated with mix time and tended to decrease with increasing total protein content. Consequently, considering significant inverse correlations between protein content and parameters of B-granules, the relationship between mix time and parameters of B-granules was expected to be positive. The positive correlation ($r=0.33^{**}$), however, was obtained only from HRW wheat. The inverse correlations between HRS wheat and parameters of B-granules were probably due in part to a positive correlation between protein content and mix time ($r=0.44^{***}$, data not shown) in HRS wheat. In HRW wheat, there was no significant correlation between protein content and mix time. It has also been reported that B-granules require shorter optimum mix time compared with A-granules (D'Appolonia and Gilles, 1971; Petrofsky and Hosenev, 1995). Chiotelli and Le Meste (2002) also reported that B-granules had a higher affinity for water than the A-granules at room temperature, resulting in faster hydration. Therefore, the present work suggests that a higher proportion of B-granules in HRS wheat may partially account for the inverse relationship between mix time and parameters of B-granules.

Farinograph absorption showed weak but significant inverse correlations to parameters of B-granules in HRS wheat, whereas in HRW wheat, diameter at Point 2 showed significant inverse correlation (Table 4). A similar reason, as given for Mixograph mix absorption, could explain these relationships. Farinograph absorption was positively correlated with wheat protein ($r=0.71^{***}$, data not shown), and parameters of B-granules were inversely correlated to protein content, resulting in negative correlations between Farinograph absorption and parameters of B-granules. Soh et al. (2006) observed a significant increase in Farinograph absorption at constant protein content (17.4–17.7%) as % B-granules increased from 17% to 32.4%. Therefore, it appears that starch granule size distribution may affect Farinograph absorption when protein content and quality are invariable. Our data suggests that starch granule size distribution may not be an important variable for mix absorption when protein content varies. The protein content in the HRS wheat flour varied widely from 10.6% to 17.8% (data not shown), and the protein composition (quality) was altered as the protein content increased (Park et al., 2006).

The relationship between Farinograph development time and starch granule size distribution was similar to the relationship between Mixograph mix time and starch granule size distribution. The same explanation would be applicable for both relationships.

Farinograph stability in HRS wheat also showed inverse correlation with parameters of B-granules. Several authors have reported the effects of starch on rheological properties. B- and non-wheat starch granules have been reported to result in large rheological differences using a constant vital gluten ratio in the reconstituted dough (Petrofsky and Hosenev, 1995). They suggested that there were strong interactions between vital gluten and non-wheat starches and wheat B-granules, resulting in less extensibility. Miller and Hosenev (1999) found that starch isolated from Kansas grown wheat gave significantly lower elastic modulus (G') and viscous modulus (G'') than starches from other Kansas and strong

Canadian wheats when dough was prepared from the same gluten source. Thus, it appears that starch from different cultivars and different size distributions makes a difference in determining the rheological properties of dough when a constant gluten source is used. The inverse relationships between Farinograph stability and parameters of B-granules, however, are hard to explain from previous reports (Miller and Hosenev, 1999; Petrofsky and Hosenev, 1995) for two reasons. First, the present study used actual HRS wheat flours, and not blends of starches and constant gluten, as did previous researches, which had various components that could affect mixing properties. Second, there is limited research to connect rheological parameters of B-granules to Farinograph stability. It is generally recognized that rheological development in dough is triggered by the formation of a continuous gluten network (MacRitchie, 1992), with starch granules embedded in the network. In addition, previous studies reported that breakdown (loss of stability) during over-mixing was caused by “depolymerization” of the protein network (Danno and Hosenev, 1982; Skerritt et al., 1999; Tanaka and Bushuk, 1973; Weegels et al., 1996). Therefore, with large variation in flour quality including protein content (10.6–17.8%, 14% moist base), Farinograph absorption (59.8–73.1%), development time (5.2–44.5 min), and loaf volume (803–1238 cm³) (data not shown), inverse relationships between Farinograph stability and parameters of B-granules could be explained by the positive correlations between protein content and Farinograph stability ($r=0.46^{***}$, data not shown). As previously discussed, protein content was inversely correlated to parameters of B-granules, possibly giving the inverse correlation between Farinograph stability and parameters of B-granules. Farinograph breakdown showed a similar relationship with stability, and a similar explanation could be applicable. Protein content and breakdown were highly positively correlated ($r=0.79^{***}$, data not shown). The HRW wheat showed a similar trend in these relationships, but was weaker with many non-significant correlations.

3.4. Relationships with breadmaking parameters

The breadmaking parameters showed significant correlations to starch granule size distribution (Table 5). A greater number of the parameters of HRS wheat, again, were significantly correlated. The trends in relationships were similar for HRW and HRS wheat, so our discussion will focus on HRS wheat.

Baking absorption and mix time were positively correlated to Mixograph absorption ($r=0.85^{***}$), mix time ($r=0.93^{***}$), Farinograph absorption ($r=0.73^{***}$) and development time ($r=0.77^{***}$) (data not shown).

Loaf volume was inversely correlated with parameters of B-granules. Differential volume at Point 1 showed the greatest inverse correlation ($r=-0.68^{***}$), followed by area range A ($r=-0.66^{***}$), A+B ($r=-0.62^{***}$) and diameter at Point 1 ($r=-0.52^{***}$). Park et al. (2005) summarized previous studies using four different concepts concerning the effects of small starch granule size on breadmaking properties: “beneficial” (Hayman et al., 1998; Sahlstrom et al., 1998; Van Vliet et al., 1992); “detrimental” (D'Appolonia and Gilles, 1971; Kulp, 1973); “little effect” (Hosenev et al., 1971); and “optimum ratio” of A- and B-granules (Lelievre et al., 1987; Park et al., 2004; Soulaka and Morrison, 1985b). More recently, Park et al. (2005) confirmed their previous observation that there seemed to be an optimum weight ratio of A- and B-granules for crumb grain score, and there was no benefit of small starch granules to loaf volume and crumb grain score when used with a constant source of gluten. Considering previous controversial results, it is difficult to state that B-granules affect loaf volume negatively. It is more appropriate to admit that the relationships were obtained due to a positive correlation between protein content and loaf volume ($r=0.91^{***}$, data not shown), and

Table 5
Correlations between starch granule size distribution and straight-dough breadmaking parameters

	Differential volume (%)						Diameter (μm)			Area range (%)								
	Point 1	Point 2	Point 3	Point 1	Point 2	Point 3	A	A + B	A + B + C									
HRW																		
<i>Breadmaking</i>																		
Absorption		0.35	**					−0.28	*	−0.26	*							
Mix time																		
Proof height	−0.46	***	0.38	***	0.31	*	−0.45	***	−0.51	***	−0.26	*	−0.28	*	−0.27	*		
Crumb grain score			0.35	**														
Loaf volume	−0.35	**	0.43	***			−0.28	*	−0.42	***	−0.40	***						
HRS																		
<i>Breadmaking</i>																		
Absorption	−0.57	***	0.30	*	0.52	***	−0.40	***	−0.57	***	−0.28	*	−0.56	***	−0.58	***	−0.37	**
Mix time	−0.52	***			0.46	***	−0.34	**	−0.43	***			−0.52	***	−0.53	***	−0.36	**
Proof height	−0.56	***	0.34	**	0.51	***	−0.37	**	−0.47	***			−0.54	***	−0.52	***		
Crumb grain score	−0.28	*			0.27	*	−0.41	***					−0.27	*				
Loaf volume	−0.68	***	0.45	***	0.60	***	−0.52	***	−0.67	***	−0.28	*	−0.66	***	−0.62	***	−0.28	*

*, **, and *** = significant correlations at $P < 0.01$, $P < 0.001$, and $P < 0.0001$, respectively.

All correlations are significant in this table at $P = 0.01$ ($>r = |\pm 0.261|$), $P = 0.001$ ($>r = |\pm 0.326|$), and $P = 0.0001$ ($>r = |\pm 0.374|$).

protein content was inversely correlated with parameters of B-granules.

Crumb grain score showed a generally weak but significant inverse linear correlation to differential volume at Point 1 ($r = -0.28^*$), diameter at Point 1 ($r = -0.41^{***}$), and area range of A ($r = -0.27^*$). These inverse relationships agreed with results from Park et al. (2005) where the authors found the lowest value of crumb grain score and fineness from the bread baked with 100% B-granules. In this study, crumb grain score was not significantly correlated to protein, consequently relationships were independent of protein content. It should be pointed out that there were no other significant correlations to crumb grain score from 68 wheat and flour quality parameters, except wheat kernel weight and size ($r = -0.36^{***}$, respectively, data not shown), which were positively correlated to parameters of B-granules. So, even though those correlation values were low, we believe it has significance.

3.5. Polynomial relationships between B-granules and crumb grain score

Several authors reported that there seemed to be an optimum weight % ratio of A- and B-granules for breadmaking (Lelievre et al., 1987; Park et al., 2004, 2005; Soulaka and Morrison, 1985b) and spaghetti production (Soh et al., 2006). A polynomial relationship was applied between B-granules and crumb grain score, resulting in improved correlations for both HRW and HRS wheats (Table 6). Correlation values of HRS wheat increased from 0.073^* to 0.154^{***} for area A, and 0.013 to 0.088^* for area A + B. In addition, we found that the linear relationships improved when the ratio of volume % of B-granules to flour protein content was used. The correlation

Table 6
Linear and polynomial correlations of B-type granule volume % and ratio with protein contents to crumb grain score

Crumb grain score vs.		Linear correlation R^2	Polynomial correlation R^2
HRW	Area A	0.004	0.018
	Area A + B	0.005	0.039
	Area A/flour protein	0.130	0.141
	Area A + B/flour protein	0.136	0.158
HRS	Area A	0.073	0.154
	Area A + B	0.013	0.088
	Area A/flour protein	0.089	0.222
	Area A + B/flour protein	0.049	0.164

*, **, and *** = significant correlations at $P < 0.01$, $P < 0.001$, and $P < 0.0001$, respectively.

improvements were more obvious for HRW wheat, which improved from 0.004 to 0.130^{**} for area A, and from 0.005 to 0.136^{**} for area A + B. The linear correlations between volume % of B-granules and crumb grain score were improved from 0.004 to 0.141^{***} and from 0.005 to 0.158^{***} for HRW wheat, and from 0.073^* to 0.222^{***} and from 0.013 to 0.164^{***} for HRS wheat after obtaining the ratios with protein content and applying polynomial correlation. Therefore, it appears that there is an optimum range of volume % B-granules, and the optimum range could vary depending on protein content. Lelievre et al. (1987) found different optimum starch size fractions for different protein concentrations when used for breadmaking. Park et al. (2005) suggested that high water absorption during baking and/or high surface area of B-granules could be responsible for gas cell stabilization and the resultant crumb grain score. It has been shown that protein–starch interactions produce different rheological properties depending on variety and granule size (Miller and Hosney, 1999; Petrofsky and Hosney, 1995). Also, bulk rheological properties of dough could affect overall gas cell stability (Van Vliet et al., 1992). Consequently, the reason for different optimum weight % of B-starch granules for different protein contents may be due to rheological properties imparted by protein–starch interactions. The higher water absorbing properties of B-granules could pull water from the attached protein matrix and liquid film in the dough system during baking. Gan et al. (1995) proposed that gas cells are stabilized by a continuous liquid film on the protein–starch matrix. Depending on how extreme the situations are, e.g. low protein content with high content of B-granules (too stiff) vs. high protein content with low content of B-granules (too viscous), the overall viscoelastic properties and gas cell stability could be changed, resulting in different crumb structures.

4. Conclusion

Starch granule size distributions of HRW and HRS wheats showed significant differences in differential volume, diameter, and volume distribution. HRW has smaller size and proportion of B-granules than HRS wheat. The reason is not clear in this study, but an explanation could be due to hot and dry growing conditions during grain filling. Parameters of B-granules showed many significant correlations with wheat and flour properties, partly due to the inverse correlation between protein content and parameters of B-granules. There appears to be different optimum ranges of B-granule weight % for flours with different protein contents. It seems that starch granule size distribution is a unique property that

affects physicochemical properties of wheat, flour, and breadmaking properties in conjunction with its counterpart, protein.

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