Size Distribution and Properties of Wheat Starch Granules in Relation to Crumb Grain Score of Pup-Loaf Bread[1]

S. H. Park,[2,3] J. D. Wilson,[4] O. K. Chung,[2,4,5] and P. A. Seib[2]

ABSTRACT

Cereal Chem. 81(6):699–704

Twelve hard winter wheat flours with protein contents of 11.8–13.6% (14% mb) were selected to investigate starch properties associated with the crumb grain score of experimentally baked pup-loaf bread. The 12 flours were classified in four groups depending on the crumb grain scores, which ranged from 1 (questionable-unsatisfactory) to 4 (satisfactory). Flours in groups 1, 2, 3, and 4 produced breads with pup-loaf volumes of 910–1,035, 1,000–1,005, 950–1,025, and 955–1,010 cm^3, respectively. Starches were isolated by a dough handwashing method and purified by washing to give 75–79% combined yield (dry flour basis) of prime (62–71%) and tailing (7–16%) starches. The prime starch was fractionated further into large A-granules and small B-granules by repeated sedimentation in aqueous slurry. All starches were assayed for weight percentage of B-granules, swelling power (92.5°C), amylose content, and granular size distribution by quantitative digital image analysis. A positive linear correlation was found between the crumb grain scores and the A-granule sizes (r = 0.65, $P < 0.05$), and a polynomial relationship (R^2 = 0.45, $P < 0.05$) occurred between the score and the weight percentage of B-granule starch. The best crumb grain score was obtained when a flour had a weight percentage of B-granules of 19.8–22.5%, shown by varietal effects.

Starch is the main component of wheat, accounting for 65–73% of dry flour mass when milling extraction is <80% (Pomeranz 1988). During baking of bread dough, starch gelatinizes and swells to absorb much of the water and form tiny particles of starch gel. Also, a small percentage of soluble starch is exuded from the granules. Most prior work on starch in breadmaking has focused on loaf volume and staling and did not consider crumb grain.

Wheat starch granules show a bi- or trimodal size distribution (Bechtel et al 1990). The large, lenticular A-granules and the small, spherical B-granules have different physical, chemical, and functional properties (Dronzek et al 1972; Kulp 1973; Nikuni 1978; Meredith 1981; Lineback 1984; Soulaka and Morrison 1985a; Morrison and Gadan 1987; Morrison 1988; Eliasson 1989; Peng et al 1999; Chiotelli and Le Meste 2002). However, there is considerable disagreement in the literature concerning the effects of different starch granule sizes on breadmaking performance.

Ponte et al (1963) reported that the weight percentage of small granules (<10 μm) was inversely correlated with the breadmaking quality of a flour. Similarly, Kulp (1973) concluded that small starch granules have an inferior baking potential compared with unfractionated starch granules regardless of the source or method of preparation of the starch. D'Appolonia and Gilles (1971) also observed a substantial decrease in loaf volume of bread containing a small starch granule fraction compared with bread baked with a large starch granule fraction. Hayman et al (1998) observed that the crumb grain of bread made from flour with large starch granules had larger cell sizes and fewer cells compared with the bread made from flours with small starch granules.

Grewe and Bailey (1927) studied various straight-grade and patent flours and found no significant correlation between granule size and breadmaking potential. Hoseney et al (1971) showed that small starch granules, when reconstituted with gluten and water-solubles, resulted in slightly higher absorption and shorter mixing time, but only slightly smaller loaf volume compared with the control flour, suggesting that granule size does not significantly affect the breadmaking potential of starch. Soulaka and Morrison (1985b) obtained the highest specific loaf volume (cm^3/g) when the proportion of B-granules was 25–35% by weight.

Much of the disagreement about the effects of starch granule size on breadmaking could be caused by different methods used for starch isolation and fractionation as well as different baking tests. The starches tested may have variations in purity, starch damage, and amylose content. Oxidation level in bread dough could also affect loaf volume and crumb structure. Under- or overoxidation would give small loaf volume and dense or open crumb grain.

Crumb grain is considered to be one of the most critical quality attributes of commercial breads in the United States. This important quality parameter is dependent on wheat cultivar, although it is affected by the baking process as well as baking formulation. Therefore, crumb grain is evaluated in experimentally baked breads for the U.S. bread wheat breeding program, and the score is one of the determinants for wheat cultivar release. The objective of this study was to investigate the effect of starch granule size of wheat cultivars on the crumb grain score of pup-loaf bread.

MATERIALS AND METHODS

Materials

Twelve hard winter wheat flours were provided by the U.S. Department of Agriculture, Agricultural Research Service, Grain Marketing and Production Research Center, Hard Winter Wheat Quality Laboratory, Manhattan, KS. Each flour was obtained by milling a composite of equal amounts of grain from different counties within a given region of Kansas and harvested in 1994 from the Kansas Winter Wheat Performance Test Nursery. Wheat and flour were stored at 4°C before milling and between uses, respectively. The experimental work on this project was completed in 1997. The 12 Kansas-grown wheat samples included seven wheat composites from the central region (C) (Public, Harvey, Reno, Stafford, and Sumner counties), KS92P0263-134EXP (C), TAM 107 (C), Ike (C), Karl (C), Karl 92 (C), Scout 66 (C), and Newton (C); two wheat composites from the western region (W) (Ellis, Thomas, Finney, and Greeley counties), Karl (W) and

---

[1] Cooperative investigations, U.S. Department of Agriculture, Agricultural Research Service, and the Department of Grain Science and Industry, Kansas State University. Contribution No. 04-007-J from the Kansas Agricultural Experiment Station, Manhattan, KS 66506. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

[2] Postdoctoral research associate and professors, respectively, Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506.

[3] Presently, research chemist, USDA-ARS Grain Marketing and Production Research Center, Manhattan, KS 66502.

[4] Research chemist and supervisory research chemist, respectively, USDA-ARS Grain Marketing and Production Research Center, Manhattan, KS 66502.

[5] Corresponding author. Phone: 785-776-2703; Fax: 785-776-2792; E-mail: okchung@gmprc.ksu.edu

Publication no. C-2004-1013-02R.

Ogallala (W); two wheat composites from the irrigated region (I) (three counties from the irrigated western region, Thomas, Finney, and Greeley, and one county from the irrigated central region, Stafford), 7853 (I) and Karl (I); and one wheat composite from the eastern region (E) (Brown, Riley, Franklin, and Labette counties), TAM 300 (E). Wheats were cleaned, tempered to 15%, and milled to 68.9–72.4% extraction rates on a Brabender Quadrumat Sr. experimental mill.

General Methods

Ash, moisture, and protein contents (N × 5.7) were determined by Approved Methods 08-01 and 44-15A (AACC 2000). Analytical data are reported on a 14% mb. Mixograph data, mixing time, and water absorption were obtained using 10 g of flour as in Approved Method 54-40A (Finney and Shogren 1972). The swelling power of starch at 92.5°C was determined according to Crosbie (1991), except that sample size was reduced from 1.0 g (db) to 0.5 g (db), and the cooling step in an ice bath was eliminated. Amylose content was determined by the modified concanavalin-A method (Yun and Matheson 1990), and starch damage was determined by an assay kit from Megazyme Int., Wicklow, Ireland (Approved Method 76-31).

Baking Test

A modified, pup, straight-dough baking method as in Approved Method 10-10B (Finney 1984) was used for an experimental breadmaking test. The bread formula contained 100 g of flour (14% mb), 11 mL of a solution containing 6 g of sucrose and 1.5 g of sodium chloride, 5 mL of aqueous malt mixture (0.25 g of dried malt), dry active yeast (1.0 g), shortening (3 g), and 1 mL of ascorbic acid solution (5 mg, 50 ppm). Bake water absorption and mixing time were estimated based on mixograph data, and finally optimized subjectively by the appearance and feel of the dough.

Doughs were fermented for 90 min at 86% rh and 30°C. They were baked at 218°C (425°F) for 18 min and were weighed immediately after removal from the oven. Loaf volumes were then measured by rapeseed displacement and specific volumes (cm^3/g).

One-day-old breads were machine-sliced and crumb grain was graded by a baking expert. Crumb grain scores were graded and recorded on a scale of 0–6, where 0 is unsatisfactory; 1 is questionable to unsatisfactory; 2 is questionable; 3 is questionable to satisfactory; 4 is satisfactory; 5 is excellent; and 6 is outstanding. The description for unsatisfactory grade (0) is a crumb grain with coarse, round cells extremely irregularly sized and shaped with thick cell walls; questionable grade (2) is a crumb grain with somewhat open, mostly round and coarse cells irregularly sized and shaped with thick cell walls; satisfactory grade (4) is a crumb grain with fine, elongated cells slightly irregularly layered with light, lacy, thin cell walls; excellent grade (5) is a bread crumb grain with very fine, elongated cells uniformly layered in with very light, lacy, thin cell walls; and outstanding grade (6) is a perfect crumb grain that is graded as excellent, with every other bread parameter such as overall shape, appearance, size of loaf, and color of crumb and crust. In this experiment, however, there were no breads graded with a crumb grain score of 0, 5, or 6. This grading system is similar to the method used by the Wheat Quality Council (0 very open, 6 very close). Instrumental methods for evaluating crumb grain using digital image analysis have been reported (Bertrand et al 1992; Zayas 1993; Sapirstein et al 1994; Rogers et al 1995; Coles and Wang 1997). However, the subjective method has been found reproducible and continues to be used as the objective methods undergo refinement.

Isolation of Wheat Starch and Its Fractionation Into Large and Small Granules

Starch was recovered from flour by the dough handwashing method of Finney et al (1982) with the modifications of Al-Obaidy (1986). Flour (100 g, 14% mb) was mixed with distilled water (≈60 mL) using a 100-g mixer (National Mfg. Co., Lincoln, NE). The dough was gently kneaded and dispersed in 100 mL of chilled, distilled water by one rubber-gloved hand with special care to keep the elastic dough intact. The kneading of dough was repeated with the following quantities of water: 2 × 100 mL, 4 × 75 mL, and then 4–6 × 50 mL. All starch washings were combined and strained through a nylon bolting cloth (75 µm opening), and the throughs were collected by centrifuging at 2,000 × *g* for 20 min. The supernatant was decanted and discarded, and the tailing fraction atop the prime starch was scraped off using a spatula. The prime starch phase was resuspended in ≈100 mL of water, centrifuged, the supernatant discarded, and the tailings collected. The purification of the prime starch fraction was repeated (usually 4×), until no tailings fraction was visible. The combined tailings were then put through the same purification procedure used above to recover a second fraction of prime starch (mostly B-granule starch). The first and second collections of starch were combined and called prime starch, while the residual starch was called tailings starch. Solutions (≈300 mL) of hydrochloric acid (0.02*M*) and sodium chloride (10 g/L) were used to resuspend the prime starch and tailings fractions during the isolation steps to inactivate enzymes and to remove protein from the starch. Finally, the starch was washed thoroughly with distilled water and collected by centrifuging at 2,000 × *g* for 20 min.

A small portion of the wet prime starch was washed with acetone followed by air-drying. The remainder of the wet prime starch portion was separated into two fractions by repeated sedimentation in water slurry (Soulaka and Morrison 1985a). The A- and B-granule fractions were separated by sedimentation for 1.5 hr in a 2-L beaker containing distilled water (≈1,800 mL) to a depth of 18 cm. The supernatant was carefully decanted and the sediment was resuspended in fresh water. This procedure was repeated 5×. The final sediment contained the A-granule fraction and the combined supernatants contained the B-granule fraction. The B-granule starch was recovered by centrifuging (4,000 × *g*, 15 min), and both fractions were then washed with acetone and air-dried. Moisture levels of the dried starches were 11–14%. All starches were assayed for residual protein, ash, amylose, and swelling power (92.5°C).

Size Distributions of Starch Granules

The starch samples, including unfractionated prime starch and A-granule and B-granule fractions, were analyzed by quantitative digital image analysis according to the method of Bechtel and Wilson (1994). A small quantity of starch (≈2 mg) was mixed thoroughly with double-distilled water (1 mL). A small drop of slurry was placed on a microscope slide, and a cover glass was placed over the suspension. The cover glass was then fixed in place with two coats of clear nail polish to prevent evaporation of water and movement of the granules. Then, the slides were viewed under bright-field illumination with a Reichert Polyvar 2 microscope equipped with an MAC 2000 (Ludl Electronics Products Ltd., Hawthorne, NY) automated stage attached. Images were captured with a Javelin Chromachip V high-resolution CCD color camera, a Javelin CVM 9 color monitor, and a Princeton Gamma-Tech Imagist II (v. 7) imaging system (PGT, Princeton, NJ). A SUN SPARC station, with a 32-bit processor, 32 MB of main memory, and 5 GB of NPI external hard disk storage, was used to acquire and process the image data.

For each sample, a total of 150 images (50 images/slide) from three slides were analyzed and stored. Starches were analyzed at 100× magnification except the smaller B-granules, which were analyzed at 250×. The stage automation software was set to collect images from 50 points of sample area. The stage moved to the specified point and the sample was focused automatically. A grayscale image was captured, stored, and converted to a binary image using color tables specifically prepared for each magnification. Data for each set of 50 images from a slide were summarized and stored. Assuming spherical shape, area-filled-equivalent diameter $(4.0 \times \text{area}/3.141)^{1/2}$ was calculated for each granule from the 150 images (10–50 granules/image) and the mean values were reported.

Statistical Analysis

A completely randomized experimental design was used with two replicates. General linear and polynomial models were analyzed by the multiple regression procedure of the Statistical Analysis System (SAS Institute, Cary, NC). Statistical abbreviations were simple correlation coefficient (r), coefficient of determinant (R^2), $P < 0.05$ (*), $P < 0.01$ (**), and least significant difference (LSD).

RESULTS AND DISCUSSION

Loaf Volume and Crumb Grain Scores

The 12 hard winter wheat flours were chosen to give three flours each (four groups) over the full range of crumb grain scores, while maintaining good to excellent loaf volumes for all flours (Table I). High loaf volume, which generally accompanies high protein content, is a necessary but not sufficient condition for a good crumb score. The range of properties of the 12 flours is given in Table II. Protein range was 11.8–13.6%, pup-loaf volumes were 910–1,035 cm^3, and specific loaf volumes were 5.8–6.8 cm^3/g. All flours had starch damage of 6.4–7.3%, and that parameter was not examined further in this study.

Isolation and Fractionation of Wheat Starches

Yields of prime starch from the 12 flour doughs ranged from 62.0 to 71.2% based on dry flour weight, whereas yields of tailings starch were 7.0–15.7%. The total starch (prime plus tailings) yield ranged from 75.4% for Karl (W) to 79.1% for Ogallala (W) (Table III). As expected, total starch yield was negatively correlated to flour protein content with an r value of –0.77** (data not shown). The weight percentage of B-granules, fractionated from the prime starch fractions, differed among the 12 samples, ranging from 15.7% for TAM 107 (C) to 27.0% of prime starch weight for Karl 92 (C) (Table III).

Figure 1 shows the typical number-size distribution of unfractionated prime starch and its A- and B-granule fractions of Scout 66 (C) separated by repeated sedimentation. Other starches showed a similar pattern of granule size distributions. The mean (number-average) equivalent diameters of the A-granule fractions were 16.6–19.6 μm and the B-granule fractions were 3.5–4.7 μm (Table IV). The unfractionated prime starches were calculated to have just a slightly larger mean size (4.1–5.2 μm) than the B-granules (Table IV), because B-granules far outnumbered A-granules in the prime starches (Fig. 1). The mean sizes of A-granules from Karl (C), Karl 92 (C), and TAM 107 (C) were smaller than those from TAM 300 (E), Newton (C), and Ogallala (W).

Different percentages of small and large granules in total wheat starch were found in the literature. Our results showed that the number of B-granules (<10 μm diameter) was 90–95% for total starch, 35–40% for A-granule fraction, and >95% for B-granule fraction (Fig. 1). Evers and Lindley (1977) prepared starch from wheat kernels by proteolysis and separated large and small granules by microsieving. They found 94.2–98.5% small granules (<10 μm diameter) by number of the total granules using Coulter counter and Quantimet image analysis. Morrison and Gadan (1987) obtained B-granules by proteolysis and repeated sedimentation that accounted for >90% of the total number of granules in mature wheats. Hayman et al (1998) reported that small granules accounted for 62.8% of total starch and 84.9% of small granule fraction. They fractionated starch from flour using a dough-washing method, separated small granules by centrifugation, and analyzed size distributions by centrifugal automatic particle analyzer. These differences in extraction and separation methods of A- and B-granules among researchers might cause different results.

Properties of Wheat Starches

The protein contents within the starch fractions were similar, indicating consistent fractionation of flours, and purification of the starches. The purified fractions contained an average of 0.23% protein for the A-granules, 0.29% for the B-granules, and 1.55% for the tailing starch fractions (data not shown).

There were significant differences between cultivars in swelling power of flours, unfractionated prime starches, and their fractions at 92.5°C and a starch-to-water ratio of 1.7:100 (w/w) (0.5 g/30 mL). Significantly higher swelling powers were observed from Ike (C) and Ogallala (W) flours, as well as unfractionated starches (A+B) and A- and B-granule fractions (Table V). Ike (C) is a double-null (*wx*-B1 and *wx*-A1) waxy mutant wheat (Graybosch et al 1998) and Ogallala (W) is a single-null (*wx*-B1) (R. A. Graybosch, *personal communication*). Partial waxy mutant wheat

TABLE I
Selection Criteria for 12 Hard Winter Wheat Flours

Group	No. of Flours	Crumb Grain Score[a]	Protein Content (%)	Loaf Volume (cm^3)
A	3	1	12.0–13.2	910–1,035
B	3	2	12.8–13.6	1,000–1,005
C	3	3	12.7–13.2	950–1,025
D	3	4	11.8–12.1	955–1,010

[a] 1 = poorest, 4 = best.

TABLE II
Protein and Ash, and Baking Data of 12 Hard Winter Wheat Flours[a]

Parameter	Range
Protein (%)	11.8–13.6
Ash (%)	0.37–0.50
Bake absorption (%)	67.9–74.1
Bake mix time (min)	5.3–13.6
Dough	
Weight (g)	175.9–181.5
Proof height (cm)	7.1–7.7
Loaf volume	
As received (cm^3)	910–1,035
Specific volume (cm^3/g)[b]	5.8–6.8
Crumb grain score	1–4

[a] Data expressed on 14% moisture base.
[b] Specific volume = loaf volume/loaf weight.

TABLE III
Yields of Starch Fractions and B-Granule Weight Percentage[a]

Cultivar or Line[b]	Starch Yield (% flour wt)			B-Granule[c] (% prime starch)
	Prime[c]	Tailings	Total	
KS92P0263-134EXP (C)	62.0	15.7	77.7	18.3
TAM 107 (C)	67.9	11.1	78.9	15.7
Karl (W)	65.7	9.7	75.4	26.0
Ike (C)	67.9	9.8	77.6	18.1
Karl (C)	69.6	7.1	76.7	24.7
Karl 92 (C)	69.9	7.0	76.9	27.0
Scout 66 (C)	71.2	7.8	79.0	21.9
7853 (I)	70.9	7.4	78.3	22.8
Karl (I)	70.6	7.4	77.9	26.7
TAM 300 (E)	65.5	13.5	79.0	22.5
Newton (C)	67.0	11.7	78.7	20.7
Ogallala (W)	67.7	11.5	79.1	19.8
Average	68.0	10.0	78.0	22.0
LSD[d]	3.6	3.6	2.0	2.5

[a] Data expressed on 14% moisture base.
[b] (C) = central region; (W) = western region; (I) = irrigated region; (E) = eastern region.
[c] Prime starch contained an overall mean protein level of 0.24%, A-starch 0.23%, B-starch 0.29%, and tailing starch 1.55%.
[d] Least significant difference ($P < 0.05$).

contains starch with reduced levels of amylose and lipids, which cause them to undergo increased hot-water swelling (Tester and Morrison 1990; Morrison et al 1993; Tsai et al 1997).

The B-granule fractions of all cultivars had higher swelling power at 92.5°C than A-granule fractions (Table V). Kulp (1973) reported that before the temperature reached ≈90°C, swelling power was higher with unfractionated wheat starch granules than with small granules (<7.5 μm). However, at >90°C, the swelling power of small-granule starch rapidly increased over that of unfractionated starch.

Amylose contents of both A-granule and B-granule starches were significantly different among samples (Table V). There were significantly lower levels of amylose for Ike (C) (A-granules, 23.4%) and Ogallala (W) (A-granules, 25.2%), compared with other cultivars. This is in agreement with previous work on Ike (C) starch showing ≈3–4 percentage points less amylose than other cultivars using the concanavalin-A method (Seib et al 2000). In agreement with previous studies (Morrison and Laignelet 1983; Morrison and Gadan 1987; Tester and Morrison 1990), the A-granule starches contained higher amylose content (average 27.8%, with a range of 23.4–30.8%) than the B-granule starches (average 24.0%, range 18.3–26.8%). As expected, starches with reduced amylose level showed increased hot-water swelling. Amylose content and starch swelling power did not show significant correlation with crumb grain score in this work (data not shown).

Wheat Starch Granule Size and Bread Crumb Grain

Starch granule size had a significant relationship with crumb grain scores. Flours that had A-granules with larger sizes tended to produce bread with better crumb grain ($r = 0.65$*, Fig. 2). If the data point of TAM 300 (E) with the highest mean diameter (19.6 μm) for A-granules was excluded, $r = 0.58$*. Hayman et al (1998) observed that bread supplemented with 25–50% (fwb) potato starch (10–100 μm diameter) showed open, round, gas cells with thick cell walls in the crumb grain. They also obtained similar results in bread made from a blend of gluten and large wheat starch granules. However, the inferior crumb grain caused by potato starch is more likely a consequence of gelatinization, swelling, and clarity properties different from those of wheat starch rather than its large granular size. Also, regarding gluten-starch bread, bread made without water-solubles could distort the baking performance of original flour due to the functional roles of water-solubles in breadmaking (Hoseney et al 1969; D'Appolonia and Gilles 1971; Miller and Hoseney 1999). A loaf of bread with

TABLE IV
Crumb Grain Scores and Equivalent Diameters of Prime Starch Granules and Their Fractions Determined by Digital Image Analysis

Cultivar or Line[a]	Crumb Grain Score	Area Filled Equivalent Diameter (μm)[b]		
		Prime Starch Granule	A-Granule	B-Granule
KS92P0263-134EXP (C)	1	4.7	17.1	4.0
TAM 107 (C)	1	4.5	16.8	3.9
Karl (W)	1	4.2	17.7	3.8
Ike (C)	2	4.3	17.7	3.5
Karl (C)	2	5.1	16.6	3.9
Karl 92 (C)	2	4.9	16.7	4.2
Scout 66 (C)	3	5.2	17.3	4.0
7853 (I)	3	4.8	17.5	4.7
Karl (I)	3	5.0	17.6	4.4
TAM 300 (E)	4	5.2	19.6	4.6
Newton (C)	4	4.2	18.2	3.5
Ogallala (W)	4	4.1	17.8	3.7
Average	2.5	4.7	17.6	4.0
LSD[c]	0.5	0.6	0.9	0.5

[a] (C) = central region; (W) = western region; (I) = irrigated region; (E) = eastern region.
[b] Equivalent diameter calculated from average area filled per starch granule assuming a spherical particle.
[c] Least significant difference ($P < 0.05$).

TABLE V
Swelling Power of Flours and Prime Starches and Amylose Contents in Prime Starches

Cultivar or Line[a]	Swelling Power at 92.5°C (g/g)				Amylose (%) Prime Starch[b]	
		Prime Starch[b]				
	Flour	A+B	A	B	A	B
KS92P0263-134EXP (C)	13.5	16.3	15.8	21.3	30.5	26.0
TAM 107 (C)	13.7	15.6	16.4	20.4	29.2	26.2
Karl (W)	14.4	15.2	16.3	20.4	27.5	23.7
Ike (C)	17.9	23.0	20.8	25.9	23.4	18.3
Karl (C)	14.2	16.1	15.1	17.7	27.5	23.1
Karl 92 (C)	14.1	16.3	15.8	18.0	27.1	23.9
Scout 66 (C)	13.2	16.8	15.5	20.3	27.2	23.7
7853 (I)	14.2	17.6	16.2	19.1	27.4	23.6
Karl (I)	14.1	18.3	15.3	18.9	28.7	24.1
TAM 300 (E)	13.6	15.6	14.9	20.4	30.8	26.6
Newton (C)	13.0	15.5	14.9	21.5	29.3	26.8
Ogallala (W)	17.2	18.4	18.7	23.8	25.2	22.1
Average	14.4	17.1	16.3	20.6	27.8	24.0
LSD[c]	0.5	1.1	0.8	1.7	0.8	0.8

[a] (C) = central region; (W) = western region; (I) = irrigated region; (E) = eastern region.
[b] A+B = unfractionated prime starch; A = large granules; B = small granules.
[c] Least significant difference ($P < 0.05$).

small loaf volume tends to have poor crumb grain and thick cell walls.

The large granules may not detrimentally affect the gas cells simply because of their size compared with small granules. Photographs of cross-sections of gas cells from dough and bread taken by Sandstedt et al (1954) showed that starch granules were poorly oriented in a freshly mixed dough. However, as gas cells expanded with increasing temperature, starch granules became aligned parallel to the film surface of a gas cell. Then, as granules were gelatinized during baking, they became more dilated and oriented even more tangentially. More recently, "film" inside a bread dough has been described as water-coated starch granules embedded in a gluten gel network (Eliasson and Larsson 1993). The large wheat starch granules contained ≈4 percentage points more amylose than the small granules (Table V), in agreement with previous findings (Seib 1994). The large granules probably release more amylose during baking, and that amylose would interact with the gluten matrix (Martin and Hoseney 1991) to provide a film that may coalesce less during baking. The result would be small gas cells that create a crumb grain with good appearance.

The weight percentage of B-granules suggest an optimum level for good crumb grain score (second-order polynomial relationship, $R^2 = 0.45^*$, Fig. 3). The flours of TAM 300 (E), Newton (C), and Ogallala (W) producing the best crumb grain contained 19.8–22.5% of B-granule starch fractions, whereas the flours of TAM 107 (C) and Karl (W), producing poor crumb grain, contained the least (15.7%) and the greatest (27.0%) proportions of B-granule starch, respectively (Table III). Because the loss of some small granules is inevitable during starch isolation, the actual weight percentage of small granules could be slightly higher than our experimental numbers. Soulaka and Morrison (1985b) indicated that the proportion of B-granules that provided the highest specific loaf volume was 25% by weight, which generally agrees with our results on crumb grain score.

These results suggest that the distribution of starch granule size may be a factor affecting bread crumb grain. If confirmed by more data, the question arises as to why there is an optimum level of small wheat starch granules that result in good appearance to the crumb grain. At a starch-to-water (w/w) ratio of 4:6, small wheat starch granules showed higher affinity for water than large granules near room temperature, and also showed much higher storage modulus up to ≈55°C (Chiotelli and Le Meste 2002). Perhaps some stiffening of starch-gluten matrix, which supports the gas cell, by 20–23% small granules prevents coalescence. However, an increasing level of small granules (>24% in this study) translates into a decreasing release of amylose from the gelatinized granules, which may weaken and rupture of the gas cell and damage to loaf volume and crumb grain. He and Hoseney (1991) suggested that the increase in viscosity of dough by interaction between swollen or gelatinized starch and gluten and simultaneous increase in gas pressure within gas cells induced the breakage of walls and accelerated the release of gas. Gan et al (1990) reported that deficiencies in the extensibility of the starch-gluten matrix and in the surface activities of the liquid film would cause the rupture of the gas cells and consequent loss of expansion.

Stoddard (1999) found a wide variation in starch granule size distribution from wheat, rye, and wild relatives and suggested the possibility for manipulating granule size distribution. If more data confirms the effects of starch granule size on breadmaking, it would be desirable to manipulate starch granule size distribution of the endosperm to meet specific needs.

CONCLUSIONS

Data from 12 wheat flours and starches suggest that starch granule size affects the appearance of bread crumb grain. It is not yet known whether such an effect is due to size or swelling

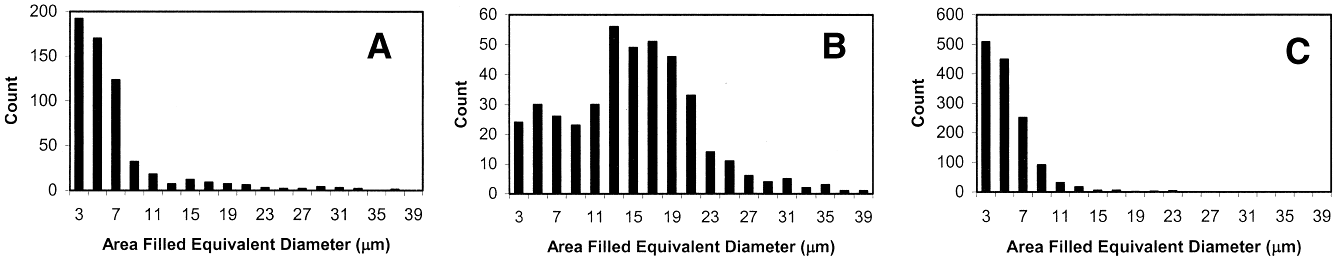

Fig. 1. Number-size distributions of Scout 66 (C) prime starch: (**A**) unfractionated (A-granules + B-granules); (**B**) A-granules; and (**C**) B-granules. Distributions were determined by digital image analysis.

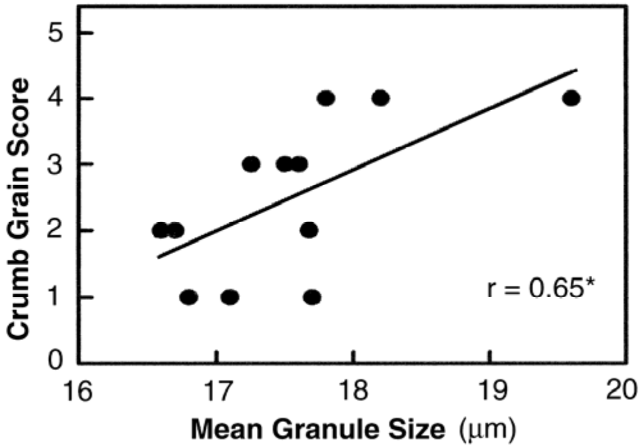

Fig. 2. Relationship between the mean size (number-average) of A-granules of prime starch and crumb grain score.

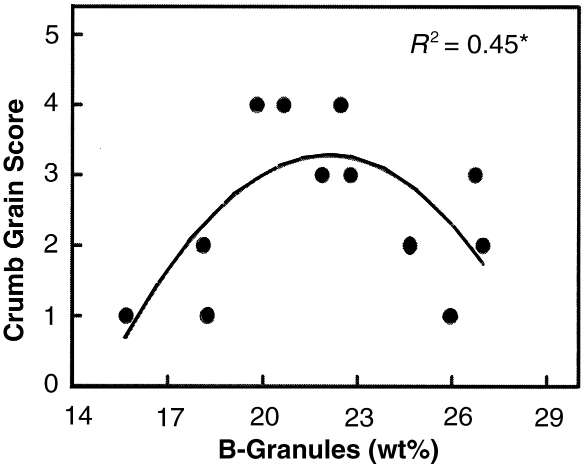

Fig. 3. Second-order polynomial relationship between the wt% of B-granules in prime starch and crumb grain score (14% mb).

behavior, or to interactions associated with granule sizes. However, we have demonstrated varietal effects on starch granule size distribution that had significant relationships with crumb grain scores of breads. The optimum range of weight percentage (≈20–23%) of B-granules in wheat cultivars produced bread with the best crumb grain scores, suggesting the need to expand studies of this potential tool for improvement of crumb grain for the U.S. bread wheat breeding programs.

ACKNOWLEDGMENTS

We thank D. B. Bechtel for technical advice in image analysis and M. S. Caley for baking and evaluating of crumb grain.

LITERATURE CITED

Al-Obaidy, K. A. 1986. Dough and gluten characteristics of good- and poor-quality flours: Lipid-protein bindings affected by mixing time, water absorption, chemicals and heat. PhD dissertation. Kansas State University: Manhattan. KS.

American Association of Cereal Chemists. 2000. Approved Methods of the AACC, 10th Ed. Methods 08-01, 10-10B, 44-15A, 46-30, 54-40A, 76-31. The Association: St. Paul, MN.

Bechtel, D. B., and Wilson, J. D. 1994. Development of an automated digital image analysis system for the study of starch size distributions in wheat. Cereal Foods World 39:588.

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

Bertrand, D., Le Guerneve, C., Marion, D., Devaux, M. F., and Robert, P. 1992. Description of the textural appearance of bread crumb by video image analysis. Cereal Chem. 69:257-261.

Chiotelli, E., and Le Meste, M. 2002. Effect of small and large wheat starch granules on thermomechanical behavior of starch. Cereal Chem. 79:286-293.

Coles, G. D., and Wang J. 1997. Objective determination of bread crumb visual texture by image analysis. Pages 153-159 in: Proceedings of International Wheat Quality Conference. J. L. Steele and O. K. Chung eds. Grain Industry Alliance: Manhattan, KS.

Crosbie, G. B. 1991. The relationship between starch swelling properties, paste viscosity and boiled noodle quality in wheat flours. J. Cereal Sci. 13:145-150.

D'Appolonia, B. L., and Gilles, K. A. 1971. Effects of various starches in baking. Cereal Chem. 48:625-636.

Dronzek, B. L., Hwang, P., and Bushuk, W. 1972. Scanning electron microscopy of starch from sprouted wheat. Cereal Chem. 49:232-239.

Eliasson, A. C. 1989. Characterization of wheat starch and gluten as related to end-use properties. Pages 177-192 in: Wheat is Unique. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

Eliasson, A. C., and Larsson, K. 1993. Dough. Page 261-314 in: Cereals in Breadmaking. Marcel Dekker: New York.

Evers, A. D., and Lindley, J. 1977. The particle-size distribution in wheat endosperm starch. J. Sci. Food Agric. 28:98-102.

Finney, K. F. 1984. An optimized, straight-dough, bread-making methods after 44 years. Cereal Chem. 61:20-27.

Finney, K. F., and Shogren, M. D. 1972. A 10-gram mixograph for determining and predicting functional properties of wheat flours. Baker's Dig. 46(2):32-42, 77.

Finney, K. F., Jones, B. L., and Shogren, M. D. 1982. Functional (breadmaking) properties of wheat protein fractions obtained by ultracentrifugation. Cereal Chem. 59:449-453.

Gan, Z., Angold, R. E., Williams, M. R., and Ellis, P. R. 1990. The microstructure and gas retention of bread dough. J. Cereal Sci. 12:15-24.

Graybosch, R. A., Peterson, C. J., Hansen, L. E., Rahmen, S., Hill, A., and Skerritt, J. H. 1998. Identification and characterization of U.S. wheats carrying null alleles at the *wx* loci. Cereal Chem. 75:162-165.

Grewe, E., and Bailey, C. H. 1927. The concentration of glutenin and other protein in various types of wheat flour. Cereal Chem. 4:230-247.

Hayman, D., Sipes, K., Hoseney, R. C., and Faubion, J. M. 1998. Factors controlling gas cell failure in bread dough. Cereal Chem. 75:585-589.

He, H., and Hoseney, R. C. 1991. Gas retention in bread dough during baking. Cereal Chem. 68:521-525.

Hoseney, R. C., Finney, K. F., Shogren, M. D., and Pomeranz, Y. 1969. Functional (breadmaking) and biochemical properties of wheat flour components. II. Role of water-solubles. Cereal Chem. 46:117-125.

Hoseney, R. C., Finney, K. F., Pomeranz, Y., and Shogren, M. D. 1971. Functional (breadmaking) and biochemical properties of wheat flour components. VII. Starch. Cereal Chem. 48:191-201.

Kulp, K. 1973. Characteristics of small-granule starch of flour and wheat. Cereal Chem. 50:666-679.

Lineback, D. R. 1984. The starch granule organization and properties. Baker's Dig. 58(2):16,18-21.

Martin, M. L., and Hoseney, R. C. 1991. A mechanism of bread forming. II. Role of starch hydrolyzing enzymes. Cereal Chem. 68:503-507.

Meredith, P. 1981. Large and small starch granules in wheat—Are they really different? Starch 33:40-44.

Miller, K. A., and Hoseney, R. C. 1999. Dynamic rheological properties of wheat starch-gluten doughs. Cereal Chem. 76:105-109.

Morrison, W. R. 1988. Lipids in cereal starches: A review. J. Cereal Sci. 8:1-15.

Morrison, W. R., and Gadan, H. 1987. The amylose and lipid contents of starch granule in developing wheat endosperm. J. Cereal Sci. 5:263-275.

Morrison, W. R., and Laignelet, B. 1983. An improved calorimetric method for the determination of apparent and total amylose in cereal and other starches. J. Cereal Sci. 1:9-20.

Morrison, W. R., Tester, R. F., Snape, C., Law, R., and Gidley, M. J. 1993. Swelling and gelatinization of cereal starches. IV. Some effects of lipid-complexed amylose and free amylose in waxy and normal barley starches. Cereal Chem. 70:385-391.

Nikuni, Z. 1978. Studies on starch granules. Starch 30:105-111.

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

Pomeranz, Y. 1988. Chemical composition of kernel structures. Page 97-158 in: Wheat Chemistry and Technology. Vol 1. Am. Assoc. Cereal Chem.: St. Paul, MN.

Ponte, J. G., Jr., Titcomb, S. T., Cerning, J., and Cotton, R. H. 1963. Studies of the high-protein fines and coarse fractions of a Southwest and an Intermountain flour. Cereal Chem. 40:601-618.

Rogers, D. E., Day, D. D., and Olewnik, M. C. 1995. Development of an objective crumb-grain measurement. Cereal Foods World 40:498-501.

Sandstedt, R. M., Schaumburg, L., and Fleming, J. 1954. The microscopic structure of bread and dough. Cereal Chem. 31:43-49.

Sapirstein, H. D., Roller, R., and Bushuk, W. 1994. Instrumental measurement of bread crumb grain by digital image analysis. Cereal Chem. 71:383-391.

Seib, P. A. 1994. Wheat starch: Isolation, structure and properties. Oyo Toshitsu Kagaki 41:49-69.

Seib, P. A., Liang, X., Guan, F., Liang, Y. T., and Yang, H. C. 2000. Comparison of Asian noodles from some hard white and hard red wheat flours. Cereal Chem. 77:816-822.

Soulaka, A. B., and Morrison, W. R. 1985a. The amylose and lipid contents, dimensions, and gelatinization characteristics of some wheat starches and their A- and B-granule fractions. J. Sci. Food Agric. 36:709-718.

Soulaka, A. B., and Morrison, W. R. 1985b. The breadbaking quality of six wheat starches differing in composition and physical properties. J. Sci. Food Agric. 36:719-727.

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

Tester, R. F., and Morrison, W. R. 1990. Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose, and lipids. Cereal Chem. 67:551-557.

Tsai, M. L., Li, C. F., and Lii, C. Y. 1997. Effects of granular structures on the pasting behaviors of starches. Cereal Chem. 74:750-757.

Yun, S. H., and Matheson, N. K. 1990. Estimation of amylose content of starches after precipitation of amylopectin by concanavalin-A. Starch 42:302-305.

Zayas, I. Y. 1993. Digital image analysis for bread crumb grain evaluation. Cereal Foods World 38:760-766.

[Received October 22, 2003. Accepted April 30, 2004.]