Granule Size Distribution and Chemical Composition of Starches from 12 Soft Wheat Cultivars

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ABSTRACT

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Granule size distribution of wheat starch is an important characteristic that can influence its chemical composition, which in turn may affect its functionality. The granule size distribution and chemical composition of soft wheat starches were characterized and compared and relationships among those properties were identified. Thirty-four starch samples from 12 soft wheat cultivars grown in the eastern half of the United States were examined. Granule size distribution was characterized using a laser light-scattering technique. Amylose and phospholipid contents were determined using colorimetric procedures. A clear trimodal distribution of granule sizes was shown by 26 out of 34 starch samples: small granules with diameters <2.8 µm, midsize granules with diameters of 2.8–9.9 µm, and large granules with diameters >9.9 µm. Volume% distribution of granules within the three size classes had ranges of 9.7–15.2% (small), 13.4–27.9% (medium), and 57.9–76.9% (large). Highly significant differences were seen among the cultivars for volume% of granules within the ranges of 9.9–18.5 µm and 18.5–42.8 µm. Cultivar specific surface area means also differed. The environment affected granule size distribution, with some cultivars exhibiting more variation than others. Pioneer 2555 was the least variable, whereas Pioneer 2550 and Geneva were the most variable cultivars. Mean total amylose (TAM), apparent amylose (AAM), and lysophospholipid (LPL) values varied significantly among cultivars. TAM was positively correlated with the volume% of granules of 9.9–18.5 µm. LPL was negatively correlated with mean starch granule diameter and positively correlated with specific surface area of granules, indicating smaller granules tended to have higher lipid contents. Results suggest that significant differences exist in granule size distribution of soft wheat starches and affect starch chemical composition. Data also suggest it is possible that lipid is preferentially associated with the biosynthesis of small starch granules.

Starch constitutes the major component of wheat flour and serves as a multifunctional ingredient for the food industry. Functionality of starch influences or controls such characteristics as texture, volume, consistency, aesthetics, moisture, and shelf stability of foods.

Many investigators have shown that starch composition (Kulp 1973, Meredith 1981, Soulaka and Morrison 1985a), gelatinization and pasting properties (Kulp 1973, Eliasson and Karlsson 1983), enzyme susceptibility (Kulp 1973), baking characteristics (D'Appolonia and Gilles 1971, Kulp 1973, Soulaka and Morrison 1985b), crystallinity and swelling properties (Wong and Lelievre 1982), and wheat class (Zayas et al 1994) were all affected by granule size. Amylose content was highest in large granules, whereas lipid content was highest in small granules. Amylose-to-amylopectin ratio and lipid content of starch contribute to its functionality. During heating, amylose leaches from starch granules and contributes both to the viscosity of the continuous phase in starch-water dispersions and to the rate of retrogradation on cooling. Various reports have linked higher peak paste viscosity, greater breakdown, and lower final viscosity of wheat starch to lower amylose contents (Moss 1980, Oda et al 1980, Moss and Miskelly 1984, Zeng et al 1997). Eating quality of *udon* noodles was negatively correlated with wheat flour amylose content (Oda et al 1980).

The role of starch lipids in determining functional properties of starch has also received considerable attention (Lorenz and Kulp 1983, Bowler et al 1985, Tester and Morrison 1990, Vasanthan and Hoover 1992). Most of those studies have concentrated on the effects of lipids on the rheological properties of starch pastes. Although there are some conflicting results, it is generally believed that surface lipids inhibit the movement of water into the granules and internal lipids (amylose-lipid complexes) reduce starch swelling and amylose leaching, and hence reduce hot paste viscosity. It was also reported that variation in the proportions of lipid-complexed amylose and lipid-free amylose could have some effect on gelatinization temperature (Morrison 1995). Those reports suggest that granule size distribution of wheat starch is an important characteristic that can influence its chemical composition, which in turn affect its functionality. Zayas et al (1994) reported that a categorized distribution of the granules of 14 hard and 10 soft wheats (representing four cultivars of each class) could distinguish between the two classes.

Accuracy of granule size distribution is dependent on both starch isolation and size-determination techniques. A good isolation method should prevent loss of small granules and a good particle size determination technique should allow one to use a large sample size and measure all the particles in the sample. Previous investigations on granule size distribution of wheat starch have reported a bimodal distribution (Brocklehurst and Evers 1977; Evers and Lindley 1977; Baruch et al 1979, 1983; Karlsson et al 1983; Soulaka and Morrison 1985a; Morrison and Scott 1986). However, Coulter counter, the size-measurement technique, used in those studies was not capable of accurately measuring granules <3 µm (Karlsson et al 1983). As a result, small starch granules were not counted. Progress in instrumentation has shown that wheat starch shows a trimodal distribution rather than bimodal (Bechtel et 1990).

In this work, a Malvern mastersizer laser light-scattering instrument was used to evaluate particle size distribution. It evaluated particles sizes down to 0.1 µm and measured a relatively large sample size. Laser light-scattering is an Approved Method of the AACC for flour particle size (AACC 1995). The starch isolation method employed prevented selective losses of small granules and produced starch of high purity. The objectives of this study were to: 1) characterize and compare granule size distribution, amylose, and phospholipid content of starches isolated from 12 soft wheat cultivars, grown at different locations of the eastern half of the United States; 2) determine relationships among those properties.

MATERIALS AND METHODS

Wheat Samples

Twelve soft wheat cultivars were evaluated: Argee, Arthur, Augusta, Blackhawk, Caldwell, Fillmore, Freedom, Geneva, Hillsdale, McNair, Pioneer 2550, and Pioneer 2555. Nonwheat material and shriveled kernels were removed from numerous preiminary nonbulked samples. Those samples were screened for elevated

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α-amylase activity using the Approved Method 22-06 (AACC 1995). Sound samples from three or four different growing locations or years were randomly combined to produce a bulked sample for each cultivar. Three or four other location samples were combined to produce another bulked sample for that cultivar. That was repeated again to produce a total of three bulked samples for each cultivar; except Blackhawk and Geneva, in which only two bulked samples were produced. Bulking samples across location and years reduced environmental differences. The same amount of wheat from each sample within each bulked group was weighed and combined to isolate starch and to determine bulk moisture, bulk nitrogen, and bulk starch content. To evaluate the association between protein content and kernel hardness, data from a set of 122 cultivars representing 13 crop years and 104 locations were evaluated for flour protein content (AACC 1995) and kernel texture (softness equivalent) using a modified Brabender Jr. mill (Finney and Andrews 1986). Those wheats were only lightly cleaned before test milling. A subset of 82 of those cultivars were thoroughly cleaned of shriveled kernels (Gaines et al 1998) and evaluated again for kernel texture (break flour) using an Allis-Chalmers mill (AACC 1995).

Moisture and nitrogen contents were determined by Approved Methods 44-15A and 46-12, respectively (AACC 1995). Total starch content was determined using a total starch analysis kit (Megazyme Pty. Ltd., Warriewood, Australia).

Starch Isolation and Purification

Starch was extracted from the wheat cultivars according to the method of McDonald and Stark (1988) with some modifications. Wheat kernels were cracked lightly by passing through a Tag-Heppenstall moisture meter roll (C. J. Tagliabue Mfg. Co., Brooklyn, NY), steeped 18 hr in 0.02*M* HCL (10 mL/g of grain) at 4°C, and neutralized with 0.2*M* NaOH. The aqueous solution was drained through a 125-µm sieve, and solids were recovered by centrifuging at 2,700 × *g* for 10 min. The steeped material was rubbed gently with a mortar and pestle in water and sieved through a 125-µm screen. Grinding with a mortar and pestle was repeated until essentially all starch granules were released. The remaining residue was homogenized in a blender with distilled water and passed through a 45-µm screen. That process was repeated three times. The combined washings were successively passed through 75- and 53-µm screens and centrifuged at 3,000 × *g* for 10 min to obtain crude starch. The solids collected were resuspended in distilled water and centrifuged at 1,600 × *g* for 20 min to separate the lower white starch layer from the top brown protein layer. The white starch layer was purified twice with a toluene shaking procedure (McDonald and Stark 1988) using 5 vol of 0.2*M* NaCl and 1 vol of toluene. The brown layer was incubated (24 hr at 25°C) twice with Protease XIV (Sigma Chemical Co., St. Louis, MO) following the procedure of McDonald and Stark (1988) and then treated twice with toluene. After toluene shaking of the brown layer, the pellet was washed with distilled water and centrifuged at 2,700 × *g* for 10 min. The solid was then resuspended with distilled water and transferred into small centrifuge containers and centrifuged at 12,000 × *g* for 14 min to scrape off the brown layer. That process was repeated until all of the brown layer disappeared. Purified starches from white and brown layers were suspended in distilled water, combined, and passed through a 53-µm screen and centrifuged. Purified starch was washed with distilled water three or four times. Finally, the starch was washed with acetone to remove water (Morrison et al 1984) and air-dried at room temperature.

TABLE I
Composition of Cultivars and Starch Recoveries and Yields

Cultivar	Bulk Sample	Protein (%, dwb)	Total Starch (%,dwb)	Starch Recovered from Grain (%, dwb)	Starch Yield (% starch wt)
Argee	1	11.9	69.5	66.8	96.2
	2	11.8	71.2	65.8	92.4
	3	12.0	68.5	67.3	98.2
Arthur	1	13.1	65.5	60.6	92.5
	2	14.3	66.5	61.6	92.7
	3	13.4	66.9	65.4	97.7
Augusta	1	10.1	69.1	62.0	89.8
	2	12.2	66.0	62.2	94.3
	3	11.5	68.4	64.5	94.3
Blackhawk	1	12.2	68.4	59.5	87.0
	2	13.4	67.4	63.7	94.5
Caldwell	1	12.0	69.2	64.9	93.8
	2	11.3	69.2	65.1	94.0
	3	11.5	69.2	65.9	95.3
Fillmore	1	10.8	69.1	66.0	95.5
	2	13.0	67.5	62.3	92.3
	3	12.6	67.7	62.7	92.6
Freedom	1	11.9	67.4	64.9	96.4
	2	10.5	69.9	64.3	92.0
	3	10.8	69.5	62.5	89.9
Geneva	1	11.4	68.2	64.1	94.1
	2	10.5	70.4	62.4	88.7
Hillsdale	1	11.3	67.2	61.8	92.0
	2	12.7	65.1	62.5	96.1
	3	14.1	65.0	59.5	91.6
McNair	1	12.4	68.3	61.3	89.8
	2	11.7	68.9	63.1	91.6
	3	12.7	67.7	61.2	90.5
Pioneer 2550	1	12.0	66.1	64.0	96.8
	2	12.0	66.6	64.8	97.3
	3	11.5	67.4	62.9	93.3
Pioneer 2555	1	11.8	68.3	66.4	97.2
	2	11.8	69.2	66.5	96.1
	3	12.3	69.1	65.1	94.2

Particle Size Analysis

Particle size characteristics of starches were determined by using a Malvern mastersizer model X, equipped with Malvern application software version 1.1a (Malvern Instruments Inc., Southborough, MA). The instrument is based on the principle of laser

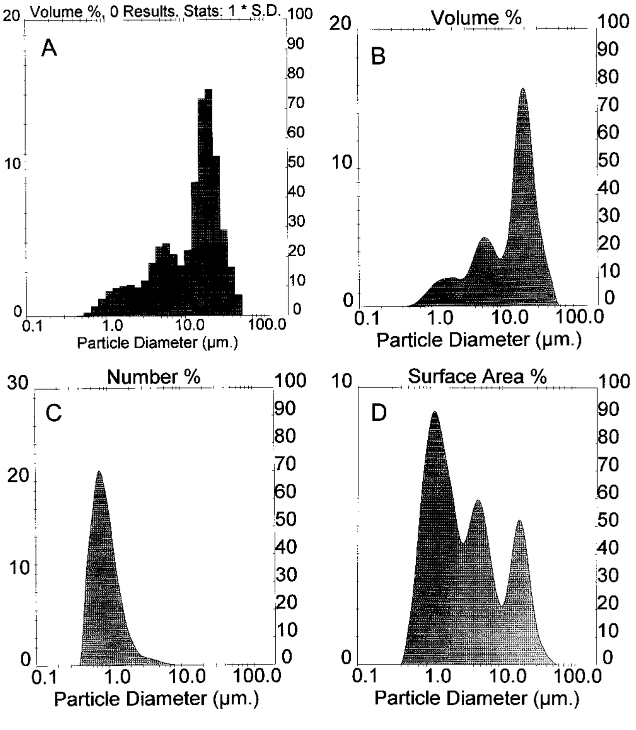

Fig. 1. Typical size (volume and number) and surface area distributions of starch granules measured with a Malvern mastersizer: histogram of volume distribution (**A**); graphical form of volume distribution (**B**); number distribution (**C**); surface area distribution (**D**).

light-scattering and capable of measuring sizes down to 0.1 μm. The size distribution is expressed in terms of the volumes of equivalent spheres and surface area and number distributions were derived from the volume distribution by use of numerical manipulation assuming spherical particles. Accuracy of the instrument was checked with standard glass particles (Duke Scientific Corp., Palo Alto, CA). For the purposes of comparing these and referenced data, it should be understood that particles measured by laser light-scattering produce data that represents the volume of equivalent spheres, with subsequent software calculation of particle diameter and surface area based, in part, on the average spherical shape of the circulating, rotating particles in suspension. Because most starch particles are not truly spherical, but rather prolate spheroids, these particle diameters (and those from Coulter counter instruments) tend to be smaller than those measured by image analysis techniques. Correlation of results of all methods of particle size measurement should be close to unity.

About 50 mg (db) of starch was weighed into glass tubes and suspended with 5 mL of distilled water. Tubes were vortexed and kept at 4°C for 1 hr. During that time tubes were gently vortexed for every 10 min. The starch suspension was transferred into the instrument's dispersion tank containing isopropyl alcohol and its size was measured.

Phosphorus Analysis

Phosphorus content of the isolated wheat starch was determined according to procedure B in the method described by Morrison (1964). Phosphorus content in the starch sample was calculated from a standard curve prepared with phosphorus standard solutions (Sigma). Starch lysophospholipid (LPL) content was obtained by multiplying phosphorus by the factor 16.5 (Morrison et al 1975).

Amylose Determination

Total and apparent amylose contents of isolated starch were determined colorimetrically according to Morrison and Laignelet (1983). Apparent amylose content (AAM) was measured before removal of starch lipids. Total amylose content (TAM) was determined on lipid-free starch prepared by precipitating starch from urea-dimethylsulfoxide solution with ethanol.

Statistical Analyses

All determinations were replicated two or three times, and data were analyzed (SAS Institute, Cary, NC). Multiple comparisons were performed after a preliminary *F*-test. When the *F*-test was significant at the 0.05 or 0.01 level, means were compared by the least significant difference test. Standard deviations of particle size parameters were ranked, and sums of the ranks were analyzed by the procedure of Wernimont (1985) to determine the variability of cultivars due to environment. Bulked samples were treated as within-cultivar replicates.

RESULTS AND DISCUSSION

Starch Recoveries and Yields

Total starch content of all samples ranged from 65.0 to 71.2% (Table I). Total starch was negatively correlated ($r = -0.662$, $P = 0.0001$) with grain protein content. The range for starch content of American wheat cultivars is reported to be from 61.2 to 72% (Pomeranz and MacMasters 1968, Cerning and Guilbot 1974). Values for soft wheat cultivars (69%) are reported to be higher than for hard wheat cultivars (64%) (Miller 1974). The cultivar Hillsdale had the lowest (65.8%) mean starch content, whereas Argee had the highest (69.7%).

Recovered starch ranged from 59.5 to 67.3%, expressed as % of dry kernel weight (Table I). Starch yields were relatively high (87–98.2%), expressed as a percentage of the total starch in the wheat.

Volume% of starch granules <9.9 μm diameter was not correlated with starch yield indicating selective losses of small granules did not occur during the isolation procedure. Therefore, contribution from small granules to the volume was accurately estimated and differences in volume% (=weight) of small granules reflected genetic or environmental differences in size distribution among wheat cutlivars.

PHYSICAL PROPERTIES

Size (volume and number) and surface area distributions of starch granules were investigated and typical distribution graphs obtained by Malvern mastersizer are shown in Fig. 1.

Granule Size Distribution

Volume distribution both in histogram and graphical forms (Fig. 1A and B) show the typical three populations of starch granules (<2.8 μm [small], 2.8–9.9 μm [midsize], >9.9 μm [large]) with peak values in the ranges of 1.7–2.3 μm, 4.3–6.5 μm, and 18.5–22.8 μm, respectively. The limits between the three populations were defined as the minimum of the curves that occurred at ≈2.8 and 9.9 μm. In eight out of 34 starch samples, the smallest size population somewhat overlapped with the midsize population (2.8–9.9 μm) and appeared as a shoulder. It is widely acknowledged that, at maturity, wheat contains two types of starch granules: large A-type granules >10 μm and smaller B-type granules ≤10 μm (Evers et al 1974, Hughes and Briarty 1976, Brocklehurst and Evers 1977, Evers and Lindley 1977, Baruch et al 1979, Meredith 1981, Eliasson and Karlsson 1983, Karlsson et al 1983, Dengate and Meredith 1984, Soulaka and Morrison 1985a). The synthesis of an A-type granule begins in an amyloplast four days after flowering (Buttrose 1963, Karlsson et al 1983, Parker 1985, Bechtel et al 1990). The diameter of these early synthesized granules stops increasing at ≈19 days after flowering, but the volume (thickness) continues to increase (Bechtel et al 1990). The synthesis of B-type

TABLE II
Mean Diameter Particle Size Distributions (volume%) of Starches[a]

Cultivar	<2.8 μm	<5.0 μm	<9.9 μm	<15.0 μm	<18.5 μm	<22.8 μm	<28.0 μm	28–42.8 μm
Argee	10.8c	19.1c	28.8bc	39.3cd	52.2e-g	69.1f-h	84.4cd	14.1bc
Arthur	13.3ab	24.6ab	35.3ab	45.9a-c	59.5b-d	75.8b-d	88.5ab	10.4de
Augusta	12.7a-c	23.7a-c	35.4ab	47.5ab	62.6ab	79.3ab	90.8a	8.2ef
Blackhawk	12.4a-c	22.6a-c	32.4a-c	45.3a-c	61.1a-c	78.0a-c	89.9a	8.9ef
Caldwell	10.4c	18.7c	26.9c	38.0d	51.4fg	68.2gh	83.5de	15.0ab
Fillmore	12.0a-c	22.1a-c	32.1a-c	41.3b-d	54.0d-g	70.1e-g	84.5cd	14.1bc
Freedom	12.6a-c	23.9a-c	34.1a-c	44.9a-d	58.0bc-e	73.5c-f	86.7bc	12.2cd
Geneva	11.2bc	19.7bc	30.2a-c	41.7bcd	55.1c-g	71.3d-g	85.3cd	13.3bc
Hillsdale	13.6a	26.3a	38.1a	50.9a	65.8a	80.9a	91.0a	7.8f
McNair	11.8a-c	22.0a-c	32.4a-c	42.2b-d	55.7cd-f	72.6d-g	86.7bc	12.0cd
Pioneer 2550	12.6a-c	23.3a-c	35.9ab	46.0a-c	58.6b-e	73.9c-e	87.0bc	11.8cd
Pioneer 2555	11.3a-c	21.7a-c	30.4a-c	37.9d	49.0g	65.1h	81.4e	17.1a

[a] Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

granules begins ≈12–14 days after flowering and increases in size up to 10 µm. In addition to A- and B- type granules, Bechtel et al (1990) indicated the existence of another distinct class of small granules (<5 µm) (C-type) that was synthesized 21 days after flowering. A-, B-, and C-type granules are more a function of when they initiated rather than their final size. Still, our observations confirm a trimodal distribution of wheat starch reported by Bechtel et al (1990). However, our cutoff points for differentiating size classes of granules are different. Bechtel et al (1990) reported cutoff points ≈5 and 16 µm, whereas ours are 2.8 and 9.9 µm. That could be explained by different techniques used during starch isolation and particle size determination, by the cultivars assessed, or by the different methods used to calculate particle size, spherical equivalent or prolate spheroid.

Cultivar means for the volume% distribution of granules in the investigated samples are given in Table II. Under these experimental conditions, 99% of the volume came from granules of 0.5–42.8 µm. Only ≈1% of the volume was contributed by granules of 42.8–52.7 µm. A greater portion of the total volume (92–97%), depending on the cultivar, was made up of granules <34.7 µm. Bechtel et al (1990) reported that the size of A-type granules continued to enlarge to a maximum diameter of 25–50 µm during endosperm development.

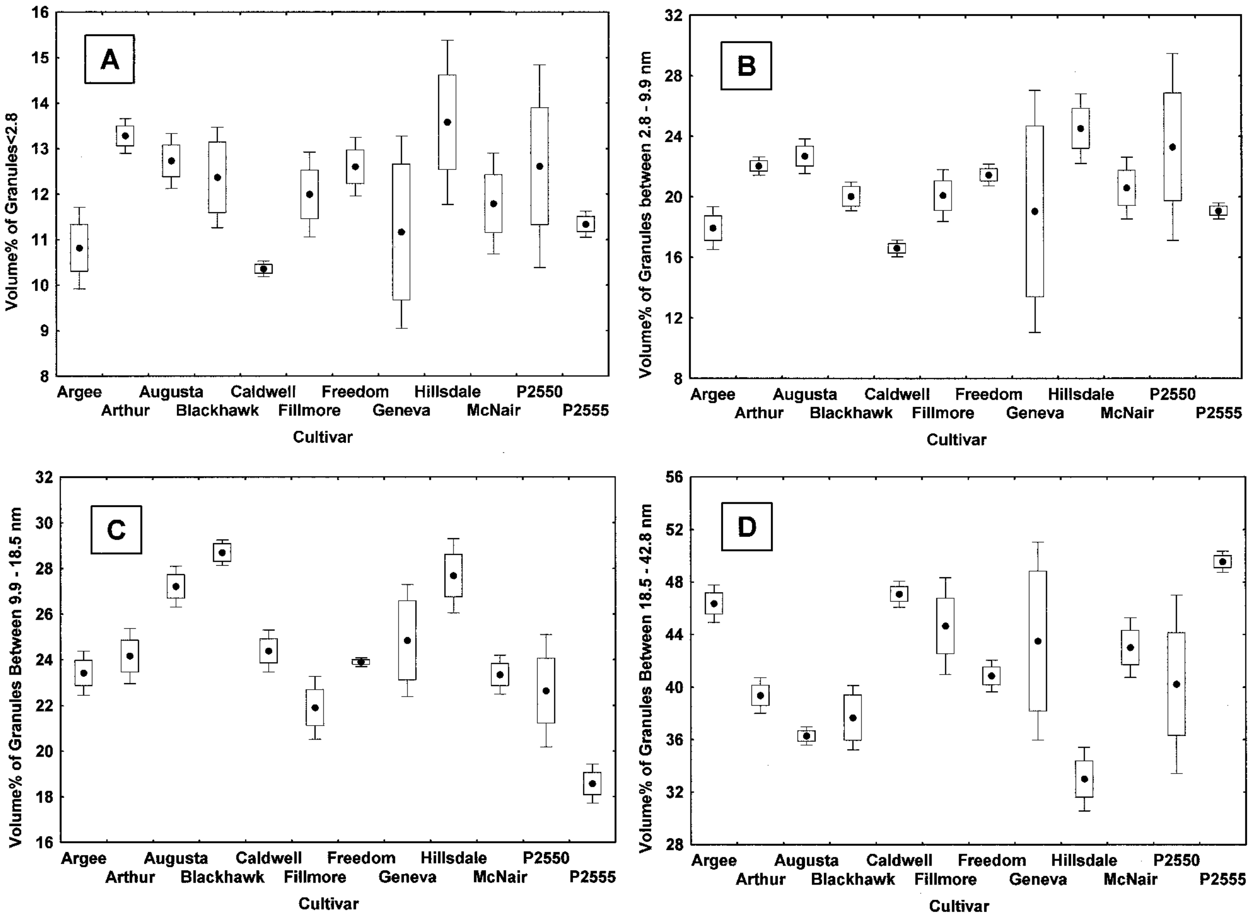

Fig. 2. Volume% of starch granules in four size classes: granules <2.8 µm (**A**); 2.8–9.9 µm (**B**); 9.9–18.5 µm (**C**); 18.5–42.8 µm (**D**). Filled circles represent data mean. Top and bottom bars of box show ± standard error of the mean. Whiskers represent one standard deviation ± from the mean.

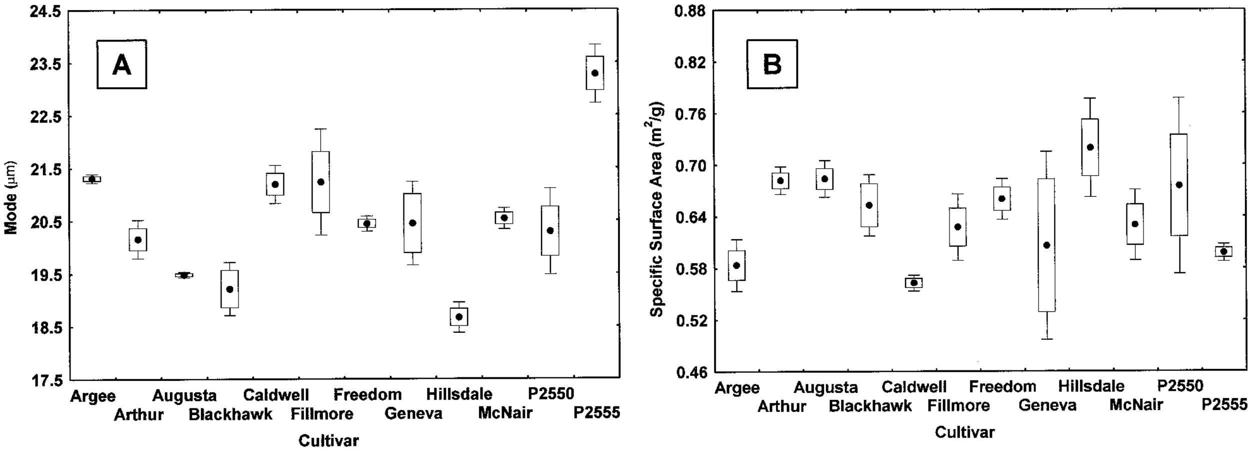

Fig. 3. Mode of the volume distribution curve for the “large granule” population (>9.9 µm) of starches isolated from 12 soft wheat cultivars (**A**). Specific surface area of starch granules from 12 soft wheat cultivars (**B**). Filled circles represent data mean. Top and bottom bars of box show ± standard error of the mean. Whiskers represent one standard deviation ± from the mean.

Contribution of the smallest granule population (<2.8 μm) to the total volume was 9.7–15.2% among 34 samples. The 12 cultivar means were 10.4–13.6% (Fig. 2A), Hillsdale had the highest mean and Caldwell had the lowest mean. Sample means for the percentage by volume of granules of 2.8–9.9 μm (midsize) were in the range of 13.4–27.9%. Cultivar means for that size group were 16.6–24.5% (Fig. 2B). Volume% of granules in that group were higher in cultivars Hillsdale, Arthur, Augusta, and Pioneer 2550; and lower in Caldwell, Argee, Geneva, and Pioneer 2555. Contributions from the large granule population (>9.9 μm) to the total volume were 57.9–76.9%. Variability within the large granule population was evaluated by dividing that group into two regions of 9.9–18.5 μm and 18.5–42.8 μm (Fig. 2C and D). Variability among cultivars within both regions were highly significant ($P <$ 0.0001). The proportion of granules >18.5 μm was higher in Pioneer 2555, Caldwell, Argee, and Fillmore.

The Malvern computer software program also computed starch granule diameter modes (location of the histogram peak). The mode diameter indicates the most frequent size class in the distribution. The mode of volume distribution ranged from 18.7 (Hillsdale) to 23.3 (Pioneer 2555) among the 12 cultivars (Fig. 3A).

Volume% of granules <5 and 9.9 μm were negatively correlated with the starch content of wheat samples (r = –0.568, P = 0.0005; r = –0.496, P = 0.0029; respectively). A positive correlation (r = 0.406, P = 0.017) was found between volume% of granules <2.8 μm and grain protein content. It is generally acknowledged that there is a positive relationship between the kernel texture (hardness) and protein content. For 212 cultivars, the correlation coefficient for harder kernel texture and flour protein content was r = 0.48. A subset of 82 of those cultivars were cleaned of all shriveled kernels, milled on a longer flow mill, and the resulting correlation was r = 0.51 between flour protein content and break flour yield.

The cultivars used in this study also differed in hardness. Because wheat hardness has a general positive association with kernel hardness, one may reason that the harder the kernel texture, the greater the number of small starch granules present in the starch. The relationship between size distribution of starch granules and wheat texture (hardness) has interested several investigators. However, because of the problems associated with sampling, results from those studies are not in agreement. Glenn et al (1992) reported that hard wheat samples had a greater number of small starch granules and a lower mean starch granule area as compared to the soft wheat cultivars, whereas Pitts et al (1989) had suggested the opposite. Bechtel et al (1993, Zayas et al 1994) were able to correctly identify wheat samples as hard or soft by visually comparing histograms of the size distribution or by using various morphometric parameters of starch. They suggested that starch morphometrical parameters may be useful to separate hard wheat from soft wheat. They found that hard wheat had a greater percentage of small granules and fewer large granules than soft wheats. They could identify wheat class by categorizing the distributions.

Volume distribution of starch granules was transformed to number distribution using Malvern application software to determine the number of granules within size classes. Figure 1C shows a typical diagram of number distribution of granules. About 10% of the total starch granules were made up of granules <0.62 μm. The number of small granules were significant although the contribution to the total volume was small. Proportions of granules <2.8 and 9.9 μm, expressed on a number basis, were in the range of 96.2–97.4% and 99.8–99.9%, respectively. Those values are greater than previously reported values. Using a Coulter counter, Evers and Lindley (1977) reported that granules <10 μm comprised 97.5% of the total population. The difference in results can be attributed to improvements in isolation techniques. An improved isolation technique prevents losses of small granules, therefore, their number increases. Also, the Malvern instrument used in our study was capable of measuring a size down to 0.1 μm as opposed to 1.7 μm, which was the limit for the Coulter counter (Evers and Lindley 1977). Bechtel et al (1990) also investigated number distribution of starch granules using a quantitative image analysis technique. They reported that the total number of starch granules was 45.7% for C-type granules (<5.3 μm), 49.5% for B-type granules (5.3–15.9 μm), and 4.8% for A-type granules (> 15.9 μm). The difference in the data may result from the methods of measurement, isolation, or methods of calculation used by the software.

Surface Area Distribution

Accumulative surface area distributions of granules for size groups are given in Table III. Figure 1D also shows a typical diagram of surface distribution of granules. As with volume distribution, surface area distribution of starches also was trimodal. The smallest granule population (<2.8 μm) occupied 51–55% of the total surface area. Contribution of size groups of 2.8–9.9 μm and 9.9–52.7 μm to the total surface area were 23–28% and 18–25%, respectively. Specific surface area of the starches (the ratio of total surface area/ total volume) ranged from 0.5288 to 0.7694 m^2/g and was highly correlated (r = 0.986, P = 0.0001) with the volume% of granules <9.9 μm. Cultivar specific surface area means ranged from 0.5628 to 0.7194 m^2/g and significantly differed (P < 0.02) among those cultivars (Fig. 3B). Within cultivar ranges differed considerably.

Variability of Physical Properties Within Cultivars

Tests for variability of physical properties within each cultivar are shown in Table IV. Significant differences between groups within cultivars indicate variability within cultivar replicate even though cultivars were bulked across location-year to reduce those effects. Fillmore did not show significant variability for any of the particle size values.

Standard deviations of size parameters (volume% of granules <2.8 μm, 2.8–9.9 μm, 9.9–18.5 μm, 18.5–42.8 μm, and specific surface area) were ranked. The sum of the ranks were analyzed to determine variability within cultivars (data not shown). The cultivars Geneva and Pioneer 2550 were consistently more variable, whereas Pioneer 2555 was less variable than the other nine cultivars. Standard deviations are also evident in Figs. 2 and 3.

TABLE III
Mean Surface Area Distribution (%)

Cultivar	<1 μm	<1.9 μm	<2.8 μm	<5.3 μm	<9.9 μm	<15.0 μm	<18.5 μm	<22.8 μm	<28.2 μm
Argee	15	40	52	67	76	82	87	93	97
Arthur	16	42	55	72	80	86	89	94	97
Augusta	17	42	54	70	80	86	91	96	98
Blackhawk	16	41	54	70	78	85	90	95	98
Caldwell	14	38	51	66	75	81	87	92	97
Fillmore	15	40	53	70	79	84	88	93	97
Freedom	15	41	54	71	80	85	90	95	98
Geneva	16	40	53	67	76	83	88	93	97
Hillsdale	17	42	54	72	82	87	92	96	99
McNair	15	40	53	69	79	84	89	94	98
Pioneer 2550	17	42	54	70	81	85	90	95	98
Pioneer 2555	14	38	52	70	78	82	87	92	96

Nitrogen Content

The nitrogen content of isolated starch often has been used to indicate starch purity. Endosperm storage proteins, LPL, and proteins located inside the starch granules contribute to the nitrogen content of isolated starch (Morrison 1981). Thus, the low nitrogen content of purified starches (0.03%) (Table V) indicates absence of endosperm proteins and, by implication, nonstarch lipids (Morrison 1981). Table V demonstrates that surface proteins were effectively removed by toluene- and salt-solution shaking and repeated centrifugation of aqueous starch slurries during the isolation procedure.

Amylose Content

Total amylose contents (TAM) of lipid-free starches and apparent amylose (AAM) contents of the starches with lipid present are given in Table V. TAM contents of cultivars were 26.7–28.8%. Although the range was small (2.1 percentage points), significant differences in TAM existed among cultivars. Fillmore had the lowest TAM, followed by Pioneer 2555, whereas Blackhawk, Freedom, McNair, and Augusta had the highest amount of TAM. Significant differences were also found in the AAM contents of cultivars. Fillmore had the lowest AAM content followed by Hillsdale and Argee, while Freedom and McNair had the highest. AAM positively correlated ($r = 0.788$, $P = 0.0001$) with TAM and negatively correlated ($r = –0.376$, $P = 0.03$) with lipid-complexed amylose in the starches. No correlations were found between volume% of granules <5–9.9 μm and AAM and TAM content of starches. However, there was a significant positive correlation ($r = 0.533$, $P = 0.001$) between TAM and volume% of granules of 9.9–18.5 μm, and a significant negative correlation ($r = –0.375$, $P = 0.029$) between TAM and volume% of granules of 18.5–42.8 μm. Some reported amylose content was higher in large granules (Kulp 1973; Duffus and Murdoch 1979; Soulaka and Morrison 1985a; Morrison 1989). Others reported the same amylose content in both small and large granules (Bathgate and Palmer 1972; Evers et al 1974).

Lipid-complexed amylose contents (ΔAM) of cultivars ranged from 5.5 to 6.6% (Table V). Argee, Arthur, Blackhawk, and Hillsdale had the highest amount of ΔAM. There was a significant positive correlation ($r = 0.7626$, $P = 0.0001$) between phospholipid and ΔAM content of starches. ΔAM content of starches positively correlated ($r = 0.437$, $P = 0.01$) with granules <2.8 μm and correlated ($r = 0.438$, $P = 0.01$) with granules of 9.9–18.5 μm due to high phospholipid content and high amylose content, in the first and second groups, respectively. The ΔAM content of starches was negatively correlated ($r = –0.49$, $P = 0.003$) with mean starch granule diameter and was positively correlated ($r = 0.42$, $P = 0.013$) with specific surface area of granules.

Phospholipid Contents

In previous reports, LPL accounted for 86–94% of total starch lipids (Morrison et al 1975, 1981; Meredith et al 1978; Hargin and Morrison 1980). Phospholipid values, calculated from total starch phosphorus, were similar to those from lipids extracted with various solvents (Morrison et al 1975, Meredith and Dengate 1978, Soulaka and Morrison 1985a). Therefore, for simplicity we used total starch phosphorus to quantify the level of phospholipid in the starch samples. Mean phospholipid contents of cultivars were 802–990 mg/100 g of dry starch. Significant differences were found among cultivars (Table V). Starches from Caldwell averaged the lowest and Hillsdale the highest phospholipid content. Phospholipid content was negatively correlated ($r = –0.56$, $P = 0.0006$) with mean starch granule diameter and positively correlated ($r =$

TABLE IV

Significance Levels from *F*-Tests for Intracultivar Variability of Volume% Distribution, Specific Surface Area, and Mode for 12 Soft Wheat Cultivars[a]

	Volume% of Granules				
Cultivars	<9.9 μm	9.9–18.5 μm	18.5–42.8 μm	Specific Surface Area	Mode
Argee	**	ns	*	**	ns
Arthur	ns	*	ns	ns	**
Augusta	**	**	ns	**	ns
Blackhawk	**	ns	**	**	ns
Caldwell	ns	**	*	ns	*
Fillmore	ns	ns	ns	ns	ns
Freedom	*	ns	*	**	ns
Geneva	**	**	**	**	ns
Hillsdale	**	**	**	**	ns
McNair	**	**	**	**	*
Pioneer 2550	**	**	**	**	ns
Pioneer 2555	ns	**	*	ns	**

[a] * and ** significant at $P < 0.05$ and $P < 0.01$, respectively. ns = Not significant $P > 0.05$.

TABLE V

Mean Nitrogen, Amylose,[a] and Lipid[b] Contents of Starches from 12 Soft Wheat Cultivars

Cultivar	Nitrogen (mg/100 g of dry starch)	AAM (%, dwb)	TAM (%, dwb)	ΔAM (%, dwb)	Phosphorus (mg/100 g of dry starch)	LPL (mg/100 g of dry starch)
Argee	29.9	21.7e[c]	28.1ab	6.4a	55.1b	909b
Arthur	31.7	22.0de	28.2ab	6.2ab	54.1bc	893bc
Augusta	31.9	22.7a-c	28.4a	5.6bc	49.8c-e	822c-e
Blackhawk	30.0	22.2c-e	28.8a	6.6a	55.1b	908b
Caldwell	32.4	22.8a-c	28.2ab	5.6c	48.6e	802e
Fillmore	32.1	20.9f	26.7c	5.8bc	50.7b-e	836b-e
Freedom	31.7	23.0a	28.8a	5.7bc	52.6b-e	868b-e
Geneva	30.4	22.3b-e	28.0ab	5.7bc	49.1de	810de
Hillsdale	33.0	21.7e	28.2ab	6.6a	60.1a	990a
McNair	30.2	22.9ab	28.8a	5.8bc	54.0bc	890bc
Pioneer 2550	27.7	22.6a-d	28.1ab	5.5c	53.5b-d	882b-d
Pioneer 2555	31.2	22.0de	27.5b	5.5c	49.3de	814de

[a] AAM = apparent amylose; TAM = total amylose; ΔAM = TAM – AAM.

[b] LPL = lysophospholipid calculated as starch phosphorus × 16.5.

[c] Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

0.53, $P = 0.0013$) with specific surface area of granules, indicating smaller granules have higher lipid contents. Those results agree with previous observations (Kulp 1973, Meredith and Dengate 1978, Soulaka and Morrison 1985a, Morrison 1989). It is possible that lipid is preferentially associated with the biosynthesis of small starch granules.

Variability of Chemical Composition Within Cultivars

Tests for variability of chemical composition within each cultivar are shown in Table VI. Variability of AAM contents within cultivars was significant for Fillmore, Freedom, Geneva and Hillsdale. For TAM content, significant variations were found within Blackhawk and Fillmore cultivars. There was significant variation of ΔAM within Fillmore, Freedom and Hillsdale cultivars.

Starch phosphorus content significantly varied within the cultivars Augusta, Caldwell, Freedom, Hillsdale, McNair, and Pioneer 2550 (Table VI). As with the granule size distribution, those results indicate that phosphorus content of starch samples is more susceptible to environmental conditions than is amylose content.

CONCLUSIONS

Analysis of starch samples from 12 soft wheat cultivars suggest that cultivar is a determining factor of starch granule size distribution and amylose and phospholipid content. However, even though we tried to reduce variation by bulking samples across location and year, variation within a cultivar, especially for starch granule size distribution and phospholipid content, indicates that environmental factors may have substantially significant influence on those properties, as well. Some cultivars (Geneva, Pioneer 2550) apparently are more responsive to the environment than others and may require multiple environment testing to predict cultivar performance.

Significant positive correlations between starch specific surface area and the volume% of granules <9.9 μm and starch phospholipid content show that smaller granules have greater surface area and higher lipid contents. In addition, higher amylose content in the 9.9–18.5 μm size group suggest that differences in starch granule size distribution among cultivars may result in differences in pasting, swelling and gelatinization properties. The relationships among those properties are currently under investigation in our laboratory.

A positive correlation ($r = 0.406$, $P = 0.017$) between volume% of granules <2.8 μm and grain protein content suggests that grain hardness, in part, may be due to starch granule size distribution between wheat classes, or even within a class.

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TABLE VI
Significance Levels[a] from *F*-Test for Intracultivar Variability of Amylose[b] and Phosphorus Contents for 12 Soft Wheat Cultivars

Cultivar	AAM	TAM	ΔAM	Phosphorus
Argee	ns	ns	ns	ns
Arthur	ns	ns	ns	ns
Augusta	ns	ns	ns	*
Blackhawk	ns	*	ns	ns
Caldwell	ns	ns	ns	**
Fillmore	*	**	*	ns
Freedom	*	ns	*	*
Geneva	*	ns	ns	ns
Hillsdale	*	ns	*	*
McNair	ns	ns	ns	**
Pioneer 2550	ns	ns	ns	*
Pioneer 2555	ns	ns	ns	ns

[a] * and ** significant at $P < 0.05$ and $P < 0.01$, respectively. ns = Not significant $P > 0.05$.

[b] AAM = apparent amylose; TAM = total amylose; ΔAM = TAM – AAM.

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