Distinguishing Selected Hard and Soft Red Winter Wheats by Image Analysis of Starch Granules

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ABSTRACT

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Starch was isolated from 24 wheat samples representing 14 hard red winter (HRW) and 10 soft red winter (SRW) wheats grown in various areas of Kansas. Samples with a wide range of near-infrared reflectance hardness values were selected from the Kansas Winter Wheat Performance Test. Isolated starch images were video-recorded using dark-field light microscopy, and the digital images were analyzed by extracting various morphometric parameters. Two of these, equivalent diameter and aspect ratio, were useful in discriminating HRW from SRW wheats. Statistical analysis procedures were used to transform the raw data (equivalent diameter and aspect ratio) into frequency percentages within defined ranges. This transformation resulted in a new set of distributional data called *counts*. A plot of data for Count 4 (equivalent diameter in the 5.5–7.0 μm range) versus data for Count 3 (aspect ratio in the 1.65–1.95 μm range) was able to distinguish HRW wheats from SRW wheats, even though the near-infrared reflectance hardness values overlapped. Distributional analyses of the starch granule size and shape descriptors proved useful in classifying hard and soft wheats.

Grain hardness is an important characteristic in the wheat industry. Although hardness is generally determined at the flour mill, the physical and biochemical parameters of hardness are not well understood. It is generally thought that hardness results from the strength of starch-protein interactions (Barlow et al 1973). In this context, a 15kDa protein called friabilin has been identified in soft starch wheats and is thought to impart "softness" to the wheat (Greenwell and Schofield 1986, Schofield and Greenwell 1987), rather than the proteins, which act as an adhesive (Barlow et al 1973, Simmonds et al 1973). The close spatial relationship between starch and storage proteins during grain development may influence starch granule morphometry. Quantitative image analysis has been used to study both isolated wheat starch (Bechtel et al 1990, 1991) and sectioned endosperm tissue (Pitts et al 1989, Glenn et al 1992). Starch granule mean area, standard deviation of granule area, and coefficient of variation were all found to be indicators of differences between hard and soft wheat endosperm cell geometry (Pitts et al 1989).

Digital image analysis of isolated starch granules, and subsequent pattern recognition, were used in this study to compare two wheat classes, hard red winter (HRW) and soft red winter (SRW), to better understand what factors contribute to the phenomenon of grain hardness. A previous report (Bechtel et al 1993), based on a visual evaluation of histogram plots of the wheat samples, indicated that size distributions differ between starches from HRW and SRW wheats. We now present a full statistical analysis of this data. The quantitative analysis supports our previous conclusions for distinguishing these two wheat classes differing in grain hardness.

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MATERIALS AND METHODS

Samples

Twenty-four wheat samples (14 HRW and 10 SRW) were selected from the 1988 Kansas Winter Wheat Performance Test, representing nine cultivars grown at different locations or environments in Kansas (Fig. 1, Table I). The samples were analyzed for near-infrared reflectance (NIR) hardness, moisture, and protein content. Starch was isolated from the grain samples as previously described (Bechtel et al 1990), with the following modification: the germ was excised, and the remainder of the grain was chopped into small pieces with a razor blade. The small endosperm pieces were then placed in the homogenization buffer and prepared as previously described (Bechtel et al 1990). Starch damage was checked by polarization microscopy.

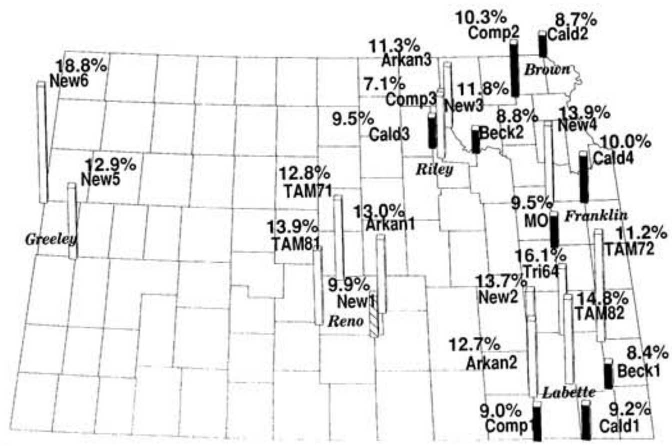

Fig. 1. Reference map of the state of Kansas showing the growing locations of each wheat cultivar studied and the percentage of starch granules with an equivalent diameter in the 5.5–7.0 μm range. See Table I for full names of cultivars. County names are in italic type.

Data Acquisition

Isolated starch was viewed as previously described (Bechtel et al 1993). Video images were analyzed by a Kontron Image Analysis System (Roche Image Analysis Systems, Elon College, NC). This system is based on a 386 AT computer with a DOS operating system, 8 Mb of video memory, and a 40-Mb hard disk. The Kontron has extensive libraries of image processing subroutines and can handle PAL (European 50 Hz) and NTSC (American 60 Hz) formats. Computed starch granule morphometrical data were analyzed using SAS (1991) and a Sun SPARC 4/50 workstation (Sun Microsystems, Mountain View, CA).

Software for Starch Evaluation

A program, written in C language to speed analysis, was designed to evaluate the images from the videotape without operator presence. The image digitization format was 512 × 512 pixels. The software program kept track of incoming images by measuring grey levels of the image and several statistical moments around the origin (zero point) of a histogram of grey levels. The discrimination subroutine computed five statistical moments, of which two were used: first moment for mean and second moment for variance. Each set of 40 videotaped images of starch granules was separated by a white image frame. The first incoming image after a white frame was stored and considered the initial reference image; the subsequent frame was subtracted from each previous reference image. The measurement portion of the program was initiated when the grey level variance between images was more than 120, a preset value determined experimentally, and picked up the differences between a current image frame and the next image. A pause command delayed the videotape recorder until image processing measurements were completed; playback then resumed until the next incoming frame was recognized and the pause command was repeated. The program also rejected images with excessive numbers of starch granules, which could be confused with the white frame that separated the samples. We evaluated the mean of grey values plus the standard deviation multiplied by 2.5. When this value exceeded 180, the images were rejected. This value was determined experimentally and was effective in recognizing images that looked nearly white to the instrument.

A second step of image preprocessing was to sharpen edge boundaries to eliminate fuzzy areas caused by dust. We used the Kontron IPS highpass filter with a 20 × 20 matrix, five loops, and no offset. The binarization of the images was determined experimentally by setting a threshold equal to the mean of grey values plus a standard deviation multiplied by 1.5. The high contrast of white starch granules on a black background was sufficient to use this threshold for discrimination, despite flaws in the videotaped images. Binarization was followed by a hole-filling step, because many of the larger starch granules had dark centers when viewed with dark-field microscopy. Granules that touched, as well as erroneously identified objects in the field of view, were eliminated using a preset value of:

$$\text{Convex perimeter}/\text{perimeter}^2 > 0.011$$

The percent of rejected objects was only about 3%. A total of 152,237 starch granules were measured for the 24 wheat samples, varying from 3,238 to 14,671 for each sample. A record of sequential image frame counts and other image processing data was displayed on the screen to ensure control of the measurement process.

Measurements were automatically converted to micrometers by comparing the aspect ratio of a pixel where $X = 1.07142$ μm and $Y = 1.33928$ μm. Microscopic images were calibrated with standard latex spheres 25 μm in diameter. Measured features of starch granules: area, perimeter, convex perimeter, maximum diameter (length), and minimum diameter, were included in a database. Eventually the morphometry of starch granules were described by derived features, such as:

$$\text{Equivalent diameter} = 2\sqrt{\text{area}/\pi} \quad (1)$$

$$\text{Aspect ratio} = \text{length}/\text{width} \quad (2)$$

$$\text{Circularity shape factor} = 4\pi\ \text{area}/\text{perimeter}^2 \quad (3)$$

The database was exported in ASCII format to the MATLAB software package, which produced graphics of equivalent diameter and other parameters.

TABLE I
1988 Kansas Wheat Starch Granules Study, Physical Characteristics

Cultivar[a]	Class	County	NIR[b] hardness	Moisture (% wb)	Protein (%)
Arkan1	HRW	Reno	54	8.9	16.7
Arkan2	HRW	Labette	102	9.9	15.2
Arkan3	HRW	Riley	75	9.6	13.5
New1	HRW	Reno	48	9.2	15.3
New2	HRW	Labette	97	10.0	14.0
New3	HRW	Riley	73	9.6	12.3
New4	HRW	Franklin	57	10.4	10.2
New5	HRW	Greeley (dry)	80	9.7	13.0
New6	HRW	Greeley (irrigated)	59	9.6	14.9
TAM71	HRW	Reno	73	9.9	13.7
TAM72	HRW	Labette	115	10.4	14.3
TAM81	HRW	Reno	48	9.9	15.5
TAM82	HRW	Labette	80	10.1	14.0
Tri64	HRW	Labette	96	10.1	15.6
Beck1	SRW	Labette	39	9.8	14.0
Beck2	SRW	Riley	16	9.7	12.7
Cald1	SRW	Labette	44	9.9	13.4
Cald2	SRW	Brown	33	10.3	9.4
Cald3	SRW	Riley	24	9.9	11.6
Cald4	SRW	Franklin	33	10.6	10.2
Comp1	SRW	Labette	55	10.0	14.8
Comp2	SRW	Brown	40	10.4	10.8
Comp3	SRW	Riley	26	9.6	13.4
MO	SRW	Franklin	21	10.2	11.0

[a] Arkan1; Arkan2; Arkan3; New1 = Newton1; New2 = Newton2; New3 = Newton3; New4 = Newton4; New5 = Newton5; New6 = Newton6; TAM71 = TAM107 (Reno); TAM72 = TAM107 (Labette); TAM81 = TAM108 (Reno); TAM82 = TAM108 (Labette); Tri64 = Triumph64; Beck1 = Becker1; Beck2 = Becker2; Cald1 = Caldwell1; Cald2 = Caldwell2; Cald3 = Caldwell3; Cald4 = Caldwell4; Comp1 = Compton1; Comp2 = Compton2; Comp3 = Compton3; MO = MO9965.
[b] Near-infrared reflectance.

RESULTS

Comparison of NIR hardness values obtained for the wheat samples shows that a certain amount of overlap occurs for the SRW and HRW samples, depending on the value chosen for

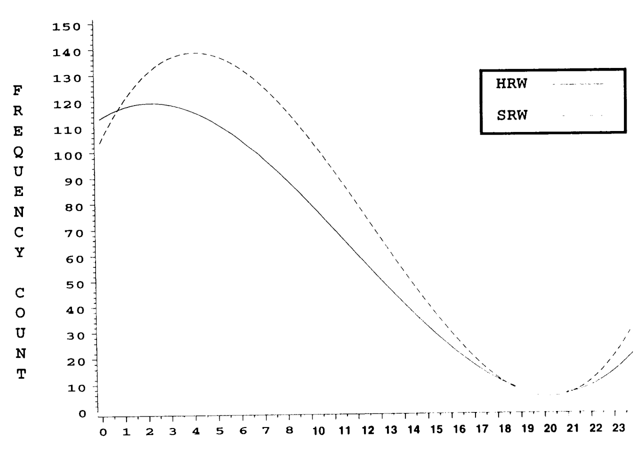

Fig. 2. Cubic regression curves for hard red winter and soft red winter wheat data plotted as frequency count (percentages within defined ranges) vs. equivalent diameter (μm).

the separation (Table I). For an NIR hardness value of 40, three SRW wheats were at or above this value. All of the HRW were above 40. An NIR value of 50 would allow the misclassification of two HRW as soft and one SRW as hard. NIR values, therefore, do not necessarily discriminate between soft HRW or hard SRW samples.

Visualization of such massive quantity of data by graphical representation, though helpful, gave little quantitative information. For example, scatter plots of individual morphometrical features of all observations, classified by cultivar, showed little clustering of the data for hard and soft wheats (data not shown). Scatter plots with wheat classified as HRW and SRW showed some clustering of soft and hard samples, but contained a large amount of overlap of individual observations (data not shown). Cubic regression curves, plotted as frequency versus equivalent diameter, show that some difference exists between the wheat samples classified as either HRW or SRW (Fig. 2).

Frequency distributions for equivalent diameter, circularity shape factor, aspect ratio, and area were determined using Proc Frequency (SAS). Derived variables (*counts*) are the transformed data (SAS Proc Transpose) representing discrete frequency classes. Table II shows the range of values for equivalent diameters and aspect ratios and the new parameter identifications. Ranges for counts were chosen arbitrarily after visual evaluation of plots of the starch granules' morphometrical variables.

TABLE II
Count Parameter for Equivalent Diameter (μm) and Aspect Ratio Ranges

Equivalent Diameter		Aspect Ratio	
Range, μm	**Count**	**Range**	**Count**
1.00–2.50	1	Low–1.35	1
2.50–4.00	2	1.35–1.65	2
4.00–5.50	3	1.65–1.95	3
5.50–7.00	4	1.95–2.25	4
7.00–8.50	5	2.25–2.55	5
8.50–10.00	6	2.55–2.85	6
10.00–11.50	7	2.85–3.15	7
11.50–13.00	8	3.15–3.45	8
13.00–14.50	9	3.45–3.75	9
14.50–16.00	10	3.75–4.05	10
16.00–17.50	11	4.05–4.35	11
17.50–19.00	12	4.35–4.65	12
19.00–20.50	13	4.65–4.95	13
20.50–High	14	4.95–5.25	14
		5.25–5.55	15
		5.55–5.85	16
		5.85–6.15	17
		6.15–High	18

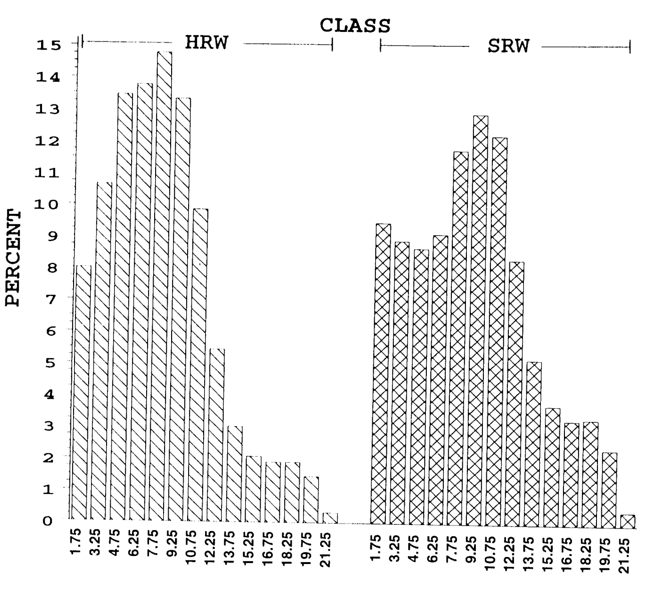

Fig. 3. Histogram of the percentage of starch granules from different wheat samples occuring within the specified ranges of equivalent diameters. HRW = hard red winter, SRW = soft red winter.

Plotting the percentage of starch granules that fall within the specified range of equivalent diameters as a histogram (Fig. 3) allows the comparison of the percentage of starch granules from different samples within the various ranges. For example, Count 4 has a midpoint value of 6.25 μm for the 5.5–7.0 μm range, representing 13.8% of the starch granules in all HRW wheat samples studied. Count 4 for the SRW wheat samples represented 9.2%. Count variables selected in Table III (equivalent diameter) and Table IV (aspect ratio) had the least degree of overlap between HRW and SRW wheats. Count 4 (equivalent diameter) values were 7.1–10.3% for SRW and 9.9–16.1% for HRW, with only one overlap value (Newton 1, 9.9%). Count 3 (aspect ratio) values showed no overlap between HRW and SRW wheat starch granules (25.9–32.0% for HRW samples and 19.9–25.4% for SRW samples). Count 3 (aspect ratio) and Count 4 (equivalent diameter) were subjectively identified as the best for class separation. A plot of Count 3 (aspect ratio) versus Count 4 (Fig. 4) showed the feasibility of separating HRW and SRW wheat classes.

DISCUSSION

One major theory regarding the source of endosperm hardness is that the starch-protein interface is stronger in hard wheats than it is soft ones (Barlow et al 1973). A close and tight interaction between these two components during development could influence the shape of starch granules. The relationship of hardness to starch-protein interaction is further substantiated by the fact that proteins from hard and soft wheats have similar in situ mechanical properties (not to be confused with rheological properties), as do the starches from hard and soft wheats (Barlow et al 1973). Also, the 15-kDa protein is consistently associated with soft wheat starch (Greenwell and Schofield 1986, Schofield and Greenwell 1987). Consequently, the interaction between starch and protein during growth and development may manifest itself in differences in shape and size of the starch between hard and soft wheats, which digital image analysis may depict. Three classes of starch granules based on size and time of initiation have been proposed: type A (large, >16 μm); type B (medium, 5–16 μm); and type C (small, <5 μm) (Bechtel et al 1990). These results show that a major difference between starch of HRW and SRW wheats is in the granules from Count 4 (equivalent diameter) with a size range of 5.5–7.0 μm, which is within the type B size class (Bechtel et al 1990).

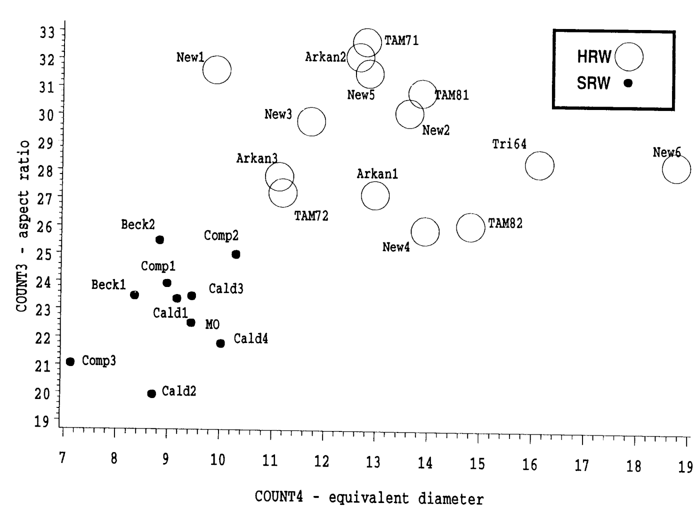

Fig. 4. Separation of hard red winter (HRW) wheats and soft red winter (SRW) wheats using Count 4 (equivalent diameter) values and Count 3 (aspect ratio) values. See Table I for full names of cultivars.

Hard wheat cultivars studied by image analysis of sectioned material had larger mean starch granule areas than did the soft wheat cultivars (Pitts et al 1989). However, analysis of the same samples using another technique revealed that the mean areas of hard wheat starch granule were lower (Glenn et al 1992). Comparing the percentage of our type A granules (sum of Counts 11–14 for equivalent diameter) between hard and soft wheats revealed that the hard wheats had a mean value of 6.45% and the soft wheats had a mean value of 9.96%. However, there were two overlaps: TAM 107-2 (16.40%) and Compton 2 (6.17%). Similar calculations from Glenn et al (1992) revealed values of 6.16 and 9.37% for hard and soft cultivars, respectively. These results suggest that soft wheats tend to have a larger proportion of starch granules in the type A size class than do hard wheats, but there are exceptions (Tam 107-2 and Compton 2). Our data for the proportion of type A granules to the total number of starch granules is consistent with several definitive studies (Morrison and Gadan 1987, South and Morrison 1990) which showed type A granules comprise between 7% and 10% of the total starch population. Our results, however, are based on starch

TABLE III
Count Values for Equivalent Diameter (%)

		Count														
Class	**Cultivar**[a]	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**SUM**[b]
HRW	Arkan1	11.35	14.76	15.51	12.99	11.24	9.14	7.91	5.30	3.52	2.23	2.00	1.87	1.70	0.48	6.05
	Arkan2	4.51	8.13	10.69	12.73	14.70	14.65	11.94	8.17	5.06	3.10	2.49	2.05	1.53	0.26	6.33
	Arkan3	8.54	9.45	11.76	11.16	13.45	15.43	12.88	5.96	2.86	2.08	1.94	2.18	1.84	0.48	6.44
	New1	6.49	8.07	10.36	9.88	13.69	16.04	13.79	8.28	3.89	2.23	2.45	2.41	1.93	0.50	7.29
	New2	9.79	11.27	13.61	13.72	14.82	12.59	8.98	4.41	2.11	1.82	2.00	2.32	2.23	0.33	6.88
	New3	9.85	9.63	10.75	11.76	13.94	13.68	12.47	7.11	3.27	2.06	1.94	1.82	1.45	0.29	5.50
	New4	6.67	10.50	13.08	13.93	16.64	15.08	10.69	5.23	2.73	1.89	1.33	1.30	0.79	0.15	3.57
	New5	6.23	8.49	10.43	12.91	18.20	18.40	12.58	5.91	2.75	1.35	1.04	0.97	0.64	0.10	2.75
	New6	8.88	15.08	21.13	18.80	12.87	7.77	4.93	2.60	1.60	1.59	1.57	1.62	1.27	0.30	4.36
	TAM71	6.92	8.02	10.28	12.81	18.14	17.40	10.51	4.83	2.51	1.90	2.27	2.17	1.82	0.42	6.68
	TAMM72	8.59	9.82	12.01	11.24	10.81	10.22	7.23	5.16	4.23	4.29	4.88	5.50	4.82	1.20	16.40
	TAM81	8.93	11.66	16.48	13.91	12.32	10.17	8.10	5.15	3.12	2.44	2.46	2.86	1.96	0.44	7.72
	TAM82	16.83	16.85	15.20	14.82	11.02	8.38	5.58	3.69	2.02	1.57	1.32	1.28	1.14	0.31	4.05
	Tri64	5.69	8.29	14.15	16.12	16.57	12.10	8.71	5.72	3.82	2.59	2.29	2.23	1.41	0.31	6.24
SRW	Beck1	10.91	10.81	10.10	8.41	10.47	9.81	9.47	6.45	5.00	3.90	4.76	4.98	4.39	0.54	14.67
	Beck2	8.14	7.08	7.68	8.84	11.58	13.87	13.94	9.84	5.97	3.95	3.53	3.18	1.82	0.59	9.12
	Cald1	14.22	12.74	11.21	9.23	11.32	8.89	6.98	4.66	3.75	4.38	3.87	4.82	3.36	0.56	12.61
	Cald2	13.26	10.51	8.31	8.70	9.78	10.59	11.35	7.55	5.10	3.87	3.53	4.26	2.67	0.51	10.87
	Cald3	13.07	10.35	9.20	9.52	11.25	11.10	9.97	6.20	4.32	3.82	4.00	3.92	2.70	0.57	11.19
	Cald4	7.87	8.28	8.10	10.04	11.64	14.45	13.66	9.19	5.49	3.48	2.81	2.67	1.94	0.40	7.82
	Comp1	11.83	11.35	9.08	9.03	11.20	11.58	10.65	6.78	4.93	3.75	3.26	3.33	2.78	0.44	9.81
	Comp2	6.25	6.91	7.94	10.31	14.96	16.94	14.44	8.75	4.32	2.99	2.12	2.14	1.55	0.36	6.17
	Comp3	8.62	7.98	8.44	7.10	9.70	11.60	13.31	12.09	7.62	4.65	3.38	3.14	2.04	0.33	8.89
	MO	7.38	7.67	8.50	9.50	12.85	14.63	13.74	8.81	5.12	3.39	3.08	2.90	2.17	0.26	8.41

[a] See Table I.
[b] SUM = Counts 11 + 12 + 13 + 14.

TABLE IV
Count Values for Aspect Ratio (%)

		Count																	
Class	**Cultivar**[a]	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
HRW	Arkan1	7.64	29.19	27.21	9.87	8.38	1.92	9.24	0.48	0.51	0.15	0.11	0.10	3.35	0.04	1.81	...	...	...
	Arkan2	9.01	36.24	32.02	9.57	5.44	1.11	3.99	0.13	0.31	0.12	0.03	0.08	0.03	1.42	0.51	...	...	...
	Arkan3	8.44	33.50	27.83	9.85	5.49	1.97	7.05	0.77	0.80	0.20	0.09	0.26	0.03	2.47	0.02	0.02	1.23	...
	New1	8.03	35.27	31.55	9.57	5.03	1.27	5.01	0.37	0.40	0.10	0.04	0.02	0.02	2.27	0.02	1.02	...	...
	New2	6.91	29.77	30.27	10.61	5.76	1.86	8.14	0.44	0.69	0.19	0.13	0.08	0.02	3.13	0.02	0.08	1.96	...
	New3	7.51	29.58	29.77	9.73	5.32	1.90	8.48	0.92	0.84	0.31	0.18	0.20	0.02	3.05	0.04	0.12	2.04	...
	New4	14.76	36.79	25.89	7.50	5.20	1.17	5.37	0.30	0.27	0.03	0.09	0.07	1.56	0.04	0.02	0.01	0.92	...
	New5	8.94	34.07	31.54	9.65	5.79	1.44	5.15	0.42	0.35	0.07	0.07	0.09	0.03	1.86	0.02	0.49	...	...
	New6	10.11	31.15	28.21	9.49	7.69	1.65	7.46	0.34	0.30	0.14	0.05	0.06	2.46	0.01	0.02	0.01	0.84	...
	TAM71	7.70	33.40	32.46	9.40	5.13	1.60	5.93	0.38	0.38	0.12	0.08	0.04	0.02	2.21	0.02	1.14	...	...
	TAMM72	8.83	35.89	27.12	8.28	5.31	2.01	6.70	0.31	0.74	0.19	0.03	0.09	0.06	2.84	0.03	1.58	...	...
	TAM81	7.77	29.31	30.83	10.80	6.95	2.00	7.44	0.04	0.53	0.09	0.04	2.90	1.30	...	...	...	...	...
	TAM82	4.73	22.03	26.04	10.88	8.70	3.40	13.41	0.77	0.82	0.20	0.22	0.18	0.08	5.28	0.06	0.08	0.02	3.12
	Tri64	11.43	37.34	28.29	8.38	4.66	1.38	4.55	0.32	0.30	0.11	0.13	0.07	0.02	1.88	0.02	0.01	1.10	...
SRW	Beck1	11.92	34.48	23.42	6.92	5.30	1.69	8.36	0.64	0.76	0.27	0.27	0.20	3.36	0.02	2.40	...	...	...
	Beck2	12.02	39.32	25.40	6.41	3.65	1.60	6.57	0.49	0.40	0.25	0.10	0.18	0.05	1.93	0.03	1.58	...	...
	Cald1	9.38	30.33	23.32	7.87	5.81	2.04	11.72	0.79	0.76	0.33	0.20	0.25	0.08	4.23	0.05	0.02	0.03	2.78
	Cald2	12.03	35.81	19.93	6.45	4.14	2.06	10.00	0.71	0.61	0.29	0.25	0.22	0.10	4.04	0.10	0.02	0.02	3.21
	Cald3	10.40	32.77	23.39	7.50	4.60	2.30	9.70	0.85	0.77	0.37	0.20	0.17	0.10	4.17	0.02	0.10	2.57	...
	Cald4	15.77	40.06	21.74	5.57	3.89	1.66	6.07	0.55	0.36	0.30	0.18	0.14	2.39	0.02	0.08	1.23	...	...
	Comp1	9.54	35.04	23.94	6.91	5.01	1.96	9.52	0.65	0.76	0.44	0.29	0.11	0.02	3.28	0.02	0.04	2.46	...
	Comp2	14.67	39.32	24.98	6.88	3.34	1.23	4.42	0.35	0.34	0.16	0.17	0.06	2.00	0.04	0.04	2.01	...	...
	Comp3	14.16	40.51	21.05	6.39	3.89	1.31	6.41	0.45	0.62	0.21	0.17	0.14	0.09	2.88	0.05	1.68	...	...
	MO	17.42	39.15	22.47	6.24	3.37	1.35	5.30	0.26	0.23	0.20	0.11	0.10	0.02	2.18	0.05	0.02	1.52	...

[a] See Table I.

granule equivalent diameter measurements made with light microscopy, while the results of Morrison and Scott (1986) are derived from Coulter Counter measurements standardized with latex spheres. Thus, the equivalent diameters were smaller. The results are comparable for data for our type A granules (>16μm diameter) and data for their type A granules (>10μm diameter).

Similar comparisons for type B granule data (Counts 4–10, equivalent diameter) in this study showed nearly identical percentages for both hard and soft wheats (61.0 and 61.6%, respectively). Type B granules, as a group, do not allow for the distinction of the two wheat classes, but Count 4 (5.5–7.0 μm equivalent diameter) in conjunction with Count 3 (aspect ratio) can distinguish between classes.

To compare other granule sizes with those of Glenn et al (1992), the data were divided into size classes as used by Glenn et al (1992). In these data, 58.1 of the hard wheat and 48.8% of the soft wheat starch granules were in the 0–50 μm^2 (0–8 μm, equivalent diameter) size class. Glenn et al (1992), however, found that 87.0 of the hard wheats and 84.5% of the soft wheats were in this size class. Substantially different percentages also were observed for the next three size classes: 50–100 μm^2: 22.67 vs. 2.76% (hard), 24.30 vs. 4.04% (soft); 100–150 μm^2: 8.65 vs. 2.10% (hard), 13.19 vs. 1.90% (soft); 150–200 μm^2: 2.22 vs. 1.70% (hard), 3.82 vs. 1.65% (soft). The large differences between size classes of 0–50 μm^2 (0–8 μm) and 50–100 μm^2 (8–11 μm) could be attributed to sample differences or sampling errors. Also, Glenn et al (1992) used sectioned material, which may underestimate the number of type B granules by visualizing only portions of these granules. Further studies are needed to clarify these differences.

Another aspect of this study was the lack of overlap of HRW and SRW samples, as well as the lack of clustering of individual cultivars when count variables were used (Fig. 4). Additional studies on a larger number of wheat cultivars are needed. There appears to be a significant amount of variation among cultivars within the HRW and SRW classes. This variation may be environmental; for example, samples of Newton were grown at the same location, but one was irrigated and the other was not (Fig. 4; Table I). The distinction of individual samples from one another also suggests a large environmental effect within each class.

CONCLUSION

Separating SRW and HRW wheat classes by using digital image analysis of isolated starch granules may provide a method for class discrimination that does not require direct physical methods of measurement, such as time to grind, particle size index, or NIR. The use of only two morphometric parameters, equivalent diameter and aspect ratio, clearly distinguished the two wheat classes. The power of class discrimination by these two descriptors was illustrated by comparing these results with those obtained from NIR hardness values where several overlapping values were obtained. Many more samples need to be analyzed to determine the effect of crop year and growing location on starch morphometric features, as well as other possible morphometric features related to endosperm texture.

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