Physical and Structural Properties of Wheat Endosperm Associated with Grain Texture

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A method of sectioning raw wheat grain was developed to characterize the physical and structural properties of endosperm tissue from hard and soft wheat varieties. The thinnest possible cross section that remained intact was taken as a measurement of cohesiveness. Hard wheat sections typically were pliable, cohesive, and could be sliced less than 1 μm thick. Soft wheat sections less than 4 μm in thickness tended to crumble. Section thickness was used to classify 152 wheat samples using classification discriminant analysis. Structural features of the endosperm matrix that influenced cohesiveness were studied in the cross-sectional surfaces of wheat using scanning electron microscopy. Differences in cohesiveness within a sample were largely accounted for by intracellular space. Differences in cohesiveness of hard and soft wheat grains generally involved continuity of the protein matrix as well as starch-protein adhesion. A 15-kDa polypeptide from sodium dodecyl sulfate-extracted starch was evident only in soft wheat samples. Nevertheless, the intensity of the 15-kDa polypeptide did not reflect the textural hardness of wheat endosperm.

Endosperm texture influences milling performance and is an important criterion for determining the end use of various wheat classes (Shellenberger 1971). Texture is a varietal characteristic (Symes 1965, 1969) that can be modified somewhat by environmental conditions (Pomeranz et al 1985, Stenvert and Kingswood 1977). In spite of its importance in processing, the basis of textural hardness in wheat is not fully understood.

Two important theories on textural hardness have stimulated considerable interest in recent years (reviewed in MacRitchie 1984). One theory attributes hardness to the degree of starch-protein adhesion (Barlow et al 1973). Starch-protein adhesion could vary in hard and soft wheat endosperm as a result of quantitative or qualitative differences in cellular products deposited at the starch-protein interface. Simmonds et al (1973) isolated a starch extract that they proposed could function in hard wheat varieties as a "cement" that binds starch and protein. Greenwell and Schofield (1986) identified a starch granule protein found predominantly in soft wheat varieties that they suggested could impair starch-protein adhesion and induce softening.

A second theory for wheat hardness is based on the physical structure of the protein matrix (Stenvert and Kingswood 1977). This theory holds that the degree of endosperm (hardness) is determined by the continuity of the protein matrix, its structure and the strength with which it physically entraps starch granules.

Failure analysis of milled, fractured, or crushed wheat samples using light and electron microscopy has been an important tool in increasing our understanding of wheat hardness (Barlow et al 1973). Further insight into the nature of wheat hardness can be obtained by using new methods that help characterize the physical properties of wheat endosperm. This report describes a simple test for classifying single grains of hard and soft wheats. Physical and structural properties of endosperm tissue determined by this and other techniques were studied in relation to textural hardness in wheat.

MATERIALS AND METHODS

Blended samples of commercially grown hard and soft wheat varieties (1987 crop year) were obtained from four USDA regional wheat quality laboratories located in Kansas, Ohio, North Dakota, and Washington. Moisture content of the samples was determined by AACC method 44-15A (1983). In addition, 41 breeder samples of Foundation Seed, 36 wheat blends from the Federal Grain Inspection Service (FGIS), and 24 samples of winter and spring wheat varieties from North Dakota were included in this study. Hardness and protein content of each sample were obtained using near-infrared reflectance (NIR) according to methods 39-70 and 39-11A, respectively (AACC 1983).

Sectioning Method
A flat surface was prepared on the germ end of an individual caryopsis by removing 2-3 mm of tissue with a metal file. The grains were secured to plastic microtome stubs using a liquid cyanoacrylate adhesive (Krazy Glue Inc., Itasca, IL). A portion (1-2 mm) of the brush end of the grain was filed away and the sample was inserted in an ultramicrotome (Sorvall MT-2). Single grains from 152 different samples were placed in number-coded containers and randomized prior to sectioning to conceal their origin. The grain face was sectioned with a glass knife at a thickness of 8 μm until the entire cross-sectional surface of the grain was in the cutting plane. Section thickness, as determined by the microtome setting, was progressively reduced and recorded when the thinnest possible intact section for each variety was achieved. Effect of moisture content on section thickness was tested for

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three hard and three soft wheat varieties equilibrated to 2, 9, 11, 14, and 17% moisture content using humidity chambers containing sulfuric acid solutions.

Classificatory discriminant analysis of the data (SAS 1985) was performed on the first observation of each variety in order to calculate a discriminant function. The discriminant function was then used in classifying the remaining data. Due to the unequal variances between wheat classes, a quadratic discriminant analysis was performed on data for thickness and hardness. A linear discriminant function using a pooled variance was used for the protein data since the variances were homogenous between wheat classes. The data were grouped into four classes (club, durum, hard, or soft) or into two classes comprised of hard (durum and hard) and soft (club and soft) wheats.

Scanning Electron Microscopy (SEM)

Single grains were mounted on plastic blocks and sectioned as described above. The same section thickness (1 μm) was used on all the samples presented in the light and SEM micrographs. Photomicrographs of sectioned wheat grains were taken with a Nikon stereoscopic microscope (model SMZ-100). The grains were remounted on aluminum specimen stubs and coated with silver paint except on the sectioned surface. The samples were sputter-coated (Polaron model E5100) with gold, desiccated for 10 min in the SEM sample chamber, and recoated (20 nm). SEM micrographs were obtained using a Hitachi S-530 SEM operated at 10 kV.

Comparisons Within Samples

The range in values for section thickness, hardness, and protein content within a sample was studied using bulk quantities of hand-sorted vitreous and nonvitreous grains. The study included three hard (Chisolm, Phoenix, and Triumph) and three soft wheat varieties (Crew, Hillsdale, and Arthur). Percent starch damage of milled samples was estimated for the six varieties according to AACC method 76-30A (1983). Water-extracted starches (1 g) from flour samples were vigorously shaken for 90 min at 50°C in 10 ml of 1% (w/v) sodium dodecyl sulfate (SDS) according to Greenwell and Schofield (1986). Following centrifugation for 15 min at 1,000 × g, the supernatant was collected, diluted with four volumes of acetone, and placed in a freezer (−20°C) overnight. Precipitated proteins were collected by centrifuging the samples for 20 min at 1,000 × g and were dried in a desiccator under reduced pressure. The pellets were solubilized in 150 μl of sample buffer, boiled for 5 min, and centrifuged at 15,000 × g for 2 min. A 20-μl sample was loaded, and proteins were separated by SDS-PAGE (Laemmli 1970) using a 17% (w/v) acrylamide slab gel. The gels were fixed and stained with Coomassie Brilliant Blue G (Sigma Chemical Co., St. Louis, MO).

RESULTS

Sectioning Method

The thinnest possible cross section that remained intact was taken as a measurement of endosperm cohesiveness. Orientation of the grain when sectioning was not important. Durum and vitreous hard wheat grains without exception were easily sectioned under 1 μm (Fig. 1A). These sections were transparent slices that remained cohesive and pliable when agitated with a camel hair brush. The cohesive properties of nonvitreous (yellow berry) hard wheat grains varied considerably both within and among varieties. The endosperm tissue centered within the cheek of the nonvitreous grains often crumbled and separated from the section (Fig. 1B). The subaleurone tissue was vitreous and remained cohesive. Thicker cuts were required to attain an intact section. Sections (1 μm) of soft wheat typically crumbled throughout the entire endosperm structure (Fig. 1C). Only small amounts of subaleurone tissue adhered to the aleurone and pericarp tissue. Thick cuts (4–12 μm) were required in soft wheat varieties to achieve sections that were relatively intact. Section thickness was not significantly affected by moisture content in the range tested (2–17%). However,

mean thickness for sections of hard wheat grains increased slightly at moisture contents above 15%.

Thickness values were similar for club and soft wheats and for durum and hard wheat varieties (Fig. 2). Club and soft wheats had a mean thickness of 11.0 and 9.0 μm, respectively. The mean
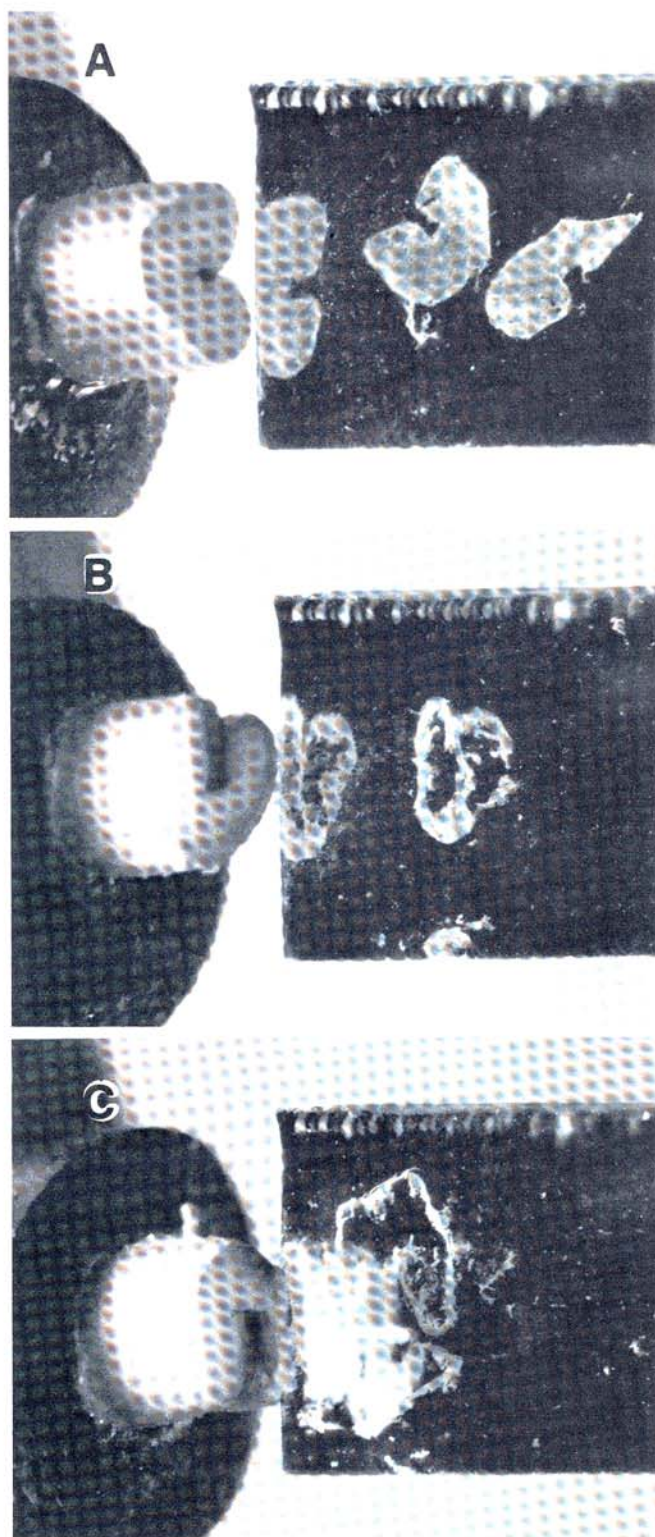

Fig. 1. Photomicrographs of wheat grain sections (1 μm) illustrating the cohesive properties of the endosperm. **A,** Sections of vitreous hard wheat grain. The sections were cohesive and remained intact throughout the entire cross section. **B,** Sections of nonvitreous hard wheat grain. The sections were less cohesive in the central cheek region than in the subaleurone region. **C,** Sections of soft wheat grain. The sections had poor cohesive properties; the entire endosperm area crumbled while sectioning.

thickness value for hard wheats was 0.8 μm and ranged from 0.4 to 1.6 μm. Sections of durum wheat had a mean thickness of 0.2 μm.

Less than 70% of the wheat samples were correctly classified by section thickness into four wheat classes (Table 1). Classification errors occurred when some soft wheat samples were misclassified as club wheat samples and vice versa. Some durum wheat samples were also misclassified as hard wheats. However, no errors occurred when the samples were simply classified as either hard or soft.

Classification by NIR hardness into four wheat classes was more accurate than by section thickness (Table I). More than 81% of the samples were correctly classified. No errors occurred in classifying durum wheats. Misclassification between club and soft wheats accounted for most of the error. When wheats were classified by NIR hardness as either hard or soft, four hard wheat samples were misclassified as soft wheat, which resulted in a 2.2% error. Protein content was not an accurate parameter for classifying wheat samples. Only 36.8% of the samples were correctly classified into four classes, and 43.3% of the samples were correctly classified into hard and soft wheat classes.

Microscopy

The surface of sectioned grain was studied using SEM to identify structural features that were typical in hard and soft endosperm. The cut surface of the grain was examined rather than the section itself, since the block face was an identical match with the section and was more stable under the electron beam. It was observed that low atmospheric pressure within the SEM sample chamber desiccated the sample, causin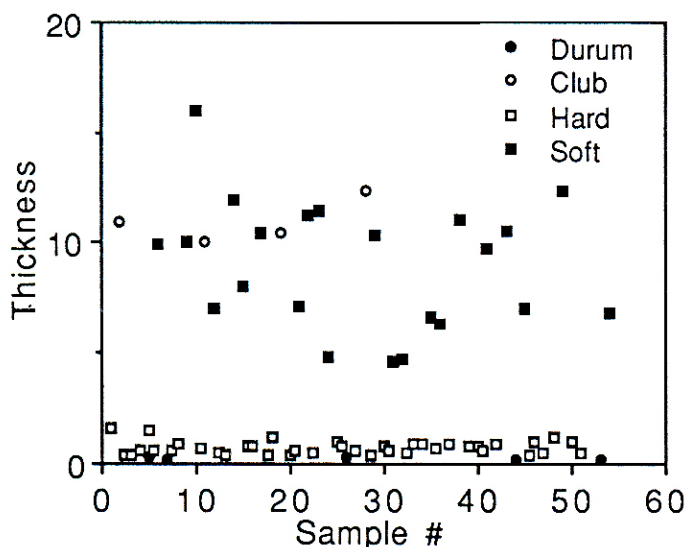g various degrees of starch and whole cell shrinkage. Shrinkage was most noticeable in vitreous grains and resulted in starch-protein and/or cell-to-cell separation. Despite some shrinkage, a close starch-protein association was evident in durum and vitreous hard wheat endosperm (Fig. 3A).

Fig. 2. Scatter plot of thickness of intact cross sections of raw grains for 70 samples representing 54 varieties.

TABLE I
Percentage of Wheat Samples Correctly Classified
Based on Discriminant Functions for Thickness, Hardness,
and Percent Protein[a]

Wheat Class	Thickness	Hardness (NIR)[b]	Protein (%)	Sample (n)	Varieties (n)
Club	61.5	53.8	0.1	13	4
Durum	63.6	100	45.4	11	5
Hard	100	95.1	17.1	82	23
Soft	54.3	76.1	84.8	46	22
Total	69.9	81.3	36.8	152	54
Club + soft	100	100	66.1	59	26
Durum + hard	100	95.7	20.4	93	28
Total	100	97.8	43.3	152	54

[a] Derived from the first observation of 54 wheat varieties.
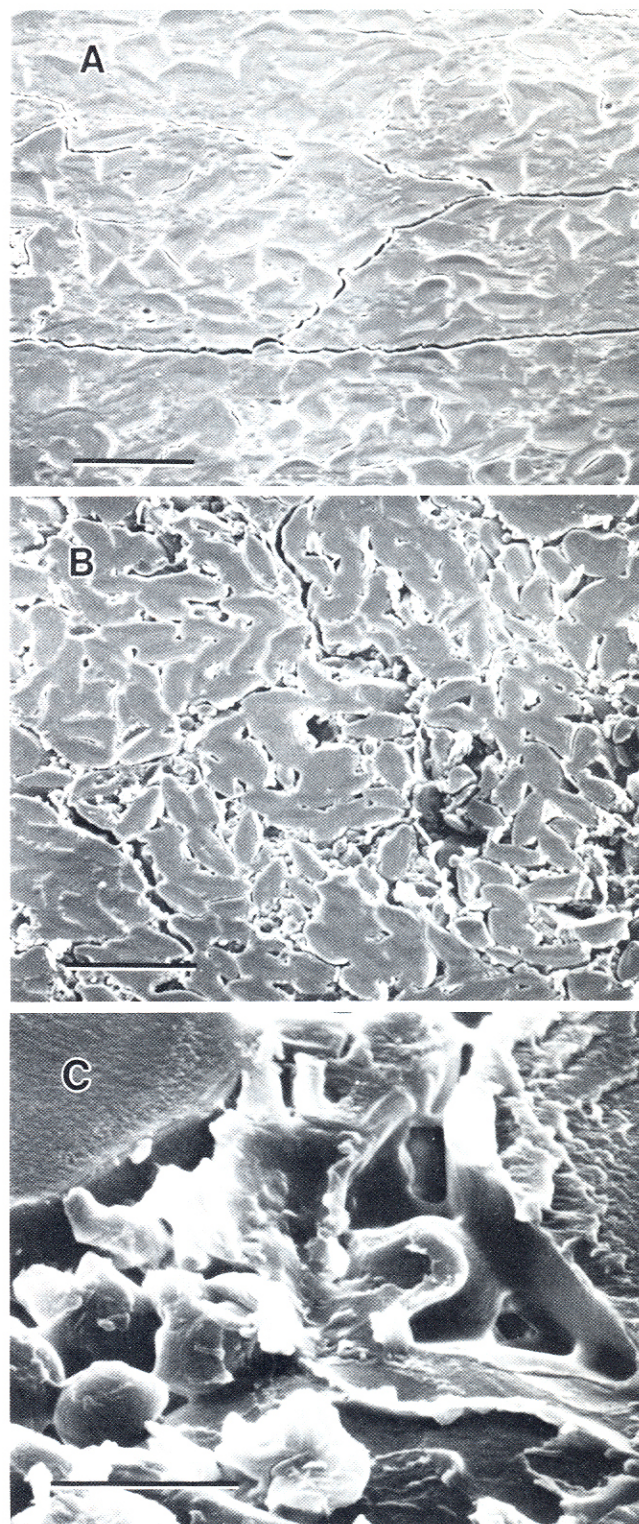[b] Near-infrared reflectance spectroscopic determination.

Fig. 3. Scanning electron micrographs of the cut surface of vitreous and nonvitreous hard wheat grains. A, The endosperm tissue of vitreous hard wheat grains was cohesive and had few, if any, air spaces. Separation between cells and around some starch granules was due to desiccation within the SEM sample chamber. B, Air spaces were visible within the central cheek region of nonvitreous hard wheat grains. Loss of cohesion occurred when aggregates of starch and protein broke out of the section. C, A film of matrix protein that surrounded and interconnected starch granules was visible in the intracellular space of nonvitreous hard wheat grains (cultivar Chisolm). Magnification bar in A and B = 50 μm; bar in C = 5 μm.

The endosperm matrix appeared continuous and lacked any visible air space.

Air space was typically interspersed throughout the central cheek region of nonvitreous hard wheat grains (Fig. 3B). This region corresponded with the floury portion of the endosperm. Most individual starch granules of the central endosperm remained intact while sectioning, although some aggregates of tissue broke free (Fig. 3B). The central endosperm matrix was characterized by an extensive network of air spaces that often had regions where a continuous layer of matrix material surrounded the starch granules (Fig. 3C).

Vitreous grains of a representative soft wheat variety were compared with samples from vitreous hard wheats. Thin sections of soft vitreous endosperm tissue had poor cohesive properties. SEM micrographs of the grain surface revealed a large number of cavities that were apparently formed when starch granules were dislodged during sectioning (Fig. 4A). Little or no air space was visible in the undisturbed regions of the endosperm (Fig. 4B). The cut surface of nonvitreous soft wheat appeared disrupted throughout its entire area (Fig. 4C). Aggregates of tissue as well as individual starch granules were dislodged while sectioning. The endosperm matrix appeared segmented and discontinuous (Fig. 4D).

Comparisons Within Samples

Three hard and soft wheat samples were selected for a wide range in vitreosity and were used as extremes to test the accuracy of section thickness, NIR hardness, and protein content for classifying wheats. Vitreous and nonvitreous grains were hand sorted for each variety, tested, and classified using the discriminant functions derived earlier. Vitreous grains within the three soft wheat varieties made up less than 1% of the wheat sample. Vitreous grains sectioned thinner, were harder, and had a higher protein content than nonvitreous grains regardless of wheat class (Table II). The hard wheat samples were correctly classified by section thickness. Classification by NIR hardness was also accurate except for the nonvitreous sample of Chisolm, a hard red winter variety.

The soft wheat varieties tested were classified correctly by section thickness except for vitreous grains of Crew and Arthur, which had cohesive properties similar to those of vitreous hard wheats. Interestingly, NIR hardness data for the soft wheat set also classified vitreous grains of Crew and Arthur as hard wheats.
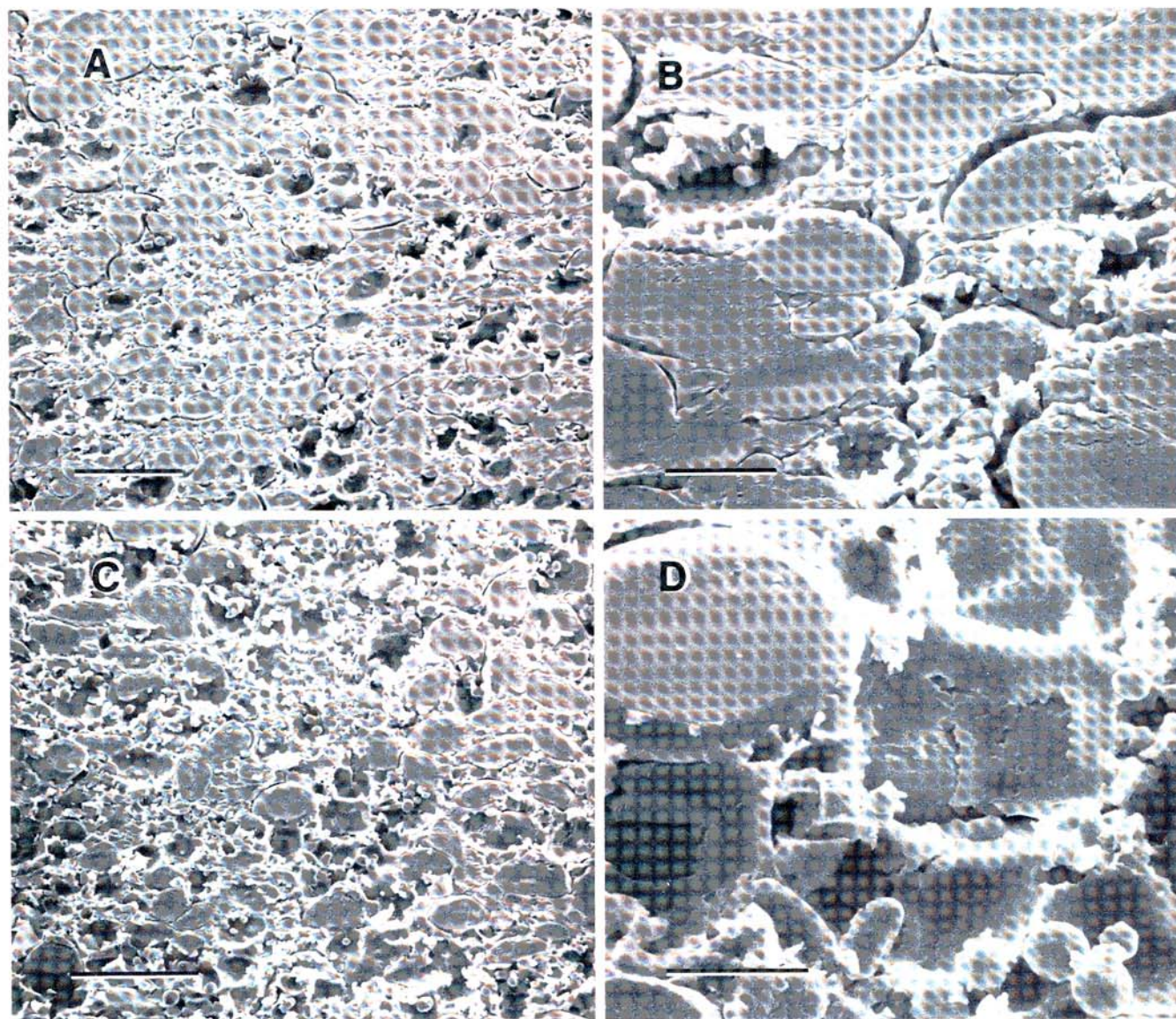

Fig. 4. Scanning electron micrographs of the cut surface of vitreous (A and B) and nonvitreous (C and D) grains of a typical soft wheat (cultivar Hillsdale). **A,** A- and B-type starch granule cavities were visible throughout the endosperm. **B,** Air spaces were not visible within the matrix in the undisturbed regions of the endosperm. Nevertheless, the matrix appeared segmented. **C,** Disruption of starch and the protein matrix structure due to the sectioning process was visible in nonvitreous grains. **D,** The endosperm matrix of nonvitreous grains contained small air spaces and appeared segmented and discontinuous. Magnification bar in A and C = 50 μm; bar in B and D = 10 μm.

TABLE II
Comparison of Hardness,[a] Thickness, and Protein Content in Vitreous[b] and Nonvitreous Wheat Grains

Wheat	Thickness (μm)	Classifi- cation	Hardness	Classifi- cation	Protein (%)	Classifi- cation	Starch Damage (%)
Hard varieties							
Chisolm							
vitreous	0.8	H	41.72	H	12.32	H	6.2
nonvitreous	1.0	H	32.62	S	9.85	S	5.1
Phoenix							
vitreous	0.4	H	71.72	H	11.48	S	8.5
nonvitreous	1.4	H	59.33	H	8.36	S	6.4
Triumph							
vitreous	0.6	H	68.35	H	13.03	H	5.8
nonvitreous	1.1	H	58.38	H	12.2	H	4.8
Soft varieties							
Crew							
vitreous	0.8	H	52.96	H	12.08	S	6.0
nonvitreous	4.6	S	11.65	S	9.51	S	4.0
Hillsdale							
vitreous	4.8	S	31.83	S	13.40	H	4.0
nonvitreous	11.2	S	23.01	S	10.64	S	3.9
Arthur							
vitreous	0.4	H	45.93	H	13.03	H	5.1
nonvitreous	4.2	S	23.03	S	10.28	S	3.9

[a] Near-infrared reflectance spectroscopy determination.
[b] Within a sample, vitreous grains have higher protein and NIR values than nonvitreous grains (paired t test at 99 and 95%, respectively).
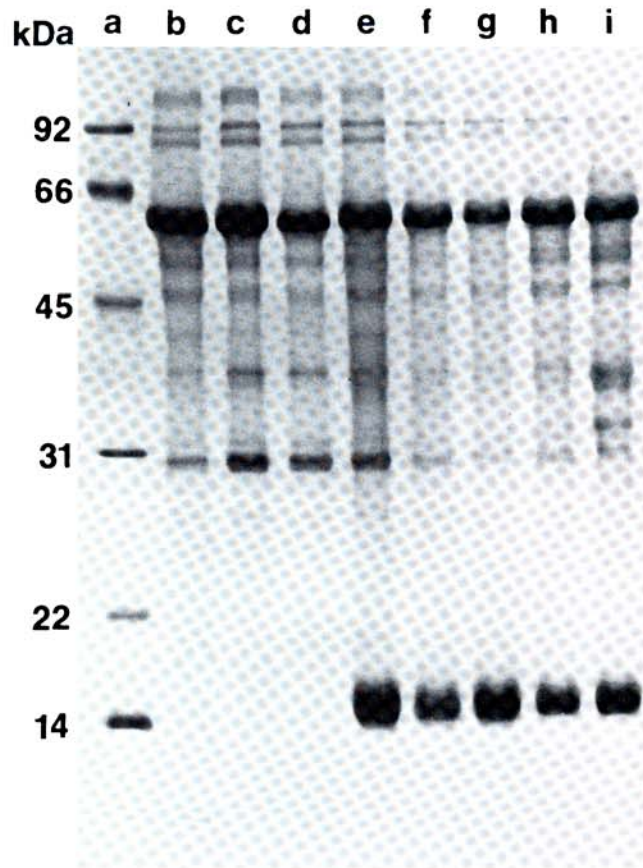

Fig. 5. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoregram of 1% SDS extracts of hard (b–d) and soft (e–i) wheat starch preparations from samples given in Table II. Molecular weight standards (a); nonvitreous sample of Phoenix (hard white wheat) (b); nonvitreous sample of Triumph (hard red wheat) (c); nonvitreous sample of Chisolm (hard red wheat) (d); vitreous sample of Hillsdale (soft red wheat) (e); vitreous (f) and nonvitreous (g) samples of Crew (Club wheat); and vitreous (h) and nonvitreous (i) samples of Arthur (soft red wheat).

The mean difference in hardness values for vitreous and non-vitreous grains excepting Crew and Arthur was 10.07. The difference in NIR hardness for vitreous and nonvitreous grains

of Crew and Arthur was 41.31 and 22.9, respectively. Protein content was not useful for classifying hard and soft wheat samples.

Starch damage within a given milled wheat sample was greatest for vitreous grains (Table II). Flour samples from vitreous grains of Crew and Arthur had starch damage levels comparable to Chisolm and Triumph hard wheat flours. The flour sample from vitreous grains of Hillsdale had nearly the same level of starch damage as the nonvitreous flour sample.

The polypeptide patterns of SDS-extractable proteins associated with starch preparations were similar for the hard (Fig. 5, lanes b, c, and d) and soft (Fig. 5, lanes e–i) wheats except for an intense 15-kDa band that occurred in soft wheat samples. The intensity of the 15-kDa polypeptide appeared slightly less in vitreous grains of Arthur and Crew (Fig. 5, lanes f and h, respectively) compared to floury grains of the same varieties (Fig. 5, lanes g and i). It is noteworthy that Chisolm (Fig. 5, lane d) had a lower NIR hardness score than vitreous grains of Arthur and Crew yet had no apparent quantities of the 15-kDa polypeptide. The floury sample of Triumph 64 (Fig. 5, lane c) had an NIR hardness score similar to the vitreous sample of Crew (Fig. 5, lane h).

DISCUSSION

Single-grain tests that accurately classify wheats of variable moisture content are being developed to identify commercial shipments of hard wheats that have been blended with soft wheats (Eckhoff 1988, Mattern 1988, Pomeranz et al 1988). Single-grain tests tend to be less accurate than bulk-sample tests since variability among wheat grains within a sample can be more than between samples (Pomeranz et al 1988). Endosperm cohesiveness as measured by section thickness may provide the basis for an effective commercial single-grain test. Section thickness values were used to accurately assign single grains that varied considerably in moisture content into hard or soft wheat classes. In cases where misclassification by section thickness occurred, classification of hand-sorted bulk samples by NIR hardness scores also failed. The positive relationship between cohesiveness and grain hardness suggests that both measure the same fundamental property. Since only a portion of each grain is needed for testing, the sectioning technique could be a useful method for discriminating grains by hardness for research or other fields where limited amounts of sample are available. Efforts to develop a rapid test for commercial purposes are currently underway.

The sectioning technique was a useful research tool for studying physical and structural properties that were associated with textural hardness. The lack of endosperm cohesion was attributable to disruption of starch granules and/or starch-protein aggregates. Cellular disassembly resulting from a loss of cell-to-cell cohesion was not observed. This supports earlier statements that cell contents rather than cell wall structure are directly responsible for textural hardness in wheat (MacRitchie 1980, Simmonds 1974).

Vitreousness of endosperm tissue is more typical of hard wheat varieties (Simmonds 1974), but its occurrence can vary depending upon environmental conditions (Parish and Halse 1968). Vitreous grains within a given sample were harder and more cohesive, had higher protein content and greater starch damage after milling (Frank 1923, Stenvert and Kingswood 1977). It was interesting to find that vitreous grains of two soft wheat varieties formed cohesive sections and were hard. It is unlikely that these samples were contaminated with hard wheat grains since the varietal characteristics of grain size and shape were constant. Furthermore, these samples contained an intense 15-kDa polypeptide, which is characteristic only of soft wheat varieties (Greenwell and Schofield 1986).

Although vitreous grains of Crew and Arthur were hard, vitreous grains of a third soft wheat variety did not form cohesive sections and were classified as soft. These results supported earlier findings (Simmonds 1974) that vitreousness alone did not account for the fundamental basis of endosperm cohesiveness and hardness. This conclusion was supported by the fact that the average hardness scores for the nonvitreous hard wheats were greater than the average hardness of the vitreous soft wheats.

Two theories describing the fundamental basis of wheat hardness have stimulated considerable discussion. Barlow et al (1973) found no difference in hardness of protein fragments or starch granules between hard and soft wheat varieties. They contended that starch-protein adhesion accounts for wheat hardness and gave little consideration to the structural features of the protein matrix. Stenvert and Kingswood (1977) attributed wheat hardness to the physical structure of the protein matrix and placed little importance on starch-protein adhesion. The results of this study support aspects of both theories.

The fact that vitreous grains were harder than nonvitreous grains within a sample clearly demonstrated that protein matrix structure can influence hardness. In addition, two distinct forms of protein matrix structure were observed. The matrix structure typical of nonvitreous soft wheat endosperm appeared segmented due to the presence of small voids and boundary lines within the matrix. This matrix structure may develop from nonfluid protein bodies that are molded and shaped by enlarging starch granules as suggested by Jennings et al (1963). Vitreous hard wheat grains and vitreous grains of Crew and Arthur were cohesive, hard, and had a continuous (unsegmented) protein matrix. This matrix structure could be formed when at some point during development the protein bodies become fluid enough to coalesce and form a continuum (Simmonds and O'Brien 1981, Stenvert and Kingswood 1977).

Stenvert and Kingswood (1977) suggested that starch granules do not adhere to protein but are merely entrapped within the protein matrix. The results of this study indicated that starch-protein adhesion occurs and is associated with a continuous matrix. This became apparent when very few of the matrix-embedded granules that became unentrapped while sectioning were dislodged in vitreous hard wheat grains or vitreous grains of Crew and Arthur. In contrast, the cut surface of similarly prepared vitreous grains classified as soft (discontinuous protein matrix) was densely impregnated by starch granule cavities, demonstrating a lack of starch-matrix adhesion.

Starch-protein adhesion has been attributed to a biochemical adhesive (Simmonds et al 1973) or "nonstick" protein (Greenwell and Schofield 1986) that is genetically linked to hard or soft wheat varieties. Greenwell and Schofield (1986) reported a 15-kDa polypeptide in SDS extracts of soft wheat starch preparations that was much less intense in hard wheat samples and was coded by a gene located on the short arm of the 5D chromosome. They

suggested that the 15-kDa polypeptide functions as a "nonstick" protein and is important in conferring endosperm softness to wheat.

Results of this study support the claim that the 15-kDa polypeptide is associated with soft wheat varieties. However, textural hardness of wheat may not be directly attributable to the presence of the 15-kDa polypeptide. This became apparent when texturally hard grains of soft wheat contained the 15-kDa polypeptide, whereas texturally soft hard wheats lacked the polypeptide. Moreover, quantitative differences in polypeptide levels among soft wheat varieties did not reflect the differences in their NIR hardness scores although quantitative differences within a variety were noted.

While the 15-kDa polypeptide observed in this study may have a modifying effect on wheat hardness within a variety, it seems clear that other factors such as protein matrix structure are also important in determining endosperm hardness.

In summary, section thickness measured a fundamental material property of wheat grains that related closely to wheat hardness. This study supports the role of protein matrix structure and starch-protein adhesion in determining wheat hardness.

ACKNOWLEDGMENTS

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