Wheat Hardness: A Microscopic Classification of Individual Grains[1]

PAUL J. MATTERN[2]

ABSTRACT

Cereal Chem. 65(4):312–315

Wheat grains placed lengthwise, crease up, on a self-adhesive tape were crushed with corrugated rollers spaced at 1.08 mm. Crushed grains were viewed with a dissecting microscope at a magnification of 15×. A hardness index was established using a rating from 1 to 10 (soft to hard). A set of standardizing photographs and a video tape were prepared as a training aid for the method. The microscopic hardness evaluation of single grains provides an inexpensive, accurate evaluation of wheat samples to determine heterogeneity of varieties, environmental influence on hardness, and the percentage mixture of soft and hard wheats. Microscopic data gave coefficients of correlation of −0.94 and −0.95 with particle size index and near-infrared reflectance, respectively.

The light microscope has been used to examine differences in endosperm breakdown between hard and soft wheats (Sandstedt et al 1952, Sandstedt 1955). Davis and Eustace (1984) and Moss et al (1980) described differences between the milling of hard and soft wheats through use of scanning electron microscopy (SEM).

Symes (1965), in a study of the particle size index (PSI) of near isogenic lines of hard and soft wheat, reported a single major gene and minor genes controlled wheat hardness. Mattern et al (1973), using maltose values and flour granularity of flour milled from chromosome substitutions lines, found that hardness in wheat, for the variety Cheyenne, was controlled by one major gene and one minor modifying gene. Greenwell and Schofield (1986) present data for the presence of an M_r-15,000 protein associated with the surface of starch granules from hexaploid bread wheat (*T. aestivum*) that controls hardness. This protein band is strong in soft wheats and faint in hard wheats when separated by gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). When the M_r-15,000 protein is present, it appears to eliminate the binding of endosperm storage proteins to starch granules.

Wheat breeders commonly make crosses between hardness types to combine desired plant characteristics. If selections are made in early segregating generations, there is significant potential for heterogeneity in grain hardness and morphology (shape). The Federal Grain Inspection Service currently identifies wheat class through the use of traditional grain morphology (shape). However, grain shape has not necessarily been maintained within hardness classes. Therefore, it has become impossible to differentiate class accurately among certain commercial wheats. Even with an individual grain test for hardness, classification becomes difficult if grains from known soft wheats overlap in hardness with known hard wheats.

Soft wheats may be blended intentionally with a hard wheat for cost advantage. A hardness test for individual grains is needed to characterize a blended sample. An inexpensive method is also needed by breeders to evaluate selections. This paper describes a microscopic test for the identification of wheat hardness in single grains.

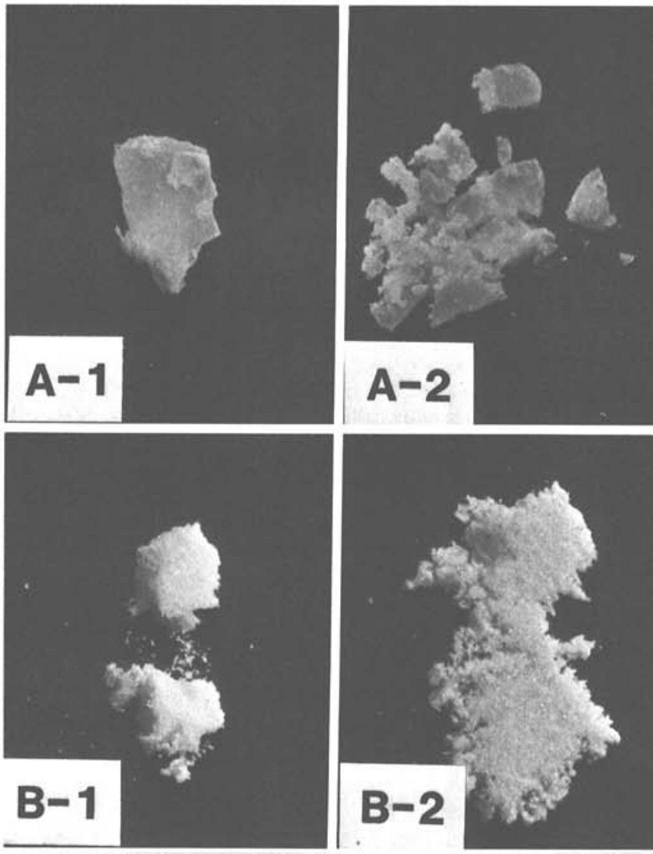

Fig. 1. A-1 and B-1 are hard and soft wheat endosperm chunks, respectively. A-2 and B-2 were produced after crushing between microscopic glass slides.

[1]Contribution of the Department of Agronomy, University of Nebraska-Lincoln, as Paper no 8489, Journal Series, Nebraska Agricultural Experiment Station. Mention of firm names or trade products does not constitute endorsement by the University of Nebraska over others not mentioned.

[2]Department of Agronomy, University of Nebraska-Lincoln, Lincoln 68583-0915.

MATERIALS AND METHODS

Preparation of Viewing Tapes

Placement of grains. A piece of ¾-in. (1.9-cm) black vinyl self-adhesive electrical tape was cut to a 24-in. (61 cm) length. After the tape had relaxed, the ends were turned back about ¾ in. (1.9 cm), and a paper strip (¼ in. [0.64 cm]) was attached along one edge to facilitate later handling with the microscope. Using tweezers, 50 wheat grains were placed longitudinally along the tape, spaced about ¼ in. (0.64 cm) apart with the crease side up. Tapes were identified with a self-adhesive label.

Although other self-adhesive tapes such as masking tape could be used, the color contrast with black tape was advantageous when video viewing and recording were to be used.

Crushing the grains. The grains were crushed by passing the tape between the two corrugated rolls of Straub Wheat Mill (model W-1), which has equal roll surface speeds and a roll spacing of 0.043 in. (1.08 mm).

Because this mill is no longer manufactured, other appropriate items could be used. One possibility is the KR-100 hand-operated mill from Kitchenetics (Campbell, CA). This mill must be operated in the roll/crack mode, which then produces an equal roll surface speed on the knurled rolls. Because of the variable roll space adjustment of the KR-100 mill, grains could be presized into three fractions using U.S. standard sieves nos. 7 and 8 to give more uniform crushing of the wheat grains.

Microscope

A dissecting microscope at a magnification of 15–20× was satisfactory for evaluating the crushed grains. An artists' X-acto knife was used to open small grains and to remove any attached bran blocking the view of the endosperm.

Moisture Level

Samples were crushed at a moisture content between 11.0 and 13.0%.

Optional Equipment

A color video camera on the microscope, a television monitor, and a video cassette recorder were useful for training, demonstrating, and maintaining permanent records.

Samples

We evaluated wheats from four U.S. Department of Agriculture

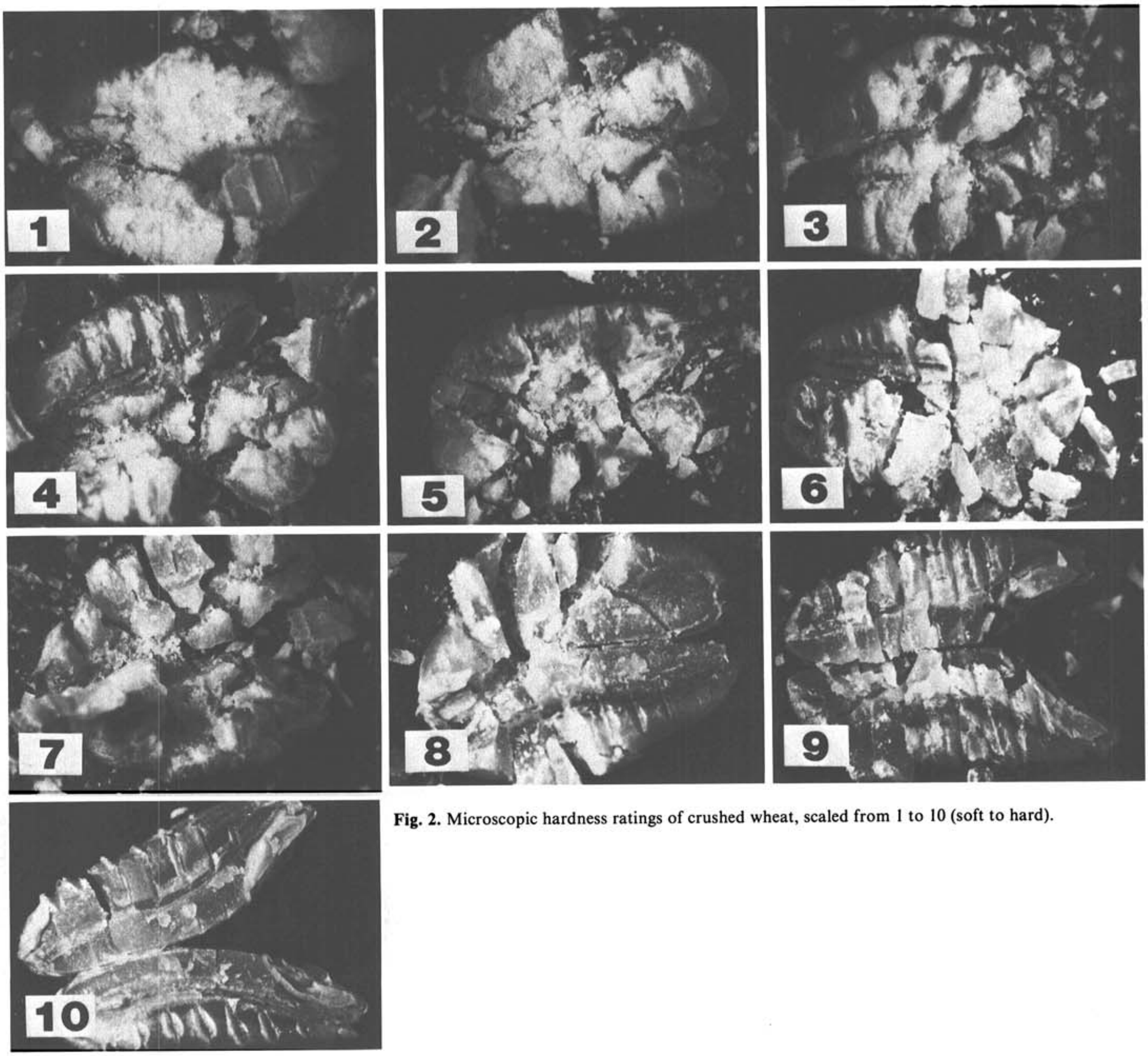

Fig. 2. Microscopic hardness ratings of crushed wheat, scaled from 1 to 10 (soft to hard).

regional nurseries grown in 1983. Of these, 29 entries grown in seven locations were from the Northern Regional Performance Nursery, 35 entries grown in seven locations were from the Southern Regional Performance Nursery, 34 entries grown in 11 locations were from the Eastern Soft Wheat Nursery, and 32 entries grown in 10 locations were from the Southern Soft Wheat Nursery.

Eighteen of the 20 test samples used by Williams and Sobering (1986) were evaluated (T-7 and T-8, Botno durum and Dirkwin soft white spring wheats, were missing). We evaluated T-1, a soft white spring, which Williams and Sobering (1986) replaced in their study with a durum sample. The "T" numbers were used in the AACC collaborative study reported by Williams and Sobering (1986).

Particle Size Index

The Symes (1961) method was modified for evaluation of particle size index (PSI). Twenty grams of wheat at 11.5% moisture was fed with a vibratory feeder at a rate of 1 g/sec to a Labconco heavy duty mill. The ground wheat was sifted 200 sec on a U.S. 70 mesh sieve, and the percent of throughs was calculated as the PSI value.

Damaged Starch

Flour residues from the PSI method were analyzed for percent damaged starch with method 76-30A (AACC 1983) using Enzeco fungal α-amylase (a replacement enzyme) containing 30,000 SKB units/g.

RESULTS AND DISCUSSION

Development of the Microscopic Technique

Original microscopic techniques (Sandstedt 1955) consisted of crushing a 0.5-mm endosperm chunk between two glass slides at a magnification of 20–30×. Hard and soft wheat endosperm break down or mill as shown in Figure 1. Soft wheat endosperm crushes with no apparent cell structure visible, whereas a hard wheat breaks sharply along cell walls and across endosperm cells to produce angular pieces. This physical appearance accounts for the well-known differences in sifting properties of hard and soft wheats.

Individual Kernel Hardness Ratings

Individual crushed grains from all classes of wheat were studied, and a relative rating scheme from 1 to 10 (soft to hard) was established (Fig. 2). An arbitrary rating of 1–4 was assigned to soft wheats, and a rating of 4–8 for the bread wheats. Durum wheats usually occupy the rating slots of 9 and 10, but occasionally certain bread wheats could be rated at this extremely hard classification.

If appropriate selection pressure were given to hardness ratings in wheat breeding programs, the number of samples with "4" ratings should decline for both traditional soft and hard wheats. However, the environment can modify hardness. Rating with the microscope is a subjective test. There is no difficulty differentiating between true hard and soft types, and a single soft grain can readily be identified in a true hard wheat sample. Yamazaki and Donelson (1983) reported that protein content did not correlate with PSI data. If higher protein is associated with a more vitreous endosperm, it could be given a harder rating with the microscopic method.

Identity of a Genotype Over Environments

Entries from the four USDA regional nurseries were evaluated for hardness by the PSI method. Cultivars or experimental lines that produced the widest ranges in PSI over locations were examined by the microscopic method to determine if a wheat maintained its hardness classification.

A problem with these samples is that we have no primary definition of hardness. Also, the assumption is made that the nursery entries for a specific class are homogenous to hardness within a sample. Individual grain analyses determined by the microscope and PSI are listed in Table I. Microscopic evaluation identified some lines that were more heterogeneous than others. This can result either from a segregating population or from a genotype × environment interaction. The Texas experimental line number TX 80A6025 from the Southern Regional Performance

TABLE I
Microscopic Data for Individual Grains for Samples from USDA Regional Nurseries Having Most Diverse Particle Size Index Ratings

Nursery/ Entry, Variety, Location	Score (1–10, Soft–Hard) 1	2	3	4	5	6	7	8	9	10	Microscopic Mean	Particle Size Index
Southern Soft Wheat Nursery												
1, FL301												
Queenstown, MD	62	28	10	...	...	...	...	...	...	...	1.5	26.4
Tifton, GA	1	16	53	25	5	...	...	...	...	...	3.2	19.6
8, Sc770164,												
Queenstown, MD	83	10	7	...	...	...	...	...	...	...	1.2	32.0
Tifton, GA	27	37	34	2	...	...	...	...	...	...	2.1	23.9
Eastern Soft Wheat Nursery												
20, Ar96-1-8,												
Lexington, KY	95	4	1	...	...	...	...	...	...	...	1.1	32.3
Ithaca, NY	18	63	19	...	...	...	...	...	...	...	2.1	21.7
6, Ar48-7-4,												
Highland Rim, TN	68	23	9	...	...	...	...	...	...	...	1.4	21.7
Ithaca, NY	1	25	66	8	...	...	...	...	...	...	2.8	17.0
Southern Regional Performance Nursery												
23, TX80A6025,												
Dallas, TX	2	10	32	27	18	10	1	...	...	...	3.8	20.0
Bozeman, MT	...	...	8	27	20	14	27	4	...	...	5.4	14.5
29, OK80019,												
Columbia, MO	...	...	...	39	50	10	1	...	...	...	4.7	15.8
Clovis, NM[a]	...	...	...	...	...	42	40	18	...	...	6.8	10.6
Northern Regional Performance Nursery												
20, CO745775-4,												
Mead, NE	...	...	...	...	4	62	34	...	...	...	6.3	15.3
Moccasin, MT	...	...	...	1	5	26	68	...	...	...	6.6	11.1
25, WT166,												
Casselton, ND	...	...	18	50	32	...	...	...	...	...	4.1	17.5
Moccasin, MT	...	...	...	...	6	55	39	...	...	...	6.3	12.5

[a] Irrigated.

TABLE II
Microscopic Data Compared with Particle Size Index (PSI) Data

Wheat Type	Score (1-10, Soft-Hard) 1	2	3	4	5	6	7	8	9	10	Microscopic Mean	PSI[a]	Near-Infrared Reflectance	Damaged Starch
Hard red spring (Glenlea)	···	···	···	···	1	9	41	49	···	···	7.4	46.3	49.6	9.3
Hard red winter	···	···	1	···	22	55	22	···	···	···	6.0	60.6	58.4	6.0
Hard red winter	···	···	4	7	34	36	19	···	···	···	5.6	64.6	60.8	5.8
Soft red winter	92	6	1	1	···	···	···	···	···	···	1.1	75.9	73.7	2.5
Soft white winter	26	18	29	18	4	4	1	···	···	···	2.7	66.5	68.0	6.2
Soft red winter	38	32	28	2	···	···	···	···	···	···	1.9	73.9	70.1	3.8
Soft red winter	74	23	3	···	···	···	···	···	···	···	1.3	71.8	69.3	3.6
Hard red spring	···	···	···	···	1	12	62	25	···	···	5.1	58.1	54.4	6.3
Soft white winter	59	29	12	···	···	···	···	···	···	···	1.5	72.0	71.8	3.8
Hard red spring	···	···	···	···	19	24	42	15	···	···	5.3	58.6	56.8	6.3
Soft red spring (HY320)	24	10	31	9	10	16	···	···	···	···	3.2	68.3	67.4	4.8
Hard red spring	···	···	···	6	38	39	13	4	···	···	5.7	59.4	57.8	6.6
Soft white spring	38	46	16	···	···	···	···	···	···	···	1.8	70.8	69.3	4.3
Hard red winter	···	4	10	27	43	16	···	···	···	···	4.6	63.9	64.2	6.2
Hard red spring	···	···	1	6	18	37	35	3	···	···	6.1	55.0	54.5	7.3
Hard red winter	1	1	3	14	33	46	2	···	···	···	5.2	65.9	64.2	5.8
Durum	···	···	···	···	1	2	1	11	45	40	9.2	39.8	43.3	9.9
Soft white spring[b]	38	46	16	···	···	···	···	···	···	···	1.8	71.5	···	4.3

[a] Particle size index, from Williams and Sobering (1986).
[b] Dropped from Williams and Sobering (1986) paper but included here.

TABLE III
Relation of Particle Size Index (PSI), Near-Infrared Reflectance (NIR), and Damaged Starch to Grain Hardness by Microscopic Method[a]

Parameter	PSI[b]	Mean NIR[b]	Damaged Starch
Microscopic hardness	−0.94	−0.95	0.93
PSI		0.98	−0.93
NIR			−0.93

[a] All values are coefficients of correlation.
[b] Williams and Sobering (1986).

Nursery showed the greatest range in individual grain heterogeneity. The grain hardness variation between the Dallas, TX, and Bozeman, MT, locations was large. Therefore, the sample was heterogeneous in hardness and was moderately influenced by environment. The correlation between the average microscopic data and PSI test was $r = -0.90$ (Table I).

Williams and Sobering (1986) reported on a set of 20 test samples. The individual grain hardness data for 18 of the samples is given in Table II.

On an individual grain hardness basis, several of the samples were heterogeneous in hardness. The microscopic technique permits one to screen samples for hardness studies and to select those with the narrowest range. The coefficient of correlation for the microscopic hardness means versus the PSI and NIR (Williams and Sobering 1986) and damaged starch were −0.94, −0.95, and 0.93, respectively. The coefficients of correlation for the damaged starch versus PSI and NIR were both −0.93 (Table III).

ACKNOWLEDGMENTS

The author gratefully acknowledges financial support form the Nebraska Wheat Board for a special project to develop a microscopic method for wheat hardness. The dedication and competence of Jane Clegg of the Wheat Quality Laboratory staff is appreciated. Samples were provided by P. C. Williams, Canadian Grain Research Laboratory, and by 35 cooperators of the four USDA-ARS regional nurseries.

LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC. Method 76-30, approved May 1960, revised November 1972 and October 1982. The Association: St. Paul, MN.

DAVIS, A. B., and EUSTACE, W. D. 1984. Scanning electron microscope views of materials from various stages in the milling of hard red winter, soft red winter, and durum wheat. Cereal Chem. 61:182–186.

GREENWELL, P., and SCHOFIELD, J. D. 1986. A starch granule protein associated with endosperm softness in wheat. Cereal Chem. 63:379-380.

MATTERN, P. J., MORRIS, R., SCHMIDT, J. W., and JOHNSON, V. A. 1973. Location of genes for kernel properties in the wheat variety 'Cheyenne' using chromosome substitution lines. Page 703 in: Proc. 4th Int. Wheat Genet. Symp. E. R. Sears and L. M. S. Sears, eds. University of Missouri: Columbia, MO.

MOSS, R., STENVERT, N. L., KINGSWOOD, K., and POINTING, G. 1980. The relationship between wheat microstructure and flour. Scanning Electron Microsc. 3:613-620.

SANDSTEDT, R. M. 1955. Photomicrographic studies of wheat starch. III. Enzymatic digestion and granule structures. Thomas Burr Osborne Medal Address. Cereal Chem. (Suppl.) 32:1-47.

SANDSTEDT, R. M., FLEMING, J., and ALLRED, J. D. 1952. Disintegration of the wheat kernel endosperm during milling. (Cinemicrograph available for loan.) Instructional Media Center, University of Nebraska-Lincoln, Lincoln, NE.

SYMES, K. J. 1961. Classification of Australian wheat varieties, based on the granularity of their whole meal. Aust. J. Exp. Agric. Anim. Husb. 1:18-23.

SYMES, K. J. 1965. The inheritance of grain hardness in wheat as measured by the particle size index. Aust. J. Agric. Res. 16:113-123.

WILLIAMS, P. C., and SOBERING, D. C. 1986. Attempts at standardization of hardness testing of wheat. II. The near-infrared reflectance method. Cereal Foods World 31:417-420.

YAMAZAKI, W. T., and DONELSON, J. R. 1983. Kernel hardness of some U.S. wheats. Cereal Chem. 60:344-350.

[Received November 19, 1987. Accepted January 7, 1988.]