Relation Between Wheat Kernel Hardness, Environment, and Gliadin Composition

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ABSTRACT

Variation in wheat kernel hardness has recently increased, making wheat classification more difficult. To assess effects of growing conditions on protein composition and hardness, wheat grown in a greenhouse and commercial field-grown wheats were examined. Mature kernels from greenhouse plants were harvested and segregated according to origin from wheat heads. Individual kernels were tested for hardness with a particle size analyzer, and gliadins were extracted and analyzed by high-performance liquid chromatography. Chromatograms were integrated, and amounts of fractions varying in hydrophobicity were determined. For greenhouse-grown wheats, hardness correlated with at least one gliadin fraction but not with original head positions of kernels. This also was true for commercial wheats, but such samples also varied in hardness due to multiple genotypes. Thus, differences in hardness among single kernels of a cultivar may result from variation in protein synthesis in kernels from different head locations, from variation between heads of the same plant that develop at different dates, and from multiple biotypes within cultivars.

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Wheat kernel texture differentiates cultivars of hard and soft wheat classes. Such classification is useful since it predicts milling times and energy requirements (Miller et al 1982, 1984, Pomeranz et al 1985) and possibly loaf volumes (Symes 1969). Many ways can be used to determine hardness and softness of bulk wheat (Pomeranz and Miller 1983, Pomeranz et al 1985). Location of growth also affects texture (Pomeranz et al 1985), and individual kernels from a cultivar grown in one location also vary in hardness (Mattern 1988).

The Federal Grain Inspection Service (FGIS) must classify wheat on a texture basis. Kernels of some new cultivars vary in hardness, making them difficult to classify. This has prompted much research on texture of spring and winter wheat kernels and searches for suitable classification methods. New devices have been developed to determine average sample hardness (Osborne and Fearn 1983, Reichert et al 1986, Eckhoff et al 1988, Mattern 1988), and methods and devices have been compared (Williams and Sobering 1986, Gaines et al 1987, Wu et al 1990).

Causes of hardness variation among wheat kernels are difficult to determine unless specific components causing such differences can be identified. Since storage proteins surround and fill (or, for soft wheats, partially fill) spaces between starch granules in the kernel, it seems likely that these proteins largely explain differences in kernel texture and hardness (Simmonds et al 1973). Minor proteins, such as those associated with soft but not hard wheat starch (Greenwell and Schofield 1986), also may be involved with endosperm texture. These do not fully explain differences in hardness, however, since hardness may vary greatly among cultivars of the same class or even among kernels of a cultivar.

We therefore used high-performance liquid chromatography (HPLC) (Bietz 1983) to analyze quantitative protein distributions of individual kernels of hard red winter (HRW), hard red spring (HRS), and soft red winter (SRW) cultivars, which differ in hardness, and we related results to the hardness of these same kernels.

MATERIALS AND METHODS

Wheat Samples

Five wheats (Stoa [HRS], Arkan and Vona [HRW], and Titan and Hart [SRW]) were obtained from the USDA-ARS Soft Wheat Quality Laboratory. Two HRS cultivars (Butte and Coteau) were grown in a greenhouse at the Northern Regional Research Center (Huebner et al 1990) to eliminate environmental variation. For each cultivar, kernels of two plants were analyzed. On the date each spike flowered (anthesis), it was tagged. When kernels were mature and dry, they were removed by forceps from top, middle, and bottom locations on the spike (for the top and bottom, only two or three spikelets were used from either end of the spike) and stored until analysis.

Individual kernels were ground in 200 ml of 99% ethanol in a metal 500-ml blender jar (model 8520, Eberbach, Ann Arbor, MI) with a two-speed blender (model PB-5, Waring, New Hartford, CT) at high (15,000 rpm) speed (Gaines 1986). The resulting meal suspension was analyzed in a Microtrac 7991-0 particle size analyzer (Leeds & Northrup, North Wales, PA) to determine average particle size. Particles were collected on filter paper (ethanol was discarded), transferred to 10-ml centrifuge tubes (Huebner and Bietz 1984), and extracted with 0.75 ml of 70% ethanol. After centrifugation at 15,000 rpm for 15 min, the clear solution was applied to a reversed phase (RP)-HPLC column to analyze gliadins. Some samples were first extracted with 0.5*N* NaCl for 10 min and centrifuged (as for gliadins) to yield salt-soluble proteins for analysis.

HPLC

We analyzed samples by RP-HPLC, using a Spectra-Physics (San Jose, CA) apparatus including a SP8700 solvent delivery system and a SP8780XR autosampler (Huebner and Bietz 1987). Proteins were detected at 210 nm (0.1 full-scale absorbance units per 10 mV) with a SF770 Spectroflow monitor (Kratos, Ramsey, NJ). A Vydac (Separations Group, Hesperia, CA) C_4 column (250 × 4.1 mm, 5-μm particle size, 300-Å pore size) was used, preceded by a 22- × 3.5-mm SynChrom RSC guard column and a 0.5-μm in-line prefilter (A-103, Upchurch, Oak Harbor, WA). The column was maintained at 60°C with a CH-20-C column heater (Scientific Systems, State College, PA) or at 57°C with a CH-460 column heater (FIAtron Laboratory Systems, Oconomowoc, WI).

Acetonitrile (ACN) and trifluoroacetic acid (TFA) were HPLC-grade. Water was purified with a Barnstead NANOpure system (Dubuque, IA). Solvents A (water containing 0.11% TFA) and B (90% ACN containing 0.09% TFA) were filtered through a 0.45-μm HVLP Millipore filter (Bedford, MA). Solvents then were deaerated under vacuum and initially sparged rapidly with helium for 5–10 min (Huebner and Bietz 1987). Slow purging with helium continued during analyses. Samples of 10–30 μm generally were analyzed (in duplicate) at 1.0 ml/min. Typically, proteins were applied to the column equilibrated with 25% solvent B and eluted with a gradient that increased to 31% B at 3 min and to 49% B at 50 min. The column then was held at 49% B for 5 min before reequilibration for the next sample. Slight adjust-

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ments in gradient conditions were occasionally made to optimize column performance.

Data Analysis

Data were recorded on an Omniscribe recorder (Houston Instruments, Austin, TX) and stored in a ModComp computer system (Fort Lauderdale, FL). Data then could be plotted to any convenient scale and automatically integrated between specified times or by Gaussian deconvolution after correction for baseline shifts due to the gradient.

RESULTS

RP-HPLC of Gliadins from Developing Kernels

Gliadin RP-HPLC chromatograms were divided into fractions varying in surface hydrophobicity (Fig. 1, fractions A–H). Amounts of each fraction (as a percentage of all gliadins eluting from the column) were determined by integration and correlated with particle size, as determined by Microtrac analysis. Particle size relates to kernel hardness (Miller et al 1984, Gaines 1986, Gaines et al 1987, Wu et al 1990): larger meal particle size values (mean diameter) denote harder kernels.

Each of the first HRW and SRW wheat cultivars analyzed consisted of two or three genetically different biotypes with different HPLC patterns (e.g., note difference in chromatograms for kernels 1, 2, and 6 in Fig. 1). This was discouraging at first because only a few kernels remained for comparison from each biotype. Each biotype (e.g., chromatograms of kernels 5 and 6, fractions E and H, Fig. 1) had areas that were significantly different; this in turn can influence apparent relative amounts of other fractions. This would be accented if smaller fractions or individual peaks were used.

First results for the cultivars Stoa, Titan, and Vona suggested relationships between amounts of some protein fractions and kernel hardness. However, some kernels did not fit such relationships, putting the accuracy of meal particle size determination or HPLC results into question. Accuracy of the method was confirmed, however, by splitting several Hart and Arkan kernels longitudinally and analyzing each half by particle size and HPLC analyses. Chromatograms were divided into smaller fractions, giving extra fractions, and correlations again were determined. Results (not shown) were similar to the earlier work and considered satisfactory. The two halves gave equal results.

To eliminate possible quantitative variation due to environment, we also analyzed kernels grown in a greenhouse (Huebner et al 1990). Figure 2 relates flowering dates, kernel head origins, and kernel weights for kernels from Butte and Coteau wheats. Data (averages for 4–5 kernels from each spike location) also are shown in Figure 2. Kernel weights vary both with flowering date and with head position: the average weight of middle kernels is largest and top kernels smallest. However, individual kernels from each

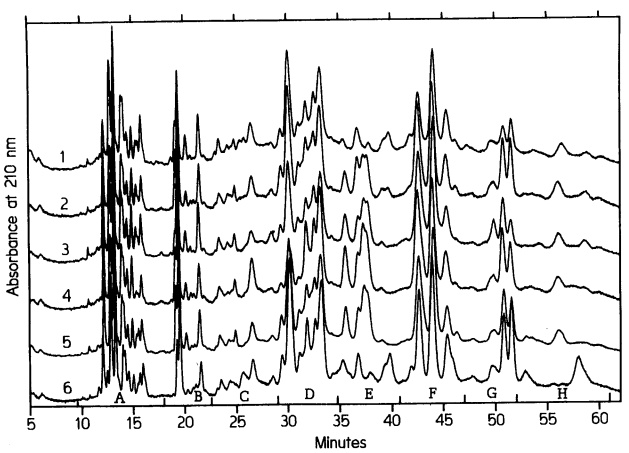

Fig. 1. Reversed-phase high-performance liquid chromatography of single kernels (numbered 1–6) of Vona gliadin after first determining hardness by particle size. Samples were from bulk supply at the USDA-ARS Soft Wheat Quality Laboratory, Wooster, OH. The gradient began at 23% acetonitrile (ACN) and increased linearly to 28% ACN at 3 min and to 43% ACN at 50 min. The column was maintained at 43% ACN for 4 min before reequilibration for 10 min with 23% ACN. Other conditions given in Materials and Methods.

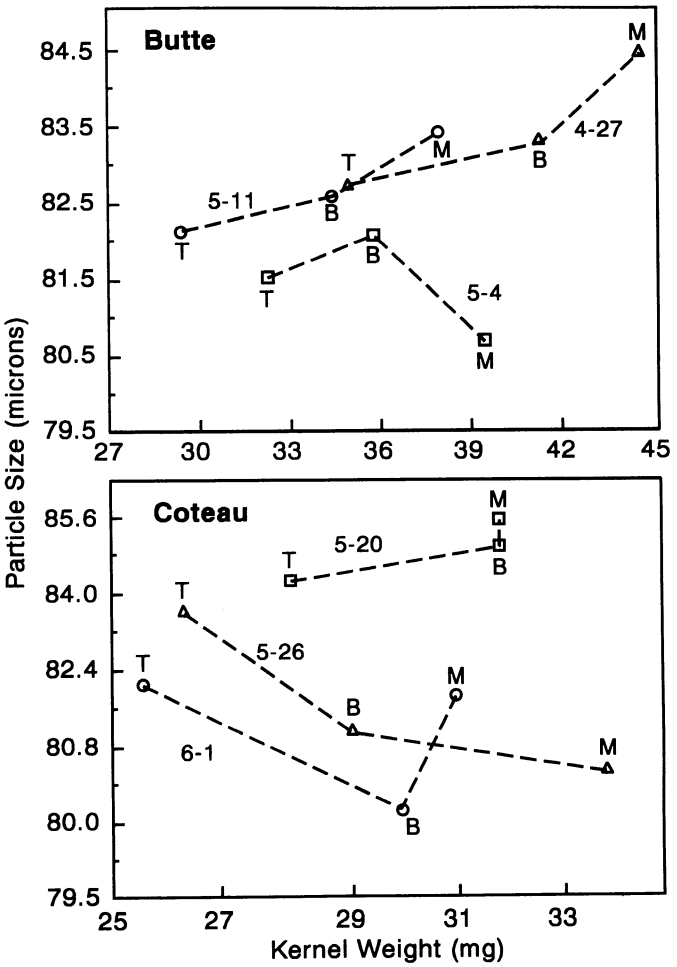

Fig. 2. Relationship between wheat kernel weight, location of kernels on spikes, flour particle size (an indicator of hardness), and developmental stage. Hard red spring cultivars Butte and Coteau were grown in a greenhouse. Butte was harvested on April 27 (4-27), May 4 (5-4), and May 11 (5-11), and Coteau on May 20 (5-20), May 26 (5-26), and June 1 (6-1). Kernels were harvested from the top (T), middle (M), and bottom (B) of three spikes for each cultivar on each date. Kernel weights and flour particle size were determined for each; averages are reported.

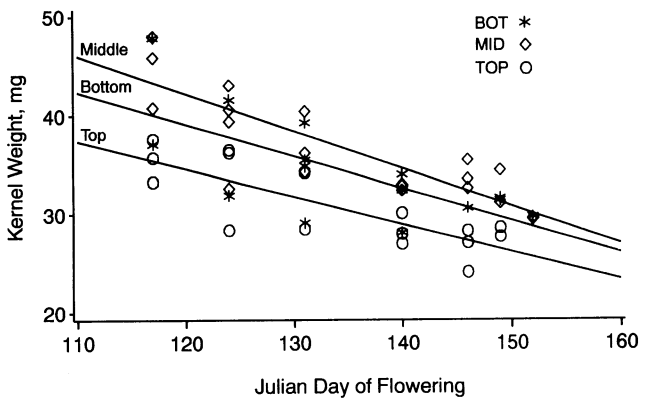

Fig. 3. Individual kernel weights of Butte and Coteau wheats as related to flowering date (Julian calendar: days numbered consecutively from January 1 to December 31) and spike location. Butte flowered on days 117, 124, and 131 (April 27 and May 4 and 11) and Coteau on days 140, 146, 149, and 152 (May 20, 26, and 29 and June 1).

location vary considerably (Figs. 2 and 3) (Huebner et al 1990). Figure 3 plots kernel weights by head locations according to flowering date. Slopes of each regression line are not significantly different for each head location, possibly indicating that kernel size is affected by day length (Marshall et al 1989) or an increase in temperature (Tashiro and Wardlaw 1989, Darroch and Baker 1990, Harding et al 1990, Randall and Moss 1990). Since the daily high temperatures in the greenhouse were not recorded, this cannot be confirmed. However, the day length was approximately 12 hr, which is less than normally found in the spring wheat-growing area. Coteau was evidently sensitive to day length and therefore flowered later than Butte. For 55 Butte and Coteau kernels, the correlation between flowering date and kernel weight is -0.70 ($P < 0.01$).

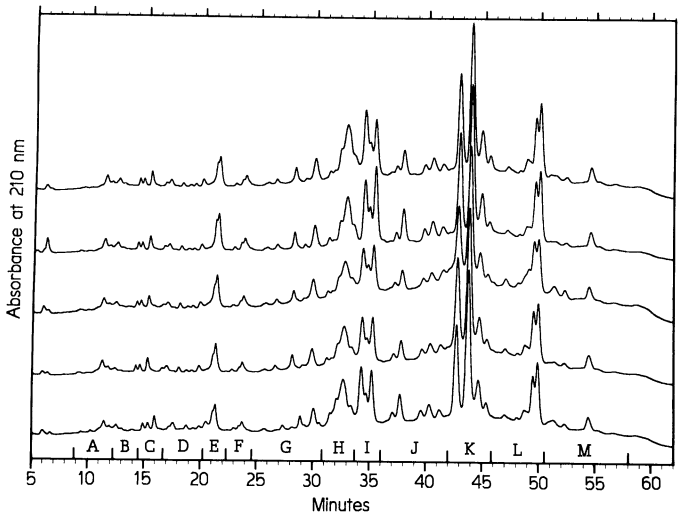

Fig. 4. Reversed-phase high-performance liquid chromatography (1.0 ml/min) of gliadins from six Butte wheat kernels. The chromatogram was divided into 13 fractions (A–M); amounts of fractions were correlated with particle size.

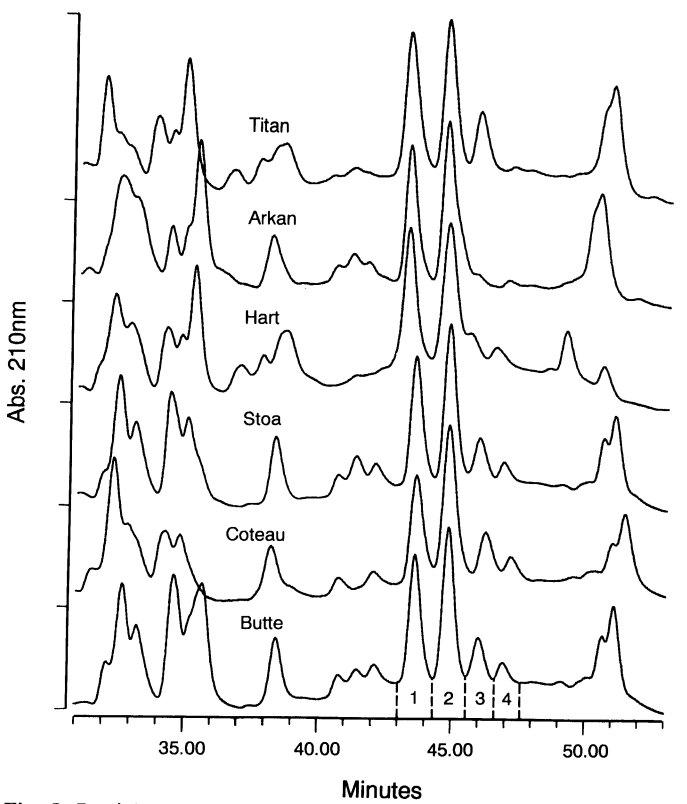

Fig. 5. Partial reversed-phase high-performance liquid chromatograms of gliadins from six wheat cultivars. Fractions 1–4 were divided approximately at the lines designated. Peak area percentages were correlated with particle size (Table I).

Particle Size and Kernel Weight

Analysis of variance was used to simultaneously consider effects of cultivar, date, and head position on particle size and kernel weight. The difference in kernel weight between Butte (average = 37.7 mg, standard deviation = 5.4) and Coteau (average = 30.5 mg, standard deviation = 2.6) in this data set was negatively correlated to the flowering date.

When cultivar, flowering date, and spike location were considered simultaneously in an analysis of variance, differences in average kernel weights between head locations were significant ($P < 0.01$); averages were 33.9, 36.9, and 30.7 mg for bottom, middle, and top kernels, respectively. Means after simultaneous consideration for differences in date, however, were nonsignificant ($P = 0.41$) (34.4 and 36.4 mg for Butte and Coteau, respectively). Date had a similar influence on weights of kernels from all head positions (Fig. 3), but after simultaneous consideration for date within Butte, the cultivar difference was no longer significant ($P = 0.08$).

Meal Particle Size

No significant differences were found between head positions and average particle size (bottom = 82.4, middle = 82.6, top = 83.6). Date and cultivar did affect particle size ($P < 0.05$). Particle size of Coteau was higher than that of Butte for the first flowering date, but that difference decreased as flowering date became later. However, the considerable difference in flowering dates between the two cultivars makes direct comparisons of particle size impossible. The overall correlation between particle size and kernel weight was $r = 0$. A previous study of particle size and kernel weight (Gaines 1986) produced the same results among hard wheats, but a correlation of 0.38 was observed among soft wheats.

RP-HPLC of Butte and Coteau

Chromatograms of Butte gliadins from top, middle, and bottom kernels of three or more spikes were divided into 13 fractions (Fig. 4, fractions A–M), and percentages of each fraction were related to meal particle size. While amounts of some fractions appeared to vary, no significant correlation to particle size was found. Fractions H and K (Fig. 4) seemed to show weak correlations, so we also examined individual peaks in these fractions. Amounts of individual peaks were subjected to analysis of variance in search of components correlated with kernel hardness. While there was a positive correlation of one peak between 31 and 36 min, it did not hold up for other cultivars. However, a significant negative correlation was found for some peaks between 43 and 47.5 min (Fig. 5).

Table I shows the correlations for four peaks between 43 and 47.5 min (Fig. 5) with particle size. The best correlations were peak 2 for Butte and Coteau, peak 3 for Stoa, and peak 1 for Arkan. For Hart, the correlation between peak amounts and particle size varied with biotype; however, when data for one atypical kernel were eliminated, both peaks showed a correlation of -0.76 ($P < 0.05$).

The raw data for the Vona samples were lost and could not be recalculated as was done for the other samples included in Table I. Peaks 3 and 4 for Arkan, Hart, and Titan were too small or nonexistent and therefore were not calculated.

Salt-Soluble Proteins

Albumins and globulins from Coteau kernels from each head location also were analyzed by RP-HPLC. Peak and fraction areas analyzed were not found to be correlated with particle size (results not shown).

DISCUSSION

Our data suggest a correlation between at least one gliadin peak (eluting between 43 and 47.5 min) and particle size (hardness).

TABLE I
Correlations of Meal Particle Size of Individual Wheat Kernels to Relative Percentage of Individual Gliadin Peaks

Cultivar	Biotype	n	Correlation with Particle Size (Peaks[a]) 1	2	3	4
Butte		18	−0.03	−0.75***[b]	−0.10	−0.22
Coteau		17	−0.06	−0.51*	−0.34	−0.42*
Stoa		6	−0.39	−0.59	−0.81**	
Arkan	A	9	−0.54	−0.42		
Arkan	A[c]	8	−0.68*	−0.51		
Arkan	B	3	−0.94	0.02		
Arkan	A + B	12	−0.47	−0.25		
Hart	A	9	−0.56	−0.34		
Hart	A[c]	8	−0.76**	−0.76**		
Hart	B	6	−0.04	0.75*		
Hart	A + B	15	−0.46	−0.10		
Titan	A	4	−0.21	0.00		
Titan	B	6	0.74	0.52		

[a] Peaks 1–4 occur at 43.5, 45, 46, and 47 min, respectively, in Figure 5.
[b] $P < 0.10$, 0.05, and 0.01 for *, **, and ***, respectively.
[c] Correlations after removing data for one atypical kernel.

Research in progress shows that some high molecular weight gliadins (HMWGs) also elute in a broad area from 35 to 55 min, with maxima at approximately 44 and 49 min. Thus, hardness-related low molecular weight gliadin components in this area may be partially obscured by the presence of HMWGs, which also may cause slight variation in elution time. Alternatively, HMWG proteins eluting in this area may themselves relate to hardness, with low molecular weight gliadins interfering with the correlations. Additional studies are in progress and should answer this question.

Protein composition varies greatly among kernels from spikes that flowered on different dates. In late-developing kernels, differences may result from early termination of synthesis of some proteins, since all proteins are not synthesized at the same rate (Huebner et al 1990). Kernels from different spike locations also vary in hardness and protein composition, but not as much as with flowering date. The finding that kernel weight correlates well with flowering date was unexpected. This conclusion must be regarded as tentative, however, since it is based on analysis of only two samples. Differences in protein composition, resulting from a combination of factors, probably affect kernel texture. Alternatively, it cannot be ruled out that other factors could interfere in the extraction of certain gliadins due to possible variation in structure related to hardness. To date there is no known evidence for this (e.g., partial extraction of gliadins produces a similar gliadin pattern).

Another unexplained phenomenon was that, during this study and others involving single kernels, occasionally a chromatogram would be obtained that had all the same gliadin peaks as other chromatograms did (indication of the same cultivar); however, there would be a significant difference in the quantity of a fraction of gliadins. This would be such a large difference that it would affect the relative percentage of other fractions. This was the reason for removing the data for one atypical kernel for Arkan and Hart in Table I.

Other factors may affect the relationship between protein composition and endosperm texture. For example, average flour particle size may not reveal subtle structural features only apparent from particle size distributions (Wu et al 1990). Protein amount (e.g., soft wheats have less protein and more air spaces between starch granules) (Evers 1988) and composition also may affect kernel texture. These and other possibilities merit further study.

In conclusion, our results clearly emphasize the complexity of wheat kernel hardness, show that protein composition varies with kernel size, flowering date, and spike location, and demonstrate that kernel texture can be correlated with protein composition.

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