

Polyacrylamide Gel Electrophoresis and High-Performance Liquid Chromatography Patterns of Gliadins from Wheat Sections and Milled and Air-Classified Fractions

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ABSTRACT

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Gliadins were extracted from hard red winter wheat samples, cultivar Vona, that ranged in protein contents from 9.5 to 14.8%; from laboratory-milled and hand-dissected fractions from the hard red winter wheat; and from air-classified flours from a commercial blend of wheat flours. The gliadin extracts were obtained both from bulk wheat samples and from individual kernels. The gliadin fractions were analyzed by polyacrylamide gel electrophoresis (PAGE) and high-performance liquid chromatography (HPLC). Extract aliquots were analyzed (by PAGE and HPLC) both on a constant sample weight and constant sample protein basis. In evaluating the electrophoregrams and elution patterns, relative PAGE

band intensities and HPLC elution peaks and their ratios were studied. Small differences in the gliadin PAGE and HPLC patterns were found in flour mill stream fractions and by-products of a single wheat variety, Vona, and in air-classified wheat flour fractions with wide variations in wheat protein (bulk samples). Considerable differences in PAGE and HPLC patterns were found in hand-dissected fractions. Qualitative and quantitative variations in PAGE and HPLC patterns were observed among single kernels of this wheat cultivar, which indicates gliadin heterogeneity (biotypes).

The relation between composition and functional properties of wheat storage proteins continues to be one of the most researched and most elusive areas in cereal chemistry and technology (Pomeranz 1968, 1980, 1985). To establish the reliability for identification, characterization, and determination of end-use properties of wheat cultivars and selections on the basis of polyacrylamide gel electrophoregrams (PAGE) and high-performance liquid chromatography (HPLC) patterns, it is important to determine the consistency of those patterns in samples of different origin and/or history (Jones et al 1982; Lookhart et al 1982b, 1983). Alternatively, the genotypic, phenotypic, and processing effects should be measured and recorded.

The gliadin electrophoregram is not affected qualitatively by the growth environment of the grain, by its protein content, by sprouting, dusting, fumigation, or by heat treatment up to and beyond that required to destroy baking quality (Wrigley et al 1982). Lookhart et al (1982a) reported that PAGE patterns of gliadin proteins extracted from homozygous wheat plants were identical even if the kernels showed large variations in kernel size, shape, and color. When wheat was germinated or severely frosted during maturation, PAGE patterns of the gliadin proteins were unaltered, even though dough mixing properties and loaf volumes were greatly impaired.

Significant changes occur in the relative intensities of gliadin bands when sulfur is severely deficient during growth (Wrigley et al 1980, Lookhart and Pomeranz 1985). The latter condition rarely, if ever, exists in commercial crops. Changes in PAGE and HPLC patterns of gliadin proteins in bread crumb and cookies were reported by Menkovska et al (1987, 1988) and Pomeranz et al (1986). Characterization by PAGE of individual wheat kernels with relatively uniform protein contents was reported by Lookhart et al (1985) and Mecham et al (1985) and by PAGE and HPLC by Lookhart et al (1986). However, the PAGE patterns of wheat kernels from bulk samples that vary widely in protein contents are very limited in the literature. We are aware of no published results on the effects of conventional milling and air classification

on HPLC patterns of gliadin proteins. Such studies are the subject of this report. Included are determinations and comparisons of PAGE and HPLC patterns of gliadins extracted from bulk samples and extracted from individual kernels from bulk samples that varied widely in protein contents.

MATERIALS AND METHODS

Protein Series of the Hard Red Winter Wheat Cultivar Vona

The tested samples had wheat protein contents of 9.5, 10.9, 12.4, 12.8, and 14.8% (14% moisture basis [mb]). The wheat samples were ground to pass a Wiley micromill (20-mesh sieve). In addition, three sound, large kernels (each) that were selected from bulk wheat samples with protein contents of 9.5, 12.4, and 14.8% were ground in a mortar and pestle for PAGE and HPLC analyses.

Hand-Dissected Fractions of Wheat Kernels

Twenty whole kernels (protein content 12.4%, 14% mb) of Vona wheat were manually dissected into germ (protein 31.9%), starchy endosperm (protein 8.8%), and remainder of the kernel (protein 12.4%).

Laboratory-Mill Fractions of Vona Wheat

The wheat sample (protein 12.4%, 14% mb) was a composite of wheats of the following stations in Kansas: St. John (1981), Hesston (1984), Belleville (1985), Ottawa (1985), and St. John (fallow 1985). The wheat was conditioned 24 hr to 15% moisture before milling on an experimental mill (Allis Chalmers Mfg. Co., Milwaukee, WI) to produce a (72%) patent flour (Finney et al 1974). Flour was rebolted over a 9XX flour cloth. The protein contents of the flour, coarse bran, fine bran, red dog, and shorts were 10.3, 14.3, 15.6, 14.7, and 16.4%, respectively. The coarse bran was ground on a Wiley micromill to pass a 20-mesh sieve before extraction and analysis of gliadin proteins.

Air-Classified Flours

Air-classified flour fractions and parent flour were obtained from D. R. Goforth, Pillsbury Company, Minneapolis, MN. The protein contents (N \times 5.7, 14% mb) of the parent flour and its constituents, low-protein, bread, and high-protein fractions, were 10.9, 6.8, 11.4, and 26.8%, respectively.

Extraction and Analysis

Flours, milling fractions, and hand-ground dissected wheat fractions were each extracted with 750 μ l of 70% (v/v) aqueous ethanol by constant weight (250 mg) and by constant total protein (250 mg \times 12%, divided by the protein content of fraction to be analyzed equals the weight of the fraction to be extracted). This formula adjusts the amount of material extracted so that

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the protein content would be equivalent to that extracted from a 12% protein material. For single kernels, 100 μ l of 70% ethanol was added to the crushed kernel, agitated in an ultrasonic bath for 30 min, and centrifuged as for bulk extracts. PAGE and HPLC of 70% ethanol extracts were done as described by Lookhart and Pomeranz (1985) and Lookhart et al (1986). All samples were analyzed at least twice by applying a constant sample weight and by applying a constant amount of protein in PAGE and in HPLC fractionations.

Analytical Methods

Moisture and protein were determined by AACC methods 44-15A and 46-11, respectively (AACC 1983).

PAGE

Two drops of glycerin containing the tracking dye methyl green were added to the 400 μ l of gliadin extracts used for electrophoresis. For single-kernel extracts, one drop of glycerin containing the tracking dye was added to each extract before electrophoresis. The electrophoresis apparatus, power supply, staining, and destaining procedures have been described (Lookhart et al 1982b, 1986). The gel acrylamide concentration (6%), temperature (20°C), electrophoresis time (2 hr), and voltage (500 V) were those that optimized separations (Lookhart et al 1986).

HPLC

The remaining 100 μ l of gliadin extract from the bulk samples and 35 μ l (one half) of the single-kernel extract was available for analysis, and small aliquots (10 μ l) were analyzed by a modification to the HPLC procedure of Bietz (1983), Lookhart (1985), and Lookhart et al (1986). The HPLC system was composed of a Varian Associates (Walnut Creek, CA) model 5060 microprocessor-controlled pumping system, a Waters Associates (Milford, MA) model 710 A autosampler (10 μ l), a Tracor (Austin, TX) model 970 variable wavelength detector (210 nm), and a Hewlett-Packard (Avondale, PA) model 3388A printer-plotter automation system. Samples were eluted at 1-ml/min on a SynChropak RP-P (C18) 6.5- μ m particle column (250 \times 4.1 mm i.d., SynChrom Inc., Linden, IN) at 45°C using a series of linear binary gradients. Solvent A was acetonitrile

FRACTION	A ₂	B ₂	C ₂	D ₂	E ₂	A ₁	B ₁	C ₁	D ₁	E ₁
LANE	1	2	3	4	5	6	7	8	9	10

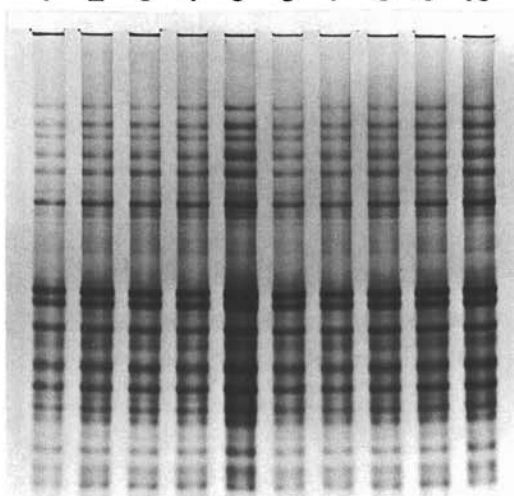


Fig. 1. Polyacrylamide gel electrophoresis patterns of gliadins extracted from bulk flour samples of the hard red winter wheat cultivar Vona varied in protein content. Lanes 1 and 6 are extracts of flour from 9.5% (A) protein wheat, 2 and 7 from 10.9% (B) protein wheat, 3 and 8 from 12.4% (C) protein wheat, 4 and 9 from 12.8% (D) protein wheat, and 5 and 10 are from 14.8% (E) protein wheat. Extracts in lanes 1-5 were applied on a constant sample weight basis; samples in lanes 6-10 were applied on a constant protein basis.

with 0.1% trifluoroacetic acid, and solvent B was water containing 0.1% trifluoroacetic acid. The multistep gradient conditions were as follows: 25% A at time 0, A held constant for 5 min, then a linear increase of A to 35% at 13 min, to 38% at 17 min, to 50% at 25 min, to 75% at 26 min, to 85% at 27 min, and a return to initial conditions (25% A) at 28 min. Reinjections were made at 45-min intervals.

RESULTS AND DISCUSSION

Protein Series of Vona Wheat

Gliadins were extracted for constant weight and for constant protein content from five bulk flour samples of Vona with varied wheat protein contents of 9.5% (A), 10.9% (B), 12.4% (C), 12.8% (D), and 14.8% (E), and A-E were analyzed by PAGE (Fig. 1)

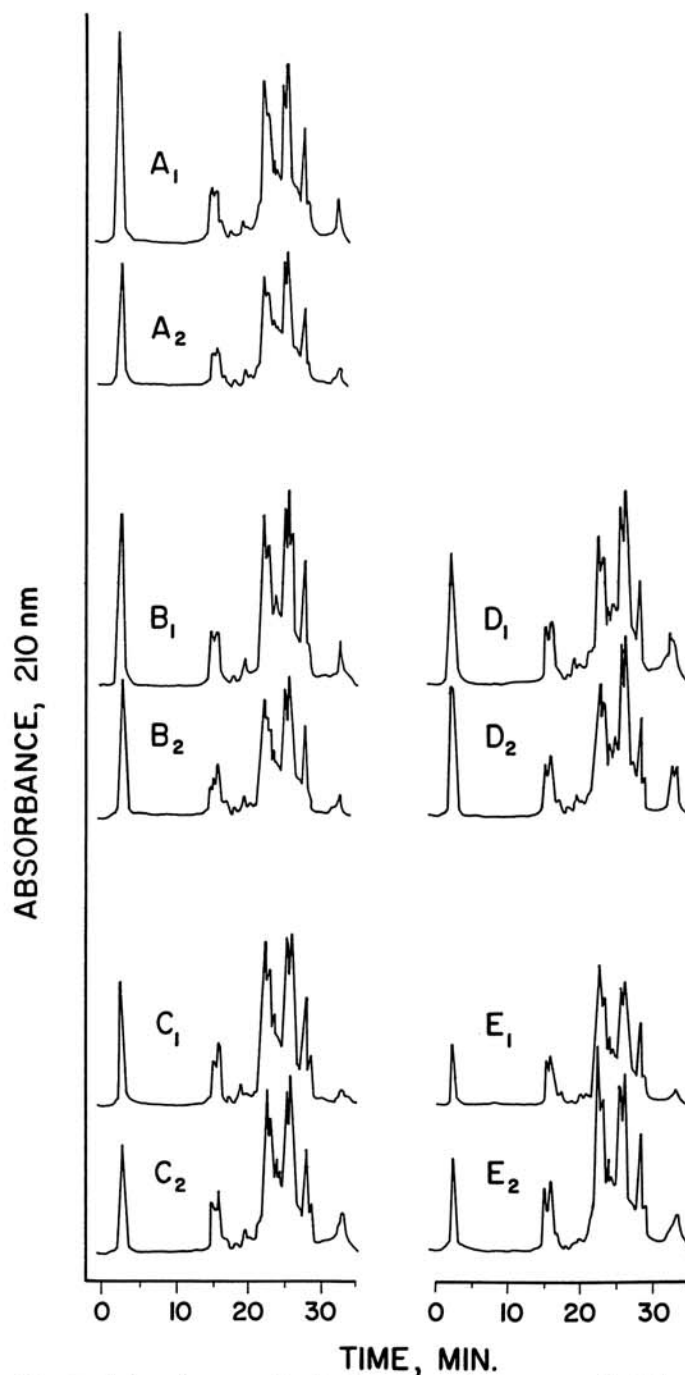


Fig. 2. High-performance liquid chromatography patterns of gliadins extracted from bulk flour samples (hard red winter wheat cultivar Vona) that varied in protein content: A (9.5%), B (10.9%), C (12.4%), D (12.8%), and E (14.8%). The subscripts 1 and 2 denote constant protein and constant weight basis, respectively.

and HPLC (Fig. 2). The constant protein extracts are identified by subscript 1 and the constant weight extracts by subscript 2. Lanes 1-5 (A_2 - E_2) of Figure 1 show the result of homogeneous volume application of samples extracted on a constant weight basis, whereas in lanes 6-10 (A_1 - E_1) the same volumes of samples extracted on a constant protein basis were applied. Differences in the relative amounts of the gliadins at each protein level are due to the contribution of nongliadin (albumin, globulins, glutelins) nitrogenous compounds. The most intensely stained gliadin bands are found in lane 5 (E_2) (Fig. 1) corresponding to the sample with the highest protein content (14.8%). Lanes 2 (B_2), 3 (C_2), and 4 (D_2), with protein contents of 10.9, 12.4, and 12.8%, appear of similar stain intensity, and lane 1 (A_2) shows the lightest bands.

In the constant-protein/volume set, the bands in lanes 6 (A_1) and 7 (B_1) appear of similar stain intensity and lighter than the bands in lanes 8 (C_1) and 9 (D_1), but the bands in the highest protein sample (14.8%) of lane 10 (E_1) are noticeably darker. The intensities of the analogous bands in lanes 6-10 theoretically should have been similar, but the gradually increased intensities of the bands in lane 8 (C_1) to 10 (E_1) might be due to a higher percentage of gliadins present in samples with very high protein content (Lasztity 1984). The band positions of all Vona bulk extracts (lanes 1-10 [A_2 - E_1], Fig. 1) were identical.

FRACTION	C_2	A_{21}	A_{22}	A_{23}	C_{21}	C_{22}	C_{23}	E_{21}	E_{22}	E_{23}
LANE	1	2	3	4	5	6	7	8	9	10

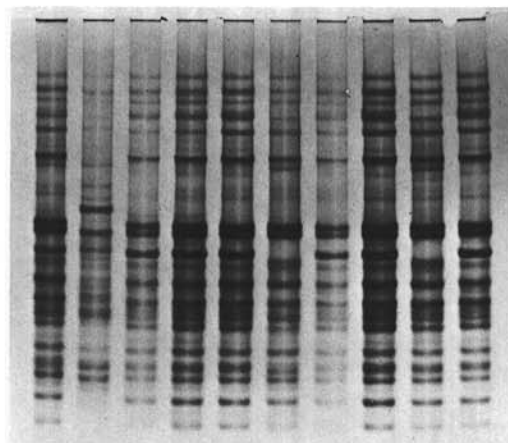


Fig. 3. Polyacrylamide gel electrophoresis patterns of gliadins extracted from bulk flour from wheat (lane 1) and from single kernels (lanes 2-10) of Vona hard red winter wheat bulk samples that varied in protein content. Lanes 2-4 (A_{21} - A_{23}) are extracts of single kernels from a 9.5% protein wheat sample; lanes 5-7 (C_{21} - C_{23}) are extracts of single kernels from a 12.4% protein wheat sample; and lanes 8-10 (E_{21} - E_{23}) are extracts of single kernels from a 14.8% protein wheat sample.

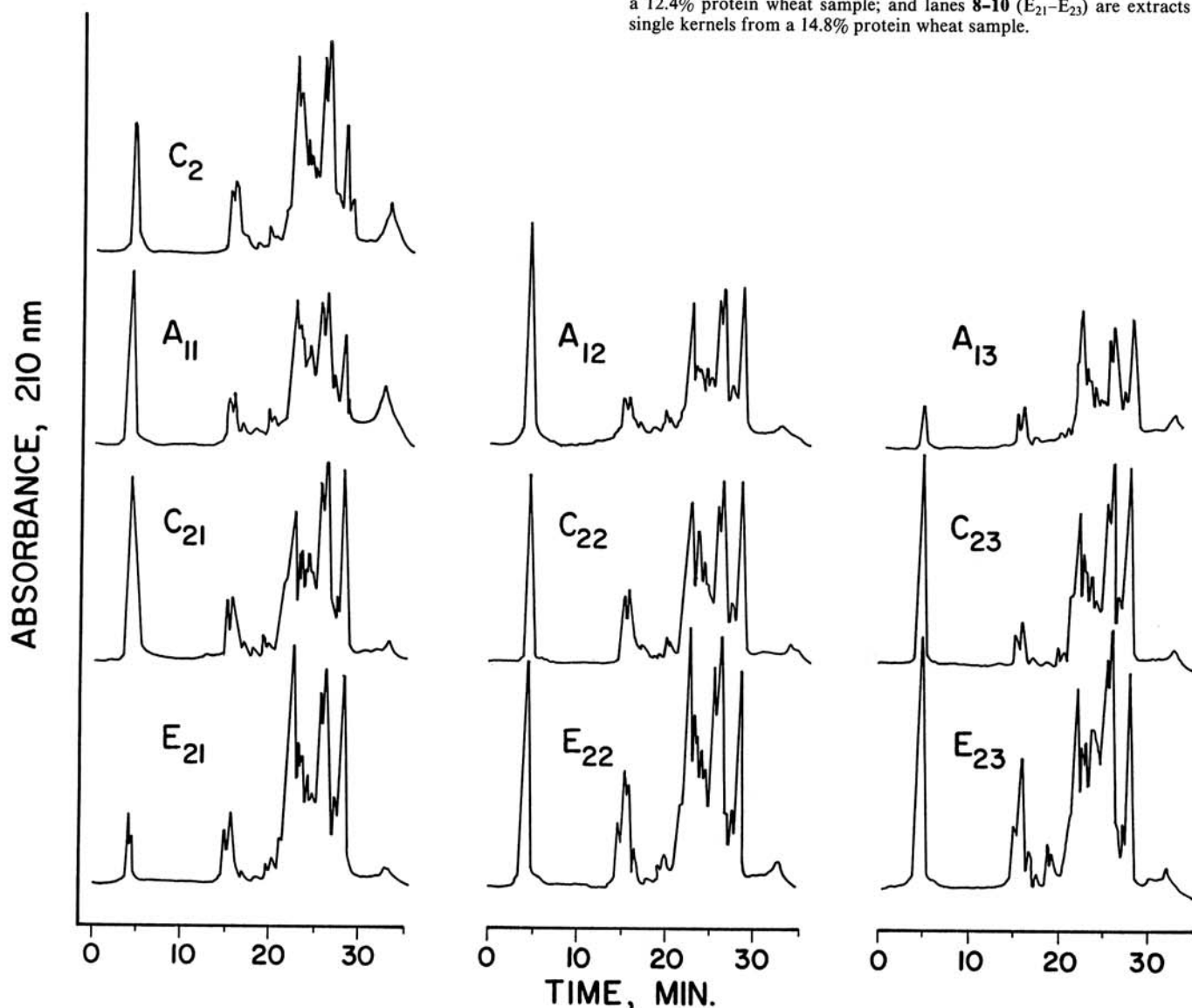


Fig. 4. High-performance liquid chromatography patterns of gliadins extracted from single kernels of Vona wheat bulk samples that varied in protein contents. A is the extract of flour from bulk wheat (protein content 12.4%), A_{1n} , C_{2n} , and E_{2n} are extracts of single seeds from bulk wheat with protein contents of 9.5, 12.4, and 14.8%, respectively.

The HPLC-patterns of all Vona bulk extracts (Fig. 2) are similar. However, the chromatograms of the extracts of the highest protein wheat (14.8%, E) were slightly different from those of other extracts in that the largest peak was at 22.5 min for E but at 27 min for A through D. The increase in protein content increases the relative amount of gliadin as reported by Fullington et al (1983) and appears to affect the amounts of particular gliadins. PAGE patterns of constant weight extracts of single kernels taken from bulk wheat samples of protein contents 9.5%

(A₂₁-A₂₃, lanes 2-4), 12.4% (C₂₁-C₂₃, lanes 5-7), and 14.8% (E₂₁-E₂₃, lanes 8-10) and of a flour from a bulk wheat sample of 12.4% protein (C₂, lane 1) are shown in Figure 3. There are noticeable differences from the bulk pattern (lane 1) in band position and intensity to the single-kernel patterns in most other lanes. Since three single seeds were analyzed for each protein level and band differences (gliadin polymorphisms) are found in all lanes, the Vona cultivar must be highly heterogeneous with respect to gliadin content and/or composition. Recently, F. Huebner (*personal communication*, 1988) also found heterogeneity in gliadin HPLC patterns of extracts of single kernels of Vona. The differences in intensity are due to variations in protein content from kernel to kernel. The band intensities of the highest protein set, lanes 8-10, appear darker overall than those of the lower protein extracts. However, variations in protein intensities and patterns are evident throughout the 10 lanes. This variation in intensity with protein content has been described earlier (Branlard and Triboni 1983, Borghi et al 1983) as due to protein content, amount of gliadin present and extracted, as well as relative amounts of each gliadin fraction.

The HPLC patterns of an analogous set of single-kernel samples are found in Figure 4. Small variations from the bulk wheat chromatographic pattern were found in the chromatograms of all single kernels. The intensities of all peaks in the 15-30 min range increased with protein content. In two of the three single-kernel extracts in the highest protein level (E₂₁-E₂₃), the peak at 22.5 min was larger than any of the other peaks, whereas the height of the 24- or 27-min peak was largest in the single-kernel extracts of all the other protein levels (A_{1n} and C_{2n}) except A₁₃. We do not know why it appears that with increasing protein levels, certain gliadin proteins are produced in relatively larger amounts than found in the normal protein concentration range.

Hand-Dissected Fractions and Laboratory-Milled Fractions

A comparison of the gliadins by PAGE is shown in Figure 5. The intensity of PAGE patterns of gliadins extracted from the laboratory-milled fractions did not consistently follow differences in protein contents because of the presence of different amounts of nongliadin nitrogenous compounds in the various fractions. Thus, applied on a constant protein (subscript 1) or constant weight (subscript 2) basis, PAGE patterns of coarse bran (C), shorts (E), and red dog (F) extracts were lighter than PAGE patterns of flour (A), ground wheat meal (B), and of fine bran (D) extracts. The ground wheat meal extract (B₁, 12.1% protein) had the darkest set of bands, and the shorts extract (E₁, 16.4% protein) had the lightest bands of the milled fractions. Gliadin patterns of shorts were even lighter than those of coarse bran or red dog. The very low intensity of the PAGE bands of hand-dissected germ can also be attributed to the low "gliadin" content of that wheat tissue. The PAGE pattern of the germ extracts (G) differed significantly and consistently from that of the starchy endosperm (H) extracts.

Lane G₁ (Fig. 5), an exception to the homogeneous volume application definition, is particularly interesting because 50 μ l was required in slot G to see the proteins in the germ fraction extract, whereas only 8 μ l was used for other slots. In lane G₂, only 8 μ l was used and no bands were found. Those gliadin bands found in G result from contamination of the germ by the endosperm in the separation procedure. The protein bands in that slot were slightly less mobile (lane G₁) than in the other lanes due to the increased volume of material applied to lane G₁. The hand-dissected starchy endosperm (H₁) and remaining material (I₁) were of similar intensity due to extraction at constant protein, even though their protein contents and intensities were different for the constant weight extracts (subscript 2).

The large differences in the PAGE patterns (Fig. 5) between the hand-dissected starchy endosperm and germ are confirmed by the very large differences in their respective HPLC patterns (Fig. 6). The germ fractions (G) possessed only proteins rich in highly hydrophilic early-eluting (near void volume) proteins. Similarly, there were some differences in intensity among the HPLC elution patterns of the extracts of the flour (A) and various

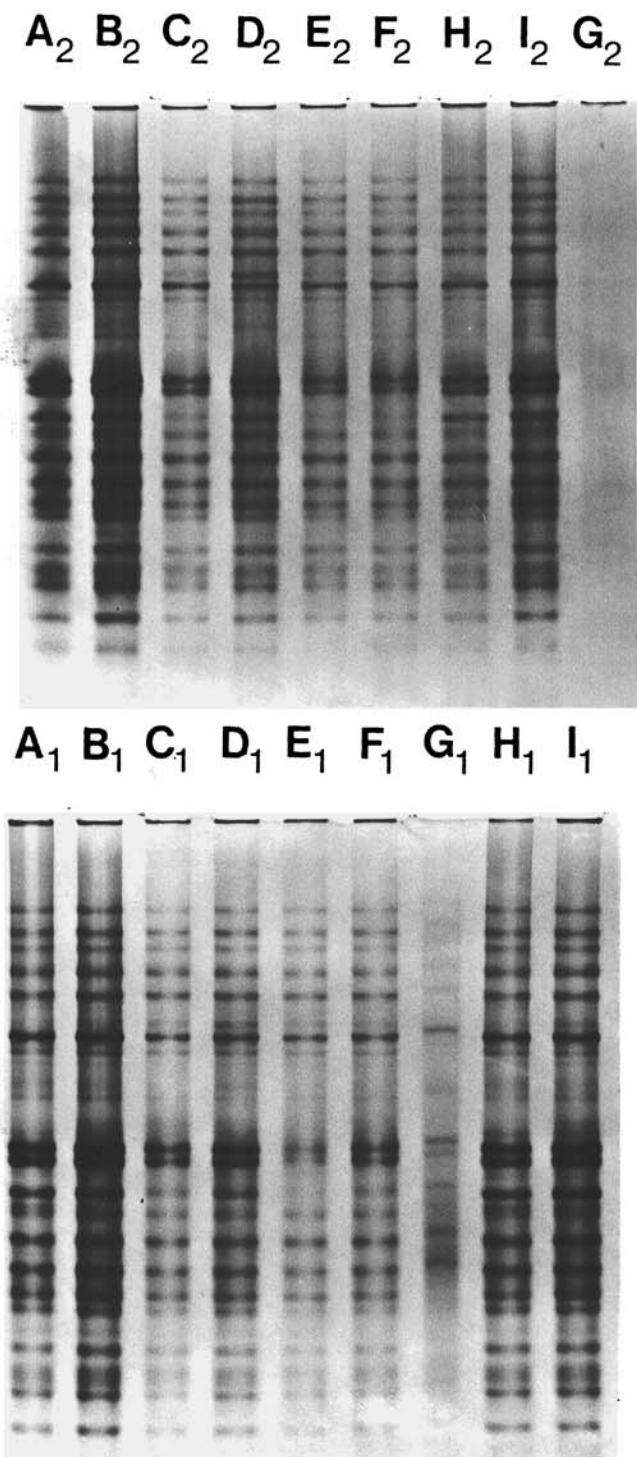


Fig. 5. Polyacrylamide gel electrophoresis patterns of gliadins extracted from 12.4% protein Vona wheat: its experimentally milled fractions—flour (A), ground wheat meal (B), coarse bran (C), fine bran (D), shorts (E), and red dog (F)—and its hand-dissected fractions—germ (G), starchy endosperm (H), and the rest (I). The subscripts 1 and 2 denote constant protein and constant weight basis, respectively, except that G was applied at 6.25 times the volume (concentration) of all others.

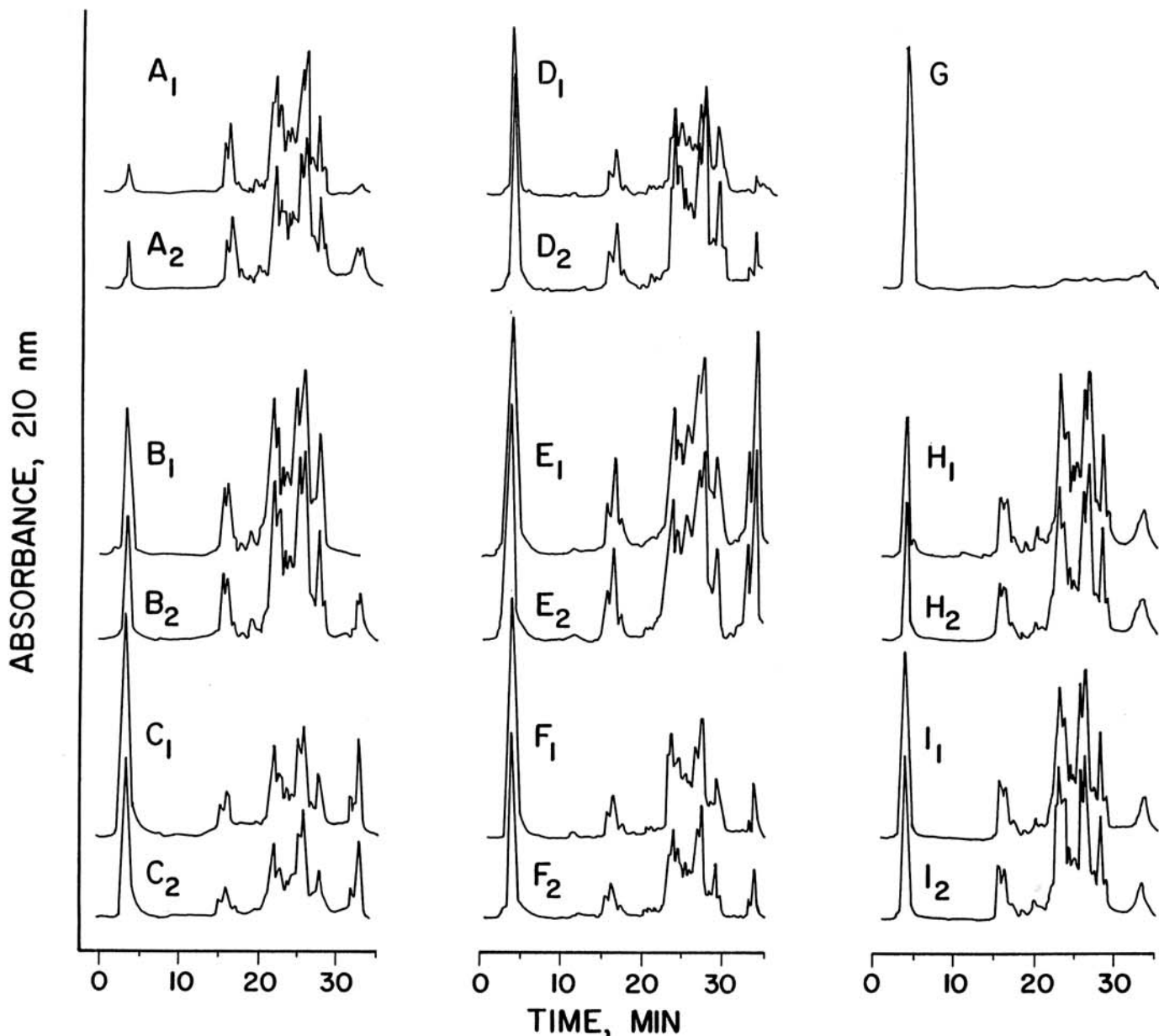


Fig. 6. High-performance liquid chromatography patterns of gliadins extracted from Vona wheat: its experimentally milled fractions—flour (A), ground wheat meal (B), coarse bran (C), fine bran (D), shorts (E), and red dog (F)—and its hand-dissected fractions—germ (G), starchy endosperm (H), and the rest (I). The subscripts 1 and 2 denote constant protein and constant weight basis, respectively.

FRACTION	A ₂	B ₂	C ₂	D ₂	A ₁	B ₁	C ₁	D ₁
LANE	1	2	3	4	5	6	7	8

milling by-products, especially in the 13–16 min range. The results confirm the expected differences in types or amounts of gliadin proteins in fractions from experimental milling.

Air-Classified Flours

Electrophoregrams of gliadins extracted from the series of air-classified flours are compared in Figure 7. As expected, in the four samples applied on a constant weight basis (A₂–D₂, lanes 1–4) the intensity of bands generally increased as protein contents increased. We expected that in the four samples applied on a constant protein basis (A₁–D₁, lanes 5–8) the intensity of bands would be comparable; however, this was not the case. The intensities of bands in the parent (B₂ and B₁, lanes 2 and 6) and bread flour (C₂ and C₁, lanes 3 and 7) were comparable (due

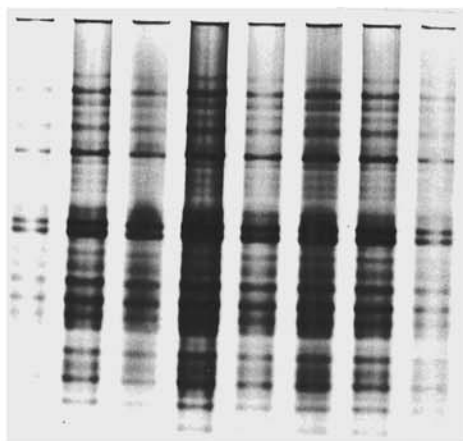


Fig. 7. Polyacrylamide gel electrophoresis patterns of gliadins extracted from air-classified flours: low-protein (A_n, lanes 1 and 5), parent flour (B_n, lanes 2 and 6), bread (C_n, lanes 3 and 7), and high-protein (D_n, lanes 4 and 8). Lanes 1–4 were extracted at constant weight basis ($n = 2$) and lanes 5–8 were extracted at constant protein basis ($n = 1$).

to similar protein concentration), but the intensity of bands in the low-protein flour (A_2 and A_1 , lanes 1 and 5) was much lower than in the corresponding parent flour. The intensities of bands in the high-protein flour fraction were high for the constant weight (D_2 , lane 4) extract, as expected, but low for the constant protein (D_1 , lane 8) extract. The results seem to point to a shift (decrease) in the gliadin composition in the high-protein fraction. Fullington et al (1983) reported that the gliadin contents increased with protein content up to 15% total protein; this air-fractionated (26.8%) high-protein fraction shows the opposite effect due to the method of fractionating the proteins (air classification). The results for PAGE were confirmed by the HPLC data in Figure

8 but the intensity differences were small.

HPLC analyses of the parent and three air-classified fractions were made (Fig. 8) to determine if increasing protein content increases particular fractions or types of proteins or if all increase linearly. Six peaks (I–VI) were chosen to be representative of peaks over the entire chromatogram. Peak heights were measured and ratios of the heights of the individual peaks II through VI to peak I were calculated for each flour protein level. The ratios were averaged to help reduce run-to-run variability, and their standard deviation was calculated to determine the variation in the HPLC system. These values were found to vary from one peak ratio to another (e.g., II:I, etc.), but the ratios within a

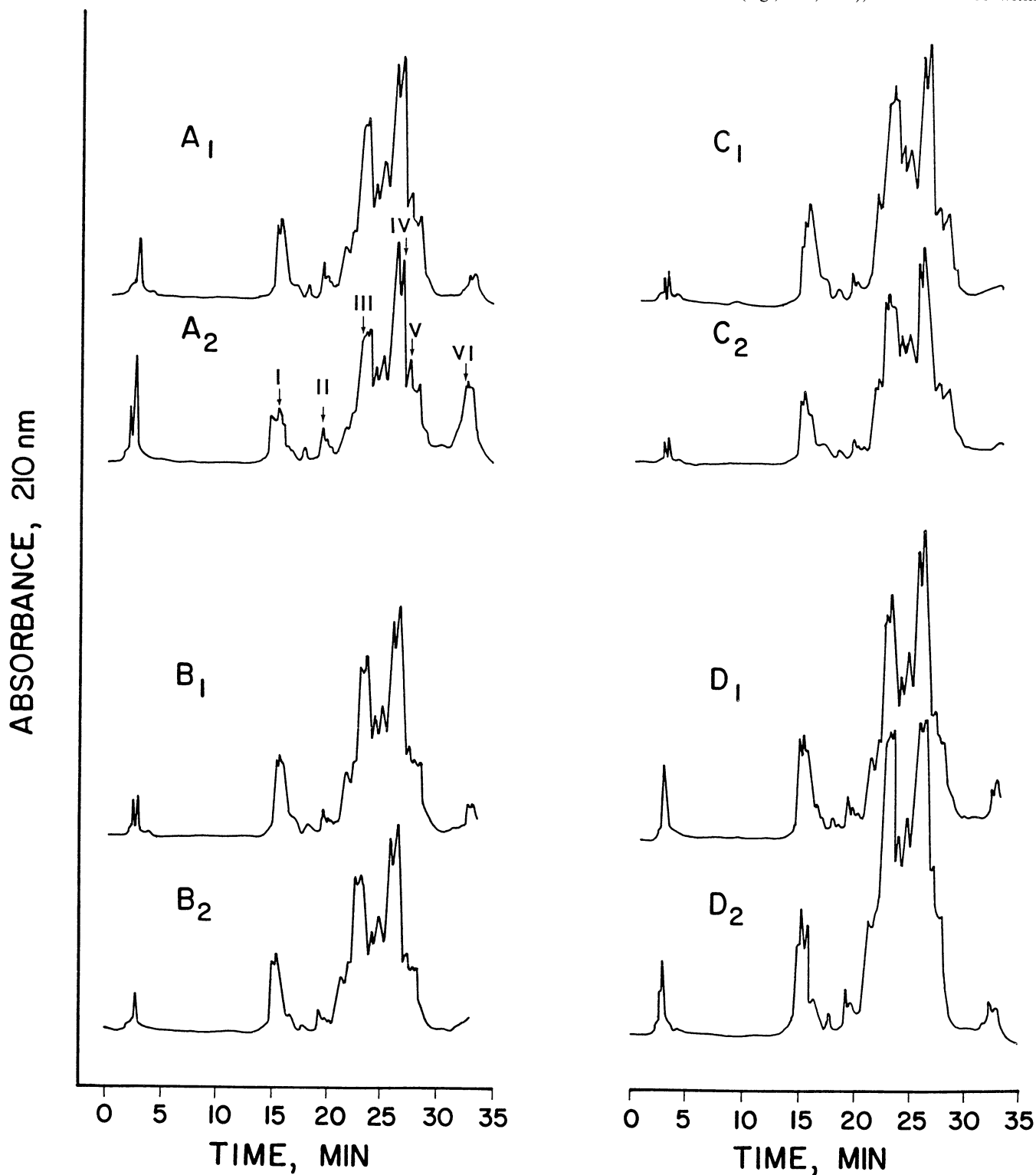


Fig. 8. High-performance liquid chromatography patterns of gliadins extracted from air-classified flours: low-protein (A), parent flour (B), bread (C), and high-protein (D). The subscripts 1 and 2 denote extraction on a constant protein and constant weight basis, respectively.

TABLE I
Height Ratios of High-Performance Liquid Chromatography Peaks^a

Air-Classified Flour Fraction	Protein Content (%)	Peak Height Ratio ^b				
		II:I	III:I	IV:I	V:I	VI:I
Low-protein	6.8	0.56 ± 0.01	2.50 ± 0.2	3.82 ± 0.33	1.50 ± 0.18	1.00 ± 0.1
Parent	10.9	0.26 ± 0.04	2.00 ± 0.08	2.67 ± 0.14	0.95 ± 0.06	0.27 ± 0.04
Bread	11.4	0.23 ± 0.06	2.30 ± 0.45	3.00 ± 0.74	0.91 ± 0.18	0.33 ± 0.12
High-protein	26.8	0.34 ± 0.06	2.60 ± 0.4	2.80 ± 0.84	0.93 ± 0.27	0.40 ± 0.41

^aPeak retention times: I, 15.5 min; II, 19.5 min; III, 23.0 min; IV, 27.0 min; V, 27.7 min; VI, 33.0 min.

^bAverage value ± standard deviation of heights from constant weight and constant protein chromatograms each analyzed in duplicate.

peak ratio set for similar protein levels (e.g., parent flour and bread flour) were similar (Table I). However, as protein contents change, so do the amount of gliadin and the relative amounts of individual proteins. The largest variation was for the ratio of peak VI to I, where the difficulty of accurately measuring the small peak (VI) at the end of the chromatogram is possibly the reason for the large variability. The standard deviations listed are measures of the reproducibility of the HPLC instrument combined, with the variation between extracting the protein on a constant weight and constant protein basis. The largest standard deviations were for bread and high-protein flour extracts. They are confirmed by comparing the peak height ratios of those fractions (in separations of gliadins applied by constant weight or constant protein content). Consistent differences are indicated in Table I.

The ratio values for each peak II to VI for the low-protein flour are higher than for the corresponding peak height ratios of the other flours, but since the absolute height of each peak is lower, relatively less of peak I is present in the low-protein flours. Peak I should be an omega gliadin, because they are the most hydrophilic of the gliadins and first gliadins eluted by HPLC (Lookhart and Albers 1988). These data suggest that smaller amounts of some omega gliadins are found in this low-protein air-classified flour than in the other fractions.

CONCLUSION

The PAGE method appears more sensitive to qualitative pattern analysis, whereas the HPLC method is more sensitive to quantitative differences. The PAGE gliadin patterns (qualitative) are nearly identical in all Vona wheat fractions examined (except germ). Differences in PAGE and in HPLC gliadin patterns were greater among the single kernels analyzed than among the fractions.

Large differences in protein contents affect not only the amount of gliadin (and nongliadin) proteins but the amount particular gliadin proteins vary as well.

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