

Protein Distributions Among Hard Red Winter Wheat Varieties as Related to Environment and Baking Quality¹

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ABSTRACT

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Previous size-exclusion high-performance liquid chromatography (SE-HPLC) studies of hard red spring (HRS) wheat proteins reported correlations of amounts of certain gliadin and glutenin fractions with general score, a quality descriptor based on loaf volume, mixing time, and other parameters. We now extended these studies to 12 hard red winter (HRW) wheats, each grown at six Midwestern locations. Gliadin and reduced glutenin fractions were isolated and fractionated by SE-HPLC. Amounts of fractions corresponding to specific molecular weight (MW) ranges, determined by integrating peaks from SE-HPLC elution profiles, were statistically correlated with various baking quality parameters. Amounts of one fraction rich in γ -gliadins correlated well with loaf volume; correlations were better than found previously for HRS wheats. Correlations

adjusted for overall protein (partial correlations) were >0.5 for 10 of the 12 samples, and >0.84 for six samples when estimated among samples, among locations, among samples at each location, and among locations for each sample. Because γ -gliadins appeared well correlated to loaf volume, the gliadin fraction was also analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC), which provides better separations. Correlations of γ -gliadin peak areas from RP-HPLC with loaf volume were then 0.93–0.99 for different varieties grown in different locations. Narrow-bore columns with shorter run times gave slightly higher correlations than did standard RP-HPLC columns. It is evident that both genetics and environment affect the relationship of quantitative protein distribution to loaf volume.

Recent reversed-phase high-performance liquid chromatography (RP-HPLC) studies have shown that baking or cooking quality differences among wheat samples can be predicted from protein compositions (Burnouf and Bietz 1984, Huebner and Bietz 1994). Van Lonkhuijsen et al (1992), using multiple linear regression to analyze RP-HPLC data for wheats with the same high molecular weight glutenin subunits, showed that amounts of specific gliadins account for up to 82% of differences in loaf volume. However, others found little correlation between protein composition and wheat flour baking quality, although actual loaf volume was not presented (Wieser et al 1994). So far, these methods have not been practical to use on a regular basis.

Molecular weight (MW) distribution and protein composition have shown some correlation to baking quality factors (% protein, loaf volume, and hardness) in HRS wheats (Huebner et al 1994). HRS wheat gliadins eluting late upon RP-HPLC correlated negatively with baking quality, as represented by a general score (Huebner 1989). This general score is not determined for breeding HRW wheats, however.

SE-HPLC can reveal variations in amounts of wheat protein MW fractions (Batey et al 1991, Graybosch et al 1994) and quantities of glutenin subunits, as well as amounts of four fractions of 70% ethanol-soluble proteins (gliadins) varying in MW (Huebner et al 1994). To further clarify relationships of hard red winter (HRW) wheat protein composition to baking quality, we analyzed a well-characterized set of wheat samples grown in different locations during the same year. Gliadin and glutenin fractions were

isolated and analyzed by size-exclusion high-performance liquid chromatography (SE-HPLC), and gliadins by RP-HPLC. Presence or amounts of MW fractions or individual polypeptides, as determined by SE-HPLC or RP-HPLC, respectively, were correlated with measurements of baking quality.

METHODS AND MATERIALS

Samples

HRW wheat flour samples were obtained from the Hard Winter Wheat Quality Laboratory (HWWQL), Grain Marketing and Production Research Center (GMPRC), Manhattan, KS. Samples consisted of 12 lines grown at six locations throughout the HRW wheat growing area (Chillicothe, TX; Lahoma, OK; Hutchinson, KS; Colby, KS; Akron, CO; and Clay Center, NE) for the 1990 crop evaluation of Uniform Growout Samples for the Wheat Quality Council. Seven varieties (Karl, Quantum 549, Pioneer 2163, Abilene, Eagle, Newton, Tam 107) were commercial cultivars, and five were breeding lines in the testing stage. All wheats were milled by an experimental Brabender Quadrumat Senior mill, and flour samples were analyzed at the HWWQL, GMPRC for many quality parameters, including percent protein, mixograph water absorption, mixograph mix time, bake water absorption, bake mix time, loaf volume, and loaf volume potential.

Mixograms for each flour sample (10 g, 14% mb) were obtained using a 10-g bowl mixograph with optimum water absorptions (Finney and Shogren 1972), which is basically the same procedure as Method 54-40 (AACC 1995). For baking, the optimized procedure described by Finney (1985) and in Method 10-10B (AACC 1995) was used.

HRS wheat flour samples were obtained from Gary Hareland, USDA/ARS Wheat Quality Laboratory, Fargo, ND. These wheats, grown as nursery samples in 1992, included three experimental lines plus Stoa and Butte 86 (standard cultivars). Spring wheats were grown at nine locations in Washington, Montana, North Dakota, South Dakota, Minnesota, and Wisconsin.

Protein Extraction

Gliadins. Flour samples (50 or 60 mg) were extracted at room temperature with 1.7 mL of 60, 70, 75, or 80% ethanol (extracted twice, first with 1.0 mL and then with 0.7 mL, and the two extracts combined) in 10-mL polypropylene tubes for 30 min with

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vortex mixing. Different sized samples were needed for optimum results with the different HPLC systems and column sizes used. Different ethanol concentrations were tried to optimize results. After centrifugation at $15,000 \times g$ for 10 min, clear supernatants were stored in vials before automatic injection for RP-HPLC (Huebner and Bietz 1986), and the residue was stored in the freezer until used for glutenin extraction.

Glutenins. Residue from the gliadin extraction was extracted with 1 mL of 5M urea in 0.05M sodium phosphate buffer, pH 7.7, containing 0.1% dithiothreitol. After 2 hr of vortex mixing at 33°C, 5 μ L of 50% 4-vinylpyridine in 60% propanol was added. Following brief vortex mixing, samples were centrifuged at $17,000 \times g$ for 10 min, and clear supernatants were removed to small autosampler vials. A mixture of 50% trifluoroacetic acid (TFA) in concentrated acetic acid ($\approx 3 \mu$ L) was then added to each sample to reduce the pH to 3.2 ± 0.2 .

RP-HPLC

For standard-sized Vydac C₄ (4.6 mm i.d. \times 150 mm) and Zorbax C₈ (4.6 mm i.d. \times 150 mm) RP-HPLC columns, an SP8700 solvent delivery system and an SP8780XR autosampler (Spectra-Physics, San Jose, CA) were used (Huebner and Bietz 1987). For best accuracy, 10- μ L full loop aliquots extracted from 60-mg flour samples were analyzed. Columns were protected with 2- \times 20-mm guard columns (C-130B, Upchurch, Oak Harbor, WA) containing

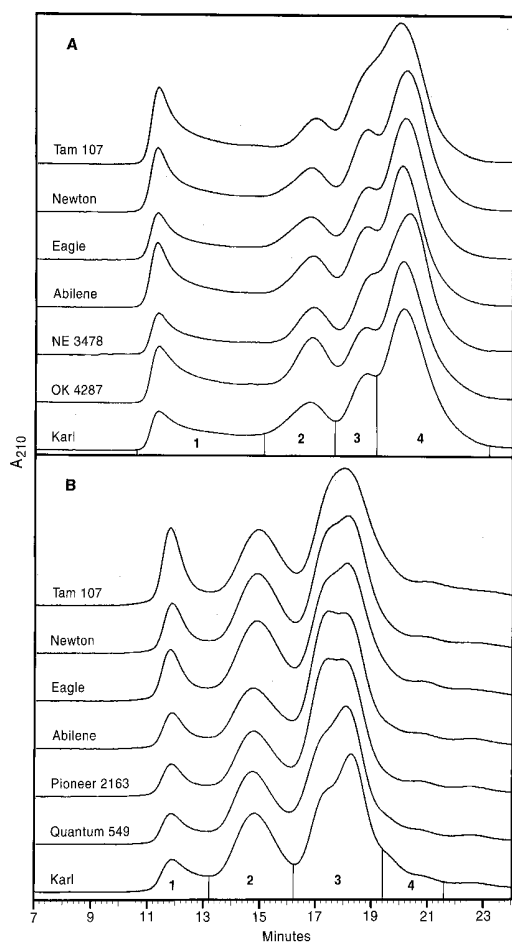


Fig. 1. Size-exclusion high-performance liquid chromatography (SE-HPLC) chromatograms (Superose 12 column, 1 \times 30 cm) of hard red winter wheat gliadins (A) and glutenins (B). Fractions 1-4 (A) correspond to ethanol-soluble glutenin subunits, ω -gliadins, γ -gliadins, and α - and β -gliadins (plus a small amount of albumins and globulins), respectively. Fractions 1-4 (B) are unreduced glutenin, reduced high molecular weight glutenin subunits (HMW-GS), reduced LMW-GS, and albumins and globulins, respectively.

0.5- μ m end filters and filled with Vydac reversed-phase packing. To enhance resolution, columns were maintained at 60°C with a CH-460 column heater (FIAtron Laboratory Systems, Oconomowoc, WI) (Bietz and Cobb 1985). Flow rates were 0.9 mL/min. Narrow-bore (2.1 mm i.d. \times 150 mm) Vydac C₄ and Zorbax C₈ columns, protected with 4- \times 12.5-mm guard columns (MAC-MOD Analytical, Chadds Ford, PA), were used with a P4000 solvent delivery system and an AS3000 autosampler (Thermo Separations Products, Fremont, CA). Columns were maintained at 60°C, and flow rates were generally 0.4 mL/min. Because 10- μ L samples extracted with high concentrations of ethanol may not be completely retained on small columns (Marchylo and Kruger 1988), 4.5- μ L aliquots of an extract from 50 mg of flour was used.

SE-HPLC

Gliadin and reduced glutenin fractions were analyzed by SE-HPLC on a 1- \times 30-cm Superose-12 column (Pharmacia LKB Biotechnology, Inc., Piscataway, NJ) with a 2.5- \times 25-mm guard column packed with prep-grade Superose-12. The same solvent delivery system and autosampler were used as for RP-HPLC on standard columns. Solvents were 45% acetonitrile (ACN) containing 0.095% TFA for gliadins, and 36% ACN with 0.12% TFA for glutenin subunits (Huebner et al 1994). (Since this work was completed, we found that solvents containing 0.095-0.12% TFA slowly degrade Superose-12 columns. While this column matrix can be cleaned even at pH 1.0, it should not be run for extended periods below pH 3.0.) Flow rate was 0.6 mL/min, and column temperature was 33°C. Proteins were detected at 210 nm (0.1 absorbance units full scale/10 mV) with a Schoeffel 770 UV monitor (Kratos, Ramsey, NJ).

Data Analyses

RP-HPLC and SE-HPLC data, expressed as voltage output of the UV monitor, were displayed on an Omniscrite recorder (Houston Inst., Austin, TX) and stored in a ModComp computer (Ft. Lauderdale, FL). Chromatogram peak areas were determined by integration and related statistically to baking quality factors using SAS (1989) software.

Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis (PAGE) was performed using pH 3.1 sodium lactate buffer (Khan et al 1985). A 6% polyacrylamide gel was produced as in Formulation C, except that catalyst (ascorbic acid and FeSO₄) concentrations were reduced by 20% to retard polymerization.

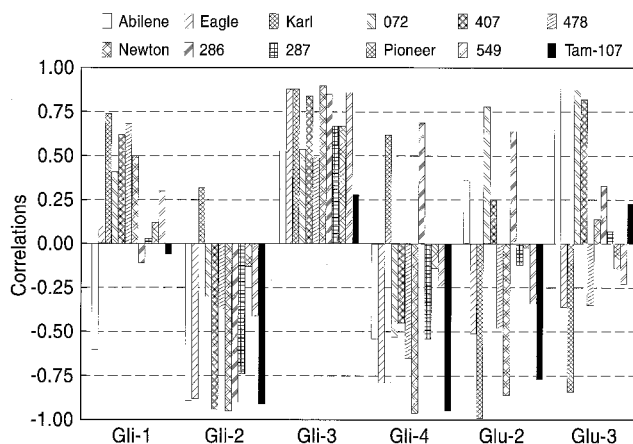


Fig. 2. Partial correlation coefficients between amounts of fractions of size-exclusion high-performance liquid chromatography and loaf volume for 12 wheat varieties grown at six locations.

RESULTS

SE-HPLC

Gliadin fractions 1–4 (Fig. 1A) correspond to ethanol-soluble glutenin subunits, ω -gliadins, γ -gliadins, and α - and β -gliadins (plus a small amount of albumins and globulins), respectively. Glutenin fractions 1–4 (Fig. 1B) are unreduced glutenin, reduced high molecular weight glutenin subunits (HMW-GS), reduced LMW-GS, and albumins and globulins, respectively. Areas of peaks were determined and statistically correlated (SAS 1989) with loaf volumes and other flour quality data.

Because actual percentage of HRW wheat flour protein correlates quite highly with loaf volume, amounts of most fractions also correlated positively with loaf volume (data not shown). However, no fraction gave a consistently high correlation for all varieties. We therefore examined the SE-HPLC data by partial correlation analysis, which eliminated the influence of total protein content on correlations between loaf volume and amounts of individual protein fractions (Fig. 2). For all cultivars, amounts of Gli-3 (γ -gliadin) correlated positively with loaf volume. Of the 12 samples examined, fractions Gli-2 (ω -gliadin) and Gli-4 (α - and β -gliadins) correlated negatively with loaf volume in samples 11 and 10, respectively. Correlations varied considerably among cultivars, however. For example, Gli-3 correlations with loaf volume ranged from 0.3 to >0.9 (Fig. 2). Low correlation values for some samples are most likely due to poor separation of γ -gliadins from α - plus β -gliadins by SE-HPLC (Fig. 1).

The amount of protein in different fractions was not highly correlated with other quality measurements, such as water absorption and mix time (data not shown). Thus such correlations from SE-HPLC data seem of little value. In part, this may be due to similar MW and incomplete chromatographic resolution of some fractions, such as Gli-3 (γ -gliadin) and Gli-4 (Tam 107 and NE 3478, Fig. 1A). Accurate integration of such data is also difficult and might contribute to poor correlations, as might the relatively low number (six) of samples of each variety. Multiple linear regression analysis of these data provided no improvement over simple and partial correlations.

RP-HPLC

While previous RP-HPLC studies did not reveal good correlations between specific gliadins and quality parameters, the correlations for γ -gliadins from SE-HPLC (Figs. 1–2) suggested the desirability of RP-HPLC analyses of γ -gliadins, which resolve

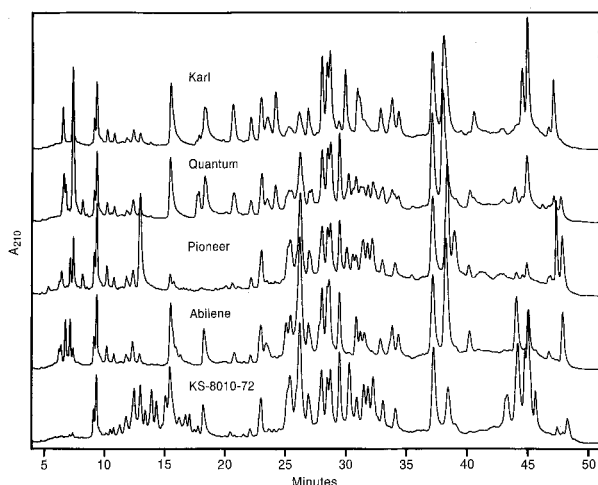


Fig. 3. Reversed-phase high-performance liquid chromatography (RP-HPLC) of gliadins from hard red winter wheats grown in Colorado. Eluting proteins were detected at 210 nm at 0.1 AUFS. Fractions were collected from Pioneer 2163 at 6–17 min; 22.5–30 min; 30–35 min; 36.5–39.5 min; and 41–49 min. Fractions from KS 8010-72 were collected at 8–19 min; 22.5–29.7 min; 30–35 min; 36.5–39.5 min; and 42–49 min.

well upon RP-HPLC (Huebner and Gaines 1992, Van Lonkhuijsen et al 1992). RP-HPLC results for gliadins from several wheat lines grown in Colorado are shown in Figure 3.

Previous studies (Bietz and Burnouf 1985) revealed general RP-HPLC elution characteristics of each type of gliadin, and suggested that γ -gliadins most likely elute at 36.5–39.5 min (Fig. 3). However, since elution patterns vary somewhat due to column type, solvent composition, and conditions used, we confirmed elution positions of γ -gliadins of selected lines by PAGE. Gliadin RP-HPLC fractions from Pioneer 2163 and KS 8010-72 (Fig. 3) were collected and combined from a series of runs. These lines were chosen because Pioneer 2163 has an extra peak at 39 min, and KS 8010-72 has a very small peak at 38.3 min plus additional peaks between 43 and 49 min. Collected fractions were lyophilized and analyzed by PAGE (Khan et al 1985) (Fig. 4).

Results in Figure 4 confirmed that fraction d from both samples is highly enriched in γ -gliadins (Woychik et al 1961). As expected, small amounts of proteins with mobilities corresponding to γ -gliadins also occur in adjacent fractions, and fraction d contained small amounts of faster migrating α - or β -gliadins. Thus, these results indicate that, regardless of compositional differences between varieties, the 36.5–39.5 min RP-HPLC range contains most γ -gliadins. This time range was, therefore, integrated in RP-HPLC data of all varieties to quantify γ -gliadins.

Loaf volume and γ -gliadin (36.5–39.5 min) areas from the same amounts of flour were highly correlated. The initial correlation

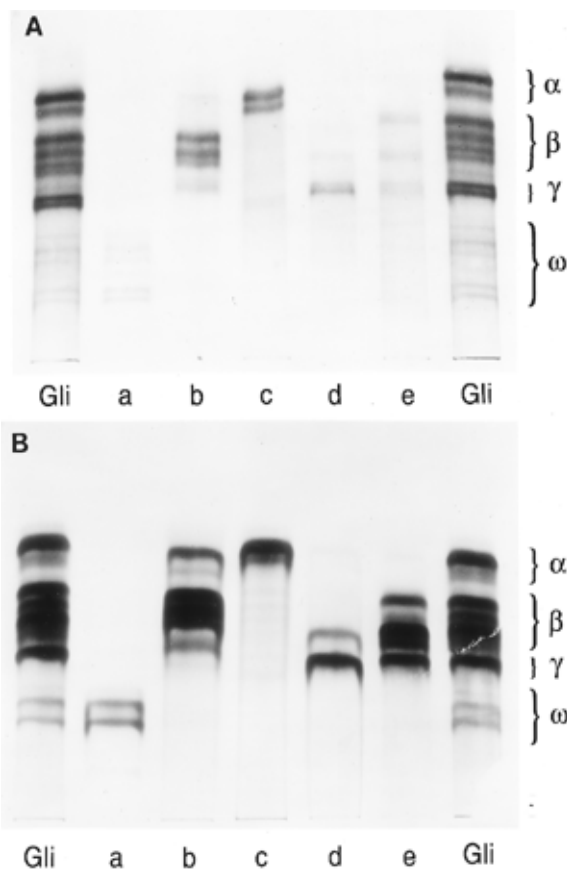


Fig. 4. Gel electrophoresis of gliadin reversed-phase high-performance liquid chromatography (RP-HPLC) fractions from Pioneer 2163 (A) and KS 8010-72 (B). Fractions in (A) correspond to the elution ranges 6–17 min (a); 22.5–30 min (b); 30–35 min (c); 36.5–39.5 min (d); and 41–49 min (e). Fractions in (B) correspond to the elution ranges 8–19 min (a); 22.5–29.7 min (b); 30–35 min (c); 36.5–39.5 min (d); and 42–49 min (e). Gli = whole gliadin extracts.

coefficients were as high as 0.97 from single analyses. Possible system errors were reduced by averaging results of two or more analyses.

We also examined the relationship between percentage of ethanol used for extraction and the consistency of the data. In most previous studies, gliadins have been extracted with 70% ethanol. We found that correlation coefficients using 75% ethanol extracts were slightly higher and more consistent than those with 60, 70, or 80% ethanol. Accordingly, we routinely used 75% ethanol.

To decrease analysis time, we tested a narrow-bore (Zorbax SB C₈ 2.1 × 150 mm) column, using a multistep gradient with a steep initial slope, followed by a shallower slope when γ -gliadins elute. The gradient began at 25% ACN, increased to 31.7% ACN at 1 min, to 38.4% ACN at 5 min, to 44% ACN at 14 min, and to 46.5% ACN at 15 min. These conditions were maintained for 1 min, and the column was then reequilibrated with initial solvent for 6 min. Flow rate was 0.4 mL/min. This gradient improved resolution of γ -gliadins, decreased analysis time to 23 min/sample, and used only one-sixth as much solvent. Using these conditions, more than 62 analyses could be performed per day. A Vydac C₄ column gave similar results.

Correlation coefficients between amounts of γ -gliadins and loaf volume for each wheat line, across multiple locations, revealed by standard (4.6 mm i.d. column, 55 min) and narrow-bore (2.1 mm i.d. column, 23 min) RP-HPLC, are listed in Table I. The average correlation coefficient was higher for results from the 2.1-mm column (0.97) than for those obtained with the standard column (0.93). This allows increased output and decreased solvent use while still yielding excellent results. Correlation coefficients were also more uniform using the 2.1-mm column. Most significant, however, is that these data show a high correlation between amount of γ -gliadins and loaf volume among many varieties. With six samples $P < 0.05$ for $r > 0.811$ and $P < 0.01$ for $r > 0.917$.

It is interesting to note how much the percentage of γ -gliadins can vary with location of growth as well as with genotype. Figure 5 shows that environmental factors at specific locations may change protein synthesis of all HRW varieties in comparison to other locations. Similar results were previously noted for HRS wheat protein fractions (Huebner et al 1994). Perhaps more interesting is that these data reveal varieties that differ in response, or lack of response, to environmental factors. This is best shown by lines for varieties "crossing over" in Figure 5. Such differential quantitative responses, as revealed by RP-HPLC of storage proteins, may be useful during breeding for selecting varieties either responsive or relatively insensitive to effects of environment.

While this information shows an excellent correlation of the actual amount of γ -gliadins to loaf volumes for each variety grown at different locations, it would not be of much practical use unless

it can be correlated with other varieties grown at one location. This has been done for the 12 varieties grown at three different locations (Fig. 6). While 11 varieties are close to a line for that location and give good correlations to loaf volumes, the KS-8010-72 is consistently off to the side and, when included with the other 11 samples, significantly reduces the correlations. For example, at Hutchinson, KS, the correlation decreased from 0.93 to 0.61 with KS-8010-72 included). This then would make it possible to reject that sample as not suitable for release. In fact, this line was dropped from breeding programs because it exhibited poor quality in a subsequent year. Recognition of the unusually low amount of γ -gliadins in this genotype, possible because of the small quantity of sample needed for analyses, might have indicated its unsuitability for release one or two years earlier during the breeding program. This could reduce the time, effort, and expense in developing new varieties.

HRS Wheats

We also attempted to determine whether the observed relationship between γ -gliadin amount and loaf volume were valid for HRS as well as HRW wheats. Five HRS cultivars, grown at up to

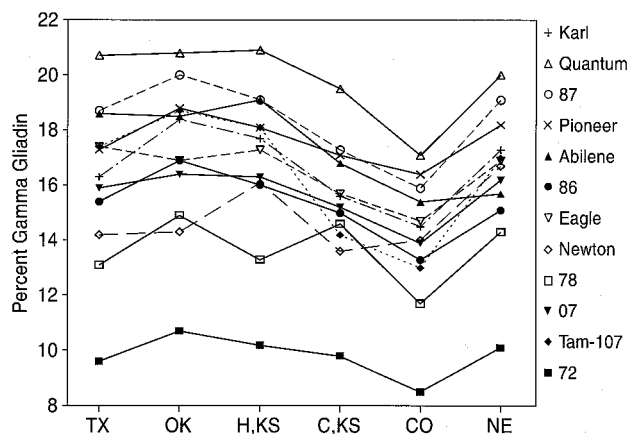


Fig. 5. Average percentages of γ -gliadins, extracted with 75% ethanol and analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC), in wheat varieties grown at six locations. TX = Texas; OK = Oklahoma; H,K,S = Hutchinson, Kansas; C,K,S = Colby, Kansas; CO = Colorado; and NE = Nebraska.

TABLE I
Correlation Coefficients Between Amounts of γ -Gliadins as Determined by Standard and Narrowbore Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) and Loaf Volume^a

Wheat	4.6-mm Column	2.1-mm Column
Karl	0.83	0.96
Quantum 547	0.997	0.99
OK 84287	0.93	0.97
Pioneer 2163	0.95	0.93
KS 8010-72	0.93	0.93
Abilene	0.97	0.94
OK 84286	0.92	0.95
Eagle	0.98	0.99
Newton	0.90	0.98
NE 83478	0.97	0.96
NE 83407	0.91	0.97
Tam 107	0.97	0.95

^a Average of two to five analyses, determined by integration of peaks from RP-HPLC elution profiles ($n = 6$).

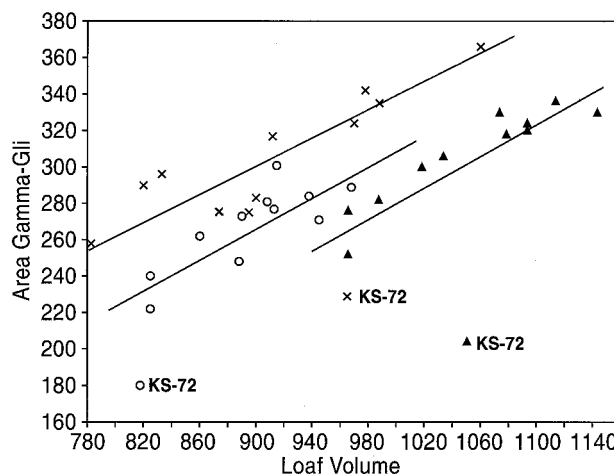


Fig. 6. Data points for 12 wheat varieties grown in Texas, Hutchinson, KS, and Colorado. Points were determined by plotting the area under the curve for the γ -gliadins (36.5–39.5 min) against the loaf volume for each variety. Only the data points for the variety KS-8010-72 are identified.

nine locations for which we had loaf volumes and other baking data, were compared. Most correlation coefficients (≈ 0.6 – 0.8 ; data not shown) were lower than for HRW wheats. For samples from one uniform nursery, however, a correlation of 0.86 was obtained.

DISCUSSION

Finney (1985) showed that loaf volume increases as protein content increases for a given cultivar. However, the relationship may not be completely linear. Our results indicate that amounts of γ -gliadins in hard wheats do not increase proportionately to other gliadins. As total protein increases, γ -gliadins also increase, but not in direct proportion to the other gliadins, so that the relative percentage of γ -gliadins is actually lower when related to the total gliadins. Except for two varieties (Abilene and Eagle), the γ -gliadins increased in area by an average of 6.75 units per each percentage point increase in protein, while the other gliadins increased at an average rate of 41.6 units per percentage point increase in protein.

The increases are averages of slopes derived from regression analysis and had a range in standard errors of 1.55 – 4.37 for the γ -gliadins and 3.81 – 20.04 for the other gliadins. The slopes for the γ -gliadins tended to be individually significant ($P < 0.10$) and the slopes for other gliadins were all individually significant ($P < 0.05$). The slope of γ -gliadin was 5.11 ± 1.44 ($P < 0.05$) for Abilene and 4.22 ± 1.95 ($P < 0.10$) for Eagle. For the other gliadins, however, the slopes were -8.91 ± 9.71 ($P < 0.40$) for Abilene and -19.14 ± 12.83 ($P < 0.20$) for Eagle. We do not know why these two varieties were different.

We are still challenged to identify wheat polypeptide determinants of loaf volume and mixing times. Many studies suggest that glutenin explains most intercultivar variation in loaf volume: it is mainly responsible for the protein network that entrains air bubbles during dough fermentation, leading to a light, porous texture. HMW-GS are especially important (Payne et al 1987), but explain only part of the variation in wheat end-use quality. Indeed, one seminal study shows that much of the variation in wheat quality can be explained by certain gliadins (Van Lonkhuijsen et al 1992) when glutenin subunits are the same.

Several studies have shown that γ -gliadins differ significantly from other gliadins in molecular size and sequence, suggesting that they have uniquely different properties. Indeed, γ -gliadins are also coded by genes at the complex Gli-1 loci on chromosomes 1 (as are ω -gliadins and LMW-GS), while α - and β -gliadins are controlled by genes at the Gli-2 loci on the short arms of the group 6 chromosomes (Payne et al 1984).

At least some γ -gliadin-like polypeptides are incorporated through intermolecular disulfide bonding into glutenin. This possibility was first suggested through sequence analyses of LMW, ethanol-soluble glutenin subunits (Bietz and Wall 1980). Subsequently, several studies have demonstrated the occurrence of γ -gliadin-like subunits in glutenin (Lew et al 1992, Köhler et al 1993, Masci et al 1995, Keck et al 1995). These studies, along with the close proximity on chromosome 1 of genes coding γ -gliadins and LMW-GS, may, in part, explain the correlations found between loaf volume and quantity of γ -gliadins. Indeed, Benedettelli et al (1992) stated that the close linkage between gliadin and LMW-GS genes at the Gli-D1/Glu-D3 loci makes it difficult to define precisely which groups of proteins are major determinants of breadmaking properties.

Thus, it may be no coincidence that both the amount of LMW-GS, as determined by SE-HPLC, and the amount of γ -gliadin, as determined by RP-HPLC, correlated well with loaf volume. Another line of evidence suggesting such a relationship is that LMW-GS have RP-HPLC elution characteristics similar to those of γ -gliadins (Huebner and Bietz 1993). Similarly, components negatively associated with baking quality elute near γ -gliadins upon RP-HPLC (Huebner 1989).

An important area of investigation in recent years has been exploring differences between U.S. HRW and HRS wheat classes. Indeed, some relationships that appear to differentiate these classes have been identified (Bietz et al 1991, Huebner et al 1991, Bietz and Schmalzried 1995). Other studies, however, show that diversity among varieties within classes may be greater than the average difference between these classes (Huebner et al 1994). The fact that the present study shows different correlations between loaf volume and γ -gliadin amounts for HRS and HRW wheats suggests a further subtle difference between these classes. Additional studies of HRS and HRW wheats are necessary to confirm and extend these observations.

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