

Differences Among Gliadins from Spring and Winter Wheat Cultivars¹

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ABSTRACT

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Accurate and reliable classification of U.S. hard red spring (HRS) and hard red winter (HRW) wheats is necessary in marketing. Such classification is difficult because kernels from many of the cultivars that are grown, even of different classes, may have similar phenotypes. Some studies suggest that HRS wheats contain more of the most hydrophobic gliadins than do HRW wheats. We used reversed-phase high-performance liquid chromatography to quantitatively analyze gliadins from HRS and HRW wheats. Diversity among cultivars was large, but statistical analyses revealed significant differences for certain gliadins. Unlike previous reports,

however, we observed no difference in amount of the latest eluting gliadins between HRS and HRW wheats. When HRS wheats were grown in California and Arizona as winter wheats, the total protein content was lower, and their gliadin compositions resembled those of the HRW wheats grown in normal HRW environments. These results suggest that quantitative differences in protein compositions of HRS and HRW wheats are primarily due to genetic and environmental, rather than to true class-related differences.

The wide variation in climate in the United States permits five main wheat classes to be grown. Two of these are hard red spring (HRS) and hard red winter (HRW) wheat classes. Many cultivars of a class are generally grown in the same region, but cultivars are generally not segregated during marketing. Because wheat classification depends on varietal identification, it may thus be difficult for grain inspectors to correctly identify wheats. This may be especially true for hard, high-protein spring and winter wheats.

Because of these considerations, finding new classification methods that are simple and reliable is highly important to the U.S. Federal Grain Inspection Service (FGIS). Many FGIS and ARS researchers have been trying to identify objective class-specific characteristics useful for grading. As part of this mission, we have examined HRS and HRW wheats in search of specific proteins that may differentiate wheat classes. Our approaches were based on knowledge that storage protein compositions, as analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC) (Bietz 1983, Burnouf et al 1983), are unique for each cultivar. Also, there have been reports that spring wheats may contain more of the most hydrophobic gliadins (that elute last upon RP-HPLC) than do winter wheats (Huebner 1989, Endo et al 1990).

As part of a search for characteristic differences among wheat classes, HRS and HRW wheats were grown over a wide U.S. geographical area. Some spring wheats were also grown as winter wheats in a typical winter wheat habitat (California). We examined these wheats in search of proteins specific to each class. The major wheat storage proteins, gliadins, were extracted and characterized by RP-HPLC. To interpret the resulting data, chromatograms were combined into patterns representative of each wheat class, and were statistically evaluated. We here present the results of this study.

MATERIALS AND METHODS

Wheat Samples

Most HRS and HRW wheats used were collected by FGIS personnel at grain elevators during unloading. Samples were milled at the USDA-ARS Wheat Quality Laboratory, Fargo, ND, divided, and sent to cooperating laboratories for analysis (e.g., percent nitrogen, milling characteristics, mixing times, rheology, water absorbance, baking characteristics, and gel electrophoresis). Other spring wheats were from the Fargo laboratory, and some winter wheats were obtained directly from breeders. Winter wheats (56 samples) were grown in Nebraska, Kansas, Oklahoma, Texas, Idaho, and Montana. Spring wheats (63 samples) were from Minnesota, North Dakota, Montana, Idaho, Utah, and California.

Other wheat kernel samples from breeders (generally 10 kernels) were pulverized using a Wig-L-Bug (Crescent Dental, Lyons, IL) (Huebner et al 1990, Kubiczek et al 1993).

Protein Extraction

Flour or pulverized kernel samples (60 mg) were extracted by 30 min of vortexing at room temperature with 1.5 ml of 70% ethanol in 10-ml polypropylene tubes. Extracted gliadins were clarified by centrifugation (8,850 × g for 10 min at 25 °C) before analysis.

RP-HPLC

Samples were analyzed by RP-HPLC (Huebner and Bietz 1986) using a Spectra-Physics (San Jose, CA) apparatus including an SP8700 solvent delivery system and an SP8780XR autosampler (Huebner and Bietz 1987). A Vydac (Separations Group, Hesperia, CA) C4 column (150- × 4.6-mm, 5-μm particle size, 300A pore size) was used, preceded by a 20- × 2-mm guard column (C-130B, Upchurch, Oak Harbor, WA) containing 0.5-μm end filters and filled with Vydac reversed-phase packing. To enhance resolution (Bietz and Cobb 1985), the column was maintained at 60 °C with a CH-460 column heater (FIAtron Laboratory Systems, Oconomowoc, WI).

Acetonitrile (ACN) and trifluoroacetic acid (TFA) were HPLC grade. Distilled water was further purified with a Barnstead NANOpure system. RP-HPLC solvent A contained 10% ACN and solvent B contained 90% ACN; solvents also contained ~0.1% TFA (slightly more in solvent A and slightly less in B to give a flat baseline [Huebner and Bietz 1987]). Solvents were filtered through a 0.45-μm Millipore HVLP filter, deaerated under vacuum, and sparged with helium during analysis. Samples of 15 μl were analyzed at a flow rate of 0.9 ml/min. The column was equilibrated with 17% solvent B and eluted with a gradient that increased to 24% B at 3 min and then to 47% B at 50 min, where it was held for 4 min before being returned to 17% B for 10 min to

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Mentioning product names is necessary to factually report data. The USDA neither guarantees nor warrants the standard of cited products, however. Mention of brand names does not imply approval of products to the exclusion of others that may also be suitable.

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re-equilibrate the column. Proteins were detected at 210 nm (0.1 absorbance units per 10 mV full scale) with a SF770 Spectroflow monitor (Kratos, Ramsey, NJ) (Huebner and Bietz 1987).

Data Analysis

Data were recorded on an Omniscrite recorder (Houston Instruments, Austin, TX) and stored in a ModComp (Fort Lauderdale, FL) computer system for subsequent plotting and calculation.

RESULTS

Figure 1 shows RP-HPLC chromatograms of gliadins from representative HRS and HRW wheats. While each cultivar contains some gliadins that elute in corresponding positions, there are also unique differences between each cultivar.

Figure 2 compares results of overlaying six typical HRS and seven HRW gliadin chromatograms. There is much similarity within each class in retention times of major protein peaks. At specific elution times, all cultivars within each class appear to either have or to lack protein peaks. Some major proteins, such as those between 43–45 min (previously shown to be γ -gliadins [Van Lonkhuijsen et al 1992]), are present in all cultivars. Also, at 17–18, 34–35 and 38–39 min, most cultivars contain relatively large protein peaks. Peaks from 48–58 min are those we refer to as the highly hydrophobic late-eluting gliadins. Previous studies (Huebner and Bietz 1986, Huebner 1989, Endo et al 1990) suggested that HRS wheats may contain more of these proteins than do HRW wheats.

Visual examination of these overlaid chromatograms appears to suggest some class-related differences in protein composition. Such comparisons are difficult to make and are subjective, however they become even more complex when the entire data set is considered; thus, a better method was needed. In search of such a method, we calculated the area under the curve of all chromatograms in 1-min segments. Results for 43 HRS and for 36 HRW cultivars were combined into two new averaged chromatograms (not shown). In these averaged chromatograms, certain areas of one class or the other have more protein. On an average for this set of samples, differences were found at 20, 21, 24, 25, 29, 34, 38–40, 43, 51, and 53–54 min. There is as much or more variation among cultivars within each class, as there is between the averaged HRS and HRW data sets. Thus, relevance of the noted differences between classes appears minimal.

The above data represent amounts of protein from equivalent amounts of grain, but HRS wheats typically contain 1–2% more

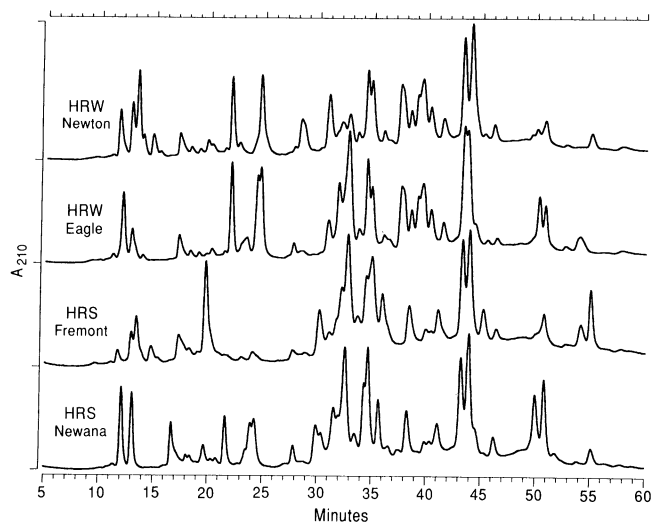


Fig. 1. Reversed-phase high-performance liquid chromatography of ethanol extracts of two hard red winter (HRW) and two hard red spring (HRS) wheats. Analytical conditions are as described in Materials and Methods.

protein than do HRW wheats. Therefore, peaks should be larger for the HRS wheats. To learn whether this might have affected our results, we also compared averaged chromatograms representing percentages, rather than absolute amounts, of protein in each 1-min segment (Fig. 3). For the 43 spring and 36 winter wheats shown in Figure 3, the percent areas (of the total) at each minute were compared between HRW and HRS wheats by *t*-tests at $P < 0.01$. At 20, 24, 33–34, and 51–54 min, the HRS percent area was significantly larger than that of the HRW cultivars. At 25, 29, and 35–40 min, the HRW percent area was larger.

Many wheats we examined were from widely different areas, so we also searched for location effects among HRS and HRW wheat classes. Figure 4 shows results for HRW wheats grown in Kansas, Nebraska, and the Northwestern states, and for HRS wheats from Minnesota, California, and the Northwestern states. For each location, 12 cultivars were compared, except for the NW HRS wheats, for which only seven cultivars were available. These data, whether expressed as absolute areas (protein amounts) (data not shown) or as relative percentages (Fig. 4) (to eliminate apparent compositional differences due to protein amounts), clearly show larger differences due to location of growth than between classes (Fig. 3). For spring wheats, those from California

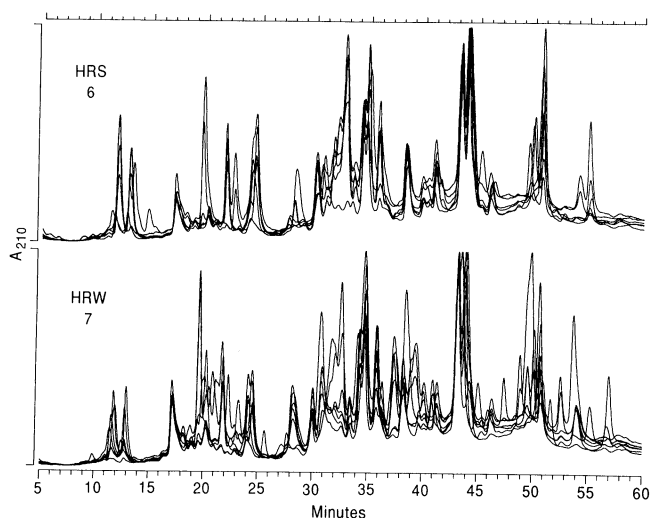


Fig. 2. Overlaid reversed-phase high-performance liquid chromatograms of 6 hard red spring (HRS) and 7 hard red winter (HRW) wheats. Analytical conditions are as described in Materials and Methods.

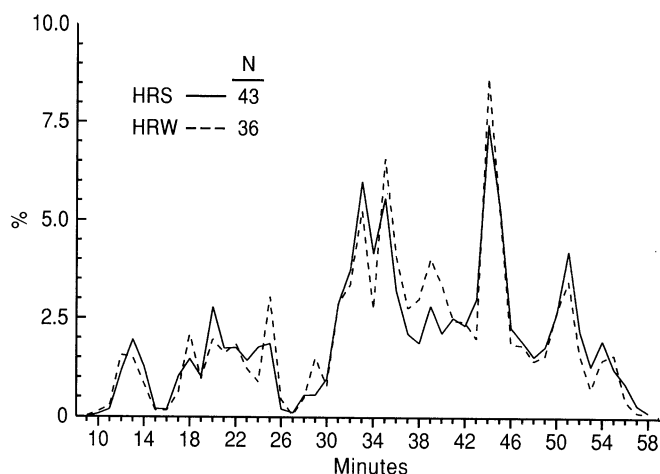


Fig. 3. Averaged reversed-phase high-performance liquid chromatography data for 43 hard red spring (HRS) and for 36 hard red winter (HRW) wheats. Individual chromatograms were divided in 1-min intervals. Combined areas for all cultivars of each class were divided by the number of samples (*N*) analyzed. Averaged data was then expressed as percentage of total area within each segment.

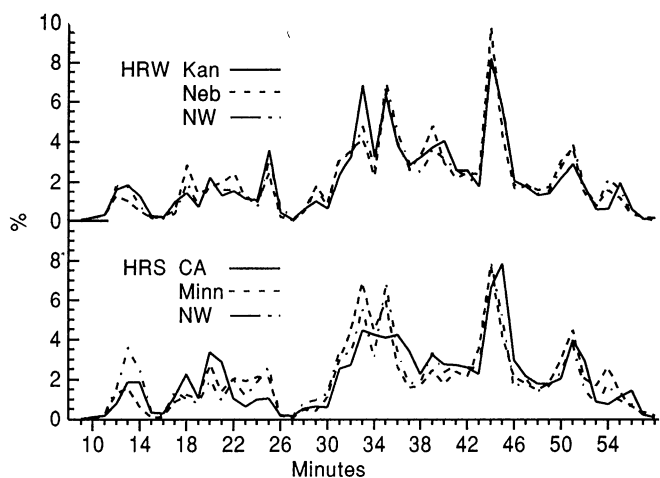


Fig. 4. Averaged gliadin reversed-phase high-performance liquid chromatography data, expressed on a relative (%) basis, of hard red winter (HRW) wheats grown in Kansas, Nebraska, and the northwestern states, and of hard red spring (HRS) wheats from Minnesota, California, and the northwestern states.

appear most different, as noted previously (Huebner 1989). For winter wheats, those from Kansas least resemble each other. These differences are most apparent when examining protein amounts (data not shown), which relate to the generally lower and higher protein contents of California and Kansas wheats, respectively. California wheats vary from 10–13% protein, while Kansas and NW wheats have 12.5–15.5% protein (data from the wheat quality laboratories at Fargo, ND, and Manhattan, KS). Differences are also readily apparent when data are compared on a relative basis (Fig. 4), showing that growing conditions significantly affect both protein composition and amount.

In part, such differences may be due to the cultivars grown in each area. Because these data represent many samples, we believe that most of the differences apparent in Figure 4 are due to growing location and to climatic differences. Graybosch (1994) and Huebner and Bietz (1994) previously reached a similar conclusion.

In one final study, the same 12 spring wheat cultivars were grown at two locations (Crookston and Morris, MN). Morris is ~160 miles south of Crookston. Gliadin compositions of wheats from these sites were only slightly different (results not shown), as might be expected as the locations were not far apart. These results suggest that protein compositions, and probably qualities, of wheat cultivars grown throughout a given region, especially their normal range of adaptation, remain fairly constant.

DISCUSSION

Our RP-HPLC data did not reveal any consistent difference between gliadin compositions of HRS and HRW wheat classes. This conclusion was reached by comparing HPLC data averaged

for many cultivars and samples of each class. Because gliadins provide reliable genetic “fingerprints” of genotypes, we might theorize that genetic differences between HRS and HRW wheats are insignificant; however, protein contents of these classes, which are influenced mostly by environment, are different.

Our results clearly show and confirm that total amounts of gliadins, as well as relative amounts of specific gliadins, vary due to location of growth or climatic conditions. Different cultivars may also respond differently to environment (Huebner and Bietz 1994). Results confirm the observation that samples of a cultivar from different locations may vary more in protein composition than samples of different cultivars of one class from a single site.

Our results do not confirm previous observations that HRS wheats (as a class) contain greater amounts of hydrophobic gliadins than do HRW wheats (Huebner and Bietz 1986, Huebner 1989, Endo et al 1990). We can only speculate that such a relationship, while valid for some cultivars, does not hold when a broader range of genetic and environmental diversity is present.

LITERATURE CITED

- BIETZ, J. A. 1983. Separation of cereal proteins by reversed-phase high-performance liquid chromatography. *J. Chromatogr.* 255:219.
- BIETZ, J. A., and COBB, L. A. 1985. Improved procedures for rapid wheat varietal identification by reversed-phase high-performance liquid chromatography of gliadin. *Cereal Chem.* 62:332.
- BURNOUF, T., BIETZ, J. A., COBB, L. A., and WALL, J. S. 1983. Chromatographie liquide haute performance des gliadines de variétés de blé tendre (*Triticum aestivum* L.) cultivées en France et possibilités d'identification variétale. *C. R. Acad. Sc. Paris* 297:377.
- ENDO, S., OKADA, K., NAGAO, S., and D'APPOLONIA. 1990. Quality characteristics of hard red spring and winter wheats. I. Differentiation by reversed-phase high-performance liquid chromatography and milling properties. *Cereal Chem.* 67:480.
- GRAYBOSCH, R. 1994. Environmental modification of gluten protein composition in North American hard red winter wheats. Pages 280–291 in: *Gluten Proteins 1993*. Association of Cereal Research: Detmold.
- HUEBNER, F. R. 1989. Assessment of potential breadmaking quality of hard spring wheats by high-performance liquid chromatography of gliadins—Year two. *Cereal Chem.* 66:333.
- HUEBNER, F. R., and BIETZ, J. A. 1986. Assessment of the potential breadmaking quality of hard wheats by reversed-phase high-performance liquid chromatography of gliadins. *J. Cereal Sci.* 4:379.
- HUEBNER, F. R., and BIETZ, J. A. 1987. Improvements in wheat protein analysis and quality prediction by reversed-phase high-performance liquid chromatography. *Cereal Chem.* 64:15.
- HUEBNER, F. R., and BIETZ, J. A. 1994. Quantitative differences among hard wheat proteins as related to environment, quality, and class. Pages 468–476 in: *Gluten Proteins 1993*. Association of Cereal Research: Detmold.
- HUEBNER, F. R., BIETZ, J. A., WEBB, B. D., and JULIANO, B. O. 1990. Rice cultivar identification by high-performance liquid chromatography of endosperm proteins. *Cereal Chem.* 67:129.
- KUBICZEK, R. P., HUEBNER, F. R., and BIETZ, J. A. 1993. Reversed-phase high-performance liquid chromatography of secalins: Application to Rye cultivar identification. *J. Cereal Sci.* 17:191.
- VAN LONKHUIJSEN, H. J., HAMER, R. J., and SCHREUDER, C. 1992. Influence of specific gliadins on the breadmaking quality of wheat. *Cereal Chem.* 69:174.

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