Quantitative Variation Among Gliadins of Wheats Grown in Different Environments

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ABSTRACT

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Reversed-phase high-performance liquid chromatography can quickly and accurately analyze small flour samples, permitting analysis of grain characteristics related to functional properties. A shortage of soil sulfur decreases wheat yield and flour quality. We therefore used reversed-phase high-performance liquid chromatography to examine quantitative differences among gliadins from the wheat varieties Shortim, Egret, and Olympic grown with different levels of sulfur fertilizer. We also examined gliadins from the varieties Butte and Coteau from a normal range of environments to better define quantitative variability within a genotype. For wheats grown at different sulfur fertilization levels, no qualitative differences among gliadins were apparent, but major quantitative variation exists, particularly for early-eluting, sulfur-poor ω-gliadins. Significant quantitative variation also exists for gliadins from different locations, suggesting a major role of weather or other environmental factors during kernel development on gliadin synthesis. This level of quantitative environmental variability is not apparent by other procedures, and may influence varietal identification through gliadin analysis.

Wheat grown on sulfur-poor soil may have reduced yield and decreased mixing and baking quality (Archer 1974, Byers and Bolton 1979, Randall et al 1981, Moss et al 1983). Amounts of cysteine and cysteine-containing proteins also decrease under conditions of low soil sulfur (Wrigley et al 1980, Moss et al 1981). Baking quality may also relate to levels of sulfur and other minerals, such as copper, potassium, and molybdenum (Douglas and Dyson 1985).

In the United States, various soils also may have low sulfur contents (Harder and Thiessen 1971, Mahler and Maples 1986, Rasmussen and Allmaras 1986). Crops besides wheat may yield poorly on such soils. Some sulfur accumulates in the soil via acid rain, resulting from burning high-sulfur fuels; because of environmental problems, however, such fuels are now being banned. This may further reduce levels of sulfur in already sulfur-deficient soils. Where soil sulfur levels are marginal, heavy addition of other fertilizers and increased crop yields also demand higher sulfur levels in soils.

Individual wheat varieties may also vary significantly in quality and functional properties from year to year, or when grown in different environments (McGuire and McNeal 1974, Baenziger et al 1985). Without simple, sensitive, accurate quantitative tests, reasons for such quality differences are not easily determined. Such tests are now being developed and used to determine sulfur contents of commodities grown on sulfur-poor soil (Skerritt and Martinuzzi 1986), to examine the influence of other conditions on quality, and to attempt to explain resulting compositional differences.

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Significant changes in percentages of high- and low-sulfur wheat proteins are apparent by electrophoresis of proteins from wheats grown on soils varying in levels of available sulfur (0–50 kg/ha of sulfur added) (Wrigley et al 1980; Moss et al 1981; Wrigley et al 1984a,b). In 1985, Lookhart and Pomeranz used electrophoresis and high-performance liquid chromatography (HPLC) to examine the relationship of gliadin composition to soil sulfur levels; however, no quantitative data were determined. Using reversed-phase (RP)-HPLC, we examined in more detail the qualitative and quantitative differences among gliadins from wheats grown on soils containing varying quantities of sulfur. We also compared gliadins from wheats from different environments, where the sulfur content of the soil was unknown. Variation in protein composition relates to soil fertility and also indicates the normal range of quantitative variability for a variety grown in different environments.

MATERIALS AND METHODS

Wheat Samples

Hard red spring (HRS) wheats and flours were from the USDA Spring and Durum Wheat Quality Laboratory, Fargo, ND. The cultivars Butte and Coteau were grown in 1984 at Carrington, Dickinson, Fargo, Hettinger, Langdon, Minot, and Williston, ND. Information concerning weather conditions (air and ground temperature, wind, rainfall, and sky conditions) and level of fertilizer added was obtained for each location. Flour samples were previously analyzed for 20 diverse characteristics, ranging from kernel size to loaf volume (Nolte et al 1985). Flours from Australian varieties (Olympic, Shortim, and Egret) were previously described (Moss et al 1983).

Protein Extraction

Flour samples (60 mg) were extracted at room temperature in 10-ml capped polypropylene centrifuge tubes with 1.5 ml of 70% (v/v) aqueous ethanol (30 min of continuous agitation on a Buchler Vortex-Evaporator [Buchler Insts., Fort Lee, NJ]). Extracts were centrifuged (15 min at 15,000 rpm [20,000 g]) in a Beckman L8-70M centrifuge (Beckman Instruments, Fullerton, CA) in a type 40 rotor (Bietz 1983, Bietz et al 1984).

RP-HPLC

RP-HPLC was performed on a Spectra-Physics (San Jose, CA) apparatus including a SP8700 solvent delivery system and SP8780XR autosampler. Proteins were detected with a SF770 Spectroflow monitor (Kratos, Ramsey, NJ). Vydac C_{18} and C_4 columns (250 × 4.1 mm; Separations Group, Hesperia, CA) and a Bio-Rad C_{18} column (250 × 4.1 mm; Bio-Rad, Richmond, CA) were used, with a 22 × 3.5-mm guard column of SynChrom RSC packing and a 0.5-μm in-line prefilter (A-103, Upchurch, Oak Harbor, WA). Eluted proteins were detected at 210 nm at 0.1 absorbance units full scale/10 mV.

Acetonitrile and trifluoroacetic acid were HPLC grade; water was purified with a Barnstead Nanopure system. Solvents were deaerated by vacuum filtration through a 0.45-μm filter and sparged with helium during use (Huebner and Bietz 1987).

Samples of 10–15 μl were analyzed at 1.0 ml/min on columns maintained at 60°C with a CH-20-C column heater (Scientific Systems, State College, PA). Proteins eluted during linear gradients of 27–45% acetonitrile (± 3%, depending on column used) during 40 min, plus 5 min at the final concentration.

Analysis of Data

Data were recorded on an Omniscribe recorder (Houston Instruments, Austin, TX), and stored in a ModComp computer system (Ft. Lauderdale, FL). Stored chromatograms were displayed on a Tektronix (Beaverton, OR) video terminal, and could be replotted to any convenient scale or normalized (as in data presented in the figures). Data from duplicate runs were integrated by an interactive program (MANCPC) in which peaks are defined by cursor position, and by an automatic program (TIMCPC), which compares chromatograms by integrating between specified time limits. Both programs can correct for baseline absorbance shifts.

RESULTS

Analysis of Wheats Grown at Different Sulfur Fertilization Levels

Gliadins from Australian wheats grown under three soil sulfur concentrations, ranging from more than adequate (50 kg S/ha) to very deficient (no S added) (Moss et al 1983), were analyzed by RP-HPLC. There were major quantitative differences for specific early- (7–22.5 min) and late-eluting (31.5–54 min) gliadins (fractions 1 and 3 in Fig. 1). Differences are emphasized in computer-generated difference plots (Fig. 1, lower), where positive peaks indicate proteins synthesized preferentially at low sulfur levels, whereas proteins enhanced by high soil sulfur give negative peaks. These differences are also readily apparent upon integration, which reveals significant variation for percentages of fractions 1 and 3 with applied sulfur, whereas fraction 2 remains relatively constant (Fig. 2). Similar results (data not shown) were obtained for the varieties Shortim and Olympic (Moss et al 1983).

To verify accuracy of this method and apparatus, four flour samples from one variety (Butte) were extracted, and each was analyzed four times (total 16 runs). Maximum variation between the runs for fractions 1 and 2, expressed as percentage of total protein, was no greater than ±0.4%, and for fraction 3, ±0.3%.

The early-eluting gliadins preferentially synthesized at low soil sulfur levels are probably ω-gliadins, which have low surface hydrophobicities and typically elute earlier than other gliadins

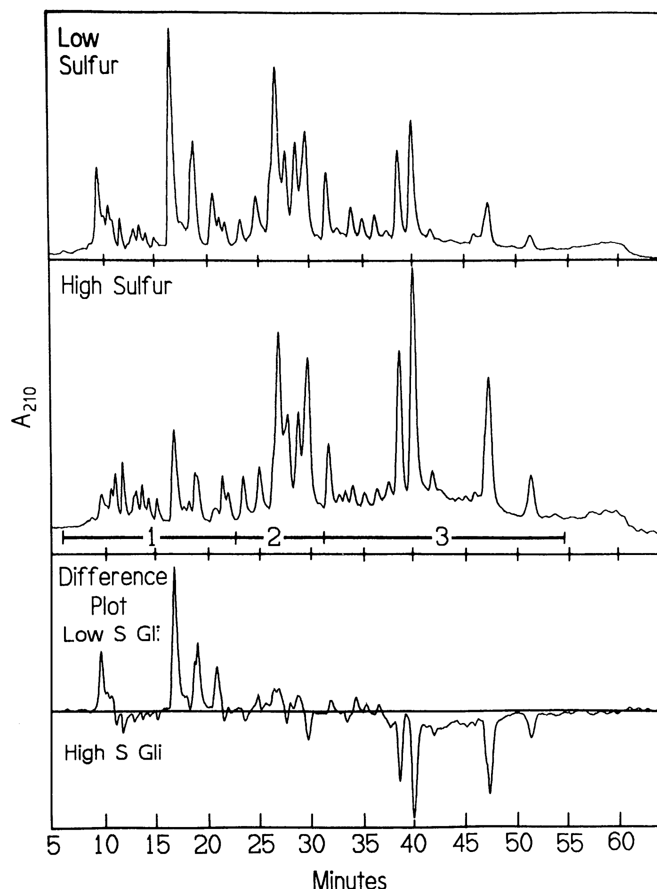


Fig. 1. Reversed-phase high-performance liquid chromatography comparison of gliadins from wheat (cultivar Egret) grown on low- and high-sulfur soils. Proteins were eluted from a Vydac C_{18} column during 40 min at 60°C with a linear gradient from 28 to 46% acetonitrile. Solvent A consisted of 0.06% trifluoroacetic acid in water, whereas solvent B was 0.045% trifluoroacetic acid in acetonitrile. Chromatograms were divided into fractions 1–3 as indicated. The difference plot is on the same scale as the absorption for the chromatograms, from which it was derived.

upon RP-HPLC (Bietz 1983, Bietz and Burnouf 1985). Electrophoresis has also shown that ω-gliadins, which lack sulfur amino acids (Booth and Ewart 1969), are preferentially synthesized at low soil sulfur levels (Wrigley et al 1980; Moss et al 1981; Wrigley et al 1984a,b). Lookhart and Pomeranz (1985) achieved qualitatively similar results using RP-HPLC. Our results thus confirm previous studies, while providing improved quantitative estimates of ω- and sulfur-rich (α, β, and γ) gliadins synthesized on soils varying in sulfur fertilization levels.

Location Effects on Gliadin Composition

Gliadin compositions of individual wheat varieties are, qualitatively, nearly constant, irrespective of conditions or location of growth. This uniformity permits varietal identification by electrophoresis (Wrigley et al 1982). Our study, and those cited by others, also clearly show quantitative differences among wheats grown under different environmental conditions, however. Furthermore, the quality of a cultivar from different environments or locations can vary widely (McGuire and McNeal 1974, Baenziger et al 1985). RP-HPLC's quantitative capabilities suggest that it may be the best method yet for revealing compositional differences among lots of a variety grown in different environments, including soils deficient in nutrients such as sulfur. RP-HPLC also permits rapid determination of wheat quality on small flour samples (Huebner and Bietz 1986).

Butte and Coteau wheats have nearly identical baking qualities (Waterer and Evans 1985). We used RP-HPLC to characterize gliadins from these varieties grown at numerous locations in North Dakota and under various conditions. Figure 3 shows a difference plot for gliadin extracted from Butte wheat grown at Williston and Langdon, ND. Clearly, there is an increased relative percentage of early-eluting low-sulfur ω-gliadins in the Williston sample. Variability also occurs for Coteau wheat from Minot, Dickinson, and Langdon, ND (Fig. 4). These results suggest that high-sulfur (late-eluting) gliadins are increased in the Dickinson and Langdon samples, compared to that from Minot, but sulfur-poor ω-gliadins, which elute early, remain relatively constant. Exact sulfur determinations were not available for soils on which these samples were grown, so we cannot absolutely relate protein composition to availability of sulfur. Since these wheat samples represent several geographical locations having soils not known to be markedly deficient in sulfur, however, our results suggest that differences among their gliadins may not result from soil sulfur deficiency.

Slight differences between runs in separation of two poorly separated major components at 16 min (Fig. 4) appear to be due to column and elution conditions. This may reflect some variation in the amount of one component between runs. Better separation of these components is usually achieved on a Vydac C_4 column.

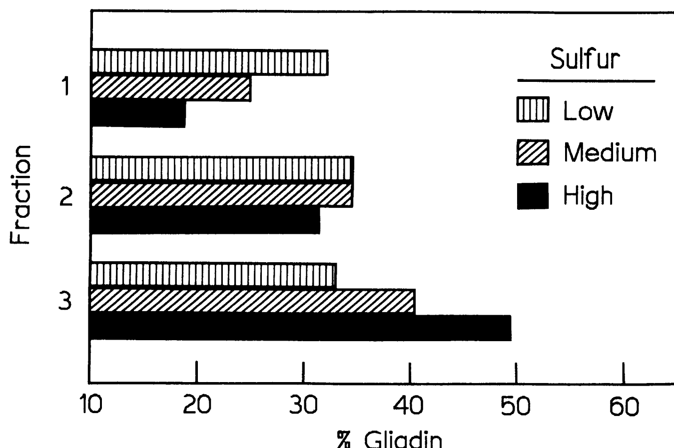


Fig. 2. Percentages of early- (7–22.5 min), intermediate- (22.5–31.5 min), and late-eluting (31.5–54 min) gliadins divided into three reversed-phase high-performance liquid chromatograpic fractions (Fig. 1) from Egret wheat grown on low-, medium-, and high-sulfur soils.

Environmental Effects on Gliadin Composition

To examine environmental effects on gliadin compositions, we obtained information on weather conditions at the time of kernel development for two varieties, Butte and Coteau, grown at seven locations (including irrigated and nonirrigated samples). RP-HPLC chromatograms were divided into three fractions (Fig. 4B), similar to that for Australian wheats, but not identical times (Fig. 1); results showing percentages of each fraction for wheats from each location are shown in Figure 5.

For both varieties, only slight variation (from <1 to 3% of total gliadin) occurred for total amount of each fraction between locations. A few individual early-eluting proteins (primarily ω-gliadins, plus albumins and globulins, which constitute only 2–5% of the total extract), however, varied by more than 100%, as in Figure 1. Amounts of these individual proteins do not appear to relate to available water, as indicated by comparison of samples from dryland and irrigated conditions.

It is interesting to consider the data in Figure 5 in terms of known weather conditions at sites where these wheats were grown. For example, at Williston, there had been little rainfall the preceding winter, poor rainfall (0.6 cm) during kernel development, and high temperature (13 days over 31°C, seven days over 32°C, and three days over 35°C) while kernels developed. As a result, kernels from Williston were shriveled due to decreased production of starch and had higher protein content. At Minot and Fargo, rainfall was also low, although temperatures were not as high. Despite such conditions, overall gliadin compositions (i.e., amounts of fractions 1–3) did not vary more at these locations than at others tested. This may partially reflect adaptation of cultivars to diverse environments; also, these two varieties differ by about five days in kernel development time, so weather conditions could have differed at critical stages of kernel development.

The number of uncontrolled variables in such a study obviously complicates interpretation of results. Regardless of cause, however, our results clearly demonstrate that environment,

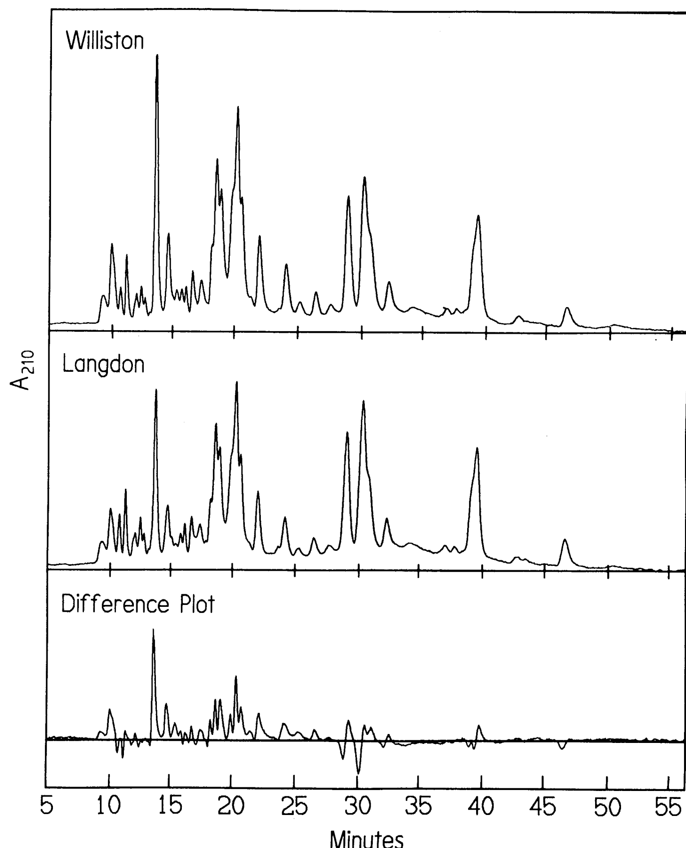
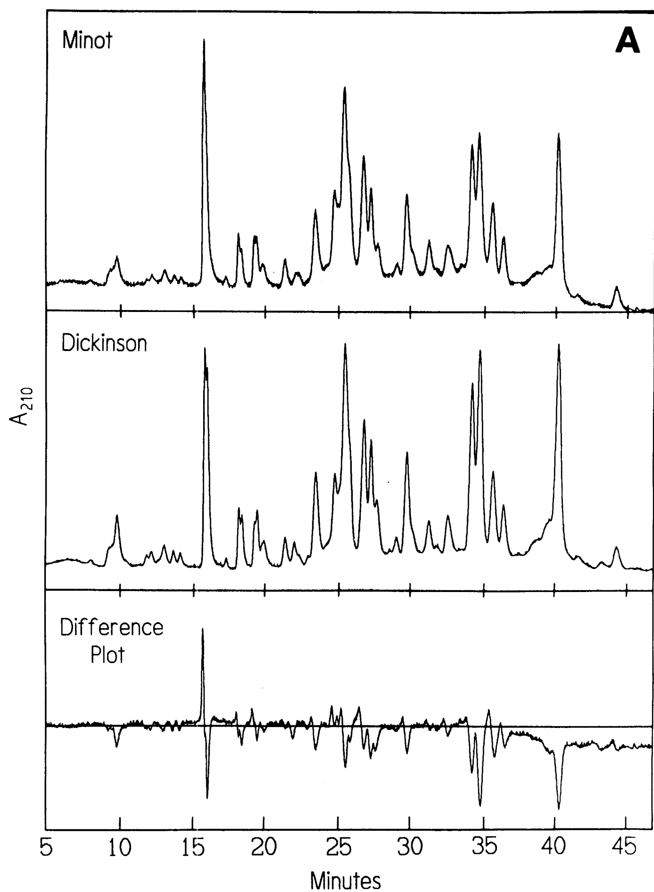


Fig. 3. A difference plot of gliadin chromatograms for Butte wheat grown at Williston and Langdon, ND. The plot is on the same scale as the absorption for the chromatograms from which it was derived.



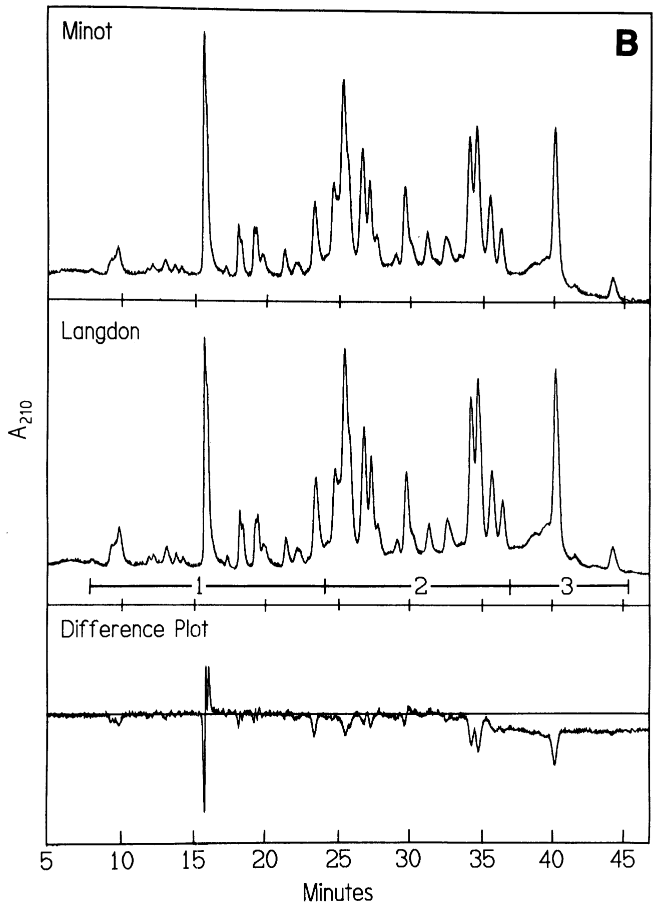


Fig. 4. Difference plots of gliadin chromatograms for Coteau wheat grown at (**A**) Minot and Dickinson, ND, and (**B**) Minot and Langdon, ND. The difference plot is on the same scale as the absorption for the chromatograms from which it was derived.

location, and soil mineral levels affect quantitative accumulation of some gliadins far more than previously recognized.

DISCUSSION

The greater surface hydrophobicities of sulfur-rich (α, β, and γ) gliadins, compared to those of sulfur-poor (ω) gliadins, make RP-HPLC well-suited for examination of quantitative protein variation of wheats grown in soils varying significantly in sulfur levels. As in all analytical studies, samples must be analyzed under equivalent conditions, using the same column to compare samples in a series.

In addition to speed, simplicity, resolution, and reproducibility, a major advantage of RP-HPLC is that it, unlike alternate methods, provides reasonably accurate quantitative information (Mahoney and Hermodson 1980). In electrophoresis, proteins are usually stained with electronegative dyes, such as Coomassie Blue, which bind ionically to basic amino acids; all such residues may not be equally accessible to dye, however, and proteins may be lost during staining or destaining. Extinction coefficients for different proteins may thus vary significantly. For these reasons, accurate quantitative evaluation of electrophoresis data is difficult.

Electrophoresis of gliadins is widely used for wheat varietal identification, since each variety has its own qualitatively similar fingerprint, even when grown at different locations. RP-HPLC, however, because it may better quantify and resolve complex protein mixtures, shows quantitative differences generally not apparent by electrophoresis. The working hypothesis that gliadin composition is nearly invariant for a genotype must clearly be reevaluated. Caution will be required in using the quantitative capabilities of RP-HPLC in varietal identification: the magnitude of normal variation within genotypes grown in different environments will have to be clearly defined. Such information may, however, lead to a better understanding of grain protein synthesis and accumulation.

Quantitative variation among gliadins from wheats grown under a normal range of environmental diversity does not preclude, however, the use of RP-HPLC for varietal identification. RP-HPLC, with computer-assisted methods of data analysis, should be highly useful to identify cultivars and predict wheat quality, and should permit totally automated identification of varieties.

Quantitative differences among gliadins may relate to moisture availability, temperature, or amount of sunshine during kernel development. These factors all may significantly influence plant growth (Sofield et al 1977, Wardlaw et al 1980). When day/night temperatures increase to 30/25°C, accumulation of dry matter decreases because of a decreased period of kernel filling (Wardlaw et al 1980, Shpiler and Blum 1986). Gliadin compositions of some of our samples may reflect such environmental variability: at Williston, the average weekly temperature for part of the grain

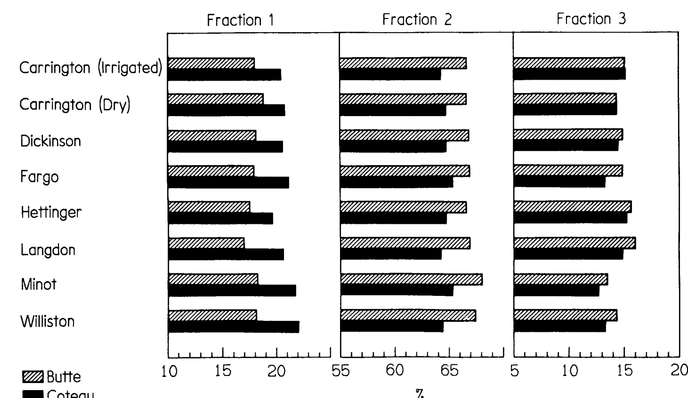


Fig. 5. Percentages of gliadins in early-, intermediate-, and late-eluting fractions (1–3, respectively, in Fig. 4B) from reversed-phase high-performance liquid chromatograms of Butte and Coteau wheats grown at seven North Dakota locations. Analyses were performed on a Bio-Rad C_{18} column. Other conditions were as in Fig. 1.

filling period was 31–33° C and reached a high of 37° C; during this period, less than 0.6 cm of effective rain fell.

Our results confirm the effect of soil sulfur level on relative synthesis of low- and high-sulfur wheat prolamins. Reasons for differences in accumulation of gliadins under less extreme conditions are not as well understood. Weather may contribute to such differences, and developmental times of varieties vary slightly. This may explain why gliadin compositions of the two wheats examined at numerous locations did not vary in exactly the same way, even at the same locations. Also, genetic differences among varieties influence protein accumulation. Probably a combination of these factors, along with soil mineral levels, affects protein accumulation and final composition. Further controlled studies are necessary to better define the effects of each factor.

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