Quantitative Variation of Wheat Proteins from Grain at Different Stages of Maturity and from Different Spike Locations

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ABSTRACT

Hard red spring wheats were grown in a greenhouse and hard red winter wheats in the field. Kernels were removed from the spring wheats periodically after flowering, and proteins analyzed by reversed-phase and size-exclusion high-performance liquid chromatography (HPLC) to reveal their compositions and rates of synthesis. Mature dried wheat kernels from the top, middle, and bottom of spikes were weighed and stored for analyses. Results revealed synthesis of both gliadin and glutenin early during maturation. HPLC patterns of gliadins (soluble in 70% ethanol) from individual kernels changed quantitatively until approximately five weeks after anthesis. Patterns also varied with spike location and kernel size. For glutenin, qualitative subunit compositions remained nearly constant during kernel development, but amounts of some subunits differed, some accumulating more rapidly than others until maturity. Synthesis of gliadin increased more rapidly than that of glutenin until the fifth week after anthesis; after this, glutenin accumulated more rapidly than gliadin. Amounts of individual high molecular weight glutenin subunits varied significantly between two varieties during maturation, suggesting differential expression of alleles at the *Glu-1* loci that code these polypeptides.

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The quality of wheat flour for bakery products has long been of interest (Shewry and Miflin 1985). There is considerable variation in flour quality, however, depending on when and where its parent wheat is grown (McGuire and McNeal 1974, Baenziger et al 1985). In part, this is because of weather and fertilizer, but some quality differences cannot be explained. For example, the cause of variation in kernel hardness, which is important to millers, is unknown (Gaines 1986, Eckhoff et al 1988).

Wheat kernels also vary in size. Kernels from different spike positions are synthesized at different times, and they have different protein contents (Levi and Anderson 1950) and compositions. Different protein classes also may be synthesized at different times (Bushuk and Wrigley 1971). Protein amino acid compositions also change during maturation (Jakher 1974, Levitskii and Vovchuk 1987). Glutenin also changes during maturation (Khan and Bushuk 1976). High molecular weight glutenin subunits, critical to formation of the gluten network through disulfide bonding, appear most important in flour baking potential (Bushuk 1985). Hydrophobic interactions have important implications in contributing to the structure and properties of gluten (Popineau and Godon 1982). Such changes in protein composition and interactions during maturation may affect breadmaking potential (Kaczkowski et al 1988).

Several methods can reveal constituents and properties of wheat proteins (Fullington et al 1983, Bushuk and Zillman 1978). Many studies of changes in protein composition during kernel development have been inconclusive, however, because of difficulties and insensitivity of the analytical methods. Today, improved methods such as high-performance liquid chromatography (HPLC) permit more rapid, sensitive, and quantitative analysis of proteins (Bietz 1983, Bietz and Burnouf 1985) and prediction of quality (Bietz and Huebner 1987; Burnouf and Bietz 1984a, 1987; Huebner and Bietz 1985, 1986, 1987), using as little as one-half kernel of wheat.

For this study, we grew wheat in a greenhouse under controlled conditions and in small field plots. Levi and Anderson (1950) had already shown that individual wheat kernels vary widely in protein content (up to 6%) within individual spikes; this may reflect differences in dates of opening of florets. Also, a study by Kaczkowski et al (1977) used reversed-phase (RP) HPLC and electrophoresis to study changes in gliadins during maturation. To extend these studies, we examined order of biosynthesis of gliadin and glutenin polypeptides during grain maturation, and ratios of proteins. We related protein compositions to kernel locations on the spike, to spike flowering dates, and to growing conditions.

MATERIALS AND METHODS

Samples

Butte and Coteau hard red spring (HRS) wheats were grown in a greenhouse using 12 hr of light, with daytime temperatures of 24–26°C and night temperatures of 19–20°C, beginning on March 3, 1987. Excess soil fertilizer was available, and a standard fertilizer mixture was added during watering at least twice during plant growth. Water was added so soil did not become dry. Wheat spikes were tagged on day of flowering (anthesis). (Since all kernels in a head do not flower simultaneously, however, one or two days' uncertainty in reported flowering times for a head is unavoidable.) Kernels were removed from spikes by forceps every two to three days. Kernels were immediately frozen and stored in sealed containers until analysis (generally within one week of harvest). During early development (14–26 days), two or three kernels were taken from the middle of a spike to obtain sufficient protein for analysis; at later maturation stages, only one to two kernels were needed.

When wheat was mature, the entire spike and stem were removed and allowed to air dry at room temperature. Individual kernels were then removed from various spike locations and stored separately at room temperature until analysis.

Two hard red winter wheat varieties, high-quality Cheyenne and medium-quality Zeta (Kaczkowski et al 1988), were grown during 1986–1987 in gray-brown podzolic soil fields at the Institute of Plant Genetics and Breeding, University of Agriculture, Warsaw, Poland. Grain was harvested at six specific times during maturation (Kaczkowski et al 1986, 1987), beginning 10–12 days after anthesis (stage 1, when grain dry matter was approximately 30% [Cheyenne] or 26% [Zeta]), and then each 7–10 days (depending on weather) until maturity (stage 6). For these samples, each maturation stage thus represents a distinct dry matter content, as described by Kaczkowski et al (1987). Kernels were hand-threshed from spikes immediately after harvest and stored at −18°C until analysis.

Protein contents of immature Cheyenne and Zeta kernels were estimated by micro-Kjeldahl nitrogen analysis using the colorimetric determination according to the official AOAC method (7.031, AOAC 1984). For Cheyenne, protein contents for kernels of maturation stages 1 through 6 were 12.1, 9.5, 9.9,

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10.3, 11.8, and 12.8%, respectively (N × 5.70). For Zeta, protein contents at stages 1 through 6 were 13.5, 10.2, 11.3, 12.4, 12.0, and 11.7%, respectively. N contents at stage 1 in both varieties apparently are composed of mainly nonprotein nitrogen and nonstorage proteins, as evidenced by small amounts of gliadins and glutenins revealed by RP-HPLC (see below). From stages 2 through 6, amounts of protein determined from nitrogen analysis appear to reflect primarily storage protein accumulation, as shown by quantitation of RP-HPLC data (see data below).

It is somewhat difficult to directly compare developmental stages of greenhouse- and field-grown samples, due to environmental variability. Thus, both developmental stages (for Cheyenne and Zeta) and days after anthesis (for Coteau and Butte) must be referred to in this communication. The two systems can therefore not be exactly compared, although similar trends in accumulation of protein are easily observed.

Sample Preparation for HPLC

Three or four mature kernels from the top, middle, or bottom of spikes were pulverized with a Wig-L-Bug (Crescent Dental Mfg. Co., Lyons, IL) (Huebner et al 1990). Flour was sieved (250 μm) to uniformity. To extract gliadins, 60 mg of each flour sample was mixed with 1.5 ml of 70% ethanol in a 10 ml polypropylene centrifuge tube (Huebner and Bietz 1984). Samples were vortexed for 30 min at room temperature using a Buchler Vortex-Evaporator (Buchler Insts., Fort Lee, NJ), and centrifuged for 15 min at 25,000 × *g*. Clear supernatants were transferred to small vials for analysis.

To isolate glutenin, the residue was again extracted with 70% ethanol (as above); the supernatant (containing residual gliadin) was discarded. Glutenins were then extracted with 1.5 ml of 6*M* urea/0.1*M* $NaPO_4$/0.8% sodium dodecyl sulfate/0.5% dithiothreitol (pH 7.7). After vortexing for 1.5 hr at 45°C, samples were centrifuged at 34,000 × *g* for 20 min and supernatants were transferred to vials for analysis. Samples were not alkylated but were generally analyzed the same day they were prepared; otherwise, they were frozen until used.

To isolate proteins from immature samples (14–26 days), 1.0 ml of 70% ethanol was added to two or three kernels in a centrifuge tube. This provided a sample dry weight similar to that of a single mature kernel. For wheat more than 26 days after anthesis, one or two kernels were used. Kernels were then crushed with a glass rod, and samples were vortexed and centrifuged as for mature kernels. The water present in the kernels did not appreciably alter the alcohol percentage in the extractant, and comparable results for replicate samples indicated good extraction efficiency. Kernels older than 26 days were dried at room temperature and then pulverized in the Wig-L-Bug. Protein contents of individual kernels were not analyzed directly but could be estimated (see below) by analysis of HPLC data.

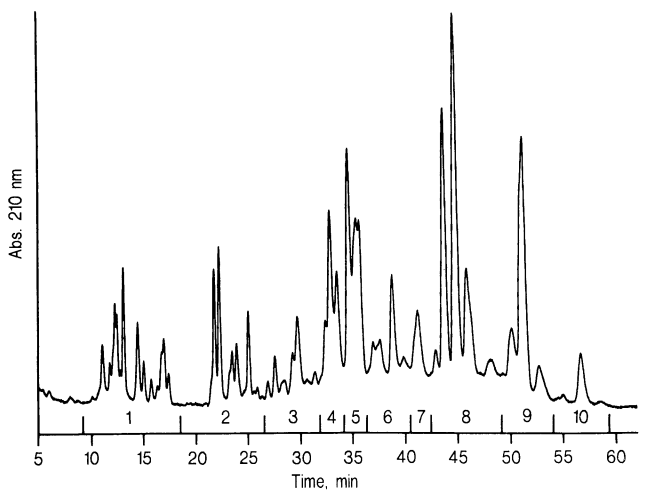

Fig. 1. Reversed-phase high-performance liquid chromatography of Butte gliadin (15–20 μl) on a Vydac C_4 (250 × 4.1 mm) column at 60°C. Solvents used were A) H_2O containing 0.06% trifluoroacetic acid and B) acetonitrile (ACN) containing 0.045% trifluoroacetic acid. The gradient, at 1.0 ml/min, was 24% ACN at 0 min, increasing linearly to 28% ACN at 2 min, and to 45% ACN at 50 min. The column was then held at 45% for 5 min and reequilibrated for 10 min with 24% ACN.

HPLC

RP-HPLC. RP-HPLC was performed on a Spectra-Physics (San Jose, CA) apparatus including a SP8700 solvent delivery system and SP8780XR autosampler (Huebner and Bietz 1987). Proteins were detected at 210 nm (0.1 absorbance units full scale [AUFS]/10 mV) with a SF770 Spectroflow monitor (Kratos, Ramsey, NJ). Absorbance at 210 nm is approximately proportional to the amount of protein present. A Vydac (Separations Group, Hesperia, CA) C_4 column (250 × 4.1 mm, particle size 5 μm, porosity 30 nm) was used. It was 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).

Acetonitrile (ACN) and trifluoroacetic acid (TFA) were HPLC grade. Water was purified with a Barnstead Nanopure system. Solvents A (water containing 0.06% TFA) and B (ACN containing 0.04% TFA) were filtered through a 0.45-μm (HVLP) Millipore filter. Solvents were then deaerated under vacuum and sparged with a rapid flow of helium for 10–15 min (Huebner and Bietz 1987). During use, slow sparging with helium continued.

Duplicate samples of 15–35 μl (volume depending on sample protein concentration) were analyzed (in duplicate or triplicate) at 1.0 ml/min. Columns were maintained at 60°C with a CH-20-C column heater (Scientific Systems, State College, PA). Proteins were eluted using linear 28–47% ACN gradients during 50 min, with an additional 5-min hold at the final concentration.

Size-exclusion HPLC. Reduced glutenin was separated by size-exclusion (SE) HPLC on a 10 × 300 mm Superose 12 column (Pharmacia, Piscataway, NJ). The separation column was preceded with a 7.5 × 75 mm TSK SW guard column (Varian Assoc. Inc., Sunnyvale, CA). The solvent was 0.1*M* Na_2HPO_4/NaH_2PO_4, pH 7.0, containing 5% ACN, 0.2% sodium dodecyl sulfate, and 0.01% dithiothreitol. Flow rate was 0.7 ml/min, column temperature was 28°C, and sample size was 6–8 μl. Molecular weight standards were bovine pancreatic chymotrypsinogen A (25 kDa) and serum albumin (67 kDa) (Pierce, Rockford, IL).

Data Analysis

Data were recorded on an Omniscribe recorder (Houston Inst., Austin, TX) and stored in a ModComp computer system (Ft. Lauderdale, FL). Data could then be plotted to any convenient scale and automatically integrated between specified time limits or by Gaussian deconvolution after correction for baseline shifts due to the gradient. To facilitate visual comparisons, plotted chromatograms in figures were normalized so that the largest peak in each had an equivalent height. In the original analyses, sample size was chosen so that the largest peak remained on-scale at 0.1 AUFS.

RESULTS

Analyses of Gliadin

RP-HPLC of gliadins from developing kernels. Figure 1 shows a typical RP-HPLC chromatogram of Butte gliadins from mature grains. Samples of different maturities were compared by dividing their chromatograms into 10 fractions, as shown. Amounts of each fraction were expressed as percentage of total area of protein peaks eluting from the column.

Figure 2 shows how three gliadin RP-HPLC fractions (1, 3, and 8) from Butte wheat grown in a greenhouse under controlled conditions vary quantitatively during kernel development. Results for Coteau wheat were similar (data not shown). Comparison to RP-HPLC patterns of isolated protein standards showed that fraction 1 contains mainly high molecular weight gliadins, fraction 3 mainly ω-gliadins, and fraction 8 mainly γ- and β-gliadins. The amount of fraction 2 (Fig. 1) also decreased with time, and fraction 5 increased; other fractions did not show any definite

quantitative progression.

These results are averages of duplicate analyses of each sample (or of more analyses if the first two varied by more than 10%). Also, since spikes flowered at different dates, samples were taken nearly every day from 14 to 38 days after anthesis; presented results are an average for three to four days. Thus, these presented results represent an average of six to eight analyses of kernels of similar ages.

Gliadins from field-grown samples varying in maturity, in which all kernels from several spikes were combined and milled, were also compared by this method. Such samples (as well as greenhouse-grown wheats) may include kernels varying slightly in maturity, since not all spikes or flowers within a spike bloom simultaneously. Figures 3 and 4 show chromatograms from Cheyenne and Zeta for each maturation stage examined. Quantitative results for Zeta (Fig. 5), a hard red winter wheat, are similar to those for Butte (Fig. 2) or Coteau in that amounts of most fractions progressively increase or decrease slightly during development. Different fractions vary somewhat in their accumulation in these two varieties. This is not surprising, however, since they are of different classes and were grown under varying conditions. Also, since wheat contains many unique gliadins that vary qualitatively with genotype, varieties cannot be easily compared using the same times to divide chromatograms into peak areas. This makes direct comparison of fractions from different varieties difficult.

Total RP-HPLC areas of gliadins (as in Fig. 1), which are approximately proportional to amounts of protein, were determined for Cheyenne and Zeta during development (Fig. 6). Amounts of gliadins increased until four or five weeks after flowering, and then leveled off, suggesting decreased synthesis of gliadin during final developmental stages. Others have also observed decreased percentage of protein during final days of maturation (Khan and Bushuk 1976). We confirmed this by determining the ratio of gliadin to glutenin (the amount of glutenin was also measured by RP-HPLC; see below) during maturation (data not shown). Results confirmed that gliadin and glutenin are synthesized at different rates; gliadin reaches a maximum at about four weeks, after which the relative rate of glutenin synthesis increases. Bushuk and Wrigley (1971) also observed changes in relative amounts of gliadin and glutenin during development.

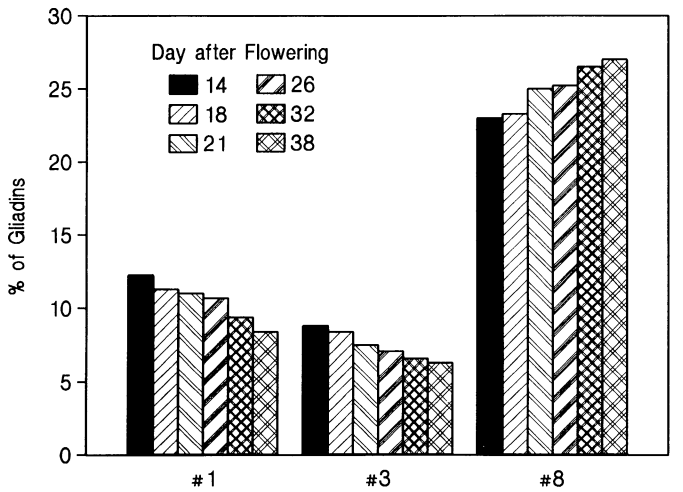

Fig. 2. Percentages of three gliadin reversed-phase high-performance liquid chromatographic fractions from immature (14–38 days after flowering) kernels of Butte wheat. Results are averages of at least two analyses. Chromatograms were divided into 10 fractions, as in Fig. 1.

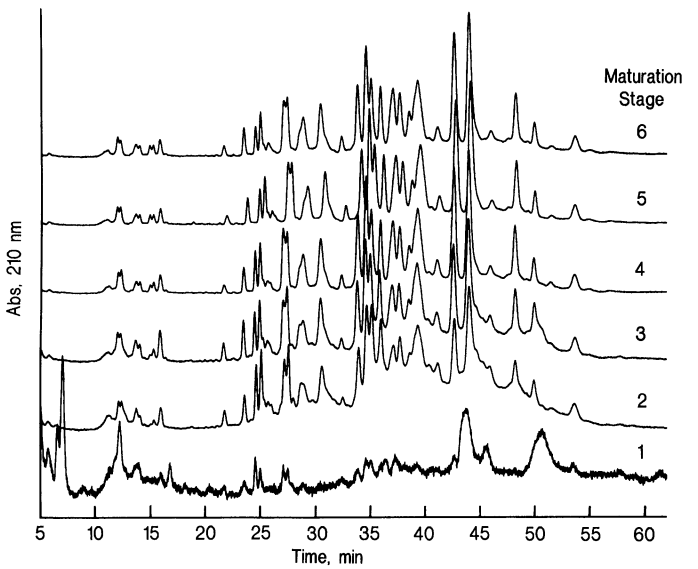

Fig. 3. Reversed-phase high-performance liquid chromatographic patterns of gliadins from Cheyenne wheat at developmental stages 1 (bottom) through 6 (top). All data were recorded at $A_{210} = 0.1$, but plots were normalized to the highest peak (as described in Materials and Methods). The noisy baseline at stage 1 indicates low protein content and detector signal for this sample (compare Table I).

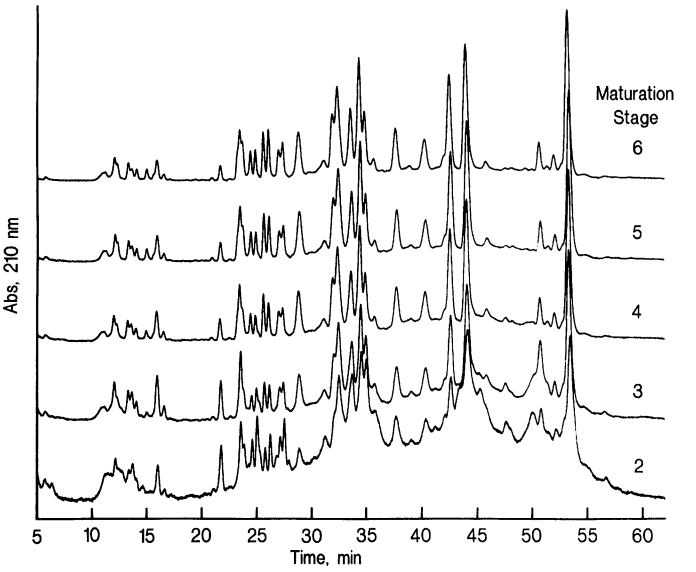

Fig. 4. Reversed-phase high-performance liquid chromatographic patterns of gliadins from Zeta wheat at developmental stages 2 (bottom) through 6 (top).

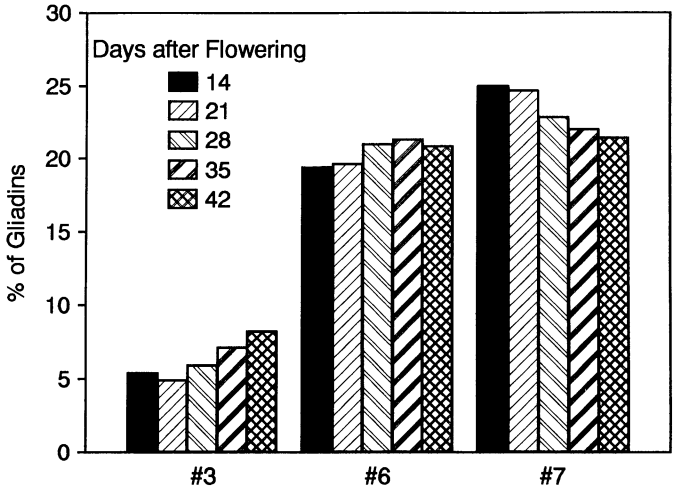

Fig. 5. Percentages of three gliadin reversed-phase high-performance liquid chromatographic fractions from immature (stages 2–6) Zeta wheat. Results are averages of at least two analyses. Chromatograms were divided into eight fractions, similar to the division shown in Fig. 1.

Gliadins at successive maturation stages of Cheyenne and Zeta wheats were also analyzed by RP-HPLC (as in Figs. 3 and 4) to study changes during maturation. Even at the first stage (about 12 days after anthesis), some gliadins (at 11–17, 24–28, and 33–40 min) are synthesized and continue to accumulate during development. Comparison of Cheyenne and Zeta gliadins reveals differences in specific peaks typical of different varieties. For each variety, gliadins at successive stages appear qualitatively identical after stage 2. However, quantitative variation in individual components continues to occur until maturity.

Percentages of individual gliadin peaks and total amounts of gliadins were also estimated by quantitation of RP-HPLC data for Cheyenne and Zeta at each maturation stage (Table I). Total peak area increased until the fourth or fifth maturation stage for both varieties. Individual gliadin peaks vary with development for wheats grown in the field as they do for those grown in a greenhouse (Figs. 2 and 5); some proteins (e.g., those at 28.9, 34.6, and 42.6 min in Cheyenne and at 28.9 min in Zeta) increase almost until maturity. Conversely, percentages of some other proteins appear slightly lower at maturity than at stages 3 and 4.

Overall, averaged over the nine gliadin peaks and both varieties, peaks increased by 58% (±8.4%) between stages 2 and 3 and by 51% (±6.7%) between stages 3 and 4. Between stages 4 and 5 and stages 5 and 6 the peak areas did not change significantly (average change −1% [±4.1] between stages 4 and 5 and +3% [±2.7] between stages 5 and 6). The three glutenin peaks did not change significantly in relative size.

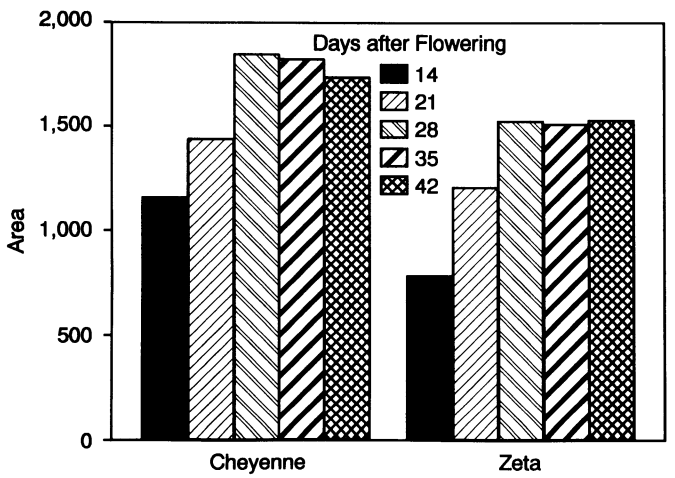

Fig. 6. Total area under reversed-phase high-performance liquid chromatographic peaks of Cheyenne and Zeta gliadins. Results for Zeta are from Fig. 5; those for Cheyenne were obtained under similar conditions.

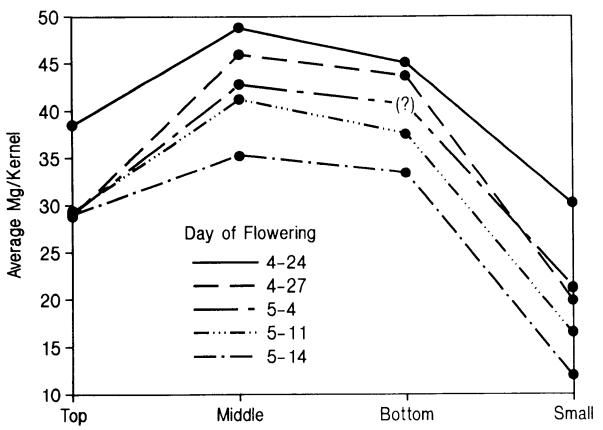

Fig. 7. Weight of kernels of Butte wheat (average of 5–10 kernels) from different spike locations as a function of date of flowering.

Relationships Among Kernel Size, Location on the Spike, Date of Anthesis, and Protein Composition

The size of individual mature wheat kernels varied with spike position, and with date of anthesis (Fig. 7). Late-flowering kernels had less time to develop and were smaller at maturity. The smallest kernels we analyzed may be supernumerary spikelets (Kadhol and Halloran 1988) between top and bottom kernels; these may be seen after threshing out mature kernels by hand.

Protein compositions of kernels from differing spike locations or from kernels flowering on different dates also may vary significantly. An example, showing amounts of fraction 6 gliadins (as in Fig. 1) in mature Coteau kernels, is shown in Figure 8. Thus, amounts of individual gliadins vary not only with developmental stage, but also, at maturity, with date of anthesis and with kernel size and location.

Analyses of Glutenin

SE-HPLC. Subunits of glutenin from wheats at different developmental stages were characterized by both SE-HPLC and RP-HPLC. Figure 9 shows typical SE-HPLC analyses. The two major peaks (B and C) correspond to high and low molecular weight glutenin subunits. In addition, higher molecular weight or unreduced protein not revealed in mature wheat by SDS-PAGE or RP-HPLC (Bietz 1983, Burnouf and Bietz 1985, Huebner and Bietz 1987) is present (peak A). This fraction could consist of polypeptides with a high tendency to aggregate (as fraction A of Huebner and Wall [1974]). Some lower molecular weight material (peak D) is also present in glutenin; this could include

TABLE I
Relative Areas (% total area) of Gliadin Peaks from Cheyenne and Zeta Wheats at Successive Maturation Stages

Cultivar	Retention Time (min)									Total Area[a]
Stage	27.1	28.9	30.5	33.8	34.6	37.1	39.3	42.6	44.0	(× 10^5)
Cheyenne										
1	3.8	···	···	3.5	···	2.3	2.1	1.4	18.3	1.30[b]
2	2.5	2.8	4.0	3.1	3.6	3.9	7.7	4.9	8.3	11.52
3	2.6	3.1	4.2	3.3	4.5	4.5	8.9	6.1	8.7	14.33
4	4.9	3.4	4.3	3.9	5.3	5.2	10.0	7.8	11.5	18.43
5	5.2	3.5	4.7	3.9	5.5	5.3	9.5	7.9	11.3	18.30
6	5.2	3.8	4.7	4.0	5.8	5.4	8.9	8.4	11.0	17.30
Zeta										
2	1.8	2.4	2.6	4.3	4.6	4.5	3.5	3.3	8.1	7.78
3	2.4	2.7	2.3	4.0	4.5	4.1	3.9	5.7	10.7	12.00
4	2.3	3.2	2.0	5.5	5.5	4.2	4.8	7.0	10.6	15.20
5	1.5	3.5	3.0	4.1	6.2	3.6	3.9	7.0	10.3	15.04
6	1.8	3.8	3.7	4.7	7.5	3.7	3.3	7.1	9.8	15.23

[a] ±15%.
[b] ±50%.

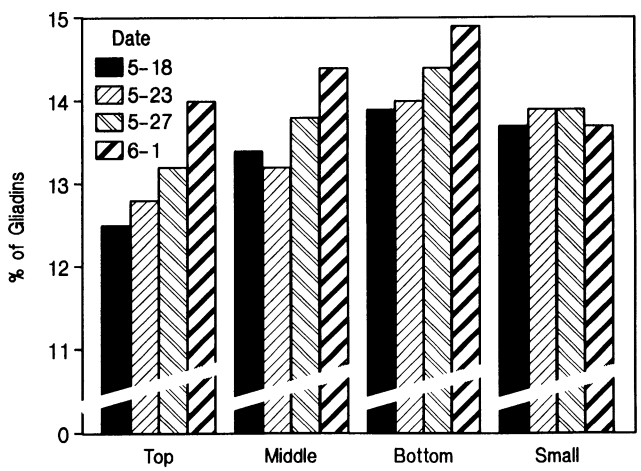

Fig. 8. Percentage of gliadin reversed-phase high-performance liquid chromatography fraction 6 in mature kernels of Coteau wheat with different flowering dates. Results are averages of at least two analyses.

albumins and globulins.

Figure 10 shows amounts of each glutenin SE-HPLC fraction at each developmental stage. The percentages of high molecular weight polypeptides (peak A, Fig. 9) and low molecular weight (<23 kDa) material (peak D) decreased significantly during maturation. Only fraction C (low molecular weight ethanol-soluble glutenin subunits) increased as wheat matured. The accumulation of these prolamins may parallel synthesis of gliadins and formation of protein bodies, where prolamins accumulate (Field et al 1983). In contrast, other glutenin subunits and constituents (Huebner and Wall 1980) may be largely nonstorage proteins. These may predominate during early kernel development, but their amounts may then remain nearly constant; this may be true for high-molecular weight subunits in fraction B. Certainly, results show that glutenin subunits are present early during kernel development and differ in rates of accumulation.

RP-HPLC. Because of its higher resolution and different mode of fractionation, RP-HPLC reveals additional developmental changes among glutenin subunits. Chromatograms for Cheyenne and Zeta glutenin subunits at stages 1–6 are shown in Figures 11 and 12, respectively. Even at the first maturation stage (about 12 days after anthesis), glutenin subunits are present. In these chromatograms, high molecular weight subunits elute between 16 and 20 min, whereas low molecular weight subunits elute between about 30 and 45 min (Burnouf and Bietz 1984b).

The three high molecular weight glutenin subunit peaks (approximately 16.7, 17.8, and 18.8 min) are incompletely resolved and are difficult to integrate accurately by normal methods. Quantitation was improved by using a Gaussian deconvolution program (GAUSDC) developed by R. Butterfield (NRRC, Peoria). Relative amounts of the three high molecular weight glutenin subunit peaks in Cheyenne and Zeta, with areas representing total amounts of these subunits, are in Table II.

Cheyenne, the better quality variety, appears to contain a larger total amount of high molecular weight subunits than does Zeta, when expressed on a weight basis. This may be due either to increased total protein content in Cheyenne or to an increased percentage of high molecular weight glutenin subunits. This agrees with the concept that both amount and composition of high molecular weight glutenin subunits govern breadmaking quality (Payne et al 1987). Also, in Cheyenne, more of these subunits

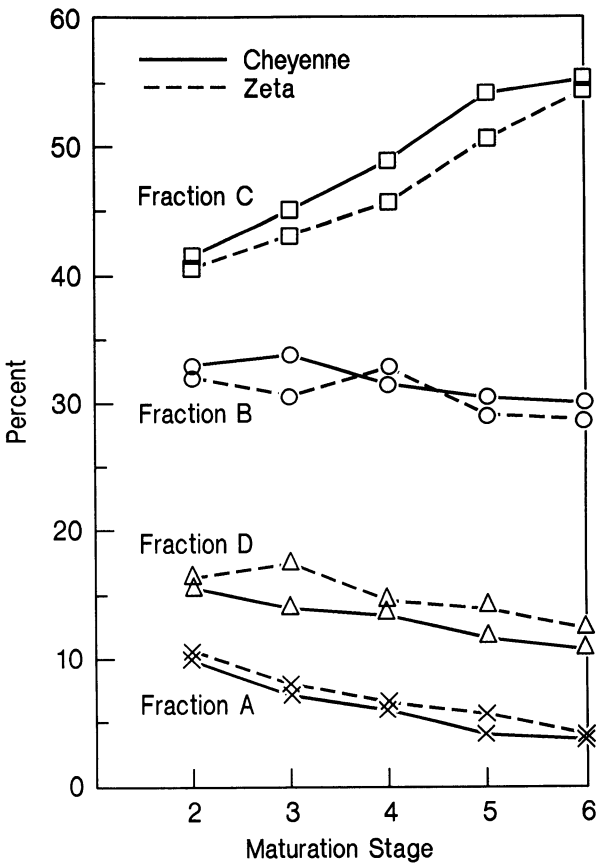

Fig. 10. Percentages of glutenin subunit size-exclusion high-performance liquid chromatographic fractions A–D (Fig. 8) in developing Cheyenne and Zeta wheats (stages 2–6). Fractions B and C correspond to high and low molecular weight glutenin subunits, respectively.

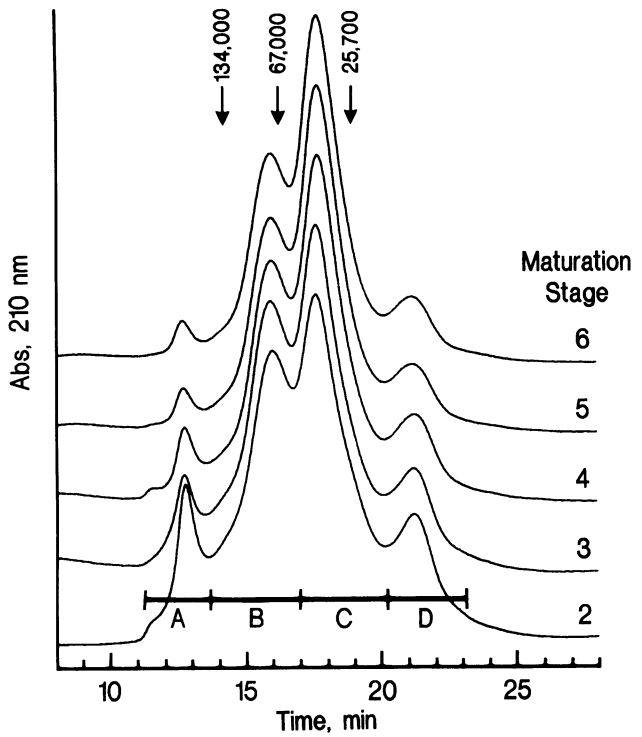

Fig. 9. Size-exclusion high-performance liquid chromatography of reduced glutenin of Cheyenne wheat of stages 2 to 6. Elution positions of molecular weight standards are shown by arrows. The 134-kDa protein was a dimer of bovine serum albumin.

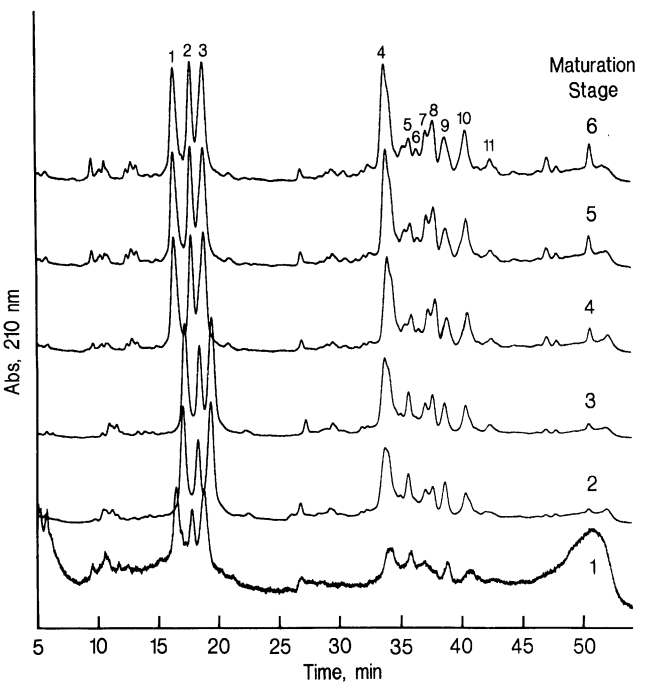

Fig. 11. Reversed-phase high-performance liquid chromatographic patterns of glutenin subunits from Cheyenne wheat at developmental stages 1 (bottom) through 6 (top). Numbers at far right designate developmental stages, whereas numbers 1–8 in the top chromatogram are peak designations.

appear to be synthesized early (until the second maturation stage). Their relative amount then decreases slowly until maturity. In Zeta, high molecular weight glutenin subunits appear to increase gradually until the fifth stage but do not reach the level of these subunits in Cheyenne. Again, this may reflect protein amount or total percentage of high molecular weight glutenin subunits.

Relative amounts of individual peaks also vary during development. In Zeta (Fig. 12), these peaks change more during maturation than in Cheyenne (Fig. 11). For example, peak 1 decreases by more than 40%, peak 2 increases by about 28%, and peak 3 remains relatively constant. The largest difference between these varieties is in peak 1, which in later developmental stages is almost twice as high in Cheyenne as in Zeta.

In both varieties, the percentage of high molecular weight glutenin subunit peak 2 appears to increase slightly during development, mainly at the expense of peak 1, but the increase in not statistically significant. These results extend observations (Burnouf and Bietz 1985) that suggest different numbers of high-molecular weight subunit coding genes at the *Glu-1* loci. Now, differences in regulation of expression of genes at these loci during development are also apparent. These results probably indicate and agree with differences in breadmaking quality associated with specific high molecular weight glutenin subunit alleles.

The total amount of glutenin in each variety during development, estimated by integration of total chromatographic peak areas (Figs. 11–12), increased until the third stage, when grain dry matter reached 50% (data not shown). Most individual components accumulate similarly, including low molecular weight subunits (30–45 min).

DISCUSSION

For any wheat variety, proteins from grain grown in different locations are qualitatively the same (Bietz et al 1984). Nevertheless, considerable quantitative variation among gliadins can result from environmental differences (Huebner and Bietz 1988). Such variation among storage proteins may have important implications in marketing and classification. Differences in protein amounts also may relate to regulation of expression of protein synthesis, to efficiency of protein deposition, to kernel hardness, and to overall quality.

Our study showed additional quantitative differences within varieties, depending on kernel position within the spike, developmental stage, and kernel size. Such changes may make varieties more difficult to identify by single-kernel RP-HPLC analysis. Qualitatively, however, different samples of a variety are still identical.

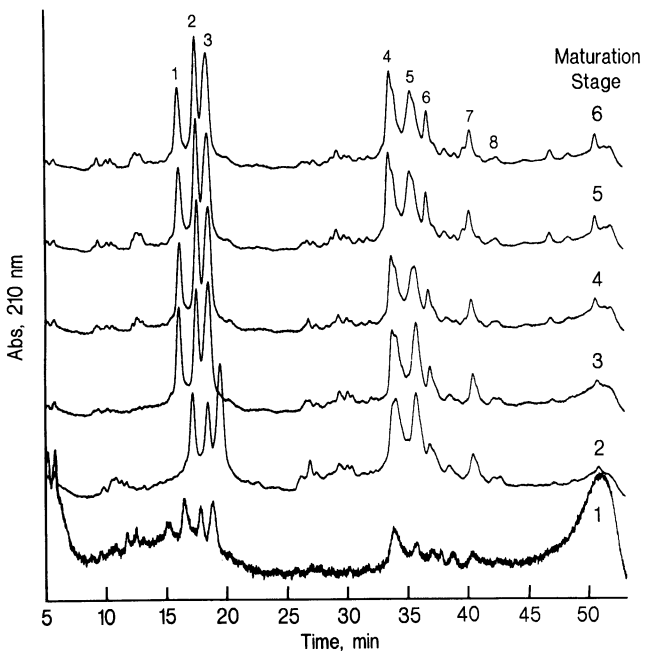

Fig. 12. Reversed-phase high-performance liquid chromatographic patterns of glutenin subunits from Zeta wheat at developmental stages 1 (bottom) through 6 (top).

TABLE II
Changes in Percentages of High Molecular Weight (HMW) Glutenin Subunit Peaks 1–3, and in Total Amount of HMW Subunits (Area), in Cheyenne and Zeta Wheat at Stages 1 to 6

	Cheyenne				Zeta			
	Minutes			Area[a]	Minutes			Area
Stage	16.7	17.7	18.8	($\times 10^5$)	16.7	17.7	18.8	($\times 10^5$)
1	36.0	25.5	38.5	0.41	34.0	27.4	38.6	0.13
2	35.0	24.5	40.5	4.49	28.5	26.5	45.0	1.41
3	35.3	24.4	40.3	3.90	23.8	32.4	43.8	1.44
4	33.4	28.1	38.5	3.29	19.8	35.1	45.1	1.55
5	33.2	30.0	36.8	2.94	21.3	35.8	42.9	1.96
6	32.8	30.2	37.0	2.77	20.4	35.1	44.5	1.86

[a] ±15%.

Analysis of wheat storage proteins by RP-HPLC clearly revealed initiation of gliadin and glutenin subunit synthesis early during kernel development (10–12 days after anthesis). Grain dry matter is then about 28% (Kaczkowski et al 1988). Previous electrophoretic analysis of glutenin in the first stage did not reveal most subunit bands, however (Kaczkowski et al 1987). Presumably this indicates the superior sensitivity of RP-HPLC or difficulties with the electrophoretic procedure. SE-HPLC comparison of total gliadin with glutenin for six weeks showed that gliadin accumulation predominated over that of glutenin up to five weeks. After that, glutenin synthesis predominated (data not shown).

In immature kernels, synthesis of individual gliadins also varied with time. Kaczkowski et al (1987) saw such changes by RP-HPLC on a broader scale. The present studies extend this finding by showing that significant changes in some gliadins occurred even late during kernel development. Such variation, resulting from nutrient availability, weather, and time available for protein synthesis during kernel filling, may largely explain quantitative differences among proteins between mature kernels and samples of any variety. The first kernels to flower will have longest times of development; later kernels may have less time, affecting protein synthesis and accumulation. This also may affect kernel size.

Our results also suggest that gliadin synthesis decreases in relation to starch synthesis after the fourth maturation stage (Fig. 6). This seems consistent with earlier results (Kaczkowski et al 1988). It also confirms the results of Finney (1954) that optimal quality occurs 10–12 days before full maturity (about 60% dry matter). By the fourth or fifth stages, gliadin accumulation is essentially complete. However, since the percentage of some gliadins increases until the sixth stage, some gliadin synthesis must still be in progress (Fig. 5).

The varieties investigated differ in baking quality, which may relate to some differences in gliadins detected by RP-HPLC. We only examined two varieties in detail, however, so such relationships are tentative. Other studies have related gliadins to quality (Bietz and Cobb 1985, Branlard and Dardevet 1985, Burnouf and Bietz 1987, Huebner and Bietz 1987). Knowledge of gliadins characteristic of high or low baking quality wheats may have practical applications.

Previous studies have shown that glutenin exists, in small amounts, even at early developmental stages (Bushuk and Wrigley 1971, Khan and Bushuk 1976, Kaczkowski et al 1987). Our RP-HPLC results confirmed these observations, extending them to a slightly earlier developmental stage, and showed that fractions increased at different rates until full maturity. In large part, the ability to measure such changes is possible only because of the better sensitivity and quantitative capabilities of RP-HPLC.

Compositions and ratios of low and high molecular weight glutenin subunits also changed significantly during development, suggesting different synthetic rates as kernels develop (Figs. 9,

11, and 12). High molecular weight subunits (peaks 1–3, Figs. 11–12) (Burnouf and Bietz 1985) especially appeared to change significantly. The total amount of high molecular weight subunits was also higher in Cheyenne than in Zeta. These high molecular weight polypeptides probably contribute to a strong gluten structure. Differences among low molecular weight glutenin subunits also exist between varieties (Burnouf and Bietz 1985) (34-40 min); these also may influence quality.

In conclusion, our results further reveal the complexity of wheat proteins and partially explain reasons for variation in protein composition and wheat quality within a variety. RP-HPLC can provide new insights into quantitative biosynthesis of polypeptides during wheat development.

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