Preliminary Assessment of a Sequential Extraction Scheme for Evaluating Quality by Reversed-Phase High-Performance Liquid Chromatography and Electrophoretic Analysis of Gliadins and Glutenins[1]

J. E. KRUGER, B. A. MARCHYLO, and D. HATCHER[2]

Cereal Chem. 65(3):208–214

ABSTRACT

After preextraction with 0.5*M* sodium chloride to remove albumins and globulins, the storage proteins of five wheats that vary widely in quality were extracted sequentially at 60° C with 50% 1-propanol, 50% 1-propanol containing 4% dithiothreitol (DTT), and 50% 1-propanol containing 4% DTT and 1% acetic acid. Proteins present in the various propanol extracts were analyzed by sodium dodecyl sulfate gradient polyacrylamide gel electrophoresis and reversed-phase high-performance liquid chromatography (RP-HPLC). The 50% 1-propanol extracted the bulk of the gliadins, whereas the other solvents extracted mainly glutenin polypeptides. The separation of related storage protein groupings was not clear-cut, however, as some overlap occurred between fractions. By choosing appropriate RP-HPLC conditions, it was possible to quantitate the relative amounts of ω-gliadins; α-, β-, and γ-gliadins; and high and low molecular weight glutenins in the different solvents. The amount of high molecular weight glutenins in 50% 1-propanol relative to the amount present in 50% 1-propanol containing DTT generally decreased with increasing dough strength. In addition, the ratio of high to low molecular weight glutenin subunits in 50% 1-propanol containing 4% DTT increased with increasingly stronger wheats.

The technique of high-performance liquid chromatography (HPLC) offers several promising possibilities for rapidly determining the breadmaking quality of a wheat cultivar. In the reversed-phased mode (RP-HPLC) and gel permeation modes (SE-HPLC), the ratio of high (HMW) to low molecular weight (LMW) glutenin subunits has been correlated with quality (Bietz 1984, Huebner and Bietz 1985). Furthermore, analysis of a specific group of gliadins has also been correlated with a "quality" score based on milling and baking quality (Huebner and Bietz 1986). In addition, researchers are continuing to ascribe specific HMW glutenin subunits, separable by electrophoresis, to good and poor breadmaking quality (Payne et al 1979, 1981, 1984). RP-HPLC has been successful in separating such HMW subunits and has the advantage that individual as well as groups of such components can be quantitated and readily manipulated with a computer (Burnouf and Bietz 1984).

The storage proteins of wheat can be categorized broadly into three groups according to Shewry et al (1986). These consist of HMW prolamins (HMW subunits of glutenin), sulfur-poor prolamins (ω-gliadins), and sulfur-rich prolamins (α-, β-, and γ-gliadins, and LMW subunits of glutenins). Wheat and barley appear to show homologous groups of such proteins with similar aggregation behavior and solubilities (Shewry et al 1984). Byers et al (1983) found that 50% 1-propanol, with or without reductant and acetic acid, was able to efficiently solubilize these storage proteins. The solvent formed the basis for a recent sequential extraction scheme that separated groups of storage proteins in barley for subsequent analysis of quality differences using RP-HPLC and electrophoresis (Marchylo et al 1986). The present paper explores the possibility of using a similar extraction scheme for fractionating wheat storage proteins into groups, i.e., sulfur-poor gliadins, sulfur-rich gliadins, HMW and LMW glutenin subunits, which would be more amenable to quantitative analyses by RP-HPLC in order to ascertain links to quality. The separations effected by the extraction scheme as well as RP-HPLC were monitored by sodium dodecyl sulfate gradient polyacrylamide gel electrophoresis (SDSGPAGE) in order to confirm the identity of such groups on RP-HPLC chromatograms.

Previous research by Bietz and Burnouf (1985) indicated that reduced and alkylated storage proteins elute during RP-HPLC in the approximate order: ω-gliadins, HMW glutenin subunits, LMW (α- and β-) gliadins, LMW ethanol-soluble glutenin subunits, and γ-gliadins. Reduced but unalkylated proteins behave similarly but generally are more hydrophobic, eluting later in HPLC chromatograms.

The sequential extraction procedure consisted initially of removing albumin and globulins with saline solution. The residue was treated with 50% 1-propanol to extract gliadins. Further extraction with 50% 1-propanol containing DTT was used then to dissociate glutenin and extract LMW and HMW subunits. Finally, 50% 1-propanol containing DTT and 1% acetic acid was used to further solubilize any remaining HMW and LMW glutenin subunits. Proteins from the various extracts were separated by RP-HPLC and SDSGPAGE, and selected groups of proteins were analyzed quantitatively by RP-HPLC. Five Canadian cultivars of wheat varying widely in quality characteristics were compared.

MATERIALS AND METHODS

Wheat Samples

The wheat cultivars selected for analysis represented a very wide spectrum of relative dough strengths. Glenlea, a Utility class wheat, is very strong. Neepawa, a Hard Red Spring (HRS) class wheat grown in large quantities in western Canada, has normal dough strength, i.e., a farinograph development time of around five minutes. Also included was a nonregistered, inferior quality (weak) HRS wheat, Prairie Pride. HY320 represented a new class of wheat, Canada Prairie Spring (CPS), grown in western Canada. It exhibits medium hardness, protein, and a slightly weaker dough strength than Neepawa. Finally, Fielder, a Soft White Spring (SWS) class wheat, was representative of an extremely weak wheat having a very low farinograph development time (about 1–1.5 min). With the exception of Prairie Pride, typical dough characteristics of these wheats are found in "Quality of Canadian Wheat 1987" (Canadian Grain Commission 1987).

The protein contents of the wheats (14% moisture basis) were as follows: Glenlea, 14.6%; Neepawa, 16.5%; Prairie Pride, 13.0%; HY 320, 13.0%; and Fielder, 11.5%.

Wheat samples were finely ground in a Udy cyclone sample mill (Udy Corp., Boulder, CO) equipped with a 1-mm sieve.

Chemicals and Reagents

All chemicals used were reagent grade, unless noted otherwise. HPLC grade acetonitrile with an ultraviolet cutoff wavelength of 190 nm was obtained from Fisher Scientific (Fair Lawn, NJ). Trifluoroacetic acid (sequanal grade) was from Pierce Chemical Co. (Rockford, IL). Water was deionized and purified with a Barnstead Nanopure II water purification system (Boston, MA).

[1]Paper no. 607 of the Grain Research Laboratory.

[2]Grain Research Laboratory, Canadian Grain Commission, 1404-303 Main Street, Winnipeg, Manitoba R3C 3G8.

RP-HPLC

A Waters HPLC and Waters 840 data and chromatography control station (Waters Associates, Inc., Milford, MA) consisted of the following components: model 501 and model 6000 pumps (solvents A and B, respectively), a Wisp 710B sample injector, a model 490 programmable multiwavelength detector, a temperature control module, systems interface module, Dec Professional 380 computer with a 33 megabyte hard disk and LC Multisystem version 3.0 software, an LA210 letter printer and LVP16 plotter. A Supelcosil LC-308 reversed-phase HPLC column [C_8, 300Å pore size, 5 μm particle size, 5 cm × 4.6 mm i.d. (Supelco Inc., Bellefont, PA)] was used, preceded by a guard column of the same packing material (Supelguard LC-308, 2 cm × 4.6 mm i.d.). A 0.5-μm precolumn filter (Upchurch Scientific, Oak Harbor, WA) was inserted before all columns or guard columns. Solvents A and B consisted of water and acetonitrile, respectively, each containing 0.1% trifluoroacetic acid. Solvents were placed in reservoirs that minimized evaporation, and were sparged continuously with helium to minimize baseline drift. Column temperature was 30° C unless otherwise stated. Chromatographic profiles were monitored at 210 nm with a detector range of 0.5 absorbance units full-scale (1 V). The gradient employed was linear from 24 to 48% acetonitrile with a flow rate of 1 ml/min and run time of 120 min. The volume injected was 15 μl, with the exception of propanol extracts for which 30 μl were injected to compensate for volume increases (2×) after reduction. It should be noted that slight losses into the solvent peak of less hydrophobic proteins may occur at these injection volumes (Marchylo and Kruger 1988). However, comparable injections were used in all cases, rendering comparisons valid.

Collection and Analysis of Peaks

An LKB model 2211 Superrac (LKB Instruments, Bromma, Sweden) was set to collect groups of peaks eluting from the HPLC detector. Several runs of 30 μl each were necessary to collect sufficient protein material for subsequent SDSGPAGE analysis. Eluted groups of peaks were concentrated to approximately 50 μl in Bio-Rad Unicep Ultracent 30 ultrafiltration cartridges (MW cutoff 30,000) (Bio-Rad, Richmond, CA). Aliquots were reduced and alkylated prior to SDSGPAGE (Marchylo 1987).

Extraction of Wheats

Ground wheat was extracted sequentially in a manner similar to that of ground barley (Marchylo et al 1986) with the solvents: 1) 0.5*M* sodium chloride; 2) 50% 1-propanol; 3) 50% 1-propanol containing 4% DTT; and 4) 50% 1-propanol containing 4% DTT and 1% acetic acid. For simplicity, these will subsequently be called: 1) salt-solubles; 2) propanol-solubles; 3) propanol-DTT-solubles; and 4) propanol-DTT-acetic acid-solubles. The procedure consisted of extraction of the solid (1 g) with 6 ml of solvent for 30 min at 60° C with vortexing every 10 min, followed by centrifugation (25,000 × *g*, 10 min). Solids were extracted twice with various solvents; however, extracts from a particular solvent extraction were not combined. A 10-min wash with deionized water of the pellet following the saline extraction was included to remove occluded salt. For comparison, a total storage protein extract was prepared by salt extraction followed by a water wash and then extraction with 50% 1-propanol containing 4% DTT and 1% acetic acid under the conditions described above. All analyses were carried out in duplicate.

TABLE I
Percent Protein[a] in Sequential Extracts from Different Wheats[b]

Wheat	0.5*M* Sodium Chloride	50% 1-Propanol	50% 1-Propanol, 4% DTT[c]	50% 1-Propanol, 4% DTT, 1% Acetic Acid	Residue
Glenlea	24.2	34.3	19.4	4.0	18.1
Neepawa	24.4	34.1	13.9	4.3	20.3
HY 320	27.4	33.8	13.9	3.3	21.6
Prairie Pride	27.4	35.4	13.3	4.6	19.2
Fielder	31.2	32.7	11.0	2.5	22.7

[a] Percent in each fraction is expressed as a percentage of the sum of the nitrogen in all fractions and the residue.
[b] Wheats are ranked in order of decreasing dough strength. In cases where the extraction is carried out twice with a particular solvent, the result shown is the combined nitrogen content. The small amount of nitrogen in the water wash is included with the salt extract. All values are the mean of duplicate determinations. Average coefficient of variation was 3.47%.
[c] DTT = Diothiothreitol.

SDSGPAGE

Total, sequential extracts (excluding the salt-solubles), and RP-HPLC peak fractions were analyzed electrophoretically by SDSGPAGE (Marchylo 1987) with the following slight modifications in sample preparation. Extracts containing acetic acid were neutralized with 2*N* NaOH prior to alkylation, and the 4-vinylpyridine concentration was increased to 0.56*M* to compensate for the higher (4%) dithiothreitol concentration. Peak fractions were rereduced and alkylated prior to electrophoresis.

Protein Content

Protein content was determined on extracts by the Kjeldahl procedure (N × 5.7) as modified by Williams (1973).

RESULTS

Sequential Extraction of Proteins

The relative percentages of protein extracted by the different extracting solutions are shown in Table I. Approximately 24–31% of the total seed nitrogen of the cultivar consists of albumins and globulins, as well as some nonprotein nitrogenous compounds. Between 32 and 37% of the seed nitrogen is extracted subsequently with 50% 1-propanol, and 11–19% more is solubilized when DTT is added to the 50% 1-propanol. Only a small amount (2.5–4.6%) of additional nitrogen is extracted when 1% acetic acid is included. The remaining 18–23% of the protein (residue) cannot be extracted easily. The insolubility of this fraction suggests that it consists largely of structural proteins (Byers et al 1983). Attempts were made to solubilize additional protein by further extraction with 50% 1-propanol and 4% DTT containing 8*M* urea; RP-HPLC revealed no proteins in this extract, however. Furthermore, the high viscosity of the extract, presumably due to solubilization of starch, makes filtering prior to analysis very difficult.

Qualitative Analysis of Protein Fractions by SDSGPAGE

Proteins extracted with 50% 1-propanol, 50% 1-propanol-DTT and 50% 1-propanol-DTT-acetic acid for the five cultivars of wheat were analyzed by SDSGPAGE (Fig. 1). For comparison, the "total" protein extracted with 50% 1-propanol-DTT-acetic acid also is shown. To render comparisons easier, proteins present in 50% 1-propanol extracts were reduced prior to analysis.

As expected, propanol-soluble proteins consisted largely of gliadins, and the propanol-DTT-solubles consisted of mainly HMW and LMW glutenin subunits. The propanol-DTT-acetic acid-solubles contained additional amounts of HMW and LMW glutenin subunits (Byers et al 1983, Shewry et al 1986).

The separation of protein groups on the basis of solubility is not clear-cut, however. First, although the bulk of sulfur-poor ω-gliadins and many sulfur-rich gliadin components were preferentially extracted with 50% 1-propanol, some HMW glutenin subunits also were extracted. Secondly, with the exception of HY320, ω-gliadins are not completely removed by 50% 1-propanol even after two extractions, and are still present in the propanol-DTT extracts. Additional extractions with 50% 1-propanol prior to 50% 1-propanol-DTT could not completely solubilize these ω-gliadins (also, presumably, α-, β- and γ-gliadins). Although the propanol-DTT-solubles contained the bulk of the HMW and LMW glutenin subunits, further amounts of such components were also extracted by 50% 1-propanol-DTT-acetic acid. The number of HMW glutenin subunits in propanol-DTT and propanol-DTT-acetic acid extracts were similar.

However, some quantitative compositional differences in HMW subunits were observed. This was most noticeable upon comparison of HMW subunits in the propanol-DTT and propanol-DTT-acetic acid-solubles of Fielder wheat.

Separation and Quantitative Analyses of Protein Fractions by RP-HPLC

SDSGPAGE indicated that all extracts in the sequential extraction scheme described above contained some overlap between wheat storage protein groups. Similar problems are encountered with fractionation schemes using other extractants (Bietz and Wall 1975). The present sequential extraction scheme, however, when applied to barley hordeins gave readily separable groups that were amenable to RP-HPLC separation and quantitation (Marchylo et al 1986). In the present study, therefore, preliminary research was carried out using RP-HPLC to determine conditions that would optimize the separation of these storage protein groups. Of particular interest was the separation of HMW glutenin from gliadins and/or LMW glutenin subunits.

Column temperature strongly influences retention times of gliadins and glutenin components (Bietz and Cobb 1985, Marchylo et al 1987) and can improve resolution of selected protein components. RP-HPLC chromatograms of the propanol-solubles (after reduction to facilitate comparisons) and the propanol-DTT-solubles from Neepawa wheat at column temperatures of 25 and 50°C are shown in Figure 2. Although there appeared to be increased resolution as indicated by a larger number of protein components at 50°C compared with 25°C, separation of storage protein groups under consideration in this paper was poorer. Comparison of the 35–45 min region of the propanol and propanol-DTT-soluble protein chromatograms indicates that overlap of HMW glutenin and gliadin components is much worse at 50°C than 25°C due to relative shifts in retention time. Because of this, column temperatures of 25 or 30°C were used in all further experiments. Several runs were carried out at 25°C, and selected chromatographic regions, designated 1, 2, 3, 4A and B, 5A and B, were collected, concentrated, and subjected to SDSGPAGE (Fig. 3). Region 1, present almost entirely in the RP-HPLC chromatogram of the propanol-solubles, consisted of protein species with molecular weights in the ω-gliadin region. Regions 2 and 3 consisted of mainly α-, β-, γ-, and some ω-gliadins. Regions 4A and B were composed largely of HMW glutenin subunits. A small amount of LMW glutenin subunits was observed in fraction 4B. However, the amount relative to HMW glutenin subunits was small. Regions 5A and 5B contained gliadins and LMW glutenin subunits, respectively. These results indicate that it is possible to separate and quantitate HMW subunits in propanol and propanol-DTT extracts by RP-HPLC, since they are reasonably free of cross-contamination by gliadin components. LMW glutenin subunits are primarily present in the propanol-DTT-solubles and elute in the region from 46 min onwards. As such, they also can be quantified by RP-HPLC. Although not shown in Figure 2, it can be anticipated that the location of HMW and LMW glutenin subunits will be analogous in chromatograms of propanol-DTT and propanol-DTT-acetic acid extracts. Experiments carried out using a column temperature of 30°C also allowed similar separations of protein subunit components to those at 25°C, but peaks were somewhat sharper.

RP-HPLC analyses at 30°C of the propanol, propanol-DTT, and propanol-DTT-acetic acid-soluble proteins of the five cultivars analyzed are shown in Figure 4. Chromatograms for the second propanol, propanol-DTT, and propanol-DTT-acetic acid extracts also were obtained but not shown. As with the electrophoretic results, it is apparent from RP-HPLC that the sequential extraction scheme does not completely resolve all subunits into clear-cut groups. For example, HMW glutenins

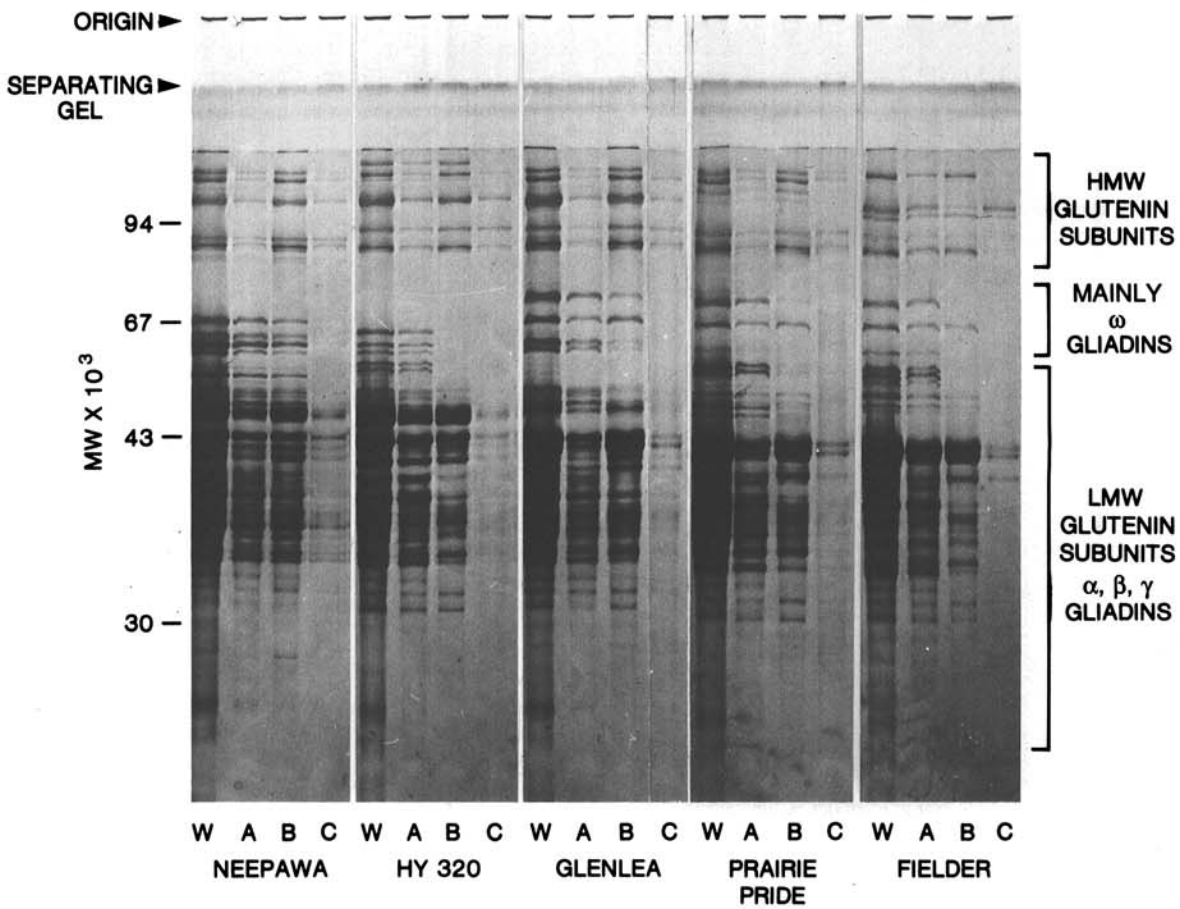

Fig. 1. Sodium dodecyl sulfate gradient polyacrylamide gel electrophoretic patterns of proteins present in sequential extracts of Neepawa, HY 320, Glenlea, Prairie Pride, and Fielder wheat. **W** = single extract of ground wheat (propanol-dithiothreitol [DTT]-acetic acid); **A** = propanol-soluble; **B** = propanol-DTT-soluble; **C** = propanol-DTT-acetic acid-soluble.

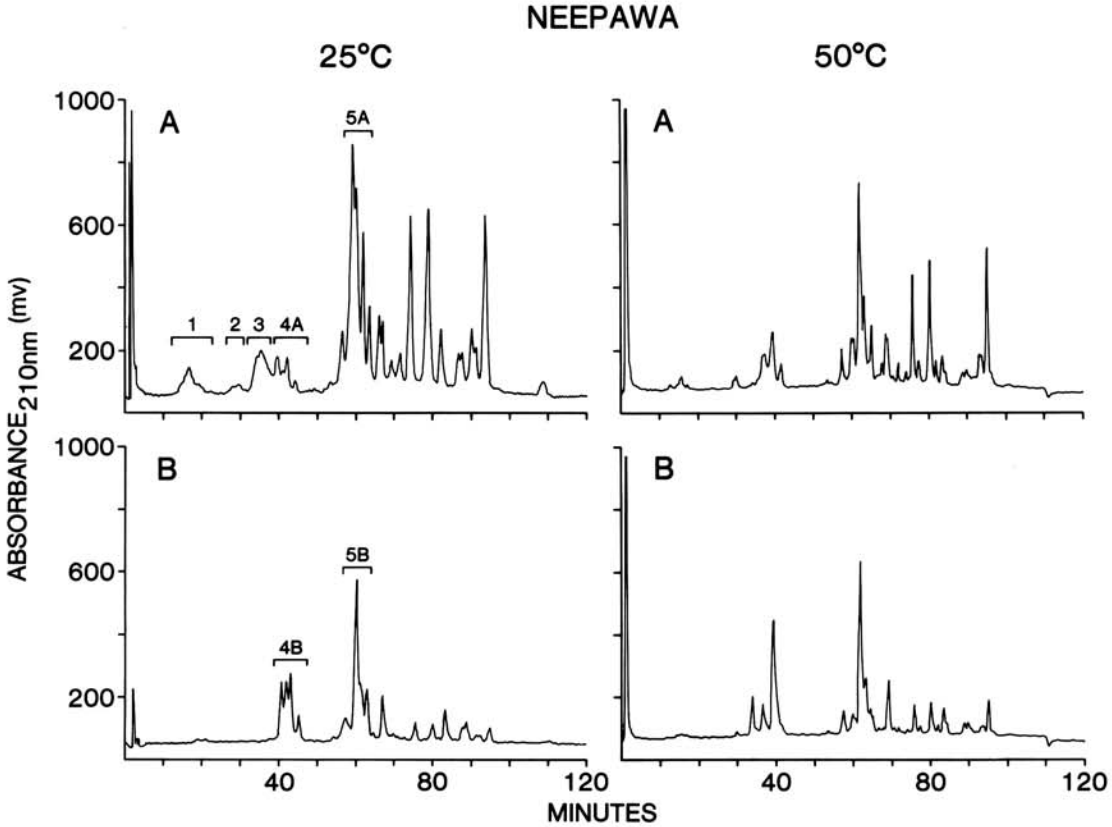

Fig. 2. Reversed-phase high-performance liquid chromatography separation of proteins present in sequential propanol (**A**) and propanol-DTT (**B**) extracts of Neepawa wheat using column temperatures of 25 and 50° C.

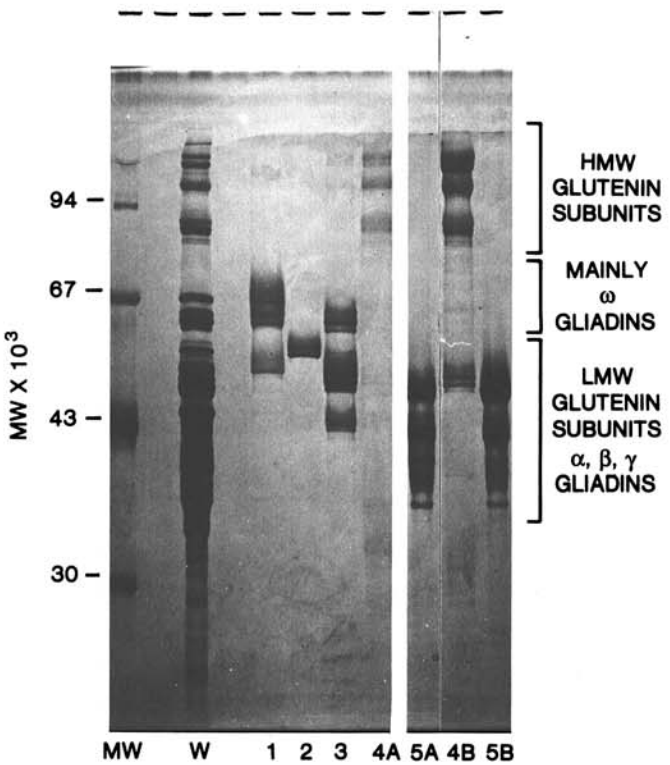

Fig. 3. Sodium dodecyl sulfate gradient polyacrylamide gel electrophoretic patterns of protein fractions from reversed-phase high-performance liquid chromatography (Fig. 2) (designated as **1, 2, 3, 4A, 5A, 4B, 5B**) and of a ground wheat extract (**W**). **MW** refers to molecular weight markers.

(elution times 35–45 min) from some wheats were extracted by propanol, with amount varying among cultivars. Secondly, ω-gliadins (elution times 12–22 min) of some wheats, i.e., Glenlea, were incompletely extracted by propanol. As also shown by electrophoresis, certain HMW glutenin subunits were extracted preferentially, as indicated by changes in relative peak areas in the propanol-DTT extracts compared with those of propanol-DTT-acetic acid. This is evident particularly with the SWS wheat Fielder. The ratio of HMW to LMW glutenin subunits in the propanol-DTT extract relative to that in the propanol-DTT-acetic acid extract also varied, depending on cultivar.

Quantitative analyses of amounts of HMW glutenin subunits, LMW glutenin subunits, ω-gliadins, and total remaining gliadins in the various sequential extracts were carried out following RP-HPLC at 25° C for the five cultivars. RP-HPLC elution ranges for quantitative analyses of a particular group of proteins varied slightly from cultivar to cultivar and were chosen to minimize cross-contamination. Areas were quantified using the scanner mode of the Waters LC Multisystem version 3.0 software. Determination of areas is reproducible within a coefficient of variation of less than 2% (Marchylo et al 1987). The results are shown in Table II. It is realized that the α-, β-, γ-gliadin fraction in the propanol-soluble fraction may contain LMW glutenins, since HMW glutenin subunits are present. In addition, alcohol-soluble aggregates, i.e., HMW gliadin or LMW glutenin (Bietz and Wall 1980), would be interpreted as gliadin. In the propanol-DTT extracts, slight contamination of the HMW and LMW glutenin subunits by gliadins may also occur. This contamination would be more prevalent where ω-gliadins are detected, such as in Glenlea wheat. Table II indicates that there are substantial differences between cultivars in the relative proportions of particular storage protein groups extracted into various solvents. For example, substantially more HMW glutenin subunits (as a proportion of the total HMW glutenin) were extracted with propanol from Fielder than the other cultivars. A small proportion of the HMW glutenin subunits is insoluble, however, requiring acetic acid in the propanol-DTT solvent to facilitate its extraction. A number of ratios were determined in order to evaluate whether they might be useful for ranking the wheats in terms of their relative dough strengths (i.e., Fielder < Prairie Pride < HY320 < Neepawa < Glenlea). The most promising ratios in this regard are shown in Figure 5. Thus, the ratio of HMW to LMW glutenin subunits in the

propanol-DTT-solubles increased with increasing strength (Fig. 5A). The exception was HY 320, which ranked as a very strong wheat. With this exception, results confirmed similar findings by Huebner and Bietz (1985) that indicated the percentage of HMW subunits in reduced glutenins generally increases with increasing mix times for different hard red winter wheat cultivars.

Another ratio that related to anticipated ranking in terms of dough strength was the ratio of HMW subunits extracted in propanol to those extracted in propanol-DTT and propanol-DTT-acetic acid (Fig. 5B). Stronger wheats (with the exception of HY320) contained progressively fewer HMW subunits extractable into the propanol solvent.

Except for Prairie Pride wheat, the amount of LMW glutenin subunits extractable by propanol-DTT and propanol-DTT-acetic acid relative to the α-, β-, γ-gliadins extracted by propanol also increased with stronger wheats (Fig. 5C). This ratio might be anticipated, because wheats with a large amount of extractable HMW glutenins in the propanol-solubles, i.e., Fielder, may

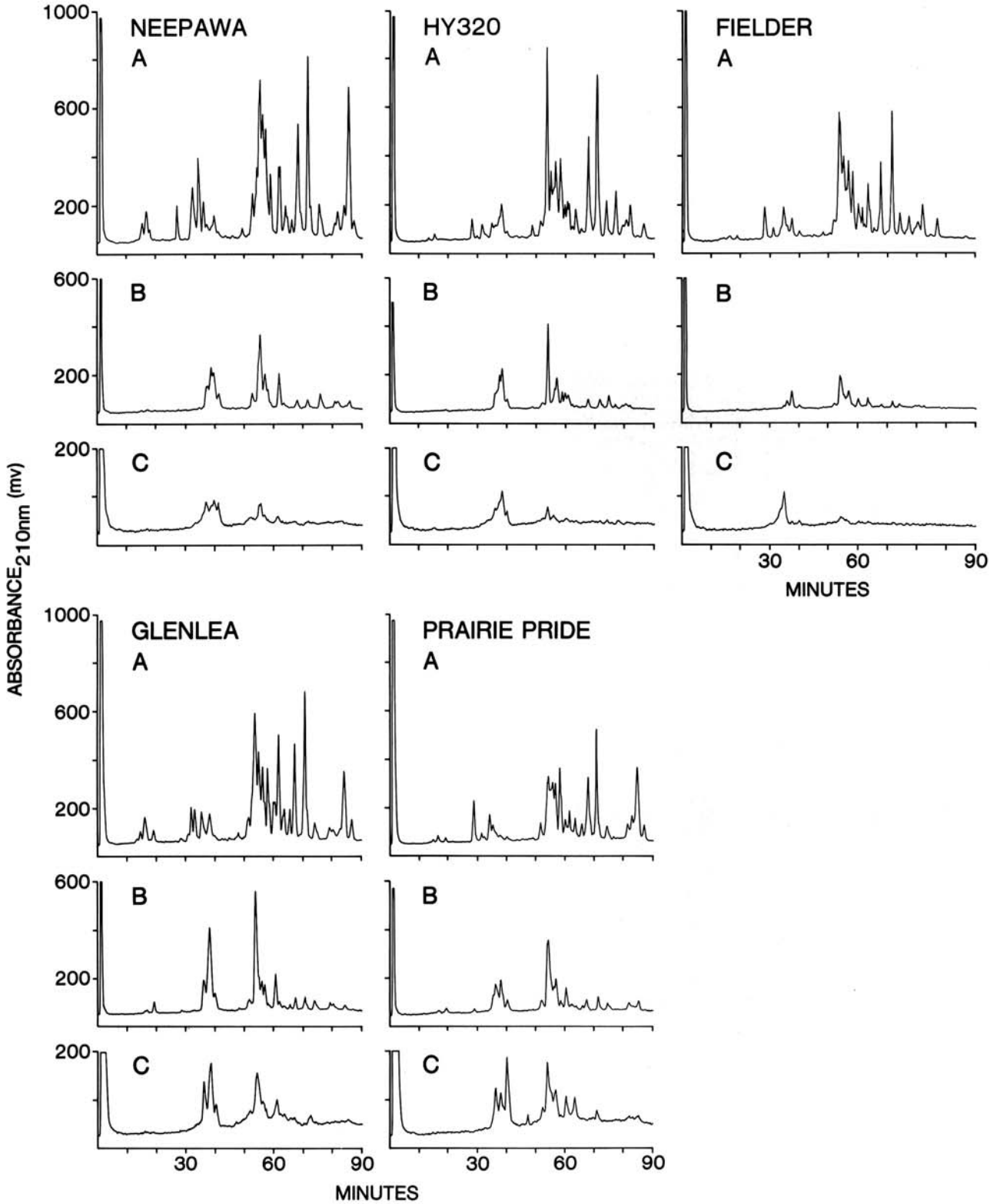

Fig. 4. Reversed-phase high-performance liquid chromatography separations of proteins in sequential propanol (**A**), propanol-dithiothreitol (DTT) (**B**), and propanol-DTT-acetic acid (**C**) extracts of Neepawa, HY 320, Glenlea, Prairie Pride, and Fielder wheat.

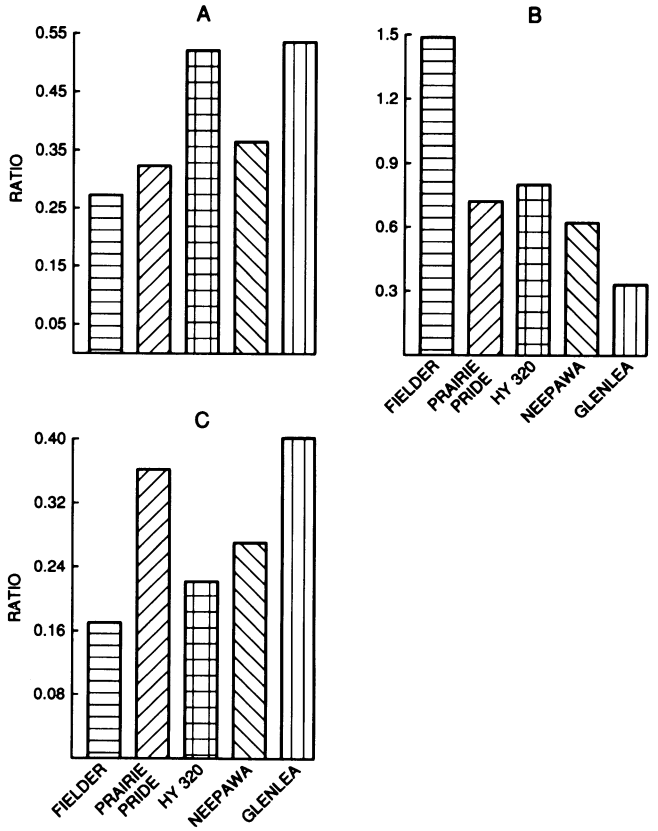

Fig. 5. Ratio of selected protein fraction areas in sequential extracts. Cultivars are ranked in order of increasing relative dough strength (Fielder → Glenlea). (**A**) Ratio of high (HMW) to low molecular weight (LMW) glutenin present in propanol-DTT extracts; (**B**) ratio of HMW glutenin subunits in propanol extracts to those present in the combined propanol-dithiothreitol (DTT) and propanol-DTT-acetic acid extracts; (**C**) ratio of total LMW glutenins (propanol-DTT and propanol-DTT-acetic acid) to total α-, β-, and γ-gliadins (propanol extracts).

contain a substantial amount of extractable LMW glutenin subunits that would be interpreted as gliadins in this paper. In the propantol-DTT solubles, relatively less LMW glutenin subunit proteins would result, leading to a low apparent ratio of LMW glutenins to gliadins. The converse would hold true for a wheat with a high ratio of LMW glutenins to gliadins such as Neepawa.

Other ratios, such as the HMW to LMW glutenin subunits in the total propanol-DTT and the propanol-DTT-acetic acid, were tested but did not properly rank cultivars in terms of strength.

DISCUSSION

Because of its efficiency in solubilizing wheat proteins (Byers et al 1983), the solvent 50% 1-propanol with and without reductant and acetic acid was assessed for its potential in extracting wheat storage proteins into groups that were more amenable to further separation and quantitation by RP-HPLC. The extraction scheme was efficient in that no further removal of storage proteins occurred with more rigorous extraction. This confirmed previous results by Byers et al (1983) in which a similar extraction procedure at 60° C exhaustively extracted all the storage proteins from wheat.

Differing amounts of HMW polypeptide material corresponding to HMW glutenin subunits were extracted from the five wheat cultivars with 50% 1-propanol at 60° C. Byers et al (1983) also found that this protein was extracted. Such HMW protein was extracted at 4° C as well as 60° C in their study, indicating that a lower temperature would not eliminate extraction of this material by 50% 1-propanol. Using a column temperature of 25° C, RP-HPLC separated and, hence, enabled quantification of the amount of HMW glutenin present in this fraction. The amount present in the 50% propanol-solubles in relation to that present in the propanol-DTT and propanol-DTT-acetic acid-solubles varied from cultivar to cultivar and, with one exception, ranked wheats on the basis of their strength.

Huebner and Bietz (1985) showed that the ratio of HMW to LMW glutenin subunits as determined by RP-HPLC or SE-HPLC can be used to determine the quality of wheats used to make bread. Their procedure for RP-HPLC consists of initially removing salt-soluble and alcohol-soluble gliadins. This is followed by extracting some glutenin, plus residual gliadins, with acetic acid, reprecipitating the glutenin by adding ethanol to 70%, and bringing the pH to neutrality, and finally, reduction and alkylation, which solubilizes all glutenin subunits. The present sequential extraction seems equally satisfactory for obtaining a fraction containing HMW and LMW subunits that are amenable to quantitation; the ratio of HMW and LMW glutenin subunits in the propanol-DTT extracts, with one exception, ranked wheats in order of anticipated relative strength. Future research is planned with a large number of cultivars to examine the extent to which such ratios are useful for predicting dough strength. The sequential extraction scheme is particularly suitable for use in this laboratory because cultivar identification of wheat and barley cultivars is performed using the same columns, gradient, etc., and with 50% 1-propanol-DTT, with or without acetic acid as extractant (Kruger and Marchylo 1985, Marchylo and Kruger 1984, Marchylo et al 1987). Furthermore, as pointed out in previous studies (Miflin and Shewry 1979, Byers et al 1983), the use of this fractionation scheme gives a more complete extraction of storage proteins than others such as the Osborne (Osborne 1924) or modified Osborne procedures (Chen and Bushuk 1970). Because there is less cross-contamination of storage protein groups, this in turn facilitates their subsequent separation by RP-HPLC.

TABLE II
Composition of Storage Proteins in Propanol (A), Propanol-DTT (B), and Propanol-DTT[a]-Acetic Acid Extracts (C) of Wheat

Wheat	Extracting Solvent	Peak Areas ($\times 10^7$)			
		Glutenin Subunits[b]		Gliadins	
		HMW	LMW	ω	α, β, γ
Glenlea					
	A	3.2	···	2.4	42.9
	B	8.7	16.4	0.4	···
	C	1.0	0.6	···	···
	Total	12.9	17.0	2.8	42.9
Neepawa					
	A	3.4	···	2.3	53.1
	B	4.9	13.5	0.3	···
	C	0.8	0.7	···	···
	Total	9.1	14.2	2.6	53.1
HY 320					
	A	4.4	···	1.0	44.7
	B	4.8	9.4	Trace	···
	C	0.8	0.4	···	···
	Total	10.0	9.8	1.0	44.7
Prairie Pride					
	A	3.4	···	0.9	39.5
	B	4.3	13.6	0.3	···
	C	0.9	0.6	···	···
	Total	8.6	14.2	1.2	39.5
Fielder					
	A	3.5	···	0.8	36.2
	B	1.6	5.9	···	···
	C	0.7	0.1	···	···
	Total	5.8	6.0	0.8	36.2

[a] DTT = Dithiothreitol.
[b] HMW = High molecular weight, LMW = low molecular weight.

ACKNOWLEDGMENTS

The authors would like to acknowledge the competent technical assistance of P. Funk and K. Handel.

LITERATURE CITED

BIETZ, J. A. 1984. Analysis of wheat gluten proteins by reversed-phase high-performance liquid chromatography. Part 1. Bakers Dig. 58:15.

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.

BIETZ, J. A., and WALL, J. S. 1975. The effect of various extractants on the subunit composition and associations of wheat glutenin. Cereal Chem. 52:145.

BIETZ, J. A., and WALL, J. S. 1980. Identity of high molecular weight gliadin and ethanol-soluble glutenin subunits of wheat: Relation to gluten structure. Cereal Chem. 57:415.

BYERS, M., MIFLIN, B. J., and SMITH, S. J. 1983. A quantitative comparison of the extraction of protein fractions from wheat grain by different solvents, and of the polypeptide and amino acid composition of the alcohol-soluble proteins. J. Sci. Food Agric. 34:447.

BURNOUF, T., and BIETZ, J. A. 1984. Reversed-phase high-performance liquid chromatography of reduced glutenin, a disulfide-bonded protein of wheat endosperm. J. Chromatogr. 299:185.

CANADIAN GRAIN COMMISSION. 1987. Quality of Canadian Wheat 1987. The Commission: Winnipeg, Manitoba, Canada.

CHEN, C. H., and BUSHUK, W. 1970. Nature of proteins in *Triticale* and its parental species. I. Solubility characteristics and amino acid comosition of endosperm proteins. Can. J. Plant Sci. 52:1021.

HUEBNER, F. R., and BIETZ, J. A. 1985. Detection of quality differences among wheats by high-performance liquid chromatography. J. Chromatogr. 327:333.

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.

KRUGER, J. E., and MARCHYLO, B. A. 1985. Selection of column and operating conditions for reversed-phase high-performance liquid chromatography of proteins in Canadian wheat. Can. J. Plant Sci. 65:285.

MARCHYLO, B. A. 1987. Identification of Canadian and American wheat cultivars by SDS gradient PAGE analysis of gliadin and glutenin subunits. Can. J. Plant Sci. 67:945.

MARCHYLO, B. A., and KRUGER, J. E. 1984. Identification of Canadian barley cultivars by reversed-phase high-performance liquid chromatography. Cereal Chem. 61:295.

MARCHYLO, B. A., and KRUGER, J. E. 1988. The effect of injection volume on the quantitative analysis of gliadins and glutenins by RP-HPLC. Cereal Chem. 65:000.

MARCHYLO, B. A., KRUGER, J. E., and HATCHER, D. 1986. High-performance liquid chromatographic and electrophoretic analysis of hordein during malting for two barley varieties of contrasting malting quality. Cereal Chem. 63:219.

MARCHYLO, B. A., HATCHER, D. W., and KRUGER, J. E. 1988. Identification of wheat cultivars by reversed-phase high-performance liquid chromatography of storage proteins. Cereal Chem. 65:28.

MIFLIN, B. J., and SHEWRY, P. R. 1979. The biology and biochemistry of cereal seed proteins. Page 137 in: Seed Protein Improvement in Cereals and Grain Legumes, Vol. 1. Proc. Ser. STI/PUB/496. Int. Atomic Energy Agency (IAEA): Vienna.

OSBORNE, T. B. 1924. In: The Vegetable Proteins, 2nd ed. Longmans, Green and Co.: London.

PAYNE, P. I., CORFIELD, K. G., and BLACKMAN, J. A. 1979. Identification of a high-MW subunit of glutenin whose presence correlates with bread-making quality in wheats of related pedigree. Theor. Appl. Genet. 55:153.

PAYNE, P. I., HOLT, L. M., and LAW, C. N. 1981. Structural and genetical studies on the high-molecular-weight subunits of wheat glutenin. I. Allelic variation in subunits amongst varieties of wheat (*Triticum aestivum*). Theor. Appl. Genet. 60:229.

PAYNE, P. I., HOLT, L. M., JACKSON, E. A., and LAW, C. N. 1984. Wheat storage proteins: Their genetics and their potential for manipulation by plant breeding. Philos. Trans. R. Soc. London Ser. B 304:359.

SHEWRY, P. R., MIFLIN, B. J., and KASARDA, D. D. 1984. The structural and evolutionary relationships of the prolamin storage proteins of barley, rye and wheat. Philos. Trans. R. Soc. Lond. Ser. B 304:297.

SHEWRY, P. R., TATHAM, A. S., FORDE, J., KREIS, M., and MIFLIN, B. 1986. The classification and nomenclature of wheat gluten proteins: A reassessment. J. Cereal Sci. 4:97.

WILLIAMS, P. C. 1973. The use of titanium dioxide as catalyst for large-scale Kjeldahl determination of the total nitrogen content of cereal grains. J. Sci. Food Agric. 24:343.

[Received June 18, 1987. Revision received December 7, 1987. Accepted December 16, 1987.]