Rapid Purification of Wheat Glutenin for Reversed-Phase High-Performance Liquid Chromatography: Comparison of Dimethyl Sulfoxide with Traditional Solvents

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ABSTRACT

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Nondefatted and defatted wheat flours were extracted with various solvents to develop a rapid procedure to isolate glutenin for reversed-phase high-performance liquid chromatography (RP-HPLC). Solvents containing 70% ethanol, 60% acetonitrile, $2M$ dimethylformamide, urea, or combinations of 70% ethanol with acetic acid and β-mercaptoethanol did not extract all albumins, globulins, and/or gliadins. Some of these proteins may co-elute with glutenin subunits upon RP-HPLC. However, acidic solvents, such as 0.7% acetic acid and aluminum lactate-lactic acid buffer, pH 3.1, efficiently extracted soluble proteins and gliadin so that remaining proteins gave RP-HPLC patterns like glutenin purified by established procedures. Glutenin could be most conveniently isolated, however, by extracting nonglutenin proteins with >90% (v/v) dimethyl sulfoxide, followed by washing the pellet with 70% ethanol. Glutenin remaining in the residue was readily soluble after reduction and pyridylethylation, and could be analyzed by RP-HPLC. Dimethyl sulfoxide extraction also largely freed glutenin of starch, thus favoring sample stability, RP-HPLC resolution, and long column lifetime. Many samples can be processed easily by this method. The procedure should help breeders and geneticists select for specific glutenin compositions that relate to quality.

Glutenin is the alcohol-insoluble high-molecular weight (HMW) class of wheat flour proteins. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) reveals that it contains many low molecular weight (LMW, approximately 10–70 kDa) and HMW (approximately 80 to 130 kDa) subunits, joined by disulfide bonds into HMW polymers (Bietz and Wall 1972). Glutenin directly influences dough strength (Wall 1979); many studies have related its composition (especially its HMW subunits) to breadmaking quality (Payne et al 1979, Burnouf and Bouriquet 1980, Moonen et al 1982).

Reversed-phase high-performance liquid chromatography (RP-HPLC) is an excellent method for separating cereal proteins, including glutenin subunits (Bietz 1983, 1986; Burnouf and Bietz 1984, 1985). RP-HPLC resolves 20 or more glutenin subunits in as little as one hour. HMW glutenin subunits elute early, and LMW polypeptides elute later. Subunit compositions of glutenins from bread wheat (Burnouf and Bietz 1984) and durum wheat (Burnouf and Bietz, *unpublished*) varieties differ, suggesting that RP-HPLC of glutenin could help identify varieties and predict wheat's technological properties (Burnouf and Bietz 1987).

To achieve reproducible high-resolution RP-HPLC separations of glutenin subunits, however, wheat albumins, globulins, and gliadins must first be removed, because some co-elute with glutenin peaks. Sequential extraction (usually based on differential solubility) and pH-precipitation (Bietz et al 1975) can isolate glutenin. These procedures are time-consuming, however, especially when is is necessary to analyze many samples. The glutenin-containing residue is also rich in starch, some of which may be solubilized after reduction and alkylation of glutenin before RP-HPLC. Such starch may precipitate upon injection of samples for HPLC, plugging columns or decreasing their lifetimes.

We therefore prepared glutenin by different methods, and compared RP-HPLC patterns of resulting reduced-alkylated subunits. Our primary goal was to devise a simple procedure for preparing glutenin essentially free of other protein classes, so its

HMW subunits could be easily analyzed by RP-HPLC. Of the procedures tested, preextraction with 90–100% dimethyl sulfoxide (DMSO) most conveniently extracted non-glutenin proteins, yielding pellets low in soluble starch from which glutenin sufficiently pure for RP-HPLC may be extracted. This procedure also permits single-kernel glutenin analysis, and should be useful to geneticists, breeders, and other researchers.

MATERIALS AND METHODS

Materials

Centurk hard red winter wheat, grown in 1979, was milled in a Brabender Quadruplex mill to approximately 70% extraction. Flour was either used as is, or defatted with 1-butanol followed by pentane-hexane (Bietz et al 1984).

Chemicals and Reagents

All chemicals were reagent-grade or better. HPLC-grade acetonitrile (ACN) was from Fisher, and trifluoroacetic acid (TFA) from Pierce. Water for HPLC was from a Barnstead Nanopure system.

Extraction

Nondefatted or defatted flour was extracted twice with 1) 0.7% acetic acid (v/v); 2) 70% ethanol (v/v); 3) 70% ethanol + 0.7% acetic acid + 1% β-mercaptoethanol (v/v); 4) 60% ACN (v/v); 5) $2M$ urea; 6) $8M$ urea; 7) $0.0085M$ aluminum lactate-lactic acid buffer, pH 3.1; 8) $2M$ dimethylformamide (DMF); or 9) 50, 90, or 100% DMSO (v/v). Flour (50 mg) and solvent (6 ml) were mixed for 30 min at room temperature, centrifuged (10 min at $27,000 \times g$), and supernatants discarded. Pellets, except those from the DMSO extraction, were directly freeze-dried. Portions of dried pellets from most procedures were further extracted with $0.05M$ Tris hydroxymethylaminomethane (Tris), pH 7.5, under the same conditions, to remove contaminants from previous extractions and to adjust sample pH to that suitable for disulfide bond reduction; pellets were then again freeze-dried. Samples were stored at room temperature until processed for RP-HPLC.

Pellets remaining after extraction of flour with DMSO were washed with the same volume of 70% ethanol. After centrifugation (10 min at $27,000 \times g$), moist pellets were stored at 4°C.

For comparison, glutenin was also isolated by sequential extraction and pH precipitation (Bietz et al 1975). In this procedure, flour is extracted with $0.04M$ NaCl and then with 70% (v/v) ethanol. The residue is then suspended in 0.7% acetic acid, ethanol is added to 70%, and the suspension is neutralized. Glutenin essentially free of other proteins precipitates upon cooling.

Protein Reduction and Alkylation

Forty milligrams of freeze-dried pellets, or the precipitate from the DMSO extraction, were suspended in 750 μl of 0.05M Tris-HCl-8M urea, pH 7.5. Disulfide bonds were reduced with 5% β-mercaptoethanol for 2 hr at room temperature, and alkylated to form pyridylethyl (PE) derivatives by adding 60 μl of 4-vinyl-pyridine (equimolar to sulfhydryl groups) for 2 hr (room temperature); the reaction was stopped by adding 500 μl of glacial acetic acid (Burnouf and Bietz 1984). This procedure quantitatively extracts all wheat flour proteins, including glutenin (Bietz et al 1975). Samples were centrifuged (15 min at 35,000 × g). Supernatants were stored at room temperature and analyzed by RP-HPLC within two days; under such conditions, such samples are stable for at least one month (Burnouf and Bietz 1984).

RP-HPLC

Instrumentation for RP-HPLC was described previously (Bietz 1983). A 250 × 4.1 mm Brownlee Aquapore RP-300 column (C$_8$, 300 Å pores, 10 μm particle size, end-capped) protected by a guard column containing a similar packing of larger particle size was used. Solvents were 0.1% TFA in water (solvent A) or in ACN (solvent B). Proteins were eluted at 1.0 ml/min with a linear gradient from 21 to 48% solvent B over 55 min. The column was reequilibrated with solvent A for 10 min between runs. The column effluent was monitored at 210 nm (0.2 absorption units full scale). Data were recorded on a Houston Instruments Omniscribe recorder (10 mV full scale). Results were also stored, compared, and plotted by a ModComp computer system. The solvent peak, eluting at the void volume (2–3 min), was not plotted. Retention times were reproducible, typically differing by less than 0.1 min for replicates within a series of runs (Bietz 1983, Burnouf and Bietz 1985).

RESULTS AND DISCUSSION

Effect of Defatting

Reduced and alkylated total protein extracts from nondefatted and defatted flours were compared by RP-HPLC to PE-glutenin purified by sequential extraction and pH precipitation (Bietz et al 1975) (Fig. 1). This was done to determine whether defatting of flour is necessary, and to find to what extent albumins, globulins, and gliadins co-elute with glutenin subunits.

Chromatograms with more than 35 peaks and shoulders resulted for PE-proteins from nondefatted (Fig. 1A) or defatted (Fig. 1B) flour. Slight qualitative and quantitative differences and differences in resolution occurred between these samples. These results suggest that some lipoproteins are extracted, and their RP-HPLC elution characteristics change upon defatting, or that interactions among gluten proteins may change upon defatting, modifying surface hydrophobicities and elution times. Overall resolution also improves upon defatting, particularly for proteins of medium (e.g., peak 1) and high (e.g., peak 2) surface hydrophobicities. Thus, defatting appears to specifically modify certain proteins.

Comparison of Total Protein Extracts with Glutenin

The effect of extracting soluble proteins and gliadins upon the RP-HPLC pattern of PE-glutenin is shown in Figure 1 (A and C). The overall patterns were quite different. Many early eluting polypeptides (groups A and B) and those of intermediate hydro-phobicity (groups C and D) are extracted, confirming that NaCl and ethanol extract albumins, globulins, or gliadins, leaving glutenin in the residue. SDS-PAGE revealed that PE-glutenin peaks a, b, c, and d contain HMW (above approximately 90,000 kDa) glutenin subunits (Burnouf and Bietz 1984, 1985); similarly, later-eluting subunits (peaks e to l) are LMW glutenin subunits. Generally, polypeptides in each major glutenin and gliadin protein class (HMW glutenin subunits, LMW glutenin subunits, ω-gliadins, α/β-gliadins, and γ-gliadins) have hydrophobicities similar to each other but different from proteins of other classes (Bietz and Burnouf 1985). Albumins and globulins, however, contain polypeptides that may co-elute with proteins of other
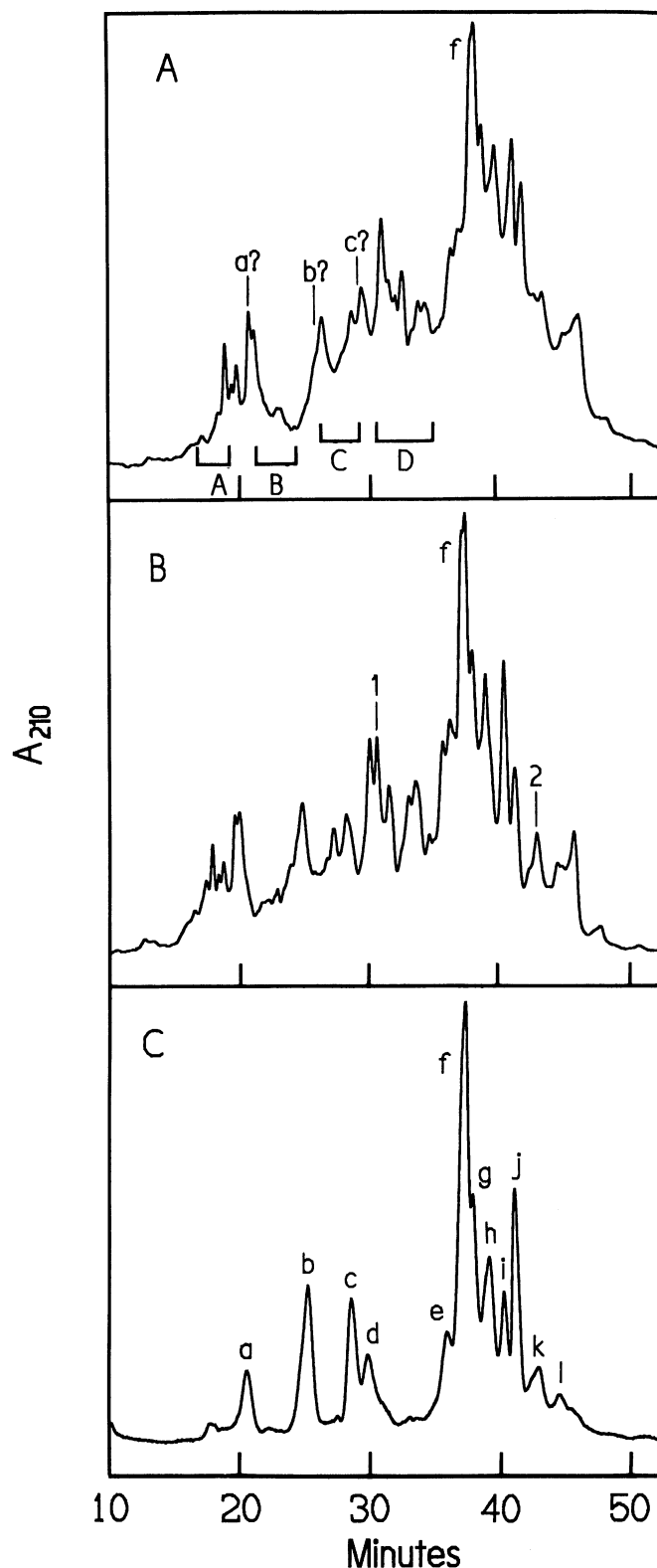

Fig. 1. Reversed-phase high-performance liquid chromatography of pyridylethyl-derivatives of total proteins extracted from nondefatted (A) or defatted (B) Centurk flour, and (C) of glutenin isolated from nondefatted Centurk flour (Bietz et al 1975). Samples were suspended in 0.05M Tris-8M urea, pH 7.5, reduced with β-mercaptoethanol (2 hr at room temperature), and alkylated with 4-vinylpyridine (2 hr, room temperature). Proteins were eluted with a linear gradient from 21 to 48% acetonitrile (+0.1% TFA) during 55 min at 1 ml/min; temperature was 30–31° C. The solvent peak (2–5 min) is not plotted. Peaks designated by lowercase letters in (A) and (B) contain probable glutenin subunits, as inferred by comparison to peaks a–1 in (C). Peaks 1 and 2 in (B) indicate regions of medium and high hydrophobicity, where resolution is significantly improved by defatting. Areas A through D designate peaks of low and intermediate hydro-phobicity, as discussed in text.

classes (Bietz 1983).

Subunits of PE-glutenin purified by sequential extraction thus appear better separated and are easier to analyze by RP-HPLC when proteins that may co-elute are absent. For example, rapid prediction of bread wheat quality by analysis of HMW glutenin
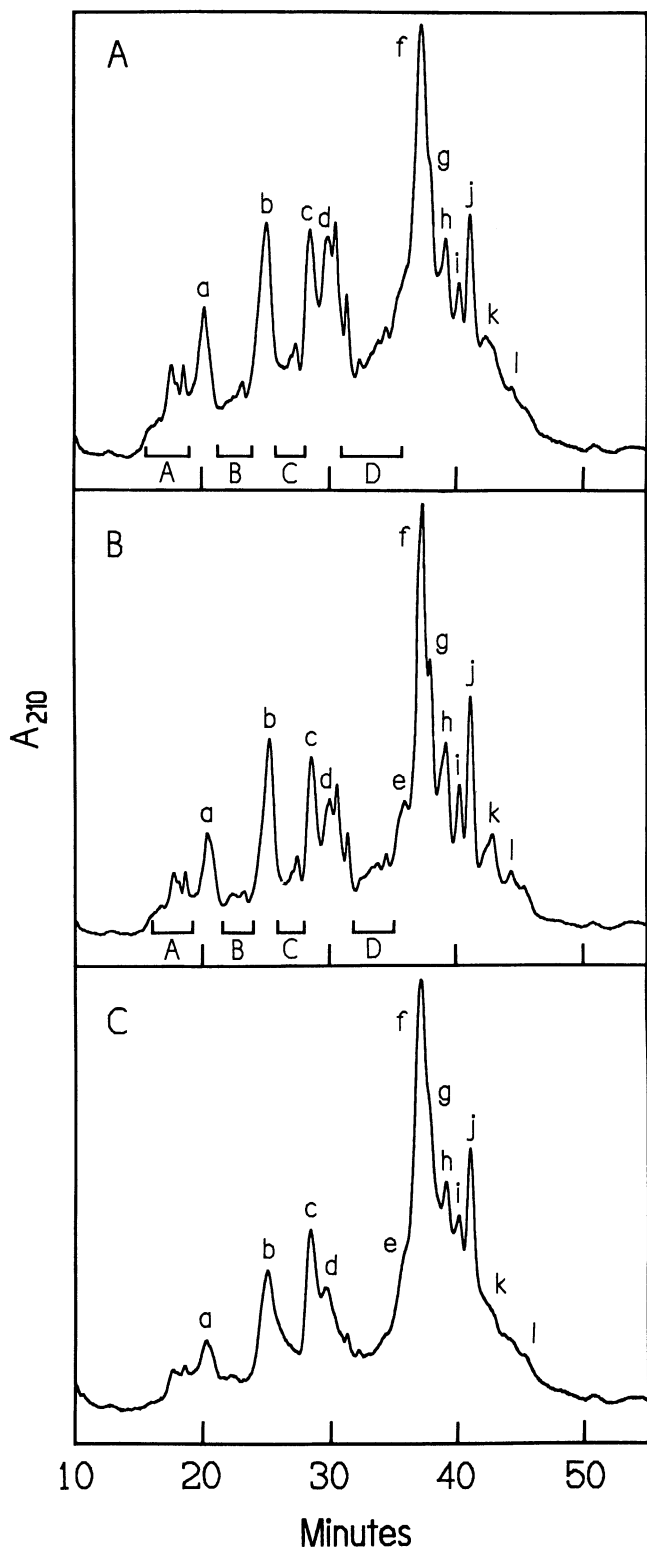
subunits in total protein extracts appears impossible by RP-HPLC since HMW subunits are obscured by co-eluting polypeptides. Similar genetic studies involving glutenin analysis require preextraction of other proteins.

Alternative Glutenin Extraction Procedures

Sequential extraction is time-consuming, especially when many samples are involved. We therefore investigated 11 procedures to purify glutenin from defatted and nondefatted flours. In addition, portions of centrifuged pellets from most procedures were extracted twice with $0.05M$ Tris, pH 7.5. This extraction removed traces of previous extractants, adjusted the pH to that optimal for disulfide bond reduction, and showed the influence of additional extraction with a low-ionic strength buffer. In each case, we compared PE-residue proteins to PE-glutenin isolated by sequential extraction and pH-precipitation (Bietz et al 1975).

70% Ethanol

Figure 2A shows the RP-HPLC separation of PE-residue proteins after extracting nondefatted flour with 70% ethanol. Defatted flour gave a similar pattern (Fig. 2B), but slight quantitative differences occurred for some peaks (e.g., c and d). In addition to prolamins, aqueous alcohols may extract lipoproteins or other proteins with high affinity for lipids, or may reduce hydrophobic interactions between proteins (Zawistowska et al 1986). Subtle differences between 70% ethanol extracts of defatted and nondefatted flours of the same variety were also observed previously (Bietz et al 1984).

Ethanol solubilizes proteins varying widely in surface hydrophobicities (compare Figs. 2A and 1A). Major early peaks in Figure 1A are absent or much reduced after ethanol extraction; many of these apparently are α-, β-, γ-, and ω-gliadins of low and intermediate hydrophobicities. Peaks a, b, c, and d (Fig. 2A) appear better separated than when ethanol extraction was not done (Fig. 1A). Comparing Figure 2A with 1C (PE-glutenin) reveals that peaks a–d in Figure 2A are HMW glutenin subunits, which co-elute with other proteins (Bietz 1983). HMW glutenin subunits constitute only 7–14% of wheat endosperm proteins, since glutenin is about 40% of all endosperm proteins and HMW subunits are 18–35% of glutenin (Burnouf and Bietz 1985). Field et al (1983) also stated that HMW polypeptides are 7–19% of total flour prolamins. Thus, HMW glutenin subunits are largely masked by other proteins during RP-HPLC of a total protein extract (Fig. 1A).

Comparison of glutenin (Fig. 1C) with proteins not extracted by 70% ethanol (Fig. 2A) also shows that resolution of late-eluting proteins is similar. These peaks contain largely LMW glutenin subunits, soluble in ethanol only after disulfide bond reduction. As expected, when ethanol-extracted flour was reextracted with $0.05M$ Tris, pH 7.5 (Fig. 2C), the resulting residue also lacked some hydrophilic proteins; the pattern was similar to that of PE-glutenin (Fig. 1C). Extraction with only 70% ethanol, however, does not remove all nonglutenin proteins.

Acetonitrile

After nondefatted and defatted flours were extracted with 60% ACN (Fig. 3A and B, respectively), residues were similar to those remaining after ethanol extraction (Fig. 2). Aluminum lactate PAGE patterns of proteins soluble in 70% ethanol and 60% ACN are also similar (unpublished). Thus, 70% ethanol and 60% ACN are comparable in their ability to extract endosperm proteins; like ethanol, ACN cannot exhaustively extract non-glutenin proteins. Reextraction of the pellet with Tris buffer (Fig. 3C) removed, as for ethanol-extracted flour, proteins of low and medium hydrophobicity.

Acidic 70% Ethanol

Figure 4A shows the RP-HPLC pattern of PE-proteins remaining after extraction of nondefatted flour with 0.7% acetic acid + 70% ethanol. The pattern is simpler than that after extraction with ethanol alone (Fig. 2A); many early-eluting proteins (near a–d in Fig. 2A) are soluble in acidic, but not neutral,

Fig. 2. Reversed-phase high-performance liquid chromatography of pyridylethyl-derivatives of residue proteins after 70% ethanol extraction of (**A**) nondefatted or (**B**) defatted flour. (**C**) illustrates the chromatographic separation of the residue protein of (A) following subsequent extraction with $0.05M$ Tris-HCl, pH 7.5. Areas A, B, C, and D show zones of elution affected by the chemical nature of the solvents. Letters a–l identify glutenin subunit peaks in "standard" glutenin. Other experimental conditions are as in Fig. 1.

70% ethanol. Results for defatted flour (not shown) were similar to those using nondefatted flour. The residue after acidic ethanol extraction (Fig. 4A) is also similar to standard PE-glutenin (Fig. 1C). Further extraction with Tris buffer did not solubilize additional proteins (Fig. 4B). Most albumins and globulins are highly charged at acidic pH, so good solubility in acidic 70% ethanol is expected. Thus, 0.7% acetic acid + 70% ethanol is a good solvent for all wheat endosperm proteins except native glutenin.
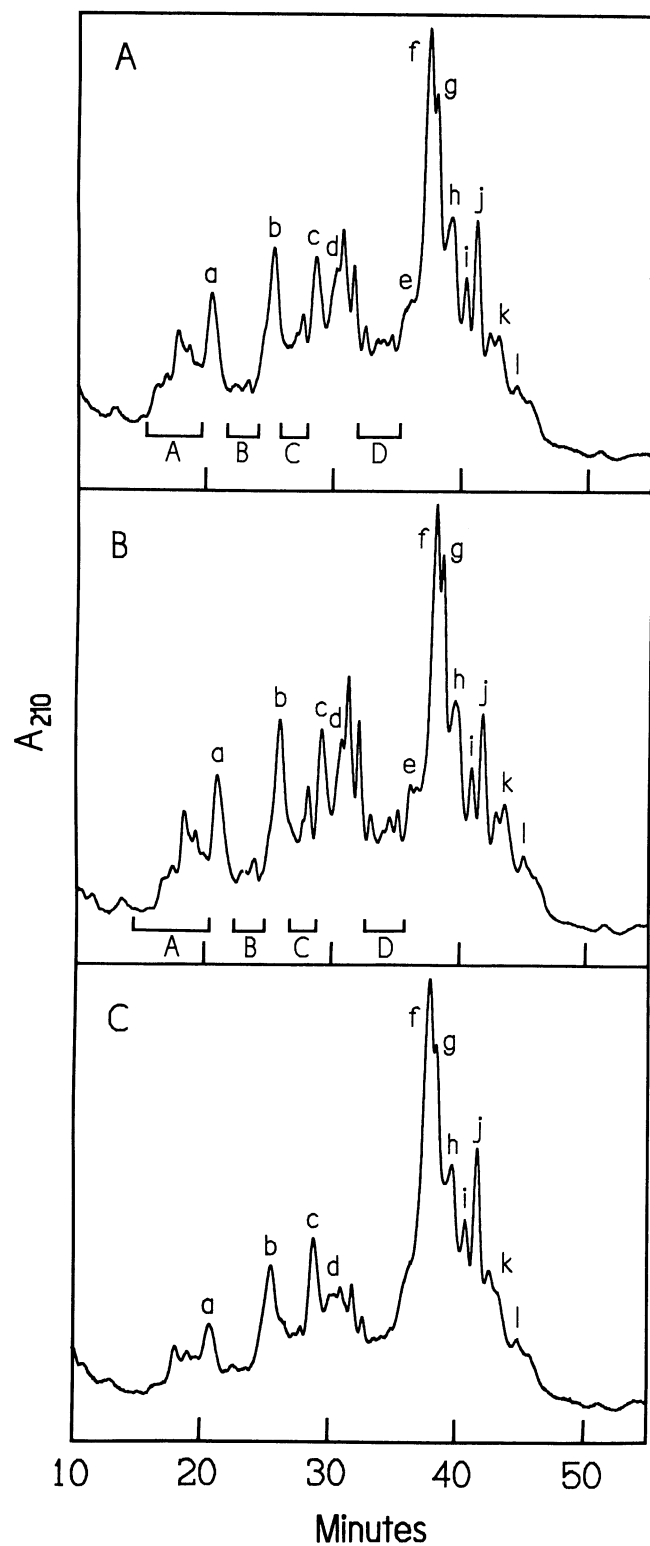
1% β-Mercaptoethanol, 0.7% Acetic Acid, and 70% Ethanol

PE-residue proteins of nondefatted flour extracted with 1% β-mercaptoethanol-0.7% acetic acid-70% ethanol (Fig. 4C) are qualitatively similar to purified glutenin (Fig. 1C). However, β-mercaptoethanol reduces disulfide bonds of glutenin, which is rich in LMW subunits (Bietz and Wall 1973, 1980; Payne and Corfield 1979), and makes these LMW subunits (peaks e–l) soluble. The result is a preparation rich in HMW subunits and an increase in the ratio of HMW to LMW subunits (i.e., peaks a–d compared with e–l). For routine analysis of all glutenin subunits, however, use of β-mercaptoethanol in 0.7% acetic acid-70% ethanol does not seem satisfactory.

Acetic Acid

RP-HPLC of residual PE-proteins after extraction with 0.7% acetic acid is shown in Figure 4D. Again, residual proteins are similar to standard glutenin (Fig. 1C), showing that 1% acetic acid is a good solvent for most nonglutenin proteins, which are soluble at low pH. Dilute acetic acid may partially solubilize glutenin upon repeated extraction, however (Bietz and Wall 1975).

Aluminum Lactate-Lactic Acid Buffer

The RP-HPLC pattern of PE-residue proteins from nondefatted flour previously extracted with 0.085M aluminum lactate-lactic acid buffer, pH 3.1, is shown in Figure 5A. The pattern is
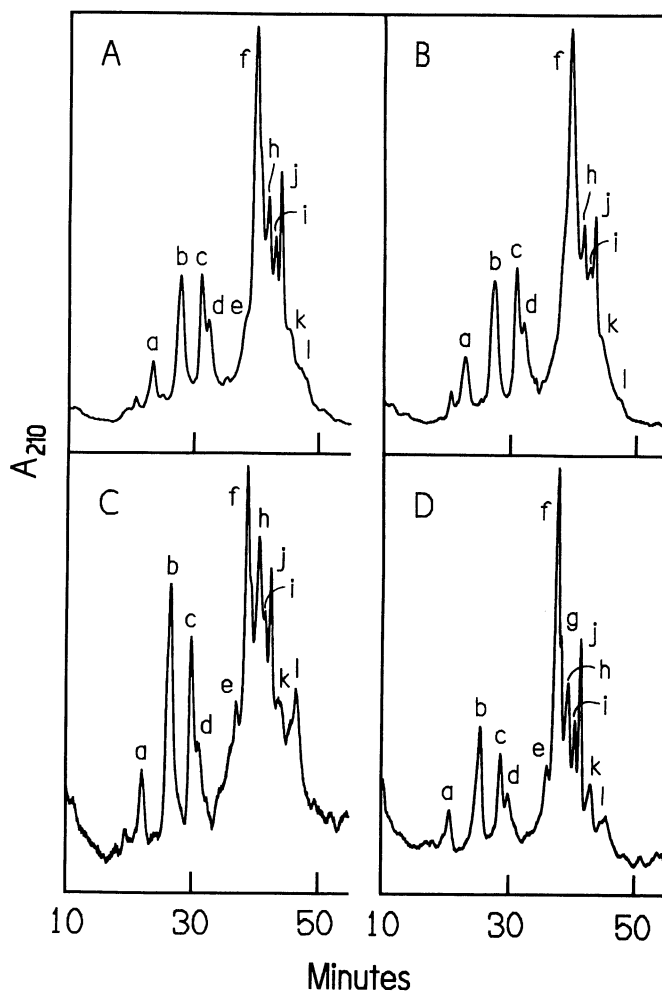

Fig. 3. Reversed-phase high-performance liquid chromatography of pyridylethyl-derivatives of residue proteins after 60% ACN extraction of (A) nondefatted and (B) defatted flour. (C) shows the residue of (A) following a second extraction with 0.05M Tris-HCl, pH 7.5. Other conditions are as in Fig. 1.

Fig. 4. Reversed-phase high-performance liquid chromatography of pyridylethyl-derivatives of residue proteins after extraction of nondefatted flour with 0.7% acetic acid-70% ethanol (A). (B) depicts proteins remaining in the residue after extraction first with 0.7% acetic acid-70% ethanol, and then with 0.05M Tris-HCl, pH 7.5. (C) depicts the chromatographic separation of pyridylethyl-derivatives of residue proteins after extraction with 1% β-mercaptoethanol-0.7% acetic acid-70% ethanol. (D) shows residue proteins remaining after extraction with 0.7% acetic acid. Other conditions are as in Fig. 1.

somewhat less complex than those for flours extracted with 70% ethanol or 60% ACN (Figs. 2A and 3A, respectively). It is similar to that of standard PE-glutenin (Fig. 1C), except that peak c is consistently smaller. Aluminum lactate buffer is a good solvent for albumins, globulins, and gliadins, as is apparent from numerous electrophoretic studies (e.g., Caldwell and Kasarda 1978). The RP-HPLC elution profile for defatted flour extracted with aluminum lactate-lactic acid buffer did not differ significantly from that of nondefatted flour (data not shown), nor from that after further extraction with Tris buffer.

2M DMF

Figure 5B shows the elution profile of PE-proteins from 2M DMF-extracted nondefatted flour. In addition to subunits characteristic of PE-glutenin (Fig. 1C), the residue contained other unextracted polypeptides (mainly in group D), suggesting that 2M DMF does not extract all nonglutenin proteins. Polypeptides that elute between peaks d and e (a–d are HMW and e–l LMW glutenin subunits [Burnouf and Bietz 1984 and 1985]) are likely gliadins, since additional extraction with Tris buffer did not remove them (Fig. 5C). Thus, 2M DMF cannot be recommended for extraction of nonglutenin proteins.

Urea

Urea, being a chaotropic agent, is a good protein solvent. We investigated the possibility that, at a suitable concentration, it might efficiently solubilize only nonglutenin proteins. Figure 5D shows an RP-HPLC pattern of PE-residue protein after extracting flour with 2M urea. Most characteristic glutenin subunit peaks
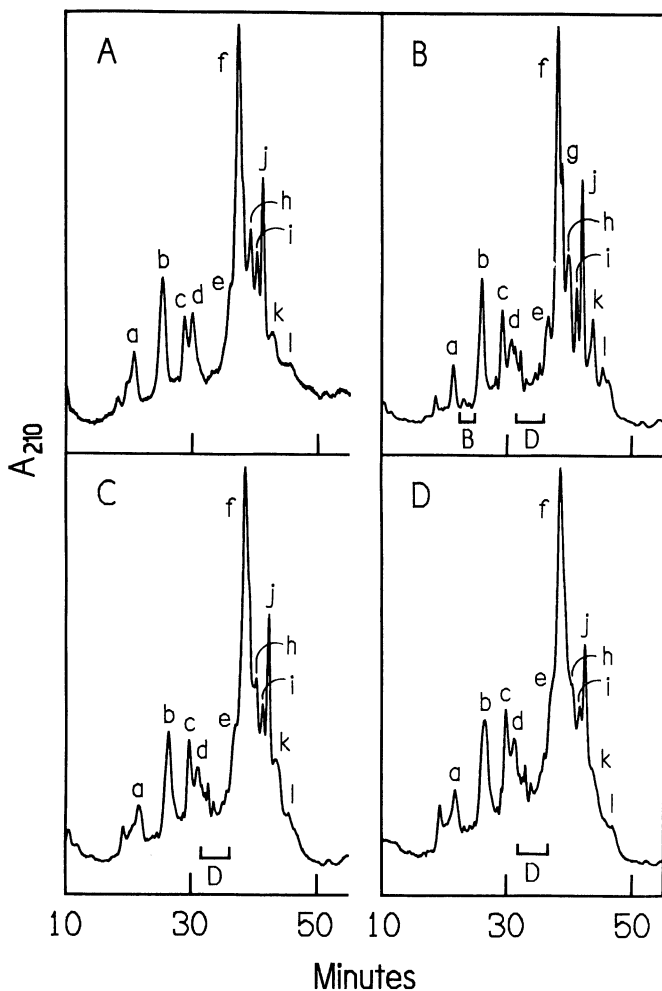
are apparent (apart from shoulder g, which did not resolve well from peak f). Additional polypeptides (e.g., between d and e in group D) were also detected, showing that 2M urea does not extract all nonglutenin protein. In addition, urea also solubilizes some glutenin (Huebner and Rothfus 1971), making urea extraction a poor choice for preparing glutenin for RP-HPLC characterization. Defining an optimal urea concentration to selectively extract nonglutenin proteins is difficult, as for all nonselective chaotropic solvents.

DMSO

Figure 6 shows RP-HPLC patterns of residual PE-proteins after extraction of nondefatted flour with 60% (A), 90% (B), or 100% (C) DMSO. Chromatograms are almost identical to that of standard PE-glutenin (Fig. 1C), except two minor peaks (between d and e) were present when 60% DMSO was used. Thus, 90 and 100% DMSO are excellent solvents to selectively extract nonglutenin proteins while isolating glutenin for RP-HPLC.

Resolution of subunits of glutenin prepared by extraction with 100% DMSO (Fig. 6C) appears better than that after extraction with 60% DMSO (Fig. 6A), or even of standard PE-glutenin (Fig. 1C) (e.g., peaks e and g). A decreased "background" of other proteins may explain this apparent improvement. DMSO may also extract polysaccharides, especially starch (e.g., Leach and Schoch 1962), promoting long RP-HPLC column lifetime. Indeed, a potential problem in glutenin RP-HPLC is that urea, which is present during reduction and alkylation of glutenin in the starchy residue, may solubilize starch as well as protein (Burnouf and Bietz 1984).
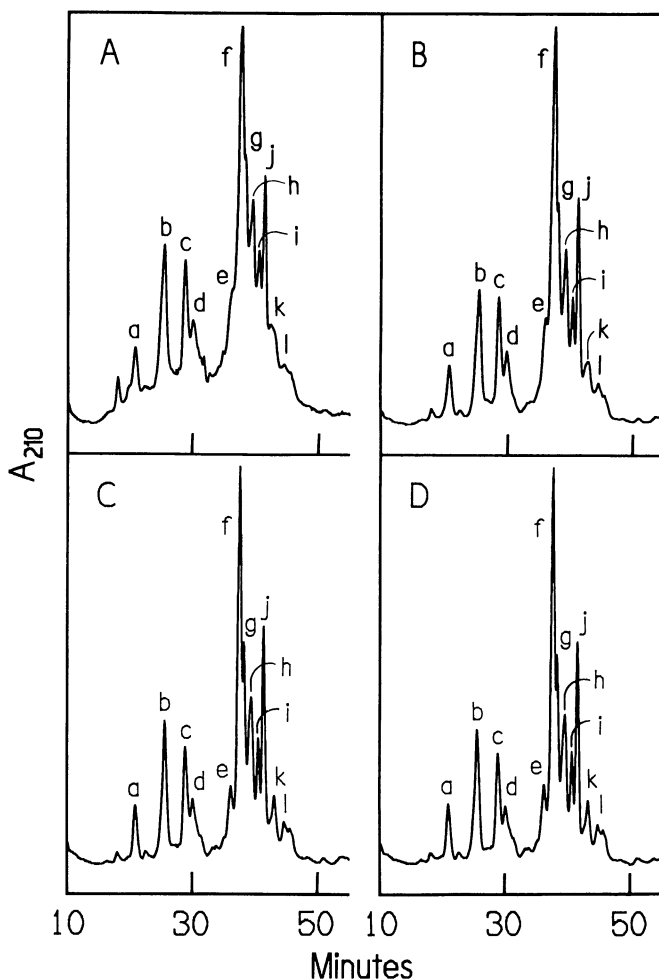

Fig. 5. Reversed-phase high-performance liquid chromatography of pyridylethyl-derivatives of residue proteins after extraction of flour with (A) aluminum lactate-lactic acid buffer, pH 3.1; (B) 2M dimethyl formamide; (C) 2M dimethyl formamide followed by 0.05M Tris-HCl, pH 7.5; and (D) 2M urea. Other conditions are as in Fig. 1.

Fig. 6. Reversed-phase high-performance liquid chromatography of pyridylethyl-residue proteins after extraction of nondefatted flour with (A) 60% dimethyl sulfoxide (DMSO), (B) 90% DMSO, and (C) 100% DMSO, and (D) of defatted flour with 100% DMSO. Extraction conditions are described in the text, and chromatographic conditions are as in Fig. 1.

If starch is injected onto an RP-HPLC column, it may accumulate there, as indicated by changing the solvent from ACN (dehydrated starch, low pressure) to H_2O (hydrated starch, high pressure) (Burnouf and Bietz 1984). Partial elution of starch during RP-HPLC gradients may also cause sloping baselines and impair resolution. Columns contaminated in this manner can be cleaned with 90% DMSO at 50°C (Burnouf and Bietz 1984). Samples can also be extracted overnight with 100% DMSO at 40°C to remove starch; the RP-HPLC pattern of the residual PE-glutenin is not altered.

Also, glutenin prepared by 100% DMSO preextraction of defatted flour (Fig. 6D) is identical to that of the nondefatted sample (Fig. 6C). Thus, defatting flour before extraction with DMSO is unnecessary.

CONCLUSION

We need a rapid procedure, applicable to large numbers of samples, to isolate glutenin for RP-HPLC. Wheat endosperm proteins are very heterogeneous and complex, however, as indicated by RP-HPLC of a total protein extract. To analyze glutenin, therefore, it is necessary to first remove nonglutenin proteins. We have therefore tested several ways to efficiently isolate glutenin free of albumins, globulins, and gliadins.

DMF and urea were not satisfactory for extracting nonglutenin proteins. These solvents extracted all classes of proteins, including some glutenin. Ethanol (70%) and 60% ACN were also not satisfactory: they did not solubilize all gliadins and/or soluble proteins. To remove such contaminants completely, additional extraction steps would be necessary.

Dilute acetic acid and aluminum lactate-lactic acid buffer were efficient solvents for extracting nonglutenin proteins. RP-HPLC patterns of the resulting residue were very similar to that of purified PE-glutenin. An advantage of these solvents is that supernatants (containing albumins, globulins, and gliadin) and residue (containing glutenin) can both be analyzed with minimum treatment from the same small sample. A disadvantage, however, may be that these solvents also partially solubilize glutenin (e.g., LMW glutenin [Bietz and Wall 1972]).

DMSO was an excellent solvent for all nonglutenin proteins. This, combined with disadvantages of other solvents tested, led us to favor use of DMSO to efficiently extract nonglutenin proteins before solubilizing glutenin.

The procedure for this DMSO extraction procedure is summarized as follows. Nondefatted flour is mixed with 90–100% DMSO at a flour/solvent ratio of 50 mg/6 ml for 30 min at room temperature (40°C may be used to speed extraction and to solubilize starch). After centrifugation (27,000 × g, 10 min), a second identical extraction may be performed. The final pellet is then washed with 70% ethanol (volume similar to that of DMSO) to wash residual DMSO from the pellet, thus avoiding any interference with subsequent reduction and alkylation. This 70% ethanol wash also prevents DMSO from eluting as a large early peak upon RP-HPLC; such a peak could interfere with detection of early eluting glutenin subunits. This 70% ethanol wash may also further purify glutenin, as shown by the white precipitate that forms when the pellet is mixed with 70% ethanol (as in Bietz et al 1975). After recentrifugation, the pellet can be stored cold before subsequent solubilization in reduction buffer.

Using the simple DMSO extraction procedure described, we can now easily isolate a glutenin fraction enriched in HMW subunits. These subunits are especially important because they can predict wheat's breadmaking quality. This and previous studies show that HMW glutenin subunits resolve well from each other and from LMW glutenin subunits upon RP-HPLC. The DMSO extraction procedure enhances this separation, and should facilitate routine use of RP-HPLC for wheat breeding, marketing, and quality control.

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APPENDIX

D. D. Kasarda and A. E. Adalsteins, during review of this manuscript, tested the dimethyl sulfoxide (DMSO) procedure for isolating glutenin, and provided additional compositional data for the resulting fraction. Their results, with permission, are reported here.

Degree of Protein and Starch Extraction

Chinese Spring flour was extracted with 100% DMSO (as summarized in Conclusions). The residue was lyophilized and weighed, and Kjeldahl nitrogen analyses were performed to evaluate the extent of protein and starch extraction. When 0.5 g of flour was extracted with 60 ml of DMSO, the freeze-dried centrifuged residue was 55% of the weight of the original flour, and contained 5.0% protein. These results indicate that about 80% of

flour proteins and 50% of starch (or other nonprotein material), by weight, were extracted. When the ratio of DMSO to protein was reduced, amounts of protein and starch extracted were less. For example, when 5 g of flour was extracted with 60 ml of DMSO, the residue weighed 4.15 g and the protein content was 4.3%; about 65% of protein was extracted under these conditions, while little starch was solubilized.

Sodium Dodecyl Sulfate-Polyacrylamide Gel
Electrophoretic Characterization
of Glutenin Prepared by this Method

Figure A-1D shows an SDS-PAGE pattern of glutenin prepared by DMSO extraction. For comparison, glutenin purified by gel filtration and ion-exchange chromatography (Shewry et al 1984) is shown (Fig. A-1A), along with a total protein extract (SDS + mercaptoethanol) from the same variety (Fig. A-1B).

Results indicate that glutenin prepared by the DMSO procedure has a very high proportion of HMW subunits. Its composition is similar to that of glutenin prepared by combined gel filtration and ion-exchange chromatography (Fig. A-1A). Thus, DMSO extraction appears to be a good method for rapidly preparing HMW subunits for RP-HPLC.

If the glutenin-containing residue after DMSO extraction is extracted with SDS without mercaptoethanol, essentially no proteins are solubilized (Fig. A-1C). This is strong evidence that the residue does not contain albumins, globulins, or gliadins, which were extracted by DMSO.

Glutenin prepared by the DMSO extraction procedure (Fig. A-1D) differs from that purified by gel filtration/ion-exchange chromatography (Fig. A-1A) by having several components with SDS-PAGE mobilities between HMW and LMW subunits. These bands may correspond to aggregated albumins (Gupta and Shepherd 1987), coded by genes on chromosome groups 4 and 5. Such polypeptides were first observed in glutenin by Bietz et al (1975). It is unknown whether these polypeptides are covalently incorporated into glutenin through disulfide bonds, associate by other forces, or are trapped within the aggregates. We do not yet know where these components elute upon RP-HPLC; they do not, however, interfere with analysis of HMW glutenin subunits (Fig. 6).
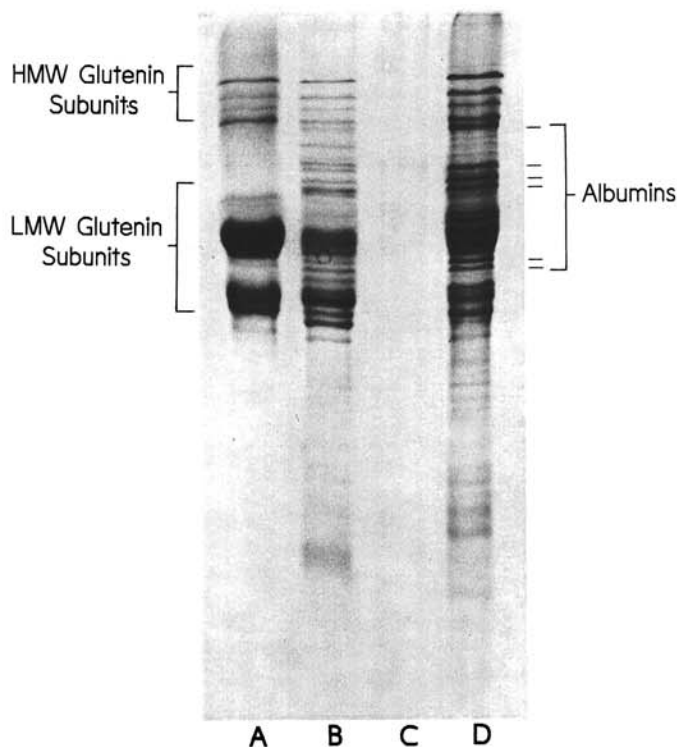

Fig. A-1. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoretic analysis (17% polyacrylamide) of (A) glutenin from Chinese Spring wheat, purified by gel filtration and ion-exchange chromatography (Shewry et al 1984); (B) total proteins of Chinese Spring, extracted from a crushed seed with SDS + β-mercaptoethanol; (C) an SDS extract (no β-mercaptoethanol) of the residue remaining after DMSO extraction of Chinese Spring flour; and (D) an SDS + mercaptoethanol extract of the residue resulting from DMSO extraction of Chinese Spring flour. Data courtesy of D. D. Kasarda and A. E. Adalsteins.

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