

# Gliadin Analysis by Reversed-Phase High-Performance Liquid Chromatography: Optimization of Extraction Conditions

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## ABSTRACT

Cereal Chem. 61(2):124-129

Reversed-phase high-performance liquid chromatography (RP-HPLC) analysis of gliadin proteins is an excellent alternative to electrophoresis for genotypic comparisons and identification of wheat varieties. This high-resolution chromatographic technique is fast, sensitive, and easily quantifiable; run-to-run reproducibility is excellent. To determine optimal conditions for extracting gliadins from wheat flour for analysis by RP-HPLC, we systematically explored the effects of the variables extraction time, defatting, prior NaCl extraction, type of extractant, and

age of extract on the resulting chromatograms. Optimal extraction was achieved by using nondefatted flour, a 30-min extraction time, and no prior NaCl extraction. Aqueous 70% ethanol or 55% 2-propanol were the most suitable extractants tested; aluminum lactate buffer (pH 3.1), 2M urea, and 2M dimethylformamide extracted much albumin, globulin, and glutenin protein in addition to gliadins, resulting in more complex patterns. The recommended extraction conditions give highly definitive RP-HPLC chromatograms with minimal expenditure of time and effort.

Reversed-phase high-performance liquid chromatography (RP-HPLC) has become an extremely powerful method for analysis of high molecular weight (HMW) proteins since the recent introduction of large pore size ( $\geq 300$  Å) columns (Regnier and Gooding 1980, Hearn et al 1982). The method is rapid (1 hr or less), sensitive, and highly reproducible; it has high resolution and is amenable to accurate quantitation. It complements other fractionation techniques by separating proteins by surface hydrophobicity, rather than by size or charge. Bietz (1983) recently demonstrated for the first time the application of this technique to cereal proteins and showed its utility in wheat breeding and genetic studies and for varietal identification, as well as for isolation, characterization, and comparison of cereal proteins.

For wheat, electrophoretic analysis of the alcohol-soluble prolamin fraction, gliadin, has been used extensively as a sensitive genotypic indicator. Various procedures for electrophoresis in starch gels or polyacrylamide gels (PAGE) in acid buffers at low ionic strengths have been described (Wrigley et al 1982) and are widely used. Resolution of gliadins by RP-HPLC generally equals or exceeds that obtained by electrophoresis (Bietz 1983), suggesting that RP-HPLC will become widely used for such analyses. Large-pore RP-HPLC columns are very new, however, and optimal conditions for their use have not yet been totally defined.

In our own laboratory, RP-HPLC is now being used extensively for analysis of different wheat genotypes; our first report of this application appears in a separate article (Bietz et al 1984). Consequently, to provide a firm foundation for our own present and future studies, as well as for those of other researchers, we here systematically compare several protocols to optimize gliadin extraction conditions for analysis by RP-HPLC.

## MATERIALS AND METHODS

### Materials

*Preparation of flours.* Centurk hard red winter wheat, grown in 1979, was milled in a Brabender Quadruplex mill to approximately 70% extraction (nondefatted flour). For preparation of defatted flour, 50-g samples were extracted with 1-butanol (three times) followed by pentane/hexane (one time); alternatively, partially defatted flours were prepared by extraction only with 1-butanol. Each extraction was performed by agitating (with a magnetic stirrer) 50 g of flour in 150 ml of extractant for 30 min, and subsequently filtering the suspension. The resulting flours were air-dried.

*Chemicals and reagents.* Reagents and solvents used in all studies were reagent grade or better. Solvents and chemicals used for RP-HPLC were previously described in detail (Bietz 1983).

### Extraction Methods

*Effect of extraction time.* Defatted Centurk flour (approximately 50 mg) was extracted with 70% ethanol (2 ml/50 mg flour), with agitation using a Buchler Vortex-Evaporator, for 5, 15, and 30 min, and for 1, 2, 3, 4, and 5 hr. Each extraction was terminated by centrifugation (10 min at  $27,000 \times g$ ); clear supernatants were analyzed without further treatment.

*Comparison of extraction conditions.* Samples of nondefatted, partially defatted, and defatted Centurk flours were first extracted one or two times with 0.1M NaCl (or alternatively, NaCl extraction was omitted) to remove albumins and globulins, and subsequently extracted separately with one of five solvents known to extract gliadins. The resulting 45 samples (three fat levels, three types of NaCl extraction, and five extractants) were subsequently analyzed at least in duplicate. NaCl extractions were performed by shaking flour aliquots with 0.1M NaCl (6 ml/50 mg of flour) for 30 min, and were terminated by centrifugation as described above. Gliadins were extracted from flour samples using 70% ethanol (v/v); 55% 2-propanol (v/v); 0.0085M aluminum lactate, pH 3.1; 2M dimethylformamide (DMF); and 2M urea. Each extraction was performed, with agitation, for 30 min using 2 ml of solvent per 50 mg of flour, and was terminated by centrifugation. Duplicate extractions were performed to verify results.

*Storage time study.* Samples of defatted and nondefatted flour were extracted with 70% ethanol as described above over a period of 28 days. Aliquots of the resulting supernatants were stored either at room temperature (20–25°C) or in a freezer (approximately –20°C) for 1–28 days before analysis.

### Chromatographic Conditions

RP-HPLC was performed on SynChropak RP-P ( $C_{18}$ ) columns (250  $\times$  4.1 mm i.d.) as described previously (Bietz 1983). Two columns of this type were evaluated: one was used for extraction time and storage-stability studies, and the other for comparison of different extraction conditions. The apparatus consisted of Waters M6000A and M45 solvent-delivery systems controlled by a model 660 solvent programmer, a WISP 710A automatic sample injector, and a model 450 variable-wavelength detector. Samples (50  $\mu$ l) were analyzed using a linear gradient from 20 to 55% solvent B (28–50.75% acetonitrile [ACN]) over 55 min (total run time = 65 min) at 1.0 ml/min. Solvent A was 15% ACN + 0.1% trifluoroacetic acid (TFA), and solvent B was 80% ACN + 0.1% TFA. Solvent pH was approximately 2.2. Components eluting from the column were detected at 210 nm (0.2 AUFS). Data were recorded on a Houston Instruments Omniscrite recorder (10 mV full-scale); simultaneously, raw data were stored by a ModComp computer system for subsequent integration and replotting.

<sup>1</sup>The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

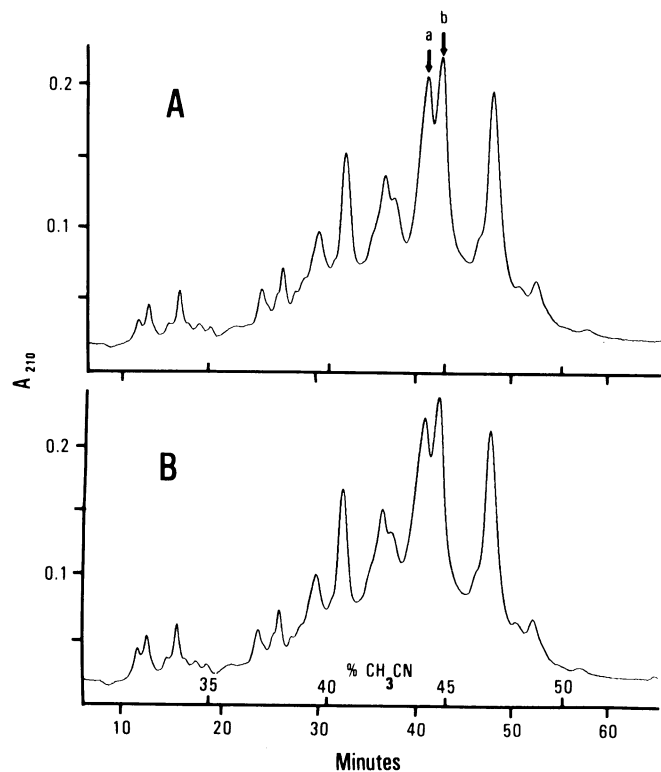
With this chromatographic system, and under these extraction and analytical conditions, good run-to-run reproducibility is obtained when any column is used. Repetitive extractions of the same sample give chromatograms that are nearly superimposable (Bietz 1983), since extracted proteins have similar solubilities and since WISP injection volumes have a precision of  $\pm 1\%$ . Most comparisons of chromatograms to date involve major qualitative or quantitative differences, but minor quantitative differences are also easily observed by comparing computer-generated replots of the data in which major peaks have equal heights, by examining difference curves of chromatograms, and by comparing relative percents rather than absolute peak areas. Slight natural quantitative variation exists within any variety, however, and the magnitude of this variability must be better understood before the significance of minor quantitative differences becomes fully apparent. As discussed previously (Bietz 1983), RP-HPLC elution times are highly reproducible, and typically differ by no more than a few seconds between runs. Certain chromatographic peaks, such as a and b in Fig. 1A, are present in chromatograms of gliadins from all hexaploid wheat varieties, and serve as useful internal standards to detect any problems with the system.

## RESULTS AND DISCUSSION

### Effects of Extraction Time

Any protein extraction must compromise speed (to minimize extraction time) and recovery (which usually increases with time). Consequently, to determine optimal extraction time, defatted Centurk flour was extracted, in duplicate, for 5 min to 5 hr with 70% ethanol.

The chromatograms (Fig. 1) comparing the extremes of extraction time, 5 min and 5 hr, are typical of those obtained by RP-HPLC for wheat gliadin proteins. Approximately 30 peaks resulted, including minor peaks and poorly resolved shoulders. To facilitate comparisons, all chromatograms have been plotted so that the largest peak of each run has the same height; absolute absorbance measurements are indicated on the ordinates. To permit replotting of absorbance data in this manner, the initial



**Fig. 1.** RP-HPLC separations of proteins extracted from nondefatted Centurk flour with 70% ethanol for: **A**, 5 min and **B**, 5 hr. Arrows in **A** indicate peaks a (40.81 min) and b (42.21 min) discussed in text.

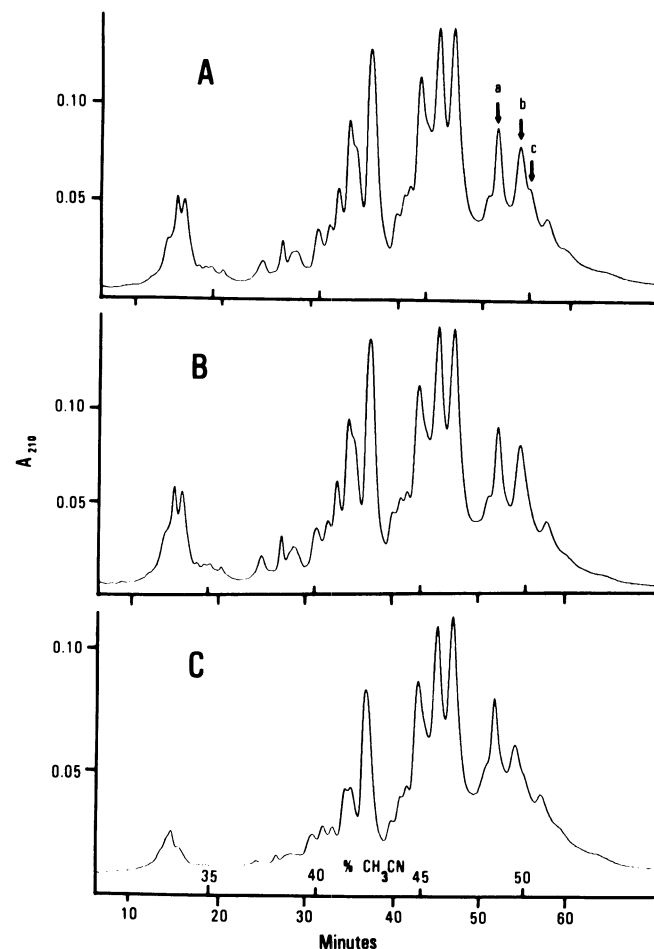
“breakthrough” peak eluting at the column’s void volume (2.0 ml) (which contains only extracted nonprotein ionic components and extraction solvent) is not shown.

Chromatograms of proteins extracted during 5 min (Fig. 1A) and 5 hr (Fig. 1B) are nearly identical, as were results for all intermediate extraction times (results not shown). Thus, extraction rates of different gliadins and any other proteins solubilized by 70% ethanol must be rapid and nearly identical. Spectrophotometric analyses based on comparison of peak heights and areas (Fig. 1) also indicate that approximately 90% as much protein is extracted in 5 min as in 5 hr, and that an extraction yield of approximately 97% is achieved in 30 min. Consequently, for RP-HPLC of gliadins from flours or ground single kernels, extraction times of 30 min are recommended when 70% ethanol is used; however, 5-min extraction times are sufficient for most qualitative comparisons of chromatograms. Since gliadins are readily soluble in all solvent systems we tested, these extraction times are probably appropriate for them as well as for 70% ethanol.

### Comparison of Extraction Conditions

In our study, we compared five protein extractants using nondefatted, partially defatted, and defatted flours, each with or without prior NaCl extraction (one and two extractions). The results are best described by presenting, for each extractant separately, representative results showing effects of defatting and salt extraction.

**70% Ethanol.** Representative chromatograms for 30-min 70% ethanol extracts of various flours are shown in Fig. 2. Extracts of nondefatted (Fig. 2A) and defatted (Fig. 2B) flours, with no prior NaCl extraction, were nearly identical; similarly, defatting did not



**Fig. 2.** RP-HPLC separations of proteins extracted by 70% ethanol from: **A**, nondefatted flour; **B**, defatted flour; and **C**, nondefatted flour following one previous NaCl extraction. The arrows in **A** indicate peaks a (51.18 min), b (53.75 min), and c (54.87 min) discussed in text.

significantly change the composition of ethanol extracts of flours previously extracted with NaCl (results not shown). Only one possibly significant difference is apparent between the nondefatted and defatted flours: an unresolved shoulder for the nondefatted flour (peak c in Fig. 2A) is absent after defatting, possibly representing a lipoprotein or a protein soluble in dry 1-butanol or in pentane/hexane. It is not surprising to find different compositions for alcohol-soluble proteins from defatted and nondefatted flours: Charbonnier (1973) demonstrated larger differences between extracts from flours defatted under somewhat different conditions. The present example illustrates the power of RP-HPLC in detecting subtle differences between samples having undergone different treatments.

Comparison of the chromatogram for the ethanol extract of nondefatted Centurk flour (Fig. 2A) to that for nondefatted flour following one salt extraction (Fig. 2C), however, reveals a number of major differences. (Results for two NaCl extractions were identical to those for one NaCl extraction.) Many early-eluting peaks in the chromatogram of the extract of nondefatted flour are either not present following salt extraction or occur in greatly reduced amounts, resulting in a somewhat simpler overall pattern. In addition, quantitative differences exist for some major later-eluting components (although elution times are not affected). These results are consistent with the previous observation (Bietz 1983) that many wheat albumins and globulins are less hydrophobic than most gliadins and elute earlier upon RP-HPLC, but that some albumins and globulins also have hydrophobicities similar to gliadins. It is also possible that protein interactions similar to those in dough formation occur during NaCl extraction, so that some gliadins incorporated into a gluten network may subsequently be insoluble upon mild ethanol extraction.

In PAGE, most albumins and globulins have greater mobilities than do gliadins. In RP-HPLC, however, albumins, globulins, and gliadins may have similar elution characteristics; this must be considered in interpreting any results. For varietal comparisons, we believe that the added complexity introduced to chromatograms by albumins and globulins is no hindrance, since water- or salt-soluble proteins are present in relatively minor amount and generally differ little between varieties, but in some cases may differentiate varieties (Wrigley et al 1982). Albumins and globulins may be less stable than gliadins, however, and the synthesis of albumins and globulins may be more subject to environmental influence. Until these factors are better understood, caution must be exercised in basing varietal identification by any method solely upon differences in albumins and globulins.

The comparison between Figs. 2A and 1A is also interesting; both represent 70% ethanol extracts of nondefatted Centurk flour not previously extracted with NaCl. Extraction times do differ (5 versus 30 min), but this does not significantly affect the results (see above); the solvent gradients for these two chromatograms were also identical. Nevertheless, a number of significant differences exist between these two chromatograms (although the total number of peaks in each is similar), which were run on two separate SynChropak RP-P columns differing in selectivity. The results of

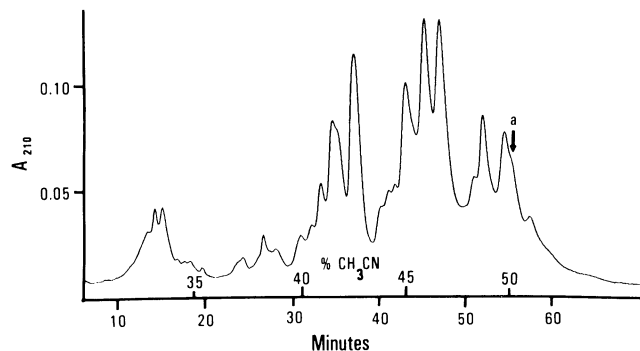


Fig. 3. RP-HPLC separation of proteins extracted by 55% 2-propanol from nondefatted Centurk flour (no previous NaCl extraction). The arrow indicates peak a (54.87 min) discussed in text.

Fig. 2 were obtained with our first RP-P column, and those of Fig. 1 with a newer column. To date we have examined several additional analytical and preparative RP-P columns and have found that they give separations essentially identical to that achieved with our second column (Fig. 1), suggesting that differences in selectivity between most columns containing the same packing will be minor. It is likely that the atypical results obtained with our first column represent a basic difference between two lots of the same packing (K. M. Gooding, personal communication); it is also possible, however, that altered selectivity was due to column age and/or condition.

Further studies are in progress to better define column-to-column variability in wide-pore RP-HPLC columns and to explore differences in selectivity (Bietz et al 1983). Initial results obtained with numerous columns having the same bonded phase reveal marked similarity in results, even when columns from different manufacturers are compared. Until differences between columns are better defined and understood, however, RP-HPLC analyses using different columns, or performed in different laboratories, must be evaluated with caution; to facilitate such comparisons, it may be desirable to use a specific reference sample as a standard. On any individual column, however, run-to-run reproducibility and long-term stability are excellent.

**55% 2-Propanol.** A chromatogram for a 2-propanol extract of nondefatted flour, with no prior NaCl extraction, is shown in Fig. 3. Qualitatively and quantitatively, these results are nearly identical to the chromatogram of an ethanol extract of the same flour (Fig. 2A). Also, the effects of defatting and of prior NaCl extraction on the chromatograms of 2-propanol extracts are identical to those observed in ethanol extracts: defatting led to no difference other than a loss of one shoulder (peak a in Fig. 3), and previous NaCl extraction led to the same differences observed for the ethanol extracts. Consequently, these results indicate that 70% ethanol and 55% 2-propanol can be used interchangeably as gliadin extractants for RP-HPLC studies.

**Aluminum lactate buffer.** A chromatogram of the proteins extracted from nondefatted Centurk flour, with no previous NaCl extraction, using 0.0085M aluminum lactate, pH 3.1, is shown in Fig. 4. The effects of defatting and of previous NaCl extraction on the chromatographic patterns of the resulting aluminum lactate extracts were similar to those observed for ethanol and 2-propanol. No difference was apparent between nondefatted and defatted flours, and NaCl extraction led to major reductions, both in type and amount, of early-eluting components in the subsequent aluminum lactate extract, as well as to some specific reductions in later-eluting components.

Comparison of the RP-HPLC pattern for an aluminum lactate extract of flour (Fig. 4) to patterns obtained for ethanol or 2-propanol extracts of the same flour (Figs. 2A and 3), however, reveal significant qualitative and quantitative differences.

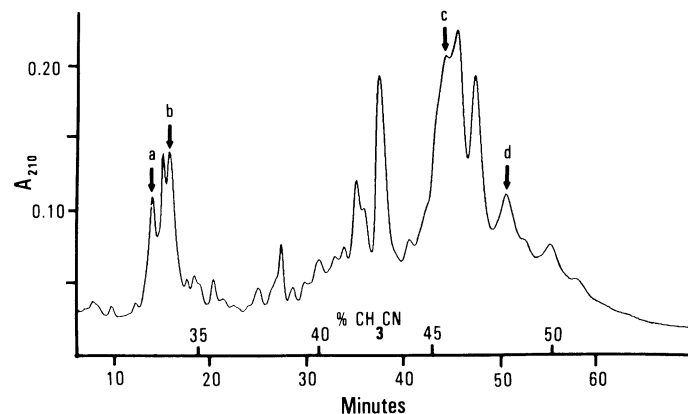


Fig. 4. RP-HPLC separation of proteins extracted by 0.0085M aluminum lactate, pH 3.1, from nondefatted Centurk flour (no previous NaCl extraction). Arrows indicate peaks a (13.80 min), b (15.61 min), c (44.18 min), and d (50.21 min) discussed in text.

Aluminum lactate buffer extracts increased amounts of early-eluting components (such as those in peaks a to b in Fig. 4); in addition, some very hydrophobic components (eg, peaks a and b in Fig. 2A) are not extracted or are extracted in low yield, whereas other late-eluting components (eg, peaks c and d in Fig. 4) are extracted in increased amounts. These effects may be due both to increased solubility of albumins and globulins in aluminum-lactate buffer and to protein aggregation through gluten formation during the extraction, with concomitant loss of solubility of some components. RP-HPLC of aluminum lactate extracts of nondefatted or defatted flours previously extracted with NaCl, however (results not shown), gave chromatograms very similar to those obtained using ethanol and 2-propanol, demonstrating that the primary difference between these extracts is increased albumin and globulin extraction by aluminum lactate buffer. Therefore, the use of aluminum lactate buffer to extract gliadins for subsequent RP-HPLC analysis cannot be recommended.

**2M Urea.** RP-HPLC patterns of flour proteins extracted with 2M urea (Fig. 5) differ significantly from those for other extractants. Urea extracts from nondefatted flour (Fig. 5A) are more complex, as evidenced by greatly increased amounts of early-eluting components (including albumins and globulins) as well as by decreased resolution and appearance of new later-eluting peaks, indicating possible extraction of glutenins. Prior NaCl extraction, however (Fig. 5B), eliminates or greatly reduces most early-eluting peaks, and the pattern of later-eluting peaks becomes sharper, resembling results for an ethanol extract after prior NaCl extraction. This similarity in patterns suggests that 2M urea does not irreversibly alter gliadin conformations. It is also possible that dough formation during NaCl extraction subsequently led to decreased glutenin extraction by 2M urea.

Defatting also affects protein extractability by 2M urea, but only without prior NaCl extraction. If NaCl extraction has occurred, patterns for 2M urea extracts of nondefatted, partially defatted, and defatted flours are identical. In the absence of NaCl extraction, however, results for defatted flour (Fig. 5C) differ quantitatively and qualitatively, suggesting that lipids markedly affect solubility or degree of association of some albumins, globulins, or glutenins (but probably not gliadins) in 2M urea.

Comparison of the 2M urea extract of nondefatted flour (no prior NaCl extraction) (Fig. 5A) to that obtained using 70% ethanol (Fig. 2A) also reveals that several major hydrophobic components of the ethanol extracts (eg, peaks a and b in Fig. 2A) are absent in the 2M urea extracts. This suggests that some ethanol-soluble proteins are aggregated forms that may be dissociated by 2M urea or that some ethanol-soluble proteins may not be extracted by 2M urea.

These results demonstrate that 2M urea is a powerful solvent for many wheat proteins in addition to gliadins. Because of this, its use as a gliadin extractant cannot be recommended when RP-HPLC is to be used for varietal identification.

**2M DMF.** As with urea, 2M DMF extracts of flours not previously extracted with NaCl (Fig. 6A) give complex chromatograms, containing, in addition to gliadins, increased amounts of both early-eluting (albumin and globulin) components and other more hydrophobic proteins (having elution times greater than approximately 30 min), presumably glutenins. Prior extraction with NaCl (Fig. 6B) markedly lowers amounts of early-eluting (albumin and globulin) components, and gives a pattern for later-eluting gliadins very similar to that obtained with ethanol under similar conditions. NaCl extraction may cause dough formation and may aggregate some components into forms subsequently insoluble in DMF.

As observed for urea, partial (Fig. 6C) or total (Fig. 6D) defatting of flour reduces the amounts of many later-eluting hydrophobic components extracted with DMF, indicating that lipids may influence protein aggregation, that lipoprotein complexes may be present, or that some hydrophobic proteins may have significant solubility in 1-butanol or pentane/hexane. Unlike with urea, however, partial and total defatting led to some differences in the composition of proteins extracted with DMF (Fig. 6C and D), indicating that lipids extractable by 1-butanol and

by pentane/hexane react differently with proteins. As with urea, DMF extracts of nondefatted and defatted flours previously extracted with NaCl were identical.

DMF, like urea, cannot be recommended for routine extraction of gliadins for varietal identification by RP-HPLC. Results indicate that DMF has potential, however, for extraction of glutenin proteins.

### Stability of Extracts

To determine the time acceptable between extraction and subsequent analysis of a sample, nondefatted and defatted flours were extracted with 70% ethanol and stored for up to 28 days before analysis. RP-HPLC revealed no differences for either defatted or nondefatted flours between fresh extracts (Fig. 7A) and those stored, either at room temperature (Fig. 7B) or in the freezer (Fig. 7C), for 18–28 days. It is likely that still longer storage periods are possible, so older extracts may be compared to fresh extracts. We have not extensively studied storage stability of gliadins in extractants other than 70% ethanol, but it is likely that stability in these extracts is similar to that in 70% ethanol.

One minor quantitative difference was consistently observed between nondefatted and defatted flours. In chromatograms of extracts of nondefatted flours (Fig. 7C), peak a was larger than in defatted flours. A similar difference was observed for a later-eluting component when these analyses were performed on a different SynChropack RP-P column (Fig. 2A and B). This further indicates that at least one 70% ethanol-soluble wheat protein also has significant solubility in 1-butanol or in pentane/hexane, or that lipids alter protein solubility or aggregation. We have noted that some precipitation occasionally occurs upon storage of samples in the freezer, suggesting that room temperature storage may generally be preferable. If visible precipitation does occur in stored

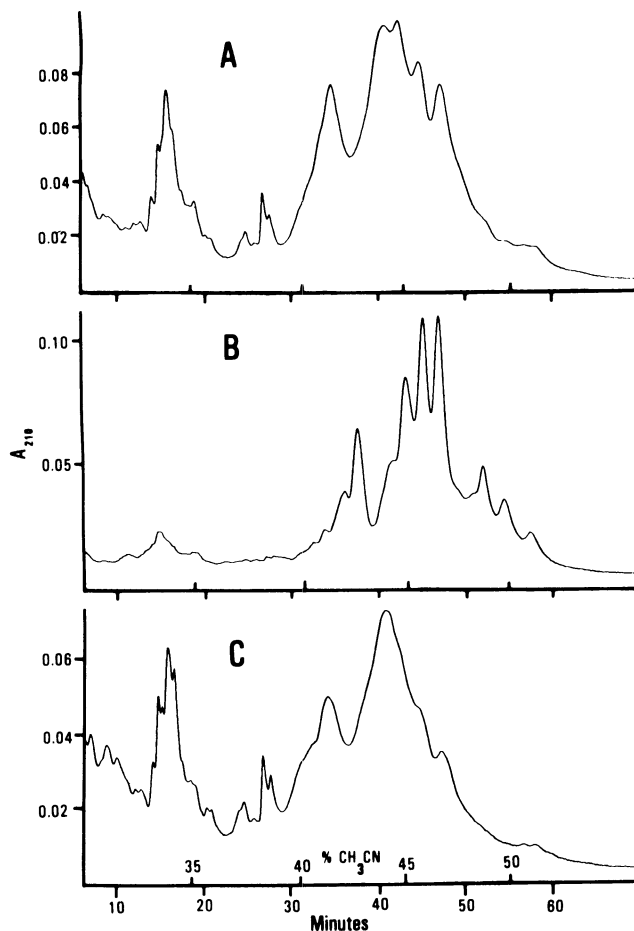


Fig. 5. RP-HPLC separations of proteins extracted by 2M urea from: A, nondefatted flour; B, nondefatted flour following one previous NaCl extraction; and C, defatted flour.

samples, the precipitate can be readily solubilized by gently heating and thorough agitation.

## CONCLUSIONS

The validity of RP-HPLC, when used for gliadin analysis, depends on the application of proper techniques of protein extraction. Since numerous extraction conditions are commonly used to solubilize gliadins prior to analysis by PAGE, we have compared several of these methods.

Variation in extraction time results in little difference in yield and composition of extracts. For most applications, 5-min extraction time gives a representative sample, and 5 hr is not excessive; 30 min is a suitable compromise. Extracts may be stored for at least 28 days either at room temperature or at  $-20^{\circ}\text{C}$  without undergoing any

apparent significant decomposition or change.

NaCl extraction of a flour before extraction of gliadins produces a chromatogram considerably simpler than that for an extract of flour not extracted with NaCl; apparently, albumins and globulins are responsible for much of the heterogeneity in the early-eluting, less hydrophobic region of the chromatogram. Indeed, albumins and globulins extracted with NaCl, which may be useful in differentiating some varieties (Wrigley et al 1982), give complex RP-HPLC chromatograms (Bietz 1983). Patterns for NaCl extracts may vary with storage time, however, due to proteolysis or protein interactions. NaCl extraction may have the additional effect, however, of promoting incorporation of some proteins into a gluten network, as in dough formation, through disulfide bond formation or through noncovalent bonding. This effect is especially predominant when stronger solvents (ie, 2M urea or 2M DMF), which apparently extract glutenins as well as gliadin and which unfold proteins and thereby promote disulfide interchange and noncovalent bonding, are used: solubility of more hydrophobic components in such solvents decreases after NaCl extraction. For most applications, especially when aqueous alcohol solutions are used as extractants, there is no advantage to NaCl extraction of a flour or ground kernel before extraction of gliadins, and a time-consuming step is thus avoided.

When aqueous alcohols or aluminum lactate were used to extract gliadins, little difference was apparent between chromatograms for nondefatted and defatted flours. When 2M urea and 2M DMF were used, however, significant differences occurred for nondefatted and

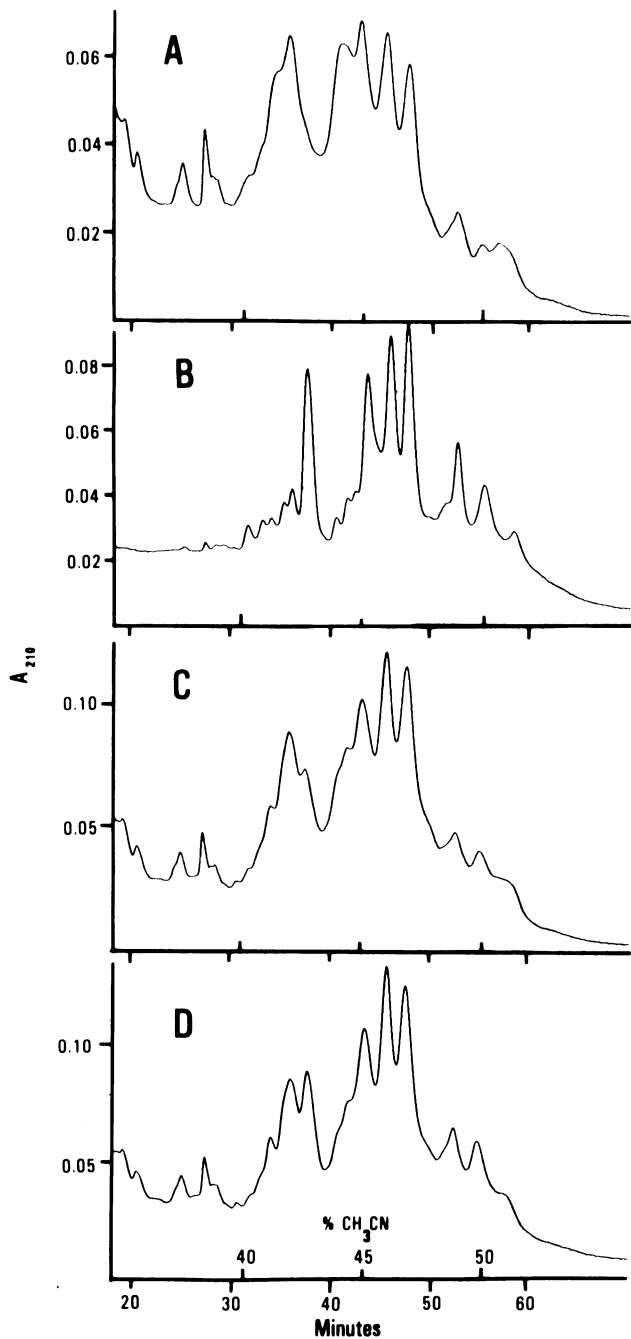


Fig. 6. RP-HPLC separations of proteins extracted by 2M DMF from: A, nondefatted flour; B, nondefatted flour following one previous NaCl extraction; C, partially defatted flour; and D, defatted flour. The DMF extractant produced an extremely large and broad breakthrough peak, so the first 18 min of these chromatograms are not shown.

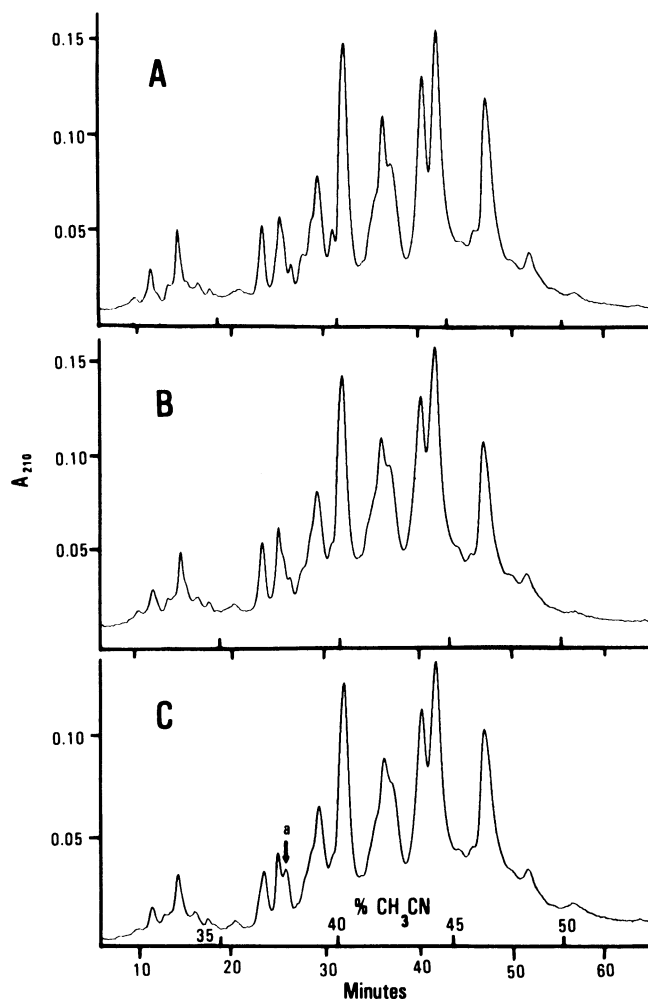


Fig. 7. RP-HPLC separations of proteins extracted with 70% ethanol from: A, defatted flour and analyzed immediately; B, defatted flour and stored for 28 days at room temperature before analysis; and C, nondefatted flour, stored for 18 days at room temperature before analysis. The arrow in C indicates peak a (25.71 min) discussed in text.

defatted flours (with no previous NaCl extraction). We interpret this to indicate either the lipoprotein nature of some extracted albumin, globulin, or glutenin proteins or the ability of lipids to otherwise promote solubility of some proteins. For routine use for varietal identification, there seems to be little, if any, justification for defatting samples before gliadin extraction.

The final, and major, variable we investigated is the protein extractant used. Ethanol (70%) and 2-propanol (55%) were very similar in their actions, and good, reproducible chromatograms resulted. Aluminum lactate buffer extracted increased amounts of albumins and globulins, and somewhat decreased or altered the apparent resolution of later-eluting gliadin components. Urea (2*M*) and DMF (2*M*) extracted not only albumins, globulins, and gliadin, but apparently also solubilized significant amounts of higher MW associated forms of glutenin. Thus, for certain specific analyses, aluminum lactate buffer, 2*M* urea, 2*M* DMF, or other solvents may be useful; but for routine use, 70% ethanol (or 55% 2-propanol) seems to be the best gliadin extractant of those we tried.

Our results demonstrate that for routine RP-HPLC analysis of gliadins, the simplest conditions of extract preparation may be the best: we recommend extracting nondefatted samples directly with 70% ethanol for approximately 30 min, and analyzing the extracts within one month. A separate article (Bietz et al 1984) details results

obtained when these extraction conditions are used to prepare gliadin samples from different wheat varieties for use in RP-HPLC analysis for varietal identification.

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[Received July 5, 1983. Accepted October 15, 1983]