Suitability of Reversed-Phase High-Performance Liquid Chromatographic Separation of Wheat Proteins for Long-Term Statistical Assessment of Breadmaking Quality[1]

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ABSTRACT

The proteins of flours of 26 widely different bread wheat varieties grown in Canada were extracted with 70% ethanol, with and without a reducing agent (20% mercaptoethanol). Replicate extracts with each solvent and duplicate injections of each extract were analyzed by high-performance liquid chromatography (HPLC) on a reversed-phase column over a period of two months. The HPLC chromatograms were compared with standards (Neepawa chromatograms) determined at the same time. For statistical analysis, each chromatogram was divided into 21 regions. The areas of these regions were used to generate prediction equations for several breadmaking quality parameters. Flour proteins of 16 different wheat varieties grown in Canada, extracted with the same two solvents, were separated using a different column of the same type. The integrated areas of the 21 regions for the 16 varieties were used to test the prediction equations. Extensigraph extensibility was the most consistently predictable breadmaking quality parameter from HPLC analyses of ethanol extracts prepared with and without the reducing agent. The extensibility appeared to be predicted from the overall protein composition rather than from individual gliadins. Prediction of dough extensibility using prediction equations derived from HPLC analyses would complement SDS-PAGE for screening varieties for breadmaking quality in early generations of breeding programs.

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In the conversion of wheat into bread, flour proteins play a major role in the unique viscoelastic properties of dough that produces bread of high quality. The main protein constituents are the gliadins and the glutenins, which together produce gluten, the three-dimensional matrix of a loaf of bread.

Statistical analyses of protein components separated by electrophoresis link certain gliadins and glutenin subunits to breadmaking quality parameters (Wrigley et al 1982; Branlard and Dardevet 1985a,b; Campbell et al 1987; Payne et al 1987; Ng and Bushuk 1988). On the other hand, doubts about the reproducibility of results with different high-performance liquid chromatography (HPLC) columns (Goldberg 1982) and their long-term stability (Glajch et al 1987), have delayed the use of reversed-phase HPLC (RP-HPLC) to predict breadmaking quality on the basis of gluten protein components separated by this technique.

In this study, the proteins of 26 diverse bread wheat varieties were analyzed by RP-HPLC and the results were used to develop equations for predicting breadmaking quality parameters. The equations were then used to predict the breadmaking quality parameters of 16 other wheat varieties analyzed on a different RP-HPLC column.

MATERIALS AND METHODS

Reagents

Chromic acid and 2-mercaptoethanol (2-ME) were of reagent grade, and dimethyl sulfoxide (DMSO) was of ACS reagent grade. All other chemicals were of HPLC grade obtained from sources indicated previously (Scanlon et al 1989).

Wheat Samples

Grain of 26 varieties of the Uniform Quality Nursery (UQN) (described by Ng and Bushuk 1988), the Canadian bread wheat variety Marquis, 10 varieties of the 1987 Saskatchewan Wheat Pool (SWP) bread wheat test, and six varieties of the 1987 Parkland Wheat Cooperative Test (PWCT) was used in the present study. Grain of the Canadian bread wheat variety Neepawa was used as the reference standard (Sapirstein et al 1989).

Sample Preparation

Grain samples were milled into straight-grade flour on a Buhler pneumatic laboratory mill. Additionally, whole meal of the variety Neepawa was prepared by grinding on a Udy cyclone mill (Udy Analyzer Co., Boulder, CO).

Two extracting solvents were used, 70% ethanol and 70% ethanol containing 20% 2-ME. The use of 20% 2-ME maximizes the amount of ethanol-soluble protein extractable from flour or ground wheat meal (Wren and Nutt 1967). Since heating the extracts did not increase protein extractability (from examination of the chromatograms, data not shown), extractions for this study were performed at room temperature. Flour (100 mg) was mixed with extracting solvent (400 μl) in 1.5-ml microcentrifuge tubes and vortexed at 5-min intervals for 15 min and then centrifuged for 15 min at 8,800 $\times g$ at room temperature. The clear supernatant was filtered through a 0.45-μm nylon filter (Millipore, Mississauga, ON) into a chromatography microvial (Hewlett-Packard, Palo Alto, CA).

Chromatography

Apparatus and solvents for HPLC were as described previously (Scanlon et al 1989). If the HPLC procedure is to be used to routinely monitor wheat quality, then changes in operating conditions should not affect (or only slightly affect) the results. Therefore two SynChropak C18, 300 Å (25 cm $\times$ 4.6 mm i.d.) reversed-phase columns were used in conjunction with guard columns (5 cm $\times$ 4.6 mm i.d.) of the same packing. The column used to generate the prediction equations (UQN varieties) was supplied by Terrochem (Edmonton, AB). Subsequently, the analogous results for 16 varieties, obtained on a similar column supplied by Phenomenex (Rancho Palos Verdes, CA), were used to test the prediction equations. The second column was used to simulate the effect of another laboratory using the prediction equations to predict breadmaking quality parameters for a variety whose proteins had been analyzed by HPLC. Column temperature was 50.0°C; solvent flow rate was 1.0 ml/min; and 10-μl aliquots were chromatographed. The elution gradients used are given in Table I.

To generate data for the prediction equations for the extracts obtained with 70% ethanol only (unreduced), the 26 UQN varieties and the variety Marquis were analyzed in random order; the extracts were analyzed in groups of six. Each extract was injected twice. The 27 varieties were reextracted and the extracts analyzed again in duplicate but in a different random order. Thus for these extracts, two replicate extracts and two duplicate injections of each were analyzed for each of the 27 varieties.

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Two replicate extracts with 70% ethanol containing 2-ME (reduced extracts) and two duplicate injections of each extract were analyzed similarly after all extracts without reducing agent had been analyzed.

Each group of six extracts (varieties) was defined as a sequence. The samples of each sequence were chromatographed in the following order: extract of Neepawa reference; extracts of the first group of six samples; two-column cleanup runs (see below); re-equilibration to the starting solvent composition (25 min); a second different extract of Neepawa; the duplicate injections of the six samples; an aliquot of a third extract of Neepawa; and finally, three cleanup runs. Each cleanup run consisted of a 25-μl injection of DMSO at a solvent composition of 55% acetonitrile/45% water (both containing 0.1% trifluoroacetic acid) at a flow rate of 0.1 ml/min for 2 hr.

For the 16 varieties used to test the prediction equations, only one replicate for each extracting solvent was prepared, and no duplicate injections were performed. The 10 SWP varieties were analyzed first. Two months later (to allow some column aging) the six PWCT varieties were analyzed similarly to obtain the data for 16 varieties for testing the prediction equations.

Quantitation of HPLC Results

Each chromatogram was divided into 21 regions (Fig. 1). Some of the regions represented distinct single peaks whereas others represented a number of peaks. Because of the selectivity and changes in retention time that occur during chromatography of a large group of samples (Sapirstein et al 1989, Scanlon et al 1989), absolute retention times were not used as limits of the regions. Rather, each chromatogram was divided into regions by comparing retention times of sample peaks with retention times of peaks in the Neepawa chromatogram acquired before and after in the same sequence (this is a form of visual normalization).

The Hewlett-Packard 79994A software was used to integrate the chromatograms (Scanlon et al 1989) and the peak areas for the regions were recorded. Since not all peaks from other varieties conveniently overlaid the demarcation limits for those of Neepawa, some peak areas had to be estimated by comparing the size of the peak of interest with similarly sized peaks for which the area had been determined.

Differences in the overall level of integration and in the amount of protein analyzed were eliminated by expressing each region as a percentage of the total peak area for each chromatogram.

Technological Tests

Farinograms were obtained according to the AACC method 54-21 (AACC 1983) using 50 g of flour to derive dough development time (in minutes) and mixing tolerance index (MTI, Brabender units). Extensigrams were obtained according to Holas and Tipples (1978) using a Brabender Extensigraph to derive extensibility (E, mm), maximum resistance (R, Brabender units), R/E (ratio of R to E), and area under the curve (A, cm^2). The remix baking test for 100 g of flour was carried out according to Kilborn and Tipples (1981). Volumes of the resulting loaves (LV, cm^3) were measured using a pup loaf volumeter (National Mfg. Co., Lincoln, NE). The baking strength index (BSI) was determined according to Tipples and Kilborn (1974). These tests gave a total of eight breadmaking quality parameters.

Statistical Analyses

Data were analyzed on the University of Manitoba Amdahl 6280 computer using Statistical Analysis System program packages (SAS 1985).

The peak areas for each of the 21 regions of the chromatogram of each of the 26 UQN varieties were the independent variables used to generate a prediction equation for each dependent variable (breadmaking quality parameter) using stepwise multiple regression (STEPWISE procedure) with the maximum r^2 improvement option. The prediction equation selected was the one that gave the maximum r^2 value with the minimum probability value. Since duplicate injections for a given extract were almost exact overlays of each other, the duplicate results were not used in the statistical analyses. Analysis of the results for the first extract of the 26 varieties produced the first set of prediction equations. The analysis was repeated for the second set of replicate extracts, and likewise for the other extracting solvent. In this way, four prediction equations were generated for each breadmaking quality parameter; two for unreduced protein extracts and two for reduced.

To test the efficacy of the 32 prediction equations (eight quality parameters, two solvents, two replicates), the linear regression procedure (REG) was used with the option of 95% confidence limits to predict the quality parameters from analogous RP-HPLC data for 10 SWP and six PWCT varieties.

TABLE I
Gradients Used to Elute Proteins of Unreduced and Reduced Samples

Unreduced Samples		Reduced Samples	
Time (min)	Acetonitrile/Water[a]	Time (min)	Acetonitrile/Water[a]
0	25:75	0	28:72
5	25:75	5	28:72
75	60:40	65	58:42
		70	58:42

[a] Both solvents contained 0.1% trifluoroacetic acid.

RESULTS AND DISCUSSION

Figure 1A and B show the separation achieved for proteins extracted from Neepawa by 70% ethanol without and with 2-ME (reducing agent), respectively. Although better separation could have been achieved in the 30–38 min region of Figure 1B (Marchylo et al 1988), the expenditure of extra time and materials was deemed unnecessary since it appears likely that the peaks in this region represent proteins of a single gliadin or glutenin type (Bietz and Burnouf 1985).

Stepwise multiple regression analysis showed highly significant relationships ($P < 0.001$) between certain gliadin components of the two replicate analyses of unreduced extracts and E, MTI, LV, and R/E. Likewise certain peak areas for the reduced extracts gave highly significant relationships ($P < 0.001$) to E, MTI, LV,

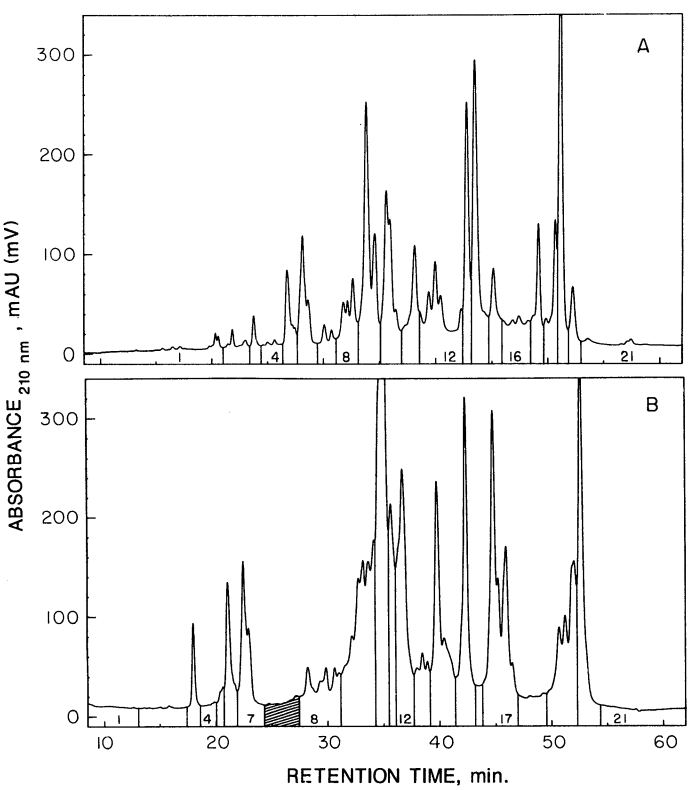

Fig. 1. Chromatograms of the variety Neepawa extracted with 70% ethanol, without (**A**) and with (**B**) 2-mercaptoethanol, showing the 21 regions. The hatched region in B was not included in the statistical analysis.

R/E, and A (data not shown). However, when the prediction equation for each quality parameter was used to predict these quality parameters for the 16 test samples, the only parameter for which predicted and actual values were within the 95% confidence limits was E, the extensigraph extensibility. Table II shows the coefficients and intercept for each extract and both solvents that allowed successful prediction of the actual extensibility of the 16 test varieties. The extensibility was obtained from a summation of the product of the coefficients and the percentage peak area of each region after addition of the intercept.

Prediction Equation Replication

Despite their success in predicting E, the equations for replicate extracts are very different, especially for the extracts obtained with 70% ethanol containing 2-ME. If individual gliadin components are statistically related to quality parameters such as E, and these components can be separated by RP-HPLC (Huebner and Bietz 1986), then the peak areas of the components should correlate well to E regardless of when the extract was analyzed. Since the same chromatographic column was used for all UQN extracts, and the flour samples were composites of four growth locations, column differences (Goldberg 1982) and location effects (Huebner and Bietz 1988) are probably not the reasons for the discrepancies. Two possible reasons for the disparity in peak quantitation between the two extracts of the same variety are random variations in the overall level of integration of chromatograms and variations in peak retention time with column use.

Percentage rather than absolute peak areas would eliminate much of the variation due to different integration levels (Brown et al 1983); the exception being for small peaks on a wide crest, where the area ascribed to a peak will be influenced by the absorbance value of the crest (Fig. 2). However, variations in peak retention time due to column aging may mean that peaks of given retention times are no longer truly correlated to peak retention times of the same extracts analyzed later on the same column. Comparing peaks relative to the peaks of the Neepawa reference chromatograms analyzed at the same time should eliminate much of the uncertainty (Sapirstein et al 1989), but variation will still occur, especially for early eluting peaks (Scanlon et al 1989).

To eliminate potentially spurious correlations with extensibility (E), the peaks most affected by column aging effects were removed so that the prediction equation generated from one set of the 26 UQN extracts would be similar to the prediction equation for the other set. To decide which peaks were most affected, the two correlation matrices generated from data for replicate extracts of a given solvent were examined. The correlation between a given pair of peaks for one extract was compared with the correlation for the same pair of peaks from the replicate extract. Peaks with a large number of unreproducible correlations with other peaks (regardless of the degree of significance of the correlation coefficients) were progressively eliminated until a low (2 or less) level of poor correlations existed for the remaining peaks between the replicate chromatograms. This led to the elimination of peaks (regions) 1, 2, 3, 7, 8, 13, 15, and 21 for the unreduced extracts and 2, 3, 5, 8, 12, and 16 for the reduced extracts.

The four modified data sets were subjected to stepwise regression analysis for each of the eight quality parameters to generate four new prediction equations. Although the new prediction equations were able to predict (within the 95% confidence limits) more LV and R/E values for the reduced extracts, they were still unable to predict these two parameters for all 16 varieties. However, of the eight quality parameters, E remained the most consistently predictable parameter.

The prediction equations for E generated from the modified data sets are given in Table III. Although the equations for different extracts are now more similar to each other, they are still substantially different. The equations for the unreduced extracts gave a satisfactory prediction for a smaller number of varieties than the unmodified data sets. Therefore, despite the fact that replicate extracts generated different prediction equations, extensibility does appear to be consistently predicted using quantitation of peaks across the full chromatograms of reduced and unreduced extracts (Table II).

TABLE II
Prediction Equations Generated for Extensibility from the Chromatograms of the 26 UQN Varieties

Peak Region	Coefficient for Peak Region			
	Unreduced Replicate 1	Unreduced Replicate 2	Reduced Replicate 1	Reduced Replicate 2
1	...	...	−2.65	−2.92
2	...	...	...	...
3	...	...	...	+1.31
4	+0.63	−0.34	...	...
5	+5.12	...	+1.50	...
6	−2.88	...	...	...
7	+2.87	...	...	−3.16
8	+1.25	+0.54	−1.54	+4.01
9	...	−0.42	...	...
10	...	+1.23	−0.92	+0.79
11	+0.31	...	−1.43	...
12	...	+0.49	...	+0.86
13	...	+2.54	...	...
14	...	...	−2.54	...
15	−2.16	+1.26	...	...
16	−1.96	...	+1.78	...
17	+2.38	+0.81	...	...
18	...	+4.35	−8.21	...
19	+1.76	+0.23	−0.94	...
20	+1.11	...	−1.53	+0.98
21	+8.57	−6.61	+9.54	...
Intercept	−34.5	−136.5	+431.1	+49.1
r^2_{max}	0.875***	0.871***	0.833***	0.700**

Unreduced Extracts and Extensibility

The results of HPLC analyses may be compared with analogous results of electrophoretic analyses. The presence of certain bands in gliadin electrophoregrams has been statistically correlated with quality in bread and durum wheats (Damidaux et al 1978, Wrigley et al 1982, Branlard and Dardevet 1985a). Likewise similar statistical relationships have been reported for gliadin peaks analyzed by RP-HPLC (Huebner and Bietz 1986, 1987). It has been suggested that the most probable cause of these relationships is closely linked associations between the gliadin genes and the gene(s)

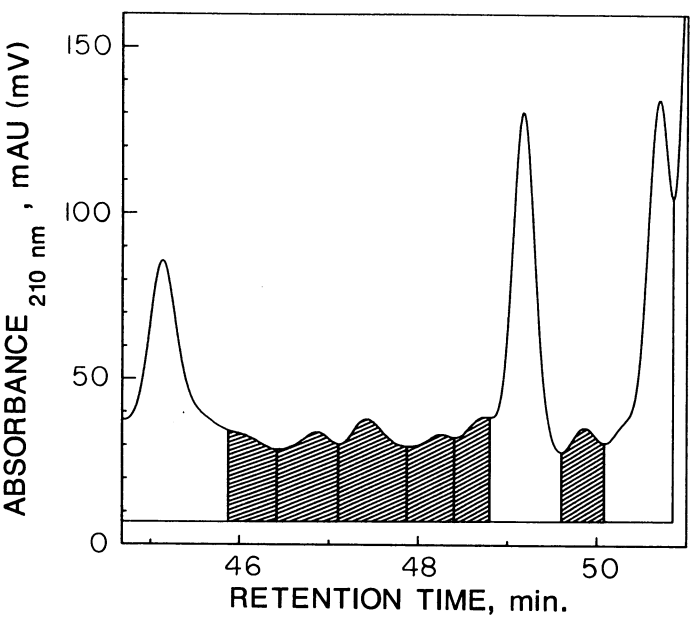

Fig. 2. Portion of chromatogram of the variety Neepawa showing those peaks (hatched) for which selection of integrator baseline or level of absorbance of the crest will have a major influence on peak quantitation.

responsible for the component(s) that contributes directly to good bread and durum wheat quality (Campbell et al 1987). Furthermore, the same authors reported no correlation between individual gliadin electrophoretic bands and extensigraph extensibility. However, the general view on the actual role of gliadins in wheat protein functionality is that they act as the plasticizing component in gluten, imparting viscous characteristics to the dough (Belton et al 1987, Graveland and Henderson 1987). Indeed, Kim et al (1988) showed an almost linear increase in extensibility when crude gliadin protein was added to wheat gluten in the 23–45% gliadin range. The results presented herein show that *E* (extensibility) may be predicted from the total gliadin composition determined by HPLC. Despite differences between the replicate prediction equations, *E* can still be predicted at the 95% confidence limits for all samples, implying that it can be predicted from the overall gliadin composition.

Reduced Extracts and Breadmaking Quality

It is well known that 70% ethanol containing the reducing agent mercaptoethanol extracts some low molecular weight (LMW) glutenin subunits (Bietz and Wall 1973, 1980) as well as some of high molecular weight (Marchylo et al 1989). Accordingly, it was thought that a greater number of breadmaking quality parameters would be related to the quantitation of HPLC peaks of reduced extracts since they contain a larger proportion of gluten proteins. However, despite the fact that the chromatograms of the reduced extracts predicted a greater number of results for LV and MTI (compared with unreduced extracts, data not shown), the only quality parameter that was unequivocally predicted was *E*. The most probable reason for this is that the presence of the large number of gliadin components confounds the correlation for other quality parameters except *E*. High molecular weight glutenin subunits (detected by SDS-PAGE) that predict loaf volume (Ng and Bushuk 1988) appear to be masked in the HPLC chromatogram by the large number of gliadin components eluted in the same elution time region.

The commments on the functionality of gliadins and their specific relationship to *E* are also applicable to the gliadins in the reduced extracts, although probably more so since the use of a reducing agent, in addition to extracting the LMW glutenins, would open up the protein structure and permit greater extraction of the gliadins. Comparison of the actual and predicted extensibility values for the 16 test varieties showed that only one value was outside the 95% confidence limits. When the data were modified by deleting spurious peaks, the *E* values predicted from both (reduced, replicates 1 and 2) prediction equations agreed with actual values within the 95% confidence limits for all 16 varieties (Table IV).

A further confounding effect that could affect the ability to relate quality parameters to HPLC chromatograms is the arbitrary demarcation of the chromatograms into a set number of regions (21 in the present study). For some varieties, two or more peaks may be present in a region (Fig. 3). The two peaks may have antagonistic effects when correlated to some of the breadmaking quality parameters. If this is the case, a higher area value ascribed to a peak region, which in some varieties may be related positively to loaf volume, may not be evident from the regression analysis. This may be caused by the fact that the higher area value in some of the varieties is due to the presence of other components that may have an opposite effect on loaf volume. Furthermore, difficulty in closely reproducing the prediction equations of replicate analyses may be explained similarly, since one of the components may be more affected by column aging than others. This would be particularly so where merged peaks on one column become separated into different regions on another or an aged column. To overcome this difficulty, it may be possible to achieve a better separation between peaks through more sophisticated gradient elution techniques, although this would require longer analysis times.

TABLE III
Prediction Equations Generated for Extensibility from the Chromatograms of the 26 UQN Varieties Using Data Sets After Peak Elimination

	Coefficient for Peak Region			
Peak Region	Unreduced Replicate 1	Unreduced Replicate 2	Reduced Replicate 1	Reduced Replicate 2
1	...	...	−2.59	−4.85
2	...	...	...	...
3	...	...	...	...
4	...	...	−0.59	...
5	+1.60	...	...	...
6	−1.83	+0.93	...	...
7	...	...	−2.12	−4.50
8	...	...	...	...
9	−0.88	−1.68	+0.35	−0.58
10	−0.53	−0.88	...	...
11	...	−0.66	−1.21	−1.10
12	−0.67	−0.68	...	...
13	...	...	...	...
14	−0.42	−0.74	−1.03	−1.61
15	...	...	...	...
16	−1.89	−2.27	...	...
17	+1.28	...	−0.55	...
18	+2.69	+2.80	−4.94	−1.69
19	....	−0.89	...	−1.42
20	−1.19	−1.79	−0.25	...
21	...	...	+5.42	...
Intercept	+344.8	+601.2	+309.4	+391.2
r^2_{max}	0.811***	0.822***	0.727**	0.716***

TABLE IV
Comparison of Actual Extensibility Values with Predicted Values[a]

Variety	Actual Extensibility	Predicted Extensibility
SWP 1	205	185.3
SWP 2	185	184.6
SWP 3	180	193.4
SWP 4	190	196.9
SWP 5	175	184.4
SWP 6	205	191.5
SWP 7	170	193.4
SWP 8	210	195.3
SWP 9	195	183.0
SWP 10	185	177.9
PWCT 1	203	146.5
PWCT 2	186	216.3
PWCT 3	186	185.3
PWCT 4	191	173.5
PWCT 5	173	160.6
PWCT 6	171	194.8

[a] Predicted values calculated from replicate no. 2 of the reduced extracts after elimination of six of the peak regions (2, 3, 5, 8, 12, and 16).

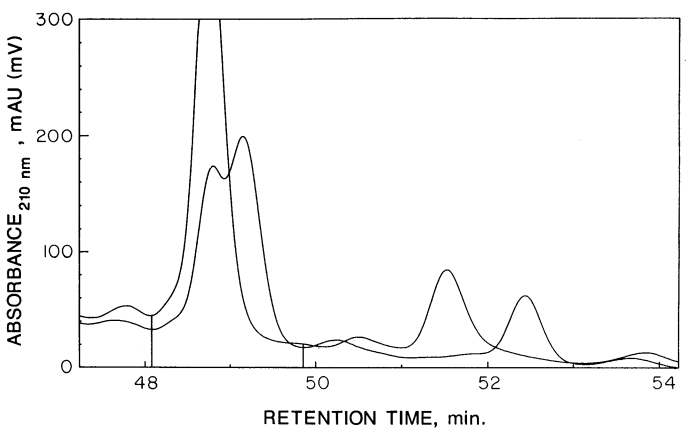

Fig. 3. Portion of chromatograms for two Uniform Quality Nursery varieties extracted without 2-mercaptoethanol. Region from 48.1 to 49.9 min represents peak region 17, a region found to correlate positively with baking strength index (additional details in text).

CONCLUSIONS

It was shown that flour proteins from one set of wheat varieties extracted by 70% ethanol without and with a reducing agent and separated by RP-HPLC can be used to predict dough extensibility determined on the Brabender Extensigraph. This finding is generally consistent with the functional role played by gliadins in wheat gluten. RP-HPLC was shown to be a sufficiently robust method; long-term column use and change of column did not diminish the predictive capacity of the separation method. However, if the method is to be used to predict breadmaking quality parameters other than extensibility, more specific fractionation procedures may be required to obviate the masking effect of passive protein components present in the extracts that are analyzed.

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LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC. Method 54-21, approved October 1982. The Association: St. Paul, MN.

BELTON, P. S., DUCE, S. L., and TATHAM, A. S. 1987. Solid state NMR studies of wheat gluten. Page 456 in: Proc. Int. Workshop on Gluten Proteins 3rd. R. Lasztity and F. Bekes, eds. World Scientific: Singapore.

BIETZ, J. A., and BURNOUF, T. 1985. Chromosomal control of wheat gliadin: Analysis by reversed-phase high-performance liquid chromatography. Theor. Appl. Genet. 70:599-609.

BIETZ, J. A., and WALL, J. S. 1973. Isolation and characterization of gliadin-like subunits from glutenin. Cereal Chem. 50:537-547.

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. 57:415-421.

BRANLARD, G., and DARDEVET, M. 1985a. Diversity of grain proteins and bread wheat quality. I. Correlation between gliadin bands and flour quality characteristics. J. Cereal Sci. 3:329-343.

BRANLARD, G., and DARDEVET, M. 1985b. Diversity of grain protein and bread wheat quality. II. Correlation between high molecular weight subunits of glutenin and flour quality characteristics. J. Cereal Sci. 3:345-354.

BROWN, A. C., III, WALLACE, D. L., BURCE, E. L., and MATHES, S. 1983. Data handling for LC detectors. Page 355 in: Liquid Chromatography Detectors. T. M. Vickrey, ed. Marcel Dekker: New York, NY.

CAMPBELL, W. P., WRIGLEY, C. W., CRESSEY, P. J., and SLACK, C. R. 1987. Statistical correlations between quality attributes and grain-protein composition for 71 hexaploid wheats used as breeding parents. Cereal Chem. 64:293-299.

DAMIDAUX, R., AUTRAN, J. C., GRIGNAC, P., and FEILLET, P. 1978. Mise en evidence de relations applicables en selection entre l'electrophoregramme des gliadines et les proprietes viscoelastiques du gluten de *Triticum durum* Desf. C.R. Acad. Sci. Ser. D. 287:701-704.

GLAJCH, J. L., KIRKLAND, J. J., and KOHLER, J. 1987. Effect of column degradation on the reversed-phase high-performance liquid chromatographic separation of peptides and proteins. J. Chromatogr. 384:81-90.

GOLDBERG, A. P. 1982. Comparison of columns for reversed-phase liquid chromatography. Anal. Chem. 54:342-345.

GRAVELAND, A., and HENDERSON, M. H. 1987. Structure and functionality of gluten proteins. Page 238 in: Proc. Int. Workshop on Gluten Proteins 3rd. R. Lasztity and F. Bekes, eds. World Scientific: Singapore.

HOLAS, J., and TIPPLES, K. H. 1978. Factors affecting farinograph and baking absorption I. Quality characteristics of flour streams. Cereal Chem. 55:637-652.

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. 4:379-388.

HUEBNER, F. R., and BIETZ, J. A. 1987. Improvements in wheat protein analysis and quality prediction by reversed-phase high-performance liquid chromatography. Cereal Chem. 64:15-20.

HUEBNER, F. R., and BIETZ, J. A. 1988. Quantitative variation among gliadins of wheats grown in different environments. Cereal Chem. 65:362-366.

KILBORN, R. H., and TIPPLES, K. H. 1981. Canadian test baking procedures. I. GRL remix method and variations. Cereal Foods World 26:624-628.

KIM, J.-J., KIEFFER, R., and BELITZ, H.-D. 1988. Rheologische Eigenschaften von rekonstituierten Weizenklebern mit variierenden Anteilen an Prolaminfraktionen verschiedener Getreidearten. Z. Lebensm. Unters. Forsch. 186:16-21.

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. 65:28-40.

MARCHYLO, B. A., KRUGER, J. E., and HATCHER, D. W. 1989. Quantitative reversed-phase high-performance liquid chromatographic analysis of wheat storage proteins as a potential quality prediction tool. J. Cereal Sci. 9:113-130.

NG, P. K. W., and BUSHUK, W. 1988. Statistical relationships between high molecular weight subunits of glutenin and breadmaking quality of Canadian-grown wheats. Cereal Chem. 65:408-413.

PAYNE, P. I., NIGHTINGALE, M. A., KRATTINGER, A. F., and HOLT, L. M. 1987. The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. J. Sci. Food Agric. 40:51-65.

SAPIRSTEIN, H. D., SCANLON, M. G., and BUSHUK, W. 1989. Normalisation of HPLC peak retention times for computerised comparison of wheat prolamin chromatograms. J. Chromatogr. 469:127-135.

SAS. 1985. SAS User's Guide: Statistics, Version 5. SAS Institute Inc.: Cary, NC.

SCANLON, M. G., SAPIRSTEIN, H. D., and BUSHUK, W. 1989. Evaluation of the precision of high-performance liquid chromatography for wheat cultivar identification. Cereal Chem. 66:112-116.

TIPPLES, K. H., and KILBORN, R. H. 1974. "Baking strength index" and the relation of protein content to loaf volume. Can. J. Plant Sci. 54:231-234.

WREN, J. J., and NUTT, J. 1967. The effect of three thiols on the extractability of wheat-flour proteins. J. Sci. Food Agric. 18:119-123.

WRIGLEY, C. W., LAWRENCE, G. J., and SHEPHERD, K. W. 1982. Assocation of glutenin subunits with gliadin composition and grain quality in wheat. Aust. J. Plant Physiol. 9:15-30.

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