

Rapid Size-Exclusion Chromatography Analysis of Molecular Size Distribution for Wheat Endosperm Protein

O. R. Larroque^{1,2,3} and F. Bekes^{1,2}

ABSTRACT

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A quick method for the separation of the main size classes of wheat endosperm proteins using size-exclusion HPLC is presented. Separations achieved in a 10-min run showed high correlation with the reference method of our laboratory (35-min) using the same type of column. The clear separation obtained allows a quick analytical determination of important parameters such as the proportion of the main classes of wheat

endosperm protein, the glutenin-to-gliadin ratio, and the percentage of unextractable (SDS-soluble with sonication) polymeric protein, all of which have been closely correlated with breadmaking quality parameters. The improved method offers the advantages of better utilization of valuable resources such as HPLC equipment, quicker analysis of large sample sets, and collection of eluted fractions.

Size-exclusion (HPLC) has been extensively used for the study of cereal endosperm proteins, particularly in wheat, since pioneer analyses in the mid 1980's (Bietz 1983, 1986). The methodology accurately separates the three main classes of wheat endosperm proteins: glutenins (polymeric protein), gliadins, and albumins+globulins (monomeric protein) (Batey et al 1991). It is also used for the analysis of the molecular size distribution of polymeric protein (Gupta et al 1993). Parameters such as percentage of polymeric protein (PPP), percentage of gliadins (PG), glutenin-to-gliadin ratio (GLU/GLI), polymeric protein in the flour (FPP), and percentage of unextractable polymeric protein (%UPP) are obtained after those analyses and are useful markers for assessing breadmaking quality (Huebner and Wall 1976, Dachkevitch and Autran 1989, MacRitchie and Lafiandra 1997).

According to standard procedures (Batey et al 1991, Gupta et al 1993), each sample that is injected into the column is prepared from a weighed amount of 10 mg of flour or whole meal that is extracted with 0.5% SDS phosphate buffer with sonication (for total protein and unextractable protein) or without it (extractable or soluble protein). Running these different extracts requires two weighings (because unextractable protein is obtained from the pellet after the extractable protein has been removed) and three injections for each material. This will require, in terms of time, ≈ 105 min (35 min each) without considering sample preparation time and replicates. Therefore, the screening of large sample sets implies a lot of equipment time and consumables usage.

The methodology of SE-HPLC has been upgraded in terms of hardware (e.g., HPLC systems and columns). These developments have resulted in improvements in the resolution of components, especially in the region of polymeric glutenin (Larroque et al 1996, 1997a). However, few attempts have been made to make the methodology faster (Larroque et al 1997b). Recently, Bean et al (1998) developed a faster alternative to SE-HPLC methods based on nitrogen combustion that allowed rapid quantitation (3 min) of insoluble polymeric protein in flour.

The aim of this research was to develop a SE-HPLC method for the screening of large sample sets and for semipreparative work that could give results as reliable as those obtained using the standard procedure in shorter times.

MATERIALS AND METHODS

Different wheat endosperm protein extracts were subjected to SE-HPLC using a Beckman System Gold HPLC, configured with two 126 Pumps, a 166 Detector and a 507E Autosampler.

Samples were extracted using 0.5% SDS and phosphate buffer (pH 6.9) according to the procedure of Batey et al (1991) for total protein and Gupta et al (1993) for SDS-soluble (without sonication) polymeric protein and SDS-soluble (with sonication) polymeric protein. Supernatants were filtered through 0.45- μ m PVDF filters before HPLC analysis.

A Phenomenex BIOSEP-SEC 4000 (5 μ m, 500 \AA , 7.8- \times 300-mm) column (Phenomenex, Torrance, CA) was used in the experiment. A running time of 10 min was used (flow rate 2 mL/min), instead of the standard 35 min run (flow rate 0.5 mL/min), which was included for comparison purposes. A Zorbax BIO GF 450 (6 μ m, 300 \AA , 9.4- \times 250-mm) column (Rockland Technologies, Inc., Chadds Ford, PA) was used for certain comparisons using the 10-min run. For analyzing the reproducibility of the results, three different extracts from two flours were injected three successive times for 10-min and 35-min running times. Eluted protein was detected at 214 nm. Injections were always 20 μ L. Acetonitrile 190 grade (Ajax Laboratory Chemicals, Auburn, Australia), TFA (sequanal grade from Pierce, Rockford, IL) and highly purified water (Hi-Pure Water Systems, Australia) were used for solvent preparation. Integration of chromatograms was performed using Beckman Gold Nouveau software (vers. 1.5). MSUSTAT and Excel v. 5.0 packages were used for the statistical analysis of the data and graphics, respectively.

RESULTS AND DISCUSSION

Figure 1 shows how elution from a particular wheat endosperm protein extract appears when using a 10-min run (Fig. 1b) or the standard 35-min run (1a). The total elution time corresponding to polymeric protein (P1) was 2 min for 1b and 8 min for 1a. Although profiles are similar, in the shorter run there is no resolution for the small peak eluting just before the main peak (in height) in the standard run. The materials eluting there have been identified as ω -gliadins (Larroque et al 1997a). No other discrepancy is noted. In SDS-soluble protein without and with sonication (SOL and UNEXT) profiles, the resolution for the main size classes, and particularly for glutenins, is similar between the short and long running procedure. Glenlea (Canada western extra strong red spring wheat) and Chinese Spring (soft wheat) are shown in Fig. 1c and 1d as examples of two sample extracts that show clear differences in the amount of SDS-soluble (with sonication) protein using either procedure.

¹ CSIRO Plant Industry, Grain Quality Research Laboratory, North Ryde, NSW 1670, Australia.

² Quality Wheat CRC Limited, North Ryde, NSW, 1670, Australia.

³ Corresponding author. E-mail: larroque@pi.csiro.au

The method is also valid for analyzing samples with narrower differences, as is the case when analyzing total polymeric protein from the same pair (Glenlea and Chinese Spring). Statistically significant differences between both samples were found for the percentage of total polymeric protein using the 35-min run (Glenlea 49.6%, Chinese Spring 53.0%) or the 10-min run (Glenlea 48.5%, Chinese Spring 51.8%).

Correlation between parameters obtained from both runs was highly significant for total polymeric protein (P1) ($R^2 = 0.94$, $n = 26$); gliadin (P2) ($R^2 = 0.92$, $n = 26$); albumins+globulins (P3) ($R^2 = 0.90$, $n = 26$); unextractable polymeric protein ($R^2 = 0.95$, $n = 17$) and total area under the chromatogram ($R^2 = 0.96$, $n = 24$).

In terms of absorbance units recorded, the short run yielded 23% of the absorbance units recorded in the longer run, which is consistent with the reduction in time (faster pass of the sample through the detector cell).

TABLE I
Reproducibility of Results Using Two Size-Exclusion HPLC Methods for Separating Wheat Proteins^a

	Injection		
	1	2	3
Sample 1			
10 min			
Extract 1	48.67	48.59	48.56
Extract 2	48.04	48.00	48.02
Extract 3	48.79	48.83	48.67
35 min			
Extract 1	49.97	49.47	49.76
Extract 2	48.99	49.40	48.87
Extract 3	50.06	49.71	50.03
Sample 2			
10 min			
Extract 1	51.88	51.94	52.02
Extract 2	51.44	51.39	51.39
Extract 3	52.14	52.15	52.02
35 min			
Extract 1	53.47	53.11	53.37
Extract 2	52.69	52.22	52.65
Extract 3	53.40	53.28	53.05

^a Percentage of Peak 1 from total protein extracts.

In Table I, the reproducibility of results using both methods is given. As indicated previously (Larroque et al 1997b), different extracts from one sample may give statistically different results. This could be due to experimental errors or sample instability. Regarding the first possibility, sources of error can be found in sample weighing, supernatant transferring (for unextractable protein), sample suspension in buffer (for SDS-soluble without sonication protein), sonication procedure, integration, and detector response. In terms of sample instability, changes in polymeric protein can occur due to the enzymatic activity of proteinases, particularly when analyzing whole meal samples (results not shown). Nevertheless, no significant differences were found between successive injections using both methods, which is in agreement with previous results using a Zorbax BIO GF 450 column (Larroque et al 1997b).

The working pressures (measured during monitor baseline) obtained at different flow rates for the columns under study were within manufacturer's limits. For the Phenomenex column, pressures of 835, 640, 450, and 245 psi were recorded at flow rates of 2, 1.5, 1, and 0.5 mL/min, respectively. For the Zorbax column, values were 950, 740, 540, and 325 psi for 2, 1.5, 1, and 0.5 mL/min flow rates, respectively.

CONCLUSIONS

The main size classes of wheat endosperm protein (glutenins, gliadins, and albumins + globulins) are very well separated when subjected to SE-HPLC using a Phenomenex BIOSEP SEC-4000 column in a 10-min run with a flow rate of 2 mL/min. This column performed clearly better than the Zorbax BIO GF 450, which had acceptable resolution for polymeric protein. The clear separation obtained allows a quick analytical determination of important parameters such as the proportion of the different types of wheat endosperm protein in total protein, the glutenin-to-gliadin ratio, and the percentage of unextractable (insoluble) polymeric protein, all of which have been closely correlated with breadmaking quality parameters (MacRitchie and Lafiandra 1997). The capability of doing this type of analysis in a quick and accurate manner is especially important when large sample sets have to be screened. Also, for preparative purposes, whole fractions of the polymeric or mono-

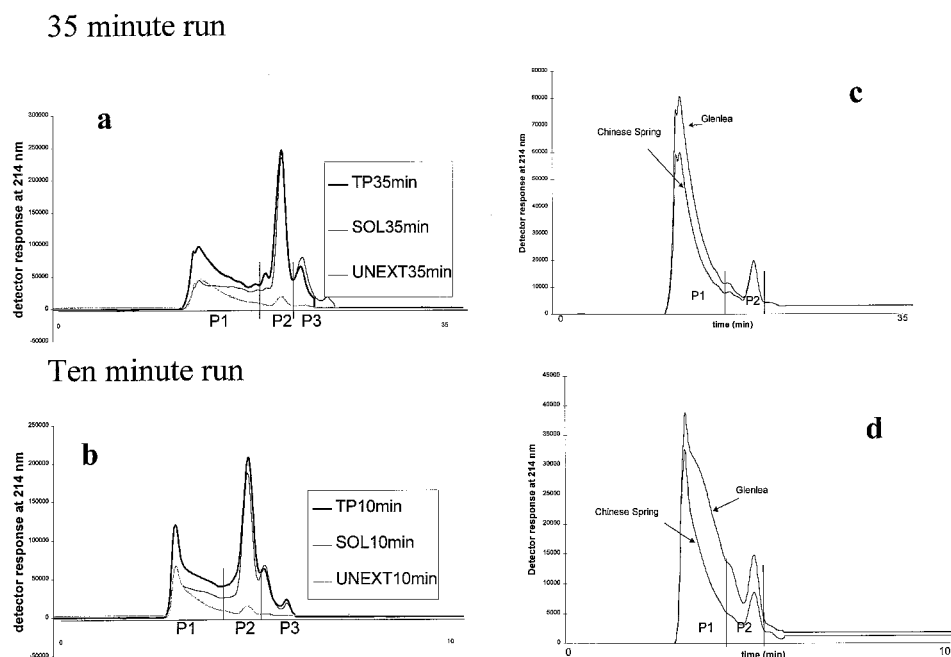


Fig. 1. Size-Exclusion HPLC separation of total protein (TP), SDS-soluble protein without sonication (SOL) and SDS-soluble protein with sonication (UNEXT) from wheat flour using 10-min rapid method (b) and 35-min reference method (a). Samples of SDS-soluble protein with sonication from Glenlea and Chinese Spring flours 35 min and 10 min procedure (c and d).

meric protein peaks are collected faster, providing adequate source material for reconstitution studies where these type of collected fractions show no changes in functional properties. Finally, the efficiency in the use of highly valuable resources such as a HPLC system is dramatically increased.

These findings, along with sample-preparation protocols that enhance sample solubility, stability, and minimum alteration in the molecular size distribution due to sonication, would help developing a better methodology for the study of SE-HPLC fractionation of wheat endosperm protein.

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