

Impact of Cultivar and Environment on Size Characteristics of Wheat Proteins Using Asymmetrical Flow Field-Flow Fractionation and Multi-Angle Laser Light Scattering^{1,2}

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ABSTRACT

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The use of multi-angle laser light scattering (MALLS) in conjunction with asymmetrical flow field-flow fractionation (A-FFFF) was investigated for the determination of the molecular weight distribution (MWD) of wheat proteins. The wheat flour proteins were dissolved by sonication in 0.1M sodium phosphate (pH 6.9) containing 2% SDS. The results presented make it evident that efficient separation and size characterization of monomeric ($M < 10^5$ g/mol) and polymeric protein ($10^5 \leq M < 10^8$ g/mol) wheat proteins can be achieved with A-FFFF/MALLS/UV in a single run. Therefore, this method appears to be able to detect

significant modifications of MWD of wheat protein, whatever the factor inducing these alterations (i.e., genetic or environmental) and whatever the nature of these alterations (i.e., monomeric-to-polymeric ratio or MWD of polymeric protein). In the present study, we have indeed demonstrated that the MWD of wheat proteins can be altered from one cultivar to another in three main ways: by changing the relative amounts of monomeric and polymeric proteins, by changing the MWD of polymeric protein, and then by changing both the monomeric-to-polymeric ratio and the MWD of polymeric protein.

It has long been known that the properties of hydrated gluten or wheat flour dough depend on two main types of protein, the glutenins and gliadins. Hydrated gliadin (monomeric protein) exhibits plasticity (Wall 1979), whereas hydrated glutenin (polymeric protein) has strong elastic properties (Wall 1979; Bietz and Huebner 1980). The commercially desirable viscoelastic properties required for good performance in processing wheat flour doughs result from the combined contributions of these two main types of protein.

In recent times, research has increased our knowledge of the complex mixture of wheat proteins. However, the view that two main groups of proteins control dough properties has not changed. In that way, the molecular weight distribution (MWD) of wheat proteins is becoming recognized as the main determinant of physical dough properties (Southan and MacRitchie 1999). However, in theory, the MWD can be altered from one sample of wheat (or one cultivar) to another by changes in the relative proportions of monomeric proteins and polymeric proteins or by changes in the size distribution of polymeric proteins (MacRitchie and Lafiandra 1997). To estimate wheat flour dough properties, it is necessary to develop methods that can measure these two main parameters (monomeric-to-polymeric ratio and MWD of polymeric proteins) at the same time and with accuracy. With the application of size-exclusion HPLC (SEC) to wheat proteins pioneered by Bietz (1984) and developed by other workers (Dachkevitch and Autran 1989), it has become possible to measure the relative quantities of monomeric and polymeric proteins. Indeed, the methodology that accurately separates gliadins and glutenins is also used for analysis of the molecular size distribution of polymeric proteins (Gupta et al 1993). However, these methods are unsuitable for measurement of the true MWD of the largest glutenin molecules because the upper size limit could not be determined accurately in the SEC studies due to the column exclusion limits (Bean and Lookhart 2001; Carceller and Aussenac 2001). On the contrary, flow field-

flow fractionation (FFFF) is not impeded by an exclusion limit (Giddings et al 1977) and has been used successfully to separate a number of these HMW fractions (Stevenson and Preston 1996; Wahlund et al 1996; Ueno et al 2002). The principle of FFFF has been reviewed (Giddings 1993) and its theoretical and experimental basis described (Giddings et al 1977, 1992; Wahlund and Giddings 1987; Litzén et al 1993). Furthermore, the MALLS technique is one of the most effective means for determining molecular weight, size, and conformation of polymers without reference to standards (Wyatt 1992). It has been applied to glutenin polymers (Egorov et al 1998; Carceller and Aussenac 2001; Stevenson et al 2003; Arfvidsson et al 2004). Moreover, the need for a direct molecular mass determination, such as light scattering, in combination with the FFF method to accurately measure size of glutenin has been pointed out by Arfvidsson and Wahlund (2003). In all these previous studies, the FFF method has been used to characterize the purified polymeric fraction (ultra large wheat protein glutenin) using different glutenin extraction and dissolution methods. However, to characterize the MWD of wheat proteins, it is necessary to measure the monomeric-to-polymeric ratio and the MWD of polymeric proteins at the same time.

Consequently, the aim of the present work was to investigate the potential of an online coupling of A-FFFF and MALLS to characterize monomeric and polymeric proteins in the same run, and to reveal the effect of cultivar and environment on the MWD of these storage proteins.

MATERIALS AND METHODS

Wheat Samples and Protein Extraction

Sixteen common wheats (Table I) were grown in 2001 and 2002 at eight locations. At maturity (53 DAA), grains were collected and milled on a laboratory mill (Brabender Sr). Flour samples (30 mg) were dispersed and incubated at 60°C for 15 min with 1 mL of 0.1M sodium phosphate buffer (pH 6.9) containing 2% (w/v) SDS. The extracts were then sonicated for 15 sec at a power setting of 50% (output 10W, 23 kHz) using a stepped microtip probe (3 mm diameter) (Branson sonifier, model B-12). Total solubilized protein extracts were obtained by gathering the supernatants after 30 min of centrifugation at $12,500 \times g$ at 20°C. The extracts were then filtered through 0.45- μ m filters (GHP AcroPrep filter plate, Gelman Sciences, France) before injection (20 μ L) into the A-FFFF/MALLS system. All samples were stored at room temperature and were analyzed within 12 hr after extraction. Protein content was determined for each extract by combustion nitrogen analysis ($N \times 5.7$) (AOAC 7.024) (Table II).

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Polymeric Protein Preparation for A-FFFF

Flour samples (30 mg) were stirred for 1 hr at room temperature (25°C) with 1 mL of 0.080M Tris-HCl buffer (pH 7.5) containing 50% (v/v) propan-1-ol according to Fu and Sapirstein (1996). Extraction was followed by centrifugation at $15,900 \times g$ for 30 min at 15°C. The supernatant (mainly monomeric proteins) was eliminated, and the residue (polymeric proteins) was then extracted with 1 mL of a sodium phosphate buffer 0.1M (pH 6.90) containing 2% (w/v) SDS and sonicated as previously described.

A-FFFF/MALLS Procedure

The A-FFFF channel was supplied by Consensus (Germany). The channel has a trapezoidal geometry where the length is 28.6 cm, the trapezoidal breadths were 2.12 and 0.47 cm, respectively, the area cut off at the inlet end was 2.25 cm², and the total area enclosed by the spacer was 36.9 cm². The channel thickness was 100 μm and the resulting channel volume 0.36 mL. The accumulation wall consisted of a CM05Y ultrafiltration membrane of regenerated cellulose (Millipore, Germany). The software for the flow control was WinFFF 3.0 supplied by Consensus. The flow in the channel was generated by a Knauer pump (MiniStar K501) and the injection of a sample was made with a variable autoinjector (model AS3000, ThermoQuest SA, Les Ulis, France). The cross-flow was generated by a Pharmacia pump (P500). Sodium phosphate buffer (0.1M, pH 6.9) containing 0.1% (w/v) SDS was used as a mobile phase. It was filtered through 0.1-μm membrane (Gelman Sciences, France) and degassed before entering the channel by a ERC 3215α degasser. An inline filter (0.1 μm) was installed between the pump and the A-FFFF channel.

TABLE I
Allelic Composition of HMW-GS in Wheat Cultivars

| Cultivar | <i>Glu-A1</i> | <i>Glu-B1</i> | <i>Glu-D1</i> |
|-----------|---------------|---------------|---------------|
| AJ 536 | null | 7+0 | 3+12 |
| Eole | 2* | 6+8 | 2+12 |
| Genial | null | 7+0 | 5+10 |
| HA 83 | 1 | 7+0/7+9 | 5+10 |
| HA 87 | 1 | 7+8 | 5+10/3+12 |
| Isengrain | null | 7+8 | 5+10 |
| Louvre | null | 7+9 | 2+12 |
| NH 535 | 1 | 7+8 | 5+10 |
| Precia | null | 6+8/7+9 | 2+12 |
| RE 204 | 1 | 7+0 | 5+10 |
| Renan | 2* | 7+8 | 5+10 |
| Rista | 2* | 7+8 | 5+10 |
| Rival | 1 | 7+9 | 5+10 |
| Soissons | 2* | 7+8 | 5+10 |
| Tremie | null | 6+8 | 3+12 |
| Valea | 2* | 7+0/6+8 | 5+10/2+12 |

TABLE II
Amount of Protein Extracted (% of total) for Wheat Cultivars

| Cultivar | Protein Extracted | SD (<i>n</i> = 16) |
|-----------|-------------------|---------------------|
| AJ 536 | 91.0 | 2.0 |
| Eole | 91.0 | 1.0 |
| Genial | 92.0 | 2.0 |
| HA 83 | 90.0 | 2.0 |
| HA 87 | 88.0 | 2.0 |
| Isengrain | 90.0 | 2.0 |
| Louvre | 92.0 | 2.0 |
| NH 535 | 90.0 | 2.0 |
| Precia | 93.0 | 2.0 |
| RE 204 | 93.0 | 2.0 |
| Renan | 88.0 | 2.0 |
| Rista | 92.0 | 1.0 |
| Rival | 90.0 | 1.0 |
| Soissons | 90.0 | 2.0 |
| Tremie | 92.0 | 1.0 |
| Valea | 89.0 | 2.0 |

The fractionated molecules from A-FFFF were monitored by a variable wavelength UV detector (model UV 2000, ThermoQuest SA, France), a MALLS photometer (model Dawn-F, Wyatt Technology, Santa Barbara, CA) and a differential refractive index detector (model ERC 7512, Erma, ThermoQuest SA, France). Pure toluene with a known Rayleigh ratio and NaCl solutions were used to calibrate MALLS and RI, respectively. MALLS photodiode coefficients were normalized using bovine serum albumin monomer as reference. Calculations of molecular weight number-average (M_n), weight-average (M_w), and z-average (M_z), and mean square radius number-average $\langle (R_g^2)^{0.5}_n \rangle$, weight-average $\langle (R_g^2)^{0.5}_w \rangle$ and z-average $\langle (R_g^2)^{0.5}_z \rangle$ were performed using the ASTRA 4.72 and Corona software (Wyatt Technology, Santa Barbara, CA). The Zimm extrapolation (first-order) (KC/R0) was used to calculate M and R_g . Value for dn/dc was measured as described in Wittgren and Wahlund (2000). A value of 0.250 mL/g was used as a refractive index increment (dn/dc) for wheat proteins. To determine the monomeric-to-polymeric ratio, the UV signal (214 nm) was integrated for each protein region ($M < 10^5$ g/mol and $M \geq 10^5$ g/mol) using the ASTRA 4.72 software.

Characterization of A-FFFF Protein Fractions

Five A-FFFF fractions were collected 10 times to obtain sufficient protein material and assigned respectively to the fractions F₁ to F₅. For each protein fraction, the 10 replicates were pooled and concentrated to a 100 μL final volume using ultra-filtration cells (5 kDa cut-off, Ultrafree, Millipore Corp.) by centrifugation at $5,000 \times g$ at 20°C. Each concentrated protein fraction (12.5 μL) was added to 12.5 μL of the sample buffer (0.5M Tris-HCl buffer, pH 6.8; 10% [w/v] SDS; 0.1% [w/v] bromophenol blue; 20% [v/v] glycerol; and 5% [v/v] 2-mercaptoethanol) and were incu-

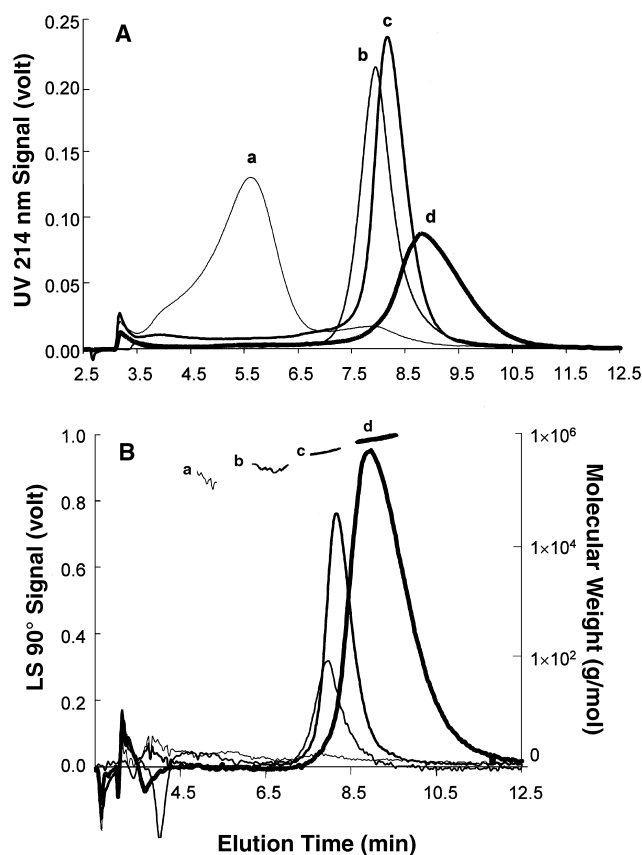


Fig. 1. Asymmetrical flow field-flow fractionation (A-FFFF) profiles of four standard proteins: lysozym (a), bovine serum albumin (b), gamma-globulin (c), thyroglobulin (d). **A**, UV response as a function of elution time. **B**, Light scattering at 90° (LS) and molecular weight as a function of elution time.

bated at 100°C for 5 min. SDS-PAGE of reduced proteins (25 µL) was performed at pH 8.8 in 12% separating gel 1 mm thick for 50 min at a constant current of 40 mA. Gel was stained with a colloidal blue staining kit (Invitrogen).

Statistical Analysis

Tests of between-subject effects (cultivar and environment), Student-Newman-Keuls tests, and Pearson correlations were computed with SPSS v. 11.5 software. Statistical significance was calculated for each analysis of tables or figures. Sixteen cultivars grown at eight locations and for two years (2001 and 2002) were used. Data of 2001 and 2002 were separately treated for variance analysis tests because the effects of principal factors were studied for differences among the two analyzed years. Because there was no agronomical replication, the part of variation due to interaction (cultivar × environment) had not been appreciated.

RESULTS AND DISCUSSION

Fractionation of Wheat Proteins by A-FFFF

Protein standards with M_w of 14.3×10^3 (lysozym), 67.0×10^3 (bovine serum albumin), 150.0×10^3 (gamma-globulin), and 665.0×10^3 g/mol (thyroglobulin) prepared in 0.1M sodium phosphate buffer (pH 6.9) containing 2% (w/v) SDS were used to assess the efficiency of the A-FFFF/MALLS system and to aid in designing conditions for separating wheat storage proteins. Optimum A-FFFF resolution of the standard molecular size marker proteins

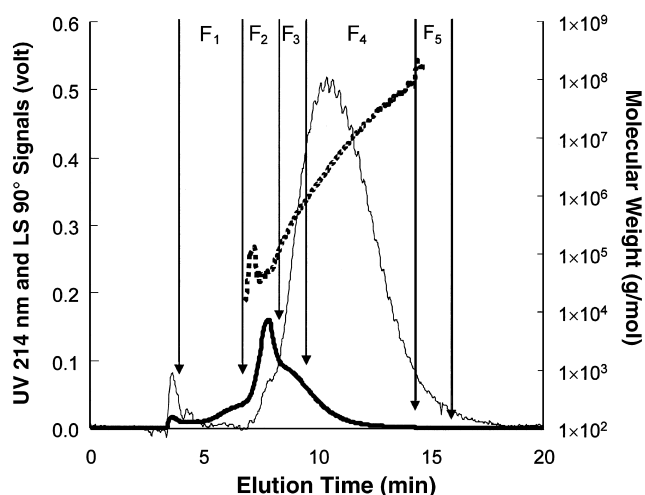


Fig. 2. Asymmetrical flow field-flow fractionation (A-FFFF) profiles of total solubilized storage proteins of Soissons. UV (bold line), light scattering at 90° (fine line), and molecular weight as a function of elution time (dotted line). Arrows define A-FFFF size fractions F₁ to F₅. A-FFFF conditions: sample focusing (120 sec), $V_{in} = 3$ mL/min, $V_c = 3.25$ mL/min; fractionation phase 1 (200 sec), $V_{in} = 4$ mL/min, $V_c = 3$ mL/min, and $V_{out} = 1$ mL/min; fractionation phase 2 (100 sec), linear reduction of V_c to 3 mL/min up to 0 mL/min with $V_{out} = 1$ mL/min; fractionation phase 3 (550 sec), $V_{in} = 1$ mL/min, $V_c = 0$ mL/min, and $V_{out} = 1$ mL/min.

(Fig. 1A) was obtained using a programmed cross-flow rate. Protein samples were focused during 120 sec at a constant inlet flow rate (V_{in}) of 3 mL/min and a constant cross-flow rate (V_c) of 3.25 mL/min. During the first 200 sec of the fractionation, V_{in} and V_c were maintained constant (4 and 3 mL/min respectively). After that, V_{in} was varied between 4 and 1 mL/min for 100 sec at a constant V_{out} at ≈ 1 mL/min, thus giving a cross-flow rate (V_c) in the interval of 3–0 mL/min. Then, V_{out} was maintained constant at 1 mL/min for 550 sec.

The use of a MALLS photometer enables the molecular weight and the root mean square radius to be calculated for each fraction eluted. Figure 1B shows the molecular weight calculated from the intensity and the angular dependence of the scattered light for the four protein standards. The values for all protein samples are plotted against the elution time. The polydispersity is very low and increases with molecular weight, indicated numerically by the ratios M_w/M_n and M_z/M_n in Table III. This table also lists the average values for molecular weight obtained by light scattering compared with the manufacturers' values ($M_{nominal}$). There is a good agreement between the light scattering data and the nominal results.

Figure 2 shows a typical fractogram of total solubilized wheat storage proteins. The molecular weights determined by A-FFFF/MALLS/UV had a range of roughly 10^4 to 10^8 g/mol and the M_w was 1.40×10^6 g/mol. The light scattering signal and the UV signal do not coincide, which is an effect of the large protein sample polydispersity. The determined molecular weight ranges of storage protein dissolved by sonication using A-FFFF/MALLS/UV were in total agreement with those determined by SEC/MALLS (Bean and Lookhart 2001; Carceller and Aussenac 2001) but the upper size limit could not be determined accurately in the SEC studies due to the column exclusion limit. With A-FFFF/MALLS, even the highest molecular weight can be separated and the complete MWD can be obtained.

Five A-FFFF fractions (F₁ to F₅) were collected and subjected to SDS-PAGE (Fig. 3). The two main fractions (F₂ and F₄) showed almost no overlap in their peptide composition and clearly represented monomeric and polymeric proteins, respectively (Fig. 3A). Fraction F₃ showed some overlap with other fractions, but it is mainly constituted of polymeric proteins. The banding patterns of reduced fractions (Fig. 3B) revealed that fractions F₃ and F₄ included polymeric proteins, mostly low molecular weight (LMW-GS) and high molecular weight glutenin subunits (HMW-GS). Fraction F₂ included gliadins, albumins, and globulins. Figure 4A shows A-FFFF profiles of total solubilized storage proteins (monomeric and polymeric proteins) and purified polymers (50% propan-1-ol insoluble glutenins) of Soissons. For these two extracts, the differential weight fraction versus the molecular weight is plotted in Fig. 4B, where the molecular weights determined by A-FFFF/MALLS/UV had a range of roughly 2×10^5 to 2×10^7 g/mol for polymeric proteins at 8.0–13.0 min. These results are in total agreement with some recent measurements obtained by A-FFFF/MALLS (Stevenson et al 2003; Arfvidsson et al 2004).

The results presented here make it evident that efficient separation and size characterization of monomeric ($M < 10^5$ g/mol) and polymeric protein wheat proteins can be achieved with

TABLE III
Calculated Mean Values of Molecular Weight for Protein Standards Determined by A-FFFF/MALLS/UV^a

| Molecular Weight ^b (g/mol) | Lysozym | Bovine Serum Albumin | Gamma-Globulin | Thyroglobulin |
|---------------------------------------|--------------------|----------------------|---------------------|---------------------|
| $M_{nominal}$ | 14.3×10^3 | 67.0×10^3 | 150.0×10^3 | 665.0×10^3 |
| M_n | 22.0×10^3 | 72.4×10^3 | 175.0×10^3 | 659.0×10^3 |
| M_w | 22.0×10^3 | 72.4×10^3 | 175.0×10^3 | 667.0×10^3 |
| M_z | 22.0×10^3 | 72.4×10^3 | 175.0×10^3 | 675.0×10^3 |
| M_w/M_n | 1.000 | 1.000 | 1.000 | 1.012 |
| M_z/M_n | 1.000 | 1.000 | 1.001 | 1.025 |

^a Asymmetrical flow field-flow fractionation (A-FFFF); multi-angle laser light scattering (MALLS); ultraviolet light (UV).

^b Molecular weight number-average (M_n), weight-average (M_w), z-average (M_z).

A-FFFF/MALLS/UV in a single run. This method offers the means of a rapid procedure for the measurement of the MWD of wheat storage proteins (monomeric-to-polymeric ratio and MWD of polymeric protein), which are becoming recognized as the main determinant of physical dough properties.

Molecular Weight Distribution of Wheat Proteins

After proving the applicability of A-FFFF connected online to MALLS detection in the fractionation of wheat storage proteins, our objective was to use the combination of A-FFFF/MALLS/UV to investigate potential variations in the MWD (monomeric-to-polymeric ratio and MWD of polymeric protein) induced by genetic or environmental factors.

Significant differences among cultivars were observed for all MWD measured traits. Environmental factors also were significant for some of these protein attributes. Relative influences of cultivar and environment on molecular weight distribution of storage proteins were compared by calculation of ratios of variance components (Table IV). As shown in Table IV, components associated with genetic factors exceeded environmental variances for protein monomeric-to-polymeric ratio ($\sigma^2_G > \sigma^2_E$ for 2001 and 2002). At the same time, components associated with genetic factors for the MWD of the polymeric protein [M_n , M_w , $\langle(Rg^2)^{0.5}_n\rangle$ and $\langle(Rg^2)^{0.5}_w\rangle$] can be larger ($\sigma^2_G > \sigma^2_E$) or smaller ($\sigma^2_G < \sigma^2_E$) than environmental components as a function of the year of the experiment (2001 vs. 2002). All these observations demonstrated

that MWD of wheat proteins is controlled by both genetic and environmental factors and are in total agreement with previous works (Gupta et al 1992; MacRitchie and Gupta 1993; Ciaffi et al 1996; Uhlen et al 1998).

Mean monomeric-to-polymeric ratio and M_n of polymeric protein for the different wheat cultivars is shown in Fig. 5A,B for the first experiment (experiment 2001). The M_n of wheat proteins can be altered from one cultivar to another in two methods. The first method involves changing the relative amounts of monomeric and polymeric proteins. For example, the cultivars NH535 and RE204 have the same M_n for polymeric protein (Fig. 5B) but are significantly discriminated by the monomeric-to-polymeric ratio (Fig. 5A). The second method involves changing the M_n of polymeric protein. For example, the cultivars Eole and RE204 have the same monomeric-to-polymeric ratio (Fig. 5A) but are significantly discriminated by the M_n of the polymeric protein (Fig. 5B). The same results were obtained with M_w (data not shown). These results are illustrated in Fig. 6 in which the differential weight fraction versus the molecular weight is plotted for three cultivars (NH535, RE204, and Eole). From these observations, we can confirm, in particular, the relationship between the allelic composition of HMW-GS and the MWD of polymeric proteins. As shown previously by Gupta and MacRitchie (1994), lines characterized by the subunit pair 5+10 (*Glu-D1*) have a MWD shifted to higher molecular weights than do those with the subunit pair 2+12 (*Glu-D1*) (Table I and Fig. 5B).

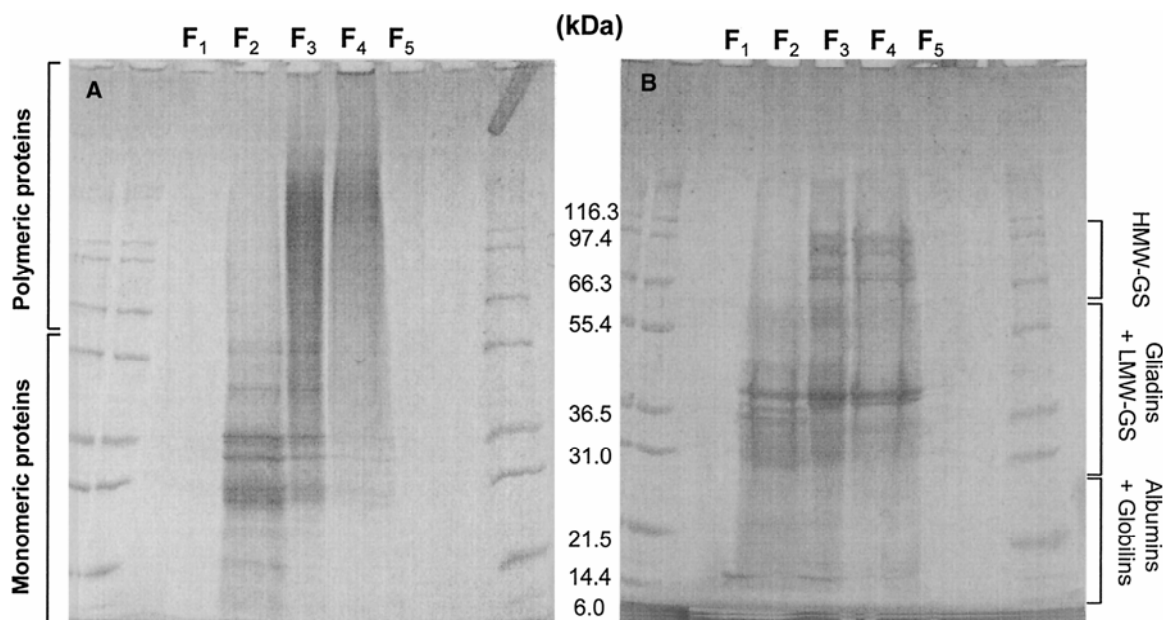


Fig. 3. One-dimensional SDS-PAGE patterns of asymmetrical flow field-flow fractionation (A-FFFF) protein fractions shown in Fig. 2 under unreduced (A) and reduced (B) conditions.

TABLE IV
Genetic (G) and Environmental (E) Influence on Molecular Weight Distribution of Storage Proteins Determined by Analysis of Variance (*F*-test) for Two Experiments

| Molecular Weight ^a | 2001 Experiment | | 2002 Experiment | |
|--|-------------------------|-------------------------|-------------------------|-------------------------|
| | σ^2_G/σ^2_R | σ^2_E/σ^2_R | σ^2_G/σ^2_R | σ^2_E/σ^2_R |
| Monomer (M_n) | ns ^b | 8.178* ^c | ns | ns |
| Monomer (M_w) | ns | 4.227* | ns | 2.472* |
| Polymer (M_n) | 6.280* | 2.210* | 2.840* | 10.980* |
| Polymer (M_w) | 2.030* | ns | ns | 4.782* |
| Polymer $\langle(Rg^2)^{0.5}_n\rangle$ | 2.950* | 2.566* | 1.950* | 13.210* |
| Polymer $\langle(Rg^2)^{0.5}_w\rangle$ | 2.110* | ns | ns | 9.918* |
| Monomer-to-polymer ratio | 7.470* | 6.483* | 4.700* | 2.168* |

^a Molecular weight number-average (M_n) and weight-average (M_w). Mean square radius number-average $\langle(Rg^2)^{0.5}_n\rangle$ and weight-average $\langle(Rg^2)^{0.5}_w\rangle$.

^b Not significant.

^c *, indicates *F*-test significance at 5% level of probability.

Moreover, the results from the present study indicate that the MWD of wheat proteins can be also altered by changing both the monomeric-to-polymeric ratio and the average molecular weight (M_n or M_w) of polymeric protein. For example, RE204 and Rival are significantly discriminated by these two parameters (Fig. 5A,B). All these observations are in total agreement with the hypothesis presented by MacRitchie and Lafiandra (1997) and demonstrated that A-FFFF/MALLS is able to detect significant modifications of MWD of wheat protein, whatever the factor inducing these alterations (genetic or environmental) and whatever the nature of these alterations (monomeric-to-polymeric ratio or M_w of polymeric protein). Moreover, our results demonstrated that A-FFFF/MALLS is able to detect the alteration of MWD of wheat protein even if sonication, used to dissolve storage protein, can experimentally induce degradation of these proteins (lowering the observed M_w and root mean square radius) (Arfvidsson et al 2004). However, to estimate the MWD of undegraded polymeric proteins, one possible approach is to mathematically model the degradation of these proteins by sonication. This would enable extrapolation of the MWD, which is measured on the SDS solubilized material (Lemelin et al *unpublished data*).

CONCLUSIONS

The results from the present study indicate that A-FFFF/MALLS offers a rapid means of fractionation and size characterization of the wheat storage proteins. Laser light scattering size analysis using MALLS/UV in conjunction with A-FFFF appears to be able to discriminate differences in the MWD of storage proteins (monomeric-to-polymeric ratio and MWD of polymeric pro-

teins) in wheat flours of different cultivars grown at different locations. To our knowledge, it is the first time that both the quantification and the molecular size characterization of monomeric and polymeric proteins can be done at the same time with accuracy (i.e., without any limitation in the determination of the MWD of polymeric proteins). Because the commercially desirable viscoelastic properties required for good performance in processing wheat flour doughs result from the combined contributions of the

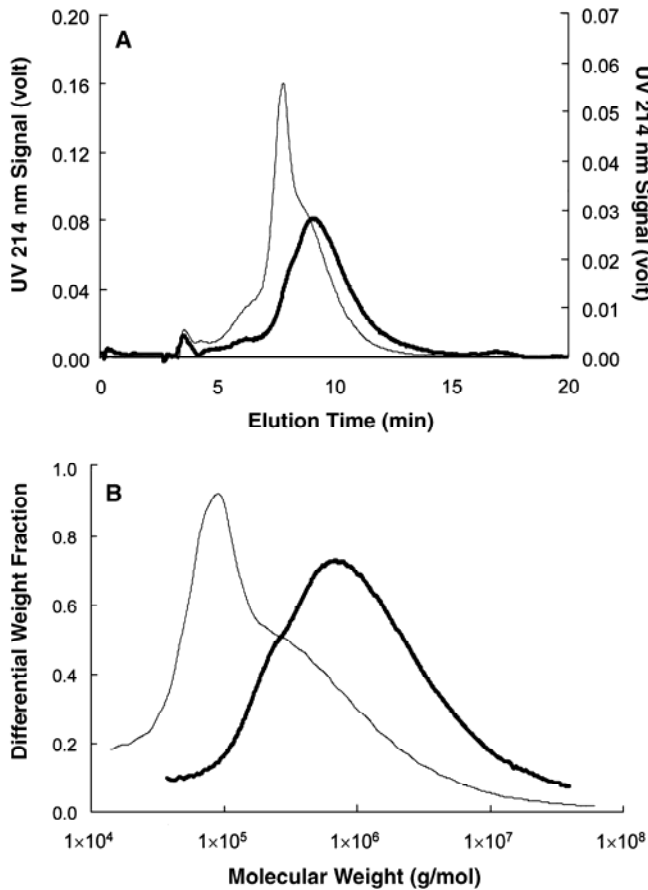


Fig. 4. Asymmetrical flow field-flow fractionation (A-FFFF) profiles of total solubilized storage proteins (fine line) and purified polymeric proteins (bold line) of Soissons. **A**, UV response as a function of elution time; **B**, differential weight fraction vs. molecular weight.

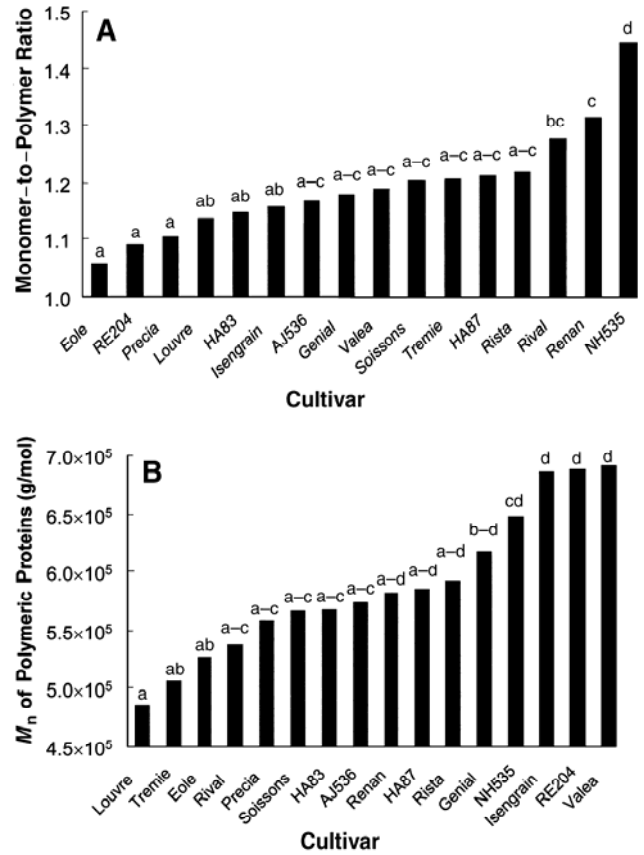


Fig. 5. Main effect of genetic factor as determined by the Newman-Keuls homogeneity test for monomeric-to-polymeric ratio (**A**); number average molecular weight (**B**) of polymeric proteins for the 2001 experiment. Bars with the same letters are not significantly different ($P < 0.01$).

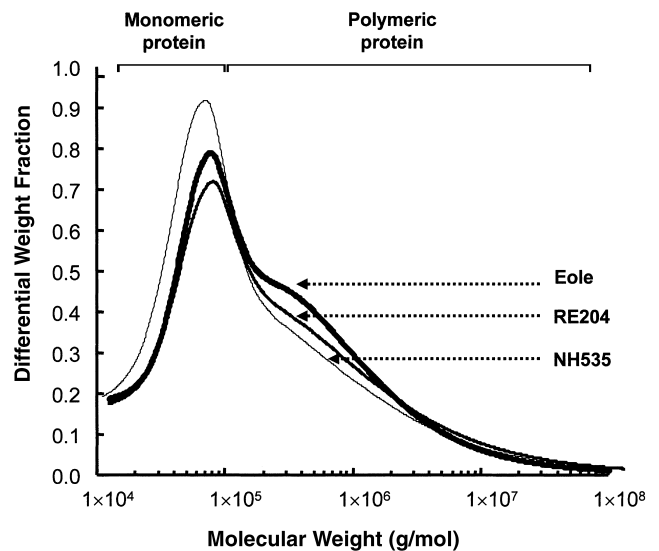


Fig. 6. Differential distribution of molecular weight of total solubilized flour proteins of three wheat cultivars (Eole, RE204, and NH535).

two main types of flour proteins (contribution of gliadins and glutenins to MWD), the procedure used in the present study can be very useful to predict the technological potential of various wheat flour samples. Further research is underway in our laboratory to explore the use of A-FFFF/MALLS/UV in determining the effects of MWD of flour proteins on physical dough properties.

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