

# Changes in SDS Solubility of Glutenin Polymers During Dough Mixing and Resting<sup>1</sup>

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## ABSTRACT

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An online coupling of high-performance size-exclusion chromatography (HPSEC) combined with multiangle laser-light scattering (MALLS) and a reverse-phase HPLC procedure were used to characterize and reveal the polydispersity of the glutenin polymers of doughs during mixing and resting. Experiments involved doughs prepared from several samples of a common French wheat cultivar (Soissons) differing in total amount of SDS-unextractable glutenin polymers. During dough mixing, the amounts, size distribution of protein, and glutenin subunit composition within the SDS-unextractable polymers changed. However, the major changes in SDS-unextractable glutenin content and size distribution occurred before the peak mixing time (MT) was reached, whereas detectable changes in subunit composition also occurred after the peak MT. Even if sonication, which was used to solubilize the total wheat glutenin, can narrow the

glutenin size distribution, HPSEC-MALLS revealed a close relationship between the SDS solubility of the glutenin polymers and size distribution, confirming a depolymerization and repolymerization hypothesis. During the depolymerization of the SDS-unextractable polymers, glutenin subunits were released in nonrandom order, which indicated that the polymers have a hierarchical structure. Some HMW glutenin subunits (HMW-GS), especially 1Dx5, were particularly resistant to the depolymerization mechanism. This suggested that the subunit plays a major role in forming the backbone of the SDS-unextractable polymers, consistent with the potential to form branched structure. These studies suggest that the SDS-unextractable polymers in flours have a well-ordered structure that can be modified by dough mixing and resting.

Proteins are recognized as the most important component governing breadmaking quality (Schofield and Booth 1983; Shewry and Mifflin 1985; Wrigley and Bietz 1988). Indeed, the large variation in dough strength and breadmaking performances of wheat flours can, to a large extent, be ascribed to variations in the level and quality of gluten proteins (Finney 1943; Finney and Barmore 1948). However, among the different flour protein groups, the glutenin fraction (a complex group of polypeptides joined together by interpolypeptide disulfide bonds) is the most important fraction related to breadmaking quality (MacRitchie et al 1990). For this reason, in the last decades, the polymeric glutenin fraction has been investigated intensively (Weegels et al 1996). Although the precise structure of these polymers is still a matter of speculation, interchain disulfide (S-S) bridges link HMW glutenin subunits (HMW-GS) and LMW glutenin subunits (LMW-GS) into polymers with varying molecular weight (MacRitchie 1992). The total glutenin polymer quantity is correlated with various technological parameters (Huebner and Wall 1976; Field et al 1983; Dachkevitch and Autran 1989; Singh et al 1990a,b). Moreover, a certain amount of these polymers remain unextractable in various extracting systems (e.g., acetic acid solution or SDS phosphate buffer). Those unextractable polymeric proteins appear also to be correlated with baking performance (Kurowska and Bushuk 1988; Gupta et al 1993; Jia et al 1996b). In addition, Gupta et al (1992) showed that the unextractable polymer quantity is more directly linked with certain technological parameters (especially those correlated with mixing) than the total glutenin quantity. The proportion of unextractable polymer fraction among the glutenin polymers appears, likewise, to be an important ratio for technological response (Gupta et al 1993; Jia et al 1996a). The breadmaking process phase where gluten functionality can be critical is dough development. Several researchers have found that the quantity of unextractable glutenin polymer decreases during dough mixing and increases again during dough resting, although the nature of the polymer before mixing is different from that afterwards (Mecham et al 1962; Tsen 1967; Tanaka and Bushuk 1973a–c; Graveland et

al 1980; Danno and Hosoney 1982a; Weegels et al 1994, 1995; Skerrett et al 1999; Veraverbeke et al 1999). Different mechanisms have been proposed to explain these observations. It has been suggested that, during mixing, the size of protein aggregates decreases (Mecham et al 1965; Tsen 1967) by physical separation of the aggregates (Tsen 1967) or by breaking of noncovalent (Tsen 1967) or covalent bonds (Tanaka and Bushuk 1973c; MacRitchie 1975; Graveland et al 1980; Danno and Hosoney 1982b). Hamer and Lichtendonk (1987), who reported a decrease in extractability of glutenin during resting, have assumed that repolymerization of glutenin polymer takes place.

This study used several samples of a common French wheat cultivar (Soissons) differing in total amount of unextractable glutenin polymers to identify possible relationships between the stage of dough mixing and resting and changes in the content and molecular size distribution of glutenin polymers. An online coupling of high-performance size-exclusion chromatography (HPSEC) with multiangle laser-light scattering (MALLS) was used to reveal the polydispersity of glutenin polymers during the process. Changes in the glutenin subunit composition of these unextractable polymers were also investigated by reversed-phase HPLC during dough mixing and resting.

## MATERIALS AND METHODS

### Flour Samples and Mixing Properties

Several grain samples of the common wheat cultivar Soissons (harvest 1996) were milled into flours on a Miag Multomat mill and used for mixing experiments. The compositions of flours are given in Table I.

TABLE I  
Composition and Properties of Flours

Soissons Sample	Moisture Content (% w/w)	Protein Content (% w/w) <sup>a</sup>	SDS-UG Content (% w/w) <sup>b</sup>	Brabender Water Absorption (% <sup>c</sup> )
T1	13.55	7.9	1.9	53.4
T2	13.84	8.6	2.2	53.4
T3	13.57	10.6	2.6	54.8
T4	13.97	10.1	2.3	54.5
T5	13.42	11.5	2.7	55.5
T6	13.26	11.3	2.8	55.6
T7	13.08	12.9	2.9	55.5

<sup>a</sup> N × 5.7, dry-matter basis.

<sup>b</sup> SDS-unextractable glutenin content.

<sup>c</sup> ICC Standard 115, flour basis.

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Mixing properties were determined with a recording mixograph using 10 g of flour. The same amount of water was added as needed to give a farinograph consistency of  $500 \pm 15$  BU (standard 155, ICC 1998) according to Weegels et al (1995). To follow the behavior of the unextractable glutenin polymers, doughs were mixed for various times (1, 2, 3, 4, 5, 6, 8, or 10 min) at  $25 \pm 1.0^\circ\text{C}$ . Directly after mixing, or after 45, 90, or 135 min of resting at  $30^\circ\text{C}$  in capped bottles to prevent drying, doughs were frozen in liquid nitrogen. All doughs that were frozen in liquid nitrogen were freeze-dried. The freeze-dried doughs were finely ground in a Janke A10 grinder fitted with a 200- $\mu\text{m}$  screen.

### SDS-Unextractable Glutenin Polymer Quantification

Freeze-dried doughs (240 mg) were stirred for 2 hr at  $60^\circ\text{C}$  in 30 mL of 0.1M sodium phosphate buffer (pH 6.90) containing 2% (w/v) SDS. These extractions were followed by centrifugation during 30 min at  $12,500 \times g$  at  $20^\circ\text{C}$  in a Jouan centrifuge (model MR 1822). Clear supernatants (soluble proteins) were eliminated and pellets (unextractable glutenin polymers) were freeze-dried. The Dumas method (method 7.024, AOAC 1995) was used to determine the nitrogen concentration of freeze-dried pellets. Three or four replicates were done and combined for analysis. Protein concentration was determined on a Leco apparatus (model FP 428) using  $N \times 5.7$ .

### Extraction and Purification of Glutenin Polymers

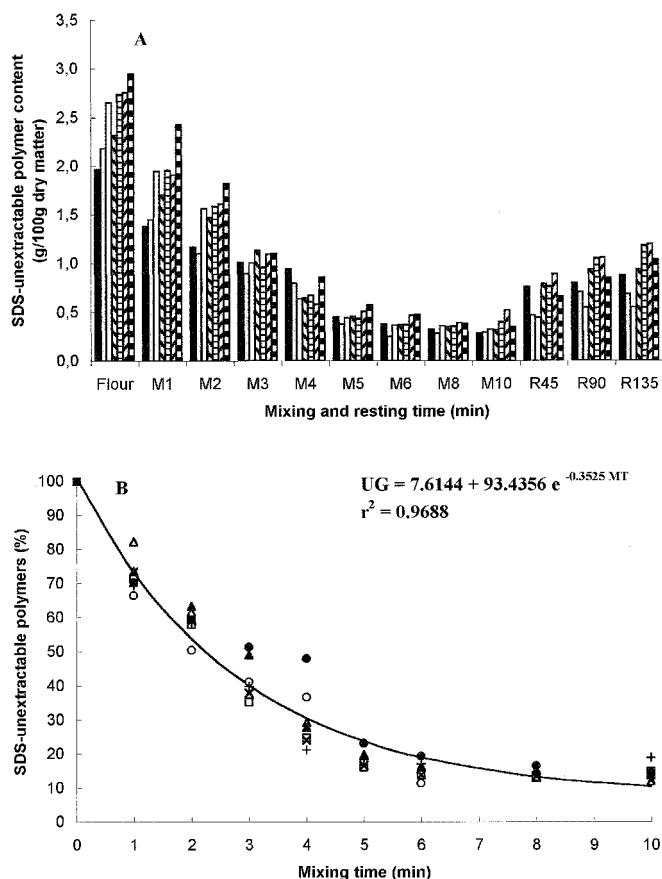
Freeze-dried doughs (0.1 g) were stirred for 1 hr at room temperature ( $25^\circ\text{C}$ ) with 2 mL of 0.3M sodium iodide (NaI) in 7.5% (v/v) of propan-1-ol according to Fu and Kovacs (1999). Extrac-

tion was followed by centrifugation at  $15,900 \times g$  for 15 min at  $15^\circ\text{C}$ . The supernatant (mainly monomeric proteins) was eliminated. The glutenins remain unextractable in the sodium iodide. The pellet was washed twice with the solvent of extraction and was then used for HPSEC-MALLS procedure.

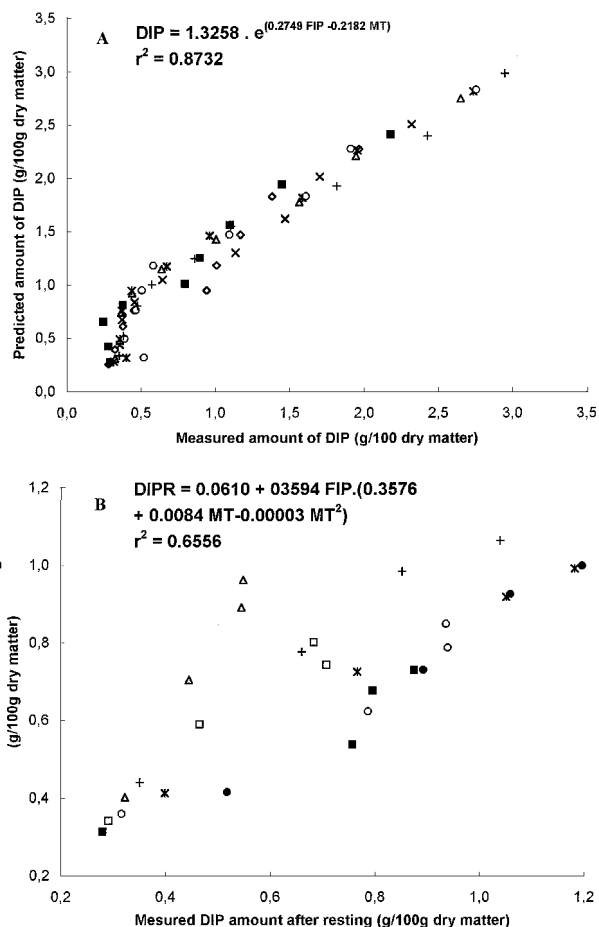
### Size Distribution of Glutenin Polymers by HPSEC-MALLS

The residue (0.1 g) obtained during the purification procedure was extracted with 1 mL of a sodium phosphate buffer 0.1M (pH 6.90) containing 2% (w/v) SDS and was sonicated for 15 sec at power setting of 50% (10W, 23 kHz) using a stepped microtip probe (3 mm diam.) (Branson Sonifier, model B-12) and centrifuged for 30 min at  $12,500 \times g$ .

The HPSEC system comprised a SpectraSYSTEM LC (Thermo Separation Products S.A., Les Ulis, France) consisting of online degasser (model SCM 400), isocratic pump (model P 4000), variable autoinjector (model AS 3000), and variable wavelength detector (model UV 2000). Refractive index of solutes was determined with a differential refractive index detector (Erma ERC-7512). Light scattering was measured on a Wyatt multiangle laser light-scattering detector (laser wavelength = 632.8 nm) (model Dawn-F; Wyatt Technology Corporation, Santa Barbara, CA). Analog signals from the 15 photodiode channels were sent to a PC with an onboard 16-channel A/D converter. The 16th channel was used for the signal of the mass-sensitive detector. Photodiode coefficients were normalized using bovine serum albumin (BSA) monomer as a reference.



**Fig. 1A**, Effects of mixing (M) and resting (R) on SDS-unextractable glutenin polymer content, and **B**, level of SDS-unextractable glutenins (UG) in dough as a function of mixing time (MT) of several Soissons flours differing in total amount of SDS-unextractable glutenins. Soissons T1 through T7 (left to right in each group for A) and (●, ○, ×, ▲, □, +, △, respectively for B).



**Fig. 2A**, Predicted dough SDS-unextractable polymer (DIP) content by mixing time (MT) and flour SDS-unextractable polymer content (FIP) and **B**, predicted amount of SDS-unextractable polymers in dough after resting (DIPR) by resting time (RT) and flour SDS-unextractable polymer content (FIP). Soissons T1 through T7 (●, ○, ×, ▲, □, +, △, respectively).

The size-exclusion system comprised two columns in series: a PL aquagel-OH 60 (300 × 7.5 mm i.d., 8 μm) and a PL aquagel-OH 40 (300 × 7.5 mm i.d., 8 μm) (Polymer Laboratories Ltd., Shropshire, UK). The eluent was sodium phosphate buffer 0.1M (pH 6.90) with 0.1% (w/v) SDS, carefully degassed and filtered before use through 0.1-μm membranes (Gelman Sciences, France). The flow rate was 0.7 mL/min. During the fractionation, the columns were thermostated at 25°C. Samples (100 μL) were injected into the HPSEC system.

Multiangle laser-scattering means measuring the intensity of the scattered light emitted by the sample molecules at different scattering angles (θ). With a modern MALLS photometer, it is possible to continuously monitor the scattering by means of several detectors mounted at different angles. For each elution slice, a weight-average molar mass ( $M_w$ ) can be as:

$$P(\theta) = 1 - a_1 [2k \sin(\theta/2)]^2 + a_2 [2k \sin(\theta/2)]^4 - \dots \quad (1)$$

$$\frac{Kc}{R_\theta} = \frac{1}{M_w P(\theta)} + 2A_2 c + \dots \quad (2)$$

$$k = \frac{4\pi^2 n_0^2}{N_A \lambda_0^4} \left( \frac{dn}{dc} \right)^2 \quad (3)$$

where  $K$  is a light-scattering constant containing the wavelength  $\lambda_0$  of the incident light, the refractive index  $n_0$  of the pure eluent, and the refractive index increment  $dn/dc$ ;  $c$  is the concentration,  $A_2$  is the second virial coefficient,  $R_\theta$  is the excess Rayleigh ratio, and  $P(\theta)$  is a general form of a scattering function. For very low con-

centrations, the second and higher order terms in equation 1 can be neglected, and  $R_\theta$  becomes directly proportional to  $M_w P(\theta)$ . Plotting  $R_\theta/Kc$  against  $\sin^2(\theta/2)$  gives  $M_w$  from the intercept with the ordinate; from the angular dependence of the intensity of the scattered light, which is included in  $a_1$  and higher order terms of the equation 2, a  $z$ -average root mean square radius  $\langle R_G^2 \rangle_z^{0.5}$  can be derived. This latter quality is defined in terms of the distribution of the volume elements of the molecule with the respect to the square of the distance from its center of gravity:

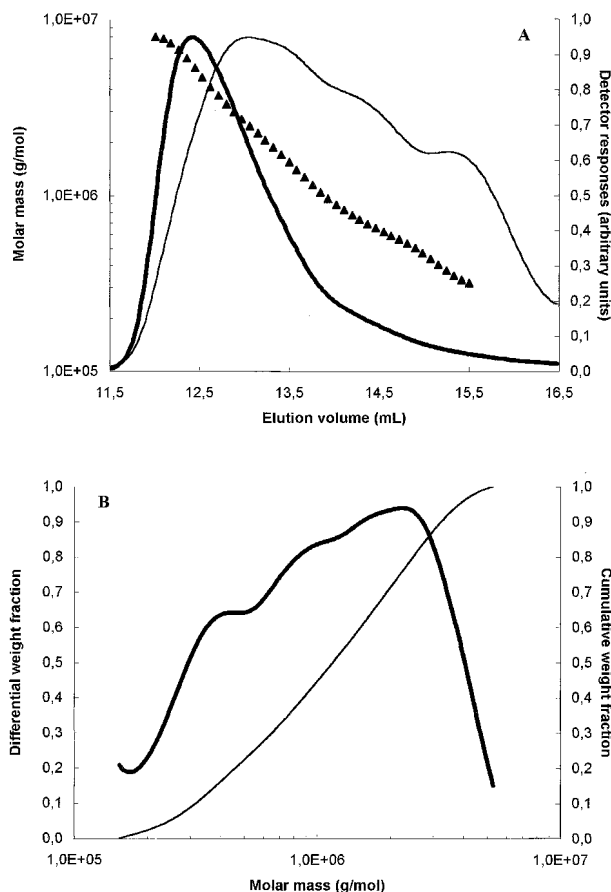
$$\langle R_G^2 \rangle_z^{0.5} = \frac{1}{V} \int r^2 dV \quad (4)$$

The calculation of the values  $\langle R_G^2 \rangle_z^{0.5}$  is independent of  $dn/dc$ ,  $M_w$ , and even  $c$  and, therefore, is insensible to errors. Number-average ( $\langle M \rangle_n$ ), weight average ( $\langle M \rangle_w$ ), and  $z$ -average ( $\langle M \rangle_z$ ) molecular weights,  $R_G^2$ , and polydispersity index ( $P = \langle M \rangle_w / \langle M \rangle_n$ ) were established with ASTRA software using a Zimm extrapolation ( $Kc/R_\theta$ ). Normalization of the photodiodes (i.e., the process by which the various photodiode signals are related to the 90° detector signal) was obtained using a relatively narrow (i.e.,  $\langle M \rangle_w / \langle M \rangle_n \approx 1$ ) LMW standard (BSA monomer,  $M_w = 67,000$ ,  $\langle M \rangle_w / \langle M \rangle_n = 1.01$ ,  $c = 2$  mg/mL). BSA was also used to determine the interconnection volume between detectors to 0.172 mL. A value of 0.190 mL/g was employed as refractive index increment ( $dn/dc$ ) for glutenins.

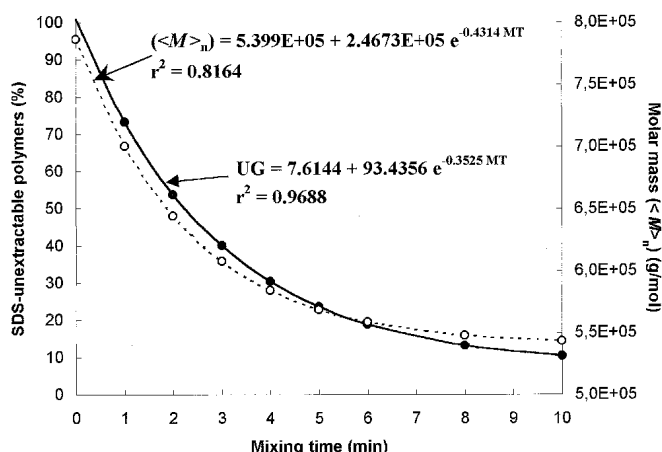
#### Quantification of Glutenin Subunits by RP-HPLC

Freeze-dried unextractable glutenin polymers (0.5 g) were extracted successively with 3.0 and 1.5 mL of 50% (v/v) propan-1-ol containing 1% (w/v) dithiothreitol (DTT) by sonication for 2 min at power setting 50% (25W, 23 kHz) using a stepped microtip probe (3 mm diam.) followed by continual stirring for 30 min at 60°C. After centrifugation (10,000 × g, 10 min), the first and second extracts were pooled. Precipitation, resolubilization, and alkylation of HMW-GS were performed as described previously (Marchylo et al 1989).

A Spectra-Physics HPLC and a PC1000/Spectranet data and chromatography control station (Spectra-Physics Analytical Software, San Jose, CA) in conjunction with a Zorbax 300 SB-C8 column (C8, 300-Å pore size, 5-μm particle size, 15 cm × 4.6 mm i.d. [Supelco Inc., Bellefont, PA]) preceded by a guard column of the same packing material (Zorbax 300 CB-C8, 2 cm × 4.6 mm i.d.) were used for analysis of glutenin subunits. Column temperature was maintained at 50°C; the column effluent was monitored at 210 nm. Glutenin subunits were resolved as described previously (Jia et al 1996b). Injection volume was 30 μL in conjunction with a multiple 5-μL injection technique (Marchylo and Kruger 1988).



**Fig. 3A**, Steric-exclusion chromatography (refractive index detection [fine line], light scattering at 90° [bold line]), and molar mass as a function of elution time [▲] and **B**, hydrodynamic parameters of total glutenin polymers of Soissons T7 (cumulative [fine line] and differential [bold line] distribution of molar mass).



**Fig. 4**, Level of SDS-unextractable glutenins (UG) and changes in number-average ( $\langle M \rangle_n$ ) molecular weight of these polymers as a function of mixing time (MT).

## RESULTS AND DISCUSSION

### Changes in SDS-Unextractable Polymer Content During Mixing and Resting

It is well known that extractability of flour proteins increases when mixing flour with water into a dough (Weegels et al 1996). This can be observed in Fig. 1A as a decrease in the amount of the SDS-unextractable glutenins from dough during mixing as determined by the Dumas protein analysis of the SDS-unextractable residue. Furthermore, the amount of SDS-unextractable glutenin polymer decreases exponentially during the mixing, and this decrease is independent of the flours used (i.e., from the SDS-unextractable glutenin polymer content of the flour). All the curves can indeed be plotted well ( $r^2 = 0.9688$ ) by using a unique exponential regression (Fig. 1B). A 70% reduction of the level of SDS-unextractable glutenins was observed during mixing to peak consistency (first 4 min). Further mixing resulted in no significant decrease in the level of SDS-unextractable proteins. However, the decrease in SDS-unextractable glutenin content began well before mixograph peak time was reached. These results suggest, as previously presented by Skerritt et al (1999), that the reduction in quantity of these polymers may not be linked directly to dough breakdown (the period after peak mixing resistance is reached and during which time dough resistance shows a continuous decrease with further mixing). Similar results were obtained by Pritchard and Brock (1994). Notwithstanding, as has been previously shown (Graveland et al 1980; Danno and Hosoney 1982a; Weegels et al 1994; Skerritt et al 1999; Veraverbeke et al 1999), the extractability of glutenin polymers decreased during resting (Fig. 1A). Although there is some restoration

of the level of the SDS-unextractable glutenins, a level similar to that found in flour is not attained during resting. Even if no definite conclusions have been reached, the depolymerization and repolymerization mechanism have been proposed to explain these observations (Mecham et al 1965; Tsen 1967; Tanaka and Bushuk 1973c; MacRitchie 1975; Hamer and Lichtendonk 1987).

As previously shown by Weegels et al (1994), the amount of SDS-unextractable glutenin polymers during the process (mixing and resting) can be very well predicted on the basis of SDS-unextractable glutenin polymer content of flour and mixing and resting time only. The data presented in Fig. 2A and B confirms this for Soissons doughs. The amount of SDS-unextractable glutenins in dough directly after mixing (DIP) can indeed be described by an exponential function of the amount of SDS-unextractable glutenins in flour (FIP) and the MT (Fig. 2A). With this equation, 87% of the variation in polymer content can be explained. Using the results obtained from resting, a relationship between the amount of SDS-unextractable glutenins in dough and the amount of SDS-unextractable glutenins in flour can be found. The amount of SDS-unextractable glutenins in dough after resting (DIPR) can be well described (66% of variation) by the amount of SDS-unextractable glutenins in flour (FIP) and resting time (RT) (Fig. 2B). According to Weegels et al (1994, 1996), who used different genotypes, it is clear that all the modifications of the glutenin polymers occurring during the process are mainly governed by quantity and far less by quality differences. This probably indicates that glutenin subunits and, notably, HMW-GS are related indirectly to breadmaking quality by the quantity and molecular weight distribution of glutenins polymers.

### Changes in Molecular Weight Distribution of the Glutenin Polymers

Even if no definite conclusions have been reached to explain the modifications in glutenins extractability during both dough mixing and resting, different mechanisms have been proposed. All these hypotheses mainly were based on a decrease of the glutenin polymer size by physical separation of the aggregates (Tsen 1967) or by breaking of noncovalent (Tsen 1967) or covalent bonds (Tanaka and Bushuk 1973; MacRitchie 1975; Graveland et al 1980; Danno and Hosoney 1982a,b). In the current study, a one-line coupling of HPSEC-MALLS was used to reveal changes in molecular size distribution of the glutenin polymers during mixing.

The results of HPSEC-MALLS experiments on the polymeric glutenins of Soissons T7 flour are shown in Fig. 3A and B. A MALLS/RI chromatogram of total polymer separated by HPSEC illustrates the remarkable power of light-scattering detection for studying the HMW fraction (Fig. 3A). In the same figure, the molar masses of the polymeric glutenins determined by means of light scattering are represented as a function of the elution volume. The molar mass distributions (differential and cumulative representation) represented in Fig. 3B were calculated by including the concentration of each respective species. From these distributions, it was possible to determine the  $\langle M \rangle_n$ ,  $\langle M \rangle_w$ , and z-average  $\langle M \rangle_z$  molar mass, and also the polydispersity index ( $P = \langle M \rangle_w / \langle M \rangle_n$ ). All of these measurements of polymeric glutenins show that the MALLS photometer is suitable for monitoring the eluate from the HPSEC on the condition that any exclusion limit happens on the gel matrix, as is the case with the SEC stationary phases (PL aquagel-OH 40 and 60) used here. Consequently, the angular-dependent scattered light readings yield the absolutely radius of gyration and molar mass and, by including the respective concentrations, they also yield the distributions for these two variables.

The increase in SDS solubility of the unextractable glutenin polymers was highly correlated with the reduction of the  $\langle M \rangle_n$  and  $\langle M \rangle_w$  molar mass of these proteins during mixing (Fig. 4). In fact, both the variables (UG and  $\langle M \rangle$ ) can be plotted well by using exponential regression as a function of MT. From these results, it appears that the SDS extractability of the glutenin polymers during mixing to the dough peak consistency (first 4 min) can be

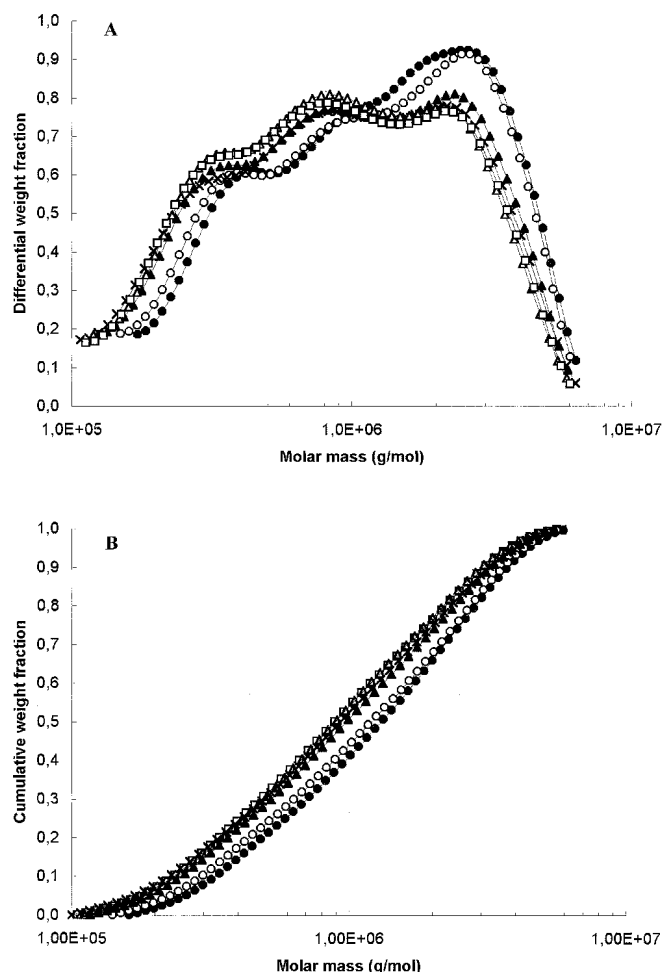


Fig. 5A, Differential and B, cumulative distribution of molar mass of total glutenin polymers during mixing. Flour (●); 2, 3, 4, 5, and 8 min of mixing (○, ▲, \*, Δ, □, respectively).

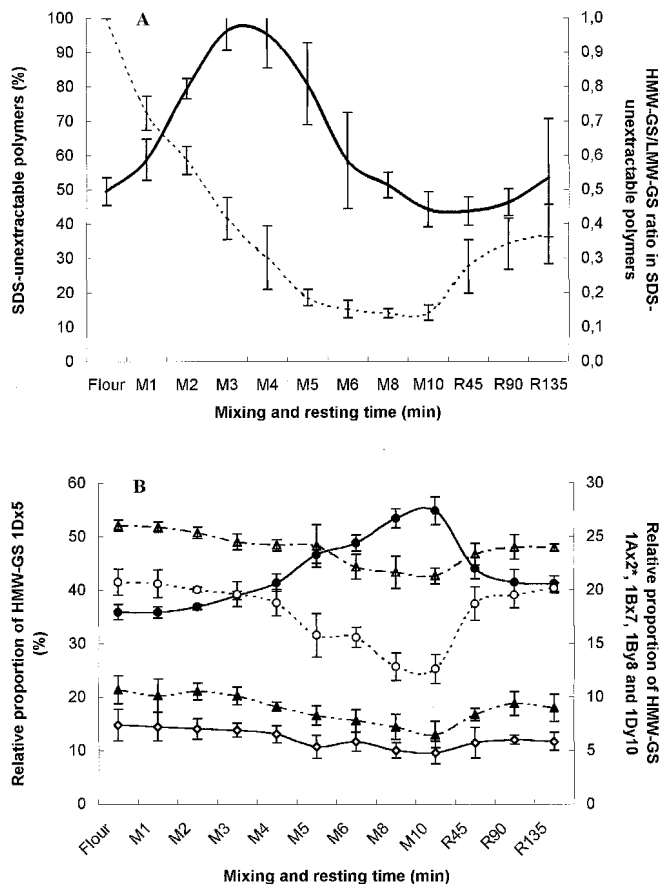
the result of the size reduction of these glutenin polymers, confirming the depolymerization hypothesis. In flour, the molar masses and the radii of gyration of the total polymers are roughly in agreement with a size distribution of glutenin calculated by Ewart (1987) based on standard theory of high polymers assuming linear molecules. The most probable weight fraction had a molecular weight of  $\approx 1.0 \text{ E}+6 \text{ g/mol}$ , and molecular weights extended to  $>5.0 \text{ E}+6 \text{ g/mol}$ . Recent measurements obtained by flow FFF have also pointed to molecular weights in millions (Stevenson and Preston 1996; Wahlund et al 1996; Stevenson et al 1999). These authors utilized the observed hydrodynamic diameters ( $d$ ) to estimate the upper and lower limits for the molecular mass of glutenins. The lower limit was defined as  $d = 0.0542M^{0.498}$  for flexible random coil polymer, and the upper limit was defined as  $d = 0.159M^{0.333}$  corresponding to a spherical shape. Values for the upper limit were in the range of 440,000 to  $11 \text{ E}+6 \text{ g/mol}$ . As in the present study, because no solvent has been found for solubilizing the total wheat glutenin, the solutions have been sonicated so that the very largest glutenins would have been reduced in size (Southan et al 1998, Carceller 2000). However, as we have already shown (Carceller 2000), even if sonication can narrow the glutenin size distribution, the protocol applied in the present study is largely insufficient to reach a molecular distribution limit. Consequently, it enables modifications in molecular distribution directly linked to dough mixing.

During mixing, the relative amount of the highest molecular weight fraction decreased, while the lowest molecular weight fraction, possibly corresponding to the oligomers, increased (Fig. 5A and B). Of the flour total polymers, 59.1% have a molar mass  $>1.0 \text{ E}+6 \text{ g/mol}$ , although they represent only 47% in the dough after 4 min of mixing. At the same time, oligomers characterized by a molar mass  $<4.0 \text{ E}+5 \text{ g/mol}$  represent 14.8 and 24.2% in flour and dough, respectively,

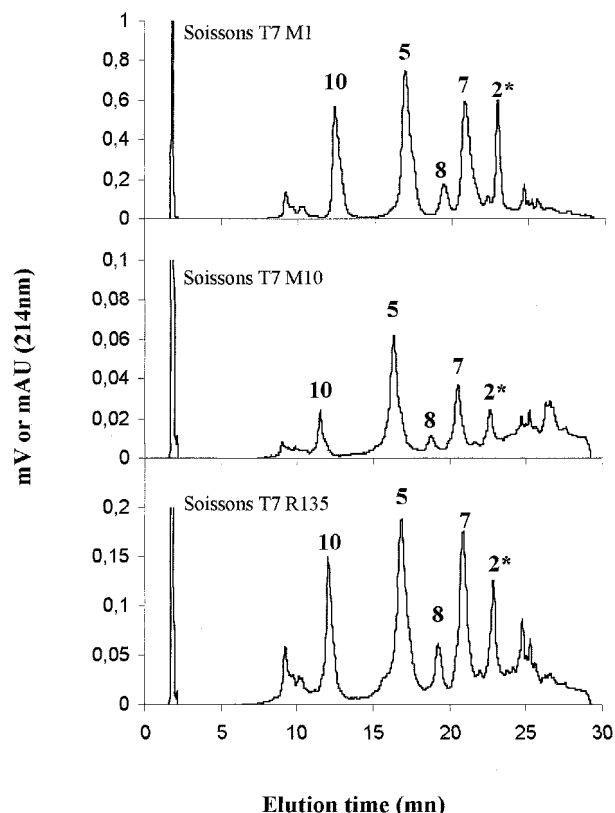
after 4 min of mixing. These results are in total agreement with the observations made by Borneo and Khan (1999) and Skerritt et al (1999) using a multilayer SDS-PAGE procedure. After 4 min of mixing (peak MT), the results revealed a depolymerization limit ( $\langle M \rangle_n$  and  $\langle M \rangle_w$  limit) (Fig. 4). Indeed, the  $\langle M \rangle_n$  and  $\langle M \rangle_w$  molar mass of the glutenin polymers were not significantly different between 4 and 10 min of mixing. These observations suggested that a critical size distribution had been reached. The changes in size distribution that have been observed for glutenin polymers correspond closely to those predicted by the Bueche theory (1960). In fact, the theory of Bueche predicts that for a polymer with a wide molecular weight distribution the chain degradation by shear only occurs at practical shear rates if entanglements are present. Breakage of bonds arises because molecular chains cannot disentangle sufficiently rapidly in response to the stress shear. The highest tension occurs at the center of the chain and, as a result, chains break preferentially at their centers. That way, all chains with a molecular mass higher than a critical size will be broken down and none of the low molecular mass material will be broken.

Even if the study of this depolymerization phenomenon deserves greater attention, the overall observations made, which are in total agreement with previous works (Gupta et al 1992, 1993; Bangur et al 1997; MacRitchie and Lafiandra 1997), nevertheless, confirm the existence of a close link between glutenin molecular distribution and rheological dough properties. Therefore, in agreement with the first remarks made by Bangur et al (1997), it seems that only polymeric proteins above a certain molecular size contribute to dough properties such as dough strength. This behavior is also in line with the theory of Bersted and Anderson (1990) (Eq. 5). In this theory, based on a modified version of the Flory equation (1945), Bersted and Anderson suggest that, for polydispersed polymers, only those molecules that formed effective entanglements contribute to tensile strength ( $\sigma$ ):

$$\sigma = \sigma_0 \left(1 - M_T / M_n^*\right) \phi \quad (5)$$



**Fig. 6A,** Changes in HMW and LMW glutenin subunit ratio (bold line) **B,** Relative proportions of HMW-GS in SDS-unextractable glutenins during mixing (M) and resting (R). 1Dx5 (●), 1Dy10 (○), 1Bx7 (△), 1Ax2\* (▲), and 1By8 (◇).



**Fig. 7.** Reversed-phase HPLC patterns of HMW glutenin subunits in SDS-unextractable polymers during mixing (M) and resting (R).

where  $\sigma_0$  = the limiting tensile strength at high molecular weight;  $M_T$  = the threshold molecular weight for effective entanglements;  $\phi$  = the fraction with molecular weight  $>M_T$ ; and  $M_n^*$  = the number-average molecular weight of this fraction (fraction with molecular weight  $>M_T$ ).

### Changes in Glutenin Subunit Composition During Mixing and Resting

In the present study, the decrease in the amount of SDS-unextractable glutenin polymers during dough mixing was accompanied by changes in the composition of these polymers (relative proportions of different glutenin subunits) (Fig. 6A and B). A significant increase in the proportion of HMW-GS in SDS-unextractable polymers was observed during mixing to peak dough consistency (first 4 min) (Fig. 6A). Further mixing resulted in a decrease of the HMW-GS level in the SDS-unextractable polymers. The proportion of HMW-GS in the unextractable glutenins that was reached at the end of mixing was not significantly different to the proportion observed in flour. Finally, this HMW-GS proportion remained closely constant during dough resting. These observations seem to demonstrate that the polymers more resistant to solubilization by dough mixing were enriched in HMW-GS. Therefore, the HMW-GS proportion in SDS-unextractable polymers decreases after peak MT, suggesting the reduction of these subunits may be linked to dough breakdown. Then, during dough resting, the glutenins repolymerization did not induce any modification of the relative subunit composition.

Apart from the changed HMW-GS/LMW-GS ratio, the different HMW-GS did not disappear at the same rate from the SDS-unextractable glutenins, resulting in changed related proportions of the various HMW-GS. These observations were well illustrated in Fig. 7, which shows reversed-phase HPLC patterns of HMW-GS from dough at different mixing and resting time. Skerritt et al (1999) observed similar effects. However, their observations that the x-type HMW-GS solubilize more readily than y-type HMW-GS could not be confirmed in the present study. Indeed, with Soissons flours the relative proportion of HMW-GS 1Dy10 decreased, while the proportion of HMW-GS 1Dx5 increased during mixing (Fig. 6B). At the same time, the proportion of HMW-GS 1Bx7, 1By8, and 1A2\* remained relatively constant. These observations were in total agreement with other works (Veraverbeke et al 1999). However, in our study, all the significant modifications of relative proportions observed for HMW-GS 1Dx5 and 1Dy10 occurred after the peak MT (dough breakdown) and not during mixing to peak dough consistency (first 4 min).

Interpretation of the changes in HMW-GS composition of the SDS-unextractable glutenin polymers during dough mixing and resting is difficult because the mechanisms responsible for the changes in the amount of SDS-unextractable glutenin are poorly understood. However, from our results, it appears that glutenin subunits (HMW-GS, LMW-GS, x- and y-type HMW-GS) were released from the polymers in a nonrandom order, which was indicative of the polymers having a hierarchical structural organization. The data obtained is in keeping with a proposed model for polymers, in which a backbone of HMW-GS exists to which LMW-GS are bound (Graveland et al 1985). According to Skerritt et al (1999), we cannot exclude the fact that differences in depolymerization rates between LMW-GS and HMW-GS can be explained by the presence of polymers with differing composition or branching within SDS-unextractable polymers; or within SDS-unextractable polymers by the presence of different classes of glutenin subunits inducing an unevenly distributed branching. Furthermore, our results, which demonstrate the major role of HMW-GS or x-type HMW-GS to the formation of the SDS-unextractable polymers, are very consistent with the observations made by Lindsay and Skerritt (1998). These authors, using stepwise reduction to examine the structure of SDS-unextractable glutenins, suggest that these polymers have a well-ordered structure in which some HMW-GS (particularly HMW-GS 1Dx5) play a major role in forming the backbone to them. Kasarda (1999)

has indeed proposed a hierarchical arrangement of the glutenin subunits that represents their relative potentials for the glutenin polymer formation. This hierarchical arrangement of glutenin subunits, in which x-type HMW-GS are defined as the most important constituent, is based on a combination of two factors relating to 1) the potential of a subunit to form branched polymers, as opposed to linear polymers, and 2) the length of the repeating sequence domain.

### CONCLUSIONS

During dough mixing the amounts, the size distributions of protein and the glutenin subunit composition within the SDS-unextractable polymers changed. However, the major changes in SDS-unextractable glutenin content and size distribution occurred before the peak MT was reached, while detectable changes in subunit composition also occurred after the peak MT. HPSEC-MALLS, which was very sensible to changes in polymer molecular weight distribution, revealed a close relationship between the SDS solubility of the glutenin polymers and their size distribution. Consequently, modifications of the SDS solubility of the glutenin polymers during the process (mixing and resting) can be attributed, to a large extent, to modifications of size distribution confirming the depolymerization and repolymerization hypothesis.

Depolymerization of SDS-unextractable polymers during mixing to peak consistency (first 4 min in the present study), which was characterized by a significant variation of the HMW-GS/LMW-GS ratio, was indicative of the polymers having a hierarchical structural organization. In fact, HMW-GS were apparently more resistant to the depolymerization than LMW-GS, consistent with them forming a backbone to the SDS-unextractable glutenin polymers. Furthermore, from our results, it appears that the LMW-GS released may be unevenly linked with HMW-GS. During mixing to peak consistency, the HMW-GS 1Dx5 was indeed not released at the same rate as the other HMW-GS from the SDS-unextractable polymers consistent with them, forming mainly HMW-GS linkage. After peak MT, the decrease of the HMW-GS/LMW-GS ratio induced with further mixing may be related directly to dough breakdown (over-mixed doughs). During this step, the unextractable glutenin polymers become enriched in some HMW-GS (specially HMW-GS 1Dx5), suggesting that this subunit plays a major role in forming the backbone of the SDS-unextractable polymers consistent with its potential to form branched structures (Kasarda 1999).

The data obtained is in keeping with a proposed model for polymers, in which a backbone of HMW subunits exists to which LMW subunits are bound (Graveland et al 1985) but in which clusters of glutenin polymers are linked by rheologically effective disulfide bonds (Gao et al 1992). This may be relevant to the observation reported by several workers that a relatively small fraction of the total number of disulfide bonds presents only some disulfide contribute to rheological properties.

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