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**Changes in SDS Solubility of Glutenin Polymers during
Dough Mixing and Resting^{1,2}**

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Thierry Aussenac^{3,4}, Jean-Luc Carceller³, and Didier Kleiber³

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INTRODUCTION

1
2 Proteins are recognized as the most important component governing bread-making
3 quality (Schofield and Booth 1983, Shewry and Miflin 1985, Wrigley and Bietz 1988). The
4 large variation in dough strength and bread-making performances of wheat flours can indeed,
5 to a large extent, be ascribed to variations in the level and quality of gluten proteins (Finney
6 1943, Finney and Barmore 1948). However, among the different flour protein group, the
7 glutenin fraction (a complex group of polypeptides joined together by interpolypeptide
8 disulfide bonds) is the most important fraction related to bread-making quality (MacRitchie *et*
9 *al* 1990). For this reason, in the last decades, the polymeric glutenin fraction has been
10 investigated intensively (Weegels *et al* 1996). Although the precise structure of these
11 polymers is still a matter of speculation, it has been shown that interchain disulfide (S-S)
12 bridges link high molecular weight glutenin subunits (HMW-GS) and low molecular weight
13 glutenin subunits (LMW-GS) into polymers with varying molecular weight (MacRitchie
14 1992). The total glutenin polymer quantity is known to be correlated with various
15 technological parameters (Huebner and Wall 1976, Field *et al* 1983, Dachkevitch and Autran
16 1989, Singh *et al* 1990a,b). Moreover, a certain amount of these polymers remain
17 unextractable in various extracting systems (e.g. acetic acid solution or SDS phosphate
18 buffer). Those unextractable polymeric proteins appear also to be correlated with baking
19 performance (Kurowska and Bushuk 1988, Gupta *et al* 1993, Jia *et al* 1996a). Besides, Gupta
20 *et al* (1992) showed that the unextractable polymer quantity is more directly linked with
21 certain technological parameters (especially those correlated with mixing) than the total
22 glutenin quantity. The proportion of unextractable polymer fraction among the glutenin
23 polymers appears likewise to be an important ratio for technological response (Gupta *et al*
24 1993, Jia *et al* 1996b). The bread-making process phase where gluten functionality can be
25 deemed to be critical is that of dough development. Several researchers have found that the

1 quantity of unextractable glutenin polymer decreases during dough mixing and increases
2 again during dough resting, although the nature of the polymer present before mixing is
3 different from that present afterwards (Mecham et al 1962 ; Tsen 1967 ; Tanaka and Bushuk
4 1973a,b,c ; Graveland et al 1980 ; Danno and Hosoney 1982a ; Weegels et al 1994,1995 ;
5 Veraverbeke et al 1999 ; Skerritt et al 1999). Different mechanisms have been proposed to
6 explain these observations. It has been suggested that, during mixing, the size of protein
7 aggregates decreases (Mecham et al 1965, Tsen 1967) by physical separation of the
8 aggregates (Tsen 1967) and/or by breaking of non-covalent (Tsen 1967) or covalent bonds
9 (Graveland et al 1980, Danno and Hosoney 1982b, Tanaka and Bushuk 1973c, MacRitchie
10 1975). Hamer and Lichtendonk (1987), who have reported a decrease in extractability of
11 glutenin during resting, have assumed that re-polymerisation of glutenin polymer takes place.

12 The current study, which used several samples of a common French wheat cultivar
13 (Soissons) differing in total amount of unextractable glutenin polymers, aimed to identify
14 possible relationships between the stage of dough mixing and resting and changes in the
15 content and molecular size distribution of glutenin polymers. An on-line coupling of HPSEC-
16 MALLS (Multiangle Laser Light Scattering) was used to reveal the polydispersity of the
17 glutenin polymers during the process. Changes in the glutenin subunit composition of these
18 unextractable polymers were also investigated by RP-HPLC during dough mixing and resting.

19

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.

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 ± 15 BU (ICC standard 115, see Table I) 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 a temperature of $25 \text{ }^{\circ}\text{C} \pm 1.0 \text{ }^{\circ}\text{C}$. Directly after mixing, or after 45, 90 or 135 min resting at $30 \text{ }^{\circ}\text{C}$ 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 $200 \text{ }\mu\text{m}$ screen.

SDS unextractable glutenin polymer quantification

Freeze-dried doughs (240 mg) were stirred for 2 hr at 60°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°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 (AOAC 7.024) 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) by multiplying N values by 5.7.

1 **Extraction and purification of glutenin polymers**

2 Freeze-dried doughs (0.1g) were stirred for 1 h at room temperature (25°C) with 2 mL
3 of 0.3 M Sodium Iodide (NaI) in 7.5% (v/v) of propan-1-ol according to Fu and Kovacs
4 (1999). Extraction was followed by centrifugation at 15 900 x *g* for 15 min at 15°C. The
5 supernatant (mainly monomeric proteins) was eliminated. The glutenins remain unextractable
6 in the sodium iodide. The pellet was washed twice with the solvent of extraction and was then
7 used for HPSEC-MALLS procedure.

8

9 **Molecular size distribution of glutenin polymers by HPSEC-MALLS**

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

15 The HPSEC system comprised a SpectraSYSTEM LC (Thermo Separation Products
16 S.A., Les Ulis, France) consisting of on-line degasser (model SCM 400), isocratic pump
17 (model P 4000), variable auto-injector (model AS 3000) and variable wavelength detector
18 (model UV 2000). Refractive index of solutes was carried out with a differential refractive
19 index detector (Erma ERC-7512). Light scattering was measured on a Wyatt multi angle laser
20 light scattering detector (laser wavelength = 632.8 nm) (model Dawn-F, Wyatt Technology
21 Corporation, Santa Barbara, CA). Analog signals from the 15 photodiode channels were sent
22 to a PC with an on-board 16 channel A/D converter. The 16th channel was used for the signal
23 of the mass-sensitive detector. Photodiode coefficients were normalised using bovine serum
24 albumin monomer as a reference. The size-exclusion system comprised two columns in
25 series : a PL aquagel-OH 60 (300 × 7.5 mm I.D., 8 µm) (Polymer Laboratories Ltd.,

1 Shropshire, UK) and a PL aquagel-OH 40 (300 × 7.5 mm I.D., 8 μm) (Polymer Laboratories
 2 Ltd., Shropshire, UK). The eluant was sodium phosphate buffer 0.1 M (pH 6.90) with 0.1%
 3 (w/v) SDS, carefully degassed and filtered before use through 0.1 μm membranes (Gelman
 4 Sciences, France). The flow rate was 0.7 mL/min. During the fractionation, the columns were
 5 thermostated at 25 °C. Samples (100 μL) were injected into the HPSEC system.

6 Multi-angle laser scattering means measuring the intensity of the scattered light
 7 emitted by the sample molecules at different scattering angles (θ). With a modern MALLS
 8 photometer, it is possible to continuously monitor the scattering by means of several detectors
 9 mounted at different angles. For each elution slice, a weight average molar mass (M_w) can be
 10 calculated using the following equations :

11

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

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

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

12 where K is a light scattering constant, containing the wavelength λ_0 of the incident light, the
 13 refractive index n_0 of the pure eluent, and the refractive index increment dn/dc , c is the
 14 concentration, A_2 is the second virial coefficient, R_θ is the excess Rayleigh ratio, and $P(\theta)$ is a
 15 general form of a scattering function. For very low concentrations, the second and higher
 16 order terms in equation 1 can be neglected, and R_θ becomes directly proportional to $M_w P(\theta)$.
 17 Plotting R_θ/Kc against $\sin^2(\theta/2)$ gives M_w from the intercept with the ordinate ; from the
 18 angular dependence of the intensity of the scattered light, which is included in a_1 and higher
 19 order terms of the equation 2, a z -average root mean square radius $\langle R_G^2 \rangle_z^{0.5}$ can be derived.

1 This latter quality is defined in terms of the distribution of the volume elements of the
2 molecule with the respect to the square of the distance from its center of gravity :

$$\langle R_G^2 \rangle_z^{0.5} = \frac{1}{V} \int r^2 dV \quad \text{Eq. 4}$$

4
5 The calculation of the values $\langle R_G^2 \rangle_z^{0.5}$ is independant of dn/dc , M_w and even c and therefore
6 is insensible to errors. Number-average ($\langle M \rangle_n$), weight average ($\langle M \rangle_w$), z -average ($\langle M \rangle_z$)
7 molecular weights, $\langle R_G^2 \rangle_z^{0.5}$ and polydispersity index ($P = \langle M \rangle_w / \langle M \rangle_n$) were established with
8 ASTRA® software using a Zimm extrapolation (Kc/R_θ). Normalisation of the photodiodes (i.
9 e., the process by which the various photodiodes' signals are related to the 90° detector
10 signal) was obtained using a relatively narrow (i. e., $\langle M \rangle_w / \langle M \rangle_n \approx 1$) low molecular weight
11 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
12 to determine the interconnection volume between detectors to 0.172 mL. A value of 0.190
13 mL/g was employed as refractive index increment (dn/dc) for glutenins.

14

15 **Quantification of glutenin subunits by RP-HPLC**

16 Freeze-dried unextractable glutenin polymers (0.5 g) were extracted successively with
17 3.0 mL and 1.5 mL of 50% (v/v) propan-1-ol containing 1% (w/v) dithiothreitol (DTT) by
18 sonication for 2 min at power setting 50% (output 25W, 23 kHz) using a stepped microtip
19 probe (3 mm diameter) followed by continual stirring for 30 min at 60°C. After centrifugation
20 (10,000 × g , 10 min), the first and second extracts were pooled. Precipitation, resolubilization,
21 and alkylation of HMW-GS were performed as described previously (Marchylo et al 1989).

22 A Spectra-Physics HPLC and a PC1000/Spectranet data and chromatography control
23 station (Spectra-Physics analytical software, San Jose, CA) in conjunction with a Zorbax 300
24 SB-C8 column (C8, 300-Å pore size, 5-µm particle size, 15 cm (4.6 mm i.d. [Supelco Inc.,

1 Bellefont, PA]) preceded by a guard column of the same packing material (Zorbax 300 CB-
2 C8, 2-cm, 4.6 mm i.d.) were used for analysis of glutenin subunits. Column temperature was
3 maintained at 50°C ; the column effluent was monitored at 210 nm. Glutenin subunits were
4 resolved as described previously (Jia et al 1996a). Injection volume were 30 µL in
5 conjunction with a multiple 5 µL injection technique (Marchylo and Kruger 1988).

6

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 (see review by Weegels *et al.* 1996). This can be observed in figure 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 ; Veraverbeke *et al.* 1999 ; Skerritt *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 similar level to that which is to be found in flour is not attained during resting. Even if no definite conclusions have been reached, the depolymerization and re-polymerization mechanism have been proposed to

1 explain these observations (Mecham et al 1965, Tsen 1967, Tanaka and Bushuk 1973c,
2 MacRitchie 1975, Hamer and Lichtendonk 1987).

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

20

21 **Changes in molecular weight distribution of the glutenin polymers**

22 Even if no definite conclusions have been reached to explain the modifications in
23 glutenins extractability during both dough mixing and resting, different mechanisms have
24 been proposed. All these hypotheses were mainly based on a decrease of the glutenin polymer
25 size by physical separation of the aggregates (Tsen 1967) and/or by breaking of non-covalent

1 (Tsen 1967) or covalent bonds (Danno and Hosoney 1982, Tanaka and Bushuk 1973,
2 MacRitchie 1975, Graveland et al. 1980). In the current study, a one-line coupling of HPSEC-
3 MALLS was used to reveal changes in molecular size distribution of the glutenin polymers
4 during mixing.

5 The results of HPSEC-MALLS experiments on the polymeric glutenins of Soissons
6 T7 flour are shown in figure 3a,b. Figure 3a shows a MALLS/RI chromatogram of total
7 polymer separated by HPSEC illustrating the remarkable power of light scattering detection
8 for studying the high molecular weight fraction. In the same figure the molar masses of the
9 polymeric glutenins determined by means of light scattering are represented as a function of
10 the elution volume. The molar mass distributions (differential and cumulative representation)
11 represented in figure 3b were calculated by including the concentration of each respective
12 species. From these distributions it was possible to determine the number-average ($\langle M \rangle_n$),
13 weight-average ($\langle M \rangle_w$) and z-average ($\langle M \rangle_z$) molar mass, and also the polydispersity index
14 ($P = \langle M \rangle_w / \langle M \rangle_n$). All these measurements of polymeric glutenins show that the multi-angle
15 laser light-scattering photometer is suitable for monitoring the eluate from the HPSEC on the
16 condition that any exclusion limit happens on the gel matrix, as is the case with the SEC
17 stationary phases (PL aquagel-OH 40 and 60) used here. Consequently, the angular-dependent
18 scattered light readings yield the absolutely radius of gyration and molar mass and, by
19 including the respective concentrations, they also yield the distributions for these two
20 variables.

21 The increase in SDS solubility of the unextractable glutenin polymers was highly
22 correlated with the reduction of the number-average ($\langle M \rangle_n$) and weight-average molar mass
23 ($\langle M \rangle_w$) of these proteins during mixing (Fig. 4). In fact, both the variables (UG and $\langle M \rangle$) can
24 be plotted well by using exponential regression as a function of mixing time (MT). From
25 these results it appears that the SDS extractability of the glutenin polymers during mixing to

1 the dough peak consistency (first 4 min) can be the result of the size reduction of these
2 glutenin polymers confirming the depolymerisation hypothesis. In flour, the molar masses and
3 the radii of gyration of the total polymers are roughly in agreement with a size distribution of
4 glutenin calculated by Ewart (1987) based on standard theory of high polymers assuming
5 linear molecules. The most probable weight fraction had a molecular weight of $\approx 1.0 \text{ E}+6$
6 g/mol, and molecular weights extended to $> 5.0 \text{ E}+6$ g/mol. Recent measurements obtained by
7 flow FFF have also pointed to molecular weights in millions (Wahlund *et al.* 1996, Stevenson
8 and Preston 1996, Stevenson *et al.* 1999). These authors utilized the observed hydrodynamic
9 diameters (d) to estimate the upper and lower limits for the molecular mass of glutenins. The
10 lower limit was defined as $d=0.0542M^{0.498}$ for flexible random coil polymer, and the upper
11 limit was defined as $d=0.159M^{0.333}$ corresponding to a spherical shape. Values for the upper
12 limit were in the range of 440,000 to $11 \text{ E}+6$ g/mol. Like in the present study, because no
13 solvent has been found for solubilizing the total wheat glutenin, the solutions have been
14 sonicated so that the very largest glutenins would have been reduced in size (Southan *et al.*
15 1998, Carceller 2000). However, as we have already shown (Carceller 2000), even if
16 sonication can narrow down the glutenin size distribution, the protocol applied in the present
17 study is largely insufficient to reach a molecular distribution limit. Consequently, it does
18 enable the following of modifications in molecular distribution directly linked to dough
19 mixing.

20 During mixing, the relative amount of the highest molecular weight fraction
21 decreased, while the lowest molecular weight fraction, possibly corresponding to the
22 oligomers, increased (Fig. 5a,b). 59.1% of flour total polymers have a molar mass which is
23 greater than $1.0 \text{ E}+6$ g/mol while they represent only 47% in the dough after 4 min of mixing.
24 At the same time, oligomers characterized by a molar mass lower than $4.0 \text{ E}+5$ g/mol
25 represent 14.8% and 24.2% in flour and dough after 4 min of mixing respectively. These

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

15 Even if the study of this depolymerisation phenomenon deserves greater attention, the
16 overall observations made which are in total agreement with previous works (Gupta *et al.*
17 1992, Gupta *et al.* 1993, MacRitchie and Lafiandra 1997, Bangur *et al.* 1997), nevertheless,
18 confirm the existence of a close link between glutenin molecular distribution and rheological
19 dough properties. Therefore, in agreement with the first remarks made by Bangur *et al.* (1997),
20 it seems that only polymeric proteins above a certain molecular size contribute to dough
21 properties such as dough strength. This behavior is also in line with the theory of Bersted and
22 Anderson (1990) (Eq. 5). In this theory, based on a modified version of the Flory equation
23 (1945), Bersted and Anderson suggest that for polydispersed polymers only those molecules
24 that formed effective entanglements contribute to tensile strength (σ) :

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

Eq. 5

1

2 where σ_0 = the limiting tensile strength at high molecular weight; M_T = the threshold
3 molecular weight for effective entanglements; ϕ = the fraction with molecular weight $>M_T$;
4 M_n^* = the number-average molecular weight of this fraction (i.e. the fraction with molecular
5 weight $>M_T$).

6

7 **Changes in glutenin subunit composition during mixing and resting**

8 In the present study, it was found that the decrease in the amount of SDS-
9 unextractable glutenin polymers during dough mixing was accompanied by changes in the
10 composition of these polymers (i.e. relative proportions of different glutenin subunits) (Fig.
11 6a,b). A significant increase in the proportion of HMW-GS in SDS-unextractable polymers
12 was observed during mixing to peak dough consistency (first 4 min) (Fig. 6a). Further mixing
13 resulted in a decrease of the HMW-GS level in the SDS-unextractable polymers. The
14 proportion of HMW-GS in the unextractable glutenins that was reached at the end of mixing
15 was not significantly different to the proportion observed in flour. Finally, this HMW-GS
16 proportion remained closely constant during the dough resting. These observations seem to
17 demonstrate that the polymers more resistant to solubilization by dough mixing were enriched
18 in HMW-GS. Therefore, the HMW-GS proportion in SDS-unextractable polymers decreases
19 after peak MT, suggesting the reduction of these subunits may be linked to dough breakdown.
20 Then, during dough resting the glutenins re-polymerization did not induce any modification
21 of the relative subunit composition.

22 Apart from the changed HMW-GS/LMW-GS ratio, the different HMW-GS did not
23 disappear at the same rate from the SDS-unextractable glutenins resulting in changed related
24 proportions of the various HMW-GS. These observations were well illustrated in figure 7,

1 which shows reversed-phase HPLC patterns of HMW-GS from dough at different mixing and
2 resting time. Skerritt *et al.* (1999) observed similar effects. However, their observations that
3 the x-type HMW-GS solubilise more readily than y-type HMW-GS could not be confirmed in
4 the present study. Indeed, with *Soissons* flours it was found that the relative proportion of
5 HMW-GS 1Dy10 decreased while the proportion of HMW-GS 1Dx5 increased during mixing
6 (Fig. 6b). At the same time, the proportion of HMW-GS 1Bx7, 1By8 and 1A2* remained
7 relatively constant. These observations were in total agreement with other works
8 (Veraverbeke *et al.* 1999). However, in our study, all the significant modifications of relative
9 proportions observed for HMW-GS 1Dx5 and 1Dy10 occurred after the peak MT (dough
10 breakdown) and not during mixing to peak dough consistency (first 4 min).

11 Interpretation of the changes in HMW-GS composition of the SDS-unextractable
12 glutenin polymers during dough mixing and resting is difficult since the mechanisms
13 responsible for the changes in the amount of SDS-unextractable glutenin are poorly
14 understood. However, from our results it appears that glutenin subunits (HMW-GS, LMW-
15 GS, x- and y-type HMW-GS) were released from the polymers in a non-random order, which
16 was indicative of the polymers having a hierarchical structural organization. The data
17 obtained is in keeping with a proposed model for polymers, in which a backbone of HMW-
18 GS exists to which LMW-GS are bound (Graveland *et al.* 1985). According to Skerritt *et al.*
19 (1999), we cannot exclude the fact that differences in depolymerization rates between LMW-
20 GS and HMW-GS can be explained by the presence of polymers with differing composition
21 or branching within SDS-unextractable polymers; or within SDS-unextractable polymers by
22 the presence of different classes of glutenin subunits inducing an unevenly distributed
23 branching. Furthermore, our results, which demonstrate the major role of HMW-GS and/or x-
24 type HMW-GS to the formation of the SDS-unextractable polymers, are very consistent with
25 the observations made by Linsay and Skerritt (1998). These authors, using stepwise reduction

1 to examine the structure of SDS-unextractable glutenins, suggest that these polymers have a
2 well-ordered structure in which some HMW-GS (particularly HMW-GS 1Dx5) play a major
3 role in forming the backbone to them. Kasarda (1999) has indeed proposed a hierarchical
4 arrangement of the glutenin subunits that represents their relative potentials for the glutenin
5 polymer formation. This hierarchical arrangement of glutenin subunits in which x-type
6 HMW-GS are defined as the most important constituent, is based on a combination of two
7 factors relating to a) the potential of a subunit to form branched polymers, as opposed to
8 linear polymers, and b) the length of the repeating sequence domain.

9

CONCLUSIONS

1
2 During dough mixing the amounts, the size distributions of protein and the glutenin
3 subunit composition within the SDS-unextractable polymers changed. However, the major
4 changes in SDS-unextractable glutenin content and size distribution occurred before the peak
5 MT was reached, while detectable changes in subunit composition occurred also after the
6 peak MT. HPSEC-MALLS, which was very sensible to changes in polymer molecular weight
7 distribution, revealing a close relationship between the SDS solubility of the glutenin
8 polymers and their size distribution. Consequently, modifications of the SDS solubility of the
9 glutenin polymers during the process (mixing and resting) can be attributed to a large extent
10 to modifications of size distribution confirming the depolymerization and repolymerization
11 hypothesis.

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

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

7

LITERATURE CITED

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FIGURE CAPTIONS

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Fig. 1 (A) Effects of mixing [M] and resting [R] on the 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. (■,●) SoissonsT1, (□,○) SoissonsT2, (□,×) SoissonsT3, (□,▲) SoissonsT4, (□,□) SoissonsT5, (□,+) SoissonsT6, (□,Δ) SoissonsT7 for (A) and (B) respectively.

Fig. 2 (A) 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). (●) SoissonsT1, (○) SoissonsT2, (×) SoissonsT3, (▲) SoissonsT4, (□) SoissonsT5, (+) SoissonsT6, (Δ) SoissonsT7.

Fig. 3 (A) Steric-exclusion chromatography and (B) hydrodynamic parameters of total glutenin polymers of SoissonsT7. (A) RI detection (fine line), light scattering at 90° (bold line) and molar mass as a function of elution time (▲). (B) 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).

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

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5 **Fig. 6** (A) Changes in HMW-GS/LMW-GS ratio (bold line) and, (B) relative proportions of
6 the different HMW-GS in total HMW-GS in the SDS-unextractable glutenins during mixing
7 [M] and resting [R]. (●) 1Dx5, (○) 1Dy10, (Δ) 1Bx7, (▲) 1Ax2* and, (◇) 1By8.

8

9 **Fig. 7** Reversed-phase high-performance liquid chromatography patterns of HMW-GS in
10 SDS-unextractable polymers during mixing [M] and resting [R].

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TABLE I
Composition and properties of flours

Samples	Moisture Content (%, w/w)	Protein content ^a (%, w/w)	SDS-unextractable glutenin content ^a (%, w/w)	Brabender water absorption ^b (%)
Soissons T1	13.55	7.9	1.9	53.4
Soissons T2	13.84	8.6	2.2	53.4
Soissons T3	13.57	10.6	2.6	54.8
Soissons T4	13.97	10.1	2.3	54.5
Soissons T5	13.42	11.5	2.7	55.5
Soissons T6	13.26	11.3	2.8	55.6
Soissons T7	13.08	12.9	2.9	55.5

^a N × 5.7 ; results expressed on a dry-matter basis.

^b On flour basis according to ICC Standard 115.

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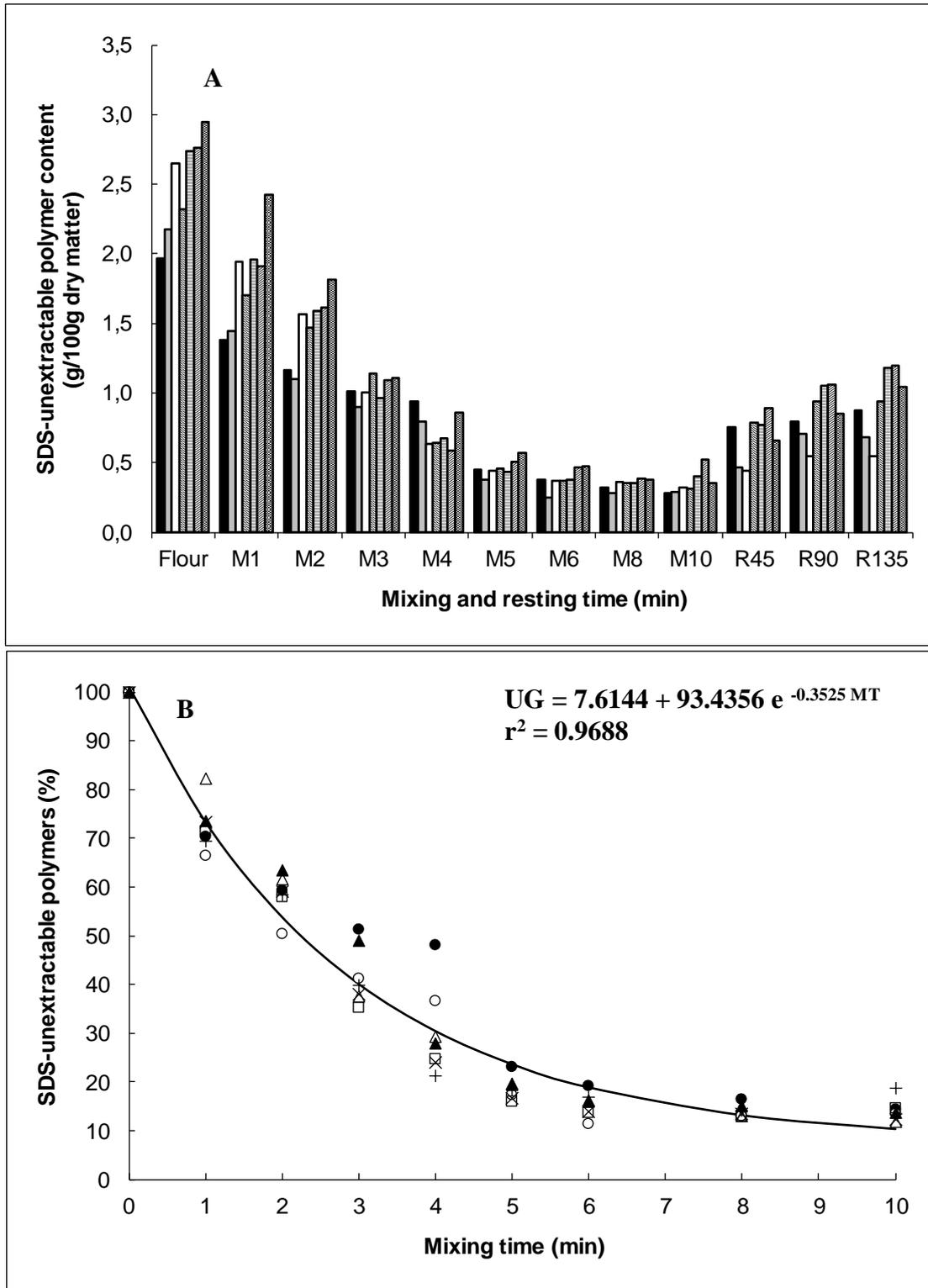
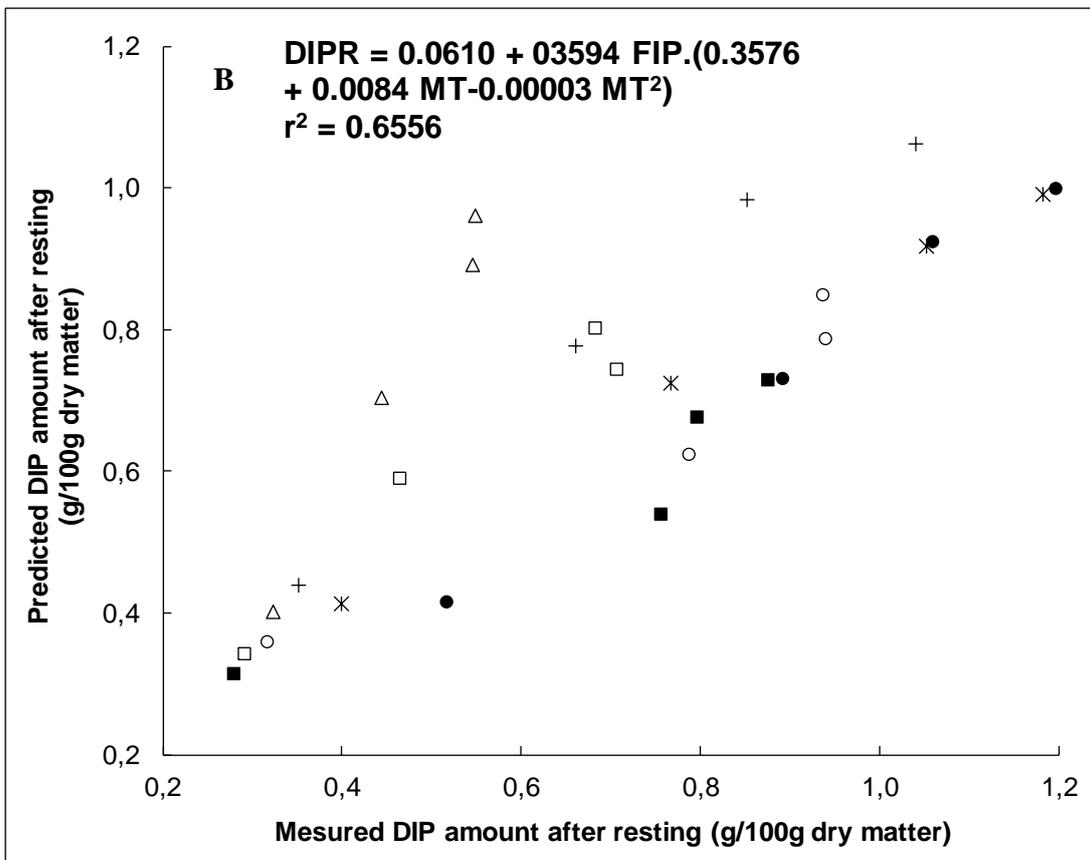
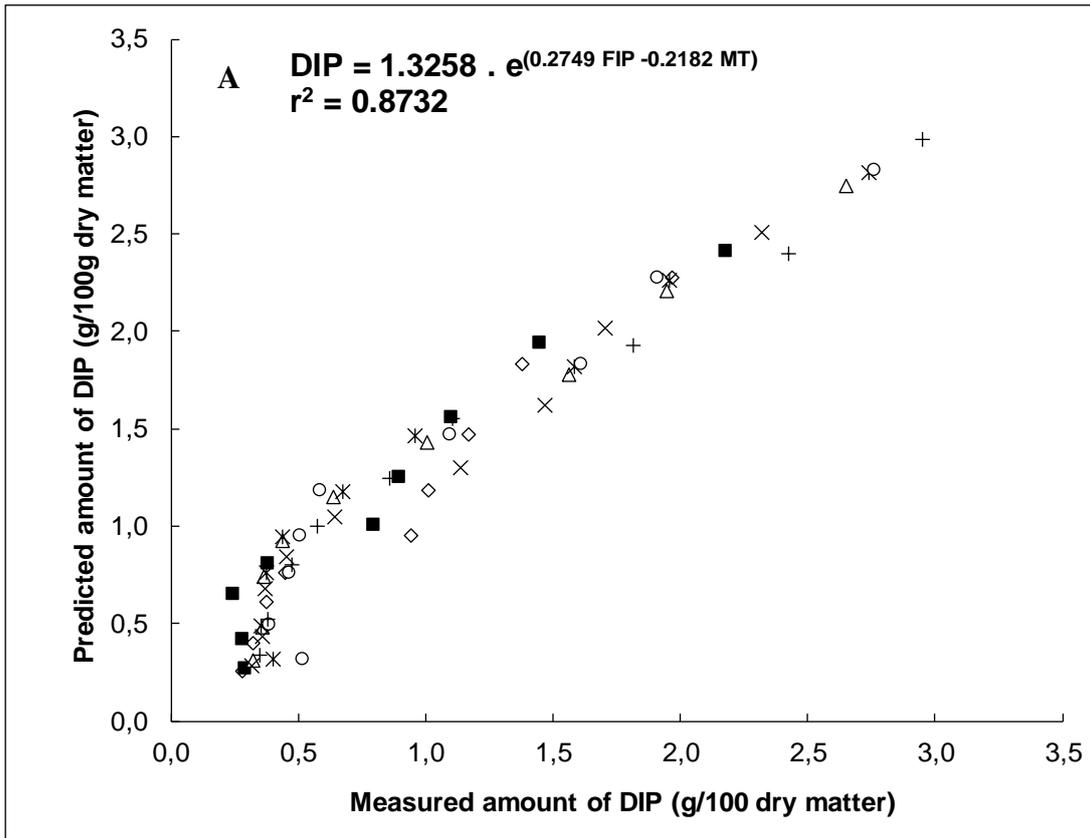


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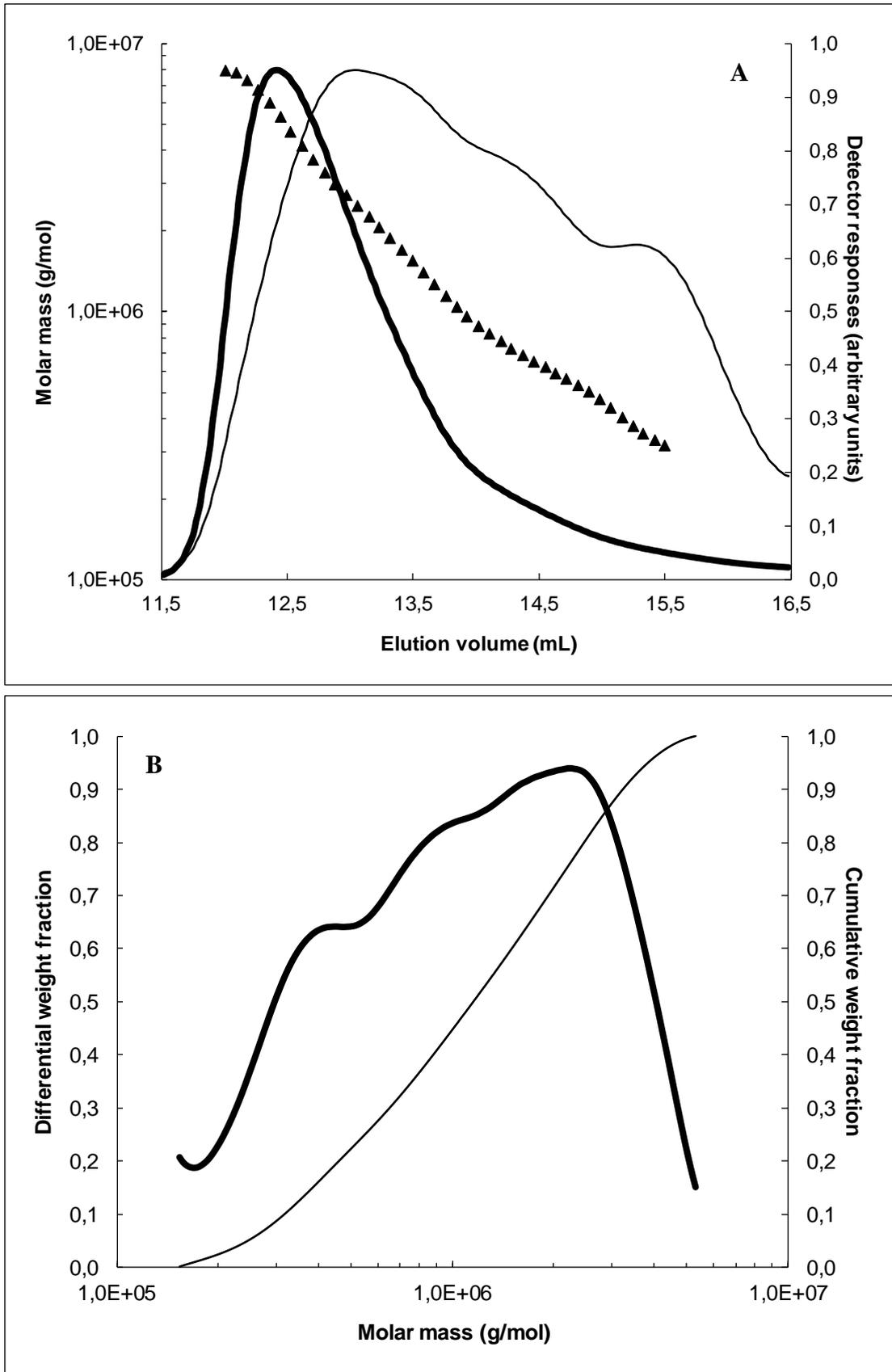


Fig. 3

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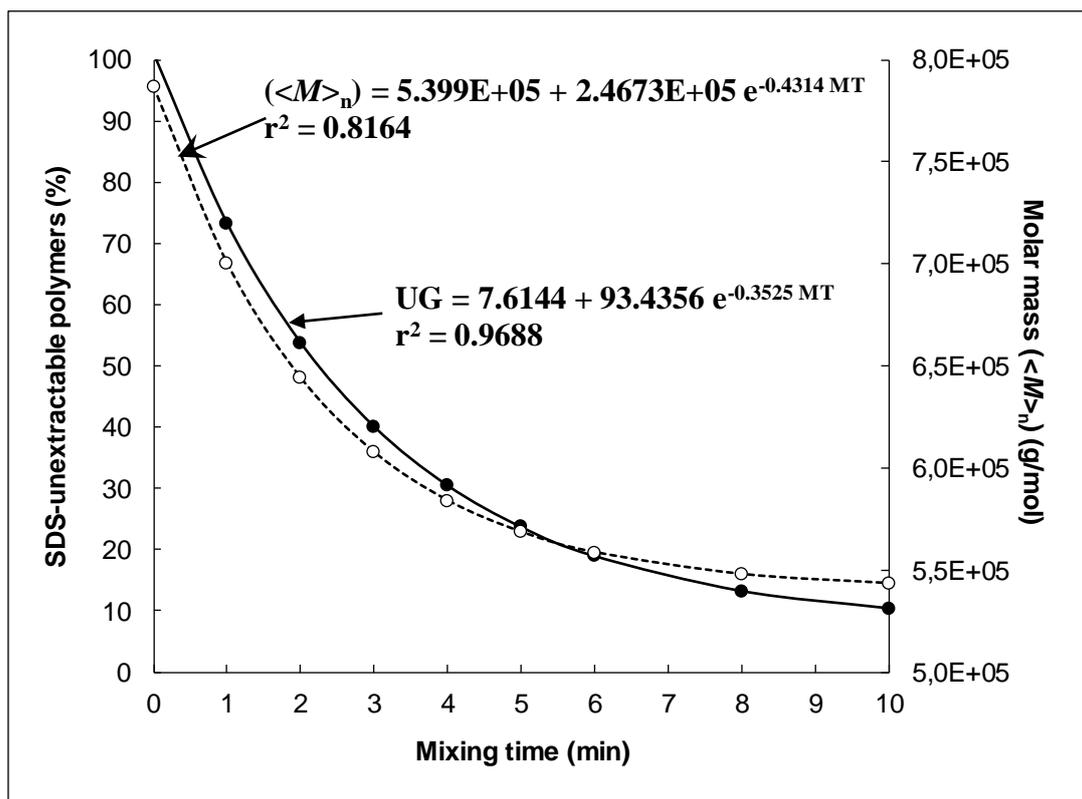


Fig. 4

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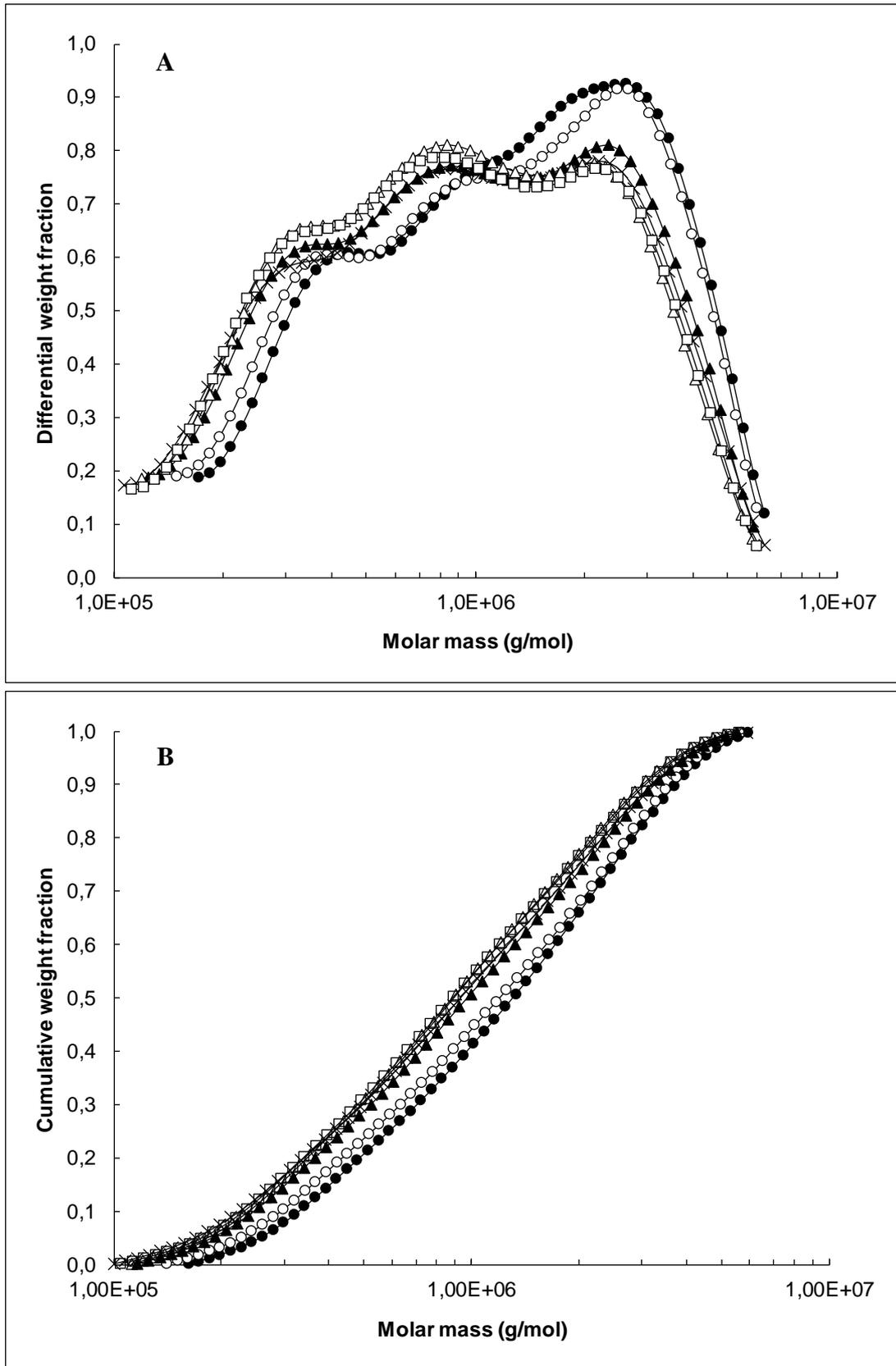


Fig. 5

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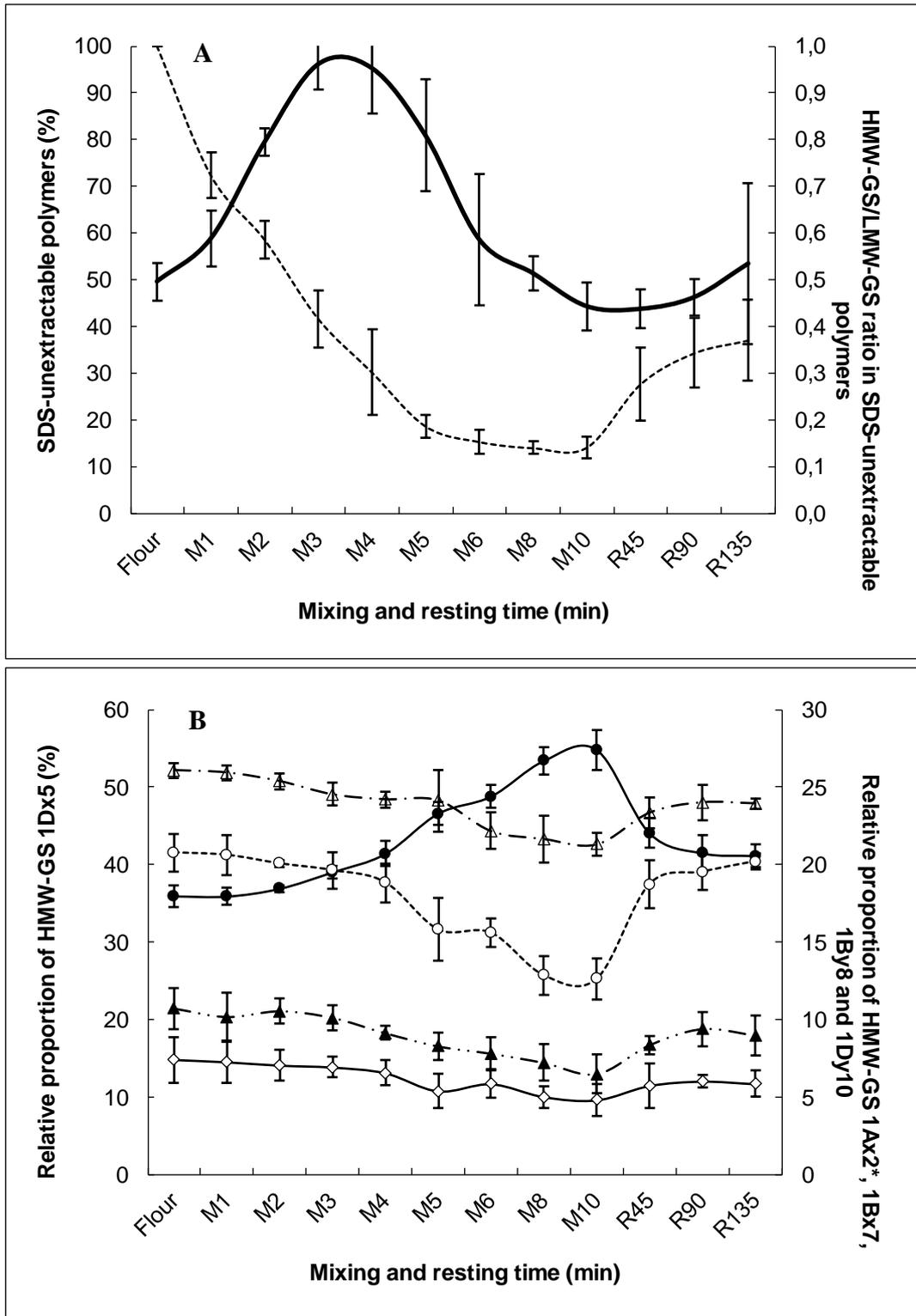


Fig. 6

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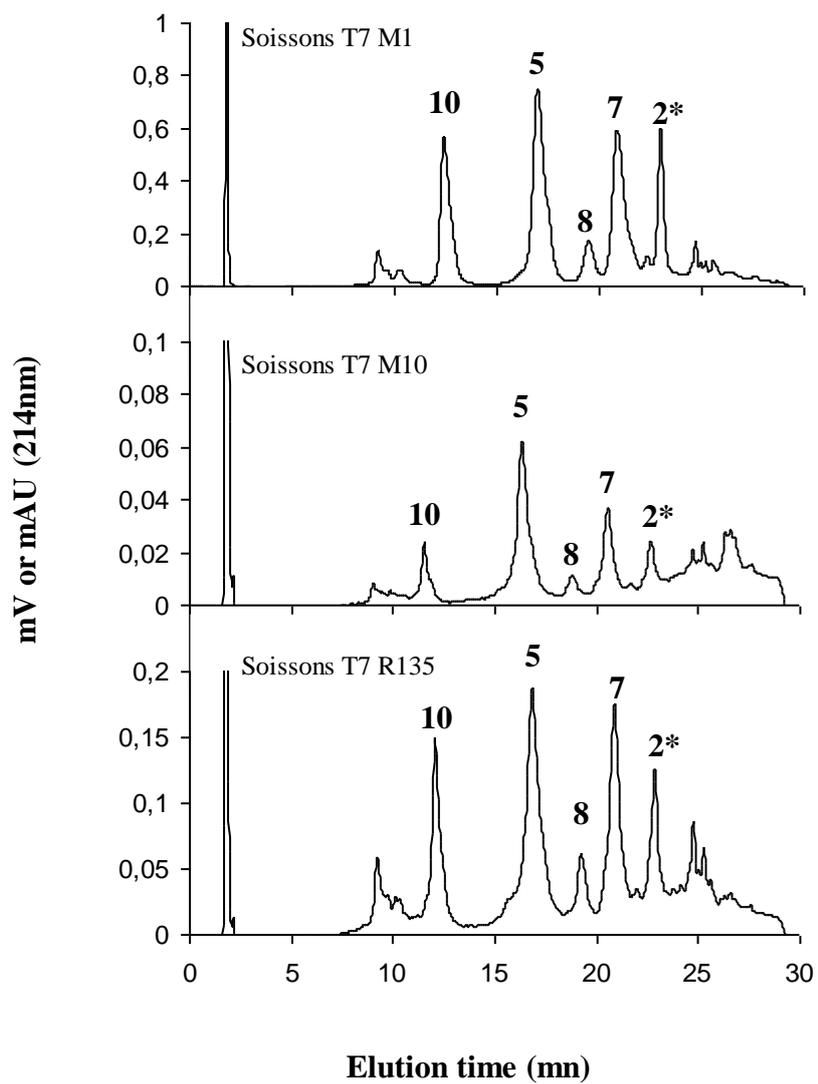


Fig. 7

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