

Depolymerization of the Glutenin Macropolymer During Dough Mixing:

I. Changes in Levels, Molecular Weight Distribution, and Overall Composition

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ABSTRACT

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Changes in the amounts, molecular weight distributions, and levels of major groups of subunits in the glutenin macropolymer (GMP) of doughs during mixing were investigated. The GMP (gel protein) is the unreduced fraction of gluten protein that remains as a layer on top of the starch after extraction of SDS-soluble proteins and centrifugation. Experiments involved doughs prepared from flours derived from one weak and one strong cultivar and lines derived from cv. Olympic that were null for specific high molecular weight glutenin subunits (HMW-GS). During mixing, the amount of GMP decreased; the major changes occurred before peak mixing time (MT, achievement of peak resistance). In addition, the average apparent molecular weight of GMP (determined by both size-exclusion HPLC and multilayer gel electrophoresis) decreased during mixing, but in this case, the major changes were seen later in the mixing process,

during dough breakdown. Even after extensive mixing, polymers and oligomers were released, not free glutenin subunits. During dough breakdown, the composition of GMP also changed, such that the proportion of HMW-GS decreased but β -amylases/D low molecular weight glutenin subunits (LMW-GS) increased. Changes in the total amounts of other LMW-GS typically were smaller with a decrease in the proportion of B subunits and an increase in the proportion of C subunits. The major changes in GMP composition were observed after peak MT (peak resistance) occurring earlier and to a greater extent in the weaker dough. Our results suggest that dough breakdown during mixing may be triggered by loss of HMW-GS, leading to changes in the molecular weight distribution and composition of the disulfide-bonded GMP.

Covalent and noncovalent interactions between the major polypeptide constituents in dough underly the unique ability of wheat flours to form elastic, extensible dough. After initial hydration of flour through mixing with water and other components, the next step in dough development is formation of a gluten network. Gluten-forming proteins deposited in protein bodies in the endosperm disrupt and become fully hydrated, forming a continuous gluten network in which starch granules are embedded. At optimal development, aggregates unfold, and a physical network of gluten polypeptides bound by both disulfide and noncovalent bonds forms (Kasarda 1989, Weegels et al 1996a). Any attempt to understand the biochemical basis for genetic and environmental differences in dough rheological behavior requires an understanding of the composition of subunit polypeptides and how they interact. Both covalent bonds (intermolecular disulfides) and noncovalent interactions are important in the formation and behavior of doughs (Ewart 1977, Graveland et al 1985, Ng et al 1991). However, although knowledge of the effects of variation in the allelic composition of some individual gluten polypeptides on dough quality recently has improved (reviewed by Skerritt 1998), our understanding of how polypeptides specifically interact to determine dough properties is poor.

To determine the relationship between such interactions and the rheological changes that occur during dough mixing, we have examined changes in the amount and composition of polypeptides that comprise the glutenin macropolymer (GMP) during dough mixing. GMP can be assayed through analysis of the translucent SDS-insoluble gel protein fraction (Graveland et al 1979) that forms on top of the starch layer of SDS extracts from flour or dough after high-speed centrifugation. The rheological behavior of this fraction has been studied (Pritchard et al 1994) with a Bohlin small-strain oscillatory rheometer to mirror the properties of the original flour. In addition, variation in the GMP content of flours may form the basis of the widely used Zeleny sedimentation screening test for prediction of dough strength (Moonen et al 1982).

Several studies have suggested that the arrangements or interactions between subunits in polymer are nonrandom. Some disulfide bonds in glutenin may be more subject to cleavage than others (Jones et al 1974, Moonen et al 1985, Ewart 1988, Ng et al 1991); indeed, it has been proposed that clusters of glutenin subunits may be linked only by single disulfide bonds (Kawamura et al 1985, Ewart 1988), which suggests that the GMP is largely linear. It has been suggested that high molecular weight glutenin subunits (HMW-GS) form the backbone of the polymer, with branches of low molecular weight glutenin subunits (LMW-GS) (Graveland et al 1985, Moonen et al 1985). Graveland et al (1979) demonstrated that after overmixing, the solubility of glutenins in SDS increased, with polymers as small as M_r 800,000 being formed. Depolymerization could be partially reversed by resting the dough and did not occur when doughs were mixed under nitrogen. Weegels et al (1996b) extended their studies to include quantitation of total HMW- and LMW-GS using reversed-phase HPLC, and demonstrated that, in three cultivars, the ratio of HMW-GS to LMW-GS decreased as doughs were overmixed. Bushuk et al (1997) recently demonstrated that the amounts of acetic acid-insoluble glutenin and high molecular weight glutenin decreased during mechanical dough development of flours from six wheat cultivars.

The current study, which used cultivars differing in mixing properties and lines that were null for specific HMW-GS, aimed to identify possible relationships between the stage of dough mixing and changes in the content and molecular weight distribution of GMP during dough mixing. Because a central role in the structure of GMP has been proposed for HMW-GS, the composition of different polypeptide families in GMP at different stages of dough mixing were investigated.

MATERIALS AND METHODS

Flour Samples and Mixing

In initial experiments, the behavior of the GMP from a strong-dough cultivar (Suneca: 13.1% protein, HMW-GS composition 1, 17+18, 5+10) and a weak-dough cultivar (Rosella: 11.6% protein, HMW-GS composition 2*, 7+8, 2+12) were compared. In subsequent experiments, the role of HMW-GS was evaluated by studying six genetic lines prepared from cv. Olympic (Lawrence et al 1988; HMW-GS composition 1, 17+18, 5+10). The lines tested were null at either zero, one, or two HMW-GS encoding loci and were denoted as ABD, -BD, A-D, AB-, -B-, and -D, with loci indicated in alphabetic order. The lines were grown at Narrabri

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(northern New South Wales, Australia) in 1990; the dough properties of the flours used from these lines are described in Gupta et al (1995). All lines were milled as straight-run flours in a Buhler mill to 71–75% extraction. The lines that contained either only the single 1A HMW-GS or were null at each HMW-GS locus were not studied because large-scale dough testing has shown that these lines are unable to form a normal dough; for example, the resulting doughs are insufficiently cohesive to be tested in an extensigraph (Gupta et al 1995).

Mixing tests were conducted with a prototype 2-g mixograph, using a modification of the standard method for 35 g of flour scaled to 2 g (Bekes and Gras 1992). Water absorption of the flours was determined by Approved Method 54-40A (AACC 1995). Mixing experiments with Suneca and Rosella flours were performed in duplicate; data shown for each mixing and biochemical analysis are means of replicates that differed by <5%. Although several parameters were measured, for brevity, only effects on mixing time (MT) and resistance breakdown (described by Bekes and Gras 1992) are reported. Each of the lines was mixed as flour-water dough for seven periods ranging from 0.5 to 10 min before dough was snap-frozen in liquid nitrogen and freeze-dried. After grinding in a mortar and pestle, dried dough was stored at -20°C until analysis. Control experiments with wetted and freeze-dried flour showed that the mortar and pestle step did not cause measurable changes in GMP solubility or molecular weight distribution.

To prepare GMP, flour or lyophilized dough (100 mg) was extracted with 1.5 mL of 1.5% (w/v) SDS in water in a 2-mL microfuge tube by vortex mixing and shaking for 1 hr at room temperature. After extended centrifugation in a microfuge (30 min, 15,600 × g), the pellet and gel were resuspended with a small spatula, washed by shaking for 30 min in 1.5% SDS, and centrifuged again. The supernatant was removed, leaving a starch pellet with a layer of GMP. The method of removal of the GMP layer depended on the nature of the next experimental step.

Analysis of GMP Molecular Weight Distribution

The molecular weight distributions of gluten proteins in doughs were analyzed by several techniques because no single method can analyze both soluble and insoluble materials over the full size range.

Determination of total GMP by modified bicinchoninic acid protein assay (Smith et al 1985). A mixture of SDS (1.5%, w/v, 1.5 mL) and dithiothreitol (DTT) (10 mM) was added to the GMP and starch residue, and the samples were vortexed overnight at 37°C. The tubes were centrifuged at 15,400 × g for 8 min, and 7.5–30 μL of supernatant was assayed for protein after addition of 40 μL of 0.1M iodoacetamide (BDH, Poole, England) in 0.1M Tris-HCl, pH 8, and incubation for 15–30 min at 37°C (Hill and Straka 1988).

Size-exclusion HPLC analysis of SDS-soluble and SDS-insoluble fractions of doughs. A Bio-Sep S-4000 column was used according to the manufacturer's specifications to fractionate globular protein complexes to M_r 2 million (Phenomenex, Torrance, CA). The GMP on top of the starch pellet was suspended by 30 sec of sonication (Singh et al 1990) in 1.5 mL of 1.5% SDS in water/100 mg of flour (or freeze-dried dough) before filtration (PVDF Acrodisc filter, Gelman, Ann Arbor, MI) and chromatographic analysis at 30°C. The mobile phase (0.5 mL/min) consisted of 50% (v/v) acetonitrile (Mallinckrodt, Paris, KY) and 0.1% (v/v) trifluoroacetic acid (Pierce, St. Louis, MO) in water (Batey et al 1991). The detection level was 214 nm.

Multilayer SDS-PAGE. The multilayer SDS polyacrylamide gel electrophoresis (MLGE) method adapted from Khan and Huckle (1992) contained multilayer gels with separate zones of 4, 6, 8, 10, 12, and 14% polyacrylamide. Recipes for buffers are given in Wrigley et al (1993). Gels were stained with 0.1% (w/v) Coomassie G250 in 40% methanol and 10% acetic acid in water. Densitometric analysis (Panasonic digital color CCTV camera, Matsushita, Japan, and VideoPro digital image analysis Software, Leading Edge Pty, Adelaide, Australia) was carried out on electrophoregrams of dough

extracts prepared by sonication in SDS buffer but without further pre-fractionation. The average molecular size (AV in weighted absorbance units) was calculated as:

$$AV = 100 \sum_{i=1}^5 i \cdot a$$

where a is absorbance (% total area scanned, i)/100. The AV value, therefore, can vary between 5 and 500. The method of analysis assumes that glutenin polymers of differing sizes bind a constant proportion of Coomassie blue dye.

Subunit Composition of GMP and SDS-Soluble Protein Fractions

The content of the major groups of GMP polypeptides from each dough at each stage of mixing were analyzed by SDS-PAGE after reduction of proteins in sample extracts by adding an equal volume of a mixture of 25% glycerol and 100 mM DTT in 1.5% SDS containing a trace of bromophenol blue, heating for 30 min at 95°C, and fractionating by SDS-PAGE on 12% T and 2.6% C slab gels (Skerritt and Underwood 1986). We quantified the total amounts of HMW-GS, D-LMW-GS/β-amylases, B-LMW-GS, and C-LMW-GS in the GMP fraction by laser densitometry (LKB Ultrosan, Bromma, Sweden; set at smoothing 2; peak width 1, peak number 35, peak area 0.1, baseline 1, and x-width 1). In addition, subunit composition of the size fractions of sonicated GMP, after fractionation by MLGE, was analyzed by excision of each zone corresponding to the interfaces between different concentrations of polyacrylamide and equilibration of the gel piece for 1 hr with 4% 2-mercaptoethanol in 2% SDS, 20% glycerol, and 125 mM Tris-HCl, pH 7.5 (the same buffer was used with stacking gels in subsequent separation). The equilibrated gel piece and surrounding buffer were loaded into the sample lane, and proteins were analyzed by the 12% T and 2.6% C gel system described above.

RESULTS AND DISCUSSION

Changes in GMP Content During Mixing

Initial examination of flours revealed that approximately one-quarter of the protein was insoluble in the solvent containing SDS, which was in keeping with previous findings (Danno et al 1974). A stepwise decrease in GMP protein content (i.e., SDS-insoluble protein) occurred during mixing, suggesting the protein macropolymer was converted into smaller, SDS-soluble polymers. Increases in protein solubility during mixing previously had been noted by Tanaka and Bushuk (1973a,b). The proportion of the largest polymers, evaluated by measuring the proportion of protein that was insoluble in 1.5% SDS, was greater in Suneca flour, the genotype that produced stronger doughs (Fig. 1). Depolymerization occurred later (in keeping with longer MT) and to a lesser extent in Suneca compared with Rosella, although the difference in amount of GMP in flours was relatively minor (Fig. 1A). No further decreases in GMP occurred after 6 min of mixing (data not shown).

Measurement of protein in GMP was achieved after solubilization of polymeric protein with SDS-DTT, followed by blocking of the sulfhydryl groups of the reductant with iodoacetamide to prevent interference in the bicinchoninic acid protein assay (Hill and Straka 1988). Other studies have quantified GMP (gel protein) by scraping the gel off the starch layer and either measuring the protein present by Kjeldahl analysis (Graveland et al 1979) or weighing (Pritchard and Brock 1994). Our approach enabled quantification of protein (relative to the response of a standard protein) rather than hydrated gel, allowing the proportion of protein that forms part of the GMP to be calculated by assaying both the SDS-soluble and SDS-insoluble protein fractions. In addition, the method is more suitable to the analysis of large numbers of small samples than other methods of measuring gel protein (Pritchard 1993). Although use of a colorimetric protein assay requires use of a standard protein, which has a different color response per microgram of protein than glutenin polypeptides, both the SDS-soluble and SDS-insoluble protein

fractions were determined relative to the corresponding protein standards, such that the calculated ratio of the protein fractions was relatively independent of the standard chosen.

The decrease in GMP content began well before mixograph peak time was reached, suggesting the reduction in quantity of GMP 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) who assessed gel protein weight at 0, 1, 2, and 5 min of mixing and noted decreases in the amount of gel protein from the beginning of mixing. Pritchard and Brock (1994) found that the weight of gel protein in flour was poorly correlated with loaf volume and that correlations between breakdown rate and loaf volume were somewhat better: low loaf volumes were associated with higher rates of breakdown. However, Weegels et al (1996b) found that variation in flour GMP content could explain 80–90% of the variation in loaf volumes for flours from 14 cultivars.

Changes in Molecular Weight Distribution

Changes in the molecular weight distribution of polymers was assessed by two methods: size-exclusion (SE) HPLC with a 400-Å bead-size column and MLGE. In the SE-HPLC experiments, the SDS-soluble protein fractions from flours and doughs were analyzed directly, and the SDS-insoluble protein fractions were analyzed after sonication to solubilize material in the absence of reducing agents (Singh et al 1990). The flour or freeze-dried doughs also were sonicated in SDS and analyzed by MLGE without prior fractionation into SDS-soluble and SDS-insoluble fractions. Analysis of 1.5% SDS in water-soluble material by SE-HPLC showed an increase in both amount and average molecular weight of protein, although the major changes occurred after peak MT. The increases were due to the fact that the solubilized protein was composed of soluble polymers (presumably derived from the insoluble macropolymer). Using viscometric analysis to assess changes in molecular weight and disulfide bond formation, Danno and Hosoney (1982) obtained similar results. The extractability of protein by SDS from a dough was greater than extractability from the corresponding flour, but at MT, Danno and Hosoney (1982) observed an increase in viscosity prior to extractability that decreased during overmixing, suggesting there initially was an increase in the molecular weight of the polymer at MT. Our evidence suggests that this conclusion is incorrect and that the initial increase in viscosity was due to solubilization of the highest molecular weight SDS-insoluble fraction.

SE-HPLC of GMP, after solubilization by sonication, also revealed changes in molecular weight distribution during mixing. Although sonication may break certain bonds (Khan et al 1994, Weegels et al 1994) during solubilization of protein, the expected trends were apparent. In this case, the relative amount of the highest molecular weight fraction decreased, while the lowest molecular weight fraction, possibly corresponding to the oligomers, increased (Fig. 2A–B). Similar changes in apparent molecular weight distribution were noted with MLGE (Fig. 3A) for both the proportion of protein in polymers that was unable to enter the 6% polyacrylamide gel layer and for a calculated relative average molecular weight (equation above) of the polymer (based on distribution across different polyacrylamide gel zones). Major decreases in apparent molecular weight occurred after peak MT and were larger for the weaker Rosella flour.

Composition of GMP at Different MT

Possible changes in GMP composition (i.e., relative proportions of different gluten polypeptides) were monitored by SDS-PAGE and densitometry after chemical reduction to glutenin subunits of the macropolymer obtained from doughs at different mixing stages. The most pronounced changes were in the relative proportions of HMW-GS, which decreased with mixing as the size of the polymer decreased. There was also a marked decrease in the absolute levels of HMW-GS. The proportion of a minor group of M_r 50,000–

60,000 polypeptides increased in Suneca dough (Fig. 4A), and the proportions of B- and C-LMW-GS remained relatively constant, with only minor decreases in the proportion of B-LMW-GS and C-LMW-GS. Similar trends were seen with Rosella dough, except they occurred earlier (in keeping with the shorter MT) and were of a greater magnitude (in keeping with the weaker nature of the Rosella dough) (Fig. 4B). Although termed “sulfur-rich albumins,” both the D-LMW-GS (Jackson et al 1985) and S-rich albumins (Gupta et al 1991) have similar electrophoretic mobilities. One possibility is that as disulfide bonds are broken during mixing, the polypeptides bind to free sulfhydryls in glutenin proteins. This finding may be in keeping with a proposed “chain-terminating” role for the polypeptides. Interestingly, both the albumins (Gupta et al 1991) and D-LMW-GS (Masci et al 1993) have been postulated to have a weakening effect.

Results with Null Lines

Because one of the major phenomena during GMP depolymerization appeared to be a decrease in the proportion of HMW-GS, lines lacking one or more HMW-GS were used to more closely examine the role of different HMW-GS in disaggregation of gluten polymers during mixing. The lines exhibited significant variation in peak MT, which was assessed with the 2-g mixer: ABD 3.6 min, A-D 3.2 min, -BD 3.3 min, AB- 2.8 min, --D 2.5 min, and -B- 2.1 min. Thus, deletion of the 1D locus caused a larger decrease in MT than deletion of 1A or 1B; this dominant effect agreed with earlier studies with larger mixers (Lawrence et al 1988) and analyses of cv. Chinese Spring nullisomic-tetrasomic lines (Rogers et al 1991). Lines with only 1A HMW-GS and the triple null line could

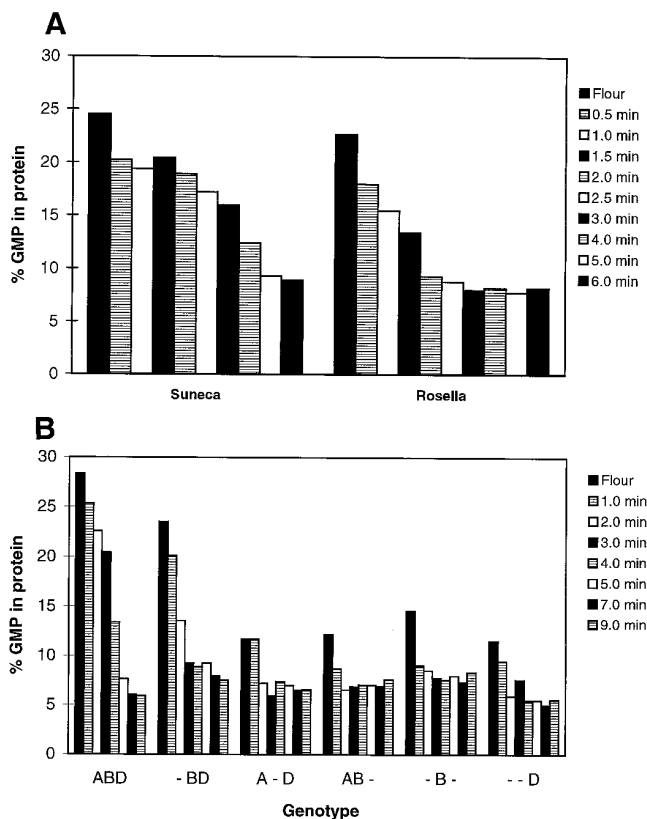


Fig. 1. Changes in glutenin macropolymer (GMP, SDS-insoluble protein) content during mixing of **A**, a strong-dough cultivar (Suneca; mixing time [MT] = 3.8 min) and weak-dough cultivar (Rosella; MT = 3.1 min) and **B**, null high molecular weight glutenin subunit genetic lines from cv. Olympic, expressed as percent GMP in protein = (SDS-insoluble protein)/(SDS-insoluble + SDS-soluble protein), determined by bicinchoninic acid protein assay. Data shown are means of two experiments; results differed by <10% of the mean.

not be mixed in the smaller mixer into a true dough and were not included in the earlier studies. The ABD line had a very high dough strength and long MT in standard laboratory rheological testing: a mixograph development time of 8.9 min and a maximal resistance of 380 BU, with a protein content of 15% (Gupta et al 1995). Thus, the control flour was stronger than Suneca or Rosella.

The relative proportion of GMP in flour protein was higher ($\approx 25\%$) for ABD and -BD flours, but deletion of either the B- or D-genome HMW-GS reduced the proportion of GMP to between 12 and 15%.

Deletion of both the B and D genomes had a roughly cumulative effect, whereas the A-genome HMW-GS had little effect (Fig. 1B). Gao and Bushuk (1993) noted similar differences in the proportion of glutenin protein for this series of flours, although their fractionation method, which was quite different, produced higher proportions of glutenin. Other studies on the flours of these HMW-GS null lines revealed that between 40 and 50% of the protein in the lines tested was polymeric, but there were much greater differences between flours in the proportion of insoluble polymers (Gupta et al 1995).

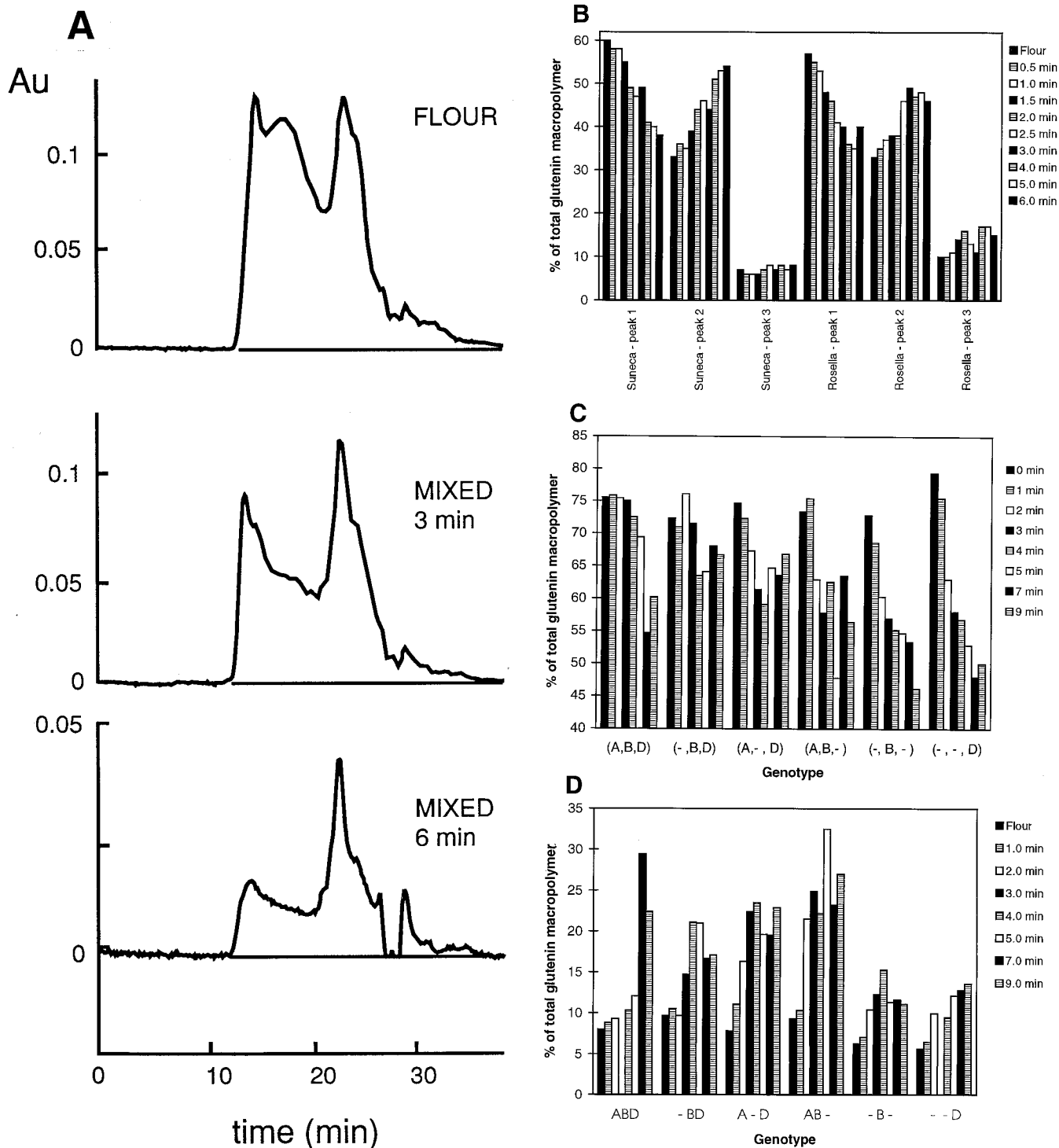


Fig. 2. Analysis of glutenin macropolymer (GMP) at various stages of mixing by size-exclusion (SE) HPLC. **A**, SE-HPLC profile for sonicated GMP from cv. Rosella flour and doughs at 3 and 6 min of mixing. **B**, Proportion of GMP in highest (peak 1, retention time <21.5 min), medium (peak 2, retention time 21.5–27 min), and lowest (peak 3, retention time >27 min) molecular weight peaks in cv. Suneca and Rosella doughs. **C and D**, Proportion of GMP in highest (peak 1) and medium (peak 2) molecular weight peaks, respectively, in doughs mixed from null high molecular weight glutenin subunits lines from cv. Olympic. Data shown are means of two experiments; results differed by <10% of the mean.

For each of the lines, there was a decrease in GMP content in dough during mixing (Fig. 1B). Like the Suneca and Rosella flours, much of the decrease in GMP content occurred before peak MT was reached. Although decreases in GMP content occurred steadily in ABD doughs and were not complete until 5 min, the decrease in GMP content was faster in doughs lacking one or two HMW-GS. In the dough with the second longest MT (-BD), most of the decrease in GMP content occurred by 3 min, whereas for the other flours decreases were complete by 2 min of mixing. The final amounts of polymer after extended (9 min) mixing were similar (5–8% of that found in flour) in all samples.

The timing of decreases in the size distribution of GMP was different from that of GMP amounts. Most of the changes in size distribution occurred after peak MT, and there were differences between the lines in the extent of depolymerization. Although MLGE (Fig. 3B) and SE-HPLC (Fig. 2) both revealed that significant decreases in molecular weight occurred during dough mixing, only MLGE showed differences in the molecular weight distribution between unmixed flours with different subunit compositions; the ABD and the -BD lines had the largest polymer sizes, and the lines lacking both the A- and D-genome HMW-GS had the smallest polymer in flour based on MLGE. The ABD control line, which had the longest MT, also had the largest relative proportion of large polymers (i.e., polymers unable to enter the 6% T phase in MLGE). Although the amount of polymer decreased by >60% during the 9-min mixing period, the changes in average molecular weight,

whether measured by MLGE or SE-HPLC, were smaller. Decreases in average molecular weight for the ABD line were seen only at 7 and 9 min of mixing, whereas deletion of subunits encoded by specific loci caused much more substantial decreases in GMP average molecular weight during mixing, and the changes occurred earlier (Fig. 3B). Changes measured in size distribution of GMP during mixing by SE-HPLC were similar to those described for MLGE; depolymerization was assessed as the proportion of the largest molecular weight peak in the chromatogram of sonicated GMP. In ABD dough, the proportion decreased from $\approx 75\%$ in flour to 60% in overmixed dough. Decreases in molecular weight were greatest for the single null doughs and the double null lacking the D genome (i.e., relative effects of subunits encoded by homologous chromosomes were $D > B > A$).

Null lines were analyzed by SDS-PAGE, and proportions of major polypeptide groups were determined (Fig. 5). In keeping with the results for Suneca and Rosella, decreases in the relative proportion of HMW-GS in the GMP were observed in each dough, with major changes occurring after peak dough development times had been reached. Although quantitation of GMP had shown that after extended mixing the final amounts of polymer were similar in all samples, the proportions of HMW-GS in the GMP differed considerably. Initially, densitometric analysis showed that HMW-GS comprised $\approx 40\%$ of the polypeptides in the GMP in the ABD and AB- lines and 20–25% in the other lines. However, although the ABD and AB- lines had a similar initial proportion of HMW-GS, the decrease in the line that lacked the D genome occurred earlier in mixing and was of a much greater magnitude. The line lacking the D

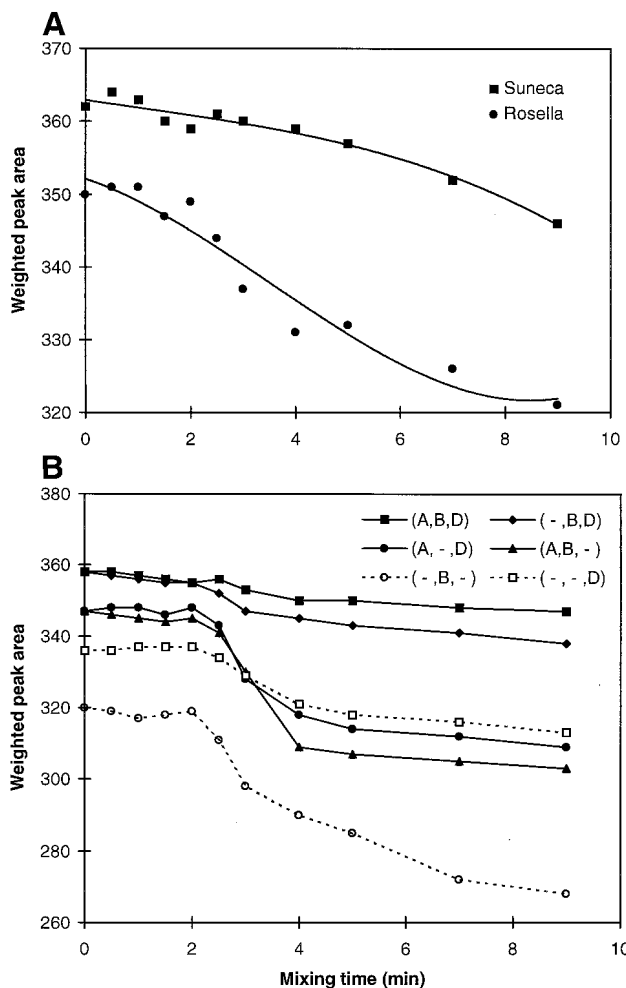


Fig. 3. Changes in notional average molecular weight of proteins extracted from doughs during mixing and analyzed by multilayer gel electrophoresis. **A**, Cvs. Suneca and Rosella. **B**, Null high molecular weight glutenin subunit genetic lines from cv. Olympic. Data shown are means of two experiments; results differed by <10% of the mean.

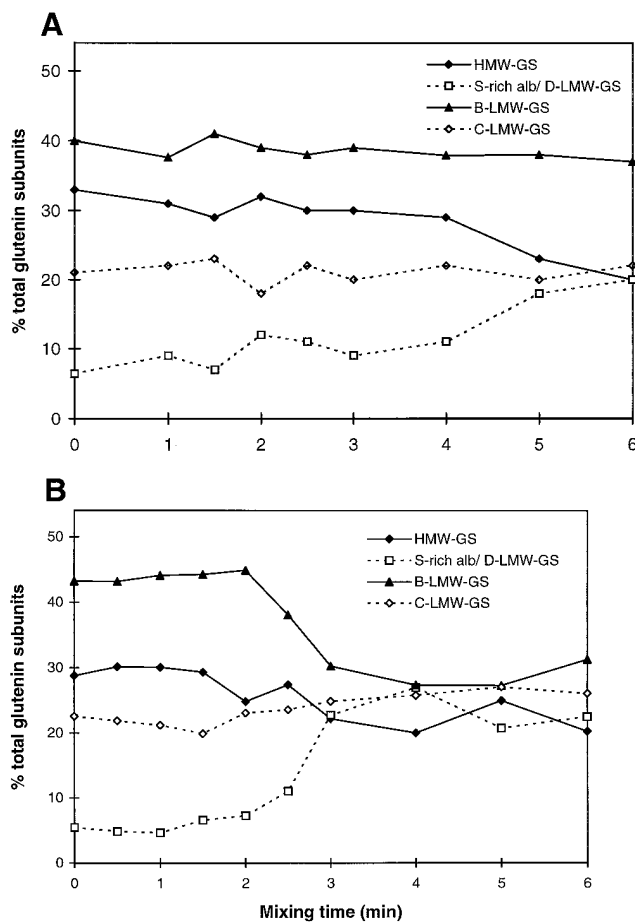


Fig. 4. Changes in proportions of total high molecular weight glutenin subunit (HMW-GS), B and C low molecular weight glutenin subunit (LMW-GS), and S-rich albumins/D-LMW-GS in **A**, cv. Suneca and **B**, cv. Rosella doughs during mixing. Data shown are means of three experiments; results differed by <10% of the mean.

genome had a correspondingly shorter MT and greater dough breakdown. The -BD and --D lines had a lower proportion of HMW-GS in flour than did the ABD and AB- lines, but there were relatively small decreases in the relative proportions of HMW-GS during mixing. The proportions of HMW-GS in the A-D and the -B- flours were lowest, and additional marked decreases (to 7–10% of the subunits in GMP) occurred with mixing. These results suggest that deletion of specific HMW-GS not only influences the rates of dough breakdown and changes in molecular weight distribution of GMP in mixing, but also the proportion of HMW-GS at various stages of dough mixing.

The observed trends in changes during mixing in the relative proportions of the other major polypeptide families in GMP, D-LMW-GS/S-rich albumins, B-LMW-GS, and C-LMW-GS were less consistent than those observed in flours from normal wheats with the full complement of HMW subunits, even though each of the flours had an identical composition of non-HMW-GS. The typical pattern for a strong-dough flour, namely an increase in D-LMW-GS/S-rich albumins, little overall change in B-LMW-GS, and an increase in C-LMW-GS, was seen with ABD flour. Similar trends were seen with both flours that lacked HMW-GS at two loci, even though these had higher proportions of subunits other than HMW-GS in the flour. However, each of the three lines that lacked a single HMW-GS exhibited decreasing proportions of D-LMW-GS and an increased proportion of B-LMW-GS. These results suggest that loss of some of the HMW subunits in these genetic lines may have perturbed the structure of the GMP, causing it to depolymerize in an abnormal manner during dough breakdown.

CONCLUSIONS

During dough mixing, both the amounts and size distributions of protein within the GMP changed; however, the major changes in GMP content occurred before peak MT was reached, while detectable changes in size distribution and composition occurred after peak MT. Thus, only the latter phenomenon may relate to dough breakdown. It is likely that the changes measured by MLGE and SE-HPLC in GMP content and molecular weight distribution actually reflect a continuum of changes in the molecular weight distribution of GMP. MLGE and SE-HPLC were sensitive to changes in polymer molecular weight distribution below approximately M_r 10 million, but decreases in the molecular weight of large GMP components would be manifest as a change in the solubility of the polymer. Even considering this caveat, it appears that after peak MT has been reached and dough breakdown begins, marked decreases in GMP molecular weight occur.

Depolymerization of GMP into smaller polymers, which under our analytical conditions became sufficiently small to be soluble in SDS detergent, may actually be a critical step that affects mixing performance because other studies have shown that very low

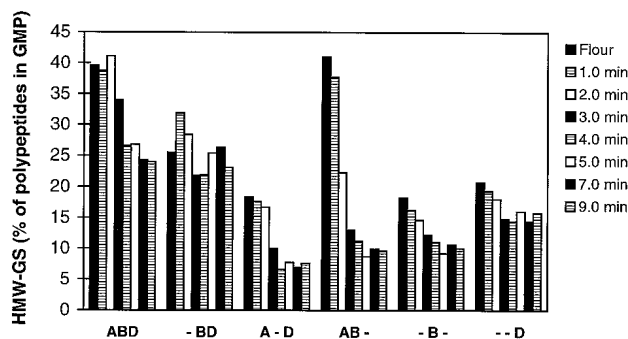


Fig. 5. Changes in proportion of total high molecular weight glutenin subunits (HMW-GS) during mixing of null HMW-GS genetic lines from cv. Olympic. Data shown are means of two experiments; results differed by <10% of the mean.

concentrations of reductants have major effects on mixing without releasing free subunits (Ng et al 1991). The study of changes in structural organization below this level (i.e., polymer to oligomer to subunit transitions) may be useful in establishing the connections between subunits within the polymers that associate to form GMP. Other data suggest that some of the breakdown of GMP can be reversed if dough is rested prior to snap-freezing (Weegels et al 1994, 1997). Because a proofing stage is incorporated commercially before baking, it will be important to further examine changes in polymer formation during the full process to better understand changes that occur in commercial processing of doughs. It also will be important to consider changes in glutenin polymerization and amounts of specific polypeptides together with measurement of free thiols (including glutathione) (Schofield and Chen 1995) and oxidoreductants, such as ascorbic acid-dehydroascorbic acid (Every 1997).

The data obtained are also in keeping with a proposed model for polymers, in which a backbone of HMW subunits exists on which LMW subunits are bound (Graveland et al 1985) but in which clusters of glutenin polymers are linked to a macropolymer (Gao and Bushuk 1993). As polymer size and content decreased, the proportion of HMW-subunits decreased, while groups of subunits such as C-LMW-GS and D-LMW-GS/ β -amylase fractions that may have chain-terminating activity, increased. However, differences in depolymerization rates between HMW- and LMW-GS could indicate two other possibilities: there are polymers present with differing composition or branching within GMP; or within the GMP, there are different classes of glutenin subunits, and their postulated branching is unevenly distributed.

To obtain a clearer validation of the model, studies on the association and dissociation behavior of individual HMW-GS and other polypeptides such as specific LMW-GS and gliadins are required. In the accompanying article (Skerritt et al 1999), we evaluate the depolymerization behavior of individual HMW- and LMW-GS from the GMP during dough mixing and breakdown. In addition to using a range of cultivars and null lines, biotypes that differ at single HMW- or LMW-GS-encoding loci are evaluated as to whether the depolymerization behavior of particular subunits occurs consistently in related lines that differ in mixing properties.

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