

# Quantitative Determination of High Molecular Weight Glutenin Subunits of Hard Red Spring Wheat by SDS-PAGE. II. Quantitative Effects of Individual Subunits on Breadmaking Quality Characteristics<sup>1</sup>

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## ABSTRACT

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High molecular weight (HMW) glutenin subunits were investigated in terms of “good” or “poor” subunits for determining the breadmaking quality of hard red spring wheat. Relationships between quantitative changes of individual HMW glutenin subunits and rheological properties of doughs and aggregation properties of native (nonreduced) glutenin proteins were investigated. Native glutenin proteins were separated by multistacking SDS-PAGE procedures. Seven genotypes with the same HMW glutenin subunits (2\*, 7+9, 5+10) were used in correlation studies between solubilities of individual HMW glutenin subunits and physical properties of doughs, while three genotypes with diverse baking qualities were used to investigate solubility changes of HMW glutenin subunits during dough overmixing in the mixograph. Native glutenins in total flour proteins extracted with SDS buffer were separated based on their migrations at stacking gel origins of different acrylamide concentrations. After reduction of glutenin aggregates from the different origins with  $\beta$ -mercaptoethanol, compositions of HMW glutenin subunits were analyzed by densitometry. It was found that aggregates with the largest molecular weight at the 4% origin of stacking gels had much higher proportions of subunit 5 (18.8%) than those at the 12% origin (9.2%). For correlation

studies, total flour proteins were fractionated into three fractions: salt-extractable (0.5M NaCl), SDS-soluble (2% SDS sodium phosphate buffer, pH 6.8), and insoluble residues. HMW glutenin subunits solubilized in SDS-soluble proteins were analyzed quantitatively by densitometry of SDS-polyacrylamide gels in relation to flour strength. The total amount of HMW glutenin subunits, x-type subunits, and proportions of subunits 5 and 2\* in SDS-soluble proteins were found to have negative correlations with dough mixing strength ( $-0.87^*$ ,  $-0.76^*$ ,  $-0.94^{**}$ , and  $-0.86^*$ , respectively, with dough stability in the mixograph). Proportions of subunit 10 solubilized in SDS buffer had a positive correlation with flour strength (0.86\*). In extended mixing studies, doughs with optimum mixing and doughs overmixed to 2.5 min after the peak in the mixograph were fractionated, and their protein compositions were compared using same procedures as for flours. During extended mixing, HMW glutenin subunits with the highest molecular weight (subunit 5) increased in solubility in SDS buffer, while subunit 10 (with lower molecular weight) decreased in solubility. These results indicated that functionalities and aggregation properties of individual HMW glutenin subunits may depend mainly on their molecular weights.

One of the most important questions asked about the functionality of high molecular weight (HMW) glutenin subunits is whether these subunits function differently from other glutenin subunits. Moreover, are HMW glutenin subunits intrinsically and functionally different from each other? Ever since Payne et al (1979) published their findings that the presence or absence of HMW glutenin subunits was related to breadmaking quality, numerous investigations have been made to find out why some HMW subunits have a “better quality” than others.

The chemical characterization of HMW glutenin subunits reported so far shows that subunits do not differ substantially from each other in their overall molecular structures (MacRitchie et al 1990, Shewry et al 1992). Little molecular basis could be found to differentiate their functionality by comparing their molecular structures. Greene et al (1988) reported that subunits 5 and 2 were 98.7% identical and subunits 10 and 12 were 97.8% identical in DNA-derived sequence homology. Some reports (Pogna et al 1987) showed that subunit 10 was solely responsible for “good quality” in the subunit 5+10 combination, while others (Rogers et al 1991) provided evidence that subunit 5 might have an influence on baking quality similar to that of subunit 10.

Screening for “good quality subunits” is important for wheat quality improvement in wheat breeding programs. Since Payne’s scoring system of HMW glutenin subunits did not work for hard red spring (HRS) wheat (Khan et al 1989), it is important to iden-

tify quality factors that control breadmaking quality. Our previous investigations have established that total amounts of HMW glutenin subunits of the total flour proteins play major roles in determining the breadmaking quality of hard red spring wheat (Huang and Khan 1996, 1997a). But the question still remains: which HMW glutenin subunits are better quality subunits in HRS wheat?

The purpose of this study was to determine the quantitative properties of individual HMW glutenin subunits in relation to the breadmaking quality of HRS wheat. This article reports experiments conducted on the solubilities of glutenin proteins, rheological changes during mixing and extended mixing, and aggregation properties of native glutenin proteins in relation to compositions of HMW glutenin subunits.

## MATERIALS AND METHODS

### Fractionation of Total Proteins

Total proteins of seven flour samples of HRS wheat with the same HMW glutenin subunit composition (2\*, 7+9, 5+10) were fractionated into three protein fractions: salt-solubles in 0.5M NaCl, SDS solubles in 2% SDS-0.05M Na-Phosphate buffer (pH 6.8), and residue proteins (insoluble in SDS buffer) as described by Huang and Khan (1997b).

### Physical Dough Tests

Mixing tolerance (stability) on the farinogram (50-g farinogram) and mixing time and tail width on the mixogram (10-g mixogram) of the seven samples were recorded following AACC methods 54-21 and 54-40 (AACC 1983).

### Dough Mixing

Three samples with the same HMW glutenin subunits (2\*, 7+9, 5+10) representing different baking qualities were used. Dough samples taken at the peak of the mixogram and samples mixed 2.5

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min beyond the peak were lyophilized and fractionated into three protein fractions as described above: salt-soluble, SDS-soluble, and residue proteins (Huang and Khan 1997b). SDS-soluble proteins were analyzed by SDS-PAGE (four determinations), and the proportions of individual HMW glutenin subunits (in relation to their total amounts solubilized in SDS buffer) were determined by densitometry. Statistical tests and comparisons of means were made between the percentage of each individual HMW glutenin subunit at the peak of dough mixing (optimum mixing) and after 2.5 min overmixing beyond the peak of the mixogram.

### Multistacking Gel Analysis

Flour proteins were extracted with 0.5% SDS sodium phosphate buffer (pH 6.8) for 20 hr. Total extracts were loaded on preparative multistacking SDS-PAGE gels (3 mm thickness) as described by Khan and Huckle (1992). Five stacking gels (4, 6, 8, 10, and 12% acrylamide concentration) and separating gels with 14% acrylamide were used. Nonreduced glutenins or glutenin polymers were separated based on their sizes and migrations along the stacking gels of different concentrations of acrylamide of various pore sizes. Glutenins at each origin were extracted in an SDS sample buffer containing the reducing agent 5% β-mercaptoethanol. Quantitative determinations of the compositions of individual HMW glutenin subunits were conducted using densitometry procedures as described by Huang (1995).

### Statistical Analysis

All statistical analyses were conducted using SAS programs (SAS 1995). Comparisons of quantities of HMW glutenin subunits between 4 and 12% origins were performed using a *t*-test. Correlations between proportions of HMW glutenin subunits solubilized in SDS buffer and dough mixing properties were conducted by comparing their correlation coefficients. Changes in the extracted amount of individual HMW glutenin subunits solubilized in SDS buffer during extended mixing were compared using an *F*-test.

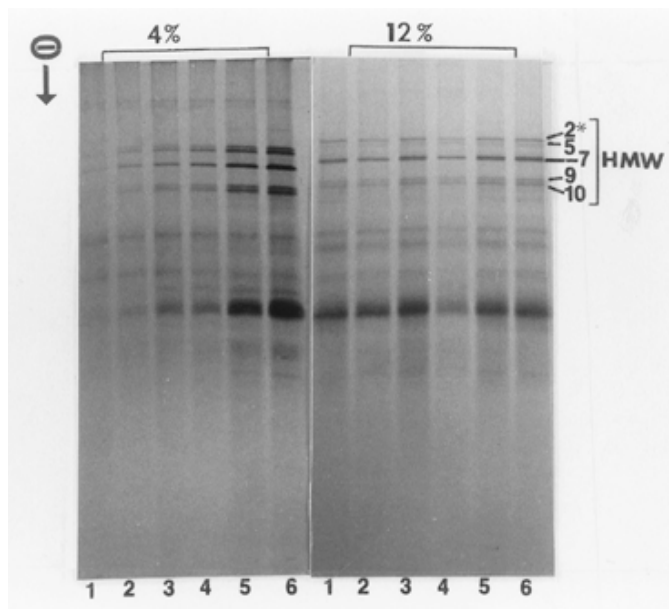
## RESULTS AND DISCUSSION

### SDS-PAGE Analysis of Glutenin Aggregates

Glutenin aggregates have been successfully separated under nonreducing conditions into six fractions based on their migration on multistacking gels (Khan and Huckle 1992). Huang and Khan (1997a) used high-resolution and high-sensitivity gel electrophoresis to characterize these aggregates from different origins. In the present study, we used the same approach to investigate the subunit composition differences between the largest aggregates at the 4% origin and the relatively small aggregates at the 12% origin by characterizing these aggregates by SDS-PAGE (Fig. 1). In the protein extraction experiments, as stirring time increased, more HMW glutenin subunits were found (shown as an increase

in staining intensity) in the largest glutenin aggregates from the 4% origin, while the small aggregates from the 12% origin were less affected (the staining intensity was relatively constant) (Fig. 1). The most significant difference in HMW subunits between the 4 and 12% origins was found in the comparison of subunits 5 and 2\* (Fig. 1). For the largest glutenin aggregates (the 4% origin), subunit 5 was substantially darker in staining intensity than subunit 2\*. For the smaller glutenin aggregates at 12% origin, subunit 2\* became much darker than subunit 5 for all stirring hours. This indicated that the size of glutenin aggregates was related to the quantities of individual HMW glutenin subunits.

HMW glutenin subunits were quantified with an imaging densitometer using volume analysis (Huang and Khan 1997a). Proportions of individual HMW glutenin subunits relative to their total amount and a statistical analysis are listed in Table I. A *t*-test of the averages of the two origins showed that the percentages of subunits 5 and 7 from the 4 and 12% origins were significantly different. The other three subunits (2\*, 9 and 10) showed no differences for the two origins. The largest glutenin aggregates (from 4% origins) contained twice the amount of subunit 5 compared to the amount in the smaller aggregates from 12% origins. On the other hand, proportions of subunit 7, which occurs in the greatest quantity among the five subunits, increased markedly as glutenin aggregates became smaller (Table I).



**Fig. 1.** SDS-PAGE of reduced glutenin proteins from 4 and 12% origins of the multistacking gel electrophoresis (Khan and Huckle 1992). HMW = high molecular weight glutenin subunits. Lane numbers from 1 to 6 correspond to protein extraction times of 0, 0.5, 1, 2, 4, and 8 hr.

**TABLE I**  
Proportions of High Molecular Weight Glutenin Subunits (HMW-GS) at 4 and 12% Origins

HMW-GS	Gel Origins (%)	Extraction Time (hr)					Average	<i>t</i> -Test <sup>a</sup>
		0.5	1	2	4	8		
5	4	17.7	16.8	19.0	19.7	21.0	18.8	a
	12	6.2	9.2	8.8	10.4	11.4	9.2	b
2*	4	15.5	15.3	13.9	14.7	14.2	14.7	a
	12	15.7	13.9	11.7	15.8	15.7	14.6	a
7	4	24.4	25.4	27.4	28.6	28.9	26.9	b
	12	39.2	37.3	35.9	35.3	35.0	36.5	a
9	4	21.7	22.6	19.7	18.4	17.8	20.0	a
	12	19.3	18.0	21.1	19.0	18.2	19.1	a
10	4	20.8	19.9	20.0	18.7	18.2	19.5	a
	12	19.6	21.6	22.5	19.5	19.7	20.6	a

<sup>a</sup> Values in a pair (same subunit) with different letters are statistically different at  $\alpha = 0.05$ .

Molecular weights of individual HMW glutenin subunits determined by SDS-PAGE analysis were quite different from those calculated from DNA sequences (Shewry et al 1992). Subunit 5 had a faster mobility on the electrophoresis gel than subunit 2\* but had a higher molecular weight from DNA sequence than subunit 2\*. These anomalous mobilities of HMW subunits were found to disappear in the presence of 4M urea as a result of some very stable residual structures. The order of molecular weight of individual subunits determined by our SDS-PAGE analysis was 2\* (116,800), 5 (113,500), 7 (101,300), 9 (88,300), and 10 (86,000), while the true molecular weight based on DNA sequence analysis should be 5 (88,100), 2\* (86,300), 7 (82,900), 9 (73,500), and 10 (67,500) (Shewry et al 1992).

These results show that the quantity of subunit 5, which has the largest molecular weight, seems to play more important roles than other subunits in extending polymer chains during glutenin synthesis, which is in agreement with our previous results (Huang and Khan 1997a). Table I shows that when glutenin aggregates become smaller, smaller subunits such as subunit 7 are incorporated in larger quantities into the glutenin polymer molecules.

### Total HMW Glutenin Subunits in SDS-Solubilized Proteins

To determine the quantitative effects of HMW glutenin subunits without the interference of qualitative effects (i.e., different subunit compositions), seven genotypes with the same HMW glutenin subunit composition (2\*, 7+9, 5+10) and diverse breadmaking quality were selected to compare their differences in protein compositions and their relationships with wheat quality. Total flour proteins were fractionated into proteins soluble in dilute salt solution, proteins soluble in 2% SDS buffer, and insoluble residue proteins. Previous studies had established that protein solubility was related to dough mixing strength and breadmaking quality (Huang and Khan 1997b). In the present study, we analyzed the protein compositions of SDS-soluble proteins by SDS-PAGE. Three groups of proteins, i.e., HMW glutenin subunits, medium molecular weight (MMW), and low molecular weight (LMW) proteins (data reported) separated by SDS-PAGE (Huang and Khan 1997b) were compared among seven genotypes. Among these seven genotypes of HRS wheat, two samples had strong dough mixing characteristics, three samples had good breadmaking quality, and two samples had poor baking quality.

Correlations between proportions of groups of proteins, combinations of HMW glutenin subunits, and individual subunits (relative to their total amounts) solubilized in SDS buffer and dough mixing properties are listed in Table II. The proportion of

total HMW glutenin subunits that was solubilized in SDS buffer correlated significantly and negatively with dough mixing strength ( $-0.87^*$  with mixing time from the mixograph). HMW glutenin subunits may affect dough strength by providing strong interactions with other flour components including proteins through quantitative effects; that is, the degree of interaction between gluten polymer molecules may be dependent on how much total HMW glutenin subunits are present in the flour. These results confirmed our previous observations that the more HMW glutenin subunits there were in the total flour proteins, the larger were the number and size of glutenin polymers in the gluten polymer network (Huang and Khan 1997a).

### Individual HMW GS Solubilized in SDS Buffer

Table II also shows the solubility of individual HMW glutenin subunits in SDS buffer and their correlations with dough mixing strength. Since *x*-type subunits, which include subunits 2\*, 7, and 5, have a lower mobility than their counterpart *y*-type subunits (9 and 10) on the SDS-PAGE gel (both *x* and *y* subunits are controlled by one gene locus), *x*-type subunits have a higher molecular weight than their respective *y*-type subunits. Correlation showed that the amount of *x*-type subunits had significant negative correlations with dough mixing strength ( $-0.76^*$  with mixing time and  $-0.81^*$  with tail width of the mixogram). These results were similar to the correlations with total HMW glutenin subunits shown above. They indicate that higher proportions of *x*-type subunits solubilized in SDS buffer are associated with weak dough mixing of a flour. We have shown so far that total HMW glutenin subunits within the total flour proteins and *x*-type subunits within total HMW glutenin subunits may have a greater effect on flour strength than their lower molecular weight counterpart proteins or subunits (the *y*-type subunits). The next step was to investigate the relationships of the quantity of individual subunits solubilized in SDS buffer to flour quality.

Subunits 5 and 2\* have the largest molecular sizes (827 and 794 amino acid residues, respectively) among all gluten protein subunits identified so far (Shewry et al 1992). Subunits 5 and 2\* showed negative correlations (Table II) with mixing tolerance ( $-0.91^{**}$  and  $-0.80^*$ , respectively), mixing time ( $-0.94^{**}$  and  $-0.86^*$ ) and tail width of the mixogram ( $-0.95^{**}$  and  $-0.76^*$ ). Subunit 10 (with smaller molecular size, 627 amino acid residues) had positive correlations with flour dough strength ( $0.83^*$  with stability,  $0.86^*$  with mixing time, and  $0.83^*$  with the tail width of the mixogram). These results indicate that the quantitative effects of individual HMW glutenin subunits on gluten protein solubility are similar to the quantitative effects of total HMW glutenin

TABLE II  
Correlation Coefficients Between Relative Quantities  
of Glutenin Subunits (Relative to Their Total Amounts)  
Solubilized in SDS Buffer and Dough Mixing Properties ( $n = 7$ )<sup>a</sup>

Glutenin Subunit <sup>b</sup>	Stability (min) <sup>c</sup>	Mixing Time (min) <sup>c</sup>	Tail Width (cm) <sup>c</sup>
HMW	ns	$-0.87^*$	$-0.79^*$
MMW	ns	ns	ns
LMW	ns	ns	ns
<i>x</i> -type	ns	$-0.76^*$	$-0.81^*$
<i>y</i> -type	ns	ns	ns
Subunit 5	$-0.91^{**}$	$-0.94^{**}$	$-0.95^{**}$
Subunit 2*	$-0.80^*$	$-0.86^*$	$-0.76^*$
Subunit 7	$0.80^*$	ns	ns
Subunit 9	ns	ns	ns
Subunit 10	$0.83^*$	$0.86^*$	$0.83^*$

<sup>a</sup> Proportion solubilized. \* and \*\* refer to significance at probability levels of 0.05 and 0.01, respectively. ns = not significant.

<sup>b</sup> HMW = High molecular weight, MMW = medium molecular weight, LMW = low molecular weight.

<sup>c</sup> Mixing properties were represented by mixing tolerance (stability) of the farinogram and mixing time and tail width of the mixogram.

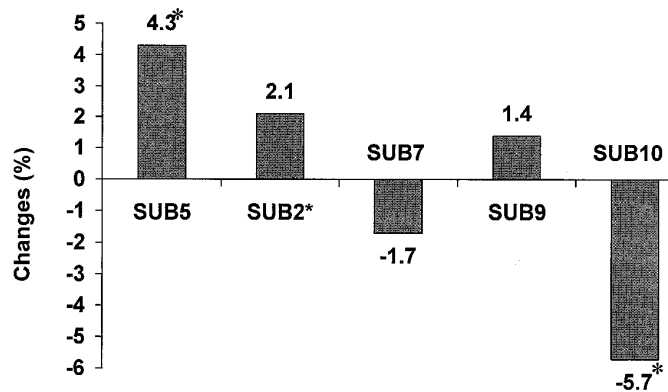


Fig. 2. Changes (%) in relative quantity of individual high molecular weight glutenin subunits relative to their total amounts solubilized in SDS buffer during extended mixing beyond the peak. SUB = subunit. \* refers to significance at the probability level of 0.05. Plus (+) sign means increase while minus (-) means decrease.

subunits and  $\alpha$ -type glutenin subunits (Table II). Based on these correlations, subunits with a relatively higher molecular weight, such as subunits 5 and 2\*, seem to contribute more to the strength of the gluten polymer network than those with a lower molecular weight, such as subunit 10. Comparing the two subunits with the highest molecular weight, 5 and 2\*, we can also see the effects of differences in molecular size of these two subunits on flour strength. Subunit 5 has a higher molecular weight than subunit 2\* and a stronger correlation ( $r > 0.9$ , significant at 0.01 level) with flour strength than subunit 2\* ( $r = \sim 0.8$ , significant at 0.05 level). Therefore, the functionality (mixing properties) of individual HMW glutenin subunits seem to be related to their molecular chain length.

### Extended Mixing Studies

Three samples used in this study had the same HMW glutenin subunits (2\*, 7+9, 5+10) but different dough strength and bread-making quality properties. During overmixing (mixing extended beyond the peak of the mixogram), subunit 5 increased significantly (4.3%) while subunit 10 decreased (5.7%) (Fig. 2). These changes in the proportions of individual HMW glutenin subunits in SDS-soluble proteins indicated that more subunits with a higher molecular weight, such as subunit 5, were solubilized in SDS buffer than subunits with a relatively lower molecular weight, such as subunit 10. The proportions of individual HMW glutenin subunits corresponded to compositional changes of the solubilized glutenin proteins in SDS buffer, i.e., the higher the molecular weight of the glutenin polymer, the more susceptible the polymer molecules to breakdown by extra shearing stress during extended mixing. Based on our previous report (Huang and Khan 1996), the increased solubility of subunit 5 was not due to the cleavage of this glutenin subunit, as determined by SDS-PAGE under non-reducing conditions, but most likely due to the increase in the amount of glutenin molecules with the largest molecular weight that were depolymerized or disaggregated by the stress of extended mixing and hence solubilized into the SDS buffer. We are not aware of any published reports indicating that mixing or overmixing can cause the release of individual HMW glutenin subunits. Glutenin molecules with the largest molecular weight contained more subunit 5, which is the largest among all subunits, and resulted in the proportions of subunit 5 increasing significantly during overmixing.

According to our previous results showing that glutenin subunits including HMW and LMW might be linked randomly (Huang and Khan 1997a), HMW glutenin subunits seem to be the most important components in this network in determining breadmaking qualities of wheat and flours. The evidence so far seems to indicate that the degree of aggregation or molecular sizes of these native (nonreduced) glutenin proteins may depend on their quantity and on the ratio of HMW glutenin subunits to total glutenin subunits (Huang and Khan 1997b). Large glutenin molecules or aggregates contain higher proportions of HMW glutenin subunits, and, within the HMW glutenin subunits, larger glutenin aggregates contain more subunit 5 than smaller glutenin aggregates. From this study, we postulate that quantities of subunit 5 synthesized during polymerization of glutenin proteins may be one of the most important factors in determining wheat grain quality. These results raise the question of how important their local structures are in terms of their contributions to the strength of the gluten polymer network. The effects of molecular weight on the functionality of HMW glutenin subunits may follow the rules of randomly linked amorphous polymers (Graessley 1993).

### CONCLUSIONS

From study of the functional properties of individual HMW glutenin subunits, solubility of glutenin subunits in SDS buffer, rheological changes of dough during extended mixing and aggregation behaviors of glutenin aggregates, it seems that individual

HMW glutenin subunits function according to their molecular sizes and their quantities in the gluten and dough system. To eliminate possible interactions and complications of qualitative effects, seven HRS wheat genotypes with the same HMW glutenin subunits (2\*, 7+9, 5+10) were used in solubility studies. Investigations on protein solubilities of flours and doughs showed that total quantities of HMW glutenin subunits,  $\alpha$ -type glutenin subunits (which have higher molecular weight than  $\gamma$ -type subunits), and individual HMW glutenin subunits of relatively higher molecular weights such as subunits 5 and 2\* had positive effects on the gluten polymer network and dough mixing properties. To our knowledge, this is the first comprehensive study to indicate that glutenin subunits may follow the same universal rules as other synthetic polymers in that molecular weight is one of the major factors determining their aggregation (polymerization) behavior and rheological properties.

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