

Quantitative Determination of High Molecular Weight Glutenin Subunits of Hard Red Spring Wheat by SDS-PAGE. I. Quantitative Effects of Total Amounts on Breadmaking Quality Characteristics¹

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ABSTRACT

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Thirteen hard red spring wheat genotypes in which seven genotypes had the same high molecular weight (HMW) glutenin subunits (2*, 7+9, 5+10) were compared for their physical-chemical and breadmaking properties. These samples were categorized into three groups based on their dough mixing and baking performances as follows: the strong dough (SD) group (six genotypes), characterized by the strongest dough mixing (average stability, 35 min); the good loaf (GL) group (four genotypes), characterized by the largest loaf volume; and the poor loaf (PL) group (three genotypes), characterized by the smallest loaf volume. Total flour proteins were fractionated into 0.5M salt-soluble proteins, 2% SDS-soluble proteins, and residue proteins (insoluble in SDS buffer). SDS-soluble proteins, residue proteins, and total flour proteins were analyzed by SDS-PAGE and densitometry procedures to determine the proportions of HMW glutenin subunits, medium molecular weight proteins, and low

molecular weight proteins in relation to the total amount of proteins. No differences in the amount of salt-soluble proteins were found among the different groups of samples. Solubilities of gluten proteins (total proteins minus salt-soluble proteins) in SDS buffer were related to the differences in dough strength and baking quality among the three groups. The SD group had the lowest solubility and the PL group had the highest. SDS-PAGE analysis showed that SDS-soluble proteins of the SD group contained a smaller amount of HMW glutenin subunits than those of the GL and PL groups. The highest proportions of HMW glutenin subunits in total flour proteins were found in the SD group, while the PL group had the lowest percentage of HMW glutenin subunits in their total flour proteins. These results showed that the total quantities of HMW glutenin subunits played an important role in determining the dough mixing strength and breadmaking performance of hard red spring wheats.

Wheat proteins have been classified into five classes, i.e., water-soluble albumins, dilute salt-soluble globulins, 70% ethanol-soluble gliadins, dilute-acid-soluble glutenins, and insoluble proteins (Osborne 1907). There have been numerous investigations on the relationships of the composition of each class of proteins to breadmaking quality, especially for gliadins and glutenins, which were found to be related more than albumins and globulins to quality differences of wheats. It is now well established that the glutenin fraction contributes more to breadmaking quality differences than the gliadin fraction (MacRitchie et al 1990, Weegles et al 1996). There were also reports on the relationships of gliadin fractions to bread wheat quality (Branlard and Dardevet 1985). The presence or absence of certain high molecular weight (HMW) glutenin subunits in relation to baking quality have been well documented (Payne et al 1987, Shewry et al 1992), while only limited investigations have been made on the functionality of low molecular weight (LMW) glutenin subunits (Gupta et al 1995) because of the difficulties of identifying LMW subunits from gliadin proteins on SDS-PAGE gels of total protein extracts.

Using the quality evaluation system of Payne et al (1987), attempts were made to explain variations in baking quality by the absence or presence of certain HMW glutenin subunits in many of the strong wheats, including hard red spring (HRS) wheats (Khan et al 1989) and some of the Australian wheats (Lawrence 1986), but they were not successful. Efforts have been made to evaluate breadmaking quality potentials of wheats by investigating the ratio of HMW polymeric proteins to other monomeric proteins with size-exclusion HPLC (Singh et al 1990, Cornec et al 1994), the absence or presence of LMW glutenin subunits and gliadin

proteins (Gupta et al 1992, 1995), and quantitative determinations of HMW glutenin subunits (Seilmeier et al 1991, Kolster et al 1992). These results indicated that size distributions of flour proteins and quantities of HMW and LMW glutenin subunits may also contribute significantly to the quality of bread wheats.

One of the many challenges facing cereal protein chemists is to fully solubilize gluten proteins in a proper solvent without destroying the native character of the wheat proteins. Protein solubility in different solvents such as dilute acetic acid or SDS solution was found to be a good indicator of breadmaking quality or gluten strength (Orth and Bushuk 1972, He et al 1991). The stronger the wheat, the more proteins (residue proteins) are left after extraction (Orth and Bushuk 1972) or the longer it takes to get the same extraction rate as that of weak wheat (He et al 1991). Glutenin proteins were also found in the ethanol-soluble gliadin fractions (Huebner and Wall 1976), which made it very difficult to completely separate and accurately determine the total quantities of glutenin proteins. Results from multistacking SDS-PAGE of HRS wheat showed good potential in characterizing native glutenin polymers (Huang and Khan 1997).

Because study of the qualitative variations in HMW glutenin subunits of HRS wheat has provided limited information on the biochemical basis of their large quality differences (Khan et al 1989), the quantitative effects of HMW glutenin subunits on dough mixing and breadbaking properties, in comparison with those of other wheat proteins, were investigated in the present study. SDS-PAGE was used to separate wheat proteins into three groups: HMW glutenin subunits, medium molecular weight (MMW) proteins, and low molecular weight (LMW) proteins. The LMW proteins were mixtures of LMW glutenin subunits, gliadins, and other soluble proteins. Statistical analysis showed that differences in MMW and LMW proteins among HRS wheat genotypes were either too small to be significant using least significant differences tests or their differences had opposite effects to those of HMW glutenin subunits. Therefore, only HMW glutenin subunits data are reported in this article.

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MATERIALS AND METHODS

Materials

A group of 13 HRS wheat genotypes grown in North Dakota was used. These HRS wheat genotypes represented diversified genetic backgrounds in wheat breeding programs at North Dakota State University and had large variances in dough and breadmaking properties. Among those 13 wheat samples were seven with a HMW glutenin subunit composition of 2*, 7+9, 5+10; two genotypes with 1, 7+8, 5+10; two genotypes with 2*, 7+9, 2+12; one genotype with 2*, 7+8, 5+10, and one with 2*, 17+18, 5+10 (Table I). All wheat samples were milled on a Buhler laboratory mill according to AACC method 26-20 (AACC 1983).

Physical-Chemical Analysis

Protein content of flours was determined using the Kjeldahl procedure as described in method 46-11 (AACC 1983). Physical dough tests were performed on a Brabender Farinograph with a 50-g sample bowl following the procedure of method 54-21 (AACC 1983). Mixing properties of flours were tested on a mixograph with a 10-g sample bowl according to method 54-40 (AACC 1983). The mixing time, the angles between the ascending and descending lines of the curve, and the width of the curve tail after 2.5 min beyond the peak were recorded on the mixogram.

Bread Baking Procedures

A straight-dough procedure based on method 10-09 (AACC 1983) was used to evaluate the breadbaking quality of the different flours. The baking formula contained 100% flour (14% moisture), 1% salt, 5% sugar, 3% compressed yeast, 2% shortening, 0.1% ammonium phosphate, and 1 ml of fungal amylase (= 15 SKB α -amylase units) (AACC 1983). The amount of water added varied depending on the flour water absorption obtained on the farinograph. The dough samples were mixed to the optimum consistency. Two-step punching procedures were adopted during the 180-min fermentation. Doughs were proofed for 55 min before being baked for 24 min in the oven at 470°F. Proof height was recorded immediately before the dough was placed into the oven. Loaf volume was measured after cooling by the rapeseed displacement method. Four loaves of bread were baked from each flour sample.

Fractionation and Solubility Studies

Flour samples were fractionated into three protein fractions: salt-solubles in 0.5M NaCl, SDS-solubles in 2% SDS-0.05M

sodium phosphate buffer (pH 6.8), and residue proteins (insoluble in SDS buffer) as described by Huang and Khan (1996) except that no yeast protease inhibitors were added in the buffer solutions.

Protein contents of each fraction were analyzed by the Kjeldahl procedure (method 46-11, AACC 1983). SDS-soluble proteins and residue proteins were further reduced and analyzed by SDS-PAGE following procedures described earlier (Huang and Khan 1997). The same amount (10 μ g) of total flour proteins of each fraction was loaded onto each lane of the SDS-PAGE gels. High molecular weight (HMW) glutenin subunits were expressed as a percentage of the sum of the total optical densities of the total proteins on the SDS-PAGE gel profile.

Statistical Analysis

A randomized complete block design was chosen for the SDS-PAGE analysis. The gel was taken as a block (replicate) in variance analysis and the 13 genotypes as treatments that were applied across the gel randomly. Five gels each were run for the analysis of total flour proteins and SDS-soluble proteins, and four gels each were run for the residue proteins. The coefficients of variances in the estimation of HMW glutenin subunits by SDS-PAGE were <5% between lanes on the same gel. Based on quality characteristics in dough mixing and breadbaking performances, the 13 genotypes were categorized into three groups for comparison (Table I): a strong dough mixing (SD) group (six genotypes), which contained genotypes characterized by the strongest dough mixing; a good loaf volume (GL) group (four genotypes), which had the largest volumes; and a poor loaf volume (PL) group (three genotypes) containing the smallest loaf volumes. Two of the seven genotypes with the same HMW glutenin subunits (2*, 7+9, 5+10) belonged to the SD group; three belonged to the GL group; and two belonged to the PL group. Least significant differences tests were performed to compare the differences in quality parameters among the different groups.

RESULTS AND DISCUSSION

Physical-Chemical Properties

Table I shows various quality characteristics of the 13 HRS wheat genotypes and their HMW glutenin subunit compositions. Protein contents of flours used in this study ranged from 12.1% to 13.3% on a 14% moisture basis. The average protein content was about 12.5%. Therefore, the quality differences among these genotypes were largely due to differences in "protein quality" rather than to the protein content of flours. The quality parameters

TABLE I
High Molecular Weight (HMW) Glutenin Subunit Compositions of 13 Hard Red Spring Wheat Genotypes and Their Physical-Chemical and Breadbaking Performances^a

Sample	HMW-GS	Pro%	ABS%	Stability	MT	Angle	Width	Proof	LV	Group
1	2*,7+9, 5+10	12.8	69	31	4	145	2	7.8	871	SD
2	2*,7+9, 5+10	12.8	68	33	3.5	145	1.5	7.4	834	SD
3	2*,7+9, 5+10	12.5	69	7	2.5	100	0.5	8.2	944	GL
4	2*,7+9, 5+10	12.6	74	5.5	2	105	0.3	8.4	978	GL
5	2*,7+9, 5+10	13.3	73	6	2	110	0.5	8.7	946	GL
6	2*,7+9, 5+10	12.1	69	11.5	2.5	120	0.8	7.4	795	PL
7	2*,7+9, 5+10	12.2	68	12.5	3	125	1.5	7.4	764	PL
8	2*,7+8, 5+10	12.1	63	40	4	145	2	7.7	845	SD
9	1, 7+8, 5+10	12.4	65	33.5	4.5	145	2	7.6	890	SD
10	1, 7+8, 5+10	12.2	64	30.5	3.5	145	1.5	7.4	794	SD
11	2*,17+18,5+10	13.1	69	41.5	4.5	150	1.8	7.5	823	SD
12	2*,7+9, 2+12	12.8	75	6	2	115	0.3	8.4	916	GL
13	2*,7+9, 2+12	12.2	71	9	1.5	125	1	7.2	740	PL

^a HMW glutenin subunits of wheat following the nomenclature of Payne et al (1987); HMW-GS = high molecular weight glutenin subunits, Pro% = protein content (%), ABS% = absorption (%), stability = mixing tolerance (min) on the farinograph, MT = mixing time (min) on the mixograph, angle = angle (°) between the ascending and descending lines of the mixogram, width = width of the mixograph curve tail after 2.5 min beyond the peak (cm), proof = proof height (cm), LV = loaf volume (cm³). Group corresponds to different groups of genotypes: SD = strong dough group, GL = good loaf group, PL = poor loaf group.

that represent dough mixing strength (such as stability or mixing tolerance from the farinograph and mixing time and angles from the mixogram) varied remarkably. Stability ranged from 5.5 to 41.5 min, whereas mixing time in the mixogram varied from 1.5 to 4.5 min.

In the baking test, proof height and loaf volume had large variations among these samples. Proof height ranged from 7.2 to 8.7 cm, whereas loaf volume ranged from 740 to 978 cm³. Distinctive differences in dough properties and breadmaking performances were observed among these genotypes (Table I). Some genotypes had very strong dough mixing strength (with a stability of over 30 min on the farinograph and a mixing time of over 3 min on the mixograph), but their baking performances were not among the best. Other genotypes had relatively weaker dough mixing strength (with a stability of less than 13 min and a mixing time of 3 min or less). The loaf volumes of this group ranged from the poorest (740 cm³) to the best (978 cm³). Clearly, it is difficult to attribute these quality differences to qualitative differences in HMW glutenin subunits since seven of these genotypes had the same subunit composition of 2*, 7+9 and 5+10 but still had large variations in dough and baking quality. The molecular basis of these quality differences was the focus of this investigation. To better understand the mixing characteristics and baking performances of these genotypes, we grouped genotypes with similar quality characteristics such that we could compare and test their differences in protein compositions as a group statistically. Comparisons of quality characteristics among those groups are shown in Table II.

Comparisons of Quality Characteristics

Table II compares the average values of the quality parameters of three groups of genotypes. The SD group had very good mixing tolerance (35 min). Bread doughs from this group were dry and tough during fermentation. The SD group had limited dough expansion during proof (proof height was 7.6 cm) and smaller loaf volume (843 cm³) than the GL group.

The GL group had the largest proof height (8.4 cm) and loaf volume (946 cm³). Even though protein contents were slightly higher than those of the other groups, the loaf volume per unit protein content was also larger than for the other groups.

TABLE II
Comparisons Between Breadmaking Quality Characteristics of Three Groups of Genotypes Based on the Least Significant Difference (LSD) Test^a

Quality Parameter	Group of Genotypes		
	Strong Dough (6)	Good Loaf (4)	Poor Loaf (3)
Stability, min	34.9a	6.1c	11.0b
ABS%	66.2b	72.9a	69.2ab
Mixing time, min	4.0a	2.1b	2.3b
Angle, °	145.8a	107.5c	123.3b
Tail width, cm	1.8a	0.4c	1.1b
Proof height, cm	7.6b	8.4a	7.3c
Loaf volume, cc	842.7b	945.9a	766.3c
Protein, %	12.6ab	12.8a	12.2b
Loaf volume per unit protein, cm ³ /%	67.2b	74.1a	63.1c

^a Means on the same line followed by different letters are significantly different at the level of $\alpha = 0.05$. Strong dough group refers to genotypes with characteristics of strong dough mixing; good loaf group includes genotypes with a good loaf volume and medium dough mixing; poor loaf group includes genotypes with a poor loaf volume and medium dough mixing. Number of genotypes included in each group is in parentheses after the group name. Stability and ABS% (water absorption) are from dough test on the farinograph; mixing time, the angle between ascending and descending line, and the tail width of the mixogram curve are from the test on the mixograph. Loaf volume per unit protein was calculated as loaf volume divided by protein content.

The PL group had smallest proof height (7.3 cm) and loaf volume (766 cm³). The mixing requirements and mixing tolerance of this group fell between those of the SD group and the GL group.

Solubility Studies of Flour Proteins

We further investigated the solubility differences of total flour proteins among these groups. Flour proteins were fractionated into three fractions: salt-soluble proteins, including both albumin and globulin proteins; SDS-soluble proteins, including both gliadin and glutenin proteins; and residue proteins (insoluble glutenins). Table III compares the three solubility fractions among the three groups (SD, GL, and PL) of all 13 genotypes and of the seven genotypes with the same HMW glutenin subunit composition (2*, 7+9, 5+10). No significant differences were found in the salt-extractable proteins among the three groups (Table III). However, major differences were found in SDS-soluble and residue protein fractions. The SD group had 17% SDS-soluble proteins. This amount was significantly lower than those of the other two groups, which had relatively weaker dough mixing strengths than the SD group (Table III). When protein solubilities of the seven flour samples with the same HMW glutenin subunit composition were compared, the differences in SDS-soluble and residue proteins between the GL group and the PL group were shown to be significant, with the PL group having the highest solubility in SDS buffer (26.9% for the PL group compared to 21.9% for the GL group).

It is clear from these results that gluten proteins from the weak dough mixing group (GL and PL) were more soluble in SDS buffer than gluten proteins from the strong dough mixing group. Gluten proteins from the SD group might either have different molecular compositions than the weak dough groups or have the same molecular composition but different conformations that result in a stronger interaction between various proteins. The highest protein solubility of the PL group (Table III) may partially explain why the PL group had the smallest loaf volume among all genotypes. It might also explain why some flours that gave acceptable curves on the farinograph and mixograph still failed in the baking performance tests. Therefore, it is normal practice to do both the physical dough tests and baking performance tests in order to determine the baking quality potential of wheats and flours. To investigate what protein components were responsible for protein solubility differences among wheat genotypes, we analyzed the protein compositions of solubilized fractions, insoluble fractions, and total flour proteins by SDS-PAGE.

SDS-PAGE Analysis of SDS-Soluble and Residue Proteins

SDS-soluble proteins obtained from the fractionation studies were treated with a reducing agent and subjected to SDS-PAGE and densitometry analysis to estimate the proportion of HMW glutenin subunits (Tables IV). The amounts of HMW glutenin subunits solubilized in SDS buffer in the form of nonreduced glutenin aggregates during extraction were related to the dough strength of the flour. The SDS-soluble proteins of the GL group (the weakest dough mixing among the three groups) contained significantly higher proportions of HMW glutenin subunits than the SD group (the strongest dough mixing group). Based on our previous observations that large glutenin aggregates contain higher proportions of HMW glutenin subunits than smaller aggregates (Huang and Khan 1997), the higher ratio of HMW subunits in SDS-soluble proteins in weak flours might indicate that more of the large glutenin aggregates in the weak flours and fewer large glutenin aggregates in the strong flours were solubilized in SDS buffer. Therefore, interactions among glutenin aggregates in strong flours seem to be greater than those in weak flours.

Similar results were obtained when the HMW glutenin subunits in the residue proteins of the three groups of genotypes were compared to those in the SDS-soluble proteins. The residue proteins of the PL group contained significantly less HMW

glutenin subunits than the residue proteins of the SD group (Table IV). It is known that residue proteins play an important role in breadmaking quality (Orth and Bushuk 1972) of different wheat varieties. Since the PL group had the least amount of residue proteins among all genotypes (Table III) and the residue proteins of the PL group also contained the smallest proportions of HMW glutenin subunits (Table IV), the PL group would be expected to have the smallest amount of the largest glutenin aggregates in the residues and therefore the poorest protein quality among all genotypes.

It is evident from the comparisons of different groups of proteins among the three groups of genotypes that HMW glutenin subunits played important roles in determining protein solubility differences among different flours. For the strong flours, the interactions among gluten proteins are stronger, so more small rather than large aggregates are released into SDS buffer, whereas in the weak flours, large aggregates are easily released due to the weaker interactions among gluten proteins. To determine what components of total flour proteins controlled the interactions among gluten proteins that resulted in solubility differences among HMW glutenin subunits, we investigated the compositional differences in the total flour proteins among the three groups of genotypes.

SDS-PAGE Analysis of Total Flour Proteins

Total flour proteins were reduced, and three groups of proteins, including HMW glutenin subunits as well as MMW proteins and LMW proteins (data not reported), were separated by SDS-PAGE and analyzed by densitometry procedures (Huang and Khan 1997). Table IV compares the proportions of HMW glutenin subunits of total flour proteins of the 13 genotypes of HRS wheat. The SD group had the highest content of HMW glutenin subunits, while the PL group had the least. The GL group had an intermediate amount of HMW glutenin proteins. It would seem that the content of HMW glutenin subunits in the total flour proteins is important not only in determining dough strength but also in determining the breadbaking performances of wheat flours. Although qualitative differences between HMW glutenin subunits may play a role in breadmaking quality, the total amounts of these subunits seem to be more critical for HRS wheats, as indicated

from the results of the present study. From the physical dough tests and breadbaking performance tests, it was clear that the SD group had overstrong dough mixing characteristics, reflected in its lower protein solubility in SDS buffer. The bread doughs were so strong that proof height was small and dough expanded slowly during oven spring before the dough was fully baked into bread. For the PL group, the opposite was true. Protein solubilities and content of HMW glutenin subunits in SDS buffer of the PL group were high, which indicated a weak gluten polymer network. The total HMW glutenin subunit content of the total flour proteins of the PL group was too low to meet the requirements of good breadmaking quality. Similar results were obtained by Lawrence et al (1988), who observed a good correlation between the content of total HMW glutenin subunits determined by SDS-PAGE analysis and breadmaking quality of special wheat lines with varying numbers of HMW glutenin subunits.

Although the percentage of HMW glutenin subunits in total flour proteins is not high compared to those of MMW and LMW proteins, the roles they play in dough rheology and breadbaking performance seem to be significant, which could be the result of their much larger molecular sizes than other proteins. As in any other polymer system (Graessley 1993), the viscoelasticity of the gluten polymer also depends on the quantities of polymers of high molecular weight. This study showed that functional differences of the various varieties or lines are likely due to differences in the amount of HMW glutenin subunits, as indicated by solubilities of wheat flour proteins, dough strength, and baking performances. The total quantity of the HMW glutenin subunits seems to dictate the interactions of the gluten polymer system. These results strongly support previous results of Huang and Khan (1997) that HMW glutenin subunits join randomly with LMW glutenin subunits to form large glutenin polymers and that the quantities of HMW subunits play an important role in determining the size distributions of glutenin aggregates (Cornec et al 1994).

These results were based on a limited number of genotypes of HRS wheat. We also noticed that relationships between dough strength, loaf volume, protein solubility, ratios of different classes of proteins, and subunit compositions of glutenin aggregates among the three groups of genotypes were not always statistically

TABLE III
Comparisons of Protein Solubilities of Total Flour Proteins of the Three Groups of 13 Genotypes^a

Protein Type	Group of Genotypes ^b			Group of Genotypes ^c		
	SD (6)	GL (4)	PL (3)	SD (2)	GL (3)	PL (2)
Salt-solubles, %	14.7a	14.3a	14.7a	14.1a	14.4a	14.9a
SDS-solubles, %	17.1b	22.1a	23.7a	16.8c	21.9b	26.9a
Residue proteins, %	68.1a	63.6b	61.6b	69.0a	63.7b	58.2c

^a LSD test was performed based on the probability level of significance $\alpha = 0.05$. Means with the same letter across are not significantly different from each other. Strong dough (SD) group refers to genotypes with characteristics of strong dough mixing; good loaf (GL) group has a good loaf volume and medium dough mixing; poor loaf (PL) group has a poor loaf volume and medium dough mixing. Numbers indicate the number of genotypes in each group.

^b Thirteen genotypes with different high molecular weight (HMW) glutenin subunits.

^c Seven genotypes with the same HMW glutenin subunit composition of 2*, 7+9, 5+10.

TABLE IV
Proportions of High Molecular Weight Glutenin Subunits (HMW-GS) in Different Protein Fractions
Extracted from Flours of 13 Genotypes Analyzed by SDS-PAGE^a

Protein Fractions	HMW-GS% ^b			HMW-GS% ^c		
	SD (6)	GL (4)	PL (3)	SD (6)	GL (4)	PL (3)
Reduced SDS-soluble	8.5b	10.4a	9.3b	7.9b	10.4a	9.0ab
Reduced residues	16.0a	15.3a	13.5b	15.6a	15.5ab	13.8b
Reduced total proteins	11.1a	10.5b	9.5c	10.9a	10.5b	9.5c

^a LSD test was based on the probability level of significance $\alpha = 0.05$. Means with the same letter across are not significantly different from each other. HMW-GS% = proportions of HMW glutenin subunits in different fractions of flour proteins (%). Strong dough (SD) group refers to genotypes with characteristics of strong dough mixing; good loaf (GL) group has a good loaf volume and medium dough mixing; poor loaf (PL) group has a poor loaf volume and medium dough mixing. Numbers indicate the number of genotypes in each group.

^b For 13 genotypes with different high molecular weight (HMW) glutenin subunit.

^c For seven genotypes with the same HMW glutenin subunit composition of 2*, 7+9, 5+10.

significant as between groups which might share certain common protein characteristics. However, among the various protein components, including LMW glutenin subunits, gliadins, and soluble proteins, proportions of HMW glutenin subunits seem to have a major impact on protein solubility, dough mixing strength, and breadmaking performances of HRS wheat. It is clear that investigations on more wheat genotypes and protein compositions, especially native glutenin aggregates, are needed before any definitive predictions of dough mixing properties and breadmaking qualities can be made by using any of these protein characteristics of HRS wheat.

SUMMARY AND CONCLUSIONS

HRS wheats seem to have a unique genetic background in terms of dough-mixing properties and breadmaking qualities, especially the overwhelmingly high percentage of HMW glutenin subunits 5+10. In this study, 11 out of 13 genotypes contained subunits 5+10 and seven of them contained the same three HMW subunits (2*, 7+9, 5+10). Solubilities of total flour proteins were found to be extremely important to dough mixing strength. The weaker the dough, the more soluble were the gluten proteins. By studying the compositions of SDS-soluble proteins, it was found that HMW glutenin subunits seem to be the major components responsible for these solubility differences among all protein components. In weak flours, more of the large glutenin aggregates, which contain higher proportions of HMW glutenin subunits, were solubilized in SDS buffer than in the strong flours. Solubility reflects the degree of long-range interactions among flour proteins, and quantities of HMW glutenin subunits seem to be the factor that controls these interactions, through their effects on molecular size distributions of native glutenin aggregates. Relationships between the total amount of HMW glutenin subunits in the flour and dough mixing strength and bread loaf volume strongly support the conclusion that it is the quantity of HMW glutenin subunits that determines wheat protein quality differences of HRS wheats.

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LITERATURE CITED

American Association of Cereal Chemists. 1983. Approved Methods of the AACC, 8th ed. Method 10-09, approved September 1985; Method 26-20, approved April 1961; Method 46-11A, approved October 1976, revised October 1982; Method 54-21, approved April 1961, reviewed October 1982; Method 54-40A, approved April 1961. The Association: St. Paul, MN.

Branlard, G., and Dardevet, M. 1985. Diversity of grain proteins and bread wheat quality. I. Correlation between gliadin bands and flour quality characteristics. *J. Cereal Sci.* 3:329-344.

Cornec, M., Popineau, Y., and Lefebvre, J. 1994. Characterisation of gluten subfractions by SE-HPLC and dynamic rheological analysis in shear. *J. Cereal Sci.* 19:131-139.

Graessley, W. W. 1993. Viscosity and flow in polymer melts and concen-

trated solutions. Pages 97-143 in: *Physical Properties of Polymers*, 2nd ed. J. E. Mark, A. Eisenberg, W. W. Graessley, L. Mandelkern, and J. L. Koenig, eds. American Chemical Society: Washington, DC.

Gupta, P. B., Batey, I. L., and MacRitchie, F. 1992. Relationships between protein composition and functional properties of wheat flours. *Cereal Chem.* 69:125-131.

Gupta, P. B., Popineau, Y., Lefebvre, J., Cornec, M., Lawrence, G. J., and MacRitchie, F. 1995. Biochemical basis of flour properties in bread wheats. II. Changes in polymeric protein formation and dough/gluten properties associated with the loss of low *Mr* or high *Mr* glutenin subunits. *J. Cereal Sci.* 21:103-116.

He, H., Feng, G. H., and Hosoney, R. C. 1991. Differences between flours in the rate of wheat protein solubility. *Cereal Chem.* 68:641-644.

Huang, D. Y., and Khan, K. 1997. Characterization and quantification of native glutenin aggregates by multistacking sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) procedures. *Cereal Chem.* 74:229-234.

Huang, D. Y., and Khan, K. 1996. Unexpected solubility changes in wheat proteins during fermentation and oven stages of the breadbaking process. *Cereal Chem.* 73:512-513.

Huebner, F. R., and Wall, J. S. 1976. Fractionation and quantitative differences of glutenin from wheat varieties varying in baking quality. *Cereal Chem.* 53:258-269.

Khan, K., Tamminga, G., and Lukow, O. 1989. The effect of wheat flour proteins on mixing and baking correlations with protein fractions and high molecular weight glutenin subunit composition by gel electrophoresis. *Cereal Chem.* 66:391-396.

Kolster, P., Kretching, C. F., and van Gelder, W. M. J. 1992. Quantification of individual high molecular weight subunits of wheat glutenin using SDS-PAGE and scanning densitometry. *J. Cereal Sci.* 15:49-61.

Lawrence, G. J. 1986. The high-molecular-weight glutenin subunit composition of Australian wheat cultivars. *Aust. J. Agric. Res.* 37:125-133.

Lawrence, G. J., MacRitchie, F., and Wrigley, C. W. 1988. Dough and baking quality of wheat lines deficient in glutenin subunits controlled by the *Glu-A1*, *Glu-B1* and *Glu-D1* loci. *J. Cereal Sci.* 7:109-112.

MacRitchie, F., Du Cros, D. L., and Wrigley, C. W. 1990. Flour polypeptides related to wheat quality. Pages 79-145 in: *Advances in Cereal Science and Technology*, Vol. 10. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

Orth, R. A., and Bushuk, W. 1972. A comparative study of the proteins of wheat of diverse baking qualities. *Cereal Chem.* 49:268-275.

Osborne, T. B. 1907. *The proteins of the wheat kernel*. Publ. 84. Carnegie Inst.: Washington, DC.

Payne, P. I., Nightingale, M. A., Krattiger, A. F., and Holt, L. M. 1987. The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. *J. Sci. Food Agric.* 40:51-65.

SAS. 1995. *SAS User's Guide: Statistics*. SAS institute Inc.: Cary, NC.

Seilmeyer, W., Belitz, H.-D., and Wieser, H. 1991. Separation and quantitative determination of high-molecular-weight subunits of glutenin from different wheat varieties and genetic variants of the variety Sicco. *Z. Lebensm. Unters. Forsch.* 192:124-129.

Shewry, P. R., Halford, N. G., and Tatham, A. S. 1992. High molecular weight subunits of wheat glutenin. *J. Cereal Sci.* 15:105-120.

Singh, N. K., Donovan, G. R., Batey, I. L., and MacRitchie, F. 1990. Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. I. Dissolution of total proteins in the absence of reducing agents. *Cereal Chem.* 67:150-161.

Weegels, P. L., Hamer, R. J., and Schofield, J. D. 1996. Functional properties of wheat glutenin (critical review). *J. Cereal Sci.* 23:1-18.

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