Characterization and Quantification of Native Glutenin Aggregates by Multistacking Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) Procedures[1]

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ABSTRACT

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Native glutenin aggregates of two different quality flours containing the same high molecular weight (HMW) glutenin subunit compositions were investigated by multistacking sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) procedures. Five stacking gels (4, 6, 8, 10, and 12%) with a separating (resolving) gel of 14% were used to separate nonreduced glutenin aggregates solubilized from flour by SDS sodium phosphate buffer. There were large differences in protein solubility of the two flours. It took 8 hr to extract 91% of total proteins from the strong flour (variety Len) while it took only 2 hr for the weak flour sample 205. Total glutenin proteins and the proportions of glutenins at the different origins (including the origin of the 14% separating gel) were quantified by high-resolution densitometry procedures. As the duration of extraction increased, both total glutenins and glutenins at the 4% origins increased. The good quality flour Len had a higher total glutenin protein (3% more) and higher proportion of glutenins with the largest molecular sizes (also 3% more) at the 4% origins than the poor quality flour sample 205. After glutenin aggregates from each origin were reduced and analyzed by SDS-PAGE, the largest glutenins at the 4% origin contained twice the amount of total HMW glutenin subunits when compared to the smaller aggregates at the 12% origin. Among the total HMW glutenin subunits, the proportion of subunit 5 (which published literature reports to be the largest molecular weight based on calculations of DNA-derived amino acid sequence analysis) was twice that at the 12% origin. A randomized structure of native glutenins is proposed based on the results of our investigations.

Native glutenin molecules have a molecular weight ranging from a few hundred thousand to millions estimated by gel-filtration chromatography (Huebner and Wall 1976, Payne and Corfield 1979, Graveland et al 1982). Huebner and Wall (1976) separated glutenin proteins into two fractions by using Sepharose 4B and 2B: a very high molecular weight fraction (>20 million) eluted at void volume and a lower molecular weight fraction with a broad peak. The ratio of the first glutenin peak to the second peak was greater for strong than for weak flours.

Payne and Corfield (1979) found that glutenin proteins excluded from Sepharose CL-4B consisted of three groups of subunits by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE): group A with apparent molecular weights from 136,000 to 95,000 (high molecular weight [HMW] glutenin subunits); group B from 42,000 to 51,000; and group C from 35,500 to 31,500. Both groups B and C were referred to as low molecular weight (LMW) glutenin subunits. Glutenins of different molecular weights, eluted from an agarose gel column, had similar subunit composition but different proportions of the three groups of glutenin subunits. As the size of the glutenin increased, the proportion of group A subunits (HMW) became larger relative to both the group B and C subunits (LMW), and the ratio of group B to group C also increased.

Graveland et (1985) fractionated glutenin proteins into three fractions by differential solubility in SDS and 70% ethanol: an SDS-insoluble glutenin I fraction (MW >10 million), an SDS-soluble but ethanol-insoluble glutenin II fraction (MW 700,000–10 million), and both SDS and ethanol soluble fraction III (MW 120,000–600,000). But they found that both glutenins I and II, which consisted of groups A, B, and C subunits, had the same ratio of the three groups of subunits (A-B-C = 3: 3.4:1.7).

Because gel filtration offers limited resolutions for separation of large native glutenins, it is difficult to investigate the differences in the sizes of glutenin aggregates among wheat and flours with different baking quality. This could be seen from the similar profiles obtained by gel-filtration chromatography of glutenin proteins of wheat lines with varying numbers of HMW glutenin subunits (from 0 to 5 HMW subunits) (Gao and Bushuk 1993). Glutenin aggregates that contained all five HMW glutenin subunits had gel-filtration profiles very similar to those aggregates that contained only LMW glutenin subunits.

Size-exclusion high-performance liquid chromatography (SE-HPLC) was also used to characterize native glutenin proteins (Singh et al 1991) by separating total glutenin proteins from nonglutenin proteins. To solubilize glutenins, the procedure involved the use of sonication which might cleave disulfide bonds of large glutenin aggregates to smaller aggregates (Khan et al 1994). The SE-HPLC procedure is limited in characterizing the molecular distributions of native glutenin proteins.

A multistacking gel electrophoresis procedure (Khan and Huckle 1992) was devised to characterize native glutenin proteins by fractionating the glutenin aggregates into six fractions based on their sizes and mobilities on the multistacking gel. In this procedure, five stacking gels with various acrylamide concentrations (4, 6, 8, 10, and 12%) on top of a 14% separating gel were used to separate the different glutenin aggregates at the various origins of the stacking gels. The larger the size of the aggregates, the more difficult for the aggregates to move into the higher concentration stacking gels. We used this procedure to investigate the compositions of native glutenin proteins of two hard red spring wheats differing in breadmaking quality.

MATERIALS AND METHODS

Wheat and Flour Samples

Two genotypes of hard red spring wheat were used for this study: Len, which has a good breadmaking quality, and the experimental line 205, which has a weak dough mixing quality. Len is commonly used as a good quality reference variety in evaluations of hard red spring wheat in the wheat breeding program at

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North Dakota State University. The experimental line 205 was not selected for release due to its weak mixing in the screening process and was chosen for this study to represent weak mixing flour samples. Wheat samples were milled on a Buhler laboratory mill according to the AACC Approved Method 26-20 (AACC 1995). The protein content of the Len flour was 13%, while that of 205 was 14% on a 14% moisture basis. The dough mixing tolerances determined by the farinograph for Len and sample 205 were 17 and 6 min, respectively. The peak time of the farinograph for Len and sample 205 were 10 and 5 min, respectively. The mixing time of the mixograph for Len and sample 205 were 4 and 2 min, respectively. The two genotypes had the same HMW glutenin subunit composition (2*, 7+9, 5+10) based on the nomenclature of Payne et al (1987).

Protein Extractions

Total flour proteins were extracted with 0.5% SDS sodium phosphate buffer (pH 6.8) as described by Khan and Huckle (1992) for various stirring times (0, 0.5, 1, 2, 4, and 8 hr) on a magnetic stirrer after 1 g of flour was wetted and suspended in 20 mL of buffer. The suspension was centrifuged at 15,000 × g. The residue was freeze-dried for Kjeldahl analysis. The supernatant was used for multistacking gel analysis after determination of its protein content by the Kjeldahl procedure (Approved Method 46-11, AACC 1995).

Multistacking Gel Electrophoresis

A gel thickness of 1.5 mm was used for analytical multistacking SDS-PAGE. A gel thickness of 3 mm was used for the preparative studies following the procedures of Khan and Huckle (1992). A 100 μg sample of protein extract, based on Kjeldahl test, was loaded onto each lane of the analytical multistacking gels. To determine variations of the glutenin proteins at different origins, the same amount of proteins was loaded on the same gel, and the coefficients of variances of the OD of each origin was estimated by the imaging densitometer (model GS-670, BioRad). Different amounts of proteins (10–250 μg) were also loaded to investigate the linearity of the OD response of the densitometry procedures (Huang 1995).

Quantification of Glutenin Proteins

Volume analysis by densitometry procedures (BioRad 1995, Huang 1995) was performed to quantify wheat proteins on both multistacking SDS-PAGE gels and regular (single-stack) SDS-PAGE gels. Volume analysis takes advantage of the three-dimensional data (consisting of signal intensity, vertical, and horizontal positions) and gives more accurate qualitative and quantitative analysis than does the traditional profile analysis, which uses only two-dimensional data (BioRad 1995). A blank lane was used to obtain the background signals. The volume of a protein band is represented by mean intensity subtracted from the background multiplied by the area of the band (BioRad 1995).

The proportions of the total polymeric glutenins (glutenin aggregates as indicated in Fig. 1) relative to total proteins in the respective lane were quantified. Also the proportions of polymeric glutenins at the individual origins relative to the sum of the total OD of all origins were quantified (Fig. 1). Origins refer to the beginning of each stacking gel. Cuts refer to the protein of the stacking gel that was excised for analysis. This cut was made ~1 mm below each origin. Cut pieces were extracted with sample buffer containing reducing agent (reduced), and then extracts were analyzed by SDS-PAGE. For quantification of origins, each origin plus the rest of the stacking gel were included in the volume

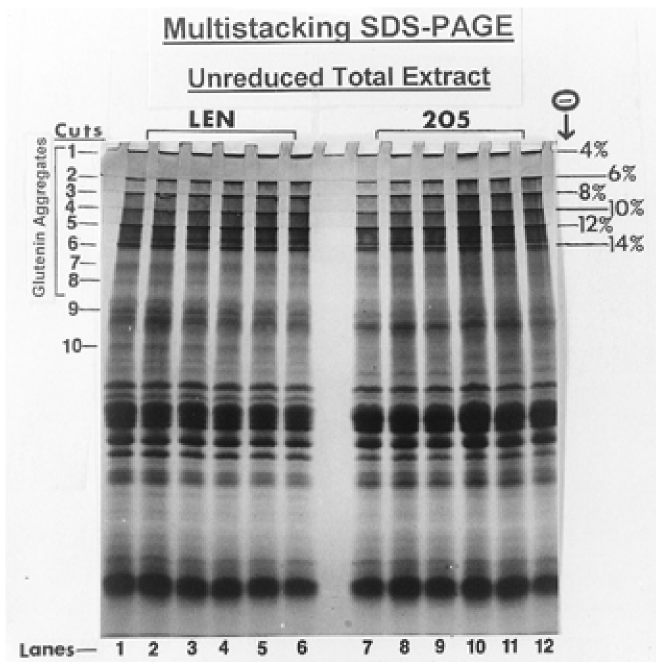

Fig. 1. Multistacking sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of total extract of flour proteins without reducing agent. Lanes 1–6 (Len) and 7–12 (205): total extract at 0, 0.5, 1, 2, 4, and 8 hr of extraction, respectively. Glutenin aggregates refer to the nonreduced glutenins. Cuts 1–10 refer to section of gel from which proteins were eluted and reduced for subsequent analyses. Percentages refer to acrylamide concentrations of the stacking gel.

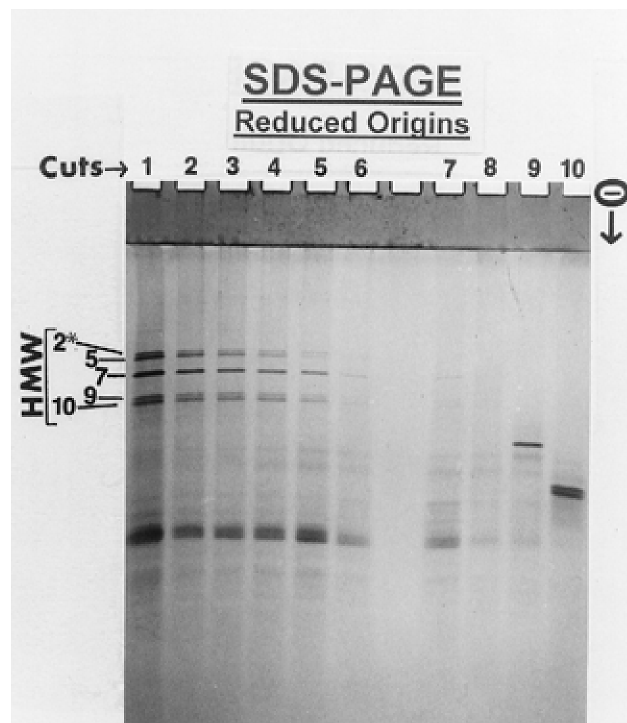

Fig. 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of reduced origins from multistacking SDS-PAGE gels. Lanes 1–10 (l to r): Cuts 1–10 labeled at top of the gel photo correspond to cuts 1–10 in Fig. 1. High molecular weight (HMW) glutenin subunits include subunits 2*, 5, 7, 9, and 10 according to the nomenclature of Payne et al (1987).

analysis procedures (Fig. 1). For the 14% separating gel, the origin and those proteins that entered into the separating gel (cuts 7–8) just below the origin were included for analysis because the latter proteins were found to be polymeric glutenins (Khan et al 1994) (Fig. 2).

Subunit Compositions of Glutenin Aggregates

Subunit compositions of glutenin aggregates from the different origins were analyzed by SDS-PAGE procedures of Neuhoff et al (1988) as modified by Huang (1995). Glutenin proteins at different origins of the preparative multistacking gels (each cut) were reduced and eluted in a Tris-HCl sample buffer (pH 6.8) containing 2% SDS (w/v) and 1% DTT at 60°C for 1 hr. Proportions of HMW glutenin subunits were estimated using volume analysis relative to LMW glutenin subunits (Fig. 2). Proportions of individual HMW glutenin subunits were determined relative to the sum of HMW glutenin subunits.

Statistical Analysis

To compare changes of glutenin proteins at different origins and subunit compositions of those origins with extraction rate, all samples with different extraction rates were loaded on the same gel. For the comparison of subunit composition of glutenins at different origins, reduced glutenin subunits from all six origins were loaded on the same gel. Both Len and 205 were loaded on the gel for the comparison of strong and weak flour samples. Randomized complete block design with two replicates were used for statistical analysis. Statistical analyses including variances analysis were made using the SAS programs (SAS 1995).

RESULTS AND DISCUSSION

Distinguishing Polymeric Glutenin from Monomeric Proteins

When proteins from the different origins of the stacking gels (Fig. 1), including the origin of the separating gel (14% origin), were analyzed by SDS-PAGE (Fig. 2), it was found that those proteins were broken down into multiple subunits after reduction of disulfide bonds. It was also noted that the streaking after the origin of the separating gel (cuts 7–8) were also broken down into multiple subunits after reduction until cut 9, in which a protein band had no change in apparent molecular weight after reduction. Most proteins below this region seem to be monomeric. For the purpose of quantification of glutenin proteins, glutenins that are above cut 8 were referred to as the total glutenin aggregates (Fig. 1).

Determining Variations in Quantification of Glutenin Proteins

Equal amounts of proteins were loaded on the same gel in different lanes to calculate the coefficients of variances (CV) of total glutenin aggregates (proteins of all stacking gels), and proportions of glutenins at the different origins (Table I). The CV% of total glutenin aggregates and of the different origins were <5% except the 8% origin which was 5.9%. These results indicated a good reproducibility within the gel compared to previously published data of ~12% (Kolster et al 1992). The variations between two gels were larger. Some origins on one gel were different from those of the other gel, while other origins were the same. The results indicated that it is better to load all treatments on the same gel and eliminate the variations between gels through statistical methods such as randomized complete block design.

The linearity of OD response to different amounts of proteins was also estimated (Table I). The total OD of different origins had

TABLE I
Comparison of Variables that Affect Quantification of Glutenin Proteins on Multistacking Polyacrylamide Gels

Gel Origins (%)	*F*-Test (%)[a]	CV(%)[b]	Correlation Coefficients[c]
Total[d]	0.5000	1.42	0.993**
4	0.0002	3.64	0.981**
6	0.0302	3.22	0.975**
8	0.3216	5.93	0.996**
10	0.0010	1.70	0.996**
12	0.0010	1.61	0.993**
14	0.1410	1.98	0.997**

[a] *F*-test of the quantities of glutenin aggregates between two gels. P (%) < 0.05 means significant difference between two gels.
[b] CV(%) = coefficient of variance in OD of glutenins within the same gel.
[c] Correlations between quantities of proteins loaded (10–250 μg) on each lane and total OD of different origins. ** = Significance at $P < 0.01$ level.
[d] Total = total glutenins including the sum of all origins.

TABLE II
Comparison of Extraction Rate on Protein Solubility of Two Flours Differing in Breadmaking Quality

	Extraction Rate (Protein %)	
Extraction Time (hr)	Len	205
0	63.3±0.3	74.3±0.6
0.05	72.9±1.1	85.7±0.9
1	75.2±0.3	87.9±0.7
2	79.6±1.0	92.6±1.3
4	85.4±0.5	95.7±1.0
8	91.4±0.9	96.1±1.1

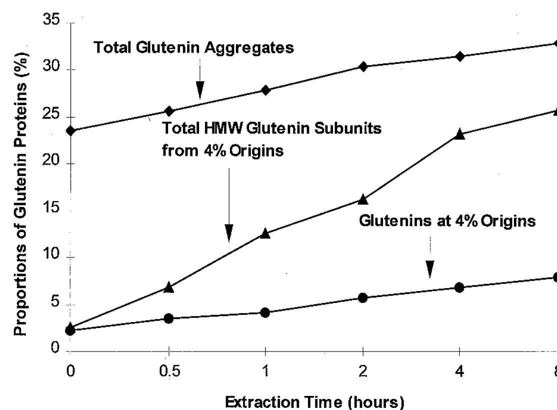

Fig. 3. Changes at different extraction times in the proportions of total glutenin aggregates (nonreduced), glutenin aggregates at 4% origins (nonreduced), and total high molecular weight (HMW) glutenin subunits from reduction of aggregates at 4% origins.

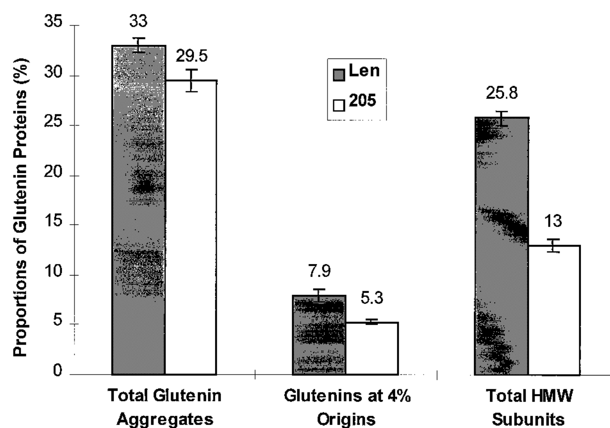

Fig. 4. Comparison of two hard red spring wheat genotypes (Len and 205) in total glutenin aggregates from all 6 origins (nonreduced), glutenins at 4% origins (nonreduced), and total high molecular weight (HMW) glutenin subunits from 4% origins at the same extraction rate (92%). Error bars represent standard deviations of means.

good responses to changing protein concentrations from 10 to 250 μg with the correlation coefficients ranging from 0.98 to 0.99. These results indicate that multistacking SDS-PAGE can be used as a reliable method to quantify native glutenin proteins at different origins.

Extraction Differences of Two Hard Red Spring Genotypes

The extraction rates were different between the flours of the strong (Len) and weak (205) genotypes. Extraction rate of Len after 8 hr was 91.4%; extraction rate of 205 was 92.6% after only 2 hr (Table II). In contrast to 205, only 79.6% of proteins of Len were solubilized in 2 hr. The protein solubility differences between the strong and weak flour is consistent with previous results (He et al 1991). It takes a longer time to solubilize the proteins of strong flours than it does for weak flours because of the stronger interactions among gluten proteins of strong flours. Stronger flours need more energy of longer mechanical stirring to disrupt those interactions. When samples were just suspended in the buffer with a spatula without intensive stirring (zero time), 74.3% of wheat proteins of 205 flour were solubilized in SDS buffer compared to 63.3% of the Len flour. The 10% difference in protein solubility between Len and 205 is, perhaps, a reflection of the compositional differences of their gluten proteins. To investigate which components may have caused these solubility differences, protein extracts were analyzed by the high-resolution multistacking SDS-PAGE procedures.

Changes in Solubility of Glutenin With Extraction Time

Total glutenin aggregates were estimated by quantifying total proteins of all origins of the stacking gels and streaking top portions of the separating gel (14%) (Fig. 3). Total glutenin aggregates increased as the extraction rate increased. This indicated that proteins that were more difficult to solubilize were largely glutenin proteins. As the stirring time increased, more glutenins from the insoluble proteins were released into the SDS solution.

When glutenin aggregates at different origins were compared, only glutenins with larger molecular sizes at the 4% origin increased as the extraction time increased (Fig. 3) (other origins were not shown in the figure). These results showed that residue proteins remaining after initial solubilization by mere suspension in buffer, contained mainly larger glutenin aggregates. These large aggregates, because of strong interactive forces, needed more stirring time and energy to gradually disrupt those interactions to solubilize them. Glutenins at the 4% origin were the largest aggregates based on their migration on the multistacking gel. Glutenins at 4% origins were more than doubled after 8 hr of extraction from ~2% (zero time) to 8% (Fig. 3). This implied that glutenin aggregates in the residue proteins after initial (zero time) suspension in the SDS buffer were mainly composed of the largest aggregates. The differences in solubility between the polymeric glutenin and monomeric proteins and between glutenins of different molecular sizes were probably caused by their differences in molecular sizes.

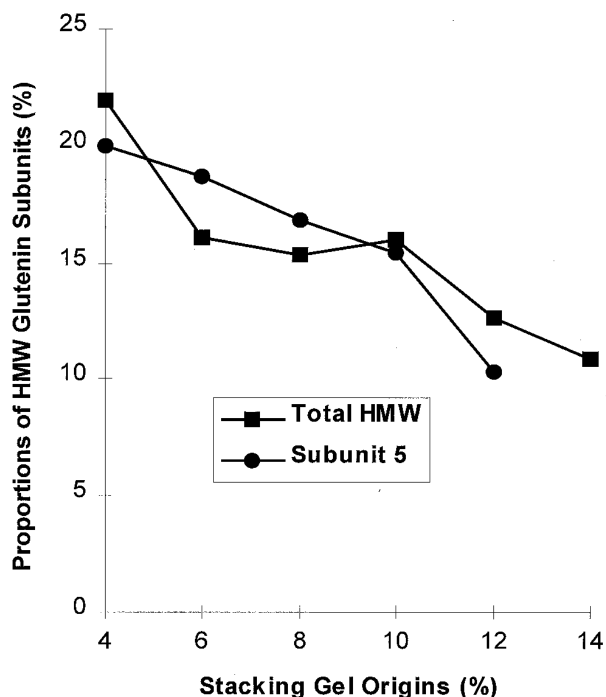

Fig. 5. Comparison of proportions of total high molecular weight (HMW) glutenin subunits and subunit 5 of glutenin aggregates at various origins of multistacking gels after 8 hr of extraction.

Subunit Compositions of Glutenins at 4% Origins

The glutenins at 4% origins of the multistacking gel which represented the largest aggregates were reduced, and their subunit compositions were analyzed by SDS-PAGE. Total HMW glutenin subunits of glutenins from 4% origins increased from ~3% before stirring (zero time) to 25% (after 8 hr of stirring) as the extraction rate increased (Fig. 3). This indicated that glutenin proteins at 4% origins were not a single molecular size species, but mixtures of varying large molecular size glutenin aggregates. As the extraction rate went higher, these largest glutenin molecules obtained after prolonged stirring contained more and more HMW glutenin subunits. In other words, glutenins that were solubilized later during extraction contained more HMW glutenin subunits. As discussed earlier, the largest glutenin aggregates at 4% origins increased markedly by mechanical stirring. These results indicated that total HMW glutenin subunits played an important role in the glutenin polymer aggregates.

Comparison of the Strong and Weak Flours

Comparison of glutenin proteins of the strong and weak flours was made at the 92% extraction rate. Len had a higher ratio of total glutenin aggregates than did 205 (~3% more) (Fig. 4). Glutenin proteins are large polymers whose molecular weights are in the millions (Huebner and Wall 1976, Payne and Corfield 1979). A higher glutenin content could make a large difference in the gluten polymer network. The higher total glutenin protein content in Len flour may contribute to its stronger dough mixing properties than 205.

The strong flour Len had more glutenins at the 4% origin (7.9%) at 91.4% extraction rate (8 hr) than did the weak flour sample 205 (5.3% at 4% origin) at the extraction rate of 92.6% (2 hr) (Table I). The weak flour 205 had more glutenin aggregates of the smallest size than Len. Differences in glutenins of other origins seemed to be more random. Similar to other polymer systems (Graessley 1984), the strength of gluten polymer network should depend on the polymers of the largest sizes, that is, the glutenin proteins. Among the glutenin aggregates, the differences between the weak and strong flours may be dependent upon the quantity of the largest glutenin molecules, that is, the glutenin aggregates at 4% origins. In this study, it was found that the good quality sample Len not only had higher total glutenin proteins but also had a higher proportion of the largest glutenin aggregates (4% origin) in total glutenin proteins than the poor quality sample 205.

The difference in subunit compositions of glutenins from 4% origins between the weak and strong flour was that Len had a much higher ratio of total HMW glutenin subunits of glutenins at 4% origins than did 205 when compared at similar extraction rates (Fig. 4). Total HMW glutenin subunits of glutenins at 4% origins of Len was ~26% at 91% extraction rate. This was twice the amount of 13% for 205 at the 92% extraction rate. The compositional differences in total HMW glutenin subunits implied that Len had a much higher proportion of largest glutenin aggregates among the mixtures of glutenin aggregates at 4% origins than did 205, though the exact molecular weight distributions of glutenins at 4% origins was unknown.

Comparison of Subunit Composition of Glutenins at Different Origins

Even though the exact molecular weight of glutenins at different origins was difficult to estimate by using multistacking gels, the relative sizes of these glutenin molecules could be compared based on their mobilities on the multistacking gel. As the concentration of stacking gel increased, the pore size of the stacking gel became smaller, that is, the pore size of the 4% gels should be larger than those of 14% gels. Proteins at each origin represented those whose molecular sizes were too large to migrate to the next stacking gel, that is, the proteins reached their molecular pore-size limit of the gel. Therefore, the molecular sizes of glutenin aggregates at the 4% origins should be much larger than those at 14% origins.

Subunit compositions of glutenins at different origins were compared using the extract after 8 hr (Fig. 5). Total HMW subunits of glutenins decreased as the concentration of stacking gel increased. For example, total HMW glutenin subunits in glutenin aggregates decreased to about half, from ~22% at the 4% origin to ~11% at the 14% origins. Payne and Corfield (1979) first reported this phenomenon using gel-filtration chromatography. These results indicated that the higher the proportions of total HMW glutenin subunits in the glutenin molecules, the larger the glutenin aggregates. The present study showed more clearly that HMW glutenin subunits are related to glutenin aggregates by using a better resolution gel electrophoresis method to separate native glutenin proteins into several molecular species.

These results are not consistent with the models proposed so far about the compositional characteristics of glutenin proteins to explain wheat quality (Graveland et al 1985, Kasarda 1989). In those models, HMW and LMW glutenin subunits are linked in a specific manner to form glutenin polymers. Results of the present study and those of other reports (Lawrence et al 1988, Seilmeier et al 1991, Halford et al 1992, Huang 1995) seem to indicate that quantitative effects of HMW glutenin subunits play a more important role than qualitative effects in determining wheat quality. Based on our quantitative data of glutenin subunits in glutenin aggregates of different sizes, the results of the present study would seem to indicate that all glutenin subunits are joined linearly (Ewart 1979) by interchain disulfide bonds not in a specific but in a random manner depending on the quantity of each subunit available at the time of polymerization. Molecular weights (both HMW and LMW subunits) and relative quantities of these subunits are, perhaps, the most important factors in determining properties of glutenin aggregates.

Proportions of Individual HMW Glutenin Subunits at Different Origins of Multistacking Gels

Five HMW glutenin subunits (2*, 7+9, and 5+10) made up the total HMW subunits in the glutenin aggregates of the two HRS wheat genotypes. The proportions of individual HMW glutenin subunits were compared among different origins relative to their total amount (Fig. 5). The most remarkable difference between the large and small glutenin aggregates was in the proportion of subunit 5 (data for other subunits not reported). As the size of the aggregates decreased from the 4% to the 12% origin, the proportion of subunit 5 decreased in half from 22% to ~11%. Subunit 5 is the largest HMW glutenin subunit based on the calculations of cDNA sequence analysis (Shewry et al 1992), even though it migrates faster on the SDS-PAGE gel than subunit 2* due to its abnormal configurations. Results on the proportions of individual HMW glutenin subunits of the glutenin aggregates seem to indicate that molecular weight of HMW glutenin subunits is also a contributing factor in determining the size differences among glutenin aggregates. At the same concentration of total HMW glutenin subunits, higher proportion of subunit 5 should produce more of the larger glutenin aggregates, which is in agreement with previous findings (Huang 1995).

Molecular Size Distributions of Glutenins

Proportions of glutenin aggregates at various origins of the multistacking gel were compared (Fig. 6). The proportions of glutenin aggregates increased progressively as the concentration of stacking gels increased. The quantities of glutenin aggregates increased linearly as their sizes became smaller (Fig. 6). In other words, there seems to be a continuous molecular size distribution of glutenin aggregates from the smallest aggregates of the 14% origins to the largest aggregates of the 4% origins. The larger the aggregates, the lesser the quantity of these aggregates.

These results of molecular size distributions of glutenin aggregates can be explained based on the model of random glutenin structures. The availability of glutenin subunits for polymerization depends statistically on their total quantity, i.e., the total glutenin subunits and the ratio of HMW subunits to LMW glutenin subunits. The availability of large amounts of LMW glutenin subunits would provide more chances to form smaller glutenin aggregates. If the proportions of HMW subunits is high, there will be more chances for them to be incorporated into the glutenin chain through interchain disulfide bonds to form more and larger aggregates. Since there are more LMW glutenin subunits than HMW subunits (Payne and Corfield 1979, Graveland et al 1985, Huang 1995) in the total flour proteins, more small glutenin aggregates are formed than large aggregates, e.g., the proportions of glutenins for Len increased from ~8% at the 4% origin (largest aggregates) to >30% at the 14% origin (smallest aggregates) (Fig. 6).

CONCLUSION

Multistacking SDS-gel electrophoresis was used to investigate differences in native (unreduced) glutenin aggregates between a good and poor quality wheat. This procedure is faster and pro-

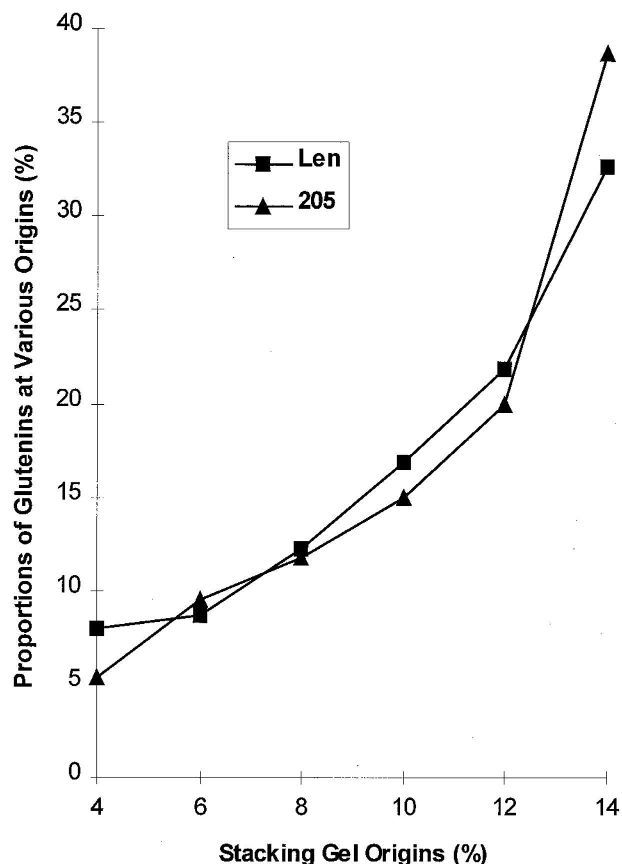

Fig. 6. Comparison of distributions of glutenin aggregates at various origins of multistacking gels at the extraction rate of 92%.

vides better resolution to separate native glutenin proteins than gel-filtration chromatography that is commonly used. The coefficients of variances were <5% for total glutenin and glutenins separated at most of the multistacking gel origins. The glutenins at the origins of the stacking gel can be conveniently eluted for further characterization.

Two flours with different breadmaking quality were compared for their protein solubilities in SDS buffer. The strong flour from variety Len took longer than the weak flour from sample 205 to solubilize wheat proteins to the same extraction rate. Continued stirring solubilized more of glutenin aggregates, especially the largest glutenin aggregates that remained at the 4% origins, the largest molecular weight species.

Len, the good quality flour sample, had more total glutenin aggregates (which were estimated by summing the amount of proteins remaining at all origins of the stacking gels) than did 205, the poor quality sample. Among glutenin proteins, Len had more aggregates of the largest sizes at the 4% origins than sample 205. These results indicated that quantity of glutenin proteins and proportions of glutenins with largest molecular sizes as determined by gel electrophoresis play an important role in determining breadbaking quality differences.

When glutenins at different origins were reduced and eluted, glutenin aggregates at the 4% origin contained twice the amount of HMW glutenin subunits as compared to the smaller aggregates at the 12% origins. Among HMW glutenin subunits, proportions of subunit 5, which has the largest molecular weight, decreased at the 12% origins to half of the amount at the 4% origins. These results suggest that molecular weight and molecular weight distributions of glutenin aggregates and the composition of their subunits play an important role in determining breadmaking quality differences of HRS wheat.

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