Quantitative Glutenin Composition from Gel Electrophoresis of Flour Mill Streams and Relationship to Breadmaking Quality

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ABSTRACT

Three samples of Nekota (hard red winter wheat) were milled, and six mill streams were collected from each sample. The 18 mill streams were analyzed separately as well as recombined to form three patent flours. The methods of multistacking (MS)-SDS-PAGE and SDS-PAGE were used to separate the unreduced SDS-soluble glutenins and the total reduced proteins, respectively. The separated proteins were quantified by densitometry. The quantity of unreduced SDS-soluble proteins was significantly different among the mill streams at the 4% (largest molecular weight polymeric glutenins) and at the 10 and 12% (smaller molecular weight polymeric glutenins) origins of the MS-SDS-PAGE gels. The quantities of total HMW-GS, LMW-GS, 2*, 7+9, and 5+10 subunits and the ratio of HMW-GS to LMW-GS in polymeric protein samples isolated using preparative MS-SDS-PAGE and in total reduced protein extracts were significantly different among mill streams. The quantities of HMW-GS, LMW-GS, 2*, 7+9, and 5+10 subunits from total reduced proteins were positively and significantly correlated with loaf volume. The quantities of glutenin subunits (both HMW-GS and LMW-GS) from unreduced SDS-soluble proteins were positively or negatively correlated with loaf volume at the various MS-SDS-PAGE gel origins but the levels of correlation were not significant. These results showed that the glutenin protein composition was different among the various mill streams and demonstrated that electrophoretic analysis of the proteins in these fractions is a useful tool for studying the variation in functional properties of flour mill streams.

The breadmaking quality of a wheat cultivar depends mainly on the protein content (Finney and Barmore 1948) and the gluten composition (Wall 1979; Weegels et al 1996). The gliadins and glutenins, major components of gluten, play an important role in dough properties and in breadmaking quality. When glutenin is treated with reducing agents and analyzed by SDS-PAGE, two groups of proteins are obtained based on molecular weight: high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) (Payne et al 1979). Efforts have been made to explain variations in baking quality based on the presence or absence of certain HMW subunits and to establish correlations between particular HMW-GS and breadmaking quality (Payne et al 1979, 1981, 1987; Moonen et al 1982; Lawrence 1986; Campbell et al 1987; Ng and Bushuk 1988; Gupta et al 1989; Lukow et al 1989; Khan et al 1989, 1992; Sutton 1991; Kolster et al 1992; Huang and Khan 1997; Ivanov et al 1998; Faergestad et al 2004; Tohidfar et al 2004). However, the presence or absence of certain subunits does not fully explain the differences observed in breadmaking quality among wheat cultivars. Several factors including the presence of particular subunits of HMW-GS and LMW-GS, the ratio of HMW-GS to LMW-GS, and the ratio of glutenin to gliadin are important but the exact contribution of each of these factors to end use functionality is still largely unknown (Uthayakumaran et al 2003).

Flour mill streams are obtained through gradual reduction of the endosperm in the milling process after bran and germ fractions are removed. Flour ash, gluten content, and sedimentation value increased with increasing numbers of breaks in the flour streams (Indrani 2003). Other characteristics in flour mill streams such as protein composition, dough rheology, dough mixing traits, baking characteristics, and falling number have also been studied (Menkovska et al 2002; Every et al 2002; Indrani et al 2003) but little is known about the quantity and distribution of glutenin subunits in flour mill streams.

A multistacking gel electrophoresis procedure (Khan and Huckle 1992) has been used to characterize native glutenin proteins by fractionating the polymeric aggregates into six fractions based on their sizes and mobilities on the multistacking gel. In this study, we used this procedure to investigate the composition of native glutenin proteins in flour mill streams and the relationship between composition of the protein fractions and breadmaking quality.

MATERIALS AND METHODS

The mill streams used in this study came from three samples of a hard red winter wheat (*Triticum aestivum* L.) 'Nekota' grown in 2004. Each wheat sample was tempered to 16.5% moisture content, conditioned for 12–18 hr, and milled in a Bühler laboratory experimental mill (MLU-202) according to Approved Method 26-21A (AACC International 2000). The tempered wheat was metered at a feed rate of ≈175 g/min to obtain three break streams (B1, B2, and B3) and three reduction streams (R1, R2, and R3) for an overall 70–75% extraction of straight-grade flour. The roll gaps were 0.380 mm for the three break rolls and were <0.038 mm for the three frosted reduction rolls. For comparison purposes, a patent flour (PF) was prepared by recombining the six mill stream fractions of each sample according to the weight percentage of each fraction. A total of 18 mill streams and three patent flours was used in this study.

Approved Method 46-30 (AACC International 2000) was used to determine protein content and Approved Method 10-09 was used to determine breadmaking quality. Loaf volume (measured by rapeseed displacement) and crumb grain score were used to compare the bread quality (Table I).

Internal crumb grain score was a subjective average score (1 = poor, 6 = excellent) for each property based on a combination of texture of crumb (roughness or silkiness), thickness or thinness of the internal cell structures, and color (degree of whiteness) of internal crumb grain according to a procedure (*unpublished*) currently used in the USDA/ARS Wheat Quality Laboratory, Fargo, ND.

Sample weights of flour (50.0–85.1 mg) containing equivalent amounts of total protein were extracted with 1.0 mL of nonreducing sample buffer (0.0625*M* Tris-HCl, pH 6.8, 2% w/v SDS, 20% v/v glycerol) by vortexing for 3 hr at 50°C (Khan et al 2003). After centrifuging at 13,000 × *g* for 20 min in an Eppendorf 5415C microcentrifuge with an 18-place rotor (F-45-18-11), the supernatants (unreduced protein) were separated by MS-SDS-PAGE (Khan and Huckle 1992). Five stacking gels (4, 6, 8, 10, and 12%)

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were layered above a separation gel of 14% acrylamide. A gel thickness of 1.5 mm was used for analytical MS-SDS-PAGE, and a gel thickness of 3 mm was used for the preparative studies. The samples were extracted and analyzed in triplicate. A flow chart of the analytical steps used in this study is presented in Fig. 1.

After preparative electrophoresis, the gel origins (4, 6, 8, 10, 12, and 14%) were excised and each was reextracted with reducing sample buffer (1% dithiothreitol [DTT] in 0.0625*M* Tris-HCl pH 6.8, 2% w/v SDS, 20% v/v glycerol) at 60°C by vortexing for 4 hr. The samples were centrifuged for 20 min at 13,000 × *g*. Equal volumes of extracts were then loaded onto 12% acrylamide gels (0.75 mm thick) for SDS-PAGE. The reduced proteins isolated from all six origins of a given sample were loaded side-by-side for comparison. This data provides information concerning the distribution of glutenin subunits in the native glutenin polymers trapped at the gel origins (Huang and Khan 1997).

Sample weights of flour (50.0–85.1 mg) containing equivalent amounts of protein were used to extract total proteins with 1.0 mL of reducing sample buffer (1% DTT in 0.0625*M* Tris-HCl pH 6.8, 2% w/v SDS, 20% v/v glycerol) by vortexing for 1 hr at 50°C. After centrifugation for 20 min at 13,000 × *g*, the supernatant was transferred to a clean tube, and the proteins were alkylated at 60°C for 30 min by adding 1.4%, v/v, vinylpyridine in 50% v/v isopropyl alcohol + 0.08*M* Tris-HCl. Equal volumes of supernatant (total reduced and alkylated proteins) were loaded onto 12% acrylamide gels (0.75 mm thick) for SDS-PAGE. Five replicates of each sample were analyzed.

The 1.5-mm MS-SDS-PAGE gels were stained with 0.25% (w/v) Coomassie Brilliant Blue-R (CBB-R) dye for 1 hr in methanol-acetic acid-water (50:10:40 v/v), then destained with methanol-acetic acid-water (20:7:73 v/v) (Khan et al 2003). SDS-PAGE gels were stained with CBB-G dye using a colloidal par-

TABLE I
Protein Content, Loaf Volume, and Crumb Grain Score of Nekota Flour Mill Streams[a]

Mill Streams[b]	PC1 (%)	PC2 (%)	PC3 (%)	Avg PC (%)	Avg Loaf Vol (cm^3)	Avg Crumb Grain Score[c]
B1	12.2	11.6	12.2	12.0	857	4.3
B2	14.8	13.7	14.8	14.4	998	6.0
B3	17.4	16.0	17.5	17.0	945	4.7
R1	10.5	10.3	10.3	10.4	653	3.0
R2	10.5	10.1	10.4	10.3	577	2.3
R3	11.0	10.8	11.1	11.0	490	1.0
PF	10.8	10.5	11.1	10.8	735	3.0

[a] Protein content was reported at 14% moisture. PC1, PC2, PC3 represent protein content of the three Nekota samples.
[b] B1, B2, B3: break streams; R1, R2, R3: reduction streams; PF: patent flour.
[c] Subjective score based on a combination of texture, fineness, and whiteness of internal crumb characteristics (1 = poor, 6 = excellent).

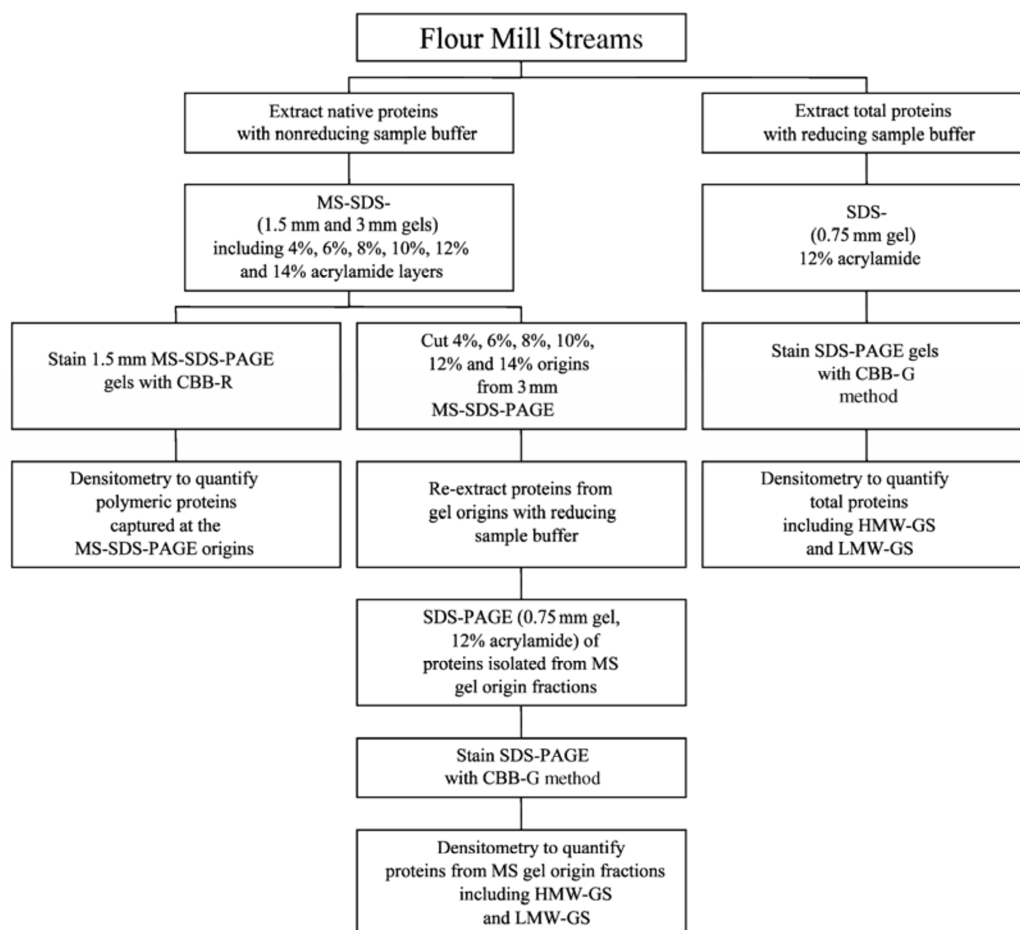

Fig. 1. Flow chart of experimental procedures of protein fractionation.

ticle method (Candiano et al 2004), where the gels were fixed with 12% trichloroacetic acid (TCA), stained overnight with 0.12% CBB-G in 10% H_3PO_4 + 10% $(NH_4)_2SO_4$ + 20% methanol, rinsed with methanol-water (25:75, v/v), and destained briefly in distilled water as needed to decrease background color.

All gels were photographed using a ChemiGenius gel documentation and analysis system (Syngene, Frederick, MD). Volume analysis by densitometry was performed to quantify the protein bands on both the MS-SDS-PAGE and SDS-PAGE gels.

Statistical Analysis

Data was analyzed using analysis of variance (ANOVA) in the general linear model (GLM) procedure of the Statistical Analysis Systems software package (SAS Institute, Cary, NC). Significant differences between group means were analyzed by Duncan's multiple-range test. A level of significance of $P \leq 0.05$ was used throughout the analysis. Pearson's correlation coefficients were used to measure the strength of the linear correlation between two variables.

RESULTS AND DISCUSSION

Quantity and Relative Size of Unreduced Polymeric Proteins in Mill Streams

Unreduced polymeric proteins, reduced proteins, and total proteins of various mill streams were obtained to study the quantity of proteins and their relationship to breadmaking quality. The unreduced polymeric proteins of the mill streams were extracted using nonreducing sample buffer by vortexing for 3 hr at 50°C. The average extraction rate of proteins in nonreducing sample buffer was ≈85% and the protein concentration in the extracts was similar within break streams (B1, B2, B3) and within reduction streams (R1, R2, R3) (data not shown). The unreduced polymeric proteins were separated with MS-SDS-PAGE (Khan and Huckle 1992). In this procedure, five stacking gels with increasing acrylamide concentrations (4, 6, 8, 10, and 12%) on top of a 14% separating gel were used to fractionate polymeric glutenin protein aggregates. Very large proteins are trapped at the interfaces (origins) of the stacking gel layers because successive layers have sharp changes in pore sizes. Separation results in a series of molecular weight species with the largest polymeric proteins corresponding to the 4% stacking layer, and successively smaller polymers in each higher percentage acrylamide layer. The proportion of unreduced SDS-soluble proteins at the various MS-SDS-PAGE origins as determined by densitometry (Huang and Khan 1997) is shown in Table II.

Within the break streams (B1, B2, B3), the quantities of unreduced SDS-soluble proteins did not show significant differences at the 4, 6, 8, and 14% origins but showed significant differences at the 10 and 12% origins. Comparing B1, B2, and B3, the quantity of protein in B1 trapped at the 10 and 12% origins was the largest. Because these higher % acrylamide layers (10 and 12%) contain smaller pore sizes, this result indicates an increased presence of lower molecular weight proteins in mill stream fraction B1. Interestingly, B1 also exhibits a smaller loaf volume than B2 or B3 (Table I), indicating that the baking quality may also be correlated with the sizes of protein polymers in the mill stream fractions. The average of the loaf volumes of the six mill streams (752 cm^3) is very close to the loaf volume of the blended patent flour (735 cm^3). Within the reduction streams (R1, R2, R3), the quantity of proteins trapped at all origins did not show significant differences.

Comparing all six mill streams or comparing the patent flour (PF) plus the six mill streams, the quantities of unreduced polymeric proteins were not significantly different at the 6, 8, and 14% origins but were significantly different at the 4% (the largest polymeric glutenin species) and at the 10 and 12% (containing smaller polymeric glutenin species) origins (Table II).

Because the polymeric proteins trapped at the various gel origins are separated according to the size of the acrylamide pores, the difference in quantity at the various origins demonstrate that the glutenin proteins are composed of polymers with a range of different structures and molecular sizes and that the size and composition of glutenin polymers in the various mill streams must be different. Based on the wheat samples used in this study, the break flours gave much better loaf volume responses than the reduction flours. The magnitude of these loaf volume differences between break and reduction streams alone cannot be attributed to the higher protein content of the break flours because the average protein content of B1 is only 1% higher than R3, yet the loaf volume of B1 is almost twice as large as R3 (Table I). The crumb grain scores (Table I) follow the trend of the loaf volume scores, that is, B2 has the highest score, R3 has the lowest, and PF has a midrange score. In summary, the structures and molecular sizes of the protein components of the mill streams seem to affect functional properties as indicated by the wide range of loaf volume and crumb grain responses of bread made from the various mill stream flours.

Quantity of HMW-GS and LMW-GS in Polymeric Glutenin Species Fractionated at Various MS-SDS-PAGE Origins

When unreduced proteins from the various origins of MS-SDS-PAGE were reextracted with reducing agent and analyzed by SDS-PAGE, two major protein groups were observed on the gels: high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS). The quantities of total HMW-GS and LMW-GS were determined by densitometry and the results are presented in Table III.

HMW-GS composition has a strong effect on breadmaking quality (Payne et al 1981, 1987; Ivanov et al 1998; Tohidfar et al 2004) and LMW-GS composition also is associated with breadmaking quality (Payne 1987; Gupta et al 1989, 1993). The gluten complex (a polymeric protein network made up of the HMW-GS, LMW-GS, and gliadins) confers viscoelasticity to dough that is necessary for producing high-quality bread. Therefore, studying the quantity and distribution of HMW-GS and LMW-GS may help us to understand the relationship between the molecular weight distribution of polymeric proteins and breadmaking quality of wheat.

TABLE II
Proportion of Unreduced SDS-Soluble Proteins in Mill Streams Fractionated by MS-SDS-PAGE[a]

	Gel Origin (% Acrylamide)					
Mill Streams	4%	6%	8%	10%	12%	14%
B1	0.43ab	0.43a	0.44a	0.48a	0.50a	0.29a
B2	0.38ab	0.40a	0.39a	0.42ab	0.44ab	0.23a
B3	0.36b	0.36a	0.35a	0.36b	0.40b	0.20a
R1	0.40ab	0.37a	0.37a	0.40ab	0.44ab	0.23a
R2	0.46a	0.35a	0.35a	0.39b	0.43ab	0.23a
R3	0.38ab	0.34a	0.35a	0.38b	0.43ab	0.24a
PF	0.40ab	0.41a	0.41a	0.46a	0.49ab	0.28a

[a] Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

Within break streams (B1, B2, and B3), the quantity of total HMW-GS was not significantly different at the 4, 6, 8, 10, and 14% origins but significantly different at the 12% origin, where the quantity of B2 was the largest. The quantity of LMW-GS was not significantly different at the 6, 8, 10, and 14% origins but significantly different at the 4% (B3, largest quantity) and 12% (B2, largest quantity) origins. The ratio of HMW-GS to LMW-GS was not significantly different at the 4, 10, 12, and 14% origins but significantly different at the 6 and 8% origins where the quantity of B2 was the largest. The differences in quantity at the various origins within B1, B2, and B3 were a result of the different structure and composition, and different molecular size of glutenin polymers. These differences seem to have an effect on breadmaking quality as shown in Table I.

Within reduction streams (R1, R2, and R3), the quantity of total HMW-GS was not significantly different at the 4, 6, 8, and 10% origins but significantly different at the 12 and 14% origins. The quantity of LMW-GS was not significantly different at the 4, 8, and 10% origins but significantly different at the 6, 12, and 14% origins. The ratio of HMW-GS to LMW-GS was not significantly different at the 10, 12, and 14% origins but significantly different at the 4, 6, and 8% origins. These results showed that the quantity and distribution of HMW-GS and LMW-GS in reduction streams (R1, R2, and R3) was different.

Comparing the patent flour and six mill streams, the quantity of HMW-GS was not significantly different at the 4, 6, 8, and 10% but significantly different at the 12 and 14% origins. The quantity of LMW-GS was not significantly different at the 8 and 10% origins but different at the 4, 6, 12, and 14% origins. The ratio of HMW-GS to LMW-GS was not significantly different at the 10%, 12, and 14% origins but significantly different at the 4, 6, and 8% origins.

TABLE III
Proportion of Total HMW-GS, LMW-GS, and the Ratio of HMW-GS to LMW-GS at Various MS-SDS-PAGE Origins in Mill Streams[a]

		Gel Origins (% Acrylamide)					
	Mill Streams	4%	6%	8%	10%	12%	14%
Total HMW-GS	B1	5.22a	4.40a	4.25a	3.96a	4.48b	3.24b
	B2	5.57a	4.51a	4.40a	4.49a	5.26a	3.69ab
	B3	5.84a	4.28a	4.09a	4.09a	4.20b	2.96b
	R1	5.71a	4.95a	4.10a	4.34a	4.92ab	3.92a
	R2	5.81a	4.50a	4.30a	4.15a	4.03b	3.04b
	R3	5.44a	4.20a	4.23a	4.62a	5.54a	4.12a
	PF	4.94a	4.31a	4.57a	4.54a	5.05ab	4.24a
LMW-GS	B1	8.18ab	6.58ab	6.41a	6.51a	9.25ab	6.84ab
	B2	7.64b	5.51ab	5.67a	7.12a	10.96a	7.76ab
	B3	10.12a	6.80ab	6.44a	6.95a	8.68b	5.51b
	R1	7.92b	5.66ab	5.22a	6.83a	10.10ab	8.07a
	R2	9.68ab	7.23a	6.70a	6.90a	7.54b	4.80b
	R3	6.58b	5.01b	5.40a	7.19a	10.84a	7.90a
	PF	6.70b	5.66ab	6.44a	7.25a	10.34a	8.58a
HMW-GS/LMW-GS	B1	0.68b	0.72ab	0.69b	0.62a	0.49a	0.49a
	B2	0.80ab	0.86a	0.81a	0.65a	0.49a	0.50a
	B3	0.60b	0.63b	0.65b	0.59a	0.50a	0.56a
	R1	0.75ab	0.85a	0.82a	0.65a	0.49a	0.51a
	R2	0.61b	0.64b	0.65b	0.60a	0.55a	0.65a
	R3	0.87a	0.91a	0.82a	0.64a	0.51a	0.52a
	PF	0.77ab	0.80a	0.73ab	0.65a	0.52a	0.55a

[a] Values followed by the same letter in the same column are not significantly different for HMW-GS, LMW-GS, and HMW-GS/LMW-GS, respectively ($P < 0.05$).

TABLE IV
Proportion of 2*, 7+9, and 5+10 Subunits at the Various MS-SDS-PAGE Origins in Mill Streams[a]

		Gel Origins (% Acrylamide)					
	Mill Streams	4%	6%	8%	10%	12%	14%
2* subunit	B1	3.55ab	2.96a	2.85a	2.62a	3.06a	2.20a
	B2	3.51ab	2.89a	2.73a	2.83a	3.19a	2.13a
	B3	3.95a	2.92a	2.71a	2.78a	2.86a	2.04a
	R1	3.56ab	2.85a	2.44a	2.64a	2.83a	2.20a
	R2	3.97a	3.07a	2.79a	2.70a	2.71a	1.96a
	R3	3.52ab	2.78a	2.62a	2.66a	3.12a	2.35a
	PF	3.29b	2.82a	2.70a	2.72a	2.94a	2.33a
7+9 subunits	B1	8.13b	7.23a	7.08a	6.75a	7.86a	5.47a
	B2	8.51ab	7.29a	7.01a	7.21a	8.13a	5.62a
	B3	9.35a	7.37a	7.13a	7.30a	7.48a	5.19a
	R1	8.85ab	7.33a	6.59a	7.07a	7.56a	5.77a
	R2	9.15ab	7.46a	7.12a	7.11a	6.94a	5.07a
	R3	8.12b	6.69a	6.51a	7.18a	8.05a	5.89a
	PF	7.73b	7.05a	7.35a	7.27a	7.82a	5.96a
5+10 subunits	B1	8.07ab	7.36a	7.03a	6.38a	6.77ab	4.90a
	B2	7.86ab	6.84a	6.45a	6.28a	6.90ab	4.62a
	B3	8.37ab	6.71a	6.67a	6.50a	6.22ab	4.46a
	R1	8.44ab	7.19a	6.50a	6.49a	6.67ab	5.03a
	R2	8.80a	7.28a	6.96a	6.53a	5.92b	4.61a
	R3	7.98ab	6.62a	6.49a	6.81a	7.30a	5.32a
	PF	7.42b	6.77a	6.96a	6.61a	6.93ab	5.32a

[a] Values followed by the same letter in the same column are not significantly different for 2*, 7+9, and 5+10 subunits, respectively ($P < 0.05$).

Based on the nomenclature of Payne et al (1987), the HMW-GS composition of Nekota includes the 2*, 7+9, and 5+10 subunits. The relative proportion of these subunit types found in the isolated MS-SDS-PAGE polymeric protein fractions was determined by densitometry and the results are shown in Table IV. Within break streams and at all origins, the quantities of the 2* and 5+10 subunits showed no significant differences. The quantity of 7+9 subunits did not show significant differences at the 6, 8, 10, 12, and 14% origins but was significantly different at the 4% origin where the quantity in B3 was the largest. Within reduction streams and at all origins, the quantities of the 2* and 7+9 subunits showed no significant differences. The quantity of 5+10 subunits did not show significant differences at 4, 6, 8, 10, and 14% origins but was significantly different at the 12% origin where the quantities in R1, R2, and R3 exhibited a wide range of values.

Comparing the patent flour and all six mill streams, the quantities of 2* and 7+9 subunits did not show significant differences at the 6, 8, 10, 12, and 14% origins but were significantly different at the 4% origin where the quantity in patent flour was much lower than that in the break and reduction streams. The quantities of 5+10 subunits were not significantly different at the 6, 8, 10, and 14% origins but were significantly different at the 4 and 12% origins (Table IV). The quantities of HMW-GS and LMW-GS and the ratio of HMW-GS to LMW-GS were different in the polymeric protein fractions isolated at the various origins of MS-SDS-PAGE gels, especially at the 4% (largest polymeric glutenin species) and at 12 and 14% (smallest polymeric glutenin species) origins. These results suggest that the quantity and distribution of HMW-GS and LMW-GS trapped at these origins are different among the various flour mill streams. The differences in glutenin composition would be expected to influence its functional properties as discussed above.

Quantity of Total Reduced Proteins in Mill Streams

Total proteins from the Nekota mill streams and patent flour were extracted with reducing agent and separated by SDS-PAGE. Two major protein groups (HMW-GS and LMW-GS) were observed, and the total quantities of HMW-GS and LMW-GS in these two regions of the gels were determined by densitometry (Table V). The relative proportion of the 2*, 7+9, and 5+10 HMW-GS were also determined by densitometry, and the results are shown in Table V.

Within break streams (B1, B2, and B3), the quantity of total HMW-GS was significantly different but the quantities of LMW-GS, 2*, 7+9, and 5+10 subunits, as well as the ratio of HMW-GS to LMW-GS, did not show significant differences. In contrast, within reduction streams (R1, R2, and R3), the quantities of HMW-GS, LMW-GS, 2*, 7+9, and 5+10 subunits were significantly different but the ratio of HMW-GS to LMW-GS was similar.

Comparing the patent flour and the six streams (Table V), the quantities of HMW-GS, LMW-GS, ratio of HMW-GS to LMW-GS, 2*, 7+9, and 5+10 subunits were all significantly different,

TABLE V
Proportion of Glutenin Subunits from SDS-PAGE of Total Reduced Protein Extract of Mill Streams[a]

Mill Streams	Glutenin Subunits					
	HMW	LMW	HMW/LMW	2*	7+9	5+10
B1	1.87ab	5.57a	0.33ab	2.98a	7.47a	10.07a
B2	1.74bc	5.38ab	0.32bc	2.83ab	7.20ab	9.97a
B3	1.68c	5.25ab	0.32bc	2.70ab	7.00ab	9.70ab
R1	1.68c	5.26ab	0.32bc	2.64bc	6.84bc	9.55ab
R2	1.61c	5.02bc	0.32bc	2.39c	6.44c	9.00b
R3	1.44d	4.71c	0.31c	2.09d	5.86d	8.00c
PF	1.91a	5.49a	0.35a	2.93a	7.43a	10.35a

[a] Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

TABLE VI
Correlation Coefficients Between Loaf Volume and Quantity of Glutenin Subunits from Different MS-SDS-PAGE Origins and from SDS-PAGE of a Total Protein Extract

Glutenin Subunits	Gel Origin (% Acrylamide)						Total Protein Extract
	4%	6%	8%	10%	12%	14%	
Break streams							
HMW-GS	0.42	0.18	0.34	0.51	0.38	0.26	–0.46
LMW-GS	–0.03	–0.30	–0.05	0.39	0.37	0.16	–0.32
HMW/LMW	0.25	0.30	0.29	0.19	0.04	0.06	–0.56
2*	0.09	–0.06	0.02	0.48	0.05	–0.21	–0.31
7+9	0.43	0.13	0.23	0.43	0.23	–0.03	–0.38
5+10	–0.18	–0.40	–0.43	–0.20	–0.23	–0.36	–0.27
Reduction streams							
HMW-GS	0.31	0.37	0.10	–0.61	–0.33	–0.11	0.80**
LMW-GS	0.44	0.31	0.27	–0.52	–0.10	0.01	0.81**
HMW/LMW	–0.51	–0.16	–0.27	–0.05	–0.39	0.02	0.57
2*	0.09	–0.06	0.02	0.48	0.05	–0.21	0.80**
7+9	0.43	0.23	–0.04	–0.54	–0.47	–0.13	0.62
5+10	0.46	0.52	0.22	–0.59	–0.36	–0.07	0.70*
All mill streams							
HMW-GS	–0.02	0.05	0.13	–0.26	–0.16	–0.32	0.59**
LMW-GS	0.29	0.18	0.25	–0.08	0.07	–0.06	0.65**
HMW/LMW	–0.29	–0.21	–0.24	–0.09	–0.37	–0.18	0.41
2*	0.07	0.16	0.28	0.21	0.23	–0.12	0.62**
7+9	0.13	0.20	0.34	–0.07	0.13	–0.15	0.64**
5+10	–0.22	–0.03	0.06	–0.36	–0.12	–0.31	0.69**

[a] * and ** Indicate significance at $P < 0.05$ and $P < 0.01$, respectively.

and the overall quantities generally decreased in the order: patent flour > B1 > B2 > B3 > R1 > R2 > R3. Loaf volume, however, decreased in a very different order: B2 > B3 > B1 > PF > R1 > R2 > R3 (Table I). This data provides further evidence that the quantitative glutenin composition and the structure of the glutenin proteins are both important factors in determining breadmaking quality differences.

Relationship Between Quantity of Glutenin Subunits of Mill Streams and Breadmaking Quality

Based on the 18 flour mill streams (nine break streams and nine reduction streams) of Nekota, using the quantitative compositional data obtained from MS-SDS-PAGE protein fractionation and the data of loaf volumes, Pearson's correlation coefficients between the quantity of glutenin subunits and loaf volume were calculated (Table VI). For the break streams, none of the correlations were significant but the quantity of HMW-GS and the ratio of HMW-GS to LMW-GS from the six origins showed positive correlations with loaf volumes while the quantities of 5+10 subunits were negatively correlated with loaf volume at all origins. The quantities of LMW-GS, 2*, and 7+9 subunits from the six origins showed a mixture of positive and negative correlations with loaf volume but none of the correlation coefficients were significant. Similarly, for the three reduction streams or for the combined data of all six mill streams, the quantities in all protein categories (HMW-GS, LMW-GS, 2*, 7+9, 5+10 subunits, and the ratio of HMW-GS to LMW-GS) showed positive correlations with loaf volume at some origins and negative correlations at other origins but the levels of correlation were not significant.

Based on the quantitative compositional data of total reduced extracts of flour protein separated on SDS-PAGE in 18 mill streams, correlation coefficients between the quantity of glutenin subunits from this extract and loaf volume were calculated (Table VI). For the break streams, the quantity of total HMW-GS, LMW-GS, 2*, 7+9, 5+10 subunits, and the ratio of HMW-GS to LMW-GS were all negatively but not significantly correlated with loaf volume. For the reduction streams, the quantities of HMW-GS, LMW-GS, 2*, and 5+10 subunits were positively and significantly correlated with loaf volume. The ratio of HMW-GS to LMW-GS and the quantity of 7+9 subunits were also positively correlated with loaf volume but not at a significant level. For the combined data of all six mill streams, the quantities of HMW-GS, LMW-GS, 2*, 7+9, and 5+10 subunits were positively and significantly correlated with loaf volume. These results are similar to previously reported research (Gupta et al 1989, 1993; Faergestad et al 2004; Tohidfar et al 2004).

In the current study, loaf volume had a wide range of correlation coefficients (including both positive and negative values) with the quantity of glutenin subunits isolated from the different MS-SDS-PAGE gel origins. This data provides evidence for the important role of gluten polymer structure in affecting breadmaking quality, as influenced by the quantitative composition of the subunits that form the various molecular weight polymeric glutenin species of the mill streams. However, the variation in breadmaking quality cannot be explained only by the variation in HMW-GS composition. The influence of the LMW-GS and gliadins and their interactions must also be considered. Wheat cultivars containing the same LMW-GS but different gliadin blocks can show contrasting gluten characteristics (Pfluger 2003) and it has been suggested that there are interactions between the gliadin/LMW-GS allele pairs and the HMW-GS alleles (Flaete and Uhlen 2003). The contributions of LMW-GS and gliadins to protein quality characteristics should be further studied in mill streams.

CONCLUSIONS

The protein composition data, and the loaf volume and crumb grain data, demonstrate that the milling process can fractionate wheat flour into mill streams of different functional properties. Based on the flour samples studied here, if high protein and better quality fractions of flour are needed, the break mill streams can be collected to obtain flour of superior functional properties such as loaf volume. In contrast, the reduction mill streams are lower in protein and functional quality, including loaf volume and crumb grain. The patent flour, which is a combination of break and reduction mill streams, exhibits an average protein content and quality of the two types of mill streams. The milling process, therefore, can be used as a tool to obtain mill streams of different protein composition and the effects of this changed protein composition distribution, as shown by MS-SDS-PAGE and SDS-PAGE, are statistically correlated with functional properties such as loaf volume.

Further work is in progress to elucidate specific changes in protein composition that affect gluten/glutenin structure (such as molecular weight distribution and composition of HMW-GS and LMW-GS) and, hence, affect the overall functional properties of the various mill streams.

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