

Distribution of Protein Composition in Bread Wheat Flour Mill Streams and Relationship to Breadmaking Quality

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ABSTRACT

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Wheat protein quantity and composition are important parameters for wheat baking quality. The objective of this study was to use fractionation techniques to separate the proteins of flour mill streams into various protein fractions, to examine the distribution of these protein fractions, and to establish a relationship between protein composition and breadmaking quality. Nine break streams, nine reduction streams, and three patent flours obtained from three samples of Nekota (a hard red winter wheat) were used in this study. A solution of 0.3M NaI + 7.5% 1-propanol was used to separate flour protein into monomeric and polymeric proteins. The protein fractions, including gliadin, albumin+globulin, HMW-GS, and LMW-GS, were precipitated with 0.1M NH₄Ac-MeOH or acetone. The fractions were statistically analyzed for their distribution in the mill

streams. The quantities of total flour protein and protein fractions in flour were significantly different among mill streams. The ratio of polymeric to monomeric proteins in break streams was significantly greater than in the reduction streams. The relationship between protein composition and breadmaking quality showed that the quantities of total flour protein, albumin+globulin, HMW-GS, and LMW-GS in flour were significantly and positively correlated with loaf volume. The ratio of HMW-GS to LMW-GS had little association with loaf volume. The gliadin content in total flour protein was negatively and significantly correlated with loaf volume. These results indicated that the quantity and composition of protein among the mill streams was different, and this resulted in differences in breadmaking quality.

Wheat proteins can be divided into monomeric fractions, which include gliadin, albumin+globulin, and polymeric glutenins, which include high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) (Singh et al 1990). Gluten proteins consist of two major fractions: monomeric gliadins and polymeric glutenins (Sapirstein and Fu 1998). When wheat flour is mixed with water, wheat gluten proteins form a continuous and viscoelastic network, and this can influence the characteristics of dough and breadmaking quality (Aussenac et al 2001). 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 more related to wheat quality than albumins and globulins (Huang and Khan 1997). The relationship of total protein content and the amounts and proportions of gluten protein types to breadmaking quality have been well documented (Haddad et al 1995; Sapirstein and Fu 1998; Wesley et al 1999; Wang and Kovacs 2002; Cuniberti et al 2003; Uthayakumaran et al 2003) although the exact contribution of each of these factors to end-use functionality is largely unknown (Uthayakumaran et al 2003).

Several methods of separating protein fractions from bread wheat flour have been published (Melas et al 1994; Verbruggen et al 1998; Fu and Kovacs 1999; Suchy et al 2003; DuPont et al 2005). In previous research (Wang et al 2006), we have used the multistacking (MS) SDS-PAGE method to separate unreduced SDS-soluble glutenins in mill streams and to study the glutenin composition and its relationship to breadmaking quality. DuPont et al (2005) reported a method to separate and determine albumin+globulin, gliadin, and glutenin from flour samples. Melas et al (1994) used a method to precipitate HMW-GS and LMW-GS from glutenin using different concentrations of acetone. In the present study, the objective was to use these fractionation techniques to separate the protein fractions from flour mill streams, to study the distribution of protein composition in mill streams, and establish relationships between protein composition and breadmaking quality.

MATERIALS AND METHODS

Flour Mill Streams

The mill streams used in this study came from three samples of a hard red winter wheat (*Triticum aestivum* L.) Nekota grown in 2004 at three different locations. Each wheat sample was tempered to 16.5% moisture content and milled on a Bühler laboratory experimental mill (MLU-202) to obtain three break (B1, B2, B3), and three reduction (R1, R2, R3) flour mill streams according to Approved Method 26-21A (AACC International 2000). For comparison purposes, a patent flour was prepared by recombining the six mill streams according to weight percentage of each fraction. A total of 18 mill streams and three patent flours were used in this study.

Breadmaking Quality of Flour

Breadmaking quality was determined by Approved Method 10-09 (AACC International 2000). Loaf volume (LV, measured by rapeseed displacement) and crumb grain score were used to compare bread quality. Internal crumb grain score was a subjective average score (1 = poor, 6 = excellent of each property) based on a combination of the texture (roughness or silkiness) of the crumb, thickness or thinness of internal cell structures, and color (degree of whiteness) of internal crumb grain according to the procedure (unpublished) currently used in the USDA/ARS Wheat Quality Laboratory, Fargo, ND.

Chemicals and Reagents

Sodium Iodide (NaI) was purchased from J. T. Baker (Phillipsburg, NJ). Ammonium acetate (NH₄Ac), 1, 4-dithiothreitol (DTT), Tris, and 1-propanol were purchased from EMD (Gibbstown, NJ). The 4-vinylpyridine was purchased from Sigma Chemical (St. Louis, MO). Methanol was purchased from VWR International (West Chester, PA). All chemicals were of analytical grade.

Extraction Solutions

Solution A was 0.3M NaI + 7.5% (v/v) 1-propanol; solution B was 1% (w/v) DTT in 50% (v/v) 1-propanol containing 0.08M Tris-HCl buffer, pH 8.0; solution C was 1.4% (v/v) 4-vinylpyridin in 50% (v/v) 1-propanol containing 0.08M Tris-HCl buffer, pH 8.0; 0.1M NH₄Ac-MeOH 0.1M in 100% methanol.

Extraction of Protein Fractions

The protocol for extraction of protein fractions in mill streams is outlined in Fig. 1. The sample flour (100 mg) was extracted

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three times with 1.0 mL of solution A by vortexing for 1 hr at room temperature and then centrifuging in an Eppendorf 5415 C microcentrifuge for 10 min at $13,000 \times g$ (Fu and Kovacs 1999). The supernatant fractions (monomeric proteins) were pooled and pellet 1 was saved. In this study, samples were extracted simultaneously in duplicate. The supernatant used for the precipitation of gliadin was cold (-20°C) 0.1M $\text{NH}_4\text{Ac-MeOH}$ added to the supernatant and mixed well, then cooled at -20°C for 48 hr, and centrifuged at $4,100 \times g$ for 10 min at room temperature to obtain the gliadin fraction (DuPont et al 2005). Another supernatant was used for precipitation of all monomeric proteins (gliadin, albumin+globulin) where the supernatant was mixed with four volumes of acetone, stored at -20°C for 24 hr, and centrifuged as above to obtain gliadin together with albumin+globulin.

The polymeric protein contained in pellet 1 was extracted three times with 1.0 mL of solution B at 60°C by vortexing 30 min and then centrifuging in an Eppendorf 5415 C microcentrifuge for 10 min at $13,000 \times g$ to obtain glutenin subunits. The three supernatants were pooled. The residue was dried for measuring protein

content. The protein in the supernatant was alkylated at 60°C for 30 min by adding solution C. Acetone (40%, final concentration) was added to the supernatant and cooled at -20°C for 30 min to precipitate the HMW-GS. After centrifuging at $4,100 \times g$ for 10 min, the HMW-GS pellet was recovered and dried. Additional acetone was then mixed with the supernatant to final concentration of 80% (v/v), cooled at -20°C for 30 min, and centrifuged as above. The LMW-GS pellet was recovered and dried (Melas et al 1994).

Determination of Protein Content in Samples

Protein content of the dried protein fractions was determined by Dumas nitrogen combustion using a nitrogen analyzer (FP-528, Leco Corp., St Joseph, MI), an EDTA standard, and a protein-to-nitrogen ratio of 5.7 (Approved Method 46-30) (AACC International 2000). Flour protein content was determined by the same method and expressed on a 14% moisture basis. Based on the dry weight and protein content, the quantity of each protein fraction was calculated and used for statistical analysis.

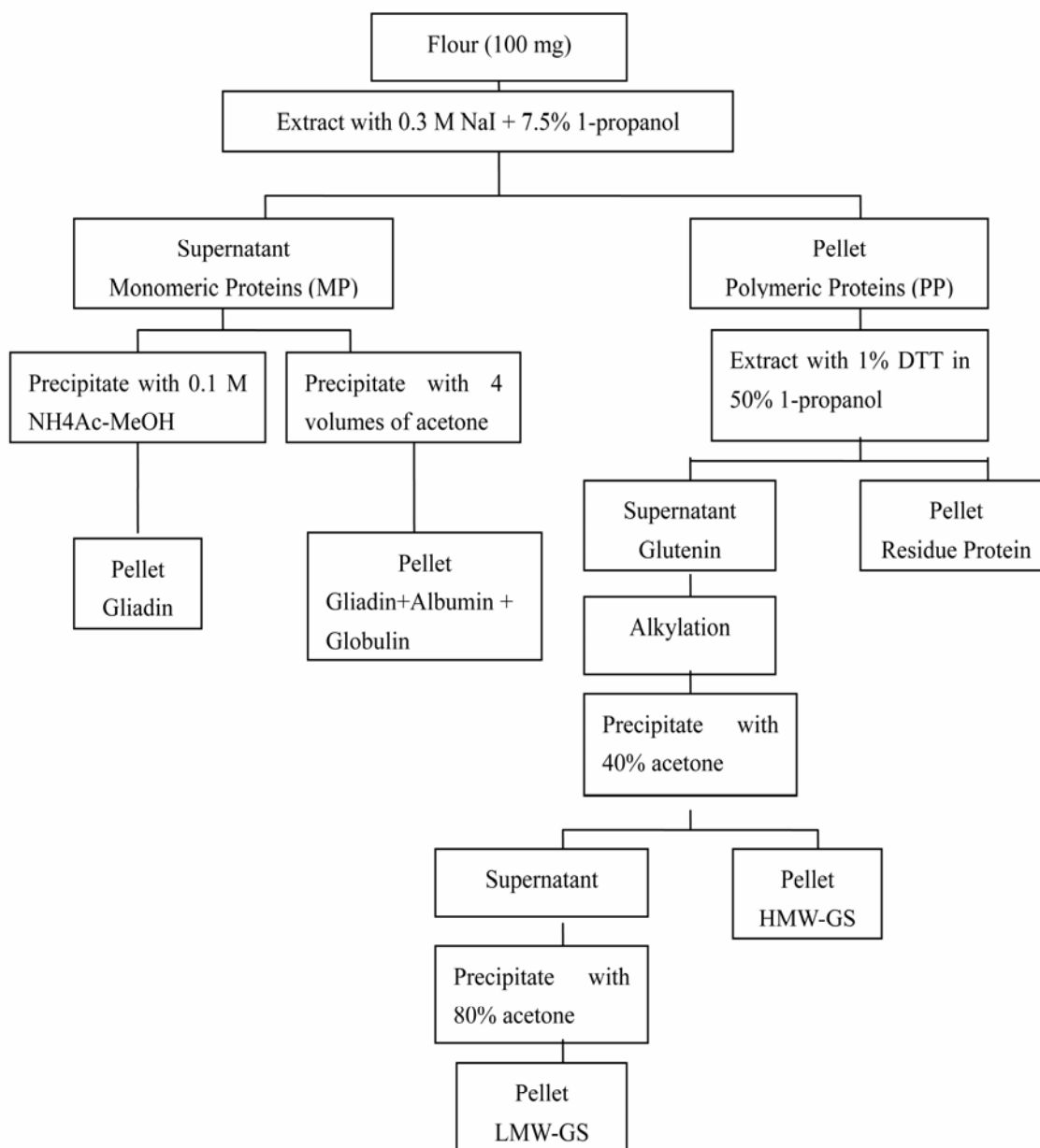


Fig. 1. Isolation procedures of protein fractions.

Statistical Analyses

Statistical analyses including variance analysis were made using the SAS program (v.9.1, SAS Institute, Cary, NC). Significant differences in characteristics of protein fractionation among mill streams 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 test was used to measure the strength of the linear correlation between two variables.

RESULTS AND DISCUSSION

Quality Characteristics of Flour Mill Streams

Differences occurred in loaf volume and crumb grain score among the flour mill streams (Table I). Average bread loaf volume (cm^3) of three samples of Nekota was highest among the break flours (B1 = 857, B2 = 998, B3 = 945) and lowest among the reduction flours (R1 = 653, R2 = 577, R3 = 490). Average crumb grain score was also highest among the break flours (B1 = 4.3, B2 = 6.0, B3 = 4.7) and lowest among the reduction flours (R1 = 3.0, R2 = 2.3, R3 = 1.0). Average loaf volume of the patent flour was 735 cm^3 and average crumb grain score was 3.0. Table II showed that the ash in various mill streams was different. Differences in quality characteristics of the mill streams likely can be attributed in part to differences in bran contamination, which will affect protein content, loaf volume, and crumb grain score.

Distribution of Total Protein and Protein Composition in Flour

In this study, the sample flour protein was separated into five fractions: gliadin, monomeric proteins, HMW-GS, LMW-GS, and residue proteins. The quantity of albumin+globulin was calculated as monomeric protein minus gliadin. The purity of the fractions extracted was tested by SDS-PAGE (12% acrylamide gels, 0.75 mm thick) and the results showed good separation of fractions (gel patterns not shown). To obtain larger quantities of each fraction, 1 g of flour from the mill streams was used. The quantity of total protein and fractions in 1 g of flour from the mill streams was

statistically analyzed for distribution in mill streams. The results are presented in Table II. Within break streams (B1, B2, and B3), the quantity of total protein was significantly different among the break streams and showed an increasing trend in the order of B1 < B2 < B3. Similar results were reported by Prabhasankar (2000). Differences in protein content of the mill streams can be affected partly by the differences in bran contamination (Table II). Within reduction streams (R1, R2, and R3), the quantity of total protein also was significantly different. The reduction streams also showed an increasing trend from R1 to R3, but the degree of difference was less than for the break streams. Within all streams (break streams, reduction streams, and patent flour), statistical analysis showed that the quantity of total protein was significantly different among mill streams. Protein quantity in break streams was significantly greater than that in reduction streams. This could be caused by the milling procedure. The break streams were increased in concentration of peripheral endosperm, which is rich in protein (Pomeranz 1988). Perhaps this increased protein caused the loaf volume in break streams to be larger than in reduction streams. Crumb grain score was also higher in break streams (Table I). These results indicated that protein quantity is associated with breadmaking quality. Patent flour (PF) was a recombined flour of break streams and reduction streams according to the weight percentage of each mill fraction. The protein quantity in PF did not show a significant difference between B1 and R3. However, the loaf volume showed a significant difference among PF, B1, and R3. The loaf volume in PF was much larger than that in R3 (Table I). Therefore, there must be other factors besides protein content associated with breadmaking quality; for example, protein composition or protein structure.

In Table II, within break streams, the quantity of gliadin, albumin+globulin, HMW-GS, and LMW-GS showed a significant increasing trend in the order B1 < B2 < B3. This may be caused by the increased total protein in these streams. Within reduction streams, the quantity of gliadin showed a significant increasing trend from R1 to R3, and the quantity of albumin+globulin showed a significant decreasing trend from R1 to R3. The quantities of

TABLE I
Loaf Volume and Crumb Grain Score of Nekota Flour Mill Streams

Mill Streams ^a	Loaf Volume (cm^3) of Three Nekota Samples			Average LV (cm^3) ^b	Average Crumb Grain Score ^{b,c}
	LV1	LV2	LV3		
B1	825	845	900	857a	4.3ad
B2	990	990	1,015	998b	6.0b
B3	930	950	955	945b	4.7bd
R1	600	650	710	653c	3.0ae
R2	500	610	605	577d	2.3ce
R3	435	480	555	490e	1.0c
PF	680	760	765	735f	3.0ae

^a B1, B2, B3, break streams; R1, R2, R3, reduction streams; PF, patent flour.

^b Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

^c Subjective score based on a combination of texture, fineness, and whiteness of internal crumb characteristics (1 = poor, 6 = excellent).

TABLE II
Amount (mg) of Ash, Total Protein, and Protein Fractions in 1 g of Flour^{a,b}

Mill Streams	Ash	TP	Gli	Alb+Glob	HMW	LMW	RP
B1	4.24aa	118.83a	23.89a	23.20ad	14.80ac	25.68a	17.36a
B2	4.36a	142.33b	28.49bd	26.57a	18.83bc	34.63b	18.61bd
B3	6.48b	170.08c	37.75c	31.35b	23.34b	43.59c	19.67b
R1	4.17a	102.09d	22.43a	24.55ad	12.03ac	19.51de	18.21ad
R2	5.38c	103.49de	26.29d	21.65cd	11.48a	17.72d	18.91bd
R3	8.01d	110.59ef	31.46b	18.64c	11.05a	16.33d	21.28c
PF	4.61a	111.88af	25.50ad	22.80d	12.71ac	22.03e	19.00bd

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

^b TP, total protein; Gli, gliadin; Alb+Glob, albumin+globulin; HMW, high molecular weight glutenin subunits; LMW, low molecular weight glutenin subunits; RP, residue protein.

HMW-GS and LMW-GS also showed a decreasing trend from R1 to R3, but it was not significant. The loaf volume in reduction streams (Table I) also showed a decreasing trend from R1 to R3, suggesting that polymeric proteins (HMW-GS and LMW-GS) play an important role in influencing breadmaking quality differences.

Residue proteins (RP) are the proteins that remain in the pellet after polymeric proteins (PP) were reduced and extracted (Fig. 1). In Table II, within all streams, the range of differences was small, and for most of the residue proteins it was not significantly different.

Percentage of Protein Fractions in Total Flour Protein in Mill Streams

To compare the distribution of the protein composition in mill streams, the percentage of each protein fraction relative to total flour protein was calculated and the results are plotted in Fig. 2. Within all streams, the protein fractions percentage showed a different distribution in various mill streams. Gliadin percentage showed an increasing trend within break streams (not significant) and reduction streams (significant). Albumin+globulin showed a decreasing trend within break streams (not significant) and reduction streams (significant). HMW-GS and LMW-GS percentage

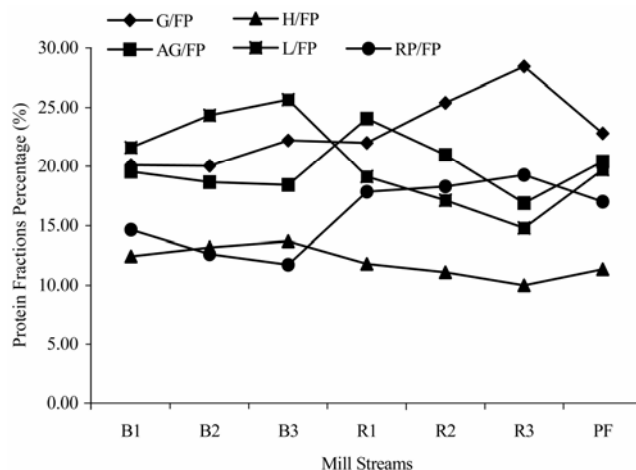


Fig. 2. Distribution of percentage of protein fractions in mill streams. G/FP = gliadin/total flour protein, AG/FP = albumin+globulin/total flour protein, H/FP = HMW-GS/total flour protein, L/FP = LMW-GS/total flour protein, RP/FP = residue protein/total flour protein.

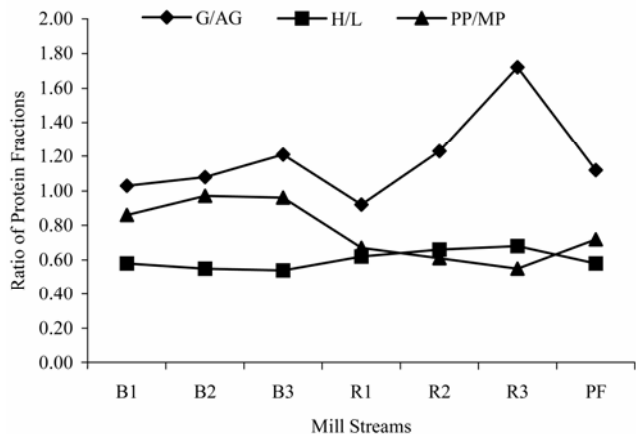


Fig. 3. Distribution ratios of various protein fractions. G/AG = gliadin/albumin+globulin, H/L = HMW-GS/LMW-GS, PP/MP = polymeric protein/monomeric protein (polymeric protein = HMW-GS and LMW-GS; monomeric protein = gliadin and albumin+globulin).

showed an increasing trend within break streams and a decreasing trend within reduction streams. The differences in HMW-GS were not significant, whereas the LMW-GS showed significant differences. Residue protein percentage showed a decreasing trend within break streams (significant) and an increasing trend within reduction streams (significant). These results indicated that the compositions of the various protein fractions in flour are different among mill streams.

Ratio of Protein Fractions

It has been shown that the proportions of various subunits are responsible for quality characteristics (Vensel et al 1997). In the present study, the ratio of protein fractions was statistically analyzed and the results are shown in Fig. 3. The ratio of gliadin/albumin+globulin showed an increasing trend within break streams (not significant) and within reduction streams (significant). The ratio of HMW-GS to LMW-GS did not show significant differences, but the ratio of polymeric to monomeric proteins (PP/MP) showed significant differences among mill streams. The PP/MP ratio in break streams was greater than in reduction streams. These results seem to indicate that the size differences (molecular weight distribution) of the polymeric proteins play a significant role in influencing breadmaking quality differences.

Relationship Between Protein Composition and Breadmaking Quality

The correlation coefficients between protein composition and breadmaking quality (loaf volume) were calculated, respectively, for break streams, reduction streams, and all streams. The results listed in Table III showed that some correlation coefficients were opposite for break streams compared with reduction streams. For example, for break streams, loaf volume was positively correlated with total protein content in flour, but for reduction streams, loaf volume was negatively and significantly correlated with total protein content. The quantity of protein composition in the break streams and the reduction streams were compared (Table II). Although total protein quantity showed an increasing trend in both break streams (from B1 to B3) and reduction streams (from R1 to R3), the loaf volume response was different (Table I). For example, loaf volume increased from B1 to B3 and decreased from

TABLE III
Correlation Coefficients Between Protein Composition and Loaf Volume^{a,b}

	Break Streams	Reduction Streams	All Mill Streams
TP	0.54	-0.80**	0.76**
G	0.44	-0.75*	0.24
AG	0.48	0.59	0.73**
H	0.51	0.74*	0.74**
L	0.53	0.41	0.84**
RP	0.42	-0.78**	-0.43*
G/FP	0.11	-0.71*	-0.77**
AG/FP	-0.47	0.66	-0.15
H/FP	0.37	0.84**	0.58**
L/FP	0.48	0.57	0.89**
RP/FP	-0.56	-0.68*	-0.92**
G/AG	0.30	-0.65	-0.44*
H/L	0.15	0.34	-0.25
PP/MP	0.76*	0.93**	0.96**

^a TP, total protein content in flour; G, gliadin content in flour; AG, albumin+globulin content in flour; H, HMW-GS content in flour; L, LMW-GS content in flour; RP, residue protein content in flour; LV, loaf volume; FP, flour protein; G/FP, gliadin content in flour protein; AG/FP, albumin+globulin content in flour protein; H/FP, HMW-GS content in flour protein; L/FP, LMW-GS content in flour protein; RP/FP, residue protein content in flour protein; G/AG, gliadin/albumin+globulin; H/L, HMW-GS/LMW-GS; PP/MP, polymeric protein/monomeric protein.

^b *,** Indicate significance at 0.05 and 0.01 level, respectively.

R1 to R3. This suggested that changes in protein composition are especially important for breadmaking quality. For gliadin and residue proteins, the correlation for break streams and reduction streams was similar to that for total protein content. However, HMW-GS and LMW-GS were positively correlated with the loaf volume for break streams and for reduction streams. Again, these results show that protein compositional differences influence breadmaking quality differences.

For all mill streams, LV was positively and significantly correlated with total protein content (Table III). Comparing the protein fractions, gliadin percentage in flour protein (G/FP) was negatively and significantly correlated with loaf volume. Albumin +globulin in flour was positively and significantly correlated with the loaf volume. HMW-GS and LMW-GS were positively and significantly correlated with loaf volume. Furthermore, the ratio of polymeric to monomeric proteins gave the highest correlation coefficient (0.96**). However, the ratio of HMW-GS to LMW-GS showed a negative, but not significant, correlation with loaf volume. Residue protein was negatively and significantly correlated with the loaf volume. These results show that the glutenin polymers, formed from distribution of HMW-GS and LMW-GS in their structure, make a significant positive contribution to breadmaking quality differences.

CONCLUSIONS

The milling process produces several mill streams in which the protein content and protein composition are different. For example, the flour of break streams has high protein content and high content of polymeric proteins, similar to a strong protein flour. In contrast, the flour of reduction streams has low protein content and low content of polymeric proteins, similar to a weak protein flour. As a result, the breadmaking quality of the respective mill streams is affected such that the loaf volume from break stream flours is much larger than that from reduction stream flours. These results can provide a guide for blending flours for use for different purposes.

Generally, a high protein quantity in flour is needed for good breadmaking quality. However, as shown in this study, the type of protein composition is more important than the protein quantity. The polymeric proteins (formed from HMW-GS and LMW-GS) play an important role in breadmaking quality differences. Gliadin, albumin+globulin, and residue proteins also are associated with breadmaking quality. Therefore, for improving wheat quality, the wheat breeder should pay attention to breeding for protein composition, for example, increasing polymeric protein quantity in grain to increase the polymeric protein content in flour and the ratio of polymeric to monomeric proteins. As shown in this study, the correlation coefficient between the ratio of polymeric to monomeric proteins and loaf volume was very high (0.96**). This information may be used as a tool to predict wheat quality in a wheat breeding program.

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