

# Relationships Between Protein Composition and Functional Properties of Wheat Flours<sup>1</sup>

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## ABSTRACT

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Relationships between protein composition (measured by size-exclusion high-performance liquid chromatography of sonicated flour suspensions in sodium dodecyl sulfate-buffer solution) and various flour quality parameters were investigated for a set of 15 hexaploid wheat cultivars grown at six different nitrogen fertilizer levels. As the flour protein content increased, the proportion of glutenin (peak 1) remained constant, the proportion of gliadin (peak 2) increased, and the proportion of albumin-globulin (peak 3) decreased. Only the glutenin measurements (i.e., the percentage of glutenin in the protein, PG, and the percentage of glutenin in flour, FG) showed consistent relationships with different quality parameters. Flour quality parameters depended in different ways on these two measurements. Extensigraph extensibility, farinograph dough development time, and loaf volume in a long fermentation baking test correlated

better with FG than with PG, accounting for 68-80% of the variation in these parameters. These correlations were higher than those with flour protein, indicating that the long-established relationships between these quality parameters and flour protein may reflect more fundamental relationships with flour glutenin. Other quality parameters (extensigraph resistance, mixograph dough development time, loaf volume in a rapid baking test) correlated better with the PG and thus appeared to depend on the balance between polymeric and monomeric proteins. However, less of the variation (38-56%) could be ascribed to PG. Evidence was obtained that an additional contribution to the variation in the latter parameters originated in the glutenin subunit composition, in particular, in the balance between the high and low molecular weight subunits.

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A cause-and-effect relationship has been established between the glutenin fraction and rheological and baking properties of wheat flour (Dimler 1965, Orth and Bushuk 1972, MacRitchie 1989). Glutenin represents a heterogeneous mixture of polypeptides linked together by intermolecular disulfide bonds. The polymeric nature of glutenin gives it its viscoelastic properties. Several

aspects of glutenin composition have been studied in attempts to explain variation in flour quality of bread wheats.

Much of the effort has been directed toward relating qualitative variation in the polypeptide composition of glutenin, particularly the high molecular weight (HMW) subunits, with variability in flour quality (Payne 1987). Recently the low molecular weight (LMW) subunits of glutenin also have been associated with flour quality in bread wheat (Payne 1987), and one survey showed that LMW and HMW subunits of glutenin together could account for about 70% of the variation in extensigraph resistance (Gupta et al 1991a).

Second, certain studies have indicated a relationship between the quantity of these two groups of subunits and flour quality

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parameters (Bietz 1984, Huebner and Bietz 1985, Gupta et al 1991b). Lawrence et al (1988), using near-isogenic lines, demonstrated that the amount of HMW glutenin related very strongly with dough properties. These studies have been useful in understanding the relationship between individual subunits and bread-making potential of wheat flour, and they have opened the possibility of modifying the functionality and/or amounts of the subunits in breeding programs using conventional or novel techniques.

A third approach involves analysis of glutenin in the native state (polymeric state). The proportion of glutenin or polymeric protein fraction has proved important in explaining the variation in flour quality (Huebner and Wall 1976, Bietz 1984, Dachkevitch and Autran 1989). Relationships, however, varied from significantly negative to significantly positive, probably because different levels of protein or glutenin extraction were obtained. Recently Singh et al (1990a) showed that sonication of flour suspensions in sodium dodecyl sulfate (SDS) solution can provide close to complete extraction of proteins and hence of glutenin. These authors found a strong positive relationship between the proportion of glutenin as measured by size-exclusion high-performance liquid chromatography (SE-HPLC) and several flour quality parameters in a set of 15 cultivars (Singh et al 1990b). The SE-HPLC procedure applied, however, gave considerable overlap between separation of different size class proteins. This procedure has since been improved to provide an almost baseline resolution of all three main classes (glutenins, gliadins, and albumins-globulins) of wheat protein in flour (Batey et al 1991).

In the present study, we applied the modified SE-HPLC procedure to analyze the protein composition of the same set of 15 cultivars, each grown at six nitrogen fertilizer levels. This enabled us to study the effect of varying protein content and to more fully explore the nature of the correlations.

## MATERIALS AND METHODS

### Flours

Flours were Buhler-milled from 15 cultivars grown at six nitrogen fertilizer levels corresponding to additions of 0, 12.5, 25, 50, 100, and 200 kg of nitrogen per hectare applied as  $\text{NH}_4\text{NO}_3$ . The cultivars were chosen to include a wide range of dough properties and baking potential. The HMW glutenin scores (Payne 1987) ranged from 5 to 10 and have been reported previously (Singh et al 1990b). Cultivars were grown at the Victorian Crops Research Institute, Horsham, Victoria, in the 1986 season and have been described previously (McCormack et al 1991). In total, 85 flour samples were analyzed (five samples were omitted because of insufficient material). Mean flour protein (FP) contents were 8.14, 8.89, 8.82, 9.76, 10.82, and 11.65% for the increasing nitrogen levels, respectively. These values differed significantly ( $P < 0.001$ ) between treatments with a standard error of the mean of 0.26%. Flour samples were stored in sealed containers below 5°C.

### Quality Measurements

Results from three different baking procedures were considered in the survey. One was a rapid microbaking test using 30.2 g of dry flour (MacRitchie and Gras 1973, MacRitchie 1976). Mixing time and water addition were optimized, and the formulation included 30 ppm of bromate and 1% sucrose. The other two baking tests were long fermentation procedures in which doughs were mixed for a fixed time of 2.5 min (Orth and Mander 1975), one without potassium bromate and the other with 20 ppm of bromate.

Mixograms were obtained with a 10-g mixograph using two procedures. The first used the same full formulation as the microbaking test. The second used only flour and water as ingredients, and the water addition corresponded to the farinograph water absorption. The purpose of this was to test whether the mixograph showed a different relationship with protein than the farinograph did because of a different formulation or because of the different mixing action. Although individual measurements differed between the two procedures, mixograph correlations with protein

composition data were similar and were different from those of the farinograph.

Farinograph and extensigraph data were determined by AACC standard methods 54-21 and 54-10 (AACC 1983), respectively. FP was measured by the Kjeldahl method, using a factor of 5.7 to calculate protein from nitrogen.

### Analysis of Protein Composition

Protein composition in terms of proportions of the three main groups of proteins (polymeric, gliadins, and albumins-globulins) were analyzed using SE-HPLC as described previously (Singh et al 1990a, Batey et al 1991). The flour samples (10 mg) were sonicated for 30 sec in 1 ml of 2% SDS-buffer solution (pH 6.9) and centrifuged for 20 min at  $15,900 \times g$ , and supernatants were filtered through 0.45- $\mu\text{m}$  filters (Singh et al 1990a). The supernatant containing total unreduced proteins was separated on a Protein Pak 300 column (Waters, Millipore, Milford, MA) using an elution buffer of 50% aqueous acetonitrile with 0.1% trifluoroacetic acid (Batey et al 1991). Three main peaks are resolved corresponding to polymeric proteins, gliadins, and albumins-globulins, respectively. The polymeric proteins (peak 1) are mainly made up of glutenins with minor amounts of HMW albumins and triticins. For simplicity, the proteins eluted in the first peak will be referred to as glutenins.

Two main measurements of composition are used in this study, percentage of glutenin in protein (PG) and percentage of glutenin

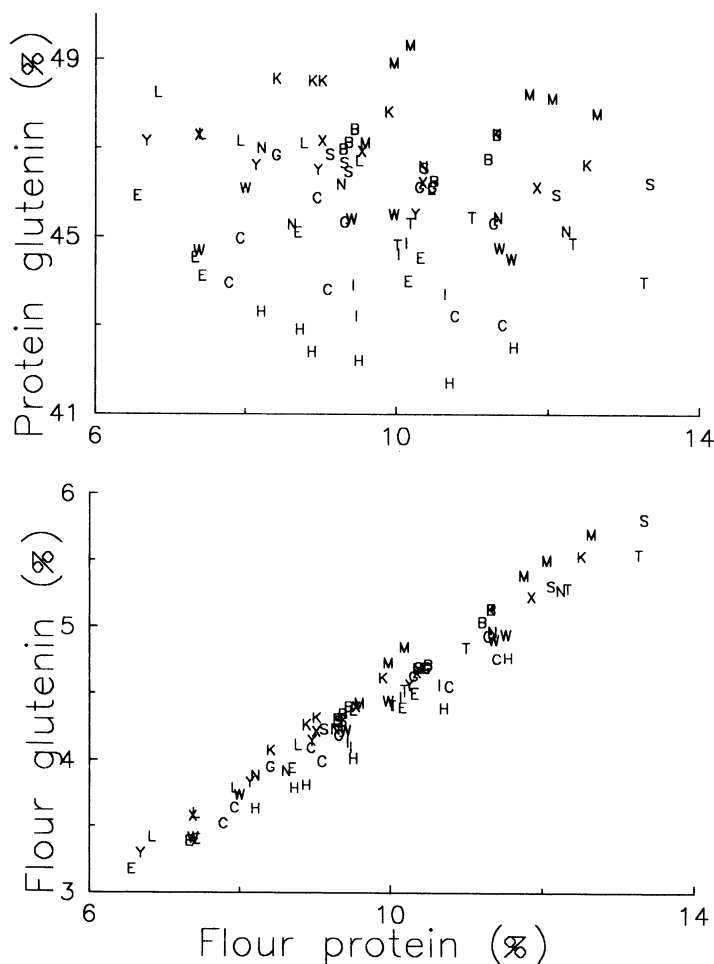


Fig. 1. Percent glutenin in protein (top) and percent glutenin in flour (bottom) as a function of percent protein in flour for  $n = 85$ . Correlation coefficients are  $-0.0924$  (top) and  $0.973^{***}$  (bottom). Wheat cultivars used: Chile 1B (C), Condor (N), Cook (K), Egret (E), Gabo (B), Gamenya (G), Halberd (H), Israel M68 (I), Mexico 8156 (M), Olympic (L), Osprey (S), Oxley (X), Timgalen (T), Wyuna (Y), WW 15 (W).

in flour (FG). They are defined as follows:  $PG = (\text{peak 1 area} / \text{total area of chromatogram}) \times 100$  and  $FG = (\text{percentage of protein in flour} \times \text{percentage of glutenin in protein}) / 100$ .

The composition of the polymeric protein (peak 1) in terms of its subunits was measured by densitometry of Coomassie blue-stained electrophoretic patterns in 12% acrylamide one-dimensional SDS-polyacrylamide gel electrophoresis gels (Lawrence and Shepherd 1980). For fraction collection, 30 mg of flour was sonicated in 1 ml of SDS-buffer, 50  $\mu$ l of the supernatant was loaded on the column, and peak 1 elutions from a total of five runs were pooled for each sample. The peak 1 protein fraction from the HPLC was collected, dialyzed against distilled water, freeze-dried, and redissolved in Tris-HCl buffer (pH 6.8) containing 2% SDS and 1.5% 2-mercaptoethanol before it was loaded onto the gels. Densitometry was carried out using a Shimadzu dual wave-length scanner CS-910 (Kyoto, Japan) and a Hewlett-Packard integrator model 3390A (Palo Alto, CA).

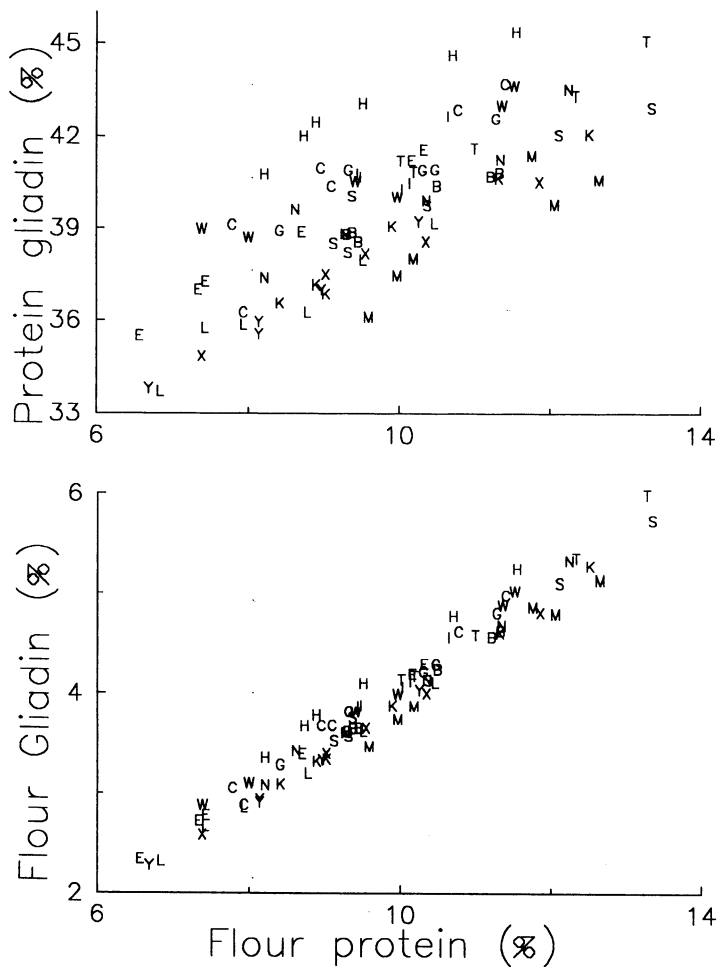
#### Statistical Analysis

The MSUSTAT statistical package (Lund 1986) was used for correlation analyses based on linear regression. The symbols \*, \*\*, and \*\*\* represent significant correlation at 5, 1, and 0.1% probability, respectively.

### RESULTS AND DISCUSSION

#### Variation of Protein Composition with Protein Content

As FP increases, no trend in PG is apparent (Fig. 1, top).

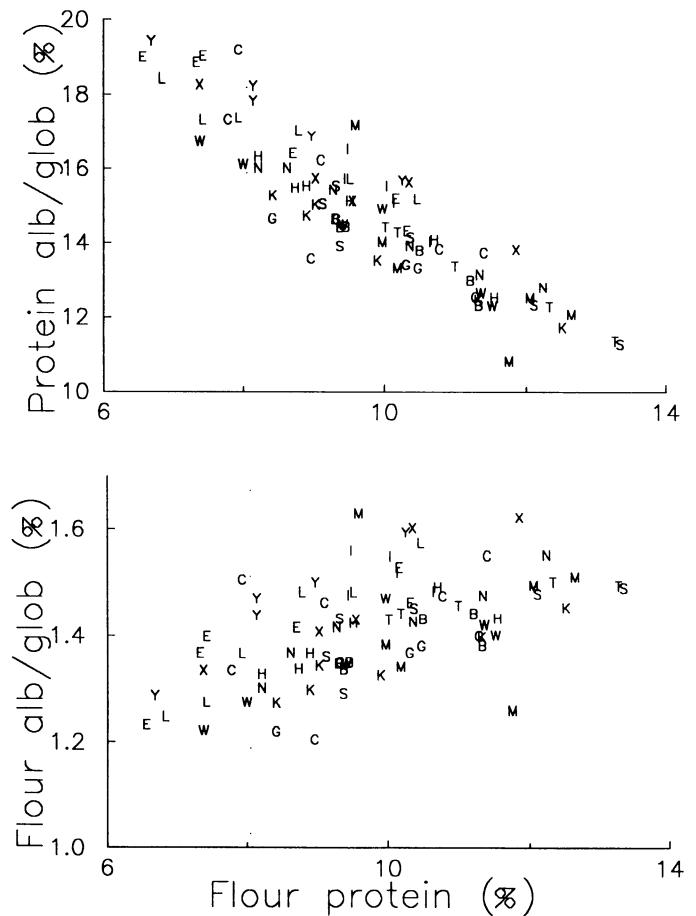


**Fig. 2.** Percent gliadin in protein (top) and percent gliadin in flour (bottom) as a function of percent protein in flour for  $n = 85$ . Correlation coefficients are 0.760\*\*\* (top) and 0.981\*\*\* (bottom). Wheat cultivars used: Chile 1B (C), Condor (N), Cook (K), Egret (E), Gabo (B), Gamenya (G), Halberd (H), Israel M68 (I), Mexico 8156 (M), Olympic (L), Osprey (S), Oxley (X), Timgalen (T), Wyuna (Y), WW 15 (W).

However, FG shows a very strong linear relationship with FP (Fig. 1, bottom). By contrast, the percentage of gliadin in protein increases with increasing protein content (Fig. 2, top), which is in agreement with previous work employing fractionation procedures (Doekes and Wennekes 1982). The more precise quantitative measurement of gliadin by the present method provides a more reliable confirmation of the result. Similarly to glutenin, the percentage of gliadin in flour is highly correlated with FP. In the case of the albumin-globulin group of proteins, the percentage in the protein decreased with increasing FP (Fig. 3, top). However, its total amount increased generally with increasing FP but over a narrow range (Fig. 3, bottom).

Examination of the correlation matrix of protein composition data with quality parameters showed that only glutenin is important. Therefore, only correlations with this group of proteins are considered in this paper. Where high correlations were found with gliadins and albumins-globulins, this occurred because of the close relationship with FP. As shown in Figure 1, the latter is highly correlated with FG, and in all cases the correlation of the given parameter with FG was higher than with flour gliadin or flour albumin-globulin.

Another clear result was that where significant correlations were found between quality parameters and the FG, these were always higher than correlations with the glutenin-gliadin ratio. This indicates that the balance between glutenin and all of the monomeric proteins is a more relevant measurement than the glutenin-gliadin ratio.



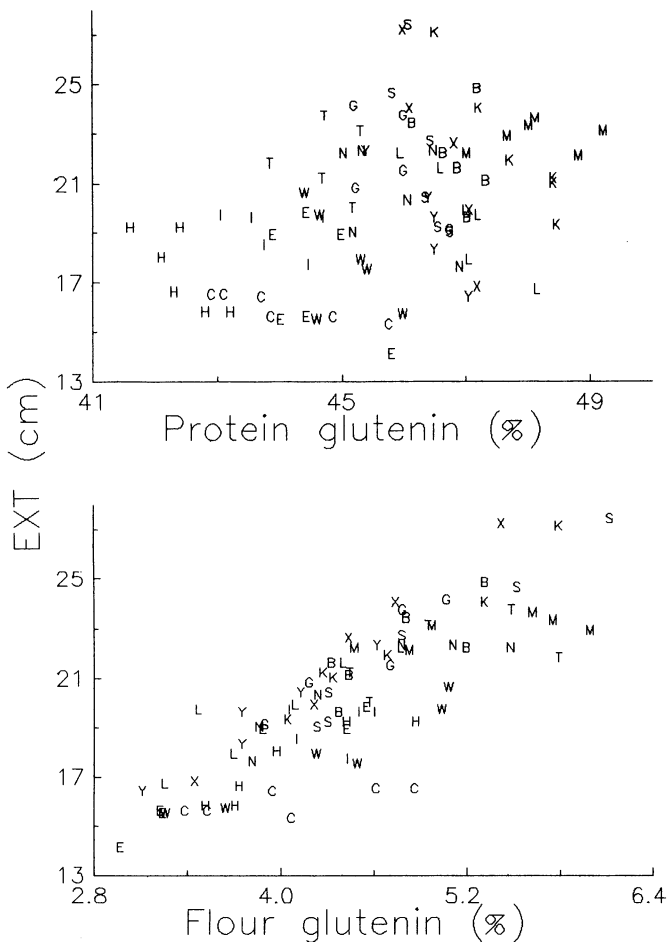
**Fig. 3.** Percent albumin-globulin in protein (top) and percent albumin-globulin in flour (bottom) as a function of percent protein in flour for  $n = 85$ . Correlation coefficients are  $-0.862$ \*\*\* (top) and  $0.547$ \*\*\* (bottom). Wheat cultivars used: Chile 1B (C), Condor (N), Cook (K), Egret (E), Gabo (B), Gamenya (G), Halberd (H), Israel M68 (I), Mexico 8156 (M), Olympic (L), Osprey (S), Oxley (X), Timgalen (T), Wyuna (Y), WW 15 (W).

### Extensigraph Parameters

Dough extensibility and PG ( $r = 0.392^{***}$ ) are significantly correlated, but dough extensibility is more highly correlated with FG ( $r = 0.831^{***}$ ) (Fig. 4). The latter accounts for about 70% of the variation in extensibility. In the case of maximum dough resistance ( $R_{max}$ ), the trends are reversed (Fig. 5).  $R_{max}$  is more highly correlated with PG, i.e., with the protein balance rather than with the total amount of glutenin. The  $r$  value is lower than that for the dough extensibility-FG relationship, and only about 45% of the variation in resistance is explained by PG.

Closer inspection of the data (Fig. 5, top) shows a clustering of points for different cultivars. Six cultivars in particular may be chosen to illustrate this point. Three cultivars (Halberd, Timgalen, and Mexico 8156) have higher  $R_{max}$  than would be predicted from the line of best fit, whereas others (Israel M68, Chile 1B, and WW15) have lower  $R_{max}$ . It therefore appears that some additional varietal characteristic is contributing to  $R_{max}$ . This is likely to be the variability in composition of the polymeric protein.

One way to assess this characteristic is to apply the HMW glutenin score (Payne 1987) for each of the six cultivars. The scores for the six cultivars do in fact parallel the behavior. The three cultivars with points falling above the line of best fit all have high scores: Halberd (8.5), Timgalen (8), and Mexico 8156 (10). The three cultivars with clearly lower values of  $R_{max}$  than predicted by the line of best fit have low scores: Israel M68 (5), Chile 1B (6.5), and WW15 (5). This scoring system has proved valuable for selection in breeding, but it is basically empirical and does not provide a real cause-effect explanation of the results.

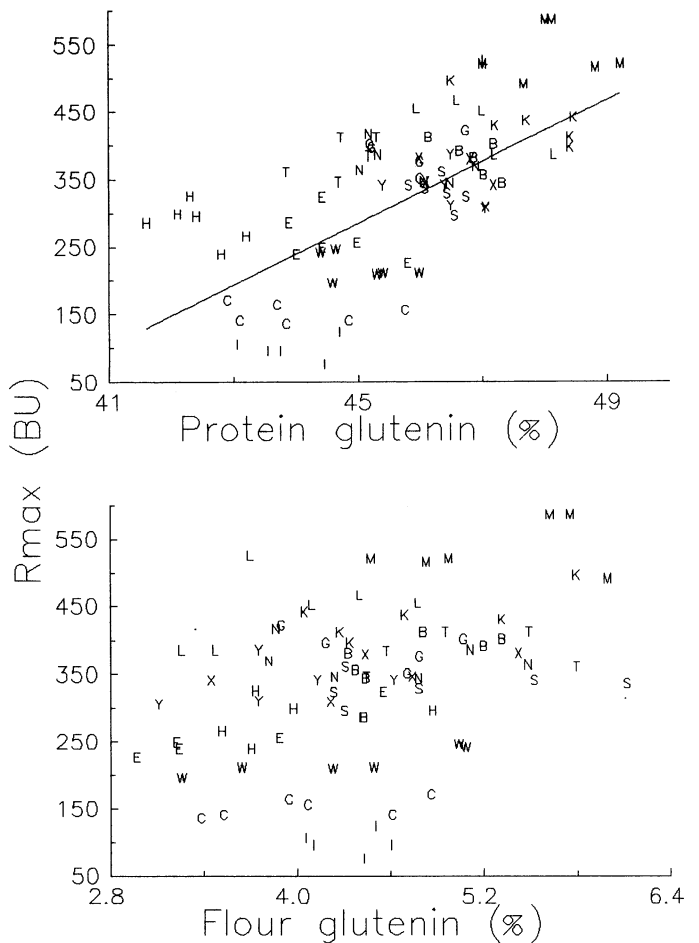


**Fig. 4.** Extensigraph extensibility (Ext) as a function of percent glutenin in protein (top) and percent glutenin in flour (bottom) for  $n = 85$ . Correlation coefficients are  $0.392^{***}$  (top) and  $0.831^{***}$  (bottom). Wheat cultivars used: Chile 1B (C), Condor (N), Cook (K), Egret (E), Gabo (B), Gamenya (G), Halberd (H), Israel M68 (I), Mexico 8156 (M), Olympic (L), Osprey (S), Oxley (X), Timgalen (T), Wyuna (Y), WW 15 (W).

One of the reasons that the three low  $R_{max}$  cultivars have low HMW glutenin scores is that they are all null at the *Glu-A1* locus; none of the three has HMW glutenin subunits encoded by the 1A chromosome. This suggests that there might be a dearth of HMW compared with LMW subunits. This was confirmed by densitometry of one-dimensional SDS-polyacrylamide gel electrophoresis patterns of the polymeric (peak 1) protein of three flour samples of Halberd (i.e., three different N treatments) and three of Israel M68 (Table I). The table shows values for the percentages of HMW glutenin subunits, HMW nonprolamins, LMW glutenin subunits, and LMW nonprolamins, and for the ratio of HMW- to LMW-glutenin subunits. The data indicate that the ratio of HMW- to LMW-glutenin subunits is consistently greater for Halberd than for Israel M68. In an earlier study, using genetic lines differing in varying amounts and number of HMW or LMW subunits of glutenin, we have shown that the HMW subunits have more pronounced effects on  $R_{max}$  and mixograph peak dough development time (MDDT) than do the LMW subunits when compared on an equal weight basis (Gupta et al 1991a). This parallels the properties of the cultivars: Halberd has a higher  $R_{max}$  than would be predicted on the basis of its protein glutenin content, whereas Israel M68 shows the opposite behavior.

### Dough Mixing Characteristics

In Figure 6, farinograph peak dough development time (FDDT) is plotted as a function of PG (top) and FG (bottom). Figure 7 shows corresponding plots for MDDT, measured using the full microbaking formulation. FDDT is found to be highly correlated



**Fig. 5.** Extensigraph maximum resistance ( $R_{max}$ ) as a function of percent glutenin in protein (top) and percent glutenin in flour (bottom) for  $n = 85$ . Correlation coefficients are  $0.665^{***}$  (top) and  $0.392^{***}$  (bottom). The line of best fit to the points is shown. Wheat cultivars used: Chile 1B (C), Condor (N), Cook (K), Egret (E), Gabo (B), Gamenya (G), Halberd (H), Israel M68 (I), Mexico 8156 (M), Olympic (L), Osprey (S), Oxley (X), Timgalen (T), Wyuna (Y), WW 15 (W).

with FG (Fig. 6, bottom) and not with PG (Fig. 6, top). On the other hand, MDDT correlates much better with PG than with FG (Fig. 7). A clustering of points for cultivars was observed for MDDT similar to that for  $R_{max}$ .

### Baking Performance

Figure 8 shows plots of loaf volume as a function of PG (top) and FG (bottom) for a long fermentation baking test using 20 ppm of bromate. Figure 9 shows corresponding plots for loaf volumes obtained in the optimized rapid microbaking test. For the long fermentation baking test without bromate, the correlation

coefficients for loaf volume against FG, PG, and FP were 0.450\*\*\*, 0.224\*, and 0.404\*\*\*, respectively.

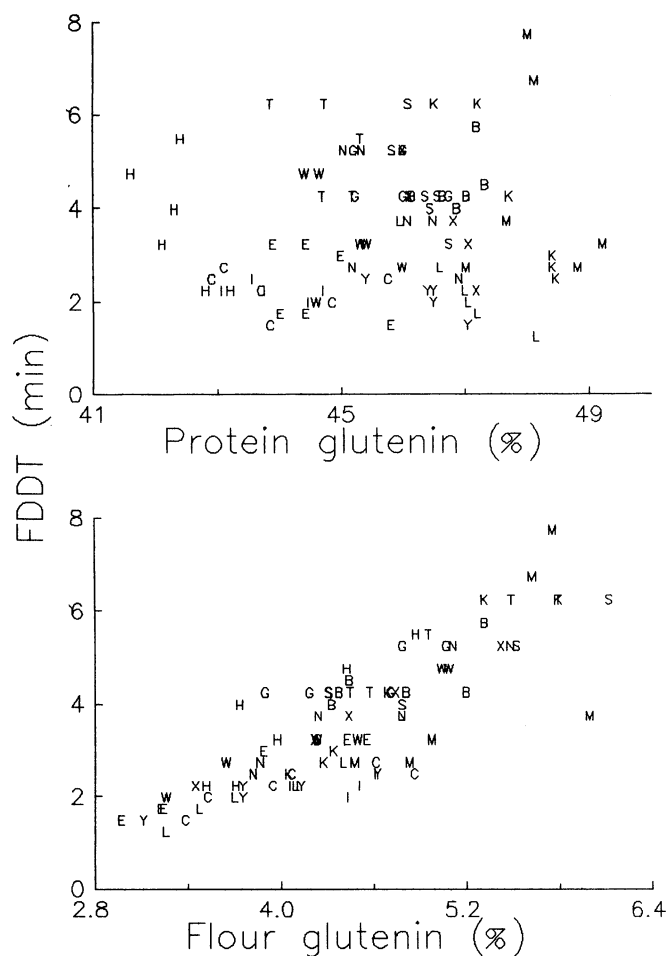
For the traditional long fermentation baking test with bromate, loaf volume correlates highly with FG (Fig. 8, bottom) and not with PG (Fig. 8, top). The rapid optimized baking test showed the reverse behavior. Loaf volume is better correlated with PG (Fig. 9, top) than with FG (Fig. 9, bottom). In Figure 8 (bottom), a number of points fall well below the general trend. These correspond to Mexico 8156, which has very long dough mixing requirements. This cultivar gave the highest loaf volumes in the optimized baking test but obviously performed below its potential

**TABLE I**  
Composition of Peak 1 Polymeric Protein as Determined by Densitometry of Electrophoretic Patterns

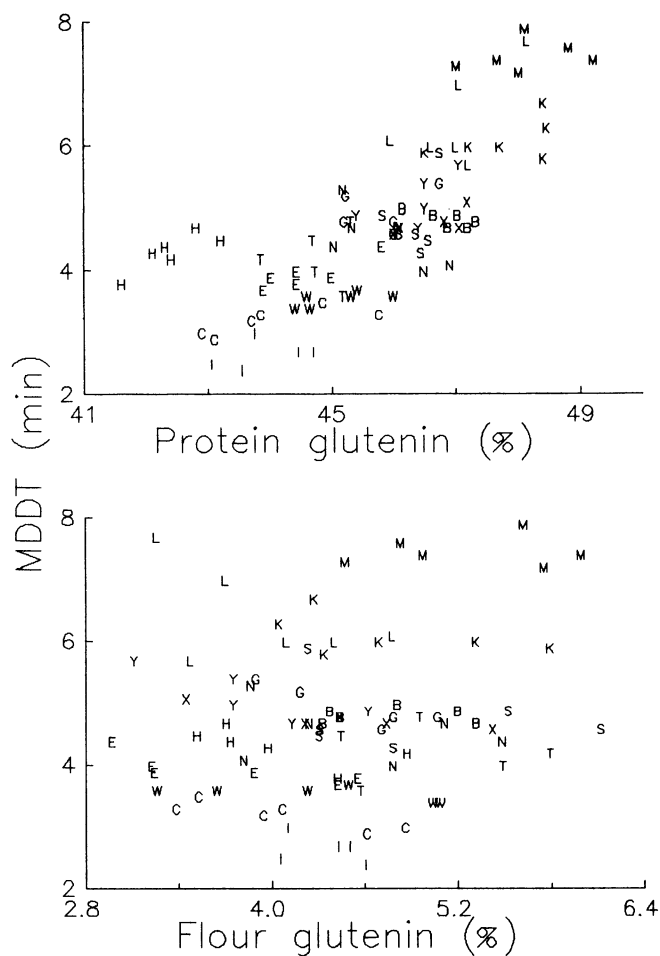
Cultivar <sup>a</sup>	HMW-Glu <sup>b</sup> (%)	HMW-NP (%)	LMW-Glu (%)	LMW-NP (%)	Ratio of HMW-Glu to LMW-Glu
Halberd N-2	20.4	10.5	63.1	6.0	0.32
Halberd N-4	21.6	11.4	60.0	7.0	0.36
Halberd N-5	20.6	9.6	62.7	7.1	0.33
					Average 0.34
Israel M68 N-1	11.8	9.8	70.6	7.8	0.17
Israel M68 N-2	11.4	8.6	74.3	5.7	0.15
Israel M68 N-3	14.9	8.5	68.1	8.5	0.22
					Average 0.18

<sup>a</sup> Each cultivar grown at three nitrogen fertilizer levels.

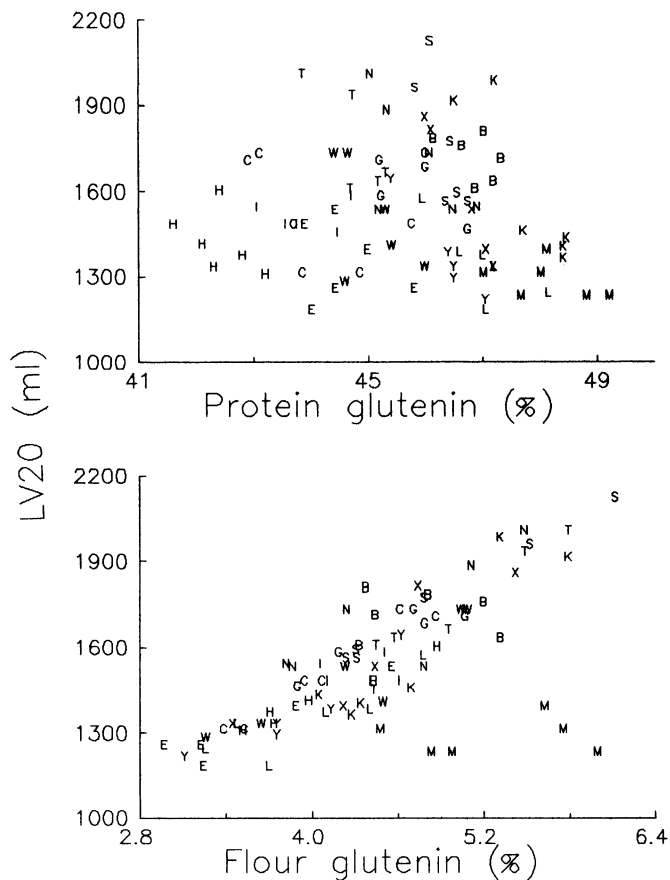
<sup>b</sup> HMW-Glu = high molecular weight glutenin subunits, LMW-Glu = low molecular weight glutenin subunits, HMW-NP = high molecular weight nonprolamins, LMW-NP = low molecular weight nonprolamins.



**Fig. 6.** Farinograph peak dough development time (FDDT) as a function of percent glutenin in protein (top) and percent glutenin in flour (bottom) for  $n = 85$ . Correlation coefficients are 0.083 (top) and 0.826\*\*\* (bottom). Wheat cultivars used: Chile 1B (C), Condor (N), Cook (K), Egret (E), Gabo (B), Gamanya (G), Halberd (H), Israel M68 (I), Mexico 8156 (M), Olympic (L), Osprey (S), Oxley (X), Timgalen (T), Wyuna (Y), WW 15 (W).



**Fig. 7.** Mixograph peak dough development time (MDDT) as a function of percent glutenin in protein (top) and percent glutenin in flour (bottom) for  $n = 85$ . Correlation coefficients are 0.748\*\*\* (top) and 0.159 (bottom). Wheat cultivars used: Chile 1B (C), Condor (N), Cook (K), Egret (E), Gabo (B), Gamanya (G), Halberd (H), Israel M68 (I), Mexico 8156 (M), Olympic (L), Osprey (S), Oxley (X), Timgalen (T), Wyuna (Y), WW 15 (W).



**Fig. 8.** Loaf volume (LV20) in a long fermentation baking test (20 ppm of bromate) as a function of percent glutenin in protein (top) and percent glutenin in flour (bottom) for  $n = 85$ . Correlation coefficients are  $-0.135$  (top) and  $0.685^{***}$  (bottom). Wheat cultivars used: Chile 1B (C), Condor (N), Cook (K), Egret (E), Gabo (B), Gamenya (G), Halberd (H), Israel M68 (I), Mexico 8156 (M), Olympic (L), Osprey (S), Oxley (X), Timgalen (T), Wyuna (Y), WW 15 (W).

**TABLE II**  
Correlation Coefficients for Quality Parameters<sup>a</sup>

Parameter	PG <sup>b</sup>	FG	FP
Extensibility	0.392 <sup>***</sup>	0.831 <sup>***</sup>	0.744 <sup>***</sup>
Farinograph development time	0.083	0.826 <sup>***</sup>	0.809 <sup>***</sup>
Loaf volume			
(long fermentation), 15 cvs.	$-0.135$	0.685 <sup>***</sup>	0.725 <sup>***</sup>
14 cvs.	$-0.007$	0.897 <sup>***</sup>	0.877 <sup>***</sup>
Maximum resistance	0.665 <sup>***</sup>	0.392 <sup>***</sup>	0.241 <sup>*</sup>
Mixograph development time	0.748 <sup>***</sup>	0.159	$-0.012$
Loaf volume (rapid)	0.616 <sup>***</sup>	0.436 <sup>***</sup>	0.297 <sup>**</sup>

<sup>a</sup> Based on linear regression.  $P = 0.05, 0.01, \text{ and } 0.001$  for \*, \*\*, and \*\*\*, respectively.

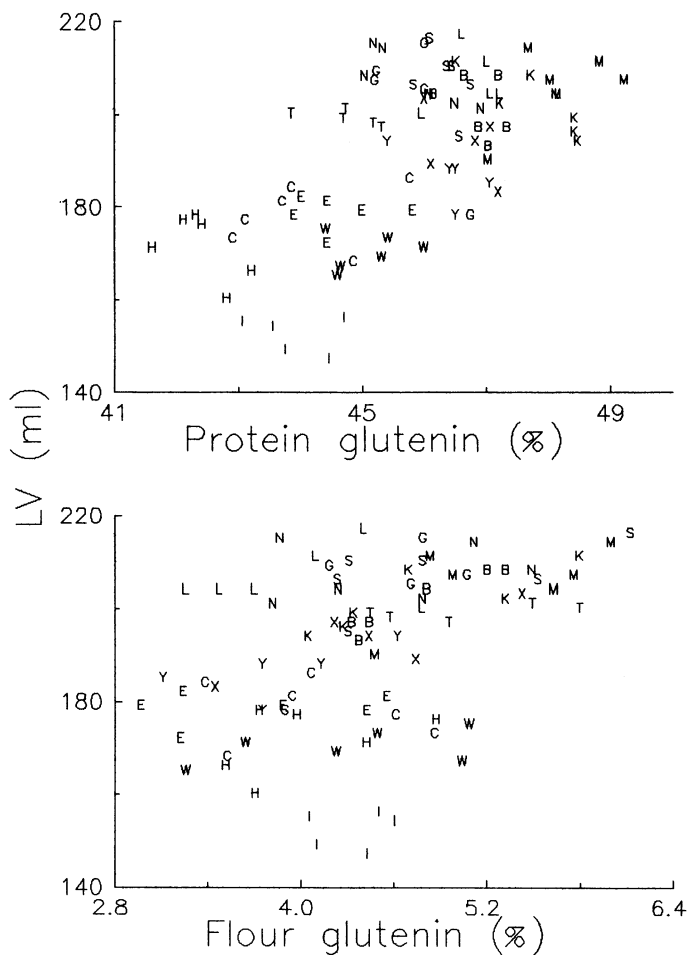
<sup>b</sup> PG = Percentage of glutenin in protein, FG = percentage of glutenin in flour, FP = percentage of protein in flour.

in the fixed mixing time test because of inadequate dough development. When Mexico 8156 was omitted (i.e., 14 cultivars considered), the correlation coefficient for the loaf volume-FG regression increased from 0.685 to 0.897.

## CONCLUSIONS

The survey showed that as FP increased, the proportion of glutenin did not vary systematically, the proportion of gliadin increased, and the proportion of albumin-globulin decreased. The total amount of each group increased, however, as both flour glutenin and flour gliadin contents were highly correlated with FP.

Table II summarizes the correlation coefficients obtained for



**Fig. 9.** Loaf volume (LV) in an optimized rapid microbaking test as a function of percent glutenin in protein (top) and percent glutenin in flour (bottom) for  $n = 85$ . Correlation coefficients are  $0.616^{***}$  (top) and  $0.436^{***}$  (bottom). Wheat cultivars used: Chile 1B (C), Condor (N), Cook (K), Egret (E), Gabo (B), Gamenya (G), Halberd (H), Israel M68 (I), Mexico 8156 (M), Olympic (L), Osprey (S), Oxley (X), Timgalen (T), Wyuna (Y), WW 15 (W).

each of the linear regression plots of Figures 4–9. It is evident that certain quality parameters (dough extensibility, FDDT, and loaf volume in a long fermentation baking test) correlate with FG. This accounts for about 68–80% of the variation in these parameters. If we compare the  $r$  values for these parameters against FG and FP (Table II), it is seen that they are higher for FG in the case of extensibility and FDDT. In the case of loaf volume, it also is higher if we omit the cultivar that underperformed due to insufficient mixing. Therefore, we conclude that the relationships that have been found between certain quality parameters (such as loaf volume) and FP probably are reflecting a more fundamental relationship with flour glutenin content.

For the quality parameters ( $R_{\max}$  and MDDT) that correlate better with the protein balance, i.e., PG rather than FG, it is notable that the  $r$  values are all similar (0.6–0.75) and lower compared with  $r$  values of over 0.8 for those parameters that relate better to FG. For these parameters, another factor is influencing the relationships: the composition of the polymeric protein, which varies between cultivars (Table I). Of particular importance is the ratio of HMW- to LMW-glutenin subunits. For a fixed glutenin content, the higher this ratio, the higher appears to be the magnitude of these flour quality parameters.

The data presented are for a set of cultivars grown at one location in one year. Further surveys are in progress to test the generality of the results. The information gained from this type of study should prove valuable as a basis for devising breeding strategies to manipulate flour properties for desired end uses.

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