

# Relationship Between Flour Protein Composition Determined By Size-Exclusion High-Performance Liquid Chromatography and Dough Rheological Parameters

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## ABSTRACT

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The relationship between protein composition, measured by size-exclusion high-performance liquid chromatography (SE-HPLC), and rheological dough properties, evaluated by alveographic test, were investigated in bread wheat cultivars grown at two locations in North and Central Italy. Total polymeric proteins were separated into two size groups on the basis of their solubility in 2% sodium dodecyl sulfate-phosphate buffer with and without reducing agents. Relative and absolute amounts of total polymeric proteins, determined by SE-HPLC after sonication, showed variable (nonsignificant to significant) relationships with rheological

properties, particularly dough tenacity (alveographic index *P*) and strength (alveographic index *W*). On the other hand, absolute or relative amounts of insoluble polymeric proteins correlated positively with dough strength and tenacity and accounted for up to 30–40% more variation in these parameters than did total polymeric proteins. Correlations of both absolute and relative amounts of soluble polymeric proteins with dough strength and tenacity were not significant and significantly negative, respectively, whereas the correlation with dough extensibility (alveographic index *L*) was significantly positive.

Gliadins and glutenins, the main gluten components, are responsible for dough extensibility and elasticity; these differences are very likely related to their different aggregative states. Gliadins are, in fact, monomeric proteins that can associate by hydrogen bonds and hydrophobic interactions, whereas glutenins are high molecular weight polymers formed by high molecular weight (HMW) and low molecular weight (LMW) subunits, held together by disulfide bonds. Reconstitution studies have shown that physical properties of doughs are determined primarily by the balance between these two types of proteins (MacRitchie 1987, Kim et al 1988), and relationships between the amount and ratio with flour technological properties have been established.

Size-exclusion high-performance liquid chromatography (SE-HPLC) has been used to relate the quantity of polymeric and monomeric protein fractions to breadmaking characteristics (Huebner and Bietz 1985, Dachkevitch and Autran 1989). Depending on the variety, it is usually found that a different proportion of gluten proteins (large aggregates) remains insoluble regardless of the solvent system used (Orth and Bushuk 1972, MacRitchie 1987). Therefore, comparison of the composition of only the extractable proteins from different cultivars can be misleading. Some results with SE-HPLC have indicated, in fact, that the magnitude of the first peak (polymeric proteins) was inversely related to flour properties of different cultivars (Bietz 1984, Orsi et al 1987, Dachkevitch and Autran 1989). However, other results with gel filtration chromatography (Huebner and Wall 1976, Bottomley et al 1982) and with SE-HPLC (Huebner and Bietz 1985) tended to show the opposite trend. These discrepancies may be explained by the condition of protein extraction (type and efficiency of the solvent) and by the extraction rate obtained because less of the larger-sized aggregates is solubilized from strong flours than from weaker ones (Orth and Bushuk 1972, Dachkevitch and Autran 1989).

A simple way to extract unreduced flour proteins, using mechanical shear with an ultrasonic probe, was proposed by Singh et al (1990a). In this system, the largest polymers break

down into smaller polymers, which facilitates their extraction; however, they are still sufficiently large to be separated from the monomeric proteins on the basis of the size. Consequently, a very strong correlation was found between the absolute and relative quantities of polymeric proteins, as measured by SE-HPLC, and several flour quality parameters in some wheat sets (Singh et al 1990b,c; Gupta et al 1992). A different procedure to quantitate insoluble aggregates and their relationships with alveographic parameters is reported in this article.

## MATERIALS AND METHODS

### Plant Material

Four bread wheat cultivars (Pandas, Maestra, Oderzo, and Spada), grown in two locations, St. Angelo Lodigiano (S.A.L.) and Viterbo (North and Central Italy, respectively) were used. In each location, four different temperature profiles were imposed during grain filling by anticipating or delaying sowing date or by covering the plots with plastic tunnels as described by Borghi et al (1995). In total, 32 flour samples were used for rheological and chromatographic analyses.

### Quality Assessment

Grains from each cultivar, tempered overnight (15% moisture), were milled on a Buhler experimental mill. Percent protein content ( $N \times 5.7$ ) was determined photometrically after micro-Kjeldahl digestion using the indophenol method performed automatically by an autoanalyzer (Carlo Erba, Milan). Alveographic tests were performed under conditions of constant dough water content using the ICC procedure (ICC 1980). Three alveographic indices were recorded for each sample: *P* corresponds to the height (mm) of the alveograph curve and is related to the tenacity of dough; *L* is an index of dough extensibility that corresponds to the length of the alveograph curve (mm); *W* ( $\times 10^{-4}$  J) corresponds to the total area of the alveograph curve and is related to the strength of the dough.

### Sample Preparation for SE-HPLC

**Protein extraction with sonication.** Total unreduced proteins were extracted from flour according to the procedure of Singh et al (1990a). To verify whether complete extraction of proteins was obtained with the microsonication technique, the residue was

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resuspended, using a vortex, in 1 ml of 0.1M Na-phosphate buffer + 2% sodium dodecyl sulfate (SDS), and centrifuged for 15 min (12,000 × g). The supernatant was discarded, and the residue was subjected to two further washings with distilled water. Washed residue was suspended in 1 ml of 0.1M Na-phosphate buffer containing 2% SDS + 0.3% dithiothreitol (DTT). After centrifugation, the clear supernatant was used for SE-HPLC separation.

**Protein extraction without sonication.** Total unreduced proteins were extracted according to the procedure of Dachkevitch and Autran (1989) with minor modifications. Flour samples (40 mg) were stirred for 2 hr at 60°C in the presence of 5 ml 0.1M Na-phosphate buffer (pH 6.9) containing 2% SDS. The supernatant obtained by centrifuging the samples (30 min at 3,7500 × g) was filtered through a 0.45-µm filter and then used (120 µl) for SE-HPLC separation.

Proteins that were insoluble in 0.1M Na-phosphate buffer containing 2% SDS were extracted from the residue with 5 ml of 0.1M Na-phosphate buffer containing 2% SDS + 0.3% DTT for 2 hr at 60°C. After centrifugation, the supernatant was filtered and used for SE-HPLC separation.

### HPLC Instrumentation

SE-HPLC was performed using an HPLC system (Waters, Milford, MA) comprising a model 650 pump and a model 484 variable wavelength detector. Samples, prepared as described above, were applied to a prepacked (10 × 30-cm) column of Superose 12 HR 10/30. A 0.05M Na-phosphate buffer (pH 6.9) containing 0.1% SDS and 0.08M NaCl was used as eluent at a flow rate of 0.4 ml/min. Absorbance was measured at 214 nm. Chromatograms were recorded on a C-R 3A integrator (Shimadzu, Kyoto).

When the extraction procedure without sonication was used, the proportion (percent) of area of each peak was calculated, considering the sum of the chromatographic areas from soluble and insoluble proteins to be the total chromatographic area. From chromatographic data, the relative proportion of soluble (peak 1 area) and insoluble (peak 4 and 5 areas) polymeric proteins present in the total protein extracted (sum of chromatographic areas of

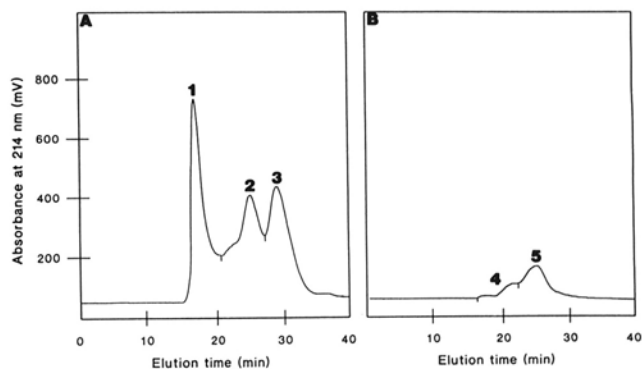
soluble and insoluble proteins), as well as in the total polymeric proteins, was calculated.

### Electrophoretic Analyses.

After collection of the fractions from each peak, proteins were precipitated with 12% trichloroacetic acid (TCA). After centrifugation (20 min at 12,000 × g), the supernatant was discarded and the pellet was washed with acetone at -20°C to remove the residual TCA. After centrifugation, acetone was removed by evaporation with nitrogen gas. Freeze-dried fractions were dissolved in 0.125M Tris-HCl buffer, pH 6.8, containing 2.75% (w/v) SDS, 10% (v/v) glycerol, and 10% (v/v) dimethylformamide. For reduced samples, the buffer also contained 1% DTT (w/v). Electrophoresis was done in 11% polyacrylamide gel using the Mini-protean II apparatus (Bio-Rad, Richmond, CA) at a constant current of 12.5 mA per gel for about 2 hr.

### Statistical Analyses

The data were analyzed using analyses of variance in which locations, cultivars, and temperature treatments were considered to be fixed effects. Correlation analyses based on linear regression between the chromatographic and technological parameters were also performed. Relationships between chromatographic and rheological parameters were estimated by a stepwise multiple regression procedure, considering the rheological parameters to be dependent variables and the chromatographic measurements to be independent ones. The prediction equation selected was the one that gave the maximum  $r^2$  value with the minimum probability value. All the statistical analyses were performed on a personal



**Fig. 1.** Size-exclusion high-performance liquid chromatographic separation of proteins from cultivar Pandas grown at Viterbo, Italy. **A**, unreduced proteins extracted in sodium dodecyl sulfate (SDS)-phosphate buffer solution using sonication. **B**, reduced proteins extracted in SDS-phosphate buffer containing 0.3% dithiothreitol from the residue of the same sample. Chromatograms for unreduced and residue proteins were divided into three and two peaks, respectively, as indicated.

**TABLE I**  
Range of Variation for Quality Characteristics of Four Cultivars Grown at Two Locations

Quality Parameters <sup>a</sup>	Environments (n = 2)	Cultivars (n = 4)	Treatments (n = 4)	Mean
FP, %	11.5–14.8	12.2–14.1	12.4–13.8	13.2
W	207–363	227–358	257–311	285
P	63–76	57–79	64–74	70
L	105–153	122–137	109–140	129

<sup>a</sup> FP = flour protein content; alveograph indices: W = strength, P = tenacity, L = extensibility.

**TABLE II**  
Protein Recoveries in the Supernatants After Sonication and Residues of Flours from Four Cultivars Grown at Two Locations, Monitored by Size-Exclusion High-Performance Liquid Chromatography (SE-HPLC)<sup>a</sup>

Cultivar	Location	Percent Flour Protein	Absolute HPLC Area × 10 <sup>6</sup>				Residue	Percent Total Proteins Not Extracted	Percent Polymeric Proteins Not Extracted
			Peak 1	Peak 2	Peak 3	Total			
Pandas	Viterbo	16.3	116.048	98.319	102.056	316.423	40.297	11.3	25.8
	S.A.L.	12.8	90.023	63.830	62.855	216.708	15.266	6.6	14.5
Spada	Viterbo	14.4	110.476	86.698	87.122	284.296	16.477	5.5	13.0
	S.A.L.	11.4	87.226	58.416	53.327	198.969	9.763	4.7	10.1
Maestra	Viterbo	16.0	108.840	100.490	102.685	312.015	32.467	9.4	23.0
	S.A.L.	12.8	83.033	64.761	68.361	216.155	13.083	5.7	13.6
Oderzo	Viterbo	13.4	89.435	87.338	80.919	257.692	9.625	3.6	10.0
	S.A.L.	12.1	80.510	70.760	63.662	214.932	6.760	3.0	7.7

<sup>a</sup> Values presented are averages of duplicate measurements with coefficient of variation <3%.

sonal computer using the STATGRAPHICS program package (STSC 1987).

## RESULTS

### Variation in Flour Protein Content and Rheological Parameters

Total protein content and alveographic indices were significantly affected by location and cultivars except for *L*, which was not significantly different among cultivars. The effect of temperature treatments was also significant for all the qualitative parameters considered but only at the 5% level (data not shown). A wide range for total protein content and the rheological parameters was observed (Table I). Ranges in flour protein content and *L* and *W* were notably larger across locations than those established among genotypes. In contrast, range in dough tenacity (*P*) was higher among cultivars than among locations. On the other hand, ranges for all the qualitative parameters considered across temperature treatments were generally lower than those detected among genotypes and environments.

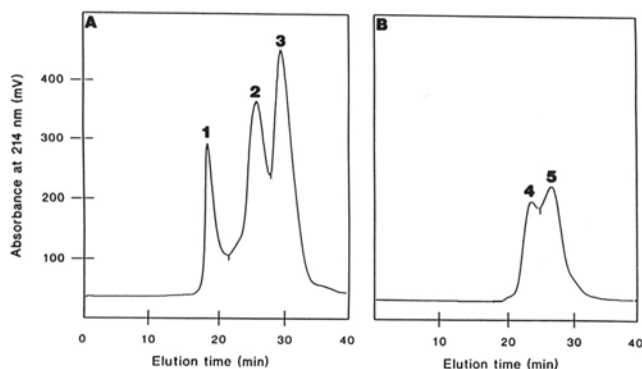
### Chromatographic Separations

**Evaluation of micro-sonication extraction.** Sonication of flour (Singh et al 1990a), and subsequent SE-HPLC separation, produced a chromatogram with three major peaks, as shown in Figure 1A. The residue remaining after microsonication was treated with a buffer containing DTT, and the resulting extract, which can be assumed to correspond to the polymeric glutenin, was analyzed by SE-HPLC. An elution profile (Fig. 1B) formed by two close peaks was obtained for all the samples analyzed, indicating that the extraction of protein was incomplete. The amount of insoluble material remaining in the residue after microsonication was calculated for the four bread wheat cultivars grown at two different locations and subjected to the same temperature treatments (Table II). A significant amount of protein was unextracted, ranging from 3.0 to 11.3%, with the efficiency of extraction of protein ranging from 88.7 to 97.0%. These extraction efficiencies were significantly lower in some cases than those previously reported by Singh et al (1990a,b), although the conditions of extraction were similar. The method of protein assay used by Singh et al (1990a,b) (directed assay by BCA method) was, however, different from the method reported in this article (indirect assay by SE-HPLC after solubilization of protein remaining in the residue). Also, the values of total protein content in the samples used in the present study were much higher (ranging from 11.4 to 16.3%) than those of the cultivars utilized by Singh et al (1990b).

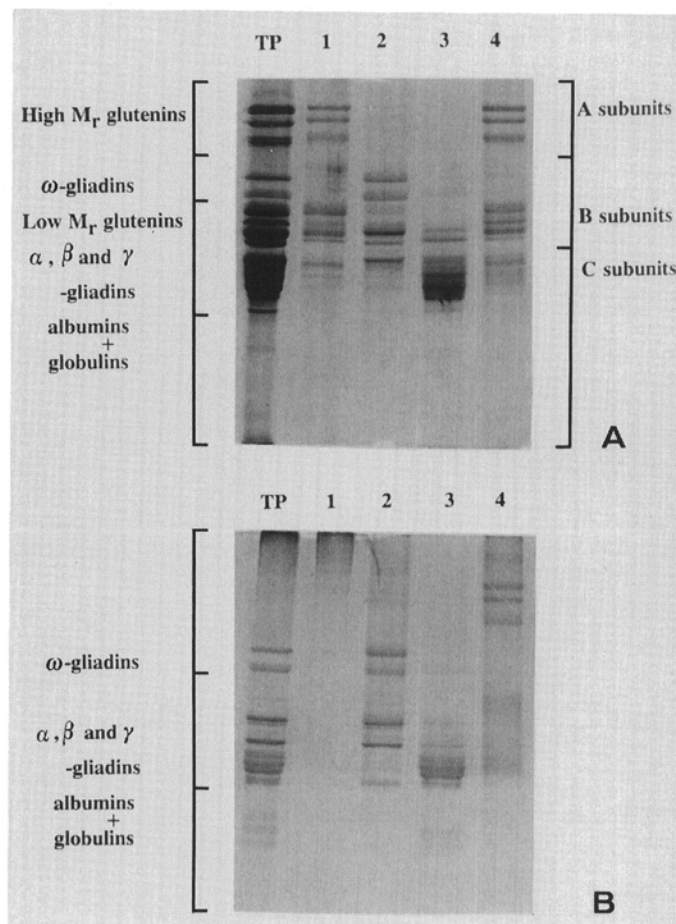
Extraction efficiency varied also among the cultivars. The proportion of total protein not extracted was, in fact, higher for culti-

vars Pandas and Maestra, which possessed excellent dough properties, than for cultivars Spada and Oderzo, with weaker dough properties. The differences between the relative and absolute quantities of protein not extracted by sonication from the sample with high (grown in Viterbo) and low (grown in S.A.L.) protein content were also higher in the cultivars Pandas and Maestra than in Spada and Oderzo, indicating that the effect of total protein content on the extraction efficiency was higher for cultivars possessing strong flours than for those with weak flours. The most likely explanation for these results is that, in the cultivars with high dough tenacity and strength, the increment of total protein content may affect the quantity and size of the polymer.

**Separation of polymeric proteins into two solubility groups and electrophoretic characterization of SE-HPLC peaks of soluble and insoluble proteins.** The flour proteins easily soluble in SDS buffer and those soluble only after reduction of disulfide bonds (residue proteins) were fractionated by SE-HPLC in three and two peaks, respectively (Fig. 2). Fractions corresponding to the three main peaks from the first extraction (soluble proteins) and the fourth fraction (peaks 4 and 5) corresponding to the insoluble proteins of cultivar Pandas (Fig. 2) were collected and subjected to SDS-PAGE in the presence or absence of reducing agents (Fig. 3). The electrophoretic separation of reduced proteins showed that the first peak of soluble proteins contained polypeptides of high and low molecular weight (lane 1 of Fig. 3a) that formed large aggregates, which, in absence of reducing agents, could not penetrate the pores of the separating gel (lane 1 of Fig. 3b). The banding patterns of reduced and unreduced proteins revealed that the other two peaks of soluble proteins comprised monomeric proteins; peak 2 (lane 2 of Fig. 3a and b) contained mostly



**Fig. 2.** Size-exclusion high-performance liquid chromatography separation of soluble (A) and insoluble (B) proteins in sodium dodecyl sulfate-phosphate buffer from the cultivar Pandas. Chromatograms for soluble and insoluble proteins were divided into three and two peaks as indicated.



**Fig. 3.** Sodium dodecyl sulfate-polyacrylamide gel electrophoretic separation of reduced (A) and unreduced (B) proteins contained in the fractions shown in Fig. 2. TP = total proteins; 1 = peak 1 proteins; 2 = peak 2 proteins; 3 = peak 3 proteins; 4 = peak 4 and 5 proteins.

gliadins with higher molecular weight ( $\omega$  and  $\gamma$  types), and peak 3 (lanes 3 of Fig. 3a and b) possessed gliadins with lower molecular weights ( $\beta$  and  $\alpha$  types) and albumin/globulins. However, the fractions of peaks 2 and 3 showed some overlap with each other, with peak 2 also containing some proteins with a range in molecular weight characteristic of  $\alpha$ - and  $\beta$ -gliadins (lane 2 of Fig. 3a and b) and with peak 3 comprising some components possessing a range in molecular weight characteristic of  $\gamma$ -gliadins, in agreement with previous results (Pasarihu et al 1992).

Composition of the protein fractions contained in peaks 1–3 extracted from sonicated flours were the same as those described previously for the three peaks of proteins extracted only by stirring (soluble proteins). Finally, the electrophoretic separation of the fourth fraction (peaks 4 and 5 in Fig. 2) showed the same banding pattern as peak 1 from soluble proteins (compare lanes 1 and 4 of Fig 3a), indicating that these two peaks comprised HMW and LMW glutenin subunits that formed larger aggregates, which were soluble in Na-phosphate buffer containing SDS only after chemical reduction of disulfide bonds by DTT.

### Relationships Between Quality Parameters and SE-HPLC Measurement of Protein Fractions

Correlation coefficients between quality attributes and SE-HPLC measurements for the two extraction procedures with and without sonication (soluble and insoluble proteins) are shown in Tables III and IV, respectively. Total protein content was highly positively correlated with absolute areas of individual peaks and in particular with total chromatographic area for each of the two extraction procedures used, although the correlation coefficient was relatively lower in that with sonication ( $r = 0.909$ ) than in the one without sonication ( $r = 0.957$ ). In fact, in the extraction procedure with sonication, the correlation coefficient between flour protein content and total polymeric proteins (peak 1) was lower than those detected between flour proteins and the amounts of the two fractions corresponding to the monomeric proteins (peaks 2 and 3, Table III). This could indicate that, in samples with higher protein content, incomplete extraction of large polymers was achieved by sonication.

In the extraction procedure of monomeric and insoluble proteins without sonication, on the contrary, polymeric proteins easily soluble in SDS buffer (peak 1) did not show very strong correlation with flour protein content (Table IV). This indicates that the total amount of these proteins increased generally with increasing flour protein content but over a narrower range than other protein fractions, in particular the insoluble ones. Studying the relationships between relative quantities of different protein fractions and flour quality parameters (detailed results not shown), we found significant positive correlations between the percent area of both monomeric and insoluble proteins and flour protein content, whereas there was a negative correlation between relative quantity of soluble polymeric proteins and total protein content ( $r = -0.487$ ). The most likely explanation for these results is that an increment of flour protein content may also positively

affect the formation of large polymers (insoluble polymeric proteins). Consequently, the relative amount of polymeric proteins easily extracted in SDS buffer decreases with increasing flour protein content. Thus, high-protein wheat should have a relatively higher proportion of larger aggregates insoluble in SDS buffer.

In both extraction procedures, total chromatographic area, which gives an indication of flour protein content, correlated significantly with all the alveographic indices (Table III and IV), but higher correlation estimates were obtained with  $L$  (extensibility) than with  $P$  (tenacity) and  $W$  (strength) of the dough.

Stepwise multiple regression was used to identify the protein fraction or combination of different monomeric and polymeric protein fractions that explained the largest amount of variation (i.e., had the highest  $r^2$  value) for each of the qualitative parameters in the utilized material. As shown in Tables III and IV, for both extraction procedures used,  $L$  was more strongly associated with the amount of monomeric proteins and particularly with absolute area of peak 2 ( $r = 0.832$  and  $0.867$ , with and without sonication, respectively) than with the amount of polymeric protein fractions and total chromatographic area. The model produced by stepwise regression analysis for  $L$  included only the quantity of monomeric proteins contained in peak 2 that explained 69 and 75% of the variability in extensibility index in the extraction procedures with and without sonication, respectively (Table V). Thus, it appears that positive correlations between polymeric protein and  $L$  are indirectly due to the effect of flour protein content because of the close relationship with these protein fractions.

Examination of the correlation matrices of protein composition data with alveographic parameters showed that polymeric protein fractions were particularly important for the determination of some other physical properties of doughs, such as tenacity and strength (Tables III and IV). In fact, for both the extraction procedures,  $W$  and  $P$  were more strongly correlated with the quantity of total polymeric and insoluble proteins than with the other protein fractions or total chromatographic area. Both these fractions have more effect on  $P$  than on  $W$ . However, there were significant differences between the relationships found with the

TABLE IV  
Correlation Coefficients Between Absolute Size-Exclusion High-Performance Liquid Chromatography Measurements of Unsonicated Flours and Quality Attributes<sup>a</sup>

Quality Parameters <sup>b</sup>	Peak 1	Peak 2	Peak 3	Residue	Total
FP	0.480	0.924	0.929	0.890	0.957
W	0.186	0.669	0.659	0.851	0.692
P	0.126	0.477	0.427	0.915	0.579
L	0.612	0.867	0.789	0.405	0.788

<sup>a</sup> Least significant values of correlation coefficient for  $n = 32$ : 0.339, 0.436, and 0.539 at 5, 1, and 0.1% probability, respectively.

<sup>b</sup> FP = flour protein content; alveograph indices:  $W$  = strength,  $P$  = tenacity,  $L$  = extensibility.

TABLE III  
Correlation Coefficients Between Absolute Size-Exclusion High-Performance Liquid Chromatography Measurements of Sonicated Flours and Quality Attributes<sup>a</sup>

Quality Parameters <sup>b</sup>	Peak 1	Peak 2	Peak 3	Total
FP	0.723	0.917	0.936	0.909
W	0.699	0.609	0.654	0.642
P	0.729	0.513	0.532	0.606
L	0.458	0.832	0.766	0.777

<sup>a</sup> Least significant values of correlation coefficient for  $n = 32$ : 0.339, 0.436, and 0.539 at 5, 1, and 0.1% probability, respectively.

<sup>b</sup> FP = flour protein content; alveograph indices:  $W$  = strength,  $P$  = tenacity,  $L$  = extensibility.

TABLE V  
Stepwise Regression Analysis Estimating the Relative Contribution ( $r^2$ ) of Size-Exclusion High-Performance Liquid Chromatography Measurements To Quality Attributes

Quality Parameters	Variable Entered	$r^2$
W	Sonicated flour	Peak 1 0.488**
	Unsonicated flour	Residue 0.724**
P	Sonicated flour	Peak 1 0.531*
	Unsonicated flour	Residue 0.837**
L	Sonicated flour	Peak 1 0.692**
	Unsonicated flour	Residue 0.752**

<sup>a</sup> Level of significance: \* =  $P < 0.01$ , \*\* =  $P < 0.001$ .

<sup>b</sup> Alveograph indices:  $W$  = strength,  $P$  = tenacity,  $L$  = extensibility.

alveographic indices in relation to the extraction procedures used. Absolute quantities of insoluble polymeric proteins gave, in fact, much higher correlation values with  $W$  and  $P$  than did the total polymeric proteins (Tables III and IV). Stepwise regression analysis indicated that the insoluble polymeric proteins explained 84 and 72% of the total variation in dough tenacity ( $P$ ) and strength ( $W$ ), respectively, whereas total polymeric proteins accounted for only 53 and 49% of the total variation in these two alveographic indices (Table V). These results indicate that the size distribution of polymeric proteins had a larger effect than the total quantity on the tenacity and strength of dough. This was confirmed by the fact that polymeric proteins easily soluble in SDS buffer (peak 1 without sonication) did not show significant correlation with  $P$  and  $W$ , whereas they were significantly correlated with  $L$  (Table IV), indicating that the flours with a lower amount of SDS-soluble polymeric proteins gave doughs with higher elasticity than extensibility. These results were confirmed by study of the relationship between the relative quantity of different proteins fraction and flour quality parameters (detailed results not shown). In fact, while the percentage of total polymeric proteins was not significantly associated with dough strength and tenacity ( $r = 0.102$  and  $0.144$  with  $W$  and  $P$ , respectively), the proportion of insoluble proteins in total protein ( $r = 0.440$  and  $0.568$  with  $W$  and  $P$ , respectively) or the proportion of insoluble proteins in total polymeric proteins ( $r = 0.524$  and  $0.678$  with  $W$  and  $P$ , respectively) were strongly correlated with these two physical properties of the dough. On the other hand, the proportion of soluble proteins in total proteins ( $r = -0.611$  and  $-0.633$  with  $W$  and  $P$ , respectively) and in total polymeric proteins were significantly negatively correlated with dough strength and tenacity.

$P$  was plotted as function of absolute quantity of total polymeric proteins (Fig. 4a) and absolute quantity of insoluble polymeric proteins (Fig. 4b) to show the different relationships between these two polymeric fractions and dough tenacity. Close inspection of the data in Fig. 4a shows a clustering of points for different cultivars. Two cultivars (Pandas and Maestra) had generally higher values of  $P$  than would be predicted from the line of best fit, whereas the other two (Spada and Oderzo) had lower values of  $P$ . This implies that some samples from the two groups of cultivars, even though they possessed similar quantity of total polymeric proteins, showed highly significant differences in dough tenacity and, hence, strength. It therefore appeared that some additional varietal characteristic, other than total polymeric proteins, was contributing to  $P$  and  $W$ , such as the ratio of HMW to LMW subunits of glutenin or the different types of individual subunits, which affected the molecular weight distribution of polymeric proteins and thus the quantity of insoluble polymeric fractions (Fig. 4b).

## DISCUSSION

Present results indicate that, even with sonication, a significant amount of protein could remain unextracted, with the extraction efficiency being related to the total protein content and the gluten characteristics of the samples. As previously shown (Gupta et al 1993), quantitative variation in total polymeric proteins may not be significantly related to dough strength parameters of different cultivars if there are large compositional differences in the polymeric proteins. Because the gluten network, on which dough rheological properties depend, is built up through interactions between protein chains during dough mixing and development, it is not surprising that polymer size fractionation of unmixed protein systems does not correlate fully with dough rheological properties.

An ideal method to study the relationships between the size of polymeric proteins and flour quality attributes should provide complete extraction of polymeric proteins in their native state and direct measurement of their sizes. Unfortunately, there is no such method available at present. However, it is possible to obtain an indication of the relative size distribution of polymeric proteins using solubility, which has been shown to reflect size (Huebner and Wall 1976, Graveland et al 1982, Gupta et al 1993). The easily soluble polymers have a smaller average size than those remaining in the residue, which are solubilized only by chemical reduction of disulfide bonds.

The results presented in this article show that the size of polymeric proteins has a greater effect than total quantity on tenacity and strength of dough. Unlike insoluble polymeric proteins, those easily soluble in SDS-buffer without DTT did not show relationships with dough strength and tenacity, but they were significantly related to dough extensibility. These results could indicate that polymeric proteins below a certain size do not contribute to tenacity and strength properties. Below a certain size range, they probably have a larger effect on dough extensibility than on dough tenacity. This is in accord with a study of the relationship between the physical properties of synthetic high polymers and molecular structure, where it was shown that molecules below a certain size limit (threshold level) do not participate in effective entanglements and thus do not contribute to tensile strength (MacRitchie 1992).

In contrast to dough strength and tenacity, dough extensibility showed a greater positive relationship with monomeric proteins than with polymeric ones. In particular, the model produced by stepwise regression analysis for  $L$  included, for both the extraction procedures utilized, only the monomeric proteins with higher molecular weight ( $\omega$ - and  $\gamma$ -gliadins) contained in peak 2. Although the functional role of these two gliadin classes in regard to extensibility properties is not known, it can be hypothesized

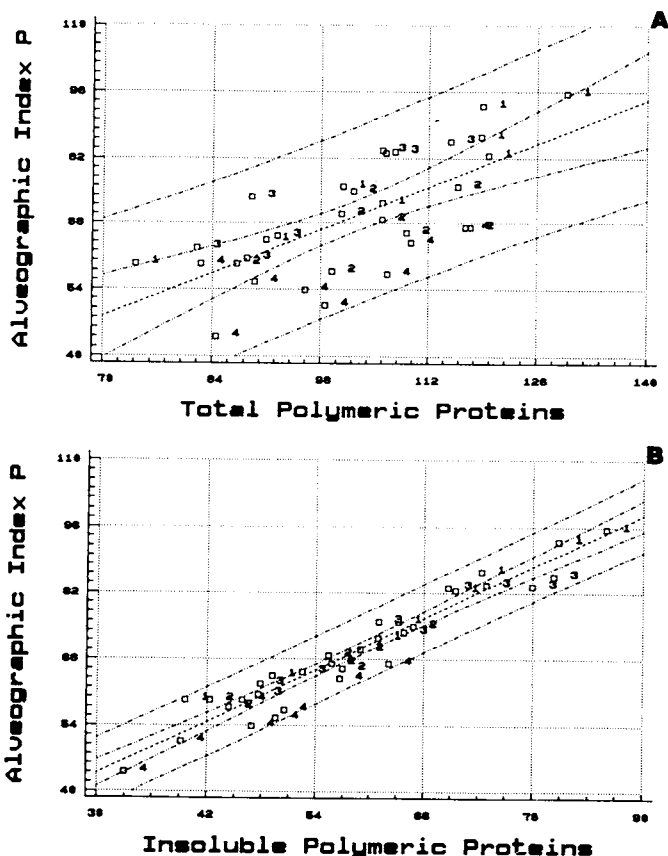


Fig. 4. Relationships between alveographic index  $P$  (tenacity) and absolute quantity of total polymeric (A) and insoluble polymeric (B) proteins. The arbitrary unit of the measurements for chromatographic parameters is millivolt  $\times$  centiminutes  $\times 10^6$ . 1 = Pandas; 2 = Spada; 3 = Maestra; 4 = Oderzo.



that the positive relationship found is mainly due to the  $\gamma$ -gliadins, which because of their particular hydrophobic properties can play an important role in the interactions among gluten proteins, positively affecting extensibility parameters. This hypothesis is based on results of Lafiandra et al (1994), who used RP-HPLC for separation and quantification of seed storage proteins and showed that dough extensibility was mainly affected by the amount of late-eluting, and hence very hydrophobic, gliadins, represented by  $\beta$  and  $\gamma$  components encoded by genes at *Gli-1* loci.

In conclusion, this article details the development of a new method for the extraction and subsequent fractionation of wheat flour proteins by SE-HPLC on a hydrophilic matrix, which can be used as a tool in breeding programs and in studies of environmental effects on wheat quality.

#### ACKNOWLEDGMENTS

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