Use of Sonication and Size-Exclusion High-Performance Liquid Chromatography in the Study of Wheat Flour Proteins. II. Relative Quantity of Glutenin as a Measure of Breadmaking Quality[1]

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ABSTRACT

Total unreduced proteins from flours of 15 wheat cultivars of diverse origin and breadmaking quality were fractionated by size-exclusion high-performance liquid chromatography (SE-HPLC) into three fractions of decreasing molecular size range, representing predominantly polymeric glutenin (including minor albumin and globulin aggregates), monomeric gliadins, and albumin/globulins, respectively. Relative quantity of glutenin (percent area of peak 1) was highly positively correlated with loaf volume, extensigraph dough resistance and extensibility, and mixograph peak development time of these flours ($r = 0.72$, 0.84, 0.84, and 0.84, respectively; $P = 0.003$–0.0001). Electrophoretic analysis of gliadins and glutenin subunits indicated that cultivars with a lower proportion of peak 1 usually have additional gliadin bands and/or a deficiency of glutenin subunits, providing a biochemical genetic basis for the SE-HPLC differences. A very strong negative correlation was found between relative quantity of albumin/globulin and flour protein content ($r = -0.92$, $P = 0.0001$). Absolute quantity of glutenin (area of peak 1 per milligram of flour) also strongly correlated with quality attributes, particularly extensibility, farinograph dough development time, and dough breakdown (r = 0.76, 0.89, and −0.65, respectively; $P = 0.009$–0.0001). Based on earlier studies with purified and enriched glutenin and gliadin fractions, we conclude that the proportion of these proteins, particularly glutenin, has a direct effect on functionality. Because very little flour is required (11 mg, half an endosperm), this method should provide a rapid small-scale, early generation test for breadmaking quality in wheat breeding.

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It has long been accepted that breadmaking quality of wheat flour is primarily determined by its proteins (reviewed by MacRitchie 1984). Hence, considerable effort has been made to elucidate which protein constituents are responsible for the quality differences. One early explanation was that flour quality varies with the glutenin-gliadin ratio (Fleurent 1896, Guess 1900). However, other cereal chemists believed this ratio to be of little significance (Grewe and Bailey 1927). Increasing evidence that both gliadin and glutenin are heterogeneous, plus confusion concerning methodology, caused use of this ratio to be abandoned (Sullivan 1965).

Although attempts to correlate glutenin-gliadin ratio with flour qualities of wheat cultivars produced conflicting results, reconstitution studies demonstrated that rheological properties of the reconstituted flours are highly influenced by relative amounts of these fractions (Lee and MacRitchie 1971, MacRitchie 1980). MacRitchie (1980) separated gluten proteins from strong and weak flours into two fractions by their differential solubility in dilute acid solutions. Although a sharp separation was not possible, the supernatant contained mainly gliadin, whereas the sediment contained mainly glutenin. By altering the ratio of these fractions and keeping total protein level constant, it was shown that the reconstituted flour strength, as measured by mixograph peak development time, was directly related to the proportion of sediment protein (glutenin) in both strong and weak flours. Recently, Kim et al (1988) obtained similar results using 70% ethanol to extract gliadins from gluten, considering the residue as glutenin. They showed that it is possible to dramatically modify gluten quality by altering the gliadin-glutenin ratio (22–56% gliadin), leading to a 20-fold variation in resistance and a 2.5-fold variation in extensibility.

These classic reconstitution studies—supported by early research on physical properties of purified gliadin and glutenin, which when hydrated produce weak-viscous and tough-elastic masses, respectively (Dimler 1965)—strongly suggest that the

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physical properties of doughs are determined primarily by the balance between these two types of proteins. A failure to correlate the glutenin-gliadin ratio with baking quality of cultivars is most probably due to inconsistent solubility of proteins from different cultivars. Furthermore, there is a variable overlap of components between protein fractions, depending upon the isolation procedure (Bietz and Wall 1980, Chakraborty and Khan 1988). To avoid these difficulties, attempts have been made to completely extract all flour proteins without reduction and to fractionate them by size using gel filtration. Two of the most successful solvents for this purpose have been the acetic acid/urea/cetyltrimethylammonium bromide (AUC) system (Meredith and Wren 1966) and sodium dodecyl sulfate (SDS) (Danno et al 1974). Thus, Huebner and Wall (1976) and Bottomley et al (1982) separated total flour proteins into four main fractions of decreasing size range: high molecular weight (HMW) glutenin, low molecular weight (LMW) glutenin, gliadin, and albumin/globulin. They found a direct relationship between the relative quantity of HMW glutenin and breadmaking quality in a few wheat cultivars. However, gel filtration is tedious and slow, and therefore not suitable for routine screening. Lack of reproducibility and quantitation are the other major problems associated with gel filtration.

Recent use of high-performance liquid chromatography (HPLC) to analyze wheat flour proteins has been more successful. Due to its speed, automation, quantitative capabilities, and small sample size requirement, it provides an ideal system for rapid screening of many wheat samples (reviewed by Bietz 1986). Bietz (1984) analyzed unreduced SDS extracts from wheats of differing breadmaking quality by size-exclusion (SE)-HPLC, and found an inverse relationship between amount of easily extracted HMW native glutenin and flour quality. He explained this on the basis that strong wheats have less acetic acid or SDS soluble glutenin, and more insoluble glutenin in their flours. The amount of insoluble fraction, often called residue or gel protein, correlates positively with flour strength, and it is the basis of residue protein and SDS sedimentation tests for breadmaking quality (Orth and Bushuk 1972, Moonen et al 1982). However, the inverse relationship between amount of HMW glutenin and flour quality (Bietz 1984) is affected by the level of protein extraction. In another set of wheats, Huebner and Bietz (1985) found this relationship to be direct, similar to the gel filtration results of Huebner and Wall (1976) and Bottomley et al (1982). Orsi et al (1987) and Dachkevitch and Autran (1989) again showed an inverse relationship between the amount of HMW glutenin and flour quality. Thus, although SE-HPLC is an excellent system for size-fractionation of wheat proteins, variable extractability of unreduced proteins is a major problem that has prevented its regular use (Bietz 1986).

Recently, we developed a simple way to completely extract unreduced flour proteins using mechanical shear with an ultrasonic probe (Singh and MacRitchie 1989, Singh et al 1990). In this system, the largest glutenin polymers break down into smaller polymers, facilitating their extraction. However, they are still sufficiently large enough to be separated on the basis of size from monomeric gliadin and albumin/globulin fractions. After a consistent complete extraction of proteins by sonication, the relative quantity of glutenin positively and significantly correlated with breadmaking quality of many diverse wheat genotypes. This also occurred with gluten fractions used in reconstitution studies. Here, we describe our results with 15 cultivars of diverse origin and breadmaking quality.

MATERIALS AND METHODS

Wheat Flour Samples

Wheat cultivars used varied widely in origin and quality attributes, including endosperm texture and dough strength (Table I). They were field grown at the Victorian Crop Research Institute, Horsham, Victoria, Australia. Flours were milled on a laboratory Buhler mill from grains of individual cultivars.

Quality Assessments

Protein content was determined by the Kjeldahl method (N × 5.7). Farinograph and extensigraph data were obtained by standard methods (Kent-Jones and Amos 1967). The test baking procedure was a short-time optimized process using 30.2 g of dry flour (MacRitchie and Gras 1973, MacRitchie 1976). Mixographs were performed with the full formulation used for the baking test.

Protein Extraction and SE-HPLC

Total unreduced proteins from normal and defatted flours were extracted with 2% SDS in 0.05*M* sodium phosphate buffer (pH 6.9) by sonication for 30 sec at power setting 5 (output 10 W) using a stepped microtip probe (3 mm diameter) as described in the preceding paper (Singh et al 1990). Sonication was done directly in 1.5-ml Eppendorf tubes with 11 mg of flour dispersed in 1 ml of solvent buffer. Samples were centrifuged at 12,000 × *g* for 20 min immediately after sonication. Clear supernatants were used for protein determination by bicinchoninic acid (BCA)

TABLE I
Quality Attributes[a] of 15 Wheat Cultivars

Cultivar	Flour Protein (%)	Milling Yield (%)	Loaf Volume (cm^3)	Extensigraph E (cm)	Extensigraph R (BU)	Extensigraph A (cm^2)	Farinograph DT (min)	Farinograph DB (BU)	Farinograph WA (%)	Mixograph MDT (min)
Chile 1B	9.0	67.2	179	16.0	148	30.4	2.00	141	55.2	3.0
Condor	10.3	75.1	192	21.9	328	91.3	3.50	75	62.8	3.8
Cook	9.8	76.0	206	21.5	420	116.5	4.00	66	62.5	5.8
Egret	8.6	71.4	177	18.5	240	58.0	2.75	116	54.8	3.7
Gabo	10.4	72.1	202	23.0	395	120.2	4.00	72	63.3	4.8
Gamenya	10.2	70.6	203	21.1	335	92.8	4.00	89	60.4	4.4
Halberd	9.4	75.8	175	17.6	283	63.2	3.00	59	59.1	4.1
Israel M68	10.0	66.4	145	17.3	60	15.8	1.75	139	60.2	2.5
Mexico 8156	11.7	75.3	202	23.3	510	157.4	6.50	35	59.8	7.7
Olympic	8.7	71.0	209	19.5	435	108.6	2.00	79	51.0	5.8
Osprey	10.3	76.6	208	22.3	313	91.6	3.75	71	63.8	4.1
Oxley	9.5	73.9	192	22.2	363	98.6	3.50	82	57.4	4.6
Timgalen	10.9	73.8	195	22.7	395	117.8	5.25	75	60.4	4.6
WW 15	9.3	75.6	167	17.5	193	45.1	3.00	74	62.5	3.4
Wyuna	8.1	74.9	176	19.2	295	72.1	1.75	124	51.0	4.8
Mean	9.8	73.1	189	20.2	314	85.4	3.38	86	59.0	4.5
SD	0.9	3.2	7	2.4	118	38.0	1.32	30	4.2	1.3
SE	0.2	0.8	5	0.6	30	9.8	0.34	8	1.1	0.3

[a] E = Extensibility; R = maximum resistance; A = area; BU = Brabender unit; DT = dough development time; DB = dough breakdown; WA = water absorption; MDT = mixograph peak development time.

method (Smith et al 1985) and for SE-HPLC fractionation (Singh et al 1990).

SDS-Polyacrylamide Gel Electrophoresis

SDS-polyacrylamide gel electrophoresis (PAGE) was similar to that described before (Singh and Shepherd 1988, Singh et al 1990). Gliadin and glutenin were extracted from 50 mg of flours using 1 ml of 70% aqueous ethanol in order to minimize albumin/globulin contamination (Gupta 1989). However, instead of overnight high-temperature treatment (Gupta 1989), we used 30-sec sonication as described above for HPLC samples. Supernatants were dried in a rotary vacuum evaporator and redissolved in 0.5 ml of SDS-PAGE sample buffer.

Gliadins were fractionated by one-dimensional (1-D) SDS-PAGE of unreduced proteins in a 7.5–15% gradient acrylamide gel with 1% cross-link. Glutenin subunits were fractionated by a modified two-step 1-D SDS-PAGE (Singh and Shepherd 1988). In the first step of this two-step procedure, gliadins and albumin/globulins were separated away from glutenin by a short (90 min) SDS-PAGE of unreduced proteins in a 8% acrylamide gel (0.8% cross-link). Then cathodal 10-mm wide strip of the separating gel (containing unreduced glutenin) was cut out using a long razor blade, and equilibrated for 1 hr at 55–60°C in 90 m*M* Tris-HCl, pH 8.0, containing 2.4% SDS, 10% glycerol, 0.001% bromophenol blue, and 10 m*M* dithiothreitol, to reduce the disulfide bonds. Thiol groups were then alkylated by a further 1 hr incubation of the gel strip at 55–60°C, after adding 4-vinylpyridine to a 20 m*M* concentration in the same equilibration solution. In the second step SDS-PAGE, the gel strip (now containing reduced subunits of glutenin) was carefully loaded onto a stacking gel of the second-step gel (8–16% gradient acrylamide gel, 1% cross-link) and electrophoresed at a constant 160 V for 16 hr. Other details were identical to the procedure in Singh and Shepherd (1988).

Statistical Analysis

All quality and HPLC data were analyzed by standard statistical methods. Means, standard deviations, coefficients of variation, correlations, and simple regressions were analyzed on an Apple MacIntosh computer system using the Statview program package. Two replicates were done for all the measurements; errors were usually less than ±2.0% of means, except for the BCA protein measurements which showed errors of up to ±6.0%

RESULTS

Variation in Grain Quality Attributes

Cultivars used in this study were chosen to represent a broad spectrum of breadmaking quality (Table I). Although most are Australian cultivars, they have diverse pedigrees (A. J. Rathjen, *personal communication*). Origins of other cultivars (Chile 1B, Israel M68, and Mexico 8156) are indicated by their names. The cultivars varied in endosperm texture (from very soft Israel M68 to Australian prime hard Cook and Osprey), as indicated by the range of milling yields (Table I). All quality attributes were highly variable, except for flour protein content, which had a narrower range (8.1–11.7%) representing only low to medium protein levels.

Relationships among quality attributes of these cultivars are shown in Table II. Flour protein content (FP) was highly significantly positively correlated with extensigraph dough extensibility (E), farinograph dough development time (DT), and water absorption (WA), and was significantly negatively correlated with dough breakdown. Importantly, dough resistance (R) was independent of flour protein content. Milling yield (MY), which reflects grain hardness, was strongly negatively correlated with dough breakdown but this could be because most soft wheats in this study produced weak doughs. Correlations of MY with other characters were weak or not significant (Table II). Bread loaf volume (LV) was highly positively correlated with extensigraph E, maximum resistance (R), area (A), and mixograph peak development time (MDT) but was weakly although significantly correlated with farinograph DT and dough breakdown. FP, MY, and WA were not significantly associated with LV. The lack of correlation between LV and FP suggests that differences in breadmaking quality of these cultivars were mainly due to protein quality, rather than quantity. Extensigraph E and R were highly positively correlated with each other (Table II), indicating that flours with higher resistance also have better extensibility. Extensigraph data (E, R, and A) were positively correlated with DT and MDT and negatively correlated with dough breakdown. A very strong correlation between R and MDT ($r = 0.94$) reflects the fact that both basically measure the dough strength. Finally, WA was significantly correlated only to FP. Most of these relationships among quality attributes are consistent with general observations in earlier studies (e.g., Campbell et al 1987).

Protein Extractability

Controlled sonication is an effective means to completely solubilize unreduced proteins from strong and weak wheat flours (Singh and MacRitchie 1989, Singh et al 1990). The extraction procedure used here was similar, except that most analyses were of nondefatted flours. For comparison, proteins were extracted without (simple stirring) and with sonication and results are depicted in Figure 1. The average protein extractability without sonication was only 72.1% (range 60.6–78.1%; coefficient of variation = 6.5). Furthermore, there was a significant negative correlation between protein extractability and LV, although the correlation coefficient ($r = -0.61$, $P = 0.016$) was not as high as previously reported between these two characters when dilute acetic acid (Orth and Bushuk 1972) and SDS (Moonen et al 1982) solutions were used as extractants. However, correlation of protein extractability with extensigraph R was much stronger ($r = -0.75$, $P = 0.0013$). After sonication, protein extractability averaged 100%, with a range of 90.4–109.8% and a coefficient of variation of 5.2. This variation did not correlate significantly with LV or R ($r = -0.22$ and -0.12; $P = 0.44$ and 0.68, respectively), and was probably due to higher standard errors

TABLE II
Correlation Coefficients Among Quality Attributes[a] of 15 Cultivars

				Extensigraph			Farinograph		
Attribute	FP	MY	LV	E	R	A	DT	DB	WA
MY	0.19								
LV	0.35	0.40							
E	0.67**[b]	0.49	0.75**						
R	0.42	0.56*	0.86***	0.80***					
A	0.57*	0.51	0.84***	0.88***	0.98***				
DT	0.86***	0.47	0.56*	0.78***	0.70**	0.79***			
DB	−0.59*	−0.74**	−0.62*	−0.67**	−0.77***	−0.77***	−0.76**		
WA	0.72**	0.33	0.14	0.43	0.06	0.19	0.54	−0.48	
MDT	0.37	0.45	0.69**	0.62*	0.91***	0.89***	0.64*	−0.68*	−0.09

[a] FP = Flour protein; MY = milling yield; LV = loaf volume; E = extensibility; R = maximum resistance; A = area; DT = dough development time; DB = dough breakdown; WA = water absorption; MDT = mixograph peak development time.
[b] *, **, *** = Significantly correlated at 5, 1, and 0.1% probability, respectively.

with the BCA method. Complete protein extraction was also indicated by a very small variation (after sonication) among cultivars for total HPLC area per milligram of flour per unit protein (range 18.5–20.3; CV = 2.8), which is another, probably more accurate measure of protein extractability. Comparison of protein extractability from defatted (Singh et al 1990) and nondefatted Mexico 8156 flour (Fig. 1) indicated that in either case 30 sec of sonication extracted more than 95% of the flour proteins.

SE-HPLC Measurements

Proteins from the wheat cultivars were fractionated by SE-HPLC into three peaks, representing predominantly glutenin, gliadin, and albumin/globulin (Singh et al 1990). Normal and defatted flours gave very similar results. For simplicity, averaged data from normal flours are shown in Table III. The lowest points on the SE-HPLC curves (valleys) were used as cutoff points for calculating areas under individual peaks. Effect of flour defatting and using fixed times as cutoff points between peaks is considered later. The relative quantities (termed percent areas) of peaks 1, 2, and 3 (Table III) represent proportions of glutenin, gliadin, and albumin/globulin, respectively, in the total protein. Areas per milligram of flour represent absolute quantities of these proteins per unit weight of flour. Variations for relative and absolute quantities of protein fractions among cultivars were small but statistically significant (Table III). The highest variability was for the relative quantity of glutenin (27.1–36.4%), whereas absolute quantity of albumin/globulin varied least (3.8–4.9 units). Typical SE-HPLC chromatograms are shown in Figure 2.

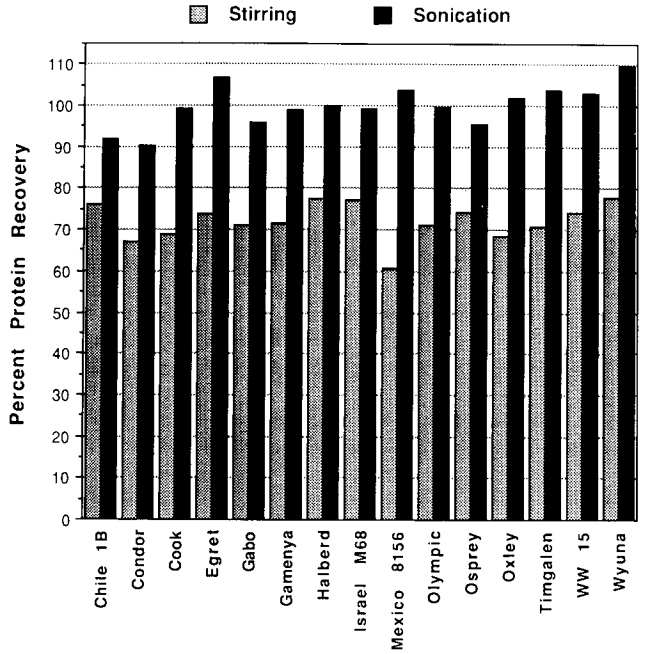

Fig. 1. Extractability of unreduced proteins (based on bicinchoninic acid protein determination on the extracts) from 15 wheat flours without and with sonication. Both control and sonicated samples were stirred by rotating at 45 rpm for 30 min before centrifugation. Standard errors were as large as 4% (stirring) and 6% (sonication) of the means.

Relationships among different HPLC parameters are shown in Table IV. Percent area of peak 1 was significantly negatively correlated with percent areas for peaks 2 and 3, but correlation between percent areas for peaks 2 and 3 was not significant. In contrast to percent areas, absolute areas of individual peaks were highly significantly positively correlated with each other and total area, which gives an indication of flour protein content since proteins were completely extracted by sonication (Fig. 1). There was a very strong negative correlation between percent area of peak 3 (relative quantity of albumin/globulin) and absolute peak areas (Table IV). This is because amount of albumin/globulin per unit weight of flour is quite stable (Table III, last column; also Doekes and Wennekes 1982). Consequently, relative amount of albumin/globulin in the protein decreases with increasing flour protein content. Thus, high-protein wheats should have relatively higher proportions of glutenin and/or gliadin in their seed proteins.

Associations Between HPLC Parameters and Quality Attributes

Following initial study by Bietz (1984), several authors reported significant associations between SE-HPLC parameters (particularly proportion of first peak) and breadmaking quality (Huebner and Bietz 1985, Orsi et al 1987, Dachkevitch and Autran 1989). However, correlations varied from strongly negative to strongly positive with increasing levels of protein extraction (Bietz 1986, Dachkevitch and Autran 1989). No significant correlation was found with an intermediate level of protein extraction (Dachkevitch and Autran 1989). In the present study, sonication removed the variation caused by incomplete protein extraction.

Correlation coefficients between SE-HPLC measurements and quality attributes are shown in Table V. FP was highly negatively correlated with the proportion of albumin/globulin in total protein (percent area of peak 3). This agrees with the conclusion drawn from HPLC data alone (Table IV and text), where absolute peak areas reflect FP. Furthermore, FP was highly positively

TABLE III
Size-Exclusion High-Performance Liquid Chromatography of 15 Wheat Cultivars

Cultivar	Percent Area			Area[a] (per milligram of flour) $\times 10^{-5}$		
	Peak 1	Peak 2	Peak 3	Peak 1	Peak 2	Peak 3
Chile 1B	28.7	41.8	29.5	4.4	6.5	4.5
Condor	32.2	40.7	27.0	5.4	7.1	4.6
Cook	33.9	39.6	26.5	5.2	6.6	4.3
Egret	30.0	40.9	29.1	4.1	5.6	3.9
Gabo	31.9	40.8	27.3	5.3	7.2	4.5
Gamenya	31.3	41.8	26.9	5.5	7.8	4.9
Halberd	27.1	43.9	29.0	4.5	7.1	4.5
Israel M68	28.0	43.1	28.9	4.8	7.2	4.7
Mexico 8156	36.4	38.9	24.7	6.6	8.3	4.9
Olympic	31.9	38.2	30.0	4.5	5.7	4.1
Osprey	31.6	40.6	27.8	5.2	7.0	4.7
Oxley	33.0	38.7	28.4	4.9	6.2	4.3
Timgalen	32.4	40.7	26.9	5.5	7.6	4.8
WW 15	29.4	41.8	28.6	4.5	6.2	4.1
Wyuna	31.4	37.7	30.9	4.0	5.1	3.8
Mean	31.3	40.6	28.1	5.0	6.7	4.4
SD	2.4	1.8	1.6	0.7	0.9	0.4
SE	0.6	0.5	0.4	0.2	0.2	0.1

[a]The arbitrary unit of this measurement is millivolts × centiminutes.

correlated with absolute area under each peak and total area, though variation for area of peak 3 was very small (Table III). This indicated a consistent solubilization of flour proteins from the diverse cultivars by sonication. As expected, the extensigraph E and the farinograph DT, dough breakdown, and WA, which significantly correlated with FP (Table II), also significantly correlated with total HPLC area (Table V). However, HPLC could separate the total protein quantity effect into three components, permitting determination of the actual fraction responsible for these associations. As shown in Table V, absolute quantities of gliadin and albumin/globulin (areas per milligram of flour peaks 2 and 3, respectively) were correlated significantly only with DT and WA. These two characters were also strongly correlated with the absolute quantity of glutenin (area per milligram of flour peak 1), suggesting a general relationship of FP to DT and WA. WA is also affected by starch damage (represented by MY), but this relationship was not significant here (Table II). Amount of albumin/globulin was only weakly associated with either character. In contrast to gliadin and albumin/globulin, absolute quantity of glutenin was significantly correlated with all other quality attributes except MY (Table V). Thus, it appears that positive correlations between FP and flour quality attributes (particularly E, A, DT, and dough breakdown) were mainly due to the glutenin fraction.

In addition to studying associations with absolute quantities of protein fractions, SE-HPLC was extremely useful for studying the relationship between relative quantities of these proteins and grain/flour quality. The relative quantity of glutenin (percent area of peak 1) highly significantly positively correlated with LV, extensigraph E, R, and A, farinograph DT, and mixograph MDT (Table V). Also, it significantly but negatively correlated with dough breakdown, a measure of dough weakness (Table II). We emphasize that although peak 1 is predominantly glutenin, it also includes significant amounts of polymeric triplet proteins and HMW albumins (Singh et al 1990). So in fact peak 1 represents total polymeric proteins, rather than only glutenin. However, there is little variation in the amounts of these minor proteins between cultivars as judged from their staining intensities in the gels (Singh and Shepherd 1985, Gupta 1989).

Relative quantity of gliadin (percent area of peak 2) did not show very strong correlations with quality attributes; however, it was significantly negatively correlated with LV and parameters of dough resistance (R, A, and MDT in Table V). These results agree with flour and gluten reconstitution studies showing that an increase in proportion of gliadin gives weaker dough gluten, whereas an increased proportion of glutenin has the opposite effect (Lee and MacRitchie 1971; MacRitchie 1980, 1987; Kim et al 1988). The relative quantity of albumin/globulin (percent area of peak 3) significantly correlated with E, R, A, DT, dough breakdown, WA, and MDT (Table V). However, these relationships followed an inverse trend to that observed between FP and these same quality attributes (Table II). Because percent area of peak 3 is very strongly negatively correlated with FP ($r = -0.92$, Table V), it is most likely that correlations between the proportion of albumin/globulin and quality attributes are indirectly due to effect of FP. However, there is evidence to suggest that correlation with dough breakdown may be real (MacRitchie, *unpublished*).

Percent heights of the HPLC peaks gave similar associations with quality attributes, as did percent areas, but due to slightly different peak shapes (Fig. 2 and Singh et al 1990) it is thought that percent areas would more accurately estimate the relative quantities of proteins. For simplicity, data on peak heights are not included here.

Biochemical Basis for SE-HPLC Differences

To provide a biochemical basis for the variation in relative quantities of HPLC peaks, proteins from 15 cultivars were fractionated by SDS-PAGE (Fig. 3). Because the amount of albumin/globulin per unit weight of flour is very stable (Table III, last column; also Doekes and Wennekes 1982), most variation is expected be due to gliadins and glutenins. It was postulated that cultivars with a lower proportion of glutenin (percent area of peak 1) would have additional gliadins and/or fewer glutenins (HMW and/or LMW subunits) in their proteins. In fact, Figure 3A demonstrates that the poor quality cultivars Chile 1B, Egret,

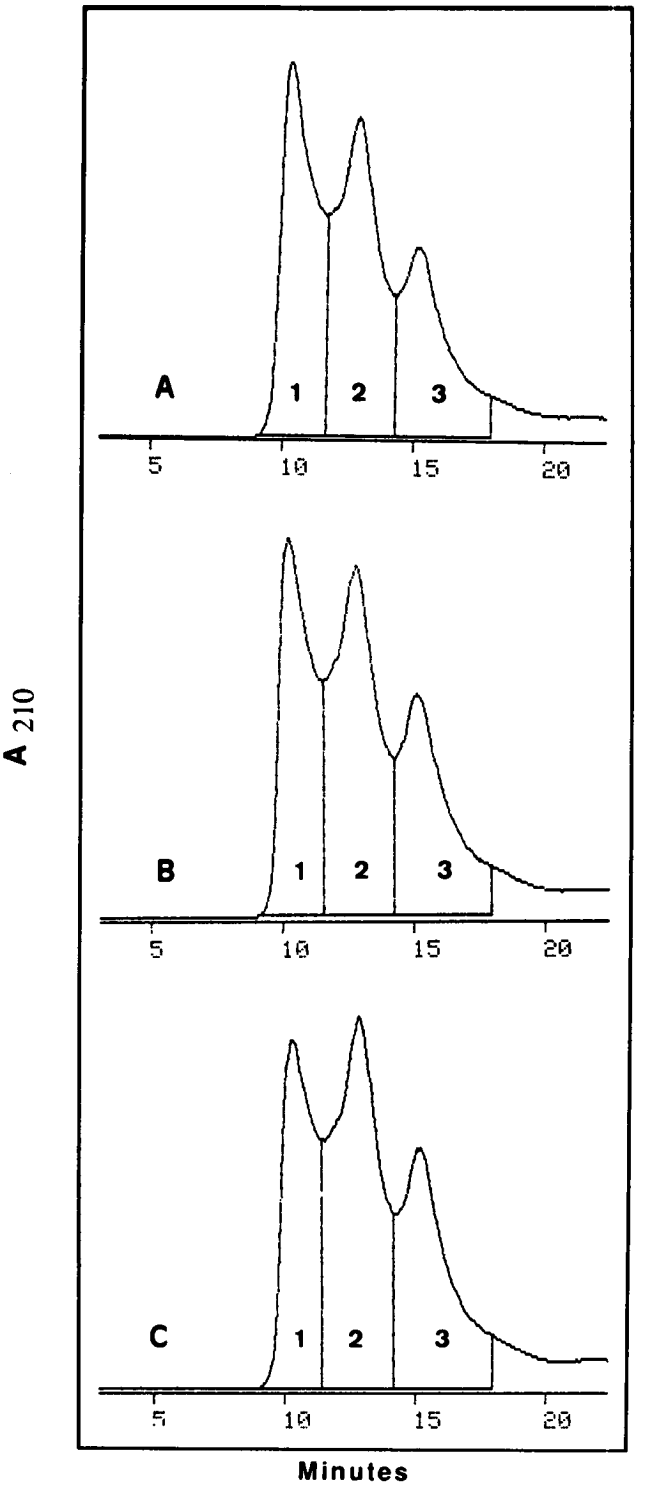

Fig. 2. Size-exclusion high-performance liquid chromatography comparison of unreduced total protein extracts from flours of wheat cultivars **A**, Mexico 8156; **B**, Timgalen; and **C**, Israel M68—producing very strong, medium, and very weak doughs, respectively. Proteins were extracted with 2% sodium dodecyl sulfate (SDS) in 0.05*M* sodium phosphate buffer (pH 6.9) by sonication and were eluted from a Waters Protein-Pak 300 column during 40 min with the same buffer but containing 0.1% SDS. Chromatograms were divided into peaks 1–3 as indicated. A_{210} = Absorbance at 210 nm; Minutes = elution time. Peak elution times for standard proteins were galactosidase (116 kDa) 10.4 min; ovalbumin (45 kDa) 12.5 min; trypsin inhibitor (20.1 kDa) 15.0 min; and cytochrome c (12.1 kDa) 15.3 min. Vo = 9.0 min, Vt = 18.0 min.

Halberd, Israel M68, and Wyuna do have additional gliadin bands compared to standard quality cultivars Cook and Gabo. Furthermore, Figure 3B shows that the poor quality cultivars either have a low HMW glutenin subunit score (Chile 1B, Israel M68, and WW 15 in Table VI) and/or a deficiency of LMW glutenin subunits (Egret, Halberd, Israel M68, and Wyuna). Good quality cultivars have the opposite features. Thus, the best quality cultivar Mexico 8156 not only has fewer gliadin bands, but also it has the best HMW glutenin subunit score and additional LMW glutenin subunits (i in Fig. 3A and B). However, gel electrophoresis provides a rather complex way of analyzing the overall balance of glutenin, which depends on three main components: gliadins, HMW glutenin subunits, and LMW glutenins subunits. At least two gel systems, acid PAGE or unreduced SDS-PAGE for gliadins and two-step SDS-PAGE for glutenin subunits, have to be used in combination with densitometry for a complete quantitative analysis. Unexpected variation in the amount of albumin/globulin may further complicate the situation. On the other hand, SE-HPLC provides a rapid one-step quantitation of glutenin, taking into account variation for all the protein fractions. However, gel electrophoresis can provide useful information on segregation of individual polypeptides among progeny of crosses between known parents.

Effect of Defatting and Alternative HPLC Cutoff Points on Glutenin Quantity Versus Flour Quality Associations

The correlations presented between HPLC measurements and flour quality were obtained with nondefatted flours using valleys (lowest points) between peaks as cutoff points to calculate peak areas. We also tested defatted flours, similar to that described earlier (Singh et al 1990), and fixed elution times as cutoff points between peaks. Only percent area of peak 1 is compared because it correlated best with most useful quality attributes. Comparisons of simple regressions between percent area of peak 1 and quality attributes showing significant correlations are presented in Table VII. Correlation coefficients, intercepts, and slopes of the regression lines for nondefatted and defatted flours were very similar, except for LV which showed different intercepts (Table VII). Although, Bekes et al (1983) showed a significant level of lipid mediated aggregation of gliadins/LMW glutenins, this may not be significant in the presence of high detergent (SDS) concentration. For simplicity of the procedure, use of nondefatted flours is recommended.

Differences between regressions with lowest point and fixed elution time cutoff points were also quite small or statistically insignificant (Table VII). We therefore prefer to use the lowest point cutoff because peaks 1 and 2 overlap considerably on the present column (Waters Protein-Pak 300; Singh et al 1990). As a consequence, fixed elution time cutoff resulted in an underestimation of glutenin in high-glutenin cultivars and their overestimation in low-glutenin cultivars, thus narrowing the glutenin range. This problem may be overcome by using larger pore-size HPLC columns (e.g., TSK 4000 SW), which should minimize the overlap between glutenin and gliadin peaks (Bietz 1984, Dachkevitch and Autran 1989); then fixed elution times could be used as cutoff points.

DISCUSSION

Due to its polymeric structure, glutenin has long been thought of as the strength protein of wheat flour. Recent flour reconstitution studies have shown that for a particular cultivar relative quantity of glutenin is directly related to strength (resistance) of dough or gluten (MacRitchie 1980, 1987; Kim et al 1988). However, due to inconsistent solubility of gliadin and glutenin fractions, it has been difficult to demonstrate this relationship in a group of cultivars using differential solubility (Grewe and Bailey 1926, Sullivan 1965). The alternative total solubility approach, involving size-exclusion chromatography for protein fractionation, has been more successful, but it has also given conflicting results due to incomplete extraction of unreduced proteins (Bietz 1986). In this study, the extractability problem was overcome by controlled sonication (Singh et al 1990). Consequently, very strong correlations were found between relative quantity of glutenin and breadmaking quality.

The role of native glutenin must be assessed in light of studies relating certain HMW glutenin subunit alleles to breadmaking quality (Payne et al 1979, 1984; Moonen et al 1982; Branlard and Dardevet 1985; Campbell et al 1987; Lawrence et al 1987, 1988). Similar associations also exist for allelic LMW glutenin

TABLE IV
Correlation Coefficients Among High-Performance Liquid Chromatography Measurements for 15 Wheat Cultivars

	Percent Area			Area (per milligram of flour)		
Measurement	Peak 1	Peak 2	Peak 3	Peak 1	Peak 2	Peak 3
% Area 2	−0.75**[a]					
% Area 3	−0.67**	0.01				
Area 1	0.70**	−0.10	−0.95***			
Area 2	0.27	0.38	−0.81***	0.87***		
Area 3	0.20	0.37	−0.69**	0.81***	0.96***	
Total area	0.42	0.21	−0.87***	0.94***	0.98***	0.95***

[a] *, **, *** = Significantly correlated at 5, 1, and 0.1% probability, respectively.

TABLE V
Correlation Coefficients Between High-Performance Liquid Chromatography Measurements and Quality Attributes

	Percent Area			Area (per milligram of flour)			
Attribute[a]	Peak 1	Peak 2	Peak 3	Peak 1	Peak 2	Peak 3	Total
FP	0.51	0.14	−0.92***[b]	0.95***	0.93***	0.87***	0.97***
MY	0.43	−0.30	−0.33	0.23	0.02	−0.11	0.08
LV	0.72**	−0.52*	−0.49	0.52*	0.24	0.21	0.35
E	0.84***	−0.48	−0.72**	0.76**	0.46	0.39	0.58*
R	0.84***	−0.63*	−0.56*	0.60*	0.26	0.15	0.42
A	0.89***	−0.59*	−0.68**	0.72**	0.39	0.28	0.51
DT	0.72**	−0.14	−0.92***	0.89***	0.74**	0.60*	0.80***
DB	−0.57*	0.17	0.68**	−0.65**	−0.48	−0.32	−0.53*
WA	0.14	0.44	−0.70**	0.58*	0.67**	0.59*	0.65**
MDT	0.84***	−0.66**	−0.52*	0.57*	0.22	0.11	0.34

[a] FP = Flour protein; MY = milling yield; LV = loaf volume; E = extensibility; R = maximum resistance; A = area; DT = dough development time; DB = dough breakdown; WA = water absorption; MDT = mixograph peak development time.
[b] *, **, *** = Significantly correlated at 5, 1, and 0.1% probability, respectively.

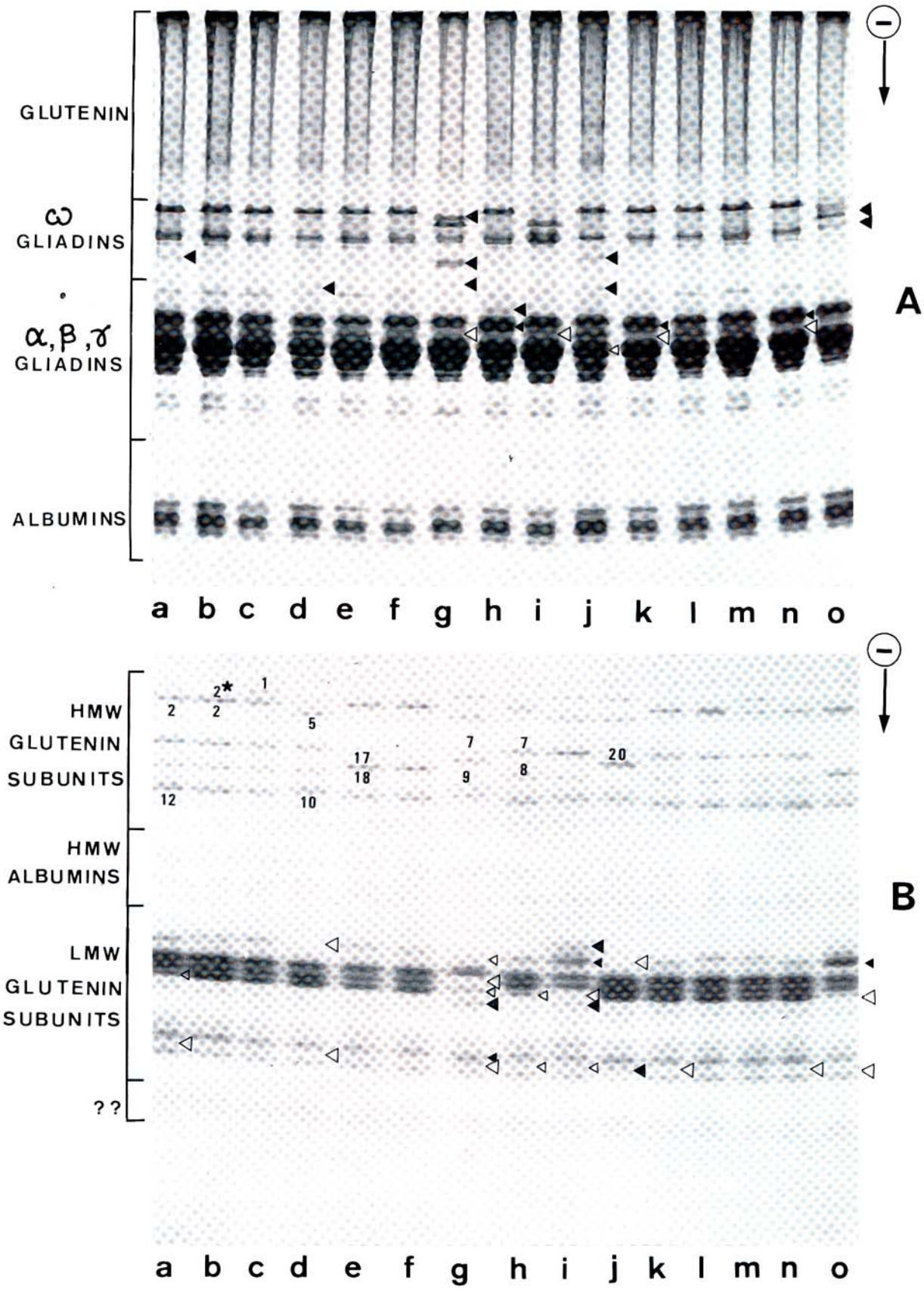

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic (SDS-PAGE) patterns of proteins extracted with 70% aqueous ethanol using sonication from the following cultivars: Chile 1B (**a**), Condor (**b**), Cook (**c**), Egret (**d**), Gabo (**e**), Gamenya (**f**), Halberd (**g**), Israel M68 (**h**), Mexico 8156 (**i**), Olympic (**j**), Osprey (**k**), Oxley (**l**), Timgalen (**m**), WW 15 (**n**), and Wyuna (**o**). Occurrence of additional protein bands (large solid triangles), increased band intensity (small solid triangles), absence of bands (large open triangles), and decreased band intensity (small open triangles) are indicated. **A,** One-dimensional (1-D) SDS-PAGE of unreduced proteins in a 5–15% gradient acrylamide gel (0.8% C). **B,** Two-step 1-D SDS-PAGE of glutenin subunits in an 8–16% gradient acrylamide gel (1% C). Presence of HMW subunit 2* was determined by SDS-PAGE in 8% acrylamide gel. Trace amounts of HMW albumins were soluble in 70% ethanol. ?? = New protein bands resolved after alkylation of -SH groups.

subunits (Payne et al 1987, Gupta 1989, Gupta and Shepherd 1988, Gupta et al 1989). Although genes for LMW glutenin subunits are closely linked with gliadins (Singh and Shepherd 1988), recombinants in durum wheat provide firm evidence that LMW glutenin subunits, rather than linked gliadins, positively affect the dough strength (Pogna et al 1988).

In most studies relating individual glutenin alleles to flour quality, quantitative aspects are not adequately emphasized. Thus, in two studies involving LMW subunits (Payne et al 1987, Gupta et al 1989), comparisons were made with null alleles (absence of bands). Hence, the positive effect of LMW glutenin subunits might have been entirely due to increased total glutenin rather than qualitative superiority of specific subunits. Similar possibilities occur for HMW glutenin subunits, whose association with breadmaking quality has been studied extensively (Payne et al 1984, Lukow et al 1989). Among allelic HMW subunits controlled by chromosome 1A (*Glu-A1* locus) bands 1 and 2 have an equal positive effect over the null allele, suggesting a quantitative effect. Similarly, among several alleles at the *Glu-B1* locus on chromosome 1B, those producing double bands or intensely staining bands (e.g., subunits 7+8, 13+16, and 17+18) are associated with superior breadmaking quality compared with those with single or faint bands (e.g., subunits 7, 20, and 6+8).

An exception to the quantitative basis of allele superiority is chromosome 1D controlled *Glu-D1* locus: HMW subunits 5+10 are better than 2+12, but there is no drastic difference in their staining intensities. Two different views prevail regarding the basis for superiority of 5+10. Greene et al (1988) suggested that it is due to the presence of an extra cysteine residue in subunit 5, whereas according to Goldsbrough et al (1988) it is due to a more regular β-turn conformation in the central repetitive region of subunit 10. No attempt has been made to accurately measure the quantity of glutenin associated with these alleles in an isogenic background. Hence the possibility that 5+10 is linked with higher glutenin quantity cannot be ruled out. The other possible way in which glutenin subunits might have different effects is by influencing the MW distribution. Polymer theory would predict that stronger dough properties could arise by either more peak 1 protein at a constant MW distribution or the same amount of peak 1 protein but with MW distribution shifted towards a higher range (i.e., higher average MW). Our present method could not test the latter possibility. Better aggregating ability of specific subunits and higher ratio of HMW to LMW glutenin subunits could be important in this respect.

More quantitative studies are needed; nevertheless, this study shows that relative quantity of total glutenin is a prime factor determining dough strength. The quantitative importance of LMW glutenin subunits linked with gliadin 45, which relates to good durum pasta-making quality, has been clearly demonstrated (Autran et al 1987). Using near-isogenic bread wheat lines, Lawrence et al (1988) showed that the percentage of densitogram

TABLE VI
HMW[a] Glutenin Subunit Composition of 15 Wheat Cultivars

Cultivar	HMW Glutenin Subunits[b]			*Glu-1* Score[c]
	Glu-A1	*Glu-B1*	*Glu-D1*	
Chile 1B	null, 1[d]	7+8	2+12	6.5
Condor	2*	7+8	2+12	8
Cook	1	7+8	2+12	8
Egret	null	7+8	5+10	7
Gabo	2*	17+18	2+12	8
Gamenya	2*	17+18	2+12	8
Halberd	1	20, 7+9[d]	5+10	8.5
Israel M68	null	7+8	2+12	5
Mexico 8156	1	7+8	5+10	10
Olympic	1	20	5+10	8
Osprey	2*	7+8	2+12	8
Oxley	2*	7+8	2+12	8
Timgalen	1	7+8	2+12	8
WW 15	null	7+8	2+12	5
Wyuna	2*	17+18	2+12	8

[a] High molecular weight.
[b] Presence of band 2* was determined using an 8% acrylamide gel.
[c] After Payne et al (1984) and Lukow et al (1989).
[d] *Glu-1* scores were determined by averaging the scores of individual mixed alleles.

TABLE VII
Effect of Defatting and Alternative High-Performance Liquid Chromatography Cutoff Points on Regressions Between Relative Quantity of Glutenin and Quality Attributes

Quality Attribute[a]	Regression Parameter[b]	Relative Quantity of Glutenin (percent area of peak 1)			
		Nondefatted Flour		Defatted Flour	
		Lowest Point Cutoff	Fixed Time Cutoff	Lowest Point Cutoff	Fixed Time Cutoff
LV	*r*	0.72**[c]	0.71**	0.72**	0.74**
	i	17.3	−82.2	−1.9	−76.0
	s	5.5±1.5	9.0±2.5	5.9±1.6	8.6±2.2
E					
	r	0.84***	0.84***	0.83***	0.86***
	i	−6.2	−21.8	−8.7	−20.4
	s	0.9±0.2	1.4±0.3	0.9±0.2	1.4±0.2
R					
	r	0.86***	0.84***	0.79***	0.77***
	i	−1,104	−1,872	−1,123	−1,599
	s	46±7	73±13	45±10	62±14
DT					
	r	0.72**	0.65**	0.60**	0.62**
	i	−9.1	−14.5	−8.1	−12.7
	s	0.4±0.1	0.6±0.2	0.4±0.1	0.5±0.2
DB					
	r	−0.57*	−0.59*	−0.53*	−0.53*
	i	313	458	320	403
	s	−7.3±2.3	−12.4±4.7	−7.2±3.2	−10.3±4.5
MDT					
	r	0.84***	0.79***	0.75**	0.69**
	i	−9.5	−16.6	−9.4	−12.8
	s	0.5±0.1	0.7±0.2	0.4±0.1	0.6±0.2

[a] LV = Loaf volume; E = extensibility; R = maximum resistance; DT = development time; DB = dough breakdown; MDT = mixograph peak dough development time.
[b] *r* = Correlation coefficient; i = intercept; s = slope.
[c] *, **, *** = Significantly correlated at 5, 1, and 0.1% probability, respectively.

area under HMW subunits (in SDS-PAGE gels) was strongly associated with dough strength. There are many exceptions to the qualitative basis of allele superiority. Some bread wheat cultivars contain HMW subunits 5+10 but produce weak doughs (e.g., Egret and Halberd in this study). Furthermore, 1BL-1RS wheat-rye translocation lines consistently produced weak-sticky doughs irrespective of their HMW subunit composition. In contrast, many good breadmaking quality cultivars (e.g., Cook, Gabo, Oxley, and Timgalen in this study) possess HMW subunits 2+12. These apparent anomalies disappear when relative quantity of total glutenin rather than presence of any specific subunit is considered as a basis of dough strength.

Although our results represent only 15 cultivars, the present approach has given consistent results with a range of experimental materials including wheat-rye translocation lines, wheat lines deficient for HMW glutenin subunits, the same cultivars at different grain protein level, sprout-damaged wheats, and gluten protein fractions for reconstitution studies (*unpublished*). We have found no significant deviation from the relationship between relative quantity of glutenin and flour strength. Hence, this method should provide a rapid small-scale test for predicting breadmaking quality in wheat breeding. A single-kernel analysis is also feasible as discussed earlier (Singh et al 1990). However, we need to widen the base of this test by analyzing many more genotypes.

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