published elsewhere (Singh et al 1990).

A number of wheat lines were used to assess the specificities of antibody combinations, including chromosome 1 and 6 nullisomic-tetrasomic lines derived from Chinese Spring (Sears 1954), near-isogenic lines prepared from the variety Olympic but lacking one or more HMW-GS (Lawrence et al 1987), and single and multiple wheat-rye chromosome 1 (short arm) translocation lines, Gabo and/or Chinese Spring mixed backgrounds (Gupta and Shepherd 1987).

Antibodies

Methods used for producing and screening monoclonal antibodies to gluten proteins and some of the specificity properties of individual antibodies are described elsewhere (Skerritt and Underwood 1986, Donovan et al 1989, Skerritt and Robson 1990). Briefly, antibodies 218/17 (IgG) and 237/24 (IgM) bound high and low (B-subclass) molecular weight (LMW) glutenin subunits and at high antibody concentrations, bound to γ- and ω-gliadins on immunoblots. High concentrations of the other three antibodies used (304/13, 405/7, and 412/01, all IgG) bound γ-gliadins and HMW-GS on immunoblots. Antibodies 412/01 and 405/7 also bound LMW-GS (Skerritt and Robson 1990). Using the two-site enzyme-linked immunosorbent assay (ELISA) format, these antibodies were highly glutenin-selective (Table I). For these studies, LMW-GS, HMW-GS, and gliadins were prepared from Timgalen variety flour, as described elsewhere (Skerritt and Robson 1990). The specificities of these antibody combinations (assessed using purified protein fractions) in the two-site assay format is often narrower than that noted on immunoblots after SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Hill and Skerritt 1989).

TABLE I
Relative Reaction of Different Antibody Combinations with Gluten Protein Fractions[a]

Antibody Combinations	SDS (2.5%)-DTT (50 mM) LMW-GS	SDS (2.5%)-DTT (50 mM) Gliadin	SDS (0.5%) LMW-GS	SDS (0.5%) Gliadin
C218/17-T412/01	340	410	170	1,200
C237/24-T237/24	5	20	4	3
C243/11-T243/11	43	350	62	160
C304/13-T304/13	6	25	3	3
C405/7-T237/24	330	1,400	150	300
C412/01-T412/01	5	19	3	2

Column heading "Solvent[b]" spans all solvent columns.

[a] Data are cross-reactions relative to high molecular weight glutenin subunits (= 100) measured using sandwich enzyme-linked immunosorbent assays with the antibody combinations shown.
[b] SDS = sodium dodecyl sulfate, DTT = dithiothreitol, LMW-GS = low molecular weight subunits.

Extraction of Gluten Fractions

In initial experiments, 500 mg of flour was dispersed in 10 ml of solvent by high-frequency homogenization using an Ultraturrax (Janke and Kunkel, Dottingen, Germany) at 15,000 rpm for 1 min and then centrifuged at 2,500 *g* for 10 min. Solvents examined included 1) acids (1 and 10 m*M* HCl), 2) alkalis (10 and 100 m*M* KOH), 3) acidified alcohol (50%, v/v, 1-propanol plus 1%, v/v, of acetic acid), 4) urea (8*M*), 5) SDS (0.5, 1.5, and 2.5%, w/v), and 6) combinations of 1) and 3) or 1) and 4). In some cases these were used in combination with the reducing agent dithiothreitol (DTT) at 10 or 50 m*M*.

In initial studies (Tables II and III) using sequential extractions with differing solutions, the first extraction was performed by vortex mixing for 1 min and then mixing by end-over-end rotation at 45 rpm for 15 min before centrifugation at 50,000 *g* for 30 min. The second extraction was performed by high-frequency homogenization, as described above. In some of these experiments (indicated), a sonifier microtip probe (Branson B-12, Danbury, CT) was used instead at 60% maximal power for 30 sec for the second extraction. Gluten proteins were routinely extracted (Tables III-V) by vortex mixing 50 mg of flour with 1 ml of solvent in a 2-ml microtube (Sarstedt, Germany, Cat. No. 72708). Although standard 1.5-ml microtubes can be used, suspension of flours was faster and more reproducible in the 2-ml tubes, which

TABLE II
Effect of Flour Extractant on Binding of Two Antibody Combinations

Extractants[a]	C304/13-T304/13 A 414[b]	C304/13-T304/13 Potency[c]	C412/01-T412/01 A 414	C412/01-T412/01 Potency
A				
Urea (8*M*)	0.196	1	0.164	1
HCl (10 m*M*)	0.355	1.5	0.252	2
Urea (8*M*)-HCl (10 m*M*)	0.407	2	0.300	2.5
Urea (8*M*)-HCl (10 m*M*)-DTT[c] (50 m*M*)	1.071	15	1.866	15
B			0.232	1
HCl (10 m*M*)	0.350	1	0.381	2
HCl (10m*M*)-DTT (10 m*M*)	0.631	3	0.485	3
HCl (10m*M*)-DTT (50 m*M*)	0.660	3		
C				
SDS (1%)	0.141	1	0.122	1
SDS (1%)-DTT (10 m*M*)	1.141	24	1.365	16
SDS (1%)-DTT (50 m*M*)	1.077	20	1.520	24
D				
1-Propanol (50%)-acetic acid (1%)	0.253	1	0.167	1
1-Propanol (50%)-acetic acid (1%)-DTT(50 m*M*)	0.672	6	0.667	6
E				
KOH (10 m*M*)	0.999	1	1.335	1
KOH (100 m*M*)	0.786	0.8	1.395	1

Column heading "Antibodies" spans both antibody combinations.

[a] DTT = dithiothreitol, SDS = sodium dodecyl sulfate.
[b] Absorbance obtained with a 1:25,000 dilution of flour extract. Similar trends were observed between 1:5,000 and 1:100,000 dilutions.
[c] Reciprocal of the wheat extract producing 50% maximal response in a wheat-extract dilution curve relative to that of the first extractant of each group (A--E).

TABLE III

Effect of Extraction Method on ELISA Absorbances and Discrimination Between Flour Samples[a,b]

Method		Low (9%) Protein Flour		High (13%) Protein Flour	
First Extractant	**Second Extractant**	**Cook A 414[c]**	**Absorbance Ratio[d]**	**Cook A 414[c]**	**Absorbance Ratio[d]**
High-frequency homogenization					
1.5% SDS + 50 m*M* DTT	None	0.63	1.24	0.77	1.21
1.5% SDS	1.5% SDS + 50 m*M* DTT	0.45	1.52	0.59	1.40
Vortex mixing					
1.5% SDS + 50 m*M* DTT	None	1.16	1.19	1.34	1.11
1.5% SDS	1.5% SDS + 50 m*M* DTT	0.98	1.23	1.17	1.40
2.5% SDS + 50 m*M* DTT	None	1.15	1.20	1.40	1.20
2.5% SDS	2.5% SDS + 50 m*M* DTT	0.70	1.16	0.95	1.18
0.5% SDS	2.5% SDS + 50 m*M* DTT	1.16	1.29	1.35	1.22

[a] Data are means of two experiments using C412/01 and T412/01; similar results were obtained with C304/13 and T304/13.
[b] ELISA = enzyme-linked immunosorbent assay, SDS = sodium dodecyl sulfate, DTT = dithiothreitol.
[c] ELISA absorbances using flour extracts diluted 1:50,000.
[d] Ratio of ELISA absorbances obtained with Cook to those obtained with Halberd, over the linear part of the concentration-response curve.

TABLE IV

Analysis of Antibody Binding to Extracts of Chinese Spring Nullisomic (n) Tetrasomic (t) Lines[a]

		Antibody[b]				
Line	**Protein (%)**	**C237/24 T237/24**	**C304/13 T304/13**	**C412/01 T412/01**	**C218/17 T412/01**	**C405/7 T237/24**
Chinese Spring	14.4	100[a]	100	100	100	100
Chinese Spring, 2x[b]	~(28.8)	265	158	162	NT[c]	NT
n 1A− t 1B	20.9	198	140	160	140	118
n 1A− t 1D	20.8	249	157	211	140	149
n 1B− t 1A	19.2	58	63	51	60	77
n 1B− t 1D	19.1	195	142	135	107	111
n 1D− t 1A	20.5	5	16	16	26	25
n 1D− t 1B	20.9	82	89	92	102	71
n 6A− t 6B	19.8	138	113	117	99	102
n 6B− t 6D	23.8	158	134	133	118	126
n 6D− t 6A	20.9	152	123	129	133	127

[a] Data are enzyme-linked immunosorbent assay absorbances relative to those obtained with Chinese Spring sodium dodecyl sulfate extracts (second extraction) diluted 1:40,000 C and T 237/24, C and T 304/13, or 1:400 C218/17 + T412/01, C405/7 + T237/24; C = solid phase, T = labeled.
[b] Analysis of Chinese Spring extracts at twice the antigen concentration in footnote "a". Thus the apparent protein concentration of the grain would be 28.8%.
[c] Not tested.

TABLE V

Relationships (Correlation Coefficients) Between Antibody Binding and Quality Parameters in 15 Diverse Wheat Cultivars

	Quality Parameter[a]									
		Mixograph	Farinograph			Extensigraph			Loaf Volume	
Antibody[b]	**Protein (%)**	**DT (min)**	**DT (min)**	**DB (BU)**	**WA %**	**E (cm)**	**R5 (BU)**	**Rmax (BU)**	**(−Bromate) (ml)**	**(+Bromate) (ml)**
C304/13-T304/13										
1-step (SDS-DTT)	0.518*[c]	0.508*	0.542*	−0.440	0.185	0.465	0.399	0.544*	0.584*	0.014
2-step (SDS)	0.230	−0.277	0.214	−0.044	0.297	−0.015	−0.173	−0.178	−0.228	0.268
(SDS-DTT)	0.496	0.739**	0.734**	−0.857***	0.229	0.726**	0.872***	0.854***	0.662**	0.246
C412/01-T412/01										
1-step (SDS-DTT)	0.839***	0.679**	0.856***	−0.770***	0.504*	0.814***	0.605*	0.759***	0.637**	0.280
2-step (SDS)	0.261	−0.219	0.233	−0.112	0.360	−0.014	−0.127	−0.145	−0.189	0.308
(SDS-DTT)	0.657**	0.754***	0.834***	−0.859***	0.414	0.894***	0.825***	0.880***	0.744***	0.395
C218/17-T412/01										
1-step (SDS-DTT)	0.487	0.771***	0.752***	−0.476	0.054	0.748***	0.692**	0.792***	0.581*	0.139
2-step (SDS-DTT)	0.545*	0.525*	0.618*	−0.608*	0.281	0.798***	0.549*	0.679**	0.733**	0.310
C405/7-T2377/24										
1-step (SDS-DTT)	0.540*	0.735**	0.595*	−0.412	0.079	0.708***	0.605*	0.720**	0.586*	0.066
2-step (SDS-DTT)	0.532*	0.758***	0.670**	−0.704**	0.060	0.731**	0.844***	0.865***	0.764***	0.263
Protein										
Total	(1.0)	0.366	0.860***	−0.598*	0.721**	0.667**	0.304	0.460	0.139	0.501*
SDS-extractable, %	−0.646**	−0.518	−0.641**	+0.500	−0.529	−0.758***	−0.469	−0.585*	−0.333	−0.465
SDS-DTT extractable, %	0.368	0.904***	0.639**	−0.630**	−0.032	0.729**	0.857***	0.916***	0.374	−0.023

[a] DT = mixograph development time, DB = dough breakdown, BU = Brabender units, WA = water absorption; E = dough extensibility; R5 = resistance to extension at 5 cm, Rmax = maximal resistance.
[b] SDS = sodium dodecyl sulfate, DTT = dithiothreitol.
[c] * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

have a wider conical base. SDS extracts were prepared by extraction with 0.5% SDS at 20°C by vortex mixing for 30 sec, sitting for 15 min, then mixing for an additional 30 sec before centrifugation in a bench-top microfuge (Beckman, Fullerton, CA) for 15 min at 15,000 *g*. The pellet from centrifugation was re-extracted with 1 ml of 2.5% (w/v) SDS-50 m*M* DTT solution by vortex mixing, as described above. In some cases, the SDS-DTT extracts were instead incubated overnight at 45°C before vortex mixing for an additional 30 sec. In other experiments, the SDS-DTT extraction was performed without initial extraction with SDS alone.

The protein contents of the sequential 0.5% SDS and 2.5% SDS-50 m*M* DTT extracts of selected flours were determined colorimetrically using bicinchoninic acid (BCA, Pierce, Rockford, IL) (Smith et al 1985). Since reducing agents interfere with this assay, DTT was removed by extensive dialysis of the extracts against 0.05% SDS-0.1% NaN_3. The protein contents of each fraction were expressed relative to the total (Kjeldahl) protein in the flour.

SDS-PAGE

SDS-PAGE analysis was performed on selected extracts using either 10% polyacrylamide gels run for 1,000 Vhr or 15% polyacrylamide gels run for 1,400 Vhr; other details are described elsewhere (Skerritt and Underwood 1986). All samples were reduced with a final concentration of 5% 2-mercaptoethanol, SDS was added to a final concentration of 2.5% and glycerol to 20%, and extracts were boiled for 5 min before analysis. Acidic and alkaline samples were neutralized first.

ELISA

Microwell ELISA plates (Immulon B, Dynatech, Chantilly, VA) were coated with 1 μg of solid phase-bound "capture" (C) monoclonal antibody (per 100-μl microwell) diluted in 50 m*M* sodium carbonate buffer, pH 9.6, for 60 min at 20°C. After three washes with 50 m*M* sodium phosphate—154 m*M* NaCl, pH 7.2 (PBS) containing 0.05% (v/v) Tween 20—microwells were postcoated with 1% (w/v) bovine serum albumin (BSA, Boehringer-Mannheim, Germany) in PBS for 60 min at 20°C. These steps can be performed either immediately before the ELISA, or batches of plates may be prepared for up to one year before use. Either gluten protein fractions (Skerritt and Robson 1990) at fourfold dilutions starting from 50 μg/ml or clarified extracts of flour samples were diluted serially in 1% BSA-PBS 0.05% Tween, and 100-μl aliquots were added to replicate microwells. After 30 min of incubation at 20°C, microwells were washed four times with PBS-0.05% Tween. Then 100 μl of horseradish peroxidase-labeled "tag" (T) monoclonal antibody per well, diluted in 1% BSA-PBS-0.05% Tween, was incubated for 30 min at 20°C. In certain cases, the same antibody could be used as C and T, without and with peroxidase label, respectively. In other cases, different monoclonal antibodies were used. The wells were washed five more times with PBS-0.05% Tween, then 150 μl of substrate (3-azino bisthiazolin-ethylsulfonic acid-0.003% H_2O_2) per well was added. After 20 min of incubation at 20°C, further color development was stopped by adding 50 μl of 6% oxalic acid per well.

RESULTS AND DISCUSSION

Specificities of Antibody Combinations

The specificities of several antibody combinations, which from preliminary experiments had been demonstrated to function in the two-site assay format with gluten proteins (Hill and Skerritt 1989), were investigated using purified gliadin, LMW-GS and HMW-GS (Table I). To analyze the cross-reaction of different antibody combinations with the three protein fractions, the displacements of the linear sections of the (logarithm) antigen dilution-absorbance curves were calculated for gliadin and LMW-GS relative to HMW-GS. To ascertain whether the solvent affected the specificity of the assay, fractions were dissolved (with sonication) both in 2.5% SDS-50 m*M* DTT and in 0.5% SDS. Effects on specificity were minor, although many antibodies showed a trend toward greater relative binding to HMW-GS compared with the other fractions in SDS. Because dough strength has been related to the proportion and composition of glutenin subunits (Payne et al 1987, Gupta et al 1989), especially the HMW-GS, antibody combinations binding strongly selectively to these subunits (e.g., C237/24-T237/24, C304/13-T304/13, and C412/01-T412/01) were selected for further study. Certain combinations (C218/17-T412/01 and C405/7-T237/24) bound relatively well to LMW-GS and were included for comparison. Other antibody combinations (e.g., 222/5-221/23) (Hill and Skerritt 1989), bound specifically to gliadin or bound each gluten protein fraction roughly equally (e.g., 243/11-243/11) (Table I) and therefore were not examined further in the current study.

Effects of Different Extractants and Extraction Methods

Initially, a number of different solvents known to extract gliadins and/or glutenins were examined. A relatively vigorous extraction technique (1-min treatment with a high-frequency homogenization probe) was chosen; flour from Suneca, a strong-gluten variety, was used. Full wheat extract dilution-response curves (Table II) were prepared for each extractant, using two antibody combinations (C304/13-T304/13 and C412/01-T412/01), shown in Table I to bind selectively to HMW-GS. Adding the reducing agent (DTT) to each extractant produced higher absorbance values, which suggested greater extraction of glutenin antigens. This was confirmed by electrophoretic analysis of the extracts (not shown). Extractants producing the greatest absorbance values in the ELISA were SDS-DTT, HCl-DTT, urea-HCl-DTT, and KOH (Table II). Because the presence of urea caused marked swelling of the flour (probably due to gelatinization of starch granules), which made collection of the centrifugation supernatants difficult, it was not studied further.

The three remaining extractant combinations were studied using samples of medium strength flour (Halberd) and a higher strength flour (Cook) blended to yield samples of approximately 9 and 13% protein for each variety. For both farinograph development time and loaf volume, the flours were ranked in decreasing order: Cook 13%, Halberd 13%, Cook 9%, and Halberd 9%. Flours that differed only moderately in strength were chosen to ensure that the discriminatory power of the test under development would be sufficient for practical use. SDS-DTT was the only extractant that discriminated the flours in the correct order, with the strongest Cook sample yielding the highest ELISA absorbances (Table III).

Table III shows a comparison of the results obtained with high-frequency homogenization of 0.5 g of sample with 10 ml of extractant and the results obtained with the small-scale method (vortex mixing of 0.05 g of sample and 1 ml of extractant) using various SDS concentrations and combinations with 50 m*M* DTT. In each case, the Cook sample gave higher absorbances in the ELISA than did Halberd, as indicated by the absorbance ratios greater than 1.0. Discrimination between the two varieties was slightly greater when preextraction with 1.5% SDS was used for both methods of extraction. This may be due to removal of the gliadin fraction (present in greater amounts than HMW-GS), with which the antibody combinations cross-react weakly (Table I). The higher protein samples of each variety gave higher absorbance values for each treatment. Vortex mixing, although less rigorous, was more efficient than homogenization, as indicated by the higher absorbance values obtained with each Cook sample. Vortex mixing is faster, and because it is suitable for use with small samples, it is a more appropriate method than high-frequency homogenization for wheat breeding quality assessment. Therefore, vortex mixing was studied further using differing SDS concentrations. With 1.5% SDS-50 m*M* DTT and 2.5% SDS-50 m*M* DTT, a preextraction step using the same concentration of SDS (i.e., 1.5 and 2.5%, respectively) reduced the absorbance values obtained with the second extraction, indicating that glutenin antigen was being removed by the initial treatment, which was undesirable. This was confirmed by analysis of these SDS extracts by SDS-PAGE under reducing conditions (not shown). Pretreatment with 0.5% (w/v) SDS did not result in the loss of significant amounts of glutenin, whereas it enhanced discrimination between the Cook

nations, very high correlation coefficients were found between antibody binding and extensigraph dough resistance (Fig. 4); loaf volumes (from loaves baked with and without bromate) and extensibility were also correlated. The binding of antibody combinations C304/13-T304/13, C218/17-T412/01, and C405/7-T237/24 were only weakly correlated with flour protein content; binding of C412/01-T412/01 was more closely correlated with protein, especially for the one-step assay. In addition to the selectivity of this antibody combination for HMW-GS (Table I), the closer correlation with protein content may be partially responsible for the superior correlations between antibody binding and quality parameters that were seen for C412/01-T412/01 using the one-step extraction method. As protein content is itself positively correlated with each of the quality parameters, its effect is hard to dissociate from that of quality-related differences in antibody binding. However, the following points should be noted:

1. Quite large alterations in protein content in samples of each of two varieties (Cook and Halberd) caused only minor increases in antibody binding.
2. The protein contents in the series of 15 wheats showed only moderate variation (8.1–11.7%).
3. In general, the correlations between the various quality parameters and antibody binding were higher than they were with protein content (Table V).

Protein contents of the sequential 0.5% SDS and 2.5% SDS-50 m*M* DTT extracts from the set of 15 flours were also determined. The relationship between these amounts and the various quality parameters are shown in Table V. Significant positive correlations between the proportion of protein that is SDS-DTT extractable (comprising both HMW-GS and LMW-GS) and several of the strength parameters and dough extensibility were seen. However, the farinograph development time and breakdown values were better correlated with antibody binding (e.g., C+T 304/13, C+T 412/01), and, unlike antibody binding, correlations between loaf volume and SDS-DTT-extracted protein were not seen. Variation in strength was associated with greater differences in antibody binding than in the amount of SDS-DTT-extracted glutenin subunits. For example, a doubling in maximal resistance was associated with a 48% increase in C+T 412/01 antibody binding; that is, a 75% difference in the amount of HMW-GS reaction (Fig. 3), but only a 35% increase in SDS-DTT-extracted glutenin subunits. Some statistically significant negative correlations were observed between certain strength and extensibility parameters and the proportion of SDS-extracted protein. However, the magnitudes of the differences between the fractions were small; a doubling in maximal resistance was associated with only a 9% decrease in SDS-extracted protein.

Thus it is quite unlikely that differences in the amount of total protein or in the amount of protein extracted by the (second) SDS-DTT extraction were the main origin of quality-antibody binding correlations. This aspect is further examined in another study (Skerritt 1991), first by statistical separation of protein content and antibody binding contributions to quality variation using multiple linear regression analysis, and then by analysis of a number of sets of wheat samples that did not vary significantly in protein content.

GENERAL DISCUSSION

An ELISA assay format and several antibody combinations were identified that have the potential to discriminate wheat flour samples differing in dough properties. The most useful antibodies bound selectively to the particular HMW-GS thought to exert a major influence on dough strength. However, since other gluten proteins (e.g., LMW-GS) can influence dough strength (Gupta and Shepherd 1988), the extent of the correlations between antibody binding and strength may vary in different sets of wheats (Skerritt 1990). The solvent used for flour extraction also had a major effect on the performance of the assay. Although acids, alkalis, and urea solutions were efficient extractants of HMW-GS, especially in the presence of a reducing agent, they did not discriminate sufficiently between samples in the ELISA despite findings by different groups that the proportions of acetic acid-insoluble (Mecham et al 1962, Orth and Bushuk 1972), dilute HCl-insoluble (MacRitchie 1987), or SDS-insoluble protein fractions (Orsi and Pallagi 1989) correlated positively with dough strength. SDS, the detergent that is widely used in wheat protein research for gluten fractionation and dissolution, was also the most useful solvent in these studies. Measurement of the amount of protein aggregates formed in the presence of SDS is the basis of the small-scale sedimentation volume test for predicting dough quality (Axford et al 1978). SDS solutions have been reported to solubilize a large proportion of glutenin in the absence of a reducing agent (Danno 1981, Bottomley et al 1982), especially after high-frequency sonication of extracts (Singh et al 1990). Although the proportion of glutenin polymers in such extracts has been demonstrated to be higher in flours with strong glutens (Singh et al 1990), quality discrimination was not observed with the current ELISA test unless a reducing agent was used. The ability of reducing treatments to enhance antibody binding to gluten proteins may arise from exposure of additional antibody-binding sites on individual HMW-GS, unavailable to the antibody on the unreduced disulfide-bonded glutenin complex. It is relevant that many of the subunit-specific regions of amino acid sequence on HMW-GS are located near cysteine residues (Anderson and Greene 1989), which would be exposed only after reduction. Another possibility is that the most suitable antibody combinations bind to repeat motifs within the HMW-GS sequences. This is supported by the observation that the antibody combinations providing the greatest HMW-GS specificity (Table I) and dough strength correlations were those based on pairs of identical monoclonal antibodies. For such a monoclonal antibody pair to be able to bind an individual polypeptide, it follows that a repeating or multiple peptide sequence is recognized. It is likely that HMW-subunit-related differences in dough strength arise from differences in the number and location of repeat motifs within HMW-GS (Flavell et al 1989).

Extraction of antigen by vortex mixing was found to be as efficient as high-frequency probe homogenization and more efficient in terms of exposure of antibody-binding sites (when a reducing agent is present) than ultrasonic probe treatment. It is also more suited to handling large numbers of small flour samples, since all the extraction manipulations can be performed using microcentrifuge tubes. Sample suspension using a vortex-type mixer is also fast and can be done in a sealed tube. The results indicate that the extractant as well as the antibody combination chosen had major effects on ELISA specificity. For example, results from analysis of extracts of Olympic-derived near-isogenic wheats lacking particular HMW-GS indicated that binding of the three antibody combinations based on 237/24, 304/13, and 412/01 was roughly equal to A-, B-, and D-genome-encoded subunits present in SDS extracts, especially if the relative amounts of these subunits (Lawrence et al 1987) were taken into account. However, selective binding to B- and D-HMW-GS was found in SDS-DTT extracts, and especially pronounced binding to D-HMW-GS after SDS preextraction was performed.

These results, obtained with the modified Olympic lines, were confirmed after the analysis of chromosome 1 aneuploids of Chinese Spring. Analysis of these lines, using the SDS-sedimentation test (Rogers et al 1988), also showed that the line with highest antibody binding, nulli 1A-tetra 1D, had potentially the best quality, and lines with depressed antibody binding (lacking 1D) also had decreased SDS-sedimentation volumes compared with euploid Chinese Spring. These results, while also indicating that chromosome 1D had the greatest influence on quality, must be interpreted with some caution, since Chinese Spring is null for the Glu-A1 allele. Analysis of these lines also showed that chromosome 6-encoded gliadins did not affect antibody binding to specific antigens in these SDS-DTT extracts. It was harder to ascertain whether antibody binding to LMW-GS played a significant role. With the antibody combinations 237/24-237/24, 304/13-304/13, and 412/01-412/01, some binding to purified LMW-GS was seen. However, effects of rye proteins complicated

the interpretation of the results with extracts of genetically modified stocks, such as the double and triple chromosome 1 (short arm) translocation lines. The decreases in binding observed with the 1AS and 1BS translocation may be due to a less extensive glutenin polymer being formed in the absence of certain LMW-GS (Graveland et al 1985). Indeed, others have demonstrated significant effects of LMW-GS on physical dough properties (Gupta and Shepherd 1988, Gupta et al 1989). The relationship between HMW-GS and dough strength is well documented in earlier genetic studies (Payne et al 1979, 1984, 1987; Burnouf and Bouriquet 1980; Branlard and Dardevet 1985), and the specificities of and results obtained with the antibody combinations used in this study correlate with these genetic findings.

In conclusion, a simple antibody-based test was developed for predicting strength-related properties of wheat dough. Several antibody combinations were assessed, using a set of 15 wheat varieties grown at a single site. In general, a two-step extraction method and use of the same antibody on the solid phase and in solution gave the best results. The companion article (Skerritt 1991) discusses in greater detail the effects of environment, fertilizer treatment, genotype, and glutenin subunit composition on the performance of the test with the different antibody combinations.

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