A Simple Antibody-Based Test for Dough Strength. II. Genotype and Environmental Effects

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ABSTRACT

The ability of simple antibody-based tests to assess and predict specific aspects of dough quality was evaluated using flours from several sets of wheat lines and varieties. Highest correlations between the binding of glutenin-specific antibodies and dough parameters were observed with dough strength (maximal resistance) and dough mixing (development time and breakdown). Differentiation between stronger and weaker lines was greatest when the sodium dodecyl sulfate (SDS)-insoluble protein fraction was examined. Significant correlations between dough quality parameters and antibody binding were obtained with sets of varieties grown at different levels of nitrogen fertilization at the same site or in a range of environments. The test was equally useful for analyzing a diverse range of unrelated genotypes and for examining progeny from related crosses. Finally, in a set of wheats having similar high molecular weight glutenin subunits, differences in antibody binding were obtained that were highly correlated with differences in dough properties.

The companion article (Skerritt 1991) describes the development of a simple test for prediction of dough strength by use of a monoclonal antibody-based enzyme-immunoassay. The assay used pairs of antibodies that bound well to particular high molecular weight glutenin subunits (HMW-GS) extracted from small wheat flour samples using a detergent, sodium dodecyl sulfate (SDS), and a reducing agent, dithiothreitol (DTT). Several factors, such as choice of antibody pairs, extractant, and assay conditions, were analyzed for their ability to distinguish between samples of a flour of a strong-dough variety, Cook, and similar flours from the variety Halberd, which produced doughs of intermediate strength. Using a set of 15 varieties of diverse quality, correlations were seen between antibody binding and a number of quality parameters including loaf volume, mixing time, and aspects of extensigraph behavior.

However, interactions between antibody binding or other biochemical parameters and various dough properties found with a particular set of varieties or samples may not always be observed on examination of other sets of genotypes or analysis of grain grown under different environmental conditions. In the set of lines examined in the companion article (Skerritt 1991), protein content was relatively uniform (9–11%). Some positive correlations that were obtained between parameters such as extensibility and dough resistance may not be seen when, for example, material having less variation in dough quality is analyzed. For these reasons, it was important to study in detail the performance of the antibody method using a number of sets of flour samples differing in properties. Since protein content can affect dough properties and loaf volumes (Finney and Barmore 1948, Bushuk et al 1969), sets of the same varieties grown under different levels of nitrogen fertilization, parents and progeny of a specific cross grown at both a high- and a low-protein site, and a set of varieties chosen at random from a world collection grown at one site were included in this study. The effect of growth environment and protein content was first studied in a set of flour varieties grown at five sites with different climatic conditions, and then in a set of nine cultivars grown at 5–7 sites but blended to yield single flours of three different protein levels. A random set of lines from a breeding program was also analyzed. Finally, a set of wheats with identical HMW-GS compositions but differing in dough properties was examined to investigate whether antibody binding-quality correlations were maintained. These studies enabled choice of antibody combination and experimental conditions to be established that were suitable for analysis of a wide variety of bread wheats.

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MATERIALS AND METHODS

Wheat Samples

Several sets of wheat varieties and breeding lines were used. First, the set of 15 wheat varieties of diverse pedigree and quality type used in the study described in the companion article (Skerritt 1991) were grown at one site (Victorian Crops Research Institute, Horsham, Victoria, Australia) in the 1986-87 season with zero nitrogenous fertilizer, standard nitrogenous fertilizer (NM, 50 kg N/ha), or excess nitrogenous fertilizer (200 kg N/ha). These wheats were milled on a laboratory Buhler mill to yield white flours (mean extraction rate, 74%). Farinograph and extensigraph tests were performed using standard AACC methods 54-21 and 54-10, respectively (AACC 1983), and the flours were test-baked using optimally mixed short-time doughs containing 30.2 g of flour (MacRitchie and Gras 1973). Mixographs for these flours were obtained with the full baking formulation (MacRitchie and Gras 1973).

In the second series of experiments, nine wheat varieties grown at up to seven sites throughout Australia were bulked to form composite (pure variety) blends of 10, 12, and 14% grain protein. These composite grain samples (27) were milled to a uniform 75% extraction using a Buhler mill. The nine varieties studied (Cook, Halberd, Kiata, Meteor, Miskle, Oxley, RAC 557, Wilgoyne) were all hard wheats. Doughs from these flours underwent full farinograph and extensigraph testing, and test loaves were baked using 1% malt extract and bromate and 120 g of flour (Moss 1980).

In a further set of experiments, 28 wheat varieties with markedly differing pedigrees from 18 countries were grown in 1982 at one site (Strathalbyn, South Australia) (Campbell et al 1987); grain was analyzed for HMW-GS composition by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Fifteen of these varieties had chromosome 1D-encoded subunits 2 and 12: Chris, Crespo, Currawa, C-273, C-518, Free Gallipoli, Giza-160, Hezera-2152, Huelquen, Kalayansona-227, Nabawa, Tainui, Toquifen S, Victor-1, and Zambezi (Campbell et al 1987), and 13 had 1D-encoded subunits 5 and 10: Ariana-66, Bina, BT-2288, Carazinho, Gaboto, Haruhikari, Jufy-1, Lerma-Rojo-64A, Manitou, Mexicana-1481, Potam-70, Roque-66, and Sondra-64A. Quality analysis methods used for this set of wheat flours were described by Campbell et al (1987).

Buhler-milled flour samples were also obtained from a set of 42 hard red spring wheats grown in 1986 at the same location (Hettinger) in North Dakota. These wheats all had HMW-GS indicative of good breadmaking quality; however, they differed in baking performance and dough properties (Khan et al 1989). Two wheats (ND-606 and Shield) had subunits 1, 7, 9, 5, and 10; five (Apex 83, Bronze Chief, Challenger, Glenman, and Norak) had subunits 1, 7, 8, 5, and 10; 12 (Cutlass, Lancer, Leif, ND-618, ND-628, Nordic, Norseman, PR-2369, Success, Solar, Walera, and Wheaton) had subunits 2*, 7, 8, 5, and 10. The

remaining 23 (Alex, Butte, Butte-86, Celtic, Columbus, Coteau, Guard, Katepwa, Len, Lew, Marshall, ND-617, ND-622, ND-623, ND-624, ND-625, ND-626, ND-627, ND-629, Olaf, Stoa, Telemark, and Waldron) had subunits 2*, 7, 9, 5, and 10. Wet gluten contents of these flours were determined using a glutomatic instrument (Falling Number AB, Stockholm, Sweden) and 10 g of flour. Farinograph dough development and bakery mixing times were determined. The latter was determined as the time required to have a dough of definite consistency and development by an experienced baker (Khan et al 1989).

The flours used to determine the performance of the assay in analyzing progeny of a specific cross consisted of 12 F_2-derived F_5 progeny selected from a cross between the varieties Kite and MKR-111/8, which had good and poor breadmaking quality, respectively, as well as two samples of each parent. For each sample, material grown at a high protein site (Roseworthy, South Australia) and a low protein site (Bordertown, South Australia) were examined. These parents differed in HMW-GS composition (Kite: 2*, 2, 17, 18, and 12; MKR-111/8: 2, 7, 8, and 12) and differed in low molecular weight glutenin subunit (LMW-GS) composition, especially in those encoded by chromosome 1A (Gupta et al 1989). Extensigraph properties were determined using doughs mixed to 500 BU on a farinograph.

Sample Preparation

An optimized method developed from experiments described in the companion article (Skerritt 1991) was used. This involved preextraction of flour with 0.5% SDS followed by extraction of the residue with 25% SDS-50 m*M* DTT. Each SDS-DTT sample extract was diluted (1:40,000) for analysis. A 100-μl sample was added to 20 ml of diluent buffer (1% bovine serum albumin-0.05% Tween 20 in 50 m*M* sodium phosphate-0.9% NaCl, pH 7.2), mixed well by shaking, and then diluted further by adding 100 μl of this dilution to 20 ml of diluent buffer in a second tube and mixed.

RESULTS AND DISCUSSION

Analysis of Wheats Grown Under High, Medium, or Zero Nitrogen Fertilization

Under nonstandard growth conditions, such as those resulting from either excessive or zero applied nitrogen, certain flours may exhibit dough quality characteristics atypical of those expected for the particular variety. Flours from wheats grown under different fertilization conditions were analyzed using several antibody combinations in the enzyme-linked immunosorbent assay (ELISA) test (Table I) (see also Table V in companion article, Skerritt 1991). As expected, in the absence of nitrogen fertilizer, flour protein contents were lower, doughs weaker and less extensible, and loaf volumes lower than under normal or excess nitrogen.

In general, similar trends were seen with correlations between antibody binding and quality parameters for each set of wheats. Correlations between antibody binding and the strength parameters, dough breakdown, and resistance were maintained for most antibody combinations; at zero nitrogen, development time and loaf volume correlations were also seen. Significant antibody binding-quality correlations were seen for several antibody combinations when flours were not preextracted with SDS before SDS-DTT extraction (not shown). However, high correlations between antibody binding and protein content were usually seen. Since each of the farinograph parameters under study was correlated with protein content (Table I), it was necessary to analyze whether variation in antibody binding (independent of variation in total protein) was correlated with quality. On the other hand, when there was little or no correlation with protein content, binding of the 412/01 (solid phase-bound, "capture" [C])-412/01 (labeled, "tag" [T]) combination in the assay following flour preextraction yielded good correlations with the key strength parameters (Table I). At high nitrogen fertilizer, development time and loaf volume correlations with antibody binding were either weaker or absent, probably because other factors were limiting on loaf volume rather than glutenin content. In support of this explanation, neither flour protein nor development time values were significantly correlated with loaf volume for this particular set of flours (not shown). Interestingly, whereas addition of bromate improved loaf volume values for the medium and high N treatments but resulted in the loss of antibody binding-loaf volume correlations (Skerritt 1991), loaf volume was not increased by addition of bromate for the zero N fertilizer samples. However, in this case, certain antibody binding correlations with loaf volume were maintained.

TABLE I
Relationships (Linear Correlation Coefficients) Between the Binding of Selected Antibodies and Quality Parameters, in Sets of 15 Wheat Varieties, Each Grown with High or Zero Nitrogen Fertilizer

	Quality Parameter[a]								
	Farinograph				Extensigraph			Baking Data	
Treatment, Antibody Combination	Protein (%)	DT (min)	DB (BU)	WA (%)	E (cm)	R5 (BU)	Rmax (BU)	LV_0 (ml)	LV_{20} (ml)
A. Zero nitrogen fertilizer									
Mean value of parameter	8.1	2.4	100	57.4	17.6	207	294	1,470	1,380
Flour protein (*r*)	...	0.743**[b]	−0.540*	0.702**	0.867***	−0.035	0.176	0.568*	0.781***
Simple regression[c]									
C412/01-T412/01 (*r*)	0.680*	0.815***	−0.734**	0.638*	0.745**	0.348	0.522*	0.866***	0.766***
Multiple regression									
C412/01-T412/01 (*rp*)	...	0.631*	−0.594*	0.308	0.424	0.508	0.557*	0.795**	0.513
B. High nitrogen fertilizer									
Mean value of parameter	11.7	4.5	74.6	61	22.3	210	345	1,615	1,701
Flour protein (*r*)	...	0.687***	−0.548*	0.800***	0.412	0.127	0.188	0.133	0.529*
Simple regression									
C412/01-T412/01 (*r*)	0.444	0.434	−0.671**	−0.059	0.587*	0.785***	0.761***	0.479	−0.035
Multiple regression									
C412/01-T412/01 (*rp*)	...	0.192	−0.570**	−0.551**	0.085	0.819***	0.770***	0.473	−0.355

[a] DT = development time, DA = dough breakdown, WA = water absorption, E = extensibility, R5 = resistance to extension at 5 cm, BU = Brabender units, Rmax = maximal resistance, LV_0 = loaf volume, LV_{20} = loaf volume using 20 ppm bromate improver.

[b] * = $P < 0.05$, ** = $P < 0.01$, *** = < 0.001.

[c] Linear correlation coefficients (*r*) for simple linear regressions $Q = ax_1 + c_1$, and partial correlation coefficient (*rp*) for Q/x_1 relationship in multiple linear regressions $Q = ax_1 + bx_2 + c$, where Q = quality parameter, x_1 = antibody binding (enzyme-linked immunosorbent assay absorbance), x_2 = protein, and a, a_1, b, c, c_1 are constants.

Multiple Linear Regression Analysis

The contribution of independent variation of antibody binding and protein content can be assessed by generating a series of multiple linear regression equations of the type: $Q = ax_1 + bx_2 + c$, where Q = quality parameter under study; x_1 = antibody binding (ELISA absorbance); x_2 = protein content; and a, b, and c are constants. The correlation between specific variation in antibody binding and the quality parameter under study can be assessed in the partial regression statistic. A reanalysis of the data from Table V of the companion article (Skerritt 1991) allows for better analysis of the effect of variation in antibody binding as a measure of quality assessment (Table II).

Comparison of the simple linear correlation coefficients between antibody binding and quality parameters for the 412/01 antibody combination (Table V, Skerritt 1991) and the partial regression statistic for the antibody binding contribution (Table II) revealed that correlations between strength parameters and antibody binding that had been found using simple linear regression were maintained following separation of the protein and antibody "contributions" in the multiple regression equation. This finding is very important, given that many of the quality parameters (development time, dough breakdown, water absorbance, extensibility) (Table II) were significantly correlated with protein content. Highest correlations were usually seen for the 412/01 C + T combination. Some significant correlations, which had been obtained by simple linear regression and could not be explained on the basis of differences in gluten protein composition, were shown to simply correlate with variation in protein content. For example, the antibody binding partial regression statistic was not significant for water absorption in the wheats fertilized with zero nitrogen.

Wheat Flours Blended to Predetermined Protein Levels

To further investigate the performance of the test at different protein levels, samples of wheat flours from nine varieties, each grown at eight sites, were blended to produce pure-varietal flours of 9, 11, and 13% protein content (Table III). Correlations between extensigraph resistance or farinograph development time and antibody binding were seen at the medium and high protein levels and between resistance and binding of antibody at low protein levels, for 412/01 only. At the medium protein level, correlations were also seen between antibody binding and loaf volume, indicating that factors other than glutenin composition may be critical at low and high protein contents. When the set of 27 flours was taken together, significant correlations were seen in simple linear regression analysis for binding of each antibody with protein content, farinograph development time, resistance, extensibility, and loaf volume. Multiple linear regression analysis to determine the relative contribution of antibody binding to each quality parameter (separate from the effect of variation in protein content) showed that resistance and development time were significantly correlated with binding of each antibody combination, but extensibility was no longer correlated.

Analysis of an International Wheat Collection

A subsample of 28 varieties was chosen at random from a larger set of wheat varieties of unrelated pedigrees, collected from various countries but grown at a single site. This set had earlier been thoroughly characterized in a wide range of physical dough tests and small-scale quality and baking tests; in addition, their HMW-GS compositions had been analyzed by SDS-PAGE (Campbell et al 1987). The 28 wheats selected fell approximately equally into two groups—those having chromosome 1D-encoded HMW-GS 2 and 12 and those having HMW-GS 5 and 10. The two sets did not differ significantly in mean protein level, hardness, or extensibility, but as shown earlier, wheats with HMW-GS 5 and 10 gave stronger doughs, more residue protein, and greater SDS sedimentation volumes and test loaf volumes (Campbell et al 1987) (Table IV).

Similar trends were noted with the binding of each of three antibody combinations based on antibodies 237/24, 304/13, and 412/01 (Table IV). Highly significant correlations were noted on simple linear regression of antibody binding data (ELISA absorbances) with dough resistance (R5 and Rmax), baking work input, and the microquality tests of "residue" protein measurement after solvent fractionation (Campbell et al 1987) and SDS sedimentation volume. Because significant correlations between antibody binding and protein content were also seen, multiple linear regression analysis was performed. Significant correlations between the antibody-binding contribution to the regression and quality were maintained for each of the parameters except for loaf volume. The latter is not unexpected, as an oxidizing improver was used for these baking tests.

Splitting the wheats into the two groups on the basis of HMW-GS composition revealed that excellent correlations were maintained between antibody binding and quality parameters such as baking work input, extensigraph resistance, and SDS sedimentation volume on either simple or multiple linear regression analysis. However, correlations between antibody binding and residue protein and mixograph development time were not seen for wheats with HMW-GS subunits 5 and 10. Therefore, the antibody method has the advantage over SDS-PAGE analysis of HMW-GS in being able to quantify differences in dough strength over and above those related to chromosome 1D-encoded HMW-GS composition. An unexpected finding was that mixograph development time correlated very well with antibody binding to extracts of the HMW-GS 2 and 12 wheats but not the HMW-GS 5 and 10 wheats. This may arise from the lower variation in development time values for the latter set of samples. With this set of samples, correlations between extensibility and antibody binding were not seen. Unlike the material described

TABLE II
Contribution of Variation in Antibody Binding to Quality Differences, Linear Regression of Results for 15 Wheat Cultivars Grown Under Standard Fertilization Conditions

	Quality Parameter[a]							
	Mixograph	Farinograph		Extensigraph				Loaf
Antibody Combination[b]	DT (min)	DT (min)	DB (BU)	WA %	E (cm)	R5 (BU)	Rmax (BU)	Volume (ml)
Simple linear regressions								
Mean value of parameter	4.5	3.4	86.3	59.0	20.2	0.195	0.318	0.189
Correlation with flour protein	3.66	0.860*** [c]	−0.598*	0.721**	0.667*	0.304	0.460	0.364
Multiple linear regressions								
C412/01-T412/01	0.731**	0.701**	−0.772**	−0.115	0.812***	0.869***	0.863***	0.719**
C304/13-T304/13	0.689**	0.692**	−0.806***	−0.215	0.611*	0.872***	0.811***	0.595*

[a] DT = development time, DB = dough breakdown, WA = water absorption, E = extensibilty, R5 = resistance to extension at 5 cm, BU = Brabender units, Rmax = maximal resistance.

[b] Values are correlation coefficients for antibody binding contributions to the multiple regression equation $Q = ax_1 + bx_2 + c$, where Q = quality parameter, x_1 = antibody binding (enzyme-linked immunosorbent assay absorbance), x_2 = protein, and a, b, c are constants.

[c] * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$; mean protein = 9.8%.

TABLE III
Relationship Between Antibody Binding and Quality Parameters for Wheat Flours Blended to 9, 11, and 13% Protein

Antibody Combination[b]	Quality Parameter[a]				
		Farinograph	Extensigraph		
	Protein (%)	DT (min)	E (cm)	Rmax (BU)	Loaf Volume (ml)
Simple linear regression					
9% protein					
C237/24 — T237/24	0.138	−0.127	−0.373	0.486	−0.018
C304/13 — T334/13	−0.094	−0.338	−0.289	0.410	0.210
C412/01 — T412/01	0.352	−0.184	−0.089	0.657*[c]	0.228
11% protein					
C237/24 — T237/24	0.383	0.673*	−0.042	0.844**	0.607
C304/13 — T304/13	0.415	0.717*	0.510	0.687*	0.735*
C412/01 — T412/01	0.309	0.745*	0.337	0.904***	0.876***
13%					
C237/24 — T237/24	0.051	0.846**	−0.180	0.839**	0.105
C304/13 — T304/13	0.100	0.822**	−0.258	0.776**	−0.062
C412/01 — T412/01	0.157	0.736*	−0.083	0.784**	0.338
All blends					
C237/24 — T237/24	0.822***	0.828***	0.495**	0.414**	0.754***
C304/13 — T304/13	0.754***	0.791***	0.512**	0.438*	0.760***
C412/01 — T412/01	0.842***	0.834***	0.584**	0.405*	0.840***
Multiple linear regression					
All blends					
C237/24 — T237/24	—	0.483*	−0.267	0.708***	0.228
C304/13 — T304/13	—	0.467*	−0.083	0.652***	0.366
C412/01 — T412/01	—	0.477*	−0.080	0.732***	0.471*

[a] DT = development time, E = extensibility, Rmax = maximal resistance.
[b] Linear correlation coefficients for simple linear regressions, $Q = ax_1 + c_1$, and partial correlation coefficient for Q/x_1 relationship in multiple linear regressions $Q = ax_1 + bx_2 + c$, where Q = quality parameter, x_1 = antibody binding (enzyme-linked immunosorbent assay absorbance), x_2 = protein, and a, a_1, b, c, c_1 are constants.
[c] * = $P < 0.05$, ** = < 0.01, *** = $P < 0.001$.

TABLE IV
Analysis of Extracts of 28 Unrelated Wheat Varieties Differing in Composition of High Molecular Weight Glutenin Subunits

Group, Antibody Combination[b]	Quality Parameter[a]									
				SDS Sedimentation	Extensigraph			Mixograph	Baking	
	Protein (%)	Residue (%)	Hardness (PSI)	Volume (ml)	E (cm)	R5 (BU)	Rmax (BU)	DT (min)	WI (Whr/kg)	LV (ml)
Full set (28)										
Mean value	12.3	31.6	17.6	70.8	21.5	224	330	4.5	8.9	15.2
C237/24-T237/24 (*r*)	0.538**[c]	0.687***	−0.268	0.677***	0.214	0.674***	0.717***	0.387*	0.711***	0.407*
(*rp*)	...	0.537***	−0.247	0.541***	−0.054	0.617***	0.644***	0.171	0.620***	0.205
C304/13-T304/13 (*r*)	0.530**	0.744***	−0.343	0.750***	0.166	0.830***	0.866***	0.500**	0.826***	0.408*
(*rp*)	...	0.626***	−0.336	0.647***	−0.114	0.813***	0.837***	0.328	0.775***	0.210
C412/01-T412/01 (*r*)	0.478**	0.687***	−0.235	0.725***	0.141	0.726***	0.809***	0.554**	0.752***	0.473**
(*rp*)	...	0.542***	−0.205	0.613***	−0.117	0.682***	0.767***	0.422*	0.677***	0.308
HMW-GS 2 + 12 wheats (15)										
Mean value	11.7	30.4	17.4	66.4	21.1	182	225	4.1	6.5	13.9
C237/24-T237/24 (*r*)	0.265	0.601*	−0.481	0.550*	−0.025	0.712**	0.812***	0.853***	0.668**	0.433
(*rp*)	...	0.563*	−0.536	0.499	−0.255	0.725**	0.800***	0.792***	0.664**	0.361
C304/13-T304/13 (*r*)	0.231	0.662**	−0.548**	0.662**	−0.127	0.686**	0.718**	0.704**	0.612*	0.185
(*rp*)	...	0.621*	−0.587*	0.591*	−0.370	0.700**	0.712**	0.685**	0.605*	0.265
C412/01-T412/01 (*r*)	0.306	0.568*	−0.455	0.643**	0.151	0.643**	0.727**	0.702**	0.719**	0.308
(*rp*)	...	0.517	−0.519	0.630*	−0.058	0.660*	0.728**	0.676**	0.722**	0.337
HMW-GS 5 + 10 wheats (13)										
Mean value	12.8	32.8	17.3	75.7	21.9	273	449	5.0	11.8	16.3
C237/24-T237/24 (*r*)	0.120	0.465	−0.179	0.647*	0.286	0.570*	0.631*	0.190	0.646*	0.489
(*rp*)	...	0.530	−0.257	0.655*	0.284	0.604*	0.606*	0.082	0.646*	0.580*
C304/13-T304/13 (*r*)	0.142	0.503	−0.333	0.685**	0.265	0.710**	0.792***	0.331	0.768**	0.347
(*rp*)	...	0.562	−0.315	0.691*	0.234	0.765**	0.820***	0.303	0.770**	0.321
C412/01-T412/01 (*r*)	0.149	0.413	−0.169	0.619*	0.336	0.529	0.580*	0.145	0.598*	0.522
(*rp*)	...	0.470	−0.146	0.625*	0.308	0.579*	0.652*	0.151	0.598*	0.507

[a] PSI = particle size index, SDS = sodium dodecyl sulfate, E = extensibility, R5 = resistance to extension at 5 cm, BU = Brabender units, Rmax = maximal resistance, DT = development time, WI = work input, LV = loaf volume.
[b] Linear correlation coefficients (*r*) for simple linear regressions $Q = ax_1 + c_1$, and partial correlation coefficient (*rp*) for Q/x_1 relationship in multiple linear regressions $Q = ax_1 + bx_2 + c$, where Q = quality parameter, x_1 = antibody binding (enzyme-linked immunosorbent assay absorbance), x_2 = protein, and a, a_1, b, c, c_1 are constants.
[c] * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

TABLE V
Relationships (Linear Correlation Coefficients) Between Antibody Binding and Quality Parameters for a Set of North Dakota Wheats Bearing High Molecular Weight Glutenin Subunits 5 and 10

Group, Antibody Combination	Quality Parameter				
	Flour Protein (%)	Wet Gluten Content (%)	Farinograph Development Time (min)	Bakery Mixing Time (min)	Loaf Volume
Complete set, n = 42					
Mean value	13.8	35.8	12.1	2.9	906
C412/01-T412/01	0.130	−0.384*[a]	0.767***	0.841***	0.054
C304/13-T304/13	0.011	−0.464**	0.770***	0.828***	0.098
8/10 Quality score set[b] (n = 12) (subunits 2*, 7, 8, 5, 10)[c]					
Mean value	13.6	35.3	10.7	2.4	882
C412/01-T412/01	−0.055	−0.519	0.778**	0.726**	−0.130
C304/13-T304/13	−0.267	−0.636*	0.794**	0.818***	−0.287
9/10 Quality score set[b] (n = 23) (subunits 2*, 7, 9, 5, 10)[c]					
Mean value	14.0	37.0	11.7	2.8	920
C412/01-T412/01	0.324	−0.102	0.649***	0.762***	0.204
C304/13-T304/13	0.264	−0.147	0.662***	0.720***	0.327

[a] * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.
[b] Quality scores allocated according to Payne et al (1987). Samples analyzed without SDS preextraction, since considerable discrimination between stronger and weaker doughed samples was seen.
[c] There were seven wheats with subunit 1 that were were excluded from analysis in the 8/10 and 9/10 quality score sets.

in Tables I and II, extensibility was not correlated with flour protein or dough resistance for this set of wheats.

Analysis of North American Wheats Bearing HMW-GS 5 and 10

The North Dakota hard red spring wheats had HMW-GS 5 and 10 (Khan et al 1989) and chromosome 1A-encoded subunits 1 or 2* (claimed by Payne et al [1987] to have an equivalent effect on dough quality). However, they had either 1B-encoded subunits 7 and 9 or 7 and 8. The latter combination is usually associated with greater dough strengths, and thus a higher quality score was allocated by Payne and coworkers. However, with most of the wheats (the 35 having 1A subunit 2*), the group of 12 lines or varieties having subunit 8 did not have longer mixing times or higher loaf volumes than did 23 wheats with subunit 9 (Table V, Fig. 1). The flour protein contents of this set of 42 wheats showed little variation (mean, 13.8 ± 0.7% SD), and no significant correlation between binding and protein content was seen for any of the antibodies tested. Therefore, multiple linear regression analysis was not performed.

The binding of each of the four antibody combinations studied (304/13 C + T and 412/01 C + T) (Table V) and C218/17-T412/01 and C407/5-T237/24 (not shown) was significantly correlated with both farinograph development time and baking mixing time. For this set of samples, Khan et al (1989) also noted significant correlations between the amount of glutenin (measured using three different solvent extraction methods) and mixing time. For the complete set of wheats, wet gluten contents correlated negatively with antibody binding. Khan et al (1989) suggested that wet gluten content was primarily determined by the amount of gliadin rather than differences in glutenin content. No correlation between antibody binding and loaf volume was seen. This may be because the wheats are already reasonably strong-doughed, and glutenin content is not the limiting factor influencing loaf volume. However, with a set of strong wheats, the method could be used to reject lines or samples with overly long mixing times. For example, flours with extracts yielding ELISA absorbances above 1.2 had farinograph development times of over 16 min (Fig. 1) and bakery development times of over 4 min, which is unsuitable for acceptable commercial throughput. The ELISA absorbance-development time correlations seemed to hold for different HMW-GS combinations (Fig. 1); there were too few of the 1, 7, 9, 5, and 10 types to draw a conclusion.

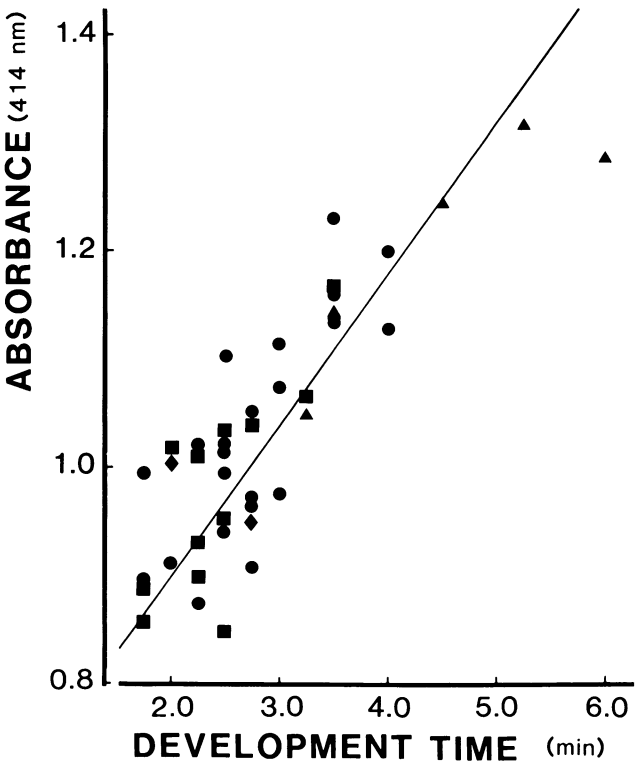

Fig. 1. Relationship between farinograph dough development time and the enzyme-linked immunosorbent assay (ELISA) absorbance for analysis of 42 wheats having high-quality subunits using antibody combination 412/01-412/01. Subunits are indicated as follows: ● = 2*, 7, 9, 5, 10; ■ = 2*, 7, 8, 5, 10; ▲ = 1, 7, 8, 5, 10; ◆ = 1, 7, 9, 5, 10.

Progeny of a Specific Cross

To investigate the usefulness of the test in segregating progeny on the basis of dough strength, three antibody combinations were investigated (Table VI) using two replicates of each parent and 12 progeny from a wheat cross of high quality (Kite) and low quality (MKR). The material was grown at a high (14%) and a low (7%) flour protein site (Gupta et al 1989). For each antibody tested and at both sites, highly significant correlations were found between antibody binding and maximal resistance but not extensibility (Table VI).

Comparison of Antibody Combinations—Differentiation of Wheats Varying in Dough Properties

As described above, good correlations were obtained between

several strength parameters of dough quality and the binding of several antibody combinations. However, equally important to good antibody binding-quality correlations is the need for good differentiation between high- and low-strength wheats by the antibody test. That is, the relative slope of the plot of ELISA absorbance vs. quality must be steep enough to enable wheats having a meaningful strength difference to give readily distinguishable ELISA absorbances. The slopes of these plots were divided by the mean absorbance value for that experiment in order to obtain a measure of relative slope. Data for three series of experiments examining the correlation between the binding of several antibody combinations with dough development times were analyzed:

1. The set of 15 varieties of diverse quality (standard fertilizer conditions, Tables II and VI in companion article [Skerritt 1991]).
2. A set of four varieties with differing pedigrees (Cook, Oxley, and Skua breadmaking type; Egret, biscuit type) grown at five Australian sites.
3. Twenty advanced breeders' lines chosen at random from one site (Horsham, Victoria).

Similar analysis using protein in place of absorbance can give an indication of the effect of protein content alone on quality.

The greatest discrimination, as seen by higher relative slopes and larger spread (coefficient of variation) of ELISA absorbance values was provided using antibody combinations such as 412/01 C + T and 237/24 C + T. Antibody combination 304/13 C + T usually gave high correlation coefficients between antibody-binding and quality parameters, but the slopes of the plots were only 60–75% those of 412/01 C + T. Deletion of the SDS preextraction step gave less discrimination of higher and lower quality wheats. This was reflected in poor correlations being obtained with the antibody combinations when the range of quality within the set of wheats under study was relatively low, as in the latter two sets of wheats. On the basis of discrimination of samples, and of correlations between absorbances and key quality characteristics, 237/24 C + T and 412/01 C + T provided the most reliable results.

As indicated from studies described in the companion article (Skerritt 1991) using genetically modified wheat varieties, these combinations as well as 304/13 C + T had similar specificities, with most binding to HMW-GS, especially those encoded by chromosomes 1D and 1B. The combinations involving either 237/24 or 412/01 as both solid-phase bound and labeled antibody bound more strongly to 1D-encoded glutenins. Accordingly, for each set of wheats examined, significant correlations were seen between the binding of different pairs of antibodies; the highest correlations were seen with 237/24 and 412/01 combinations.

Generality of the Relationship Between Antibody Binding and Quality

Although several different sets of wheat varieties provided significant correlations between antibody binding and several gluten strength parameters, it is important to ascertain whether the arithmetic relationship between these parameters and binding of a given antibody was consistent when different sets of wheats were studied. This aspect was investigated for several antibodies and quality parameters. For a particular set of varieties, both the slope and the intercept of the quality parameter-ELISA absorbance plot were very reproducible for assays performed by different operators. Some differences (up to twofold) in the slopes were noted for different wheat sets; this may be due to differences in ranges of protein contents and quality types. More important, the dough tests were performed at different laboratories, thus these data are not free from systematic error. Nevertheless, it should prove possible to predict the approximate dough properties of wheats in a test set using calibration standards, especially if they are derived from similar pedigrees and environments.

TABLE VI
Analysis of Parents and Progeny of a Kite × MKR Cross-Grown at a High Protein (14%) and a Low Protein (7%) Site

	Antibody Combination		
Site	C237/24-T237/24	C304/13-T304/13	C412/01-T412/01
Low-protein site			
Absorbance range	0.51–1.18	0.35–0.55	0.46–0.82
Maximal resistance, r[a]	0.579*[b]	0.728***	0.692**
Extensibility, r	0.201	0.232	0.256
High-protein site			
Absorbance range	0.87–2.03	0.36–0.57	0.50–1.03
Maximal resistance, r	0.781***	0.727***	0.772***
Extensibility, r	0.086	0.077	0.098

[a] Linear correlation coefficient. Quality data from Gupta et al (1989).
[b] * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

GENERAL DISCUSSION

Breeding new wheat varieties requires analysis of yield, disease resistance, and quality in new lines. Quality of wheat may be defined as suitability of a particular wheat line, variety, or sample for use in a particular product. The quality objectives of different wheat breeding programs vary, depending on the nature of products required by the intended market for the wheat varieties and local environmental conditions. Each product requires a certain wheat quality type, with grain hardness, protein content, and storage protein composition having major effects on dough properties of the lines. The strength of the gluten network can be measured as several rheological factors, including dough resistance to extension, dough development or mixing time, and breakdown time. Each of these parameters can be measured physically using standard AACC methods and equipment. However, initial crosses within breeding programs may produce hundreds or thousands of progeny bearing only small amounts (500 mg–20 g) of grain. Thus, the larger scale rheological methods, which require many grams of flour and are very labor-intensive, cannot be used in early generation quality screening. Early generation (e.g., F_2) quality screening would enable lines of unacceptable quality to be rejected earlier in the breeding process.

Some small-scale quality tests have been used for many years. These include assessment of the sedimentation volume after shaking a flour or whole-meal slurry in lactic acid (Zeleny 1947) or SDS-lactic acid solution (Axford et al 1978) or the assessment of the cohesiveness under water of a yeasted whole-meal dough ball (Pelshenke 1933). Finally, a protein solubility method, in which the proportion of acetic acid-insoluble protein in flours is measured by Kjeldahl nitrogen determination, has been proposed (Orth and O'Brien 1976). However, these methods are suitable only for simultaneous analysis of up to 20–40 samples, require several grams of flour, and usually take 20–120 min. Some methods are not equally applicable to whole meals and flours.

A new method was recently developed for quantifying glutenin aggregates by high-performance liquid chromatography (HPLC) following sonic disruption of the glutenin complex (Singh et al 1990a). Like the antibody test, good correlations were obtained with the HPLC method and several aspects of gluten strength (Singh et al 1990b). Indeed, results obtained with the two methods for the set of 15 varieties correlated well; $r = 0.74$, $P < 0.001$ for 412/01 C + T. Other HPLC methods for predicting dough strength have also been described (Huebner and Bietz 1985, 1986; Krueger et al 1988; Dachkevitch and Autran 1989; Sutton et al 1989). The HPLC method provides useful information on gliadin monomer-to-glutenin aggregate ratios, but for routine use the antibody test offers the following advantages:

1. Much lower capital cost.
2. Lower per-sample cost (extraction of samples is simpler).
3. Much higher throughput. (The ELISA method can analyze several hundred samples per day, as opposed to 20–25.)
4. Easier to interpret, as more color development in the ELISA equates with higher dough strength.
5. Greater differentiation of samples differing only moderately in strength.

Flours producing a 50% difference in ELISA absorbance may differ only by 15% in HPLC peak area. However, the HPLC method has the potential of being dependent not on the protein content of the sample but on the glutenin-to-gliadin ratio. The method also has greater potential for automating all steps of the operation after sample extraction, although similarly powerful ELISA "robots" are now common in clinical laboratories.

The need for a rapid test for dough strength also arises from the need to control the quality of flour purchased by processors (e.g., in applications used in baking or by starch-gluten manufacturers). Although these particular tests are not required to be small-scale, baking and rheological test methods are still labor-intensive and time-consuming. The procedure described is adaptable to analysis of large numbers of small (50 mg) samples, is suitable for use with either flour or whole meal (Skerritt, *unpublished*), does not require expensive equipment or skilled operators, and allows such assays to be performed by small or remote laboratories.

In summary, the method predicts dough-mixing requirements and strength with high reliability in a diverse range of wheat varieties and lines. Loaf volume is not uniformly predicted, as it is only sometimes correlated with dough strength or glutenin content and composition, especially when oxidizing improvers have been used in baking. For example, significant (protein-independent) correlations between antibody binding and loaf volumes were obtained for the flours analyzed in Tables I–III but not for those in Tables IV and V. This inconsistency of correlation between a dough strength measurement and loaf volume has been seen directly in physical dough tests and with medium-scale biochemical tests such as SDS sedimentation. On most occasions, extensibility was either not correlated (Tables IV and V) or correlated only in a protein-dependent manner (Tables I and III), with binding of antibodies such as 412/01. The molecular basis of dough extensibility is poorly established, but it probably is not dependent on the quantities of specific HMW-GS as measured by this antibody test. In its current form, the antibody method is a small-scale test suitable for use by wheat-breeding laboratories. Further modification of the method is underway to simplify sample handling by eliminating the need to weigh samples or to perform extensive dilutions of the flour extracts.

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