A Simple Antibody-Based Test for Dough Strength. III. Further Simplification and Collaborative Evaluation for Wheat Quality Screening

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ABSTRACT

An antibody-based method for discrimination of wheat flours or whole meals on the basis of differences in dough strength (Skerritt 1991b), as measured by extensigraph maximum resistance for example, was simplified for use in large-scale screening to predict dough quality. The major modification was the addition of unlabeled antibody to the diluted grain extract being analyzed, which simplified sample handling. It reduced the dilution of the grain extracts being tested and improved the differentiation between flours of different strengths. A method was also developed for simultaneous extraction and testing of sets of 96 unweighed whole meal samples. The method was tested in a collaborative trial in eight laboratories (none with significant prior immunoassay experience) using two separate analyses of a set of 16 flours, including three blind duplicates. Each laboratory reported highly significant correlations between color developed in the assay and rheological measurements of dough strength, such as farinograph development time and extensigraph maximum resistance. Good estimates of within- and between-laboratory precision were also obtained, indicating that the method was suitable for quality assessment in wheat breeding.

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Direct, objective measurement of wheat quality for a particular end product requires preparation on a test scale. Test baking is not always possible or practical, especially for a large number of samples where only small amounts of whole meal are available. Therefore, quality is determined later in the breeding process by measuring particular rheological properties of dough samples or particular biochemical components of the flour related to the desired end-use quality (Rasper 1991). These rheological properties have been positively correlated with the glutenin content of natural and reconstituted flours as well as with the glutenin subunit composition of flours (MacRitchie et al 1990, MacRitchie 1992). Measuring these rheological parameters or protein components can be time-consuming and, in the case of the dough tests, could require up to 50 g of flour. Thus, these procedures are not amenable to analyzing the large number of small (whole meal) samples produced in the early generations of a breeding program.

Breeders favor selection for protein quality, as distinct from protein content, thus possibly avoiding any yield penalty (O'Brien et al 1989, Fischer et al 1989). Recently, enzyme-linked immunosorbent assay (ELISA) technology, with its significant advantages of low capital, low running costs, and simple sample handling, has been used for quality screening. Detection and screening applications include sulfur-deficiency-related extensibility loss (Skerritt et al 1987), 1BL.1RS rye and *Agropyron* chromosomal translocations (Howes et al 1989b; N. Howes, *unpublished data*), specific gluten protein products of allelic genes (Skerritt et al 1988, Howes et al 1989a, Kovacs et al 1991), and less well-defined aspects of baking quality (Chan et al 1990).

We reported an assay to predict strength-related properties of wheat dough (Skerritt 1991a). The method employed antibodies that recognized high molecular weight glutenin subunits (HMW-GS) and gave high correlations between antibody binding and dough-strength parameters such as extensigraph maximum resistance and good discrimination between samples from different genetic backgrounds and agronomic environments (Skerritt 1991b). The method was suitable for small-scale testing. However, its capacity to handle large numbers of samples was restricted by the extremely high sensitivity to glutenin antigens. This complicated sample handling by requiring an extract dilution of 1:40,000.

The aim of the present study was to modify the assay to allow simpler sample preparation by eliminating the need to weigh the flour and the preextraction step. In addition, we aimed to reduce the sample dilution to a level sufficient to take advantage of commercially available multiple sample handling equipment while maintaining the discrimination between samples of differing dough strength. To assess the reliability and convenience of the method, we subjected it to a collaborative trial in eight laboratories.

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MATERIALS AND METHODS

Flour Samples

Assessment of modifications to the assay were made using the set of flours from 15 diverse wheat cultivars grown at a single site (Horsham, Victoria, Australia) as described previously (Singh et al 1990, Skerritt 1991a). This is termed the development set. Variations in extraction conditions and the performance of the final method were also tested on other sets of wheat flour or whole meal samples (Table I).

We tested two sets from Australian interstate wheat variety trials (IWVT): 1) a set of 27 flour samples (75% extraction rate) comprising three sets of pure varietal blends (10, 12, and 14% grain protein) from nine varieties grown at several Australian sites (Skerritt 1991b); 2) a set of 78 flours comprising 26 varieties and advanced lines grown at three sites (Narrabri, New South Wales; Dooen, Victoria; and Wongan Hills, Western Australia) in the 1989-90 season. The baking and rheological data for these samples were obtained from P. Gore of the Bread Research Institute of Australia.

We also tested a world set of 43 wheat varieties from various countries grown at a single site (Strathalbyn, South Australia) (Campbell et al 1987, Skerritt 1991b) containing both hard and soft wheats with a range of dough strengths.

Four sets of breeders' random lines were tested: two sets of 22 and 24 samples, respectively (Horsham); one set of 39 samples (Lincoln, New Zealand); and one set of 50 samples (Narrabri). The quality data for each of these sets were obtained from the laboratories of the breeding programs. The New Zealand set was rheologically tested using a mechanical dough development mixer (Greenwood 1989). The first two sets of breeders' lines contained both soft and hard low-medium protein lines; the third and fourth sets of breeders' lines were all hard, medium-high protein wheats.

Sample Preparation

Initial experiments employed only minor modifications to the original extraction procedure (Skerritt 1991a), including a reduced concentration of reductant in the extraction solution, decreased centrifugation time, and lower extraction temperature. Flour samples (50 mg) were extracted in capped 2-ml polypropylene tubes (Bio-Rad Laboratories, Richmond, CA) by vortexing the sample for 30 sec before and after incubating for 16 hr at 37°C with 1 ml of 2.5% (w/v) sodium dodecylsulfate (SDS) and 10 m*M* dithiothreitol (DTT). The extracts were clarified by centrifugation at 15,000 × *g* for 20 sec in a microfuge (Beckman, Fullerton,

CA). Extractions were performed either directly (one-step) or after preextraction with 1 ml of 0.5% (w/v) SDS (two-step). Preextraction involved vortexing for 30 sec before and after a 5-min rest period at room temperature, followed by centrifugation.

Experiments compared short (1 hr) and long (16–24 hr) extraction times and accurately weighed or tightly packed fixed-volume flour samples in an attempt to avoid weighing the samples. Volume-dispensed flour pellets were produced using a small cylinder and piston as a syringe. The pellets were broken up and dispersed by shaking the dry-capped tubes briefly before adding the preextraction solution. This procedure was tested at two volume levels: 1) 50-mg samples extracted in individual 2-ml tubes using vortex mixing; and 2) 25-mg samples extracted using 500 μl of extractant in individual wells of a deep-well microplate (Beckman). These deep-well microplates are an 8 × 12 array of 1.1-ml tubes molded into a single piece of polypropylene. The samples in the deep-well microplates were extracted by end-over-end mixing, after preextraction and centrifugation in a bench-top centrifuge (Hettich, Tuttlingen, Germany) at 1,200 × *g* for 5 min.

Enzyme-Immunoassay Procedures

The monoclonal antibodies (mAb) employed, 237/24 (IgM) and 412/01 (IgG), have been described previously (Skerritt 1991a,b). Initial experiments to reduce the sensitivity of the earlier method (Skerritt 1991a), involving manipulation of antigen-antibody interaction conditions, as well as direct modification of the antibodies, included the following procedures. Antibody Fab fragments, possessing only a single antigen-binding site, were produced by proteinase digestion (Bidlack and Mabie 1986, Boguslawski et al 1989) and then used either alone or in combination with whole immunoglobulins in the sandwich ELISA format. The mAbs were chemically modified by carbamylation or metal-ion coordination. The pH was varied between 4 and 10 for several of the ELISA incubation steps. Reagents, such as protein denaturants (urea, guanidine hydrochloride, and SDS) and a reductant (2-mercaptoethanol), were included in solutions employed at various stages of the ELISA procedure.

The addition of soluble mAb to the sample incubation step of the ELISA was followed through to the final format of the assay. Polystyrene microplates (Maxisorp, Nunc, Roskilde, Denmark) were coated with mAbs 237/24 or 412/01 as described previously (Skerritt 1991a). The plate wells were washed three times with 10 m*M* sodium phosphate, 150 m*M* NaCl buffer (pH 7.2) (PBS) containing 0.05% (v/v) Tween-20 (PBS-T). Residual protein-binding sites were blocked on each well with 150 μl of 1% (w/v) bovine serum albumin (BSA) (Boehringer, Mannheim, Germany) in PBS for 60 min at ambient temperature. These plates were used immediately in experiments or were freeze-dried and vacuum-sealed for use in test kits.

To determine the effects of competition between microwell-bound and soluble mAb on flour-sample dilution and discrimination of flours differing in strength, the wells of prepared plates were incubated for 60 min at room temperature with 50 μl of the mAb used for microwell coating in a range of concentrations and 50 μl of flour extracts serially diluted into a sample diluent of 0.1% (w/v) fish skin gelatin (Sigma, St. Louis, MO) in PBS-T. The optimized method used a fixed concentration of soluble mAb and a constant sample dilution.

After the sample incubation step, the plates were washed three times with PBS-T, and then each well was incubated with 100 μl of horseradish peroxidase (HRP)-labeled antibody for 30 min at room temperature. The same antibody, either 237/24 or 412/01, was used throughout each assay (microwell coating, sample incubation step, and detection of bound HMW-GS). The plate was washed four times with PBS-T, then each well was incubated with 150 μl of color developer, containing 2 m*M* diammonium 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) and 0.003% (w/v) hydrogen peroxide in 50 m*M* sodium citrate, pH 4.5. After 30 min incubation, the color development was terminated by adding 50 μl of 3% (w/v) oxalic acid to each well. Solution absorbance in the microwells was determined at 414 nm.

Collaborative Trial

The mAb 412/01 method was formatted into compact test kits containing four antibody-precoated plates, sample diluent (5× concentrate), unlabeled antibody (additive to sample diluent in microwells), wash buffer (10× concentrate), preextractant and extractant, enzyme-labeled antibody, peroxidase substrate-chromogen, stopping solution, and detailed protocol instructions.

TABLE I
Quality Data of Wheat Cultivars

	Quality Parameter								
	Flour Protein, %			Development Time, (min) or Work Input (Wh/kg)			Extensigraph Maximum Resistance (R_{max}) (BU)		
Wheat Set (*n*)[a]	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Development (15)	9.8	0.9	8.1–11.7	3.4	1.3	1.8–6.5[c]	318	126	60–570
				4.5	1.3	2.5–7.7[d]			
IWVT1[b]									
Total (27)	11.0	1.7	8.4–13.2	4.8	1.4	2.1–8.0[c]	310	62	155–425
10% Protein (9)	9.0	0.3	8.4–9.4	3.4	0.9	2.1–5.3[c]	319	46	270–400
12% Protein (9)	11.0	0.2	10.8–11.4	4.8	0.9	3.4–6.7[c]	296	77	155–425
14% Protein (9)	13.0	0.2	12.7–13.2	6.2	0.9	5.2–8.0[c]	317	63	240–420
IWVT2									
Total (78)	9.9	2.1	6.3–13.3	3.8	2.3	0.9–10.8[c]	277	77	70–450
Narrabri (26)	11.8	0.7	10.7–13.3	5.1	2.1	2.4–10.8[c]	289	81	160–450
Dooen (26)	7.1	0.4	6.3–7.9	1.5	0.4	0.9–2.4[c]	282	75	120–400
Wongan Hills (26)	10.7	0.8	9.0–12.0	4.8	1.9	2.0–9.5[c]	262	75	70–370
Breeders' random lines									
1 (22)	9.4	0.7	8.2–10.8	3.1	0.8	2.0–5.0[c]	311	82	191–487
2 (24)	10.1	0.7	8.4–11.6	3.6	1.7	1.5–9.0[c]	300	102	106–481
3 (39)	12.8	1.5	10.4–16.2	13.4	4.2	6.7–22.5[e]	nt[f]	nt	nt
4 (50)	13.8	0.8	11.9–15.2	4.4	1.1	2.4–6.6[d]	nt	nt	nt
World (43)	12.3	1.0	10.4–14.0	4.6	1.6	2.0–8.0[c]	354	174	120–770
				9.4	4.6	2.8–23.7[e]			
Interlaboratory trial (16)	10.9	0.9	8.9–12.3	4.4	1.6	2.0–7.5[c]	324	116	80–570

[a] Number of samples in the set.
[b] Australian interstate wheat variety trials.
[c] Farinograph.
[d] Mixograph.
[e] Work input from mechanical dough development mixer.
[f] nt = Not tested.

A two-step extraction of 50-mg (weighed) samples of the provided flour samples was performed for the trial. The extracts were diluted in two steps to 1:440 by adding 50 μl of extract to 1 ml of diluent, mixing thoroughly, and adding 50 μl of this dilution to a further 1 ml of diluent. Immediately after 0.6 μg of the unlabeled 412/01 in 50 μl of diluent was pipetted into each well, 50 μl of the diluted sample was added to the microwells. The wells were incubated at ambient temperature for 1 hr, washed three times in PBS-T, incubated for 30 min at ambient temperature with HRP-labeled 412/01, and washed four times in PBS-T. The color developed for 30 min.

Each trial participant was provided with a set of 16 numbered Buhler-milled flour samples of 13 different flours. Three of these were represented twice in the set to determine within-assay precision. The 13 wheat varieties were: Chile, Condor, Cook, Egret, Gabo, Gamenya, Israel, Mexico, Osprey, Oxley, Timgalen, Wyuna, and WW15. They were a subset of the cultivars in the development set and were grown in the same season at a slightly higher level of nitrogen fertilizer (100 kg/ha) (McCormack et al 1991, Gupta et al 1992). Two separate experiments, including extractions and analyses in duplicate microwells, were performed by each collaborator.

Data were examined for three standard measures of precision (Youden and Steiner 1975): within-lab replicate assays (repeatability), between-lab comparisons (reproducibility), and blind duplicates. The correlation coefficients for the linear regression of each set of ELISA data with specific rheological parameters were also calculated.

RESULTS

Simplification of the Assay

Minor changes were made to the original extraction procedure (Skerritt 1991a). Preextract centrifugation time was reduced from 15 min to 20 sec. DTT concentration in the extraction solution was reduced from 50 to 10 m*M*. Extraction temperature was reduced from 45 to 37°C. These reductions did not significantly alter the results obtained with four flour samples (extensigraph maximum resistance [R_{max}] 148, 240, 395, and 570 Brabender units [BU]) from the development set.

Several method modifications (SDS, guanidine hydrochloride, ferric ions, mAb carbamylation, and Fabs) reduced assay sensitivity, as desired; however, they also resulted in lower reproducibility and decreased discrimination of flours of differing dough strength. Adding soluble mAb to the diluted sample was effective in reducing the sensitivity of the assay; flour extracts could be analyzed in a dilution range of 1:200–1:1,000 because of the competition between the immobilized mAb and the soluble antibody for HMW-GS antigens in the added samples. Reducing the dilution from 1:10,000–1:50,000 to only 1:200–1:500 enabled the use of multichannel pipets and 96-well dilution blocks. This significantly reduced assay time for large numbers of samples. Furthermore, adding competing mAb also increased the slope of the linear section of the curve for both antibodies, which resulted in enhanced discrimination between samples of differing dough strength. For example, with mAb 412/01, the 1:40,000 flour extract dilution without competing antibody (Skerritt 1991b) was reduced to 1:3,000 and 1:400 with 1 and 10 μg of competing antibody per ml, respectively. The difference in assay absorbance values increased significantly (Fig. 1) between the strong (Mexico), moderately strong (Gabo), and weak flour (Egret).

Performance with Sets of Flours

The relationships between the binding of the two antibodies and dough quality parameters for the development set were established using the modified method (Table II). Correlations between antibody binding and several rheological dough-strength measurements, such as farinograph development time, dough breakdown, or R_{max}, were maintained at the level of the original method (Skerritt 1991a,b) using extracts prepared with or without preextraction. As noted earlier, high correlations between antibody binding and flour protein content were obtained with this set of flours. The significance of this protein effect was tested in two ways. First, the antibody response (absorbance) was normalized for the flour protein content; the correlations (r_n in Table II) between antibody binding and strength-related parameters were of the same order as the unnormalized response correlations. Second, multiple linear regression was applied to quality parameters with respect to antibody binding and protein content to determine the partial correlation coefficient of the antibody response (r_p in Table II). The significance of the antibody correlation was also apparent with this data treatment. These data confirm the results obtained using the original method (Skerritt 1991b), showing that the antibody response in the assay was dependent on dough strength and not simply the protein content.

The method easily discriminated flours with only moderate differences in dough strength with an increase in absorbance of 0.25 for each 100 BU of R_{max}. The discrimination of one-step extraction equaled that of the two-step extraction, which was an improvement from the original method (Skerritt 1991a). However, the predictive value of one-step extraction remained poorer than two-step extraction. (See correlation coefficients in Table II.) Therefore, single SDS-DTT extraction would save labor in screening very large sets of lines, but more of the poorer lines would be retained and more lines of the target strength would be culled.

Data for analyses of several sets of wheats using mAb 412/01 are shown in Table III. Although the development set exhibited a closer relationship between mAb binding and farinograph development time than between mAb binding and R_{max}, this was not a general finding. The analyzed flour sets displayed more consistent correlations between ELISA data and dough-strength parameters such as R_{max}. Where an insignificant correlation coefficient did arise, there was generally a high correlation between antibody binding and protein content. In these cases, significant correlations between the dough-strength parameter and the antibody data resulted when multiple linear regression was applied, as with the data in Table II. In Table III the correlation between antibody binding and R_{max} for the complete set of IWVT set 1 changed from 0.353 to 0.633 ($P < 0.001$). This correlation changed from 0.413 to 0.552 ($P < 0.01$) for breeders' set 1.

Extraction Conditions

The effect of the duration of the SDS-DTT extraction step was tested for both antibodies with several sets of wheat flours.

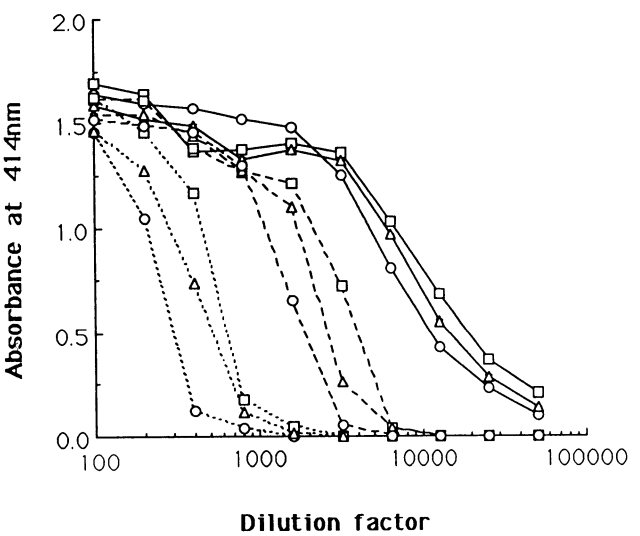

Fig. 1. Discrimination of different varieties with the new competition-sandwich ELISA. Relationship between assay absorbance and dilution factor (reciprocal of dilution) for SDS-DTT extracts (after SDS preextraction) of three flours from the development set with monoclonal antibody 412/01. Flour samples: □ = Mexico, strong; △ = Gabo, moderate; and ○ = Egret, weak. Competing antibody concentrations: —— = no mAb competition; - - - = 1 μg/ml; and ··· = 10 μg/ml. Data points are means of duplicates with error bars of one standard deviation.

TABLE II
Relationships Between Antibody Binding and Quality Parameters in 15 Diverse Wheat Cultivars Using Modified Method

Antibody[a]	Dilution	Linear Correlation[b]	Quality Parameter: Mixograph Development Time (min)	Farinograph: Development Time (min)	Farinograph: Dough Breakdown (BU)[c]	Extensigraph: Dough Extensibility (cm)	Extensigraph: R_{max}[d] (BU)	Flour Protein (%)
237/24								
One-Step extraction	1:500	r	0.543*[e]	0.819***	−0.587*	0.778***	0.651**	0.816***
		r_p	0.455	0.398	−0.215	0.542*	0.536*	
		r_n	0.524*	0.770***	−0.565	0.773***	0.639*	
Two-Step extraction	1:300	r	0.561*	0.861***	−0.570*	0.807***	0.677**	0.772***
		r_p	0.471	0.607*	−0.213	0.617*	0.571*	
		r_n	0.514*	0.730**	−0.474	0.749***	0.639*	
412/01								
One-Step extraction	1:300	r	0.528*	0.899***	−0.728**	0.798***	0.694**	0.814***
		r_p	0.426	0.674**	−0.520*	0.590*	0.619*	
		r_n	0.507	0.853***	−0.732**	0.788***	0.689**	
Two-Step extraction	1:250	r	0.725**	0.949***	−0.756***	0.850***	0.812***	0.852***
		r_p	0.849***	0.808***	−0.588*	0.722**	0.905***	
		r_n	0.744**	0.919***	−0.770***	0.872***	0.844***	

[a] Competition antibody concentration was 40 μg/ml for 237/24 and 20 μg/ml for 412/01.
[b] r = Linear correlation coefficient (simple linear regression); r_p = r part for antibody binding contribution (multiple regression equation) $Q = ax_1 + bx_2 + c$, where Q = quality parameter, x_1 = antibody binding, x_2 = protein; r_n = linear correlation coefficient of antibody response normalized for protein.
[c] BU = Brabender units.
[d] R_{max} = maximal resistance.
[e] * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

TABLE III
Relationships (Linear Correlation Coefficients) Between Antibody Binding[a] and Quality Parameters

Wheat Set (n)[b]	Quality Parameter: Protein (%)	Farinograph: DT[c] (min)	Farinograph: DB[d] (BU)	MDD[e] work input (Wh/kg)	Extensigraph: E[f] (cm)	Extensigraph: R_{max}[g] (BU)
Development set (15)	0.852***[h]	0.949***	−0.756***	nt[i]	0.850***	0.812***
IWVT[j]						
Set 1						
Total (27)	0.849***	0.780***	nt	nt	0.848***	0.353
10% protein (9)	0.049	0.398	nt	nt	0.089	0.678*
12% protein (9)	0.419	0.694*	nt	nt	0.640*	0.789**
14% protein (9)	0.026	0.547	nt	nt	0.725*	0.721*
Set 2						
Total (68)	0.332**	0.480***	nt	nt	0.443***	0.439***
Narrabri (26)	0.423*	0.467*	nt	nt	0.111	0.601***
Dooen (26)	0.363	0.482*	nt	nt	0.383*	0.671***
Wongan Hills (26)	0.676***	0.685***	nt	nt	0.797***	0.828***
Breeders' random lines						
Set 1 (22)	0.764***	0.366	−0.700***	nt	0.609**	0.413
Set 3 (39)	0.764***	nt	nt	0.724***	nt	nt
World (43)	0.482**	0.400**	nt	0.588***	0.459**	0.593***
Interlaboratory trial (16)	0.713**	0.930***	−0.725***	nt	0.745***	0.851***
Breeders' random lines (24)						
Set 2 flour						
Weighed sample	0.214	0.236	−0.576**	nt	0.403	0.746***
Fixed volume	0.093	0.322	−0.544**	nt	0.486*	0.779***
Set 2 whole meal						
Weighed sample	0.240	0.408*	−0.605**	nt	0.538**	0.712***
Fixed volume	0.132	0.263	−0.496**	nt	0.509*	0.654**

[a] Competition antibody concentration 412/01 was 20 μg/ml with two-step extraction of flours diluted 1:200.
[b] Number of samples in the set.
[c] Development time.
[d] Dough breakdown in Brabender units (BU).
[e] Mechanical dough development (MDD) mixer.
[f] Dough extensibility.
[g] R_{max} = maximal resistance.
[h] * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
[i] Not tested.
[j] Australian interstate wheat variety trials.

Only slight differences in correlations between quality parameters and absorbance values were observed between incubations at 1 hr and incubations at 16–24 hr (data not shown). Therefore, rapid results could be obtained using the two-step extraction method by reducing the second extraction to 1 hr. However, for large numbers of samples, the procedure was standardized using a two-step extraction, with an overnight second extraction.

The method was adaptable to both flour and whole meal samples, whether they were accurately weighed or presented as a tightly packed fixed-volume by use of a simple syringe device. Each method of sample presentation produced similar correlations between ELISA color and dough-strength parameters (Table III). Tightly packed flour volumes were necessary to maintain a constant weight (coefficient of variation [CV] approximately 5%), especially when the sample population included both hard and soft wheats. Sampling by volume, rather than by weight, was also adapted to simultaneous small-scale extractions performed in deep-well microplates. Correlations between mAb binding and rheological measurements were 0.670 ($P < 0.001$) for farinograph development time and 0.407 ($P < 0.001$) for R_{max} with IWVT set 2 ($n = 68$) and 0.793 ($P < 0.001$) for mixograph development time with breeders' set 4 ($n = 50$). The small-scale extraction method has the drawback of requiring an appropriate centrifuge and rotor at the preextraction step, but it has the distinct advantage of allowing simultaneous extraction and dilution of large numbers of samples because each of these deep-well plates has the capacity for 96 samples. Using this format, which would be optimal for high sample throughput, an estimated 1,000 samples per day could be analyzed by a single operator, excluding the sampling process.

Collaborative Trial

A collaborative trial of the method was performed by eight operators (predominantly chemists from wheat breeding programs) to assess the method in the hands of individuals who were untrained in ELISA techniques. Interlaboratory variation in the absorbance values for the test set was caused by use of a variety of photometers and detection wavelengths. Although most of the laboratories had access to ELISA plate readers, some laboratories reported absorbance data using 405 and 450 nm rather than the optimum wavelength of 414 nm. Thus, absorbance data was normalized to the mean of the set by dividing the value for an individual sample by the mean. Some individual sample analyses (10 out of 256) were rejected because absorbance values obtained were equivalent to the blank values, which indicated a missed sample dilution or failure to transfer the diluted sample to the microwell.

The precision parameters for the analyzed samples are presented in Table IV. Within-laboratory repeatability (RSD_r) was 7–43% and between-lab reproducibility (RSD_R) was 10–43%. Only two cultivars, which gave low absorbance values, had poorer precision values. The majority were 20% or lower. The relatively low precision between microwell plate readers, including microwell positional bias (Harrison and Hammock 1988), could cause up to a 20% variation at low absorbance readings (Skerritt and Hill 1991). The values obtained in this trial were as good, or better, than other collaborative trials of ELISA methods for food proteins (Olsman et al 1985, Skerritt and Hill 1991). The RSD_r was studied for blind duplicate sample results of low ($R_{max} = 270$ BU), moderate ($R_{max} = 330$ BU), and high ($R_{max} = 413$ BU) dough strength. The mean normalized absorbances were 0.490 ± 0.146, 0.917 ± 0.190, and 1.525 ± 0.118, respectively. RSD_r were excellent at 15, 13, and 9%, respectively.

Each of the laboratories obtained highly significant correlations between their absorbance data and strength-related parameters ($P < 0.001$). Correlation coefficients for farinograph dough development time were 0.842–0.959 and extensigraph R_{max} were 0.746–0.848. The ability to discriminate between samples, as measured by the slope of the regression line, varied very little for five of the laboratories (R_{max} vs. normalized absorbance, slope $= 200 \pm 5$, CV = 3%). The other three laboratories, with slightly lower discrimination, raised the CV to 15%. The precision of the slopes obtained should be viewed in relation to the 10–15% variation for determining rheological parameters (Bloksma et al 1962).

DISCUSSION

The antibody-based test for prediction of gluten strength (Skerritt 1991a,b) has been modified to further simplify the method. Sample preparation was simplified with good performance for samples presented on a volume basis as well as accurately weighed samples. The extraction procedure was adapted to smaller volume samples to handle multiple samples simultaneously. Significant correlations between antibody binding and dough-strength parameters were obtained using a direct extraction with SDS-DTT together with short extraction times, although brief preextraction with SDS gave consistently higher correlation values and improved strength prediction. Dilution of sample extracts was simplified by changing the assay from a standard sandwich ELISA to a new competition-sandwich format. This had a dual effect: 1) improving sample handling by reducing the sample dilution required, and 2) maintaining, and improving,

TABLE IV
Interlaboratory Trial Set Quality Data and Precision Parameters

Cultivar	Sample Number	Protein Content (%)	FDT[a] (min)	R_{max}[b] (BU)	Mean Normalized Absorbance	RSD_r[c] (%)	RSD_R[d] (%)
Condor	1	11.3	5.00	370	1.245	22	22
Oxley	2	10.3	4.00	330	0.942	27	13
Cook	3	11.3	6.00	413	1.598	12	12
Osprey	4	12.1	5.00	325	1.448	8	10
Egret	5	10.1	3.00	270	0.471	43	43
Mexico	6	12.0	7.50	570	1.886	12	16
Egret	7	10.1	3.00	270	0.505	31	43
Chile	8	10.7	2.50	125	0.333	41	41
Israel	9	10.6	2.25	80	0.379	26	29
Wyuna	10	8.9	2.00	325	0.447	19	24
Gamenya	11	10.4	5.00	360	0.967	22	22
Gabo	12	11.1	4.00	375	0.962	20	30
Cook	13	11.3	6.00	413	1.471	7	14
Timgalen	14	12.3	6.00	395	1.495	11	13
WW15	15	11.3	4.50	230	0.727	22	29
Oxley	16	10.3	4.00	330	0.879	24	25

[a] FDT = farinograph development time.
[b] R_{max} = extensigraph maximum resistance in Brabender units.
[c] RSD_r = relative standard deviation of repeatability (within-lab precision).
[d] RSD_R = relative standard deviation of reproducibility (between-lab precision).

dough-strength differentiation.

A collaborative trial using blind coded samples is the most appropriate test of the performance of a new method. It was performed in laboratories with no significant prior experience in ELISA methods. The collaborative trial revealed good precision, both within and between laboratories, with consistent correlations of results for dough-strength parameters.

This method also has the potential for rapid format performance in cereal processing laboratories for predicting mixing times, as well as quality testing for segregation at grain receival, if the need arises. The assay kits could be used in early generation testing to cull unwanted strength characteristics from the breeding program. The method integrates with other antibody-based tests and complements alternative techniques for measuring quality characteristics, such as the small-scale mixograph (Gras and O'Brien 1992) and SDS-PAGE analysis of protein components.

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