SCREENING FOR BREADBAKING QUALITY BY THE SDS-SEDIMENTATION TEST AND SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS

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ABSTRACT

There are many small-scale tests for protein quality which, for one reason or another, have proved unsatisfactory in quality wheat breeding programmes. This paper describes a new test, the sodium dodecyl sulphate sedimentation test which avoids most of the problems experienced with the others. The test requires only a small sample, is very rapid, repeatable and shows a high correlation with test baking results in both pure lines and early segregating generations. It is dependent upon protein quantity.

The biochemical basis of breadbaking is complex, with different protein and non-protein fractions all showing some association with quality. High molecular weight glutenin subunits, separated by polyacrylamide gel electrophoresis, are simply inherited and are associated with protein quality measured by the SDS-sedimentation test. The effects of the good quality subunits are additive, so the procedure is a useful additional test for breadbaking quality.

Additional Keywords: wheat, Triticum aestivum, breeding, protein

INTRODUCTION

Breeding for breadbaking in wheat depends upon identifying genetic components of quality and devising simple rapid tests to measure them. For such a test to be useful in early generations, it must require a small sample size, have a high repeatability, show a strong correlation with later full-scale measurements and have a low genotype-environment interaction.

There are four major aspects of breadbaking ability which must be considered in a quality breeding programme. These are the milling texture of the grain, and the α -amylase content, protein content and protein quality of the flour. However, this paper will concentrate upon the application of a new test for protein quality and some aspects of its biochemical control. The work was carried out while the author was on study leave at the Plant Breeding Institute (PBI), England.

There have been many small-scale tests for protein quality which have shown good correlation to loaf volume and high heritabilities. Measurements of dough rheological properties, the Zeleny sedimentation test and the Pelshenke test, are still used in many breeding programmes (Zeleny, 1978) although they do not fulfil all of the above requirements for an efficient screening test. A more recent test, the residue protein test (Orth and O'Brien, 1976) also has problems of throughput and sample size. Another new test, the sodium dodecyl sulphate (SDS) sedimentation test (Axford *et al.*, 1978), has no such problems. This test has been refined at the PBI (Blackman and Gill, 1980) and this paper reports on its value to a quality wheat breeding programme.

There is still confusion over the biochemical basis of baking quality and close associations between both the glutenin and gliadin protein fractions and quality have been claimed. Payne *et al.* (1981) correlated specific high molecular weight (HMW) glutenin subunits with SDS-sedimentation volume whereas Wrigley *et al.* (1981) claimed a stronger association with gliadin subunits. HMW glutenins are investigated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and this paper gives some evidence supporting the association between HMW glutenins and protein quality and an indication of how such studies can benefit a wheat breeder.

MATERIALS AND METHODS

The SDS-sedimentation test:

6 g of wholemeal, ground in a Udy cyclone mill with a 1 mm sieve, is added to 50 ml of distilled water in a 100 ml graduated measuring cylinder, stoppered and shaken for 15 seconds. The cylinder is shaken again after 2 and 4 minutes. After the third shaking, 50 ml of a SDS-lactic acid reagent (2% (w/v) SDS and 0.196% (v/v) lactic acid) is added and mixed by inverting the cylinder five times. This is repeated after 2, 4 and 6 minutes. After the last inversion, the suspension is left to settle for 15 minutes and the volume of the sediment recorded. A rack is used so that 10 samples can be tested at once. The test should be carried out in a laboratory with controlled temperature. The quantity of the ingredients may be altered if less than 6 g wholemeal is available. For example, the return from the single spring wheat plants grown in this study was insufficient for the 6 g test and the quantities were halved. This half test is very highly correlated with the full test (Griffin, 1982).

SDS-PAGE:

The SDS-PAGE procedure used is described by Payne *et al.* (1981). Ten percent polyacrylamide gels are made upon which up to 33 samples can be run. A general protein stain, Coomassie Brilliant Blue R, is used to distinguish the protein subunits, the HMW glutenins being the slowest moving.

Test baking:

At the PBI, a mechanical dough development process is used to bake loaves from 280 g of flour, mixed with 11 wh/kg dough work input (Blackman and Gill, 1980). Loaf volume is determined by rape seed displacement and the crumb characteristics recorded are a visual assessment of loaf appearance and crumb colour, texture and resilience. In New Zealand, test baking at the Wheat Research Institute (WRI) follows essentially the same procedure (Mitchell, pers. comm.) except that smaller loaves are made (125 g flour) and an optimum work input used.

Test material:

The wheats used in this study were grown as yield plots or short rows between 1979 and 1982 at the PBI as part of a larger study by the author. A trial was grown by Crop Research Division (CRD) at Palmerston North, New Zealand in 1980 and 1981. The material was completely protected from disease by regular Bayleton spraying, but otherwise received the normal cultural treatments given to wheat at the PBI.

RESULTS AND DISCUSSION

Early results with the SDS-sedimentation test suggested that it was more independent of protein content than other small-scale protein quality tests (Blackman and Gill, 1980). However, the correlation between SDS-sedimentation volume and protein content was highly significant within cultivars (Table 1) so, when comparing material with variable protein content, an adjustment should be made to a constant protein level using regression analysis. The regression and correlation coefficients in Table 1 vary significantly, although the real significance of this is uncertain as 'b' for individual cultivars differed between experiments and over years (Griffin, 1982). After four years experience, the PBI has found no evidence of a differential genotypic effect for SDS-sedimentation volume (Blackman, pers. comm.).

TABLE 1: Correlation and regression coefficients relating protein content and SDS-sedimentation volume within 13 Wheat Lines.

Lines	Correlation coefficient (r)	Regression coefficient (b)	F ratio, difference between b's
Rongotea	0.827	2.88	
Sandown	0.764	3.44	
TW 275/7	0.766	2.18	
Timmo	0.784	3.07	
TW 37/34	0.757	2.42	
TW 269/9	0.759	3.33	
TW 355/1/191	0.805	3.66	
Oroua	0.659	2.33	
Sicco	0.681	1.63	
Peko	0.706	3.00	
Pataka	0.446	2.40	
TGS 706/15	0.639	2.06	
TGS 39/79	0.681	1.86	2.421**

All correlation coefficients highly significant (34 d.f.)

Six NZ wheats and six European wheats were grown for two seasons at both the PBI and Palmerston North. The correlations of test baking with SDS-sedimentation volume and protein content for these trials are given in Table 2. Test baking was highly correlated with SDS-sedimentation volume but showed a lower and more variable association with protein content. At least half of the variation in baking results was explained by the SDS-sedimentation test, even in the trials with the lower correlations. The test adequately predicted baking performance in NZ and European wheats irrespective of where the test baking was done or where the wheats were grown. A similar high correlation between SDS-sedimentation volume and baking quality has been measured in Canada (Preston *et al.*, 1982) and the Netherlands (Moonen *et al.*, 1982). However, in Germany only a low correlation could be obtained although this improved when a sample of English rather than German wheats was used (Bolling and Munzing, 1979).

The good correlation of SDS-sedimentation volume and the poor correlation of protein content with test baking

TABLE 2: Correlation coefficients of test baking with SDS-sedimentation volume and protein content in 6 New Zealand and 6 European wheats grown at Plant Breeding Institute, England and Palmerston North, New Zealand.

Trial	PBI loaf volume score		PBI crumb characteristics		WRI loaf volume score	
	SDS	Protein	SDS	Protein	SDS	Protein
1980 PBI	0.894**	0.286	0.920**	0.420	0.706*	0.280
1981 PBI	0.920**	0.752**	0.825**	0.278	0.820**	0.793**
1980/81 Palmerston North	0.837**	0.682*	0.631*	0.188	0.735**	0.668*
1981/82 Palmerston North	0.876**	0.165	0.823**	0.182	0.753**	0.686*

TABLE 3: Correlation coefficients of F5 test baking with SDS-sedimentation volume and protein content in F2 - F5 for 20 lines from crosses WBG1 and WBG5.

Cross	Generation	Loaf volume		Crumb characteristics	
		SDS	Protein	SDS	Protein
WBG 1	F2	0.654**	-0.038	0.616**	-0.146
(Alcedo x Brigand) winter	F3	0.609**	0.121	0.561*	0.078
	F4	0.712**	0.044	0.660**	-0.059
	F5	0.818**	0.191	0.779**	0.146
WBG 5	F2	0.575**	0.158	0.426	0.205
(Sicco x TGS 39/79) spring	F3	0.612**	0.107	0.571**	0.280
	F4	0.545*	0.146	0.622**	0.302
	F5	0.792**	0.134	0.818**	0.100

TABLE 4: The segregation of HMW glutenin subunits in four wheat crosses.

Cross	Subunits		Chromosomal location	Number progeny tested			Mendelian ratio			Chi-square
	x	y		x	xy	y	x	xy	y	
WBG 1	7+9	6+8	1B	34	13	23	3	2	3	3.848
(Alcedo x Brigand)	5+10	2+12	1D	32	12	26	3	2	3	2.990
WBG 5	7+9	6+8	1B	70	49	51	3	2	3	4.157
(Sicco x TGS 39/79)	5+10	3+12	ID	70	35	65	3	2	3	1.961
	1	2*	1A	63	43	64	3	2	3	0.390
WBG 6	7+9	6+8	1B	20	38	21	1	2	1	0.139
(TW 275/7 x Sicco)	5+10	2+12	1D	20	40	19	1	2	1	0.038
	1	null	1B	55	24		3	1		1.219
WBG 7	1	2*	1A	21	14	13	3	2	3	2.222
(Pataka x Sicco)										

All chi-square tests non-significant.

was confirmed with 20 lines from two crosses in the F_2-F_5 generations (Table 3). The correlations in the spring cross were not significantly lower than those in the winter cross, despite a greatly reduced range of loaf volumes in the spring lines. SDS-sedimentation volume in the early generations was also highly correlated with the later baking results so early generation selection of high SDS-sedimentation lines would have been effective in retaining the best baking quality lines. On the other hand, selection for high protein content would not necessarily have retained these good quality lines. The high heritability of SDS-sedimentation volume implied in these results confirms those from other genetical analyses (Griffin, 1982). For the 20 lines of Table 3, the correlation between the SDS-sedimentation volume of individual F_2 plants and their F_5 bulks was 0.655** and 0.627** for crosses WBG1 and WBG5 respectively.

Grain from individual F_3 plants or bulk F_3 plots in four crosses was characterised by SDS-PAGE. The HMW glutenins were analysed following Payne *et al.* (1981) and in each case the observed ratio conformed to the expected Mendelian ratio for a single gene (Table 4). The effects of particular HMW glutenin subunit groups on SDS-sedimentation volume in the four crosses are shown in Table 5. The comparisons confirm the results of Payne *et al.* (1981); subunits 1, 5 + 10 and 7 + 9 are associated with good quality and subunits 2 + 12, 3 + 12, 6 + 8 and the null alternative of 1 or 2* are associated with poor quality. In addition, Table 5 suggests that subunit 2* is at least the equivalent of 1 and perhaps associated with even slightly better quality. However, despite the significant differences, the range in volumes within each phenotypic class is very wide and many progenies with good subunits give low SDS-sedimentation volumes and *vice versa*. Baking quality cannot be explained soley in terms of one gluten protein fraction or another but rather it results from complex interactions between the subunits of several protein and non-protein fractions. A clear understanding of how these subunits influence quality is not possible until their physical structure is fully understood. Presumably the good glutenin subunits have amino acid sequences which allow increased bonding between these and other fractions thus producing a stronger and more elastic gluten.

TABLE 5: Comparison between the presence of HMW glutenin subunits and SDS-sedimentation volumes in four wheat crosses.

Cross	Subunits			Mean SDS sedimentation volume		Significance
	x	vs	y	x	y	
WBG1 (Alcedo x Brigand)	7+9		6+8	25.8	18.7	t=4.300***
	5+10		2+12	25.5	19.1	t=3.926**
WBG5 (Sicco x TGS 39/79)	7+9		6+8	31.1	28.7	t=2.275*
	5+10		3+12	31.8	27.9	t=4.041***
	1		2*	30.3	30.3	t=0.000
WBG6 (TW 275/7 x Sicco)	7+9		6+8	31.6	28.7	t=3.190**
	5+10		2+12	31.7	28.8	t=3.108**
	1		null	30.4	28.9	t=2.305*
WBG7 (Pataka x Sicco)	1		2*	33.4	36.9	t=2.183*

TABLE 6: Mean SDS-sedimentation volumes of lines with an increasing number of good quality subunits.

Cross	Number of good quality subunits				LSD .05
	3	2	1	0	
WBG 1 (Alcedo x Brigand)		27.6	23.3	13.6	3.7
WBG 5 (Sicco x TGS 39/79)		32.3	29.8	25.3	2.7
WBG 6	33.3	31.9	28.6	25.8	2.8

In each cross, the addition of a good quality subunit group caused an increase in the mean SDS-sedimentation volume (Table 6). A comparison of the different combinations of allelic 1B and 1D subunits also indicated that the 1D subunits 5 + 10 had a relatively greater influence upon quality than the 1B subunits 7 + 9 (Griffin, 1982). Thus SDS-PAGE is a useful additional test for breadbaking quality although it should be emphasised that selection for improved quality must not be based solely upon HMW glutenin subunits. The procedure's greatest value in a breeding programme is for indicating crosses from which good combinations of subunit's will result, increasing the chances of subsequently recovering high quality lines. SDS-PAGE will also indicate the wheats most likely to have consistently high quality, so helping selection between related lines for which possibly only limited test baking data are available.

Agronomic considerations are also very important in any quality breeding programme, so an understanding of the yield-quality relationship is important, particularly in this study the correlations with SDS-sedimentation volume. The correlations between some agronomic and quality characters using pooled data from three crosses are given in Table 7. The only significant agronomic-quality correlation was the negative relationship between grain yield and protein content and between harvest index and protein content. This type of relationship has been reported many times and is a major limiting factor in breeding high yielding wheats with good breadbaking quality. The other quality components showed no highly significant associations with agronomic characters so are compatible with high yielding ability. The yield of wheats with good quality often lag behind those with poor quality but Bingham and Blackman (1978) stress that this is due to the complication of having to screen for the extra quality components which greatly reduces the number of lines remaining for selection on yield.

CONCLUSIONS

The SDS-sedimentation test for protein quality fulfils all the requirements for an efficient small-scale screening test except for its dependence upon protein quantity. However, protein content can be rapidly assessed by an infra-red reflectance method and its effects removed from the SDS-sedimentation volumes by regression analysis. The test allows protein quality to be evaluated in the early generations, on single F_2 plants if necessary, meaning that only good quality lines should progress into later generation yield trials where space is at a premium. SDS-sedimentation volume shows no substantial correlations with agronomic characters so is compatible with high yield.

Protein quality can be associated with specific HMW glutenin subunits and the effects of the good quality subunits are additive. Therefore, SDS-PAGE is a useful additional test for breadbaking quality. However, the variance in quality for each HMW glutenin subunit is large and other protein and non-protein fractions are also involved. The major value of SDS-PAGE is in indicating the parental combinations from which high quality segregants are most likely.

TABLE 7: Correlation coefficients between agronomic and quality characters, pooled from three wheat crosses (Alcedo x Brigand, Alcedo x Virtue, Sicco x TGS 39/79) in the F3 and F4 generations.

		1	2	3	4	5	6	7
1	Ear number/plant							
2	Height	-0.026						
3	Biological yield/plant	0.512	0.480					
4	Grain yield/plant	0.549	0.266	0.865				
5	Harvest index	0.038	-0.396	-0.280	0.337			
6	Protein content	-0.080	0.066	-0.267	-0.382	-0.505		
7	Grinding time	-0.164	0.048	0.040	-0.019	-0.099	0.219	
8	SDS-sedimentation	0.058	-0.034	-0.084	-0.125	-0.125	0.554	0.222
		368 df						
		Levels of significance 5% 0.103 1% 0.134						

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