PROJECT REPORT No. 99

IMPROVEMENT OF METHODS FOR MEASURING THE QUALITY OF BREADMAKING WHEAT

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IMPROVEMENT OF METHODS FOR MEASURING THE QUALITY OF BREADMAKING WHEAT

by

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Abstract

We have improved the procedures in the gel-protein test to make it more reliable and cost effective, and it has now become an established measure of quality in the Recommended List trials. Evaluation of a simple-to-use Dynamic Shear Rheometer, showed that this could be used as a much cheaper alternative to the VOR model Bohlin rheometer for the gel-protein test.

The monoclonal antibody, 0610-IFRN, may be of potential value in wheat breeding as it was shown to discriminate 1) Mercia from other breadmaking varieties, and 2) between breadmaking and biscuit-making varieties.

The gluten index test, as performed on the Glutomatic, was evaluated and found to be indicative of quality, but it lacked robustness as a rapid test and was unable to distinguish between the main UK breadmaking varieties. It indicated a low level response to heat damage in flours.

Optimal conditions were developed for resolving gliadin fractions by capillary electrophoresis, and preliminary analysis was made of water-soluble metalloproteins which may be potential quality markers.

Upon evaluation of the SDS test, we found a poor relationship with loaf volume for the 1992 Recommended List trial samples, however, prediction of loaf volume can be improved by taking into account the Falling Number, protein content, and damaged starch levels.

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1. INTRODUCTION

A number of instances in recent years suggest that traditional methods of assessing quality such as the sodium dodecylsulphate (SDS) sedimentation volume did not always truly reflect the baking potential of a flour. Two varieties, Fresco and Pastiche, despite their successful passage through the United Kingdom National and Recommended List Trials, were found to perform poorly for breadmaking.

Fresco was identified as an "extra-strong" wheat that required high levels of work-input during mixing for optimum dough development which was not commercially acceptable (Pritchard and Brock,1994). This aspect was discussed in detail in Project Report No. 36 (Bent *et al.*,1991) in a study of the similar variety Torfrida.

The glutenin fraction of Pastiche was, in contrast, shown to be too weak for optimum development in a mechanical dough development system such as the Chorleywood Bread Process (CBP). The baking performance of this variety was discussed in Project Report No. 31 (Osborne *et al.*, 1991). An additional observation concerning this variety was that, contrary to traditional belief, loaf volume did not increase with increasing protein content.

These two examples emphasise the importance of work-input requirement of wheat for bread-making in the UK and underline the need to establish new or improved methods of measuring wheat quality. It is recognised that such tests would have to retain the simplicity of the SDS test for it to be widely adopted by all sectors of the grain trade.

The remit of the project was broad based to include all aspects of wheat quality from genetically inherited protein species through traditional quality tests to the rheological character of doughs and dough liquors and the small-scale testing of work-input requirement. The principal objectives of this project were:-

* To develop a small-scale method to determine the optimum work-input of single varieties.

* To identify protein fractions which influence baking quality using techniques such as two-dimensional gel electrophoresis and capillary electrophoresis.

* To investigate means of improving the predictive ability of the SDS test by correcting for other flour properties.

* To establish whether the lack of a relationship between protein content and loaf volume applies to other varieties.

* To develop the gel-protein test for wider application in the grain trade.

* To determine whether surface rheological properties of dough liquors and bulk rheological properties of doughs could be used as quality tests.

* To evaluate the gluten index test using the Glutomatic.

* To test the use of monoclonal antibody technology in the assessment of wheat quality.

2. MATERIALS AND METHODS

2.1 Small-scale measurement of work-input requirement

Mixing was carried out using the Compudomixer system consisting of a Constant Power instrument (Dalgety) and a DCE 330 Torque Power machine / Brabender Do-corder (Calibre Control Instruments). The Compudomixer affords precise computer control of dough mixing speed and time and can also provide work-input data. Doughs were mixed from 50 g of a commercial CBP breadmaking flour.

2.2 Identification of protein fractions which influence baking quality

2.2.1 Two-dimensional polyacrylamide gel electrophoresis

At least 40% of the variability in breadmaking quality of UK-grown wheat is not explained by allelic variation in high molecular weight glutenin subunits. Two samples of the variety Torfrida differing in baking performance and in other properties were used to investigate non-glutenin sources of variation.

One dimensional (1-D) SDS-PAGE analysis (Laemmli, 1970) of these samples had produced virtually identical gel band patterns. A more detailed examination was necessary. Two dimensional (2-D) PAGE can provide much higher resolution. In the first dimension proteins are separated by isoelectric focusing (IEF) (molecular charge), and in the second by SDS-PAGE (molecular size). Around 500 components can be resolved and detected on a single polyacrylamide gel. To overcome problems with reproducibility and insensitivity of staining the gels, procedures were modified to include dimethylpiperazine and sodium thiosulphite in the second dimension PAGE (Hochstrasser and Merril, 1988). Silver staining was by the method of Mellish and Tkachuk, 1991.

2.2.2. High performance capillary electrophoresis (CE)

A preliminary analysis of wheat by CE was conducted by Spectra Physics, prior to purchase of our own instrument. Following on from that preliminary study, the gliadin fraction was extracted from grains using the following solvents: 60% isopropyl alcohol; 6% urea; 75% ethanol; 75% methanol, and separated in the capillary zone electrophoresis CZE) mode (Grossman *et al.*, 1989; Lee & Heo, 1991) in 150 mM phosphoric acid, at 60°C. The resolution in the CZE mode was unsatisfactory as only minor non-reproducible differences were observed within the 20 mins separation time between several of the varieties which were examined.

Some of the parameters which influence resolution include: buffer composition, ionic strength, pH, temperature, sample injection volume, capillary conditioning and washing procedures, capillary dimensions, and voltage (Li, 1992). These factors were all explored systematically. Running buffers which were tried included 50 - 200 mM phosphoric acid, 50 mM citric acid, 50 mM CHES pH 8.8, and 50 mM sodium borate pH 8. Samples were extracted in various alcohol solutions, water, and SDS to fractionate gliadins, globulins and albumins, and the total proteins respectively.

2.3 The predictive ability of the SDS sedimentation volume test

The SDS sedimentation volume test (Redman, 1979) has been widely adopted in the grain trade as a measure of the baking quality of wheat. Generally, it is reliable and a major advantage lies in its simplicity and speed, but there have been occasions where the test has failed to predict baking performance. This study set out to investigate whether the predictive ability of the SDS test could be improved by corrections for other flour properties such as protein content, Falling Number and damaged starch. All these additional parameters are known to influence baking performance.

Data collected on the 1992 Recommended List (RL) trial samples were subjected to analysis of variance (Genstat 5 software). SDS volume, protein content (P), Falling Number (FN) and damaged starch (DS) were independent variables, and loaf volumes achieved in spiral mixing and CBP processes were dependent variables.

Each of P, FN, and DS was added to SDS to test whether there was an improvement in predictability and by establishing the regression with all four independent variables, removal of each one in turn allowed the relative importance of each variable to be assessed.

2.4 The relationship between protein content and loaf volume

Traditionally, it has been believed that there is linear relationship between protein content and loaf volume. Application of nitrogen fertilisers to breadmaking wheat has been based upon the belief that, the higher the protein content, the better the quality and higher the premium. There is much evidence to suggest that in the past, and for specific varieties, this was indeed true (Hoseny, 1986).

Recent work (Sylvester-Bradley, 1990) has suggested that in the Chorleywood Bread Process (CBP), the relationship between protein content and loaf volume may not be linear, and indeed that there may be little advantage in aiming for higher protein contents.

Work at FMBRA (Osborne *et al.*, 1991) on the then new variety Pastiche showed that there was no significant increase in loaf volume with increasing protein content. It appeared, therefore, that modern varieties were not responding to additional nitrogen in the same way that their forebears had done. The environmental and the cost pressures to reduce fertiliser treatment, made a detailed examination of the relationship between loaf volume and protein content for existing breadmaking varieties in the UK a necessary objective of this project.

Flour samples: 52 samples of Mercia, 27 samples of Hereward and 19 other samples of 12 different varieties were collected as part of the HGCA Wheat Quality Survey (1992). The sampling is based upon both regional and variety sowings within in the UK. All samples were baked by the CBP and protein content was measured by NIR corrected to 14% moisture basis.

2.5 Development of the gel-protein method

Work carried out under MAFF and HGCA contract (Pritchard and Bhandari, 1992; Pritchard and Brock, 1994; Pritchard *et al.*, 1992), has demonstrated that the weight, breakdown rate

during mixing, and elastic modulus of the fraction of wheat protein termed gel-protein by Graveland *et al.* (1979), are useful additional measures of wheat quality. The methods are now used as part of the protocol for evaluation of NIAB RL samples.

The methods as devised require sophisticated equipment such as an ultracentrifuge, and a small-strain oscillatory rheometer such as the Bohlin VOR model. For measuring the breakdown rate, time is also an important consideration as it requires making doughs. Because of the time factor, these have concentrated on gel-protein weight and elastic modulus.

The study considered four aspects:-

2.5.1 Reproducibility

This had two aims: a) to investigate the influence of % SDS, mixing time, mixing temperature, and centrifugation time on the generation of gel-protein; and b) to determine the effect of duplicate measurements, equilibrium time and temperature, stirring and removal of residual SDS on the measurement of elastic modulus.

2.5.2 Bohlin operating conditions

Dynamic oscillatory rheological measurements, such as those used to measure gel-protein rheology, should be carried out at a strain within the linear viscoelastic region for the sample being tested. Within the linear viscoelastic region the measured rheological parameters, e.g. elastic modulus (G′) and viscous modulus (G″) are independent of the applied strain. When the gel protein rheological test was developed it was found that an applied strain of 142×10^{-3} was in the middle of the linear region and this strain has been used for all subsequent measurements. In the current study we have tested the linear viscoelastic region of a wider range of U.K. and European bread and feed wheat varieties.

2.5.3 Miniaturisation of the gel-protein tests

The standard method of producing gel-protein from defatted flour as used in this project is rather cumbersome and time consuming in two ways. Firstly, the heavy angled ultracentrifuge rotor in our possession which generates the requisite g-force holds only ten samples, and centrifugation takes about one hour, thus limiting the throughput. Secondly, the gel layer is produced as a thin layer at a sloping angle and this awkward tube geometry makes it difficult to measure both the weight and properties of the gel *in situ*. The gel must be scraped out carefully in a labour-intensive process.

These drawbacks derive from the fact that a rather dilute dispersion of flour in SDS (5g/90ml) is used which necessitates the large volume in order to produce enough gel material for measuring purposes. The Bohlin requires at least 3 g of gel-protein. This is achieved in the large capacity (10 x 100 ml) angled rotor, since the alternative lighter swing-out rotors generally available take fewer and smaller tubes which have to be light enough to spin at the high speeds to produce the adequate g-force. Such rotors produce gel layers which are perpendicular to the tube walls and hence are more amenable for measuring *in situ*.

Scaling down experiments were performed to generate non-sloping gel-protein layers in various small tubes, with a relatively high ratio of (gel) height to diameter by adjusting the levels of water, flour and SDS, whilst maintaining the saturation binding of about 1.4g/g SDS/protein to yield comparable amounts of gel-protein to that in the standard assay. Additional studies were conducted to characterise the scaled down level of gel-protein *in situ* using alternative methods to rheology.

2.5.4 Bohlin Dynamic Shear Rheometer

The dynamic shear rheometer (DSR) is a controlled stress instrument that was designed initially for testing asphalt to ensure it conformed to US Highway regulations. It is less versatile than the VOR and has a much lower price, ~ 1/4 - 1/3 of the price of the VOR. The software is simple to use and can be configured to indicate pass or fail, making it unnecessary to study complex rheological data. These features would make it particularly appropriate as a quality control instrument. See Appendix 4.

We have consulted with Bohlin, who have adapted a DSR to take a cup-and-bob fitting, suitable for gel-protein testing and we have evaluated this alongside the VOR on the 1993 RL samples. We currently use the VOR in oscillatory mode to measure gel-protein rheology. An oscillatory sweep is carried out over the range 0.1 - 20 Hz and the data at 1 Hz is used to compare samples. The measurement is carried out with a cup-and-bob, internal diameter 14 mm, at an applied strain of 0.142. This is in the linear region for gel-proteins, although it is towards the upper end and we intend to reduce the strain applied to a lower level. However, the 1993 RL samples were tested at a strain of 0.142 in keeping with previous work.

2.6 Rheological tests

2.6.1 Bulk rheology of bread doughs

Earlier work at FMBRA on the stress relaxation of bread doughs had found a relationship between the initial and secondary relaxation gradients and loaf volume (Collins and Pritchard, 1992). This relationship has been used to produce a model to predict loaf volume from the rheological parameters derived from the Bohlin VOR rheometer.

2.6.2 Surface rheology of dough liquors

Studies at FMBRA had suggested that the observed varietal differences in the capacity to retain gas within the baking dough may involve surface phenomena (Sahi and Sonecha, 1991). This difference may be attributed to the non-glutenin fraction of wheat and the property of the films formed by water-soluble components. The surface characteristics of films of water-soluble components were determined on a number of selected current UK varieties including Torfrida.

2.7 Gluten index using the Glutomatic

The protein content (N x 5.7) is generally regarded as an important indicator of wheat quality, and has been shown to correlate with wet gluten content. In some countries, such as Austria, the quantity and quality of gluten measured in the flour are used for the commercial evaluation of wheat. The "swelling number" (Quellzahl) test is performed to

measure the influence of lactic acid on gluten, and can be useful in detecting certain slimy characteristics of gluten caused by insect damage (Aberham, 1969). The gluten index test, a rapid measurement of the same basic characteristics of wet gluten, has been reported recently using the Glutomatic (Perten, 1989; 1990).

2.8 Monoclonal antibody study

The applicability of a monoclonal antibody (Mab) in developing a quantitative assay for measuring the quality of a wide range of bread and biscuit-making wheat flours was assessed using a recently available rapid filter-membrane format ELISA technique.

Working in collaboration with the Institute of Food Research (IFR), Norwich, we have studied a broad-specificity monoclonal antibody, IFRN-0610. This Mab has been raised against glutenins and recognises the total gliadin and glutenin fractions of wheat, as well as exhibiting a very broad specificity towards prolamins from rye, barley and oats (Brett *et al.*, 1990).

A one-step extraction of prolamins, based on a method previously described by Mills *et al.*, 1990, was carried out, in duplicate, on a large number of de-fatted samples from the 1993 harvest of the National List (NL1 and NL2) trials. The sample extracts, prolamin standard and the antibody conjugate were stored in aliquots at -20°C. A modified 96-well microtitre plate ELISA (enzyme-linked immunosorbent assay) technique, originally developed by Dr Clare Mills of IFR, Norwich, was employed (Fig. 1, Appendix 5). Each plate contained, in triplicate, the following: 1) reagent blank; 2) 1.56, 3.13, 6.25, 12 and 25 μg/ml prolamin standard points; 3) duplicate extracts of Mercia 19, the control sample; 4) duplicate extracts of up to 12 of NL trial flour samples. Thus each flour sample had a total of six data points. Mercia 19 (NL2, Cambridge site) was included as an internal control sample, and was extracted each time with every set of 12 NL samples extracted, from a total of 158 flour samples, over a period of several weeks.

The total prolamin fraction of Avalon, comprising the gliadin and glutenin group of proteins, was used for the standard curve. The data were analysed using the Kineti-Calc software (Anachem Ltd), featuring a cubic spline curve-fitting programme for generating and analysing the prolamin standard curve which enabled the calculation of the relative prolamin concentration of each flour extract (Fig. 2, Appendix 5).

The monoclonal antibody IFRN-0610, which had been conjugated with horse-radish peroxidase, and the chromatographically purified prolamin standard were both supplied by Dr. Clare Mills of IFR, Norwich. The Chromogen and substrate buffer were purchased from Cambridge Veterinary Sciences Ltd, the Multiscreen vacuum manifold and the Multiscreen HA (0.45μm membranes made of mixed esters of cellulose) filtration plates were from Millipore Ltd. Other reagents were of standard laboratory grade.

The end-point of the assay, measured as an absorbance at 450 nm, was determined photometrically on a Bio-Tek EL311 microplate reader (Anachem Ltd) connected to a computer for data storage and processing. The rapid-flow nitrocellulose membrane filter-plate immunoassay is facilitated by a rotary vacuum pump, which sucks the reactants through the membrane in each well. As this method does not require any standing time for the

antibody/antigen reaction and washing procedures, it allows the ELISA protocol to be performed in under 30 minutes for each plate, compared to the several hours normally required using the conventional format.

The following solutions were used:

PAM*	50% propan-1-ol, 2% acetic acid, 100 mM DTT (dithiothreitol). M* denotes mercaptoethanol which was originally employed but later replaced with DTT
TPD	50 mM Tris, 50% propan-1-ol, 50 mM DTT, pH 7.4.
TBS	50 mM Tris, 0.14 M sodium chloride, pH 7.4.
PBST	Phosphate buffered saline, Tween. 20 mM phosphate, 0.15 M sodium chloride, 0.05% Tween 20, pH 7.4.
Stop soln.	2 M sulphuric acid.
Antibody conjugate	IFRN-0610. horse-radish peroxidase, diluted x1000 in PBST.
Substrate	50% Chromogen, 50% Buffer.
Prolamin standard	Protein was dissolved in PAM, and diluted to give a standard curve in the range of 1.56 - 25 μg/ml.

All the sample extracts were diluted x100 in PAM prior their addition to the ELISA plates. Care was taken to minimise the time between making dilutions of the working prolamin standards and the antibody conjugate from their respective frozen stock solutions, and applying them onto the assay plates.

3. RESULTS AND DISCUSSION

3.1 Small-scale measurement of work-input requirement

The objective of this project was to develop new or improved methods of assessing wheat quality. In the changed circumstances of the 1990's, increased dependence on home-grown wheat, rapid changes of variety, variable growing climate and more targeted breeding programmes have resulted in traditional measures such as protein content and SDS test not always adequately reflecting baking quality.

In the late 1980's in the particular circumstances of the CBP, promising new varieties such as Fresco failed in the market place because of a high work-input requirement. The desirability of a small-scale test to determine this quality attribute is apparent. In this project some progress was made towards such a test; in particular into the mixing of doughs with the Compudomixer.
Fig.1 shows a mixing speed versus mixing time plot for a commercial CBP bread flour. The mixing time of this material in the CBP would be just over 2 minutes. If an arbitrary mixing time of 130 seconds were used, the mixer speed would be 163 rpm. This confirmed that the Compudomixer system was appropriate for small-scale CBP mixing and for the study of work-input requirements of wheat varieties. Further work was terminated since considerable method development was required for test baking, and other aspects of the project were deemed to have greater potential for success.

3.2 Identification of protein fractions which influence baking quality

3.2.1 Two-dimensional polyacrylamide gel electrophoresis

Visual inspection of the 2-D PAGE patterns of SDS-extracted samples revealed no differences between the two samples of Torfrida. However, water-soluble components of these samples had a common subunit pattern, but some differences in relative staining intensity of several spots were observed (Fig.2). Some day to day variation in spot patterns and staining intensities were inevitable given the sensitivity of the technique. The availability of the CE technology which was also aimed at identifying quality related protein fractions resulted in 2-D gel electrophoresis studies being shelved.

3.2.2 High performance capillary electrophoresis (CE)

CE technology has the ability to resolve rapidly and identify a wide range of bio-molecules within an automated system, and therefore has a potential application in the routine analysis of key components as markers of breadmaking quality. However, CE is a rapidly developing technique which has been available in a commercial form only in the last few years (Schomburg, 1990). Published reports on cereal analysis by CE have been very limited (Bietz & Schmalzreid, 1992; Bietz, 1992). A study was undertaken to optimise sample extraction and CE running conditions, and look for markers of wheat quality.

Separation of gliadins by MECC

The best results were obtained employing the micellar electro-kinetic capillary chromatography (MECC) mode (Terabe *et al.*, 1984 & 1985; Li, 1992) with 20 - 60 mM sodium tetraborate, 1% SDS, 20% methanol, pH 9 as the resolving buffer, on samples extracted with 75% methanol. The varieties examined were: Avalon, Mercia, Hereward, Riband, Minaret, Slejpner, and Jerico, as well as Galahad and Brock which are difficult to distinguish by acid-PAGE. The gliadin fraction analyses of some of these varieties are shown in Fig.3. Here several differences between the profiles of Avalon, Riband, Hereward, and Mercia extracts can be observed, in addition to their common peaks.

Reproducibility of CE runs

A gliadin fraction of Mercia extracted with 70% methanol was subjected to seven consecutive runs, with a capillary wash using 0.1 M sodium hydroxide between each run to remove any adhering material.

Table 1

#	Buffer used	Run no.	A	B	C	F	K	L	O	R	V
			Peak Migration Times (mins)								
Mer 1	x0	1	9.34	10.46	11.47	12.70	13.67	14.14	14.66	15.32	16.73
Mer 2	x1	2	9.09	10.21	11.16	12.35	13.28	13.70	14.24	14.85	16.19
Mer 3	x0	1	(----- fault developed, run aborted ------)								
Mer 4	x1	2	8.96	10.08	11.01	12.16	13.06	13.48	14.01	14.62	15.89
Mer 5	x2	3	8.94	10.09	11.02	12.18	13.09	13.51	14.04	14.65	15.93
Mer 6	x3	4	8.89	10.11	11.04	12.20	13.10	13.51	14.05	14.80	15.95
Mer 7	x0	1	9.06	10.18	11.14	12.31	13.23	13.63	14.18	14.80	16.08
	average		**9.05**	**10.19**	**11.14**	**12.31**	**13.24**	**13.66**	**14.20**	**14.82**	**16.13**
	± (%)		2.5	1.9	2.1	2.9	2.3	2.4	2.3	2.4	2.6
			Peak Areas								
Mer 1	x0	1	777	2,202	2,222	3,128	7,984	4,529	11,998	2,836	1,850
Mer 2	x1	2	709	2,620	1,498	7,943	3,315	4,081	8,256	2,016	1,830
Mer 4	x1	2	1,263	2,258	1,976	10,134	3,807	5,495	10,342	2,760	2,212
Mer 5	x2	3	793	1,641	1,767	9,469	3,519	4,824	10,066	2,495	2,344
Mer 6	x3	4	989	1,854	2,313	7,319	3,352	4,839	9,489	2,365	2,295
Mer 7	x0	1	964	1,103	2,103	9,175	3,506	4,392	5,668	1,980	2,213
	average		**916**	**1,946**	**1,980**	**7,861**	**4,247**	**4,693**	**9,303**	**2,409**	**2,124**
	± (%)		3	39	21	38	55	15	34	18	12

Fig.4 shows two typical runs, Mercia 1 and Mercia 2, where peaks selected for analysis are labelled, and where most of the migration times are included. Comparison of the peak migration times and areas for six runs indicate that the migration times are more reproducible than peak areas. The migration times vary by 1.9% to 2.9%, whereas the areas vary by 12% to 55% (Table 1).

Analysis of water-soluble metalloproteins from Torfrida 1 & 2 samples

The two samples of Torfrida (see section 2.2.1) were further examined by CE. Metalloprotein complexes (T1 & T2) which had been previously purified from the water-soluble fraction from Torfrida 1 and 2, and had been shown to differ in their ability to depolymerise gel-protein were subjected to CE. As the two Torfrida flours had distinct baking and dough rheological properties, these metalloprotein complexes could be regarded as quality markers (Pritchard & Bhandari, 1992). CE analysis was performed in the MECC mode and T1 produced a single peak at 16 minutes, while T2 produced multiple peaks at around the same place (Fig. 5). This preliminary investigation supports the earlier study in that the two metalloprotein complexes are distinguishable by another technique and may contain unique components which influence the breadmaking quality of the Torfrida flours.

The search for protein markers of quality involving the fractionation of wheat proteins (sections 2.2 and 3.2) indicated that both 2-D polyacrylamide gel electrophoresis and CE are capable of detecting and identifying key individual species. The latter is a very rapid (typical run times 10 - 20 mins), requiring no staining/destaining steps and offers greater resolution than most of the existing techniques. The reports of capillary array electrophoresis where multiple CE separations are performed in parallel using bundles (of up to 24) microcapillaries demonstrate that this technology may be adapted to give routine high-speed, high-throughput analysis of wheat samples (Mathies and Huang, 1992).

In our study, an optimal procedure was developed for resolving the gliadin fraction, in which the peak migration times did not vary by more than 3%. This compares favourably with gel electrophoresis where subunit migration can be difficult to control precisely and reproducibly. Much larger variations were observed for the peak areas, especially the smaller ones. This was due to the drifting baseline which could be reduced by further method development and improved data-handling computer software.

The fraction collection facility on the instrument would make it possible for the characterisation of components within the resolved peaks by other sensitive techniques such as 2-D polyacrylamide gel electrophoresis. A preliminary study of the separation of high molecular glutenins based on size, by employing a viscous sieving material in a capillary, has recently been reported (Applied Biosystems Newsletter, 1993). However, much more work would be required to examine this and other fractions, and to develop the software for improving the analysis of the electrophoretic profiles. We felt that such a labour-intensive study was beyond the scope of the present project.

The metalloprotein complexes from the two near-isogenic samples of Torfrida have been resolved by CE. Because of their putative involvement in wheat redox systems (Pritchard and Bhandari, 1992), they are likely to play an important role in quality. These components may serve as quality markers in CE analysis and antibody assays.

3.3 The predictive ability of the SDS sedimentation volume test

The proportion of the variability in loaf volume explained by each of the independent variables; protein (P), Falling Number (FN) and damaged starch (DS) is listed in Table 2.

Table 2

Predicting loaf volume: the (%) influence of measured flour properties

Parameter	SDS	SDS + P	SDS + FN	SDS + DS	SDS + All	SDS + FN+DS
Mixing method						
Spiral	26	32	40	55	63	62
CBP	28	30	30	41	48	NA

When each parameter was removed in turn from the overall predictability, it was found that for the spiral mixer, protein content was having no influence in the analysis of variance. For the CBP, interactions between P, FN, and DS meant that the influence of each parameter was dependent upon those remaining in the equation.

The low level of predictive ability for SDS alone is probably a reflection of the samples studied. Only those varieties that are suitable for breadmaking are now tested by methods appropriate for breadmaking wheat. Thus the quality range was limited in this study.

It was apparent from examination of the raw data that the association between loaf volume and protein content is not necessarily linear. For this reason, quadratic terms were introduced. They had the effect of raising the predictive ability of the SDS test to 74% for spiral mixing, and to 66% for the CBP. For spiral mixing P and FN were included in the relation. Again, interactions between parameters in the CBP prevented any judgement of the importance of individual parameters.

A recent study in Canada (Ayoub *et al.*, 1993) concluded that the SDS test seemed unable to differentiate between eastern Canadian wheats with different performance in the remix test and that it was not suitable for predicting wheat strength.

Our study of the effect other quality parameters have on the predictive ability of the SDS test in terms of loaf volume, has shown that all three parameters: protein content, Falling Number (a measure of α-amylase) and damaged starch (influenced by grain hardness and a large influence on water absorption) can improve the correlation between SDS volume and loaf volume. The value of the SDS test, which lies in its simplicity and speed, would be compromised by the need to carry out other quality tests. However, protein content (by NIR) and Falling Number are rapid tests. Damaged starch can be predicted from grain hardness by NIR.

These results were obtained using samples from one season and require data from future harvests to establish the relationships observed and their long term viability. It is clear that some modification to the SDS test, provided that it retained speed and simplicity, could make it more applicable in current circumstances.

3.4 The relationship between protein content and loaf volume

Figs. 6 and 7 show the relationship between protein content and loaf volume for Mercia and Hereward respectively. There was insufficient data for the other varieties. From the scatter of the data points it is not clear as to whether the relationship for Mercia is linear or non-linear. The data set do not allow a judgement to be made as to whether loaf volume falls at protein contents greater than c.13% or whether it is at a plateau above 12% protein level.

The correlation coefficient between protein content and loaf volume was 0.21, suggesting that for this sample set, no significant correlation existed between the two. This set of samples did not, of course, come from a controlled experiment. They were selected merely on the basis of region and varietal balance within a region. They did, however, represent the practical situation faced by the milling industry in its everyday purchasing of wheat. The results obtained here add credence to the finding of Sylvester-Bradley (1990) and confirm the impression gained with the variety Pastiche (Osborne *et al.*, 1991).

However, other studies at Chorleywood (Salmon *et al.*, 1990) suggested that a controlled experiment with the variety Mercia, application of nitrogen fertiliser (as ammonium nitrate or as foliar applied urea) increased grain protein content with concomitant improvement in baking quality (loaf volume). These relationships, have of course, been deduced from one season's grains, covering two varieties, albeit those most likely to be grown for breadmaking purposes. To validate them it will be necessary to repeat the study on other annual harvest samples, covering as wide a range of samples / varieties as possible.

Protein content is a very important yardstick in the buying and selling of grain. These results could have major implications for the grain trade as there may be little purpose in buying the more expensive high protein wheat for white breadmaking. For wholemeal, high protein contents are required (Pritchard *et al.*, 1992). These data also have implications for the use of nitrogen fertilisers. If high protein contents are no longer necessary, there could be scope to reduce fertiliser applications, which would be of environmental and financial benefit.

3.5 Development of the gel-protein test

3.5.1 Reproducibility

Our preliminary results suggest that while the extraction of gel-protein is tolerant of changes in experimental method, the centrifugation stage is important. If the time dropped below 20 minutes the speed did not reach 25,000 rpm, resulting in starch retention in the gel.
The standard rheological test of gel-protein requires 3 - 5 g of material, therefore, only ~50% of the extracted protein is examined. Duplicate measurements on each sample resulted in a 50% reduction in standard deviation. Duplicate measurements have now been incorporated into the standard protocol for the elastic modulus of gel-protein. Although stirring the gel-protein layer was shown to reduce variability, the agitation caused a

significant reduction in elastic modulus (from 37.9 Pa to 30.6 Pa, mean data). This has not been adopted.

3.5.2 Bohlin VOR operating conditions

All varieties tested show a linear region for G′ and G″ that begins at a strain of $\sim 20 \times 10^{-3}$ (Fig. 8a - d). For strong varieties, such as Cadenza (Fig. 8a), the linear region continues until a strain of 200×10^{-3}. For weaker U.K. wheat varieties (e.g. Mercia and Riband, Figs. 8b+c) the linear viscoelastic region begins to fail at strains of $\sim 150 \times 10^{-3}$. For the European varieties (eg Rinconada Fig. 8d) studied the linear region is completed at lower strains ($\sim 80 \times 10^{-3}$).

These results suggested that carrying out oscillatory measurements at a strain of $\sim 70 \times 10^{-3}$ may be more appropriate for gel-protein samples. At the lower strain samples would be measured at a strain closer to the centre of the linear viscoelastic region. This would reduce the possibility for variation in the results of sample measurements.

A sample of Mercia was then measured in oscillatory mode at a range of different strain levels. The variability of the measured gel-protein elastic modulus was found to be least at a strain of 63×10^{-3} (Table 3). Both above and below this level of applied strain the variability in the results increased. The elastic moduli values fall with increasing strain, although there is only a small change after the strain exceeds 50×10^{-3}. This confirms the presence of the linear viscoelastic region above this strain.

Table 3
Elastic Moduli and Standard Deviations of Mercia Gel-Proteins Measured at Different Levels of Applied Strain.

Applied Strain	Mean Elastic Modulus at 1 Hz, Pa	Standard Deviation	Standard Deviation/ Mean Elastic Modulus
0.0247	62.58	12.19	19.48
0.0371	56.1	8.13	14.49
0.051	47.17	8.07	17.11
0.0628	45.53	3.06	6.72
0.0751	43.18	4.67	10.82
0.142	39.48	5.21	13.20

Further work with the varieties Spark, Hereward and Rinconada at the applied strain levels of 62.8×10^{-3} and 14.2×10^{-2} confirmed that there was less variation in results at the lower strain. At the strain level of 14.2×10^{-2} the pooled standard deviation was 12.2% of the sample mean. When the strain of 62.8×10^{-3} was applied in the oscillatory test the pooled standard deviation fell to 5.4% of the sample mean.
These results indicate that when gel-protein is subjected to oscillatory measurements to determine their elastic modulus an applied strain of 62.8×10^{-3} will produce more accurate

results for a wider range of samples than a higher strain of 14.2×10^{-2} which has previously been used.

3.5.3 Miniaturisation of the gel-protein test

A 3 x 25 ml capacity swing-out rotor used at a speed of 27,000 rpm (100,000 x g), which produced a clearly defined gel layer within a total run time of 40 minutes. Satisfactory gel layers were obtained using 2.5, 3.0, 5.0 g flour and 1.5, 7.5, 15 ml 9% (w/v) SDS respectively with the 25 ml transparent polycarbonate centrifuge tubes. Measuring both the thickness and weight of scraped out gel layer showed that the gel-protein was produced in variety-dependent manner, when Soissons, Mercia, Hereward, Galahad and Riband were examined (data not shown). Further scaling down was achieved with 0.5 g Fresco flour and 1.5 ml 9% SDS in 2.0 ml microcentrifuge tubes which were supported in a cushion of water to prevent them from collapsing during spinning.

Attempts were made to characterise these "mini-" gel-protein preparations by examining the force/penetration curves obtained by using a Stevens CR Analyser penetrometer with a 5 mm diameter flat-ended cylindrical stainless steel probe. This instrument applies the probe to the gel layer at a constant rate and measures the variation in resistance force with depth of penetration. It was found that such a system lacked the sensitivity required to record the depth and rheological nature of the gel-protein inside the centrifuge tube.

3.5.4 Bohlin Dynamic Shear Rheometer

These samples were also tested on the DSR rheometer in oscillatory mode, the frequency sweep was over the range 0.1 - 2 Hz. A cup-and-bob, internal diameter 14 mm, was also used in this instrument. A shear stress of 1.4 Pa was applied to the sample and this generated a strain of ~ 0.07, this value of strain is close to the level that it is intended to start using on the VOR.

The elastic modulus (G′) of gel-protein is the rheological parameter that we currently use as a quality measurement. The two rheological instruments showed good agreement in their measured G′ values. Fig. 9a shows the relationship between the two measurements with a fitted linear regression of 0.9. There is little difference in the magnitude of the G′ values, suggesting the lower strain applied on the DSR has had little effect on the measured elastic properties.

The viscosity (η) and viscous modulus (G″) of gel-proteins measured in the two instruments show good agreement (Figs. 9b+c) with a correlation coefficients of 0.89 in both cases. However the magnitude of the viscous properties are slightly lower when measured in the DSR compared to the VOR. This suggests that the lower strain applied in the DSR is influencing the measurement of the viscous properties of the gel-protein.

Fig. 9d shows the relationship between phase angle measured on the two instruments. The phase angle of gel-proteins measured on the VOR are typically in the region 25 - 30° C, and the samples studied here fell in the range ~22° - 33° C. The range of phase angles recorded by the DSR was much broader ~20° - 57° C, with the bulk of the samples falling between ~20° C and 30° C. The differences may in part be attributed to the lower strain

in the DSR and the subsequent effect on the measured viscous properties.

The phase angle measured on the DSR decreases exponentially with increasing elastic modulus (Fig. 10). This suggests that in samples with high elastic moduli values the elastic properties dominate rheology to a greater extent than in samples with low elastic moduli. This relationship cannot be seen so clearly from the VOR data (Fig. 10) because their is not such a wide spread in phase angle values.

These findings suggest that the DSR or other simpler rheometers could be used to routinely measure gel-protein rheology without a great loss in sensitivity of the measurement. Both rheometers require only minimal training to measure the rheology of gel-proteins in a cup-and-bob system. The major difference between the two instruments is the greater range of operating conditions that can be applied using the VOR instrument. The DSR holder assembly for the cup-and-bob requires some modification. In addition, the circulating water bath has to be turned off after each measurement to allow removal of the cup for washing and sample loading. However, this should be easy to rectify.

In conclusion, we have fully evaluated and improved on the gel-protein test, and investigated ways in which it may be successfully performed on less complex and cheaper instrumentation.

It was found that the generation of gel-protein material from the SDS solution/flour mix is tolerant of changes in extracting conditions such as time, temperature and sample handling. However, the centrifugation stage must be rigorous enough to separate starch components from gel-protein.

The reproducibility of the elastic modulus method using the Bohlin VOR can be improved by replicate analysis of each gel-protein sample, and by stirring the gel prior to loading the rheometer. Temperature and time of equilibration prior to measuring have little influence on absolute values or on the standard deviation.

The Bohlin measuring protocol for gel-protein was originally set up using a strong flour. This study has shown that when gel protein is subjected to oscillatory measurements to determine its elastic modulus an applied strain of 62.8×10^{-3} will produce more accurate results for a wider range of samples than a higher strain of 14.2×10^{-2} which has previously been used.

The improvement in the reliability of the elastic modulus (G') test has implications for the testing of Recommended List (RL) samples. At present, the RL protocol allows for two tests of gel protein quality: the breakdown rate and the elastic modulus. The former is time consuming and expensive. During the course of the present study, improvements to the elastic modulus method have been adopted and the technique is now used as the sole quality test of the gel-protein fraction for the assessment of RL samples with consequent savings in cost. From 1994 and onwards, the gel-protein of the NL samples will also be tested only for the elastic modulus, which will provide breeders with more information to help them assess varieties with greater confidence in the predictive stages.

However, there may be occasions when in the event of an unexpected elastic modulus result, measurement of the breakdown rate would provide additional information. The elastic

modulus of gel-protein has become a useful quality test for UK wheat. Recent work has shown that it is also suitable for quality testing of German varieties for use in the Rapid Mix Test (RMT) (Pritchard and Abel, 1994). At present it is a laboratory test which requires an ultracentrifuge and a sophisticated rheometer. We have attempted to miniaturise the test to make it more applicable throughout the grain trade.

Attempts at miniaturisation of the gel-protein succeeded in: a) developing a method of increasing the relative proportion of gel layer in each centrifuge tube; b) producing gel layers perpendicular to the tube axis which can be measured more easily and more accurately. We have produced measurable levels of gel-protein in 2 ml tubes, which will allow the processing of large number of flour samples. The technical challenges of rheologically assessing gel-proteins *in situ*, may be met by exploiting emerging techniques. Instrumentation such as those which measure the density variations in colloidal materials by sound echo, or by applying an alternating voltage, causing particles to move back and forth and generating sound waves, as featured by the Acoustosizer (Dayton, 1994), may be suitable. Small scale measurement of gel-protein would be very useful for analysing large numbers of samples of limited material which may be available at the time of selecting varieties.

The present requirement an expensive research grade rheometer may be addressed by the development of the Bohlin DSR. We have shown that the quality control instrument can be used to measure accurately the gel-proteins extracted from wheat flour. This instrument costing between a third and a quarter of the price of the Bohlin VOR can produce equivalent results. There remains a possibility that the cost may be reduced even further with the development of a wholly dedicated instrument.

3.6 Rheological tests

3.6.1 Bulk rheology of bread dough

Predicted values of loaf volume derived from relaxation slopes of stress-relaxation curves of bread dough made from a number of current UK varieties are plotted in Fig.11.

The stress relaxation curves of bread dough can be used to predict loaf volume ($r=0.91$). By comparison, the yeasted dough expansion test is able to predict CBP loaf volume with $r=0.87$ (Pritchard, 1987). This was marginally better than the SDS test for wholemeal flours. The rheological test would have one major advantage; it is carried out on yeasted bread doughs and, therefore, truly reflects the processing performance. All these studies have been carried out on the Bohlin VOR. It is probable that, as with the elastic modulus measurement in the gel-protein test, a simplified rheometer could be developed.

3.6.2 Surface rheology of dough liquors

The amount of dough liquor extracted from the various flours is listed in Table 4. Haven produced very little liquor and therefore could not be studied. Fresco yielded the greatest quantity. The dough liquors differed in viscosity; the extremes being Dean (very viscous) and Fresco (water-thin). The other varieties were intermediate.

Table 4

Wheat variety	Dough weight centrifuged g	Yield of dough liquor g	% dough liquor on dough weight	Comments on dough liquor
Dean	68.1	3.02	4.43	Thick, v. viscous
Fresco	66.0	10.17	15.41	Watery
Hereward	88.0	8.25	9.35	Viscous
Haven	84.0	0.20	0.24	-
Riband	86.0	7.36	8.56	Slightly viscous
Mercia	88.5	10.26	11.59	Viscous
Torfrida 1	88.0	11.7	13.3	Runny
Torfrida 2	88.0	7.5	8.5	Viscous

The degree of variation in dough liquor quantity was unexpected, since all the doughs had been mixed with water additions equivalent to the measured water-absorption level. This data would suggest that water-absorption does not reflect the true water-requirement of a flour. The surface tension of reconstituted freeze-dried dough liquor (1 g/100 ml water) also varied between the samples (details may be obtained from FMBRA). Of the current UK varieties, Fresco reduced the surface tension of water least. With the exception of Dean, the order of surface tension values followed breadmaking performance. Plots of changes in surface viscosity are shown in Fig. 12. Again Fresco and Dean were extremes. The two (near isogenic) Torfrida samples were similar to Mercia. The surface elasticity data (not shown) ranked Fresco, Hereward and Mercia in the same order as surface viscosity. Riband and Dean did not display any surface activity.

Dough liquor represents the free water of a fully developed dough in which bubble nuclei are occluded during mixing. It is composed of flour constituents which have been solubilised by the limited amount of water available after flour has been fully hydrated. These materials are thought to exert their influence at the gas bubble / dough liquor interface and dough liquor / starch and / gluten interface. The mechanical properties of the thin films formed in the dough are thought to influence the stability of the gas bubbles and hence their gas retaining properties. A film possessing good rheological properties such as elasticity and viscosity would be expected to stabilise gas bubbles against rupture for a longer period of time relative to gas bubbles surrounded by film of poor rheological properties.

The surface measurements performed determine the properties of the non-gluten water-soluble fraction of flour. Thus they provide additional information to that obtained by conventional flour and dough testing methods. In conjunction with 2-D PAGE and CE, this technique could be exploited to further understand the function of the less well known non-gluten proteins and other components in wheat quality.

3.7 Gluten index using the Glutomatic

3.7.1 Analysis of selected samples

The Glutomatic was used to measure the gluten index for a range of different flours. The samples were tested over a period of time during which changes were made both to the design of the machine and the way in which it was operated after consultation with the instrument suppliers: Calibre Control Instruments Ltd.

The gluten index of a sample, as measured by the Glutomatic, is defined as the average of the two readings corresponding to the left and right chambers. The manufacturer claims that the readings from the two chambers are acceptable only if they are within 8 units of each other. The manufacturer has claimed that the standard deviation of repeatability is 5.2, and the standard deviation of reproducibility is 8.3, where

Repeatability refers to the variation in the measurements when conditions in which the machine is used are kept constant.

Reproducibility refers to the variation in the measurements when the readings are made on different occasions, by different operators, etc.

For each of the following flours Mercia, Torfrida and Hereward, four consecutive readings were taken to determine the instrument repeatability. The results are as follows:

Table 5

Sample	Mercia	Torfrida	Hereward
1	68.48	89.92	68.38
2	67.99	88.31	68.49
3	65.89	85.34	71.04
4	60.16	79.53	71.22
Mean	**65.63**	**85.76**	**69.78**
Std.dev.	3.82	4.58	1.56

In all cases, the observed variation in the readings was less than the standard deviation of repeatability claimed by the manufacturer.

Full details of individual determinations of gluten index are not given and may be obtained from FMBRA. Table 5 gives the mean value of the gluten index, for each flour type, together with the observed standard deviations.

In all cases, the observed variation in the readings was less than the standard deviation of reproducibility claimed by the manufacturer. However, it can be seen from the range of standard deviations, that the variation is greater at the lower than upper end of the range of values for the gluten index, i.e. the readings are less reproducible at low values. Riband was

a typical example of a flour having a gluten index at the lower end of the range; in many cases, readings were unattainable due to the operator experiencing problems with the machinery flooding or the starch not washing out.

The mean results show that the flours under study have been classified into several groups according to the gluten index. Strong flours like CWW, Torfrida and Talon have a gluten index of 80 or more, while breadmaking flours like Hereward, Estica and Mercia have a gluten index in the range of about 50 to 60. Pastiche, whose performance was found to be too variable for commercial use (Osborne *et al.*, 1991), has a gluten index less than 50, the lowest of all the flours under study (Table 6).

Table 6

Gluten Index Values

	N	Mean	Std.dev.	Min	Max	cv
CWW	6	88.3	2.04	85.4	90.8	2.3%
Tor	16	84.5	5.95	76.6	95.9	7.0%
Tal	6	83.9	2.83	79.0	86.8	3.4%
Dea	7	67.7	8.60	56.4	80.0	12.7%
Her	15	53.8	7.28	48.3	71.1	12.5%
Est	7	55.3	6.53	40.6	59.0	11.8%
Mer	21	54.4	7.37	41.3	68.5	13.5%
Pas	8	47.5	3.26	43.4	54.4	6.9%

To look at differences between flour types and between wheat varieties, a boxplot is used (Fig. 13). Boxplots display the main features of a data batch and permit simple comparisons of several batches. Here, the boxes represent the middle 50% of values for each flour type, i.e. the range of values between the lower and upper quartiles. The median, i.e. the value for which the 50% of the values are lower, is indicated by a cross within each box. The tails attached to the boxes join values which lie above and below the upper and lower quartiles respectively. Possible outliers are indicated by an '*' and 'O' depending on their extremity.

On the basis of these results, the gluten index does not distinguish between flours within the same group, i.e. strong (G.I. > 80), medium (G.I. range of about 50-60) or very weak (G.I. < 50).

3.7.2 Samples grown on different sites

The varieties Torfrida, Hereward, Mercia and Pastiche which had been grown on 5 different sites were examined for their gluten index values. No significant differences were observed between sites for each variety (Fig. 14), suggesting that the gluten index lacks sensitivity in determining any effects of the environment on gluten quality. This could have some advantage for breeders who require a measure of quality independent of site or of season. However, for end use quality, where seasonal and site variation are known to be important, this feature could be a disadvantage.

3.7.3 Analysis of the 2 sieved fractions of gluten by gel electrophoresis

In an effort to understand the basis of the gluten index test, and in particular the mechanism underlying the partitioning of the gluten material through the sieve, the composition of the retained and non-retained portions were examined. SDS-PAGE (gel electrophoresis) analysis of the retained and non-retained portions of six varieties revealed no apparent differences between the two fractions. (Data not shown)

3.7.4 Gluten index values of heat damaged flours

The gluten index values of Mercia flour of varying degrees of heat damage, was determined from samples produced by blending untreated flour with 100% heat damaged flour (Fig. 15). The mean values of the heat damaged samples were lower than the control samples (0% heat damage), exhibiting sensitivity even at the 5% heat damage level. This preliminary result suggests that gluten index may be an useful indicator of heat damaged flour.

It was concluded that the gluten index as not particularly suitable as a rapid test because it lacks robustness, at least in our hands. We did, however, find that analysis of a large number of samples enabled the ranking of varieties based on the gluten index that is generally consistent with rankings based on gel-protein parameter and loaf volume ranking. It was also able to differentiate Pastiche from the strong flours. The method is very sensitive to a number of factors including sample and buffer temperature, humidity, the frequent clogging-up of the nylon sieves which led to having to use new sieves, handling of the washed gluten (i.e. taking care not to tear it).

We found that a significant number of values were invalid because of the large differences between left and right chambers, and in the case of weaker flours, clogging-up of the sieves was a major problem. Therefore, as this method requires a substantial number of replicate determinations, it is too labour intensive in its present form for the rapid assessment of U.K. breadmaking wheats. Our preliminary studies indicate that it may provide some information in analysing heat damaged wheat flours. Taylor *et al.* (1994) have also briefly reported a relationship between heat damage and the gluten index. Our overall findings are not inconsistent with a recent report of a collaborative study to evaluate the gluten index of a limited number of samples, within as well as between laboratories (McDonald, 1994).

3.8 Monoclonal antibody study

Details of ELISA data generation and assessment are given the Appendix 5.

3.8.1 Analysis of National List Trials I

Samples

Breadmaking potential
Mercia, Trafalgar, ICI 13088, VDH 1138, Elsoms, CWW 92/6, Semu.

Biscuit-making potential
Riband, NFC451, NFC403.

All wheat varieties were grown in 1993 at the same five sites (Cambridge, Wye, Harper Adams, Headley Hall, NFC).

Test methods
The samples were tested once by each of the following test methods:

Sedimentation Volume, SDS
Gel-protein weight
Falling Number
Elastic modulus
NIR Protein
CBP loaf volume

Quality parameters and baking results

Mean results for each variety are presented in Table 7. Varieties are ranked in decreasing order of mean value. Lines are used to join together varieties which are not significantly different at the 5% level.

Varieties were ranked in a similar order by SDS sedimentation volume and elastic modulus with breadmaking varieties having higher scores than biscuit-making varieties. The rankings obtained by the other four methods were less comparable although almost all of the methods ranked Trafalgar either highest or lowest of the varieties.

All methods detected significant differences between the varieties although the classification of the varieties into groups was different. Trafalgar had a significantly lower mean falling number than the other varieties and significantly higher mean values of NIR protein and gel-protein weight. The breadmaking varieties Trafalgar, VDH, Elsoms, Mercia and CWW had significantly higher mean values of SDS sedimentation volume and elastic modulus than either of the biscuit-making varieties Riband, NFC451 and NFC403.

Antibody results

Experimental design
Varieties from a single site were all tested on the same plate, and a different plate was used for each site. This permitted more precise comparisons to be made between varieties since variations due to different plates and different growing site are removed from the comparison.

A comparison between varieties
Mean results of absorbance and concentration for each variety are presented in Table 8a. Since the comparison between varieties did not depend upon growing site the results are an average of duplicate measurements for the 5 sites. Varieties are ranked in decreasing order of mean value. Lines are used to join together varieties which are not significantly different at the 5% level.

Varieties were ranked in a similar order by absorbance and concentration with the highest ranking being assigned to Trafalgar and the lowest to Riband.

The method detected significant differences in absorbance and concentration between varieties. Trafalgar had a significantly higher mean values of these quantities than Mercia, and in turn Mercia had significantly higher mean values than the other varieties. The mean absorbances and concentrations of the other varieties varied over a much smaller range.

A comparison between breadmaking and biscuit-making varieties
For 5 out of the 6 plates used for testing, breadmaking varieties had a significantly higher mean absorbance and mean concentration than biscuit-making varieties (see Table 8b).

Relationship between absorbance (concentration) and baking results
There was a poor correlation between absorbance (concentration) and baking quality.

3.8.2 Analysis of National List Trials 2

The approach adopted to evaluate this monoclonal antibody was to treat all the samples as members of one population, work through them in variety order, site by site and to express the optical density and calculated concentration (response to the epitope) sample by sample. This approach was deemed appropriate to test how an ELISA test would be used within the grain trade if our studies showed that such tests had commercial potential. No attempt was made to arrange the samples by site or variety on a particular plate. The results obtained showed very large plate to plate variation; a matter which will have to be addressed with the manufacturer if this technique is to be commercially viable.

There were 20 varieties in NLT 2 which meant that more than one plate was required for each site. Because of the plate to plate variation it was therefore not possible to carry out meaningful statistical analysis on the NLT 2 data in the way that the smaller NLT 1 set had been. It was however, possible to make some appropriate observations.

The results obtained for each plate have been ranked in order of optical density (Tables 9 and 10) and calculated concentration (Tables 11 and 12). It is clear that the varieties are not

being ranked consistently. For example in Table 12, plate 15 variety NFC 344/1 (1018.9) was significantly greater than Sennet (587.5), (LSD,5% 243.2). On plates 18 and 20 NFC 344/1 and Sennet were not significantly different. This variability is a matter of concern and could involve the antibody-antigen response, the inherent inconsistency of the plates and to the limited quality range within this sample set. NLT 2 did not include an obvious outlier like Trafalgar!

As the project proceeded, the possibility of using monoclonal antibodies (Mab) became a reality as a consequence of the collaboration with the Institute of Food Research at Norwich. The validation of their antibody IFRN-0610 formed the principal activity of the third year of the project and was a logical development from the studies on protein fractions and improvements in the gel-protein test. This antibody was raised against glutenin and was known to respond to the repeat motif of both gliadin and glutenin. It was, therefore, expected to respond to quantitative aspects of protein, rather than to qualitative factors.

The statistical analysis showed that the Mab data clearly distinguished Trafalgar from other varieties, as did protein content, gel-protein weight and elastic modulus. The average protein content of Trafalgar from five sites is 16.08 %, and it is not surprising that the antibody responded so strongly to the high level of protein. The IFRN-0610 Mab was able to distinguish between Trafalgar and Mercia, which itself was distinct from the remaining varieties by this criteria. It did not clearly discriminate between the other varieties. The average protein content of the five samples of Mercia was 10.36 %, which is similar to many of the other varieties examined, excluding Trafalgar. It would appear that the Mab was responding to some qualitative elements in the protein content as well as quantitative ones which enabled it to pick out the current premium breadmaking variety grown in the UK.

The lack of discrimination in most of the varieties may be a function of the relatively small range of the Mab data. In Table 7 the mean values for NLT 1 samples show that the ratios of highest to lowest for each of the protein related parameters were SDS, 2.48; protein content, 1.73; gel-protein weight, 3.4; elastic modulus 11.0; Mab absorbance, 1.27 and Mab concentration, 1.99. The Mab range was of a similar order to that of protein content.

The sample set selected (National List trials, 1993) contained varieties that had already passed some screening procedures by breeders, i.e. they were expected to achieve a certain performance in the field (yield, disease resistance) or in breadmaking. Thus they represented the sort of population for which the new quality test was intended. The lack of discrimination between breadmaking varieties, with the exception of Trafalgar and Mercia, means that under the conditions of the test in this study sufficient discrimination was not possible.

However, in the NLT 1 sample set it was possible to discriminate on most plates between breadmaking and biscuit making varieties. This particular antibody might be of potential value in wheat breeding, especially as ELISA multi-well format permits large scale screening of very small samples. It is possible that such tests would allow better discrimination between breeding lines at an earlier generation where a wider range of material is evaluated, than is at present possible with the SDS test. Evaluation of the full potential of Mab IFRN-0610 requires further study, where substantial improvements in the methods involving the nitrocellulose filter-plates will be necessary.

It is known that other monoclonal antibodies have been raised to wheat storage proteins but were not available for this study. One or more of these might have more potential for discriminating between samples that are quite similar in performance.

4 CONCLUSIONS

1) Optimal conditions were developed for resolving gliadin fractions by capillary electrophoresis, and preliminary analysis was made of water-soluble metalloproteins which may be potential quality markers.

2) Our evaluation of the SDS test showed that there was a poor relationship between this test and loaf volume for the 1992 RL trial samples, however, prediction of loaf volume can be improved by taking into account the Falling Number, protein content, and damaged starch levels.

3) We have improved the procedures in the gel-protein test to make it more reliable and cost effective, and it has now become an established measure of quality in the RL trials. The elastic modulus of gel-protein has the potential for discrimination between varieties and samples. Evaluation of a simple-to-use rheometer, (Dynamic Shear Rheometer), showed that this could be adapted to become a much cheaper alternative to the Bohlin VOR and thus offer the prospect of a more widely applicable quality test.

4) The gluten index test, as performed on the Glutomatic, was evaluated and found to be indicative of quality, but lacked robustness as a rapid test and was unable to distinguish between the main UK breadmaking varieties. It indicated a low level response to heat damage in flours.

5) The monoclonal antibody, 0610-IFRN may be of potential value in wheat breeding as it was shown to discriminate 1) Mercia from other breadmaking varieties, and 2) between breadmaking and biscuit-making varieties.

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Table 7 Baking results for NLT1 varieties

	SDS		NIR Protein		Gel Protein Wt		Elastic Modulus		Falling Number		Loaf Volume
Elsoms	92.0	Trafalgar	16.08	Trafalgar	13.498	Trafalgar	43.86	Mercia	374.2	Mercia	1397
VDH	88.8	VDH	10.52	Elsoms	12.006	VDH	42.96	NFC451	344.8	Trafalgar	1391
CWW 92	83.0	Mercia	10.36	CWW	11.704	Elsoms	24.72	Semu	335.2	Elsom	1386
Trafalgar	73.2	NFC451	10.16	VDH	11.122	Mercia	14.22	ICI	327.6	CWW	1348
Mercia	63.6	NFC403	10.08	Mercia	10.282	CWW	14.02	CWW	314.4	ICI	1288
ICI	61.8	Elsoms	10.02	Semu	10.004	ICI	11.78	VDH	297.4	VDH	1246
Semu	61.4	Semu	9.92	ICI	9.682	NFC451	8.42	Riband	293.0	Semu	1227
NFC451	56.0	CWW	9.80	Riband	9.270	Semu	5.58	Elsom	285.2		
Riband	45.0	Riband	9.60	NFC451	7.398	Riband	4.98	NFC403	248.0		
NFC403	37.2	ICI	9.30	NFC403	5.358	NFC403	4.02	Trafalgar	137.0		
LSD 5%	6.64		0.857		1.184		3.85		54.88		80.21

Table 8a Antibody results for NLT1 varieties

Absorbance		Concentration	
Trafalgar	0.8911	Trafalgar	898.16
Mercia	0.8350	Mercia	731.69
Elsoms	0.7620	Elsoms	580.28
ICI	0.7610	ICI	576.60
VDH	0.7449	CWW	533.74
CWW	0.7447	VDH	533.41
NFC403	0.7304	NFC403	530.00
Semu	0.7290	NFC451	504.60
NFC451	0.7160	Semu	503.80
Riband	0.7010	Riband	452.01
LSD 5%	0.0409		86.03

Table 8b A comparison of breadmaking and biscuit-making varieties

Absorbance				Concentration		
Plate	Bread	Biscuit		Bread	Biscuit	
1	0.8388	0.8413	n.s	744.63	746.67	n.s
2	0.8863	0.8189	*	725.25	527.00	*
3	0.7615	0.6473	***	412.50	274.33	***
4	0.7007	0.6579	*	626.25	516.00	*
5	0.6940	0.6147	*	542.25	413.00	*
Overall Mean	0.7763	0.7160		610.18	495.40	

Table 9 Absorbance results for NLT2 breadmaking varieties

Plate	14		15		16		17		19
Turpin	0.8799	Ctrl	0.8590	Cebeco	0.9907	Turpin	1.0069	Turpin	0.9438
Ctrl	0.8365	Mercia	0.8465	Piccadilly	0.9850	CWW 91/7	0.8960	Piccadilly	0.9093
CWW 91/2	0.8192	Turpin	0.7816	Ctrl	0.9240	Cebeco	0.8218	Ctrl	0.8780
Cebeco	0.8175	CWW 91/7	0.7728	Wykeham	0.8772	Ctrl	0.8183	Gondola	0.8751
Bercy	0.7667	CWW 91/2	0.7280	Gondola	0.8478	Tjalk	0.8064	Cebeco	0.8651
Flash	0.7596	Bercy	0.7165	Flash	0.8338	CWW 91/2	0.7788	Bercy	0.8598
CWW 91/7	0.7485			CBP W13	0.7782	Mercia	0.7407	Flash	0.8560
Piccadilly	0.7420			Tjalk	0.7495	Bercy	0.7172	Tjalk	0.8483
Gondola	0.7411					Gondola	0.6795	CWW 91/2	0.8125
Tjalk	0.7383					Flash	0.6673	Wykeham	0.7936
Wykeham	0.7223							CBP W13	0.7187
CBP W13	0.6705								
LSD 5%	0.072		0.1523		0.0854		0.1409		0.1069

Table 9 (continued)

Plate	20		21
Mercia	0.9348	Ctrl	0.8178
Ctrl	0.9237	CBP W13	0.8070
CWW 91/7	0.8305	Cebeco	0.7779
CWW 91/2	0.8246	Wykeham	0.7707
Bercy	0.8070	Flash	0.7633
Turpin	0.7952	Gondola	0.7261
Piccadilly	0.7882	Tjalk	0.7035
LSD 5%	0.12		0.745

Table 10 Concentration results for NLT2 breadmaking varieties

Plate	14		15		16		17		19
Turpin	833.3	Ctrl	770.8	Cebeco	1359.3	Turpin	1074.9	Turpin	1030.3
Ctrl	779.7	Mercia	741.2	Piccadilly	1348.4	CWW 91/7	876.4	Piccadilly	968.2
CWW 91/2	740.5	Turpin	538.2	Ctrl	1214.8	Cebeco	747.9	Ctrl	912.5
Cebeco	736.6	CWW 91/7	563.8	Wykeham	1107.4	Ctrl	742.0	Gondola	906.2
Bercy	628.0	CWW 91/2	453.4	Gondola	1032.4	Tjalk	723.3	Cebeco	889.0
Flash	614.2	Bercy	426.9	Flash	1002.3	CWW 91/2	674.9	Bercy	877.8
CWW 91/7	591.9			CBP W13	853.9	Mercia	611.5	Flash	872.3
Piccadilly	580.9			Tjalk	769.9	Bercy	571.8	Tjalk	856.7
Gondola	575.5					Gondola	509.7	CWW 91/2	790.9
Tjalk	569.9					Flash	490.2	Wykeham	754.8
Wykeham	538.8							CBP W13	604.4
CBP W13	444.1								
LSD 5%	147.1		371.9		203.8		241.05		198.5

Table 11 Absorbance results for NLT2 biscuit-making varieties

Plate	15		16		17		18		20		21
NFC 344/1	0.9650	Riband	0.7828	Cobet	0.8212	NFC 251/1	1.1205	CWW 91/3	1.0497	NFC 251/1	0.8055
CWW 91/1	0.9647	NFC 344/1	0.7190	Sennet	0.7962	CWW 91/3	1.0149	NFC 251/1	0.9364	Riband	0.7986
NFC 251/1	0.9313	CWW 91/1	0.7140	Kontiki	0.7898	CWW 91/1	1.0119	Kontiki	0.8700	CWW 91/1	0.7549
Combat	0.9120	NFC 251/1	0.6925			Combat	0.9920	Sennet	0.8565	CWW 91/3	0.7287
CWW 91/3	0.8825	CWW 91/3	0.6828			Sennet	0.9828	NFC 344/1	0.8147	Combat	0.7045
Kontiki	0.7938					NFC 344/1	0.9696	Combat	0.7549	NFC 344/1	0.6903
Sonnet	0.7828					Riband	0.9246				
						Kontiki	0.8212				
LSD 5%	0.1025		0.1167		0.0723		0.1072		0.1045		0.1477

Table 12 Concentration results for NLT2 biscuit-making varieties

Plate	15		16		17		18		20		21
NFC 344/1	1018.9	Riband	870.0	Cobet	746.8	NFC 251/1	1037.4	CWW 91/3	959.4	NFC 251/1	718.5
CWW 91/1	1018.2	NFC 344/1	654.2	Sennet	704.1	CWW 91/3	878.2	NFC 251/1	726.5	Riband	704.9
NFC 251/1	940.2	CWW 91/1	639.9	Kontiki	693.5	CWW 91/1	873.5	Kontiki	593.6	CWW 91/1	627.7
Combat	895.3	NFC 251/1	550.5			Combat	843.6	Sennet	567.2	CWW 91/3	581.9
CWW 91/3	826.2	CWW 91/3	508.5			Sennet	830.1	NFC 344/1	488.0	Combat	540.3
Kontiki	614.8					NFC 344/1	809.9	Combat	379.8	NFC 344/1	515.9
Sonnet	587.5					Riband	742.0				
						Kontiki	584.2				
LSD 5%	243.2		465.7		122.8		161.6		211.0		260.6

Figure 1

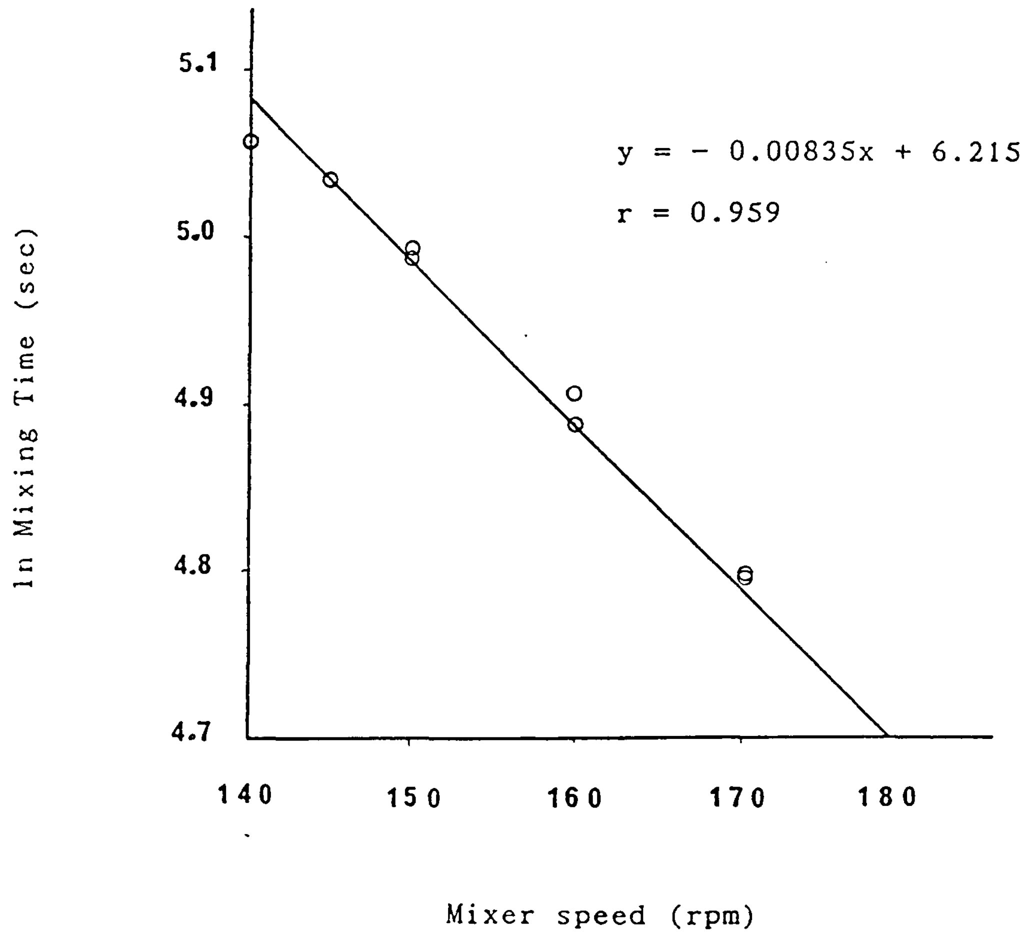
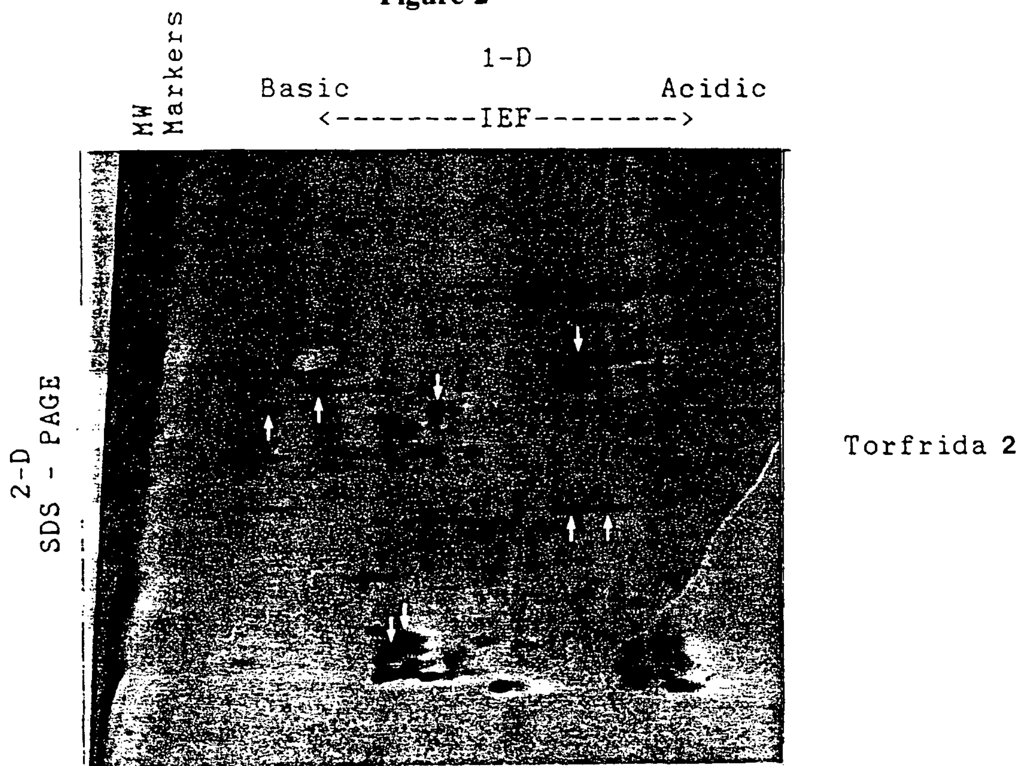
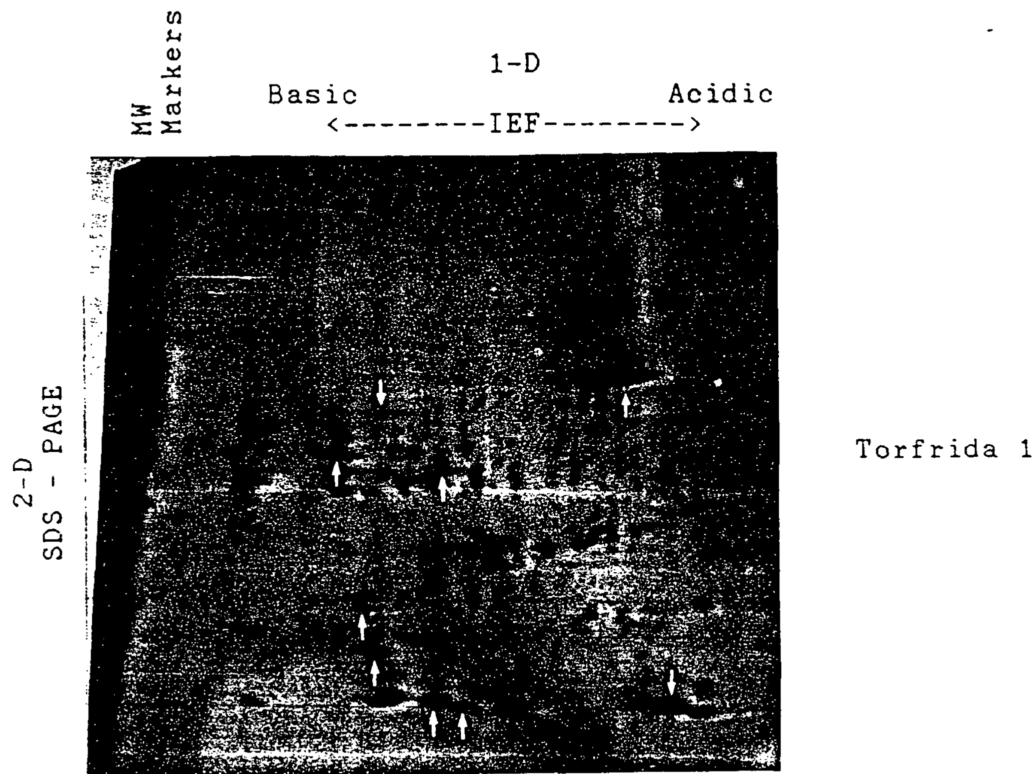


Fig. 1 Plot of (natural)log of mixing time versus mixer speed for dough made from 50g of flour.

Figure 2





2-D PAGE of water-soluble extracts of Torfrida 1 & 2

Figure 3

Absorbance ------>

AVALON

E-gram Display: \CEDATA\BORATE\RIBAND4.BFF

MERCIA

E-gram Display: \CEDATA\BORATE\HEREWARF.BFF

RIBAND

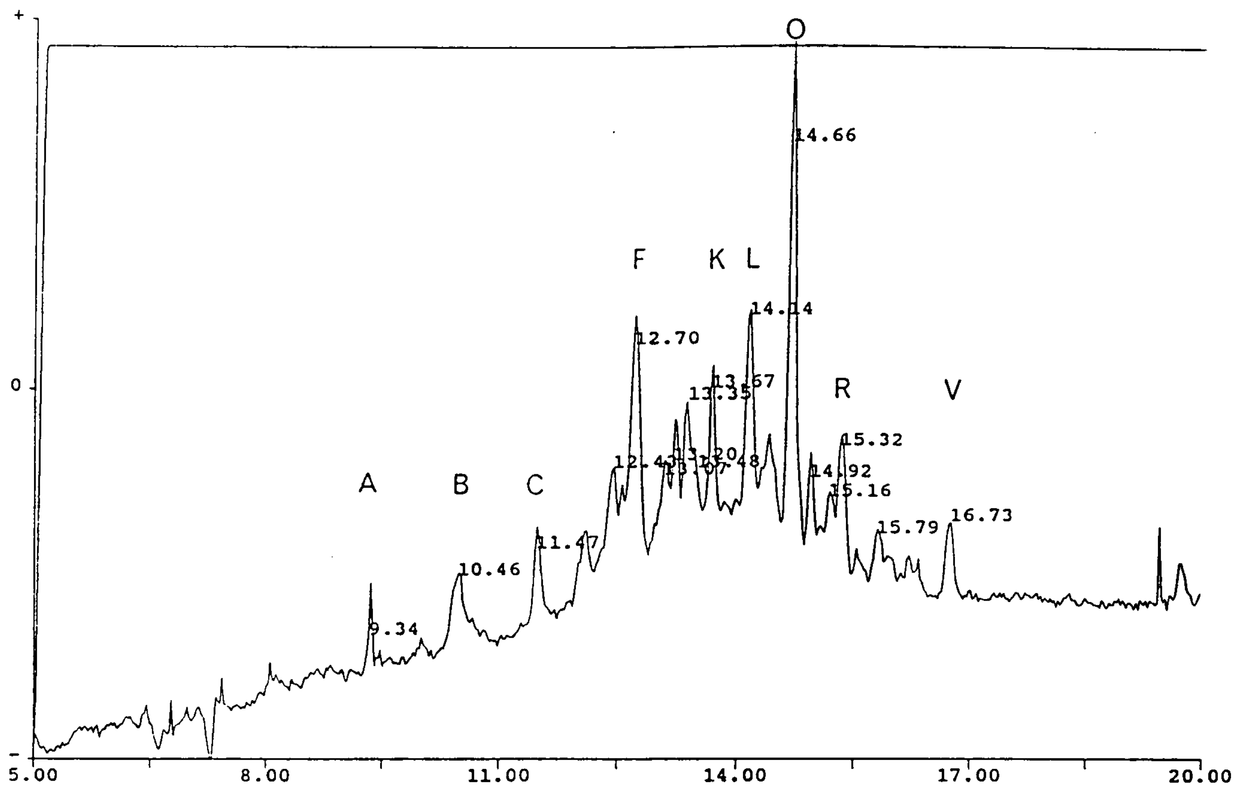
E-gram Display: \CEDATA\BORATE\AVALON15.BFF

HEREWARD

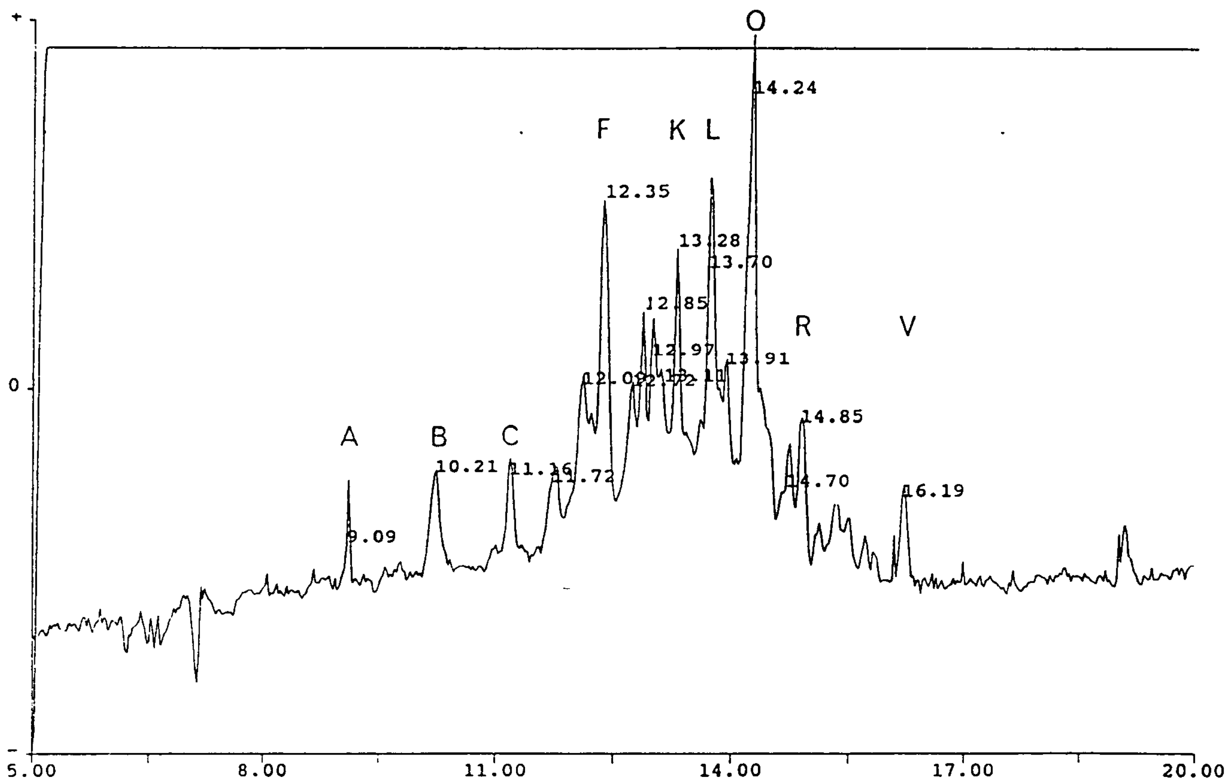
E-gram Display: \CEDATA\BORATE\MERCIA6.BFF

Time (minutes) ------>

Figure 4

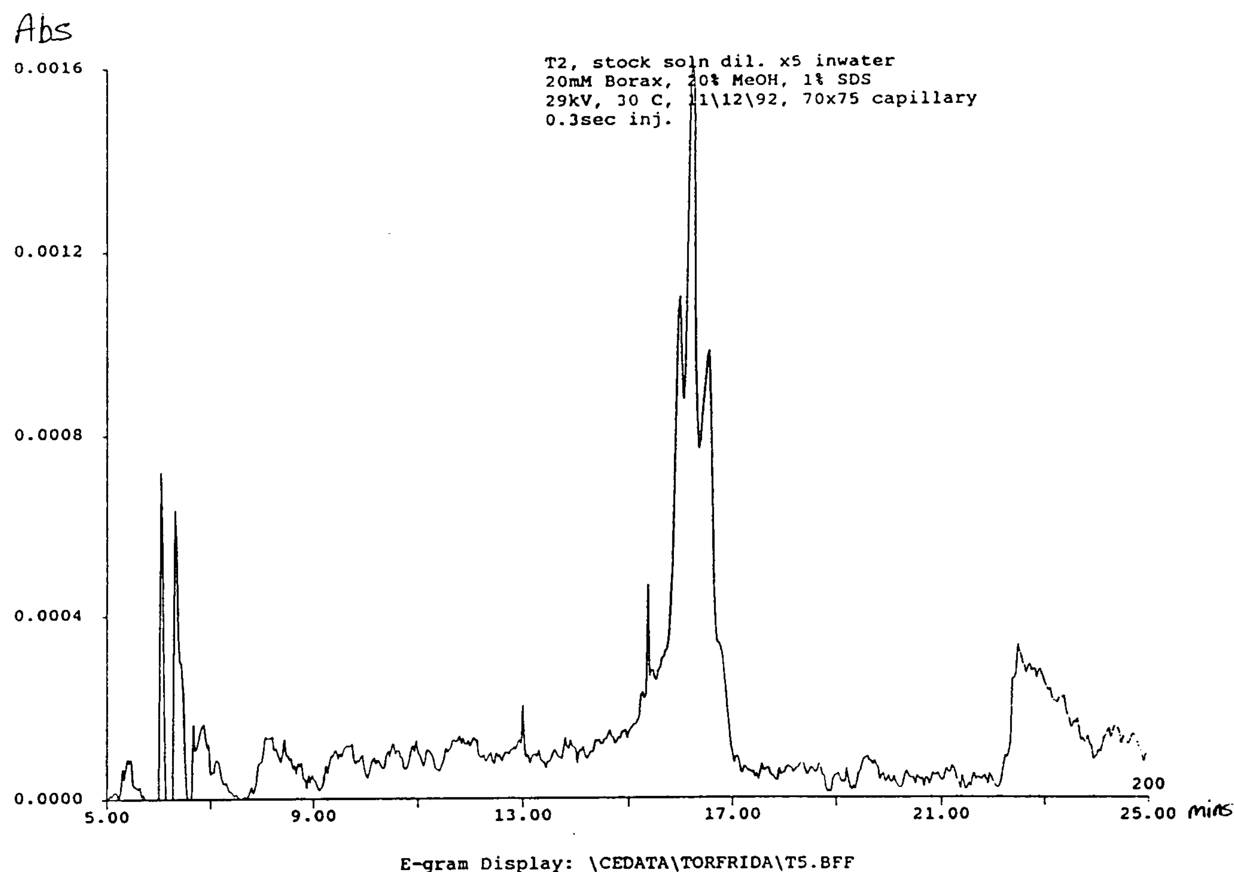



```
C:\CEDATA\MERCIA\MERCIA1.REP - 200 nm  Aux: kV (abs)
Buffer 1.04 buffer ( 5.00min)
Sample 1.05 mercia Hydro    0.3sec
Average  28.81kV  82.40uA   0.35gO
```




```
C:\CEDATA\MERCIA\MERCIA2.REP - 200 nm  Aux: kV (abs)
Buffer 1.04 buffer ( 0.00min)
Sample 1.05 mercia Hydro    0.3sec
Average  28.81kV  82.42uA   0.35gO
```

Figure 5



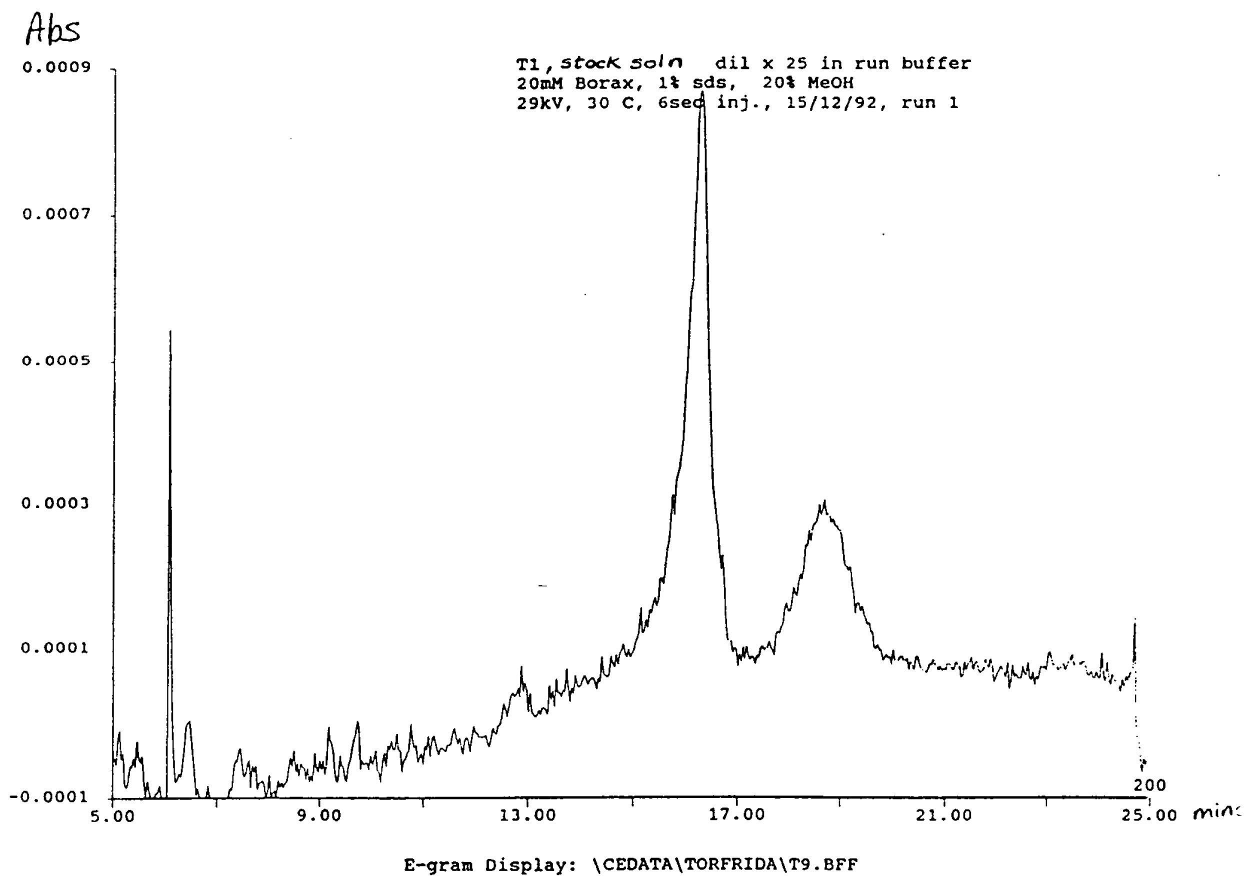


Figure 6

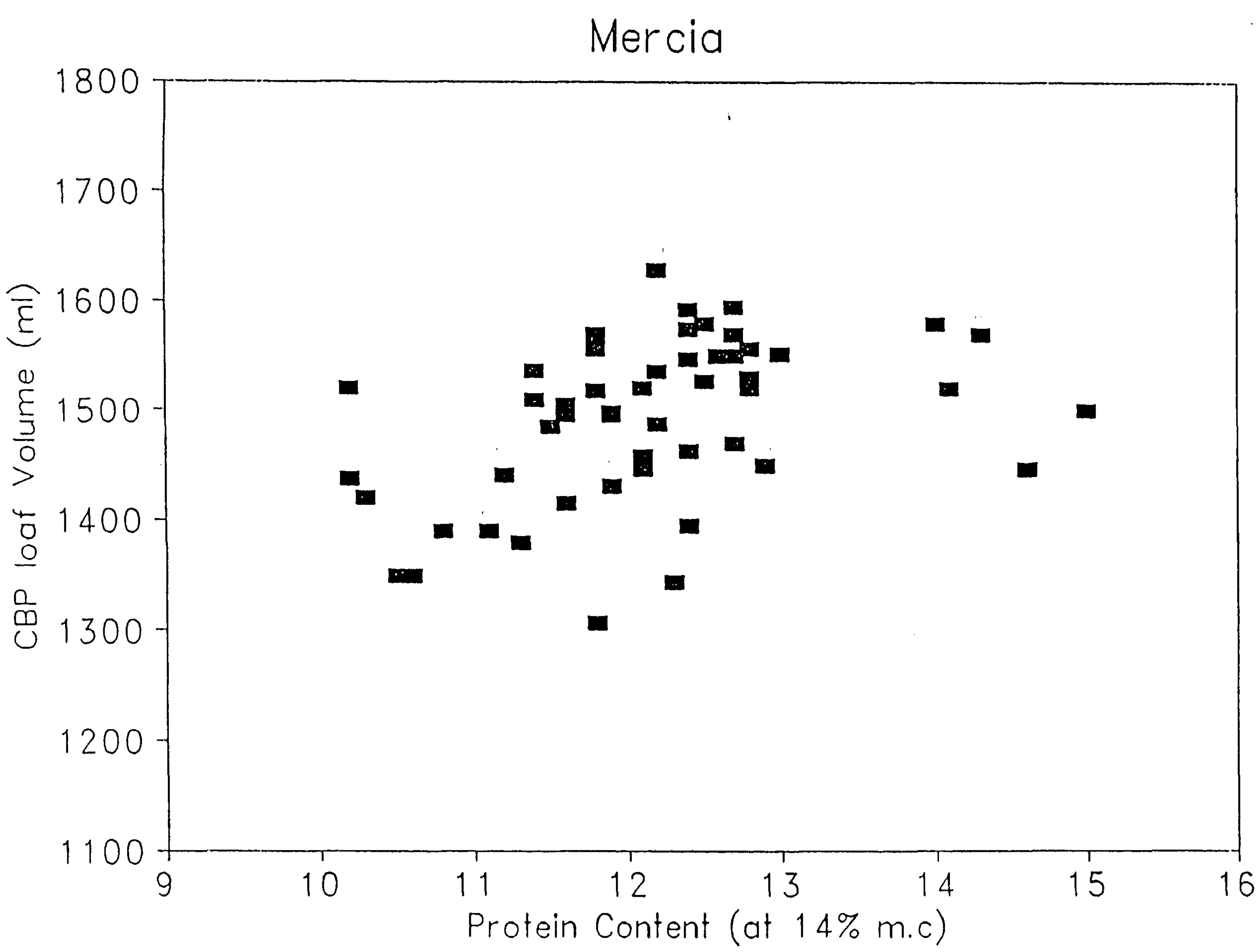


Figure 7

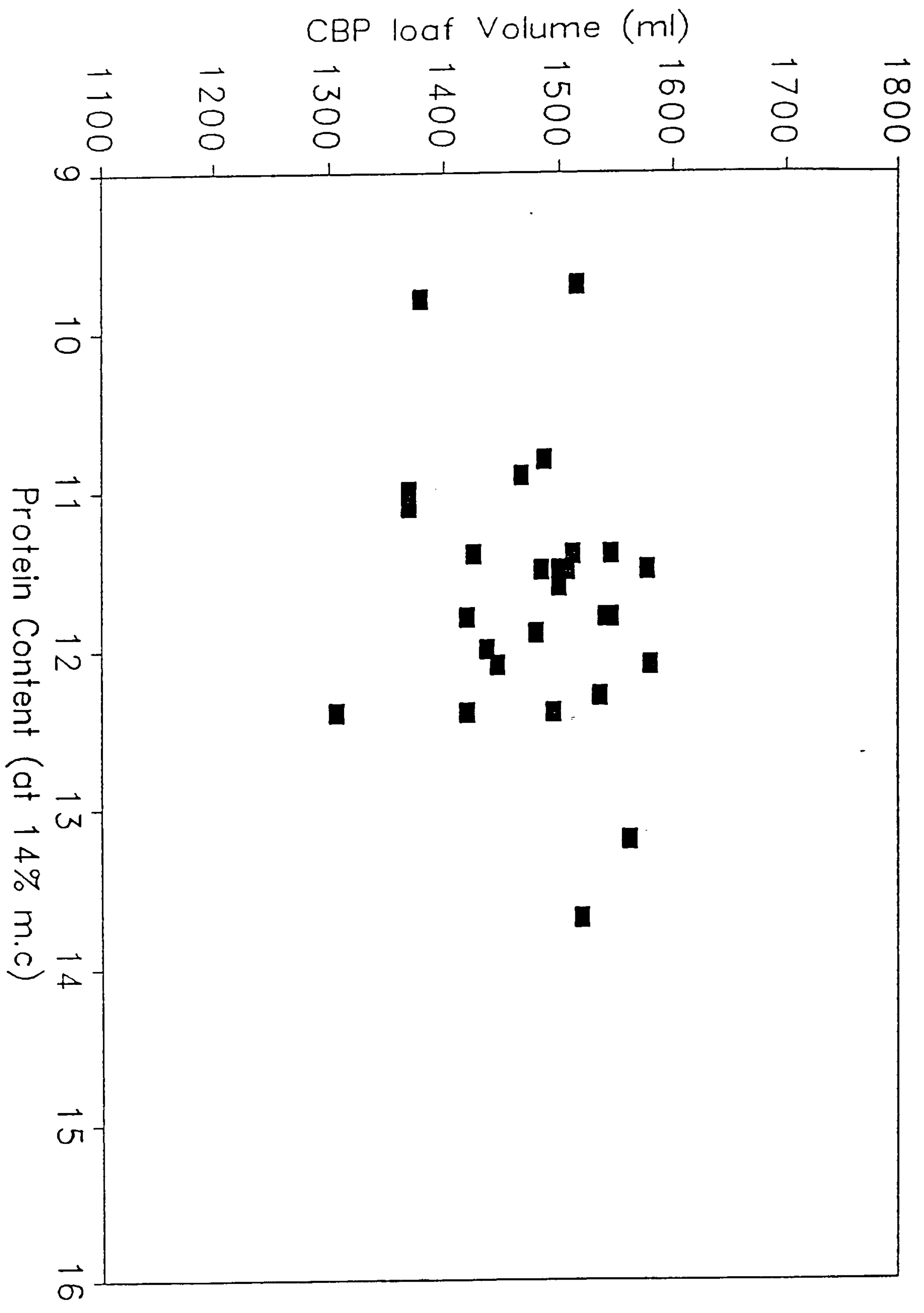
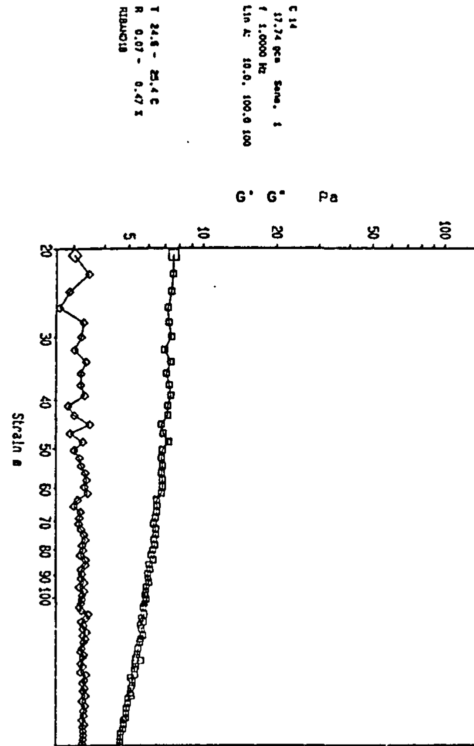
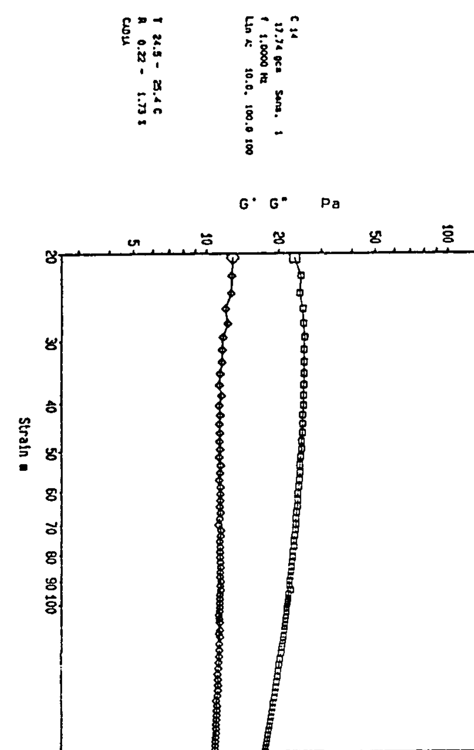
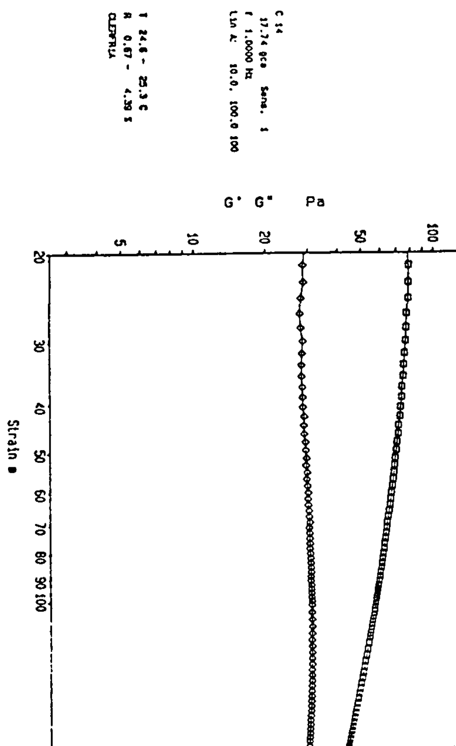


Figure 8







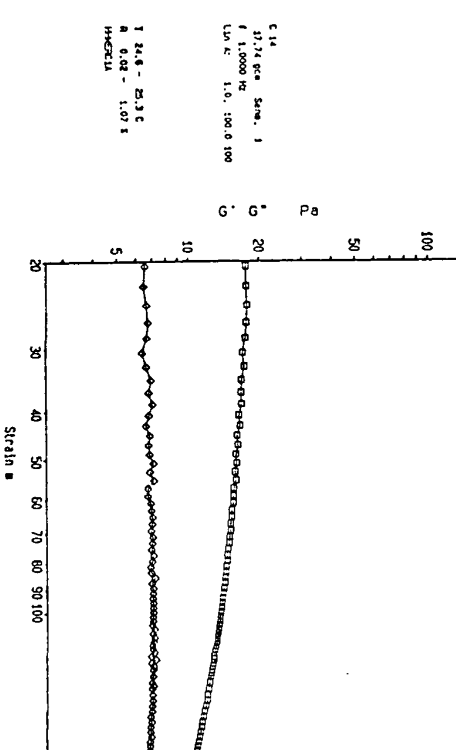


Figure 9

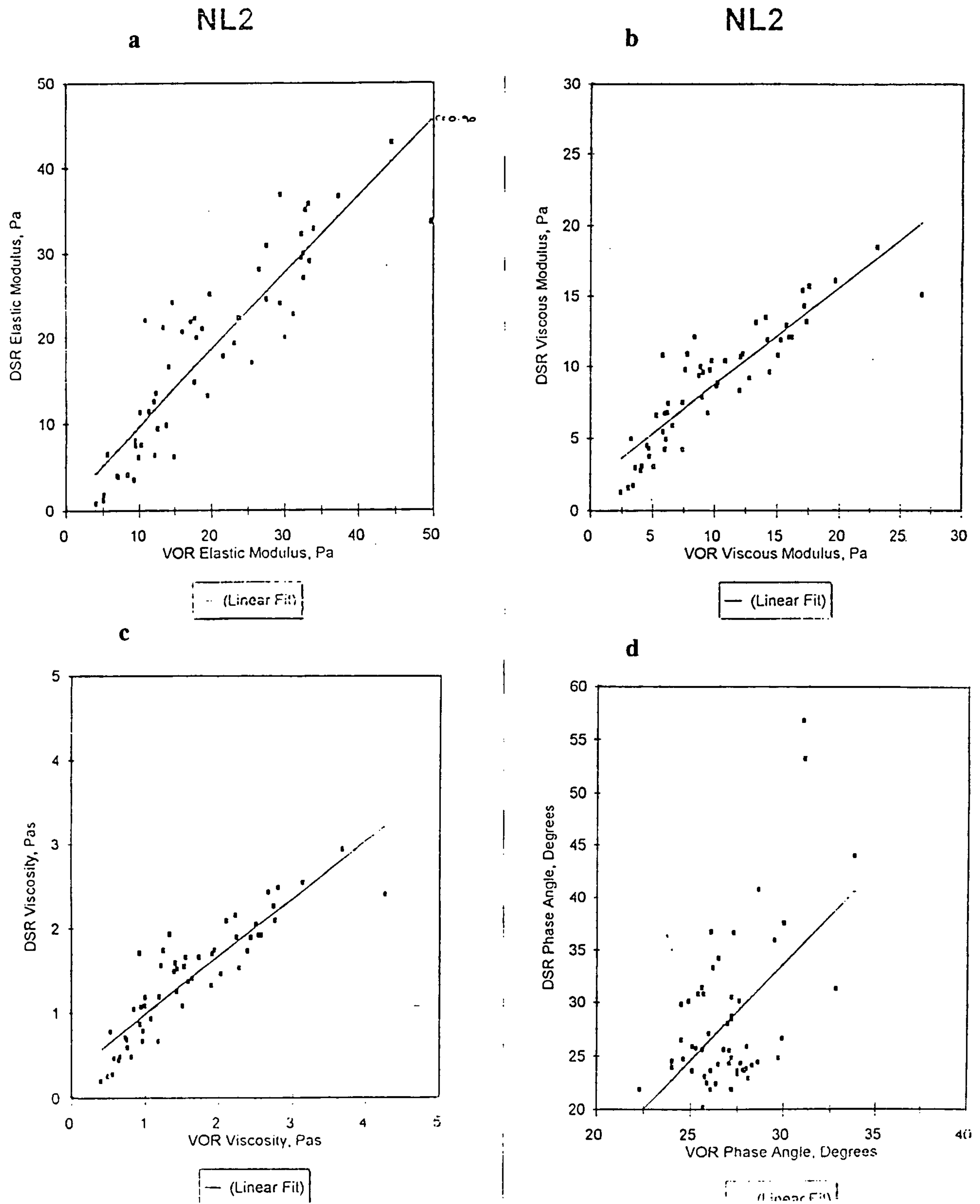


Figure 10

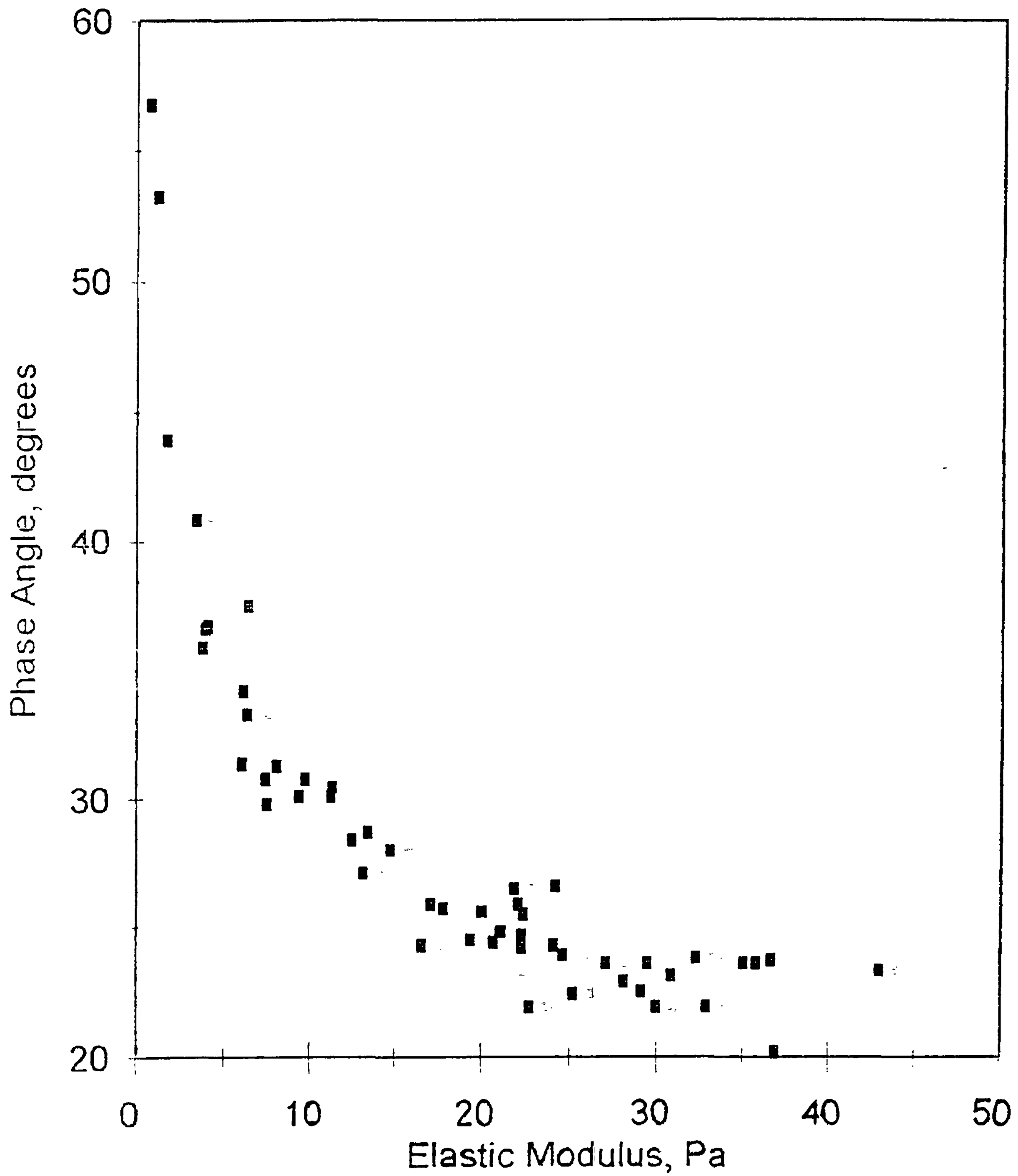


Figure 11

Predicted Volume, ml

1650
1600
1550
1500
1450
1400
1350
1300
1250
1200

1250 1350 1450 1550 1650 1750
1300 1400 1500 1600 1700

Loaf Volume, ml

Denotes ave data points
Riband
Hereward
Mercia
Dean
Haven
Fresco

Prediction Used:
Loaf Vol = 0.14M1 + 12.162M2 + 1843.5

Fig 2. Loaf Volume Prediction from Initial and Secondary Relaxation Slopes

Figure 12

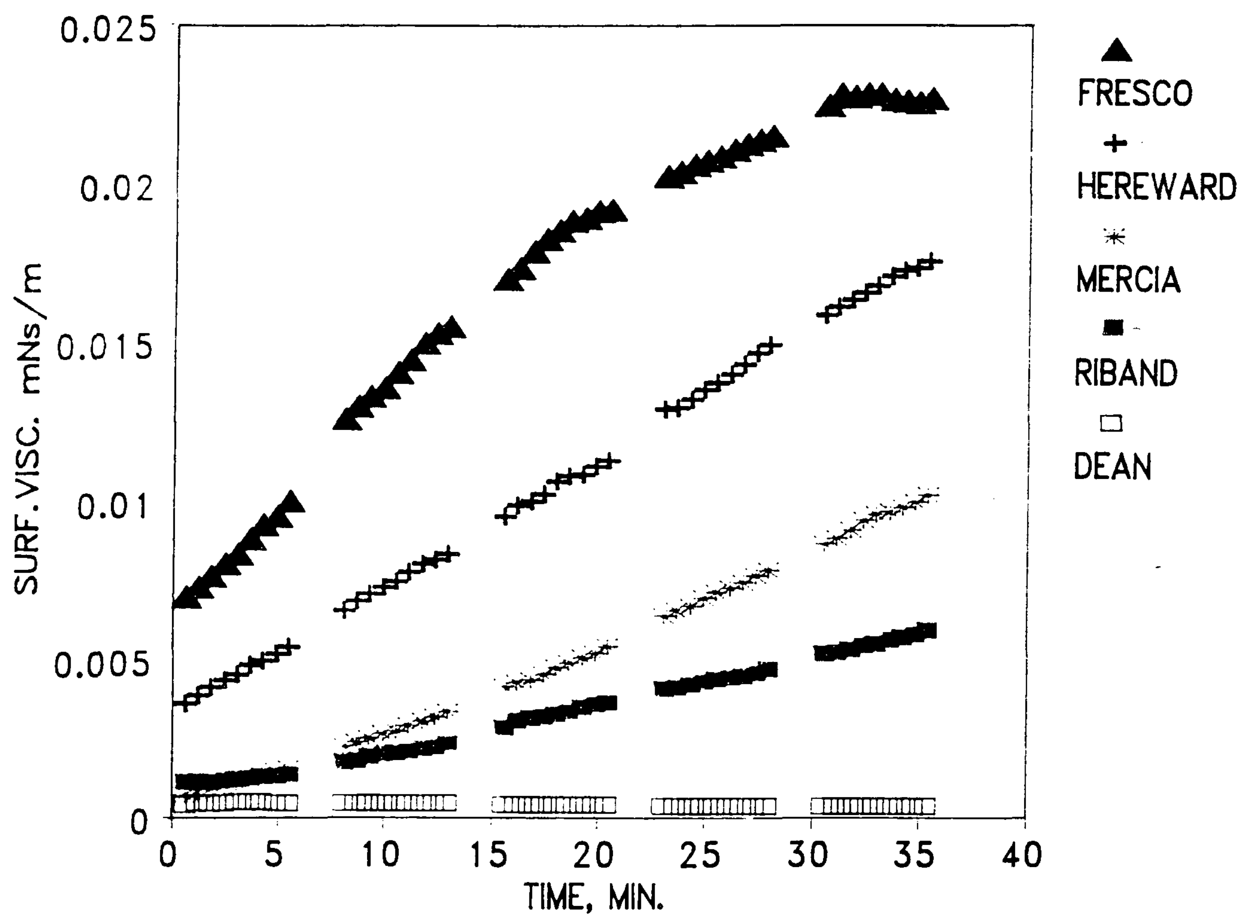
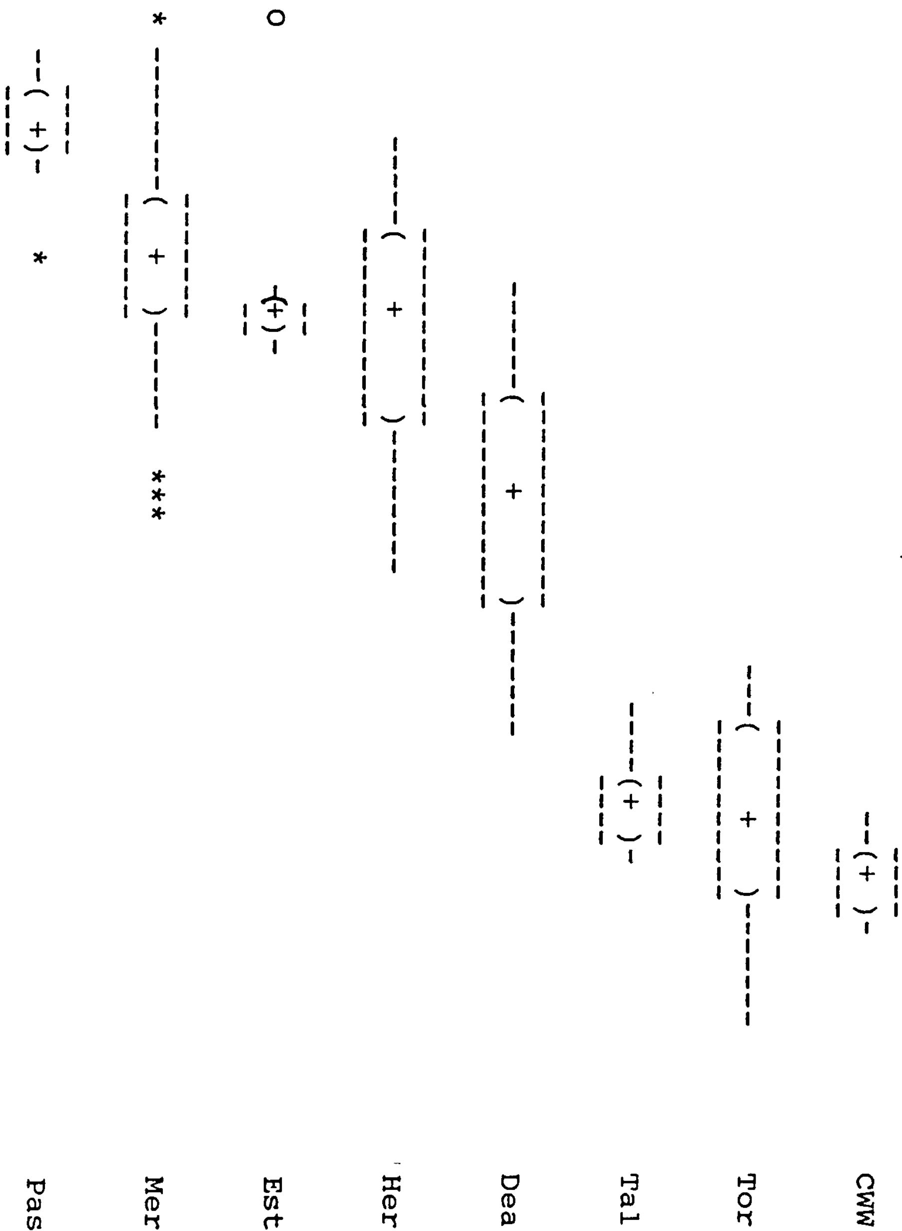


Fig. Surface Viscosity of Dough Liquor Material

Figure 13

Boxplot of Gluten Index for different flour types



Range of obs. :40.600 - 95.900

Figure 14

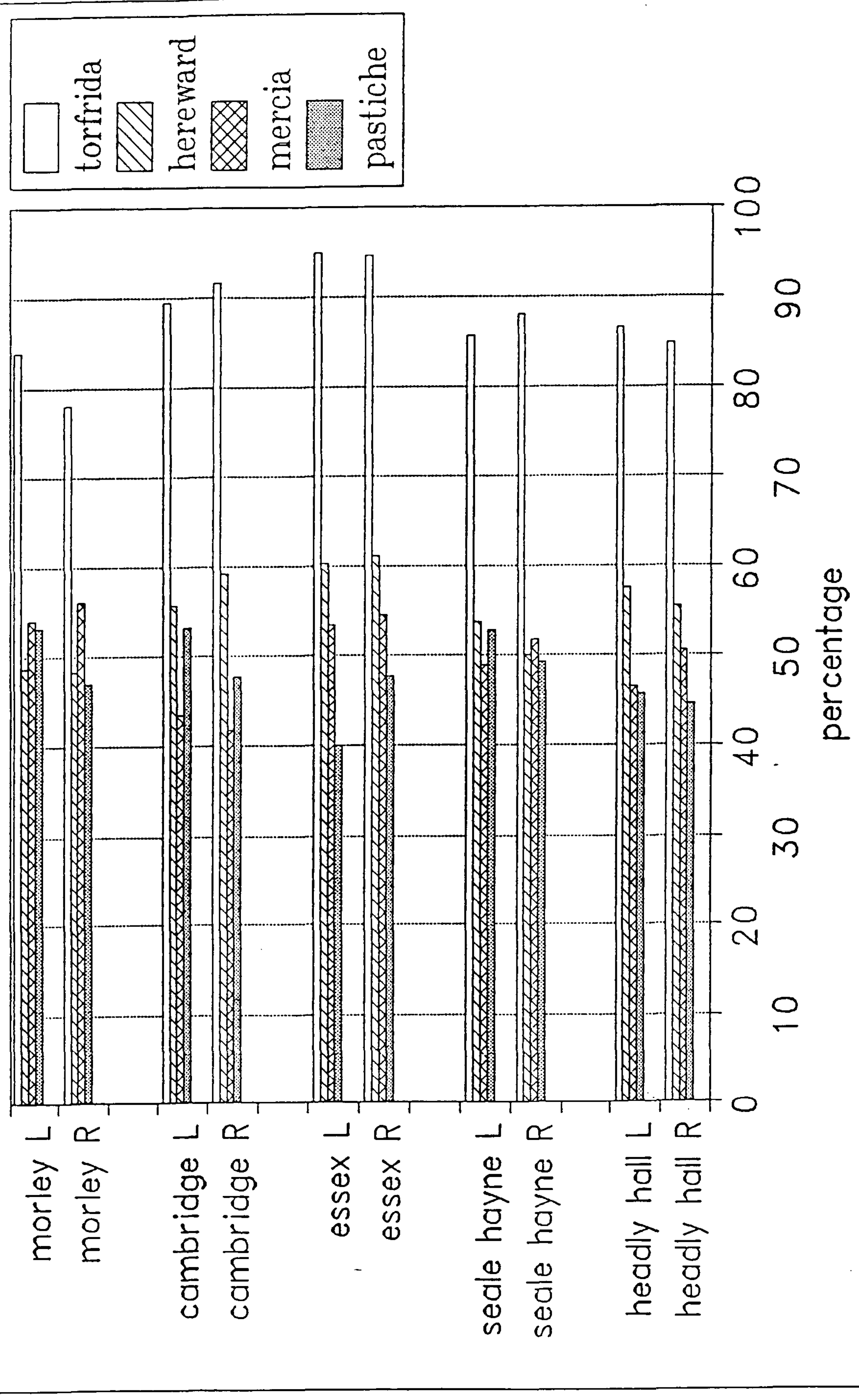
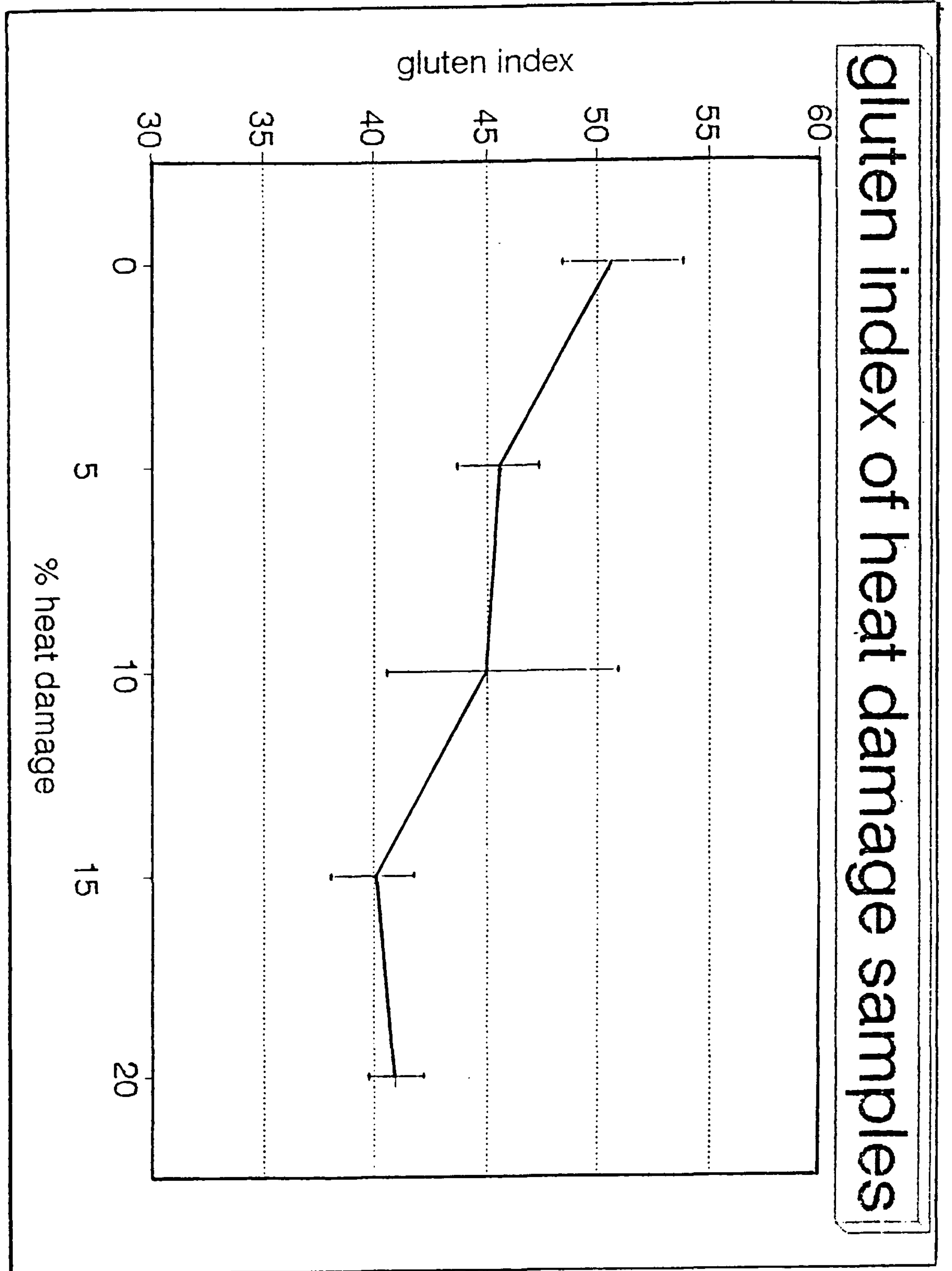


Figure 15



Appendix 1

Wheat storage and milling procedure

Storage

Wheat samples were checked to ensure moisture contents were below 14.5% before storage at ambient temperature and humidity of 53% until required for milling.

Conditioning and milling

18 to 25 hours before milling samples were conditioned by adjusting moisture content to 15.5%.

Milling was carried out using a laboratory Buhler mill, model 202, in a room at controlled temperature of 20°C and a relative humidity of 65%. First and third roll gaps were set at 0.6 and 0.4mm and first and third reduction roll gaps 0.3 and 0.2 mm respectively. The scalpers from the first and second reduction roll sifters were removed. These roll gap settings and the sifter cloth sizes employed are such that the flour meets requirements of EEC Regulation No. 1628/77. A feed setting of 2 was used during milling to obtain a flow rate of approximately 6kg/h.

Bran and offal were retreated twice on a Buhler 302 laboratory impact finisher.

Using different wheats these milling conditions have previously given flours with commercial levels of starch damage and extraction (Osborne et al, 1991).

Extraction rate was calculated on a total product basis, with a requirement that 98.0% of feed was recovered from the mill.

For wholemeal flours the bran was ground in a Christy Norris 8" laboratory hammer mill with 1.6mm screen.

All flours were blended for 30 minutes in a ribbon blender to ensure uniformity before entry into the test programme.

Appendix 2

Recipe, breadmaking procedure and loaf assessment methods

Breadmaking process: CBP

Bread type: 400g, white

Control recipe:

	% of flour weight	g/mix
Flour	100	1400
Yeast (compressed)	2.5	35
Salt	2.0	28
Water	As determined by Simon Extrusion Meter 10 min. method	
Ascorbic acid (100 ppm AA)	0.1	0.14

The alpha-amylase activity of the flour is adjusted to 80 FU by the addition of fungal alpha-amylase.

Dough processing:

Mixing machine	:	Morton double Z-blade
Beater speed (normal)	:	300 rev/min
Beater speed (variable)	:	250, 300 to 600 in 100 rev/min increments
Work input	:	Variable in Wh/kg dough
Pressure	:	Atmospheric
Dough temperature	:	30.5 +/- 1°C
Scaling	:	By hand to 454g
First moulding	:	Cylinder using Mono moulder
First proof	:	10 min at ambient temperature
Final moulding	:	Single-piece cylinder, (R7, W5.5, P1.25)
Pan size	:	Top 160mm x 98mm, 83mm deep
Shape	:	Unlidded
Proving conditions	:	43°C humidity to prevent skinning
Proving height	:	10cm
Baking temperature	:	244°C
Oven type	:	Direct gas-fired reel
Baking time	:	25 min
Baking humidity	:	No steam injected
Cooling	:	Open rack at room temperature
Storage	:	Closed cupboard overnight at 21°C

Assessment

Loaf volume measured by seed displacement

Crumb structure score, max 10 points, high points awarded for close uniform structure. Structure recorded by colour photograph.

Whole loaf score, max points 10, high marks awarded for large and uniform oven spring breaks.

Selection of optimum work input

Optimum work input was judged to be the lowest work level which gave loaves of high volume combined with high scores for crumb structure and whole loaf appearance.

Appendix 3

Estimation of gel-protein (Glutenin 1) from flour and dough and its rheological assessment

Laboratory assessment of gel-protein breakdown during mixing

280g flour + 5g sodium chloride and water level appropriate for flour water absorption (Simon Extrusion Meter 10min method) were mixed in a Simon Majorpin. 20g samples of dough were removed after time intervals up to 12 min, immediately frozen and subsequently freeze-dried.

Bread dough assessment of gel-protein

Immediately after mixing in each of the machines used for breadmaking, see Appendix 2, a sample of dough was frozen and subsequently freeze-dried. For BFP and SMM doughs were also sampled after bulk fermentation periods.

Processing of freeze dried dough

Freeze-dried dough was milled to pass through a 250 micron sieve before defatting for one hour with 40:60 petroleum ether, (20g to 50ml). Flours were also defatted.

5g of defatted dough, or flour, were sprinkled into 90ml 1.5% sodium dodecyl-sulphate solution at 10°C with magnetic stirring. After 10 minutes extraction time under gentle agitation, the mixture was centrifuged at 63,000 x g for 40 minutes.

Gel-protein was determined by the weight of the gel layer between the supernatant SDS soluble material and the starch pellet. The weight was recorded as weight of gel per 5g flour or dough.

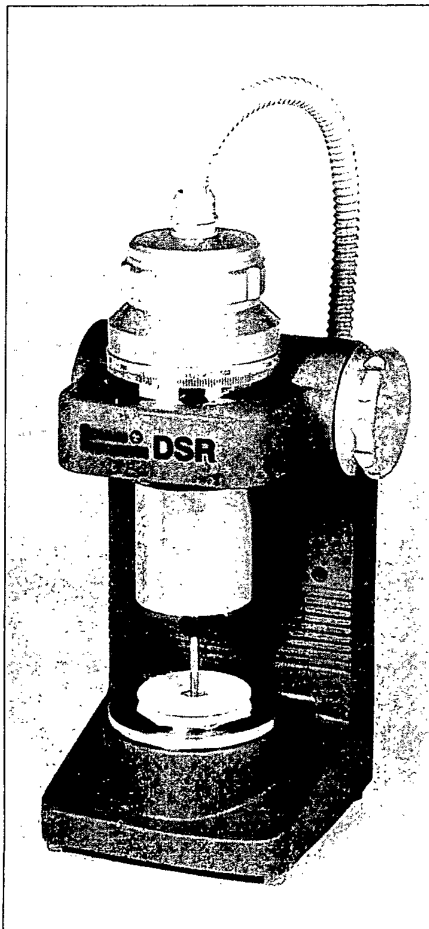
The method is based upon Graveland, et al, 1979.

Rheological assessment of gel-protein

Laboratory prepared gel-protein from the Majorpin mixer was separated from the residue starch and supernatant SDS solution and evaluated by oscillatory stress rheometry on the Bohlin VOR rheometer. A frequency sweep from 0.1 to 20 Hz was carried out. Data at 1 Hz for elastic modulus (G') and viscosity were collected.

Introducing the Bohlin DSR Rheometer

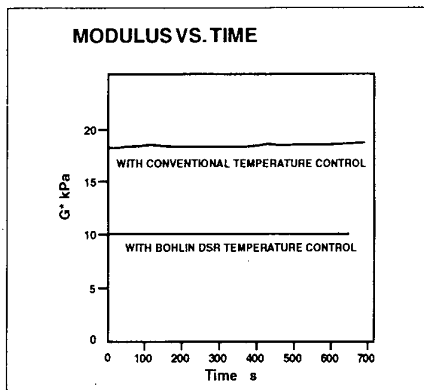
The Bohlin DSR rheometer was launched late in 1992 following demand from the U.S. funded SHRP (Strategic Highway Research Program) for a rheometer for work on Asphalts and other bitumous materials. The SHRP project thoroughly examined all aspects of highway technology and part of the program involved establishing relevant rheological parameters and the development of new test methods to predict rutting resistance and other mechanical attributes of the asphalt binder materials used in highway construction. These new methods have led to a set of binder specifications based upon linear viscoelastic properties. After consultation between Bohlin development engineers and researchers in SHRP, the Bohlin DSR was developed to meet the stringent demands placed upon instrument performance from the SHRP program. Since its launch, the DSR has rapidly become the industry standard unit for Asphalt binder testing and is being used not only in the USA but throughout the world by asphalt producers, subcontractors and governmental testing facilities. An innovative aspect of the DSR, which is based on proven Bohlin CS technology, is the temperature control system. With an accuracy of +/- 0. IC and negligible gradient (better than 0.1 C/mm) across the sample, it is ideal for testing bitumous samples where the Modulus can vary by as much as 20% per degree centigrade making conventional rheometers unsuitable. As standard the DSR is supplied with a Microsoft Windows based SHRP specification software which is designed for routine sample testing against the SHRP binder specifications. The SHRP specification software is designed to be as simple to use whilst retaining the flexibility for which Bohlin software is renowned. Features such as automatic test numbering, operator and sample ID fields and a note pad make it ideal for routine use. The measurement result is augmented by a simple PASS/FAIL message to indicate a samples status with respect to the SHRP test. For general purpose research work, Creep/Recovery, Oscillation, and Viscometry software are available as well as a Data Processing package. More information on the DSR Rheometer and its options can be obtained from any Bohlin Instruments office.



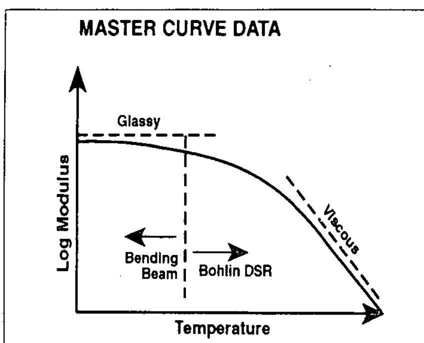
United States FHWA Chooses the Bohlin DSR

Bohlin Instruments was recently awarded a contract to supply the DSR rheometer to the United States Federal Highway Administration. The contract was awarded to Bohlin on technical merit after a bidding process in which Bohlin was not the lowest bid. 17 of the DSR units with the SHRP specification software have now been supplied to the FHWA and are being used throughout the United States to further highway construction technology.

'FHWA had specific and very demanding technical specifications, Bohlin was able to work with them and supply units tailored to their needs' commented John Casola at Bohlin's U.S .office.



This diagram shows typical oscillatory asphalt data obtained with a conventionally heated lower plate vs. the DSR water jacket. It demonstrates the extreme temperature sensitivity of the asphalt. A measurement error of approximately50% is seen in the measured data due to the existencs of temperature gradients wnhen the DSR temperature control isn't used.



Using research grade software, data from the Bohlin Dynamic Shear Rhometer is used to help generate a master curve describing binder performance over a wide range of conditions. Low temperature data can be obtained from a bending beam test.

Appendix 5

(From 3.8) ELISA data generation and assessment

Each 96-well ELISA plate contained, in triplicate, a reagent blank, 5 prolamin standard points, duplicate extractions of the Mercia internal control, and the duplicate extractions of up to 12 flour samples (Table 1B). The Kineti-Calc software processed the raw absorbance data after subtracting the averaged reagent blank value (Table 1A).

Standard curve analysis

An example of a log plot of the prolamin standard absorbance values are give in Fig. 1A. The data set out in Table 1C was then processed by a cubic curve-fitting method (Fig. 1B). Data points were screened to remove outliers which contributed to a coefficient of variation greater than 15%.

Sample analysis

The Kineti-Calc software calculated the statistical values of the absorbance data (Table 2A), and presented the concentration in μg/ml (Table 2B) using the fitted-curve for the prolamin standard. Data was screened to reject obvious outliers from the raw data which had produced coefficient of variations of greater than 15%.

Repeatability and reproducibility

Fig. 3 shows the variation between ELISA plates in mean absorbance and concentration for the Mercia control variety (NLT 2, Cambridge site). A period of 8½ weeks lapsed between the first and last plate tested. The standard deviations of repeatability, based on the variability between duplicates within a plate, were 0.036 and 56.789 for absorbance and concentration respectively.

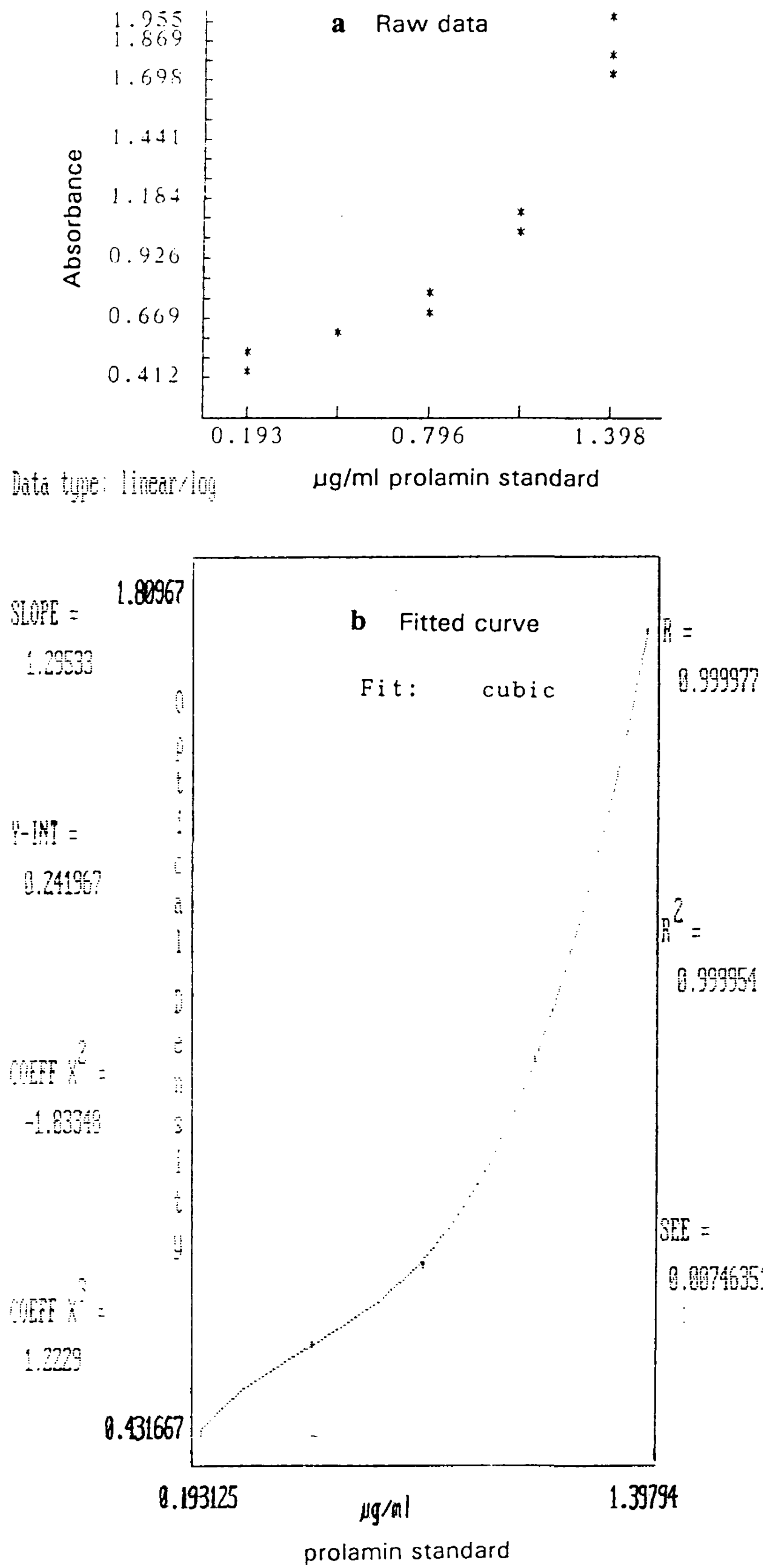
Mean values of absorbance and concentration tended to decrease with time, particularly for concentration. The standard deviations of reproducibility, based on the variability within and between plates, were 0.106 and 218.443 for absorbance and concentration respectively.

Much of the variation in the absorbance values was due to the flow characteristics of the ELISA filter plates used in this study. It was found that liquid was sucked through at a considerably slower rate in some of the 96 wells compared to others within a single plate, during the antibody/sample application and washing stages. This resulted in higher absorbance values for the slower wells, presumably due to greater reaction time within these wells. The reason for the differences in flow through the membranes is unclear, it may be due to lack of uniformity in the thickness or the pore size of the 0.45 μm nitrocellulose membrane discs attached to the bottom of each plate well. We also found a significant batch to batch variation in the filter-plates, whereby, whole plate results were rejected on the grounds of unacceptably high variation in the replicate raw data (not shown). This was seen in the latter stages of our study. Viable alternatives to the Millipore filter-plate could not be evaluated within the scope of this project.

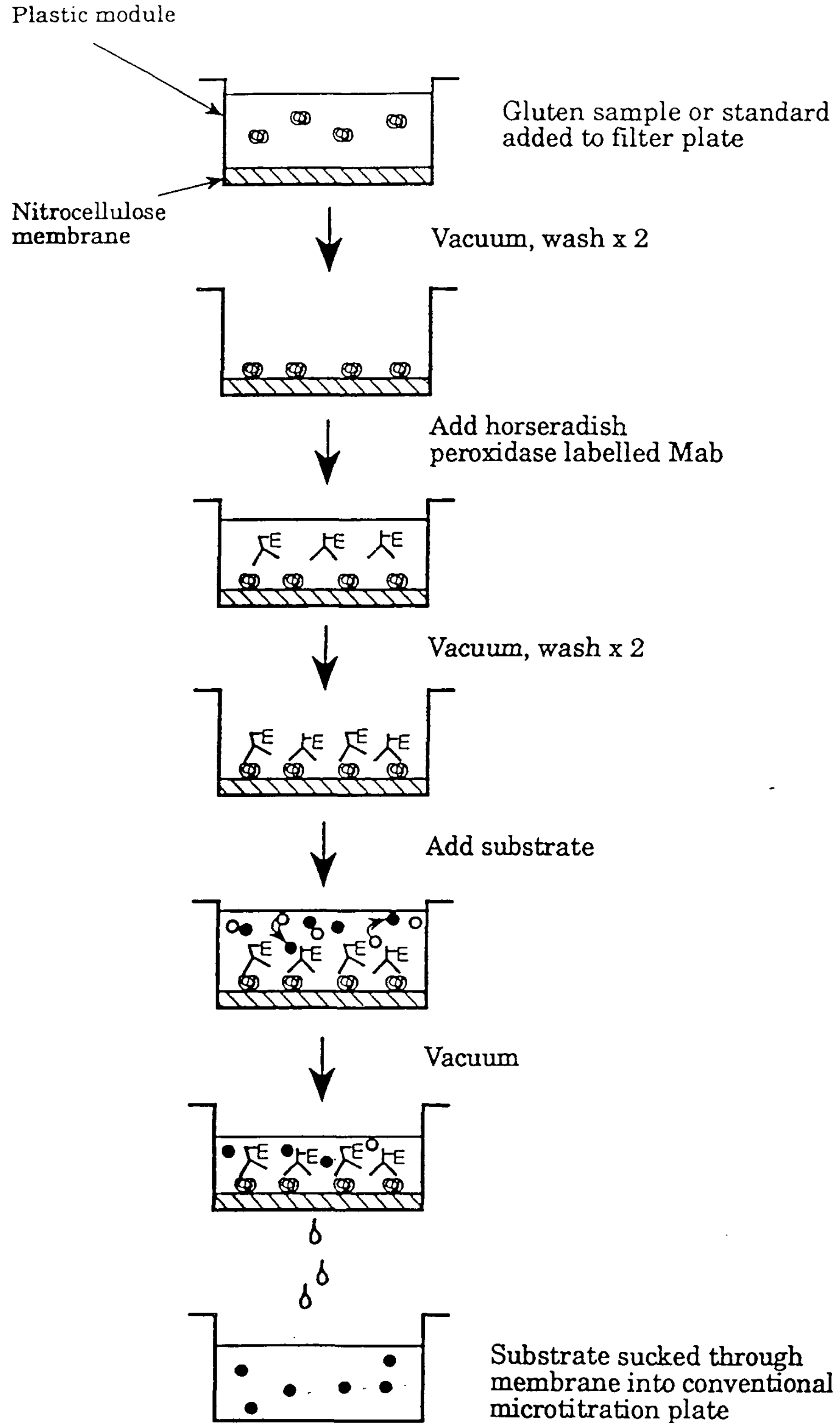
The concentration values of the flour extracts were calculated from the fitted standard curve in which there is an exponential relationship between the absorbance and concentration. It was found that relatively small variations in the shape and position of the standard curve had a significant effect on the calculated concentration value for a given absorbance value of a sample extract. One disadvantage of the cubic fit method is that the extreme ends of the prolamin standard values, which tend to exhibit the greater variation, will influence the curve shape in a disproportionate manner.

We experienced a significant variation in results generated by different operators in our preliminary studies (data not shown). Therefore, all the results presented here have been obtained by a single trained operator, using freshly prepared reagents where necessary.

Appendix 5, Figure 1 Standard curve analysis by Kineti-Calc software

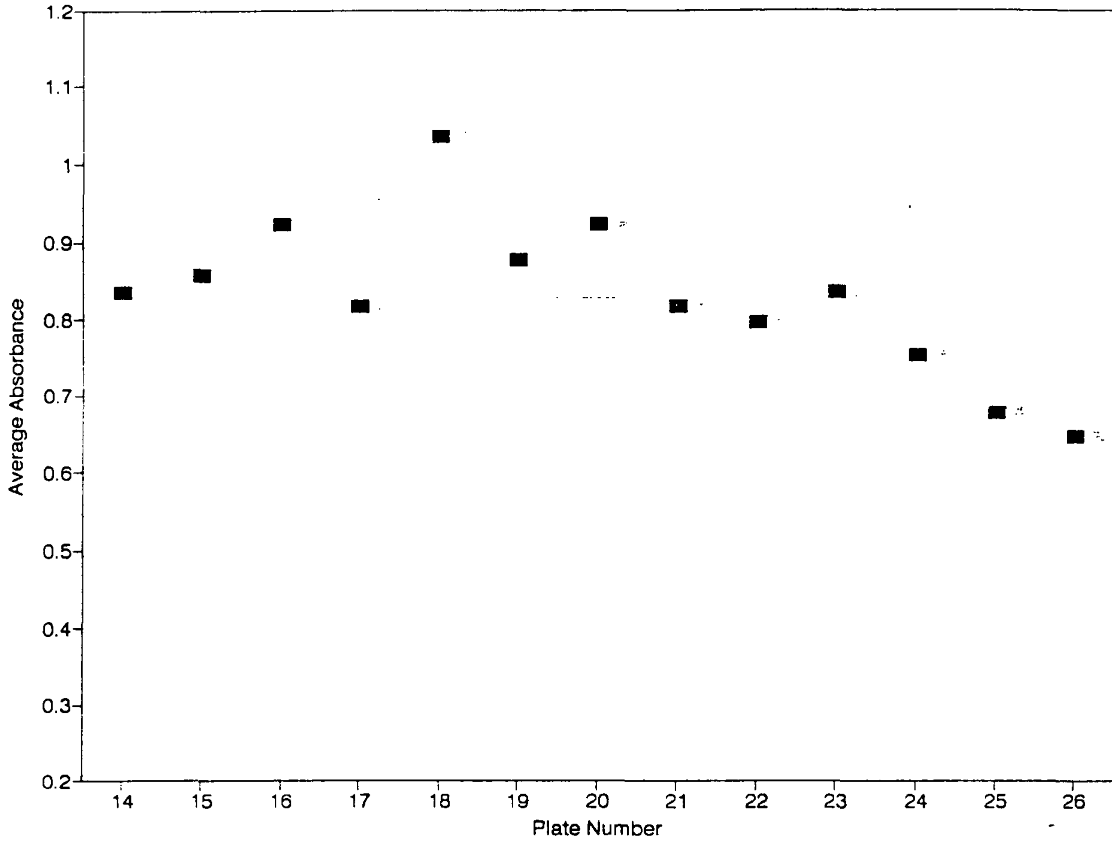


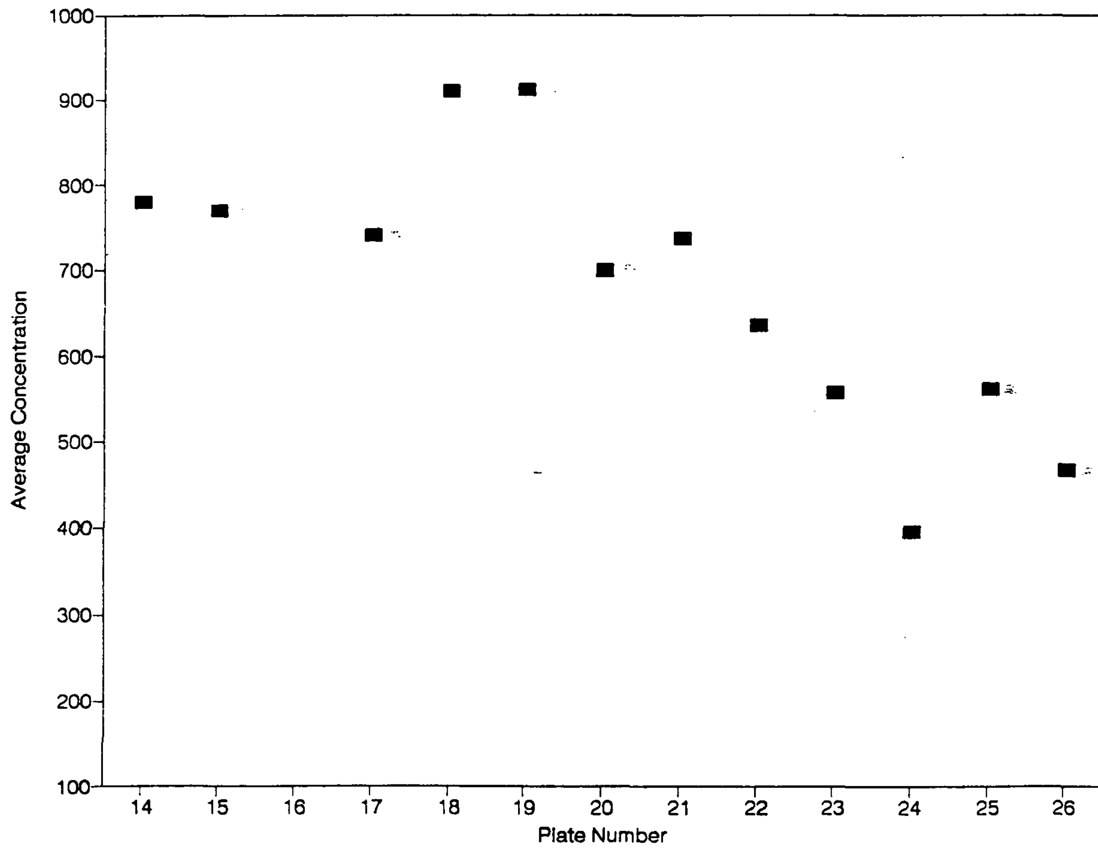
Appendix 5, Figure 2



Steps involved in the filter-plate immunoassay for quantification of gluten in wheat flour.

Appendix 5, Figure 3





Appendix 5

Platefile: GLIA19A
Plate/Reading #: 1

Table 1a

03/09/94 13:03:23

	1	2	3	4	5	6	7	8	9	10	11	12
A	0#000	0#000	0#000	.469	.412	.414	.571	.581	.606	.722	.710	.736
B	1.044	1.079	1.100	1.782	1.955	1.692	.828	.969	.871	.849	.806	.945
C	.772	.820	.784	.839	.848	.812	.879	1.023	.912	.926	.990	.933
D	.896	.770	.752	.930	.920	.891	1.000	.912	.956	.892	.880	.816
E	.911	.816	.805	.878	.984	.797	.836	.866	.836	.844	.917	.837
F	.830	.745	.817	.953	.865	.880	.869	.929	.978	.826	.756	.893
G	.883	.786	.782	.805	.760	.746	.755	.710	.791	.703	.701	.652
H	.786	.807	.765	.725	.752	.767	.784	.749	.747	.837	.876	.930

Template: GLIA19.MAP

Table 1b

	1	2	3	4	5	6	7	8	9	10	11	12
A				S1.56	S1.56	S1.56	S3.13	S3.13	S3.13	S6.25	S6.25	S6.25
B	S12.5	S12.5	S12.5	S 25	S 25	S 25	1	1	1	2	2	2
C	3	3	3	4	4	4	5	5	5	6	6	6
D	7	7	7	8	8	8	9	9	9	10	10	10
E	11	11	11	12	12	12	13	13	13	14	14	14
F	15	15	15	16	16	16	17	17	17	18	18	18
G	19	19	19	20	20	20	21	21	21	22	22	22
H	23	23	23	24	24	24	25	25	25	26	26	26

Platefile: GLIA19A # 1 Correlation coefficient: 1
Fit: cubic Coefficient of determination: 1
Data type: linear/log Standard error of estimate: 0.0074635
Equation: Y = 0.24197 + 1.2953X -1.8335X^2 + 1.2229X^3

Table 1c Standards Report

Standard Value	Location	Absorbance	Mean Absorbance	Predicted Conc	Standard Deviation	Coefficient of Variation
1.56	A04	0.469				
1.56	A05	0.412				
1.56	A06	0.414	0.4317	(1.556)	0.03235	7.494%
3.13	A07	0.571				
3.13	A08	0.581				
3.13	A09	0.606	0.5860	3.19852	0.01803	3.076%
6.25	A10	0.722				
6.25	A11	0.710				
6.25	A12	0.736	0.7227	6.13966	0.01301	1.801%
12.5	B01	1.044				
12.5	B02	1.079				
12.5	B03	1.100	1.074	12.5608	0.02829	2.633%
25	B04	1.782				
25	B05	1.955				
25	B06	1.692	1.810	24.9846	0.1337	7.386%

Appendix 5

Table 2a

Description:

Measure of central tendency for plate #1 Platefile: GLIA19A

Set#	Set Name	Mean	Standard Deviation	Variance	Coefficient of Variation
1	mercia 19	0.889	0.072	0.005	8.1%
2	mercia 19	0.867	0.071	0.005	8.2%
3	cww 91/2 167	0.792	0.025	0.001	3.2%
4	cww 91/2 167	0.833	0.019	0.000	2.2%
5	turpin 168	0.938	0.075	0.006	8.0%
6	turpin 168	0.950	0.035	0.001	3.7%
7	bercy 169	0.806	0.078	0.006	9.7%
8	bercy 169	0.914	0.020	0.000	2.2%
9	piccadil1170	0.956	0.044	0.002	4.6%
10	piccadil1170	0.863	0.041	0.002	4.7%
11	cebec943 171	0.844	0.058	0.003	6.9%
12	cebec943 171	0.886	0.094	0.009	10.6%
13	flash 172	0.846	0.017	0.000	2.0%
14	flash 172	0.866	0.044	0.002	5.1%
15	tjalk 173	0.797	0.046	0.002	5.7%
16	tjalk 173	0.899	0.047	0.002	5.2%
17	gondola 174	0.925	0.055	0.003	5.9%
18	gondola 174	0.825	0.069	0.005	8.3%
19	wykeham 175	0.817	0.057	0.003	7.0%
20	wykeham 175	0.770	0.031	0.001	4.0%
21	cpb w13 176	0.752	0.041	0.002	5.4%
22	cpb w13 176	0.685	0.029	0.001	4.2%
23	riband 177	0.786	0.021	0.000	2.7%
24	riband 177	0.748	0.021	0.000	2.8%
25	cww 91/1 178	0.760	0.021	0.000	2.7%
26	cww 91/1 178	0.881	0.047	0.002	5.3%

Table 2b

Sample #	Sample Name	Avg. Absorbance	Dilution	Concentration
1	mercia 19	0.8893	100	933.091
2	mercia 19	0.8667	100	891.924
3	cww 91/2 167	0.7920	100	752.072
4	cww 91/2 167	0.8330	100	829.767
5	turpin 168	0.9380	100	1019.98
6	turpin 168	0.9497	100	1040.56
7	bercy 169	0.8060	100	778.881
8	bercy 169	0.9137	100	976.769
9	piccadil1170	0.9560	100	1051.69
10	piccadil1170	0.8627	100	884.605
11	cebec943 171	0.8440	100	850.221
12	cebec943 171	0.8863	100	927.671
13	flash 172	0.8460	100	853.924
14	flash 172	0.8660	100	890.705
15	tjalk 173	0.7973	100	762.322
16	tjalk 173	0.8993	100	951.102
17	gondola 174	0.9253	100	997.543
18	gondola 174	0.8250	100	814.794
19	wykeham 175	0.8170	100	799.735
20	wykeham 175	0.7703	100	709.935
21	cpb w13 176	0.7520	100	673.595
22	cpb w13 176	0.6853	100	535.109
23	riband 177	0.7860	100	740.486
24	riband 177	0.7480	100	665.576
25	cww 91/1 178	0.7600	100	689.535
26	cww 91/1 178	0.8810	100	918.014