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DETECTION OF HEAT DAMAGE IN WHEAT

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FINAL REPORT: Detection of Heat Damage in Wheat

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FINAL REPORT - DETECTION OF HEAT DAMAGE IN WHEAT

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Start date: 1.10.95 **Duration: 3 years (extra 4 months)**

ABSTRACT

The aim of this project was to develop a quick, reliable and simple test for detecting heat damaged wheat samples at mill intake. The selected test should be capable of providing results within the standard 20-30 minute turnaround time at mill intake and reliably reject loads of heat damaged wheat which, if included in a bread grist, would have a deleterious effect on breadmaking performance.

Heat damage is caused when wheat, that has been harvested in wet conditions, is dried for prolonged periods using air temperatures above 60°C. The most important effect of excessive heat is to inflict damage to functional wheat proteins that are responsible for the production of the visco-elastic gluten network required for bread production. When gluten of the right quality is present, this network entraps air that is introduced during mixing and carbon dioxide developed during fermentation, enabling the baker to produce a loaf of the required volume and texture. Damage to the vital protein framework results in structural weaknesses and hence inability to retain gas bubbles, with consequent reduction in loaf volume and crumb structure, i.e. an overall decrease in breadmaking quality. Heat damage also reduces the germination capacity of a wheat sample.

Within this study, a standard 7-day germination test was used to determine the level of heat damage inflicted on a moist wheat sample when subjected to different temperature/time regimes. The results of germination studies provided the "reference" results for heat damage against which all "potential" heat damage methods were compared. A standard Chorleywood Bread Process (CBP) test was used to determine the effect of differing levels of heat damage on end-use quality. Loaf volume was used to identify the critical point at which heat damage produced a significant effect on breadmaking performance and provide conclusive proof of the serious effect heat damage can have on grain destined for the flour milling market.

Since it is primarily the proteins that are damaged by heat, quantification of proteins was carried out by polyacrylamide gel electrophoresis (PAGE) and capillary electrophoresis (CE). The identification of a protein entity that was eliminated or produced when heat damage occurred, could have led to the development of a test kit suitable for use at mill intake laboratories. Such a marker was not found.

Several methods for the detection of heat damaged wheat were investigated:

- ✧ Near UV absorbance of salt-soluble proteins
- ✧ Protein solubility test
- ✧ Rapid germination testing (Germograph)
- ✧ Rapid visco analyzer
- ✧ Turbidity test
- ✧ Durotest
- ✧ Gel protein
- ✧ Gluten quality (testing extensibility)
- ✧ Mixograph
- ✧ Image analysis
- ✧ Surface tension measurements

Initially, these were tested by comparing the results of severely heat treated and control samples for a range of wheat varieties. Those methods showing the most promise were subsequently evaluated using a range of heat treated samples to check their sensitivity to milder levels of heat damage.

The Turbidity test gave consistently reliable results under the conditions used for the study and has been shown to relate better to germination test results than the currently used gluten washing technique. The method was modified to use a simpler spectrophotometer (colorimeter) suitable for use in a mill or grain store situation.

1. INTRODUCTION

Heat damage to wheat in the UK is caused by excessive post-harvest drying to reduce moisture content and ensure safe storage or by the storage of moist wheat in bins for extended periods of time which can result in "bin burning" and thus a darkening of the seed coat (Every *et al.,* 1987). The methods used to dry large quantities of wheat on the farm tend to involve the slow movement of grain through an air-heated chamber, followed by cooling and cleaning. However, grains passing through dryers are often not uniformly heated, which leads to parcels of wheat which contain both normal and heat damaged areas of grain (Harrison *et al,* 1969). Heat damage due to grain drying is particularly serious in high quality wheat for milling production situations, where early harvesting is often recommended to protect quality attributes.

Excessive heat will alter several fundamental constituents of the wheat, but the proteins involved in gluten development are the most important. These enable the development of a visco-elastic structure during breadmaking that enables the gas produced during fermentation to be retained, creating the opportunity to produce typical high volume UK bread. Previous studies had shown that performance in a small-scale bulk fermentation baking test was progressively worse as temperatures were increased from 50 to 70°C (Bruce, 1992). By 75°C, drastic changes in protein structure and conformation had occurred and protein functionality was lost. Grain viability or germination capacity is used to provide a "reference" measure of the impact of heat damage. However, both germination and baking tests are unsuitable for use in a mill intake situation and more rapid, small-scale tests are required.

Consideration of the components that suffer from overheating of grain suggests a number of approaches to the detection of heat damage. A primary consequence of heat damage involves changes to wheat proteins. Loss of protein solubility (within both the salt soluble and glutenin fractions) has been used by Every (1987) and Harrison et al. (1969) respectively as the basis for a test of heat damage. Changes in UV absorbance due to changes in the structural characteristics of specific amino acids that are sensitive to heat, deactivation of enzymes, surface tension properties and foaming capacity of protein solutions and various dough rheological techniques have all been explored. Heat damage is traditionally measured at mill intake by washing out gluten and subjectively assessing its quality. However, the test is rather slow and lacks sensitivity to low levels of heat damage.

Most potential methods for detection have focussed on grain protein, but heat may also create changes in the starch composition and properties or affect water uptake through the bran layers. These approaches were also explored in this project.

2. PROJECT OBJECTIVES

- To create different levels of heat damage in a range of samples differing in terms of variety and quality to act as a test set for method development and optimisation.
- To develop a simple, rapid and sensitive test for heat damage that can be used by grain store laboratories and at mill intake.
- To investigate wheat protein biochemistry to seek a new marker for heat damage.
- To investigate whether critical drying temperatures differ between varieties.

3. AREAS OF STUDY

3.1 Production and confirmation of heat damage

3.2 Evaluation of methods to detect heat damage

3.3 Protein studies to identify a novel marker for heat damage

3.4 Optimisation of the Turbidity test

Each area is reported in greater detail in a separate paper.

3.1 Production and confirmation of heat damage

In order to test the effectiveness of methods for the detection of heat damage in wheat, it was necessary to induce different levels of heat damage in test samples. Methods were developed to create a range of different levels of heat damage by conditioning grain to various moisture contents and exposing it to different temperature/time combinations.

To measure the amount of heat damage generated by these individual heat treatments, a standard germination test was carried out to provide a reference point for heat damage that could be used to compare the performance of any new test developed for this purpose.

The combinations of initial moisture content, temperature and time used to create heat damage produced the range of heat damage levels that could be used to measure the effectiveness of potential tests. The standard germination test was effective in differentiating between different levels of heat damage created by individual treatments. Depending on the original moisture content of the samples, temperatures in excess of 68°C for 40 minutes were required to generate measurable heat damage (<90% germination capacity) under the experimental conditions used in this work. Farmers should still adhere to current recommendations of a maximum air temperature of 60°C for safe drying of wheat for the milling industry as this study is based on a limited data set and laboratory-scale dryers.

CBP loaf volume, used to indicate effect on end-use quality, was less sensitive to heat damage with temperatures in excess of 83°C required to produce a measurable difference in this breadmaking quality attribute.

There appeared to be no difference between the temperature required to induce heat damage to proteins of the soft biscuit and hard bread wheats examined in this study.

3.2 Evaluation of methods to detect heat damage

A wide range of potential methods for the detection of heat damage were evaluated in terms of sensitivity, "ease of use", speed and suitability for use at a mill intake.

3.2.1 Protein solubility test (PST)

A primary consequence of heat damage is the loss of protein solubility (particularly the salt-soluble albumins and globulins) and is the basis for the PST test developed by Every (1987).

Comparisons between the Bio-Rad and an "in-house" Bradford reagent, mechanical and hand shaking methods, plus wholemeal versus a "sieved wholemeal" were carried out to provide a suitable compromise in terms of speed and sensitivity.

Increasing heat damage was shown to produce a decrease in Absorbance at 595nm, but wheat variety was found to affect PST results. In addition, there was considerable overlap between varieties at critical temperature/time points i.e. the test appeared to be insensitive to mild heat damaged samples. On this basis, it was decided that this test would not be reliable for the detection of overheated grain at mill intake.

3.2.2 Near UV absorbance of salt-soluble proteins

Changes in the structural characteristics of individual amino acids affects their ability to absorb radiation at 275-280nm. The vital amino acids are present in minute quantities in the wheat grain and low Absorbance levels for sound wheat samples combined with poor repeatability and minor reductions as a result of heat damage suggested that this approach was not sensitive enough.

3.2.3 Surface tension measurements and foam testing

Proteins have specific surface and foaming properties that may be altered by heat treatment. Comparisons between surface tension measurements for sound and heat damaged samples were not consistent whilst differences in foaming were too small to be of value.

3.2.4 Gel protein rheology

The fraction of glutenin protein, which is insoluble in sodium dodecyl sulphate (SDS), and is separated as a gel layer during centrifugation of a de-fatted flour and SDS mixture, is known as "gel protein". The quantity and quality of this functional protein is critical for good breadmaking performance. During initial studies, the SDS-insoluble glutenin fraction appeared to be very sensitive to heat damage. However, strong varietal effects were indicated for all measures of gel protein rheology that would limit the use of this technique to only severely damaged samples of known varieties.

3.2.5 Durotest

This immunoassay test kit, normally used to detect the presence of non durum wheat in pasta products, contains a monoclonal antibody that is specific for the protein friabilin present in *Triticum aestivum* but not *T. durum*. Since heat treatment during processing of pasta is known to affect the absolute values produced in this test, its use in the detection of heat damage was investigated. Unfortunately, the approach was unsuccessful, as the measured Absorbance values could not be brought on scale.

3.2.6 Gluten quality

One of the standard ways, used by UK millers, to measure the rheological properties of dough is the Brabender Extensograph test. Recently a small-scale instrument has been developed by Stable Microsystems (the SMS Keiffer test rig) that can be used to measure the elasticity and extensibility of prepared dough or gluten. Problems with the gluten washing apparatus limited the amount of useful information that could be gathered from this test. However, even the limited data available suggests that the test is unlikely to detect low levels of heat damage and is sensitive to variety.

3.2.7 Mixograph

The 2g Mixograph provides an alternative small-scale measure of the rheological properties of dough. This instrument was effective in measuring severe levels of heat damage, but was found to be of limited use for lower levels of heat damage due to trace inconsistency and the need for prior knowledge concerning the sample under test.

3.2.8 Germograph (Easi-Twin) 2 pot

Alternative ways of measuring grain viability to the time-consuming germination test exist. Tetrazolium salt solutions are routinely used, but require a soaking period of 16 hours. This instrument claimed to provide an indication of germination capacity within 10-15 minutes. The embryo of sound, healthy grains stained a deep crimson-red, but unfortunately embryos of severely heat damaged grains also took up some stain and therefore the Germograph was not found to be a suitable method for the reliable, objective detection of heat damage under the conditions used in this study.

3.2.9 Image analysis

Heat treatment may be expected to influence the water uptake properties of wheat grains. Image analysis can be used to measure the difference in grain dimensions resulting from a fixed soaking period. Unfortunately, differences in water uptake, as measured by changes in grain size, failed to provide a suitable means of differentiating between sound and severely heat damaged samples.

3.2.10 Turbidity test

Heat treatment of grain reduces the amount of heat-precipitable protein that can be extracted by saline solution. Subsequent measurement of the turbidity has been related to the extent of heat damage. Initial results were very encouraging and led to the establishment of a putative cut-off point of $0.3A_{500nm}$, below which heat damage was certain to have occurred.

3.2.11 Rapid Visco Analyser (RVA)

The RVA provides a convenient means of measuring changes in the pasting properties of starch. Differences between treated and untreated samples were relatively small and inconsistent and therefore this was not progressed as a means of detecting heat damage.

3.3 Protein studies to identify a novel marker for heat damage

Since many methods for the detection of heat damage rely on changes in protein solubility and structure, wheat protein composition was investigated in a search for a novel biochemical marker for heat damage. If such a marker were to be discovered, this could lead to the development of a specific test kit for heat damage in wheat. Protein fractionation and separation of protein entities by gel electrophoresis or capillary electrophoresis was used to compare the protein composition of severely heat damaged and control, untreated wheat samples.

Protein studies indicated a shift in the amount of protein extracted using different solvents, i.e. changes in protein solubility rather than the removal or appearance of a new protein entity that would be required to develop a novel marker. This confirmed our focus on protein solubility as a means of detecting heat damage.

3.4 Optimisation of the Turbidity test

Following success in the initial screening phase, attempts were made to evaluate the effect of flour type and the replacement of the spectrophotometer with a low-cost colorimeter more suitable for a mill intake situation. Using wholemeal flour and a fixed filter colorimeter, the cut-off of 0.3 for A_{520nm} could be

retained and the test appeared to be relatively insensitive to variety. The performance of the test was assessed against other measures of heat damage as shown in Table 1.

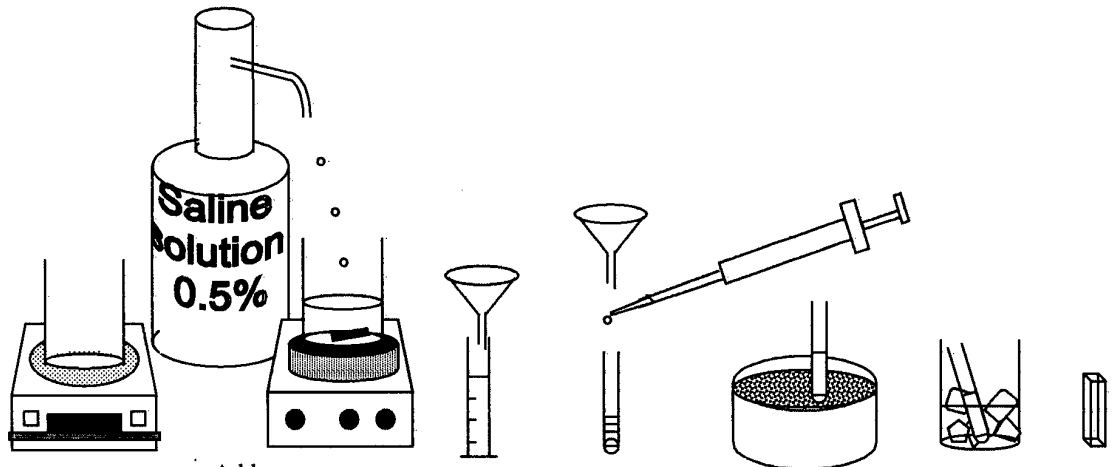
Table 1: Correlation coefficients between breadmaking quality - loaf volume (LV), Turbidity test (A_{520nm}), wet gluten content (Glu) and germination capacity (%G).

	Hereward				Soissons			Riband		
	LV	A_{520}	Glut	%G	LV	A_{520}	%G	A_{520}	Glut	%G
LV	1.000				1.000			-		
A_{520}	0.805	1.000			0.935	1.000		1.000		
Glu	0.886	0.815	1.000		-	-	-	0.838	1.000	
%	0.707	0.947	0.710	1.000	0.857	0.968	1.000	0.915	0.754	1.000

All correlations between Turbidity test results and germination capacity were above 0.9, suggesting a good relationship between heat damage levels and A_{520nm}. For the three varieties tested, this correlation appears to be significantly better than either the currently used mill intake test of wet gluten washing or breadmaking quality, the final arbiter of end-use quality. Thus, the Turbidity test provides a better means of detecting heat damaged wheat at mill intake than the previous slower and less sensitive wet gluten measurement.

A standardised version of the Turbidity test for use in the detection of heat damaged wheat is provided in Figure 1.

Figure 1: Schematic of the Turbidity test



The authors recommend that the minimum requirement for quality control of the method should be regular checks using a standard stock of well-mixed sound wheat. In addition, to provide greater confidence in detecting low levels of heat damage, a range of samples varying in heat damage levels and referenced to germination test results should be prepared. All method check samples should be replaced at least annually. When replicate tests were carried out by two operators using the same equipment and the same samples over a short period of time, A_{520nm} results were within ±3%.

4. IMPLICATIONS FOR HGCA LEVY PAYERS

- A simple, rapid test for heat damage that could be used by a farmer, grain store or mill intake laboratories to detect heat damage has been developed.
- This provides a simpler, more sensitive and quicker means of detecting heat damaged wheat than the previously used wet gluten methodology. Turbidity test results have been shown to be variety independent for the limited range of samples tested and to relate better to reference method data (germination capacity).
- Use of the Turbidity test, by the farmer or grain storeman, to monitor the impact of grain drying on wheat quality would provide improved control of the drying process and better segregation of stocks.
- Increased confidence and capability to reject slightly heat damaged grain at mill intake will result in improved consistency in the miller's major raw material with consequent effect on product quality for his customer, the baker.

5. REFERENCES

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HARRISON, K.R., DOARKES, P.F. and GREER, E.N. (1969). Detection of heat damage in dried wheat. *Milling* **151**, 37-38.

FINAL REPORT: Detection of Heat Damage in Wheat
PAPER 1: Production and Confirmation of Heat Damage

INTRODUCTION

In order to test the effectiveness of methods for the detection of heat damage in wheat, it was necessary to induce different levels of heat damage in test samples. To measure the amount of heat damage generated by these individual heat treatments, a standard germination test was carried out on all untreated and heat treated samples. Results of germination tests also provide a reference point for heat damage that can be used to compare the performance of any new test developed for this purpose.

Initially, severe heat treatment was used to produce extreme samples, i.e. undamaged and totally heat damaged, that were expected to react very differently in potential methods for detecting heat damage. This material was used in primary screening of potential heat damage tests. Any test that was unable to differentiate between these extremes was deemed unsuitable for detecting heat damage in wheat samples. This preliminary assessment also provided the first opportunity to assess the accuracy, speed and "ease of use" in a mill intake situation of potential heat damage methods. In addition, where the difference in test results for this material was small, the test could be discarded as lacking the required sensitivity to detect low levels of heat damage. Large differences would indicate a potentially sensitive test that may be worthy of further investigation.

Sensitivity of the tests, selected as having potential for the detection of differing levels of heat damage from the preliminary screen with extreme samples, was evaluated using a range of heat treated samples prepared in later phases of the work.

MATERIALS & METHODS

Grain samples

Phase 1: Severe heat damage

Four varieties were selected to cover a range of hardness and protein quality characteristics: Hereward (hard, **nabim** Group 1), Soissons (hard, **nabim** Group 2), Hunter (soft, **nabim** Group 4) and Riband (soft, **nabim** Group 3). The percentage grain protein (Kjeldahl, N x 5.7 at 14% moisture content) was determined by Near Infra-Red Spectroscopy (NIR) (Table 1).

Table 1: NIR grain protein content of sound grain

Variety	Hereward	Soissons	Hunter	Riband
Grain protein at 14% moisture (%)	11.8	12.0	9.8	9.6

Phase 2: Differing levels of heat damage

The same four varieties were used, but samples were larger and had different quality attributes. Bulk samples were cleaned and tested for specific weight, grain protein, Falling Number, wet gluten %, sodium dodecyl sulphate (SDS) sedimentation volume and varietal purity by electrophoresis (Table 2).

Table 2: Basic wheat quality attributes of test samples

Specified Variety	Specific weight (kg/hl)	Protein @ 14mb (%)	Falling No. (s)	Wet gluten (%): quality	SDS Volume (ml)	Purity (No. of grains of specified variety found in the 14 tested)
Hereward	81.7	10.2	312	24.7: satisfactory	86	12/14
Soissons	84.2	10.3	311	23.1: rather tough	93	14/14
Hunter	74.9	10.5	345	24.9: weak	55	14/14
Riband	75.6	8.9	226	18.8: slightly weak	46	11/14

Phase 3: Differing levels of heat damage

The three varieties Hereward, Soissons and Riband were used (a suitable sample of Hunter could not be sourced). Bulk samples were cleaned and tested for specific weight, grain protein, Falling Number, wet gluten % and varietal purity by electrophoresis (Table 3).

Table 3: Basic wheat quality attributes of test samples

Specified Variety	Specific weight (kg/hl)	Protein @ 14mb (%)	Falling No. (s)	Wet gluten (%): quality	Purity (No. of grains of specified variety found in the 14 tested)
Soissons	75.8	12.0	236	28.9 : strong	14/14
Hereward	79.9	11.4	375	28.1 : satisfactory	49/56
Riband	78.2	10.6	279	26.0 : slightly	13/14

Generation of heat damage in wheat

Phase 1: Severe heat damage

Each bulk sample was divided into two; one was stored as the control, the other was subjected to a temperature of 130°C for 30 minutes in a fan-assisted oven to induce severe damage (see Appendix 1).

Generation of differing levels of heat damage

Phase 2: Differing levels of heat damage

Full details of the methods used are given in Appendix 10.

A sub-sample was retained for use as a control and the remaining bulk was conditioned to 25% moisture content (in two stages over consecutive days) and stored for approximately 24h prior to heat treatment. Sinar (AP 6060 Moisture Analyzer) moisture content readings were verified by oven drying sub-samples of the conditioned grain immediately before heat treatment. Results were found to be 25±0.5% for all varieties. Sub-samples of the bulk were subjected to a range of temperatures (actual recorded oven temperature of 55, 68, 83, 91, 103 or 116°C) for a period of 40 or 80 minutes. In order to produce the volume of heat treated grain necessary for larger-scale (Chopin) milling of white flour and subsequent breadmaking, and to ensure consistency of heat treatment, a larger Mitchell drying oven was used and heat damaged samples were produced according to a strictly defined protocol. Samples were removed from the oven and transferred to a controlled temperature room to dry until their moisture content was 15.0% or below as determined by Sinar moisture meter. Samples were stored for at least 72h storage prior to the production of white flour.

Phase 3: Differing levels of heat damage

The procedures adopted were equivalent to Phase 2 except that samples of 5.3kg were used and conditioned to either a) 18% moisture content or b) 25% moisture content (carried out in two stages on consecutive days) for a fixed period of 60 minutes. Sinar moisture readings on the conditioned wheat were verified by oven drying. Values of 18.4±0.5% and 25±0.4% were found for all varieties. Approximately 24h after conditioning, one set of samples were dried at each temperature used (65, 75, 77 and 85°C) for 1 hour. The results from Phase 2 suggested that the temperature/time combinations used would include the lowest level of heat damage that would impair breadmaking performance. Perforated trays were used to improve the air circulation through the samples during heating. Treated samples were removed from the oven and allowed to cool. The moisture content was checked using a Sinar moisture meter prior to storage for at least 72h.

Wholemeal preparation

Since heat treatment, particularly the high temperatures used to generate extreme heat damage, resulted in a reduction in grain moisture content, the samples were cooled, conditioned to suitable moisture contents (~15%) and stored for 16 hours before grinding. This conditioning period produced grain that milled to a similar particle size to the untreated (control) grain when milled using a standard laboratory mill (KT 3100). This mill is used to produce a finely ground wholemeal for other wheat intake tests, such as Hagberg Falling Number. The ground wholemeal was thoroughly blended and stored for at least 8 hours before any tests of

heat damage were carried out. To grind grain without heat generation and thus moisture loss, a Bühler MIAG mill was used to produce a coarse ground wholemeal. This type of mill is used routinely to grind samples for moisture determination.

Chopin Milling to produce white flour

Grain samples were conditioned to 15.0±0.5% for soft and 16.0±0.5% for hard wheat before Chopin milling. The mill was set according to a standard protocol and the following sieves used on the break and reduction sides of the mill:

	Break		**Reduction**
	1st sieve	2nd sieve	
Aperture (micron)	800	140	160
Wire diameter (micron)	315	90	112

Using this mill set-up, a white flour with a minimum extraction of 55% should be produced. For the samples tested an extraction rate of approximately 70% was obtained. The flour was blended to produce a homogeneous mix of the individual flour fractions.

Bühler milling to produce "commercial style" white flour

Each sample was conditioned to a suitable moisture content (15.5% for soft and 16.5% for hard wheat) and milled using a Bühler laboratory mill (MLU 202) under standard conditions with the aim of producing white flour extraction rates between 75% and 80%. The roller settings used to mill the hard wheats were designed to produce a high level of starch damage to mimic commercial milling, whilst for the soft sample of Riband low starch damage levels were desirable as breadmaking performance was not to be carried. Tables 4 and 5 provide details of the roller settings and sifter covers used on the Bühler mill. Bran and offal by-products from the milling process were passed through a Bühler (MLU 203) bran finisher twice to remove adhering endosperm from these fractions. All flour streams produced were then blended to give a uniform white flour for subsequent testing.

Table 4: Roll gap settings

	Hard		**Soft**	
	1st	**3rd**	**1st**	**3rd**
Break rolls	0.06mm	0.04mm	0.1mm	0.07mm
Reduction rolls	0.03mm	0.02mm	0.07mm	0.03mm

Table 5: Sifter covers used, listed from top down (µm)

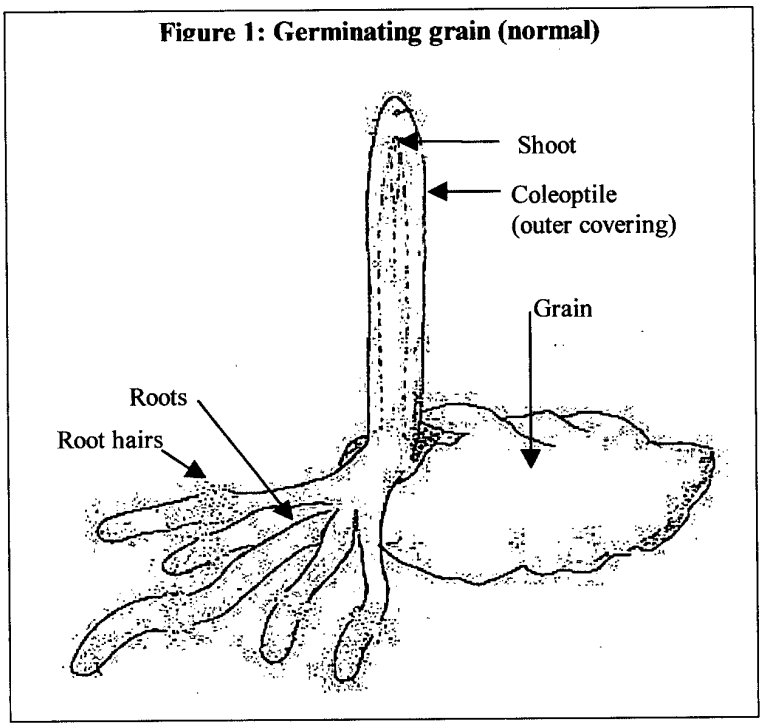
	1st	**2nd**	**3rd**
Break rolls	710	600	530
	140	140	140

	1st	**2nd**	**3rd**
Reduction rolls	180	180	140
	180	180	140

Confirmation of heat damage: Germination testing

A standard germination test was used to check the viability of each treated sample. Four samples of 100 grains were taken from each heat treatment of each variety and tested on moistened paper towels. After a cold-shock period, the samples under test were moved to a temperature-controlled room at 20.4±2.0°C for a further seven days.

Figure 1: Germinating grain (normal)



Germination was assessed visually and seeds were classified into three groups:

- **Normal:** Normal development of roots and shoots within the 7-day incubation period (see Figure 1).
- **Abnormal:** Abnormal growth included the leaf breaking through the end of the coleoptile, absence of roots or shoot and retention of the coleoptile under the pericarp (outermost covering of the grain).
- **No growth:** No growth included grains which appeared to have begun to germinate but did not form roots or a shoot and, in some cases, were infected with fungal or bacterial growth.

Germination results were calculated as the mean of the four replicates for each treatment and quoted as a percentage. Full details of the germination test are provided in Appendix 11.

Germination testing was carried out on control and heat treated samples generated under conditions outlined in Phase 2 and 3. They were not used for Phase 1 samples as this treatment was designed to produce complete heat damage.

Breadmaking

A standard Chorleywood Bread Process (CBP) method was used to evaluate the effect of varying levels of heat damage on end-use quality. A Morton Z-blade mixer was used to mix a bread dough from the following ingredients to a total work input of 11 watt hours kg^{-1} at a fixed rate of work input.

Ingredient	**Weight (g)**	**%(by flour weight)**
Flour	840	100
Yeast	21	2.5
Salt	16.8	2.0
Fat (Ambrex, slip point c. 45°C)	8.4	1.0
Ascorbic acid (100ppm)	0.084	0.01

Fungal *alpha*-amylase to supplement all samples to 40 Farrand units of activity.
Water level as determined by Farinograph 600 line.
Further details are provided in Appendix 26.

Duplicate (400g, single piece) loaves were produced from each Chopin-milled flour and assessed for loaf volume by seed displacement and crumb structure score after overnight storage at 21°C.

Biscuit-making quality

This method was designed to test the rheological properties of biscuit flour by mixing it into a semi-sweet dough under controlled conditions.

Ingredient	**Weight (g)**	
Flour	200	
Fat	32	(kept at 26°C)
Pulverised sugar	42	
Salt	0.7	
Cream powder	0.7	
Skimmed milk powder	5.0	
Sodium bicarbonate	1.1	
Ammonium bicarbonate	1.1	(kept in fridge)
Tap water	40	(at room temperature) depending on flour used (an extra 2g water reduces the extrusion time by approximately 35s, 2g less water extends the extrusion time by approximately 35s).

Instead of baking biscuits, the dough was tested for extrusion time and the rheological properties of the full recipe dough measured using the Bohlin VOR or CS 50 rheometer. Such measurements can be used to make comparisons between the suitability of different flour samples for semi-sweet biscuit production. Full details of the procedures used are shown in Appendices 8.24 and 8.25.

RESULTS AND DISCUSSION

Germination test

Phase 2: Differing levels of heat damage

Correlations between the mean results from each variety tested at each temperature and time combination used in Phase 2 were calculated (Table 6). For 11 degrees of freedom, a value of 0.553 or higher would indicate a close similarity between the sets of data being compared.

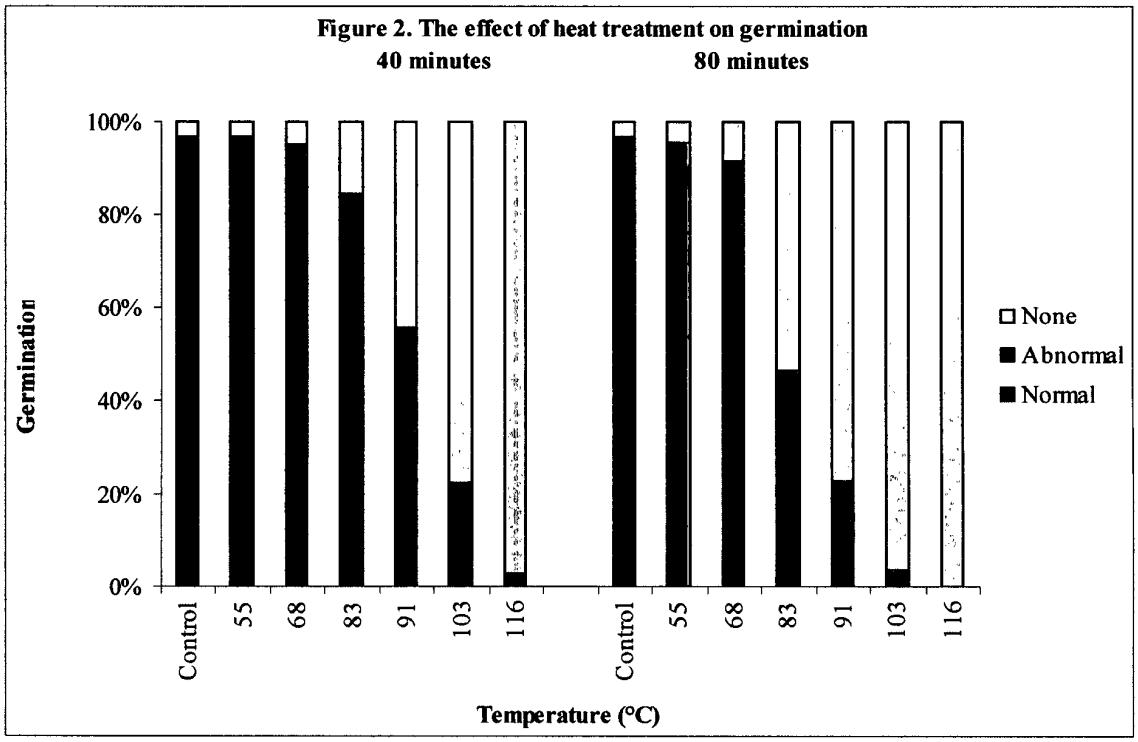
Table 6: Correlation coefficients between germination test results for individual varieties

	Hereward	**Soissons**	**Hunter**	**Riband**
Hereward	1.000			
Soissons	0.987	1.000		
Hunter	0.967	0.941	1.000	
Riband	0.991	0.975	0.974	1.000

A high level of agreement was found for all varieties, suggesting that there is no significant difference between the varieties tested. This suggests that endosperm texture and protein quality have little effect on the degree of heat damage induced under the controlled conditions of temperature and time used in this experiment. Due to the high correlation coefficients observed between varieties, the results shown in Figure 2 are the averages over all four varieties tested.

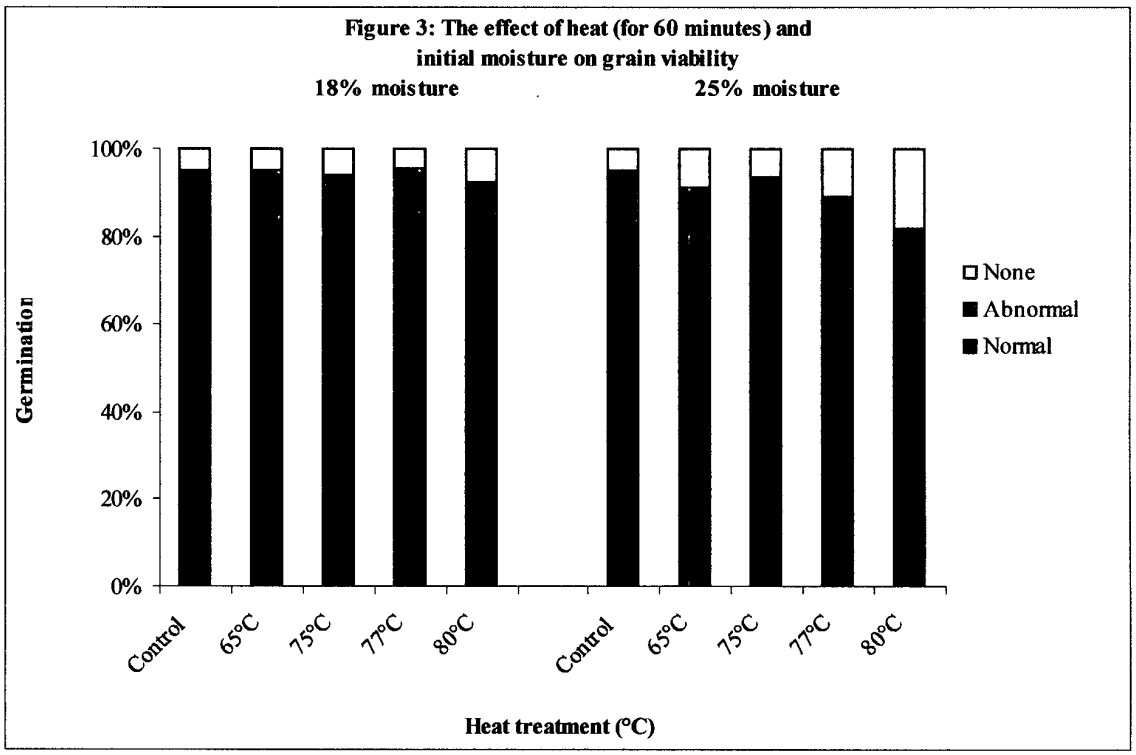
Heat treatment at 55°C for 40 or 80 minutes produced high germination scores (above 90%) which were not significantly different from the control untreated samples. The maximum drying temperature recommended to avoid heat damage in wheat destined for flour milling is 60°C. Germination results confirmed that the laboratory-scale drying produced the expected results. Treatment at 68°C for 40 minutes created slight heat damage, i.e. increased the amount of non-viable grain. Extending the time of treatment from 40 to 80 minutes significantly affected the level of heat damage generated. Samples heated at higher temperatures than these showed a marked decrease in normal germination until those

heated to 103°C for 80 minutes and 116°C for both 40 and 80 minutes produced virtually no viable grains, i.e. the sample was completely damaged by these treatments.



Phase 3: Different levels of heat damage

The results illustrated in Figure 3 represent an average over the three varieties used in order to enable comparison with data shown in Figure 2. All three gave similar scores for each of the three germination classes, although Riband gave rise to slightly more normal seedlings than Hereward or Soissons. Similar results were obtained from the control and the heated samples, although the percentage of abnormal seedlings was higher than in samples generated under the conditions used in Phase 2, even for the control samples. Where the starting material was held at 18% moisture content prior to heat treatment, no significant heat damage occurred when the grain was exposed to temperatures of up to 80°C for a period of 1hour. This confirms the long held view that higher temperatures can be used to dry wheat that is closer to the desired storage moisture level of <15%. For the samples conditioned to 25% moisture before heat treatment a reduction in germination capacity was indicated at 77°C, and at 80°C less than 60% of grain germinated normally. Temperatures of 65°C and 75°C did not have any effect on germination capacity under either conditioning regime or in any variety used in this experiment. This finding is in contrast to current recommendations for drying wheat for the milling industry and these temperatures should not be used as a guide for safe drying of wheat as this study is based on a limited data set and under laboratory procedures.

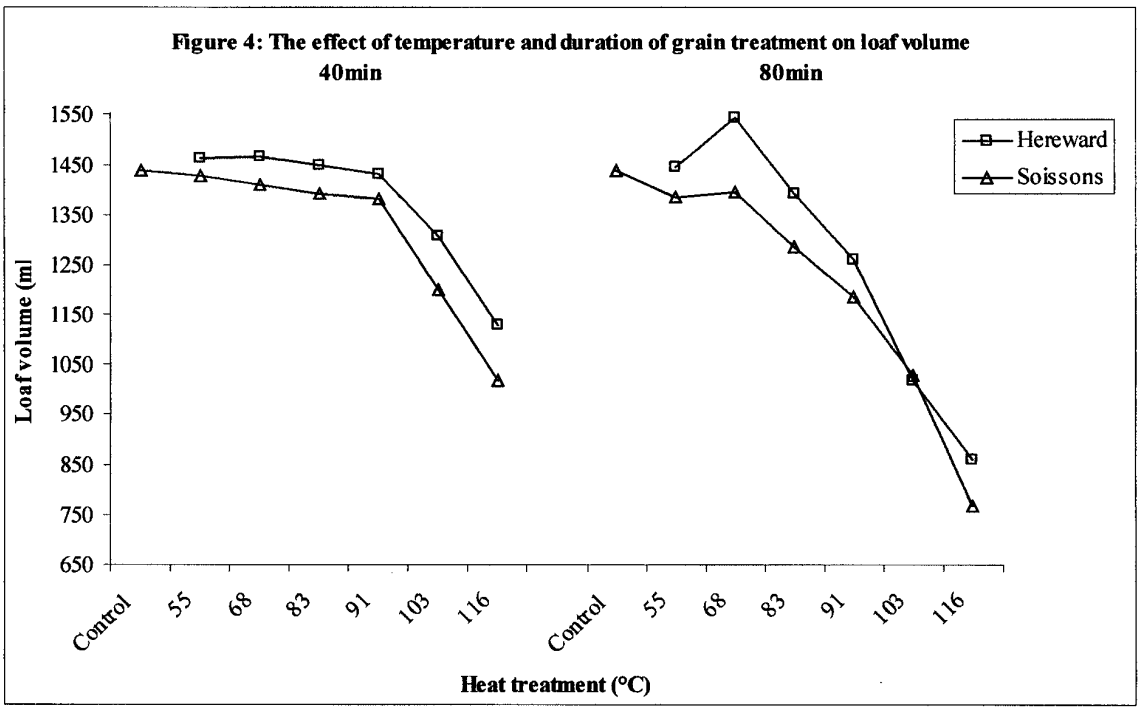


Breadmaking quality

CBP loaf volumes for control and heat treated samples produced under Phase 2 are illustrated in Figure 4 for the breadmaking varieties Hereward and Soissons studied. Poor loaf volumes were produced for the control samples. This could be partly due to the use of the Chopin mill, which produces flour of lower starch damage than that generated in a Bühler or commercial flour mill. Comparison of 40 minute heat treatments shows that breadmaking performance, as measured by loaf volume, was not significantly affected by temperatures of up to 91°C. Thus, the levels of heat damage detected by the germination test when grain was treated at 68°C and 83°C for 40 minutes failed to have a measurable effect on bread quality. A sharp drop in loaf volume was apparent when temperatures in excess of 91°C were used for a period of 40 minutes. This treatment produced less than 60% normal germination and thus reflects significant heat damage.

For the 80 minute treatments, significant reductions in loaf volume were not seen for either variety until a temperature of 83°C was attained. Beyond this point loaf volume decreased dramatically as the temperature of heat treatment increased. The lowest volumes, achieved at the highest temperature time combination, were approximately half that of the control loaves. The results for treatments involving the 80 minute duration are in better agreement with the germination test results, i.e. both loaf volume and percentage germination decrease significantly for the samples treated at temperatures of 83°C or above.

Whilst the CBP loaf volume of control samples was below that expected for high quality UK bread wheat (1550 to 1650ml is more typical for Soissons and Hereward respectively), reductions of the order of 200ml observed for 40 minutes at 91°C are very significant and reflect bread that would be totally unacceptable to the typical consumer.



Rheology of biscuit dough

The method used for preparing dough samples for rheological testing depended heavily on the water absorption of the flour sample and the amount of work put into mixing the dough. Rheological results of dough samples, that satisfied the extrusion criteria, did not follow a repeatable trend between varieties and therefore are not presented here. The reliability of this method for evaluating absolute quality of biscuit flours in relation to heat damage was found to be questionable under the circumstances used in this study. The method requires variation of dough mixing to achieve a constant extrusion time. This may be expected to "correct" for some of the variability induced by heat damage in rheological properties. Thus, this test was found to be an unsatisfactory means of detecting heat damage in biscuit dough. Rheological testing of basic wheat flour dough or prepared gluten is likely to be more effective.

CONCLUSIONS

The combinations of initial moisture content, temperature and time used to create heat damage produced the required range of different heat damage levels. The reference germination test was effective in differentiating between heat treated samples which were slightly and severely heat damaged. Depending on the original moisture content of the samples, temperatures in excess of 68°C were required to generate measurable heat damage under the experimental conditions used in this work. CBP loaf volume, used to indicate effect on end-use quality, was less sensitive to heat damage, with temperatures in excess of 83°C required to produce a measurable difference in this breadmaking quality attribute.

FINAL REPORT: Detection of Heat Damage in Wheat
PAPER 2: Evaluation of Methods to Detect Heat Damage

INTRODUCTION

When harvest conditions are damp, the resulting wheat must be dried to optimise the moisture content for long term storage. If heated air is used for drying, careful temperature control is necessary to avoid damage to the grain by overheating or failure to cool the bulk adequately before storage.

Serious heat damage may cause a darkening of the seed coat, giving grain an obviously different appearance when damaged. However, gluten quality may be adversely affected at lower temperatures than that which causes obvious external change. Heat damage is traditionally measured at mill intake by washing the starch and water-soluble proteins from a prepared dough and subjectively assessing the gluten which remains. Due to protein quality differences between varieties that are destined for different end-uses, the subjective element of the test makes it difficult to produce quantitative results and therefore the test lacks sensitivity to low levels of heat damage.

Changes in protein structure may affect the activity of some wheat enzymes; as a result, germination viability is significantly affected by overheating and can give an accurate indication of heat damage within a sample. However, this test is very time consuming and unsuitable for use in a mill intake or grain store situation. A more rapid tetrazolium staining procedure for testing grain viability is based on the fact that the enzyme dehydrogenase is deactivated when grain is exposed to excessively high temperatures.

Consideration of the components that suffer from overheating of grain suggests a number of approaches to the detection of heat damage. A primary consequence of heat damage is the loss of protein solubility (particularly the salt-soluble albumins and globulins). This is used as the basis for the protein solubility test (PST) developed by Every (1987). In addition, individual amino acids absorb UV radiation at 275-280nm due to their structural characteristics and are sensitive to heat. Therefore, potentially their measurement may be used to determine levels of heat damage in samples. The insoluble glutenins have been found to be the most sensitive to heat and are rendered even more insoluble by heat treatment. Thus, measures of the turbidity of protein extracts and the rheology of the SDS-insoluble "gel protein" fraction were considered to be worthy of investigation.

Protein changes may also affect the rheological properties of dough. Traditional methods of measuring dough rheology are slow and require significant quantities of white flour. Rheological techniques which have recently been introduced into the UK, such as the 2g Mixograph (Dobraszczyk & Schofield, 1999) and the Stable Microsystems Keiffer rig, have the advantage of using small amounts of material and the potential for use with wholemeal samples. Protein surface properties and their relationship with water may also be affected by changes in structure. Therefore, surface tension and foaming capacity were evaluated in terms of their ability to measure heat damage.

Most potential methods for detection have focussed on grain protein in some shape or form. However, heat may also create changes in the starch component and thus alter its pasting properties, or alter the permeability of the bran layers, thus influencing water uptake properties.

This study aimed to develop a simple, rapid and accurate test for heat damage that is independent of wheat variety.

MATERIALS

For initial screening of all potential heat damage tests, samples of untreated (control) and totally heat damaged material of the varieties Hereward, Soissons, Hunter and Riband were tested. Failure to differentiate between these extremes in terms of heat damage was cause for rejection of the test. A preliminary assessment of repeatability, convenience (speed) and "ease of use" in a mill intake situation was made. Where a test passed this first screen, it was evaluated more fully using further samples (Hereward, Soissons, Hunter and Riband) from a different harvest which had been subjected to a range

of temperatures (actual recorded oven temperature of 55, 68, 83, 91, 103 or 116°C) for a period of 40 or 80 minutes.

Details of the samples used and heat treatment employed are given in "Paper1: Production and confirmation of heat damage".

METHODS, RESULTS AND DISCUSSION

Since this paper is concerned with an evaluation of the performance of a number of individual methods, each procedure will be presented and results discussed in sequence.

1. Protein solubility test (PST)

1.1 Introduction and method

A primary consequence of heat damage is the loss of protein solubility (particularly the salt-soluble albumins and globulins). This was used as the basis for the protein solubility test (PST) developed by Every (1987). Particular emphasis was placed on this test as it appeared to provide a rapid and simple procedure to quantify heat damage in wheat. The PST was assessed in terms of accuracy and speed.

Full details of the method used are provided in Appendix 2. In summary, saline solution and wholemeal flour were mixed and allowed to stand. A sample was then removed from the top of the mixture, added to Bradford reagent solution and allowed to stand for a specified length of time before the absorbance at 595nm was read against a blank of Bradford reagent solution.

1.2 Factors affecting results

1.2.1 Bradford reagent

Bradford staining reagent is available commercially (Bio-Rad Protein Assay Dye Reagent Concentrate, Bio-Rad Laboratories Ltd, Hemel Hempstead, Herts, UK) for ~£100, or may be prepared "in-house". Due to the high purchase cost of this reagent, an equivalent mixture was prepared from individual ingredients "in-house" according to the procedure set out by Bradford. Eight individual tests were carried out for each Bradford reagent solution using two of the test varieties, Soissons and Hunter. Mean results and standard deviations are given in Table 1.

Table 1: Effect of Bradford reagent on PST results from sound wheat samples (A_{595nm}).

	SOISSONS		HUNTER	
	In-house	Bio-Rad	In-house	Bio-Rad
Mean	0.451	0.733	0.343	0.844
S.D.	0.060	0.063	0.052	0.056

The Absorbances of sound samples of both Soissons and Hunter were significantly lower for the "in-house" reagent than the purchased Bio-Rad equivalent. It appeared that the two reagents absorbed light differently. The "in-house" prepared Bradford reagent had a blue colour and a maximum absorbance at 640-645nm, whereas the Bio-Rad reagent appeared browner and had a maximum absorbance at 650nm. One possible cause of this difference was the use of methanol in the Bio-Rad reagent instead of 95% ethanol in the "in house" reagent as suggested by Bradford.

The Bio-Rad Bradford reagent was used in all further experiments as this potentially provided a greater range of Absorbance values for the detection of heat damage and removed one possible variable when attempting to standardise the test.

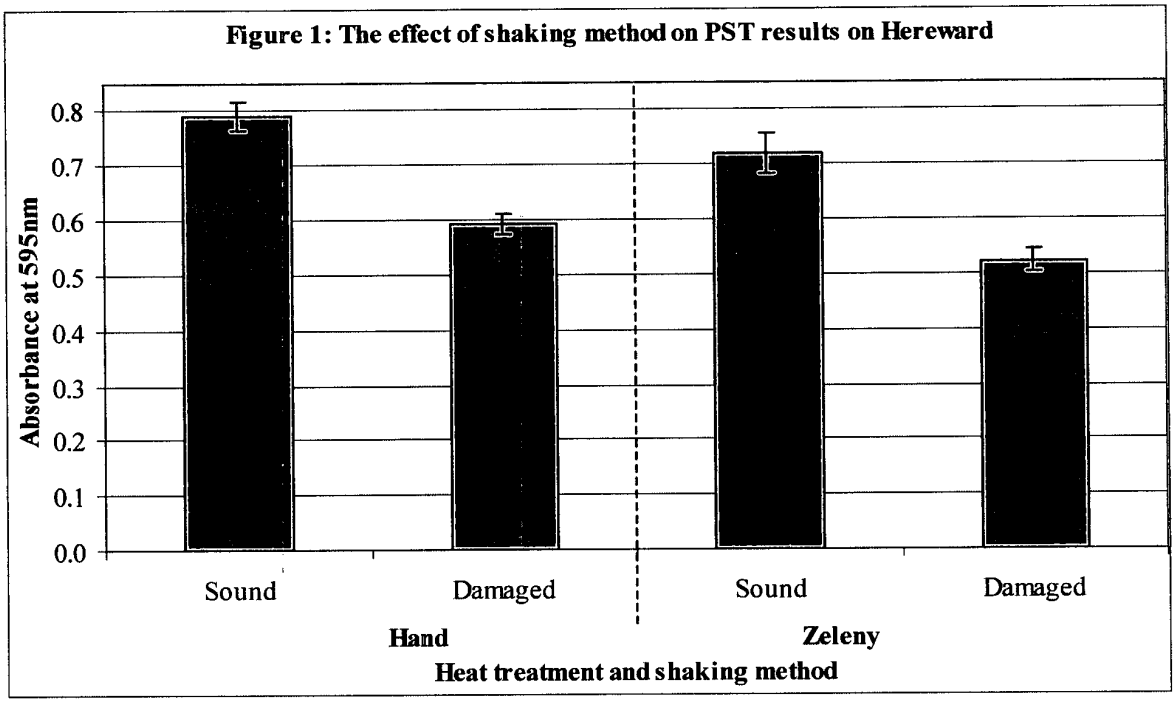
1.2.2 Shaking method

Protein extraction was carried out by inverting the cylinder containing the mixture of flour and salt solution by hand. Each sample took several minutes to test because inverting several cylinders

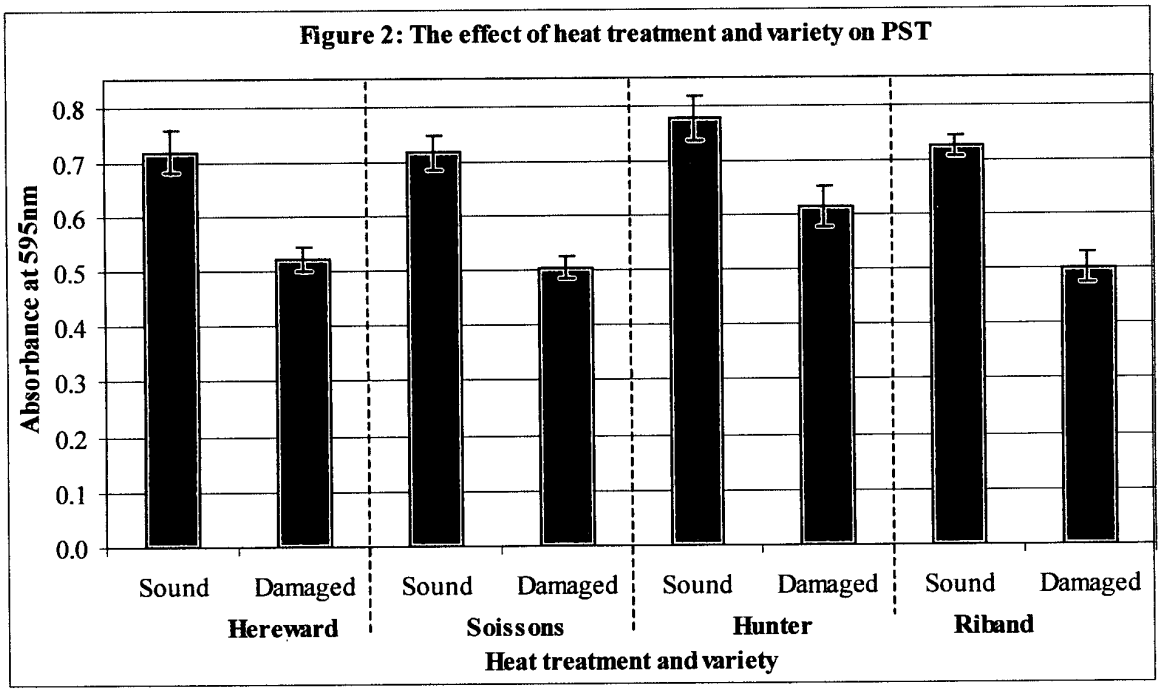
simultaneously by hand was not possible. Therefore, the performance of a mechanical shaker was investigated.

The use of a Zeleny shaker (mechanical inverter) increased the speed of testing several samples simultaneously, allowing eight samples to be analysed in approximately 20min (compared with 32min for the manual method). Also, the extraction stage of the method was standardised. Each bar in Figure 1 represents the mean value of eight individual tests; error bars represent the standard deviation for each set of results. Both sound and heat damaged samples of Hereward were tested using manual and mechanical inversion. Results were slightly higher when extracts were hand- rather than mechanically-shaken. The Zeleny shaking technique was used in all subsequent experiments due to the large numbers of samples to be tested.

Figure 1: The effect of shaking method on PST results on Hereward

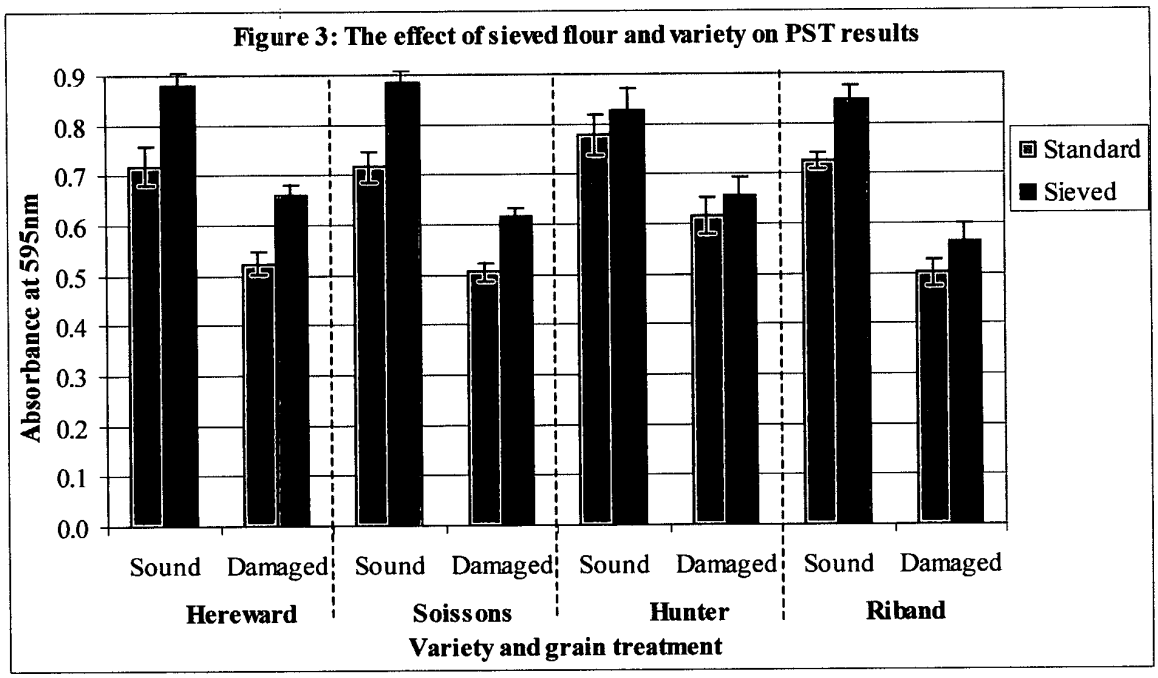


The mean results from eight tests on sound and heat damaged samples of Hereward, Soissons, Hunter and Riband are shown in Figure 2. Error bars represent the standard deviation within each set of results. In all four varieties, severe heat damage resulted in a consistent decrease in the measured Absorbance at 595nm of approximately 0.2. Thus, this test appeared to be capable of detecting severe heat damage.



1.2.3 Sieved wholemeal

To remove most of the bran particles, wholemeal flour was sieved using a 150μm sieve. The PST was carried out on the "white flour" obtained. The effect of flour sieving on PST results from sound and heat damaged samples of each variety is illustrated in Figure 3. Each bar on the chart represents the mean of eight tests; standard deviations are shown as error bars.

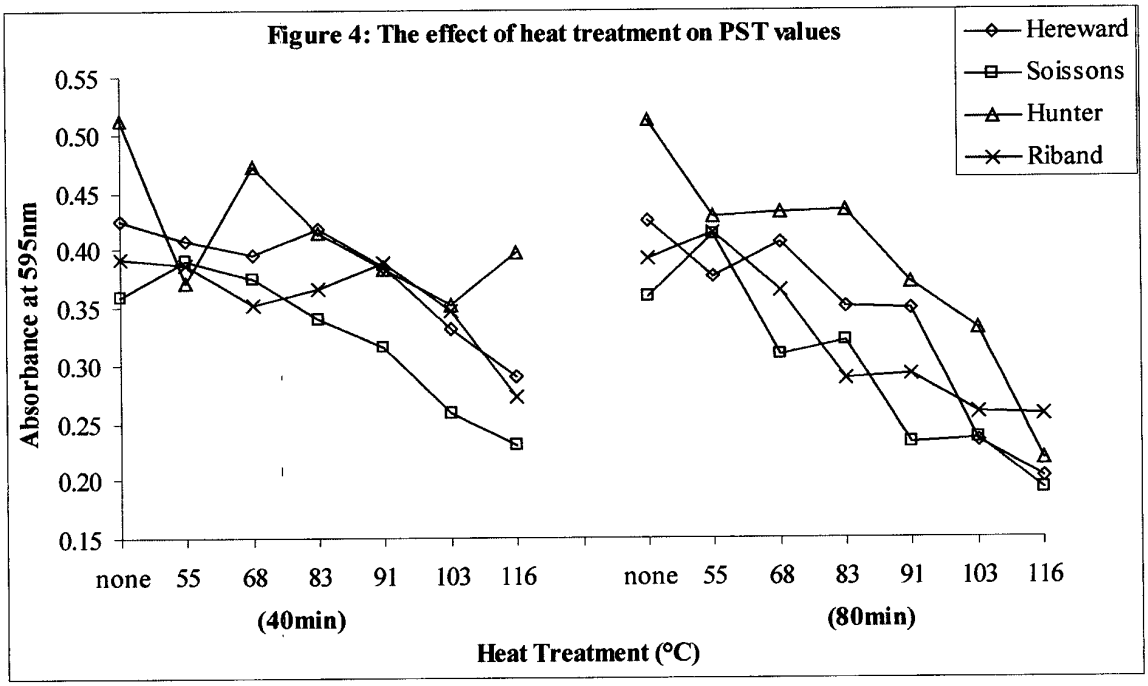


Sieving the flour gave increased Absorbance at 595nm for all four varieties tested. The standard deviation (averaged over the varieties used) was reduced using sieved flour and slightly larger differences between

sound and severely heat damaged samples were obtained when the PST was performed on sieved flour. Thus, sieving improved the repeatability of the test and should contribute to improved differentiation between varying levels of heat damage. However, sieving through a 150μm sieve added significantly to the time taken to carry out the test and the small advantages were outweighed by time constraints in a mill intake situation.

1.2.4 Sensitivity to variety

Every (1987) reported that PST results did not depend on wheat variety. T-tests were carried out on results from pairs of varieties obtained in this study. For sound wheat samples, no significant (P=0.05) differences were found between Riband and Soissons, Hereward and Soissons, or Hereward and Riband. There were significant differences between Hunter and each of the other three varieties. Results from damaged samples showed significant differences between results of all varieties except between Riband and Soissons. The preferred method for detection of heat damage should be relatively insensitive to wheat variety.



1.2.5 Performance of PST on samples containing different levels of heat damage

Sieving wholemeal through a 150μm sieve was discontinued as it was considered too time consuming for rapid testing and did not make a major improvement to the existing method. Each test was carried out in duplicate for each sample: the mean results are plotted in Figure 4.

Again, there was a noticeable effect of variety on test results although all four varieties followed a similar trend. Control and mildly heated samples gave the highest Absorbance readings for each variety. As the heat treatment increased in severity, so the Absorbance reading decreased. The Absorbance values for different varieties subjected to the same heat treatment regimes varied markedly; e.g. values ranging from 0.23 to 0.4 were observed for the most severely damaged samples in this set. In addition, there was considerable overlap in Absorbance values between one heat treatment and the next, preventing a clear cut-off point from being established.

1.3 Discussion

Initially, the PST was standardised by using the Bradford reagent produced by Bio-Rad. The use of a Zeleny shaker allowed several samples to be analysed in a short time. Sieving wholemeal samples before testing slightly improved PST accuracy. The differences between Absorbance values for sound and severely heat damaged were large enough to suggest that the sensitivity of the test was suitable for use on a range of heat treated samples.

Every (1987) found that sound Oroua (1986 harvest) ground on a KT Mill gave a PST value of around 0.53. In previous work, Absorbance values for sound samples of the varieties Brock, Hornet and Yecora ranged from 0.60 to 0.65 (Brown, 1989). Absorbance values obtained in this study for sound samples ranged from 0.66 (Soissons) to 0.83 (Hunter), providing some confidence in the technique.

Absorbance values for severely heat damaged material are likely to be dependent on the exact heat treatment imposed. Previous authors (Every, 1987 and Brown, 1989) damaged wheat after conditioning it to a high moisture content. In our studies, severely heat damaged wheat was initially produced by heating unconditioned grain to 130°C and therefore reliable comparisons cannot be made. Wheat variety was found to affect PST results; generally Hunter gave higher results than Hereward, Soissons or Riband. Less varietal difference was found when sieved flour was used.

When samples from a range of artificially heat treated samples were tested, increasing heat damage was shown as a decrease in Absorbance at 595nm. Results from germination and breadmaking tests have indicated that heat damage would be a problem when grain was heated above 83°C for 80min or above 91°C for 40min prior to use (see Paper 1: "Production and confirmation of heat damage"). There was a considerable overlap between the four varieties at these critical heat treatments and the test appeared to be insensitive to mild heat damaged samples.

On the basis of these results, it was decided that this test would not be reliable for the detection of overheated grain at mill intake.

2 *Near UV absorbance of salt-soluble proteins*

2.1 Introduction and method

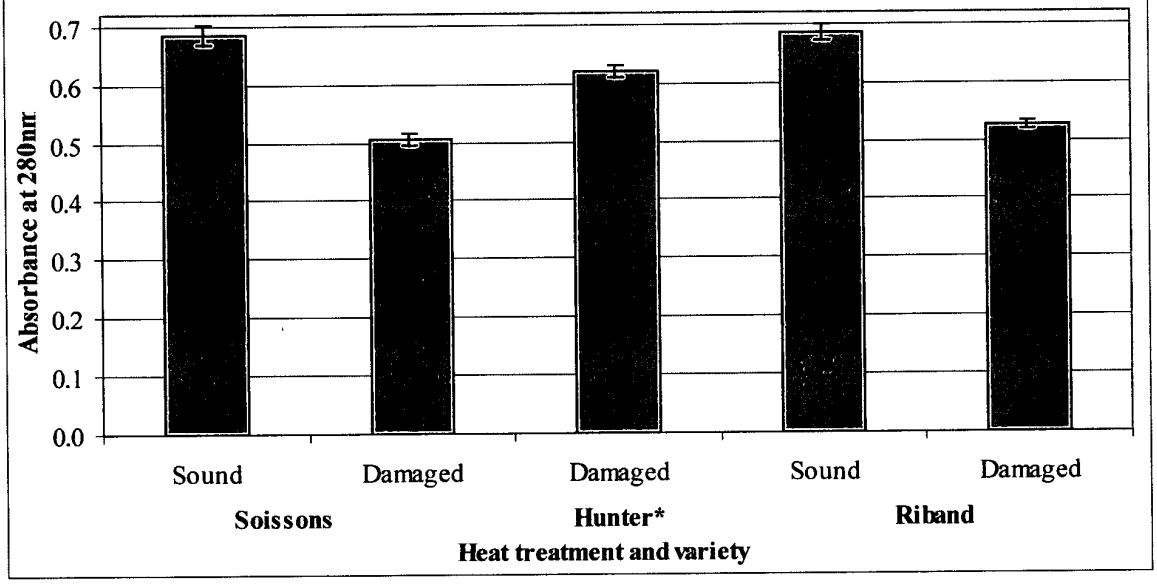
Some amino acids, including tryptophan, tyrosine and phenylalanine, absorb radiation at 275-280nm due to their structural characteristics. These aromatic amino acids are sensitive to heat and therefore their measurement may be used to determine heat damage in wheat samples. Absorbance measured at 280nm accounts for approximately 80% of the nitrogen present in salt-soluble proteins and should provide a reliable indication of the amount of such proteins present.

Full details of the method are given in Appendix 3. In summary, wholemeal and saline solution were mixed, then centrifuged. The supernatant was diluted with saline solution and Absorbance was read at 280nm.

2.2 Results

Results of tests carried out on severely heat damaged samples are presented in Figure 5. The mean values of eight measurements are presented; error bars represent the standard deviation of each set of results. Sound samples of both Soissons and Riband produced Absorbance values over 0.1 units higher than the corresponding value for the severely heat damaged samples. These results suggested that the test was relatively insensitive to variety. In addition, the low standard deviation of the Absorbance results (approximately 0.012) indicated that this test was more repeatable than the PST. Thus, initial screening suggested that this test had some potential for the detection of heat damage. In addition, the test is simple, rapid and easy to use, thus fulfilling the basic requirements for a mill intake test.

Figure 5: The effect of heat treatment (130°C for 30 minutes) on the absorbance at 280nm of salt-soluble proteins extracted from wheat



*Insufficient flour to analyse sound Hunter.

In order to further evaluate the method, selected samples exposed to a range of different temperature and time combinations were tested in duplicate. Three samples, namely the control, 55°C for 40min and 116°C for 80min were selected for each variety. The results are summarised in Table 2. Small varietal differences were seen, whilst differences between minimum and maximum heat treatments were also small and in the case of Hunter, almost negligible.

Table 2: Absorbance at 280nm of salt-soluble proteins

	Hereward	Soissons	Hunter	Riband
Control	0.368	0.311	0.345	0.303
40min @ 55°C	0.336	0.325	0.302	0.308
80min @ 116°C	0.254	0.222	0.307	0.259

As for screening tests, Absorbance values reduced as the level of heat damage increased. Significant differences were observed between sound samples (see Figure 5 and Table 2 for comparisons). In addition, it is clear that even severe heat damage only produced small differences in Absorbance. This suggests that the test is likely to be insufficiently sensitive to detect low levels of heat damage. Absorbance values for severely heat damaged Hunter (80 min at 116°C) were significantly higher than that obtained for other varieties. This observed inconsistency between the limited varieties examined would make it very difficult to define a simple cut-off point and would prevent the test from being of use at mill intake.

2.3 Discussion

The amino acids responsible for UV absorbance are present in minute quantities in the wheat grain. To counteract this, the measurement would need to be very sensitive to heat damage. Results confirmed that Absorbance levels were often low for sound wheat samples and significant differences existed between sound samples tested from different seasons. Absorbance values decreased as the level of heat damage increased, but differences were relatively small. Therefore, the test did not appear sensitive enough to warrant further investigation.

3 Surface tension measurements and foam testing

3.1 Introduction and methods

Proteins have specific surface properties that are altered by heat treatment. Tests that measure the surface properties of mixtures containing salt-soluble wheat proteins were evaluated in terms of their ability to measure heat damage. The ability of a solution to foam is due to the amphiphilic nature (conferred by phospholipids or hydrophobic residues of proteins) that causes them to migrate to an interface between water and air. Chemical bonds may be broken by heat treatment, unmasking hydrophobic regions of particular and thus leading to increased foaming ability.

Salt-soluble proteins were extracted from samples of wheat by mixing flour and saline solution, then centrifuging. Surface tension measurements were carried out on the supernatant using the ring method.

Foaming capacity was measured by shaking an extract of flour protein and reading the volume of liquid and foam immediately. A second volume reading was made after allowing the mixture to settle. Full details of the methods used are provided in Appendix 4.

3.2 Results

Heat treatment resulted in lower surface tension readings for Hunter and Hereward, but higher surface tension readings for Soissons and Riband. In a second set of experiments, 2g flour were dissolved in 50ml of 2% sodium chloride solution. From this set of tests, all surface tension measurements from heat damaged samples were higher than from sound samples.

Foam testing performed on Hunter and Hereward showed a slight increase in foaming ability for the damaged wheat samples compared with the controls.

3.3 Discussion

Heat is known to reduce the solubility of salt-soluble proteins; however, the relationship between protein solubility and surface tension is not clear. Comparisons between sound and heat damaged samples did not produce consistent differences; both positive and negative differences were observed. Flour lipids are known to influence surface tension measurements. Therefore, it is likely that the use of de-fatted flour would improve the reliability of such measurements. However, the need for a pre-testing treatment such as de-fatting would render this test unsuitable for use at mill intake.

The ability of a protein extract to foam may be expected to increase with increasing levels of heat damage. Only slight differences were found between sound and severely heat damaged samples, suggesting that the test would not be sufficiently sensitive to detect small differences in heat damage. In addition, the test used relatively expensive and sensitive equipment that would be unsuitable for routine use at mill intake. For these reasons this test was abandoned at the screening stage.

4 Gel protein rheology

4.1 Introduction and methods

The fraction of glutenin protein, which is insoluble in sodium dodecyl sulphate (SDS), and is separated as a gel layer during centrifugation of a de-fatted flour and SDS mixture, is known as "gel protein". The weight of this protein fraction has been shown to correlate with other measurements of protein quality and breadmaking performance, such as SDS sedimentation volume and loaf volume respectively (Graveland *et al*, 1982). Rheological testing of the gel protein fraction provides detailed information on the characteristics of this functional protein which is crucial for breadmaking performance (Oliver & Pritchard, 1993). Elastic modulus (G') is the most commonly measured parameter and provides a reliable indication of protein strength. G' values are known to be affected by variety: this is the basis of the gel protein test, therefore only comparisons between sound and damaged samples of the same variety can be made reliably. Full details of the methods used are presented in Appendices 5 and 6.

In the initial phase of testing, wholemeal flour samples were de-fatted, accurately weighed and mixed with a solution of SDS for a specified length of time. The resulting mixture was then ultracentrifuged to separate the starch and gel protein components. After gently pouring off the supernatant, the gel protein layer was carefully removed, weighed and tested for viscosity and elasticity using a Bohlin VOR rheometer. Parameters studied were: G' (elastic modulus), G" (viscous modulus) and the phase angle δ [with $\delta = \tan^{-1}(G''/G')$].

After this initial screening, white flour produced from a range of heat treated grain samples (milled on a laboratory scale Chopin mill) was used in place of wholemeal. White flour samples are preferable for gel protein measurement as any interference from bran particles is removed.

4.2 Results

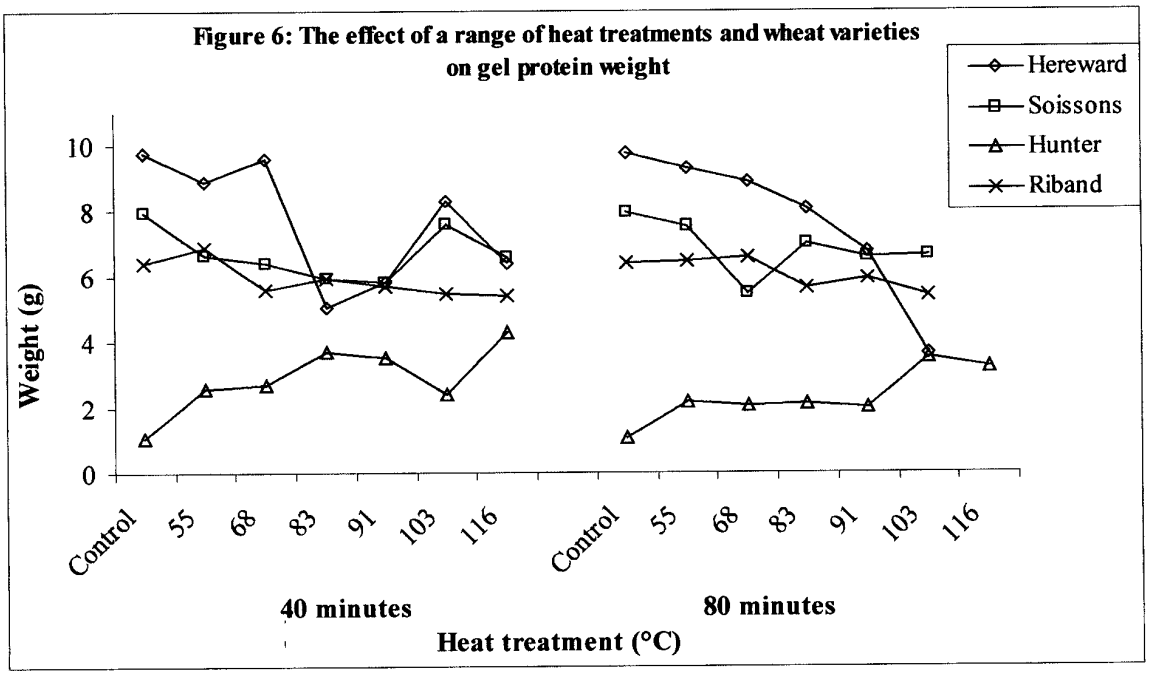
The weight of gel protein obtained from severely heat damaged samples of both Hunter and Hereward were significantly lower than that from the relevant controls (Table 3). Such low gel protein weights have never been encountered in normal UK wheat samples and therefore it appeared that measurement of gel protein weight alone may be used as an indicator of heat damage. For the variety Soissons, it was difficult to produce good duplication of gel protein weight data and therefore no mean value is quoted. Whilst these results are not totally consistent with data from the other two varieties, all show a significant change in functional protein resulting from heat damage. Rheological measurements of G' and G", where these were possible, were also significantly altered by heat damage. Whilst gel protein measurements in the current form cannot be considered to be suitable for use in a mill intake situation, it was still deemed worthy of further investigation as parallel work was being conducted in an attempt to simplify this technique (Whitworth, 1999). It is also clear from preliminary studies that if this test is to be used for the detection of heat damage then it will be necessary to expose sub-samples of unknown test samples to a specific heat treatment in order to pick up differences.

Table 3: The effect of heat damage on weight and rheological parameters of gel protein

	Sound wheat				Heat damaged wheat			
Variety	**Weight (g/5g)**	**G' (Pa)**	**G" (Pa)**	**δ**	**Weight (g/5g)**	**G' (Pa)**	**G" (Pa)**	**δ**
Hereward	9.45	14.8	9.4	32.5	1.62	4.6	5.1	48.4
Soissons	6.21	69.0	48.7	33.3	NA	2.6	3.0	48.3
Hunter	5.12	6.7	3.9	30.3	2.63	NA	NA	0.6
Riband	6.16	5.8	3.8	33.7	ND	ND	ND	ND

δ = phase angle, G' = elastic modulus and G" = viscous modulus at a frequency of 1Hz.
NA = not available, ND = not determined.

When samples exposed to differing temperature/time combinations were examined, sufficient gel protein was obtained from Hereward, Soissons and Riband for the rheological analysis of most samples (Figures 6, 7, 8 and 9). Gluten weights for sound Hereward (9.45g/ 5g of flour) and Soissons (6.21g/ 5g of flour) were comparable with typical values obtained from the 1996 Recommended List trials of 11.9 and 9.6g/5g flour respectively. (It is not possible to provide an equivalent value for Riband or Hunter as gel protein measurements are not carried out for biscuit or feed wheats.)



Insufficient weight of gel protein was obtained from the variety Hunter to carry out rheological testing, therefore this variety is included in Figure 6 only. Unfortunately, results were not consistent across all varieties. As the level of heat treatment increased, gel protein weight decreased for Hereward, increased for Hunter and remained fairly constant for Soissons and Riband. In addition, since the weight of gel protein is a varietal characteristic (based on the high molecular weight glutenin content of the sample), the starting point for each sample tested was different. Thus, unless the operator had prior knowledge of the variety under test and typical gel protein weight values for that variety, the results could be misleading.

In spite of these perceived limitations of this test procedure, rheological measurements were still analysed as these may provide an insight into fundamental changes occurring in protein characteristics as a result of heat treatment.

For the three varieties studied, significant increases in protein strength (G') were observed for heat treatments of 116°C for 40 minutes or above 91°C for 80 minutes (Figure 7). Such heat treatments relate to severe heat damage and failure of the test to pick up lower levels, where protein damage would be expected to occur, suggests that this test may not be sensitive enough for routine use in screening intake samples. In addition, significant differences were observed between the control results for the three varieties studied. The G' values for sound Hereward (~ 25Pa) and Soissons (~ 62Pa) were consistent with typical results from sound samples of these varieties from the 1996 Recommended List trials, i.e. 21.9 and 67.2Pa respectively. As expected from previous experience of these varieties, Soissons was over twice as strong as Hereward and, in turn, Hereward was twice as strong as Riband. Such varietal differences in G' limit the suitability of such a test for the detection of even the most severe heat damage in an unknown sample.

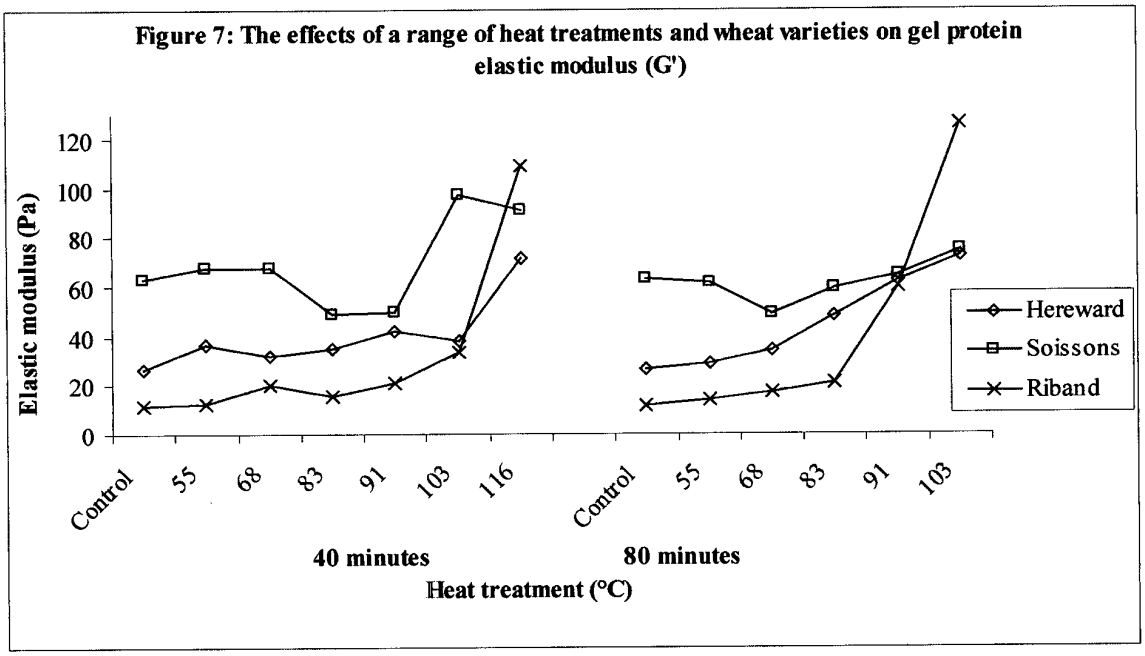
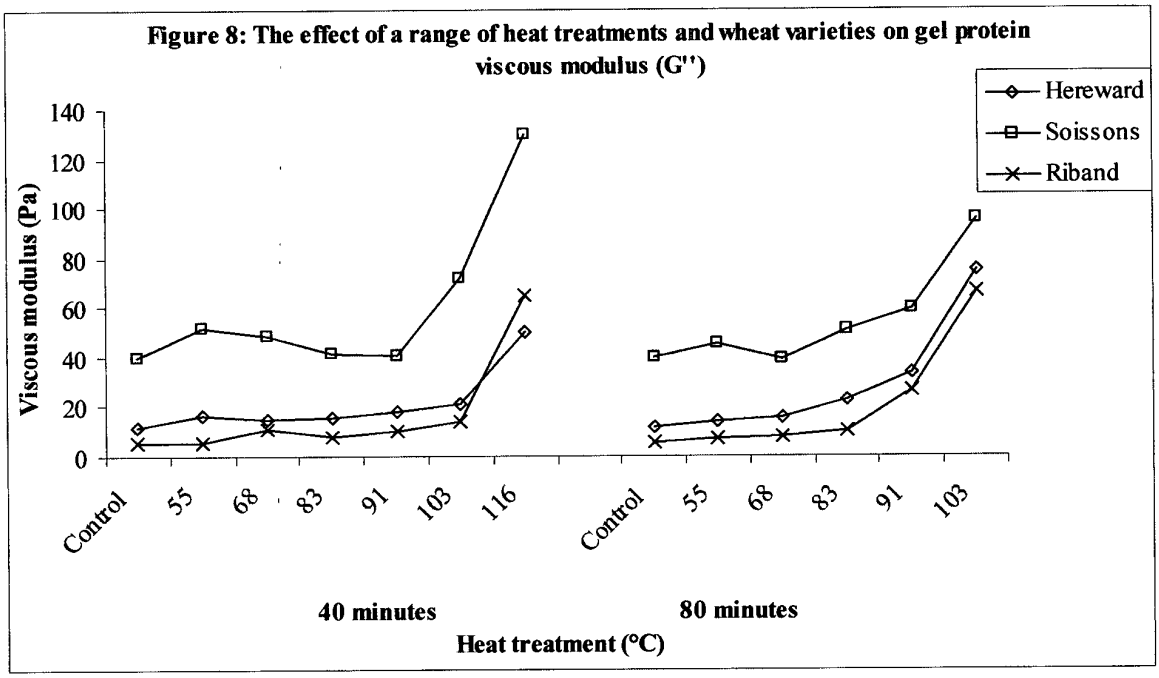


Figure 8 presents data for the viscous modulus of gel protein. A very consistent pattern is seen across all varieties with increases in G" occurring when samples were exposed to high temperatures (116°C for 40 minute treatment and 91°C for the longer time period). The 116°C for 80 minute treatment could not be measured as no gel protein was produced i.e. the SDS-insoluble protein was destroyed by this heat treatment.

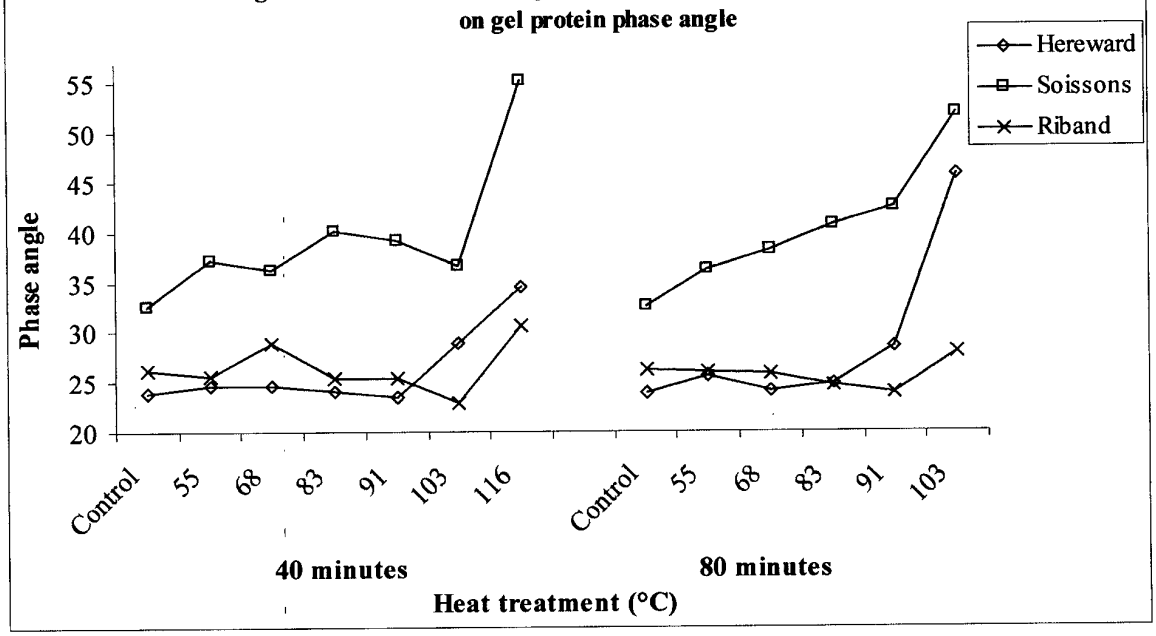


Again, prior knowledge of the varietal identity would be required to enable detection of this level of heat damage as a strong varietal effect was observed in the data. Significant reductions in CBP loaf volume were observed when heat treatments of 103°C or above were applied for 40 minutes or when 83°C or above was applied for 80 minutes. G" measurements failed to detect changes in SDS-insoluble protein at these

critical temperature and time combinations, suggesting that the method lacks the necessary sensitivity for use at mill intake.

As for the other rheological properties of gel protein, phase angle increased at the more extreme temperature/time combinations used in this study and a strong varietal influence was indicated (Figure 9).

Figure 9: The effect of a range of heat treatments and wheat varieties on gel protein phase angle



4.3 Discussion

During initial studies, the SDS-insoluble glutenin fraction appeared to be very sensitive to heat damage. Significantly lower gel protein weights were observed for severely heat treated Hereward and Hunter samples. In addition, preliminary rheological data for gel protein suggested that significant effects on the quality and fundamental rheology of this functional protein occurred under conditions of severe heat treatment. These two observations indicate denaturation of SDS-insoluble proteins under this extreme heat treatment and suggested this research technique may offer a route to heat damage detection that subsequently could be simplified for use in a mill intake situation.

When the gel protein methodology was used to examine a wider range of heat treated material, gel protein weight values were erratic and variety dependent. The elastic modulus values, G', which are a measure protein strength, suggest an increase in toughness, but this only reached significance for individual varieties when samples were exposed to high temperature and time combinations. Similarly, other measures of gel protein rheology (G" and δ) increased markedly when samples were exposed to high temperatures for a long time (80 minute treatment time). Strong varietal effects were indicated for all measures of gel protein rheology that would limit the use of this technique to severely damaged samples of known varieties where prior knowledge is held on typical results for a particular growing season.

Given these limitations plus the time required to perform a standard gel protein test (average 24 hours from supply of sample) and the expensive and sensitive equipment used, this test is not suitable for the evaluation of heat damage in commercial samples.

5 Durotest

5.1 Introduction and method

This is a commercially available immunoassay test kit that is used to verify the purity of durum wheat products by detecting any common (i.e. non-durum) wheat that may be present. A monoclonal antibody that is specific for the protein friabilin is used. This protein is present only in non-durum wheat. Since it was known that heat treatment of pasta during production influences the absolute values produced, it was considered that a preliminary screen of a slightly modified Durotest procedure may detect differences between sound and severely heat damaged samples.

Samples were extracted and adsorbed onto the surface of a microtitre plate overnight. The wells were washed, and the monoclonal antibody-labelled enzyme added, which binds to the protein friabilin. The wells were washed again and a clear coloured substrate added. A coloured product formed in the presence of a friabilin/antibody immune complex. The reaction was stopped with acid and the Absorbance of each sample read at a wavelength of 450nm. The test takes two days to carry out, but many samples may be tested in a single batch. Full details of the method used are provided in Appendix 16.

5.2 Results

All results from the heat treated and control samples gave Absorbance values of greater than 4.00 at 280nm, i.e. were off the scale used in this assay. Therefore, no differences between the heat treatments could be determined.

5.3 Discussion

This method was not suitable for the detection of heat damaged wheat samples.

6 Gluten quality

6.1 Introduction and methods

One of the standard ways, used by UK millers, to measure the rheological properties of dough is the Brabender Extensograph test. The commonly measured parameters are resistance to dough stretching and the extensibility or distance the dough can be stretched. Many flour specifications include Extensograph data and severe heat damage is known to influence the balance of resistance and extensibility (increasing the former and decreasing the latter). However, the test requires the production of a white flour (minimum quantity 1kg) and, for reasons of speed and size of sample required, cannot be used at mill intake. Recently a small-scale instrument has been developed by Stable Microsystems (the SMS Keiffer test rig) that can be used to measure the elasticity and extensibility of prepared dough or gluten without the need for small-scale white flour production.

Dough was prepared from white flour and sodium chloride solution; the starch was washed from the dough to leave a sample of wet gluten. Following removal of excess water, the gluten produced was weighed, placed into a Teflon dough form and shaped into several strips. A 40 minute resting period was required to permit the gluten to relax after "work hardening" that occurs during mixing and handling. A tensile test was carried out in which the centre of the strip of gluten was caught by a hook and lifted a pre-determined distance. During extension the gluten reaches its elastic limit (peak force) after which it breaks. At this point the distance was noted and used as an indication of gluten extensibility. A plot of the force measured over time was recorded for each sample.

Full details of the methods used are given in Appendices 18 and 19

6.2 Results

The automated method (Glutomatic) used to obtain gluten from samples of flour was in many cases unsuccessful due to flooding of the instrument. The samples of wet gluten that were recovered were subjected to testing of elasticity and extensibility.

Due to the lack of samples for comparison, it is not possible to draw many conclusions on the effect of heat treatment on gluten quality. Only a few of the mildly heat treated samples yielded gluten samples and, within these, little difference was observed in the values obtained for peak force or maximum distance for a particular variety. In summary, the maximum distance (extensibility) achieved was similar, i.e. this parameter showed little discriminative power. The maximum force (elasticity) was related to protein strength. For example, Soissons, the strongest breadmaking variety under test, was able to bear the greatest load before breaking, followed by Hereward.

6.3 Discussion

Problems with the gluten washing apparatus limited the amount of useful information that could be gathered from this test. A less automated system, e.g the Simon gluten washer, may have improved gluten extraction from some of the more seriously heat damaged grain. However, even the limited data available suggests that the test is unlikely to detect low levels of heat damage and is sensitive to variety. Severe heat damage does lead to reduced amounts of wet gluten and, in extreme cases, lack of a coherent gluten: therefore wet gluten measurements have a role to play in the detection of heat damage. However, the rheological measurements described here did not add to the ability to detect heat damage and the required 40 minute resting period made the test itself too slow for inclusion in a mill intake testing protocol.

7 Germograph (Easi-Twin) 2 pot

7.1 Introduction and methods

The reference method used to determine the amount of heat damage present in a sample of wheat used in this study is a standard germination test (see Appendix 11 for details of the method used). Whilst this test gives a reliable indication of grain viability, it is slow (taking 7 to 9 days depending on whether a heat- or cold-shock is required prior to germination). Alternative, standard grain viability tests, based on the use of tetrazolium salt solutions, have been used, but require a soaking period of 16 hours.

The Germograph Easi-Twin 2 apparatus was designed for three major purposes:

- germination testing of seeds using a saturated solution of tetrazolium salts for embryo staining to give an indication of their germination capacity;
- pre-germination using copper sulphate solution;
- acrospire profile testing on malt.

It is the first application that is of interest in the context of heat damage. The use of heat and vacuum were intended to significantly speed up the staining process and as a test period of 10-15 minutes was claimed, the instrument appeared to have potential for detecting heat damage quickly and simply.

Fifty grains were each split longitudinally to facilitate penetration of the solution into the embryo tissue. The apparatus required a warming up period to ensure that the tetrazolium salt solution was at 40°C before adding the split grains. A vacuum was applied to accelerate the process of tissue infiltration. After running for 10min, the apparatus was switched off and the grains were removed. Full details of the method used can be found in Appendix 17.

7.2 Results

Under test conditions, the living embryo region of sound and healthy grains was stained a deep crimson-red. Unfortunately, the embryo of the most severely heat damaged grains also took up some colour under the test conditions, staining pale pink. This effect complicated the picture, suggesting that it would be difficult to distinguish between controls and low levels of heat damage. No varietal effects were noted, i.e. similar results were observed for each of the varieties tested in the preliminary phase of this work.

7.3 Discussion

Although differences were seen between test samples, they were not distinct enough between the most extreme treatments (control and severely heat damaged). This was due to partial staining that was apparent in the most severely damaged samples. Without an objective measurement of the degree of staining of control samples, moderately damaged ones would be impossible to differentiate, thus making the test highly impractical. The preparation of the grains was time consuming and complex, even though the test itself was short. In summary, the Germograph Easi-Twin 2 was not found to be a suitable method for the reliable and objective detection of heat damage for the conditions used in this study.

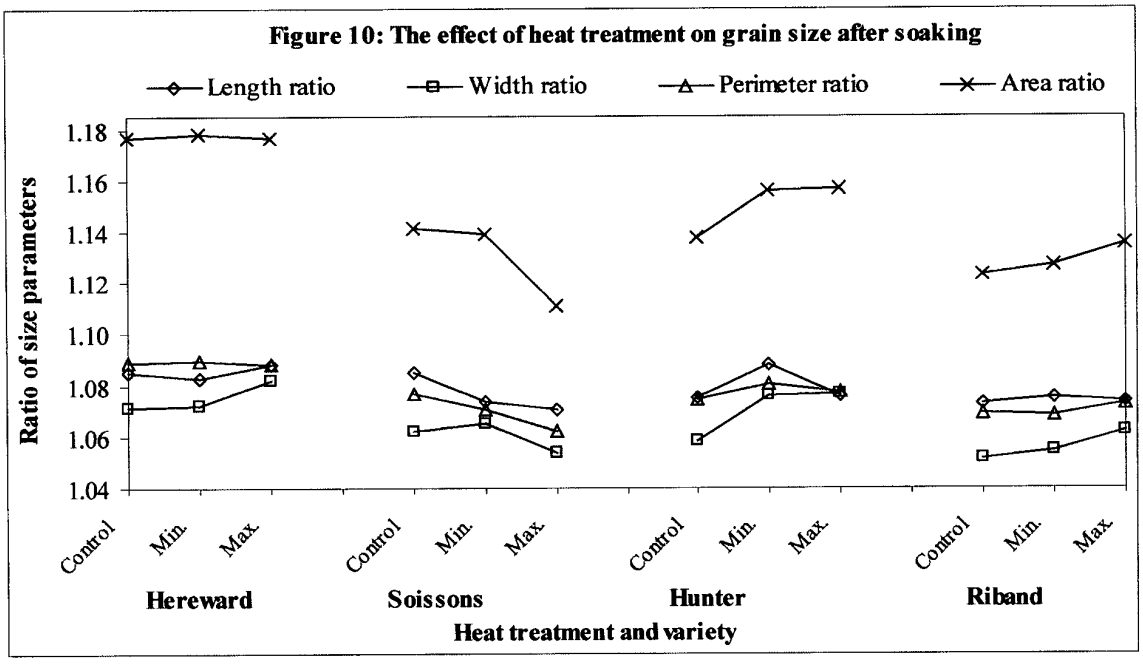
8 Image analysis

8.1 Introduction and method

Heat treatment may be expected to influence the water uptake properties of wheat grains. Water penetration is known to be affected by the initial moisture content of the grain and the permeability of the bran layers that influence the speed of water uptake. Water is absorbed quickly by empty pericarp cells providing a reservoir in the bran layers for transference into the endosperm. The testa contains impermeable components that restrict the rate of access of water. Most water actually gains access through the grain embryo, where overlying layers are thinnest, and advances through the endosperm towards the beard end of the grain (Kent & Evers, 1994). For these reasons an attempt was made to use image analysis to measure changes in size that resulted from soaking grain at elevated temperatures for a fixed period of time. It was expected that heat damaged grains would produce little or no increase in grain size within the test period.

Grains were spread out over a back-lit transparent background, a video camera and digitising hardware represented the differences in brightness between grains and background as higher and lower values in computer memory. Image analysis software identified values considered to represent grains and determined their number and arrangement. Grain dimensions were calculated by calibrating the scaling between actual distances and numbers of pixels on the computer screen. A representative 200 grains from each of the most extreme heat treatments and controls were measured by image analysis. Samples were soaked in distilled water at 40°C for 2h and 200 grains were measured again to investigate any differences in size induced by soaking. Full details of the method used are presented in Appendix 15.

Figure 10: The effect of heat treatment on grain size after soaking



8.2 Results

Results are expressed as the ratio of "before soaking" to "after soaking" for each size parameter measured and are plotted in Figure 10. In each case there was a marked increase in grain size after soaking, but the magnitude of this differed between varieties. Comparing the control sample with heat treated (Min. and Max. shown on the graphs) indicated that, over the four varieties tested, there was no consistent pattern of size change between samples that were subjected to different heating regimes. For the same parameter, both increases and decreases in ratio occurred between the control and most severely heat damaged samples.

8.3 Discussion

Differences in water uptake, as measured by changes in grain size, failed to provide a suitable means of differentiating between sound and severely heat damaged samples. The image analysis technique has been used routinely in other research (Whitworth, 1999) to measure differences in grain dimensions in a repeatable and effective manner. The simplicity of this test made it appealing, although the length of soaking time would have to be reduced for practical use had the test been shown to have potential as a means of detecting heat damage. Grain size is influenced by many factors including variety plus husbandry and climatic conditions during cultivation and thus the population of grain within a bulk sample may be expected to show significant natural variation. Undoubtedly, this contributed to the lack of differences seen between extremes of heat treatment, i.e. any difference generated by heat treatments were small compared with natural variations in grain size. Thus, measurement of grain size was not considered to be suitable for the detection of heat damage in wheat.

9 Mixograph

9.1 Introduction and method

As discussed under Section 6 of this paper, severe heat damage is known to influence the rheological characteristics of wheat flour dough, as measured by a range of empirical methods commonly used by millers such as Brabender Extensograph or Farinograph and Chopin Alveograph. All these tests require a minimum of 250g of white flour. Much research has been focussed on developing small-scale methods of measuring dough rheology.

The 2g Mixograph is one such development. A suitable instrument was available at the University of Reading and following a period of training, selected flour samples were tested. Due to limited availability of the instrument, tests were carried out on white flour from each variety for three heat treatments only (control, slightly heat damaged and the most severe treatment, i.e.116°C for 80 minutes).

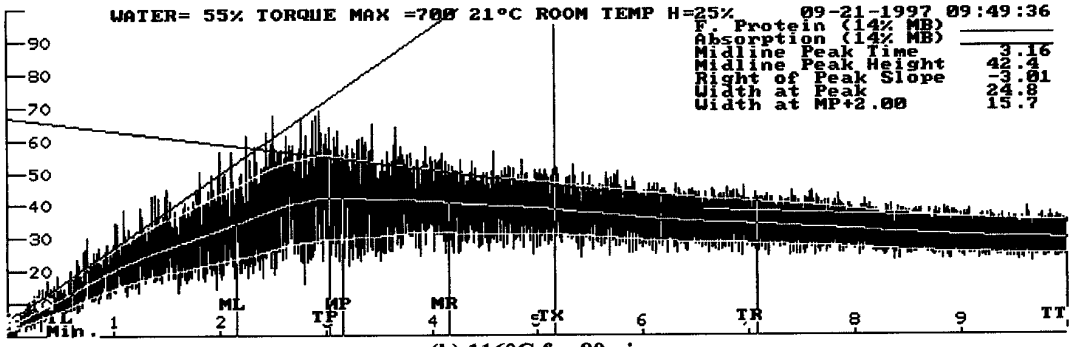
The instrument produces a mixing curve that represents the torque on the motor as a sample of flour is mixed with water to form a dough. For each sample tested, 2g of flour was weighed into the mixer bowl, an appropriate amount of distilled water (calculated in relation to the water absorption of the flour) was added (~ 1ml), and the bowl was secured in place. Mixing causes combination of the basic ingredients, followed by protein hydration to form gluten which under conditions of continued mixing will reach its optimum consistency or development. Continued gentle mixing, as carried out in a Mixograph, will start to break down the dough with consequent reduction in torque measurements. The Mixograph used automatically plotted a curve on a computer screen over the complete time course of 10 minutes. Full details of the test procedure are provided in Appendix 22.

9.2 Results

Mixograph traces, comparing control and the most extreme heat treatment (116°C for 80 minutes) for each of the four varieties are shown in Figures 11a&b for Hereward, Figures 12a&b for Soissons, Figures 13a&b for Hunter and Figures 14a&b for Riband. In each case, severe heat treatment resulted in a significant change in the mixing curve produced. Sound samples produced a peak which varied in its position (midline peak time) and height (midline peak height) according to the variety under test, whilst all the severely heat damaged samples showed a characteristically low and straight trace.

Figure 11: The effect of heat damage in Hereward on the 2g Mixograph

(a) Control



(b) 116°C for 80min

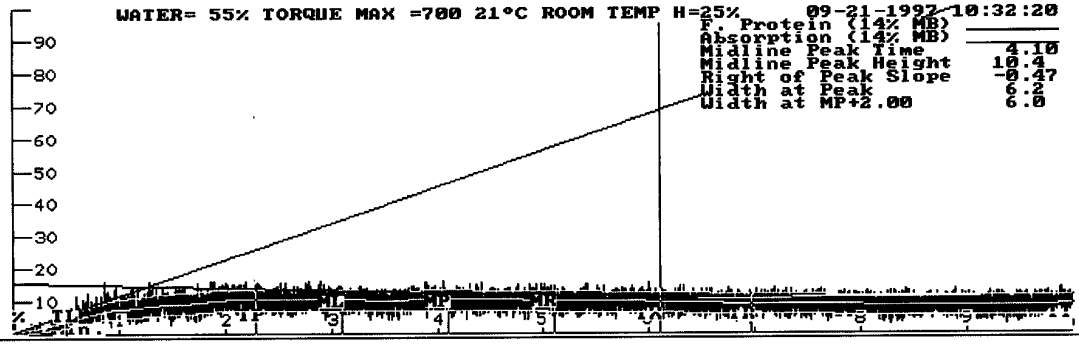
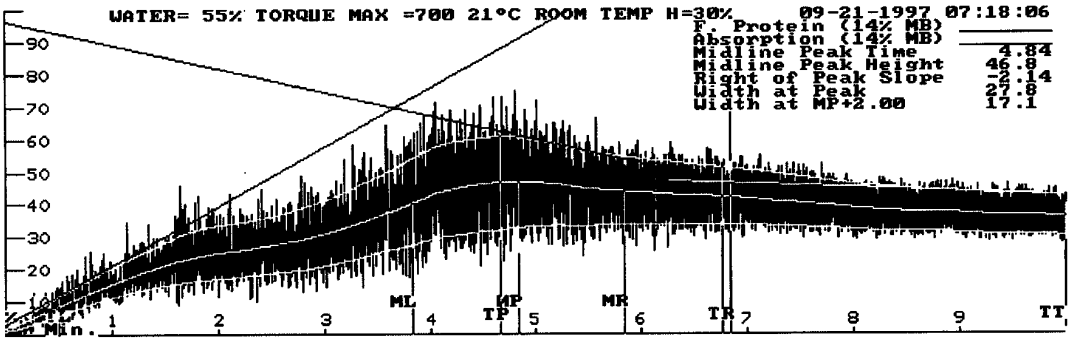


Figure 12: The effect of heat damage in Soissons on the 2g Mixograph

(a) Control



(b) 116°C for 80min

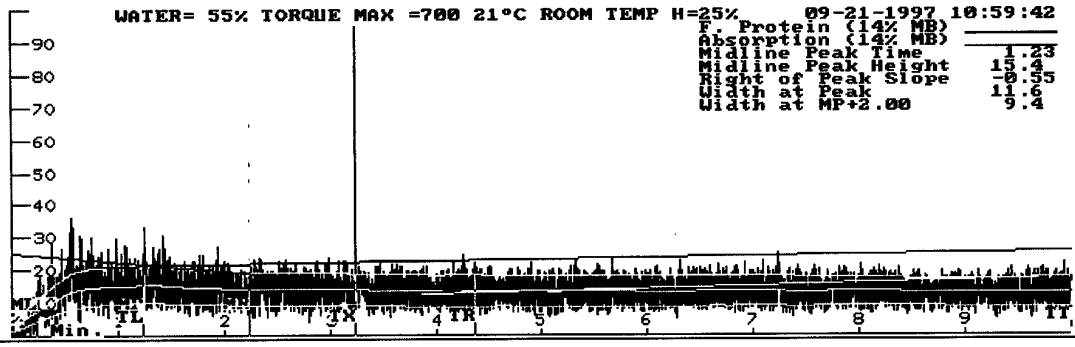
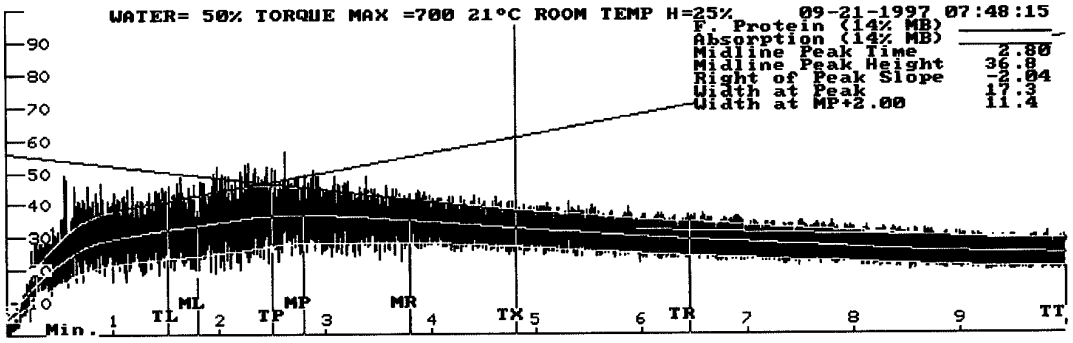


Figure 13: The effect of heat damage in Hunter on the 2g Mixograph

(a) Control



(b) 116°C for 80min

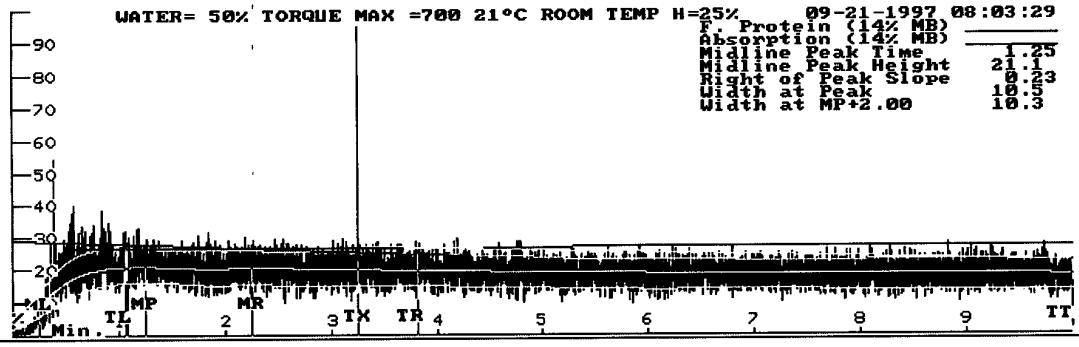
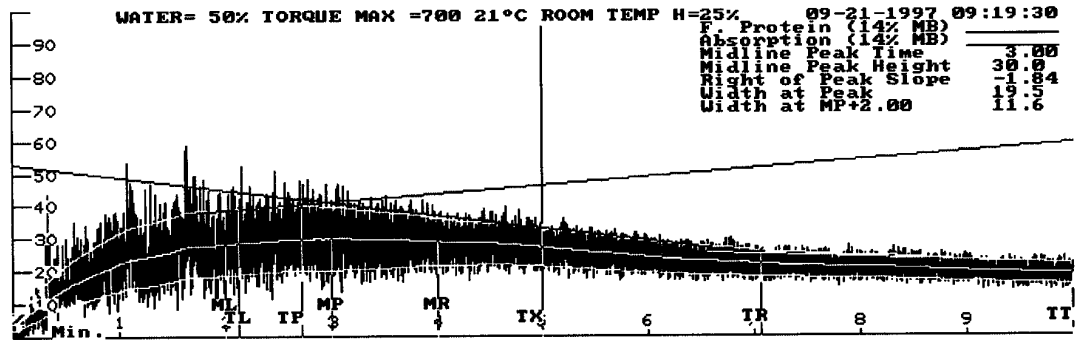
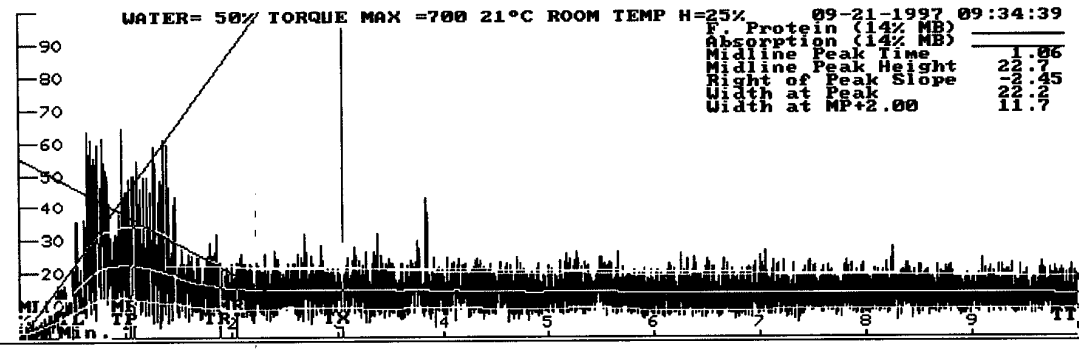


Figure 14: The effect of heat damage in Riband on the 2g Mixograph

(a) Control



(b) 116°C for 80min



The instrument tried to calculate a peak time for severely heat damaged samples, but this probably represents the incorporation of flour and water into a dry dough rather than true development of a cohesive dough. The temperature/time combination (116°C for 80 minutes) resulted in severe heat denaturation and the production of a very dry dough that was very short and inextensible. Thus, this test appeared to be capable of detecting severe heat damage very effectively. However, differences observed between the traces produced by sound samples of individual varieties indicated that prior knowledge of the variety plus typical Mixograph data for this variety would be required to enable decisions to be made regarding detection of low levels of heat damage.

When the lower heat treatment samples were examined by this technique (curves not shown here), small differences in trace data were observed but these were not consistent across the four varieties tested.

9.3 Discussion

The Mixograph was effective in measuring severe levels of heat damage. However, it would be of limited use for lower levels of heat damage for two main reasons: i) inconsistency in terms of the effect of slight heat damage on Mixograph traces and ii) typical results for each variety in a particular year would need to be available for comparison. The test could be carried out within an acceptable timescale, but uses white flour which is inconvenient and time consuming if the test was to be employed at mill intake. It may be possible to use sieved wholemeal flour, but this was not investigated as the presence of bran fragments is likely to add to the current variability in test results. In summary, this technique was not suitable for use at mill intake to detect heat damage levels that will have an impact on end-use quality.

10 Turbidity test

10.1 Introduction and method

Heat treatment of grain reduces the amount of heat-precipitable protein that can be extracted by saline solution from a flour or wholemeal produced from that grain. An estimate of heat precipitable proteins can be made by heating a saline extract in the presence of a protective colloid (gum arabic solution). The turbidity of the resulting solution has been shown to be inversely related to the extent of heat damage (Harrison *et al* 1969)

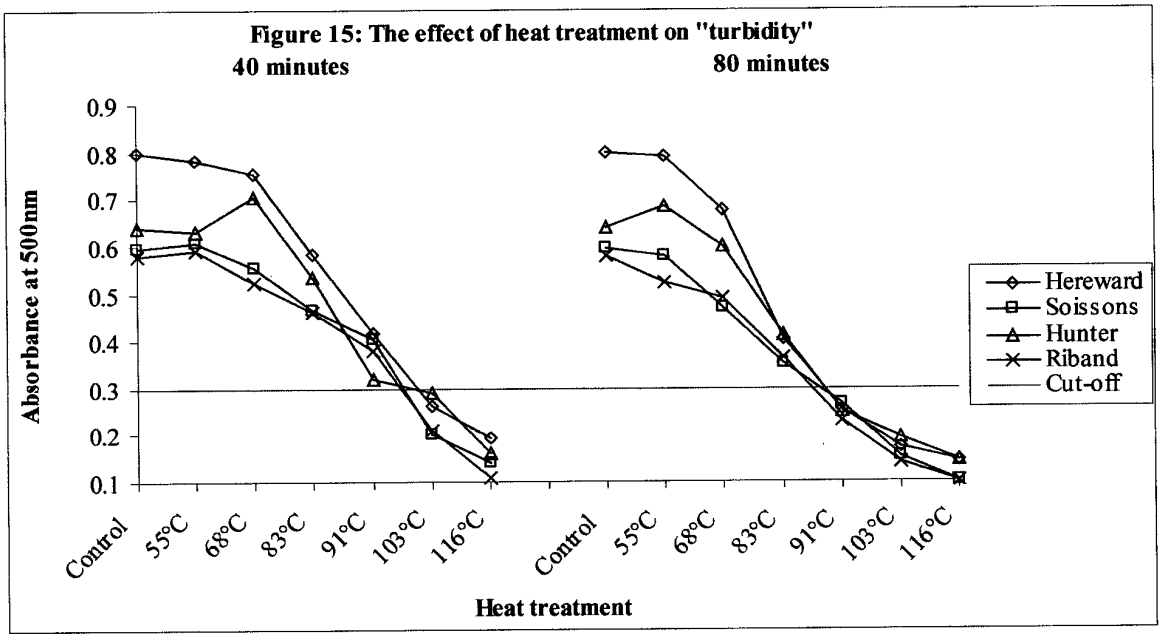
Initially, the original test method was evaluated in terms of performance. The following summarises the basic method used.

- Mill approximately 50g grain to produce wholemeal.
- Weigh 5.0g of wholemeal into a 300ml beaker.
- Add 100ml of 0.5% saline and stir vigorously for 3min.
- Filter the mixture, rejecting the first few ml of filtrate.
- Collect the next 10ml of filtrate in a boiling tube.
- Add 1ml of acaciae (gum arabic) solution and mix by shaking.
- Place the tube in a boiling water bath for 3min.
- Cool the tube in cold water.
- Read the Absorbance at 500nm (to mimic using a neutral disc filter on an absorptiometer)

Each stage of the test was carefully standardised to make it as reliable as possible.

10.2 Results

Initial results, using severely heat treated samples, were very encouraging as were those from the range of heat treatments (Figure 15). Whilst there was some indication of varietal differences within the sound wheat samples, the differences narrowed as heat damage became more serious. This led to a cut-off point of $0.3A_{500nm}$ being imposed, below which heat damage was certain to have occurred (see "Paper 1: Production and confirmation of heat damage" for results of germination tests).



10.3 Discussion

Following the success of initial screening of the method, the procedure was revised to improve accuracy and repeatability and full details of the final Turbidity test developed within this study are provided in Appendix 21. Further experimental work carried out to optimise the Turbidity test is given in "Paper 4: Optimisation of the Turbidity test."

11 Rapid Visco Analyser (RVA)

11.1 Introduction and methods

In addition to changes in the protein component of wheat flour, heat damage may also be expected to affect the pasting properties of starch. The RVA provides a convenient means of measuring starch paste viscosity, gelatinisation and "setback" on a small scale. Before beginning sample testing, a temperature regime must be selected, either from those used previously, or by programming a new one. For this work, an existing regime for wheat flour analysis and one described in the RVA literature for the detection of heat damage in gluten samples were used (Table 4).

Table 4: Temperature and time regimes used for analysis of heat treated samples

Programme 1		Programme 2	
Temperature (°C)	**Time (minutes)**	**Temperature (°C)**	**Time (minutes)**
50	1.0	50	2.0
95	4.8	85	6.5
95	7.3	85	8.5
50	11.1	20	16.0
End	15.4	End	20.4

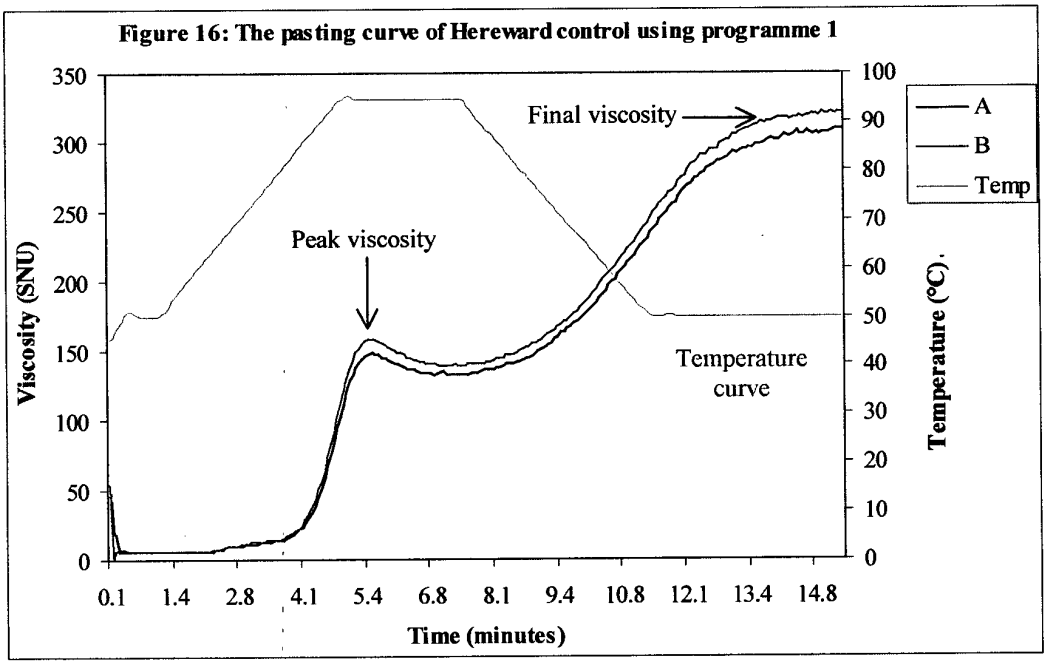
25g of distilled water was weighed into a metal canister, 4g of wholemeal added and a plastic paddle used to prepare a homogeneous slurry by moving through the mixture 10 times. The canister, with the paddle inside it, was then inserted into the instrument and the motor tower depressed to begin the heating cycle. During the test, the starch became gelatinised, resulting in a rise in viscosity. The paste was subjected to high temperature and controlled shear during which its stability was measured. A further

cooling cycle provided an indication of setback during gelation. Each sample was tested in duplicate. Full details of the method used are given in Appendix 23.

11.2 Results

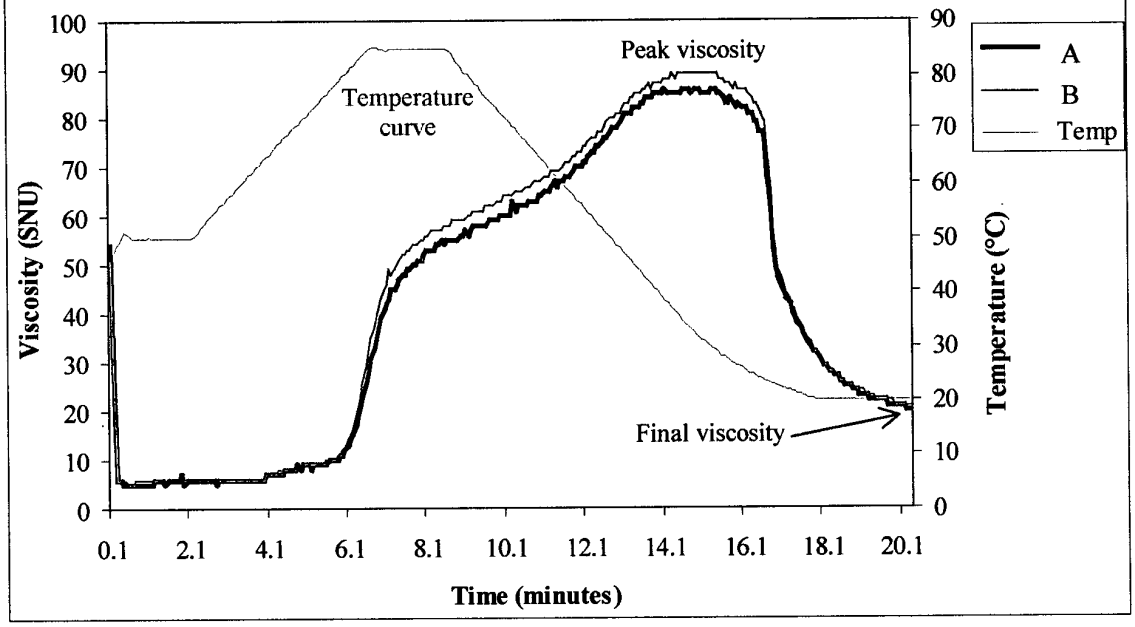
Curves all followed a similar overall shape, but at slightly higher or lower y-axis (viscosity) values. The different temperature and time regimes used gave rise to very different profiles. For Programme 1: as the temperature approached 95°C (right hand y-axis), a peak in viscosity occurred (left hand axis) due to gelatinisation of the starch in the paste. Paste viscosity further increased as the mixture cooled until it finally set (Figure 16).

Figure 16: The pasting curve of Hereward control using programme 1



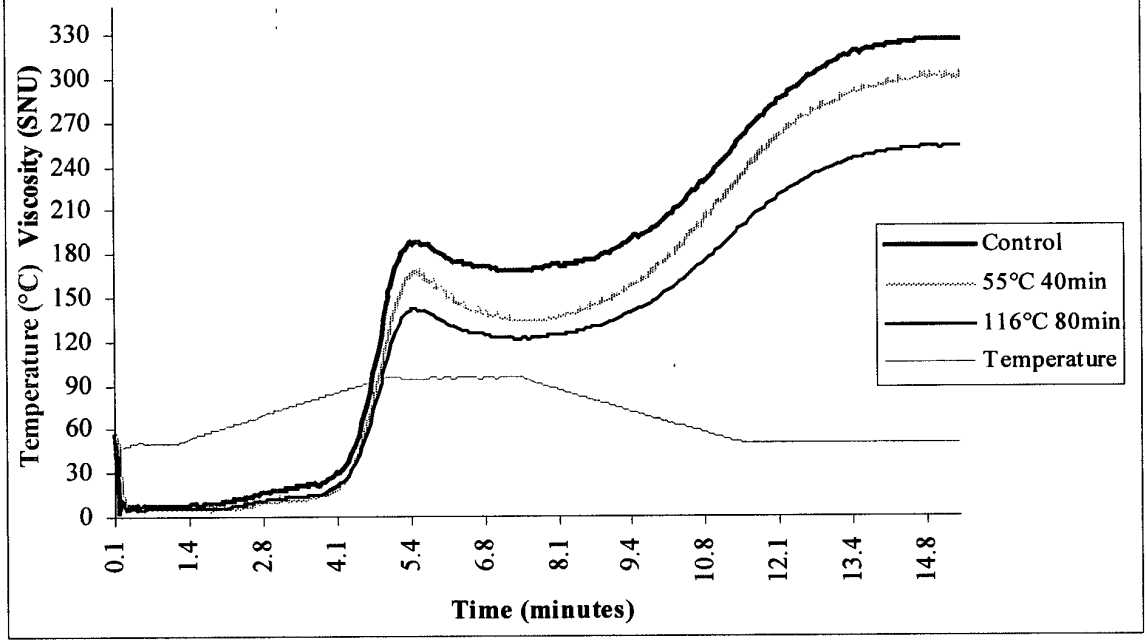
With Programme 2 (Figure 20), the flour mixture required a longer period of time at the lower peak temperature (85°C) before gelatinisation occurred. It appears from the subsequent decline in viscosity that true gelatinisation was not achieved, although the sample did thicken significantly. On subsequent cooling, the paste lost viscosity rather than forming an almost solid mass, as for the previous temperature programme.

Figure 17: The pasting curve of Hereward control using programme 2



Duplicate pasting curves for individual samples were in good agreement, indicating the repeatability of the test. However, comparison of controls and heat treated samples showed small and inconsistent differences which could not be relied upon to provide detection of heat damage.

Figure 18: The effect of heat treatment on pasting curves



11.3 Discussion

If more pronounced differences between treated and untreated samples had been observed, this test could have found a place at mill intake as it is quick, simple to use and highly automated. However,

pasting properties are also affected by *alpha*-amylase activity that liquefies starch, causing paste thinning, and thus works against any increase in paste viscosity resulting from heat damage. Whilst it may be possible to develop an RVA programme with temperature and time regimes that would differentiate between heat treatments, it is unlikely that such a procedure would be sufficiently sensitive to detect all but the most severe heat damage. For the above reason, this method was not considered to be suitable for the detection of heat damage.

CONCLUSIONS

A range of potential tests for heat damage have been screened, using undamaged and severely heat damaged wholemeal or white flour samples, for suitability of use in a mill intake situation. The performance of the most promising methods was further evaluated using a range of samples that had been exposed to differing levels of heat damage. Methods were assessed in terms of sensitivity to low levels of heat damage [on the basis of comparisons with the reference germination test], "ease of use" and insensitivity to variety. The Turbidity test was shown to be the best predictor of heat damage on this basis. Detailed studies, presented in "Paper 4: Optimisation of the Turbidity test", were therefore undertaken to fully evaluate the performance of this test and develop it as a mill intake procedure.

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INTRODUCTION

Since many methods for the detection of heat damage rely on changes in protein solubility and structure, wheat protein composition was investigated in detail in an attempt to identify a novel biochemical marker for heat damage. If such a marker were to be discovered this could lead to the development of a specific test kit for heat damage in wheat. Protein fractionation, followed by separation of protein entities by gel electrophoresis and capillary electrophoresis, was used to compare grain protein composition of severely heat damaged and control (untreated) wheat samples of the same variety. Visual and computerised analysis of the protein patterns produced was carried out to search for significant differences between damaged and undamaged samples.

MATERIALS AND METHODS

Total protein was extracted from wholemeal flour following the procedure of Laemmli (1970). Full details of the method used are presented in Appendix 7. Flour proteins were also fractionated according to a modified Osborne classification into three basic fractions:

- combined albumins and globulins (extracted with a dilute salt solution),
- followed by gliadins (extracted in alcohol),
- and lastly reduced glutenins [extracted with a solution containing the detergent sodium dodecyl sulphate (SDS) and a reducing agent such as dithiothreitol (DTT)].

Each fraction was examined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using the Bio-Rad Electrophoresis apparatus that separates protein mixtures into a series of discrete bands on the basis of molecular weight. When proteins are denatured with excess SDS, the detergent micelles bind to the polypeptides at a constant mass ratio and the polypeptide is organised into a rod-like structure. The bound SDS micelles each impose a net negative charge that effectively swamps the intrinsic charge of the polypeptide. The SDS-polypeptide complexes have, with few exceptions, the same charge/mass ratio. Electrophoretic migration is therefore logarithmically proportional to the molecular weight of the polypeptide chain. Samples are treated with SDS under reducing conditions, i.e. 1,4-dithiothreitol (DTT), resulting in cleavage of the disulphide bonds between and within proteins. In their native form unreduced, insoluble glutenin proteins would not enter the gel and therefore could not be fractionated.

The properties of the gel slab can be tailored to meet different separation requirements by manipulating the proportions of the ingredients used. By using gradient gels, a molecular sieving effect is achieved such that protein entities of high molecular weight become trapped in a specific region of the gel where the pores are too small to permit their passage. For further details of the method used see Appendix 9.

Table 1: Molecular weight standard mixture

Protein	Obtained from	Certified Molecular weight (Mw) in kDaltons
Myosin	Rabbit muscle	200
ß- Galactosidase	*E. coli*	116
Phosphorylase-b	Rabbit muscle	97.4
Transferin	Human	78
Bovine serum albumin	Cattle blood	67
Ovalbumin	Hen egg	45
Lactate dehydrogenase	Cattle heart	35.5
Carbonic anhydrase	Cattle erythrocyte	29.5
Trypsin inhibitor	Soy bean	21.5
Myoglobin	Sperm whale	17.2
Cytochrome C	Horse heart	12.3
Trypsin inhibitor	Cattle pancreas	6.2

Optimum sample loading was found to be approximately 25mg protein per well for the Bio-Rad gels. In each case, two extracts were applied to each gel as follows: (i) a fixed sample weight/ml of solution and

(ii) weight of sample adjusted to compensate for differences in protein content as measured by a Lowry protein assay (Lowry method, Appendix 8). This combination provides the opportunity to detect differences in extractability in the first gel and differences in the proportions of individual proteins within a fraction in the second. All illustrations included in this paper are of the latter type of gel. Each gel included a track containing a mixture of molecular weight standards (Table 1).

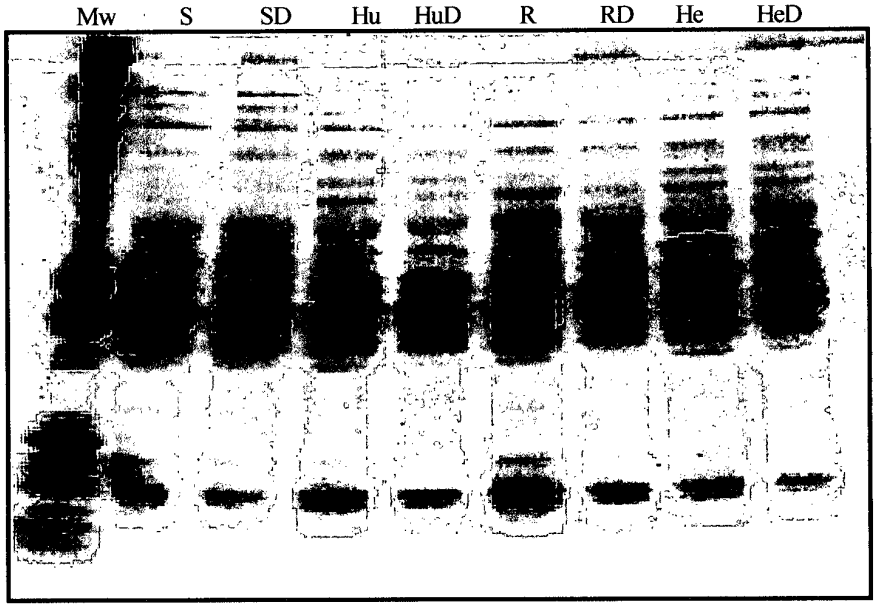
Images of the final gels produced were captured using a video camera and analysed using proprietary software (UVP Phoretix) to quantify electrophoretic band patterns in terms of band position and intensity. Details of the method used are given in Appendix 13.

RESULTS

Total protein extracts

Results obtained from SDS-PAGE separation of total protein extracts on Bio-Rad gradient gels (7.5-25%) for 80 minutes at 150 volts are shown in Figure 1. 20μg of protein was loaded for all extracts, according to the results of Lowry assays. Total protein content altered between varieties, as would be expected from their different genetic backgrounds. For each varietal pair, the electrophoretic pattern produced is identical in terms of band position. Differences in both the intensity of individual bands and the intensity of the background staining between the control and heat-damaged sample of each variety are evident, i.e. severely heat damaged grain produced more background staining and reduced levels of some of the faster moving proteins (Mw <30kd). However, there is no evidence of the complete disappearance of any band within the electrophoretic pattern for any variety.

Figure 1: Total protein extracts of control and heat damaged (D) samples of the varieties Soissons (S), Hunter (Hu), Riband (R) and Hereward (He). (Sample volume was adjusted to compensate for differences in protein content)



Mw = Molecular weight markers

The samples under examination were extremes of heat damage, i.e. untreated and severely heat damaged, and therefore complete elimination of a band or bands would be necessary to enable the detection of lower levels of heat damage.

Total protein extracts provide an opportunity to overview the effect of heat damage on total wheat protein composition. Due to the size limitations of the separating gel, many bands are not well resolved and it would therefore be difficult to detect small differences in band pattern from total protein extract separation.

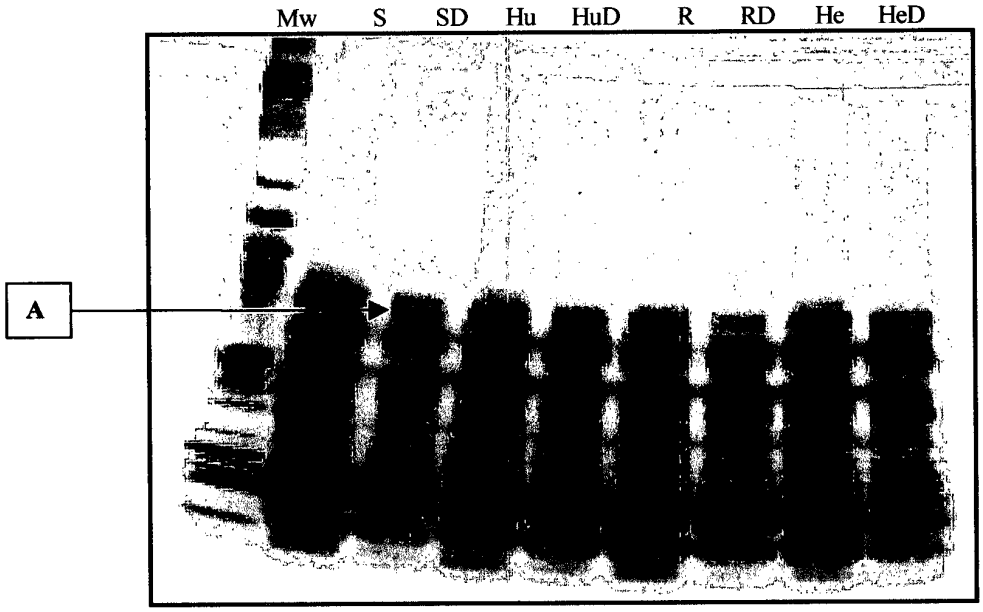
However, it appears that heat treatment produces a shift in molecular size of the extractable proteins rather than total removal of a particular band or bands.

Combined albumin/ globulin protein fraction

Protein fractionation followed by electrophoretic separation provides an opportunity to examine the effect of heat damage on the various wheat protein classes in some detail.

The albumin/globulin fraction extracted with dilute salt solution from control and heat damaged wheat was studied using SDS-PAGE and a separation time of 80 minutes.

Figure 2: Extracted albumin/globulin proteins from control and heat damaged (D) samples of the varieties Soissons (S), Hunter (Hu), Riband (R) and Hereward (He). (Sample volume was adjusted to compensate for differences in protein content).

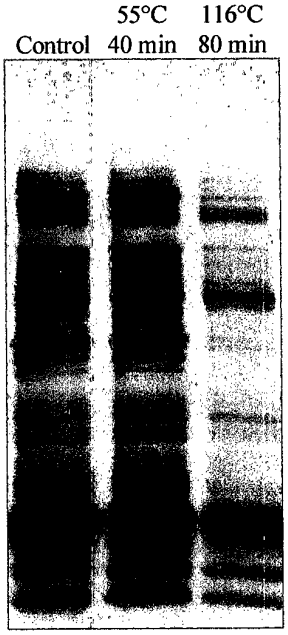


Mw = Molecular weight markers

For all varieties, reduced band intensity was observed across the entire band pattern when severely heat damaged samples were compared with the equivalent control samples. In some varieties, e.g. Soissons, there was an indication of changes in some of the slower-moving bands (region marked A), but this did not appear to be consistent across all varieties and therefore would probably not form the basis of a marker for heat damage.

In Figure 3 the effect of selected heat treatments (control, 55°C for 40 minutes and 116°C for 80 minutes) on the albumin/globulin protein fraction of Hereward wheat samples is presented. Whilst the individual tracks suggest that the most severe heat treatment produced a slightly different band pattern, the gel scans confirm that only a slight change in the arrangement of bands in the slowest moving region has occurred. In all other areas, the basic pattern is identical to the control and once again there has merely been a significant shift in band intensity. This pattern difference is consistent across the other three varieties. However, the change in band arrangement is very small. Total absence of a particular band would be needed under these conditions of severe heat treatment to enable development of a specific marker that could detect low levels of heat damage.

Figure 3: Comparison of albumin/globulin protein extracts of Hereward from control and selected heat treatments.



Gliadin protein fraction

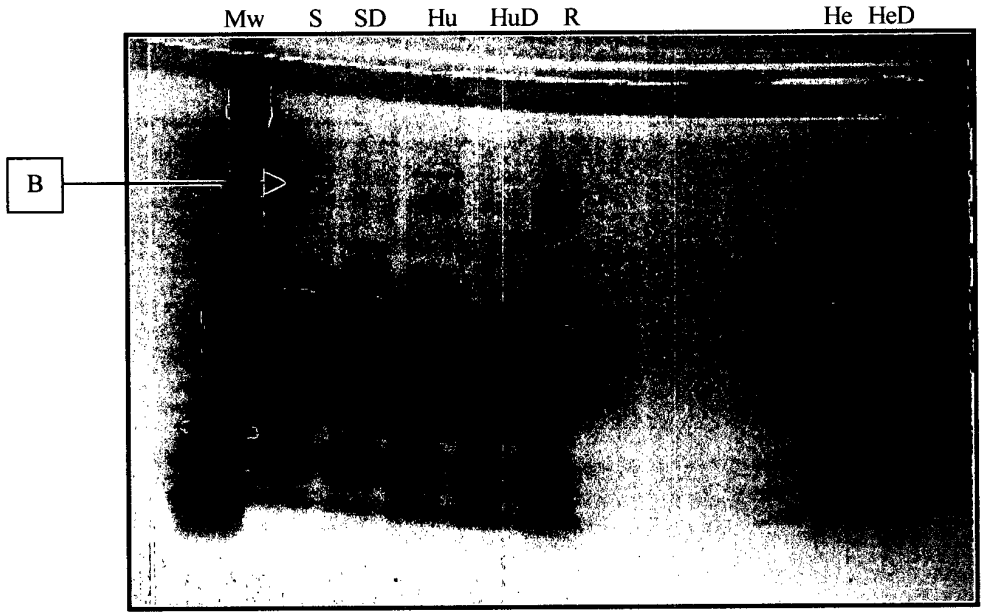
Following salt extraction to remove the albumin/globulin fraction, the remaining material was extracted with propan-2-ol. Analysis of the gliadin fraction was very difficult as bands tended to be rather distorted.

This may be a feature of the high alcohol content of the samples that made them difficult to load effectively onto the SDS-PAGE gel. For liquid extracts, it is recommended that double strength Laemmli buffer (100µl of extract were added to 100 µl of double strength Laemmli buffer) is used but this failed to completely solve the issue of pattern distortion which was observed in all SDS-PAGE gels for separation of the gliadin fraction. In fact, the Riband heat damaged sample could not be loaded due high alcohol content and therefore 4 lanes on the gel shown in Figure 4 are blank. A running time of 75 minutes was used for separation in this case.

There was a suggestion that some of the slower-moving material in the gliadin fraction disappeared when the sample was severely heat damaged (see region marked B, just below 97.4kd in molecular weight). The loss of gliadins (~100kd) in heat damaged samples has been documented, but in all gels examined, bands in this region were very faint for sound wheat and it was difficult to decide whether total disappearance of this band had occurred. The effect seemed most pronounced in Soissons.

Several attempts were made to run gliadin extracts on SDS-PAGE gels, but it was not possible to produce a consistent or clear pattern in this slow-moving region. (Acid-PAGE gels of gliadins extracted with 1M urea were also attempted, but not shown here. Under these conditions, the gliadin proteins could be separated more clearly into discrete, straight bands. However, there was no apparent difference in the slow-moving region of the gel, suggesting that differences in extraction may result in differential removal of some of the low-molecular-weight glutenins in the sequential extraction procedure that would not be separated on the acid-PAGE gel. In fact, heat damaged samples produced lower intensity across the entire band pattern, but no evidence of total disappearance of any band or groups of bands.)

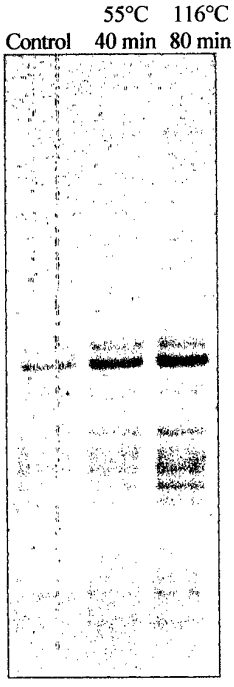
Figure 4: Extracted gliadin proteins from control and heat damaged (D) samples of the varieties Soissons (S), Hunter (Hu) , Riband (R) and Hereward (He). (Sample volume was adjusted to compensate for differences in protein content).



Mw = Molecular weight markers

In Figure 5 the effect of selected heat treatments (control, 55°C for 40 minutes and 116°C for 80 minutes) on the gliadin protein fraction of Hereward wheat samples is presented. In summary, no consistent differences could be detected between the control and heat treated samples and therefore this apparently promising approach was abandoned.

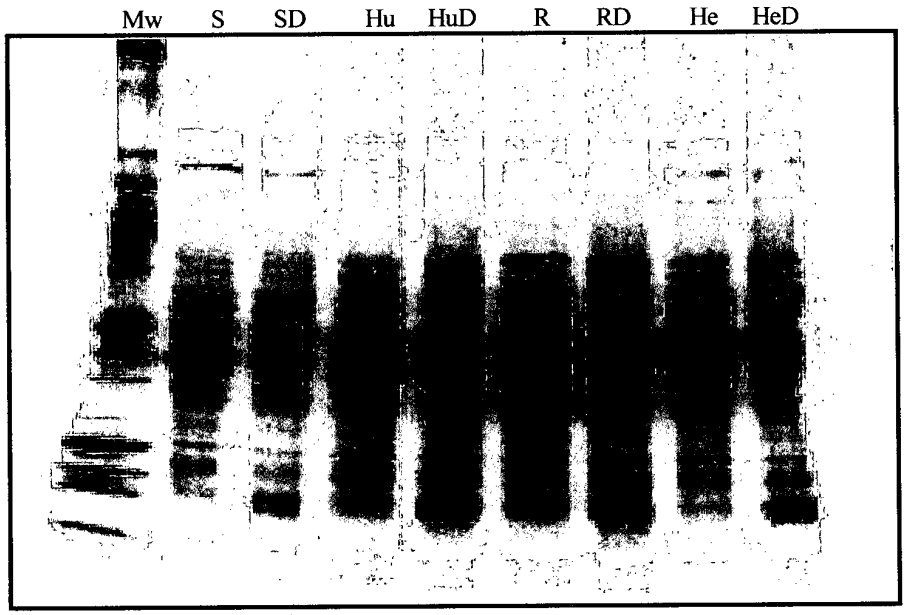
Figure 5: Comparison of gliadin protein extracts of Hereward from control and selected heat treatments.



Glutenin protein fraction

Following sequential extraction with salt and alcohol, the material remaining in the centrifuge pellet was extracted using SDS plus the reducing agent dithiothreitol. As for the gliadin fraction, double strength Laemmli buffer was added to the prepared extract. A running time of 80 minutes was used.

Figure 6: Extracted glutenin proteins from control and heat damaged (D) samples of the varieties Soissons (S), Hunter (Hu) and Hereward (He). (Sample volume was adjusted to compensate for differences in protein content).



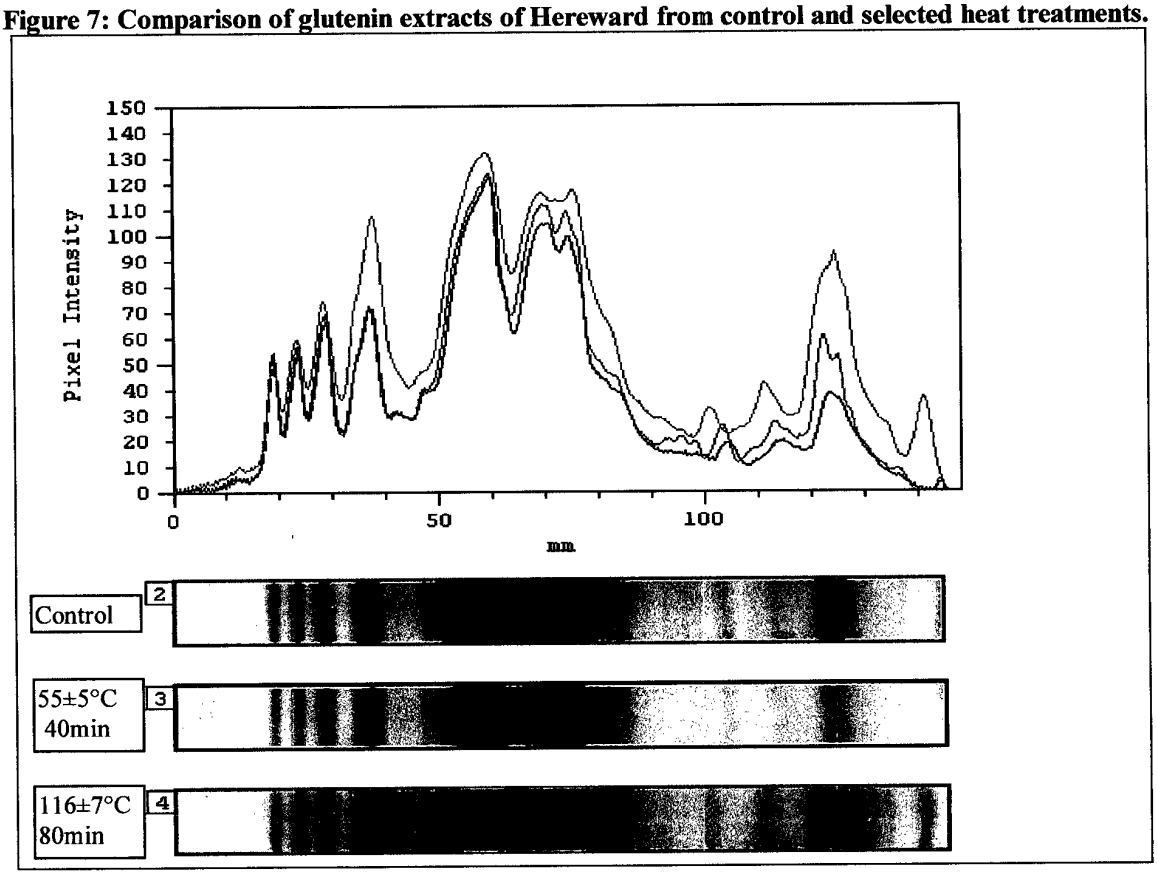
Mw = Molecular weight markers

Band intensity for low-molecular-weight glutenins (10-20 kD) increased in heat damaged samples of all varieties. In addition, high-molecular-weight components (>50 kD) were found to increase with heat damage across all varieties. There was no evidence of the presence of new bands in severely heat damaged grain or the absence of bands present in sound grain.

Densitometry confirmed that the basic band pattern remains the same and heat damage merely causes a change in intensity. Separation of glutenin protein extracts (Figure 7) shows individual gel tracks and results of densitometric scans of total protein extracts from three Hereward treatments (control, 55°C for 40 minutes and 116°C for 80 minutes). The patterns for the control and 55°C treatment were virtually super-imposable with a suggestion of reduced intensity in the faster-moving, low molecular weight material. Background staining was also similar in the control and mild heat treatment sample. This heat treatment also had a negligible effect on the germination capacity of the sample (see "Paper 1: Production and confirmation of heat damage" for details).

However, when the more extreme treatment of 116°C for 80 minutes that produced significant heat damage and reduction in germination capacity was examined, there appeared to be an upward shift in terms of band intensity in all but the slowest moving region of the pattern. Background staining of the gel was also significantly increased. Increases in band intensity were most pronounced in the fastest moving region of the pattern. This pattern was repeated for this treatment in all four varieties.

Figure 7: Comparison of glutenin extracts of Hereward from control and selected heat treatments.



Capillary electrophoresis (CE)

This method employs a similar principle to that of PAGE, except that separation takes place in a capillary, i.e. charged entities are separated in an electrical field. Sample molecules are introduced hydrodynamically (utilising pressure differences) or electrokinetically (using electrical pull to draw in the sample). Separation is based on the charge to mass ratios of the constituent molecules, with the most positively charged species migrating fastest.

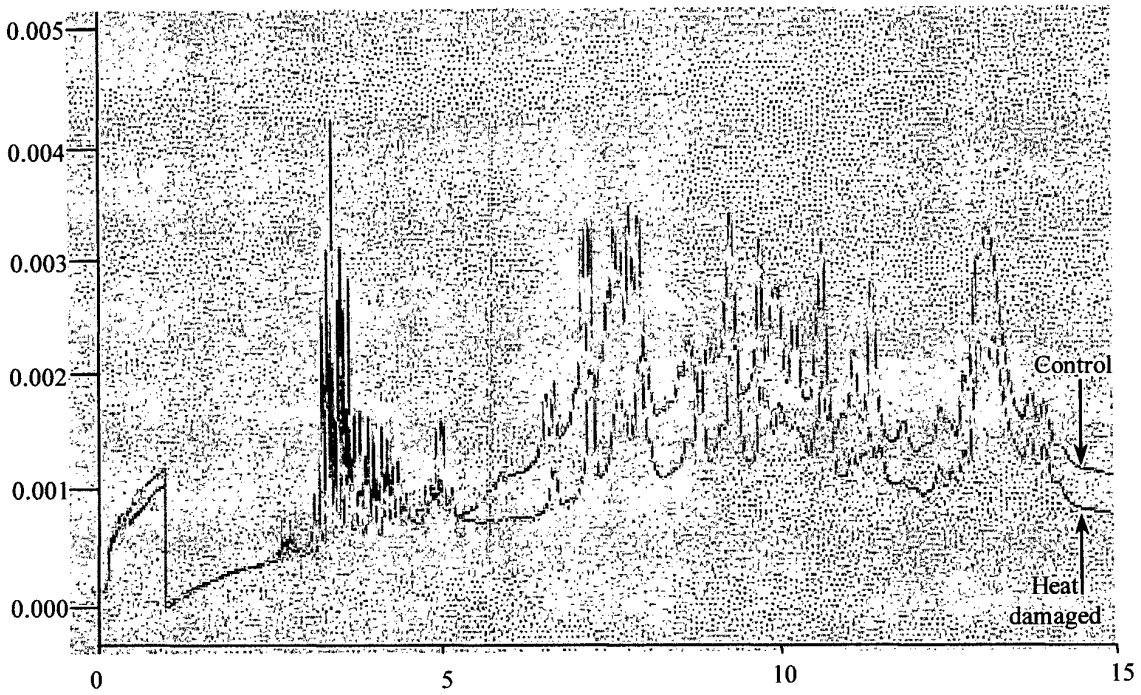
Once a sample is injected, the end of the capillary is removed from the sample reservoir and immersed in the running buffer. As the sample migrates through the capillary, an in-line detector monitors the presence or absence of migrating constituents. In an automated capillary electrophoresis system, the detector is built-in, which eliminates the need for connections to external monitors and prevents the re-mixing of closely migrating bands.

A chart, consisting of a series of peaks and troughs, is recorded which represents the separation of the sample into a series of constituents.

Each sample was prepared immediately before testing by adding ethanol to ground grain in a centrifuge tube, mixing thoroughly, centrifuging and filtering. Each filtered supernatant was then placed into a plastic sample vial and tested. Further details of the method used are provided in Appendix 14

Previous experience of capillary electrophoresis has suggested that due to the different manner of separation, this technique may be capable of identifying additional components not readily detected using a PAGE system.

Figure 8: Capillary electrophoretic separation of wheat proteins for control and heat damaged Hereward



A typical comparison between the control and severely heat damaged Hereward is shown in Figure 8. As expected, many peaks are visible on the CE-trace and resolution in the control sample is significantly better than in the heat damaged sample. For example, a series of 5 peaks are resolved at about 7-7.5 minutes for sound grain whereas only two are separated for heat damaged. In addition, Absorbance values or intensity is higher across all peaks in the region 5-15 minutes for sound grain than for heat damaged. This pattern of changes due to heat damage of proteins was reasonably consistent across the four varieties tested. Significant differences were observed between varieties, indicating the potential of the technique as a means of identifying individual wheat varieties, but the very large number of components detected and differences in baseline made traces difficult to interpret.

In summary, gel capillary electrophoresis results indicated a shift in the amount of protein extracted using different solvents, i.e. changes in protein solubility rather than the removal or appearance of a new protein entity.

The Lowry assay, details of which are provided in Appendix 8, was used to measure the protein content of individual fractions. Results presented in Table 2 indicate that, for the four varieties studied, more protein was measured in the heat damaged samples compared with the sound controls of the same variety. This data confirms findings from gel and capillary electrophoresis studies that heat damage causes a shift in extractable protein. There was a suggestion that the magnitude of the increase was dependent on variety, but results are based on limited data.

The increase in protein content is expressed as:

$$\frac{\text{The difference between protein content of sound wheat and heat damaged wheat}}{\text{Protein content of sound wheat}}$$

Within the individual protein fractions, albumins/globulins plus gliadins were consistently decreased by heat damage whilst the glutenin fraction was very significantly increased. Of the three fractions

investigated, the glutenin fraction appeared most increased by heat damage. As shown in Table 2, changes within the individual fractions were relatively consistent over all four varieties tested and were confirmed by repeat assays on a different set of extracts.

Table 2: Change in protein content, as measured by the Lowry assay, between control and severely heat damaged samples.

	Total proteins - Laemmli	Albumins and globulins	gliadins	glutenins	Total proteins in DTT/ SDS
Hereward	+22.1%	-25.30%	-39.5%	+ 75.1%	+2.2%
Soissons	+22.3%	-26.6%	-37.5%	+47.2%	+5.2%
Hunter	+30.8%	-15.4%	-27%	+69.7%	+0.03%
Riband	+28.8%	-24.6%	-46.6%	+120%	+0.84%

DISCUSSION

The search for a novel marker for heat damage failed to identify a single entity that could be used as the basis of a test kit.

Proteins from heat damaged wheats were studied using Osborne fractionation and gel filtration by Preston *et al.* (1989) who found that the insoluble glutenin protein fraction was the most sensitive to heat. Current protein fractionation and separation studies confirm this. Studies by Schofield *et al.* (1983) indicated that heat reduced mainly the amount of gluten proteins and high molecular weight glutenins extracted in SDS buffer. Heating provokes the unfolding of glutenin polypeptides that facilitates SH/S-S interchange between exposed S-S and SH groups in adjacent molecules. The level of total available sulphydryl (SH) groups remains constant up to 100°C but there is a transfer of SH groups from an SDS-extractable to SDS-unextractable form.

This confirmed our focus on the characteristics of wheat protein, and particularly protein solubility, under conditions known to cause heat damage in "Paper 2: Evaluation of methods to detect heat damage".

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INTRODUCTION

Heat treatment of grain reduces the amount of heat-precipitable protein that can be extracted by saline from milled white flour or wholemeal produced from that grain. An estimate of heat precipitable proteins can be obtained by heating a saline extract in the presence of a protective colloid (gum arabic solution). Previous research (Harrison *et. al.*, 1969) has shown that the turbidity of the resulting solution is inversely related to the extent of heat damage. Earlier studies by the current authors had indicated that a simple Turbidity test provided a means of separating severely heat damaged from sound grain and encompassed the elements of speed and "ease of use" required in a mill intake or grain handling and storage situation. The existing test used an expensive spectrophotometer that would make the method more applicable to a fully equipped laboratory; therefore the use of a low-cost fixed filter colorimeter was investigated. In addition, further work was required to investigate the sensitivity of the method to low levels of heat damage.

MATERIALS & METHODS

Grain samples

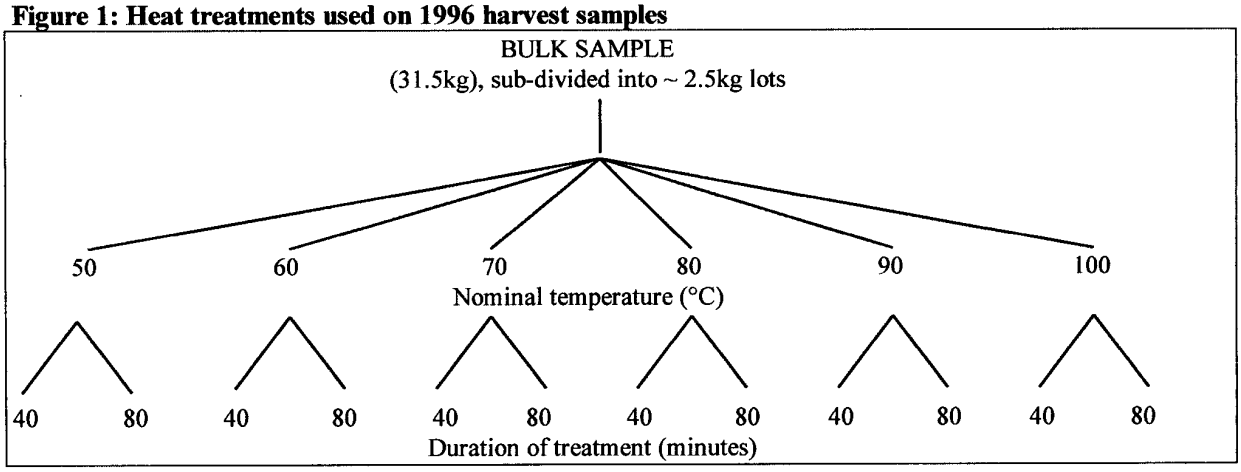
Commercial grain samples were selected to represent hard, breadmaking wheats in **nabim** Groups 1 and 2 (Hereward and Soissons) plus examples of soft endosperm textured, biscuit and feed wheats (Riband and Hunter) as found in **nabim** Groups 3 and 4 (**nabim**, 1997). Typical intake measures of specific weight, grain protein (Kjeldahl, N x 5.7) at 14% moisture, Falling Number, wet gluten, and SDS sedimentation volume were carried out according to standard methods (Salmon, 1997). These ensured that the samples were sound, i.e. acceptable in terms of *alpha*-amylase levels, had not suffered from heat damage and had no major defects that would cause rejection for flour milling.

Further samples of Soissons, Hereward and Riband were selected from the 1997 harvest to confirm the sensitivity of the Turbidity test and to examine safe drying temperatures. See "Paper 1: Production and confirmation of heat damage" for basic quality attributes of the samples used in both seasons.

Heat treatment

In order to produce samples that are more typical of commercial experience and to challenge the Turbidity test in terms of sensitivity to heat damage, a range of damage was induced by subjecting the above four wheat samples to different temperatures for different times (Figure 1). Nominal temperatures are those set on the dryer, the actual temperature during each drying regime was recorded and is used in all subsequent results. Since heat damage is caused by a combination of initial sample moisture content, temperature and the length of time of exposure to raised temperature, bulk wheat samples were conditioned to 25.0% moisture content in two stages on consecutive days prior to heat treatment.

Figure 1: Heat treatments used on 1996 harvest samples

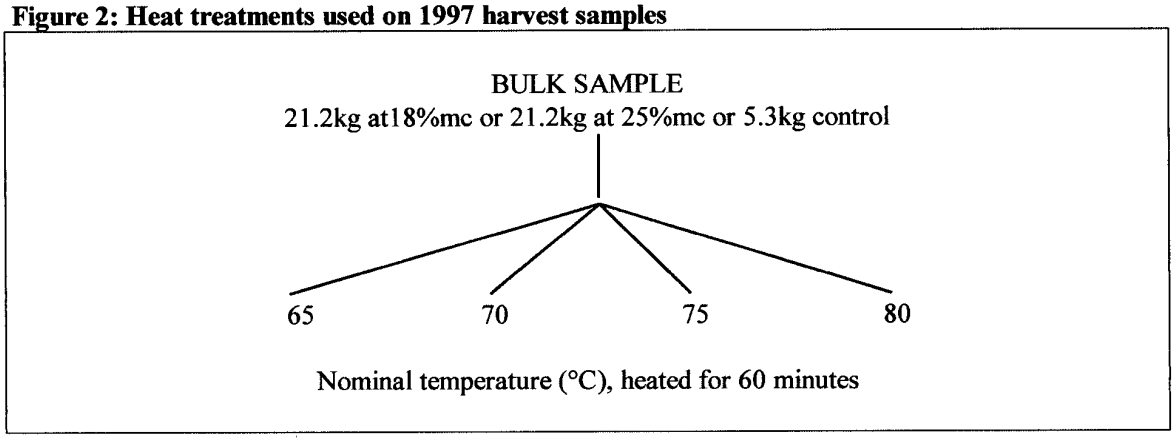


As both 40 and 80-minute heat treatments were effective in producing different levels of heat damage, it was decided to use a single holding time, but to study more than one initial moisture level. For confirmatory work on samples ex 1997 harvest, heat damage was induced by subjecting wheat samples

to different temperatures for a period of an hour. One hour was chosen after consideration of the results obtained from the previous studies (Figure 1).

Heat treatment of samples is summarised in Figure 2 below. Control samples did not receive any conditioning or heat treatment.

Figure 2: Heat treatments used on 1997 harvest samples



Turbidity test

The original method used wholemeal flour, ground to pass through a 0.8mm sieve using a standard laboratory mill. Mixing was carried out using a magnetic stirrer, with variable speed and reverse action options. After some experimentation, mixing using a relatively low speed and reverse action approximately every 3s was found to completely mix the flour and saline solution and produce the most repeatable results. Initially, Absorbance at 500nm (an arbitrary wavelength for measuring turbidity) was measured, but subsequently Absorbance at 540nm was adopted as this wavelength is used for other measurements in a cereals testing laboratory, which removed the necessity to re-set the spectrophotometer and hence a potential source of error.

Experiments to optimise the method for use in a mill intake laboratory included:

- Effect of flour type on Turbidity test results
- Use of a low cost, fixed filter colorimeter

In an attempt to reduce the cost of setting up the test, a low-cost, fixed filter colorimeter (CO75 obtainable from Walden Precision Apparatus Ltd, Cambridge) was used in place of the expensive variable wavelength spectrophotometer used in earlier studies. This instrument is supplied with eight fixed wavelength filters (typical bandpass 40nm) to cover the range 400 to 700nm. The 520nm filter was chosen as this was closest to the previously used wavelength of 540nm. A liquid dispenser, capable of delivering 100ml aliquots, was also used to increase the speed and accuracy of saline solution delivery.

Germination test

Given appropriate conditions of moisture, temperature and time, all healthy (undamaged) grains should germinate. Thus, the germination test is used as a reference method for heat damage and comparisons are made with this to measure the effectiveness of rapid tests such as the Turbidity test.

From each sample, four representative sub-samples of 100 grains were selected. Each was spread evenly over a folded paper towel and secured before the addition of ~ 15ml of distilled water. Trays of samples were sealed in a large plastic bag and placed in a refrigerator at 4°C for 40h. Following this pre-germination cold-shock, samples were moved to a temperature controlled room at 21±5°C for a further seven days. Germination was assessed visually by classifying individual grains as: normal, abnormal or no growth and expressed as % germination.

Wet gluten weight

Using a standard test protocol [2], a dough is produced from a fixed amount of flour or ground wheat and a buffered solution of sodium chloride. Wet gluten is isolated by washing this dough with buffered sodium chloride solution to remove the starch and water-soluble proteins. After removal of the excess water, the wet gluten is weighed. Sound wheat samples normally produce a certain wet gluten level. As a rough rule of thumb more than 2.7 times the protein content of the sample is expected in terms of wet gluten weight. Significant failure to obtain this level of gluten from a fixed amount of sample indicates that there is something wrong with the sample e.g. heat or insect damage. Subjective assessment of the rheological properties of the prepared wet gluten from a heat damaged sample would confirm that damage to wheat proteins had resulted in a short and tough product lacking the visco-elastic properties required for effective breadmaking. Such samples would be rejected at a mill intake.

Despite major use of this test by the milling industry, it lacks the sensitivity required to detect low levels of heat damage that may still have an impact on breadmaking performance.

Test baking

A standard Chorleywood Bread Process (CBP) method was used to evaluate the effect of varying levels of heat damage on end-use quality. A Morton Z-blade mixer was used to mix a bread dough from the following ingredients to a total work input of 11 watt-hours kg^{-1} at a fixed rate of work input.

Ingredient	**Weight, g**	**%(by flour weight)**
Flour	840	100
Yeast	21	2.5
Salt	16.8	2.0
Fat (Ambrex, slip point c. 45°C)	8.4	1.0
Ascorbic acid (100ppm)	0.084	0.01

Fungal *alpha*-amylase to supplement all samples to 40 Farrand units of activity.
Water level as determined by Farinograph 600 line.

Four hundred gramme, single piece white bread was produced and, after overnight storage at 21°C, was assessed for loaf volume by seed displacement and crumb structure score by an experienced bakery technician.

RESULTS AND DISCUSSION

Effect of flour type on Turbidity test results (A_{540nm})

To assess the performance of the Turbidity test with wholemeal and white flour, samples of all four varieties were tested in duplicate. A Perkin Elmer Lambda 3 UV/visible variable wavelength spectrophotometer was used. A number of principal Absorption bands, including 536±0.5nm, were checked weekly before the instrument was used. For wholemeal, a 5g sample was used (as in previous experiments) and for Chopin-milled white flour an 8g sample as recommended by Harrison *et al.* (1969) was used. Figures 3 and 4 present Turbidity test results (Absorbance at A_{540nm}) obtained for wholemeal and white flour respectively.

The wholemeal flour data showed good agreement between varieties i.e. hard and soft endosperm textured varieties from **nabim** Groups 1 to 4 (**nabim**, 1997) were not significantly different over the range of heat damage tested. A cut-off point of $0.3A_{540nm}$ appeared to enable differentiation between sound and heat damaged samples: A_{540nm} values below 0.3 indicating that heat damage had taken place under the temperature and time regimes tested. This was confirmed by germination and breadmaking quality test results (data not shown here).

The results for Chopin-milled white flour showed much greater variability. In particular, sound (control) samples of individual varieties were significantly different e.g. sound samples of Riband and Hereward produced A_{540nm} values of 0.4 and 0.8 respectively. Given such varietal differences, it was more difficult

to separate sound samples from heat damaged ones. For example, the sound sample of Riband produced an A_{540nm} value equivalent to a slightly heat damaged (treated at 91°C for 40 minutes) sample of Soissons. Due to the wider spread of results from the Chopin-milled white flour, a lower A_{540nm} cut-off point was required. An A_{540nm} value of 0.25 was selected. However, this does not provide the margin of error obtained in wholemeal samples. For example using the white flour test, some heat damaged samples may not be detected. Thus treatment at 103°C for 40 minutes, which has been shown to produce detectable effects on germination and breadmaking performance (data not shown here), would be misclassified as producing sound samples for the varieties Hereward, Soissons and Hunter used in this study.

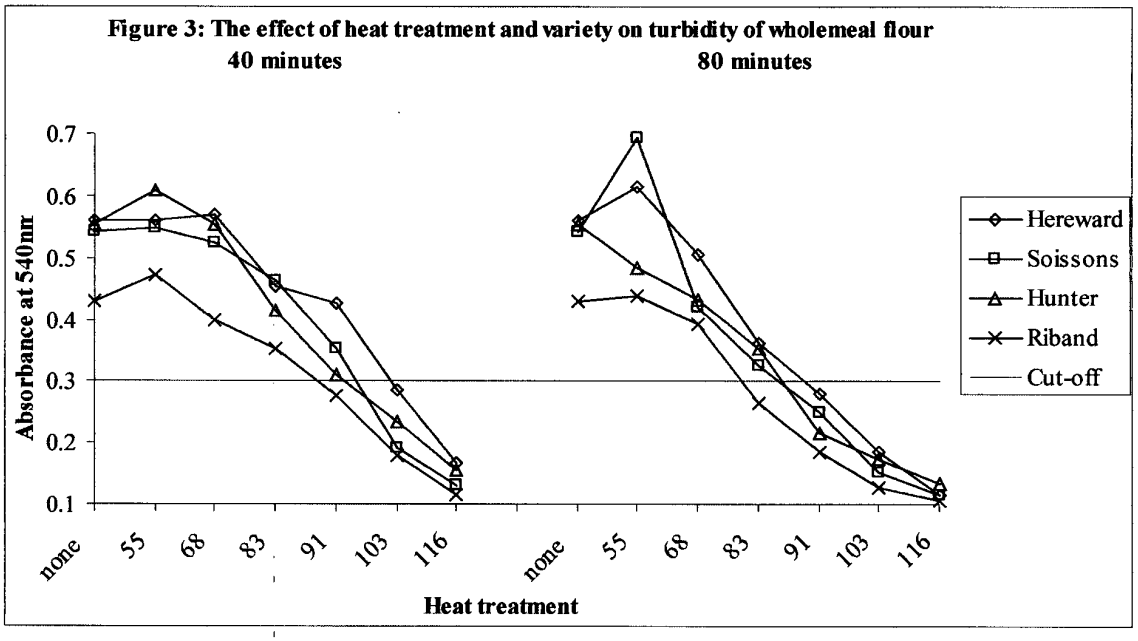


Figure 3: The effect of heat treatment and variety on turbidity of wholemeal flour

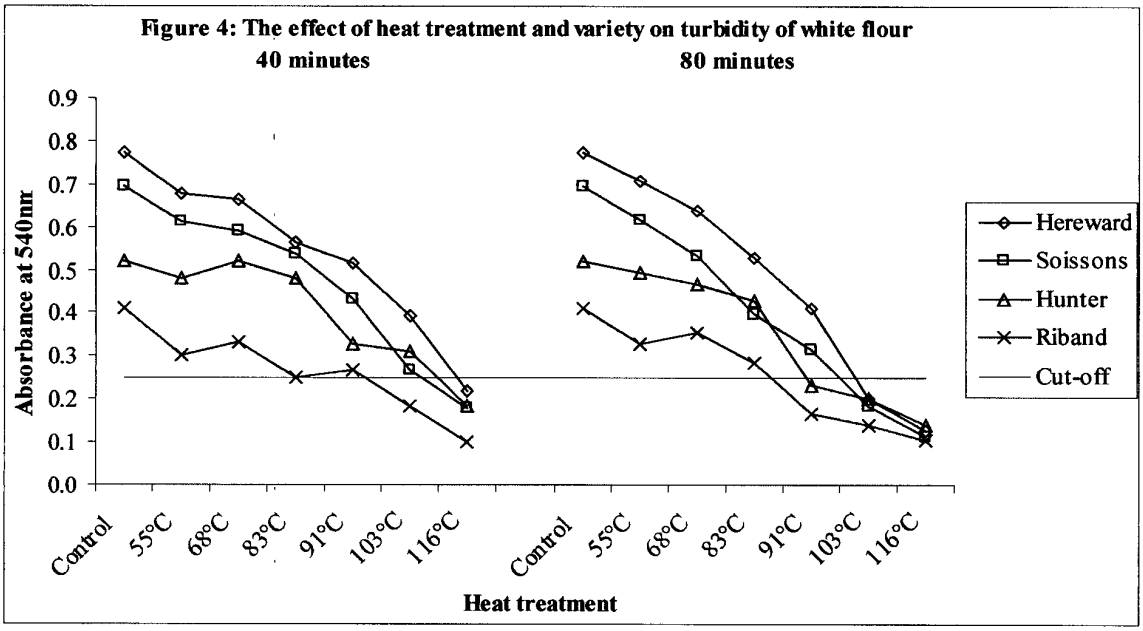


Figure 4: The effect of heat treatment and variety on turbidity of white flour

In conclusion, wholemeal samples produced more reliable results in the Turbidity test. Bran particles did not interfere with the measurement of turbidity and Chopin milling appeared to separate heat-precipitable protein differently in varieties that differed in terms of hardness and protein quality. The

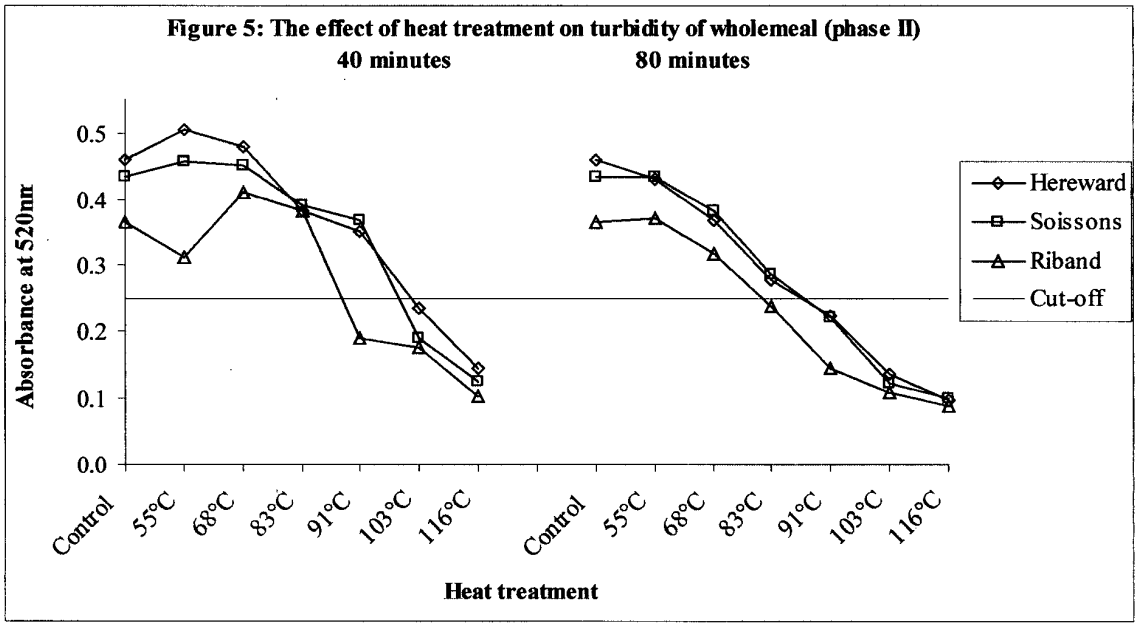
wholemeal used was typical of that routinely produced in mill intake and grain storage situations for the Falling Number test and therefore provided a significant advantage in terms of speed and potential uptake of the test. Laboratory-milled wholemeals were used in all other test optimisation experiments.

Turbidity test modification : use of a low cost fixed filter colorimeter

The modifications to the basic method described earlier were intended to increase its "ease of use" and minimise operator input, thus making it more suitable for use at mill intake.

Results produced for wholemeal samples of the varieties Hereward, Soissons and Riband (Figure 5) using the colorimeter (A_{520nm}) were significantly lower than equivalent values (A_{540nm}) obtained from the more expensive spectrophotometer (Figure 3). However, the previously observed relationships between individual varieties and different heat treatments were essentially unaltered. A drop of ~0.1 Absorbance units was observed in the control and mildly heat damaged samples. Severely heat damaged samples produced similarly low Absorbance values under each test protocol.

Figure 5: The effect of heat treatment on turbidity of wholemeal (phase II)



Subsequent Turbidity tests carried out on three of the same varieties from a different harvest year and exposed to slightly different heat treatments confirmed the lower A_{520nm} values obtained with this method. An A_{520nm} cut-off point of 0.3 still appeared to be suitable, despite the lower readings obtained for control samples using the fixed filter colorimeter. In addition, there appeared to be a sample influence on measured Absorbance values for the untreated controls for all varieties in Figure 3 compared with Figure 5. This suggests that some season-to-season variation may occur within a variety resulting from basic differences in protein content or quality.

Performance of the Turbidity test as a measure of heat damage.

Results were obtained from a series of control and heat damaged samples produced in three varieties (Hereward, Soissons and Riband) from the 1996 harvest. These samples were conditioned to 18 and 25% moisture content and then exposed to differing temperature and time combinations as shown in Figure 1, they produced very high correlations as shown in Table 1. These results suggest that variety does not have a major effect on Turbidity test results i.e. the test is relatively insensitive to variety. However as indicated for comparisons between 1996 and 1997 harvest samples above, there may be a seasonal effect. Therefore, it is recommended that if the Turbidity method is to be used routinely for the detection of heat damage that a set of standard wheat samples, containing known undamaged and severely heat damaged samples be used for direct comparison purposes.

Table 1: Correlation coefficients between varieties using the Turbidity test

	Hereward	Soissons	Riband
Hereward	1.000		
Soissons	0.979	1.000	
Riband	0.920	0.930	1.000

The gluten washing method, which is currently used for detecting heat damage at mill intake, was compared with loaf volume, germination testing and the Turbidity test results for the three varieties Hereward, Soissons and Riband. Breadmaking performance (represented by loaf volume) was carried out on the hard Group 1 and 2 wheat types only. Due to limitations of sample size, gluten washing was carried out for Hereward and Riband only. Comparisons were, therefore, made within individual varieties. The correlation coefficients for each comparison are summarised in Table 2.

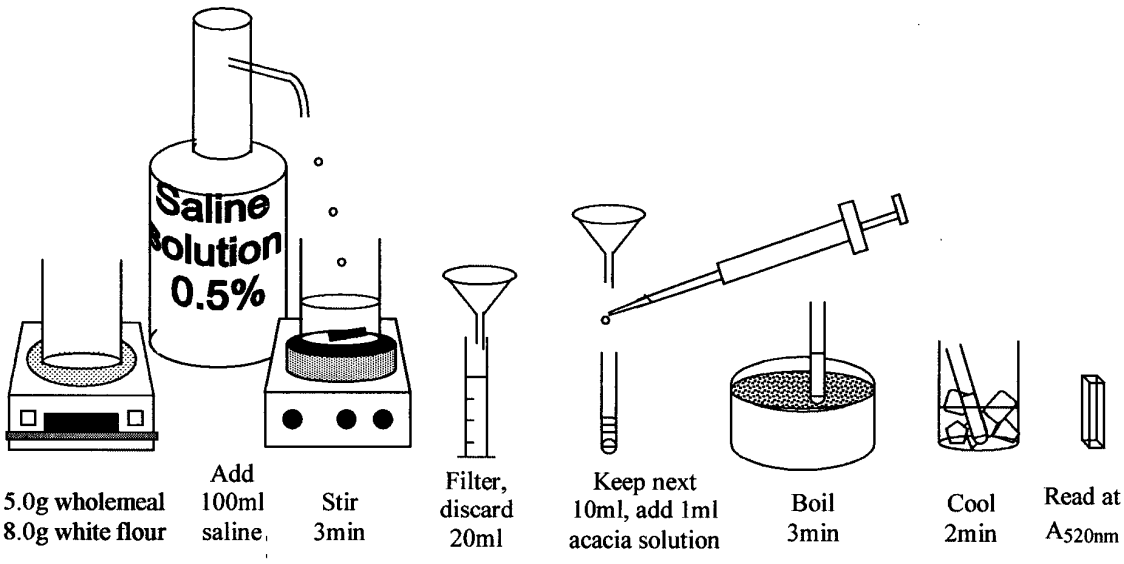
Table 2: Correlation coefficients between tests for each variety

	Hereward				Soissons			Riband		
	LV	A_{520}	Glut	%G	LV	A_{520}	%G	A_{520}	Glut	%G
LV	1.000				1.000			-		
A_{520}	0.805	1.000			0.935	1.000		1.000		
Glut	0.886	0.815	1.000		-	-	-	0.838	1.000	
%G	0.707	0.947	0.710	1.000	0.857	0.968	1.000	0.915	0.754	1.000

Where LV= CBP loaf volume, A_{520} = results from the modified Turbidity test, Glut = wet gluten and % G = germination capacity.

In all three varieties, the correlation between Turbidity test results and germination capacity is above 0.9 suggesting a good relationship between heat damage levels and Turbidity as measured by A_{520nm}. For the limited varieties tested, this correlation appears to be significantly better than the currently used mill intake test of wet gluten washing and the final arbiter of end-use quality i.e. test baking. This data suggests that the Turbidity test should provide a better means of detecting heat damaged wheat at mill intake than the previous slower and less sensitive wet gluten measurement.

Figure 6: Schematic of the Turbidity test



It is important that the method is followed very carefully with attention to detail at each stage (Figure 6). The authors recommend that the minimum requirement for quality control of the method should be

regular checks using a standard stock of well-mixed sound wheat. In addition, to provide greater confidence in detecting low levels of heat damage a range of samples varying in heat damage levels and referenced to germination test results should be prepared. All method check samples should be replaced at least annually. When replicate tests were carried out by two operators, using the same equipment and the same samples over a short period of time, A_{520nm} results were within ±3%.

CONCLUSIONS

Initial Turbidity test results appeared to be very promising. Further developments were made in an attempt to produce a simple, reliable method that was not variety dependent and employed low-cost equipment that could be used for the detection of heat damaged wheat in a mill intake or grain storage situation. A cut-off point of $0.3A_{520nm}$ appears to provide a simple means of detecting the presence of heat damage in wheat samples. Further automation e.g. development of a test rig that would enable consecutive testing of intake samples with the minimum of human intervention would be possible if the test became routinely used by UK millers, but would significantly increase the cost of implementing the test.

Correlation coefficients between commonly used measures of heat damage and the Turbidity test suggest that the latter relates well to germination test results and is more effective at detecting heat damage than wet gluten or CBP loaf volume.

Further validation of the test in routine use by mill intake laboratories would be necessary before this test could be considered for approval as a standard method e.g. a Flour Testing Working Group Method (Salmon, 1997). In addition, it would be necessary to generate further heat treated samples from a wider range of varieties, seasons and locations to calculate the effect of such parameters on test performance and make appropriate recommendations regarding the standards for method control.

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HARRISON, K.R., DOARKS, P.F. AND GREER, E.N. (1969). Detection of heat damage in dried wheat. *Milling* **151(7)**, 37-38.)

nabim (1997) **nabim** Wheat Guide 97. National Association of British & Irish Millers, 21 Arlington Street, London.

SALMON, S. (1997) Guideline No. 3 Manual of Methods for Wheat and Flour Testing. Campden & Chorleywood Food Research Association, Chipping Campden, Glos. UK.

FINAL REPORT: Detection of Heat Damage in Wheat - Appendices of Methods

1. PREPARATION OF HEAT DAMAGED SAMPLES FROM THE 1995 HARVEST

1.1 Scope

Heat treatment applied to different wheat varieties in order to induce serious heat damage.

1.2 Principle

In order to study the effects of heat damage on wheat, several varieties of different hardness and gluten quality were selected: Soissons, Riband, Hunter and Hereward. Wheat samples were each divided into two lots, one was stored as a control sample, the other was heat treated under defined conditions to induce heat damage. Samples of both control and heat damaged wheats were then ground into wholemeal flour for testing.

1.3 Material

Wheat samples of Soissons, Riband, Hunter and Hereward.

Table 1: Moisture and protein contents of the sound (control) and heat damaged (HD) wheats under study as determined by NIR

	Hereward		Soissons		Hunter		Riband	
	control	HD	control	HD	control	HD	control	HD
Moisture (%)	9.5	5.4	9.9	5.3	11.5	6.3	11.7	6.2
Hardness	50		48		1		15	
Protein at 14% (%)	11.8		12.0		9.8		9.6	

1.4 Apparatus

1.4.1 Laboratory balance (accurate to ±0.1 g)
1.4.2 Laboratory oven (Gallenkamp, Oven 300)
1.4.3 Hot air dryer
1.4.4 Metal trays
1.4.5 Sealable plastic bags
1.4.6 Falling number 3100 Mill (or other Laboratory Mill)

1.5 Procedure

1.5.1 *Heat treatment to induce severe heat damage*

Weigh 300 ± 1g of each wheat sample. Spread the wheat on aluminium foil placed on an oven tray. Set temperature of the oven to 130°C. Switch fan on to ensure even distribution of heat in the oven. Do not overload the trays as overloading results in uneven heat distribution and it is important to ensure that the amount of heat received by all varieties is similar. Once the temperature is stable, place the oven trays into the oven and leave for 30min. before removing.

1.5.2 *Handling of samples following heat treatment*

Wheat samples are conditioned to 15% moisture content in plastic bags and left in the wheat store at 13°C for 16 hours before grinding.

1.5.3 *Grinding*

Grind 300g of both sound and heat damaged samples. Place the resulting wholemeal flour into sealable plastic bags and keep in the wheat store at 13°C for at least 8 hours before testing.

2. PROTEIN SOLUBILITY TEST (PST)Based on the work of Every (1987) on detection of heat damage.

2.1 Scope

This method is used to determine the loss of solubility in the soluble wheat protein fraction as an indicator of heat damage.

2.2 Principle

Coomassie Brilliant Blue G-250, contained in the Bradford reagent, reacts with proteins to form a coloured compound which absorbs at 595nm. Coomassie Brilliant Blue G-250 exists in two different colour forms: red and blue. The red form is converted to the blue form when the dye binds to protein; a shift in the absorption maximum of the dye occurs, from 465nm to 595nm, and it is the increase in absorption at 595nm which is monitored. Absorbance of the salt-soluble protein solutions has been related to heat damage (Bradford, 1976).

2.3 Apparatus

2.3.1 Standard laboratory glassware
2.3.2 Automatic pipettes (capacity: 0 to 200µl and 1 to 5ml)
2.3.3 Analytical balance (accuracy ± 0.01g)
2.3.4 Spectrophotometer (capable of reading absorbance at 595nm) e.g. Perkin-Elmer Lambda 3, UV/VIS
2.3.5 Zeleny shaker (optional)
2.3.6 Bench centrifuge (capable of spinning at 2000rpm)
2.3.7 Glass fibre filter papers
2.3.8 Semi-micro glass cuvettes

2.4 Reagents

2.4.1 Solution of 2% (w/v) NaCl: dissolve 20g sodium chloride (Analar) in 1 litre of distilled water.

2.4.2 Bio-Rad Protein Assay Dye Reagent Concentrate (Bio-Rad Laboratories Ltd, Hemel Hempstead, Herts, UK) stored in refrigerator: before use, dilute 1 part concentrate with 4 parts distilled water, e.g. 20ml concentrate + 80ml distilled water.

NB Bio-Rad Protein Assay Dye Reagent should be shaken each day before use to achieve consistent results. This reagent should be stored at:

- room temperature for use within a month
- or 4°C for use within two months.

2.4.3 In-house prepared Bradford reagent as described by Bradford (1976): dissolve 100mg Coomassie Brilliant Blue G-250 in 50ml Absolute Alcohol (99.7% v/v min in C_2H_5OH). Add 100ml 85% w/v phosphoric acid. Make up to one litre with distilled water.

2.5 Procedure

(Modifications which were made during this project are shown in parentheses and italics.)

2.5.1 Add 50ml of 2% (w/v) NaCl to 1g wholemeal flour in a 100ml measuring cylinder. *(Pass the wholemeal flour through a 150 micron sieve).*

2.5.2 Mix by inverting the cylinder once every second for 30s. *(Place the cylinder on a Zeleny shaker and run for 30 seconds).*

2.5.3 Allow the mixture to stand for 30s then remove 100µl of extract from the top layer of liquid. *(a. Filter 5ml extract from the top layer of liquid through glass fibre and remove 100µl or b. Centrifuge the entire mixture using a bench centrifuge for 20 minutes at 2000rpm then remove 100µl of supernatant).*

2.5.4 Mix the extract (100µl) with 3ml of a solution of Bradford Reagent. Allow it to stand for at least 2min (spectrophotometer readings can be made from 2min to 1h).

2.5.5 Transfer solution to semi-micro cuvette using a Pasteur pipette and read the absorbance at 595nm against a blank of the protein assay dye reagent.

2.6 Expression of results

Absorbance at 595nm is related to % of heat damage. In the first stage of this study, only differences in Absorbance between sound and heat damaged wheat were considered.

2.7 References

BRADFORD, M.M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilising the principle of protein dye binding. *Analytical Biochemistry* **72**, 248-254.

EVERY, D. (1987). A simple, four-minute, protein-solubility test for heat damage in wheat. *Journal of Cereal Science* **6**, 225-236.

3. NEAR-UV ABSORBANCE OF SALT-SOLUBLE PROTEINS AS AN INDICATOR OF HEAT DAMAGE

3.1 Scope

Investigation of the loss of solubility of salt-soluble proteins as an indicator of heat damage in wheat.

3.2 Principle

Due to the structural characteristics of some amino acids such as tryptophan, tyrosine and phenylalanine they absorb at 275-280nm. These aromatic amino acids are sensitive to heat and thus their quantification could be used as an evaluation of heat damage. Though the proportion of these amino acids is not very important in wheat, absorbance in UV was investigated.

Tryptophan represents 1.5% of total protein content in flour and 6.9% (1.1g/16g nitrogen) of salt-soluble proteins. Its maximum absorption occurs at 280nm. Tyrosine represents 2.8% of total protein content in flour and 18.1% (2.9g/16g nitrogen) of salt-soluble proteins. Its maximum absorption occurs at 275nm at pH7 or 295nm at pH13. Phenylalanine represents 4.8% of total protein content in flour and 21.9% (3.5g/16g nitrogen) of salt-soluble proteins. Its maximum peak of

absorption occurs at 267nm. Absorbance was measured at 280nm as this accounted for approximately 80% of the nitrogen present in salt-soluble proteins.

3.3 Apparatus

3.3.1 Standard laboratory glassware
3.3.2 Automatic pipettes (capacity: 1 to 5 ml)
3.3.3 Analytical balance (accuracy ± 0.01g)
3.3.4 Zeleny shaker (optional)
3.3.5 Bench centrifuge (capable of spinning at 2000rpm)
3.3.6 Centrifuge tubes (capacity: 100ml)
3.3.7 Spectrophotometer (capable of reading absorbance at 280nm)
3.3.8 Quartz cuvettes

3.4 Reagents

3.4.1 Solution of 2% (w/v) sodium chloride. Dissolve 20g of sodium chloride (Analar) in 1 litre of distilled water.

3.5 Procedure

3.5.1 Weigh 1g wholemeal flour, add to a 100ml measuring cylinder containing 50ml of 2% (w/v) sodium chloride.

3.5.2 Place measuring cylinder on a Zeleny shaker and allow to run for 30s or invert cylinder once every second for 30 seconds.

3.5.3 Transfer the mixture into a centrifuge tube and spin using a bench centrifuge for 20min at 2000rpm.

3.5.4 Remove 2ml supernatant and dilute with an equal amount of 2% sodium chloride solution to obtain absorbance values within the range 0 to 1 at 280nm.

3.5.5 Read Absorbance at 280nm against distilled water (using quartz cuvettes).

3.6 Expression of results

Absorbance at 280nm can be related to the amount of heat damage. A 100% heat damaged wheat sample should be generated and checked using a standard germination test (see Appendix 11 for details).

4. SURFACE TENSION MEASUREMENTS AND FOAM TESTS ON FLOUR PROTEIN EXTRACTS

4.1 Scope

Proteins have specific surface properties that are altered by heat treatment. Tests that measure the surface properties of extracts containing salt-soluble wheat proteins were evaluated in terms of their ability to detect heat damage.

4.2 Principle

A platinum ring connected to a torsion balance is immersed into a solution, then withdrawn slowly through the air-liquid interface. The force necessary to withdraw the ring against the interfacial tension is measured.

Foaming ability can also provide an indication of surface properties of protein extracts.

4.3 Apparatus

4.3.1 Standard laboratory glassware
4.3.2 Analytical balance (accuracy ± 0.01g)
4.3.3 Zeleny shaker (optional)
4.3.4 Bench centrifuge (capable of spinning at 2000rpm)
4.3.5 Centrifuge tubes (capacity: 100ml)
4.3.6 Kruss K8 Tensiometer

4.4 Reagents

4.1 Solution of 2% (w/v) sodium chloride. Dissolve 20 g of sodium chloride (Analar) in 1 litre of distilled water.

4.5 Procedure

4.5.1 *Extraction of salt-soluble proteins*

4.5.1.1 Weigh 1g of flour and pour into a 100ml cylinder (if using optional Zeleny shaker, a standard Zeleny cylinder should be used). Add 50 ml of 2% (w/v) sodium chloride. Place the cylinder on the Zeleny shaker and run the apparatus 30 seconds or invert cylinder every second for 30 seconds. Pour the entire flour mixture into a centrifuge tube. Centrifuge at 2000 rpm for 20 minutes. Surface tension measurements are carried out using the Kruss K8 tensiometer and employing the ring method.

4.5.1.2 Clean the ring thoroughly by washing in distilled water, then acetone followed by heating in a bunsen burner. The sample vessel is also carefully cleaned by a 5 minute wash in distilled water then dried using acetone.

4.5.1.3 The torsion balance is locked before inserting the pin of the ring into the ring guidance. Prior to each measurement, the measuring dial is set to zero. In this state the balance will swing freely around its zero position. The tension of double distilled water at 20°C equals 72.6mN/m. A sample of double distilled water should be measured prior to testing salt- soluble protein extracts. If the value for water differs from 72.6 a correction factor should be applied to all test results.

4.5.2 *Foam test*

4.5.2.1 Pour about 20 ml of flour protein extract into a 100 ml measuring cylinder, seal the cylinder with a hermetic stopper.

4.5.2.2 Shake the cylinder by hand 10 times (in a repeatable manner e.g. one shake per second).

4.5.2.3 Read the volume of liquid and foam immediately after shaking.

4.5.2.4 Read the volume of liquid and foam after 10 minutes after the end of shaking.

4.6 Expression of results

4.6.1 For the surface tension measurements using the Kruss K8 tensiometer and employing the ring method, results are expressed in mN/m.

4.6.2 For the foam test, the volume of liquid and foam are expressed in ml. The percentage overrun (ml of foam over liquid) may also be used to describe the foaming capacity of protein extracts.

5. WEIGHT OF GEL PROTEIN

5.1 Scope

This method is applicable to commercial flour and laboratory milled white flour (Buhler, Chopin or Quadrumat Junior) and to laboratory milled wholemeal.

5.2 Principle

The fraction of glutenin protein, which is insoluble in sodium dodecyl sulphate (SDS) and is separated as a gel layer during centrifugation of a flour/SDS mixture, is known as gel protein. The weight of this protein fraction is primarily genetically controlled, although, environmental factors e.g. protein content, also influence results. Gel protein weight has been shown to correlate with other measures of protein quality e.g. SDS sedimentation volume.

5.3 Apparatus

All measuring equipment should be calibrated.

5.3.1 Balance capable of weighing at least 15 ± 0.01g.
5.3.2 Ultracentrifuge capable of a speed of 40,000rpm.
5.3.3 Ultracentrifuge tubes with a capacity of at least 90ml with screw-top lids.
5.3.4 Tube rack for the above.
5.3.5 Magnetic stirrer.
5.3.6 Multiple point magnetic stirrer.
5.3.7 Cooling bath at approximately 10°C.
5.3.8 Whatman No. 1 filter paper or equivalent (24cm diameter).
5.3.9 Timer capable of reading in intervals of at least 10 seconds.
5.3.10 Thermometer with a working range of -10 to 100°C and graduated in 1°C intervals.
5.3.11 Measuring cylinder, 50ml.
5.3.12 Double pan swing balance.
5.3.13 Wide-necked conical flasks, 250ml.
5.3.14 Conical flasks, 500ml.
5.3.15 Plastic funnels, minimum 6cm diameter.
5.3.16 Plastic sieve (2.5cm internal diameter, 1mm aperture sieve).

5.4 Reagents

5.4.1 Petroleum ether (General Purpose Reagent grade), boiling point 40-60°C.

5.4.2 Sodium dodecyl sulphate (SDS) solution (1.8% w/v). Dissolve 18g SDS in distilled water and make up to 1litre.

5.5 Procedure

(Prepare samples in batches depending on the number of tubes which can be spun at one time in the ultracentrifuge).

5.5.1 *Preparation of wheat samples*

5.5.1.1 Mill representative samples of wheat according to a standard procedure using a Buhler, Chopin, Quadrumat Junior or Falling Number 3100 Mill. The method of milling is known to affect results and, therefore, for comparative work, the same milling process must be used.

5.5.2 *Defatting of flour samples*

5.5.2.1 Weigh approximately 15g of each flour into a separate wide-necked conical flask. Place the flasks in a fume cupboard and add 40ml petroleum ether to each. Seal each flask using aluminium foil and shake the flour/solvent mixture at 15min intervals (i.e. at 0, 15, 30, 45 and 60min).

5.5.2.2 At the end of the extraction period, filter the mixtures through labelled Whatman No. 1 filter papers into 500ml conical flasks. (Collect the waste petroleum ether in a labelled Winchester for solvent disposal).

5.5.2.3 Allow the defatted flour remaining on the filter papers to dry in the fume cupboard (with full extraction) for approximately 1h.

5.5.2.4 Remove any lumps in the flour by pressing them with a plastic spatula before packaging the samples in labelled, sealable plastic bags.

5.5.2.5 If the defatted samples are to be kept for longer than a day, they should be placed in double, sealed bags and stored at 4°C.

5.5.3 *SDS extraction of defatted flours*

5.5.3.1 Prepare a cooling bath (approximately 10°C) by adding ice to distilled water in a tray and placing it on top of the multiple point magnetic stirrer.

5.5.3.2 Measure 90ml of 1.5% SDS solution into numbered 150ml beakers with magnetic followers and place on the multiple point stirrer to equilibrate to temperature.

5.5.3.3 Weigh 5.00g of defatted flour into numbered paper cases.

5.5.3.4 At 1min intervals, place one 150ml beaker containing SDS solution into a small cooling bath on top of a magnetic stirrer. Set the stirrer to a fast speed so that the vortex in the centre almost reaches the bottom of the beaker.

5.5.3.5 Add a flour sample through a plastic sieve (held in place by a clamp on a stand) to the SDS solution. Record the starting time for each sample, as soon as the flour has been mixed in, replace the beaker onto its original position on the multiple point magnetic stirrer and repeat the process for the next beaker and sample.

5.5.3.6 After a total of 10min mixing, decant each sample into a numbered centrifuge tube. Use a double pan balance to ensure that each pair of samples is accurately balanced for centrifuging.

5.5.3.7 Place balanced pairs of tubes adjacent to one another in the rack ensuring that the lids are securely fastened. The balancing of pairs of tubes should be carried out rapidly to prevent overrunning of the sample stirring times.

5.5.4 *Ultracentrifuge settings*

5.5.4.1 Switch on the water supply to the centrifuge. Check that the following settings have been selected: Rotor temperature 14°C; Maximum temperature 30°C; Time 35min; Rpm 40 x 1000; Brake on.

5.5.4.2 Place the samples in the centrifuge, using the appropriate rotor head, ensuring balanced pairs are opposite each other.

5.5.4.3 Secure the rotor lid in place. Switch on the centrifuge and allow to run. At the end of the spin, carefully transfer the tubes back to the rack and place in a refrigerator at 4 ± 1°C for 30min.

5.5.4.4 Carefully decant off the supernatant then gently scrape off the gel layer, using a rounded spatula, into a previously tared plastic dish, taking care to avoid contamination with starch.

5.5.4.5 Bread wheat varieties normally produce a thick, opaque gel layer whilst biscuit/feed wheats tend to have a fluid, translucent layer. Record the weight of the gel protein fraction in g to 2 decimal places.

5.5.4.6 If the gel protein is to be examined rheologically (see Appendix 6), load 2.5g sample into the concentric ring C14 of the Bohlin rheometer as soon as possible after weighing.

NB. If the weight of the gel protein obtained in the test is less than 7g and the gel is required for rheological assessment, a second gel protein weight determination must be carried out.

5.6 Expression of results

Results are expressed as g (to the nearest 0.1g) of gel protein per 5g flour.

5.7 Quality control

A check sample should be included in each batch of samples. For example, 15 single determinations of gel protein weight carried out on a check sample of defatted flour produced a mean value of 10.0g/5g flour and a standard deviation of 0.56. A control chart should be constructed with control limits set at ±1.1 about a mean value of 10g/5g.

5.8 Reference

GRAVELAND, A., BONGER, P. and BOSVELD, P. (1979). Extraction and fractionation of wheat flour proteins. *Journal of the Science of Food and Agriculture* **30**, 71-84.

6. MEASUREMENT OF GEL PROTEIN RHEOLOGY

6.1 Scope

To measure the elastic modulus (G′) of the gel protein fraction of wheat flour prepared according to Appendix 5. The Bohlin VOR controlled strain rheometer is used configured with the Fluids head and operating in oscillation mode.

6.2 Principle

The vibration of the machine at a set frequency is measured through a sample of the material to be tested. A number of parameters are derived from the measurements taken, the most important with respect to gel protein are the viscosity, phase angle, viscous modulus (G″) and elastic modulus (G′) at a frequency of 1Hz. Larger values of G′ imply that the material is more resistant to deformation.

6.3 Apparatus

6.3.1 Temperature control unit
6.3.2 Computer and monitor loaded with appropriate software.
6.3.3 Compressed air, set at 2bar
6.3.4 Electronic interface unit relating information between the computer and the rheometer.
6.3.5 Bohlin VOR rheometer

6.4 Procedure

6.4.1 Connect the equipment and set up for use with the Fluids head, cup and bob geometry.

6.4.2 Switch on the power and compressed air supply. Select the oscillation mode from the computer and alter the settings to give a suitable temperature and range of frequencies for testing. Allow the equipment to warm up for approximately 30min.

6.4.3 Ensure the cup part of the cup and bob assembly is clean and weigh 2.50g of extracted gel protein into it.

6.4.4 Align the cup and bob correctly in the Bohlin Rheometer and adjust the torque setting to zero.

6.4.5 Set the equipment running through the selected frequencies of vibrations. Measurements are recorded at each one frequency of vibration.

6.4.6 Print, record or store the data as required.

6.4.7 Discard the sample, clean the cup and continue with another sample as necessary. Duplicate readings should be taken for each gel protein sample.

6.5 Expression of results

From the recorded data, select individual results for elastic modulus (G′) at 1Hz. This frequency has been found to produce reliable results and be suited to sample comparison for gel protein. The mean value, provided replicates agree within stated limits are reported in Pascal (Pa).

7. EXTRACTION AND FRACTIONATION OF WHEAT FLOUR PROTEIN

7.1 Scope

Different types of protein present in flour may be fractionated dependent upon their relative differences in solubility. Comparison of the composition of similar fractions from different samples may then be made using electrophoresis.

7.2 Principle

The different flour protein components are separated by sequential extraction in different solvents. Albumins and globulins are extracted with a dilute salt solution, gliadins using alcoholic and glutenins with a solution of containing the detergent sodium dodecyl sulphate (SDS) and a reducing agent such as dithiothreitol (DTT). Total protein can be extracted using a standard Laemmli buffer.

7.3 Apparatus

7.3.1 General analytical glassware
7.3.2 Screw-capped hydrolysis tubes (for large sample >1 ml)
7.3.3 Eppendorf microcentrifuge tubes (1.5ml)
7.3.4 Automatic pipettes (capacity: 0 to 200µl, 200 to 1000µl and 1 to 5ml)
7.3.5 Analytical balance (accuracy ± 0.0001g)
7.3.6 Shaker bath set at room temperature
7.3.7 Boiling water bath
7.3.8 Timer
7.3.9 Vortex
7.3.10 Centrifuge capable of spinning samples at 300 x g
7.3.11 Microcentrifuge capable of spinning samples at 12,000 x g

7.4 Reagents

7.4.1 Sodium chloride (0.5M). Dissolve 2.922g of sodium chloride in 100ml distilled water

7.4.2 Propan-2-ol (60% v/v). Mix 60ml of 100% (v/v) propan-2-ol with 40ml distilled water.

7.4.3 Sodium dodecyl sulphate (SDS) (1.5%), 5mM 1,4-Dithiothreitol (DTT) Mix 75ml of 1.5% SDS solution (1.5g sodium dodecyl sulphate (SDS) + 100ml distilled water) and 375µl of 1M 1,4-Dithiothreitol (DTT) solution. This solution must be prepared freshly each day.

7.4.4 Laemmli extraction buffer. Mix 62ml of distilled water with 10ml of 15% SDS solution , 10ml of glycerol, 8ml of 1M Tris/HCl pH6.8 and10ml 50mM DTT

Note: Laemmli extraction buffer has been modified to be suitable for Lowry assays: the concentration of SDS and DTT in the extract is reduced to 1.5% and 5mM respectively.

7.5 Procedure

(Extraction based on 750mg of flour)

7.5.1 *Albumin and globulin extraction*

7.5.1.1 Add 7.5ml of 0.5M of sodium chloride (7.4.1) to each pre-weighed flour sample into screw-topped hydrolysis tubes, shake the tubes until pellets dissolve and then vortex.

7.5.1.2 Place the samples horizontally in the shaker bath for 1h at room temperature to keep the mixture in suspension.

7.5.1.3 Centrifuge in the Mistral centrifuge at 1000rpm (300 x g) and 25°C for 10min.

7.5.1.4 Pour off supernatants into labelled tubes and drain the pellets.

7.5.2 *Gliadin extraction*

7.5.2.1 Add 7.5ml of 60% v/v propan-2-ol to each pellet. Shake vigorously by hand until the pellet breaks up and dissolves.

7.5.2.2 Place samples horizontally in a shaker bath for 1h at room temperature to keep the mixtures in suspension.

7.5.2.3 Centrifuge in the Mistral at 1000 rpm (300 x g) and 25°C for 10min.

7.5.2.4 Pour off supernatants into labelled tubes and drain the pellets.

7.5.3 *Glutenin extraction*

7.5.3.1 Add 7.5ml of 1.5% SDS, 5mM DTT. Shake the tube vigorously to break the pellet and vortex.

7.5.3.2 Place the samples horizontally in shaker bath for 55min at room temperature to keep the mixtures in suspension.

7.5.3.3 Centrifuge in the Mistral for 10min at 1000rpm and 25°C. Pour off the supernatant.

7.5.4 *Total SDS/DTT extraction*

7.5.4.1 Weigh 750mg of each flour sample into a screw-topped hydrolysis tube. Add 7.5ml extraction solution and vortex.

7.5.4.2 Place the samples on a shaker bath for 45min at room temperature.

7.5.4.3 Centrifuge for 10min at 1000rpm then pour off the supernatant.

7.5.5 *Total Laemmli extraction*

7.5.5.1 Weigh 100 ±2mg flour (wholemeal) into 1.5ml microcentrifuge tubes. Add 1.0ml of extraction solution and vortex.

7.5.5.2 Place in a shaker bath for 45min at room temperature and vortex.

7.5.5.3 Boil the extracts for 3 to 5min (100°C) and cool to room temperature.

7.5.5.4 Centrifuge for 5.5min at maximum speed (12) in a microcentrifuge for small samples, or 20min at 2000 in a bench centrifuge. Pour off supernatant.

7.5.5.5 Store extracts in the laboratory freezer (in hydrolysis tubes) until use.

Note: Lowry results may be confirmed by Kjeldahl determination. In this case, more protein extract is needed: 500 ± 2mg of flour are weighed and dispersed into 5ml of extraction solution, these larger samples require boiling for 5-6min.

7.6 Reference

LAEMMLI, U.K., 1970. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. Nature, **227**, 680-685.

8. LOWRY ASSAYS FOR FLOUR PROTEIN QUANTIFICATION

8.1 Scope

Quantification of protein present in flour extracts as prepared in 8.7

8.2 Principle

Under alkaline conditions, copper ions (Cu^{2+}) form a complex with the peptide bonds of proteins and becomes reduced (to Cu^{+}). The Cu^{+} as well as the R groups of tyrosine, tryptophan and cysteine residues then react with the Folin Reagent. The reagent reacts by first producing an unstable product which is slowly reduced to become molybdenum/tungsten blue. The absorbance of the solution is read at 750nm (630nm for the micro-titre plate assays). Protein content of the sample is given by the Beer-Lambert relationship

$$A_x = \varepsilon * c * l$$

where: x is the wavelength in nm, ε is the molar extinction coefficient, c is the molar concentration of the substance analysed and l is the length of the cuvette (usually 1cm).

8.3 Apparatus

8.3.1 *Test-tube assays*
8.3.1.1 Small volume disposable cuvettes for readings at 750nm
8.3.1.2 Standard laboratory glassware (Analytical grade)
8.3.1.3 Automatic pipettes (capacity: 0 to 200µl, 200 to 1000µl and 1 to 5ml)
8.3.1.4 Timer
8.3.1.5 UV/Visible Spectrophotometer (e.g. Perkin-Elmer Lambda 3)
8.3.1.6 UV cuvettes for readings at 280nm
8.3.1.7 Heated magnetic stirrer
8.3.1.8 Thermometer suitable for measuring between 0°C and 100°C

8.3.2 *Micro-titre plate assays*
8.3.2.1 Micro-titre plates (96 well)
8.3.2.2 Micro-titre plate reader (e.g. Biotek) set to 630nm
8.3.2.3 Incubator / shaker

8.4 Reagents

8.4.1 *Test-tube Lowry assay*

8.4.1.1 Solution A: Weigh out 5g of anhydrous sodium carbonate, 1g of sodium hydroxide pellets, 0.4g sodium (+) tartrate and 2.5g of sodium dodecyl sulphate (SDS). Make up to 250ml with distilled water (after standing at room temperature for some time, SDS may precipitate out of solution, this can be made to re-dissolve by heating to approximately 50°C and using a magnetic stirrer).

8.4.1.2 Solution B: Weigh out 1.28g of anhydrous copper sulphate. Make up to 50ml with distilled water.

8.4.1.3 Solution C: Mix 25ml of solution A with 250µl of solution B. Make up a fresh mixture for each set of assays. (Heating at approximately 50°C may be necessary to encourage SDS to dissolve if it has precipitated out of solution).

8.4.1.4 Folin-Ciocalteu solution; freshly made from Folin-Ciocalteu's Phenol reagent 2.0 N (Sigma F-9252) and distilled water (1:1) (BDH Folin-Ciocalteu reagent may also be used). Store in a refrigerator (4°C) when not in use.

8.4.1.5 BSA (bovine serum albumin, Sigma) stored in a refrigerator (4°C).

8.4.1.6 SDS solution (1%) for making protein extract dilutions.

8.4.1.7 SDS solution (1.5%) for making sample dilutions (for albumins/globulins and gliadins):

8.4.1.8 Weigh 1.5g of SDS, add 100ml of distilled water and mix using a magnetic stirrer until dissolved, heat to approximately 50°C if necessary.

8.4.1.9 SDS/1,4-Dithiothreitol (DTT) solution (1.5% SDS + 0.1mM DTT, for SDS/DTT soluble proteins): Mix 10µl of 1M DTT and 99.990ml of 1.5% SDS solution (or 10ml 1mM DTT and 90ml 1.5% SDS solution).

8.4.1.10 Modified Laemmli buffer (for dilution of Laemmli extracts only):

8.4.1.11 Mix 62ml of distilled water, 10ml of 15% SDS, 10ml glycerol, 8ml of 1M Tris/HCl pH6.8 and 10ml 1mM DTT. (Further dilution of this standard is carried out using buffer 8.4.1.10 above.)

8.4.2 *Micro-titre plate Lowry protein assay*

8.4.2.1 BSA stock (2mg/ml in water) Weigh 44mg (this includes 10% over to allow for water content) BSA (Sigma A-7030), dissolve in 20ml of distilled water. Read the OD at 280nm in a 1cm UV cuvette against distilled water. Add water to bring the OD to 1.320.

8.4.2.2 BSA stock 0.3mg/ml in water: Mix 3.00ml stock 2mg/ml BSA in water, 7.00ml distilled water and 10.00ml of 2% sodium dodecyl sulphate (SDS) solution in water.

8.4.2.3 Lowry solution A: Weigh 6.0g of anhydrous sodium carbonate (Na_2CO_3), 1.2g sodium hydroxide (NaOH) pellets, 0.5g sodium (+) tartrate and 1.0g SDS. Make up to 100ml with distilled water (SDS may precipitate out of solution, this can be made to re-dissolve by heating to approximately 50°C and using a magnetic stirrer).

8.4.2.4 Lowry solution B: Weigh out 5.00g of hydrated copper sulphate ($CuSO_4.5H_2O$) and make up to 25ml with distilled water.

8.4.2.5 Lowry solution C prepared fresh each day: Mix 10.0ml of solution A and 100µl of solution B (for 96 wells) at approximately 50°C (to encourage SDS to dissolve if it has precipitated out of solution) using a heated magnetic stirrer, stir until dissolved, then cool to room temperature.

8.4.2.6 Dilute Folin's reagent: Weigh 1.2ml of 2N Folin's reagent (Sigma F-9252) add 4.8ml water (for 96 wells) and mix on a magnetic stirrer until dissolved.

8.5 Procedure

8.5.1 Protein extraction is performed according to the method described in Appendix 7.

8.5.2 *Protein extract dilution*

8.5.2.1 Depending on the solution used for extraction, dilute the extract with an appropriate buffer.

Note: Several compounds may interfere with the reagents used in the Lowry assay, in particular DTT used in the extraction of total proteins. To perform the Lowry assay on SDS/DTT extract and Laemmli extracts, samples have to be diluted to obtain a final concentration in DTT equal to 0.1mM; a final concentration of SDS equal to 1.5% and a final concentration of protein of between 0 and 60µl BSA.

8.5.2.2 Approximately a fifty times dilution will be necessary (i.e. 30µl extract supernatant and 1470µl 1% SDS in an Eppendorf tube), vortex the mixture.

8.5.3 *Preparation of standards*

8.5.3.1 Prepare a solution of 10mg BSA/ml in the appropriate BSA dilution buffer, depending on the solution used for the extract being tested.

8.5.3.2 Dilute with the appropriate buffer to obtain a solution of 0.2mg BSA/ml. (i.e. 1ml of the solution prepared in 8.5.3.1 and 49ml of buffer).

Note: For analysis of Laemmli extracts, BSA solution (0.2 mg/ml) must be boiled as well as the samples.

8.5.3.3 For the test-tube assay - using 0.2mg BSA/ml solution (equivalent to 200µg BSA/100µl).

Standards	S0	S10	S20	S30	S40	S50	S60
µg of BSA	0	10	20	30	40	50	60
µl 0.2mg BSA/ml solution	0	50	100	150	200	250	300
µl of BSA dilution buffer	300	250	200	150	100	50	0

8.5.3.4 For microtitre assay - using 0.3mg BSA/ml solution (see 5.2.1 and 5.2.2).

Standards	S0	S5	S10	S20	S30	S40	S50	S60
Standard conc. (µg/µl)	0	2.5	5	10	15	20	25	30
Vol. 0.3mg/ml BSA (ml)	0	0.375	0.75	1.50	2.25	3.00	3.75	4.50
Vol. 1% SDS (ml)	4.50	4.125	3.75	3.00	2.25	1.50	0.75	0

8.5.4 *Test-tube Lowry protein determination*

8.5.4.1 Add 300µl of samples/standards to 900µl of solution C.

8.5.4.2 Mix and allow to stand for 30min at room temperature.

8.5.4.3 Add 90µl (1:1) Folin-Ciocalteu solution, mix, and allow to stand at room temperature for 45min.

8.5.4.4 Read sample optical densities in small volume disposable cuvettes at 750nm against a water blank.

8.5.4.5 Draw a standard graph of optical density (OD) at 750nm against µg of BSA. Read off sample OD and convert to µg BSA. Correct for any dilution of the sample.

8.5.5 *Microtitre plate Lowry protein assay*

8.5.5.1 Use a complete set of standards in triplicate on each plate.

8.5.5.2 Transfer 100µl of each sample into each of three wells of a 96 well microtitre plate i.e. test sample in triplicate.

8.5.5.3 Add 50µl of Lowry solution C.

8.5.5.4 Mix on an incubator/shaker and allow to stand at room temperature for 10min.

8.5.5.5 Add 50µl dilute Folin's reagent whilst mixing on the shaker.

8.5.5.6 Allow to stand at room temperature for 30min.

8.5.5.7 Gently blow air over the surface of the plate with a Pasteur pipette and rubber bulb to burst the SDS bubbles.

8.5.5.8 Read the Absorbance of the samples in the plate at 630nm using the Biotek plate reader.

8.5.5.9 Ensure that there is not a floppy disk inserted into drive a: and switch on the computer.

8.5.5.10 At the C> prompt, type "BIOTEK" <enter> to begin the program.

8.5.5.11 Using the keyboard directly below the screen, press the pink "ENTER" button and type in the date in the American format (i.e. 05/13/97 for 13th May).

8.5.5.12 Press the mauve "PROMPT" button to check the settings then "OPTION" to alter any, such as the wavelength. If all are correct, press "PROMPT" repeatedly until **READY** appears.

8.5.5.13 Insert a floppy disk into drive a: to record the files produced.

8.5.5.14 Using the other keyboard and the mouse, select the "DEFAULT" menu, ensure that Template = "DS19-76" and Protocol = "endpnt".

8.5.5.15 From the "OPERATIONS" menu, select C-calc then R-reader.

8.5.5.16 Type in the filename which will be stored on the floppy disk, wait until the light above the a: drive has stopped flashing.

8.5.5.17 At the prompt: waiting for plate #1, insert the microtitre plate firmly into position and press the "START" button on the instrument keyboard.

8.5.5.18 To print out the data, press "print screen" on the other keyboard.

8.5.6 *Computer calculation of Lowry* - using the Biotek microtitre plate reader and computer.

8.5.6.1 Ensure that there is not a floppy disk inserted into drive a: and switch on the computer.

8.5.6.2 At the C> prompt, type "BIOTEK" <enter> to begin the program.

8.5.6.3 Insert the floppy disk into drive a:. From the OPERATIONS menu, select M to create a data file and <Esc> for a new file.

8.5.6.4 Type in the filename to be used then <enter>

8.5.6.5 Type in the optical densities to be used as the standards (S0 to S60) and samples in the appropriate box:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S0	S0	S0	S10	S10	S10	S20	S20	S20	S30	S30	S30
B	S40	S40	S40	S50	S50	S50	S60	S60	S60			
C	1	1	1	2	2	2	3	3	3	4	4	4
D	5	5	5	6	6	6	7	7	7	8	8	8
E	9	9	9	10	10	10	11	11	11	12	12	12
F	13	13	13	14	14	14	15	15	15	16	16	16
G	17	17	17	18	18	18	19	19	19	20	20	20

S = BSA Standards
Number = Sample Number

8.5.6.6 Hold down <ctrl> and press "S" to save data to disk, return to the main menu.

8.5.6.7 From the DATA DEFAULTS menu, select "T" to enable another template to be used. Highlight the one required and press <enter>.

8.5.6.8 To make a new template:

- From the OPERATIONS menu, select "T" to edit a template.
- Press <Esc> to make a new template, type the filename to be assigned and press <enter>.
- Standards need to be prefixed by "S" and followed by a value along the Y-axis (i.e. µgBSA).
- Samples require numbers only
- Hold down <Ctrl> and press "S" to save to disk.

8.5.6.9 From the OPERATIONS menu, select "C" to calculate and "D" from disk. Highlight the filename, <enter>. Type "P" to proceed.

8.5.6.10 Edit the standard graph if necessary. Press <Page Down> and wait.

8.5.6.11 Hold down <Ctrl> and press "P" to print the graph, press <Page Down> to step through the results.

8.5.6.12 Press <Print Screen> to print any screen with paper aligned first.

8.6 Expression of results

Convert optical density readings to µg BSA using the standard curve. Correct for dilution factors used. The protein content of samples is expressed in mg BSA/100mg flour.

9. SEPARATION AND ANALYSIS OF PROTEIN FRACTIONS USING SODIUM DODECYL SULPHATE - POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE) - BIO-RAD MINI GEL SYSTEMS

9.1 Scope

Protein extracts from flour and other wheat based products can be separated into a series of discrete bands on the basis of molecular weight on polyacrylamide gels. Each gel slab is produced by mixing certain proportions of acrylamide and polymerising agents. The properties

of the slab can be tailored to meet different separation requirements by manipulating the proportions of the ingredients used. Higher concentrations of acrylamide will reduce pore size within the gel, thus the speed at which proteins travel through it. Smaller protein components will travel faster than larger ones and not separate out clearly using homogenous gels. By using gradient gels, a molecular sieving effect is produced i.e. protein entities of high molecular weight become trapped in a specific region of the gel where the pores are too small to permit their passage.

9.2 Principle

When proteins are denatured with excess SDS (sodium dodecyl sulphate), the detergent micelles bind to the polypeptides at a constant mass ratio and the polypeptide is organised into a rod-like structure. The bound SDS micelles each impose a net negative charge which effectively swamps the intrinsic charge of the polypeptide. The SDS-polypeptide complexes have, with few exceptions, the same charge/mass ratio. Electrophoretic migration is therefore logarithmically proportional to the molecular weight of the polypeptide chain. Samples are treated with SDS under reducing conditions i.e. 1,4 - dithiothreitol (DTT) resulting in cleavage of the disulphide bonds between and within proteins. The basic recipe and method can be adapted to work with Pharmacia Vertical Gel Electrophoresis Units GE 2/4 and other electrophoresis systems.

9.3 Apparatus

It is assumed that the following range of general laboratory equipment is available: beakers, volumetric flasks, measuring cylinders, automatic pipettes (checked for accuracy on a regular basis), water bath (boiling), analytical balance (capable of weighing at least 0.001 to at least 4 decimal places), pH meter, timer (accurate to 1s), microcentrifuge (capable of spinning at a speed of at least 12,000 x g) plus microcentrifuge tubes (1.5ml). In addition, the following specialised apparatus may be required:

9.3.1 *Gel-casting equipment - Mini-Protean II Slab Cell (Bio-Rad)*

9.3.1.1 Set of glass plates (inner and outer)
9.3.1.2 Spacers (1.5mm)
9.3.1.3 Sandwich clamp assembly
9.3.1.4 Casting stand (along with rubber gaskets)
9.3.1.5 Teflon combs (15 wells x 1.5mm)
9.3.1.6 Gel casting equipment - two glass chambers connected via a plastic tube. The first chamber has at least one flow outlet. To this flow outlet, fit a set of plastic tubes (the final tube should have a narrow diameter and be placed between the inner and outer glass plates, down the side of one of the spacers), to pour the gel without making bubbles.

Note: If several outlets are available on the casting gel equipment, two sets of tubes can be fitted and thus two gels can be poured into glass plates simultaneously.

9.3.1.7. Magnetic stirrer and small follower.
9.3.1.8 Inner cooling core.
9.3.1.9 Lower buffer chamber and lid.

9.3.2 *Gel-casting Equipment- Bio-Rad Ready Gel Cell*

9.3.2.1 Clamping frame
9.3.2.2 Electrode assembly
9.3.2.3 Mini tank and lid
9.3.2.4 Sample loading guides (10 and 15 wells)

9.3.2.5 Mini Cell Buffer Dam; substituted for a Ready Gel cassette when running only one gel.

9.3.2.6 Ready Gels (Bio-Rad). 10-20% Tris-Glycine Gel, with 4% stacking gel, 10 wells, 0.375M Tris-HCl, pH8.

9.3.3 *Power supply*

Electrophoresis Constant Power Supply e.g. ECPS 3000/150 (Pharmacia).

9.4 Reagents

9.4.1 *Stock acrylamide, 40%:*

Weigh 38.96g of acrylamide and 1.04g of N,N'-Methylene bisacrylamide (Bis) into a beaker. Make up to 100ml with distilled water in a 100ml volumetric flask. Filter through Whatman No. 54 filter paper. Transfer to a labelled glass container and store at 2-8°C This solution remains stable for at least 3 months. (NB. Both acrylamide and Bis carry hazard warnings therefore should be handled with extreme care in the dry or unpolymerized state. Refer to COSHH record before use).

9.4.2 *Stock acrylamide, 30%:*

Weigh 29.22g of acrylamide and 0.78g of N,N'-Methylene bisacrylamide (Bis) into a beaker. Make up to 100ml with distilled water in a 100ml volumetric flask. Filter through Whatman No. 54 filter paper. Transfer to a labelled glass container and store at 2-8°C This solution remains stable for at least 3 months. (NB. Both acrylamide and Bis carry hazard warnings therefore should be handled with extreme care in the dry or unpolymerized state. Refer to COSHH record before use).

9.4.3 *Stock Tris/HCl, 3M pH8.8 buffer*:

Weigh 32.3g of Tris(hydroxymethyl)methylamine (Tris) into a beaker. Dissolve in approximately 80ml distilled water with the aid of a magnetic stirrer. Using a pH meter, adjust to pH8.8 with Analar grade HCl. Make up to 100ml with distilled water in a 100ml volumetric flask, transfer to a labelled glass container and store at 2-8°C.

9.4.4 *Stock Tris/HCl, 1M pH6.8 buffer*:

Weigh 12.5g of Tris(hydroxymethyl)methylamine (Tris) into a beaker. Dissolve in approximately 80ml distilled water with the aid of a magnetic stirrer. Using a pH meter, adjust to pH6.8 with Analar grade HCl. Make up to 100ml with distilled water in a 100ml volumetric flask, transfer to a labelled glass container and store at 2-8°C.

9.4.5 *Stock Tris/glycine/SDS Running Buffer, (10X concentration)*:

Weigh 72.1g of glycine, 5g of sodium dodecyl sulphate (SDS) and 15.15g of Tris(hydroxymethyl)-methylamine (Tris) into a beaker, dissolve in approximately 400ml distilled water with the aid of a magnetic stirrer. Make up to 500ml with distilled water in a 500ml volumetric flask. Dilute 50ml to 500ml with distilled water for use.

9.4.6 *Coomassie R250 stain solution*:

Dissolve 1.0g Kenacid Blue R (Coomassie R250) in 800ml methanol and 140ml acetic acid in a 2 litre beaker. Make up to 2 litres with distilled water and stir overnight. Filter through Whatman No. 54 filter paper.

9.4.7 *Destain solution*:

Add 500ml industrial methylated spirits (I.M.S.) to 140ml acetic acid. Make up to 2 litres with distilled water.

9.4.8 *Bromophenol Blue solution (BPB):*

Make a 2mg/ml solution of Bromophenol Blue into distilled water (i.e. 0.2g in 100ml).

9.4.9 *Sample solution buffer (SSB)*:

Mix 62ml distilled water, 10ml 20% SDS, 10ml glycerol and 8ml of 1M Tris/HCl pH6.8.

9.4.10 *Loading buffer (Laemmli)*:

Mix 900µl sample solution buffer (SSB), 100µl of 1M 1,4-Dithiothreitol (DTT) and 20µl Bromophenol Blue solution (BPB).

9.4.11 *Double strength Laemmli*:

Mix 200µl of glycerol, 200µl 1M of 1,4-Dithiothreitol (DTT), 160µl of 1M Tris/HCl pH6.8 buffer, 40µl Bromophenol Blue solution (BPB) and 300µl water.

9.4.12 *Decon 90, 5%:*

Add 50ml Decon 90 to 950ml distilled water. Use for cleaning and treating glass plates to prevent adhesion of the gel to the glass surface.

9.4.13 Molecular weight markers of known composition, prepared in-house.

9.5 Procedure

9.5.1 *Sample preparation*

9.5.1.1 Laemmli extracts: Weigh 10 mg of flour into Eppendorf tubes. Add 500µl of freshly made Laemmli loading buffer to each and vortex. Place the mixtures in a boiling water bath for 3min, allow to cool and vortex. Centrifuge the mixtures for 5min at approximately 12,000 x g. Transfer 45µl extract into another Eppendorf tube and add 15µl Laemmli buffer, vortex and load onto the gel when ready. Remove a microcentrifuge tube of molecular markers from the freezer, thaw, dilute 50µl with 25µl Laemmli buffer and vortex.

9.5.1.2 Liquid samples: Dilute 1:1 (v/v) with double strength Laemmli buffer (5.1.11). Vortex, boil, allow to cool, vortex and centrifuge as described above.

9.5.2 *Gel preparation*

9.5.2.1 Ready Gel Cell (Bio-Rad): Store purchased gels at 2-8°C and use within 3 months or before best before date. Follow instructions with the Ready Gels.

9.5.3 *Casting gradient PAGE gels*

9.5.3.1 Prepare 7.5% and 25% polyacrylamide gels in small beakers from the ingredients in Table 1.

Table 1

Ingredients for 7.5 + 25% gel solutions	25%	7.5%	Ingredients for 5% stacking gel	5% stacking
40% acrylamide	11.3 ml	3.4 ml	30% acrylamide	1.67 ml
3M Tris/HCl pH8.8	2.3 ml	2.3 ml	1M Tris/HCl pH 6.8	1.25 ml
Water	2.7 ml	12.3 ml	Water	7.03 ml
Sucrose	2.7 g	-		

9.5.3.2 Heat solution in boiling water bath for 3 min to enable degassing.

9.5.3.3 Degas by drawing up the warm mixture into a 50ml syringe, blocking the syringe nozzle with a rubber bung and pulling back on the syringe plunger to create a vacuum. Bubbles will appear as air is removed from the mixture, when bubbles stop appearing, return the mixture to its beaker and cool by placing into cold water.

Note: Degassing can be done by hand with a syringe for small volumes of gel (≤10ml), or with a water pump for higher volumes (>10ml). Calculate the volume of gel required to fill the glass plates. Usually prepare 10ml of each concentration.

9.5.3.4 Add the relevant quantities of the ingredients shown in Table 2.

Table 2

Gel ingredients	25%	7.5%	5% stacking
20% sodium dodecyl sulphate (SDS)	90 µl	90µl	50 µl
N,N,N',N'-Tetramethyl-ethylenediamine (TEMED)	12µl	12µl	10 µl
Fresh 60 mg/ml ammonium persulphate (APS)	45µl	68µl	83.3µl

9.5.3.5 Place the outlet of the casting equipment between the plates near the side. Place a comb into the plates and make a mark about one cm under the comb to mark the level of resolving gel required. Remove the comb.

9.5.3.6 Before adding TEMED, APS and 20% SDS, place the casting equipment onto the stirrer.

9.5.3.7 Place a clamp between the two gel chambers and one on the tube(s) leading to the plates.

9.5.3.8 Put a magnet into the first chamber and turn on the stirrer.

9.5.3.9 Add SDS, TEMED and APS.

9.5.3.10 Pour the **exact amount needed of 25% solution (7ml for 2 gels was found suitable in several experiments)** in the second chamber. Open the clamp leading to the first chamber to remove any air bubbles. Once it flows properly, close the clamp between the chambers and put back any solution of 25% into the second chamber.

9.5.3.11 Pour **the exact amount needed of 7.5% solution (7ml for 2 gels was found suitable in several experiments)** into the first chamber.

9.5.3.12 Open the clamp between chambers then the clamp on the outlet. Both chambers should empty at the same pace. If the flow is not even, gently tap on the top of the chambers.

9.5.3.13 Once the entire gel solutions have been poured between the plates, rinse the casting gel equipment with distilled water.

9.5.3.14 Using a Pasteur pipette, add enough alcohol to the top of the gel solution to prevent dehydration during polymerisation and cover with clingfilm to prevent loss of alcohol and moisture. Let the gel polymerise for 45min.

9.5.4 *Adding the stacking gel*

9.5.4.1 Prepare the amount of stacking gel required. Degas and cool.

9.5.4.2 Remove alcohol from the surface of the polymerised gel using a Pasteur pipette.

9.5.4.3 Place a comb between the plates.

9.5.4.4 Add SDS, TEMED and APS to the stacking gel solution

9.5.4.5 Using a Pasteur pipette, add the stacking gel solution, allowing it to flow down the side of one of the spacers. Ensure no bubbles are trapped under the comb.

9.5.4.6 Let the gel polymerise for 30 to 45min.

9.5.5 *Assembling the upper chamber*

Follow the instructions from the relevant Bio-Rad manual.

Note: Ensure a good seal between the gel plates and the green rubber gasket is achieved to prevent leakage of buffer from the inner chamber. Wash and wipe the gasket, then apply a thin layer of vacuum grease or petroleum jelly to form an air-tight seal.

9.5.6 *Assembling the complete apparatus*

9.5.6.1 Place the electrode assembly containing the gel slabs into the tank to form the inner buffer chamber.

9.5.6.2 Prepare 350ml running buffer (for each tank used) by diluting 35ml of 10X Tris/glycerine running buffer with distilled water.

9.5.6.3 Fill the upper chamber with buffer until it reaches a level halfway between the short and long plates.

9.5.6.4 Fill the lower (mini tank) chamber with buffer carefully, pouring it down the inside of the tank to prevent air bubbles from forming (check no air bubbles are trapped at the bottom of the gel otherwise blow out gently using a Pasteur pipette with a curved tip).

9.5.6.5 Remove the comb(s) of the gel(s) if this has not been done already and wash out the wells with running buffer using a Pasteur pipette.

9.5.7 *Loading the samples*

With the Sample Loading Guide in place, load a pre-determined volume of each sample (usually 25µl) and molecular weight markers with a glass-barrelled syringe or an automatic pipette. Rinse syringe with distilled water or running buffer twice between samples.

9.5.8 *Electrophoretic separation*

9.5.8.1 Place the lid on the tank by matching the colours on the lid with the jacks on the electrode assembly to fully enclose the apparatus.

9.5.8.2 Connect the plugs to the power supply.

9.5.8.3 Turn on the power supply and initially run for 15min at 20volts (V).

9.5.8.4 After this time, alter the voltage to 150V.

9.5.8.5 After approximately one hour, the samples should reach the bottom of the gel (indicated by the dye front), it should then be run for a further 15min before switching off the power supply.

9.5.9 *Gel removal*

Follow the instructions from Bio-Rad manual.

9.5.10 *Band detection*

9.5.10.1 Place the gel(s) into 200ml of Coomassie Blue staining solution and leave overnight on a slow shaker.

9.5.10.2 Carefully tip off the stain, rinse with tap water and add 200ml of destain solution, return to the slow shaker.

9.5.10.3 Change the destain once after 2h.

9.5.11 *Band analysis* - see Appendix 13 for details

9.6 Expression of results

Photographic images may be printed from the gels scanned into UVP Phoretix software package.

10. PREPARATION OF A RANGE OF HEAT DAMAGED SAMPLES FROM FOUR VARIETIES OF WINTER WHEAT

10.1 Scope

From the preliminary work carried out on this project, further developments included a sensitivity study to assess different degrees of heat damage on samples of grain. Work focused on glutenins for the detection of heat damage; and more detailed assessment of other tests for heat damage to assess their suitability for use at Grain Intake. The samples collected for this work were as detailed in Table 1 below.

Table 1

Code CM/23430	Specified Variety[1]	Spec.wt., kg/hl	Protein %	Fall. No., s	Wet gluten, %: quality	SDS Volume , ml	Purity (No. of grains of [1] found in 14 tested)
/1	Hereward	81.65	10.2	312	24.7: satisfactory	86	12/14
/2	Soissons	84.16	10.3	311	23.1: rather tough	93	14/14
/3	Hunter	74.86	10.5	345	24.9: weak	55	14/14
/4	Riband	75.57	8.9	226	18.8: slightly weak	46	11/14

Previously, samples of wheat used in heat damage work were either sound or seriously heat damaged. In order to produce samples that are more typical of commercial experience, a range of damage was incurred by subjecting wheat samples to different temperatures for different times. Heat damage is caused by a combination of initial sample moisture content, temperature and the length of time of exposure to raised temperature.

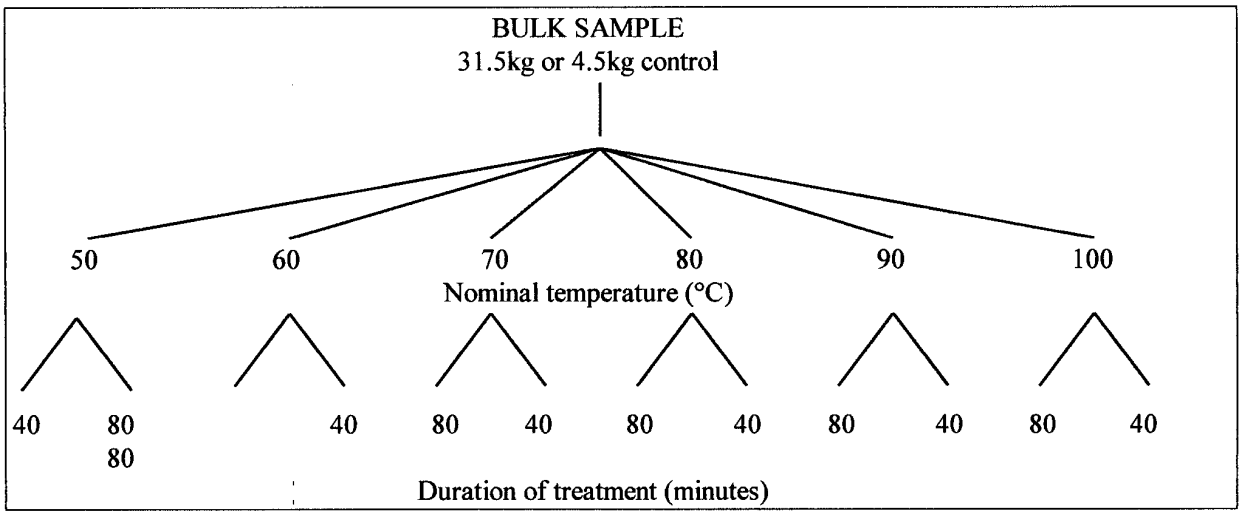
10.2 Principle

The treatment of samples is summarised in Figure 1 overleaf, the control sample received no heat treatment.

10.3 Apparatus

10.3.1 Carter - Day Dockage tester for grain cleaning
10.3.2 Sinar moisture meter (AP 6060 Moisture Analyzer)
10.3.3 Measuring cylinder
10.3.4 Re-sealable plastic bags
10.3.5 Cement mixer used for conditioning grain
10.3.6 Mitchell dryer
10.3.7 Squirrel data logger for recording actual temperature of dryer during treatment.
10.3.8 Chopin mill, small-scale mill used to produce white flour (~60% extraction)
10.3.9 Flour blender

Figure 1



10.4 Procedure

10.4.1 *Grain cleaning and conditioning*

10.4.1.1 Pass each variety through a Dockage tester to clean grain and remove extraneous matter.

10.4.1.2 Weigh out a control sample of 4.5kg and two bulk samples of 13.5kg. Condition the bulk samples to 25.0% moisture content in two stages on consecutive days. Determine the amount of water to be added per kg by measuring the moisture content of the stored bulk samples and using the following equation:

$$\text{Volume required (ml)} = \frac{(M2-M1) \times 1000}{(100-M2)}$$

M1 = % moisture content before conditioning, M2 = required % moisture content

Multiply the amount of water to be added per kg by the weight of the bulk samples to be conditioned (i.e. 13.5kg). The details of the moisture contents and amounts of water added are given in Table 2 below, a Sinar moisture meter was used to measure moisture content except for the final moisture readings which were determined using a two-stage oven drying process.

Table 2. Water required to condition samples to 25% moisture content

Samples	Variety	% moisture	Water added (ml)	Final % moisture
CM/23430/1/3-8	Hereward	14.6	2 x 936	24.7
CM/23430/1/9-14	Hereward	14.6	2 x 936	24.8
CM/23430/2/3-8	Soissons	14.6	2 x 936	24.6
CM/23430/2/9-14	Soissons	14.6	2 x 936	24.6
CM/23430/3/3-8	Hunter	15.0	2 x 900	25.4
CM/23430/3/9-14	Hunter	15.0	2 x 900	25.5
CM/23430/4/3-8	Riband	14.4	2 x 954	25.4
CM/23430/4/9-14	Riband	14.4	2 x 954	25.0

10.4.1.3 Place each bulk sample in turn into a cement mixer, switch on, and add the determined amount of water. Place a lid over the mouth of the mixer to prevent water loss and agitate the grain for 20min.

10.4.1.4 Transfer each sample into a strong plastic bag, seal with a removable clip and leave to equilibrate for a minimum of 22h at 4°C before the second conditioning.

10.4.1.5 After completion of conditioning, leave samples again to equilibrate at 4°C for approximately 24h (± 2h) before subjecting each one to a particular heat treatment.

10.4.2 *Heat treatment*

10.4.2.1 Switch on Mitchell dryer and set to the required temperature.

10.4.2.2 Use a "Squirrel" data-logger to measure and record the actual temperature within the oven. From this data, calculate the mean temperature for each treatment.

10.4.2.3 Divide each bulk sample into six smaller ones. For each of the four varieties used, spread two samples evenly onto metal trays (0.4m x 0.8m), covered with similar but inverted trays. Place

the trays into the preheated Mitchell Dryer at the first temperature setting. Remove one tray of each variety of wheat after 40min, the other after 80min.

10.4.2.4 Reset the oven to the next temperature, allow to warm up for at least 20min.
Repeat steps 10.4.2.2 and 10.4.2.3 until all treatment combinations produced have been.

Note: In an attempt to reduce variation caused by samples being at different positions within the oven, they were assigned different levels for each temperature treatment as detailed in Table 3 (The top of the table represents the top of the oven).

Table 3. Tray plan used in oven treatments

55±5°C	68±4°C	83±5°C	91±6°C	103±6°C	116±7°C
Hereward	Hunter	Riband	Soissons	Hunter	Riband
Soissons	Hereward	Hunter	Hereward	Riband	Hunter
Hunter	Soissons	Soissons	Riband	Hereward	Hereward
Riband	Riband	Hereward	Hunter	Soissons	Soissons

10.4.2.4 Remove samples from the oven and transfer them to a controlled temperature room 21±5°C until their moisture content is ≤ 15.0% according to a Sinar moisture meter.

10.4.2.5 Empty the contents of each tray into a labelled re-sealable plastic bag for ease of storage of samples at 4°C for at least 72h before milling.

10.4.3 *Sample testing*

10.4.3.1 Measure % moisture content of samples using a Sinar meter and condition 1.5kg of each one was by the addition of the amount of water to give 16.0±0.5% moisture content for hard wheats and 15.0±0.5% for soft wheats.

10.4.3.2 Chopin mill the grain at an extraction rate of approximately 70% white flour and blend for 20min to give a uniform mix of the different flour fractions produced.

10.4.3.3 Germination testing of treated whole grain will verify that heat damage has been sustained (see Appendix 11).

11. GERMINATION TESTING OF HEAT DAMAGED WHEAT SEED.

11.1 Scope

For an accurate measure of percentage seed germination, it has been recommended by the Official Seed Testing Station, Huntingdon Road, Cambridge that 400 grains should be used. From the choice of sand, sterile compost or rolled paper towels offered, the latter was used as these were most easily obtainable.

11.2 Principle

Given appropriate conditions and left for seven days, all healthy seeds should germinate. A pre-germination heat- or cold-shock may be required in order to allow germination to occur if seed used has not been over-wintered. In this study, samples had been differentially heat treated as described in Appendices 10 and 27. A germination test was performed to compare the viability of the heat treated and control seed.

11.3 Apparatus

11.3.1 Cleaned grain samples
11.3.2 Paper towels (e.g. Kimwipes™ 25 x 46cm sheets i.e. 4 x number of samples to be tested)
11.3.3 Medium rubber bands (4 x number of sample to be tested)
11.3.4 Four trays
11.3.5 Measuring cylinder
11.3.6 Four large plastic bags
11.3.7 Adhesive tape
11.3.8 Falling Number Numigral seed counter (optional)

11.4 Reagents

11.4.1 Distilled water (15ml x 4 x number of samples to be tested)

11.5 Procedure

11.5.1 From each sample, four sub-samples of 100 grains were counted manually or using grain counter (split grains were replaced with whole ones manually).

11.5.2 Paper towels were folded in half by placing the two shorter edges together, this process was repeated to give a crease mark then the second fold was reopened.

11.5.3 One sub-sample of 100 grains was emptied over half of the towel to one side of the crease, the grains were spread out evenly and covered by refolding the paper towel.

11.5.4 The folded towel was then rolled tightly enough to secure the grains held within it and fastened with a rubber band to prevent any grains escaping.

11.5.5 This process was repeated for each sub-sample i.e. each one was placed on a different tray.

11.5.6 All remaining samples were treated in a similar manner until four replicates of sub-samples were completed. The position of the rolls was recorded and they were carefully kept in order instead of labelling them.

11.5.7 Approximately 15ml of distilled water was measured in a cylinder and poured over each rolled towel. This was repeated for all of the rolls in a given replicate, the tray was placed within a large plastic bag and sealed by folding the excess bag and taping it to itself.

11.5.8 The bag was labelled and placed into a refrigerator at 4°C for 40h then moved to a temperature controlled room at 21±5°C for a further seven days.

11.5.9 Germination was assessed visually as to whether seeds showed normal, abnormal or no growth. Abnormal growth included the leaf breaking through the end of the coleoptile, absence of roots or shoot and retention of the coleoptile under the pericarp. No growth included grains which appeared to have begun to germinate but did not form roots or a shoot and in some cases were infected with fungal or bacterial growth.

11.6 Expression of results

Results were calculated as the mean of the four replicates for each treatment sample and quoted as a percentage.

12. SEPARATION AND ANALYSIS OF PROTEIN FRACTIONS USING SODIUM DODECYL SULPHATE - POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE) - LARGE DUAL SLAB APPARATUS

12.1 Scope

As for Appendix 9.

12.2 Principle

As for Appendix 9.

12.3 Apparatus

As for method shown in Appendix 9 except that the following specialised apparatus may be used:

12.3.1 *Gel cassette*

12.3.1.1 Set of glass plates (inner and outer) x2
12.3.1.2 Large gel spacer (160mm x 165mm x 1.5mm)
12.3.1.3 Bulldog clips x12
12.3.1.4 Small gel spacers (155mm x 6mm x 1.5mm) x4
12.3.1.5 Rubber tubing gaskets x2
12.3.1.6 Medium gel spacer (154mm x 35mm)

12.3.2 *Gel-casting equipment*

12.3.2.1 Two cylindrical chambers connected via a plastic tube. Only one of these chambers has an outlet connected to a hollow needle via another tube.
12.3.2.2 Magnetic stirrer and small magnet
12.3.2.3 Pump
12.3.2.4 Lower buffer chamber and lid
12.3.2.5 Gaskets x2
12.3.2.6 Combs (15 wells: 147 x 36 x 1.5mm) x2

12.3.3 *Power supply*

Electrophoresis Crosspower power supply for supplying a constant voltage across the gel.

12.4 Reagents

12.4.1 *Stock acrylamide, 40%:*

As for Appendix 9.4.

12.4.2 *Stock acrylamide, 30%:*

As for Appendix 9.4.

12.4.3 *Stock Tris/HCl, 3M pH8.8 buffer*:

As for Appendix 9.4.

12.4.4 *Stock Tris/HCl, 1M pH6.8 buffer*:

As for Appendix 9.4.

12.4.5 *Stock Tris/glycine/SDS Running Buffer, (10X concentration)*:

As for Appendix 9.4.

12.4.6 *Coomassie R250 stain solution*:

As for Appendix 9.4.

12.4.7 *Destain solution*:

As for Appendix 9.4.

12.4.8 *Bromophenol Blue solution (BPB)*:

As for Appendix 9.4.

12.4.9 *Sample solution buffer (SSB)*:

As for Appendix 9.4.

12.4.10 *Loading buffer (Laemmli)*:

As for Appendix 9.4.

12.4.11 *Double strength Laemmli*:

As for Appendix 9.4.

12.4.12 *Decon 90, 5%:*

As for Appendix 9.4.

12.4.13 Molecular weight markers of known composition (prepared in house).

12.5 Procedure

12.5.1 *Sample preparation*

12.5.1.1 Laemmli extracts: Weigh 10 mg of flour into Eppendorf tubes. Add 500µl of freshly made Laemmli loading buffer to each and vortex. Place the mixtures in a boiling water bath for 3min, allow to cool and vortex. Centrifuge the mixtures for 5.5min at approximately 12,000 x g. Transfer 45µl extract into another Eppendorf tube and add 15µl Laemmli buffer, vortex and load onto the gel when ready. Remove a microcentrifuge tube of molecular markers from the freezer, thaw, dilute 50µl with 25µl Laemmli buffer and vortex.

12.5.1.2 *Liquid samples*: Dilute 1:1 (v/v) with double strength Laemmli buffer. Vortex, boil for 3min, allow to cool, vortex and centrifuge for 5.5min at approximately 12,000 x g .

12.5.2 Assembly of glass plate sandwiches

12.5.2.1 Soak 2 x large and 2 x small glass plates in 5% Decon 90 overnight, rinse with tap water, then distilled water and wipe dry with a paper towel. Avoid touching the surfaces that are to be next to the gel. Ensure any scratched surfaces are not used as inner plate surfaces. Place the plates on paper towels on the bench and wipe the inner surfaces with I.M.S. using tissue paper.

12.5.2.2 Place the large spacer onto the large glass plate and clip into place.

12.5.2.3 Place the small spacers either side of this ensuring the rounded corners are at the top of the arrangement.

12.5.2.4 After wiping a rubber tube gasket with I.M.S., fix it to the bottom right hand corner of the large spacer with tape. Wrap the tube around the arrangement of spacers and anchor it by placing the small glass plate on top.

12.5.2.5 Clip the apparatus together with two clips on each of the left, right and top sides, ensuring that a good seal has been achieved.

12.5.2.6 Remove the large gel spacer from the unclipped side. Mark a line for the top of the gel using the medium gel spacer. Repeat the process for the second pair of glass plates.

12.5.3 *Casting gradient PAGE gels*

12.5.3.1 Prepare 7.5% and 25% polyacrylamide gels in small beakers from the ingredients in Table 1.

Table 1

Ingredients for 7.5 + 25% gradient	25% gradient	7.5% gradient	Ingredients for stacking gel	5% stacking
40% acrylamide	11.3 ml	3.4 ml	30% acrylamide	1.67 ml
3M TRIS/HCl pH8.8	2.3 ml	2.3 ml	1M TRIS/HCl pH 6.8	1.25 ml
Water	2.7 ml	12.3 ml	Water	7.03 ml
Sucrose	2.7 g	-		

12.5.3.2 Heat solution in boiling water bath for 3 min to enable degassing.

12.5.3.3 Degas by drawing up the warm mixture into a 50ml syringe, blocking the syringe nozzle with a rubber bung and pulling back on the syringe plunger to create a vacuum. Bubbles will appear as air is removed from the mixture, when bubbles stop appearing, return the mixture to its beaker and cool by placing into cold water.

Note: Degassing can be done by hand with a syringe for small volumes of gel (≤10ml), or with a water pump for higher volumes (>10ml). Calculate the volume of gel required to fill the glass plates. Usually prepare 10ml of each concentration.

12.5.3.4 Add the relevant quantities of the ingredients shown in Table 2.

12.5.3.5 Place a hollow needle at the end of the outlet tube from the casting equipment between the plates and next to the right hand side of the apparatus.

12.5.3.6 Place the casting equipment onto the stirrer. Place one clamp between the two gel chambers and another on the outlet tube leading to the prepared gel cassette.

Table 2

Gel Ingredients	25% gradient	7.5% gradient	5% stacking
20% sodium dodecyl sulphate (SDS)	90 µl	90µl	50 µl
N,N,N',N'-Tetramethyl-ethylenediamine (TEMED)	12µl	12µl	10 µl
Fresh 60 mg/ml Ammonium persulphate APS	45µl	68µl	83.3µl

12.5.3.7 Put a magnet into the chamber nearest the gel cassette and turn on the stirrer.

12.5.3.8 Add SDS, TEMED and APS to the gel mixtures.

12.5.3.9 Pour the 25% solution into the chamber furthest from the gel cassette. Squeeze the tube leading from this chamber to the clamp to expel the air in it.

12.5.3.10 Pour the 7.5% solution into the chamber nearest the gel cassette and squeeze out the air from the tube between this chamber and the clamp between the two chambers.

12.5.3.11 Open the clamp on the outlet, if the solution does not begin to flow immediately, cover the top of the chamber nearest the gel cassette to form a seal until the flow begins, then remove the clamp from between the chambers. The magnetic stirrer speed may need reducing to prevent air bubbles from entering the gel.

12.5.3.12 Repeat for the other gel cassette.

12.5.3.13 Once the entire gel solutions have been poured, rinse the gel-casting equipment with distilled water.

12.5.3.14 Gently add a layer of distilled water to the top of each gel and cover loosely with cling film. Allow polymerisation to take place for at least 40min.

12.5.4 *Adding the stacking gel*

12.5.4.1 Prepare the 5% stacking gel, degas and cool.

12.5.4.2 Remove the water previously added to the surface of the polymerised gel by tipping it into a paper towel.

12.5.4.3 Insert a comb between the plates at an angle with the left-hand side lower to avoid trapping bubbles.

12.5.4.4 Add SDS, TEMED and APS to the stacking gel solution

12.5.4.5 Using a Pasteur pipette, apply the stacking gel between the plates from the left-hand side of the gel cassette, ensuring no air bubbles are trapped.

12.5.4.6 Let the gel polymerise for at least 30min.

12.5.5 *Assembling the complete apparatus*

12.5.5.1 Put the upper buffer chamber on top of the support structures and check it is firmly in place with the drain from the top fitting into the collecting tube below.

12.5.5.2 Fill the lower buffer chambers with diluted running buffer and prime the pump.

12.5.5.3 Place each gel plate in turn into a lower chamber, one corner first to prevent trapping bubbles beneath them.

12.5.5.4 Spray both the side of the gel apparatus and a gasket with water to enable them to stick together vertically. Insert the cushioning pads on either side of the comb after dipping them in Vaseline. Spray the gasket and the side of the gel plate next to it with water and squash them together, add clips to hold in place.

Note: Place the two clips at the top of the gel simultaneously to prevent cracking the gel cassette.

12.5.5.5 Mark the bottom of the end wells on the glass. Slowly ease out the comb, first using a knife then by hand; vertically.

12.5.5.6 Fill the wells with tank buffer to the top of the gel using a Pasteur pipette.

12.5.6 *Loading the samples into the large gels*

12.5.6.1 Fill the upper buffer chamber with running buffer.

12.5.6.2 Using an accurate pipette or a Hamilton syringe, load 50µl of each sample into the relevant well.

12.5.7 *Electrophoretic separation using the large gels*

12.5.7.1 Connect the plugs from the power pack to the gel apparatus.

12.5.7.2 Turn on the power supply and check that it reads 70 volts, allow to run continuously for 17h with the pump running.

12.5.8 *Large gel removal*

12.5.8.1 Switch off the power to the gel apparatus and disconnect the plugs. Remove the pump inflow to allow the pump to empty before switching it off.

12.5.8.2 Remove the clips holding the first gel in place and place it on the bench.

12.5.8.3 Ease off the top plate and remove the small side spacers, use the cork borer to mark the running gel with a distinctive pattern for later identification.

12.5.9 *Band detection*

12.5.9.1 Carefully transfer one gel into a flat, plastic container, add 200ml of Coomassie Blue stain and put on slow shaker for 6h.

12.5.9.2 Repeat for the other gel.

12.5.9.3 Carefully discard the stain, rinse the gels with tap water then add 200ml of destain solution to each. Return to the slow shaker and leave overnight.

12.5.9.4 Repeat 12.5.9.3, leaving the gels to destain for approximately 5h.

12.5.9.5 Store the gels in a sealed container with destain containing approximately 10% glycerol indefinitely.

12.6 Expression of results

As photographic images produced by scanning the gels into Phoretix software package "Gelscan 1D Advanced" (See Appendix 13).

13. SCANNING PROTEIN ELECTROPHORETIC GELS AND ANALYSIS OF THEIR BANDING PATTERNS USING GELSCAN 1D ADVANCED

13.1 Scope

Semi-transparent materials on which banding patterns have been produced may be analysed using this method. The image capture facility of the apparatus is able to store images of completed electrophoresis gels showing protein bands (See Appendices 9 and 12). Print outs may then be made of the image. Banding pattern analysis can be made in the associated software: Gelscan 1D Advanced.

13.2 Principle

Comparisons between gel lanes are based on the optical density measured along their lengths once an image has been scanned into the computer. Light and dark areas are converted into peaks and troughs on a graph; the peaks correspond to the higher intensities of light from an inverted image of the gel. An accurate measurement is given of the locations of each band in a given lane on a gel. From the relative positions of the bands, Rf (retardation factor) values can be compared with those produced by molecular weight markers and molecular weights can be calculated.

13.3 Apparatus

13.3.1 UVP - GDS8000 integrated system comprising:
Transilluminator TW-43 white light (230V, 50Hz, 0.80amps)
UVP camera with zoom lens attached to Torch/UVP Pentium computer
Grab-It 2.5 (image capture) and Advanced gelworks 1D (image analysis) software
Sony digital graphic printer UP-D860E

13.4 Procedure

Refer to the user's guide to UPV- GDS8000 manual for detailed description of gel scanning, background correction and peak quantification.

13.5 Expression of results

Images of complete gels, comparisons of several lanes, individual lanes or individual bands can be printed out. Also, data recorded can be transferred into spreadsheets for further analysis.

14. CAPILLARY ELECTROPHORESIS FOR THE ANALYSIS OF GLIADINS IN WHEAT

14.1 Scope

This method is used to identify gliadin proteins in wheat grain extracts (0.25g).

14.2 Principle

Capillary electrophoresis involves the movement of molecules through a capillary subjected to an electrical field. Sample molecules are introduced hydrodynamically (utilising pressure differences) or electrokinetically (using electrical pull to draw in the sample). Separation is based on the charge to mass ratios of the constituent molecules, with the most positively charged species migrating the fastest.

Once a sample is injected, the end of the capillary is removed from the sample reservoir and immersed in the running buffer. As the sample migrates through the capillary, an in-line detector monitors the presence or absence of various constituents. In an automated capillary electrophoresis system, the detector is built-in, which eliminates the need for connections to external monitors and prevents the re-mixing of closely migrating bands. A prinout consisting of a series of peaks and troughs to represents separated gliadins within the samples being tested.

14.3 Apparatus

14.3.1 Spectraphoresis 1000 (Capillary electrophoresis instrumentation)
14.3.2 Capillary cassette comprising a bobbin assembly that holds the capillary, the electrodes and the lens assembly
14.3.3 Sample vials (2ml capacity)
14.3.4 Automatic pipettes
14.3.5 Vial caps
14.3.6 Conical flask with side spout
14.3.7 Sonicator

14.4 Reagents

14.4.1 For extraction: 30ml ethanol and 70ml distilled water.
14.4.2 Phosphoric acid: 1.153ml of 1M Orthophosphoric acid plus 8.847ml of distilled water. Adjust to pH 2.5 with concentrated NaOH before making up to final volume
14.4.3 Phosphate/acetonitrile buffer: 20ml acetonitrile (methyl cyanide) added to 80ml Bio-Rad HPE 0.1M
14.4.4 Phosphate buffer (catalogue no. 148-5010). Sonicated for 20min prior to use. Adjusted to pH 2.5 with concentrated HCl before making up to final volume.
14.4.5 Sodium hydroxide (0.1M): 0.08g sodium hydroxide added to 20ml distilled water.

14.5 Procedure

14.5.1 Switch on the power to the computer and interface.

14.5.2 Add 1ml 30% ethanol to 25mg grain (ground in a coffee grinder) in a centrifuge tube. Vortex briefly, hold in sonicator for 5min, centrifuge for 2min on maximum speed.

14.5.3 Slowly filter the grain extract supernatant into a plastic insert with a 1ml syringe (take apart to avoid rupturing filter) and filter through a 0.2µm filter. Place into a plastic vial.

14.5.4 Activate the electrophoresis display by pressing the "select" key on the front panel until the green light next to the lower (electrophoresis) display is lit.

14.5.5 Press "Stby" on the front panel to open the auto-sampler drawer. Wait for a loud click indicating the drawer latch has been released. Then pull the drawer out.

14.5.6 Place the vials containing buffers into the following positions:

Vial 1.01 = phosphate buffer (change every few runs as electrolytes deplete)
Vial 1.02 = 1M phosphoric acid
Vial 1.03 = 0.1M NaOH
Vial 1.04 = grain extract
Vial 1.05 = 0.1M NaOH
Vial 1.06 = water
Vial 1.0 = air

14.5.7 Place degassed phosphate buffer containing acetonitrile in main jar positioned at the front of the autosampler (sonicated for 20min prior to use).

14.5.8 Access "software 100 V1.5", "Inst. Control", "Manual".

14.5.9 For the standard wash prior to use, select "edit wash cycle" and programme the following parameters:

Vial number	Duration (min)	Temperature (°C)	Description
1.05	1	40	NaOH
1.02	30	40	1M phosphoric acid
1.03	10	40	0.1M phosphate buffer
1.01	20	40	Running buffer

Use HPLC grade water in the buffer reservoir.
Press "Return" when complete.

14.5.10 To run a set of samples, having selected "Manual", choose method "GLIA19.MCE" and "Activate". Add sample to carousel in vial number 1.04. Replace running buffer for each new sample as the electrolytes will have depleted.

14.5.11 After completing sample readings, flush with water then air for 2min each, then flush the vacuum system by initiating five 0.1min degas cycles from the "Do" line of the "Run" menu (accessed from the instrument control panel with the computer switched off).

14.6 Expression of results

Peaks observed on the trace indicate the presence of gliadin proteins. The faster moving protein molecules i.e. small and most positively charged appear first on the trace. The CE trace records Absorbance at 200mm against peak elution time.

15. DETERMINATION OF WHEAT GRAIN SIZE BY IMAGE ANALYSIS

15.1 Scope

This method is applicable to clean samples of wheat. Calibration is carried out as part of the procedure.

15.2 Principle

An image of grains against a back-lit transparent background exhibits contrast between dark grains, and a bright background. Use of a video camera and digitising hardware represents these brightnesses as values of different magnitude in computer memory. Image analysis software can identify values considered to represent grains (thresholding) and determine their number and arrangement. These can be used to calculate grain dimensions if the scaling between distance and number of pixels is known, according to a calibration.

15.3 Apparatus

15.3.1 PC computer with installed Sprynt frame grabber board and Semper software.
15.3.2 JVC video camera model TK870E with 18-108 mm zoom lens (No. 4530283).
15.3.3 Cootes vibratory feeder fitted with custom made grooved perspex sample tray.
15.3.4 Kaiser copy stand
15.3.5 Standard set of 11 calibration aluminium cylinders (always use the same set, for which the true mean size has been incorporated into the program).
15.3.6 Transparent plastic ruler
15.3.7 Soft brush
15.3.8 T-square

15.4 Procedure

In some instances, it may be necessary to stop the program, which can be achieved by pressing the *Escape* key. If it is required to re-run the program, you should first type the commands:

Deassign device 5 [*Ctrl* + *Enter*]
Delete 1,99 [*Ctrl* + *Enter*]

15.4.1 Run the application program *SEMPER* from *WINDOWS*, by double clicking on its icon with the mouse. You will be prompted for the video source in use. If the camera is connected to the computer via a Spectra decoder, type 3 to select the *Spectra-rgb* source. If it is connected directly to the computer, type 1 to select the *JVC-rgb* source.

15.4.2 Type *lib wheat* at the command prompt and press the *Ctrl* and *Enter* keys together to execute the command. You will be prompted for a file number. Enter a value, (*nnn*) in the range 1 to 999. The data will be stored in a file named *Wheat.nnn*. If this file already exists, you will be prompted for an alternative number.

15.4.3 A live colour image will be displayed on one of the monitors, with coloured bands representing positions of constant image brightness. Position the camera at the top of the copy stand. Use the T-square to adjust its angle such that it points in a direction perpendicular to the surface of the perspex sample tray, and directed at the polished part of its length.

15.4.4 Adjust the zoom of the camera lens, such that the inner 11 tracks of the tray are visible within the outline drawn on the monitor, and run horizontally across it, parallel to the top and bottom edges of the image. (If the tracks cannot be clearly seen, try placing a calibration cylinder or wheat grain in a given track to see whether it is visible on the monitor). Ensure that the image of a wheat grain placed on any of these tracks does not touch the top or bottom of the rectangular window, and has bright background between itself and both of these edges. Place the feet of the feeder on pieces of blu-tak to prevent it moving during use.

15.4.5 Focus the camera lens until the image of a calibration cylinder placed on the tray is maximally sharp as viewed on the monitor. You may need to adjust the brightness of the image using the lens aperture

adjustment to assist in judging the point of best focus. Repeat steps 15.4.4 and 15.4.5 until the criteria of both are simultaneously satisfied. Press down on the feeder to hold it firmly in place on the blu-tak. Check that the criteria of 15.4.3, 15.4.4 and 15.4.5 are still satisfied, and readjust if necessary.

15.4.6 Clean the area of the sample tray visible to the camera using a soft brush. Adjust the brightness of the image by adjusting the camera aperture until the predominant colour of the image of the back-lit tray is royal blue. There should be hardly any pink representing brighter regions (typically towards the centre of the image).

15.4.7 Click on the left mouse button to stop the live image and store it as the background.

15.4.8 If the program is being run for the first time since starting *SEMPER*, it will require calibration. Initially, the program requires a measurement of the scale of the image. Provide this by placing a ruler on grooved surface of the sample tray. Use the mouse to place the cross hair in turn over two points on the image a known distance (at least 90 mm) apart. Click the left mouse button to identify each of the points. When prompted, type the distance between the points in units of millimetres (type the number only, not the units) and press *Enter*. The computer will display a scaling factor that represents the separation of adjacent pixels in millimetres. Record this value.

15.4.9 The boundary of objects on screen typically shows a gradient of brightness, and the apparent size of an object therefore depends on the brightness identified as representing its true boundary. This is identified by a calibration procedure: Place each of the 11 aluminium calibration cylinders in a different one of the 11 grooves visible within the rectangular window, such that the images of the cylinders are centrally placed on the vertical centre line drawn on the image.

15.4.10 Click the left mouse button. The program will calculate the mean area of the cylinders, using the current threshold, and the amount by which it exceeds the true value. Type 0 to continue calibration using an adjusted threshold and continue doing so when prompted until the integer threshold is identified which gives the minimum deviation of mean area (this should be less than 0.001 mm^2).

15.4.11 When a satisfactory threshold has been adopted, type 1 when prompted to accept the current threshold.

15.4.12 The system is now ready to measure a sample. When prompted, type the minimum number of grains to be measured. If sufficient grains are available, a minimum of 400 grains should normally be chosen (see Repeatability and Reproducibility section). Fill the hopper of the feeder with the grain to be measured.

15.4.13 Switch on the feeder allowing grain to feed along the grooves into the field of view. Ensure that the grain feeds uniformly from the entire width of the hopper, at a rate such that grains lie within the grooves, and do not abut between adjacent grooves. Adjustments can be made to the width of the gate through which the grain feeds, the amplitude of vibration (for which the minimum setting is usually appropriate) and the position of the nodes of vibration according to the position of a weight, which is normally most appropriate at the end of the tray furthest from the hopper. Grains can be re-fed until the settings are appropriate. The blu-tak under the feeder should ensure that it does not move across the bench during measurement. Ensure that this is the case by checking for misalignment of the image of the tray on the monitor.

15.4.14 When the feeder is appropriately adjusted, move the grains back to the hopper, clean the tray with a soft brush, and feed grains onto the tray for measurement. Turn off the feeder when sufficient grains are in view. Some grains may move beyond the field of view. If it is required that every grain be measured (eg for small samples), return such grains to the hopper. Remove any pieces of chaff or other foreign material if necessary.

15.4.15 Click the left mouse button to make a measurement. The image on screen will change in various ways as processing proceeds. You will be presented with an image in which objects considered to be single grains, suitable for measurement are coloured green. Objects considered as possible broken or double grain images will be identified in red. (Initially, the computer will not attempt to reject any grain images automatically. However, after sufficient grains have been measured, the computer will 'learn' the characteristics of the sample and mark dissimilar objects as candidates for rejection).

15.4.16 If you wish to change the designation of any of the identified objects, click on them in turn with the left mouse button. Depending on the purpose of the measurement, it may be required either to include broken grains or to reject them. The decision made for each sample or group of samples should be recorded. When all objects required for measurement are green and all those to be rejected are red, click on the background to continue.

15.4.17 The computer will perform further processing, and will briefly colour the measured grains in distinct colours, labelled with a number (the Area / mm^2), and a cross hair identifying orientation. If the software frequently fails to separate the images of touching grains correctly, the feeder should be adjusted to ensure a lower density of feed, the analysis stopped (see start of Appendix 15.4), and the measurement repeated from step 15.4.2. In such an event, the existing calibration will be used, and you will not be prompted to repeat the calibration.

15.4.18 When a live image is displayed once more, move measured grains beyond the measurement region of the tray to prevent them being re-analysed, and repeat steps 15.4.14 to 15.4.17. Continue until the program indicates that sufficient grains have been measured. At this point, it will display some results and then stop.

15.4.19 The number of grains measured will be displayed. Record this value. The program will also display the mean and standard deviations of the grain measurements: Length (mm), Breadth (mm), Perimeter (mm) and Area (mm^2). Record these to 2 decimal places precision. The name of the output file will also be displayed. If desired, rename the file using WINDOWS or DOS. Record the filename finally adopted.

15.5 Expression of Results

The following values should be recorded for each sample:

a) Scaling factor (the separation of adjacent pixels), in units of millimetres.
b) Threshold value
c) Number of grains - an integer
d) Filename of stored data.
e) Mean and standard deviation for each of the parameters: length (mm), breadth (mm), area (mm^2) and perimeter (mm) to 2 decimal places each.

For each sample or group of samples, any policy on the inclusion or rejection of grains from the data set should be recorded. For example, this will normally be a choice between rejecting or including broken grains.

15.6 Repeatability and Reproducibility

The repeatability of the mean value of each of the measured parameters is affected by the number of grains measured. Measurements of a set of 37 UK wheat samples gave standard deviations of grain area of 2.61 to 5.01 mm^2. The majority (30) had a standard deviation $\sigma < 4.00$ mm^2, suggesting that the error in the mean resulting from sampling will be approximately

Equation 1

$$\sigma_{mean} = \frac{\sigma}{\sqrt{N}}$$

where N is the number of grains measured. A minimum of 400 grains is recommended, for which this calculation gives an estimated standard error of $\sigma_{mean} = 4.00/(400)^{1/2} = 0.2$ mm^2, which represents approximately 3% of the range of mean areas (13.38 to 20.34 mm^2) measured for the set of UK samples. Increased numbers of grains can be expected to reduce the sampling error in the mean, and decreased numbers of grains to increase it, approximately in accordance with Equation 1. In addition to sampling errors, errors due to the measurement technique can also be expected.

Repeat measurements of at least 400 grains, drawn separately on each occasion from a common 50 g sample gave the following results. Measurements were made consecutively on a single day by 2 operators, but the apparatus was deliberately realigned between measurements, and was readjusted and re-calibrated on each occasion:

Operator	Number of grains	Area / mm^2 Mean (Std. Dev. for single grains)	Length / mm Mean (Std. Dev.)	Breadth / mm Mean (Std. Dev.)	Perimeter / mm Mean (Std. Dev.)
1		18.97 (2.94)	6.56 (0.51)	3.77 (0.37)	16.68 (1.41)
	445	19.20 (2.88)	6.57 (0.46)	3.79 (0.35)	16.77 (1.24)
	425	19.04 (2.85)	6.54 (0.47)	3.78 (0.35)	16.67 (1.23)
2	420	19.48 (2.96)	6.62 (0.56)	3.81 (0.34)	16.88 (1.45)
	426	19.17 (2.68)	6.56 (0.41)	3.80 (0.34)	16.76 (1.12)
	449	19.42 (3.05)	6.60 (0.59)	3.82 (0.33)	16.91 (1.50)

One possible source of variations between operators is the subjectivity of identifying broken grains for rejection, and the possibility of failing to notice and reject such grains or double grain images not already identified automatically. For example, each mistaken inclusion of a double grain image in a sample of 400 grains affects the mean area by approximately 0.25%.

15.7 Quality Control

Unless deliberately bypassed, a calibration procedure is executed when the programme is first run. This is designed to account for magnification and illumination brightness.

16. DUROTEST P ASSAY FRIABILIN WITHIN HEAT DAMAGED SAMPLES

16.1 Scope

A rapid test to verify the purity of durum wheat products. Adulteration by *T. aestivum* wheat flour is detected using the Durotest P kit. This is possible due to the presence of the protein friabilin within common wheats but not in durum wheats. An attempt was made to extend this scope by running the assay on heat damaged samples in an attempt to detect differences between the differently treated samples.

16.2 Principle

A monoclonal antibody that is specific for the protein friabilin is used. This protein is present only in non-durum wheat. Samples of pasta are extracted and adsorbed onto the surface of a micro-titre plate overnight. The wells are washed, and the monoclonal antibody labelled enzyme is added which binds to the protein friabilin (if present within the sample). The wells are washed again and a clear coloured substrate added. A coloured product forms in the presence of a friabilin/antibody immune complex. The reaction is stopped with acid and the absorbance of each sample is read at a wavelength of 450nm. The level of adulteration of samples can be calculated by comparisons of results with those from standards.

16.3 Apparatus

16.3.1 Test kit provided contained:

• Microwell plate	x1
• Extraction buffer at working strength	100ml
• Wash buffer x10 concentrate	100ml
• Antibody - HRP concentrate	100µl **NB. Store at -20°C**
• Coating buffer x10 concentrate	100ml
• TMB reagent	15ml
• Stopping solution (2% sulphuric acid)	15ml
• Casein	3g
• Microcentrifuge tubes	x100

16.3.2 Measuring cylinders for dilution of extraction and wash buffer concentrates
16.3.3 Microcentrifuge
16.3.4 Pipettes 5ml, 1-5ml
16.3.5 Pipette tips
16.3.6 Wash bottle (for washing plate)
16.3.7 UV spectrophotometer (280nm)
16.3.8 Microplate reader (450nm)

16.4 Reagents

16.4.1 Extraction buffer (provided ready for use)

16.4.2 Coating buffer diluted 1:10 (v/v) with distilled water (64ml made up to 640ml per plate)

16.4.3 Wash buffer diluted 1:10 (v/v) with distilled water (50ml made up to 500ml)

16.4.4 Conjugate diluent buffer: 200mg casein plus 20ml diluted wash buffer

16.4.5 Conjugate: antibody - HRP concentrate diluted 1 in 3000 with conjugate diluent buffer (5µl made up to 15ml)

16.4.6 TMB reagent (provided ready for use)

16.4.7 Stopping solution (2% sulphuric acid v/v)

16.5 Procedure

16.5.1 *Day 1*

a) Make up diluted coating buffer.
b) Weigh 2mg white flour samples, with and without 98mg durum flour, into individual microcentrifuge tubes (labelled and dated).
c) Add 1ml extraction buffer to each sample and vortex to suspend sample.
d) Place the samples in a rack with a lid secured on top, on a shaker for 30min at room temperature vortex setting 4.
e) Microcentrifuge samples for 3min at high speed.
f) Transfer 400µl supernatant from each tube to a test-tube and make up to 6ml with coating buffer.
g) Read absorbance of diluted samples at 280nm in quartz cuvettes, using a solution of coating buffer as a blank. Change samples using a Pasteur pipette.
h) Normalise the protein concentration of all samples by diluting them according to the equation below:

$$\frac{\text{Lowest sample OD or 0.25}}{\text{Sample OD (to be diluted)}} \times 1\text{ml} = \text{volume of sample to be diluted}$$

The volume of coating buffer required is 1ml - volume of sample to be diluted.

1. Add 100µl sample to each well of the microtitre plate.
2. Incubate the plate overnight at room temperature.

16.5.2 *Day 2*

a) Make up diluted wash buffer, conjugate diluent buffer and conjugate.
b) Discard the plate contents to waste.
c) Wash each well with a working solution of wash buffer using the wash bottle to apply approximately the same amount to each well. Repeat the washing process a further three times then strike the plate face down several times on a paper towel placed on a flat surface.
d) Add 100µl conjugate to each well and cover the plate with cling film to prevent evaporation of the samples. Incubate at 37°C for 90min.
e) Wash the plate six times as previously before striking it face down several times on a paper towel placed on a flat surface.
f) Dispense 100µl TMB reagent into each well and incubate for 20min at room temperature.
g) Stop the reaction by adding 100µl stopping solution to each well.
h) Measure the absorbance at 450nm using a microplate reader and compare results to controls for each variety.

16.6 Expression of results

Take the means of absorbance readings for each sample.

17. USE OF THE GERMOGRAPH EASI-TWIN 2 POT FOR TESTING GRAIN VIABILITY

17.1 Scope

The apparatus can be used for germination testing of seeds using a solution of tetrazolium salts for embryo staining to give an indication of their germination percentages, pre-germination using copper sulphate solution and acrospire profile testing on malt.

17.2 Principle

By exposing the embryo of a seed to the tetrazolium salt solution, penetration of the solution into the tissue is allowed. By increasing the temperature and applying a vacuum, the process of tissue infiltration is accelerated. Therefore, instead of soaking the grains for 16 hours as in standard grain viability tests, results may be achieved in 10-15 minutes.

17.3 Apparatus

17.3.1 Germograph Easi-Twin 2 pot heating, vacuum inducing and timing device.
17.3.2 Scalpel or knife to split grain lengthways.
17.3.3 Binocular dissecting microscope capable of a total magnification of x40.
17.3.4 Magnetic stirrer.

17.4 Reagents

17.4.1 Saturated tetrazolium salt solution stored in an amber glass bottle at 2-8°C
Weigh 1.00g p-iodonitrotetrazolium violet into a 300ml amber glass bottle, add 200ml distilled water and mix on a magnetic stirrer for approx. 1h **without** heating. Add a further 100ml distilled water and mix for another minute.

17.5 Procedure

17.5.1 Switch on the power at the mains and using the switch at the rear of the instrument, the temperature will be displayed and will begin rising to 40°C or the temperature which has been set.

17.5.2 Prepare grain from the first sample to be tested. Randomly take 50 grains, cut them in half lengthways to expose the embryo, discard one half of each (or keep separately to test as a repeat set).

17.5.3 Once the set temperature has been achieved, check the settings of the instrument are 10min, 15mmHg and 40°C and alter them if they are not.

a) To check the settings, press down both the MUTE and STOP buttons simultaneously for a couple of seconds until the display shows the time setting.
b) If the time setting is not 10min, alter it by pressing the STOP button to increase the selected digit or select the next digit along by pressing the START button, then altering it using the stop button.
c) Once the time setting has been checked or set, press the MUTE button to change the display to another parameter and remain in the set mode.
d) Check or alter the other settings as necessary, exit the set mode by pressing the MUTE and STOP buttons simultaneously.

17.5.4 Remove the perforated seed holder from the pot and add approximately 15ml tetrazolium salt solution.

17.5.5 Replace the seed holder, place the lid on the pot and allow 5min for the temperature to stabilise.

17.5.6 Place the sample of split kernels into the perforated seed holder and gently lower it into the solution.

17.5.7 Replace the lid, and while gently pressing it, press the START button to begin the test, continue to hold the lid for 5s until the vacuum is established.

17.5.8 The test will then proceed automatically for 10min.

17.5.9 At the end of the run, an alarm will sound, to stop it, press the mute button.

17.5.10 To release the vacuum, depress the button on the top panel

17.5.11 Remove the samples from the pots for examination.

17.6 Expression of results

Record the number of grains that show the embryo region stained deep crimson red and convert value to a percentage. Record pink colour produced in any wheat embryo under test.

18. DETERMINATION OF GLUTEN CONTENT USING THE FALLING NUMBER GLUTOMATIC

(Derived from the manufacturer's manual and in part ICC Standard Method No. 137/1)

18.1 Scope

This method is applicable to ground wheat and wheat flours.

18.2 Principle

A dough is prepared from a sample of flour or ground wheat and a buffered solution of sodium chloride. Wet gluten is isolated by washing this dough with buffered sodium chloride solution. After removal of excess water, the gluten is either weighed or dried and weighed according to whether wet gluten or dry gluten content is required.

18.3 Apparatus

All measuring equipment must be calibrated.

18.3.1 Falling Number Glutomatic and Centrifuge
The Glutomatic is factory set according to the ICC Standard Method No. 137 i.e. A dough mixing time of 20 seconds and a washing time of 5 minutes. These must be used whenever possible, alteration will change results.
The sieve-hook distance must be 0.7 +/-0.05mm.

18.3.2 Gluten washing chambers with chromed sieve holders and interchangeable 80µm metal sieves for samples of white flour. Black chromate holders with 800µm metal sieves are also required when testing brown or wholemeal flours and ground wheat.

Note: New sieves should be cleaned with an ordinary household detergent and rinsed with water before use.

18.3.3 Adjustable dispenser for buffered salt solution, set to deliver 5.0ml.

18.3.4 Plastic container for buffered salt solution, 10 litre.

Note: After filling the container, connect to Glutomatic and run the apparatus for 1-2 minutes to ensure that the system is full of salt solution.

18.3.5 Beaker, 500 ml to collect washings.

18.3.6 Wash bottle of distilled or deionised water.

18.3.7 Balances: one capable of weighing at least 50g to at least 2 decimal places, second capable of weighing at least 200g.

18.3.8 Glutork (see * below).

18.3.9 Timer, capable of reading in intervals of at least 1 second (see * below).

18.3.10 Mill, (See♦ below). Gluten formation and washing are effected by the particle size of the ground wheat. The mill used must produce ground wheat complying with the following specification.

Aperture of sieve	% of sample passing through sieve
710 micrometers	100
500 micrometers	95 – 100
210-200 micrometers	80 or less

The particle size distribution should be checked regularly, using a well-mixed sample of ground grain, as described in Flour Testing Working Group Method No. 0011 Sieving tests using a laboratory sifter.

* The Glutork specified in 18.3.8 is only required for the determination of dry gluten content and the timer specified in 18.3.9 is only required if the Glutork is not fitted with one.

♦ The mill specified in 18.3.10 is only required when testing wheat.

18.4 Reagents

18.4.1 Sodium chloride solution, 2% w/v buffered to pH 5.95:
Dissolve 200g sodium chloride, 7.45g potassium dihydrogen phosphate and 2.46g disodium hydrogen phosphate dihydrate in 10 litres of distilled or deionised water. Check the pH and adjust if necessary.
A concentrated stock solution may be purchased. This stock solution has a stated shelf life of 12 months and is stamped with a "use before" date. A fresh working solution must be prepared daily.

18.5 Procedure

If dry gluten content is required the closed Glutork should be turned on at the same time as the Glutomatic is started.

18.5.1 *White Flour Samples*

18.5.1.1 The milling method used to prepare white flours from wheat samples influences the results of the gluten determination. Different mills produce different flour fractions of different composition. To obtain comparable results the same method of sample preparation must be used. The milling procedure used to produce white flour should be reported together with percent gluten.

18.5.1.2 Before starting the first test, use the wash bottle to place a few drops of water in the hole in the front of the Perspex body of the mixing head. This need not be done when testing further samples in the same test run providing tests are carried out without delay.

18.5.1.3 Assemble the gluten washing chamber with a fine metal sieve carefully positioned between the end of the Perspex chamber and the perforated steel base.

18.5.1.4 From the capillary water bridge to prevent flour losses by thoroughly moistening the sieve. Remove excess water by holding a cloth in one hand and knocking the gluten washing chamber gently to spread the flour evenly.

18.5.1.5 Weigh out 10.00g of flour and transfer into the gluten washing chamber. Shake the chamber gently to spread the flour evenly.

18.5.1.6 Check that the dispenser is primed with buffer salt solution; this may be done by pumping two volumes to waste.

18.5.1.7 Hold the gluten washing chamber at a slight angle and use the dispenser to add 5.0ml of salt solution of the flour sample; direct the stream of salt solution against the plastic side wall of the chamber so that it does not go through the sieve. Shake the gluten washing chamber gently so that the salt solution is spread evenly over the flour.

18.5.1.8 Place the gluten washing chamber in position and fix in the bayonet fastening.

18.5.1.9 Press the green 'start' button. The mixing and washing sequences proceed automatically.

18.5.1.10 If at any time during the washing sequence it is noticed that the sample has become attached to some part of the gluten washing chamber or kneading assembly, the program should be stopped by pressing the yellow 'hold' button, the chamber lowered and the sample freed. The washing sequence may be re-started a) from the point at which it was halted by pressing the 'hold' button again and b) from the beginning by pressing the 'ON/OFF' button.

18.5.1.11 At the end of the washing sequence the Glutomatic stops and the green light comes on.

18.5.1.12 Remove the gluten washing chamber and take out the wet gluten. Ensure that no gluten is left on the hook or the Perspex mixing body.

18.5.1.13 After each test the interchangeable metal sieves should be cleaned by rinsing under running water; this can normally be done without dismantling the gluten washing chamber. The Perspex mixing body and hook should be also be wiped with a clean cloth.

Note: It is recommended that the hook be cleaned with fresh water and dried before the apparatus is left to stand for any period of time, otherwise there is a danger that, in, time, the hook will separate at the welded joint.

18.5.2 *Brown, wholemeal flours and ground wheat*

18.5.2.1 Grind a representative sample of wheat in the same manner as for the Falling Number determination (FWTG No. 0006). The mill must be fed carefully with wheat to avoid heating and overloading. Grinding should be continued for 30-40 seconds after the last of the sample has entered the mill. Small quantities (up to 1% of the ground wheat must be carefully mixed before use.

18.5.2.2 Carry out the actions described in 5.1.1 to 5.1.7

18.5.2.3 Press the red 'wash' button and then the green 'start' button. The illuminated red 'wash' button will start to blink. After completing the mixing sequence plus 2 minutes washing the Glutomatic will stop with both the yellow 'hold' button and the red 'wash' buttons illuminated.

18.5.2.4 Remove the gluten washing chamber with the partially washed out gluten and transfer the total contents, including bran particles, into a gluten washing chamber containing a coarse sieve. This may be done by placing the test chambers open end to open end with the fine sieve uppermost under gently running water.

18.5.2.5 Place the gluten washing chamber with the coarse sieve, containing the transferred gluten ball, in the working position and fix in the bayonet fastening. Press the red 'wash' button. The remainder of the washing sequence will now be completed.

18.5.2.6 If at any time during the washing sequence it is noticed that the sample has become attached to same part of the gluten washing chamber or kneading assembly, the program should be stopped by pressing the yellow 'hold' button, the chamber lowered and the sample freed. The washing sequence may be re-started a) from the point at which it was halted by pressing the hold' button again and b) from the beginning by pressing the 'ON/OFF' button.

18.5.2.7 At the end of the washing sequence the Glutomatic stops and the green light comes on.

18.5.2.8 Remove the gluten washing chamber and take out the wet gluten. Ensure that no gluten is left on the hook or the Perspex mixing body.

18.5.2.9 After each test the interchangeable metal sieves should be cleaned by rinsing under running water, this can normally be done without dismantling the gluten washing chamber. The Perspex mixing body and hook should also be wiped clean with a cloth.

Note: It is recommended that the hook be cleaned with fresh water and dried before the apparatus is left to stand for any period of time. Otherwise there is a danger that, in time, the hook will separate at the weld joint.

18.5.3 *Removal of excess water*

18.5.3.1 Divide the wet gluten into two pieces of approximately equal weight. Open the centrifuge lid and place the pieces of wet gluten on the holding pins in front of the screens.

18.5.3.2 Close the centrifuge lid and press the green 'start' button. The centrifuge will start and run for 1 minute, then stop automatically. The yellow lamp is lit while the centrifuge is running and an audible signal indicates when centrifugation is complete.

18.5.3.3 Take out the centrifuge sieves, remove the wet gluten from them and weigh the gluten with an accuracy of 0.01g. Ensure that no gluten is left in the centrifuge.

18.5.4 *Dry gluten content*

Dry gluten content may be determined using the Glutork as follows:

18.5.4.1 Turning on the Glutork

a) Press the red button on the timer unit of the Glutork at the same tine as the Glutomatic is started; this allows time for the Glutork to warm up during the mixing and washing sequence. The timer turns off the heater after 4 minutes, but the temperature remains at approximately 150°C providing the Glutork is kept closed. The green light indicates that the operating temperature has been reached. The red light will go on and off as the thermostat operates.

b) If the Glutork is not fitted with a timer, allow 10 minutes for warming up. The Glutork should be in the closed position. Do not leave the Glutork in the open position with the power on for long periods of time. Switch off when not in use.

18.5.4.2 Open the Glutork and place the wet gluten in the centre of the lower plate. Take care not to damage the Teflon coating. Close the Glutork and press the red button again to start the timer. If a timer is not fitted, drying must be timed by means of a separate timer.

18.5.4.3 After 4 minutes, drying is completed and the red light goes out. Open the Glutork, remove the dry gluten, taking care not to damage the Teflon coating, and weigh with an accuracy of 0.01g. Close the Glutork.

18.6 Expression of results

The percent gluten content may be calculated by the use of the following equations:

Wet gluten content, % = weight of wet gluten x 10
Dry gluten content, % = weight of dry gluten x 10

18.7 Repeatability and reproducibility

Repeatability and reproducibility figures for the wet gluten content (%) of wheat and flour are as follows:

	Repeatability	**Reproducibility**
Ground wheat	4.86	10.38
Flour	2.48	4.24

No values are presented for dry gluten

18.8 References

S.SALMON (1997) Manual of Methods for Wheat and Flour Testing : CCFRA
No. 0006 - Grinding a representative sample of wheat (In: Determination of Falling Number)
No. 0011 - Sieving tests using a laboratory sifter

19. MEASUREMENT OF GLUTEN QUALITY USING THE SMS KIEFFER RIG

19.1 Scope

The elasticity and extensibility of prepared dough or gluten can be measured on a very small scale. Other rheological techniques exist for measuring protein quality, but require flour samples in excess of 250g of sample.

19.2 Principle

A sample of dough or gluten is prepared using a standard method, formed into strips and allowed to rest before being stretched. A hook catches the centre of the sample strip and moves for a predetermined distance during which the dough or gluten will reach its elastic limit (at the peak force) after which it will break. At this point (i.e. close to zero force) the distance is noted and used as an indication of dough extensibility. A plot of the force measured over time is recorded on the monitor.

19.3 Apparatus

19.3.1 *Stable Microsystems TA-XT2 Texture Analyser.*

The following settings are required:

19.3.1.1 Mode:	Measure Force in Tension
19.3.1.2 Option:	Return to Start
19.3.1.3 Pre-test Speed:	2.0mm/s
19.3.1.4 Test Speed:	3.3mm/s
19.3.1.5 Post-test Speed:	10.0mm/s
19.3.1.6 Distance:	75mm
19.3.1.7 Trigger Type:	Auto – 5g
19.3.1.8 Data Acquisition:	200pps

19.3.2 *Kieffer dough and gluten extensibility rig (A/KIE) using 5kg load cell.*

19.4 Procedure

19.4.1 *Sample Preparation*

19.4.1.1 Before using the Teflon dough form, oil both sides with a small amount of oil, to avoid sample adhesion.

19.4.1.2 Place 15g dough/gluten sample (the latter prepared according to 8.18) onto the grooved base of the form.

19.4.1.3 Place the top block of the form onto the sample and push down firmly until the two blocks come together.

19.4.1.4 Remove all excess dough cleanly from the sides, using a knife, ensuring that strips do not remain connected.

19.4.1.5 Clamp the dough form in the form press for 40 minutes (this cuts the sample into strips, allows the dough/gluten to relax and prevent loss of moisture).

19.4.1.6 Remove the dough form from the press and uncover the dough/gluten strips one by one when required, by carefully sliding the top form block over the grooved base.

Note: If the dough is soft, it may be necessary to place strips of plastic lametta in the grooves under the dough.

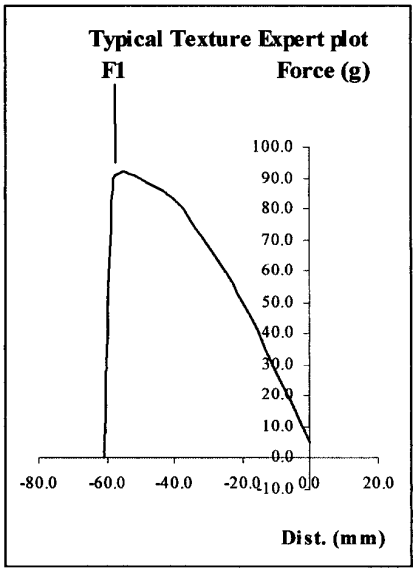
19.4.2 *Test set-up*

19.4.2.1 Ensure that the hook probe is lowered to the upper surface of the spring-loaded clamp.

19.4.2.2 Remove each strip very carefully with a spatula, taking care not to penetrate, stretch or deform the dough/gluten.

19.4.2.3 Place the strips onto the grooved region of the sample plate and, holding down the spring loaded clamp lever, insert the plate into the rig.

19.4.2.4 Release the handle slowly, then begin the tensile test.



19.5 Expression of results

Data of particular interest are the peak force (F1), and distance values. (These are shown on the typical Texture Expert plot.)

19.6 Repeatability and reproducibility

Test results obtained from 20 dough samples (of the same preparation) gave the following mean peak force and distance values (at the extension limit points), along with their respective coefficients of variation (C. V.):

Mean Peak Force (±S. D.)	C. V.	Distance Peak (±S.D.)	C. V.
97.7g±7.3	7.5%	60.8mm±3.9	6.5%

20. GLUTEN WASHING OF WHEAT USING A GLUTEN WASHER

20.1 Scope

This method is applicable to ground wheat and flour. Examination of the gluten washed out from a wheat can give an indication of the suitability of the wheat for use in a breadmaking or biscuitmaking grist and of the presence of heat damaged wheat. Gluten washed from flour can give an indication of protein quality and content.

20.2 Principle

A wet mechanical process is used to remove the starch from a dough ball, leaving behind a portion of gluten, which is then assessed for quantity and quality. A good, acceptable gluten for breadmaking should be no darker than the Reference Colour Standard, of a satisfactory quantity, be cohesive, extensible and elastic. A good, acceptable gluten for biscuitmaking should be no darker than the Reference Colour Standard, of a satisfactory quantity, be cohesive and extensible.

20.3 Apparatus

20.3.1 Top pan balance accurate to ± 0.2g.
20.3.2 Simon laboratory plansifter.
20.3.3 10N sieve, cover and receiver plus sieving aids.
20.3.4 100ml beaker or other suitable container.
20.3.5 Spatula or glass rod for mixing.
20.3.6 Device to measure approximately 5ml (e.g. 10ml measuring cylinder or 5ml grade B graduated pipette).
20.3.7 Simon gluten washer fitted with a 50GG sieve and paddles.
20.3.8 Timer accurate to ± 30 seconds at 10 minutes..
20.3.9 Numbered Reference Colour Standard, (Dulux gloss Lizard 10 B 21, to BS 4800 revised.).

20.4 Procedure

20.4.1 Mix the sample thoroughly, ensuring it has been uniformly homogenised before use.

20.4.2 Grind whole grain samples in a Falling number 3100 Mill (PRP02). If the sample is not white flour sieve the blended meal through a 10N using a Simon Laboratory Plansifter sieve to obtain just over 10g of flour.

20.4.3 Weigh 10 ± 0.2g of flour into a 100ml beaker, add approximately 5 ml of water and mix thoroughly in a Minor Pin mixer to make a tight dough ball. If the dough is not tight enough, mix in some more flour. If the dough is too dry, add a little more water. For soft or low protein wheats or flours the dough ball may need to be rolled on the palm of a hand for 30 seconds prior to gluten washing. This working of the dough helps avoid perfectly good gluten being washed away

20.4.4 Ensure that the kneading chamber of the gluten washer is clean and that the 50GG sieve is clean and free from any blockages. The 50GG sieve should be changed periodically and kept clean from dried dough. The paddles should be in good condition and pressing hard on the inside of the chamber. The chrome plating on the inside of the gluten washing chamber should be intact.

20.4.5 Thoroughly moisten the 50GG sieve and adjust the water flow to 20 ± 2ml per minute (Note 3). The water flow rate should be checked at least daily when in use. Ensure the swan neck through which the water is dispensed is close to its normal operating position when checking the flow rate

20.4.6 Place the dough ball in the washing chamber. Start the gluten washer and let it run for 10 minutes.

20.4.7 Ensure that there is a consistent flow of water to the washing chamber throughout the duration of the test.

20.4.8 After 10 minutes washing, stop the machine and collect all the pieces of dough in the chamber to produce a single piece of gluten. Switch on for a further 5 minutes or until all the starch has been washed out. If there is no evidence of a gluten forming or the gluten washes out, the machine should be cleaned and the test repeated from Appendix 20.4.3 having set the water flow to the low end of the range given in 20.4.5 and ensuring that the rolling of the dough in 20.4.3 is carried out

20.4.9 After the full duration of 15 minutes remove the gluten ball, weigh. If time permits, place it in a labelled beaker of cold tap water and leave it to rest at room temperature for approximately one hour before assessment.

20.4.10 If all the gluten has been washed through the sieve, either the water flow was too high or the protein has been denatured. This normally indicates a heat-damaged wheat sample.

20.4.11 Compare the colour of the gluten with the standard. It should be a creamy grey colour and not darker than the Reference Colour Standard. A dark gluten can indicate that the wheat has been partially heat damaged.

20.4.12 The gluten should form a smooth textured cohesive ball. If the texture is particularly rough and uneven, partial heat damage may be suspected.

20.4.13 Assess the quantity of gluten in comparison to that expected from a satisfactory wheat of a similar type and protein content. Typically, for a 10g flour sample, the weight of wet gluten recovered is approximately 2.7 times protein content (as is) of the flour. A significantly lower quantity may suggest partial heat damage.

20.4.14 Assess the gluten quality, if possible after resting for one hour.

20.4.15 Gently stretch the gluten to assess elasticity, when extended allow the gluten to spring back towards its original shape. If the gluten is very resistant to stretching, it is TOUGH. If the gluten springs back towards it original shape, it is ELASTIC. If there is no tendency to return to its original shape, it is INELASTIC.

20.4.16 Test the extensibility of the gluten by stretching it lengthways and widthways to produce a thin sheet. If the gluten breaks readily into small pieces, it is SHORT. If a thin, even sheet is produced then the gluten is EXTENSIBLE.

20.4.17 If the gluten is soft, weak and flowing then it may have been affected by proteolytic enzymes originating from insect attack

20.4.18 Clean the machine thoroughly, paying particular attention to the 50GG sieve at least once per day.

Notes: (1) If it is not possible to allow the gluten to rest for one hour (e.g. Mill intake situation), then the gluten can be assessed immediately washing is completed. The lack of resting time must be taken into account when assessing the gluten since it will be tougher.

20.5 Expression of results

The weight of the gluten ball remaining after washing should be recorded. Also the colour and subjective assessments of extensibility and elasticity.

21. THE TURBIDITY TEST AS A MEASURE OF WHEAT PROTEIN THERMAL DENATURATION

(Adapted from: Harrison, KR; Doarks, PF and Greer, EN (1969). Detection of heat damage in dried wheat. *Milling* **151(7)**, 37-38.)

21.1 Scope

To detect denaturation of wheat proteins caused by excessive exposure to heat.

21.2 Principle

Heat treatment of grain reduces the amount of heat-precipitable protein that can be extracted by saline from wholemeal produced from that grain. Although only a small proportion of the total wheat protein is soluble in neutral saline, a good correlation is shown between the loss of protein solubility and injury to germination capacity. An estimate of heat precipitable proteins is made by heating the saline extract in the presence of a protective colloid (acacia solution). The turbidity of the resulting solution has been shown to be inversely related to the extent of heat damage. A reduction in germination capacity reliably indicates that heat damage is present, although, this method for determining heat damage is rather time consuming to be suited to routine use. A test suggesting little or no reduction in germination capacity, therefore, indicates a sample is undamaged by heat. A large proportion of the protein giving rise to turbidity in this test is derived from the germ. Shrivelled and poorly matured grains will, therefore, be likely to give higher results than well filled grains. If varietal differences are found, they may be partially attributed to variable germ content.

21.3 Apparatus

21.3.1 Mill to produce a coarse wholemeal sample (e.g. Buhler-Miag)
21.3.2 Analytical balance capable of weighing accurately to ±0.1g
21.3.3 250ml beaker(s)
21.3.4 Automatic dispenser capable of dispensing 100ml
21.3.5 Calibrated timer capable of measuring 30s to 3min
21.3.6 Magnetic stirrer
21.3.7 Whatman No. 4 filter paper, 15cm diameter
21.3.8 Centrifuge capable of approximately 12,000 x g
21.3.9 Funnel
21.3.10 Measuring cylinder capable of measuring 20ml ±1ml
21.3.11 Graduated or calibrated boiling tube(s) capable of measuring 10ml ±1ml
21.3.12 Automatic pipette capable of dispensing 1.0ml
21.3.13 Boiling waterbath with test-tube holder
21.3.14 Colorimeter capable of measuring absorbance at 520nm.

21.4 Reagents

21.4.1 *Saline 0.5%:*
Weigh 5.0g sodium chloride (NaCl) into a 1litre volumetric flask, add approximately 900ml distilled water, allow to dissolve then make it up to the mark.

21.4.2 *Acacia solution 10% (w/v)*:
Weigh 5.0g acacia powder, dissolve in approximately 40ml distilled water using a magnetic stirrer (heating to approximately 50°C if necessary), make it up to 50ml in a volumetric flask, mix well and store in a suitably labelled container.

Note: This solution is stable for 5 days, so should be made fresh each week and stored refrigerated.

21.5 Procedure

21.5.1 Mill the grain sample(s) to be tested (using a Buhler-Miag or similar) to produce wholemeal and mix thoroughly.

21.5.2 Check the level of the water bath and switch on to boil.

21.5.3 Weigh 5.0g wholemeal into a 250ml beaker.

21.5.4 Switch on the colorimeter and check the correct filter is in place.

21.5.5 Dispense 100ml of 0.5% saline into the beaker containing the sample and mix to keep the wholemeal in suspension (at room temperature) for 3min using a magnetic stirrer at a specific setting (e.g. speed = 3, reverse period = 6) to keep the method constant.

21.5.6 Filter the suspension through Whatman No.4 fluted filter paper into a measuring cylinder until 20ml filtrate has been collected.

21.5.7 Exchange the cylinder for a graduated (or marked) boiling tube and collect 10ml filtrate.

21.5.8 Discard the remaining sample.

21.5.9 Add 1.0ml of 10% (w/v) acacia solution, put a stopper in the neck of the tube and invert quickly five times to mix then remove the stopper.

21.5.10 Heat the tube in a boiling waterbath for 3min, transfer immediately into a beaker containing ice and water, leave for 2min.

21.5.11 Read the absorbance of the solution at 520nm immediately.

21.6 Performance data (repeatability and reproducibility)

The detail of each stage should be as closely standardised as possible. Replicability should be ±3% and should be checked by regular reference to a standard stock of well mixed sound wheat.

22. MIXOGRAPH PROCEDURE

22.1 Scope

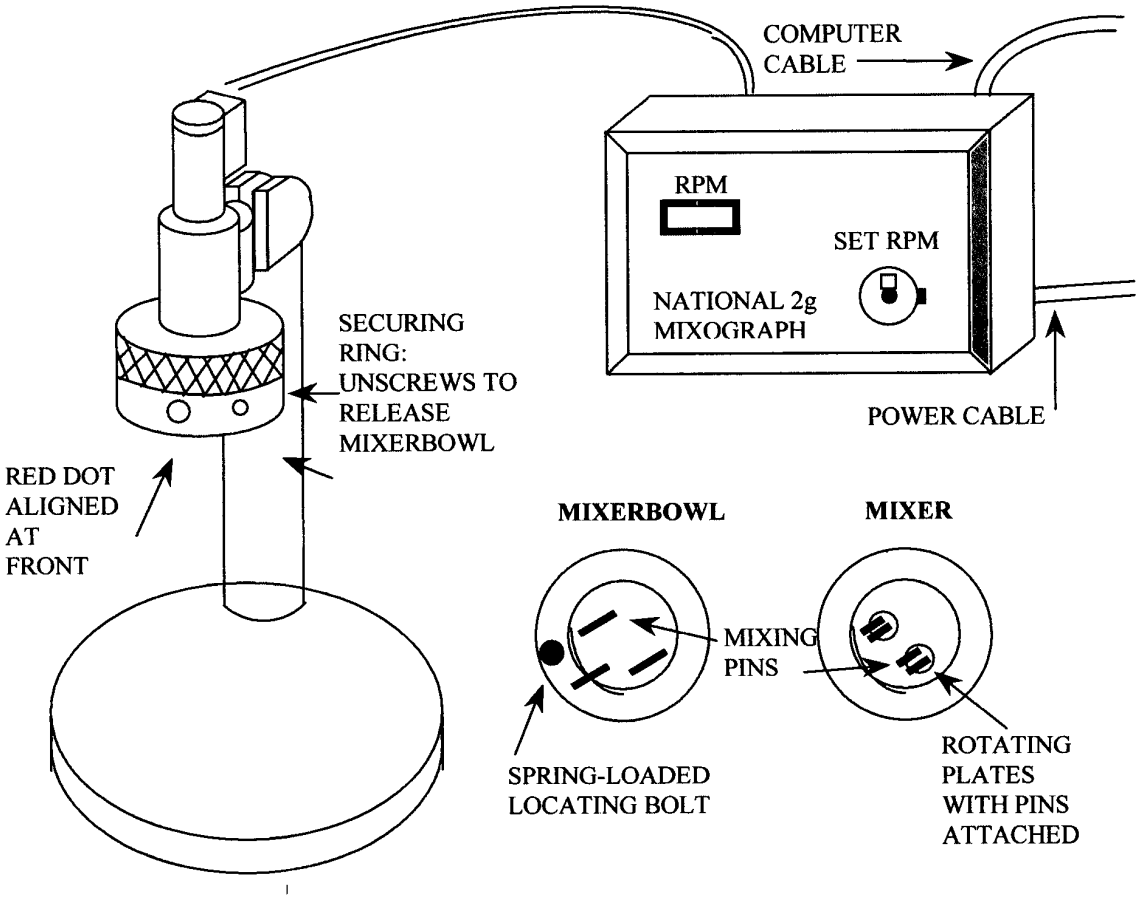
This equipment may be used to assess the protein quality in wheat flour and gluten samples.

22.2 Principle

Flour and water are mixed together to form a dough in the mixer bowl of the apparatus. The resistance to mixing is measured directly from the torque on the motor during the mixing process and plotted by computer upon completion of analysis.

22.3 Apparatus

The equipment illustrated below was attached to a computer on which the results were shown.



In addition to the equipment shown, the following are required:

22.3.1 Balance accurate to ±0.01g

22.3.2 Automatic pipette capable of dispensing 1±0.1ml

22.4 Procedure

22.4.1 Weigh 2.00g flour into the mixerbowl of the instrument.

22.4.2 Add the required amount of water depending on the water absorption of individual flour samples (usually around 50-55% i.e. add 1.0 -1.1ml distilled water to 2.0g of sample).

22.4.3 Place the bowl below the mixer, checking its orientation by ensuring the red dot is at the front of the apparatus and that the locating bolt is in place.

22.4.4 Secure the cup by tightening the outer (securing) ring until finger tight.

22.4.5 Mixing and analysis begins automatically and lasts for approximately 10min.

22.5 Expression of results

A large amount of data is generated by the computer such as: midline peak time, midline peak height, right of peak slope, width of peak, width of curve at midline peak +2min.

23. MEASUREMENT OF VISCOSITY USING THE RAPID VISCO ANALYSER (RVA)

23.1 Scope

The Rapid Visco Analyser (RVA) can be used to assess the pasting quality of any starch-based material, particularly where the cooked viscosity is important. The precise linear ramped heating and cooling abilities of the RVA, along with steady state temperature control, allow careful control of the cooking environment. Changes in viscosity are also continuously recorded. The method is applicable to any ground material including, but not limited to, cereal and other starches, flours, wholemeal and formulations (Table 1). It may also be used to assess amylase activity. The method can be used for quality control, to compare samples, to assess amylase activity in flour, to investigate effect of formulation (e.g. effect of added sugars), to determine degree of modification during starch manufacture etc. Since different test materials employ different amounts of sample plus heating and cooling regimes, it is essential that the profile used is noted on the test report.

Table 1

Material	Amount (g)
Wholemeal	4.00
Flour	3.50
Native Starch	
Cereal, Non-waxy	3.00
Cereal, Waxy	2.50
Potato	2.00
Tapioca	2.50
Modified Starch	
Acid Modified	4.00 – 22.00
Oxidised	4.00 – 22.00
Substituted	2.50
Cross-linked	2.50

23.2 Principle

The pasting properties of starch and starch-containing products are readily assessed in the RVA. During the test, the starch is gelatinised with consequent rise in viscosity, subjected to high temperature and controlled shear during which its stability is measured, then cooled to provide an indication of gel setback. Values for pasting temperature, peak paste viscosity, time to peak, temperature at peak, hot and cold paste viscosity, breakdown, setback, final viscosity and other parameters can be calculated from the sample trace.

23.3 Apparatus

23.3.1 Rapid Visco Analyser computer and accessories.
23.3.2 Balance accurate to ±0.01g

23.4 Reagents

23.4.1 Water, distilled
23.4.2 Ethanol (95%)

23.5 Procedure

23.5.1 Switch on the RVA and allow it to warm up for 30min.

23.5.2 Switch on the associated computer, run the RVA control software, and enter one of the profiles shown below:

Standard 1	Heat damaged gluten and breadmaking flour profile:
Idle temperature: 50°C	Idle temperature: 50°C
150°C @ 1.0min 1	50°C @ 2.0min
295°C @ 4.7min 2	85°C @ 6.5min
395°C @ 7.2min 3	85° @ 8.5min
450°C @ 11.0min 4	20°C @ 16min
End of test: 13.0min	End of test: 20.0min

23.5.3 Enter file name for data collection.

23.5.4 Measure 25.00± 0.05ml of distilled water (and ethanol) into a new canister by weighing 25.00g.

23.5.5 If necessary, grind a representative wheat sample on a hammer mill (0.8 mm sieve).

23.5.6 Weigh 4.00± 0.01g wholemeal (14% moisture basis) into a weighing vessel and transfer sample into the canister.

23.5.7 The amount of material to use depends on the nature of the sample, Table 1 may be used as a general guide.

23.5.8 Place a paddle into a canister and vigorously jog the blade through the sample up and down 10 times. If any lumps remain on the water surface or adhere to the paddle then repeat the mixing action.

23.5.9 Place the paddle into the canister and insert the canister into the instrument.

23.5.10 Initiate the measurement cycle by depressing the motor tower of the instrument.

23.5.11 Remove the canister on completion of the test and discard.

23.5.12 From the pasting curve, the pasting temperature, peak viscosity, time to peak, breakdown, minimum viscosity, setback and final viscosity may be measured (Appendix 23.6).

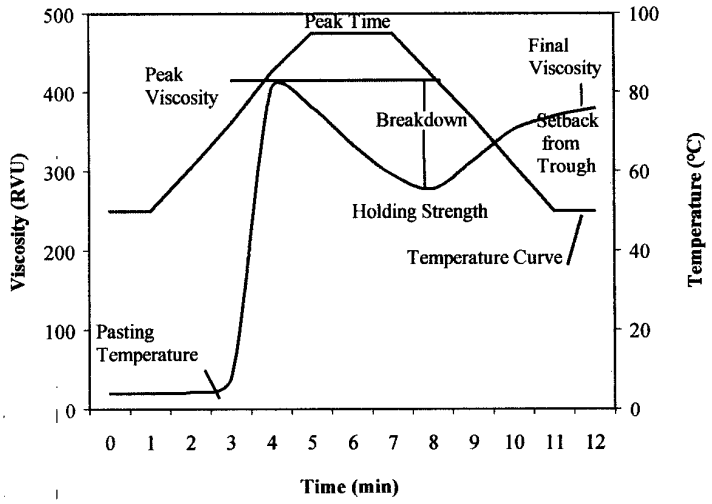
Notes:
For best results, sample weights should be corrected for the sample moisture content, to give a constant dry weight. The moisture basis normally used is 14% as is, and correction tables are available from Newport Scientific.

The correction formulae for 14% moisture basis are:

$M_2 = (100 - 14) \times M_1/(100 - M_1)$
$W_1 = 25.0 = (M_1 - M_2)$
Where M_1 = sample mass for the material listed in Table 1.
M_2 = corrected sample mass
W_1= corrected water volume

23.6 Expression of results

Example: Pasting curve of starch using the STANDARD 1 profile, showing the commonly measured parameters



24. PREPARATION OF SEMI-SWEET BISCUIT DOUGH FOR RHEOLOGICAL TESTING

24.1 Scope

This method is designed to test the rheological properties of biscuit flour by mixing it into a semi-sweet dough under controlled conditions. Instead of baking biscuits, the dough is tested for extrusion time using the apparatus detailed below and the rheological properties measured using the Bohlin VOR.

24.2 Principle

The visco-elastic qualities of full recipe biscuit doughs are measured using the Bohlin VOR. Such measurements can be used to make comparisons between the suitability of different flour samples fort his particular end-use.

24.3 Apparatus

24.3.1 Greaseproof paper
24.3.2 Balance accurate to 0.01g
24.3.3 Disposable paper cups to weigh ingredients
24.3.4 Plastic bags
24.3.5 Spoons

24.3.6 Sieve
24.3.7 Funnel
24.3.8 Scraper for fat
24.3.9 Plastic container to measure water into
24.3.10 Thermometer, capable of measuring 0-100^{0}C
24.3.11 Timers accurate to 1.0s
24.3.12 Dough former, rolling plates
24.3.13 Automatic mixer of 300g capacity
24.3.14 Modified Simon Extrusion Cahmber with water jacket for temperature control and piston plus 2375g weight
24.3.15 Waterbath at 40°C
24.3.16 Bohlin VOR or CS-50

24.4 Reagents

24.4.1	Flour	200g
24.4.2	Fat	32g (kept at 26°C)
24.4.3	Pulverised sugar	42g
24.4.5	Salt	0.7g
24.4.6	Cream powder	0.7g
24.4.7	Skimmed milk powder	5.0g
24.4.8	Sodium bicarbonate	1.1g
24.4.9	Ammonium bicarbonate	1.1g (kept in fridge)
24.4.10	Tap water	40g (at room temperature) depending on flour used

(an extra 2g water reduces the extrusion time by approximately 35s, 2g less water extends the extrusion time by approximately 35s).

24.5 Procedure

To be carried out in a temperature controlled room at 21±0.5°C.

24.5.1 *Preparation*

24.5.1.1 Ensure the equipment has been switched on at the mains, that the water bath has reached 40°C and that the Astell cabinet is at 37.8°C.

24.5.1.2 Weigh out all ingredients, store the fat in the incubator set at 26°C, allow an extra 0.15g water to allow for the amount which sticks to the container when it has been emptied.

24.5.1.3 Sieve all dry ingredients except ammonium bicarbonate onto a large sheet of greaseproof paper.

24.5.1.4 Ensure the Bohlin VOR is set up (see Appendix 25) and running in preparation for reading the first biscuit dough sample.

24.5.2 *Mixing (use some saved dough from a previous mix to warm up the mixer for 10min prior to running the first test sample)*

24.5.2.1 Place the fat and the sieved dry ingredients into the mixer.

24.5.2.2 Dissolve the ammonium bicarbonate in the pre-weighed water.

24.5.2.3 Start the mixer and slowly add the dissolved ammonium bicarbonate through the hole in the lid using a funnel.

24.5.2.4 Begin timing, mix for 6min then check the temperature (stop mixer and open lid) every 60s until the dough temperature reaches 40°C (approximately 8-12min).

24.5.2.5 Ensure that the water container is dry ready for weighing the water for the next dough.

24.5.3 *Relaxation*

24.5.3.1 Remove 60±1g dough from the mixer and roll into a cylinder using the dough former. The top of the former should slide forward from the central position approximately 12cm and backward the same distance, a total of six times in each direction.

24.5.3.2 Remove the chamber of the extruder (kept at 40°C by a water jacket), stretch the dough cylinder slightly to allow it to slide easily into the chamber. Press the top of the dough cylinder into the chamber until the dough begins to appear from the hole in the bottom of the chamber.

24.5.3.3 Remove and discard excess dough from the top of the chamber and replace it into the extruder assembly. Begin timing the relaxation for 10min.

24.5.3.4 After the 10min, drop the plunger and weight (2375g) on top of it. The time for the plunger to travel 1 complete revolution is recorded, the target extrusion time is 50±5s.

24.5.3.5 Remove the dough from the extruder by unscrewing the narrow end and pulling out the dough, the remaining dough can then easily be pushed through the wide barrel of the extruder. Reassemble the extruder ready for the next sample.

24.5.3.6 Cut two 2g samples of dough from the mixer with minimal tearing and stretching of the dough, place one into the Astell cabinet at 37.8°C to rest for 20min, take the other to test on the Bohlin VOR immediately.

24.5.3.7 Remove the remaining dough from the mixer ready for the next sample by removing the front wing nuts and removing the bowl.

24.5.3.8 Take appropriate dough sample using rheological measurements on test (see 25).

24.5.3.9 Discard any remaining dough.

24.5.3.10 At the end of the session, remove the mixing compartment and wash in the sink with warm soapy water. Ensure that any trapped dough is removed from the fixed part of the equipment as far as is possible, when it dries, it can cause machine failure. Dry the equipment and reassemble it.

24.6 Expression of results

Record extrusion times in seconds but do not accept other readings if the target extrusion time of 50± seconds is not achieved.

25. USING THE BOHLIN CS-50 FOR TESTING SEMI-SWEET BISCUIT DOUGHS

25.1 Scope

This method is used to test the rheological properties of semi-sweet biscuit dough mixing following controlled conditions. Instead of baking biscuits, the dough was tested using a Bohlin CS-50 viscometer.

25.2 Principle

The visco-elastic qualities of the dough are measured by the Bohlin CS-50. Such measurements can be used to make comparisons between the suitability of different flour samples for semi-sweet biscuit production.

25.3 Apparatus

25.3.1 Bohlin CS-50

25.4 Reagent

25.4.1 Semi-sweet biscuit dough prepared according to Appendix 24.

25.5 Procedure

25.5.1 Wind up the cone or bob.

25.5.2 Lock the sliding bar (to the left) before removing or adding any accessories (turn clockwise to undo).

25.5.3 Ensure the cone and plate are securely fitted and check the gap is 0.15mm using the feeler gauge and adjusting if necessary.

25.5.4 Turn on the air supply and check it is set at 3bar.

25.5.5 Turn on the computer and monitor.

25.5.6 Exit windows, change directory to "CS" (type "CD CS" and return).

25.5.7 Type "CS" and return.

25.5.8 Check the measuring system selected is CP 4/40 (if not, select it from the menu using key F10 then F1 - press F1 again to confirm changes).

25.5.9 Check the target strain is 0.00311.

25.5.10 Switch on the power unit (at the back).

25.5.11 Switch on the temperature control unit (turn the knob to the right and press reset).

Note: Turn off the temperature control unit before removing the plate from the Bohlin to prevent flooding.

25.5.12 Put sample on and wind down the cone as far as possible.

25.5.13 Press "Ctrl" and "s" simultaneously to begin measurement.

25.5.14 Save to disk, press pause to read off values and note them while reading is in progress.

25.6 Expression of results

Record rheology readings of elastic modulus (G′), viscous modulus (G″) and phase angle from the Bohlin CS-50.

26. TEST BAKING – CHORLEYWOOD BREAD PROCESS

26.1 Scope

400g loaves using the Morton Mixer

26.2 Apparatus

26.2.1 Morton Mixer.
26.2.2 Mono moulder, settings (18, 2).
26.2.3 Single-piece cylinder moulder, settings (R7, W5.5, P1.25)
26.2.4 Tin Size: 160 x 98 x 83 mm at top.
26.2.5 Gas fired, 12 tray reel oven.
26.2.6 Wire cooling racks.
26.2.7 Cooling cupboard at 21°C

26.3 Recipe

	% of Flour Weight	g/mix
Flour	100	840
Yeast	2.5	21
Salt	2.0	16.8
Fat (Ambrex, Slip Point c45°C)	1.0	8.4
Ascorbic Acid (100ppm)	0.01	0.084
Water (as determined by Brabender Farnograph, 600 line)		

26.4 Procedure

26.4.1 Mix ingredients for 30s at slow speed, scrape down then complete mixing at fast speed. (Work Input is 11Wh/kg)

26.4.2 Final dough temperature must be 30 ± 1°C.

26.4.3 Scale the dough by hand into 454g pieces, then mould.

26.4.4 Leave the dough for first proof for 10min under cover at 27°C.

26.4.5 Place the dough into tins and allow to prove at 43°C with humidity to prevent skinning until a proof height of 10cm has been reached.

26.4.6 Bake at 244°C in a gas reel oven for 25min.

26.4.7 Remove from tins and allow to cool on wire racks. Store in a cupboard overnight at 21°C.

27. PREPARATION OF A RANGE OF HEAT DAMAGED SAMPLES FROM THREE VARIETIES OF WINTER WHEAT CONDITIONED TO EITHER 18% OR 25% MOISTURE CONTENT

27.1 Scope

From the work carried out on material harvested in 1996, a test was developed which was able to differentiate between heat damaged and non-damaged samples of wheat. In order to look at safe drying temperatures and to confirm the sensitivity of the test, wheat from the 1997 harvest was required at different levels of heat treatment.

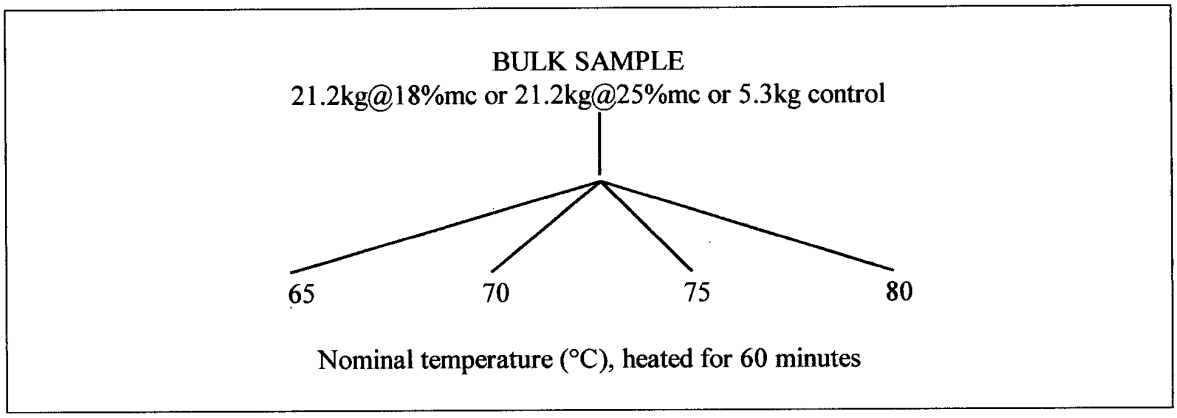
The samples collected for this work are detailed in the table below.

Code	Variety	Kg/hl	Protein %	HFN	Wet gluten % (Quality)	Purity
CMS/98/92	Soissons	75.82	12.0	236	28.9 (strong & elastic)	14/14
CMS/98/93	Hereward	79.97	11.4	375	28.1 (satisfactory)	49/56
CMS/98/156	Riband	78.16	10.6	279	26.0 (slightly weak)	13/14

A range of damage was incurred by subjecting wheat samples to different temperatures for a period of an hour. This duration was chosen after looking at the results obtained from the previous year of the study when 40 or 80 minutes were used. As both were effective in damaging the wheat, it was decided to use this intermediate time period but to study more than one initial moisture level.

27.2 Principle

The treatment of samples is summarised by the figure below, the control sample did not receive any conditioning or heat treatment.



27.3 Apparatus

27.3.1 Carter-Day Dockage tester
27.3.2 Sinar moisture meter (AP 6060 Moisture Analyzer)
27.3.3 Measuring cylinder
27.3.4 Re-sealable plastic bags
27.3.5 Cement mixer for bulk grain conditioning.
27.3.6 Timer accurate to ±1 second
27.3.7 Mitchell dryer
27.3.8 Squirrel data logger to record actual dryer temperature.

27.4 Procedure

27.4.1 *Grain cleaning and conditioning*

27.4.1.1 Pass each variety through a dockage grain cleaner to remove extraneous matter.

27.4.1.2 Weigh out two bulk samples of 21.2kg and a control sample of at least 5.3kg.

27.4.1.3 Condition one bulk sample to 18.0%, the other to 25.0% moisture content in one or two stages on consecutive days. Ensuring that both sets of samples are ready to be heat treated on the same day.

27.4.1.4 Determine the amount of water to be added per kg by measuring the moisture content of the stored bulk samples and using the following equation:

$$\text{Volume required (ml)} = \frac{(M2-M1) \times 1000}{(100-M2)}$$

M1 = % moisture content before conditioning, M2 = required % moisture content

27.4.1.5 Multiply the amount of water to be added per kg by the weight of the bulk samples to be conditioned (i.e. 21.2). The details of the moisture contents and amounts of water added are given in Table 2 overleaf. A Sinar moisture meter was used except for the final moisture readings which were determined using a two-stage oven drying procedure.

Table 2. Water required to condition samples to 18% moisture content

Sample CMS/98	Variety	% moisture	Water added (ml)	Final % moisture
92/1-4	Soissons	15.7	595	18.8
93/1-4	Hereward	15.0	2 x 388	18.1
156/1-4	Riband	14.9	2 x 400	17.9

Table 3. Water required to condition samples to 25% moisture content

Sample CMS/98	Variety	% moisture	Water added (ml)	Final % moisture
92/5-8	Soissons	15.9	2 x 1286	25.4
93/5-8	Hereward	15.1	2 x 1399	24.7
156/5-8	Riband	14.9	2 x 1428	24.9

27.4.1.6 Place each bulk sample in turn into a cement mixer, switch on, and add the determined amount of water.

27.4.1.7 Place a lid over the mouth of the mixer to prevent water loss and agitate the grain for 20min.

27.4.1.8 Transfer each sample into a strong plastic bag, seal with a removable clip and leave to equilibrate for a minimum of 22h at 4°C before the second conditioning.

27.4.1.9 After completion of conditioning, leave samples to equilibrate at 4°C for approximately 24h (±2h) before subjecting each one to a particular heat treatment.

27.4.2 *Heat treatment*

27.4.2.1 Switch on Mitchell dryer; turn the red switch clockwise. After a few seconds depress the high temperature fail button to turn it off. Set the required temperature using the arrow keys below the digital readout and switch on the oven by depressing the green start button. Use a calibrated Grant "Squirrel" data-logger to measure and record the actual temperature within the oven. Stop the oven before opening the door.

27.4.2.2 Weigh each bulk sample then mix in the cement mixer for 2min before removing 20g for oven moisture testing.

27.4.2.3 Weigh one quarter of the total bulk of each variety into each of four resealable plastic bags.

27.4.2.4 Take one of the smaller bags of each of the six different variety and moisture treatments divide equally between two perforated metal trays (0.45 x 0.75m). Spread evenly and cover with a similar inverted tray. Repeat for the other variety and moisture treatments.

27.4.2.5 Place the trays into the preheated Mitchell Dryer (approximately 65°C inside the dryer).

27.4.2.6 Remove the trays after 60min and transfer them to solid trays in a controlled temperature room 21±5°C until their moisture content is ≤ 15.0% according to a Sinar moisture meter.

27.4.2.7 Reset the oven to the next temperature (70, 75, 80°C).

27.4.2.8 Repeat steps 6.2.3 to 6.2.6 until all treatments have been made.

27.4.2.9 Empty the contents of each solid tray into a labelled re-sealable plastic bag and store at 4°C for at least 72h.

27.5 Expression of results

From the data collected by the Squirrel data logger, calculate the mean actual temperature for each treatment.