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GRAVITY TABLE SEPARATION IN THE PRODUCTION OF MILLING AND BAKING QUALITY WHEAT FROM SAMPLES CONTAINING SPROUT-DAMAGED GRAIN

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GRAVITY TABLE SEPARATION IN THE PRODUCTION OF MILLING AND BAKING QUALITY WHEAT FROM SAMPLES CONTAINING SPROUT-DAMAGED GRAIN

Project No. 0003/1/88

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Abstract

It is well-known that gravity tables are able successfully to separate wheat of acceptable Falling Number from a bulk of sprout-damaged grain. However, almost no information was available on the milling quality, and more particularly the baking quality, of wheats treated in this way. The aims of this investigation were to obtain such information and, if necessary, to develop tests for selecting appropriate wheat samples for treatment. Fifteen pairs of samples (before and after gravity separation) of wheat of breadmaking varieties were obtained from the operators of commercial gravity separators. These were subjected to a range of quality testing, including the test baking of bread. Although gravity separation was extremely successful in improving the Falling Number and specific weight of all of the samples, it was less successful in improving the baking quality of the wheats. Even the treated samples performed less well in the baking tests than would be expected of a good quality breadmaking wheat. In particular, some of the problems associated with low Falling Number were still observed to some extent in bread from the tested samples. Examination of the condition of the protein in the samples did not provide any explanation for the poor baking performance. All but one of the fifteen wheat samples were of the variety Avalon. To put the results into context, a number of samples of Avalon of naturally high Falling Number from the same (1987) harvest were also test baked. The overall quality of the bread from these samples was comparable with that from the gravity-separated samples. This result, suggesting that other problems with the quality of the wheats from the 1987 harvest (and Avalon in particular) may have been to blame, makes the results hard to interpret. Further work is needed.

OBJECTIVES

To establish the effect of gravity separation on the milling and baking quality of sprout-damaged wheat. To investigate the reasons for poor baking quality of separated wheats having acceptable Falling Number. To develop a rapid screening test for the selection of wheats that are suitable for gravity separation.

1. INTRODUCTION

When wheat has been subject to rain while still in the ear, it can suffer from premature germination leading to high levels of the enzyme *alpha*-amylase and low Hagberg Falling Numbers in the harvested crop. Such wheat samples were common in the 1987 wheat harvest resulting in a mean Hagberg Falling Number for the National crop as low as 163s[1]. A large proportion of the UK wheat crop was deemed to be unsuitable for breadmaking purposes on the basis of low Falling Number values. However, a recent pilot-scale study has shown that the Falling Number and specific weight of wheat can be improved by passing it over a gravity separating table[2].

Gravity separators fractionate wheat samples on the basis of differences in grain density; sound, ungerminated wheat grains having a higher density than sprout-damaged grains. Removing this sprout-damaged fraction, which has a low Falling Number and specific weight, thus produces improvements in these quality parameters. Therefore, it appears to be possible to upgrade a proportion of a wheat sample, of a breadmaking variety, from feed to milling quality. This would benefit farmers and increase the supply of suitable home-grown wheat to millers. However, little is known about the milling and breadmaking performance of such gravity separated wheats. To this end we have undertaken a research project to assess the quality of gravity separated wheat as offered to the milling industry. No attempt was made to examine the economics of the separation process.

In addition to the investigations described in the main body of this report we also undertook an examination of possible protein defects responsible for poor baking performance (Appendix 2), and an assessment of visual methods of detecting *alpha*-amylase in individual grains as a potential method of screening wheat samples for their suitability for gravity separation (Appendix 3).

2. MATERIALS AND METHODS

We obtained breadmaking variety wheats from operators of commercial gravity separators who were able to supply the low Falling Number, untreated wheat and the separated fraction designated as milling quality. Fifteen pairs of samples (mainly Avalon, but one of Rektor) were obtained from six different sources. Although the

exact origin of each wheat was not known, these samples came from the major wheat growing areas including Essex, Lincolnshire, Oxfordshire and Hampshire. In addition, we examined five Avalon wheat samples of natural Falling Number greater than 250s. Four of these control samples of Avalon were from the 1987 harvest and one came from the 1986 harvest.

Samples were subjected to the test procedures listed below. Due to limitations of sample size we were not able to perform all of the tests on every sample. All the tests were routine evaluation procedures performed by standard methods except where references are given.

Wheat

Hagberg Falling Number (7g)
Specific weight
Protein content (14% moisture basis)
SDS sedimentation test
Visual appearance
Heat damage[3]
Milling/Extraction rate*

*Fixed milling system

Flour

Hagberg Falling Number (7g)
Protein content (14% moisture basis)
GCF
Alpha-amylase (FU)[4]
Damaged starch (FU)
Resistance and Extensibility - Brabender Extensograph at 45 min
Gluten quality/colour - visual examination
Microbiology a) Total viable counts
b) Moulds
c) Yeasts

Bread

Long Fermentation Process (LFP) bake - standard three hour procedure with the addition of 20mg/kg potassium bromate (flour weight basis). Water absorption determined by 3h, yeasted Simon extrusion technique.

Chorleywood Bread Process (CBP) bake - standard test procedure with the addition of 30mg/kg ascorbic acid and 45mg/kg potassium bromate (flour weight basis). Water absorption determined by 10 min (CBP) Simon extrusion technique.

Loaf volume

Amylose content of crumb[5])

Dextrin content of crumb[5]) CBP bake only

3. RESULTS

3.1 Effects on wheat quality

Table 1 shows the results of specific weight and wheat Falling Number determinations. Gravity separation produced a highly significant improvement in

grain specific weight. Before treatment the mean specific weight was 74.65kg/hl and passage over a gravity separator increased this parameter by an average of 3.22kg/hl (see Table 2). In every case grain specific weight was improved by gravity separation and 80% of separated samples had specific weights in excess of 76kg/hl, the value required in most milling quality wheat contracts.

The average increase in wheat Falling Number due to gravity separation was 144.3s. Treatment produced a highly significant improvement in this quality parameter (see Table 2). Separated wheat exhibited Falling Numbers in the range 209-345s, values likely to have been acceptable at mill intake in the 1987 season.

No heat damage was detected in any sample, either untreated or separated. However, the test method was not considered to be sensitive enough to indicate levels of damage below 15% with reasonable confidence[3]. Visual examination of samples revealed grains with black-point and an overall dark and dull appearance. In some instances appearance improved after separation; this could usually be related to the removal of sprouted and shrivelled grains.

Full results of quality testing are given in Appendix 1. Table 2 summarizes quality characteristics of untreated and separated samples as mean values and differences between pairs together with an indication of their statistical significance. Only samples where both untreated and gravity separated results were available have been used to compile this table. Although the separation process did not change wheat protein content, SDS volume showed a significant mean improvement of 3.7ml. This suggested some improvement in protein quality.

3.2 Effects on flour quality

Using a fixed milling system gravity separated wheat yielded an average of 1% more flour, with an improved flour grade colour figure (GCF). These small improvements reflect the removal of shrivelled grains by the gravity separator. However, many of the flour samples were of high GCF even after gravity separation of the wheat samples. The laboratory mill was set to give near commercial starch damage levels and under these conditions gravity separation had no effect on this parameter.

As anticipated from wheat Falling Number results, flour Falling Numbers and *alpha*-amylase levels showed highly significant changes as a result of the separation process (see Table 2).

Flour protein and wet gluten contents were essentially unchanged by gravity separation. Flour protein content ranged from 10.3 to 11.7% (14% moisture content basis). Thus some samples would be considered below optimum for breadmaking

purposes, particularly in a long fermentation process. All glutens were judged to be of acceptable colour but were generally weak and extensible. We observed similar effects in the Brabender Extensograph test where most of the samples failed to produce a Resistance/Extensibility curve typical of breadmaking quality wheat. Small, but statistically significant, improvements were found in both parameters following gravity separation.

Examination of the microbiological condition of flour samples produced no conclusive evidence for a reduction in mould, yeast or total viable counts. All samples were within the normal ranges of values recorded for 1987 flours[6].

3.3 Effects on baking quality

When flour samples were baked by the LFP, gravity-separated samples had significantly higher water absorptions, but lower loaf volumes than the untreated samples. Both effects can be attributed to the reduction in *alpha*-amylase activity observed in gravity-separated flour samples. High *alpha*-amylase activity results in dough softening during the 3h yeasted water absorption and is known to increase loaf volume[7]. Thus, any treatment which reduces *alpha*-amylase can be expected to have the opposite effect. Bread baked from untreated samples, where flour Falling Numbers were less than 200, showed all the typical characteristics of high *alpha*-amylase activity: intense crust colouration; weak, fragile crumb; poor crumb colour; and soft, damp and sticky crumb lacking resilience. Loaf volume was generally good but crumb cell structure was often rather open and irregular as a result. Many loaves had sharp corners which is a sign of dough softening or weakening during processing. Gravity separation resulted in reduced crust colour and crumb stickiness, but the problems of crumb fragility, poor crumb colour and resilience plus signs of general dough weakness still remained, though to a lesser extent.

When a CBP test baking procedure was used, flour from gravity separated samples had significantly increased water absorption but loaf volume was unaffected compared with untreated samples (see Table 2). Many of the loaves baked from gravity-separated samples by this process were characterized as lacking in volume with poor oven spring. Interestingly the better quality CBP bread was produced where the Falling Number of the original, untreated wheat was greater than 140s.

In order to put the quality of loaves from gravity-separated samples into perspective, we baked selected samples alongside Avalon controls which had naturally high Falling Numbers but a range of protein contents and had not been over a gravity separator.

separation. However, even the results obtained for the control series were not as expected from a breadmaking quality wheat variety such as Avalon.

Samples of breadcrumb from all CBP loaves were examined for amylose and dextrin contents. Results (see Table 2) show very significant reductions in both measurements following gravity separation of the original wheat sample.

3.4 Protein quality

Examination of the complete spectrum of flour proteins by SDS-PAGE electrophoresis indicated no qualitative or quantitative differences between separated samples and standard Avalon or Rektor wheat from other years (see Appendix 2).

When proteolytic activity was measured on selected samples of untreated and gravity separated wheat flour, there was no evidence of excessive activity in any of the samples examined and no major reduction in activity resulting from gravity separation. Similarly, limited analysis of accessible SH-groups suggested no difference in the SH-group content of treated and untreated flour.

3.5 Visualization of *alpha*-amylase in individual grains

The examination of exposed internal grain surfaces for enzymes associated with germination by staining and fluorescent methods revealed no consistent differences between unseparated and separated samples (see Appendix 3). The inability of these techniques to screen wheat samples for their suitability for gravity separation may be due to the lack of development of the techniques.

4. DISCUSSION

A number of reports[8–11] in the trade press and one from ADAS[2] have detailed the advantages of using gravity separators to upgrade wheats of low Hagberg Falling Number and low specific weight. An earlier report in the *FMBRA Bulletin*[12] also demonstrated that gravity separation had a beneficial effect on wheat Falling Number and specific weight. In that instance a small-scale laboratory unit was used with wheats of acceptable Falling Number and specific weight. In this investigation we were able to assess further the ability of gravity separators to select high quality wheat from a starting material that would have been classified as feed quality, on the basis of normal mill intake testing, even though it was a breadmaking variety. As the wheats were obtained from a number of sources using different makes of separator, this assessment gives an overall picture rather than one that is specific to a single machine and single commercial operation.

It must be appreciated that the overall quality of the 1987 UK wheat crop was very poor. The mean value for Falling Number for the 1987 crop was 163s

Table 3 shows the main quality characteristics of the control Avalon flours. Even within these controls one of them, sample D, produced unexpectedly low CBP loaf volume for its protein content. Many of the gravity separated flours would also be considered below average for the variety Avalon. Figure 1 shows a comparison between an Avalon control (Table 3,E) and a typical 'before' and 'after' gravity separation sample pair using a CBP baking test. The protein content of these samples was 10.4% and flour Falling Number was improved from 136 to 283s by gravity separation. Obviously the control Avalon has better volume and oven lift than the loaf produced from gravity-separated wheat. However, the separation process resulted in reduced crust colour and produced a loaf with a finer and less open crumb structure than before separation. Crumb texture and resilience were also improved to a certain extent, but the overall quality of the bread was still inferior to that from the control Avalon made from a flour of lower protein content. Generally, in terms of loaf volume and quality, many of the gravity separated flours would be ranked towards the middle to lower end of the control Avalon range.

Gluten strength, as measured by the Brabender Extensograph, was generally better in the control samples of Avalon than in the samples pre- and post-gravity

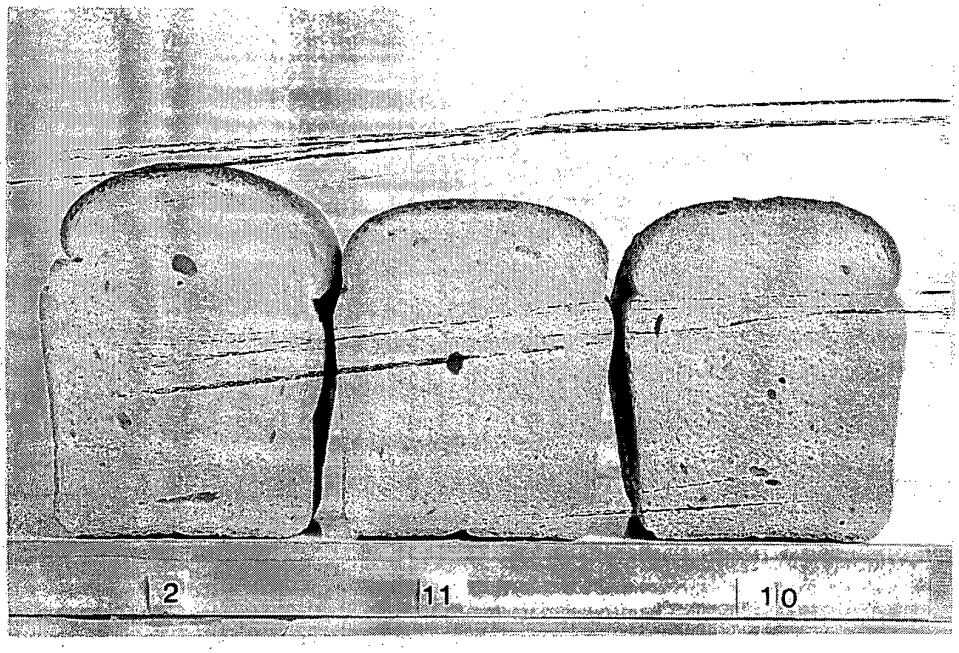

Fig. 1 Loaves baked by the CBP from (left to right) a control flour and from post- and pre- separated wheat flours

compared with 222s for the previous year and mean specific weights were 73.0 and 76.8kg/hl for 1987 and 1986 respectively[2]. The results of this investigation must be judged in the light of this overall crop quality.

This investigation confirms the previous findings that gravity separators are capable of removing a fraction of wheat of higher Falling Number and specific weight than the starting material. In this exercise we did not set out to investigate the economics of the process and did not have information on the proportion of the starting material that was upgraded. However, this can vary considerably, reported values of a 'commercial sample' ranging from 35 to 78.5% of the starting material[2]. In this work we found that most of the separated samples had specific weights that would be acceptable for milling quality wheat. Those that did not were close to the acceptance level of 76kg/hl and slight adjustment of the separator might have achieved a higher value at reduced yield. Wheat Falling Numbers of separated wheats were all considerably higher than those of the corresponding starting material. The wide range of results reflected the quality of the starting material and possibly the operator's judgement as to an acceptable level of Falling Number for milling quality wheat.

4.1 Milling performance

All wheats were milled on a laboratory mill using a fixed comparative milling system. This procedure showed that separated wheats had improved flour yields and in many cases the yield improvement was accompanied by a lower flour GCF. Removal of low density, shrivelled grains would account for both improved flour yield and better flour colour. In general flour colours were higher than would be commercially acceptable. The mean GCF value for separated flours was 3.88, approx. 1 GCF unit higher than required commercially. Using the value of 0.4 GCF/1% flour yield established by Dexter and Martin[13], extraction rates would need to be reduced by some 2.5%, if a gravity-separated wheat were to be milled on its own. The rather high GCF values of the flours produced in this investigation will have had a detrimental effect on their baking quality but also reflect the overall dirty nature of these wheats. The milling process was set to control starch damage in the flours at near commercial levels. Comparison of results shows a relatively narrow range of starch damage and essentially no differences between untreated and separated samples.

4.2 Breadmaking performance

Bread produced from gravity-separated wheat was somewhat disappointing given that all samples examined were of proven breadmaking varieties. Flour protein content

was sometimes below optimum for breadmaking at less than 10.5%. Low protein content is known to affect breadmaking performance and can manifest itself as poor volume, poor and ragged oven break, poor crumb texture, poor crumb cell structure or as a lack of crumb softness and resilience[14]. Many of these features were apparent in loaves produced from wheat pre- and post-gravity separation, but were also associated with samples from the 1987 crop where protein content was perfectly acceptable. Therefore, it seemed likely that protein quality rather than protein content *per se* was at fault.

Gravity separation certainly reduced *alpha*-amylase activity in wheat and flour and therefore markedly reduced some of the gross defects produced by this enzyme in the final bread, i.e. high crust colour and crumb stickiness. However, passage over the separating table did not cure completely all of the problems associated with wheat from the rather poor 1987 harvest.

4.3 Microbiological condition

Gravity separators did not appear to be particularly effective in improving the microbiological condition of the grain. Mould contamination, in particular, is known to lead to poor flour colour and is likely to have implications with respect to breadmaking performance[15]. German workers have observed a deterioration in baking quality resulting from *Fusarium culmorum* infection[16]. We observed reductions in sedimentation values and loaf volume together with a tendency towards slacker gluten in badly infected samples. While the values for total viable and mould counts observed in this investigation could bear some improvement, they would not be considered to represent particularly high levels of contamination and therefore are unlikely to be solely responsible for the less-than-perfect baking quality observed. When in one case the total viable count was reduced from 9.1 to 0.019×10^6 by gravity separation, the final bread still showed defects in crumb texture, structure and resilience and had a sharp-cornered appearance.

4.4 Protein quality

When the wheat grain prepares itself for germination, the levels of amylolytic enzymes, such as *alpha*-amylase, increase dramatically. However, a significant increase in proteolytic enzymes also occurs. The limited examination of flour proteins by electrophoresis showed no substantial differences between untreated and separated samples (Appendix 2). This is consistent with earlier work by Redman where observations suggested no large-scale proteolytic breakdown of high molecular weight proteins in doughs produced from high amylolytic activity flours[17]. Investigations into proteolytic activity and SH-group content of pairs of untreated and

separated flours, where essentially no differences could be found, supported this view. Thus, no major degradation of wheat endosperm proteins appears to have taken place and it is not possible at this stage to explain below-par baking performance observed in many gravity-separated samples in terms of gross changes in protein structure. Conversely, it is not possible to rule out proteolytic activity as a factor influencing the overall baking quality of such separated wheat samples and further basic biochemical work may be required to establish why some samples with improved Falling Number do not reach their full baking potential.

Many of the breadmaking faults observed in gravity-separated samples have also been found in other samples of Avalon wheat from the 1987 harvest. Since virtually all of the samples examined in this investigation were of the variety Avalon, it is possible that these problems are associated with the generally poor performance of this variety in 1987.

Selection of suitable samples for gravity separator processing is likely to be an important determinant of final product quality. The ideal sample is likely to contain a small proportion of severely damaged grain, produced by pockets of lodging in the wheat field. This type of sample should benefit substantially from gravity separation. It seems likely that many of the samples examined in this investigation came from wheat fields which had sustained fairly uniform levels of incipient sprouting. In such cases gravity separation may cure the amylase problem, but wheat quality may have been affected in other respects and this may limit the amount of separated material which can be included in a breadmaking grist.

5. CONCLUSIONS

The poor breadmaking quality of the 1987 wheat crop in general, and Avalon in particular, had an over-riding influence on this investigation. As a result, it has been difficult to obtain a clear-cut answer as to the usefulness of gravity-separated wheats for breadmaking. However, we have clearly demonstrated that gravity separators can be used to obtain wheat of satisfactory specific weight and Falling Number from a breadmaking variety that would have otherwise been marketed as feed wheat. In years when wheat supply is short, the gravity separation process could offer the possibility of increasing the supply of milling quality wheat.

In view of the low quality of all wheats available during the time-span of this study, it would be incautious to suggest that gravity-separated wheats have little value for breadmaking. Although the quality of the bread produced in this work was below par, it should be remembered that wheats were being tested as individual samples and not as part of a grist and that a simple recipe was used and not

one that incorporated sophisticated improvers. The quality of the bread was judged overall to be only slightly inferior to that of control samples of naturally high Falling Number drawn from the same crop year.

This investigation needs to be extended to wheat samples of better underlying quality to establish the full potential of gravity separation. Investigations into the biochemical condition of the wheat proteins of both untreated and separated wheats indicated no substantial differences from other Avalon samples. However, a more extensive study into the condition of the proteins of gravity separated sprout-damaged wheats is needed.

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TABLE 1: Effects of gravity separation on specific weight and wheat Falling Number

Specific weight (kg/hl)		Wheat Falling Number (s)	
Untreated	Separated	Untreated	Separated
70.8	NA	70	278
75.4	80.0	148	345
75.6	79.1	107	335
77.8	81.8	140	338
74.2	77.2	80	209
70.6	74.6	165	234
72.7	76.4	149	246
76.2	78.8	162	259
72.1	75.6	130	246
74.2	76.8	163	241
74.6	78.5	159	313
75.4	77.7	118	273
75.3	77.5	105	271
75.0	78.2	156	291
76.0	78.1	171	309

NA = not available

TABLE 2: **Effects of gravity separation on the milling and baking quality of wheat**

	Mean 1	Mean 2	Difference 2-1	Sig. level
Specific weight, kg/hl	74.65	77.87	3.22	***
Wheat Falling Number, s	134.9	279.2	144.3	***
Wheat protein content, %Δ	12.14	12.08	-0.06	NS
SDS sedimentation volume, ml	69.1	72.8	3.7	**
Flour extraction rate, %	75.02	76.05	1.03	**
Flour colour, GCF	4.79	3.88	-0.91	*
Starch damage, FU	22.0	21.2	-0.8	NS
Flour Falling Number, s	172.7	295.4	122.7	***
Alpha-Amylase, FU	39.8	10.4	-29.4	***
Flour protein content, %Δ	11.00	10.84	-0.16	*
Wet gluten content, %	30.72	30.59	-0.13	NS
Resistance, BU	179.5	208.0	28.5	*
Extensibility, cm	21.55	19.50	-2.05	**
Total viable counts ($x10^6$)	1.91	1.55	-0.36	NS
Water absorption, %) LFP	51.09	54.00	2.91	***
Loaf volume, ml) LFP	1560	1486	-74	**
Water absorption, %) CBP	54.82	56.56	1.74	**
Loaf volume, ml) CBP	1359	1349	-10	NS
Amylose content, mg/g+	5.79	3.30	-2.49	***
Dextrin content, units/g+	585.5	277.2	-308.3	***

1 Untreated
2 Gravity separated
Δ at 14% moisture
\+ of breadcrumb (CBP)

NS not significant
* p<.05
** p<.01
*** p<.001

TABLE 3: **Results of baking quality tests carried out on control Avalons from the 1986 and 1987 harvests**

	1986	1987			
	A	B	C	D	E
Flour Falling Number, s	306	326	264	328	269
Flour protein content, %$^{\Delta}$	10.5	9.2	10.8	11.1	10.2
Resistance, BU	260	200	270	245	-
Extensibility, cm	19.3	18.5	20.5	19.5	-
Water absorption, %) CBP	54.3	55.7	54.6	55.7	56.1
Loaf volume, ml)	1385	1175	1395	1295	1370

Δ at 14% moisture

APPENDIX 1

Full results of quality tests

Wheat Protein content, *%		Wheat SDS volume, ml	
Untreated	Separated	Untreated	Separated
11.3	11.7	72	72
11.8	11.8	70	73
-	-	-	-
12.55	12.4	79	88
11.8	12.0	69	75
13.0	12.9	84	82
11.9	12.1	80	86
12.0	12.15	68	69
12.3	12.2	68	71
12.6	12.7	65	68
12.45	12.25	57	61
11.45	11.5	63	68
11.6	11.55	65	68
13.05	12.1	59	70
12.1	11.8	68	68

* 14% moisture basis

Flour Extraction rate,%*		Flour Colour, GCF		Starch Damage, FU	
Untreated	Separated	Untreated	Separated	Untreated	Separated
69.5**	77.9	4.3**	5.9	21**	21
74.3	76.5	3.6	2.0	24	21
74.0	74.3	4.65	3.1	22	24
74.5	75.6	4.6	2.8	23	25
74.9	75.5	5.2	3.45	26	23
-	74.9	-	5.15	-	19
74.5	74.8	5.9	5.15	21	22
75.4	76.1	5.5	6.9	21	22
75.2	76.2	6.3	5.2	21	19
75.3	76.3	5.5	3.7	19	17
76.2	77.8	6.0	4.4	22	22
75.2	75.6	3.7	2.4	23	23
74.5	76.5	3.3	2.2	23	23
75.5	77.4	5.05	4.1	23	23
75.8	76.2	3.5	3.0	18	12

*Total products basis, SR + Finisher flours
** No Finisher flour

Flour Falling Number, sec		Alpha-amylase, FU	
Untreated	Separated	Untreated	Separated
142	380	99	9
182	331	39	10
157	342	43	8
179	356	36	6
117	247	72	14
-	271	-	11
205	271	26	11
199	283	25	10
159	243	42	12
205	268	24	13
183	300	28	11
136	283	58	11
131	297	58	10
208	310	30	11
184	309	36	9

Flour protein content, *%		Wet gluten content, %	
Untreated	Separated	Untreated	Separated
11.3	10.7	-	-
10.6	10.3	29.9	29.9
10.7	10.8	30.6	31.6
11.4	11.4	28.0	28.8
10.6	10.6	30.2	31.2
-	11.5	-	30.1
11.1	10.9	28.3	31.5
10.9	10.8	27.3	27.5
11.2	11.0	31.9	30.1
11.7	11.5	33.6	31.1
11.5	11.1	32.9	31.7
10.4	10.4	33.6	31.3
10.4	10.4	32.3	28.4
11.7	11.1	30.6	32.7
10.8	10.7	30.4	31.9

* 14% moisture basis.

Brabender Extensograph results
at 45 min

Resistance (BU)		Extensibility (cm)	
Untreated	Separated	Untreated	Separated
-	-	-	-
100	140	23.8	21.6
120	140	25.0	20.7
345	425	17.6	16.7
90	130	20.0	20.0
-	-	-	-
-	-	-	-
350	325	12.7	12.6
165	200	22.3	20.1
180	230	24.4	20.9
115	-	23.3	-
110	130	22.4	19.6
125	150	23.8	21.3
80	-	21.0	-
210	210	23.5	21.5

Flour Microbiological results

Total viable counts ($x10^6$)		Moulds ($x10^3$)		Yeasts ($x10^2$)	
Untreated	Separated	Untreated	Separated	Untreated	Separated
1.5	>10	22.0*	9.5*	*	*
2.0	0.9	26.0*	16.0*	*	*
1.0	0.22	23.0*	8.0*	*	*
1.8	2.3	4.5	2.6	8.0	5.0
2.3	3.4	5.3	6.6	4.0	3.0
-	2.5	-	3.4	-	4.0
.032	.0075	4.2	2.6	3.0	1.0
.025	.0098	3.7	2.7	1.5	2.5
1.7	2.8	5.3	3.1	3.5	3.0
2.0	0.91	4.3	3.7	<1.0	2.5
1.8	7.7	2.1	1.7	6.0	0.5
9.1	0.019	3.4	1.7	2.0	4.0
2.0	0.86	3.1	2.3	0.5	4.0
1.1	0.64	1.3	0.5	10.0	0.5
0.027	0.37	5.1	1.8	<1.0	<1.0

* Moulds and yeasts were combined for the first 3 samples

LFP Loaf characteristics

Water absorption, %		Loaf volume, ml		Specific volume, ml/g	
Untreated	Separated	Untreated	Separated	Untreated	Separated
52.5	54.3	1595	1395	4.31	3.67
50.0	52.5	1570	1380	4.29	3.68
48.6	53.6	1480	1400	3.94	3.69
51.4	57.9	1605	1485	4.25	3.84
51.1	52.9	1600	1575	4.26	4.23
-	55.7	-	1505	-	4.02
53.9	55.4	1635	1525	4.36	3.99
52.5	55.4	1190	1160	3.17	3.04
49.6	52.9	1570	1590	4.30	4.31
52.1	53.6	1710	1630	4.71	4.47
50.0	53.6	1500	1560	4.10	4.20
51.4	53.9	1640	1530	4.51	4.11
51.8	54.3	1665	1585	4.56	4.34
50.7	54.3	1465	1495	4.01	4.04
49.6	51.4	1615	1505	4.55	4.30

CBP Loaf characteristics

Water absorption, %		Volume, ml		Specific volume, ml/g	
Untreated	Separated	Untreated	Separated	Untreated	separated
55.4	59.6	1305	1260	3.35	3.25
54.6	55.4	1300	1260	3.30	3.20
53.2	56.8	1305	1275	3.30	3.22
56.4	58.9	1500	1450	3.85	3.67
53.6	56.8	1350	1350	3.46	3.38
-	57.1	-	1400	-	3.54
56.4	57.5	1400	1400	3.50	3.41
56.8	58.9	1420	1400	3.71	3.66
54.6	55.7	1420	1350	3.54	3.59
58.6	55.7	1480	1485	3.58	3.61
55.4	55.7	1320	1335	3.39	3.48
52.1	55.0	1320	1350	3.37	3.46
53.9	54.6	1320	1330	3.48	3.46
53.9	56.1	1220	1295	3.16	3.40
53.2	55.7	1365	1315	3.62	3.38

CBP crumb characteristics

Amylose, mg/g		Dextrin, units/g	
Untreated	Separated	Untreated	Separated
7.73	3.16	1257	316
5.96	4.25	655	311
6.96	3.80	717	295
4.53	2.59	472	231
7.37	3.77	832	311
-	2.77	-	230
4.46	3.12	399	271
5.04	3.67	482	302
5.30	2.87	594	293
4.42	2.69	431	232
3.82	2.09	495	268
5.41	2.73	610	252
9.01	3.90	893	294
5.10	3.48	455	261
6.02	4.07	576	291

APPENDIX 2

An examination of flour proteins of untreated and separated samples: attempts to identify defects in the gluten structure

INTRODUCTION

Although the Falling Number of the wheats was much improved by gravity separation, their breadmaking quality was only slightly improved. During processing, the doughs became over-extensible, and flowed in the baking tins to give sharp-cornered loaves which lacked oven-spring and had poor crumb texture. Since dough rheology is dominated by the viscoelastic complex of gluten proteins, an explanation was sought in terms of the protein chemistry of the flours. In principle, breakdown of the glutens could be in the peptide linkages of the protein chains, or in the disulphide cross-links of the chain aggregates. Such changes would be caused respectively by proteases, and by disulphide reductases or reducing-agent products from other redox enzymes. The source of such agents could be endogenous within the wheat, being products of a very early stage in wheat germination; this stage would have relatively low *alpha*-amylase levels and grain properties indistinguishable by the gravity separator. Alternatively, the gluten-damaging agents could be exogenous, the products of biological contamination of the wetted wheat by fungi, bacteria or insects.

The reported attempts to detect such gluten-damaging agents in the separated wheats of poor quality were preliminary in nature. They were limited by the small sample set and the lack of sound samples from the same harvest for comparison. Proteolytic activity in the flours was measured by a traditional direct method. The state of the gluten proteins was also examined by gradient SDS-polyacrylamide gel electrophoresis. This technique readily resolves the high-molecular-weight glutenin subunits (HMW-Gs), which are known to be the keystone of gluten structure. SDS-PAGE has been used elsewhere to demonstrate breakdown of these proteins by proteases from fungal infection[16] and bug saliva[18]. The free sulphydryl content of some flours was measured polarographically in an attempt to detect any unusual redox effects.

METHODS

Direct assay of saline-extractable protease activity in flours was performed by a modifed Ayre-Anderson method[19].

SDS-PAGE according to the method of Laemmli[20] was used to analyse the reduced, SDS-solubilised flour proteins according to molecular size on 7.5-25%

linear gradient gels. One set of flour samples was extracted directly to detect pre-existing damage to the HMW-glutenins. A second set was wetted with salt solution, and taken through a time/temperature programme that simulated proving and baking of dough to reveal any proteolytic damage that ocurred during the bread-making process.

An EDT Differential Pulse Polarograph was used to measure accessible thiol groups in flour suspensions[21]; the dropping mercury electrode of this instrument measured thiol groups via the amount of phenyl mercuric acetate that the flours were able to sequester.

RESULTS

Table I: Effect of gravity-table separation on protease activity in flours

Sample	Gravity Separated	Protease activity (mg N/10g flour)
Rektor	No	21.6
	Yes	17.6
Avalon	No	27.7
	Yes	25.3
	Yes	25.8
Sound 1986 Avalon	No	23.5
1982 commercial	No	19.5
bread flours	No	27.6
	No	36.3
1983 sound	No	23.3
single varieties	No	27.2
	No	14.9

From Table I it can be seen that the test flours (both untreated and gravity separated) did not exhibit excessive protease activity, relative to sound flours. A slight reduction in protease content after gravity separation (2 samples) is insignificant compared to the spread of values seen.

Table II: Integrity of HMW-glutenins in sprout-damaged flours

Sample	Gravity Separated	HMW-glutenins as % of total protein extract	
		Before heating	After heating
1987 Avalon, control	No	6.0	5.8
Rektor	No	5.5	5.7
	Yes	6.2	6.5
Avalon	No	4.9	5.3
	Yes	5.4	6.1
Avalon	No	4.6	5.3
	Yes	5.4	5.4
Avalon	No	5.8	6.1
	Yes	5.7	5.8

By visual inspection, the SDS-PAGE electrophoretic patterns of proteins extracted from the flours were all normal; in particular the distinctive HMW-glutenin patterns were present at normal intensity. Quantification of the HMW-glutenins as % of total protein extract (Table II) confirms that all samples examined were similar, including the one control flour. Neither gravity separation, nor the simulated baking treatment, made any marked difference to HMW-glutenin patterns.

Table III: Accessible SH groups in a sprout-damaged flour

Sample	Gravity-table treatment	Accessible SH (μmoles/g flour)
Avalon	No	0.95 ± 0.03 (n = 4)
	Yes	1.01 ± 0.04 (n = 2)
38 sound flours*	No	0.66 to 1.07 Mean value 0.85

* measured previously and used for comparison

The one flour that was examined had an accessible thiol content within the normal range, and it was not significantly altered by gravity separation.

DISCUSSION

In so far as comparisons were possible with data for sound wheats, none of the sprout-damaged wheats examined in this study were abnormal in terms of gross protease content, status of HMW-glutenins, or free SH content. Although only a few samples were studied, the experimental approach would have detected the major lesions found in other case histories of slack over-extensible doughs[16,18]. Clearly, the gravity separated grains of relatively high Falling Number contain some factor detrimental to breadmaking. This factor acts in a manner too subtle to have been detected in this preliminary investigation. Whether the factor was introduced by contamination or by germination, further research work is required to identify it and devise methods of control.

APPENDIX 3

Visual methods for detection of *alpha*-amylase in individual grains derived from untreated and gravity separated wheat samples

AIM

An assessment of the distribution within samples of wheat of grains with high germinative enzyme levels as a method of selecting wheats that are suitable for gravity separation.

INTRODUCTION

The degree of variation of *alpha*-amylase activity among grains cannot be assessed by bulk methods e.g. Falling Number but other measurements, such as the Phadebas method, are sufficiently sensitive. However, they are very time-consuming and are not normally considered for the comparison of large samples.

Visual methods, capable of providing an indication of germinative enzyme activity, are available. These enable relatively large numbers of grains to be assessed simultaneously. They involve mounting grains on a suitable backing, so that they can be abraded to expose an internal face. The exposed face is then treated to reveal characteristic features related to enzyme activity. Thus, the Briggs method[22] exposes cracks and fissures in the endosperm of barley brought about by digestion of components such as cell-walls and endosperm storage protein. The fissures are emphasised by coating the surface with coloured varnish which concentrates in them.

The other visual method is a histochemical one. In the method of Jensen and Heltved[23], non-fluorescent fluorescein dibutyrate is applied in solution to the abraded grain surface. Lipase present in the germinated grains catalyses the hydrolysis by which butyrate groups are cleaved from fluorescein. Since several hydrolytic enzymes, including *alpha*-amylase and lipase, are secreted in germinating grain almost synchronously, the detection of one is indicative of the presence of the others.

METHODS

Both methods were untried in our hands and therefore calibration samples were required. Grains from a sample of Avalon with a relatively high Falling Number, were set to germinate on filter paper in petri-dishes under ambient conditions. Sub-samples were taken at intervals, air-dried and fixed with rapid-setting epoxy resin to plywood backing, as described by Briggs[22]. At each time interval, one

hundred grains were taken for each method of visualisation. Germination continued for 64h, when the last samples were taken. Samples were also taken from the experimental series and fixed to backing boards. The Falling Numbers and *alpha*-amylase values were known for these and hence a relationship between visual assessment and those values could be established.

Vanishing method. Some crazing was noted but poor correlation existed between observed modification and germination period, and modification and Falling Number/*alpha*-amylase. Grain texture affected the perception of modification, cracks being more readily seen in mealy than in vitreous grains - a problem which does not arise in barley, for which the test was developed. With such a bias inherent in the method, the Briggs method was rejected in this context.

Fluorescence method. Fluorescein dibutyrate is not readily available from UK sources and fluorescein diacetate was used as an alternative. A reasonable relationship was obtainable between germination period and number of grains exhibiting fluorescence. The area of fluorescence varied and the threshold for inclusion in the 'fluorescent' category was decided somewhat arbitrarily.

Calibration of the proportion of fluorescent grains against Falling Number required a higher threshold to be selected.

Image analysis was used in an attempt to remove the subjectivity of 'thresholding'.

RESULTS AND DISCUSSION

The table shows examples of manual counts of fluorescence for samples before and after separation. Falling Numbers and Biocon *alpha*-amylase values are also included. Image analysis counting was not sufficiently well developed during the time span of this investigation.

% Fluorescence, *alpha*-amylase and Falling Number of grains before and after separation

Sample	Before/ After*	% Fluorescent**	*alpha*-amylase (FU)	Falling Number (Sec)
Rektor	B	87	26	140
	A	97	11	338
Avalon	B	84	72	165
	A	90	14	234
Avalon	B	80	42	130
	A	63	12	246
Avalon	B	88	28	159
	A	91	11	313
Avalon	B	89	30	156
	A	90	11	291

*B = Before separation *A = After separation

** Results are means of duplicate readings based on two samples

All counts were very high and the lack of discrimination between samples of varying amylase activities led to suspicion that some aspects of the technique were at fault. Experiments comparing application of the fluorescein diacetate by brush and by spray showed that considerably higher values resulted from the use of spray. Fluorescein diacetate is documented as being a somewhat unstable compound and it is possible that its stability is reduced by being finely divided into droplets.

Two main points arise from this study:

1. Substitution of the dibutyrate with diacetate led to unforeseen problems which have prevented a proper evaluation of the published method and indeed a proper comparison of the samples in the present exercise.
2. In attempting to achieve success with the modified procedure, useful methods of mounting grains, simpler than those published, have been devised and the problems associated with image analysis of the fluorescent material have been identified.

CONCLUSION

The procedures used in this work were adapted from published work that was designed to fulfill different aims. At this stage it is not possible to say whether

the inability to distinguish between grains of untreated and gravity separated wheats was due to the techniques or to insufficient differences in the samples. Further development work on these sophisticated techniques will be required if this approach is to be used to select wheats that are appropriate for gravity separation.