

PROJECT REPORT No. 2

THE MEASUREMENT OF WET GLUTEN COLOUR

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### THE MEASUREMENT OF WET GLUTEN COLOUR

Project No. 0034/1/87

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#### **Abstract**

The aims of this investigation were to devise an instrumental method of measuring wet gluten colour to replace the subjective methods in current use, and to investigate the relationship between flour colour (GCF) and wet gluten colour. A number of sample preparation methods and colour measuring instruments were The preferred method involved grinding 150g of wheat on a Glen Creston disc mill, washing out the gluten using a Glutomatic semi-automatic gluten washer, and measuring its colour on the L\* scale on a Dr Lange tristimulus colour instrument. This method gave rapid and acceptably reproducible results. Correlations between wet gluten colours measured in this way and the GCF of flours milled from the same wheats on a laboratory Buhler mill showed that although gluten colour was one of the factors affecting flour GCF the relationship was not sufficiently strong to be useful for predictive purposes. A similar correlation exercise in which dry flour colour measured by a tristimulus instrument replaced GCF was more encouraging, and the developed method of measurement of wet gluten colour may be of more use if and when the milling industry moves towards dry flour colour measurement.

### **OBJECTIVES**

To devise a method of measuring wet gluten colour that gives a numerical index that can be applied by merchants and millers. To investigate the factors that influence wet gluten colour and the relationship between flour colour (GCF) and wet gluten colour.

### 1. INTRODUCTION

### 1.1 Flour colour and gluten colour

Flour colour is used as an indication of flour quality. Millers sell white flours against a colour specification, a low colour value being deemed to be better than a high value as it indicates a whiter flour. Colour measurement of flours is presently carried out in the UK on a Kent-Jones and Martin flour colour grader using a wet batter method. The resulting measurement is referred to as grade colour figure (GCF).

Millers would like to be able to obtain an estimate of flour colour before milling. Some millers currently do this by subjectively assessing the colour of the gluten extracted from the wheat. A flour is produced on a small-scale mill and the bran and offals separated off by sieving. The gluten is washed out of the flour sample and is left to rest for approximately 30 minutes. The miller then tests for quality and looks at the colour. A gluten of a good light colour and capable of producing a very thin membrane without breaking is considered to predict a good flour quality and colour.

This assessment procedure is open to criticism. Mill laboratories use a variety of small-scale mills for producing the test samples, e.g. KT Hammer Mills, Glen Creston, Quadrumat, coffee grinders etc., each method producing flours of varying particle size and bran content which can affect the colour assessment of the sample. When the gluten is extracted from the sample, the estimate of the flour colour is purely reliant on the eye of the assessor. The eye, though sensitive and discriminating, cannot make quantitative judgements that are reproducible and suitable for records. Different eyes make different assessments.

A standard method of measuring wet gluten colour that gives a numerical index that can be used and accepted by merchants and millers across the industry is required. To be able to devise a method, it is necessary to investigate the factors that influence wet gluten colour and the relationship between flour colour (GCF) and wet gluten colour. The development of an instrumental method of measuring wet gluten colour for the prediction of flour colour, giving numerical values, would create a more realistic basis for wheat trading.

## 1.2 Tristimulus colour measurement

Nearly all industries are concerned with the appearance of their products, and colour measurement is widely used, for example for matching batches of paint, wallpaper, fabrics or foodstuffs to meet consumers' requirements. In many cases this colour measurement is based on the tristimulus technique. The three basic colour values obtained can be transformed into a number of different value scales. The CIE 1976 L\*a\*b\* (CIELAB) scale is widely used (Anon, 1987). The Lab type scales, of which this is one example, are based on the opponent-colours theory of colour vision. Figure 1 below gives a pictorial representation of the Lab colour scales.

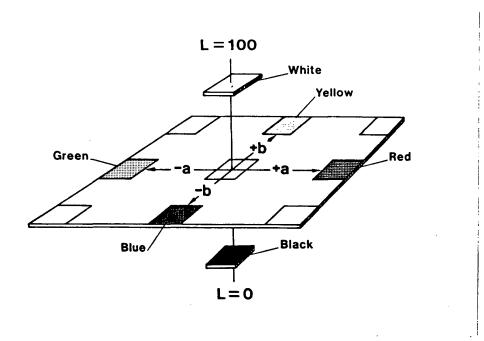


Fig. 1 Lab scale for colour measurement

When a sample is measured on a tristimulus instrument in the CIELAB colour space, three readings are taken:

L\* measures from white (100) to black (0). This indicates the lightness of the sample.

a\* measures the red/green opposites. A positive a\* indicates a red colour, a negative a\* indicates a green colour.

b\* measures the yellow/blue opposites. A positive b\* value indicates a yellow colour, a negative b\* value indicates a blue colour.

The higher value of the pair a\* and b\* is the colour or 'chroma'. The lower value indicates the hue e.g. reddish, yellowish. The preferred L\*a\*b\* scale differs from the HunterLab Lab scale, which was also used in this investigation, mainly in the darker region, where it offers greater discrimination. In both scales, values are as described qualitatively in Figure 1.

A whiteness index (WI) has also been used in this investigation. Whiteness compares the object with the preferred white. Ideal white is bluish, and departures from this towards yellow reduce whiteness rating by about four times as much as departures towards grey. The whiteness index used in this work (ASTM method E 313-73) was mathematically derived from Y and Z values, Y measuring brightness and Z giving a contribution from blue/yellow.

#### 2. MATERIALS AND METHODS

### 2.1 Wheat and flour samples

The wheat samples used for this work all originated from NIAB National and Recommended List trials and ADAS secondary trials, 1986 harvest, and were thus all of single known varieties. The Buhler-milled flour samples came from wheat from the same source.

### 2.2 Milling

2.2.1 Buhler milling White flours were produced by a fixed milling system using a Buhler Laboratory mill MLU-202 in conjunction with an impact finisher MLU-302. Wheats were conditioned by water addition 16h before milling. Wheats were milled at 15.0 and 15.5% moisture content for soft and hard varieties respectively.

2.2.2 Small scale milling Outlines of the procedures and mills are given in Section 3 for KT grinding and Quadrumat milling. The chosen procedure for rapid flour preparation is as follows:

## **Equipment**

Glen Creston Disc Mill
125

mum sieve and brass receiver

Balance

#### Method

- 1. Weigh out 150 grams of wheat.
- 2. Set the Glen Creston mill on its finest setting, 0-1.
- 3. Pass the wheat through the mill and collect all the ground sample.
- 4. Pass the ground wheat over the  $125\mu m$  sieve, preferably on a mechanical siever for 5 minutes, and collect the 'white' flour in the brass receiver.
- 5. Approximately 40 grams of 'white' flour will be extracted.

Flours produced by this method are referred to as 'rapid flours' in this report.

### 2.3 Gluten preparation

All glutens were prepared using a Falling Number Glutomatic 2100 according to the manufacturer's instructions. In cases of soft flours when flooding occurred the program was stopped and the procedure repeated using less mixing water. If difficulties still occurred the mixed sample was soaked for approximately 4 minutes in distilled water before resuming.

### 2.4 Colour measurement.

- 2.4.1 Grade Colour Figure (GCF) A Kent-Jones and Martin Series III colour grader was used for flour colour measurement.
- 2.4.2 Tristimulus colour measurement Instruments were used according to manufacturers' instructions. Details of sample presentation are given in Section 3.6.

### 3. RESULTS

## 3.1. Introduction

The investigations can conveniently be divided into five parts for reporting purposes:

- An investigation of the relationship between the GCF of Buhler-milled flours and various measurements of the colour of glutens extracted from the same flours.
- A comparison of three small-scale mills for the rapid production of flour samples for gluten preparation, and a study of the reproducibility of gluten colour measurements using the preferred mill for flour production.

- An investigation of the relationship between the GCF of Buhler-milled flours and the colours of glutens extracted from rapidly-milled flours from the same wheats.
- A repeat of the above correlation exercise but using dry flour colour measurements on the Buhler-milled flours instead of GCF.
- A comparison of four instruments for making tristimulus colour measurements.

## 3.2 Relationships between flour GCF and gluten colour for Buhler-milled flours

Relationships between flour GCF and gluten colour measured by tristimulus and NIR instruments were examined using laboratory Buhler-milled flours to establish the most useful colour scale for subsequent experiments. Glutens were extracted from the flours using a Glutomatic 2100 gluten washer. The use of the semi-automatic instrument ensured standard washing conditions.

Initially tristimulus colour measurements were made with a Hunterlab D25M-9 instrument using the Lab scale and Whiteness Index (WI). In subsequent work the CIE 1976 L\*a\*b\* scale was used. Although Lab has been used previously for bread colour measurements it was recognized that L\*a\*b is now the more widely used scale. In general there are only minor differences between Lab and L\*a\*b\* for light colours.

3.2.1 GCF vs gluten tristimulus values Using 46 Buhler-milled flours with a GCF range of 0 to 5 units the strongest simple linear relationship for GCF was with L values (see Fig. 2). The equation GCF = 33.09 - 0.48 L describing this relationship had a correlation coefficient of -0.74 and a residual standard deviation (sd) of 0.95. Neither a nor b contributed significantly to this relationship when added to the equation. Soft and hard varieties are identified in Figure 2 by 'S' and 'H'. Analysis showed that endosperm texture had no significant effect on the relationship between GCF and L. Figure 3 shows GCF vs L with two hard varieties Mercia and Avalon identified by 'M' and 'A'. For a given gluten colour, flours milled from Avalon had GCF values significantly higher on average than flours from the other varieties. Clearly variety has an influence on the relationship between gluten colour and flour colour. When a or b values for the 46 glutens were considered in single linear relationships with GCF very low correlation coefficients were found. If L\* was used in place of L the relationship with GCF was slightly altered to GCF = 39.72 - 0.53 L\* but the correlation coefficient remained the same as for L values. Figure 4 shows the relationship between flour GCF and gluten

whiteness index (WI). There is no significant linear trend and the correlation coefficient for this relationship is -0.11.

- 3.2.2 NIR for gluten colour measurement The Technicon InfraAlyzer contains a filter for colour measurements at a wavelength of 450nm. As many mill intake laboratories use this NIR instrument for protein and moisture measurements it was assessed for gluten colour measurement with the same set of 46 Buhler-milled flours. In previous work it was shown that filters 3 and 9 could be used to establish a flour GCF calibration (Osborne et al., 1982; Hook and Fearn, 1986). The same filters have been used for gluten colour measurement. The calibration equation GCF = 3.97 8.88 log 3 + 44.85 log 9 with correlation r = 0.81 and sd = 0.85 was derived from data for the 46 flours. This equation predicts flour GCF directly from reflectance measurements on the gluten. Figure 5 shows the relationship between flour colour values as predicted by NIR and the actual GCF.
- 3.2.3 Choice of colour measurement for gluten Tristimulus L\* values and NIR gave similar standard deviations when used for the prediction of flour GCF based on mean gluten colour measurements. Since the mean values were calculated from six readings per sample, it was possible to compare the reproducibility of the predictions pooled over the 46 flours. The appropriate standard deviations were 0.38 and 0.89 for L\* and NIR respectively. The greater reproducibility of the L\* values favoured this measurement over NIR. In addition the known relatively short life (~18 months) of the 450nm filter (log 9) reduces the usefulnesss of the NIR technique for gluten colour measurement. In subsequent work L\* and WI values have been used to measure gluten colour. Whiteness index (WI) was included despite its poor correlation with GCF as other work being conducted at the same time on flour colour measurement showed this scale to approximate well to human perception of dry flour colour.
- 3.3 Determination of the most effective method of rapid flour production for gluten colour measurement
- 3.3.1 Comparison of three mills A quick, small-scale method of laboratory milling was required to produce samples of flour with low bran content for gluten preparation. Such a method should be suited to a mill intake laboratory where test times should be less than 20 minutes. Three mills were investigated.

The KT Hammer Mill was assessed as a possible method of flour production as it is common to most mill laboratories for use in Falling Number testing. The

flours produced were very dark, even when passed over a  $125\mu m$  sieve. These in turn produced glutens which were too dark and bran contaminated to be of practical use.

The Brabender Quadrumat Junior produced a very clean, white flour, the bran being separated off during milling by an internal sieve. The Quadrumat milling was also performed with the addition of ½% water to the wheat just prior to grinding. This procedure produced even better results. The addition of water conditioned the bran so it sheared off the endosperm more easily, therefore producing a whiter, less contaminated flour. Unfortunately, Quadrumat mills are relatively rare in milling laboratories and would be expensive to install, so this method is unlikely to be utilized.

The Glen Creston Disc Mill produced a flour which was light coloured, although it had to be passed over a 125 µm sieve to remove the bran. This cheap and simple mill was easy to operate and produced a suitable flour. It was therefore selected as the most suitable mill for further investigations. A range of mill operating conditions were explored to establish a standard procedure for flour production.

Samples of wheat were milled on coarse, medium and fine settings. Milling on the finest setting produced the whitest flour, with least bran contamination and colour values closest to Buhler-milled flours. An assessment of the effect of moisture content of the wheat on flour production was undertaken. Samples of wheat were dried or conditioned to 12%, 14%, 16% and 18% moisture content and milled on coarse, medium and fine settings. The whitest flours produced on the Glen Creston were milled at 18% moisture on the finest setting. However, due to the high moisture content, the mill became clogged, sticky and difficult to clean. Also, as the average moisture content of grain entering a mill is likely to be 15% or less, it would take a long time to condition a sample to 18% for milling for gluten colour analysis. As the miller needs to take a sample of wheat from a lorry, test it and have an assessment of its flour quality within 20 minutes, this amount of conditioning would not be feasible. It was concluded that the most suitable conditions for milling with the Glen Creston Disc Mill were by using the finest setting and not altering the moisture content.

After conditions of milling were selected the reproducibility of this method of preparation was assessed.

3.3.2 Reproducibility Samples of two wheats (one hard, one soft) were milled in triplicate on the Glen Creston and measured for flour and gluten colour in duplicate

on each of two days. The colour measurements were taken on the Hunterlab D25M-9 on CIELAB utilizing L\*. The results are given in Tables 1 and 2.

The differences in reproducibility for the hard and soft wheats were not statistically significant, nor was there any significant day-to-day variability in any of the measurements. The flour results indicate that the mill produced flours of consistent quality. They also show the high reproducibility of tristimulus colour measurement. The gluten colour measurement was not as reproducible as that for flour, either because of extra variability introduced at the gluten washing stage or because of the greater difficulty of sample presentation. One possible reason for the poorer reproducibility of the gluten colours, the influence of storage under water before measurement, was investigated in detail.

3.3.3. The effect of excess water on gluten colour measurement An explanation for the poorer reproducibility of gluten colour measurements when compared with flour colour measurements was sought. A possible reason was thought to be the presence of excess water in the gluten presentation cell. Before presentation to the Hunterlab, glutens were stored in excess distilled water to prevent a darkened skin from forming on their surface. To investigate the effect of this, three samples of each of the two wheats used previously were milled, sieved, gluten washed in duplicate and measured for colour as one complete exercise, i.e. taking the washed gluten from the Glutomatic and immediately presenting it to the Hunterlab. The results are given in Table 3.

Comparing the means and standard deviations of the gluten colour values in Tables 1 and 2 with the corresponding values in Table 3, no significant differences were observed. The presence of excess water did not appear to affect the readings to any extent. Therefore, if necessary, it is possible to store small batches of glutens for a short time (1-2 hours) in water before taking colour measurements. This procedure was used for convenience in this investigation.

## 3.4 Relationship between GCF of Buhler-milled flours and rapid gluten colour

CIELAB is the internationally utilized colour space across many industries concerned with colour measurement, therefore it was decided to adopt L\* rather than L for gluten colour measurement. In spite of the negative results obtained with Buhler milled flours (see 3.2) Whiteness Index (WI) was also considered. A set of 18 rapid flours and their wet rapid glutens were measured on L\* and WI and correlated against the GCF of Buhler milled flours from the same wheats. Figures 6 and 7 are plots of flour GCF vs gluten L\* and WI respectively. Table 4 gives the

correlation coefficients and residual standard deviations for fitted straight line relationships for both flour and gluten.

This reappraisal of colour values based on rapid flour and glutens confirmed that L\* had the stronger relationship with flour GCF values. The relationship between gluten L\* and flour GCF (Fig. 6) was not sufficiently close to permit its use to predict flour GCF from gluten colour alone. Figure 6 suggests that there may be some influence of grain hardness on the relationship and therefore a number of additional hard milling wheats were examined.

A total of 20 hard-milling wheats were milled on the Glen Creston disc mill and measured on L\* as flours and gluten, and then correlations with GCF of the corresponding Buhler-milled flours were examined. Table 5 gives the correlation coefficients and residual standard deviations for fitted straight line relationships.

Although L\* of rapid milled flours and GCF of Buhler-milled flours correlated better for these hard wheat samples than both soft and hard combined (Table 4), gluten L\* values were less highly correlated with GCF. Hence there was no advantage gained from sub-dividing samples according to endosperm texture. Whiteness index of gluten was again shown to be a less useful indicator of GCF than L\* values. The relationships found between gluten L\* and flour GCF were not sufficiently strong for predictive purposes. This is perhaps not too surprising as it is known that flour GCF is a complex measurement that is made up of contributions from bran content, endosperm yellow pigments and protein (Barnes, 1986; Hook, 1987). Although some of the bran will be incorporated into a gluten it appears that the extracted gluten colour (as measured by L\*) does not incorporate sufficient of the GCF colour factors to be a usable predictor of GCF by itself.

# 3.5 Relationship between dry flour colour and rapid gluten colour

The current procedure for flour colour measurement based on a flour/water batter (Kent-Jones and Martin colour grader) is under review. The advent of relatively cheap tristimulus instruments offers an alternative approach to flour colour measurement. As preliminary investigations have shown that tristimulus flour colour measurements are promising, a limited study of dry flour colour versus gluten colour measurements was undertaken.

Flours were Buhler milled from 18 wheat samples and their GCF's and L\* values measured. Rapid flours and glutens were prepared using the Glen Creston mill and L\* measured. Two tristimulus instruments, the Hunterlab and the Dr Lange instruments were used for colour measurements (see 3.6). Table 6 gives the correlation coefficients and residual standard deviations for various equations

predicting flour colour from gluten colour.

L\* values for both Buhler-milled and rapid flours correlated much better with gluten L\* values than did GCF. There are obvious advantages in relating flour and gluten colour measurements using the same instrument and scale (L\*) over a comparison of GCF and gluten L\* (see Figs 8 and 9). Unfortunately whilst the milling industry continues to use GCF for flour colour measurement the usefulness of gluten colour assessment by tristimulus instruments is markedly reduced. The full potential of gluten colour measurement by tristimulus will only be realised when and if flour colour measurement is performed by the same technique.

## 3.6 Comparison of tristimulus colour measuring instruments

Four tristimulus colour measuring instruments were investigated:

- Dr Lange Microcolor LMC1
- Minolta Chromameter CR110
- Hunterlab Tristimulus Colorimeter D25M-9
- Trivector CL6000 (updated version of the Colourtronic 5000)

Instruments were loaned by suppliers (see Appendix) for a short time period. Due to the lack of time available for the use of each machine, flours rather than glutens were used for most of the assessment. A limited number of glutens were also measured to assess the suitability of the machines for measuring gluten colour.

## Dr Lange Microcolor LMC1

Design The instrument was a single compact unit comprising the measuring unit, data processor, and built in printer. It was very simple to use, the keyboard was small and clearly marked and the instruction manual concise and understandable. The instrument appeared to be well designed to stand up to the rigours of an industrial situation.

Operation No warm up time was required. Calibration using a standard white tile took about 15 seconds. Special glass 'kuvette' sample presentation cells were provided that were extremely quick and easy to use and aided good reproducibility. All colour spaces that might be required for flour or gluten colour measurement were provided by the instrument. It could also give mean readings. The measuring unit could be removed and used away from the data station, power being supplied by rechargeable batteries.

### The Minolta Chromameter CR110

Design The Minolta needed to be kept in its case to protect the optics from knocks or damage from dust or other contamination. It had to be reassembled for use each time via a number of brackets, cables and power packs. An inexperienced operator would need as much as two hours experience before being fully confident in its use.

Operation When using an AC power supply, no warm up time was necessary. Calibration via a white tile was quick but a little complicated. There was no sample presentation device for powder colour measurements. A 60mm plastic petri-dish packed solidly with flour was used, measurements being taken through the lid which was pressed to flatten the sample. This method produced adequately reproducible results but was extremely time consuming. The Minolta provided only four colour spaces: Yxy, L\*a\*b\*, L\*C\*H<sup>O</sup> and Munsell. Other colour spaces would have to be calculated from measured values. The measuring unit could be used independently from the micro-processor, power being supplied by rechargeable batteries.

### Hunterlab Tristimulus Colorimeter D25M-9

Design This was a large instrument designed to withstand industrial conditions. Its use was straightforward once learnt. The size of aperture was variable; 25mm proved the most suitable for flour and gluten measurement.

Operation The Hunterlab needed at least two hours to warm up. Calibration, via a white and a black tile was quick but slightly complicated and the instruction book would be required if not continually operating. There was no special sample presentation cell but a Technicon InfraAlyzer test cell sufficed, both for flour and gluten. The Hunterlab measured all the colour spaces required for flour and gluten evaluations.

### Trivector CL6000

Design This instrument consist of three separate units: a measuring head, a microprocessor, and a hand-held controller and printer. These were all linked by cables; two power connections were required. The measuring head could be used in a number of positions. No mounting device was supplied; samples were measured on petri-dishes hand-held below the optical head which was placed on a bench with the measuring area protruding over the edge. The handset which

controlled the microprocessor was operated by touch. The operation of the handset, although not too difficult, was quite long winded and information could be quite laborious to extract. The printer did not work automatically, only when requested. The microprocessor allowed a number of readings to be taken for each sample and calculated mean values. A moving sample could be measured; this was recommended due to the very small measuring area.

Operation The CL6000 needed no warm up period. The calibration of the instrument used a black and a white tile and was relatively simple, although the instructions would be required until the operator became accustomed to the procedure. There was no special presentation equipment. A 60mm plastic petri-dish was overfilled with flour sample, smoothed over with a spatula and left uncovered whilst being measured. The instrument was capable of producing all the measurements required for flour and gluten assessment.

## 3.6.1 Sample presentation for gluten

Dr. Lange Gluten was placed in the kuvette with a weight applied to flatten the measuring surface.

Trivector, Minolta The lack of a presentation cell for flour made gluten presentation even more difficult. In order to present gluten in a petri-dish, at least 50g white flour would need to be washed out. This would be too time consuming to be practical. No satisfactory method was found.

Hunterlab The Technicon InfraAlyzer test cell, as used for flour samples, held the gluten in place sufficiently well to get adequate readings.

3.6.2 Comparison of L\*, b\* and WI values from Microcolour, Minolta and Hunterlab instruments A set of 20 flours was measured by Dr Lange Microcolour, Minolta CR110 and Hunterlab D25M-9 instruments. The L\*, b\* and WI values from each instrument were compared. The ranges of the measurements obtained are given in Table 7. These ranges show instrument differences, some of which may be due to the lack of a standard sample presentation technique. In addition there were a few cases where the ranking orders were slightly different.

The correlations between the measurements on the three instruments are given in Table 8. The Microcolour and Minolta instruments produced measurements all three of which correlated highly. The ranges of the measurements were

different, notably for WI, but it would be possible to calibrate one instrument against the other. The Hunterlab WI values correlated highly with those from the other two instruments, and again calibration would be possible. The L\* and b\* values from the Hunterlab were less well correlated with those from the other two instruments, and on these scales the instruments would not be regarded as exchangeable. Thus, in defining a standard method for flour or gluten colour measurement there could be some scope for allowing the use of different instruments but potential problems do exist.

3.6.3 Reproducibility of the instruments To ascertain the reproducibility of the instruments, each was presented with a light and a dark flour three times and the measurements recorded. The results are given in Table 9. The Dr Lange Microcolor LMC1 was shown to be just more reproducible than the Hunterlab, but not significantly better than the others tested. However, its ease of operation and sample presentation made it the best choice for both flour and gluten colour measurement.

TABLE 1: Reproducibility of rapid milling of hard wheat (Mercia)

		Flour			Gluten	
	L*	mean	sd	L*	mean	sd
Day 1	84.80			66.24		
	84.79	84.80		64.05	65.15	
	84.58			64.75		
	84.64	84.61		63.15	63.95	
	84.43			64.90		
,	84.39	84.41	0.20	63.58	64.24	0.60
Day 2	84.70			64.06		
	84.74	84.72		63.44	63.75	
	84.71			64.16		
	84.71	84.71		62.82	63.49	
	84.62			64.36		•
	84.61	84.62	0.06	62.40	63.38	0.19
Overall		84.64	0.13		63.99	0.63

Note: pairs of results are duplicate colour measurements on samples from a single milling.

TABLE 2: Reproducibility of rapid milling of soft wheat (Galahad)

		Flour			Gluten	
	L*	mean	sd	L*	mean	sd
Day 1	85.64			64.32		
•	85.69	85.67		62.95	63.63	
	85.97			60.73		
	85.95	85.96		60.67	60.70	
	85.87			62.09		
	85.84	85.85	0.15	59.70	60.90	1.63
Day 2	85.72			60.74		
•	85.35	85.54	*	60.98	60.86	
	85.75			62.57		
	85.78	85.77		61.69	62.13	
	85.74			62.93		
	85.73	85.73	0.12	62.70	62.82	0.99
Overell		05.75	0.14		61.04	1 00
Overall		85.75	0.14		61.84	1.22

Note: pairs of results are duplicate colour measurements on samples from a single milling.

TABLE 3: L\* values of freshly prepared gluten

Wheat	L*	mean	sd
Manata	64.71		
Mercia	64.38	64.55	
	64.42		
	63.58	64.00	
	62.98		
	64.08	63.53	
•		64.03	0.51
Overall		·	
	59.58		
Galahad	60.58	60.08	
	60.51		
	61.83	61.17	
	60.79		
	59.06	59.93	
Overall		60.00	0.60
Overall		60.39	0.68

Note: pairs of results are colour measurements on duplicate gluten washings from a single milling.

TABLE 4: Statistics for fitted straight lines, GCF of Buhler flour vs colour of rapid flour or gluten, 18 wheats

		Correlation	sd (GCF units)
Flours			
	GCF vs L*	-0.53	1.25
	GCF vs WI	-0.34	1.39
Glutens			
	GCF vs L*	-0.60	1.18
	GCF vs WI	0.13	1.46

TABLE 5: Statistics for fitted straight lines, GCF of Buhler flour vs colour of rapid flour or gluten, 20 hard wheats

	Correlation	sd (GCF units)
GCF vs Flour L*	-0.76	0.85
WI	-0.14	1.31
GCF vs Gluten L*	-0.43	1.19
WI	0.35	1.23
L* Rapid Flour vs L* Rapid	0.56	0.74

TABLE 6: Statistics for fitted straight lines, flour colour v gluten colour, 18 wheats

			Correlation	sd
Dr Lange	•			
	GCF	vs L* Rapid Gluten	-0.48	1.29 (GCF units)
	L* Rapid Flour	vs L* Rapid Gluten	0.69	1.10 (L* units)
	L* Buhler Flour	vs L* Rapid Gluten	0.70	0.61 (L* units)
Hunterlab	•			
	GCF	vs L* Rapid Gluten	-0.60	1.18 (GCF units)
	L* Rapid Flour	vs L* Rapid Gluten	0.74	1.02 (L* units)
	L* Buhler Flour	vs L* Rapid Gluten	0.75	0.49 (L* units)

TABLE 7: Ranges of L\*, b\* and WI values for 20 flours measured on 3 instruments

	Microcolour	Minolta	Hunterlab
Range of L* values	89.8 - 93.9	89.6 - 93.4	88.2 - 93.4
Range of b* values	5.0 - 10.3	2.8 - 8.0	-1.3 - 9.7
Range of WI values	5.7 - 31.8	37.9 - 74.8	24.4 - 57.9

TABLE 8: Correlations between three instruments,  $L^{\star}$ ,  $b^{\star}$  and WI measurements on 20 flours

	L*	b*	WI
Microcolour vs. Hunterlab:	0.77	0.91	0.99
Microcolour vs. Minolta:	0.99	0.99	0.99
Hunterlab vs. Minolta:	0.78	0.89	0.99

TABLE 9: Reproducibility of four instruments: 3 replicates of 2 flours

Dr Lange Micro	color LMC1		Minolta CR	110	
Sample	L* value	sd	Sample	L* value	sd
I (light)	94.0 93.9 94.0	0.06	P (light)	92.92 92.82 92.70	0.10
Q (dark)	90.1 90.0 90.1	0.06	W (dark)	90.84 90.52 90.64	0.16
Trivector CL6000	D		Hunter	ab D25M-9	
Sample	L* value	sd	Sample	L* value	sd
I (light)	98.98 98.80 98.80	0.10	l (light)	90.51 90.88 90.67	0.19
Q (dark)	94.53 94.71 94.46	0.13	Q (dark)	87.21 87.14 87.32	0.09

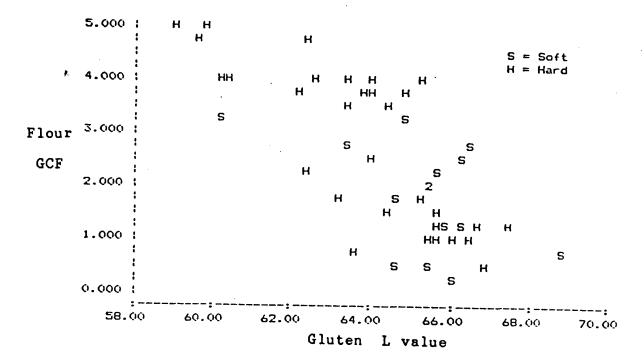


Fig. 2 GCF vs gluten L by grain hardness

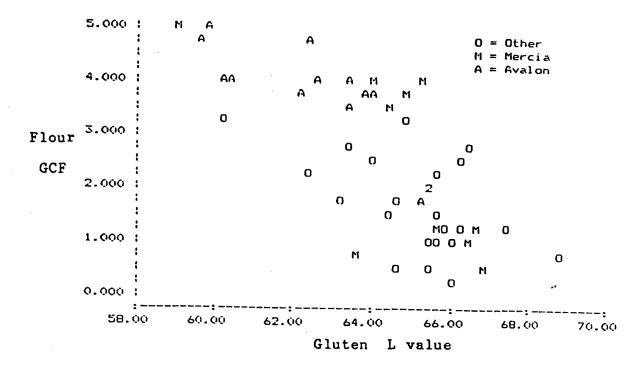


Fig. 3 GCF vs gluten L by variety

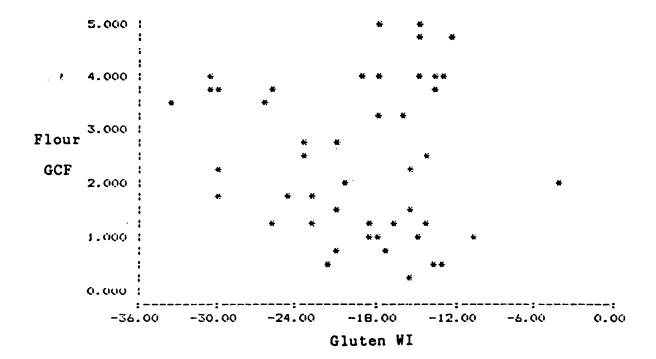


Fig. 4 GCF vs gluten whiteness index Wl.

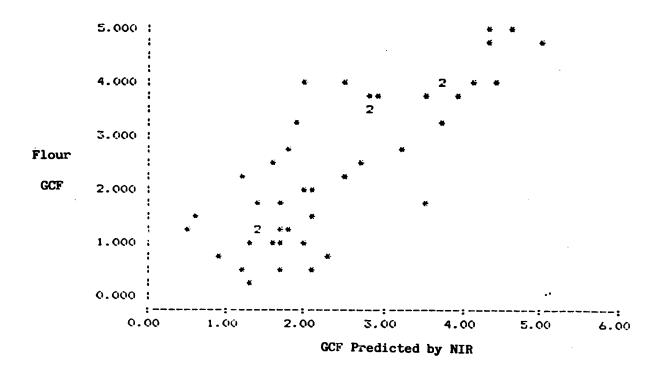


Fig. 5 GCF vs NIR Prediction.

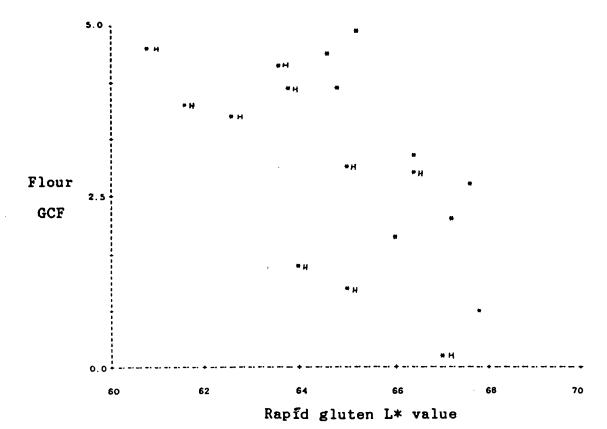


Fig. 6 GCF vs rapid gluten L\* Hard varieties denoted by H.

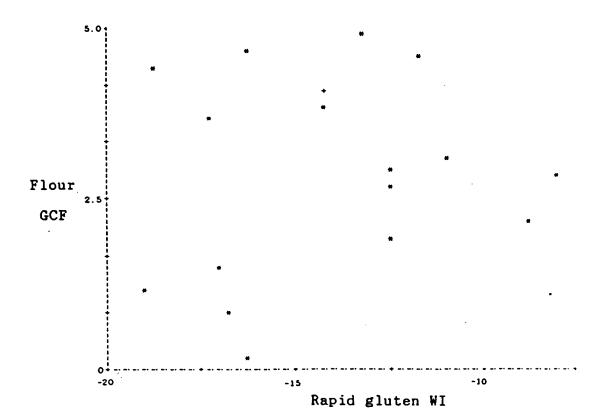
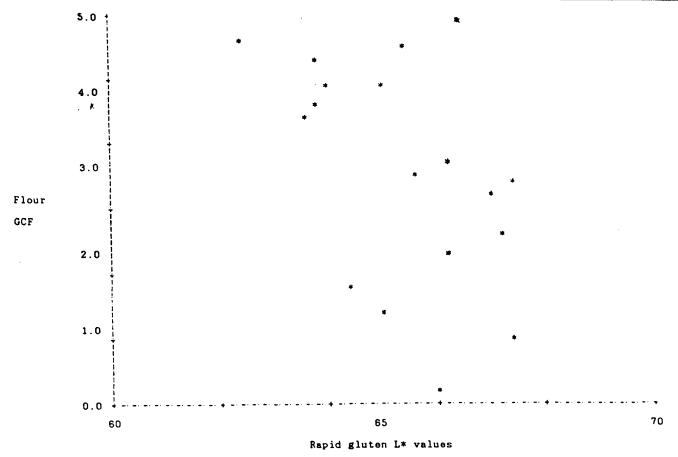
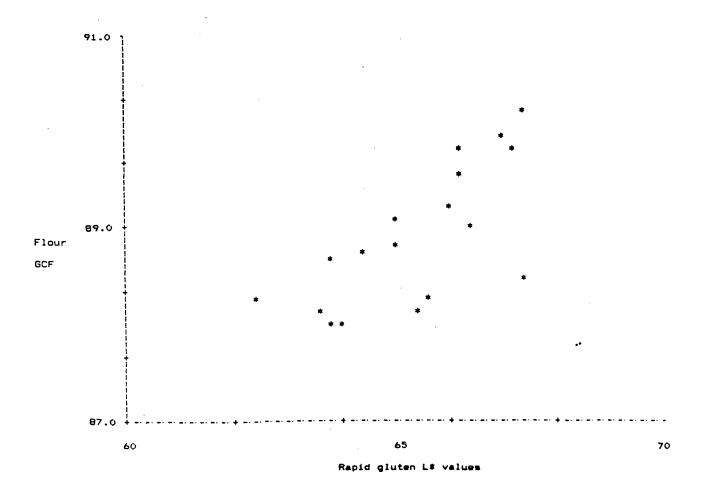


Fig. 7 GCF vs rapid gluten whiteness index WI.



GCF vs Rapid gluten L\*

Fig. 8



GCF vs Rapid gluten L\* Fig. 9

### 4. DISCUSSION

The aim of devising a rapid, reproducible method of measuring wet gluten colour on a numerical scale has been achieved. The recommended procedure is to grind 150g of wheat on a Glen Creston mill (2.2.2), wash out the gluten from the resulting white flour using a Glutomatic semi-automatic gluten washer (2.3), and measure the gluten colour on the L\* scale using a Dr Lange tristimulus instrument (3.6). This procedure can be completed in under 20 minutes by an operator familiar with the equipment.

In setting up a standard method, it would probably be necessary to specify by name the mill to be used for the grinding step. However it may not be necessary to specify the colour measuring instrument in the same way. In principle the L\* scales from different instruments should be comparable, although in practice differences were found. Some of the differences undoubtedly resulted from the variety of sample presentation methods that had to be used.

Although most of the work reported here was carried out using the Hunterlab instrument for colour measurement (because it was available throughout the whole period), more limited experience with the Dr Lange indicated that very similar results would have been obtained with that instrument. In particular the reproducibility figures for gluten colour in section 3.3 depend mainly on the variability in sample preparation and would not be expected to change. Pooling all the results on reproducibility gives a standard deviation of 0.9 L\* units. For comparison the range of L\* values for all the glutens measured was approximately 60 to 70. Given the difficulties associated with washing out and handling gluten, reproducibility of this order is probably the best that could be expected.

Where the investigations have been less successful is in using the measured wet gluten colour to predict flour GCF. With the residual standard deviations from regressions of GCF on gluten L\* being around 1.2 GCF units, predictions of GCF from gluten L\* would be accurate to ± 2 GCF units at best. This range is too wide to be of practical use. It was known at the outset that gluten colour was only one of the factors affecting GCF; what was not clear, precisely because of the lack of an objective measurement technique, was how important a factor it was. The results obtained showed that gluten colour taken by itself is not a practically useful predictor of flour GCF. Clearly gluten colour does contribute to flour GCF, and it might be possible to derive a more complex relationship in which a measurement of gluten colour was combined with other rapid measurements to predict GCF more successfully. It was not possible to investigate this in the time available.

The results obtained in predicting dry flour colour (as opposed to GCF) from gluten colour were more encouraging, although the correlations were still not very high. Research aimed at replacing GCF by a dry flour colour measurment, using a tristimulus instrument, is under way at present. There are indications that the milling industry may change to using such a measurement at some future time, although it is not yet clear which measurement scale will be selected. If and when it does, it would be worth reinvestigating the usefulness of wet gluten colour, as measured by the method described, as a predictor of flour colour.

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# **APPENDIX**

# Dr Lange Microcolor LMCI

META Scientific Ltd 7 Fosters Grove Windlesham Surrey GU20 6JZ

## Minolta Chromameter CR110

Minolta (UK) Ltd 1-3 Tanners Drive Blakelands Work Milton Keynes MK14 5BU

# Hunterlab Trisimulus Colorimeter D25119

Kirstol Ltd

Cheethams Park Estate

Park Street

Stalybridge

Cheshire

SK15 2BT

# Trivector CL6000

Vinten Scientific Systems Ltd Sunderland Road Sandy Beds SG19 1RB