The Influence of Protein and Protein Blending on Modification and Malt Specifications

P. M. Tansing, S. J. Logue, S. Roumeliotis, R. Kaczmarek, and A. R. Barr

Adelaide University, Dept. of Plant Science, PMB1, Glen Osmond, SA, 5064, Australia

Introduction

In order to achieve malting grade, Australian barley must generally have a grain protein (GP) concentration of between 9.0 – 11.8 % (ABB Grain Ltd). For overseas markets, the trend is towards an even tighter GP range (10.0 – 11.5 % GP). Management of malting segregations can be difficult for bulk handling authorities, and therefore "active stack management" of grain that allows for the inclusion of a wider range of GP to achieve an ideal "average" GP is an attractive solution.

However, blending samples from a wide range of protein contents presents a number of potential difficulties. For example,

- Malt extract and diastatic power will change markedly over the protein range
- Differences in water uptake may make optimisation of steep and germination conditions difficult
- The proportion of "steely" grains may increase as the mean protein increases

Home *et al* (1997) found that protein variation within a single barley spike can vary by 1.1% and between plants in small breeders plots from 3.7% to 9% for a single variety. As tillers are more synchronous in European crops, the variation in protein for individual kernels in a single spike found by Home *et al* (1997) probably underestimates the protein variation in Australian crops. Field variation in protein is also well known, as are the effects of environmental conditions on grain protein (Moody 1999). If variation in protein in a small breeder's experiment can be up to 9%, a sample taken from a paddock with a mean protein of 11.5%, may have a range of 7-16% protein for individual grains. Even if protein variation within a field varied by only 3.7%, if 2 samples with mean proteins of 9% and 12% were blended to obtain an overall mean GP of 10.5%, the protein of individual grains could actually range from 8.6% - 12.3%.

The first part of this study investigated the effects of blending samples with different GP concentrations prior to malting on malting specifications. The second part of this study investigated whether different micromalter protocols could improve malting specifications for samples with higher GP.

Materials and Methods

Barley Samples

Experiment 1 (Protein Blending) used Schooner and Sloop grain from the 1999 Stage 1 Trials grown at Maitland and Brinkworth in South Australia. Stage 1 trials have a control grid of a

number of commercial varieties repeated in a systematic pattern in an otherwise unreplicated experiment. For one experiment, field variation in fertility, soil water holding capacity and other factors result in a considerable range in protein content even within the same variety. The protein content of the Schooner and Sloop plots in the control grid was determined and this "natural" variation was utilised to obtain samples with a range in protein of 10.3 – 13.3 % (Maitland) and 10.8% - 12.6% (Brinkworth). Samples were blended prior to malting to achieve a standard GP of 11.8% (Table1), and also malted separately with the analysis results averaged to compare with the results of the same samples blended prior to malting.

Table 1. Samples chosen for Experiment 1 – Protein Blending from 1999 Maitland and Brinkworth field experiments

Site	Variety	GP of samples to be blended	Percentage difference in GP between samples	Mean blended GP
Maitland	Schooner	11.8 11.8	0	11.8
		11.3 12.3	1	11.8
		10.8 12.8	2	11.8
		10.3 13.3	3	11.8
	Sloop	11.8 11.8	0	11.8
		11.3 12.3	1	11.8
		10.8 12.8	2	11.8
		10.3 13.3	3	11.8
Brinkworth	Schooner	11.3 12.2	1	11.8
		10.8 12.8	2	11.8
	Sloop	11.3 12.2	1	11.8
		10.8 12.8	2	11.8

Experiment 2 (Protein Modification) used two barley varieties (Schooner, Sloop) from the 1999 Stage 1 Trials grown Maitland in South Australia. Four samples of each variety with 2 replicates, representing a 3% range in GP were malted using three different micromalter protocols differing in steep and germination regime.

Barley Quality Analysis

Grain Protein (GP) was measured using a Technicon Infraalyser 400 Near Infrared (NIR) instrument, calibrated with the Kjeldahl method of protein determination (Analysis committee of the EBC, 1998) as the reference.

Micromalting

Barley samples were screened over a 2.2mm screen. 120g of each sample in duplicate was micromalted in a Phoenix Automatic Micromalting System without the use of additives. Micromalter Schedules 1, 2, & 3 (see Table 2) with varying steep and germination cycles were used for Expt 2 (Protein Modification). Micromalter Schedule1 (see Table 2) was used for Expt 1 (Protein Blending).

Table 2 Micromalter Schedules used in Expt 1 (Schedule 1) and Expt 2 (Schedules 1-3)

Stage	**Schedule 1**	**Temp °C**	**Schedule 2**	**Temp °C**	**Schedule 3**	**Temp °C**
Wash	0.25		0.25		0.25	
Steep 1	7	15	7	15	7	15
Germination 1	8	15	8	15	10	15
Steep 2	9	15	9	15	5	15
Germination 2	94.5	15	6	15	94.5	15
Steep 3	-	15	0.5	15	-	15
Germination 3	-	15	88.5	15	-	15
Kiln 1	9	30-40	9	30-40	9	30-40
Kiln 2	4	40-60	4	40-60	4	40-60
Kiln 3	2	60-70	2	60-70	2	60-70
Kiln 4	4.5	70-80	4.5	70-80	4.5	70-80
Kiln 5	0.5	25	0.5	25	0.5	25
Total Steep + Germination (hrs)	118.5		119.0		116.5	

Malt Quality Analysis

The malt quality analyses carried out were the same for both experiments, all malt quality parameters were assessed using standard analytical methods (Barley Quality Report 1999 season) with the addition of Partly Unmodified Grains (EBC Method 4.15), and Extract Difference of Malt (EBC Method 4.5.2). Hot Water Extract (HWE) was analysed with a small scale version of the recommended EBC fine grind method (Macleod et al., 1991) using two grind sizes 0.2mm and 1.0mm. The difference between the fine and coarse HWE was then calculated to determine the F/C difference. Viscosity for the HWE samples was determined using an AMV 200 rolling ball viscometer. Density for the HWE samples was determined using DMA 58 density meter. Malt

Protein (MP) was measured using a Technicon Infraalyser 400 Near Infrared (NIR) instrument, calibrated with the Kjeldahl method of protein determination (Analysis committee of the EBC, 1998) as the reference. Diastatic Power (DP) was determined using a rapid small-scale version of a standard starch digestion followed by measurement of reducing sugars with a parahydroxybenzoic acid hydrazide reagent (PAHBAH). A spectrophotometric method recommended by the American Society of Brewing Chemists (ASBC) was used to assess soluble protein (SP) (ASBC, 1992). Kolbach Index (KI) was calculated using the ratio of soluble protein to malt protein. Friability was measured using a Pfeuffer GMBH Friabilimeter, and Partly Unmodified Grains (PUG) measured on the retained fraction.

Statistical Analysis

Experiment 1- The blended samples were compared to the mean of the samples malted individually using regression analysis where μ_{12}= mean of mixed samples, μ_{12}= mean of unblended sample for protein level 1, μ_{12}=mean of unblended sample for protein level 2. We expect μ_{12}= (1/2 μ_1+1/2 μ_2) After calculating the difference between μ_{12} and (1/2 μ_1+1/2 μ_2)we can regress these differences against protein to test if the difference is statistically different from zero. In addition, samples were compared using analysis of variance for each malt quality trait. The comparison of blended samples is shown in Table 3.

Experiment 2 - Analysis of variance of the malt quality data in experiment 2 was undertaken using the model Trait = Variety/(Protein*Micromalter)

The probability of significant variety effects, one way and two way interactions is shown in Table 4. Since GP and micromalter protocol were nested variables, main effects could not be calculated.

Results and Discussion

Experiment 1 Protein blending study

Blended vs unblended

In most cases, the performance of the blended malts was similar to that predicted by the average performance of different protein samples when tested individually (Figure 1 - Schooner; other data not shown). The exception was for partly unmodified grains (PUG). The blended samples tended to be higher than expected (Figure 1). Soluble protein and Kolbach Index also tended to be unpredictable. FC-difference showed a large deviation from expectation, although this was most noticeable in blended samples from the same protein band. In general, the same malt quality parameters show significant variation in the Sloop samples as well (data not shown), namely, PUG, Kolbach Index and F-C difference.

Whether the differences recorded are “real”, and likely to be observed in a commercial situation, are open to discussion. Grains from different protein bands may take up water at different rates, and in a commercial situation, we would expect that there may be greater competition for water than in a small micromalter sample. In addition, malt analysis on small quantities of malt may not always reflect the problems encountered in a brewhouse, and this is the case particularly in malt mixtures made of overmodified and poorly modified malts (Home *et al* 1997).

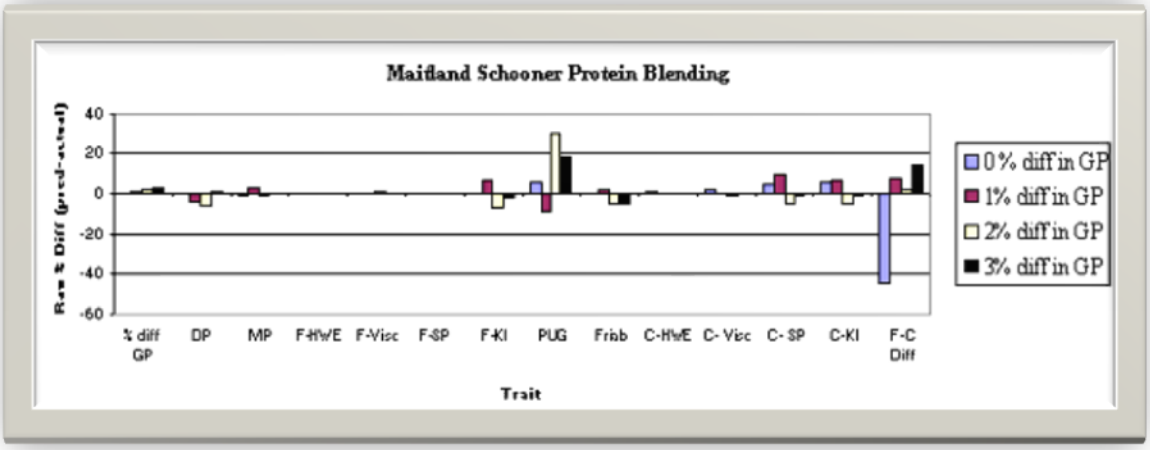

Figure 1. A comparison of the mean value of the blended samples with the calculated average of the protein bands blended, expressed as a percentage of the grand mean for each parameter, for 14 malt quality parameters

Blended

The usual expectation is that within a particular set of environmental conditions in a trial site, that variety and protein content are the prime determinants of malting quality. However, blending samples of increasingly different protein content to obtain the same average protein was shown to also affect some malt quality traits. For example, Schooner samples at Maitland had increased Fine-Coarse difference and PUG, indicating modification problems, while other malt quality traits were improved by blending (eg; Kolbach Index decreased, DP increased), or were unaffected (Fine extract) (Table 3).

Table 3. Comparison of key malt quality parameters for blended Schooner samples from Maitland (mean grain protein of 11.8%)

Protein content of each component of blend	GP difference between samples	Fine extract	DP	PUG	FC Difference	Fine Kolbach Index
11.8 11.8	0	78.3	393	9.5	1.9	44.8
11.3 12.3	1	78.1	416	14.5	3.5	41.8
10.8 12.8	2	78.3	395	14.0	3.7	37.3
10.3 13.3	3	78.3	451	11.3	3.2	40.2
LSD 5%		0.8	59	8.4	1.8	6.7

Experiment 2 Protein modification study

The analysis of the protein modification study partitioned variance into variety, variety x grain protein, variety x micromalter protocol and variety x grain protein x micromalter protocol factors. For every malt quality parameter, there were significant effects of variety, variety x grain protein and variety x micromalter protocol. However, second order interactions between variety x grain

protein x micromalter protocol were only significant for friability, fine viscosity and PUG, although fine HWE and coarse soluble protein were significant at 10% level of probability (Table 3). It is noteworthy that PUG is again responding in a different way to most other quality parameters (as per Experiment 1). The conclusion drawn is that it is possible to tailor the micromalting protocol, and presumably the commercial malting protocol, to accommodate different protein bands and achieve satisfactory specifications for most quality parameters. However, uniformity of modification may be compromised.

Table 4. Probability levels for variance components of analysis from protein modification study including 13 malt quality parameters

	Variety	**Var[3] x GP[1]**	**Var x MM[2]**	**Var x GP x MM**
Friability	0.001	0.001	0.001	0.030
PUG	0.001	0.001	0.001	0.002
FC diff	0.001	0.001	0.001	0.624
Fine HWE	0.001	0.001	0.001	0.058
Fine Visc	0.001	0.001	0.001	0.011
Fine SP	0.001	0.001	0.001	0.122
Fine KI	0.001	0.001	0.001	0.692
Coarse HWE	0.001	0.001	0.001	0.491
Coarse Visc	0.001	0.001	0.001	0.246
Coarse SP	0.001	0.001	0.001	0.072
Coarse KI	0.001	0.001	0.001	0.384
DP	0.001	0.001	0.001	0.107
MP	0.002	0.001	0.004	0.131

Footnotes; [1] = Grain protein; [2] = Micromalter protocol; [3] = variety

Conclusion

Blending of different protein bands to produce a mean protein within malt specifications results in a decrease of the homogeneity of that malt. However, blending small differences in protein had no adverse effects on malt quality and would enable an increase in the protein range accepted at storage facilities.

Changes in quality for blended samples are predictable for most traits (i.e. by calculating the mean of the performance of the individual samples before blending) but may not be for some indicators of modification, such as PUG and Kolbach Index.

Micromalter protocols can be adapted to accommodate different protein bands to achieve satisfactory malting specifications for most quality parameters. However, the ideal protocol for blended samples could not be predicted simply from the performance of the samples prior to blending.

Acknowledgements

This work acknowledges the support of the Malting Barley Quality Improvement Program, a program including the Australian university, state government research investment corporations and foundations and malting and brewing companies. The authors would like to thank Colleen Hunt from Biometrics SA for statistical analysis.

References

1. ABB Grain Ltd., (1999) South Australian Barley Delivery Specifications Season 1999/2000, Ver1.0

2. American Society of Brewing Chemists (1992) Methods of Analysis, 8th ed., The Society: St Paul, MN.

3. Analysis Committee of the EBC (1998) Analytica EBC, Verlag Hans Carl Geranke-Fachverlag.

4. Home, S., Wilhelmson, A., Tammisola, J., and Husman, J. (1997) Journal of the ASBC., 55(2):47-51.

5. Moody D(1999) Chinese Malting Barley Study Tour, Agriculture Victoria, Australia.

6. Macleod, L.C., Lance, R.C.M., Dowling, M.A., Logue, C.J., and Sparrow, D.H.B. (1991) *Proceedings of the 5th Australian Barley Technical Symposium*, Horsham, Victoria, p.80-83.

7. S.A Barley Improvement Program, (2001) *Barley Quality Report 1999 Season*, Adelaide University, Waite Campus