



## **The influence of malt quality on the colloidal stability of beer**

Louise Robinson<sup>1</sup>, Marian Sheehan<sup>2</sup>, Mont Stuart<sup>3</sup>, Andrew Barr<sup>1</sup>, Chris Ford<sup>1</sup> and Evan Evans<sup>1</sup>

<sup>1</sup>Department of Plant Science, Adelaide University, Waite Campus, Glen Osmond, SA 5064

<sup>2</sup>Lion Nathan Australia, 29 Nyrang St, Lidcombe, NSW 2141

<sup>3</sup>Joe White Maltings Ltd, PO Box 1176, Collingwood, Vic 3066

## **Abstract**

The formation of permanent haze in beer during storage and transport is a significant quality problem which places limitations on the shelf life of the product. Permanent hazes result from the interaction of proteins (or their fragments) that contain high levels of proline with polyphenols present in the beer. The most likely protein candidate is the hordein storage proteins. In this study a polymorphism for putative haze active hordeins was identified and surveyed by immunoblot in 170 Australian and international barley varieties. With the antiserum which was raised against the silica eluent protein (SE), a protein band of ~12 kDa was recognised in extracts from 150 barley and kilned malt samples. In 20 varieties, the antiserum did not immunodetect the ~12 kDa SE band. A pilot brewing trial indicated that beer brewed from varieties that did not contain the ~12kDa SE band were less prone to the formation of haze.

## **Introduction**

The formation of permanent haze in beer is a serious quality problem, which places limitations on the storage life of the product. Presently a number of stabilisation procedures are utilised in breweries to reduce and/or delay the onset of haze formation. These include the utilisation of prolonged cold treatments, fining with gelatin, isinglass, or tannic acid; addition of proteolytic enzymes and treatments with absorbents, polyvinylpyrrolidone (PVPP) or silica (Siebert and Lynn, 1998).

Most frequently, haze formation in beer is due to the cross-linking of a protein and a polyphenol (Bamforth, 1999). The protein and/or its fragments which contain high levels of proline cross react with the polyphenol to form a haze. During the storage of beer, polyphenol monomers (the proanthocyanidins; catechin, epicatechin and galocatechin) are slowly oxidised to form higher proanthocyanidin dimers. These include proanthocyanidin B3 and prodelphinidin B3 which can be more efficiently bound to the proline residues and thus form a large cross-linking protein-polyphenol

network, producing large colloidal particles and maximum light scattering (Siebert, 1999).

Haze active proteins that have been isolated from beer have been found to be derived from barley hordeins and are comprised of fragments of several different molecular weights (Asano *et al.*, 1982).

This indicates either several of the known barley hordeins are involved in haze formation or that one or more of these hordeins are degraded during proteolysis that occurs during mashing. The distribution and mole percentage of proline within the hordeins has also been shown to be directly related to haze forming potential (Ottrupp, 1987 and 1989).

The hordeins are a complex polymorphic mix which when separated by electrophoresis can be classified into four groups of polypeptides: B, C, D and  $\gamma$  according to molecular size (Shewry, 1993).

The hordein storage proteins are made up of 20-30 proteins that account for 50-60% of the total protein fraction of the barley endosperm (Shewry *et al.*, 1981). Genetic analysis shows that the hordeins are encoded by families of genes at single, linked loci on chromosome 5 (1H) (Shewry, 1993). The N-terminal sequences of hordeins are also rich in glutamine and proline repeats (Shewry, 1993). When the absorbed fraction of protein bound to silica gel used for the colloidal stabilisation of beer is analysed the mole percentage of proline is found to be 33.2 and of glutamate/glutamine 32.7 (Sheehan *et al.*, 1999), consistent with the proline/glutamine rich repeats of the hordeins. With the use of polypeptide model systems, silica gels have also been found to have high specificity for haze active protein because the silica gel binds to the same haze active proline residues as do haze active polyphenols (Siebert and Lynn, 1997). From silica gel, a silica eluent (SE) protein has been isolated and an antibody raised against the protein (Evans *et al.*, 2001). It is likely that this antibody can be used to identify barley varieties that are less predisposed to the formation of haze in beer.

In this study, a polymorphism for potentially haze active hordeins was identified with the SE antiserum and assessed by immunoblot in a selection of Australian and international barley varieties.

## **Materials and Methods**

### ***Barley samples***

One hundred and seventy Australian and international genotypes were selected and screened by immunoblot for the presence and/or absence of the SE band. Samples for the immunoblot survey were obtained from the 2000 Australian Winter Cereals Collection, Tamworth; the 2000 growing seasons breeding experiments conducted at Charlick, Port Wakefield, Tuckey; the 1998 season experiments at Yeelanna and the 1997 season experiments at Brinkworth. Additional samples were obtained from Berne Jones (WI, USA) and David Moody (VIDA). Commercial malts were obtained

from Joe White Maltings, Kirin Australia, Barrett Burston Malting, International Malting Company, Coors and Carlsberg, Denmark. Thirty of the genotypes were screened twice with samples obtained from two different sites/sources.

### ***Protein extraction, SDS-PAGE and immunoblotting***

Ground sample (20mg) was extracted in SDS-PAGE sample buffer (1M Urea, 4% SDS, Tris buffer pH 8.0) and 1% (v/v) 2-mercapto-ethanol. The extracted protein was separated by SDS-PAGE (15% gels) (Laemmli, 1970). The separated proteins were transferred to nitrocellulose (100V, 1hr) (0.2 µm pore size; Schleicher and Schuell, NH, USA) (Towbin *et al.*, 1979) and incubated overnight with the polyclonal SE antibody (1/1000 dilution). Antibody binding was detected using a HRP /conjugated goat anti rabbit antibody (1/5000 dilution) (Bio-Rad, Richmond, CA, USA).

### ***Mapping of SE trait***

The SE polymorphism was screened by immunoblot in 92 lines from the Chebec x Harrington (Kretschmer *et al.*, 1997) mapping population. QGENE (Nelson, 1997) was used to graphically display the association between molecular markers and the SE trait which was established using interval analysis (Lander and Botstein, 1989).

## **Results and Discussion**

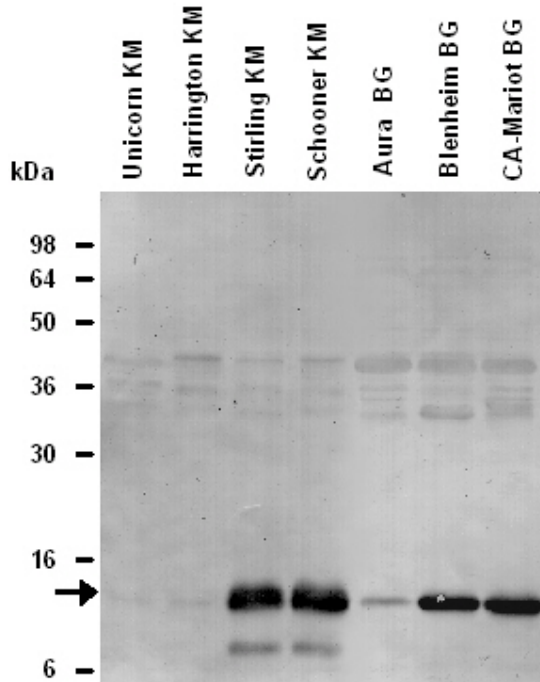
### ***Immunodetection of SE polymorphism***

The antiserum raised against the silica eluent protein (SE) was used in this study to identify a polymorphism for potentially haze active hordeins. Immunoblots of putative hordein extracts from barley and kilned malt revealed 150 varieties screened were positive (+) for the SE band (Table 1). Incubation of the blots with the antiserum at room temperature immunodetected an intense protein band with an apparent molecular mass of ~12 kDa with additional faint bands located between 32 and 98 kDa (Figure 1). The quantity of the immunodetected protein band of ~12 kDa increased in kilned malt samples along with the appearance of a less intense band with an apparent molecular mass of ~8 kDa, probably reflective that a degree of proteolysis has occurred during malting. About 30% of hordein is known to be degraded during malting (Smith, 1990). The antiserum failed to immunodetect the ~12 kDa SE band in 20 varieties (Table 1). A 50 litre pilot brewing trial found that beer made from two SE negative (-) varieties, were more resistant to haze force testing than beer made from 4 varieties containing the ~12 kDa band (Evans *et al.*, 2001). Further investigation is warranted to determine what role this ~12 kDa protein plays in the formation of chill haze. With 10 varieties which were designated SE (-) there was a faint ~12 kDa SE band immunodetected (Figure 1), although this may have been due to some cross-contamination of individual samples at this stage an explanation for this observation is not available.

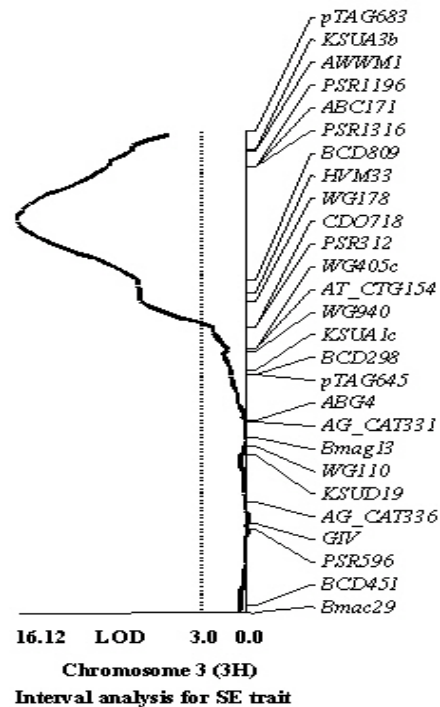
### ***Genetic mapping of the SE trait***

The SE polymorphism was further assessed by immunoblot in the Chebec (SE + band) x Harrington (SE - band) mapping population. Interval mapping analysis at the confidence level  $P = 0.0001$  showed the polymorphism mapped to chromosome 3 (3H) with the likelihood of difference (LOD) score highly significant at 16.12 (Figure 2). This demonstrates that the presence/absence of this band is controlled by the expression of a small number of major genes with at least two alleles.

**Figure 1:** Immunoblot of the SE trait. KM=Kilned Malt, BG=Barley



**Figure 2:** Interval mapping analysis of the Chebec (SE +) x Harrington (SE -) population.



## Conclusions

The hordein polypeptides that are present in both barley and malt have long been identified as having the potential to affect the colloidal stability of beer. Utilising an antibody raised against a silica eluent protein (SE) eluted from silica gel used for the colloidal stabilisation of beer, a low molecular weight ~12kDa band was identified. In this survey 150 varieties were found to be SE (+), while 20 varieties were found to be SE (-). Potentially, selection of SE (-) varieties may provide an opportunity to genetically improve the haze stability of beer produced from these varieties.

## Acknowledgements

We wish to thank the ARC, Lion Nathan Australia and Joe White Maltings for their financial support. We would also like to thank Joe White Maltings, Adelaide

Malting Company, Barratt Burston Maltings, International Malting Company and Kirin Australia for the provision of malt samples and the Australian Winter Cereals Collection, Tamworth for the provision of seed samples.

## References

1. Asano, K., Shinagawa, K., and Hashimoto, N. (1982) *J. Am. Soc. Brew. Chem.*, 40(4):147-154.
2. Bamforth, C.W. (1999) *J. Am. Soc. Brew. Chem.*, 57(3):81-90.
3. Evans, D.E., Sheehan, M.C., Robinson, L.H., Hill, A., Tolhurst, R., Gale, K., and Barr, A.R. (2001) *Proc. 11<sup>th</sup> Barley Tech. Symposium, Canberra* (in these proceedings)
4. Kretschmer, J.M., Chalmers, K.J., Manning, S., Karakousis, A., Barr, A.R., Islam, A.K.M.R., Logue, S.J., Choe, Y.W., Barker, S.J., Lance, R.C.M., and Langridge, P. (1997) *Theor. Appl. Genet.*, 94:1060-1064.
5. Laemmli, V.K. (1970) *Nature*, 227:680-685.
6. Lander, E.S., and Botstein, D. (1989) *Genetics*, 121:185-199.
7. Nelson, J.C. (1997) *Mol. Breeding*, 3:229-235.
8. Outtrup, H. (1989) *Proc. Eur. Brew. Conv. 22<sup>nd</sup> Congr., Zurich*, p 609-616.
9. Outtrup, H., Fogh, R., and Schaumburg, K. (1987) *Proc. Eur. Brew. Conv. 21<sup>st</sup> Congr., Madrid*, p 583-590.
10. Sheehan, M., Evans, E., and Skerritt, J. (1999) *Proc. 9<sup>th</sup> Barley Tech. Symposium., Melbourne, 2.9.1-2.9.4.*
11. Shewry, P.R. (1993) In: *Chemistry and Technology*. MacGregor, A.W., and Bhatti, R.S. (eds.) American Society of Cereal Chemists, Minnesota, U.S.A. p. 131-197.
12. Shewry, P.R., Wolfe, M.S., Slater, S.E., Parmar, S., Faulks, A.J., and Mifflin, B.J. (1981) *'Barley Genetics IV'*, Edinburgh University Press, U.K. p 596-603.
13. Siebert, K.J. (1999) *J. Agric. Food Chem.*, 47(2):353-362.
14. Siebert, K.J., and Lynn, P.Y. (1998) *J. Am. Soc. Brew. Chem.*, 56(1):24-31.
15. Siebert K.J., and Lynn P.Y. (1997) *J. Am. Soc. Brew. Chem.*, 55(2):73-78.
16. Smith, D.B. (1990) *Plant Varieties and Seeds*, 3:63-80.
17. Towbin, H., Staehelin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA*, 76:4350-4354.

**Table 1: Overall Classification of Varieties**

Australian and international varieties were obtained from 14 sites/sources as described in the material and methods section. Varieties identified by a (\*) are varieties in which a faint ~12 kDa SE band was detected.

SE (+)				SE (-)
Absynnia (Seln)	Elo	Manley	Semal	Aura*

Acclaim	Etu	Maresi	Shannon	Bowman
Alondra	Fatran	Maris Mink	Schooner	Brenda*
Alexis	Felicie	Maris Puma	Shonkin	Dicktoo
Amagi Nijo	Fergie	Maris Trojan	Skiff	Harrington
Arapiles	Forrest	Mars	Sloop	Haruna Nijo
Arra	Franklin	Maud	Steptoe	Kustaa*
Ayr	Gairdner	Meltan	Stirling	Landlord*
Barke	Galan	Melusine	Sultan	Moravian III
Baroness	Galaxy	Metcalf	Tallon	Morex
Barque	Galleon	Monarch	Tadmore	Onslow*
Beka	Gleam	Mundah	Terno	Osiris*
Berolina	Gobernadora	Narin	TG-Harrington-	Pirkka
Betzes	Golden	Novum	58	Polygena*
Blenheim	Promise	Oconnor	Thuringia	Pomo*
Bonanza	Grimmett	Optic	Tilga	RISO 1508
Brindabella	Grit	Orbit	Tore	TG-
Calicuchima	Guardian	Ortolan	Torkel	Harrington-06*
California_Mariot	Halcyon	Oxbow	Torrent	TR306
Carmarque	Hariot	Parwan	Tremois	Trebon*
Caminant	Jarek	Patty	Trine	Unicorn
Caruso	Jubilant	Picola	Trinity	
Cask	Kaputar	Pipkin	Triumph	
Chariot	Keel	Pitcher	Tweed	
Chebec	Kilta	Plaisant	Ulandra	
Chinook	Koral	Pokko	Vada	
Clarity	Korinna	Pompadour	Varunda	
Clipper	Koru	Prisma	Venture	
Cooper	Kredit	Proctor	Vigdis/Viqdis?	
Cork	Krona	Profit	Viktor	
Corniche	Kympii	Puffin	Volga	
Daphne	Ladik	Reggae	Waranga	
Dash	Lara	Research	Waveney	
Defra	Libra	Resibee	Weeah	
Derkada	Lindwall	Rifle	Yagan	
Derkado	Loke Abed	Rubin	Yerong	
Diva	Lumar	Sahara	Zephyr	
Ellice	Magda	Scarlett		
	Maltine			