



The influence of protein composition on beer haze and foam stability

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Abstract

Progress is reported on the development and application of immunochemical techniques including of enzyme-linked immunosorbent assays (ELISA) to predict beer quality from malt used to brew beer using pilot (50 L) and small scale (700-800 mL) brewing procedure. Polyclonal and monoclonal antibodies were raised to protein fractions of enriched beer foam proteins and proteins extracted from silica used for beer stabilisation. These antibodies were subsequently used to develop ELISAs and for immunoblotting. These ELISAs were found to measure different malt derived proteins. It is anticipated that the immunochemical methods developed may be applied to select and manipulate malt for brewing to improve beer foam stability while reducing the likelihood of storage haze formation.

Introduction

Beer foam and storage haze stability are of critical importance to brewers as they are amongst the first characteristics by which a consumer judges the quality of their beer. As such, brewers desire foam with optimum stability, quantity, lacing whiteness, 'creaminess' and strength. Of these characteristics, beer foam stability is arguably the most important. For if the foam is not stable the other characteristics are of little consequence. Not surprisingly, the most commonly used methods for commercially evaluating beer foam quality such as Rudin head retention test (HRV) and NIBEM essentially measure beer foam stability. Key malt derived foam-positive proteins including protein Z4, LTP1 and some members of the hordein storage protein family interact principally with hop acids to stabilise foam. Despite the availability of various foam enhancement options including propylene glycol alginate (PGA) or wickets, the use of malt with enhanced levels of foam promoting proteins is desirable as this option is relatively less expensive and are increasingly being perceived by the consumer as being more natural.

Brewers generally desire that there is no or minimal haze formed during the anticipated storage life of the product. Formation of haze is considered to be a sign of aging or contamination. The exception are "bottle/cask conditioned" beers (i.e., Coopers Pale Ale) where the yeast, added to the product to enable carbonation, is present to give a hazy impression. The main haze types formed during beer storage are chill or permanent hazes that result from the interaction between polyphenols and the proline residues in proteins (Siebert 1999). Beer haze stabilisation options include the general minimising of oxygen during brewing process, removing haze active proteins with silica, papain or tannic acid, while PVPP or the use of proanthocyanidin free malt seek to minimise the level of polyphenols. Silica mediated beer stabilisation procedures remove haze active protein (silica eluent, SE) with high levels of proline (>30%) and glutamine (>30%) (Sheehan et al., 1999), suggestive of hordein origin, without reducing foam stability. As proteins haze activity is dependent on the distribution of proline within the protein (Outtrup 1989), the use of malt with reduced levels of haze active proteins, presumably hordeins, is also a viable strategy for improving haze stability.

The aim of this paper is to utilise immunochemical procedures including ELISA to discriminate between malt foam promoting and haze active proteins to predict the brewing quality of malts and develop improved malting varieties.

Materials and Methods

Foam and haze protein isolation

At 4°C, 2x foam protein was isolated by first generating 500ml of foam from 500ml of beer with N₂ in a separating funnel, allowing the foam to drain for 20 min and then discarding the drainage. A further 500ml of 5% ethanol was added to the residual foam, from which 500ml of foam was generated with N₂, allowed to drain for 20min. The residual 2x foam was allowed to collapse into 50ml of water and freeze dried to concentrate. Silica eluent (SE) was isolated by adding silica (Lucilite) to "green" beer at 200mg/L to achieve colloidal stabilization. The silica was collected, washed twice with 5% ethanol and the protein eluted by washing with 2% NH₃. The eluted protein was then adjusted to pH 8.0 with HCl and then dialysed in to water. The eluted protein was concentrated by freeze drying. Obersteig layer protein was collected from a pilot scale lauter tun by Dr Chris Ford.

Antibodies were raised to these crude protein preparations by standard methods in rabbits to produce polyclonal antibodies and mice to eventually produce monoclonal antibodies (Harlow and Lane 1988).

Small scale and pilot scale brewing

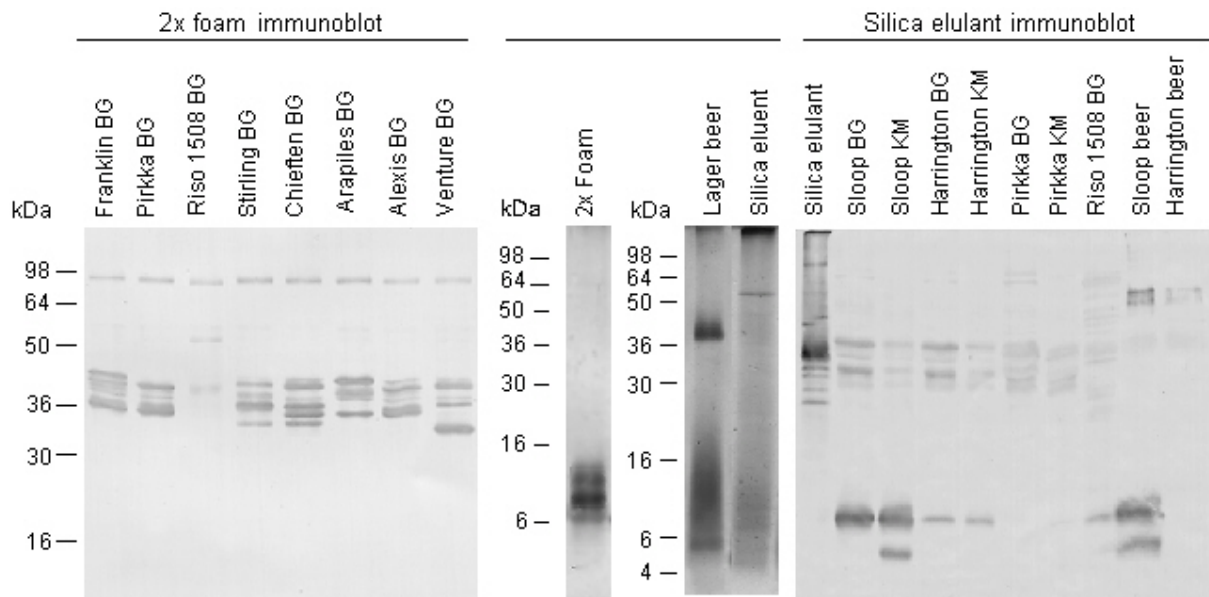
Small (600-800ml) and pilot scale brewing (50L) was conducted as described in Evans et al., (1999c). Beer foam stability was conducted by Rudin (Bishop et al. 1975) or NIBEM (Klopper 1977) methods.

Seven small and pilot scale experiments that were conducted over 4 years are as follows:

- Experiment 1: Small and pilot scale brews conducted on laboratory malted barley (Evans et al., 1999c).
- Experiment 2: Mash filtration trial on commercial malts (Evans et al., 1998).
- Experiment 3: Small scale brews conducted on laboratory malted barley in a trial investigating the effect of modification (Evans et al., 1999a). Malts were divided into those which had acceptable KI (38.0-48.7) and those that had low KI (30.2-36.7).
- Experiment 4: Small scale brews of commercial malts.
- Experiment 5: Small scale brews of commercial malts conducted by Mr Kay Schmacher (VLB, Berlin).
- Experiment 6: Small scale brews of micro-malted Schooner barley with different protein contents.
- Experiment 7: Small scale brews of commercial malts.

Bioglucanase and Biocellulase (Quest International) were added to 55 units of activity to the brews of experiments 4-7 to remove β -glucan and arabinoxylan so as to nullify the effect of viscosity of Rudin beer foam measurement.

Figure 1: SDS-PAGE of 2x foam and silica eluent silver stained for total protein and immunoblots with their antibodies.



Results and Discussion

Development of immunochemical procedures to target foam promoting and haze active proteins

To characterise foam and haze active proteins, foam protein and protein eluted from silica used for protein stabilisation were isolated. These relatively crude protein fractions were used to raise polyclonal and monoclonal antibodies. The polyclonal antibodies produced the distinctive immunoblot patterns shown in Figure 1. The patterns of bands in the 30-50 kDa and ~90 kDa range are typical of the patterns that would be expected for hordeins. Interestingly, the immunoblot for the silica eluent (SE) shows a distinct polymorphism in a low molecular weight band at approximately 12 kDa with an extra band at 5 kDa appearing in malt and beer. Sloop contains the band/s while, in Harrington the band is almost absent. In a survey of Australian and international barley varieties, it was observed that this band/s was absent in a relatively small proportion of barley varieties including Harrington and Unicorn (Robinson et al., 2001). A further antibody developed to oberteig protein recognised 3-4 bands at ~40 kDa (data not shown).

ELISAs were developed with the polyclonal and monoclonal antibodies in both the qualitative antibody capture (ac-ELISA), format: antigen//antibody//goat anti-rabbit horse radish peroxidase conjugate, and quantitative double-antibody sandwich (ds-ELISA), format: antibody//antigen//antibody//goat anti-rabbit horse radish peroxidase conjugate, formats. These were in addition to ds-ELISAs that were previously developed to the specific foam active proteins, protein Z4, protein Z7 and LTP1 (Evans and Hejgaard 1999). By a process of elimination, the newly developed ELISAs were narrowed down to the ac-ELISAs ac-2xfoam, ac-oberteig and ac-SE applied to malt extracts using 1M urea, 1% β -mercapto ethanol as recommended by Skerritt and Henry (1988) and a 80102 (monoclonal)//2x foam ds-ELISA on PBS extract. Interestingly, both the ds-80102//2x foam and ac-2xfoam ELISAs were highly correlated with the protein Z4 ELISA ($r \sim 0.8$).

Malt protein composition and quality influence beer foam stability

Small and pilot scale brewing trials demonstrate that malt source has a substantial effect on beer foam stability with ranges (min to max) between 5-20 sec HRV (5-20%) between the best and worst performing malts (Table 1). This foam difference is commercially significant as the addition of the foam stabilizer PGA is reputed to result in an increase in HRV of approximately 5-10%. Addition of Biocellulase and Bioglucanase, experiments 4-7, to the small scale mash effectively nullifies the contribution of viscosity to the Rudin measurement by removing β -glucan and arabinoxylan from the beer (data not shown). The resulting ranges of 5-18 sec HRV must therefore be solely attributed to differences in the foam protein composition of the malt used to brew these beers.

Table 1: Comparison of the effect of malt source and the ability of beer/malt characteristics to predict beer foam quality in seven brewing trials.

Experiment	1	2	3a	3b	4	5	6	7
Year malted	1997	1997	1997	1997	1998	1999	1999	2000
Number of malt	25	12	11	14	11	12	12	24

samples								
Bioglucanase/cellulase	No	No	No	No	Yes	Yes	Yes	Yes
Average KI	47.4	43.3	34.2	43.0	45.0	43.3	41.6	43.3
HRV maximum (sec)	109.0	288 ^a	115.5	113.3	111.2	115.5	99.4	112.0
HRV minimum (sec)	88.3	159 ^a	106.5	94.7	101.0	98.5	94.1	94.4
HRV range (sec)	20.7	129 ^a	9.0	18.6	10.2	17.0	5.3	17.6
Correlations with HRV (or NIBEM)								
Beer protein ^b	0.62**	nd	0.47	0.92**	0.41	0.42	0.44	0.02
Beer viscosity	0.55**	nd	0.50	0.80**	0.37	0.45	0.43	0.10
Malt KI	-0.72**	-0.31	0.19	-0.43	-0.46	-0.31	-0.46	-0.49*
Malt ds-protein Z4	0.76**	0.80**	0.74**	0.71**	-0.33	-0.07	0.67*	0.02
Malt ac-2xfoam	0.57**	nd	nd	nd	-0.11	0.01	0.03	-0.2
Malt ds-80102//2xfoam	nd	nd	nd	nd	0.15	0.33	nd	0.24
Malt ds-protein Z7	-0.1	0.14	-0.49	-0.49	0.63*	0.26	0.52	0.47*
Malt ds-LTP1	0.2	0.32	-0.59*	0.60*	0.54	0.68*	0.26	-0.06
Malt ac-oberteig	0.50*	nd	nd	nd	0.70*	0.44	0.5	0.77**
Malt ac-SE	0.51**	nd	nd	nd	0.05	0.61*	0.36	0.48*

^a Foam quality measured by NIBEM foam analysis, ^b Beer protein measured by Bradford (1976) method.

* = $P < 0.05$, ** = $P < 0.01$.

Prediction of beer foam stability from malt characteristics

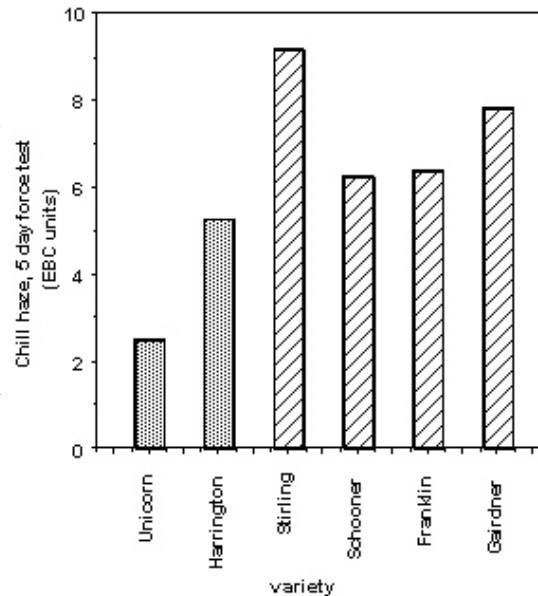
Prediction of the potential foam performance of malts and varieties would be a tool particularly to brewers. Traditionally brewers looking to improve the quality of their beer foam have relied on specifying lower malt KI (i.e. ~40 KI) and historical perceptions of varietal performance. *Ex ante* trouble shooting sub-optimal beer foam performance has been generally limited to use of the Bradford (1976) coomassie blue binding method and attempting to exclude the influence of foam negative substances such as lipids. The regression analysis of seven brewing trials presented in Table 1 demonstrates that no one malt or beer factor will on its own predict beer foam stability. This underlines the fact that prediction of beer foam quality is relatively complex. Although not all foam promoting factors can currently be measured, the objective of predicting potential malt foam stability can be progressed by a strategy of accumulation of foam promoting indicators. Table 1 indicates that these include reducing malt KI, and increasing the levels of protein Z4, LTP1, protein Z7, and the levels of proteins measured by the ac-oberteig and ac-2x foam. At this stage we would be wary of increasing the levels of ac-SE as this material is associated with potential

to form haze, although we yet to identify a significant correlation with haze formation.

The influence of malt source on beer haze stability.

A pilot brewing trial has shown that beer made from malt of the varieties Harrington and in particular Unicorn were more resistant to haze force testing than beer made from Stirling, Schooner, Franklin and Gairdner (Figure 2). Immunoblots with the SE antibody show that the malt/barley latter varieties contain a ~12 and ~5 kDa bands while in Harrington and Unicorn this band is essentially absent (Robinson et al., 2001). The neither the ac-SE or other ELISA developed against SE was correlated with the force test chill haze stability shown in Figure 2 (data not presented). Since the ~12 and 5 kDa bands are absent in immunoblots of Harrington beer (Figure 1), it is possible that this protein performs a critical role in haze formation. We are currently testing the hypothesis that genetically ensuring the absence of these low molecular weight SE bands may be an opportunity to improve the haze stability of beer produced by these varieties.

Figure 2: The colloidal stability of beer produced from 6 pilot scale brews with the Tooheys 5 day chill haze force test.



Conclusions

Pilot and small scale brewing trials were used to test the ability of immunochemical tests to predict the potential of malt samples to produce beer with superior foam and haze stability. Foam stability was predicted by lowering malt KI, and increasing the amount of protein measured by the protein Z4, protein Z7, LTP1, ac-2xfoam and ac-oberteig. Improved beer haze stability was predicted by the absence of ~12 and ~5 kDa protein bands observed in immunoblots with an antibody raised against protein eluted from silica used from beer haze stabilisation. Combined these procedures have the potential to be used to improve the foam and haze stability of future Australian malting barley varieties.

Acknowledgments

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