Improved Methods for Determination of Beer Haze Protein Derived from Malt

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Introduction

The presence of cloudiness or haze in beer is one of the more obvious quality defects discernable to the consumer. In addition, ever increasing demands are being placed on product quality with pressure to maximize output, expanding market areas and requirements for "use by date" labeling.

The most frequent cause of haze in packaged beer is due to complex formation between proteins and condensed polyphenols (tannins). It is common practice for brewers to employ beer stabilizer treatments to remove one or both of these principal haze constituents. In order to eliminate proteinaceous material that impacts on haze, beers are treated in one of the following ways: protein may be degraded (chillproofing enzyme), precipitated by addition of polyphenols (tannic acid) or adsorbed by silica gels. The effectiveness of these reagents is dependent on their capacity to specifically target the "haze sensitive" protein fraction of beer with minimal negative effects on the protein fraction involved in beer foam quality.

The precise protein fraction removed by these treatments is not clearly defined due to the analytical difficulties involved in characterization of beer proteins. This is because the original barley proteins are chemically modified and subject to proteolysis during processing resulting in a complex mixture of polypeptide fragments in beer.

When malt beers are fractionated by electrophoresis (SDS polyacrylamide gel electrophoresis) and stained with Coomassie blue reagent, polypeptides contained within high molecular weight, (Mr 40,000) and low molecular weight (Mr 5,000- 15,000) regions are seen. Two major beer polypeptides that are present within these molecular weight fractions have been characterized. These are barley – derived protein Z (Mr 40,000) (Kaersgaard and Hejgaard, 1979) and Lipid Transfer Protein (LTP, Mr 10,000) (Sorensen et al. 1993). Recently, quantitative ELISAs developed against protein Z and LTP were described (Evans and Hejgaard, 1999) and used to evaluate the impact of these proteins on beer foam quality (Evans et al. 1999).

Previously we characterized beer samples using two groups of hordein reactive antibodies (Sheehan and Skerritt, 1997) group 1 cross – reactive with subunits from B/D aggregates and group 2 cross – reactive with B/C monomers. By this approach it was seen that malt beers contained a number of hordein – derived fragments. The beer samples contained polypeptides of molecular weight >50,000 which primarily reacted with group 1 antibodies while the second group of antibodies recognized beer polypeptides of 30,000 or lower molecular weight.

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The aim of the present study was to investigate the impact of haze stabilizer treatments on beer protein content by employing the specific protein measurements described above.

Experimental

Protein
Total high molecular protein was measured using Coomassie Plus reagent- and bovine serum albumin (BSA) standard. ELISA (enzyme linked immunosorbent assays) were carried out as previously described and antibody binding to extracts was monitored by absorbance at 414nm.

Stabilizer Treatments
A small – scale study was carried out where maturation beer (16P) was diluted with deaerated water (10P) and centrifuged at 10,000 rpm for 15 minutes. Stabilizer additions were made to samples (500ml) at the following rates; tannic acid (50ppm), silica hydrogel (Lucilite PC9, 600ppm) and chillproofing enzyme (Profix, 6ppm). All samples were incubated on ice for one hour and then pasteurized to 15 PU.

For Pilot scale studies, maturation beer (10L, 10P) was treated with silica gel at a range of concentrations (0,100,300,600,1200 mg/l) and filtered through a plate and frame filter with filter sheets (Cuno) containing diatomaceous earth.

Results and Discussion

Impact of stabilizer treatments on beer protein content
A small – scale study was carried out in order to evaluate the impact of beer haze stabilizers on protein contents. Total beer protein (Bradford method) showed minor decreases (5% – 13%) following stabilizer treatments Table 1.

Table 1. Total Protein Contents of Beer (mg/l) after Stabilizer treatments

No treatment	Silica hydrogel	Tannic acid	Chillproofing enzyme
275± 5	243± 8	239± 8	263±11

In a similar way, the profiles of beer proteins separated by electrophoresis were not significantly changed following stabilizer treatments. The two major molecular weight regions (detectable after Coomassie blue staining) at (1) Mr 40,000 and (2) Mr 5000 - 15,000 were at similar levels for each of the samples (data not shown). This result is in agreement with previous studies where it was seen that a small loss of total protein following stabilizer treatment is sufficient to ensure good colloidal stability of the resulting beer.

In order to carry out a more specific assessment of the impact of stabilizers on beer protein content immunological methods (ELISA) were employed using antibodies cross- reactive with barley B/C monomers, D/B aggregate subunits, protein Z (Mr 40,000) and LTP (Mr 10,000). For the three stabilisers investigated it was apparent that decreases in ELISA absorbances were significantly greater for hordein reactive fragments than for the other beer proteins measured (Protein Z, and Lipid Transfer Protein) Fig.1. In the case of beer polypeptides cross –reactive with hordein specific antibodies (monomeric B/C) the greatest decreases occurred with silica gel 67%, followed by tannic acid 58% and chillproofing enzyme 43%. Significant decreases were also seen for the polypeptide fraction reactive with the second hordein antibody type (D/B aggregate subunits), silica gel 42%, chillproofing

enzyme 57% and tannic acid treated beer 37%. Decreases for other specific protein fractions were far lower in the range 3% to 6% for protein Z and 4% to 16% for Lipid transfer Protein.

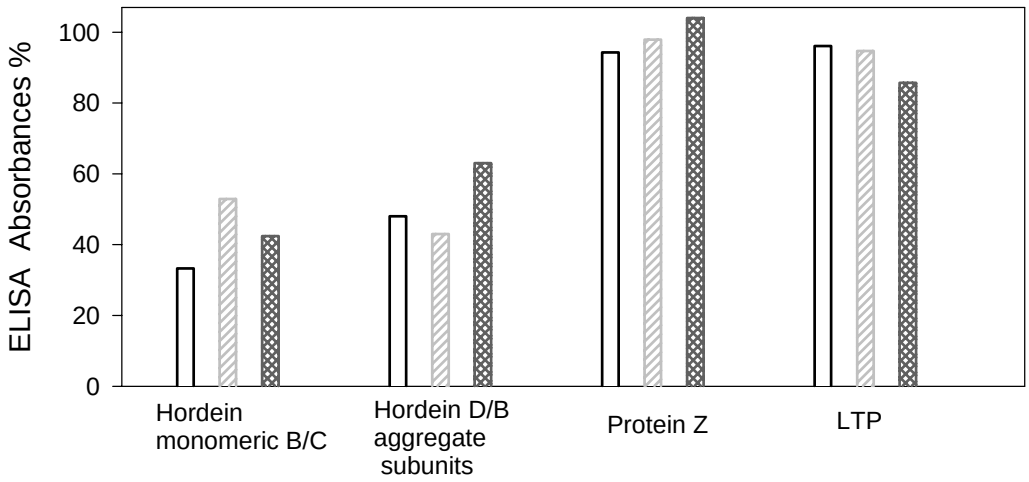

Figure 1 Comparison of relative protein levels in beers treated with stabilisers Elisa absorbances are expressed as % remaining after stabiliser treatment relative to a control (untreated beer 100%)

Influence of Stabilizer Concentration on Beer Protein Content

The relationship between stabilizer dose rates (silica hydrogel, 0, 100, 300, 600, and 1200 mg/l) and protein contents of maturation beer was studied in a pilot- scale filtration trial. At low silica concentration hordein ELISA absorbances declined essentially in proportion to the amount of silica added and reached saturation at approximately 600mg/l silica. Decreases in total protein and other specific beer proteins (Protein Z and Lipid Transfer Protein) were far less even at higher silica concentrations (600- 1200 mg/l).

Further evidence for the high specificity of the stabilizer for hordein - derived beer polypeptides was seen from amino acid composition data of the silica adsorbed fraction (eluted with 2% ammonium hydroxide solution). This fraction contained a high proportion of proline (33.2 mole %) and glutamate/glutamine (32.7 mole %) consistent with the presence of hordein repeat sequences.

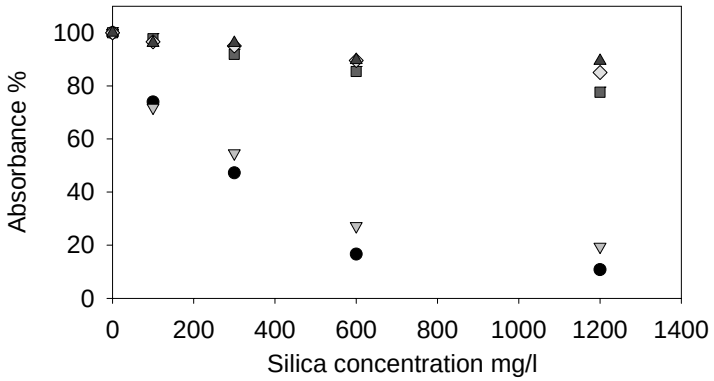

Figure 2. Protein remaining (% Absorbances) after Silica treatments at a range of concentrations (0, 100, 300, 600, and 1200 mg/l)
▲ Total protein ● Hordein B/C monomeric
▽ Hordein D/B aggregate subunits ◇ LTP
■ Protein Z

References

Evans, D.E and Hejgaard, J. (1999) J. Inst Brew 105: 159 –169.
Evans, D.E., Sheehan, M.C., and Stewart, D.C. (1999) J. Inst Brew 105:171 – 177.
Kaersgaard, P. and Hejgaard, J. (1979) J. Inst Brew 85 : 103 – 111.
Sheehan, M.C. and Skerritt, J.H. (1997) J. Inst Brew. 103 : 297 – 306
Sorensen, S.B., Bech, L.M., Muldbjerg, M., Beenfeldt, T. and Breddam, K. (1993) MBAA Tech Quart 30: 136 – 145.