An investigation of a barley protein (SE/BTI-CMe) and its influence on beer haze stability

by

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Abstract

In bright beers, the formation of haze is a serious quality problem, which places limitations on the storage life of the product. To the consumer haze often represents a sign of ageing or contamination of the product. In this study, SDS-PAGE immunoblot analysis using an antiserum that was raised against a silica eluent (SE) protein fraction (obtained from silica gel, used for the colloidal stabilisation of beer), detected a range of protein bands in barley, malt, beer and haze. A polymorphism was observed in which some barley varieties contained a MW ~12000 band (SE +ve) while in other varieties this band was absent (SE -ve). A survey of 219 Australian and international barley varieties, including a comprehensive selection of current and past malting varieties, identified 181 varieties as SE +ve, and 38 varieties as SE -ve. The genetic basis for the presence or absence of the SE protein was determined by interval mapping analysis which found that the MW ~12000 band mapped to the short arm of chromosome 3H.

Pilot brewing trials (100 L and 300 L) found that beer brewed from SE –ve malt varieties formed less haze in haze force testing trials (5 days at 55°C, 1 day at 0°C) and in natural ageing testing trials, than beer produced from SE +ve malt varieties. The interaction between the presence or absence of the SE protein and controlled atmosphere brewing was investigated by brewing under nitrogen, oxygen or air. Controlled atmosphere pilot brewing trials (10 L) indicated that both oxygen and nitrogen rich atmospheres produced beers with poorer colloidal stability compared to brewing under a normal atmosphere. Filtration trials showed that the haze stability of beer could be influenced by the filtration process. Filtration trials showed that the material used to filter the beer (cellulose sheets impregnated with DE) was capable of

removing some haze protein from the beer, thus improving the haze stability of SE +ve beers. The removal of the SE protein and other proteins during filtration from beer brewed with a SE +ve malt variety, along with a reduction in the level of total protein as measured by Commassie blue dye binding, resulted in improved haze stability.

The SE protein was characterised using comparative two-dimensional (2-D) gel electrophoresis immunoblots of barley seed extracts from both SE +ve and SE -ve varieties. The SE protein spot identified was excised and its partial sequence determined, after in-gel cleavage using trypsin and separation of the resulting fragments by reversed-phase HPLC. N-terminal sequence analysis of the tryptic peptides from SE +ve and SE -ve varieties identified the SE protein as the barley trypsin inhibitor CMe precursor (BTI-CMe). The mature BTI-CMe protein is 13.3 kDa and the functional gene is located on chromosome 3H, consistent with the information presented on the SE protein. Cloning of the BTI-CMe protein demonstrated that both SE -ve and SE +ve barley varieties contain a BTI-CMe protein family member that is similar but consistently different, primarily in the last 30 amino acid residues of their C-termini. Specific primers were designed to amplify the full-length BTI-CMe protein as well as a truncated protein (C-terminal region) in both BTI-CMe1 (SE +ve) and BTI-CMe3.1 (SE –ve) variants and four constructs were made. BTI-CMe was expressed in *E.coli*, purified and polyclonal antibodies raised to the recombinant protein. The recombinant BTI-CMe proteins proved to be poorly immunogenic and thus this experiment was not conclusive.

These results of this study suggest that the selection of SE -ve malt varieties for brewing combined with optimised stabilisation and filtration treatments, has the potential to improve beer haze stability. This would reduce the need or requirement for traditional colloidal stabilisation treatments, reducing brewery costs and environmental wastes.