

IDENTIFICATION OF THE POLYPHENOLS IN BARLEY, HOPS, AND BEER BY HPLC - ELECTROSPRAY MASS SPECTROMETRY.

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Polyphenols, in various degrees of polymerisation, are constituents of beer which are derived from the raw materials barley and hops. Polyphenols are known to influence the flavour, flavour stability, physical stability and colour of beer. Polyphenols are also advantageous in beer as polyphenols are natural antioxidants which can react with oxygen and thus protect other beer constituents against oxidation. The deleterious effect of polyphenols on the physical stability of beer results from the formation of haze which consists of polyphenol - protein complexes.

Techniques which have been previously used for the analysis of polyphenols have involved HPLC with UV-Visible and electrochemical detection as well as the isolation of individual compounds with subsequent analysis by NMR and Mass Spectrometry. It has only been recently that HPLC/Mass Spectrometric techniques have been applied to the identification of polyphenols in malt and beer.

We have developed isolation procedures specific for polyphenols so that a sample of sufficient concentration could be obtained and the possibility of interfering compounds reduced. The procedure involves adsorption onto a Sephadex LH-20 column. After washing with water the polyphenols were desorbed using an acetone/water mixture (3:1) containing acetic acid (0.1%).

Electrospray mass spectrometry was able to give molecular ion information as well as structural information from fragmentation initiated at high cone voltage in the positive ion mode. For the polymerised polyphenols a mechanism for the fragmentation of the interflavan bond has been proposed which has been confirmed by the synthesis and subsequent mass spectral analysis of various cyanidin and delphinidin dimers. The fragmentation mechanism proposed is able to discern the order of the individual catechin and gallocatechin units within the proanthocyanidin molecule.

In this work over 50 different proanthocyanidins of barley have been detected. The present work has therefore identified a much greater range of proanthocyanidins than previously known. In particular previously proposed but uncharacterised tetramers and pentamers have been identified. It was interesting to note that many of the higher polymerised proanthocyanidins were absent in the finished product. These compounds are presumably lost during kettle boil.

The method of isolation and identification discussed in this paper is currently being used to identify the polyphenols of hops.

