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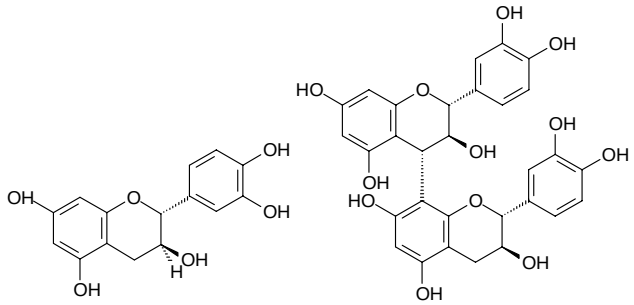
Determination and Identification of Flavonoids in Barley with HPLC-DAD-MS/MS

Flavonoids are a class of phenolic compounds ubiquitously found in plants. These so called secondary plant metabolites are known to have several pharmacological effects and health benefits in humans due to different properties such as anti-oxidant, anti-atherosclerotic, anticancerogenic etc activity. Therefore, the identification and quantification of flavonoids in barley (cf. figure) is of great interest since this type of corn is used as a raw material for the production of different food products as bread or specifically beer. From the technological perspective oligomeric flavonoids, the so called proanthocyanidins, are undesired in the brewing process of beer. Since they are able to form complexes with proteins, precipitation in beer will result.

Figure: chemical structure of selected flavonoids found in barley; monomer (+)-catechin (l.h.s.), dimer procyanidin B3 (r.h.s)



The purpose of this work is the development and validation of an analytical method (sampling, sample treatment, chromatographic seperation) for the determination and quantification of selected flavonoids in barley. Two main problems arose in the formation of an analytical method: On the one hand the overall spectrum of flavonoids occurring in barley is currently not known. On the other hand there are no proanthocyanidins commercially available as reference standard.

The main issue during the development of the HPLC-method with DAD detection was the systematic testing of several analytical separation columns. For the extraction of flanonoids of barley two sample preparation methods were used: a traditional solid/liquid extraction supported by ultra-turrax or by microwaves. In conclusion, for a quantitative determination of flavonoids in barley a chromatographic system consisting of a RP-18 separation column and a phosphate buffer/acetonitrile-gradient or an acetic acid/acetonitrile-gradient as the mobile phase were discovered to be most suitable. Finally, the newly developed and optimized HPLC-DAD method was validated according to international guidelines.

Another aim was the identification of flavonoids found in barley extracts by MS^n-detection applying an LC-ESI-ion trap system. Based on typical fragmentation reactions and characteristic product ions, respectively a systematic approach for the assignment of proanthocyanidins was formulated. By applying this approach dimer, trimer and tetramer proanthocyanidins were discovered in barley which had not been specified before.

The application of the HPLC-DAD-MS/MS-method developed was demonstrated by determining the content of flavonoids in three types of barley used for brewing. Additionally, in order to track the flavonoid flux during the brewing process, several samples taken from certain steps of the process were examined. It could be shown that the flavonoid concentration decreased from the malt as a common feedstock to the

final beer. Moreover, change of flavonoid composition could be analytically revealed in every step of the overall process. A significant decrease in the flavonoid concentration could be observed after filtration of the final beer using a polymer material (PVPP). This polymer should selectively absorb polyphenols from beer to avoid undesired precipitation. The effectiveness of this filtration step was confirmed by the fact that in the readily prepared beer only monomer and dimer, but no oligomer flavonoids could be found anymore.