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Characterization of prenylflavonoids and hop bitter acids in various classes of Czech beers and hop extracts using high-performance liquid chromatography–mass spectrometry

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ABSTRACT

Hops contain a wide range of polyphenolic compounds with antioxidant properties divided in various chemical classes. These compounds are detected in hop extracts and also in beer as its main product. Based on the careful optimization of column type, column packing, mobile phase composition and gradient steepness, two high-performance liquid chromatography–mass spectrometry (HPLC/MS) methods have been developed. The first method using Purospher Star RP-8e column and the gradient of aqueous acetonitrile containing 0.3% formic acid is optimized for the separation of low polar polyphenolic compounds, while the second one with Zorbax SB-CN column is used for more polar hops and beer components. In this work, more compounds are detected in comparison to previous reports. In total, 49 low polar and 37 polar compounds are detected in studied samples and their molecular weights are determined based on atmospheric pressure chemical ionization (APCI) mass spectra. Some compounds are identified based on the interpretation of their full scan and tandem APCI mass spectra, retention behavior and UV spectra, while the full structure elucidation of other species still requires further research. The quantitation of xanthohumol related prenylflavonoids and bitter acids is done with two detection techniques (APCI-MS and UV detection) providing comparable results. Both compound classes (i.e., prenylflavonoids and bitter acids) are separated and quantitated in a single HPLC run, where numerous other polyphenolics are detected as well.

1. Introduction

Hop plant (*Humulus lupulus* L.) is used in the beer production especially to add bitterness. The female inflorescences of the hop plant (hops) contain mainly hop resins, polyphenolic compounds, essential oils and other related compounds [1]. The main hop resins are bitter acids as a source of bitterness of beer. They are divided into two groups, humulones (α-acids) and lupulones (β-acids). Both types of these bitter acids have several homologs, such as normal-, co-, and ad-homologs (Fig. 1). Minor hop acids including post-, pre- and adpre-homologues [2] can be found in hops in addition to main types of bitter acids. During the brewing process, humulones are transformed into isohumulones (iso-α-acids) [3], which are responsible for the specific bitter taste and the stability of beer foam. The foam is one of the first qualitative signs of beer quality recognized by consumers. The foam stability can be affected by other compounds, such as proteins, metal ions, lipids and amino acids [4].

The hops contains many polyphenolic compounds in addition to bitter acids. The most important hop flavonoids (Fig. 2) are xanthohumol (XN) and related prenylflavonoids as isoxanthohumol (IXN), desmethylxanthohumol (DMX), 6-prenylnaringenin (6-PN), 8-prenylnaringenin (8-PN) and 6-geranylnaringenin (6-GN) [1,5,6]. These hop prenylflavonoids have a positive effect on the human health due to antioxidant, anticancer, antimicrobial and anti-inflammatory properties [1]. They also reduce the cholesterol level, protect against the cardiovascular diseases and inhibit many enzymes, e.g., 8-prenylnaringenin is known as a potent phytoestrogen [1]. The hops is used in the beer production, therefore one of the main dietary source of xanthohumol and related prenylflavonoids for people is beer. Nowadays, the hop extracts, prepared either by the extraction with organic solvents (methanol, ethanol, hexane or isooctane) [7] or by the supercritical fluid extraction with carbon dioxide [8], are used in the beer production. The content of prenylflavonoids in hops depends on the variety, stress factors and storage conditions. There is a possibility to distinguish the technology of beer production and find out the addition of hops

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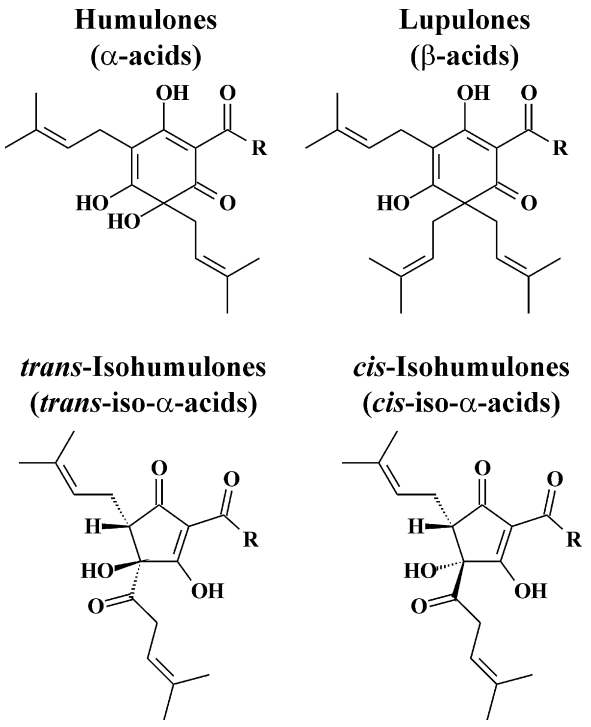

Fig. 1. Structures of individual classes of bitter acids: humulones (α-acids), lupulones (β-acids) and *cis*- and *trans*-isohumulones (iso-α-acids). R can correspond the following homologs: $-CH_2CH(CH_3)_2$ for humulone (H) and lupulone (L), $-CH(CH_3)_2$ for cohumulone (coH) and colupulone (coL), $-CH(CH_3)CH_2CH_3$ for adhumulone (adH) and adlupulone (adL), $-CH_2CH_2CH(CH_3)_2$ for prehumulone (preH) and prelupulone (preL), $-CH_2CH_3$ for posthumulone (postH) and postlupulone (postL), $-(CH_2)_4CH_3$ for adprehumulone (adpreH) and adprelupulone (adpreL). The same notation of R is used for *cis*- and *trans*-isohumulones.

after brewing process according to the amount of prenylflavonoids and bitter acids in beer. Therefore, the analysis of bitter acids and prenylflavonoids is important for the quality control of beer.

Beer samples can be analyzed without any pretreatment [3] or after the purification and preconcentration steps on solid phase extraction (SPE) columns packed with octadecylsilica [9] or octylsilica [10]. The most common separation technique to analyze flavonoids in food, drinks and other biological samples is reversed-phase high-performance liquid chromatography (HPLC) [11] with UV [9,12], electrochemical [13–15] or evaporate light-scattering [16] detection. Furthermore, gas chromatography [11] and electromigration techniques, such as capillary zone electrophoresis [11,17] or capillary electrochromatography [11], can be employed as well. To analyze hops and beer samples, the coupling of HPLC with mass spectrometry (MS) using electrospray ionization (ESI) [18–21] or atmospheric pressure chemical ionization (APCI) [2,3,6] in both polarity modes is often reported.

The qualitative and quantitative analysis of hop extracts of typical Czech varieties and Czech beer samples is presented in this paper. Two chromatographic methods are optimized for the separation of compounds with a lower polarity in beer and hops and compounds with the higher polarity in beer. To identify unknown prenylflavonoids, the APCI-MS in both polarity modes and tandem mass spectrometry (MS/MS) is used. The main prenylflavonoids, XN, IXN and 8-PN together with bitter acids (α, β and iso-α-acids) are quantified by HPLC with UV and MS detection in hop extracts and beer samples. The change in the flavonoid composition is monitored during the beer aging experiment.

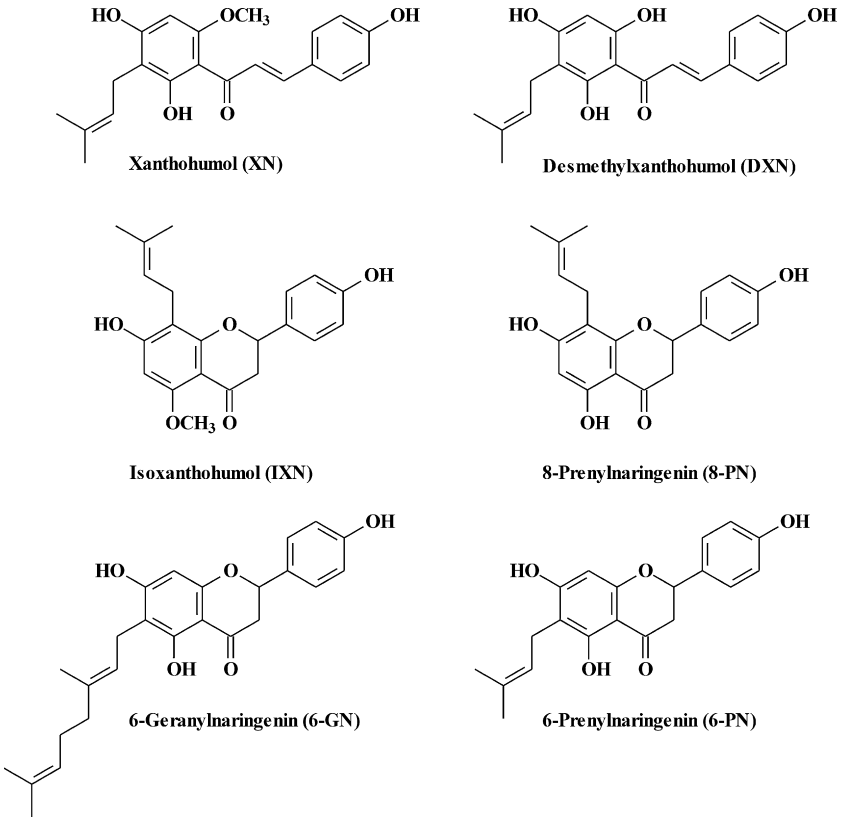

Fig. 2. Structures of common prenylflavonoids detected in hop and beer samples.