On the trail of hard resin components

ANALYSIS OF BITTER SUBSTANCES | In beer production, the development of modern analyses provides possibilities of extending the analytical spectrum. It is possible to investigate the detailed path of individual compounds from raw materials to the finished beer, using mass spectroscopy. The hard resin fraction of hops that mainly consists of polar i.e. easily water soluble bitter substances is ascribed a special role in terms of dry hopping. This makes efficient transfer of these substances from hops to beer possible. The present contribution describes the compounds involved, as well as the flavour contribution they make to beer bitterness.

IN THE CONTEXT of his thesis, Michael Dresel investigated individual, sensory-active components of the hard resin fraction [1]. It was possible to characterise 40 bitter substances from the hard resin fraction. The presence of these compounds was verified in 75 different hop varieties from all over the world and their behaviour during storage and extraction analysed [1].

Glucopyranosides and prenylated flavonoids

The most important substances identified can be divided into two different classes of compounds. On the one hand, glucopyranosides are involved; these are multifidols bonded to a residue sugar, and flavonols such as kaempherol and quercetin. On the other hand, prenylated flavonoids are described, with xanthohumol being the principal component. Fig. 1 shows the chemical structural formula of compounds of both substance classes.

Multifidol glucosides were isolated from hops for the first time in 2005 [2]. They are intermediate products of biosynthesis of alpha and beta-acids. They have the same (acyl) side chains as bitter substances. The amount in hops is about 0.5 per cent [3]. In contrast to multifidol glucosides, flavonol glucosides are widespread in nature. Amounts of these compounds in hops are described as not going above 1 per cent [3]. Both multifidol glucosides as well as prenylated flavonoids are typical for hops. Xanthohumol, the principal component of the substance class of prenylated flavonoids, was discovered already 100 years ago. The amount in dried hops can go up to 1.2 per cent and depends on hop variety [3]. More than 250 scientific contributions about properties of xanthohumol beneficial to health have been published to-date. Most of them reported results from in-vitro research using isolated body cells or enzymes. Most recent studies, however, are seen as a milestone in xanthohumol research as the effect of the substance was confirmed in first human studies [4]. In addition to xanthohumol, isoxanthohumol formed by conversion of xanthohumol during wort boiling, 8-prenylnaringenin and 6-prenylnaringenin are representatives of the group of prenylated flavonoids. The latter two compounds are formed by cyclisation of desmethylxanthohumol in a ratio 1:3 (8-PN:6-PN).

HPLC-MS/MS analysis

It is possible to analyse bitter substances such as alpha-acids or humulinones (oxidised alpha-acids) in dry hopped beers using HPLC-UV analyses. However, analysing glucopyranosides as well as prenylated flavonoids in dry hopped beers requires use of mass spectrometry. Liquid chromatography using mass spectrometry coupling is an analysis method for determining non-volatile substances in food. Quantification of even minute concentrations in the trace range is possible using the sensitive detector.

The above-mentioned investigations by Michael Dresel show an HPLC-MS/MS method for determination of various hard resin components in hop production, hop extracts and beer [1]. Following the method published, an HPLC-MS/MS method was

LIMITS OF DETERMINATION (LD) AND QUANTIFICATION (LQ)

Substance	LD	LQ
8-prenylnaringenin	0.01	0.05
Kaempferol glucoside	0.01	0.05
Quercetin glucoside	0.01	0.05
Quercetin rutinoside	0.01	0.05
Quercetin malonyl glucoside	0.01	0.05
co-multifidol glucoside	0.05	0.10
Isoxanthuhomol	0.05	0.10
Xanthohumol	0.05	0.10

Table 1

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developed which made it possible to identify and quantify initially nine individual components of the hard resin fraction from hops in dry hopped beers in just one LC-MS/MS run and using selected mass transfers. The substances included in this method are individual components of the hard resin fraction, i.e. multifidol glucoside, kaempferol and quercetin glucoside, quercetin rutinoside, quercetin malonyl glucoside, xanthohumol, isoxanthohumol as well as 6- and 8-prenylnaringenin (fig. 1). For sample preparation, the beers are degassed and diluted.

Fig. 2 shows a specific mass transfer and the corresponding retention times for each substance investigated. Dicamba, bentazone und nicarbazine were used as internal standards. These compounds are not detected in beer but cover the various retention times of the compounds analysed. Dicamba was used as an internal standard for co-multifidol glucoside, bentazone for kaempferol and quercetin glucoside, quercetin rutinoside as well as quercetin malonyl glucoside and nicarbazine for the other components. Table 1 lists the detection and determination limits of the method presented in this contribution.

A detailed description of the method parameters is found in the publication "LC-MS/MS Analysis of hop flavonoids in dry-hopped beers", published in Brewing Science – Monatsschrift für Brauwissenschaft [5].

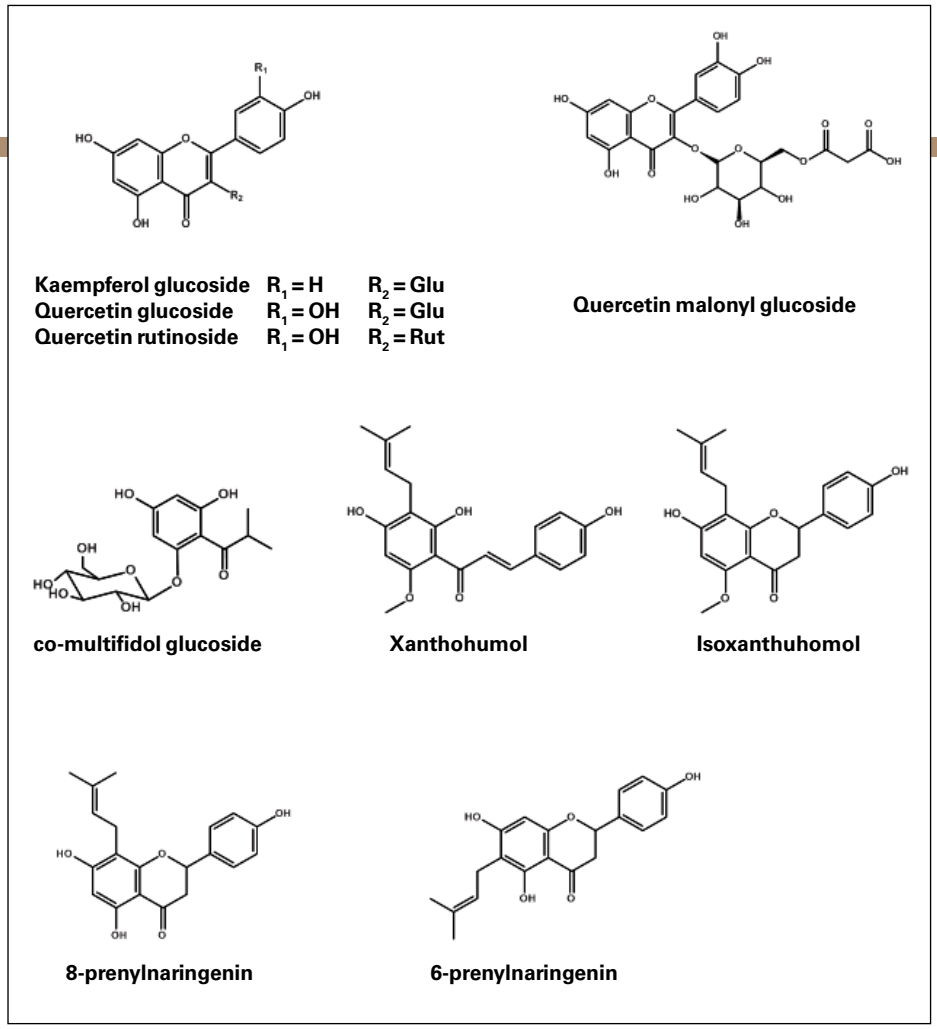

Fig. 1 Chemical structures of selected hard resin components

Influence of hop variety

Two different brewing tests were used as an investigation basis for the HPLC-MS/MS method developed.

In the first brewing test, only the "AlphaExtrakt" hop product was added to the base beer at the start of boiling in order to adjust bitterness. The AlphaExtrakt is an aqueous solution of alpha-acids [6]. Accordingly, it was not possible to detect any of the analytes investigated in the base beer from the first test. The base beer, having 4.6 per cent by volume, 21 bitterness units and a pH value of 4.0, was divided up and dry hopped with five different hop varieties. Table 2 is an overview of the dry hopping recipe. The beers were dry hopped statically in a keg for seven days at 3 °C and not filtered prior to filling.

The results for beers 1–5 show the influence of the various hop varieties on composition of specific bitter substance in dry hopped beer. Table 3 lists the quantitative data.

Beer number 2, produced with the Bravo hop variety, had the highest quantity of co-multifidol glucoside of 3.32 mg/l. The lowest amount of this substance, 0.38 mg/l, was found in beer number 4 which had been dry hopped with the Denali hop variety. Beers number 2, 3 and 4 showed significant differences for the co-multifidol glucoside individual component.

The highest concentrations of substances analysed were detected in beer number 2. Beer number 4 had the lowest amounts. None of the two compounds, 6- and 8-prenylnaringenin, were found in any of the beers analysed. The reason for this is attributed to the extremely low quantities of the two compounds in hops. 8-prenylnaringenin is described as having a quantity of less than 0.01 per cent in the plant. The concentration of isoxanthohumol did not exceed 0.11 mg/l. It is possible to obtain a higher concentration of isoxanthohumol in beer when hop addition takes place early on in the process. Xanthohumol is then converted into isoxanthohumol during wort boiling [7]. Xanthohumol concentration in the beers investigated ranged from 0.10 to 0.18 mg/l. Gahr et al. also reported xanthohumol concentrations in dry hopped beers between 0.10 and 0.17 mg/l [8].

In the second brewing test, a Pale Ale base beer was produced with Hallertau Tradition (pellets type 90 and an alpha-acid content of 3.8 %).

This Pale Ale base beer, with 6.0 per cent by volume alcohol, 24 bitterness units and a pH value of 4.3, was subsequently divided and dry hopped with the Lemondrop and Bravo hop varieties and with addition of HopGun (BrauKon GmbH). Again, it was not filtered.

An analysis of the base beer showed quantitative data for eight of the nine compounds investigated. The results point to substance transfer into the beer already during wort boiling (fig. 3). The influence of dry hopping is obvious.

Both beers, Pale Ale number 1 and number 2, show a clear rise in concentration, compared to the base beer, for the substances multifidol glucoside, quercetin glucoside, kaempferol glucoside and xanthohumol. For Pale Ale number 1, no difference in terms of the two quercetin derivatives, quercetin rutinoside and quercetin malonyl glucoside, were noted. However, these two individual components increased significantly in Pale Ale number 2.

It was possible to show a drop in isoxanthohumol in both dry hopped beers, compared to the base beer.

The ratio between quercetin glucoside and kaempferol glucoside in beer number 5 (test 1) and Pale Ale number 1 (test 2) of both beers, dry hopped with the Lemondrop hop variety, differed in terms of the ratio of these two substances in the other beers. In these two beers, concentration determined had a ratio of 1:1. All other beers had a higher concentration of quercetin glucoside compared to kaempferol glucoside. The ratio between the two glucosides is a genetic one and a function of hop variety. This could be evidenced in investigations of the World Hop Collection of 121 hop varieties [9].

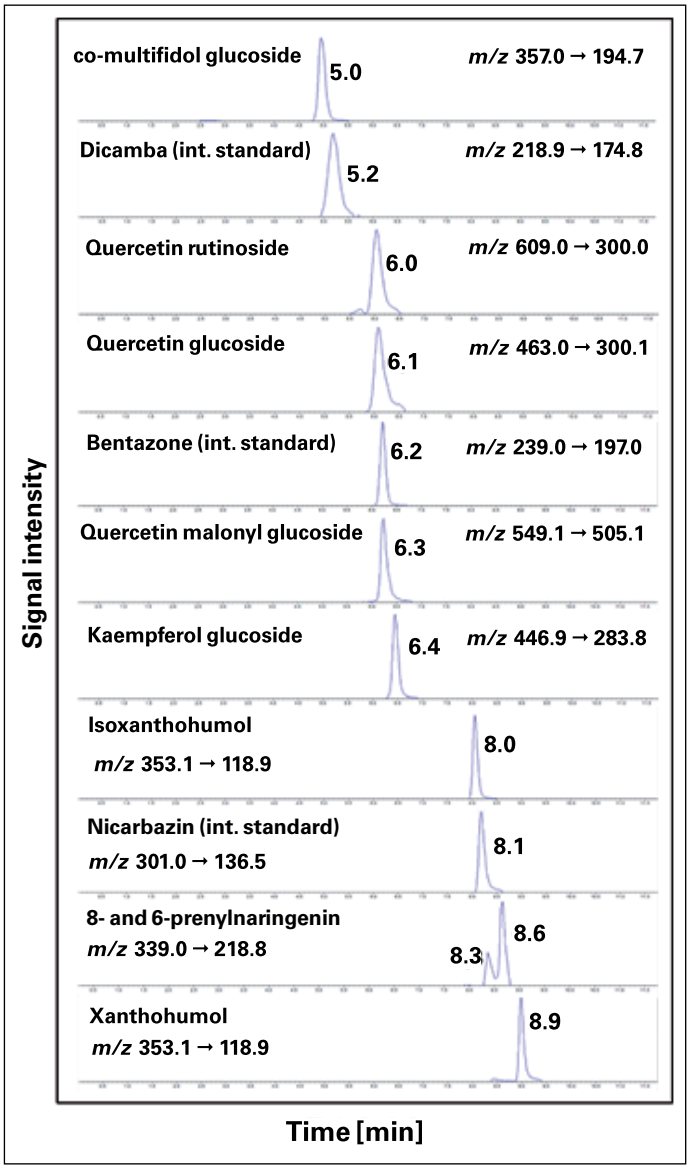

Fig. 2
LC-MS/MS chromatograms of hard resin components determined in dry hopped beers

RECIPE DRY HOPPING

	Hop variety	Hop product	Hop quantity
Test 1			
Beer no. 1	Apollo	P90	500 g/hl
Beer no. 2	Bravo	P90	500 g/hl
Beer no. 3	Calypso	P90	500 g/hl
Beer no. 4	Denali	P90	500 g/hl
Beer no. 5	Lemondrop	P90	500 g/hl
Test 2			
Pale Ale no. 1	Lemondrop	P90	500 g/hl
Pale Ale no. 2	Bravo	P90	500 g/hl

Table 2

Sensory contribution to beer bitterness

Humans perceive bitterness based on the receptor family hTAS2R, having about 25 G protein coupled receptors (GPCR). Molecular biological studies showed that iso-alpha-acids present in beer, and also the prenylated flavonoids xanthohumol, isoxanthohumol and 8-prenylnaringenin, activate the three bitter receptors hTAS2R1, hTAS2R14 and hTAS2R40 [10].

It is necessary to be familiar with the so-called taste threshold values in order to determine the contribution of a bitter substance to overall bitterness, e.g. of a dry hopped beer. A concentration in beer that is equal to or higher than the taste threshold value is regarded as a direct contribution toward bitterness. Additive effects play a part when different substances activate the same receptors. They may also be relevant below the taste threshold value for total bitterness.

In the literature, the hard resin components co-multifidol glucoside and all prenylated flavonoids are described as being exclusively bitter whereas the glucosides of quercetin and kaempferol are also perceived as astringent, in addition to bitter. The thesis of Michael Dresel shows the taste threshold values of these substances [1], listed in table 4. The threshold values were determined in 5 per cent ethanol with a pH value of 4.4 [1]. In addition to quantitative determination, brewing tests showed that hard resin components have a positive effect on the quality of beer bitterness [1].

The taste threshold value of co-multifidol glucoside amounts to 1.8 mg/l. Concentrations of co-multifidol glucoside determined are above the taste threshold value in beer number 2 from test 1 and in the three beers from test number 2. A contribution of this individual component to overall bitterness would thus be expected. Concentrations of kaempferol and quercetin glucoside in the beers tested are below their taste threshold values of bitterness perception though the

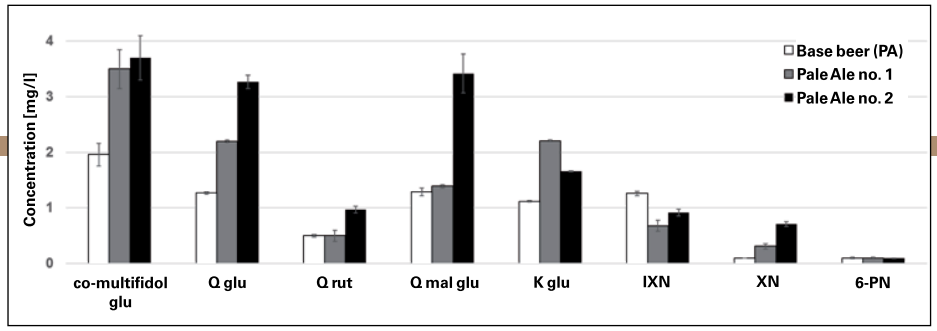

Fig. 3 Concentration (mg/l) and standard deviation (n=3) of hard resin components in dry hopped beers from test 2

taste threshold value for astringency is exceeded in the three beers from test 2 and in beer number 2 from test 1. It would thus be expected that these two compounds contribute to the overall perception of astringency in these beers. Concentrations of xanthohumol, isoxanthohumol and 6-prenylnaringenin are considerably below the respective taste threshold values. A combination of dry hopping and roasted malt in beer production may, however, result in xanthohumol concentrations of e.g. 6 mg/l in beer [11]. The taste threshold value for xanthohumol of 3.5 mg/l is thus obviously exceeded. This compound can contribute to beer bitterness.

Summary

The method presented here allows quantitative analysis of specific bitter components that are regarded as representative of the compound classes tested. The beers showed significant differences for the individual components of the hard resin fractions tested, depending on hop variety. In addition, it is possible to determine the taste contribution of individual substances to overall bitterness based on the taste threshold values published in the literature.

CONCENTRATION (MG/L) OF HARD RESIN COMPONENTS IN BEERS FROM TEST 1

Substance	Base beer	Beer no. 1	Beer no. 2	Beer no. 3	Beer no. 4	Beer no. 5
co-multifidol glucoside	n.d.	1.48	3.32	1.15	0.38	1.59
Quercetin glucoside	n.d.	0.72	1.72	0.63	0.48	0.38
Quercetin rutinoside	n.d.	0.46	0.54	0.33	0.22	0.34
Quercetin malonyl glucoside	n.d.	0.30	1.24	1.30	0.43	0.55
Kaempferol glucoside	n.d.	0.21	0.38	0.19	0.11	0.52
Xanthohumol	n.d.	0.10	0.16	0.18	0.10	n.d.
Isoxanthuhomol	n.d.	0.10	0.11	0.10	0.10	n.d.
6-prenylnaringenin*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
8-prenylnaringenin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

**6-prenylnaringenin was quantified with 8-prenylnaringenin; n.d. = not detected*

Table 3

TASTE QUALITY AND THRESHOLD VALUES IN MG/L FROM [1]

Substance	Quality	Taste threshold (mg/l)
co-multifidol glucoside	bitter	1.8
Kaempferol glucoside	bitter/astringent	13/0.5
Quercetin glucoside	bitter/astringent	13/0.9
Xanthohumol	bitter	3.5
Isoxanthuhomol	bitter	5.6
6-prenylnaringenin	bitter	3.4
8-prenylnaringenin	bitter	2.7

Table 4

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