LC-MS/MS analysis of hop flavonoids in dry-hopped beers

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Introduction

The spectrum of bitter substances in hops includes α- and β-acids but also prenyllavonoids and glucopyranosides (Figure 1). Multifidal glucosides were isolated from hops in 2005, for the first time (1). These compounds are intermediate products of the biosynthesis of α- and β-acids. Both multifidal glucosides and prenyllavonoids (e.g. xanthohumol) are typical for hops. The composition of quercetin and kaempferol glucosides in hops is suitable to differentiate hop varieties (2). New findings about key bitter compounds in hops and their contribution to the bitter profile of beer were introduced and discussed in literature recently (3,4).

Whereas HPLC-UV analysis of selected bitter substances like α-acids or humulones (oxidized α-acids) in dry-hopped beers is feasible, the analysis of hop flavonoids like multifidal, kaempferol and quercetin glucosides requires the use of HPLC-MS/MS technique. For this reason, the present study was executed to investigate a suitable HPLC-MS/MS method for monitoring hop flavonoids in dry-hopped beers.

Materials and methods

Analyses of degassed beer samples without further clean-up procedures were performed after addition of internal standards (dicamba, bentazon and nicarbazin) and dilution by LC-MS/MS. The HPLC system (Shimadzu Corporation, Kyoto, Japan) was coupled with a mass spectrometer (SCIEX, Darmstadt, Germany) running in the negative ion mode. The quantitation was done using the scheduled MRM mode of the instrument with the fragmentation parameters optimized prior to analysis. Data processing and integration was performed by using Analyst software and MultiQuant (SCIEX, Darmstadt, Germany). For chromatography, an analytical 50 x 2.0 mm Synergi 4u Fusion-RP 60A column (Phenomenex, Aschaffenburg, Germany) served as the stationary phase. Quantitation was done by external calibration in a range between 1 and 500 ng/ml. Beers tested in this study were from different brewing trials. For the first trial, the basic beer (Pale Ale) contains alpha extract only (5). This initial beer was then split into different parts and each was dry-hopped using one of the five varieties shown in Table 1. For the second trial, another Pale Ale basic beer was split too and dry-hopped with two different hop varieties (Table 1). Hallertau Tradition (pellets type 90) has been used for the Pale Ale basic beer (trial 2) during wort boiling.

Table 1. Dry hopping conditions for both brewing trials.

Results

The developed HPLC-MS/MS method allows the identification and quantitation of 9 hop flavonoids in dry-hopped beers using a single LC-MS/MS run with selective mass transitions as given in Figure 2.

The quantitative data for both brewing trials are summarized in Table 2. The basic beer of trial 1 was prepared using alpha extract only for bittering. None of the substances tested was detected by this method in the beer. The results for beers No.1-5 show the influence of dry hopping on the hop flavonoid composition. The highest amount for co-multifidal glucoside was observed in beer No. 2 with hop variety Bravo. The lowest amount was detected in beer No. 4, dry-hopped with the hop variety Denali. Beer No. 2 (with Bravo) showed the highest amounts for all substances tested. 6- and 8-prenylnaringenin were not detected in the dry-hopped beers.

The analysis of Pale Ale basic beer (trial 2) gives quantitative data for 7 of 9 hop flavonoids tested. The results show the entrance of these substances to the beer during wort boiling already. The impact of dry hopping on the hop flavonoids pattern in beer is obvious after subtraction of the results from the Pale Ale basic beer.

For the ratio of quercetin and kaempferol glucosides in beer No. 5 and Pale Ale No. 1, both dry-hopped with the hop variety Lemondrop, it could be observed that the amounts of these 2 compounds were equal in these 2 beers whereas the concentration of quercetin glucoside was higher in comparison to kaempferol glucoside in all other beers. The composition of quercetin and kaempferol glucosides is genetically determined and therefore depend on the variety (2).

Table 2. Amounts of hop flavonoids in dry-hopped beers in mg/L (both brewing trials).

Conclusions

The developed HPLC-MS/MS method allows the identification and quantitation of selected hop flavonoids in dry-hopped beers using a single LC-MS/MS run. Dry-hopped beers produced with different hop varieties showed significant differences in their hop flavonoids pattern. The contribution of these bitter substances to the overall bitter profile of beer could be evaluated using the quantitative data and the flavour thresholds known from literature.

References