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Occurence of glycosidically bound flavour compounds in hops, hop products and beer

Glycosidically bound flavour compounds of hops are considered to contribute to the hop flavour in beer. Therefore, different hop varieties and hop products were analyzed for their glycoside content. During extraction with supercritical CO_2 almost all of the hop glycosides remain in spent hops. After ethanol extraction, glycosides could be detected in both the ethanol tannin extract and the ethanol pure resin extract. In all examined varieties an enrichment of the sensorially important glycosides of monoterpene alcohols (e.g. linalool) and nor-carotenoids could be observed in the ethanol pure resin extracts. Some of these glycosides could be detected in hopped beer, where they could act as precursors for the organoleptically active linalool. This could be confirmed by a rise in free linalool in a stored beer brewed with a re-extracted ethanol pure resin extract containing linalyl glycoside, compared to a beer brewed with a glycoside-free hop preparation. The strongly flavour-active ß-damascenone could also be liberated from hop glycosides, but hops seem not to be the only source for this ketone.

Descriptors: *Humulus lupulus*, hops, glycosides, aglycones, pure resin extract, glycosidically bound flavour compounds

1 Introduction

Glycosides are odourless compounds, containing a sugar-moiety (usually ß-D-glucose) and a non-sugar-moiety (= aglycone). Numerous glycosides of aliphatic alcohols, phenols, terpene alcohols and nor-carotenoids have been recently identified in hops (1-4). From these glycosides, odoriferous aglycones like ß-damascenone or linalool could be liberated by thermal or enzymatic cleavage of their glycosides (see Fig. 1).

The orthonasal odour threshold of ß-damascenone in water is 4 ng/L (5). The odour threshold of the natural isomer (*R*)-linalool in hops was determined to be 0,14 µg/L in water (5) and 2,2 µg/L in beer (6). Because of these low threshold values, ß-damascenone and linalool show the highest calculated odour activity values in pilsner-type beers (5) along with typical fermentation-derived compounds like ethanol and some ethyl esters.

Linalool was identified as one of the most potent flavour compounds in fresh and dried hops (7), strongly contributing to the floral flavour note of hopped beer (5, 8, 9). As its concentration is considered to be useful to predict flavour intensity of hopped beers (10), linalool can be regarded as a quality indicator for hopped beer (6). A >95% decrease of the linalool concentration was observed during wort boiling (10, 11). During storage, linalool rapidly disappears from beer brewed with hop oil (12) and carbon dioxide extract (13). Linalool also disappears rapidly in the first 2 of 8 weeks storage in a beer spiked with pure linalool (14). In contrast to these findings, an increase in linalool during fermentation and aging was observed in beer brewed with hop pellets (10, 15, 16). Liberation of linalool from glycosides was considered to be responsible for this increase (10, 15).

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Tables and Figures see Appendix

The concentration of ß-damascenone increases during and after wort boiling (11) and during artificial aging of beers (17). A high ß-damascenone content seems to be important to the flavour of aged beer (18). As high yields of ß-damascenone are obtained at lower pH values and after addition of ß-glucosidase to the fresh beer, the increase in ß-damascenone was explained partially by acid hydrolysis of glycosides (17, 18).

The aim of this study was to investigate different hop varieties and hop products for their composition of glycosides and to support the assumption that these glycosides can also be found in freshly brewed beer and give rise to hop-glycoside-derived flavour compounds in beer.

2 Material and methods

Dried hops (Czech Saazer CSA, Hallertauer Tradition HHT, Hallertauer Mittelfrüh HHA, Hallertauer Hersbrucker HHE, Hallertauer Magnum HHM), hop pellets (Type 90), ethanol pure resin extract, ethanol tannin extract, carbon dioxide extract, spent solids (each of Hallertauer Perle HPE), ethanol pure resin and tannin extracts (of Hallertauer Perle PE, Hallertauer Tradition HT and Spalter Select SE) were obtained from HHV, D-84048 Mainburg. The hemicellulase preparation Rapidase was a gift of B. Heimann, DSM Food specialities, D-44319 Dortmund.

A detailed description of the isolation of glycosides and analysis of liberated aglycones in hops was published recently (3). In short, glycosides were extracted with hot water or methanol-water (4:1), after addition of the internal standard phenyl-ß-D-glucoside. The extracts were decanted, treated with Polyclar (PVPP), filtrated and concentrated to dryness. Volatile compounds were removed by extraction with pentane-dichloromethane (2:1). After fractionation on Amberlite XAD-2 resin, the methanol eluate was concentrated to dryness and the glycosidic fraction was incubated with ß-glucosidase or the hemicellulase preparation Rapidase (3) in a phosphate-citrate buffer (pH 5) at 40 °C for 1 or 3 days. Liberated aglycones were extracted with ether and investigated by gas chromatography - mass spectrometry (GC-MS). Measured peak areas are corrected by considering the area of the internal standard. Values are given for 500 ml beer, 25 g dried hops, 100 g hop pellets, 75 g spent hops, 25 g carbon dioxide extract, 25 g ethanol pure resin extract, 25 g ethanol tannin extract.

Additionally, three different pilsner beers have been brewed in a pilot brewery. Apart of the hop products, all ingredients and brewing parameters were identical. Beer B_{STA} is a standard type pilsner beer. The hop products used were ethanol pure resin extract and pellets (type 90). Beer B_{ISO} was made with an isomerized hop extract. This iso-extract is produced from a carbon dioxide extract and contains only purified isomerized α-acids. Beer B_{REX} was made with a re-extracted ethanol pure resin extract. Re-extraction has been carried out with supercritical CO_2 (280 bar, 50 °C). By such a re-extraction most of the α- and β-acids and all of the volatile essential oils are removed. So, both iso-extract and re-extracted ethanol pure resin extracts did not contain any volatile essential oils, e.g. free linalool. The compositions of the different hop products used for producing beers B_{STA}, B_{ISO} and B_{REX} are given in Table 1.

Glycosidically bound compounds in beer were analyzed by enzymatic cleavage of XAD-purified glycoside extracts. Volatile components of beers were analyzed by solid-phase extraction (13) with subsequent GC-MS anaylysis and quantification by comparison of peak areas with the area of the internal standard borneol. Beers were analyzed for their volatile components immediately after brewing and after 8 weeks storage at 28 °C, respectively.

GC-MS-analysis: a Siemens SiChromat gaschromatograph was directly coupled to a Finnigan 8222 mass spectrometer. Separation was achieved on a DB5 30 m x 0,54 mm fused-silica capillary column (1.5 µm film thickness), programmed at a rate of 5 °C/min from 100 °C to 250 °C. The GC eluent was divided by a live-T-switching device to allow simultaneous sniffing analysis and mass spectrometric identification.

Alpha-, iso-alpha- and beta-acids, soft and hard resins and essential oils of hop and hop products were analyzed according to the EBC methods 7.7, 7.8, 7.5, 7.6 and 7.10 (Analytica-EBC, Grundwerk 1990).

3 Results and discussion

3.1 Glycosides in different hop varieties

Dried hops (analytical characterization, see Table 2) were investigated for the contents of the aglycones by hot water extraction of glycosides, purification on XAD-2-resin and enzymatic cleavage with ß-glucosidase (1 day, 40 °C, pH 5). The distribution of some typical aglycones is shown in Table 3. The same aglycones were found in all hop varieties, but there are distinct differences in the absolute content of these glycosides. HHE and HHT showed highest amounts of the monoterpenes linalool and α-terpineol, whereas CSA contained remarkable amounts of glycosidically bound nor-carotenoids. The lowest amount of linalool was found with the bitter hop HHM, which is characterized by a considerable 3-methyl-pentan-2-ol content. According to these results, it could be stated that the contribution of glycosides to beer flavour is dependent on the hop variety used.

3.2 Glycosides in different hop products

Figure 2 shows the contents of some typical aglycones in different hop products from HPE (isolated with water-methanol, fractionated on XAD-2-resin and liberated by ß-glucosidase, 1 day, 40 °C, pH 5). For comparison purposes, the amounts of the aglycones in the hop pellets were set to 100 %.

Whereas most of the hop glycosides remain in the spent hops during carbon dioxide extraction, they can be extracted with ethanol in good yields. Sensorially important glycosides of terpene alcohols and nor-carotenoids are enriched in the ethanol pure resin extract. Phenolic aglycones, like methyl salicylate, tyrosol, *p*-coumaric and ferulic acid, as well as their decarboxylation products vinylphenol and vinylguaiacol, were found mainly in the ethanol tannin extract.

3.3 Glycosides in ethanolic hop extracts

Figure 3 shows the contents of some typical aglycones in ethanol pure resin extracts and ethanol tannin extracts from different hop varieties (isolated from hot water extracts after removing the volatile compounds, cleavage of glycosides with the hemicellulase preparation (3 days, 40 °C, pH 5) without purification on XAD-2-resin). These results verify the findings that there are varietal differences (e.g. PE obviously contains less linalyl glycoside than HT and especially SE). Additionally, Figure 3 shows again that most of the glycosides of monoterpene alcohols and nor-carotenoids are enriched in the ethanol pure resin extracts of each variety.

3.4 Glycosides in beer

To verify the presence of glycosides in hopped beer, a glycoside extract of a fresh brewed Pilsner beer (B_{STA}), hopped with ethanol pure resin extract and hop pellets was prepared by fractionation on the Amberlite XAD-2 resin. The results of enzymatic hydrolysis of this glycoside extract are shown in Table 4. During sniffing-GC-MS-analysis of the extract of aglycones, flavour impressions were recognized especially for 3-(Z)-hexenol (green-grassy), 1-octen-3-ol (mushroom) and the very odour-active linalool (flowery-citrus).

To verify the liberation of aglycones from hop glycosides during brewing and storage of beer, a beer brewed with an iso-extract (B_{ISO}) was compared to a beer brewed with re-extracted ethanol pure resin extract (B_{REX}). Both hop products were free of volatiles. In the freshly brewed beers, the presence of glycosidically bound compounds was verified again by enzymatic cleavage of a XAD-purified glycoside extract. Enzymatically liberated linalool and α-terpineol were found only in beer B_{REX} as compared to an enzyme-free control experiment confirming the presence of glycosidically bound hop compounds in the re-extracted ethanol pure resin extract used to produce B_{REX}. Analysis of the volatile compounds in the freshly brewed beer and after 8 weeks of storage demonstrate the enrichment of linalool and α-terpineol in beer B_{REX}, whereas ß-damascenone was enriched in both samples (Table 5).

Although the strongly flavour-active ß-damascenone (flowery-fruity) could be liberated by acidic hydrolysis from the above mentioned glycoside extract of hopped beer (4), the possible precursor of ß-damascenone, 3-hydroxy-ß-damascone, was also found in unhopped beer (4). This could indicate that hop is not the only source for ß-damascenone. In whiskey, ß-damascenone is considered to be formed during distillation from a precursor from barley (19).

4 Conclusion

Depending on the hop variety, there are considerable amounts of glycosides of monoterpene alcohols and nor-carotenoids in hop pellets and ethanol pure resin extracts, but not in carbon dioxide extracts. The same glycosides could be identified also in hopped beer. The liberation of aglycones during the brewing process and the storage of beer was demonstrated by comparing beer brewed with and without a glycoside-containing hop extract. Although their total amounts are very low, it seems possible that linalool and some nor-carotenoids, liberated from hop glycosides, may contribute to hop flavour of beer.

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Appendix

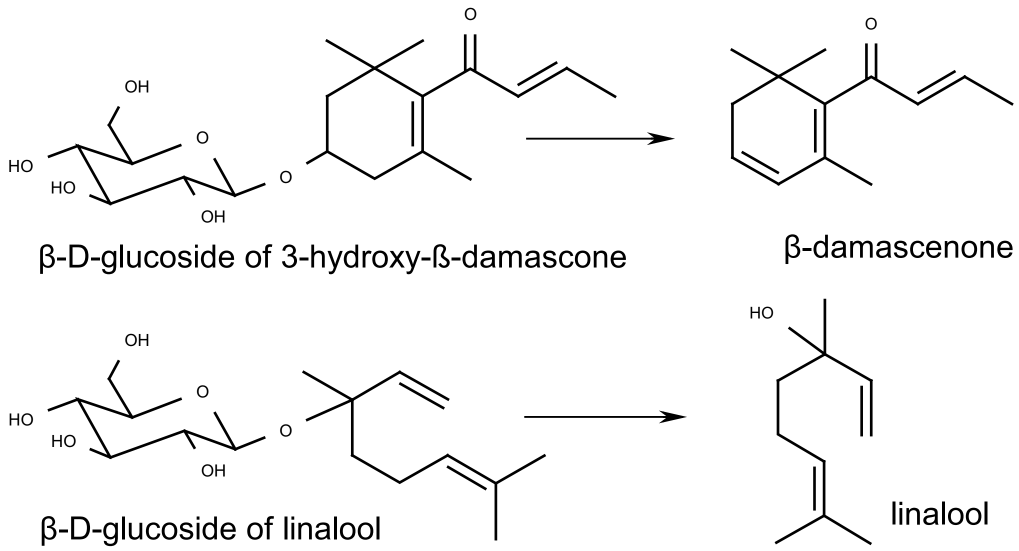

Fig. 1 Examples of typical glucosides in hops and corresponding flavour-active aglycones identified in hopped beer

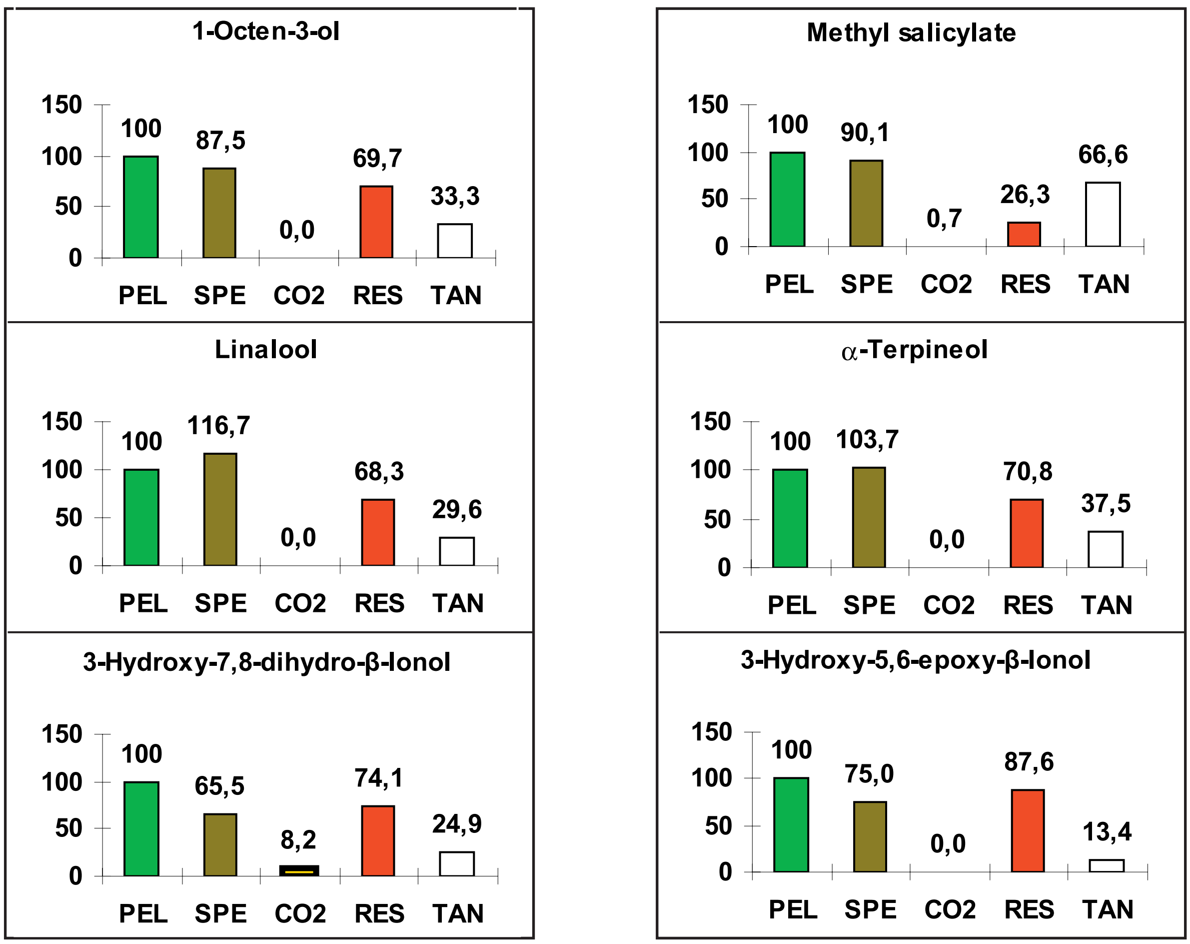

Fig. 2 Content of some aglycones, enzymatically liberated from glycosides of different hop products (variety Hallertauer Perle); PEL = pellet, SPE = spent hops, CO_2 = carbon dioxide extract, RES = ethanol pure resin extract, TAN = ethanol tannin extract

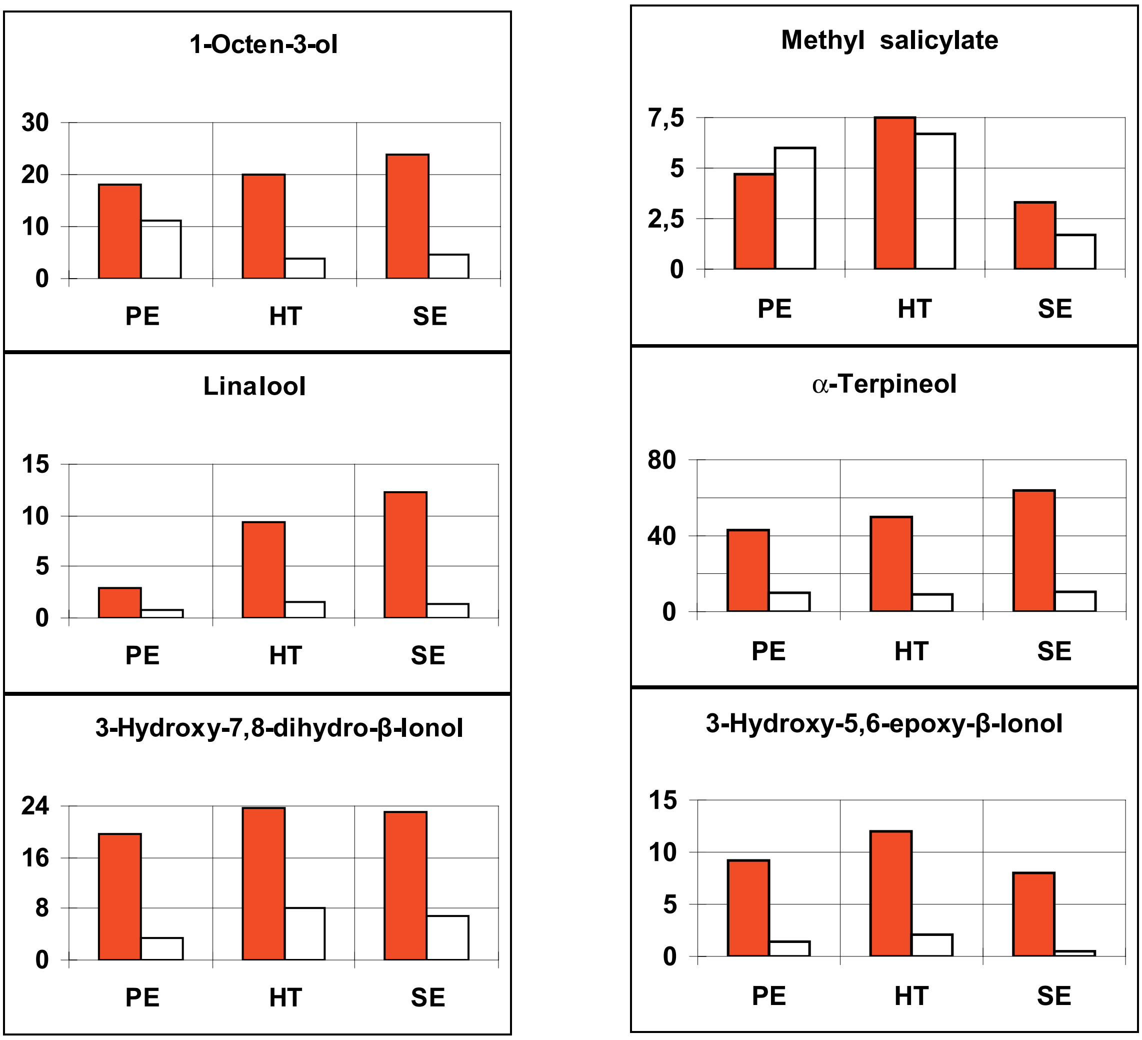

Fig. 3 Content of some aglycones, enzymatically liberated from glycosides of ethanol extracts of different hop varieties. (PE= Hallertauer Perle, HT= Hallertauer Tradition, SE= Spalter Select); pure resin extract (left bar, filled) and tannin extract (right bar, blank)

Table 1 Analytical characterization of different hop products used for beer production

Ingredients (%)	Pellet Type 90	Ethanol pure resin extract	Re-extracted ethanol pure resin extract	Iso-Extract
Alpha-acids	7.6	36.4	0.8	-
Iso-alpha-acids	-	1.3	1.4	27.9
Beta-acids	4.6	23.7	0.1	-
Xanthohumol	0.5	2.1	2.0	-
Non-specific resins	7.2	31.1	16.7	-
Essential oil	1.1	4.3	-	-
Carrier/Solvent	-	-	kieselguhr	alkaline water

Table 2 Analytical characterization of different varieties of dried hops

compound	CSA	HHA	HHE	HHT	HHM
Alpha-acids (%)	3,9	4,2	5,3	6,5	13,2
Beta-acids (%)	4,7	5,2	6,3	5,0	6,5
Essential oil (ml/100 g)	0,9	1,4	1,4	1,3	2,8
<u>**Composition of essential oil (%)**</u>					
Myrcene	38,5	14,9	28,2	19,6	35,8
Linalool	0,4	0,6	0,6	0,8	0,3
Geraniol		0,1			0,2
β-Caryophyllene	7,1	13,6	11,2	11,6	10,7
α-Humulene	21,9	42,4	24,1	35,4	32,1
E-β-Farnesene	15,8	0,5			
α-/β-Selinene	0,8	1,4	9,8	0,8	0,7
Humulene epoxide	1,0	5,7	1,0	3,6	0,9
α-/β-Eudesmol			2,2		

Table 3 Peak areas of aglycones liberated from different varieties of dried hops

Compound	CSA	HHA	HHE	HHT	HHM
3-Methylbut-2-en-1-ol	110	42	31	33	38
3-Methylpentan-2-ol	99	45	36	40	192
3-(Z)-Hexenol	175	195	211	156	73
1-Octen-3-ol	124	93	175	89	63
Benzylalcohol	228	250	188	283	98
Methyl salicylate	39	21	26	48	24
Linalool	19	17	27	25	12
α-Terpineol	41	83	123	93	64
7-Hydroxy-α-terpineol	98	212	157	128	237
3-Hydroxy-β-damascenone	4	1	2	2	2
3-Hydroxy-7,8-dihydro-β-ionol	191	113	149	110	62
3-Hydroxy-5,6-epoxy-β-ionol	200	157	132	117	76
Vomifoliol	79	28	19	10	50
Dihydrovomifoliol	57	34	22	11	31

Table 4 Peak areas of aglycones liberated from glycosides, isolated from a fresh brewed pilsner beer hopped with ethanol pure resin extract and pellets (B_{STA}).

Enzymatic hydrolysis ß-Glucosidase, pH 5, 24 h, 40 °C	Addition of enzyme	Without enzyme
3(Z)-Hexenol	9	0
1-Octen-3-ol	484	0
1,5-Octadien-3-ol	39	0
Linalool	9	0
α -Terpineol	17	0
8-Hydroxy-linalool I	6	0
8-Hydroxy-linalool II	32	0
Benzylalcohol	82	15
3-Hydroxy-7,8-dihydro-β-ionol	10	0

Table 5 Amount (ppb) of selected volatiles in fresh brewed and 8 weeks stored beers brewed with iso Extract (B_{ISO}) and re-extracted ethanol pure resin extract (B_{REX}), respectively.

compound	B_{ISO} fresh	B_{ISO} 8 weeks	B_{REX} fresh	B_{REX} 8 weeks
Linalool	0	1	3	8
α-Terpineol	0	1	0	4
β-Damascenone	0	2	0	3