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Determination of Hop Aromatisation of Beer by Headspace Solid Phase Microextraction in Combination with Gas Chromatography and Mass Spectrometry

In contrast to determination of hop utilisation in terms of beer bitterness, analytical measurement of the yield of hop aromatisation is not straightforward on account of the enormous chemical complexity of hop essential oil and hoppy aroma derived thereof. In this paper, selective determination of hop oil-derived constituents in the flavour profile of pilsner beer is achieved through headspace solid phase microextraction (HS-SPME) in combination with gas chromatography-mass spectrometry (GC-MS). Highly enriched floral and spicy hop essences were prepared by density programmed supercritical fluid extraction (SFE), followed by solid phase extraction (SPE), and subsequently added in well-defined amounts to an experimental pilsner, exclusively bittered with purified iso-α-acids extract. In view of reliable measurement of the yield of aromatisation of the pilsner with the respective hop oil fractions, characteristic marker components for the floral and spicy SFE/SPE hop essences, respectively, were selected. Reliable determination of the selected markers for floral and spicy SFE/SPE hop essences in the aromatised beers turned out to be possible on condition that HS-SPME is combined with GC-MS comprising selected fragment ion monitoring (SIM). Using this approach, application of hop oil preparations can be monitored analytically, allowing improved control and specification of hop aromatisation according to the desired beer flavour. The average yield of beer aromatisation using floral and spicy hop essences was determined at about 95 %.

On the basis of their sesquiterpenoid GC-MS/SIM pattern, fresh commercial lager beers could be differentiated, which may be useful for brand fingerprinting and identification. Upon forced ageing of the beers, levels of hop oil-derived sesquiterpenoids, in particular of caryophyllene and humulene epoxides, decrease significantly. In addition, *trans*-nerolidol is identified as a potential marker for measuring flavour (in)stability of pilsner beer.

Descriptors: hop essences, hop aromatisation, hop utilisation, solid phase microextraction, mass spectrometry

1 Introduction

Hops are used in brewing to impart beer bitterness and hoppy aroma. In conventional brews, hop cones, hop pellets or non-isomerised hop extract are added to obtain a desired bitterness level and/or hoppy beer aroma. Nowadays, in view of enhanced control of hoppy flavour attributes, different types of advanced hop products are available. In respect of hoppy aroma of beer, several types of hop oil preparations are commercially available to impart late or dry-hop aroma or to introduce citrusy, floral, fruity, spicy or herbal flavour top-notes into the beer [1–3]. The main objective of using hop oil products is to attain to the desired hoppy aroma or hop-derived aroma top note in a controlled way, aiming at enhanced consistency of hoppy aroma in the final beer.

Controlling hoppy aroma in finished beer requires detailed knowledge on the chemistry and analysis of hop oil-derived components in both hop products and finished beer. However, the analytical detection of minor hop oil compounds in the complex flavour profile of finished beer is not evident. About 35–40 years ago, the contribution of hop essential oil to beer aroma was even questioned [4,5] and this erroneous view was mainly due to the lack of sensitivity and selectivity of analytical equipment used at that time. The work of *Tressl* et al. [6]. is now considered as a milestone in hop aroma research since it resulted in the detection and partial identification of about 50 hop oil-derived constituents in a German kettle-hopped lager. Since then, as a result of increasing possibilities offered by the development of highly sophisticated analytical equipment, intensive research has been done in order to unravel the intriguing issue of hoppy aroma of beer. Many compounds have been proposed to be responsible for, or at least connected to hoppy aroma. Linalool and geraniol for example have been related with floral aspects of hoppy aroma and, at present, linalool is even considered as an analytical marker for the intensity and the quality of hoppy aroma of beer [7–11]. Special attention has also been given to the oxidation products of the major hop oil constituents. Oxygenated sesquiterpenes, such as humulene epoxides, humulol, humulenol II, and humuladienone, have been associated with hoppy flavour of beer,

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in particular with the 'spicy' or so-called 'noble' flavour attribute [12–16]. Currently, it is generally recognised that hoppy aroma of beer finds its origin in many different volatiles present in, or derived from hop essential oil. However, part of hoppy aroma has also been ascribed to the presence of hop glycosides extracted from the hop leaf material during the boil and from which flavour-active aglycones (e.g. linalool) can be released by yeast activity during subsequent fermentation/lagering [17–22]. In summary, it can be stated that hoppy aroma of beer is far from completely understood since the role of particular constituents (e.g. oxygenated sesquiterpenes) remains controversial, key character impact compounds await for their discovery, and, moreover, flavouring interactions in the complex beer matrix are almost non-explored.

Most published procedures for analytical determination of hop-derived volatiles in the flavour profile of finished beers are labour-intensive and time-consuming. Large beer volumes appear to be required for extraction of the targeted volatile hop constituents because of their trace amounts in beer. Different methodologies are used for extraction of the beer volatiles and, in many cases, extraction is followed by a concentration step and/or clean-up procedure of the extract. Beer aroma extraction procedures are traditionally based on simultaneous steam distillation-extraction [23–25] or on liquid extraction using conventional solvents [26–30]. Further concentration and/or fractionation of the obtained extract is carried out by evaporation of the extraction solvent (e.g. via rotary evaporation [31] or Kuderna Danish [30, 32] and column chromatography [12, 27, 31], respectively. An optimised extraction technique using XAD-2 resin and Kuderna Danish evaporation was proposed by *Lermusieau* et al. [32] to recover hop aroma compounds from beer. In order to avoid the formation of heat-induced artefacts upon extraction and/or evaporation, headspace techniques [33], solvent assisted flavour evaporation (SAFE) [34, 35], stir bar-sorptive extraction (SBSE) [36, 37] or solid phase microextraction (SPME) [9, 38–40] have been applied to isolate hop-derived volatiles from beer. For the detection, identification and accurate determination of extracted hop oil-derived constituents, advanced gas chromatography in combination with state-of-the-art mass spectrometry is indispensable.

Aiming at enhanced consistency of hoppy character of conventionally hopped beers, the 'Hop Aroma Component Profile (HACP)' was developed by Nickerson and Van Engel [41]. This concept is based on determination of the level of 22 selected hop oil constituents in hops to adjust hopping rates in the brewery for improved control of hop aromatisation. In this study, we aim at selective detection and quantification of hop oil-derived volatiles in the complex beer aroma profile in view of reliable determination of the yield of beer aromatisation using hop oil essences. For that purpose, SPME was chosen as the extraction technique, in combination with state-of-the art gas chromatography and mass spectrometry. Nowadays, SPME is widely used as extraction technique in the field of flavour research for instrumental evaluation of the organoleptic quality of food products and alcoholic beverages [42, 43]. The popularity of this extraction technique is attributed undoubtedly to the many advantages it has when compared to conventional extraction methods. SPME is a solvent-free extraction technique, does not require high temperatures, pressures or large sample volumes, and is easy to automate. The technique is essentially based on establishing an equilibrium between the analytes in the sample, in the headspace of the sample, and in the stationary phase of the extraction fibre. Extracted volatiles are thermally desorbed in the heated injector of a GC for further analysis. In the field of beer flavour research, for example, an SPME based procedure in combination with a stable isotope dilution assay (SIDA) was successfully applied by *Steinhaus* et al. [9] for reliable quantification of (*R*)- and (*S*)-linalool in beer.

Clearly, in addition to SPME, highly selective and sensitive detection is required for adequate determination of minor hop oil constituents in the complex beer flavour profile. Therefore, state-of-the art GC-MS based on monitoring selected fragment ions (SIM) from the compounds of interest, was applied in this paper, aiming at reliable determination of the yield of advanced hop aromatisation of beer. In addition, the optimised analytical GC-MS/SIM procedure was further implemented for hop oil constituent fingerprinting of fresh and aged commercial lagers.

2 Materials and methods

2.1 Chemicals

All reference compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were of analytical grade: β-caryophyllene (98.5 %), caryophyllene oxide (≥99.0 %), 2-decanone (≥99.5 %), α-humulene (≥98.0 %), limonene (97.0 %), 2-methylbutyl 2-methylbutanoate (90.0 %), methyl decanoate (≥99.5 %), methyl nonanoate (≥99.8 %), methyl octanoate (≥99.8 %), β-myrcene (≥95.0 %), *trans*-nerolidol (98.0 %), β-pinene (99.0 %), 2-undecanone (99.0 %).

2.2 Plant material

Hop pellets T90 (crop year 2007) from different varieties (cv. Hersbrucker Spät, cv. Saaz: Clarebout, Vlamertinge, Belgium; cv. Hallertau Tradition: HVG, Wolnzach, Germany) were stored for no longer than 1 month under recommended conditions (cold storage at 0 °C, packaged under vacuum in metallised polyethylene laminates [44] to prevent oxidative transformations of the brewing principles.

2.3 Preparation of hop oil essences

Varietal floral and spicy hop oil essences were prepared from pellets T90 cv. Hallertau Tradition and pellets T90 cv. Hersbrucker Spät, respectively, via density programmed supercritical fluid extraction (SFE) using carbon dioxide and subsequent solid phase extraction (SPE) for further purification of SFE fractions.

Floral essences were prepared from pellets cv. Hallertau Tradition because of the pleasant and pronounced flavour attributes of these varietal essences and the high level of hop oil constituents associated with floral/fruity/hop aroma in the essences. The hop variety Hersbrucker Spät was consciously selected for preparation of oxygenated sesquiterpenoid SFE/SPE essences, since pronounced spicy flavour attributes have been reported for beers aromatised with Hersbrucker Spät hops essences [45]. In addition,

it was found that Hersbrucker Spät hop pellets contain high levels of oxygenated sesquiterpenoids, i.e. hop oil components that have been associated with the spicy/noble aspect of hop and hoppy aroma, when compared to other hop varieties.

Preparation of floral hop essences. Ground hop pellets cv. Hallertau Tradition were extracted using a Dionex SFE-703 supercritical fluid extractor (Dionex, Sunnyvale, CA, USA). A carbon dioxide density of 0.29 g/mL was applied, the extracted volatiles were collected in ethanol, and further fractionation of the SFE extracts was performed via solid phase extraction. Varian Bond Elut C_{18} cartridges (500 mg)(Varian, Palo Alto, CA, USA) were employed for this purpose.

Preparation of spicy hop essences. Ground hop pellets T90 cv. Hersbrucker Spät were extracted via two-step SFE using a Dionex SFE-703 supercritical fluid extractor (Dionex, Sunnyvale, CA). The first extraction was performed using a carbon dioxide density of 0.29 g/mL and was finished when 25.0 L of gaseous carbon dioxide was collected. The remaining hop solids were further extracted by applying a carbon dioxide density of 0.50 g/mL until a volume of 25.0 L of gaseous carbon dioxide was collected, yielding the "crude" spicy SFE extract. Further fractionation of the SFE extracts was performed via solid-phase extraction. Varian Bond Elut C_{18} cartridges (500 mg) (Varian, Palo Alto, CA) were employed for this purpose.

For more details on the extraction/fractionation procedure, reference is made to *Van Opstaele* et al. [45].

2.4 Commercial and experimental lager beers

Fresh commercial lager beers (code: A, B, C, D) were obtained from four different Belgian breweries.

A reference pilot lager beer was prepared at our pilot brewery (5 hL scale). This brewing installation is a prototype for innovative wort production as described by *De Rouck* et al. [46]. At the end of wort boiling, the reference brew was bittered by the addition of pre-isomerised hop extract (Botanix, Kent, UK). Next, the brew was split up and one part of the brew was further late hopped by addition of pellets T90 cv. Saaz (33 g/hL). In this way, a non-aromatised reference beer and a late hopped beer (code: lager E) were obtained.

The following conditions were used: 84 kg fine milled Pilsner malt (wet disc mill, Meura, Péruwelz, Belgium) is mixed with 1.84 hL reversed osmosis brewing water with addition of $CaCl_2$ (80 ppm Ca^{2+}) and 120 mL lactic acid (30 %, v/v) per hL brewing water; mashing-in: temperature: 64 °C; pH 5.2; brewing scheme: 64 °C (30 min), 72 °C (20 min), 78 °C (1 min) (temperature increase: 1 °C/min); wort filtration: membrane assisted thin bed filter; sparging up to 11.5 °P sweet wort; wort boiling: 60 min atmospheric boiling using a double jacket for heating (evaporation: 5 %); at the end of boiling, 0.2 ppm Zn^{2+} ions were added, as well as iso-α-acids extract aiming at 25 ppm iso-α-acids in the finished beer (3.85 g iso-α-acids added/hL; utilisation: 65 % at end of boiling); wort clarification: decantation in combination vessel; after cooling and aeration, the wort (original gravity: 12 °P) was pitched with 10^7 yeast cells/mL (inoculum: dry yeast, strain W 34/70 (Fermentis), was hydrated for 1 hour in sterile water with a volume of 10 times the weight of the dry yeast); primary fermentation: 8 days at 12 °C in cilindroconical tanks; maturation: 10 days at –0.5 °C; beer filtration: kieselguhr/cellulose sheets (pore size 1µm); CO_2 saturation up to 5.6 g/L; packaging: 6 head rotating counter pressure filler (monobloc, CIMEC, Italy) using double pre-evacuation with intermediate CO_2 rinsing and overfoaming with hot water injection before capping (final oxygen levels: below 50 ppb).

Aromatisation of the reference pilot lager with floral or spicy hop essence was done by adding a precise volume of essence to a bottle of beer (250 mL) under carbon dioxide atmosphere. The volume of essence to be added for aromatisation was calculated on the basis of GC-FID analysis of the essence. For floral hop essences, addition levels are based on the total level of volatiles in the floral essences, whereas for spicy hop essences levels of addition are based on the level of sesquiterpenoids present in the spicy essences. In view of determination of the yield of aromatisation, essences were added at 100 ppb floral and 100 ppb spicy components, respectively. Therefore, the reference lager was aromatised in the bottle under carbon dioxide atmosphere with 37.5 µL floral essence cv. Hallertau Tradition or 49.3 µl spicy essence cv. Hersbrucker Spät. Aromatised samples were immediately crown-capped and stored at 1 °C until further analysis.

Samples of the commercial lagers A–D and the pilot lager E (late hopped beer) were forced aged in the dark at 40 °C for 10 days.

2.5 Solid phase microextraction (SPME) for extraction of beer volatiles

Solid phase microextractions were automated using a CombiPal autosampler (CTC Analytics, Switzerland). A volume of 5 mL of beer aromatised with hop essences, was pipetted into a 20 mL vial under carbon dioxide atmosphere and closed with a PTFE-coated septum. Volatile compounds were extracted by inserting a polydimethylsiloxane fibre (PDMS, 100µm, Supelco, Bellefonte, PA, USA) into the vial headspace. Extraction temperature and time were set at 40 °C and 30 min in the case of extracting volatiles originating from floral hop essence. For isolation of oxygenated sesquiterpenes, extraction temperature was set at 60 °C and total extraction time was 60 min. Before starting the actual extraction, samples were pre-incubated at the respective temperature for 5 min. During pre-incubation and extraction, samples were stirred at 500 rpm.

2.6 GC-MS conditions for separation, detection and quantification of beer volatiles

Gas chromatographic operating conditions were as follows. Extracted volatiles were thermally desorbed in the heated inlet (250 °C) of the Ultra Trace gas chromatograph (Thermo Fisher Scientific, Austin, TX) for 3 min. Helium (Alphagaz 2, Air Liquide, Belgium) was used as a carrier gas at a constant flow of 1.0 mL/min. Injection was done in the splitless mode for 3 min at 250 °C. Separation of the injected compounds was performed on a 40 m x 0.18 mm i.d. x 0.2 µm (film thickness) RTX-1 capillary column (Restek Corporation, Bellefonte, PA, USA). The oven temperature program for determination of floral hop oil compounds was as fol-

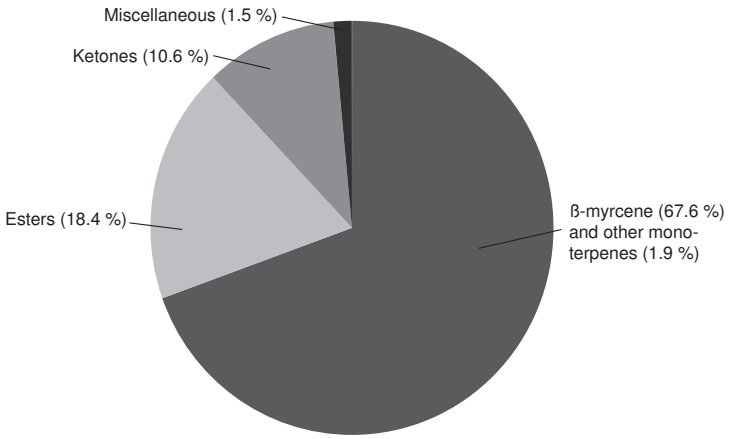

Fig. 1 Relative proportion of different chemical groups of compounds in the volatile profile of floral hop essence cv. Hallertau Tradition (based on Van Opstaele et al., [47])

lows: 3 min at 35 °C, followed by a temperature increase at 5 °C/min up to 250 °C (1 min isotherm). For determination of sesquiterpenoids following oven program was used: 1 min at 40 °C, increase of temperature at 10 °C/min up to 200 °C, then at 3 °C/min up to a final temperature of 260 °C (3 min isotherm).

Mass spectrometric detection of marker components for floral hop essence and the sesquiterpenoid hop fraction, was obtained by a dual stage quadrupole MS (DSQ I, Thermo Fisher Scientific, Austin, TX) operating in the electron ionisation mode (EI, 70 eV). The ion source temperature was set at 240 °C and the electron multiplier voltage was 1,445 V. Analyses were performed in both full scan (m/z = 40–400) and selected ion monitoring (SIM) mode. Following fragment ions were selected for determination of floral compounds: m/z = 58, 74, 87, 88, 93, and 136. For detection of sesquiterpenoids in the SIM mode, ions at m/z = 67, 79, 81, 82, 93, 138, 161, 204, 220, and 222 were selected. Selection of fragment ions was based on their high intensity in the mass spectra of marker compounds for the floral and sesquiterpenoid fraction (see further Fig. 2 and Table 2; table see page 154). The identity of marker components was confirmed by mass spectral comparison using the 'NIST98' and 'Flavor MS Library for Xcalibur, 2003'

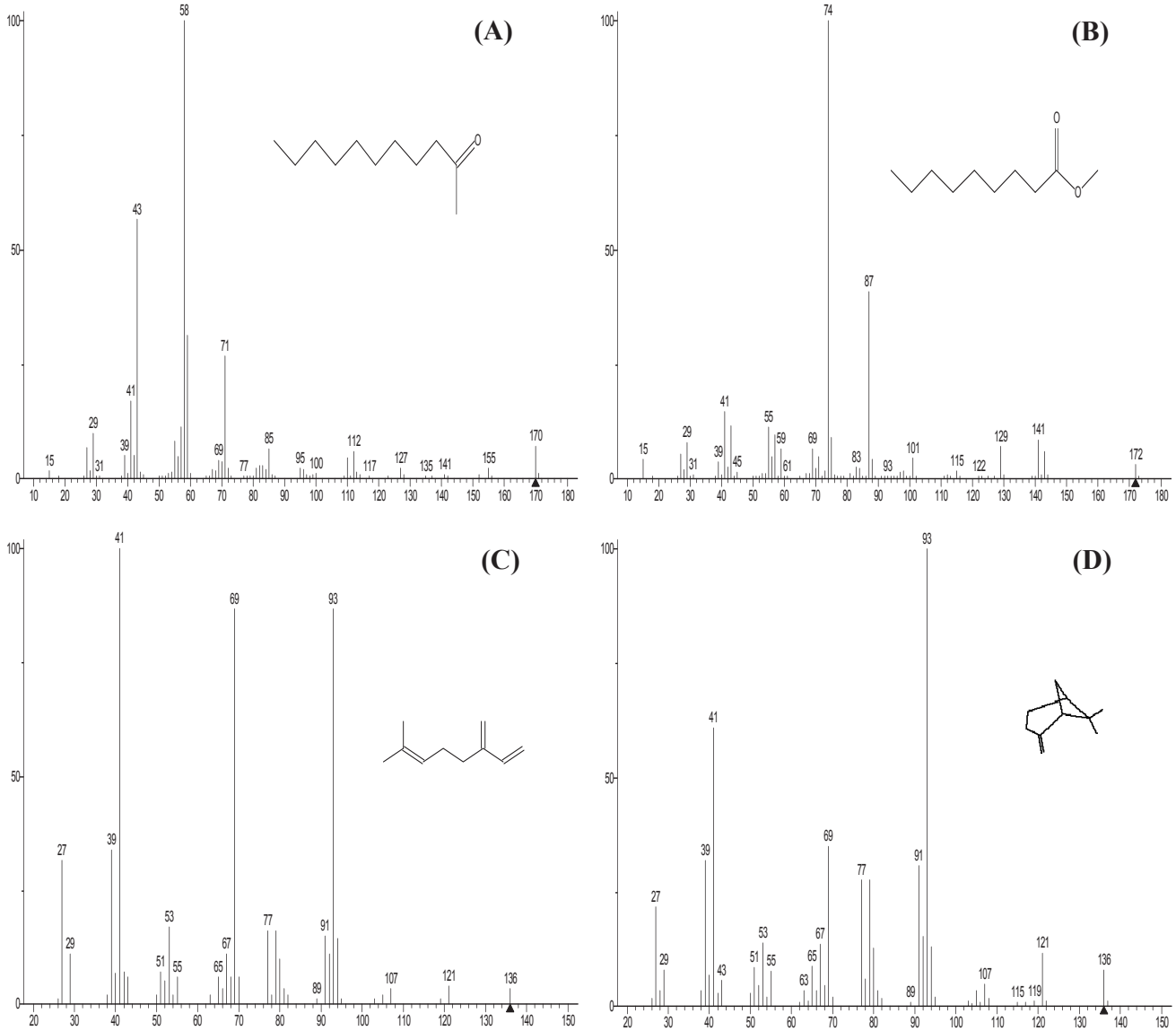

Fig. 2 Mass spectra of 2-undecanone (A), methyl nonanoate (B), β-myrcene (C) and β-pinene (D) as examples of marker compounds for respectively the ketone, ester, and monoterpene hydrocarbon group of volatiles present in floral hop essences (X-axis: m/z; Y-axis: relative abundance)

Table 1 **Marker hop oil constituents from floral SFE/SPE hop essence cv. Hallertau Tradition for determination of the yield of aromatisation. (No.: compound number; RI: Calculated Retention Index of compound on RTX-1 capillary column; CV: coefficient of variation obtained on 5 analyses of aromatised beer; Yield: ratio of the level of marker in aromatised beer to the level of marker in added volume of hop essence; [a]authentic reference compound available).**

No.	RI	Component	CV (%)	Yield (%)
1	972	β-pinene[a]	2.9	98
2	988	**β**-myrcene[a]	3.5	97
3	1023	limonene[a]	3.4	98
4	1050	methyl 2-methylheptanoate	2.9	96
5	1072	methyl 6-methylheptanoate	3.4	96
6	1091	2-methylbutyl 2-methylbutanoate[a]	3.3	99
7	1094	2-methylbutyl 3-methylbutanoate	5.4	100
8	1108	methyl octanoate[a]	6.8	99
9	1148	methyl 2-methyloctanoate	2.0	95
10	1174	2-decanone[a]	2.9	97
11	1208	methyl nonanoate[a]	3.7	84
12	1233	heptyl 2-methylpropanoate	2.6	92
13	1234	unidentified ketone	3.5	97
14	1240	unidentified ketone	2.3	98
15	1247	methyl 2-methylnonanoate	1.7	86
16	1264	methyl 4,6-methyloctanoate	2.0	83
17	1276	2-undecanone[a]	4.6	98
18	1292	methyl 4-decenoate	1.5	95
19	1308	methyl decanoate[a]	5.4	92
20	1332	octyl 2-methylpropanoate	4.3	87
21	1336	2-methylbutyl heptanoate	6.7	93
22	1443	unidentified ketone	2.2	97
23	1488	methyl 3,6-dodecadienoate	3.7	89

spectral libraries (Interscience, Louvain-la-Neuve, Belgium), retention times of authentic reference compounds, and calculation of retention indices. Retention indices were determined using a homologous series of normal alkanes (C_8–C_{18}; Sigma-Aldrich, St. Louis, MO, USA).

2.7 Semi-quantitative and quantitative determination of selected marker compounds

Semi-quantitative determination of selected marker compounds was obtained by adding as internal standard dodecane (C_{12} (≥99 %; Sigma-Aldrich, St. Louis, MO, USA)) prior to SPME extraction (30 µL of internal standard solution (1.05 µg C_{12}/mL ethanol) to 5 mL beer). The concentration of the compound of interest is then calculated on the basis of the ratio of the peak area of the marker compound to the peak area of the internal standard and to the concentration of added internal standard. In case authentic reference compounds were available, quantitative determination of marker constituents in aromatised beers was obtained by the standard addition method (addition level of the compounds: 1, 10, 20, 50, 75, 100 µg/L). The yield of aromatisation (%) upon addition of hop essence is given by the ratio of the level of marker compound measured in the beer to the level of compound in the added hop essence.

3 Results and discussion

In this study, the development of a reliable analytical method for selective determination of hop oil-derived volatiles in the beer flavour profile, and determination of the yield of hop aromatisation using hop essences, are aimed at. For that purpose, headspace solid phase microextraction (HS-SPME) is selected as extraction technique for isolation of the volatiles on account of its many advantages compared to conventional extraction techniques. Furthermore, HS-SPME is performed online with state-of-the-art gas chromatographic separation and mass spectrometric detection of the extracted volatiles. Since all volatile beer constituents, i.e. both predominant compounds (ppm level) arising from the fermentation and minor volatiles like hop oil constituents (ppb level) are extracted by HS-SPME, reliable determination of hop oil-derived aromas requires MS detection through selected ion monitoring (SIM). With this technique, high selectivity on the detector level can be obtained by monitoring only selected, typical fragment ions for the compounds of interest. Furthermore, when compared to the standard full scan operating mode, sensitivity is generally increased by a factor 10–100 when only selected fragment ions are monitored.

3.1 Determination of marker constituents for floral hop essences in beer by headspace-solid phase microextraction – gas chromatography – mass spectrometry

A pilot lager beer exclusively bittered with iso-α-acids extract, was aromatised with floral hop essence cv. Hallertau Tradition (addition rate: 100 µg floral compounds per litre beer). HS-SPME-GC-MS profiling of this particular essence showed the presence of approximately 90 different volatiles as reported by Van Opstaele et al. [47]. Figure 1 summarises these results by grouping the constituents into different chemical classes (monoterpene hydrocarbons, esters, ketones, and 'miscellaneous': a group comprising aldehydes, furans, and sesquiterpenoids) and by displaying their relative proportion (based on peak areas) in the floral hop essence. Clearly, the monoterpene hydrocarbon β-myrcene is the predominant compound since it accounts for 67.6 % of total peak area. Most of the identified compounds belong to the 'ester' group which accounts for 18.4 % of total peak area. This group comprises a whole series of ethyl esters, methyl esters, and branched, unbranched, saturated, and unsaturated esters. The ketone group accounts for 10.6 % of total peak area, while sesquiterpenoids, aldehydes, and furans represent a minor fraction.

By analysing the pilot lager beer aromatised with floral hop essence cv. Hallertau Tradition in the full scan operating mode, none of the targeted floral compounds was detected because of too low selectivity and sensitivity (data not shown). Therefore, characteristic fragment ions of typical volatiles from the floral essence had to be selected to allow performing MS detection by

Table 2 **Relative intensities (Rel. Int.) for the ten most abundant fragment ions (No. 1–10; m/z) and the molecular ion (MW) in the mass spectrum of hop-derived sesquiterpene hydrocarbons and oxygenated sesquiterpenes. (mass spectral data were obtained by MS-analysis (EI, full scan) of spicy hop essence cv. Hersbrucker Spät* and authentic references**)**

Ions	α-Humulene**		Humulene epoxide I*		Humulene epoxide II*		Humulene epoxide III*		Humuladienone*	
No.	m/z	Rel. Int.	m/z	Rel. Int.	m/z	Rel. Int.	m/z	Rel. Int.	m/z	Rel. Int.
1	79	16.1	43	23.8	43	59.5	41	34.2	67	46.2
2	80	29.3	79	18.7	**67**	**100**	67	35.3	69	19.3
3	91	21.0	80	22.8	68	42.6	68	30.0	82	20.8
4	92	19.6	91	22.8	69	36.1	69	27.6	95	41.6
5	**93**	**100**	92	19.2	93	46.0	79	38.6	96	97.5
6	94	13.0	**93**	**100**	95	55.3	**81**	**100**	109	60.7
7	105	14.1	105	20.2	96	83.4	93	38.1	110	30.8
8	107	19.4	107	25.3	109	91.5	95	38.0	123	36.3
9	121	32.5	121	41.5	123	41.7	107	27.5	137	25.6
10	147	22.2	138	23.0	138	82.5	109	23.6	**138**	**100**
MW	**204**	**6.7**	**220**	**5.4**	**220**	**6.9**	**220**	**2.4**	**220**	**14.8**

Ions	Humulenol II*		Humulol*		β-Caryophyllene**		Caryophyllene Oxide**		τ-Cadinol*	
No.	m/z	Rel. Int.	m/z	Rel. Int.	m/z	Rel. Int.	m/z	Rel. Int.	m/z	Rel. Int.
1	**67**	**100**	**82**	**100**	41	50.1	41	77.1	41	29.1
2	69	89.6	83	80.2	69	85.4	55	49.2	43	39.6
3	79	52.4	67	48.3	79	66.6	67	43.9	79	28.6
4	81	97.2	71	32.3	91	86.3	69	59.1	81	42.1
5	91	60.7	41	19.8	**93**	**100**	**79**	**100**	93	28.1
6	93	79.5	55	19.5	105	63.0	81	51.6	95	26.6
7	95	86.0	93	15.0	107	48.0	91	53.1	105	56.2
8	107	68.1	107	13.4	119	50.0	93	77.8	119	29.1
9	109	71.6	125	12.6	120	46.8	95	56.2	121	27.3
10	119	72.9	161	5.2	133	99.8	107	62.1	**161**	**100**
MW	**220**	**15.3**	**222**	**2.0**	**204**	**8.11**	**220**	**0.7**	**222**	**0.0**

selected ion monitoring. On account of high peak intensities in the EI mass spectrum (see Fig. 2) of the hop volatiles of interest, i.e. compounds belonging to the group of ketones, esters and monoterpene hydrocarbons, following ions were chosen for selective determination of hop-derived floral volatiles in the beer flavour profile: m/z = 58 (ketones); m/z = 74, 87, 88 (esters); m/z = 93, 136 for monoterpene hydrocarbons. The choice of m/z = 58 for the detection of ketones is based on the high intensity of this fragment ion in the mass spectrum of methyl ketones (see Fig. 2A), as a result of McLafferty rearrangement of the molecular ion. For the same reason, fragment ions with m/z = 74, 87, 88 are chosen for the ester group (see Fig. 2B). The high intensity of the selected fragment ions in the mass spectra of 2-undecanone and methyl nonanoate as representative compounds for the ketone group and methyl esters, respectively, is clearly illustrated in figure 2. The mass spectrum of monoterpene hydrocarbons is characterised by a highly intense fragment ion at m/z = 93, and a detectable molecular ion at m/z = 136 (Fig. 2C, Fig. 2D).

As a result of SIM analysis based on the selected fragment ions, 23 components originating from the floral essence are detected in the volatile profile of the aromatised beer (see Table 1). The predominant compound from the floral essence, i.e. β-myrcene, coelutes with a compound present in the profile of the non-aromatised beer. However, by post-processing the SIM chromatogram with the Xcalibur® software through selecting the molecular ion at m/z = 136, the peak area of β-myrcene can be determined without interference of the co-eluting compound (ethyl hexanoate). Thus, all of the 23 hop oil-derived components can be considered as candidate marker compounds for determination of the yield of aromatisation of beers with the floral hop essence of this particular variety. As shown in table 1, most of the floral hop oil volatiles in the profile of the aromatised beer belong to the ester group (15 in total), while only 5 ketones and 3 monoterpene hydrocarbons are detected. For each of the selected compounds, the yield of aromatisation can be calculated on the basis of the ratio of the semi-quantitative level of the component in the aromatised beer to the level of the component in the added hop essence. When the authentic reference compound is available, the precise level of the selected marker can be determined in both the essence and the aromatised beer through the standard addition method, and consequently, those levels are preferably used for calculating the yield of aromatisation. For example, the concentration of β-myrcene in this particular floral essence amounts to 424 µg/mL, as determined by quantitative analysis using the standard addition method. In this experiment, 37.5 µL essence was added to a bottle of beer (250 mL), which resulted in a β-myrcene concentration of 63.5 µg/L beer. Upon analysis of the aromatised beer via standard addition of the β-myrcene reference compound, the average concentration was determined at 61.7 ± 2.2 µg/L. Thus, for β-myrcene the calculated yield of aromatisation amounts to 97 %. Based on the yields obtained via the standard addition

method, it can be derived from table 1 that the average yield of beer aromatisation with the floral hop essence amounts to 96 %.

In conclusion, the presented methodology based on HS-SPME allows us to evaluate hop aromatisation through the addition of varietal floral hop essence by selecting representative markers for the essence. However, HS-SPME must be combined with selected ion monitoring (SIM) as mass spectrometric detection technique, in order to obtain selectivity and reliable (semi-)quantitative determination of marker volatiles. Evidently, since floral hop essence is part of total hop essential oil, the above proposed marker volatiles will also be useful in evaluating hop aromatisation of beer by total hop essential.

3.2 Determination of marker constituents for the oxygenated sesquiterpenoid hop oil fraction in beer through HS-SPME-GC-MS/SIM

A reliable methodology for determination of hop-derived sesquiterpenoids in the beer aroma profile is aimed at. Since all beer volatiles in the headspace are extracted by HS-SPME, selective determination of the compounds of interest should rely on mass spectrometric detection. In particular MS detection by selected ion monitoring may provide required selectivity since hop sesquiterpenoids show typical fragmentation patterns upon electron ionisation, yielding characteristic mass fragments in the resulting EI-MS spectra. Table 2 shows the relative intensities of the ten most abundant fragment ions and the molecular ion in the EI mass spectrum of typical hop sesquiterpene hydrocarbons and their oxidation products. Based on this information, following mass fragments are selected for SIM detection of the hop-derived sesquiterpenoid fraction in the aroma profile of finished beers: m/z = 67, 79, 81, 82, 93, 138, and 161. As displayed in table 2, the molecular ion for sesquiterpene hydrocarbons (m/z = 204) and oxygenated sesquiterpenoids (m/z = 220 or m/z = 222) is certainly not the most abundant ion in the respective mass spectra and is even not detected in the case of τ-cadinol. Nevertheless, the molecular ions are incorporated in the SIM method since their abundance is sufficient to provide evidence on the molecular weight of the measured compounds.

In order to evaluate the proposed detection technique for determination of (oxygenated) hop sesquiterpenes, a pilot pilsner exclusively bittered with iso-α-acids extract was aromatised in the bottle with spicy hop essence, prepared according to our in-house SFE/SPE technology (addition rate: 100 µg spicy compounds per litre beer). The spicy hop essence was used for this purpose since comprehensive characterisation of this particular fraction of total hop essential oil showed that oxygenated sesquiterpenes represent the major chemical compound class, accounting for at least 65 % of the total volatile fraction [48]. The volatiles of the aromatised and non-aromatised beer were extracted by HS-SPME and subsequently analysed by GC-MS/SIM as described in the 'Materials and Methods' section. As shown in figure 3, SPME extraction time and temperature affect the peak area of the targeted compounds. Because the combination of an extraction time of 60 min and an extraction temperature of 60 °C provided the highest response, these SPME conditions were applied in subsequent analyses. The GC-MS/SIM chromatograms as depicted in figure 5 clearly

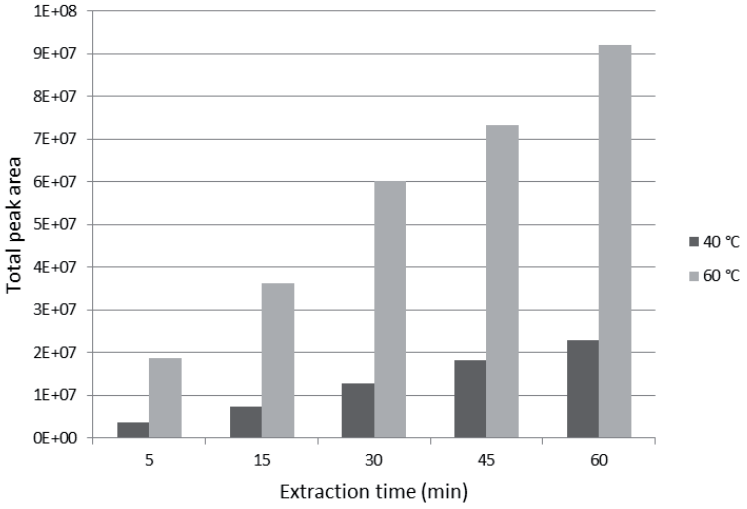

Fig. 3 Time-temperature effect on the extraction of marker sesquiterpenoids from beer by HS-SPME using a polydimethylsiloxane fibre coating (PDMS, 100 µm)

demonstrate the detection of oxidation products of α-humulene and β-caryophyllene in the volatile fraction of pilsner beers.

Real quantitative data on the oxygenated hop sesquiterpenes cannot be given since (except for caryophyllene oxide) authentic reference compounds were not available. Consequently, results in table 3 represent semi-quantitative determinations related to dodecane (internal standard). Coefficients of variation range from 2.3 to 5.8 %, implying good repeatability of the proposed method. Aromatisation yields are obtained by calculating the ratio of the semi-quantitative level of the respective marker component in the aromatised beer to the semi-quantitative level added via dosage of spicy hop essence. For all marker sesquiterpenoids, aromatisation yields appear to be higher than 90 %, except for humulene epoxide II (76 %). Higher sensitivity of humulene epoxide II towards hydrolysis in the beer matrix, compared to the other compounds, has been reported by *Deinzer* and *Yang* [14], which may explain the lower recovery for humulene epoxide II.

3.3 Determination of oxygenated sesquiterpenoids in lager beers

The presented HS-SPME-GC-MS methodologies represent a powerful tool for the determination of the yield of hop aromatisa-

Table 3 Semi-quantitative determination of marker sesquiterpenoids from spicy hop essence cv. Hersbrucker Spät in beer and corresponding yields of aromatisation (CV (%): coefficient of variation; mean of 5 analyses on aromatised pilot beer)

No.	Humulene and caryophyllene oxidation products	Concentration (µg/L)	CV (%)	Yield (%)
1	humuladienone	2.04	4.9	95
2	caryophyllene oxide*	14.3	2.3	98
3	humulene epoxide I	4.58	6.3	93
4	humulene epoxide II	12.9	5.8	76
5	humulenol II	14.5	5.3	93
6	humulene epoxide III	19.3	3.5	92

*Quantification based on authentic reference compound

Table 4 Semi-quantitative levels of sesquiterpene hydrocarbons and oxygenated sesquiterpenes in fresh commercial lagers (Lager A, B, C, D) and a fresh pilot lager (Lager E) (Values represent mean of 5 analyses ± standard deviation).

Component	RI[a]	Lager A µg/L[b]	Lager B µg/L[b]	Lager C µg/L[b]	Lager D µg/L[b]	Lager E µg/L[b]
β-farnesene[d]	1451	1.44 ± 0.10	3.56 ± 0.17	1.74 ± 0.15	2.18 ± 0.13	2.24 ± 0.13
α-humulene[d]	1459	3.18 ± 0.19	2.76 ± 0.14	2.58 ± 0.15	3.50 ± 0.19	8.34 ± 0.38
γ-cadinene	1521	0.32 ± 0.03	0.26 ± 0.03	0.12 ± 0.02	0.22 ± 0.02	0.72 ± 0.08
δ-cadinene	1532	1.16 ± 0.08	0.44 ± 0.03	n.d.	0.36 ± 0.03	0.30 ± 0.03
unidentified sesquiterpenoid (MW = 220)	1541	0.38 ± 0.03	0.18 ± 0.02	0.16 ± 0.01	0.10 ± 0.01	0.16 ± 0.02
trans-nerolidol[d]	1564	6.90 ± 0.16	11.2 ± 0.24	4.62 ± 0.13	8.86 ± 0.28	9.16 ± 0.22
caryophyllene oxide[d]	1571	3.76 ± 0.15	1.26 ± 0.07	2.44 ± 0.11	2.30 ± 0.12	9.04 ± 0.38
caryophyllene alcohol	1581	5.26 ± 0.29	1.62 ± 0.11	1.50 ± 0.12	1.08 ± 0.08	0.92 ± 0.10
humulene epoxide I	1595	19.0 ± 0.48	12.0 ± 0.36	7.86 ± 0.23	3.08 ± 0.10	4.78 ± 0.13
humulol	1597	9.72 ± 0.41	2.24 ± 0.15	1.80 ± 0.09	2.10 ± 0.12	1.14 ± 0.06
humulene epoxide II	1605	2.48 ± 0.09	3.30 ± 0.13	0.86 ± 0.04	0.66 ± 0.05	0.96 ± 0.05
unidentified sesquiterpenoid (MW = 222)	1610	2.52 ± 0.11	0.32 ± 0.02	1.86 ± 0.08	n.d.	0.34 ± 0.03
unidentified sesquiterpenoid (MW = 222)	1613	3.08 ± 0.13	1.72 ± 0.08	2.02 ± 0.09	0.68 ± 0.04	2.16 ± 0.10
unknown	1617	0.34 ± 0.02	n.d.	0.06 ± 0.01	0.12 ± 0.01	0.10 ± 0.01
unknown	1621	0.54 ± 0.02	0.90 ± 0.05	0.54 ± 0.04	0.14 ± 0.01	0.58 ± 0.04
unidentified sesquiterpenoid(s)	1624	6.04 ± 0.19	3.40 ± 0.13	3.44 ± 0.10	1.78 ± 0.07	3.98 ± 0.16
humulenol II	1627	8.76 ± 0.24	5.70 ± 0.18	3.66 ± 0.12	2.86 ± 0.08	3.80 ± 0.13
τ-cadinol	1634	14.8 ± 0.43	7.46 ± 0.17	9.04 ± 0.28	4.12 ± 0.15	12.5 ± 0.44
β-eudesmol	1637	1.60 ± 0.12	0.56 ± 0.05	0.60 ± 0.05	0.26 ± 0.03	1.42 ± 0.16
cadina-1,4-dien-3-ol[c]	1641	1.72 ± 0.08	0.74 ± 0.04	0.82 ± 0.05	0.34 ± 0.04	1.08 ± 0.09
unidentified sesquiterpenoid(s)	1646	12.3 ± 0.80	2.72 ± 0.19	9.00 ± 0.59	1.32 ± 0.13	3.50 ± 0.26
farnesol	1712	3.64 ± 0.13	11.9 ± 0.39	5.32 ± 0.24	5.04 ± 0.21	5.92 ± 0.22
Sum		**109 ± 6.0**	**74.4 ± 3.8**	**60.0 ± 4.3**	**41.1 ± 2.8**	**73.2 ± 4.3**

[a] Retention index on RTX-1 (40 m x 0.18 mm i.d. x 0.20 µm film thickness) calculated on the basis of a hydrocarbon mixture ($C_{10}C_{18}$)

[b] the concentration of volatiles (µg/L) is related to the concentration of C_{12} as internal standard (Mean of 5 analyses ± standard deviation; coefficient of variation ranges between 2.1 % (*trans*-nerolidol) and 16.7 % (γ-cadinene))

[c] tentative identification on the basis of the mass spectrum

[d] identity confirmed by authentic reference compounds

n.d.: not detected

tion using hop oil essences. However, application of the analytical protocols is not limited to the evaluation of the practices of advanced hopping using (fractionated) hop oils but also offers the possibility for accurate determination of hop oil derived constituents in conventionally hopped beers. This may be of high value for quality control, evaluation of the impact of brewing/hopping technologies on the final beer aroma and flavour (in)stability studies of the hop aromatic character of beers brewed via the more widely applied conventional hopping techniques, i.e. early/late kettle hopping and/or dry hopping. To demonstrate the potential of the proposed methodologies for investigation of conventionally hopped beers, 4 commercial (conventionally hopped) lager beers (code: Lager A–D) and one pilot lager (iso-α-acids extract bittering and late hopping with Saaz pellets, code: Lager E) were analysed according to the methodologies for (oxygenated) sesquiterpenes by the HS-SPME-GC-MS/SIM methodology as described above.

Differentiation of fresh lager beers on the basis of the sesquiterpenoid fingerprint

Table 4 displays 22 components, detected in the sesquiterpenoid part of the registered chromatograms. Fifteen constituents were identified on the basis of mass spectral information, retention indices and/or authentic reference compounds (for structures see Fig. 4). The semi-quantitative data in table 4 demonstrate that the beers clearly differ regarding their total level of hop-derived (oxygenated) sesquiterpenes (total levels range from 41 µg/L (Lager D) to 109 µg/L (Lager A)), individual levels of the detected compounds and relative composition of the total oxygenated sesquiterpenoid fraction.

An obvious explanation for the observed differences between the sesquiterpenoid profiles of the analysed beers cannot be given here since no detailed information is available on the processing of the commercial lagers. Hop variety, age of the hops, and hop dosage (amount of hops and point(s) of addition), are all affecting the pattern of hop oil oxidation products in the final beer. Nevertheless, by applying the optimised HS-SPME-GC-MS/SIM procedure, sesquiterpenoid patterns in hops and beers can be described analytically in terms of selected markers, allowing semi-quantitative profiling and technological evaluation of utilisation in the brewery of an essential part of hop oil, i.e. the oxygenated sesquiterpenoid fraction.

HS-SPME-GC-MS/SIM profiling of sesquiterpenoids in forced aged lagers

The optimised methodology for selective analysis of sesquiterpenoids in the volatile fraction of beer, should also allow to monitor the stability of individual sesquiterpenoids when beers are aged. At present, little information is available on the behaviour of these particular compounds during beer ageing. *Peacock* and Deinzer [26] reported on the (in)stability of only a few hop oil components,

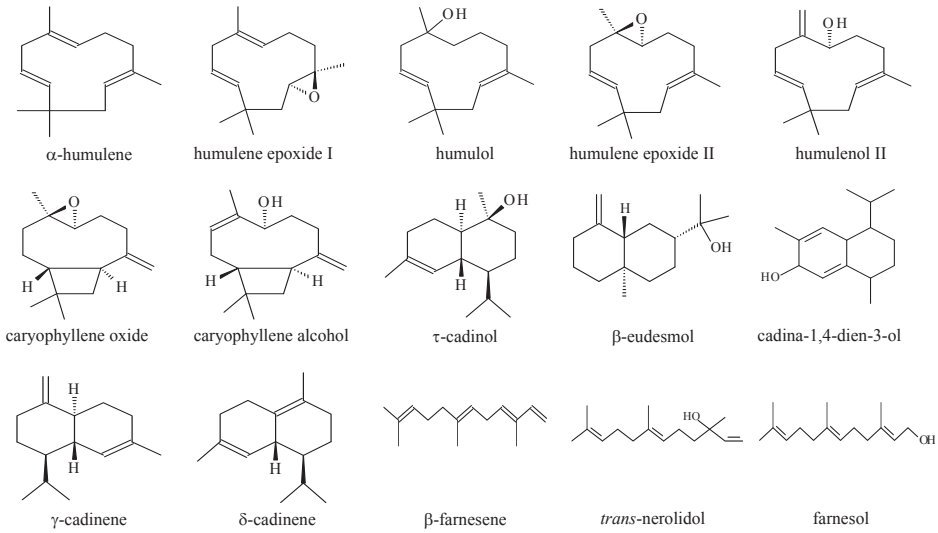

Fig. 4 Structures of sesquiterpene hydrocarbons and oxygenated sesquiterpenes (tentatively) identified in commercial pilsners and pilot pilsner

including the sesquiterpene alcohol humulenol II, in spiked beers during ageing. A decomposition of 66 % for humulenol II was noticed by these authors upon 61 days of beer ageing at room temperature. As shown in table 5, we collected analytical data on the stability of 14 sesquiterpenoids during forced ageing (10 days at 40 °C) of five different lagers. Clearly, as can be derived from the calculated ratios of the level of the selected markers in the aged beers to the level in the fresh beers, the level of the majority of compounds decreases during forced beer ageing. Furthermore, the loss-percentage for a particular compound obviously depends on the beer matrix. When comparing for example the observed losses of humulenol II, a decrease ranging from 1.6 % (commercial lager C) up to 36.2 % (late hopped experimental lager E) is noticed. According to Peacock and Deinzer [26], losses of hop sesquiterpenoids during beer storage can be ascribed to adsorption of the compounds by the crown liner. The major reason for losses, however, is considered to be chemical degradation, caused by oxygen in the headspace of the bottle and/or acid hydrolysis. Based on the total levels of typical hop oil-derived constituents (see Table 5), hop sesquiterpenoids are most stable in beer C and the least stable in the beers B and E.

Interestingly, as can be seen in table 5, levels of *trans*-nerolidol significantly increased upon forced beer ageing. When comparing the sesquiterpenoid profile of the fresh and aged commercial lager A, the increase in *trans*-nerolidol upon ageing is striking. The peak corresponding to *trans*-nerolidol is even the most abundant one in the aged beer sesquiterpenoid profile (Fig. 5B). As apparent from table 5, the increase in *trans*-nerolidol under the applied ageing conditions ranges from 231 % (lager B) to 338 % (lager C). Thus, whereas in lager C, compared to the other beers, hop-derived sesquiterpenoids are most stable during beer ageing, the increase in *trans*-nerolidol is most pronounced in beer C. The identity of *trans*-nerolidol was confirmed by comparison of the mass spectrum

Table 5 Semi-quantitative levels (mean value of 5 analyses) of hop-derived sesquiterpenoids in aged commercial lagers (Lager A, B, C, D) and a pilot lager (Lager E). (A/F (%): ratio (%) of the level of the marker compound in the aged beer (10 days, 40 °C) to the level in the fresh beer (for levels in fresh lagers, see Table 4).

Compound	RI[a]	Lager A			Lager B			Lager C			Lager D			Lager E		
		µg/L[b]	A/F (%)	CV (%)	µg/L[b]	A/F (%)	CV (%)	µg/L[b]	A/F (%)	CV (%)	µg/L[b]	A/F (%)	CV (%)	µg/L[b]	A/F (%)	CV (%)
α-humulene derived products																
α-humulene[d]	1459	2.30	72.3	5.2	2.54	92.0	7.4	1.82	70.5	7.8	2.02	57.7	5.6	2.62	31.4	6.9
humulene epoxide I[c]	1595	12.0	63.0	3.5	7.24	60.1	2.9	6.00	76.3	3.2	2.18	70.8	4.4	3.40	71.1	3.5
humulene epoxide II[c]	1605	1.46	58.9	4.8	1.98	60.0	3.5	0.48	55.8	3.3	0.50	75.8	3.8	0.62	64.6	3.5
humulol[c]	1597	8.74	89.9	5.2	1.80	80.4	4.6	1.12	62.2	3.8	1.52	72.4	5.3	0.78	68.4	4.6
humulenol II[c]	1627	7.54	86.1	3.4	5.26	92.3	4.2	3.60	98.4	5.3	2.32	81.1	3.9	2.42	63.8	3.8
β-caryophyllene derived products																
caryophyllene oxide[d]	1571	2.36	62.8	5.7	0.84	66.7	4.8	2.32	95.1	5.3	1.50	65.2	5.5	5.36	59.2	5.8
caryophyllene alcohol[c]	1581	4.92	93.5	5.3	1.28	79.0	3.4	1.24	82.6	4.2	0.92	85.2	4.7	0.86	93.5	4.8
other oxygenated sesquiterpenoids																
unidentified sesquiterpenoid (MW = 222)	1613	2.80	90.9	6.2	1.44	83.7	5.7	1.68	83.2	4.7	0.54	79.4	5.6	1.68	77.8	5.3
unidentified sesquiterpenoid(s)	1624	5.00	82.8	3.2	3.34	98.2	2.6	3.02	87.8	3.5	1.22	68.5	3.9	2.92	73.4	3.3
τ-cadinol[c]	1634	14.2	95.7	3.1	4.92	66.0	4.0	9.52	105	4.9	3.76	91.3	4.7	10.1	80.4	4.2
β-eudesmol[c]	1637	1.56	97.5	4.2	0.70	125	5.3	0.84	140	4.0	0.26	100	4.6	1.10	77.5	4.2
cadina-1,4-dien-3-ol[e]	1641	0.98	57.0	6.2	0.52	70.3	5.8	0.70	85.4	6.8	0.20	58.8	5.5	0.60	55.6	6.5
unidentified sesquiterpenoid(s)	1646	10.5	84.9	3.3	2.22	81.6	2.2	7.66	85.1	3.1	0.94	71.2	2.8	2.62	74.9	3.2
farnesol[c]	1712	4.06	111	3.0	12.1	101	2.4	7.70	145	2.5	3.12	61.9	3.2	4.58	77.4	2.8
trans-nerolidol[d]	1564	18.9	275	2.6	26.0	231	3.1	15.6	338	2.8	20.7	234	2.8	22.8	249	3.0
SUM of hop-oil derived compounds[f]		**72.0**	**80.0**	**4.5**	**31.5**	**73.8**	**4.1**	**38.18**	**88.7**	**4.1**	**15.86**	**77.1**	**4.7**	**32.44**	**71.6**	**4.2**

[a] Retention index on RTX-1 (40 m x 0.18 mm i.d. x 0.20 µm film thickness) calculated on the basis of a hydrocarbon mixture (C_{10}–C_{18})

[b] the concentration of volatiles (µg/L) is related to the concentration of C_{12} as internal standard (mean of 5 analyses; CV: coefficient of variation of A/F parameter)

[c] tentative identification on the basis of the mass spectrum and RI

[d] identity confirmed by authentic reference compounds

[e] tentative identification on the basis of mass spectral comparison

[f] Sum of oxygenated compounds minus α-humulene, farnesol, *trans*-nerolidol

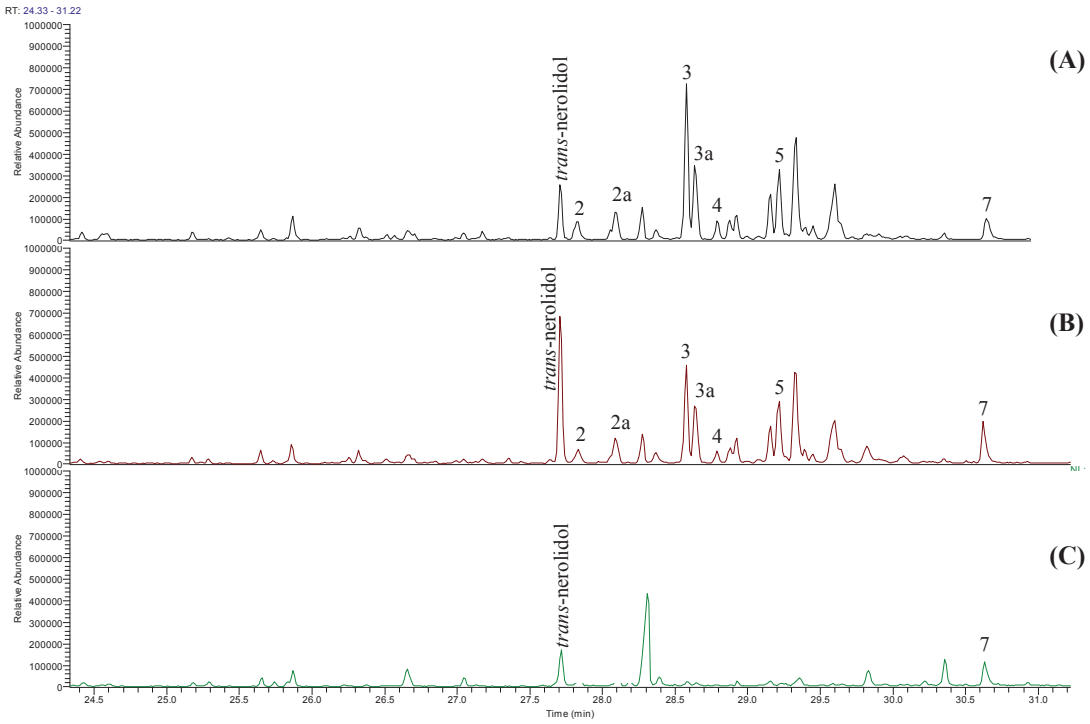

Fig. 5 HS-SPME-GC-MS/SIM profiles of sesquiterpenoids in fresh commercial lager A (A), aged (10d, 40 °C) commercial lager A (B), and non-aromatised fresh experimental lager exclusively bittered with iso-a-acids extract (C). (peak identification: 2: caryophyllene oxide; 2a: caryophyllene alcohol; 3: humulene epoxide I; 3a: humulol; 4: humulene epoxide II; 5: humulenol II; 7: farnesol)

of the component detected in beer with the mass spectrum in the reference mass spectral library 'Flavor MS Library for Xcalibur 2003', and also by calculation of the retention index (RI = 1564) via analysis of the authentic reference compound. Apparently, our results obtained upon analysis of the behaviour of hop-derived sesquiterpenoids during beer ageing, additionally have led to the identification of a particular component, i.e. *trans*-nerolidol, that might be useful in measuring flavour (in)stability of beer. This finding is in accordance with the results of *Tsuji* and *Mizuno* [37] who considered nerolidol to be a valuable marker candidate for indicating the storage period of any type of beer.

Trans-nerolidol (3,7,11-trimethyl-1,6,10-dodecatrien-3-ol) is a tertiary terpene alcohol showing floral, citrus, and woody odour notes and it is found in the essential oil of many different plants [49]. *Trans*-nerolidol is also present in the essential oil of hops [49-52] and considered as an end-product of biosynthesis and thus not as a chemical oxidation product from sesquiterpene hydrocarbons [13, 41]. Although *trans*-nerolidol is detected as a constituent in hop essential oil, its presence in beer may not be exclusively ascribed to the use of hops. Indeed, our experiments show the presence of *trans*-nerolidol in the sesquiterpenoid profile of an experimental lager exclusively hopped with iso-α-acids extract for bittering purposes (see Fig. 5C). The same observation is made for farnesol (3,7,11-trimethyl-2,6,10-dodecatrien-1-ol) (see Fig. 5C). As reported in literature, farnesol is produced by *Saccharomyces cerevisiae* from farnesyl pyrophosphate during fermentation, and subsequently secreted into the beer [53–55]. Under acidic conditions, such as those in beer, farnesol can be further transformed into *trans*-nerolidol (for structures see Fig. 4). Since farnesol and (partly) *trans*-nerolidol originate from yeast activity, these compounds cannot be used as representative markers for determination of the yield of hop aromatisation.

4 Conclusions

In this work, we optimised analytical methods based on headspace-solid phase microextraction (HS-SPME) and advanced gas chromatography-mass spectrometry (GC-MS) for selective determination of hop oil-derived volatiles in the complex beer aroma profile.

HS-SPME of beer volatiles combined with MS monitoring of selected fragment ions (SIM) of hop oil-derived constituents proved successful for reliable determination of representative markers for the floral and oxygenated sesquiterpenoid fraction of hop essential oil, respectively. Sesquiterpenoid patterning provided discrimination between fresh lagers and the proposed GC-MS/SIM procedure may be useful in view of more detailed brand characterisation. In addition, beer ageing experiments pointed to the instability of the oxygenated sesquiterpenoid hop oil fraction and led to the identification of *trans*-nerolidol as a potential marker for beer flavour (in)stability.

From the practical point of view, the optimised SPME-GC-MS methods allow adequate determination of the yield of advanced hop aromatisation using hop oil essences. Moreover, the proposed analytical procedures are particularly suitable for state-of-the-art studies on the common practice of conventional hopping which is even more difficult to understand and control than advanced hopping using well-defined hop oil preparations.

In conclusion, the established analytical methodology allows for sound technological assessment of hop aromatisation, in particular determination of hop utilisation in terms of essential oil constituents, which should be of interest for all brewers aiming at enhanced control and consistency of hop aromatic character in beer.

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