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Headspace Trap GC-MS analysis of hop aroma compounds in beer

A headspace (HS)-trap gas chromatography (GC)-mass spectrometry (MS) method was developed to investigate dry hopping aroma in beer. Analysis of monoterpenes, sesquiterpenes, terpene alcohols, esters, and ketones was performed. The analytical findings enable to elucidate the impact of the hop variety as well as the influence of hop dosage time and beer style on the aroma profile of beer. Furthermore, studies on the storage behavior of different aroma compounds in beer were carried out. The HS-trap GC-MS technique covers a broad range of hop-derived aroma compounds and is suitable for quality control during production of dry hopped beers.

Descriptors: dry hopping, hop volatiles, beer analysis, storage behavior

1 Introduction

The essential oils represent an important group of hop constituents [1]. This fraction contains a large number of aroma compounds which are important for the overall aroma of hops and have an impact on the beer aroma influenced by hops. In 2000, *Steinhaus* and *Schieberle* confirmed the volatiles linalool and myrcene as key contributors to the overall aroma of dried hop cones of the hop variety Spalter Select [2]. In addition, ethyl isobutanoate, methyl-2-methylbutanoate, (*E,Z*)-1,3,5-undecatriene, and propyl-2-methylbutanoate were described as important odorants in dry hops [2]. A further study, executed by Steinhaus et al. in 2007, revealed geraniol as well as 4-methyl-4-sulfanylpentan-2-one (MSP) as key contributors to the aroma of the US hop variety Cascade [3]. These volatiles are described to be more varietal specific. Several further aroma compounds like esters, aldehydes, ketones, and more have been reported in various hop varieties [1–4].

To evaluate the aroma impact of different hop volatiles on beer aroma, the knowledge of odor threshold values is important. The odor threshold concentration depends strongly on the matrix where it is determined. The odor threshold values of hop-derived aroma compounds reported in the literature deviate clearly. For example, the odor threshold of myrcene has been reported between 9–1000 µg/L [1]. The odor threshold value of (R)-linalool in beer is 2.2 µg/L [5] but also higher values could be found in the literature (2–80 µg/L, [1]). Linalool has been characterized as key aroma compound in beer i.e. in a Pilsner type beer [5, 6] whereas for myrcene an aroma contribution to a dry hopped beer could be observed [5]. *Takoi* et al. investigated the impact of different hop addition times on the aroma profile of beer and observed that myrcene amounts increased by the delaying of hop addition time [7]. The authors also pointed out that myrcene might be an important contributor to beer flavor in the beers tested [7]. Studies on the contribution of terpene alcohols (geraniol, β-citronellol, nerol, and α-terpineol), in addition to linalool, to beer aroma were executed and an additive effect among linalool, geraniol, and β-citronellol at amounts of 5 µg/L of geraniol and β-citronellol was determined [8].

The characterization of beer aroma affected by hops requires quantitative analysis of the final product. Headspace sampling is a suitable and widely-used technique for extraction of volatiles from complex sample matrix. To improve detection limits of analytes, the use of headspace-solid phase microextraction (HS-SPME) combined with GC-MS [7, 9, 10] as well as the application of a stir bar sorptive extraction (SBSE) method with GC-MS [11–13] are described in literature for the analysis of hop-derived aroma compounds in beer. Furthermore, a headspace (HS)-trap GC-MS technique for determination of volatile components in different hop varieties was published recently by *Aberl* and *Coelhan* [4]. Sulphur compounds were not integrated in the described method because of very low concentrations as well as the limits of detection for these substances using GC-MS technique. The comparison with conventional hop essential oil analysis showed a good correlation between the results of the HS-trap method and conventional method [4]. The use of headspace trap in combination with gas chromatography is also established for the determination of aromatic volatile organic compounds in groundwater, mineral water, and drinking water [14] as well as for the analysis of volatile constituents in spirits [15]. The characterization of hop aroma using headspace trap with GC-MS and an olfactory port is also possible through the direct analysis of hop cones or hop pellets [16]. The HS-trap approach is suitable for the analysis of both solid and liquid samples. Using this technology, most of the headspace vapor in a pressurized vial is passed through an adsorbent trap to collect the volatiles. The carrier gas is used to pressurize the headspace vial contents. The trap is then rapidly heated and the desorbed components are transferred to the GC column. Multiple cycles may be executed to effectively transfer the total vapor to the GC column.

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The purpose of the present study was to apply a headspace (HS)-trap method in combination with gas chromatography-mass spectrometry (GC-MS) for the determination of hop-derived volatile compounds in beer and to evaluate the impact of these components on the overall aroma profile in fresh and stored beer samples.

2 Materials and methods

2.1 HS-trap sampling conditions

A TurboMatrix™ HS-40 Trap (Perkin Elmer, Rodgau, Germany) was used. The HS-trap sampling parameters are given in table 1.

2.2 Sample preparation for HS-trap analysis

Beer was decarbonated by manual shaking. 5 ml of beer (direct or after dilution with water) was transferred into a HS-vial (20 ml) and spiked with the internal standard linalool-d5 (final concentration in the HS-vial = 20 µg/L). The HS-trap sampling conditions were used as given in table 1.

Table 1 HS-trap sampling conditions for hop aroma compounds

parameter	value
temperature	
oven temperature	85 °C
needle temperature	90 °C
transfer line temperature	130 °C
trap low temperature	40 °C
trap high temperature	280 °C
pressure	
column pressure	150 kPa
vial pressure	240 kPa
desorption pressure	150 kPa
time	
thermostatting time	15¹/45² min
pressurization time per cycle	1.0 min
trap load time per cycle	1.6 min
dry purge time	8 min
heating hold time	15 min
pulse cycles	1¹/2²

1 analysis of all compounds except geraniol and β-citronellol
2 analysis of geraniol and β-citronellol

Analysis of geraniol and β-citronellol was done using a modified sample preparation. Therefore, 2 g of sodium chloride (Sigma Aldrich, Steinheim, Germany) was added to the HS-vial (20 ml). 5 ml of decarbonated beer sample (direct or after dilution with water) was also transferred into the vial and spiked with the internal standard citronellol-d6 (final concentration in the HS-vial = 50 µg/L). The HS-trap parameters for the analysis are shown in table 1.

The gas chromatography-mass spectrometry analysis was performed using the conditions described below.

2.3 HS-trap calibration

A six-point standard calibration curve for each aroma compound was generated.

The calibration range for myrcene was between 2–400 µg/L and between 1–200 µg/L for all remaining volatiles. An aqueous solution for the highest concentration was prepared using the stock solutions. Further calibration levels were achieved by dilution with water. The ethanol content was kept at the same level (20 µl ethanol/5 ml sample volume) for all calibration levels. Linalool-d5 was added as internal standard (final concentration in the HS-vial = 20 µg/L).

The calibration range for geraniol and β-citronellol was between 5–200 µg/L. An aqueous solution for the highest concentration was also prepared using the stock solutions and the further calibration levels were achieved by dilution with water. Before dilution, 2 g of sodium chloride (Sigma Aldrich, Steinheim, Germany) was added to the HS-vial (20 ml). The ethanol content was kept at the same level (20 µl ethanol/5 ml sample volume) for all calibration levels. Citronellol-d6 was added as internal standard (final concentration in the HS-vial = 50 µg/L).

The linear response over the concentration range analyzed was with a R^2 value > 0.99.

2.4 GC-MS conditions

All the substances used (Table 2) were obtained from Sigma-Aldrich (Steinheim, Germany). The internal standards linalool-D5 and citronellol-D6 were obtained from VLB Berlin (Research Institute for Instrumental Beer and Beverage Analysis).

Beer analysis was performed using a Thermo Focus gas chromatograph coupled to a DSQ II quadrupole mass spectrometer (Thermo Fisher Scientific, Dreieich, Germany). The TurboMatrix™ HS-40 Trap was used as a HS sampler. A Perkin Elmer Elite-200 capillary column (60 m length x 0.25 mm i.d.; film thickness, 1 µm) was used for chromatographic separation. Helium served as carrier gas with a column head pressure of 150 kPa controlled by the HS-trap sampler. The GC temperature program was from 45 °C (held for 2.0 min) up to 100 °C at a rate of 3 °C/min (held for 0 min), then up to 200 °C at a rate of 5 °C/min (held for 0 min) and further up to 270 °C at a rate of 40 °C (held for 5 min). The MS transfer line was set at 250 °C and the ion source temperature was 230 °C. The mass spectrometer was operated in selected ion monitoring (SIM) mode using electron ionization (70 eV). The analytes were detected in time windows. The identification was done on the basis of their retention times and the fragment ions in comparison to the standard compounds (Table 2).

2.5 Beer samples

2.5.1 Influence of hop variety

19 different beer samples were analyzed. The hop varieties used for dry hopping were grown in the growing region Hallertau (Mandarina

Table 2 Compound-specific parameters for GC-MS analysis

compound	retention time (min)	fragment ions (m/z)
mono- and sesquiterpenes		
α-pinene	16.7	121, **136**
β-pinene	19.5	121, **136**
myrcene	19.7	107, **136**
β-limonene	21.4	**68**, 136
β-farnesene	36.7	**161**, 204
β-caryophyllene	37.6	**133**, 204
α-humulene	38.6	**147**, 204
terpene alcohols		
linalool	27.5	**121**, 136
α-terpineol	31.9	121, **136**
β-citronellol	32.7	123, **138**
geraniol	33.8	**123**, 154
ketones		
E,Z-1,3,5-undecatriene	26.9	79, **150**
2-nonanone	31.5	71, **142**
2-decanone	34.8	98, **156**
2-undecanone	37.7	110, **170**
2-dodecanone	40.4	126, **184**
β-damascenone	41.4	177, **192**
2-tridecanone	42.1	140, **198**
esters		
ethyl isobutanoate	13.5	88, **116**
methyl-2-methylbutanoate	14.4	88, **101**
ethyl-2-methylbutanoate	17.8	85, **102**
isoamyl acetate	20.6	**70**, 87
isobutyl isobutanoate	21.6	**71**, 114
methyl hexanoate	22.1	74, **99**
propyl-2-methylbutanoate	22.7	85, **103**
3-methylbutyl propanoate	24.3	70, **75**
2-methylbutyl isobutanoate	26.2	**89**, 158
methyl heptanoate	26.4	**101**, 144
methyl octanoate	30.1	**87**, 158
methyl nonanoate	33.4	**98**, 172
methyl decanoate	36.4	**143**, 155
ethyl dodecanoate	42.3	**157**, 228
epoxide		
caryophyllene epoxide	44.3	205, **220**
internal standards		
linalool-D5	27.5	**126**, 141
citronellol-D6	32.1	129, **162**

Quantitation ions are given in bold

Bavaria, Smaragd, Polaris, Cascade, Hallertau Blanc, and Saphir) and in the U.S. (Bravo, Lemondrop and Calypso). Hop pellets (17 beer samples) and hop oils (2 beer samples) were used for dry hopping. The amounts for pellets were between 100–300 g/hl. The amounts for hop oils were 0.8 g/hl and 2.4 g/hl.

2.5.2 Influence of hop addition time

The hop variety US Lemondrop was used for the brewing trials. The total oil content of pellets type 90 used was 1.1 ml/100 g. The amount of α-acids was 6.0 %. The brewing trials were carried out in a 20 hl pilot plant (Bitburger Brewery, Bitburg, Germany). A standard 2-mash decoction procedure was used to produce German Pilsner type beers (original wort: 12.5 %, alcohol: 5.2 vol.-%, 100 % Pilsner Malt). Wort was boiled for 75 min at 100 °C and fermented at 11 °C with a standard lager yeast strain. Maturation was done at fermentation temperature until diacetyl was below 0.1 ppm. Table 3 shows relevant differences in hop amount and hop addition time. Dry hopping was done in a static way with 7 days of contact time at 0 °C. Beers were filtered. The total oxygen amount of beers filled in bottles was below 0.07 mg/L.

Table 3 Hopping conditions for brewing trials

beer	hop variety	time of hopping	amount
German Pilsner 1	Lemondrop	early	8 g α/hl
		late	6 g α/hl
German Pilsner 2	Lemondrop	early	8 g α/hl
		late	6 g α/hl
		dry hopping	250 g/hl
Pale Ale	Lemondrop	early	10 g α/hl
		late	4 g α/hl
		dry hopping	250 g/hl

2.5.3 Influence of beer style

In addition to German Pilsner beers, a Pale Ale with the hop variety Lemondrop was brewed in the 20 hl pilot plant (Bitburger Brewery, Bitburg, Germany). The hop amount is given in table 3. For the Pale Ale a 3-step infusion mashing regime was used, boiling was comparable to the Pilsner Type beers and fermentation was done at 20 °C with an English style Ale yeast. Maturation was done at fermentation temperature until diacetyl was below 0.1 ppm. Original wort was 12.4 %, the alcohol amount was 5.8 vol.-% and the following malt mixture was used: 64 % Pilsner Malt, 16 % CARAHELL®, 10 % Munich Malt and 10 % Wheat Malt. Dry hopping was also done in a static way at 0 °C with 7 days of contact. Beers were used without a filtration step. The total oxygen content of beers filled in bottles was below 0.07 mg/L.

2.5.4 Influence of storage

To evaluate the storage behavior of beers dry hopped with the hop variety Lemondrop, German Pilsner beer 2 and the Pale Ale were stored for 3, 6 and 12 months at two different storage temperatures (5 °C and 20 °C).

3 Results and discussion

According to Aberl and Coelhan [4], the HS-trap and GC-MS parameters for the analysis of hop-derived volatiles in hop samples were transferred to the analysis of beer samples. The determination of geraniol and β-citronellol was added to the method. These two analytes were not part of the published method [4].

To monitor the relevance of the selected aroma compounds, listed in table 2, and to evaluate the impact of different hop varieties on the aroma profile of dry hopped beer, 19 fresh beer samples with 9 different hop varieties were analyzed by means of HS-trap GC-MS technique (Data not given).

Significant differences were detected for the terpene alcohols linalool, α-terpineol, geraniol and β-citronellol. In addition, significant differences between the samples tested were observed for myrcene, β-caryophyllene, α-humulene, β-farnesene as well as for the esters isoamyl acetate, 2-methylbutyl isobutanoate, and isobutyl isobutanoate. The compounds linalool, geraniol and myrcene were described in literature as key aroma compounds in hops [2, 3]. Only minor differences were found for the ketones 2-nonanone, 2-decanone, 2-undecanone, 2-dodecanone, 2-tridecanone, and β-damascenone, the 3 esters (methyl nonanoate, methyl decanoate, ethyl dodecanoate) as well as for β-limonene.

The following volatile compounds were not detectable in the evaluated beer samples: *E*,*Z*-1,3,5-undecatriene, α- and β-pinene, caryophyllene oxide and the remaining esters (methyl-2-methylbutanoate, ethyl-2-methylbutanoate, propyl-2-methylbutanoate, ethyl isobutanoate, methyl hexanoate, methyl heptanoate, methyl octanoate, 3-methylbutyl propanoate). Although *E*,*Z*-1,3,5-undecatriene and the esters methyl-2-methylbutanoate, propyl-2-methylbutanoate and ethyl isobutanoate have been characterized as key aroma compounds in hops by Steinhaus and Schieberle [2], they could not be observed in beer samples tested. These volatiles don't play a role for the aroma profile of fresh dry hopped beers.

Studies on hop aroma profile in beer as a function of hop addition time were carried out for a German Pilsner type beer with the hop variety Lemondrop. Lemondrop is an aroma-type hop. It has a lemon aroma that imparts notes of citrus, herbal, fruity and floral. Relevant differences in hop amount and hop addition time are given in table 3.

Typical hop-derived aroma compounds detectable in beer samples with Lemondrop are given in table 4. Limit of detection for all other compounds was 1 µg/L.

The comparison of late and dry hopping reveals a concentration increase for the terpenes myrcene, β-caryophyllene and α-humulene. The odor threshold of myrcene has been reported in literature between 9–1000 µg/L [1]. The conclusion can be drawn that myrcene has a higher aroma impact in dry hopped Pilsner beer 2. The amounts of β-caryophyllene and α-humulene were very low and far below the odor thresholds known from literature with 160–420 µg/L for β-caryophyllene and 747 µg/L for humulene [1].

A considerably high entry through dry hopping was also detectable for linalool and geraniol but not for α-terpineol and β-citronellol. The odor thresholds of the terpene alcohols are 2–80 µg/L for linalool, 4–300 µg/L for geraniol, 9–40 µg/L for β-citronellol and 330 µg/L for α-terpineol [1]. Therefore both, linalool and geraniol, clearly contribute to the aroma profile of dry hopped beers with the hop variety Lemondrop. Whereas the detected amounts of α-terpineol are far below the odor threshold, β-citronellol may still have some aroma contribution. Even below its threshold, some synergistic effects of β-citronellol in beer are described by Takoi et al. as soon as this aroma compound is present in coexistence with linalool and geraniol [8, 17].

Table 4 Amounts [µg/L] and standard deviation (n = 3) of hop aroma compounds in German Pilsner beer 1 and 2

compound	Pilsner 1 early and late	Pilsner 2 early, late and dry hopping
mono- and sesquiterpenes		
myrcene	45.6 ± 1.1	79.7 ± 2.8
β-caryophyllene	1.1 ± 0	2.3 ± 0.2
α-humulene	1.6 ± 0.04	5.2 ± 0.5
terpene alcohols		
linalool	62.3 ± 4.7	155 ± 8.0
α-terpineol	16.8 ± 0.9	25.4 ± 6.4
geraniol	27.4 ± 4.3	265 ± 45.8
β-citronellol	22.8 ± 1.1	26.3 ± 3.5
ketones		
2-nonanone	0.9 ± 0.02	6.9 ± 0.3
2-decanone	1.0 ± 0.01	4.4 ± 0.3
2-undecanone	23.0 ± 0.8	33.3 ± 6.1
esters		
isoamyl acetate	275 ± 54.6	330 ± 11.6
2-methylbutyl isobutanoate	7.1 ± 0.3	34.7 ± 3.2
ethyl dodecanoate	25.6 ± 1.6	31.7 ± 3.5

Also for 2-nonanone and 2-decanone significantly higher contents were observed in the dry hopped beer in comparison to late hopped beer, whereas for 2-undecanone the additional entry through dry hopping was less considerable. The odor thresholds of the two ketones 2-nonanone and 2-undecanone known from literature were determined in water, and they are 5–200 µg/L for 2-nonanone and 7 µg/L for 2-undecanone [18]. The aroma quality of the ketones is fruity. Thus especially the aroma contribution of 2-undecanone to both late hopped and dry hopped beer may be relevant, although none of these ketones could be clearly identified as key aroma compound of beer so far.

Only 2-methylbutyl isobutanoate was significantly influenced by dry hopping. The differences in isoamyl acetate and ethyl dodecanoate proved to be rather low between both beers. The odor threshold of 2-methylbutyl isobutanoate has been reported in literature with 57 µg/L in a model solution and 78 µg/L in beer [19]. However, a synergistic effect of this ester could be detected in a concentration as low as 5 µg/L [19] so that some contribution to the aroma profile of beer being dry hopped with the variety Lemondrop is expected.

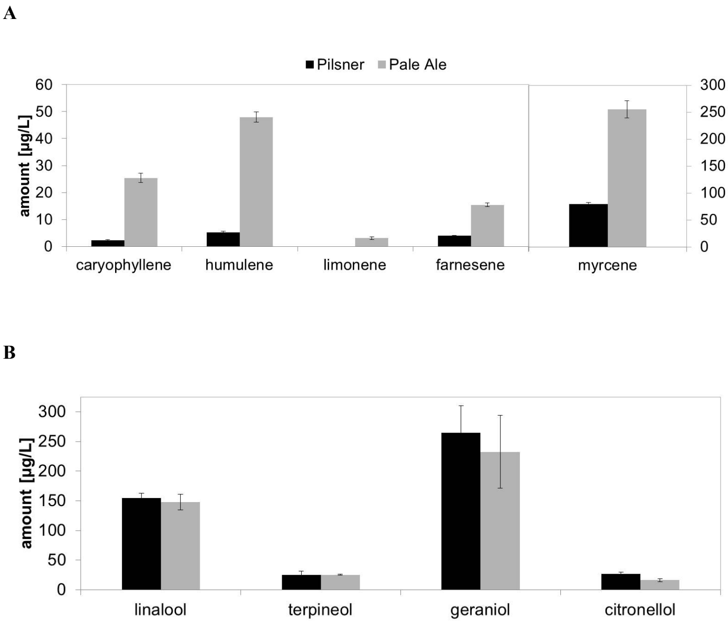

Fig. 1 **Concentrations [µg/L] and standard deviation (n = 3) of mono- and sesquiterpenes (A) and terpenes alcohols (B) in Pilsner type beer (German Pilsner 2) and Pale Ale**

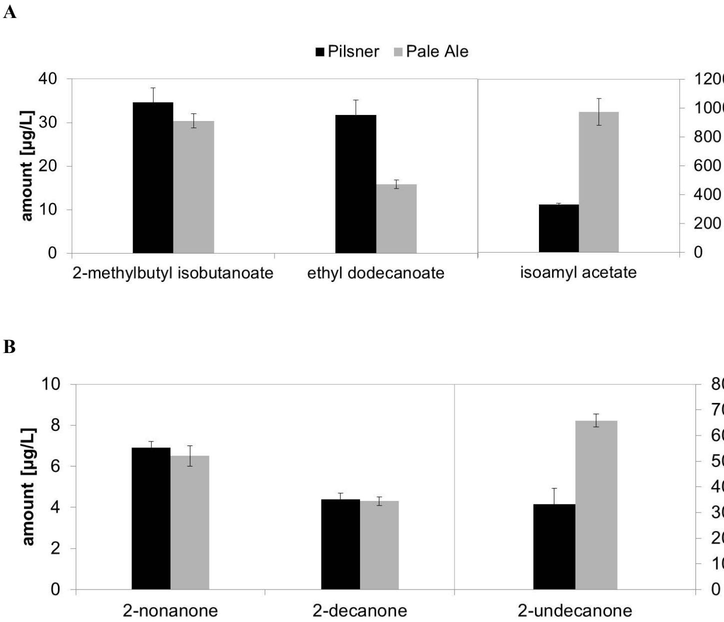

Fig. 2 **Concentrations [µg/L] and standard deviation (n = 3) of esters (A) and ketones (B) in Pilsner type beer (German Pilsner 2) and Pale Ale**

Two different beer styles, a German Pilsner beer (Pilsner 2) and a Pale Ale, produced with the same hop variety as well as the same total hop amount were compared. Both beers were early, late and dry hopped. The amount of Lemondrop used for brewing is given in table 3.

The results, presented in figure 1 and figure 2, demonstrate significantly higher concentrations of the mono- and sesquiterpenes myrcene, β-caryophyllene, α-humulene, β-farnesene and β-limonene in the Pale Ale in comparison to Pilsner beer (Fig. 1A). The filtration of the Pilsner beer might contribute to the lower amounts of mono- and sesquiterpenes in this beer. No significant differences in these two beers were determined for the more polar terpene alcohols linalool, geraniol, and α-terpineol (Fig. 1B). Only the amount of β-citronellol was lower in Pale Ale (Fig. 1B). The behavior of esters among themselves was different (Fig. 2A). Whereas for isoamyl acetate a significantly higher amount was detectable in Pale Ale, the difference in 2-methylbutyl isobutanoate content proved to be rather low between both beers. For ethyl dodecanoate a higher amount was detectable in Pilsner beer in comparison to Pale Ale (Fig. 2A). The differences for esters might be dependent on the yeast used for brewing. A higher concentration in Pale Ale was determined for 2-undecanone but no significant differences were detected for 2-nonanone and 2-decanone (Fig. 2B).

The introduced method allows the monitoring of hop aroma characteristics in different beer styles produced with the same hop variety and the same total hop amount.

The behavior of several aroma compounds in a Pilsner type beer (Pilsner 2) as well as in a Pale Ale was observed over a period of 12 months. The analysis was carried out after 3, 6 and 12 months of storage at two different storage temperatures. Figure 3 shows the results of selected compounds with the remaining amounts presented in percent of the initial concentration.

The concentration of linalool remains on a rather constant level, between 85 and 103 % of the initial amount, in both beer styles and for both storage temperature (Fig. 3, see next page). The detected amounts of α-terpineol increased with a longer storage time in both beer styles. After 12 months of storage at room temperature over 200 % of the initial concentration was detectable in Pale Ale (Fig. 3). The presence of glycosides of terpene alcohols in hopped beer and the liberation of aglycones during storage [20], explain the rising level. *Forster* et al. also observed an increase of the β-terpineol amount during storage at 20 °C for 120 days [21]. In Pale Ale, geraniol showed a stepwise decline at room temperature and was rather stable at cold temperature. It was also rather stable after 3, 6 and 12 months of storage for the Pilsner type beer. Differences between both storage temperatures were marginal in this type of beer (Fig. 3). In both beer styles a stepwise decrease was observed for β-citronellol (Fig. 3). Studies on the storage behavior of terpene alcohols executed by Forster et al. [21] revealed stable monoterpenes like linalool, geraniol and β-citronellol. However, in this study these compounds were only investigated after the storage time of 3 months [21].

Terpenes (myrcene and α-humulene), esters (isoamyl acetate and 2-methylbutyl isobutanoate) and the ketone 2-undecanone showed a clear decline over the storage time (Fig. 3). Both 2-methylbutyl isobutanoate and 2-undecanone were more stable in the Pilsner beer in comparison to the Pale Ale. The decline observed for terpenes, esters and 2-undecanone in dry hopped beers is also known from literature [22]. Because of low initial concentrations (<10 µg/L), β-caryophyllene, 2-nonanone and 2-decanone are not imaged here. They all decreased during storage.

4 Conclusions

The developed HS-trap GC-MS method proved to be reliable for the analysis of dry hopped beers on some of their most typical key

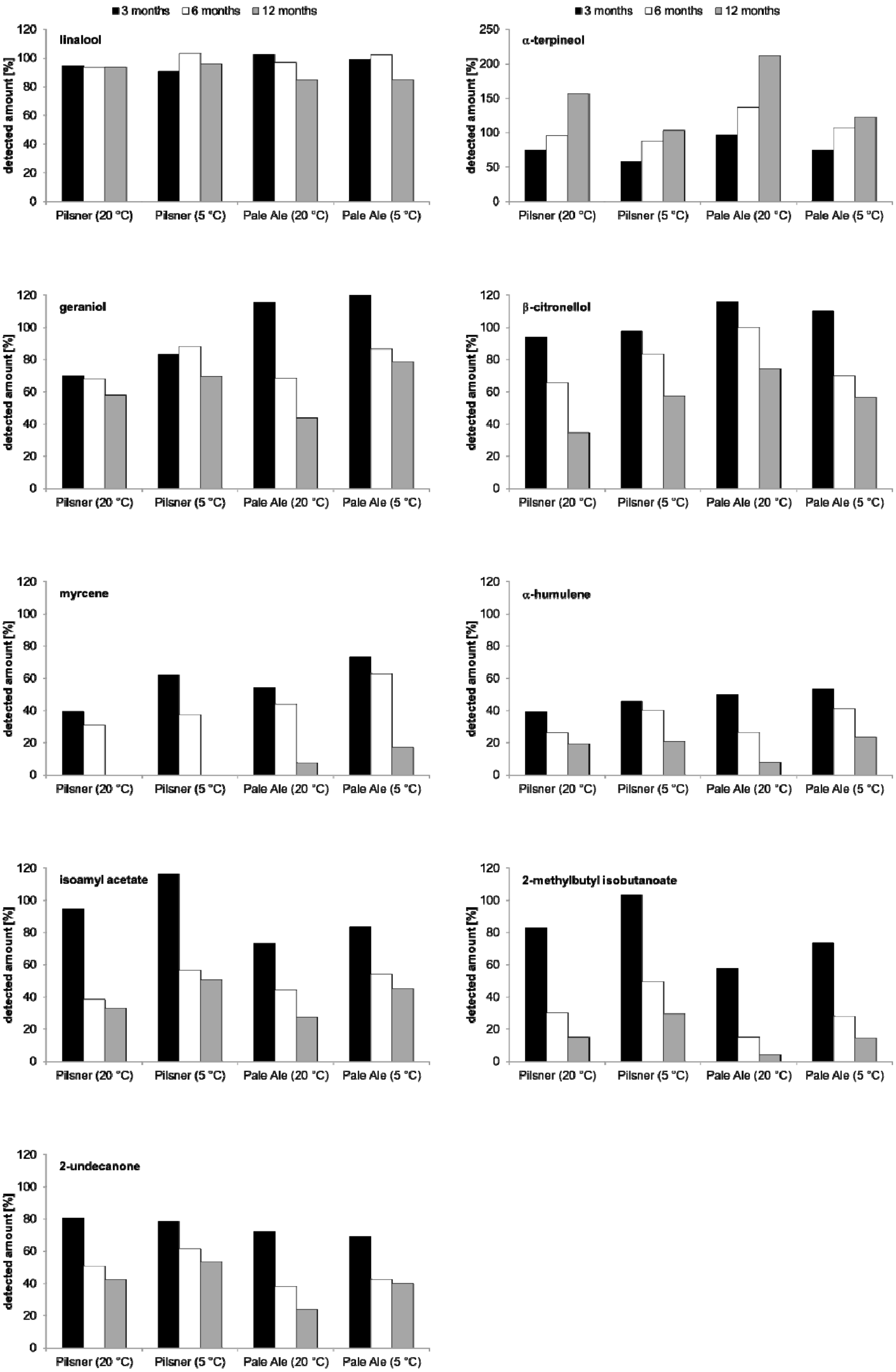

Fig. 3 Remaining amounts [%] of hop-derived aroma compounds in stored beer samples (Pilsner = German Pilsner 2)

aroma compounds known from literature, i.e. myrcene, linalool, geraniol, β-citronellol, and 2-methylbutyl isobutanoate.

The observations of two different beer styles produced with the same total hop amount and same hop variety revealed the differences on the hop aroma profile in fresh beer as well as during storage.

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