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Determination of Volatile Compounds in Different Hop Varieties by Headspace-Trap GC/MS—In Comparison with Conventional Hop Essential Oil Analysis

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ABSTRACT: A headspace (HS)-trap method in combination with gas chromatography (GC)−mass spectrometry (MS) was developed for the determination of volatile constituents in hops. The highly sensitive HS-trap system reduces the detection limit by using up to four trap enrichment cycles. Seventy hop samples of different varieties and cultivation regions, from the 2008 harvest, were examined using the HS-trap-GC-MS method and the established "European Brewery Convention" (EBC) method for hop essential oil analysis. Twenty-one different volatiles were quantified for each hop sample. For all compounds, except caryophyllene oxide, a strong correlation was found between the results of the HS-trap method and the EBC method. Experiments have revealed that the EBC method using steam distillation is not appropriate for thermolabile compounds, such as caryophyllene oxide, due to decomposition during boiling. The HS-trap method is fast and sensitive, requires small sample amounts and minimal sample preparation, and is easy to apply.

KEYWORDS: *hop, Humulus lupulus L., hop essential oil, headspace trap, gas chromatography−mass spectrometry*

INTRODUCTION

Hop (*Humulus lupulus* L.) imparts a bitter flavor and pleasant hoppy aroma to beer. Apart from the bitter substances, the essential oils are the most important group of hop constituents. The biosynthesis of essential oil takes place in the hop lupulin gland.[1,2] The total oil content is dependent on the hop type and amounts to about 0.1−2.0% by dry weight.[1] More than 400 hop aroma components have been identified.[3] They can be divided into two main classes, the hydrocarbons (40−80% by weight of the total hop essential oil) and the oxygen-containing compounds. The hydrocarbons can be subdivided into monoterpenes and sesquiterpenes. Myrcene is the most common monoterpene and comprises 10−72% of hop essential oil. The most abundant sesquiterpenes are α-humulene (15−42% of hop essential oil) and β-caryophyllene (2.8−18.2% of hop essential oil).[3]

For brewers, aroma-active hop volatiles are of prime interest with regard to developing beer with a distinct hoppy flavor. Linalool is one of the most aromatic flavor components of hop essential oil and has been considered as a primary substance for hoppy aromatic beers.[5,6] It is a very flavorful terpene alcohol, with citrus- and bergamot-like odor. Linalool is contained in hop essential oil in amounts of up to 1.1% by weight.[3,7] Myrcene and linalool are considered as the most odor-active volatiles in all analyzed hop varieties.[5,8,9] Myrcene usually does not make a contribution to hop aroma in beer, because its concentration is often far below the sensoric threshold level due to its evaporation during wort boiling.[4,10−12]

Different methods are available to analyze the essential oils in hops and quantify their flavor composition. They are based either on the principle of steam distillation or on extraction with organic solvents. Extraction with carbon dioxide and direct thermal desorption methods are also applied for this purpose.[13−17] In most of these techniques, extensive sample preparation and special equipment are required. This complex sample preparation for volatile analysis is frequently connected with a risk of indefinable analyte loss.[17] In steam distillation, a large amount of the hop sample and an extraction time of 3−4 h are necessary.[13] Furthermore, extraneous nonvolatile residues along with the essential oils can be extracted by the solvent extraction method as well as with the carbon dioxide extraction method. The gas chromatography (GC) columns may be adversely affected by these nonvolatile residues.[17]

With the headspace (HS)-trap method, analyte losses during sample handling can be reduced, and detection limits can be improved by repeating the trap enrichment cycle. This system is characterized by a good repeatability without carryover effects.[18] The principle of HS-trap technology can be explained in five steps. First, the sample is heated inside a sealed vial until equilibrium is achieved. The carrier gas is then used to pressurize the vial contents. Next, the cooled adsorbent trap is loaded by vapor extraction from the sample vial. This pressurization−decay cycling can be repeated up to four times to increase recovery rates. After vapor extraction is completed, a flow of dry carrier gas is passed through the trap to remove moisture from the sample. Finally, the analytes are thermally desorbed and transported by the carrier gas into the GC column for separation and quantification.[18−20] By using a pneumatic pressure-balanced sampling technique, the sensitivity is increased, and the performance is improved. Volatile compounds in almost any sample matrix can be determined simply and quickly using HS-trap GC.[18]

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The objective of this study was to develop an alternative and reliable method for the analysis of volatile hop constituents and to compare the results with those obtained by the EBC method and the "American Society of Brewing Chemists" (ASBC) method. The data were also subjected to a correlation analysis to determine their statistical relationship for the 70 different hop samples analyzed.

MATERIALS AND METHODS

Hop Samples. Commercial hop samples of 24 different varieties (Table 1) were supplied by Joh. Barth & Sohn GmbH & Co. KG, Nuernberg, Germany, and Simon H. Steiner Hopfen GmbH, Mainburg, Germany. All analyzed hop samples were from the 2008 harvest. Seventy samples (36 hop pellets and 34 hop cones) were stored in airtight plastic bags, in a dark deep freezer at −24 °C until analysis.

Table 1. Hop Samples

variety	abbreviation	origin	N
	aroma hops		
Aurora	Sl-AU	Slovenia	1
Bobek	Sl-BO	Slovenia	2
Golding	Sl-GO	Slovenia	1
Hallertauer	HHA	Hallertau/Germany	2
Mittelfrüher	THA	Tettnang/Germany	1
Hallertauer Tradition	FR-HT	France	1
	THT	Tettnang/Germany	1
	EHT	Elbe-Saale/Germany	1
	HHT	Hallertau/Germany	3
Lublin	PL-LU	Poland	2
Opal	HOL	Hallertau/Germany	1
Perle	HPE	Hallertau/Germany	7
	TPE	Tettnang/Germany	1
	EPE	Elbe-Saale/Germany	2
Saazer	CZ-SA	Czechoslovakia	2
	SK-SA	Slovakia	1
Saphir	HSR	Hallertau/Germany	2
Smaragd	HSD	Hallertau/Germany	1
Spalter	SSP	Spalt/Germany	1
	FR-ST	France	1
Spalter Select	HSE	Hallertau/Germany	2
	SSE	Spalt/Germany	3
Tettnanger	TTE	Tettnang/Germany	5
	bitter hops		
Columbus	US-CO	United States	1
Hallertauer Magnum	HHM	Hallertau/Germany	8
	EHM	Elbe-Saale/Germany	2
Hallertauer Merkur	HMR	Hallertau/Germany	2
	EMR	Elbe-Saale/Germany	1
Hallertauer Herkules	HHS	Hallertau/Germany	2
	EHS	Elbe-Saale/Germany	1
Hallertauer Taurus	HTU	Hallertau/Germany	1
	ETU	Elbe-Saale/Germany	1
Marco Polo	CMP	China	1
Northern Brewer	HNB	Hallertau/Germany	1
	ENB	Elbe-Saale/Germany	1
Nugget	HNU	Hallertau/Germany	1
	NEU	Elbe-Saale/Germany	1
Target	ETA	Elbe-Saale/Germany	1
Zeus	US-ZS	United States	1

Chemicals. The following substances were obtained from Sigma-Aldrich (Taufkirchen, Germany) and diluted to the designated stock solutions: α-pinene, β-pinene, myrcene, limonene, β-caryophyllene, α-humulene, *R*-(−)-linalool, *iso*-butylisobutyrate, methylhexanoate, methylheptanoate, methyloctanoate, methylnonanoate, methyldecanoate, ethyldodecanoate, 2-nonanone, 2-decanone, 2-undecanone, 2-dodecanone, 2-tridecanone, damascenone, and (−)-caryophyllene oxide. Myrcene was dissolved in a mixture of tetrahydrofuran p.a. (Merck, Darmstadt, Germany) and ethanol p.a. (Merck, Darmstadt, Germany) in a ratio of 2:1 (vol.), while all other analytes were dissolved in ethanol p.a. Pure water prepared from deionized water passed through a TKA water purification device (TKA water preparation system GmbH, Niederelbert, Germany) was used in all cases where high purity water was required. As internal standards, linalool-D3 was used for quantifying of linalool, while *n*-nonane was used for all other compounds. Linalool-D3 was purchased from Dr. Ehrenstorfer (Augsburg, Germany), and *n*-nonane was supplied by Merck (Darmstadt, Germany).

HS-Trap Calibration. A five-point standard calibration curve for each volatile hop compound was generated. Three replications were carried out for each calibration level. Calibration ranges for all substances are listed in Table 2. An aqueous solution for the highest level was prepared from the stock solutions, according to the corresponding calibration concentrations. Further calibration levels were achieved by diluting with pure water to the lower concentrations (dilution factor 1:2). During the dilution series, the ethanol content was kept at the same level. The ethanol concentration in the calibration solution and in the sample solution amounted to 20 mg per 5 mL. For analysis, 5 mL of calibration solution was pipetted into a HS vial (20 mL) and spiked with 6 μL of internal standard solution. The vial was immediately sealed with Teflon-lined silicone septa.

For hop essential oil analysis, the calibration was done in the same way. However, unlike in the HS-trap calibration, liquid samples dissolved in ethanol were injected.

Sample Preparation for HS-Trap Analysis. A 2.0 g amount of ground hops (pellets or cones) was weighed in a Schott Duran laboratory bottle (50 mL) with Teflon-lined screw cap, and then, 18.0 g of ethanol was added. The extraction was carried out in an ultrasonic bath at 55 °C for 45 min. Then, the sample was cooled in an ice bath for 30 min. A 5.0 mL amount of pure water and 20 mg of the supernatant hop extract were transferred into a HS vial (20 mL) and spiked with the 6 μL of internal standard solution. The gas chromatography/mass spectrometry (GC/MS) analyses were performed using the HS-trap method as described below.

Sample Preparation for EBC Method. The procedure is based on the EBC and ASBC methods[13,14] to determine the essential oil content of hops and hop pellets by steam distillation. A 25.0 g amount of ground hops or unground hop pellets was placed into a 2.0 L round-bottom flask, and 1250 mL of pure water was added. The distillation was continued for 3−4 h until the volume of hop essential oil remained constant. The volume of hop essential oil in the receiver was read from the scale. For conversion of volume to weight, a mean density value of 0.82 g/mL was used. For this calculation, the densities of 10 different hop essential oils were determined, and the results were averaged. An aliquot of the hop essential oil was dissolved in 20 mL of ethanol so that a concentration of 700 ng/μL resulted. The hop essential oil solution also contained internal standard *n*-nonane and linalool-D3. These GC/MS analyses were carried out according to the hop essential oil method described below.

Redistillation of Hop Essential Oils. This experimental trial was performed in duplicate for three varieties: Hallertauer Magnum, Hallertauer Mittelfrüher, and Bobek. The hop essential oil recovered from the first distillation was added back to the hop residue and steam-distilled again. Oils from the first and redistillation trials were analyzed for comparison.

GC/MS Conditions for the HS-Trap Method. Analysis of the hop extract was performed using a Thermo Trace Ultra gas chromatograph coupled to a DSQ II quadrupole mass spectrometer (Thermo Scientific, West Palm Beach, FL). A Turbo Matrix HS-40 Trap (PerkinElmer LAS GmbH, Rodgau, Germany) was used as a HS sampler. The HS-trap sampling parameters are displayed in Table 3. A DB-5MS capillary column (60 m length × 0.25 mm i.d.; film thickness, 0.25 μm; Thermo Scientific) was used for chromatographic separation.

Table 2. Parameters of HS-Trap GC/MS Calibration

target analyte	retention time (min)	formula	qualifying and quantitation ions[a] (m/z)	cal. range (μg/g)	RSD[b] (%)	calibration curve	R^2
			monoterpenes				
α-pinene	13.70	$C_{10}H_{16}$	*105*, *107*, **121**, **136**	2.5−40	6.3	linear	0.9974
β-pinene	15.13	$C_{10}H_{16}$	*105*, *107*, **121**, **136**	4.0−64	5.3	linear	0.9978
myrcene	15.63	$C_{10}H_{16}$	**93**, *107*, *121*, **136**	1000−16000	5.6	linear	0.9944
limonene	17.16	$C_{10}H_{16}$	**107**, *115*, *121*, **136**	5.0−80	5.4	linear	0.9965
			sesquiterpenes				
β-caryophyllene	30.06	$C_{15}H_{24}$	**133**, *147*, *161*, **204**	120−1920	2.7	linear	0.9980
α-humulene	31.22	$C_{15}H_{24}$	*133*, *147*, *161*, **204**	400−6400	3.0	linear	0.9975
			terpene alcohols				
linalool	19.49	$C_{10}H_{18}0$	**121**, **136**	12−192	3.7	linear	0.9980
			esters				
isobutylisobutyrate	12.83	$C_8H_{16}0_2$	**71**, *101*, *144*	2.5−40	3.3	linear	0.9993
methylhexanoate	13.25	$C_7H_{14}0_2$	**99**, *101*, *105*	1.0−16	2.1	linear	0.9953
methylheptanoate	16.74	$C_8H_{16}0_2$	*113*, *115*, **144**	2.5−40	7.6	linear	0.9948
methyloctanoate	20.17	$C_9H_{18}0_2$	**87**, *127*, **158**	4.0−64	4.8	linear	0.9949
methylnonanoate	23.42	$C_{10}H_{20}0_2$	**98**, *129*, *142*, **172**	2.5−40	3.5	linear	0.9975
methyldecanoate	26.45	$C_{11}H_{22}0_2$	**143**, **155**, *170*, *186*	2.5−40	3.9	$(\log(x))^2$	0.9989
ethyldodecanoate	33.74	$C_{14}H_{28}0_2$	*157*, *183*, **228**	3.5−56	3.7	linear	0.9922
			ketones				
2-nonanone	19.17	$C_9H_{18}0$	*85*, *127*, **142**	1.5−24	2.4	linear	0.9983
2-decanone	22.51	$C_{10}H_{20}0$	*98*, *127*, **156**	2.0−32	2.4	linear	0.9969
2-undecanone	25.70	$C_{11}H_{22}0$	*110*, *155*, **170**	10−160	2.6	linear	0.9968
2-dodecanone	28.70	$C_{12}H_{24}0$	*126*, *169*, **184**	4.0−64	5.1	$\log(x)$	0.9948
2-tridecanone	31.46	$C_{13}H_{26}0$	*140*, *183*, **198**	15−240	5.2	$(\log(x))^2$	0.9989
damascenone	28.50	$C_{13}H_{18}0$	*121*, *175*, **190**	1.25−20	4.7	linear	0.9960
			epoxide				
caryophyllene oxide	34.50	$C_{15}H_{24}0$	*177*, *187*, *205*, **220**	40−640	5.6	$(\log(x))^2$	0.9991
			internal standard				
n-nonane	12.30	C_9H_{20}	*99*, **128**	1500			
linalool-D3	19.43	$C_{10}H_{15}0D_3$	**124**, *139*	150			

[a]Quantitation ions are shown in bold, and qualifying ions are shown in italics. [b]Relative standard deviation.

Table 3. HS-Trap Sampling Conditions

parameter	value
temperature	
oven thermostatting temperature	85 °C
needle temperature	90 °C
transfer line temperature	130 °C
trap low temperature	40 °C
trap high temperature	340 °C
pressure	
column pressure	160 kPa
vial pressure	240 kPa
desorption pressure	70 kPa
time	
thermostatting time	45 min
pressurization time	1 min
trap load time	2 min
desorption time	0.6 min
GC cycle time	60 min
heating hold time	8 min
dry purge time	8 min
pulse cycles	2
split	no
dry purge	yes

Helium served as a 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 200 °C at a rate of 5 °C/min (held for 0.0 min) and further up to 300 °C at a rate of 40 °C/min (held for 3.0 min). The MS transfer line temperature was set up to 250 °C and the ion sources temperature was set up to 230 °C. The mass spectrometer was operated in selected ion monitoring (SIM) mode using electron impact ionization (70 eV). The analytes were detected in time windows and identified on the basis of their retention times and their fragment ions by comparison with standard compounds (Table 2). The data were processed using Xcalibur software (Thermo Scientific). All samples were analyzed in duplicate, and the results were averaged.

GC/MS Conditions for the Analysis of Hop Essential Oils. GC/MS conditions were the same as described above, except 1 μL aliquots of ethanolic solutions of hop essential oils were injected using splitless injection technique (splitless time, 0.5 min) maintained at an injector temperature of 220 °C. The carrier gas flow was kept constant at 1 mL/min.

RESULTS AND DISCUSSION

By means of HS-trap analysis and the conventional hop essential oil analysis, more than 65 hop volatile compounds were identified by the NIST MS library search. Twenty-one of these compounds were quantified by analyte specific calibration curves. An example chromatogram obtained by the HS-trap method for the hop variety of Hallertauer Magnum is shown in Figure 1. Peaks of the analyzed compounds are labeled on the chromatogram.

The coefficient of variation was computed for all analyzed compounds, to prove the reproducibility of HS-trap data.

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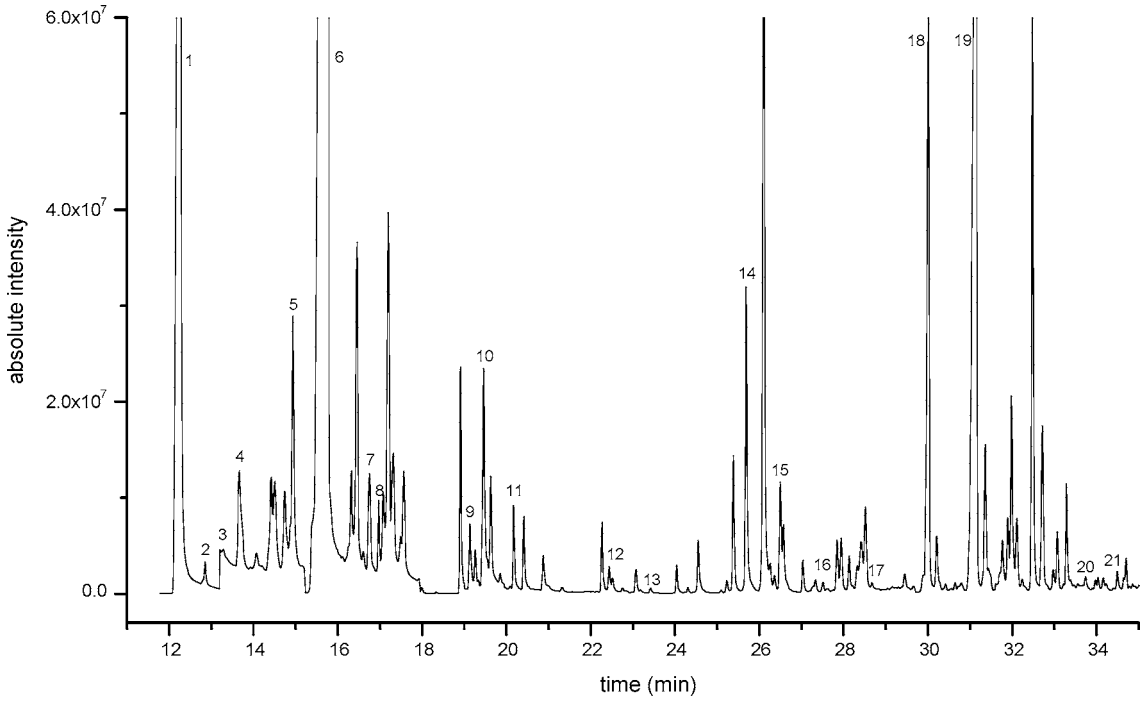

Figure 1. HS-trap GC/MS total ion chromatogram of the hop variety Hallertauer Magnum. Peaks: **1** (IS1), *n*-nonane; **2**, isobutylisobutyrate; **3**, methylhexanoate; **4**, α-pinene; **5**, β-pinene; **6**, myrcene; **7**, methylheptanoate; **8**, limonene; **9**, 2-nonanone; **10**, linalool + linalool-D3; **11**, methyloctanoate; **12**, 2-decanone; **13**, methylnonanoate; **14**, 2-undecanone; **15**, methyldecanoate, **16**, damascenone; **17**, 2-dodecanone; **18**, β-caryophyllene; **19**, α-humulene; **20**, ethyldodecanoate; and **21**, caryophyllene oxide.

For that purpose, a 10-fold determination for the hop variety of Hallertauer Magnum was conducted. The calculated values for the coefficient of variation were between 1.3 and 5.1% for all compounds. Assuming the recovery for EBC method was 100% for the sum of analyzed compounds, the values obtained by HS-trap method varied between 83.4 and 119.4%. The average value for this relative recovery was 97.0%.

Concentrations (μg/g air-dried hop) of hop compounds obtained by means of HS-trap and hop essential oil methods as well as the corresponding hop essential oil content are shown in Tables 4 and 5 for a selection of different hop varieties. The hop essential oil content of the samples was highly dependent on the hop species. The values ranged from 3878 to 23760 μg/g with a database of 70 hop essential oil samples. A classification of hop essential oil contents from 16 out of the 24 analyzed hop varieties is shown in Figure 2. The data of eight excluded hop varieties, Columbus, Marco Polo, Aurora, Golding, Opal, Smaragd, Target, and Zeus, were not shown since only one sample for each was analyzed. Bitter hops usually contained higher levels of essential oils than aroma hops. The composition of hop essential oils is genetically determined and can be used to differentiate between hop varieties.[21−23] As shown in Figure 2, only a few of hop varieties can be distinguished by the total amount of essential oils. One should be aware of the fact that essential oil amounts as well as the compositions may vary in dependence on climatic and soil conditions. Therefore, because of limited number of samples for each hop variety analyzed, the data in Figure 2 should be considered with care for purposes of hop variety discrimination.

For the majority of compounds, similar results were found between the HS-trap method and the hop essential oil analysis method as indicated by the correlation coefficients R (Table 6). The HS data and hop essential oil data of linalool correlated with R = 0.9886, while the correlation coefficient of monoterpene myrcene was 0.9843. An exception was the epoxide caryophyllene oxide, whose data correlated only with R = 0.6036. Generally, the results were dependent on the boiling point or rather the partial vapor pressure of the individual substances (Table 6).

Substances with a boiling point lower than <215 °C showed better correlations between HS-trap and hop essential oil data. Furthermore, it is apparent that in most cases as compared to the EBC hop essential oil method, the HS-trap method provided somewhat higher concentrations for those substances with relatively low boiling points (Tables 4 and 5). This applies to all monoterpenes; the terpene alcohol linalool; the esters isobutylisobutyrate, methylhexanoate, methylheptanoate, methyloctanoate, and methylnonanoate; and the ketones 2-nonanone, 2-decanone, and 2-undecanone. As for linalool, the key hoppy aroma compound in beer, the values obtained using HS-trap method were, on average, higher by a factor of 1.2.

In contrast, higher values were found for substances with a boiling point higher than 228 °C using the hop essential oil analysis method. This applied to all sesquiterpenes, esters methyldodecanoate, and especially ethyldodecanoate as well as ketones 2-dodecanone, 2-tridecanone, and damascenone. Particularly with α-humulene, concentrations obtained by oil analysis were on average 30% higher than with HS-trap method. Deviations as in the case of higher boiling point components may be reduced if isotopically labeled internal standards were available for such compounds. As mentioned before, the epoxide caryophyllene oxide, which has a high boiling point of 280 °C, opposed these findings. In this case, the results of the HS-trap analysis were on average 2.9-fold higher than with the hop essential oil method. Because of the low volatility of this compound, indeed, higher values were expected in the hop essential oil analysis method. It was assumed that an oxidation of caryophyllene to caryophyllene oxide may have taken place

Table 4. Comparison of Concentrations[a] (μg/g Air-Dried Hop) for Selected Aroma Hops

	HSE		HPE		HHA		HSR		THT		TTE	
	oil cont.: 9735 μg/g		oil cont.: 12210 μg/g		oil cont.: 9240 μg/g		oil cont.: 7755 μg/g		oil cont.: 6105 μg/g		oil cont.: 6600 μg/g	
	HS-trap analysis	hop-oil analysis	HS-trap analysis	hop-oil analysis	HS-trap analysis	hop-oil analysis	HS-trap analysis	hop-oil analysis	HS-trap analysis	hop-oil analysis	HS-trap analysis	hop-oil analysis
						monoterpenes						
α-pinene	10.1 ± 0.1	6.3 ± 0.2	10.2 ± 0.2	7.3 ± 0.1	7.3 ± 0.1	4.5 ± 0.1	8.1 ± 0.2	5.2 ± 0.1	5.7 ± 0.1	3.5 ± 0.1	7.8 ± 0.2	4.7 ± 0.3
β-pinene	19.4 ± 0.2	14.5 ± 0.3	28.3 ± 0.3	20.2 ± 0.2	15.4 ± 0.2	11.0 ± 0.1	14.3 ± 0.2	11.7 ± 0.2	10.3 ± 0.1	7.5 ± 0.1	14.7 ± 0.2	12.5 ± 0.4
myrcene	4947 ± 34	4743 ± 43	5081 ± 39	4123 ± 33	3935 ± 29	3215 ± 23	3083 ± 32	2783 ± 29	3147 ± 34	2330 ± 36	2783 ± 28	2598 ± 22
limonene	24.5 ± 0.3	17.7 ± 0.2	23.3 ± 0.2	18.8 ± 0.2	16.6 ± 0.1	10.9 ± 0.0	18.8 ± 0.1	15.3 ± 0.2	9.6 ± 0.2	6.9 ± 0.1	15.0 ± 0.1	11.7 ± 0.2
						sesquiterpenes						
β-caryophyllene	342 ± 8	521 ± 10	1339 ± 13	1674 ± 15	483 ± 9	886 ± 12	204 ± 5	520 ± 11	292 ± 7	393 ± 6	142 ± 3	295 ± 5
α-humulene	783 ± 11	985 ± 12	4464 ± 39	4512 ± 31	1855 ± 17	2537 ± 24	853 ± 15	1208 ± 16	1375 ± 13	1403 ± 14	540 ± 8	967 ± 13
						terpene alcohol						
linalool	77.7 ± 1.2	74.2 ± 0.5	23.3 ± 0.4	22.1 ± 0.2	54.8 ± 0.7	41.6 ± 0.3	57.6 ± 0.5	42.7 ± 0.3	42.0 ± 0.5	32.9 ± 0.5	23.3 ± 0.3	23.3 ± 0.2
						esters						
isobutylisobutyrate	3.5 ± 0.1	2.4 ± 0.1	21.8 ± 0.3	27.9 ± 0.3	9.3 ± 0.1	7.8 ± 0.1	8.5 ± 0.2	7.1 ± 0.1	16.9 ± 0.3	13.4 ± 0.2	1.5 ± 0.1	1.0 ± 0.0
methylhexanoate	1.6 ± 0.0	1.3 ± 0.0	3.4 ± 0.0	2.8 ± 0.0	2.1 ± 0.0	1.8 ± 0.0	3.1 ± 0.1	2.5 ± 0.0	8.3 ± 0.1	7.0 ± 0.1	0.6 ± 0.0	0.4 ± 0.0
methylheptanoate	13.4 ± 0.3	12.5 ± 0.2	13.5 ± 0.2	10.5 ± 0.3	7.3 ± 0.1	7.2 ± 0.1	12.9 ± 0.1	10.5 ± 0.1	43.0 ± 0.6	34.7 ± 0.5	9.2 ± 0.2	9.0 ± 0.1
methyloctanoate	6.5 ± 0.0	6.1 ± 0.1	15.1 ± 0.2	13.5 ± 0.1	9.7 ± 0.2	6.1 ± 0.1	22.8 ± 0.2	15.7 ± 0.5	30.1 ± 0.4	16.6 ± 0.1	5.3 ± 0.1	4.5 ± 0.2
methylnonanoate	7.8 ± 0.1	6.6 ± 0.0	15.6 ± 0.1	13.1 ± 0.1	7.8 ± 0.1	5.9 ± 0.0	22.2 ± 0.2	16.0 ± 0.3	23.9 ± 0.2	17.5 ± 0.4	4.1 ± 0.1	4.1 ± 0.1
methyldecanoate	2.3 ± 0.1	1.7 ± 0.0	3.4 ± 0.0	3.7 ± 0.1	3.2 ± 0.1	3.0 ± 0.1	10.7 ± 0.1	12.4 ± 0.1	4.8 ± 0.1	4.0 ± 0.0	2.7 ± 0.1	3.0 ± 0.0
ethyldodecanoate	12.4 ± 0.2	13.7 ± 0.2	23.3 ± 0.2	24.7 ± 0.2	18.1 ± 0.2	19.9 ± 0.1	15.2 ± 0.1	16.7 ± 0.2	8.5 ± 0.1	9.2 ± 0.1	9.5 ± 0.2	10.6 ± 0.1
						ketones						
2-nonanone	11.3 ± 0.1	10.3 ± 0.1	10.0 ± 0.0	9.2 ± 0.0	8.8 ± 0.1	7.1 ± 0.1	21.6 ± 0.4	18.5 ± 0.3	2.9 ± 0.0	2.0 ± 0.0	7.9 ± 0.0	6.8 ± 0.2
2-decanone	14.3 ± 0.2	13.5 ± 0.1	15.8 ± 0.1	13.2 ± 0.1	14.4 ± 0.1	12.7 ± 0.0	29.6 ± 0.3	26.1 ± 0.1	7.4 ± 0.1	6.8 ± 0.0	12.6 ± 0.2	11.4 ± 0.2
2-undecanone	50.9 ± 0.5	49.7 ± 0.5	49.2 ± 0.2	48.1 ± 0.3	51.3 ± 0.4	49.1 ± 0.3	111 ± 0.7	91.0 ± 0.4	29.2 ± 0.1	23.3 ± 0.2	35.8 ± 0.4	31.3 ± 0.3
2-dodecanone	18.7 ± 0.1	21.6 ± 0.1	24.9 ± 0.1	27.5 ± 0.2	17.5 ± 0.1	15.4 ± 0.1	35.8 ± 0.1	36.6 ± 0.2	14.4 ± 0.1	13.2 ± 0.1	14.9 ± 0.1	15.9 ± 0.1
2-tridecanone	86.7 ± 0.7	105 ± 0.8	91.4 ± 0.8	99.6 ± 0.9	54.8 ± 0.5	57.3 ± 0.6	123 ± 1.2	132 ± 2.0	42.6 ± 0.5	47.2 ± 0.8	71.7 ± 0.4	75.2 ± 0.7
damascenone	4.1 ± 0.1	4.9 ± 0.1	9.0 ± 0.2	9.6 ± 0.1	5.8 ± 0.0	6.3 ± 0.1	4.0 ± 0.1	4.3 ± 0.1	2.9 ± 0.1	3.1 ± 0.0	2.8 ± 0.0	3.5 ± 0.1
						epoxide						
caryophyllene oxide	103 ± 1.1	93.4 ± 0.9	238 ± 4.5	84.0 ± 0.8	294 ± 4.8	174 ± 2.0	246 ± 3.2	182 ± 1.6	161 ± 1.4	43.5 ± 0.3	172 ± 2.6	166 ± 2.3

[a]Concentrations (±standard deviations) are given as the mean of duplicates.

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Table 5. Comparison of Concentrations[a] (μg/g Air-Dried Hop) for Selected Bitter Hops

	HHS		HHM		HMR		ENU		ENB		ETA	
	oil cont.: 13365 μg/g		oil cont.: 17408 μg/g		oil cont.: 20378 μg/g		oil cont.: 17820 μg/g		oil cont.: 8745 μg/g		oil cont.: 14355 μg/g	
	HS-trap analysis	hop-oil analysis	HS-trap analysis	hop-oil analysis	HS-trap analysis	hop-oil analysis	HS-trap analysis	hop-oil analysis	HS-trap analysis	hop-oil analysis	HS-trap analysis	hop-oil analysis
						monoterpenes						
α-pinene	17.0 ± 0.2	12.6 ± 0.3	20.2 ± 0.2	15.7 ± 0.2	18.0 ± 0.2	14.3 ± 0.1	14.5 ± 0.1	10.2 ± 0.1	12.1 ± 0.2	7.2 ± 0.1	12.7 ± 0.2	10.3 ± 0.1
β-pinene	27.6 ± 0.2	17.7 ± 0.1	31.4 ± 0.2	24.6 ± 0.3	37.0 ± 0.3	31.8 ± 0.4	29.1 ± 0.2	22.8 ± 0.1	15.5 ± 0.2	12.4 ± 0.1	21.4 ± 0.2	19.9 ± 0.2
myrcene	7269 ± 64	6429 ± 88	9644 ± 71	10494 ± 75	7802 ± 69	8431 ± 62	8648 ± 82	8596 ± 75	5251 ± 45	4865 ± 33	7472 ± 78	7258 ± 81
limonene	31.7 ± 0.3	28.2 ± 0.2	42.6 ± 0.5	37.3 ± 0.1	35.2 ± 0.3	34.1 ± 0.4	38.2 ± 0.3	29.6 ± 0.4	23.2 ± 0.2	15.8 ± 0.1	31.2 ± 0.4	25.9 ± 0.2
						sesquiterpenes						
β-caryophyllene	570 ± 9	760 ± 18	966 ± 11	1518 ± 16	972 ± 10	2311 ± 31	1253 ± 12	1428 ± 21	499 ± 7	937 ± 12	379 ± 5	901 ± 9
α-humulene	2098 ± 13	2714 ± 21	3562 ± 24	4465 ± 29	3258 ± 21	4339 ± 31	2881 ± 19	3103 ± 22	1736 ± 15	2597 ± 19	1297 ± 12	2055 ± 16
						terpene alcohol						
linalool	30.9 ± 0.3	25.9 ± 0.1	40.3 ± 0.5	38.6 ± 0.5	124 ± 0.8	103 ± 0.8	87.6 ± 0.5	72.5 ± 0.7	15.2 ± 0.2	13.2 ± 0.1	73.1 ± 0.6	68.6 ± 0.7
						esters						
isobutylisobutyrate	63.5 ± 0.5	56.1 ± 0.3	7.3 ± 0.1	5.3 ± 0.0	27.8 ± 0.2	33.5 ± 0.2	55.2 ± 0.4	61.7 ± 0.5	25.0 ± 0.3	18.8 ± 0.2	58.9 ± 0.5	59.5 ± 0.3
methylhexanoate	1.7 ± 0.1	0.9 ± 0.0	2.9 ± 0.1	2.2 ± 0.1	9.2 ± 0.1	7.0 ± 0.0	19.5 ± 0.1	15.4 ± 0.2	3.9 ± 0.1	2.7 ± 0.2	3.6 ± 0.1	2.8 ± 0.1
methylheptanoate	34.2 ± 0.3	30.8 ± 0.2	27.4 ± 0.3	24.5 ± 0.2	35.7 ± 0.4	32.4 ± 0.3	56.4 ± 0.4	49.1 ± 0.5	28.3 ± 0.3	24.5 ± 0.1	24.5 ± 0.2	24.5 ± 0.2
methyloctanoate	41.8 ± 0.5	37.3 ± 0.2	33.3 ± 0.4	28.9 ± 0.3	30.4 ± 0.2	23.0 ± 0.2	70.9 ± 0.7	64.5 ± 0.8	21.6 ± 0.2	15.8 ± 0.2	40.8 ± 0.4	33.1 ± 0.5
methylnonanoate	40.8 ± 0.4	30.3 ± 0.6	32.4 ± 0.4	26.5 ± 0.5	19.8 ± 0.2	12.8 ± 0.1	40.8 ± 0.3	35.2 ± 0.1	18.3 ± 0.1	12.6 ± 0.1	34.2 ± 0.3	31.5 ± 0.4
methyldecanoate	9.6 ± 0.2	10.9 ± 0.1	21.6 ± 0.3	25.3 ± 0.2	19.3 ± 0.1	21.5 ± 0.1	29.0 ± 0.2	30.6 ± 0.4	3.9 ± 0.1	4.3 ± 0.0	19.7 ± 0.2	21.7 ± 0.1
ethyldodecanoate	10.7 ± 0.1	11.5 ± 0.0	22.9 ± 0.2	24.4 ± 0.1	22.6 ± 0.2	24.9 ± 0.1	17.1 ± 0.2	18.1 ± 0.2	12.3 ± 0.1	13.2 ± 0.1	26.5 ± 0.2	27.9 ± 0.2
						ketones						
2-nonanone	23.4 ± 0.2	20.7 ± 0.2	10.2 ± 0.1	9.3 ± 0.1	6.1 ± 0.1	5.7 ± 0.2	0.7 ± 0.0	0.8 ± 0.0	4.6 ± 0.1	4.1 ± 0.0	5.5 ± 0.2	4.9 ± 0.1
2-decanone	29.5 ± 0.1	27.8 ± 0.1	13.0 ± 0.1	10.8 ± 0.1	13.7 ± 0.2	11.5 ± 0.1	2.4 ± 0.1	2.2 ± 0.0	9.7 ± 0.1	8.9 ± 0.0	14.7 ± 0.3	11.5 ± 0.2
2-undecanone	64.6 ± 0.2	58.2 ± 0.1	48.9 ± 0.4	50.4 ± 0.1	84.8 ± 0.6	76.9 ± 0.4	49.6 ± 0.3	48.3 ± 0.2	34.4 ± 0.3	28.7 ± 0.1	75.4 ± 0.4	74.4 ± 0.3
2-dodecanone	25.4 ± 0.1	26.5 ± 0.3	34.1 ± 0.1	35.2 ± 0.2	35.5 ± 0.4	42.6 ± 0.1	30.3 ± 0.2	33.4 ± 0.3	16.7 ± 0.3	17.5 ± 0.2	40.7 ± 0.5	43.6 ± 0.3
2-tridecanone	66.3 ± 0.7	73.9 ± 0.5	138 ± 1.4	146 ± 1.3	157 ± 1.6	180 ± 1.9	165 ± 1.7	187 ± 2.2	69.9 ± 0.5	76.4 ± 0.9	176 ± 2.2	194 ± 2.4
damascenone	6.9 ± 0.1	7.1 ± 0.1	9.8 ± 0.1	10.8 ± 0.2	14.4 ± 0.2	15.3 ± 0.2	6.4 ± 0.1	7.8 ± 0.1	4.8 ± 0.0	5.3 ± 0.1	7.6 ± 0.1	8.7 ± 0.1
						epoxide						
caryophyllene oxide	194 ± 2.5	47.6 ± 0.5	359 ± 4.2	96.7 ± 1.1	529 ± 4.4	120 ± 1.5	211 ± 2.4	59.8 ± 0.7	213 ± 2.1	52.5 ± 0.4	309 ± 2.8	92.9 ± 1.0

[a]Concentrations (±standard deviations) are given as the mean of duplicates.

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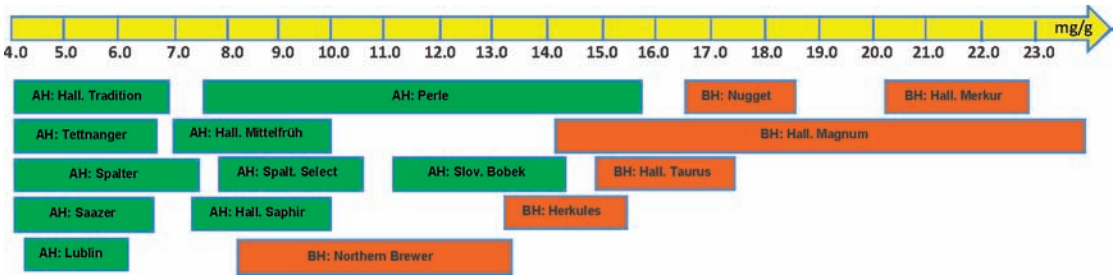

Figure 2. Classification of hop species in regard to their hop essential oil contents (mg/g air-dried hop): AH, aroma hop; BH, bitter hop.

Table 6. Correlation Coefficients (R) for Concentration Values Obtained by HS-Trap and EBC Hop Essential Oil Analysis Methods

compound	boiling point[a] (°C)	R
monoterpenes		
α-pinene	155−156	0.9828
β-pinene	164−165	0.9799
myrcene	167	0.9843
limonene	177−178	0.9803
sesquiterpenes		
β-caryophyllene	262−264[b]	0.9075
α-humulene	276	0.9577
terpene alcohol		
linalool	198−200	0.9886
esters		
isobutylisobutyrate	147−149	0.9833
methylhexanoate	151	0.9879
methylheptanoate	173	0.9858
methyloctanoate	193	0.9885
methylnonanoate	213−214	0.9818
methyldecanoate	224[b]	0.9748
ethyldodecanoate	272	0.9668
ketones		
2-nonanone	192[b]	0.9897
2-decanone	209−212	0.9876
2-undecanone	228	0.9648
2-dodecanone	247[b]	0.9698
2-tridecanone	263	0.9597
damascenone	274−275	0.9657
epoxide		
caryophyllene oxide	280	0.6036

[a]Ref 25. [b]Ref 26.

during the preparation of ethanolic hop extract or during the analysis itself. HS-trap analysis is carried out at a high desorption temperature of 340 °C to ensure complete desorption of volatiles adsorbed on trap material. However, the HS-trap module was in an inert condition, with no oxygen present in the adsorbent trap. A temperature test with a solution of caryophyllene showed that neither during sample preparation nor during the analysis did an oxidation occur.

With regard to exploring the effect of distillation on the composition of hop essential oils, three hop samples were subjected to a redistillation trial. The test revealed that caryophyllene oxide decomposed during the steam distillation by 40−60%. This result supports that the recovery of epoxide caryophyllene oxide is impaired during steam distillation. Other epoxides undergo assumingly similar decomposition rates.

In the redistillation test, it was also observed that concentrations of some highly volatile monoterpenes and esters decreased. For example, α-pinene and isobutylisobutyrate were recovered at 27 and 38%, respectively. This explains the higher HS-trap results for particular substances. These results show that in comparison to the conventional distillation, the HS-trap method is advantageous, especially for volatile compounds and epoxides.

Eri et al.[17] also obtained lower values for caryophyllene oxide with the steam distillation−extraction (SDE) method as compared with their direct thermal desorption method (DTD). Oxidation processes during the analysis were not observed. It was assumed that DTD provided better extraction efficiency and therefore enabled a better recovery of oxygenated compounds.[17] The decomposition of epoxides during the steam distillation was neither discussed and nor supported by results.

In the present work, correlations between the different hop essential oil components were also studied. For the determination of correlation coefficients, 150 hop essential oil analyses and 21 hop essential oil components were evaluated. The data revealed that compounds of the same chemical substance group correlated with each other. For instance, among the analyzed monoterpenes, the correlation coefficient was greater than 0.90. The best correlation was seen between α-pinene and limonene with $R = 0.9642$. The sesquiterpenes, α-humulene and β-caryophyllene, correlated with each other also with $R > 0.90$. If a relationship between the substances exists, it can be concluded that intermediate stages may be common during the biosynthesis or they are converted into one another. All terpenoid hydrocarbons are derived from a common biogenic origin.[21] Good correlations were also observed between all methylesters. Moreover, the ketones 2-nonanone and 2-decanone and 2-dodecanone and 2-tridecanone are well-correlated with each other. In the present study, linalool did not correlate to any other hop essential oil component. In a previous report, correlations ranging from 0.81 to 0.92 were also found within the substance classes of monoterpenes and sesquiterpenes, and no correlation was seen between linalool and other compounds.[21]

In further testing, total hop essential oil contents recovered by steam distillation and the sum of concentrations obtained by HS-trap method were compared (Table 6). A strong relationship was seen between results from the two methods (R = 0.968), although some major components of hop essential oil, like *cis*-4-decenoic acid methyl ester and farnesene, could not be quantified due to the lack of standards. By extending of number of analyzed compounds, the determination of hop essential oil content using HS-trap method may be more accurate.

Among hop volatiles, the lowest concentration measured by means of HS-trap technique was 0.6 μg/g for methylhexanoate in the hop variety TTE (Table 4). An extract amount of 20 mg was sufficient to detect such constituents with low concentration. Considering that 20 mg of extract, which is diluted with 5 mL of pure water prior to HS-trap measurement, is equivalent

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to 2 mg of hop, demonstrates high sensitivity of the method. The sensitivity of HS-trap method may be further increased by adding salt to HS-vial and by taking additional extract, up to 10-fold or more. As a result, other undetected compounds with very low concentrations may also be analyzed.

Previous studies have shown that the hydrocarbon content of the hop essential oil changed from 88 to 7% and the oxygenated components amount from 12 to 93% after 3 years of storage at 0 °C.[24] Although the storage of our hop samples was at a much lower temperature (−24 °C) and the analyses were performed around 9 months after the harvest, the compositions of hop essential oil components might have changed. Nevertheless, this circumstance was insignificant for comparison of HS-trap method with the EBC method.

From the results, it can be concluded that HS-trap method, in comparison to EBC method, gives similar results in terms of volatile compounds and does not affect heat unstable components like epoxides. Compounds present in low concentrations were also detected by HS-trap-GC/MS method. The HS-trap-GC/MS method is rapid and easy to perform. Furthermore, the amount of hop essential oil can be calculated with a high certainty from the determined concentrations of hop components. Correlation analysis showed that monoterpenes and sesquiterpenes indicate the best correlation within their substance group.

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Notes

The authors declare no competing financial interest.

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