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Comparison of the Analytical Profiles of Volatiles in Single-Hopped Worts and Beers as a Function of the Hop Variety

Being one of the most consumed beverages in the world, much effort has been made to reveal the structures responsible for the sensorial characteristics of beer. Yet, the knowledge on the precise contribution of hop-derived volatiles towards the hoppy aroma of beer is rather fragmented. For a long time, the aroma of fresh beer was believed to be mainly imparted by single compounds. However, increasing evidence showed that sensorial perception of the hoppy aroma of beer is more complex than originally assumed. Moreover, the factors responsible for the perceivable differences originating from distinct hop varieties used for late and dry hopping have not been fully revealed.

In order to understand how the choice of the hop variety affects the final aroma of beer, we investigated in a previous study with four different hop varieties how the analytical composition of the volatile fraction changes throughout the brewing process and how that affects the composition of late and dry hopped beers. However, the analysis of four different hop varieties arose more questions. Therefore, in this study, we studied 15 other hop varieties during different stages along the brewing process of single hopped beers and analyzed wort and beer samples via headspace solid-phase micro extraction and gas chromatography-mass spectrometry (HS-SPME GC-MS). Additionally, the profiles of the corresponding hop varieties were determined. This enabled the accurate determination of both the full spectrum of hop oil-derived compounds as well as of the higher esters and higher alcohols produced during fermentation. Our investigation reveals substantial changes in the volatile patterns of the wort and beer samples, in comparison with the selected hop variety, which arose from the boiling and fermentation processes, as well as the applied late and additional dry hopping techniques. Concentrations of the "floral" (e.g. oxygenated fraction of total hop essential oil composed of monoterpene alcohols, esters, ketones and aldehydes) and the sesquiterpenoid hop oil fractions changed significantly along the brewing process. As expected, concentrations of saturated esters and higher alcohols in beers were shown to be mainly influenced by the fermentation and not by the hop variety. Although the concentrations of practically all other compound classes (especially of linalool and geraniol as the most important monoterpene alcohols) were higher in the dry hopped beers, dry hopping does not affect the original intrinsic qualitative composition of hop oil constituents. Yet, substantial quantitative changes were observed.

Furthermore, special attention was paid to the influence of additional dry hopping on the transfer behavior of selected hop derived-monoterpene alcohols. Transfer rates for linalool were comparable for all 15 hop varieties, whereas the transfer rates for geraniol differed significantly which indicates that the selected hop variety is of major importance.

Descriptors: hops, beer, sesquiterpenes, volatiles, GC-MS, *Humulus lupulus*

1 Introduction

In order to add the typical bitter taste as well as an attractive aroma to beer, hops (*Humulus lupulus* L.) have been used as an essential ingredient during beer production for many centuries. Hops contribute to the aroma, taste and foam stability of beer in a unique way and, in this respect, careful attention has been paid to influences of particular hop-derived compounds. In addition, various health-beneficial activities have been described including cancer-chemopreventive, anti-inflammatory, anti-angiogenic and estrogenic properties [1–6]. Moreover, studies provided evidence that certain compounds (e.g. iso-α-acids and its reduced derivatives) are able to counteract diabetes type-2 and obesity [6–9]. Other compounds (e.g. β-acids) increase the shelf-life of beer because of their antimicrobial activity [6, 10] or have high potential to improve flavor-stability as natural antioxidants [11–13].

Recently, research has been focused on the aroma that hops add to beer, as craft brewers started to utilize specific hop varieties

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in order to produce beers with a one-of-a-kind taste and flavor profile. Accordingly, hop varieties that can introduce special flavor impressions to beer, so-called flavor hop varieties, have become very popular among brewers worldwide [14]. Furthermore, over the years the hop industry developed different hop products [15] such as pellets, extracts and special-purpose materials in order to meet brewer's expectations. For example, ethanolic and carbon dioxide hop extracts are rich in α- and β-acids, products enriched with xanthohumol differ mainly in the content and the distribution pattern of prenylated flavonoids, and special aroma formulations or oils are able to enhance flavor and taste perceptions [16–20].

However, it is not fully understood how the typical hoppy flavor of beer develops during beer production and, as a matter of fact, hop varieties are still widely selected based on previous brewing experiences. Furthermore, the aroma that hops add to beer is of greater importance than the original intrinsic hop aroma in the plant itself. Yet, in order to fully understand possible interactions of key aroma compounds and to anticipate (un-)desired effects, profound knowledge of the low-molecular-weight constituents, called sensometabolites [21, 22], has to be acquired to facilitate the use of hops according to the desired flavor perceptions. The interactions of all aroma components that reflect the sensory blueprint of a product include the unique taste and flavor signature of food and beverages such as beer and hops. Therefore, it is a necessity to systematically identify, characterize and quantify the sensory-active key compounds that are present in raw materials and/or are produced upon food processing [22, 23].

Research has identified over 450 compounds so far and much progress has been made in the field of hop flavor research [24]. However, it is believed that most of the compounds present in the essential hop oils are still ill-defined or unknown [25]. Besides, different parameters, such as the hop variety (or varieties) used for early, late and dry hopping, as well as the hopping regime, including the amount and time point of hop addition, do have a huge impact on the aroma perception [26–30]. Revelation of the effects of the hopping regime on the volatile composition of beer is so far only partly possible due to insufficient knowledge and, as a result, general conclusions cannot be drawn.

Nevertheless, it is feasible to monitor selected compounds in samples that have been taken during the manufacturing of beer by the use of GC-MS [31]. Especially the behavior of the most abundant compounds in hops, β-myrcene, α-humulene, β-caryophyllene, linalool and geraniol, during the brewing process was the aim of this study [31].

Forster et al. [32, 33] investigated the influence of the dry hopping technology of four new German hop varieties with regard to transfer rates of geraniol and linalool. Interestingly, they observed a nearly quantitative transfer of linalool from hops to beer, whereas the transfer rates for geraniol varied from 50 to 180 %. However the significance of the results of four German hop varieties is limited and we therefore are analyzing in this study 15 different hop varieties in order to draw general conclusions. Forster et al. [32, 33] suggested in their study that glycosidically bound compounds might be at least partly released due to yeast metabolism, as observed earlier by *Kollmannsberger* et al. [34]. Such enzymatically driven reactions may affect final geraniol concentrations differently, depending on initial concentrations of the corresponding esters present in hops and beer. However, linalool and geraniol are only 2 compounds that are believed to be responsible for special flavor impressions of beer. Studies indicate that a series of hop derived-compounds such as β-citronellol, geraniol and geranyl acetate, linalool oxide, α-eudesmol, α-terpineol, as well as different degradation products of humulene and many others are involved in the total flavor of beer [27, 29, 35–43]. Interestingly, only linalool showed a direct contribution to the overall aroma and, for many other compounds, it was observed that they do not directly contribute to the hoppy aroma of beer, because final concentrations did not exceed their odor threshold concentrations [44–47]. Moreover, studies indicated that synergistic effects in the aroma perception of flavor-active compounds are yet underestimated [45–47].

As it was already mentioned for linalool, geraniol and β-citronellol, studies demonstrated that volatiles can contribute to the overall aroma perception of beer below their threshold concentrations due to synergistic effects [19, 31, and 37]. However, knowledge on the impact of early, late and dry hopping on the compositions of the volatile fractions of beers and intermediate samples is rather fragmented. For this reason, it is essential to know how the brewing process, the hop variety and, above all, the hopping technology influence the volatile profiles in order to elucidate the evolution of hoppy aroma and to identify pivotal production steps during the brewing process.

Furthermore, the applied hopping technique and the kind of hop oil fraction used for advanced hopping have strong influences on the concentrations of analytical marker compounds such as linalool and sesquiterpenoids [48]. Especially conventional late and dry hopping procedures resulted in high levels of linalool and sesquiterpenoids. Recently, 5 hop varieties were used to produce 5 identically brewed late hopped and 5 identically brewed dry hopped beers. The results of this study helped to identify crucial process steps, e.g. centrifugation, regarding losses of aroma-active volatiles during beer production in general, but final conclusions were limited in view of the restricted number of samples [49].

In order to gain further insights in the full spectra of hop oil-derived volatiles and to accurately determine them, samples were taken in this study throughout the brewing process and analyzed using HS-SPME GC-MS. Comparing 15 different single late and dry hopped beers, respectively, as well as intermediate wort samples and, more importantly, the hops themselves, will help to reveal analytical differences that are undoubtedly linked to the used hop variety.

2 Materials and Methods

2.1 Chemicals

All reference compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were of analytical grade: 1. Esters, alcohols: ethyl acetate (≥ 99.8 %), ethyl butanoate (99 %), ethyl decanoate (≥ 99 %), 4-ethylphenol (≥ 98 %), ethyl hexanoate (≥ 99 %), ethyl octanoate (≥ 99 %), isoamyl acetate (98 %), isobutyl alcohol

Table 1 Information on the hop varieties (hop harvest 2012) used for the production of the single hop wort and beer samples

Hop variety	Abbre-viation	Type of hops	α-Acid [%]	Hop oil [ml/100 g]
Cascade	CAS	Aroma hops	5.57	1.15
Centennial	CEN	Aroma hops	7.98	1.59
Citra	CIT	Aroma/Flavor hops	7.61	1.53
Challenger	CHA	Flavor hops	10.8	0.84
Cluster	CLU	Aroma/Flavor hops	4.15	0.57
Columbus	COL	Flavor hops	12.0	2.24
Galena	GAL	Bittering hops	9.65	1.15
Magnum	MAG	Bittering hops	17.8	3.12
Nugget	NUG	Bittering hops	12.7	1.98
Palisade	PAL	Aroma hops	5.40	0.85
Saaz	SAA	Aroma hops	4.46	0.73
Simcoe	SIM	Flavor hops	11.6	1.62
Sorachi Ace	SOR	Flavor hops	11.1	2.09
Tettnanger	TET	Aroma hops	3.72	0.63
Warrior	WAR	Bittering hops	14.3	1.47

(≥ 99 %), isobutyl acetate (98 %), 2-methyl-1-butanol (≥ 99 %), 3-methyl-1-butanol (≥ 99.5 %), phenylethyl acetate (≥ 99 %), 1-propanol (≥ 99.5 %); 2. Hop oil compounds: geraniol (98 %), linalool (98.5 %); 3. Internal standards: d6-benzaldehyde (98 %), nonadecane (99 %). Ethanol absolute (≥ 99.8 %) was purchased from VWR International (Zaventem, Belgium); Milli-Q water was obtained from a Milli-Q purification system (Synergy 185, Millipore S.A., Molsheim, France).

2.2 Wort and beer samples

All beers were prepared on a semi-industrial scale (40 hl) using single hop technology with particular hop varieties (Table 1); all following the same hopping procedure (see Table 2). Additionally, from each variety both a dry hopped and a late hopped beer was produced. The amounts of each hop addition were standardized by weight and not by their α-contents or their hop oil concentrations. This was done to achieve an equal treatment for all hop varieties. Wort and beer samples were taken at different production steps (Table 2) and immediately stored at –20 °C and 1 °C, respectively, until further analyzed.

2.3 Quantitative GC-MS determinations of esters and higher alcohols

Extraction of volatile esters and higher alcohols as well as of 4-ethylphenol from beer has been done by headspace-solid-phase micro-extraction (HS-SPME) for 30 min at 40 °C using a 65 µm PDMS-DVB fiber coating. Therefore, 5 mL of undiluted samples were pipetted into an extraction vial (20 mL). For the analysis of the hop samples, an aliquot of grinded pellets was weight into an extraction vial (20 mL). After adding a defined amount of d6-benzaldehyde as an internal standard, the extraction vial was immediately closed with a magnetic bimetal crimp cap containing a silicone/Teflon septum (Interscience, Louvain-la-Neuve, Belgium). For the analysis of hops, defined amounts of hops were transferred into an extraction vial (20 mL) and 5 mL of water were added. Components were separated and detected by capillary gas chromatography/mass spectrometry (CGC/MS) (Ion Trap – ITQ; Thermo Fisher Scientific, Austin, TX, USA) operating in the electron ionization mode. The ITQ was coupled to a ThermoFinnigan Trace GC (Thermo Fisher Scientific, Austin, TX, USA) equipped with a CTC-PAL auto sampler (CTC Analytics, Zwingen, Switzerland), a heated split/splitless injector with a narrow glass inlet liner (0.5 mL volume), and a RTX-1 fused-silica capillary column (40 m × 0.18 mm i.d., 0.2 µm film thickness, Restek, Corporation, Bellefonte, PA, USA). Helium (Alphagaz 2, Air Liquide, Luik, Belgium) was used as carrier gas at a flow rate of 0.8 mL/min. The inlet temperature was 230 °C and the injection occurred in the split mode (split ratio 1/12). The oven temperature has been held at 40 °C for 3 min, then raised to 200 °C at 6 °C/min, followed by an increase to 250 °C at 15 °C/min and an isothermal period at 250 °C for 3 min. External calibration curves were recorded using d6-benzaldehyde as an internal standard. Concentrations of quantified compounds in hops were corrected to the amount of hops used to produce the wort and beer samples.

Table 2 Assignment of wort and beer samples and the time points of sampling

Abbreviation	Time point of sampling
Hops	Hop pellets, harvest 2012
PRE-EH	After 15 min boiling – just before early hopping
PW	After 10 min cooling – just before fermentation
Late Hopped	Bottled beer without dry hopping = Late hopped beer
Dry Hopped	Bottled beer with additional dry hopping

2.4 GC-MS profiling of hop oil volatiles

Headspace solid-phase micro-extractions (45 min at 60 °C using a 100 µm PDMS fiber coating; Supelco, Bellefonte, PA, USA) of wort and beer samples were automated using a CombiPal auto sampler (CTC Analytics, Zwingen, Switzerland). Five mL of undiluted samples were pipetted into an extraction vial (20 mL). For the analysis of the hop samples, an aliquot of grinded pellets was weight into an extraction vial (20 mL). After adding 2 g of sodium chloride (p.a., VWR International, Zaventem, Belgium), the extraction vial was immediately closed with a magnetic bimetal crimp cap containing a silicone/Teflon septum (Interscience, Louvain-la-Neuve, Belgium).

Gas chromatographic operating conditions were as follows. SPME fibers with extracted volatiles were thermally desorbed in the heated inlet (split/splitless injector, 250 °C) of the Ultra Trace gas chromatograph (Thermo Fisher Scientific, Austin, TX, USA) for 3 min. Helium (Alphagaz 2, Air Liquide, Luik, Belgium) was used as a carrier gas at a constant flow rate of 1.0 mL/min. Injection was performed in the split mode (split ratio 1/10) for 3 min at 250 °C. Separation of the injected compounds was performed on a 40 m × 0.18 mm i.d. × 0.20 µm film thickness RTX-1 capillary column (Restek Corporation, Bellefonte, PA, USA). The oven temperature program for separation of the volatiles was as follows: 3 min at 35 °C, followed by a temperature increase at 5 °C/min to 250 °C and an isothermal period at 250 °C for 1 minute. Mass spectrometric detection of volatiles was achieved by a dual-

stage quadrupole MS (DSQ I, Thermo Fisher Scientific, Austin, TX, USA) operating in the electron ionization mode (EI, 70 eV). The ion source temperature was set at 240 °C, and the electron multiplier voltage was 1445 V. Analyses were performed in the full scan operating mode (m/z 40–400). The detected compounds were identified by mass spectral comparison via Xcalibur software (v.1.4 SR1, Interscience) using the NIST98 and Flavor MS library for Xcalibur 2003 spectral libraries (Interscience), retention times of authentic reference compounds, and calculation of retention indices (RI) of the volatiles. Therefore, Kovat's retention indices were determined by using a homologous series of normal alkanes (C8–C23; Sigma-Aldrich, St. Louis, MO, USA). When no reference compounds were available, constituents were 'tentatively identified' using the following criteria: (1) MS match factor > 650 and calculated RI = literature RI ± 5 or (2) MS match factor > 750 when no literature RI was available. Compounds having a MS match factor < 750 and literature RI significantly different from the calculated RI were considered as 'unknowns'. Quantitative data for linalool and geraniol were obtained by recording external calibration curves. Semi-quantitative data of all other compounds were obtained using nonadecane as an internal standard. Concentrations of quantified compounds in hops were corrected to the amount of hops used to produce the wort and beer samples.

2.5 Data processing

Processing of the chromatographic data was performed by the Xcalibur ™ data system (Version 2.0.7, Thermo Electron Corporation, Austin, TX, USA).

3 Results and Discussion

The aim of the present study was to investigate the evolution of hop-derived volatiles throughout the brewing process. Therefore, samples were taken at different production steps (see Table 2) along the brewing process and their volatile composition was determined. The obtained data were furthermore compared with the analytical data of the hop varieties (see Table 1).

Table 3 Assignment of single analytes to compound classes.

Alcohols		
n-Propanol	Isobutanol	3-Methyl-1-butanol
2-Methyl-1-butanol	Phenylethanol	
Saturated esters		
Ethyl acetate	Ethyl hexanoate	Ethyl octanoate
Isobutyl acetate	Isoamyl isobutyrate	Phenethyl acetate
Ethyl butanoate	2-Methylbutyl butanoate	Ethyl decanoate
Isoamyl acetate	2-Methylbutyl isopentanoate	Ethyl dodecanoate
Isoamyl propionate	2-Methylbutyl pentanoate	
Unsaturated esters		
Methyl *cis*-2-decenoate	Neryl acetate	Ethyl *cis*-4-decenoate
Methyl *trans*-4,9-decadienoate	Ethyl *trans*-4-decenoate	Neryl butyrate
Methyl geranate	Ethyl 4,9-decadienoate	
Monoterpene hydrocarbons		
Beta-pinene	Beta-myrcene	
Monoterpene alcohols		
Linalool	Beta-citronellol	Geraniol
Alpha-terpineol		
Sesquiterpene hydrocarbons		
Beta-caryophyllene	Alpha-amorphene	Gamma-cadinene
Alpha-humulene	Beta-selinene	Calamenene
Alpha-copaene	Gamma-amorphene	Delta-cadinene
Beta-calarene	Alpha-selinene	(*trans*-)Cadina-1,4-diene
Gamma-muurolene	Epizonarene	
Oxygenated sesquiterpenes		
Caryolan-1-ol	Humulene epoxide II	Tau-cadinol
Caryophyllene oxide	Humulene epoxide III	Alpha-cadinol
Humulene epoxide I	Humulenol II	
Humulol	Caryophylladienol	
Others		
4-ethylphenol	2-Undecanone	

To gain first insights into the varietal dependence of early and late kettle hopping in comparison with additional dry hopping on the analytical profiles of volatile marker compounds, in total, 15 single hop beers using the same hopping regime were brewed and 59 components were assigned upon analysis of the volatile fraction of all wort and beer samples originating from all 15 hop varieties. The volatile constituents were further classified into 5 alcohols, 14 saturated esters, 8 unsaturated esters, 2 monoterpene hydrocarbons, 4 monoterpene alcohols, 14 oxygenated sesquiterpenes, 10 sesquiterpene hydrocarbons and 2 'others' (see Table 3).

3.1 Evolution of volatiles during the brewing process

HS-SPME GC-MS profiling of compound classes of different single hopped beers throughout the brewing process

In order to investigate the differences in the volatile compositions of wort and beer samples caused by the hop variety used and to estimate the importance of the hop variety on the final characteristics of late and dry hopped beers, single hopped beers of 15 different varieties were compared. Therefore, all volatiles (i.e., hop oil volatiles and volatiles produced during fermentation) were quantified and subdivided in compound classes (Table 4 and Fig. 1, see next page).

As expected no fermentation-derived alcohols were detected in the hop samples (Table 4 and Fig. 1). For all other compound classes, big differences in the patterns of volatile compounds were observed for all analyzed hop samples. Quantitative differences are most likely due to genetic differences and

Table 4 **Total concentrations (µg/L) of compound classes quantified in 15 different hop varieties as well as the corresponding wort and beer samples. The concentrations in hops are normalized to the hop amount used to produce the dry-hopped beers**

Sample type[a]	Compound class	CAS[b]	CEN[b]	CIT[b]	CHAb	CLU[b]	COL[b]	GAL[b]	MAG[b]	NUG[b]	PAL[b]	SAA[b]	SIM[b]	SOR[b]	TET[b]	WAR[b]
Hops	∑ Saturated Esters	357	236	90.3	88.0	36.2	99.4	181	359	327	179	n.d.	48.2	14.0	5.29	133
	∑ Unsaturated Esters	863	2382	1828	60.8	54.7	205	520	148	356	18.8	43.6	113	7.47	21.8	135
	∑ Monoterpene hydrocarbons	18303	6698	5896	1606	481	1576	2217	7114	4001	1523	247	709	711	361	1546
	∑ Monoterpene alcohols	1698	3024	1931	561	527	886	693	775	2167	461	709	2005	1413	1727	1026
	∑ Sesquiterpene hydrocarbons	17742	8563	8912	1387	532	2076	9747	3983	6682	2929	902	1483	1532	1425	3360
	∑ Oxygenated sesquiterpenoids	327	376	120	1,76	1.45	24,9	382	2.54	14.4	20.7	6.02	22.6	12.6	11.5	8.82
	∑ others	67.4	35.3	194	38.5	10.8	4.37	116	34.2	47.1	22.4	16.4	33.1	16.3	6.80	41.4
PRE-EH	∑ Monoterpene hydrocarbons	1.57	0.99	0.50	5.03	7.08	2.04	0.65	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	∑ Sesquiterpene hydrocarbons	1.34	3.43	1.98	3.27	3.99	1.42	2.85	n.d.	8.75	2.41	2.59	2.88	1.97	6.10	2.81
PW	∑ Saturated Esters	1.95	7.05	4.67	17.6	7.59	8.10	16.1	17.2	21.0	7.31	n.d.	41.6	3.43	n.d.	9.30
	∑ Unsaturated Esters	14.3	57.4	60.1	10.1	18.4	18.2	17.2	31.7	2.19	11.2	122	200	4.33	140.3	14.3
	∑ Monoterpene hydrocarbons	27.8	30.6	70.2	18.0	19.5	18.6	21.9	104	62.8	17.9	206	317	71.9	694	39.8
	∑ Monoterpene alcohols	507	1146	426	113	312	546	336	244	531	149	232	482	161	298	369
	∑ Sesquiterpene hydrocarbons	105	69.5	58.3	21.3	14.3	14.8	99.2	140	120	43,1	377	234	395	573	53.8
	∑ Oxygenated sesquiterpenoids	12.4	13.8	6.64	1.31	0.78	0.64	16.5	1.43	2.61	18.6	51.7	57.2	12.7	150	4.34
	∑ others	2.42	0.48	4.36	5.45	2.59	2.24	3.94	2.87	2.61	0.85	53.1	62.6	7.71	37.4	3.71
NonDry	∑ Alcohols	101	93.0	132	129	155	172	99.3	85.9	85.7	84.7	97.9	87.2	119	127	88.5
	∑ Saturated Esters	76.2	72.4	80.6	75.5	81.1	78.6	71.6	63.1	65.5	71.4	83.7	43.0	81.7	72.8	72.2
	∑ Unsaturated Esters	17.1	33.1	24.1	2.70	11.5	7.32	15.0	7.98	10.8	6.61	16.7	34.6	1.44	21.7	9.20
	∑ Monoterpene hydrocarbons	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	17.0	18.9	15.5	29.5	n.d.
	∑ Monoterpene alcohols	210	380	236	75.2	139	340	134	107	262	63.9	92.6	189	257	145	109
	∑ Sesquiterpene hydrocarbons	1.49	n.d.	0.45	0.55	1.04	3.02	0.76	2.45	1.41	0.45	n.d.	1.25	1.98	4.54	1.39
	∑ Oxygenated sesquiterpenoids	3.13	7.58	3.42	1.07	1.84	13.2	2.67	0.45	n.d.	3.90	7.65	11.5	18.4	9.72	1.67
	∑ others	43.0	32.4	34.9	45.1	33.3	41.9	29.8	38.1	33.4	27.0	36.0	39.8	39.9	38.9	33.6

Table 4 continues next page ...

environmental effects as it has been described, for instance, for the hop variety *Cascade* [50]. The most important compound classes from a quantitative point of view were the monoterpene hydrocarbons (β-pinene and β-myrcene) and sesquiterpene hydrocarbons (α-humulene, β-caryophyllene and many others) followed by the monoterpene alcohols (linalool and geraniol). Of less quantitative importance were the saturated and unsaturated esters as well as the oxygenated sesquiterpenoids. The variances of saturated ester

Dry	∑ Alcohols	176	115	104	177	120	139	131	124	101	121	150	157	150	142	98.1
	∑ Saturated Esters	125	98.0	89.3	117	95.0	99.3	103	98.8	96.3	88.7	91.9	93.1	65.3	95.3	93.0
	∑ Unsaturated Esters	39.9	128	32.7	6.20	12.0	35.5	27.8	15.0	20.3	6.90	34.0	81.4	8.13	46.7	12.9
	∑ Monoterpene hydrocarbons	n.d.	n.d.	n.d.	n.d.	0.33	n.d.	n.d.	3.79	n.d.	0.39	24.0	37.3	25.3	35.1	n.d.
	∑ Monoterpene alcohols	601	1276	685	137	303	589	280	285	792	160	235	871	508	650	321
	∑ Sesquiterpene hydrocarbons	8.57	9.26	2.66	3.34	0.99	23.3	7.98	28.4	29.5	1.60	4.55	11.3	43.7	24.8	12.4
	∑ Oxygenated sesquiterpenoids	7.95	12.2	3.48	4.23	0.16	11.4	24.3	2.34	2.33	3.22	14.4	20.9	20.3	6.82	2.58
	∑ others	65.9	32.9	38.2	51.2	43.5	40.1	42.4	42.7	46.1	40.6	48.8	62.6	57.8	45.2	42.0

[a] Sample abbreviation refers to Table 2
[b] Hop varieties according to Table 1
n.d.: not detected

concentrations are related to substantial differences for isoamyl propionate, isoamyl isobutyrate and 2-methylbutyl esters (Table 5). Unsaturated methyl esters as well as unsaturated esters derived from the monoterpene alcohols geraniol and nerol were responsible for the quantitative inter-varietal differences in the patterns of the unsaturated esters. In contrast, ethyl *trans*-4-decenoate did not affect the concentration of the unsaturated esters in total, but it was an important varietal marker as it was only detected in the varieties *Citra*, *Simcoe*, *Tettnanger* and *Warrior*. Caryophyllene oxide as well as humulene epoxides I and II (Table 5) were important compounds regarding oxygenated sesquiterpenoid concentrations. Other compounds, like caryolan-1-ol, humulol or τ-cadinol, were important markers for inter-varietal differentiation.

Surprisingly, we were able to detect the sesquiterpenes hydrocarbons α-humulene and β-caryophyllene as well as the monoterpene hydrocarbon β-myrcene in unhopped wort samples (PRE-EH; Table 6, see page 16, and Fig. 1, see page 16). Although it is not evident to identify those two sesquiterpenes in unhopped wort, our findings stand in line with the results of *De Schutter* et al. [51], *Michiu* et al. [52] and our working group [*Dresel* et al., 49], proving that sesquiterpenoids, which were normally regarded as hop oil constituents, may be present in unhopped wort. *De Clippeleer* et al. [53] were already able to show that the charge of malt can have a significant influence on the final concentration of volatiles. Therefore, the fact that compounds such as α-humulene, β-caryophyllene and β-myrcene were not detected in every unhopped wort sample might be related to the malt quality.

The quantitatively most predominant compound classes (Table 7, see page 18, and Fig. 1, see page 16) in the pitching wort samples (PW) were the monoterpene alcohols (especially linalool and geraniol) followed by the monoterpene (β-myrcene) and sesquiterpene hydrocarbons (especially α-humulene and β-caryophyllene). Compared with the monoterpene alcohols the monoterpene and sesquiterpene hydrocarbons were more volatile and may be lost during wort boiling. Furthermore, adsorption of the apolar hydrocarbons to the trub can also influence the final concentrations as the monoterpene alcohols are more polar and therefore show a better solubility. As observed for the hop samples, the saturated and unsaturated esters as well as the oxygenated sesquiterpenoids seemed to play a minor role and significantly higher concentrations of unsaturated esters and oxygenated sesquiterpenoids were only detectable for the hop varieties *Saaz*, *Simcoe* and *Tettnanger*. Especially the increased concentrations of oxygenated sesquiterpenoids for the varieties *Saaz*, *Simcoe*, *Sorachi Ace* and *Tettnanger* were noteworthy for two reasons. On the one hand, oxygenated sesquiterpenoids were present in the hop varieties *Saaz*, *Simcoe*, *Sorachi Ace* and *Tettnanger* in comparably low concentrations (see Table 5) and not detectable at all in the unhopped wort samples (see Table 6). On the other hand, oxygenated sesquiterpenoids were detected in increased concentrations in the pitching wort samples derived from those varieties (see Table 7). However, the differences of the sesquiterpene monoterpenes in the hop and unhopped wort samples could not explain the formation of oxygenated sesquiterpenoids in comparison with the other samples. A yet undiscovered mechanism seemed to result in increased concentrations of these compounds in the pitching wort samples derived from those hop varieties. In this context, one should have in mind that especially the hop varieties *Saaz* and *Tettnanger* are traditional aroma varieties which are recommended to produce beers with a so-called noble hop aroma. A similar behavior was observed for β-myrcene, which was present in the pitching wort samples derived from the hop varieties *Saaz*, *Simcoe*, *Sorachi Ace* and *Tettnanger* in unexpectedly high concentrations. A possible explanation could be that β-myrcene is present in those hop varieties in a chemically bound state and released during the boiling process.

In the late hopped beers, the monoterpene alcohols (linalool and geraniol) followed by the alcohols (especially 3-methyl-1-butanol) and saturated esters (especially ethyl acetate) were the most important compound classes. However, concentrations of the compound classes mentioned were quite comparable for all varieties. The formation of the esters and the higher alcohols could be easily explained by the yeast metabolism. As high concentrations of the monoterpene alcohols were already detected in the pitching wort samples, it was no surprise that relatively high concentrations were also present in the fermented samples. Yet, the total concentrations were lower than in the pitching wort samples. This could be due to adsorption of those compounds to the yeast cells or due to a metabolization. The same applies for β-myrcene as well, which

Table 5 **(Semi-) Quantitative data (µg/L) of the 15 used hop varieties (Hops). Concentrations are normalized to the hop amount used to produce the dry-hopped beers. Compounds highlighted with an asterisk were analyzed quantitatively**

Compound	RI[a]	CAS[b]	CEN[b]	CIT[b]	CHA[b]	CLU[b]	COL[b]	GAL[b]	MAG[b]	NUG[b]	PAL[b]	SAA[b]	SIM[b]	SOR[b]	TET[b]	WAR[b]
Esters																
Saturated esters																
Isoamyl propionate	944	86.1	46.8	48.7	6.12	2.97	19.8	31.0	62.2	40.1	22.1	n.d.	7.84	2.94	0.65	13.2
Isoamyl isobutyrate	996	57.6	9.06	2.57	9.75	6.80	12.9	9.40	40.4	66.3	21.6	n.d.	13.3	1.90	4.65	25.6
2-Methylbutyl butanoate	1003	151	48.1	11.7	63.0	22.0	48.0	53.8	220	167	98.0	n.d.	22.3	7.87	n.d.	72.8
2-Methylbutyl isopentanoate	1095	30.2	65.6	13.2	5.96	1.58	9.73	50.2	24.7	29.4	18.2	n.d.	4.74	1.30	n.d.	9.46
2-Methylbutyl pentanoate	1098	32.1	66.6	14.1	3.11	2.83	8.89	36.9	11.7	23.9	19.0	n.d.	n.d.	n.d.	n.d.	11.5
Σ		357	236	90.3	88.0	36.2	99.4	181	359	327	179	n.d.	48.2	14.0	5.29	133
Unsaturated esters																
Methyl *cis*-2-decenoate	1294	84.7	434	770	31.9	32.5	15.1	212	n.d.	171	n.d.	24.8	35.9	3.88	8.47	69.2
Methyl *trans*-4,9-decadienoate	1296	23.1	107	335	24.6	13.6	n.d.	40.0	71.7	162	n.d.	11.6	17.4	1.96	6.99	15.3
Methyl geranate	1298	170	1628	586	4.35	0.69	9.85	45.2	19.8	22.7	15.1	2.65	43.7	1.63	5.41	22.0
Neryl acetate	1359	389	33.0	27.9	n.d.	5.46	95.5	111	n.d.	n.d.	3.70	n.d.	n.d.	n.d.	n.d.	n.d.
Ethyl *trans*-4-decenoate	1364	n.d.	n.d.	24.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.21	n.d.	0.91	7.77
Neryl butyrate	1507	196	179	84.5	n.d.	2.46	84.9	112	56.1	n.d.	n.d.	4.57	13.2	n.d.	n.d.	21.0
Σ		863	2382	1828	60.8	54.7	205	520	148	356	18.8	43.6	113	7.47	21.8	135
Terpenes																
Monoterpene hydrocarbons																
Beta-pinene	958	359	394	286	27.9	10.1	43.5	80.8	156	58.7	40.1	4.28	13.2	11.4	5.29	29.6
Beta-myrcene	965	17944	6304	5610	1579	471	1532	2136	6958	3942	1483	243	696	699	356	1516
Σ		18303	6698	5896	1606	481	1576	2217	7114	4001	1523	247	709	711	361	1546
Monoterpene alcohols																
Linalool *	1085	748	1573	1813	516	286	744	409	660	2082	430	540	1158	948	1577	572
Alpha-terpineol	1170	n.d.	n.d.	n.d.	n.d.	n.d.	1.10	n.d.	n.d.	n.d.	n.d.	n.d.	4.11	n.d.	n.d.	n.d.
Beta-citronellol	1209	n.d.	10.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Geraniol *	1235	950	1441	118	44,9	241	140	284	115	85.0	31.1	169	843	465	150	455
Σ		1698	3024	1931	561	527	886	693	775	2167	461	709	2005	1413	1727	1026
Sesquiterpenes																
Sesquiterpene hydrocarbons																
Beta-caryophyllene	1419	5276	3537	4027	280	158	658	3493	1040	2366	1083	144	466	324	342	1298
Alpha-humulene	1434	10206	3694	3378	871	317	871	4239	2630	3427	1416	667	788	1026	929	1490
Alpha-copaene	1377	176	143	143	12.2	4.30	49.4	339	34.0	69,6	38.8	5.74	16.8	12.9	11.7	31.5
Beta-calarene	1474	53.5	14.2	17.4	3.16	2.24	9.75	31.9	9.90	17.8	11.4	2.88	3.48	3.94	n.d.	11.7
Gamma-muurolene	1477	297	128	122	n.d.	0.41	98.4	200	48.5	101	70.4	13.3	41.4	27.5	3.81	67.0
Alpha-amorphene	1480	0.50	n.d.	26.8	4.52	10.3	9.87	31.5	5.21	12.7	8.30	2.44	4.72	4.13	24.7	7.82

Table 5 continues next page ...

Beta-selinene	1487	375	51.9	330	35.7	0.92	63.8	259	14.8	146	64.0	5.64	10.0	15.2	3.16	100
Gamma-amorphene	1495	99.4	56.4	n.d.	n.d.	12.3	n.d.	46.9	19.9	66.8	17.1	7.61	13.1	10.8	8.47	45.2
Alpha-selinene	1498	496	118	400	122	n.d.	59.5	325	15.4	159	57.6	3.94	10.0	10.9	9.42	99.0
Epizonarene	1500	n.d.	n.d.	n.d.	n.d.	10.7	n.d.	n.d.	10.8	n.d.	14.0	3.40	7.19	7.42	6.49	8.48
Gamma-cadinene	1511	n.d.	257	134	19.1	n.d.	84.7	233	53.4	94.9	13.4	13.9	42.6	28.3	27.4	63.8
Calamenene	1523	64.1	47.9	18.6	n.d.	0.49	7.83	64.7	n.d.	16.8	5.45	4.57	6.39	4.46	4.32	8.28
Delta-cadinene	1529	624	469	285	35.7	16.1	145	430	91.2	187	118.9	24.5	65.9	50.6	49.9	117
(*trans*-) Cadina-1,4-diene	1534	73.8	47.1	31.3	3.85	n.d.	17.7	53.4	10.2	17.5	10.6	2.54	7.58	5.16	4.82	11.8
Σ		17742	8563	8912	1387	532	2076	9747	3983	6682	2929	902	1483	1532	1425	3360
Oxygenated sesquiterpenes																
Caryolan-1-ol	1541	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.66	n.d.	n.d.
Caryophyllene oxide	1566	62.6	92.3	24.8	n.d.	n.d.	8.40	69.5	n.d.	2.13	4.69	n.d.	2.97	n.d.	n.d.	1.54
Humulene epoxide I	1584	26.0	23.7	12.6	n.d.	0.27	1.20	27.1	n.d.	1.97	3.05	0.55	n.d.	n.d.	n.d.	1.05
Humulol	1588	n.d.	32.4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.62	n.d.	n.d.
Humulene epoxide II	1595	178	177	49.5	1.76	1.18	11.4	190	2.54	7.22	9.45	1.08	4.68	1.24	n.d.	2.88
Humulene epoxide III	1615	15.7	n.d.	n.d.	n.d.	n.d.	1.05	n.d.	n.d.	n.d.	n.d.	0.45	1.35	0.75	0.80	n.d.
Humulenol II	1634	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	29.3	n.d.	n.d.	n.d.	2.98	8.66	5.27	6.08	n.d.
Caryophy lladienol	1638	n.d.	14.4	n.d.	n.d.	n.d.	n.d.	23.2	n.d.	n.d.	n.d.	n.d.	2.62	0.89	1.21	1.74
Tau-cadinol	1644	30.7	25.0	23.6	n.d.	n.d.	2.02	26.0	n.d.	n.d.	1.43	0.97	2.35	1.72	2.61	n.d.
Alpha-cadinol	1655	13.7	10.7	9.62	n.d.	n.d.	0.80	17.2	n.d.	3.07	2.08	n.d.	n.d.	0.46	0.86	1.62
Σ		327	376	120	1.76	1.45	24.9	382	2.54	14.4	20.7	6.02	22.6	12.6	11.5	8.82
Others																
2-Undecanone	1285	67.4	35.3	194	38.5	10.8	4.37	116	34.2	47.1	22.4	16.4	33.1	16.3	6.80	41.4
Σ		67.4	35.3	194	38.5	10.8	4.37	116	34.2	47.1	22.4	16.4	33.1	16.3	6.80	41.4

[a] Calculated retention index (RTX-1 capillary column, 40 m x 0.18 mm i.d. x 0.20 µm film thickness.
[b] Hop varieties according to table 1
n.d.: not detected

was present in high concentrations in the pitching wort samples of the hop varieties *Saaz*, *Simcoe*, *Sorachi Ace* and *Tettnanger*. Due to lower concentrations in the remaining samples and the losses during fermentation, β-myrcene is only detectable in rather low concentrations in the late hopped beers of the hop varieties *Saaz*, *Simcoe*, *Sorachi Ace* and *Tettnanger*.

Unsaturated esters, sesquiterpene hydrocarbons and oxygenated sesquiterpenoids are detected in relatively small concentrations. Concentrations of the higher alcohols, the saturated esters and the 'others' in the late hopped beers and the dry hopped beers were rather similar, because additional dry hopping should not have an influence on the fermentation profile. However, the concentrations of all other compound classes, with the exception of the monoterpene hydrocarbons, were much higher in the dry hopped beers. Yet, the concentration of β-myrcene was slightly increased in the beers derived from the hop varieties *Saaz*, *Simcoe*, *Sorachi Ace* and *Tettnanger*, but still absent in all other samples. β-Pinene was only detectable in concentrations below 2 µg/L in the beers derived from the hop varieties *Cluster*, *Magnum* and *Palisade*. Concentrations of the monoterpene alcohols (linalool and geraniol) were strongly increased. An efficient extraction rate of these compounds during dry hopping, limited losses due to adsorption to trub and a good solubility of these compounds in an aqueous medium could account for this observation.

As the profiles of the pitching wort samples, the late hopped beers and the dry hopped beers were dominated by the concentrations of monoterpene alcohols, figure 2 shows the profiles without this compound class enabling a more detailed insight into varietal differences. The profiles of the pitching worts strongly varied. Especially the concentrations of unsaturated esters, monoterpene and sesquiterpene hydrocarbons and oxygenated sesquiterpenoids showed great varietal differences. In general, the varieties *Challenger*, *Cluster*, *Columbus*, *Palisade* and *Warrior* showed the lowest concentrations of the mentioned compound classes. Varieties *Cascade*, *Centennial*, *Citra*, *Galena*, *Magnum* and Nugget featured medium concentrations and the highest

concentrations were found for the varieties *Saaz*, *Simcoe*, *Sorachi Ace* and *Tettnanger*.

Interestingly, these characteristics were not transferred neither to the late hopped beers nor to the dry hopped beers (Fig. 2). Concentrations of the alcohols were comparable for all samples except for the samples derived from the hop varieties *Challenger*, *Cluster* and *Columbus*, which showed alcohol concentrations above 100 µg/L. The reason for this behavior is not clear. Furthermore, significant differences were found for the monoterpene hydrocarbons, which were present in the samples derived from hop varieties *Saaz*, *Simcoe*, *Sorachi Ace* and *Tettnanger*. Elevated concentrations of oxygenated sesquiterpenoids were found for the varieties Centennial and Columbus as well as for the varieties *Palisade*, *Saaz*, *Simcoe*, *Sorachi Ace* and *Tettnanger*.

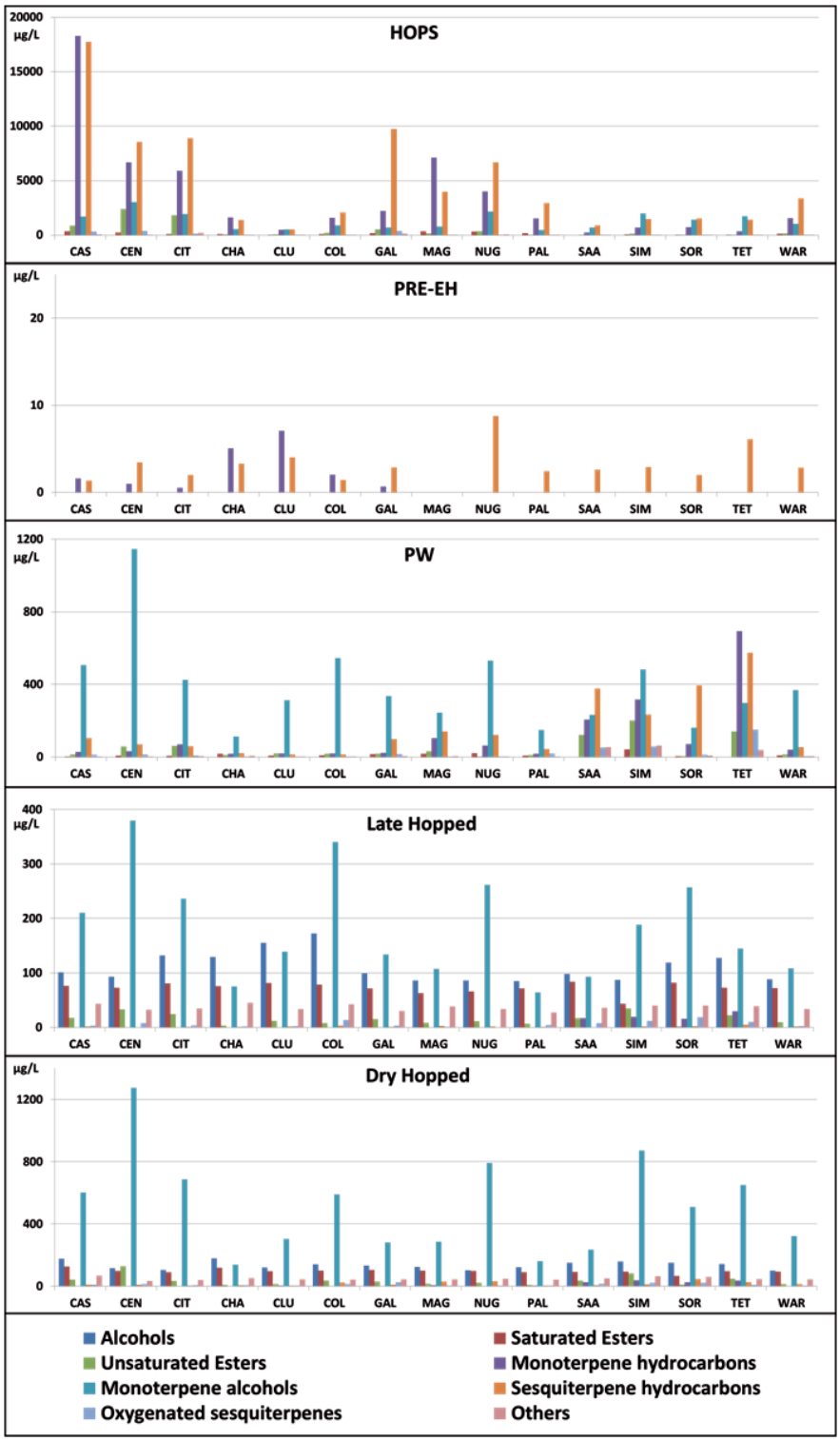

Fig. 1 Concentrations (µg/L) of various compound classes in hops as well as in wort and beer samples withdrawn along the brewing process as a function of the hop variety used

As expected, the concentrations of the alcohols, the saturated esters and the unsaturated esters of the late hopped beers in comparison with the corresponding samples of the dry hopped beers did not differ significantly. Moreover, whereas the alcohol concentrations of the late hopped samples of the varieties *Challenger*, *Cluster* and *Columbus* were slightly increased, concentrations for the dry hopped beers were only increased for the beers derived from the varieties *Cascade* and *Challenger*. Interestingly, concentrations of the saturated esters were also slightly increased in the beers derived from the hop varieties *Cascade* and *Challenger*.

Concentrations of the unsaturated esters varied, especially beers *Centennial* and *Simcoe* featured very high concentrations. Furthermore, concentrations of the monoterpene and sesquiterpene hydrocarbons and the oxygenated sesquiterpenoids differed throughout the samples, leading to a characteristic pattern for each beer. Yet, it should not be forgotten that also the concentrations of the monoterpene alcohols varied especially for the pitching worts as well as for the late and dry hopped beer sample, contributing as well to the characteristic pattern for each beer.

Detailed analysis of the inter-varietal differences of the volatiles of single hopped beers throughout the beer manufacturing process

So far, the results shown above provided an insight into the fundamental changes of different compound classes during the beer production resulting in individual single hop beers with special aroma attributes. However, it remains unclear whether these variations are related to single compounds or if these characteristics are affected by all compounds within a group. Therefore, monitoring the changes of individual compounds will help to identify varietal differences and will be the scientific basis towards a better understanding how the hopping regime and the use of a specific hop variety may affect the patterns of aroma-active volatiles of beers.

Table 5 summarizes the semi-quantitative concentrations of all compounds in all analyzed hop samples. In total, 5 saturated esters, 6 unsatu-

Table 6 **(Semi-) Quantitative data (µg/L) of the 15 unhopped wort samples (PRE-EH). Compounds highlighted with an asterisk were analyzed quantitatively**

Compound	RI[a]	CAS[b]	CEN[b]	CIT[b]	CHA[b]	CLU[b]	COL[b]	GAL[b]	MAG[b]	NUG[b]	PAL[b]	SAA[b]	SIM[b]	SOR[b]	TET[b]	WAR[b]
Terpenes																
Monoterpene hydrocarbons																
Beta-myrcene	965	1.57	0.99	0.50	5.03	7.08	2.04	0.65	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ		1.57	0.99	0.50	5.03	7.08	2.04	0.65	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sesquiterpenes																
Sesquiterpenes hydrocarbons																
Beta-caryophyllene	1419	n.d.	0.98	0.68	n.d.	1.09	0.29	0.99	n.d.	1.47	n.d.	n.d.	0.91	0.30	n.d.	n.d.
Alpha-humulene	1434	1.34	2.45	1.31	3.27	2.91	1.13	1.86	n.d.	7.28	2.41	2.59	1.97	1.67	6.10	2.81
Σ		1.34	3.43	1.98	3.27	3.99	1.42	2.85	n.d.	8.75	2.41	2.59	2.88	1.97	6.10	2.81

[a] Calculated retention index (RTX-1 capillary column, 40 m x 0.18 mm i.d. x 0.20 µm film thickness
[b] Worts derived from hop varieties according to table 1
n.d.: not detected

rated esters, 2 monoterpene hydrocarbons, 4 monoterpene alcohols, 14 sesquiterpene hydrocarbons, 10 oxygenated sesquiterpenoids and 2-undecanone were detected. Whereas isoamyl and 2-methylbutyl esters were present in varying con-centrations in almost every hop sample, these compounds were not detectable in the hop variety *Saaz*. The greatest concentration range (7.9–220 µg/L) was found for 2-methylbutyl butanoate, whereas the concentrations of all other compounds did not usually exceed 60 µg/L.

Concentrations of the unsaturated esters varied significantly within all hop varieties and it was not possible to identify a common pattern. However, concentrations of unsaturated esters were lowest in the hop varieties *Palisade*, *Sorachi Ace* and *Tettnanger*.

Interestingly, a direct correlation (R^2 = 0.9803) between the concentration of the monoterpene hydrocarbons β-myrcene and β-pinene was found. A similar behavior (R^2 = 0.6906) was observed for the monoterpene alcohols linalool and geraniol with the exception of the hop varieties *Citra*, *Nugget* and *Tettnanger*, which showed relatively low concentrations of geraniol. Furthermore, concentrations of all other sesquiterpene hydrocarbons were highest for those hop varieties with high concentrations of α-humulene and β-caryophyllene.

Table 5 shows that caryophyllene oxide, humulene epoxides I and II, as well as τ-cadinol were the predominant oxygenated sesquiterpenoids. However, concentrations of oxygenated sesquiterpenoids in hops could be correlated to the heat and oxygen load during the production of hop pellets.

As already mentioned before, the monoterpene hydrocarbon β-myrcene and the sesquiterpenes hydrocarbons α-humulene and

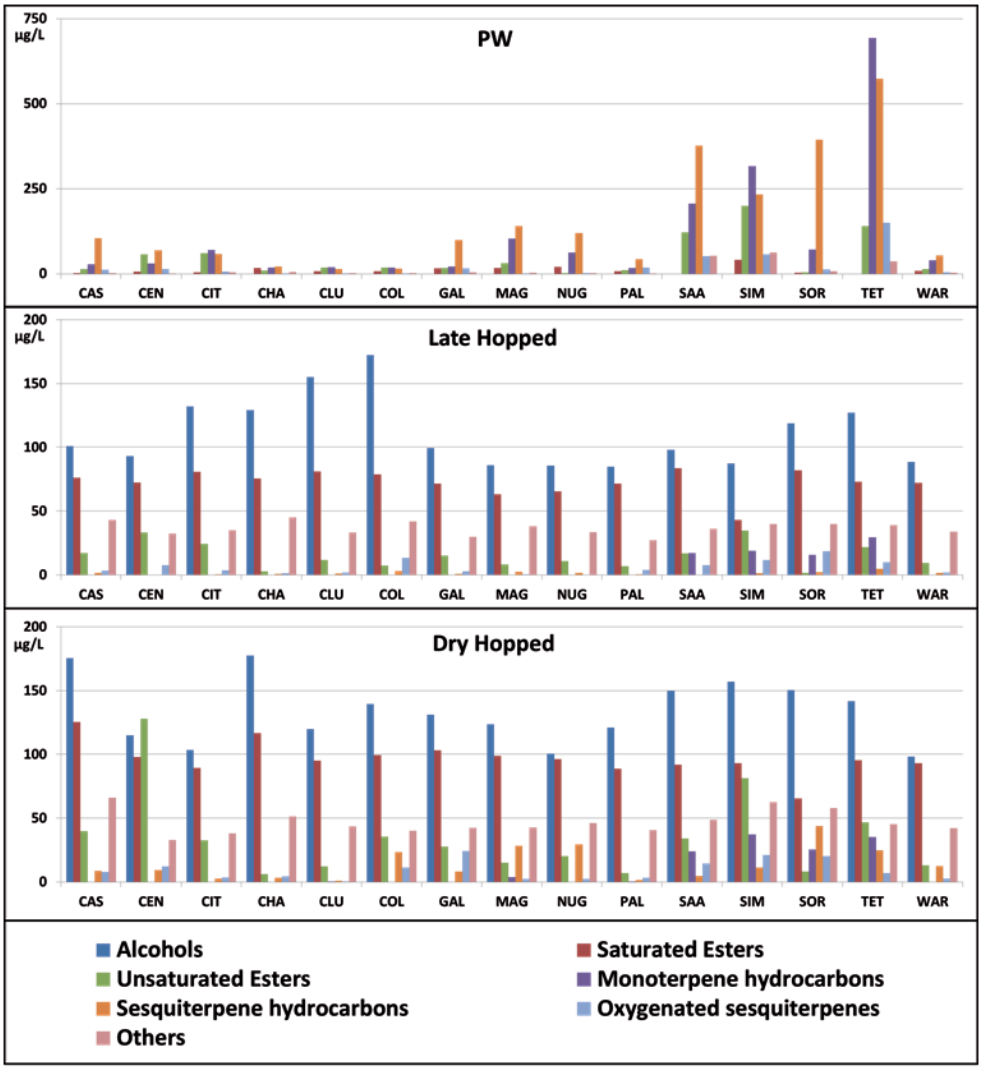

Fig. 2 **Concentrations (µg/L) of various compound classes – exclusive of the monoterpene alcohols – in hops as well as in wort and beer samples withdrawn along the brewing process as a function of the hop variety used**

Table 7 (Semi-)Quantitative data (µg/L) of the 15 pitching wort samples (PW). Compounds highlighted with an asterisk were analyzed quantitatively

Compound	RI[a]	CAS[b]	CEN[b]	CIT[b]	CHA[b]	CLU[b]	COL[b]	GAL[b]	MAG[b]	NUG[b]	PAL[b]	SAA[b]	SIM[b]	SOR[b]	TET[b]	WAR[b]
Esters																
Saturated esters																
Isoamyl propionate	944	n.d.	n.d.	n.d.	1.18	n.d.	0.71	1.06	2.06	1.55	0.99	n.d.	3.57	n.d.	n.d.	0.74
Isoamyl isobutyrate	996	n.d.	0.85	0.91	11.7	4.66	4.71	1.84	1.78	3.61	0.93	n.d.	10.2	n.d.	n.d.	1.68
2-Methylbutyl butanoate	1003	1.12	3.60	3.15	4.12	1.98	1.66	11.1	11.7	11.9	5.39	n.d.	22.6	1.51	n.d.	6.00
2-Methylbutyl isopentanoate	1095	n.d.	1.24	n.d.	0.61	n.d.	0.51	1.24	1.14	1.88	n.d.	n.d.	5.17	n.d.	n.d.	n.d.
2-Methylbutyl pentanoate	1098	n.d.	0.92	n.d.	n.d.	n.d.	0.51	0.89	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.87
Ethyl dodecanoate	1572	n.d.	n.d.	0.61	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.66	n.d.	n.d.
∑		1.95	7.05	4.67	17.6	7.59	8.10	16.1	17.2	21.0	7.31	n.d.	41.6	3.43	n.d.	9.30
Unsaturated esters																
Methyl *cis*-2-decenoate	1294	1.26	5.68	15.9	3.61	9.54	9.64	7.61	15.5	n.d.	9.58	59.6	58.1	1.20	46.3	7.76
Methyl *trans*-4,9-decadienoate	1296	1.01	2.54	15.8	5.59	8.14	8.13	2.74	7.54	n.d.	n.d.	37.1	32.7	n.d.	39.0	3.24
Methyl geranate	1298	3.29	45.1	26.0	0.94	0.69	0.47	1.51	2.50	2.19	1.59	7.86	85.0	0.69	34.1	3.25
Neryl acetate	1359	5.12	0.36	0.61	n.d.	n.d.	n.d.	1.14	n.d.	n.d.	n.d.	17.4	n.d.	n.d.	n.d.	n.d.
Neryl butyrate	1507	3.60	3.66	1.83	n.d.	n.d.	n.d.	4.21	6.10	n.d.	n.d.	n.d.	24.3	2.45	21.0	n.d.
∑		14.3	57.4	60.1	10.1	18.4	18.2	17.2	31.7	2.19	11.2	122	200	4.33	140	14.3
Terpenes																
Monoterpene hydrocarbons																
Beta-pinene	958	0.49	0.81	0.79	n.d.	0.44	n.d.	1.03	3.81	1.05	1.12	2.78	4.05	0.74	n.d.	0.97
Beta-myrcene	965	27.3	29.8	69.4	18.0	19.0	18.6	20.8	100	61.8	16.8	204	313	71.1	694	38.8
∑		27.8	30.6	70.2	18.0	19.5	18.6	21.9	104	62.8	17.9	206	317	71.9	694	39.8
Monoterpene alcohols																
Linalool *	1085	157	374	146	103	85.5	421	96.7	142	457	64.7	119	252	20.0	176	135
Alpha-terpineol	1170	n.d.	n.d.	n.d.	n.d.	0.71	0.82	n.d.	1.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Beta-citronellol	1209	n.d.	0.70	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,5	n.d.	n.d.	n.d.	n.d.	n.d.
Geraniol *	1235	350	771	280	10.1	226	124	239	101	74.5	84.2	112	230	141	121	234
∑		507	1146	426	113	312	546	336	244	531	149	232	482	161	298	369
Sesquiterpenes																
Sesquiterpenes hydrocarbons																
Beta-caryophyllene	1419	18.3	14.2	13.5	2.72	3.00	3.00	19.5	21.3	24.1	11.0	55.3	20.2	66.6	80.3	14.3
Alpha-humulene	1434	69.0	44.9	35.3	15.6	11.3	11.6	60.0	102	81.9	26.1	190	55.2	281	274	33.2
Alpha-copaene	1377	0.28	0.28	n.d.	n.d.	n.d.	n.d.	n.d.	0.54	0.31	n.d.	3.48	3.44	1.32	6.51	n.d.
Beta-calarene	1474	0.44	1.18	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.68	n.d.	8.91	4.67	1.73	5.28	n.d.
Gamma-muurolene	1477	1.89	1.01	0.99	n.d.	n.d.	n.d.	2.06	1.88	1.93	1.09	18.6	19.5	6.45	19.8	0.86
Alpha-amorphene	1480	0.40	1.08	0,6	n.d.	n.d.	n.d.	n.d.	n.d.	0.49	n.d.	5.90	4.55	1.15	6.72	n.d.

Table 7 continues next page ...

Beta-selinene	1487	3.25	0.77	2.98	1.51	n.d.	n.d.	3.73	0.83	1.90	1.29	10.6	11.2	4.66	12.7	1.58
Gamma-amorphene	1495	0.83	n.d.	n.d.	n.d.	n.d.	n.d.	0.68	n.d.	0.53	0.26	11.2	9.46	1.60	10.5	n.d.
Alpha-selinene	1498	3.81	0.85	3.73	1.53	n.d.	n.d.	7.35	n.d.	2.97	0.80	n.d.	6.05	3.24	31.6	1.26
Epizonarene	1500	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.54	n.d.	n.d.	6.68	24.0	2.52	38.5	0.24
Gamma-cadinene	1511	2.13	1.80	0.93	n.d.	n.d.	n.d.	1.82	2.82	1.63	0.81	20.4	25.2	7.64	28.2	0.89
Calamenene	1523	0.80	0.63	0.32	n.d.	n.d.	n.d.	0.93	n.d.	0.49	0.31	7.46	5.42	1.88	22.9	n.d.
Delta-cadinene	1529	3.50	2.77	n.d.	n.d.	n.d.	0.22	3.12	3.87	3.10	1.57	34.2	40.6	13.6	19.0	1.53
(*trans*-)Cadina-1,4-diene	1534	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.57	0.33	n.d.	4.43	4.56	1.33	17.1	n.d.
Σ		105	69.5	58.3	21.3	14.3	14.8	99.2	140	120	43,1	377	234	395	573	53.8
Oxygenated sesquiterpenes																
Caryolan-1-ol	1541	0.53	0.43	0.50	n.d.	n.d.	n.d.	0.64	n.d.	n.d.	0.77	6.63	n.d.	n.d.	16.5	0.23
Caryophyllene oxide	1566	1.04	1.44	0.31	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.08	3.39	5.70	1.51	11.2	0.49
Humulene epoxide I	1584	1.19	1.36	0.26	n.d.	0.12	n.d.	1.51	0.22	0.54	1.32	4.47	3.91	n.d.	11.4	0.41
Humulol	1588	0.79	0.59	0.81	0.30	0.23	0.22	1.12	0.52	0.91	0.71	4.13	5.79	1.18	n.d.	0.58
Humulene epoxide II	1595	3.11	3.38	0.60	0.62	0.43	0.43	2.90	0.69	1.17	2.36	4.44	8.27	1.69	10.9	0.76
Humulene epoxide III	1615	n.d.	0.80	1.89	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.90	4.28	3.58	0.90	n.d.	0.22
Humulenol II	1634	1.90	2.48	n.d.	n.d.	n.d.	n.d.	5.70	n.d.	n.d.	6.10	15.0	11.3	3.85	34.4	1.12
Caryophylladienol	1638	0.75	0.94	n.d.	n.d.	n.d.	n.d.	1.64	n.d.	n.d.	2.60	n.d.	4.37	0.81	11.11	n.d.
Tau-cadinol	1644	2.01	1.55	1.53	n.d.	n.d.	n.d.	1.85	n.d.	n.d.	1.76	6.86	10.3	2.01	40.4	n.d.
Alpha-cadinol	1655	1.12	0.79	0.75	0.40	n.d.	n.d.	1.16	n.d.	n.d.	0.99	2.46	4.01	0.74	14.2	0.55
Σ		12.4	13.8	6.64	1.31	0.78	0.64	16.5	1.43	2.62	18.6	51.7	57.2	12.7	150	4.34
Others																
2-Undecanone	1285	2.42	0.48	4.36	5.45	2.59	2.24	3.94	2.87	2.61	0.85	53.1	62.6	7.71	37.4	3.71
Σ		2.42	0.48	4.36	5.45	2.59	2.24	3.94	2.87	2.61	0.85	53.1	62.6	7.71	37.4	3.71

[a] Calculated retention index (RTX-1 capillary column, 40 m x 0.18 mm i.d. x 0.20 µm film thickness
[b] Worts derived from hop varieties according to table 1
n.d.: not detected

β-caryophyllene were occasionally detected in low concentrations (<8 µg/L) in the unhopped wort samples (Table 6). Although concentrations did not differ since all unhopped wort samples were produced using the same brewing regime, these compounds were not present in all samples.

The quantitative data of the compounds detected in the pitching wort samples (PW) are summarized in Table 7. Concentrations of the saturated esters were rather low in the PW samples. Only for the varieties *Challenger*, *Galena*, *Magnum*, *Nugget* and *Simcoe*, higher levels of isoamyl isobutyrate and/or 2-methylbutyl butanoate of 10–12 µg/L (with exception of variety *Simcoe*: 22.6 µg/L 2-methylbutyl butanoate) were found. In general, concentrations of the unsaturated esters were found to be below 10 µg/L. Only for the varieties *Centennial*, *Citra*, *Saaz*, *Simcoe* and *Tettnanger*, higher concentrations (15–85 µg/L) of methyl *cis*-2-decenoate, methyl *trans*-4,9-decadienoate, methyl geranate and neryl butyrate were measured.

Concentrations of the monoterpenoids β-myrcene, linalool and geraniol varied significantly (10–771 µg/L) and no correlation with other substances was evidenced. Therefore, these compounds are potential marker compounds for the used hop varieties. Also, concentrations of the sesquiterpene hydrocarbons were low except for α-humulene, γ-muurolene and δ-cadinene, which were present in rather high concentrations in the pitching worts produced with the hop varieties *Saaz*, *Simcoe*, *Sorachi Ace* and *Tettnanger*. Interestingly, variety *Tettnanger* showed high concentrations for almost all sesquiterpene hydrocarbons. Furthermore, pitching wort of the hop variety *Simcoe* was the only sample in which the concentration of δ-cadinene was higher than the concentration of α-humulene.

A similar pattern was observed for the oxygenated sesquiterpenoids which were present in rather high concentrations in the beers derived from the hop varieties *Saaz*, *Simcoe* and *Tettnanger*. Surprisingly, the hop varieties *Saaz*, *Simcoe* and *Tettnanger* featured relatively low concentrations of sesquiterpene hydrocarbons and oxygenated sesquiterpenoids. That stands in contrast to the fact that, for example, the hop varieties *Cascade*, *Centennial*, *Citra*, and *Galena* featured high concentrations of sesquiterpene hydrocarbons as

Table 8 (Semi-)Quantitative data (µg/L) of the 15 beers without dry hopping (late hopped beer – Late Hopped). Compounds highlighted with an asterisk were analyzed quantitatively

Compound	RI[a]	CAS[b]	CEN[b]	CIT[b]	CHA[b]	CLU[b]	COL[b]	GAL[b]	MAG[b]	NUG[b]	PAL[b]	SAA[b]	SIM[b]	SOR[b]	TET[b]	WAR[b]
Alcohols																
n-Propanol	545	7.08	4.76	40.7	7.21	27.3	40.7	19.8	11.9	4.32	6.59	3.67	2.14	19.5	37.3	6.32
Isobutanol	608	18.9	15.6	14.7	18.9	15.0	17.9	12.4	12.9	14.6	13.3	19.8	15.6	22.7	12.4	14.6
3-Methyl-1-butanol	725	53.3	53.6	54.7	72.7	69.0	69.7	47.6	47.0	51.7	48.7	48.2	48.6	53.6	54.2	52.4
2-Methyl-1-butanol	729	13.3	14.4	14.6	17.4	16.4	17.0	12.3	12.2	13.1	13.7	12.3	11.8	13.0	14.1	12.9
Phenylethanol	1088	8.32	4.61	7.33	12.9	27.2	26.9	7.14	1.90	1.96	2.44	14.0	9.06	9.93	9.30	2.20
∑		101	93.0	132	129	155	172	99.3	85.9	85.7	84.7	97.9	87.2	119	127	88.5
Esters																
Saturated esters																
Ethyl acetate *	600	62.5	65.8	70.3	66.6	63.3	58.6	59.0	52.6	55.4	63.2	74.5	34.2	72.7	67.1	63.1
Isobutyl acetate *	764	0.12	0.12	0.10	0.10	0.09	0.09	0.09	0.07	0.09	0.11	0.09	0.04	0.14	0.10	0.12
Ethyl butanoate *	791	0.19	0.21	0.21	0.14	0.15	0.15	0.18	0.17	0.15	0.19	0.16	0.12	0.21	0.21	0.18
Isoamyl acetate *	845	3.85	3.38	3.85	3.47	3.00	2.65	2.79	2.75	3.28	3.68	3.46	1.43	4.23	3.74	4.05
Ethyl hexanoate *	962	0.31	0.31	0.41	0.34	0.27	0.26	0.30	0.32	0.30	0.37	0.27	0.22	0.33	0.34	0.32
Isoamyl isobutyrate	996	n.d.	n.d.	0.54	n.d.	1.07	3.17	0.38	2.17	0.55	n.d.	n.d.	1.87	n.d.	n.d.	0.31
2-Methylbutyl butanoate	1003	n.d.	0.71	0.65	n.d.	n.d.	n.d.	1.23	n.d.	1.36	0.55	n.d.	3.11	n.d.	n.d.	0.58
Ethyl octanoate *	1180	0.46	0.46	0.52	0.42	0.43	0.41	0.44	0.39	0.42	0.41	0.37	0.33	0.43	0.47	0.43
Phenethyl acetate *	1224	0.81	0.64	0.81	0.77	0.66	0.65	0.56	0.60	0.78	0.66	0.71	0.40	0.94	0.74	0.85
Ethyl decanoate *	1377	0.11	0.09	0.10	0.09	0.13	0.09	0.12	0.09	0.10	0.07	0.08	0.06	0.10	0.13	0.08
Ethyl dodecanoate	1572	7.90	0.64	3.07	3.58	11.98	12.53	6.57	3.89	3.02	2.25	4.03	1.26	2.63	n.d.	2.17
∑		76.2	72.4	80.6	75.5	81.1	78.6	71.6	63.1	65.5	71.4	83.7	43.0	81.7	72.8	72.2
Unsaturated esters																
Methyl *cis*-2-decenoate	1294	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.69	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Methyl geranate	1298	0.70	15.0	6.24	n.d.	n.d.	1.27	0.44	0.59	0.36	0.35	n.d.	15.7	n.d.	2.35	0.59
Neryl acetate	1359	3.63	5.54	4.55	n.d.	4.06	n.d.	5.70	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ethyl *trans*-4-decenoate	1364	2.63	3.93	4.50	n.d.	n.d.	n.d.	4.00	2.87	3.71	2.71	2.83	5.42	n.d.	3.33	3.62
Ethyl 4,9-decadienoate	1360	6.23	4.29	5.21	n.d.	3.77	5.15	4.82	1.89	4.88	n.d.	3.15	5.52	n.d.	4.60	3.08
Ethyl *cis*-4-decenoate	1364	3.93	4.25	3.64	2.70	3.67	n.d.	n.d.	1.93	1.85	3.55	10.7	7.94	1.44	11.4	1.91
∑		17.1	33.1	24.1	2.70	11.5	7.32	15.0	7.98	10.8	6.61	16.7	34.6	1.44	21.7	9.20
Terpenes																
Monoterpene hydrocarbons																
Beta-myrcene	965	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	17.0	18.9	15.5	29.5	n.d.
∑		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	17.0	18.9	15.5	29.5	n.d.
Monoterpene alcohols																
Linalool *	1085	85.8	175	155	58.7	39.2	214	54.6	55.5	225	27.5	56.1	30.6	162	101	41.1

Table 8 continues next page ...

Alpha-terpineol	1170	n.d.	n.d.	n.d.	n.d.	n.d.	3.74	n.d.	n.d.	n.d.	n.d.	n.d.	2.67	2.23	n.d.	n.d.
Beta-citronellol	1209	n.d.	0.90	n.d.	n.d.	n.d.	7.42	n.d.	n.d.	n.d.	n.d.	n.d.	2.62	n.d.	n.d.	n.d.
Geraniol *	1235	124	204	81.2	16.5	99.8	115	79.0	51.6	36.9	36.4	36.5	152	92.7	44.3	67.5
Σ		210	380	236	75.2	139	340	134	107	262	63.9	92.6	189	257	145	109
Sesquiterpenes																
Sesquiterpene hydrocarbons																
Beta-caryophyllene	1419	0.52	n.d.	n.d.	n.d.	0.24	1.19	n.d.	0.71	0.35	0.25	n.d.	n.d.	0.63	1.22	0.51
Alpha-humulene	1434	0.98	n.d.	0.45	0.55	0.79	1.38	0.76	1.74	1.05	0.20	n.d.	1.25	1.34	3.32	0.88
Σ		1.49	n.d.	0.45	0.55	1.04	3.02	0.76	2.45	1.41	0.45	n.d.	1.25	1.98	4.54	1.39
Oxygenated sesquiterpenes																
Caryolan-1-ol	1541	n.d.	n.d.	0.64	n.d.	n.d.	0.74	n.d.	n.d.	n.d.	0.20	n.d.	1.39	1.48	n.d.	0.54
Caryophyllene oxide	1566	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.05	n.d.	n.d.
Humulene epoxide I	1584	n.d.	0.38	n.d.	0.21	n.d.	0.98	n.d.	n.d.	n.d.	0.35	0.86	0.80	n.d.	n.d.	n.d.
Humulol	1588	n.d.	2.26	0.58	n.d.	n.d.	n.d.	0.70	0.45	n.d.	0.25	1.19	2.02	1.43	n.d.	0.56
Humulene epoxide II	1595	0.76	1.60	0.26	0.86	1.55	11.0	0.64	n.d.	n.d.	0.53	n.d.	0.98	n.d.	n.d.	n.d.
Humulene epoxide III	1615	n.d.	n.d.	n.d.	n.d.	0.29	0.51	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.76	n.d.	n.d.
Humulenol II	1634	1.56	2.42	0.41	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.50	3.05	3.29	7.64	4.15	n.d.
Tau-cadinol	1644	0.81	0.93	0.99	n.d.	n.d.	n.d.	0.82	n.d.	n.d.	0.61	1.70	3.06	3.52	3.77	n.d.
Alpha-cadinol	1655	n.d.	n.d.	0.55	n.d.	n.d.	n.d.	0.61	n.d.	n.d.	0.51	0.85	n.d.	1.50	1.80	0.57
Σ		3.13	7.58	3.42	1.07	1.84	13.2	2.77	0.45	n.d.	3.95	7.65	11.5	18.4	9.72	1.67
Others																
4-ethyl phenol	1140	43.0	32.4	34.9	45.1	33.3	41.9	29.8	38.1	33.4	27.0	36.0	39.8	39.9	38.9	33.6
Σ		43.0	32.4	34.9	45.1	33.3	41.9	29.8	38.1	33.4	27.0	36.0	39.8	39.9	38.9	33.6

[a] Calculated retention index (RTX-1 capillary column, 40 m x 0.18 mm i.d. x 0.20 µm film thickness
[b] Beers derived from hop varieties according to table 1
n.d.: not detected

well as oxygenated sesquiterpenoids, whereas the corresponding pitching worts contained only small amounts of these compound groups. Interestingly, in some beers (varieties *Challenger*, *Cluster*, *Columbus*, *Magnum*, *Nugget* and *Warrior*), only very low concentrations of all oxygenated sesquiterpenoids were detected.

The concentration of 2-undecanone was comparably low in all beers, except for the beers that also exhibited high concentrations of oxygenated sesquiterpenoids (varieties *Saaz*, *Simcoe* and *Tettnanger*).

Table 8 summarizes the results of the beers without additional dry hopping (late hopped beers). Total concentrations of the fermentation alcohols do not differ substantially. However, some samples show increased levels, which are mainly due to increased concentrations of isopropanol. Concentrations of the saturated esters are quite comparable with ethyl acetate being the predominant saturated ester in all beers. As expected for hop-derived compounds, the results of the unsaturated esters are more diversified. High concentrations of methyl geranate are characteristic for the beers derived from the hop samples *Centennial*, *Citra* and *Simcoe*. Ethyl *cis*-4-decenoate is the only unsaturated ester that was measured in the late hopped beers *Challenger* and *Sorachi Ace*.

Interestingly, hardly any monoterpene hydrocarbon was detected in the late hopped beers. As mentioned before, only β-myrcene has been found in the beers (varieties *Saaz*, *Simcoe*, *Sorachi Ace* and *Tettnanger)*. The greatest concentration variance was observed for the monoterpene alcohols. With concentrations between 27.5–225 µg/L and 16.5 – 204 µg/L, respectively, linalool and geraniol showed concentration differences by a factor of 10. α-Terpineol (beers of the varieties *Columbus*, *Simcoe* and *Sorachi Ace*) and β-citronellol (beers *Centennial*, *Columbus* and *Simcoe*) were only detectable in 3 beers each in rather low concentrations. As in some cases, those compounds were not present in the corresponding hop samples, since they have been formed in the course of the brewing process. *Takoi* et al. [54] as well as *King* and *Dickinson* [55] already observed that monoterpene alcohols such as β-citronellol and α-terpineol can be formed through biotransformation by different yeast species.

α-Humulene and β-caryophyllene were the only sesquiterpene hydrocarbons detectable in the beer samples and their concentrations

Table 9 **(Semi-) Quantitative data (µg/L) of the 15 dry hopped beers (Dry Hopped). Concentrations are normalized to the hop amount used to produce the dry-hopped beers. Compounds highlighted with an asterisk were analyzed quantitatively**

Compound	RI[a]	CAS[b]	CEN[b]	CIT[b]	CHA[b]	CLU[b]	COL[b]	GAL[b]	MAG[b]	NUG[b]	PAL[b]	SAA[b]	SIM[b]	SOR[b]	TET[b]	WAR[b]
Alcohols																
n-Propanol	545	n.d.	30.3	5.56	44.7	0.44	4.83	n.d.	30.0	4.39	35.1	11.6	5.93	7.99	9.31	4.06
Isobutanol	608	37.3	9.00	16.1	16.2	19.6	11.0	25.0	15.9	17.5	11.9	28.9	32.4	33.3	20.9	17.9
3-Methyl-1-butanol	725	95.3	51.0	57.3	73.8	71.2	68.9	78.0	59.8	59.6	53.6	73.1	78.4	74.1	71.2	57.3
2-Methyl-1-butanol	729	25.5	13.0	15.3	17.2	16.3	16.0	21.2	15.2	15.0	14.6	19.3	18.8	17.3	18.1	14.3
Phenylethanol	1088	17.4	11.6	9.38	25.6	12.5	38.6	6.83	2.77	4.20	5.76	17.1	21.6	17.8	22.4	4.63
∑		176	115	104	177	120	139	131	124	101	121	150	157	150	142	98.1
Esters																
Saturated esters																
Ethyl acetate*	600	97.5	64.2	73.2	71.0	66.1	60.6	75.3	69.0	66.3	68.4	85.9	51.6	49.9	81.4	73.9
Isobutyl acetate*	764	0.66	0.08	0.09	0.10	0.09	0.09	0.10	0.08	0.09	0.09	0.14	0.07	0.08	0.12	0.12
Ethyl butanoate*	791	0.31	0.18	0.21	0.18	0.17	0.15	0.21	0.20	0.18	0.22	0.22	0.20	0.15	0.17	0.19
Isoamyl acetate*	845	4.00	2.15	3.12	3.22	2.48	1.83	2.68	2.49	2.67	3.42	4.05	1.54	1.69	3.24	3.17
Isoamyl propionate	944	0.96	n.d.	1.05	2.69	1.18	7.40	1.59	2.40	1.55	1.35	n.d.	3.69	1.70	n.d.	0.76
Ethyl hexanoate*	962	0.25	0.19	0.23	0.14	0.19	0.07	0.19	0.22	0.08	0.19	0.25	0.13	0.10	0.16	0.21
Isoamyl isobutyrate	996	1.39	3.47	1.46	22.9	6.42	19.5	3.38	15.8	4.61	1.45	n.d.	14.3	1.99	9.34	1.48
2-Methylbutyl butanoate	1003	3.09	9.35	3.73	9.58	3.07	1.58	15.2	n.d.	11.3	6.52	n.d.	19.6	7.50	n.d.	4.69
2-Methylbutyl isopentanoate	1095	n.d.	5.06	n.d.	n.d.	n.d.	n.d.	1.95	n.d.	1.30	2.23	n.d.	n.d.	n.d.	n.d.	1.61
2-Methylbutyl pentanoate	1098	n.d.	3.05	n.d.	n.d.	n.d.	n.d.	1.26	n.d.	1.74	1.56	n.d.	n.d.	n.d.	n.d.	0.76
Ethyl octanoate*	1180	0.25	0.32	0.40	0.33	0.37	0.27	0.19	0.28	0.34	0.36	0.23	0.15	0.15	0.19	0.33
Phenethyl acetate*	1224	0.79	0.47	0.66	0.74	0.67	0.43	0.46	0.47	0.59	0.56	1.05	0.36	0.45	0.67	0.66
Ethyl decanoate *	1377	0.06	0.06	0.09	0.08	0.12	0.06	n.d.	0.05	0.07	0.07	0.06	n.d.	n.d.	0.06	0.07
Ethyl dodecanoate	1572	16.0	9.44	5.01	5.69	14.2	7.29	0.72	7.85	5.50	2.25	n.d.	1.42	1.62	n.d.	5.01
∑		125	98.0	89.3	117	95.0	99.3	103	98.8	96.3	88.7	91.9	93.1	65.3	95.3	93.0
Unsaturated esters																
Methyl *cis*-2-decenoate	1294	n.d.	n.d.	0.50	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Methyl geranate	1298	4.95	80.3	15.1	1.14	0.24	3.91	1.71	1.45	1.09	0.75	4.71	46.5	1.27	12.3	1.43
Neryl acetate	1359	14.7	17.6	5.27	n.d.	0.28	20.7	14.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ethyl *trans*-4-decenoate	1364	8.50	13.7	7.16	1.34	5.01	n.d.	7.23	6.20	7.63	4.37	6.61	14.5	n.d.	8.22	6.18
Ethyl 4.9-decadienoate	1360	11.7	6.87	4.71	2.20	4.31	9.23	4.63	3.68	9.37	n.d.	8.21	14.8	2.83	13.5	3.98
Ethyl *cis*-4-decenoate	1364	n.d.	7.91	n.d.	1.52	2.17	n.d.	n.d.	1.85	1.62	1.78	14.4	5.54	4.04	12.7	1.35
Neryl butyrate	1507	n.d.	1.55	n.d.	n.d.	n.d.	1.71	n.d.	1.82	0.59	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
∑		39.9	128	32.7	6.20	12.0	35.5	27.8	15.0	20.3	6.90	34.0	81.4	8.13	46.7	12.9
Terpenes																
Monoterpene hydrocarbons																
Beta-pinene	958	n.d.	n.d.	n.d.	n.d.	0.33	n.d.	n.d.	1.04	n.d.	0.39	n.d.	n.d.	n.d.	n.d.	n.d.

Beta-myrcene	965	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.75	n.d.	n.d.	24.0	37.3	25.3	35.1	n.d.
∑		n.d.	n.d.	n.d.	n.d.	0.33	n.d.	n.d.	3.79	n.d.	0.39	24.0	37.3	25.3	35.1	n.d.
Monoterpene alcohols																
Linalool*	1085	230	518	496	111	93.9	420	115	189	718	82.4	141	430	263	453	155
Alpha-terpineol	1170	n.d.	n.d.	n.d.	n.d.	n.d.	2.78	n.d.	n.d.	n.d.	n.d.	3.55	8.22	3.59	n.d.	n.d.
Beta-citronellol	1209	1.64	3.90	0.59	n.d.	n.d.	3.38	n.d.	n.d.	n.d.	n.d.	n.d.	6.62	n.d.	n.d.	n.d.
Geraniol*	1235	370	754	189	25.7	210	163	164	95.7	74.0	77.5	89.7	427	241	196	167
∑		601	1276	685	137	303	589	280	285	792	160	235	871	508	650	321
Sesquiterpenes																
Sesquiterpenes hydrocarbons																
Beta-caryophyllene	1419	1.99	2.32	0.73	0.38	0.19	5.38	1.53	4.63	6.21	0.48	0.93	2.59	8.03	4.99	2.25
Alpha-humulene	1434	5.88	6.18	1.93	2.52	0.80	11.00	4.69	21.09	18.30	1.13	3.63	6.22	28.5	16.0	4.07
Beta-calarene	1474	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.27	n.d.	n.d.
Gamma-muurolene	1477	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.55	0.60	n.d.	n.d.	n.d.	n.d.	n.d.	0.33
Beta-selinene	1487	n.d.	n.d.	n.d.	0.43	n.d.	1.68	0.82	n.d.	1.03	n.d.	n.d.	n.d.	1.42	n.d.	0.54
Gamma-amorphene	1495	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.44	n.d.	n.d.	n.d.	n.d.	n.d.	0.73	n.d.	n.d.
Alpha-selinene	1498	n.d.	n.d.	n.d.	0.43	n.d.	1.68	0.82	n.d.	1.03	n.d.	n.d.	n.d.	1.42	n.d.	0.54
Gamma-cadinene	1511	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.81	0.70	n.d.	n.d.	n.d.	1.00	0.90	4.47
Delta-cadinene	1529	0.71	0.75	n.d.	n.d.	n.d.	1.51	0.50	1.28	1.50	n.d.	n.d.	0.90	2.02	1.46	0.43
(*trans*-) Cadina-1.4-diene	1534	n.d.	n.d.	n.d.	n.d.	n.d.	3.70	n.d.	n.d.	0.34	n.d.	n.d.	1.55	0.75	1.49	n.d.
∑		8.57	9.26	2.66	3.34	0.99	23.3	7.98	28.4	29.5	1.60	4.55	11.3	43.7	24.8	12.4
Oxygenated sesquiterpenes																
Caryolan-1-ol	1541	n.d.	n.d.	0.28	n.d.	n.d.	0.77	6.97	n.d.	0.33	0.47	0.57	1.49	1.19	1.63	0.38
Caryophyllene oxide	1566	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.39	2.34	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Humulene epoxide I	1584	n.d.	1.32	n.d.	n.d.	n.d.	1.20	n.d.	n.d.	0.29	0.64	1.33	0.79	n.d.	1.94	0.21
Humulol	1588	n.d.	1.22	n.d.	n.d.	n.d.	n.d.	11.8	n.d.	0.39	0.29	1.33	1.65	1.18	n.d.	0.30
Humulene epoxide II	1595	1.73	4.41	0.25	1.53	n.d.	8.77	0.98	n.d.	0.69	1.00	1.19	1.12	3.19	3.25	0.89
Humulene epoxide III	1615	n.d.	n.d.	n.d.	2.27	0.16	0.66	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.68	n.d.	n.d.
Humulenol II	1634	3.10	n.d.	0.81	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5.66	6.49	7.58	n.d.	n.d.
Tau-cadinol	1644	3.12	3.61	1.42	n.d.	n.d.	n.d.	2.10	n.d.	n.d.	n.d.	3.20	7.04	4.35	n.d.	n.d.
Alpha-cadinol	1655	n.d.	1.60	0.72	n.d.	n.d.	n.d.	1.01	n.d.	0.63	0.82	1.10	2.4	2.14	n.d.	0.79
∑		7.95	12.2	3.48	4.23	0.16	11.4	24.3	2.34	2.33	3.22	14.4	20.9	20.3	6.82	2.58
Others																
4-Ethylphenol	1140	63.9	31.6	36.8	47.2	42.3	40.1	40.9	41.8	44.9	40.1	46.2	55.4	51.9	40.7	40.8
2-Undecanone	1285	1.99	1.37	1.38	3.97	1.24	n.d.	1.53	0.92	1.15	0.58	2.67	7.15	5.92	4.52	1.20
∑		65.9	32.9	38.2	51.2	43.5	40.1	42.4	42.7	46.1	40.6	48.8	62.6	57.8	45.2	42.0

[a] Calculated retention index (RTX-1 capillary column, 40 m x 0.18 mm i.d. x 0.20 µm film thickness

[b] Beers derived from hop varieties according to table 1

n.d.: not detected

Table 10 Transfer rates (%) of linalool into pitching wort (PW), late hopped beers and dry hopped beers for each hop variety

Transfer rate[a] into	CAS[b]	CEN[b]	CIT[b]	CHA[b]	CLU[b]	COL[b]	GAL[b]	MAG[b]	NUG[b]	PAL[b]	SAA[b]	SIM[b]	SOR[b]	TET[b]	WAR[b]
PW	36.0	40.8	13.8	34.1	51.3	96.9	40.6	36.8	37.6	25.8	37.9	37.3	3.61	19.2	40.5
Late hopped beers	19.6	19.1	14.6	19.5	23.6	49.2	22.9	14.4	18.5	11.0	17.8	4.53	29.3	10.9	12.3
Dry hopped beers	46.2	52.4	45.1	24.3	46.0	66.6	35.6	48.7	56.8	30.7	37.9	82.7	25.6	53.7	47.7

[a] Transfer rates for each wort and late hopped beer sample are calculated on the basis of the used hop amount used during early and late kettle hopping. Transfer rates for the dry hopped beer are calculated on the basis of the hop amount additionally used during the dry hopping step
[b] Beers derived from hop varieties according to table 1

rarely exceeded 1.00 µg/L. Low concentrations of those compounds in beers are mainly due to a low solubility and, most importantly, to losses during fermentation including adsorption to yeast cells [49]. In general, concentrations of the oxygenated sesquiterpenoids were comparably low and no clear concentrations or distribution patterns were observed. However, increased concentrations were detected in the late hopped beers derived from the hop varieties *Saaz*, *Simcoe*, *Sorachi Ace* and *Tettnanger* as it was already the case for the pitching worts. Although the concentrations measured in the late hopped beer samples were lower than the concentrations of the pitching worts, the results underline once again that the hop varieties *Saaz*, *Simcoe*, *Sorachi Ace* and *Tettnanger* behave differently from the other hop varieties.

In contrast to the pitching wort samples, 2-undecanone was not detectable in any sample. Instead, 4-ethylphenol as a fermentation product was observed in all beer samples in comparable concentrations (36.5 µg/L ± 9.50 µg/L).

Table 9 summarizes the results of the beers with additional dry hopping. In general, the concentrations of all compound groups are increased compared to the late hopped beers. For the alcohols, the concentrations of especially 2-methyl-1-butanol and 3-methyl-1-butanol increased. This could be due to a slightly higher yeast activity during fermentation thereby releasing those alcohols from the corresponding hop-derived butanoate esters as dry hopping was already started at the end of fermentation. For the saturated esters, only ethyl acetate showed a pronounced concentration increase, whereas the concentrations of all other saturated esters remained at low levels. Again, a higher yeast activity may account for a slightly higher ethyl acetate concentration. This effect resulted either randomly or induced by compounds present in the hop used for dry hopping.

In case of the unsaturated esters, an increase of the total concentrations was especially noticeable for beers derived from the hop varieties *Centennial*, *Columbus* and *Simcoe*, which was caused by increased concentrations of methyl geranate and/or neryl acetate.As observed for the late hopped beers, β-myrcene was found in the beers of the hop varieties *Saaz*, *Simcoe*, *Sorachi Ace* and *Tettnanger*. Yet, concentrations in the dry hopped beers where slightly higher ranging from 24.0 to 37.3 µg/L. Additionally, a low concentration of β-myrcene (2.75 µg/L) was found in the beer derived from the hop variety Magnum. The monoterpene alcohols are the group that was influenced most by the dry hopping process and especially the concentrations of linalool and geraniol were boosted (see paragraph 3.2). α-Terpineol (beers of the varieties *Columbus*, *Saaz*, *Simcoe* and *Sorachi Ace*) and β-citronellol (beers of the varieties *Cascade*, *Centennial*, *Citra*, *Columbus* and *Simcoe*) were only detectable in some beers in rather low concentrations (0.59 – 8.22 µg/L). α-Humulene was the predominant sesquiterpene hydrocarbon in the dry hopped beers (up to 28 µg/L). Besides, low concentrations of several other sesquiterpene hydrocarbons, including β-caryophyllene, were measured in the dry hopped beers. However, their concentrations were always below 5 µg/L. Several oxygenated sesquiterpenoids were detected in all beers albeit in rather low concentrations (< 10 µg/L). Only for the beers derived from the hop varieties *Centennial*, *Columbus*, *Galena*, *Saaz*, *Simcoe* and *Sorachi Ace* elevated concentrations (up to 24.3 µg/L) were observed. Although it could be expected that the concentrations of the oxygenated sesquiterpenoids increased by dry hopping, such effect was clearly not evident. A possible explanation could be the limited solubility of those compounds in the beer matrix.

Besides slightly increased concentrations of 4-ethylphenol, low concentrations of 2-undecanone (< 6 µg/L) were detected in all beer samples (except beer derived from the variety *Columbus*). Elevated concentrations of 4-ethylphenol once again indicated that the yeast performance could have been slightly different. The same behavior was observed for the components ethyl acetate as well as for 2-methyl-1-butanol and 3-methyl-1-butanol. The presence of 2-undecanone was clearly caused by the dry hopping process as this volatile is already present in hops.

Table 11 Transfer rates (%) of geraniol into pitching wort (PW), late hopped beers and dry hopped beers for each hop variety

Transfer rate[a] into	CAS[b]	CEN[b]	CIT[b]	CHA[b]	CLU[b]	COL[b]	GAL[b]	MAG[b]	NUG[b]	PAL[b]	SAA[b]	SIM[b]	SOR[b]	TET[b]	WAR[b]
PW	63.2	91.7	407	38.7	160	151	144	150	150	464	113	46.8	52.0	139	88.3
Late hopped beers	22.5	24.3	118	63.0	71.0	141	47.7	77.0	74.4	200	37.0	31.1	34.2	50.7	25.5
Dry hopped beers	62.0	91.5	219	49.1	109	81.3	72.1	91.9	104	317	75.6	78.0	76.7	244	52.4

[a] Transfer rates for each wort and late hopped beer sample are calculated on the basis of the used hop amount used during early and late kettle hopping. Transfer rates for the dry hopped beer are calculated on the basis of the hop amount additionally used during the dry hopping step
[b] Beers derived from hop varieties according to table 1

3.2 Transfer rates of hop oil-derived compounds

The obtained results indicate that the total concentrations increased during dry-hopping for each compound group. Recently, Forster et al. reported that the levels of linalool in beers can be explained by the linalool content derived from hops whereas the geraniol contents in beers exceeded the introduced amounts [32, 33, 56]. The authors were able to show that the presence of geranyl acetate in hops could account for the high levels of geraniol in beers [57]. Therefore, transfer rates of the most important hop-derived compounds are calculated for the pitching wort, the late hopped beers and the dry hopped beers. Surprisingly, linalool and geraniol were the only compounds for which significant transfer rates were found.

Transfer rates for the dry hopped beers were calculated on the basis of the amount of hops used. The pitching wort samples and the late hopped beers were calculated on basis of the amount of hops used to produce these samples. Transfer rates for the dry hopping minus the amount present in the late hopped beers served to focus on the effect of the dry hopping on the transfer rates.

Transfer rates of linalool

Table 10 summarizes the transfer rates of linalool in the pitching wort, the late hopped and the dry hopped beer samples for all hop varieties. In general, linalool levels of the pitching wort samples ranged from 20 to 40 %. For some samples, lower transfer rates of 3.61 % (variety *Sorachi Ace*) and 13.8 % (variety *Citra*) were observed, whereas variety *Columbus* displayed an almost quantitative transfer rate (96.9 %) of linalool. During subsequent process steps, about 50 % of linalool, present in the pitching wort, was lost, resulting in transfer rates of about 10 to 20 %. Some exceptions were observed at this stage: for the late hopped beer derived from hop variety *Columbus*, a relatively high transfer rate of almost 50 % was observed. Yet, the initial transfer rate compared with the pitching wort samples was reduced up to 50 % as well. More interestingly, the transfer rate of the beer derived from the hop variety *Citra* did not change significantly compared with its pitching wort sample. Surprisingly, for the beer derived from hop variety *Simcoe*, a significant loss was recorded (from 37.3 % to 4.53 %) and for beer derived from the variety *Sorachi Ace*, an increase was observed (from 3.61 to 29.3 %). The reason for this unexpected and contradictory behavior of samples derived from the hop varieties *Citra*, *Simcoe* and *Sorachi Ace* remained unclear.

In general, between 35 and 65 % of linalool present in the hops used for dry hopping were transferred into the final beers. For beers of the varieties *Challenger* and *Palisade*, however, a lower transfer rate of 24.3 and 30.7 %, respectively, was detected. Interestingly, for the beer of the variety *Simcoe* a transfer rate of 82.7 % was observed. Lower concentrations could be due to a more pronounced conversion of linalool to nerol and geraniol [58]. Higher concentrations could be explained by the release of linalool from its corresponding glycosides [59].

Transfer rates of geraniol

Table 11 summarizes the transfer rates of geraniol in the pitching wort samples, the late hopped and the dry hopped beers. Transfer rates for the pitching worts ranged from 38.7 % to 464 %. Interestingly, the hop varieties seemed to be separated in three groups. One group consists of the varieties *Cascade*, *Centennial*, *Challenger*, *Simcoe*, *Sorachi Ace* and *Warrior* with rates up to 91.7 %. Another group comprises the varieties *Cluster*, *Columbus*, *Galena*, *Magnum*, *Nugget*, *Saaz* and *Tettnanger* with rates between 139 and 160 %. The third group is formed by the varieties *Citra* and *Palisade* with transfer rates above 400 %. In general, a reduction of the yield of geraniol is observed for the late hopped beers. Transfer rates are varying between 22.5 % (variety *Cascade*) and 77.0 % (variety *Magnum*). Only the varieties *Citra*, *Columbus* and *Palisade* show transfer rates above 100 %. The additional dry hopping step reveals transfer rates between 49.1 % and 317 %. Forster et al. [32, 33, 56] provided a possible explanation for transfer rates above 100 %. In an initial study on 4 hop varieties, they found evidence that the transfer rates of geraniol are either around 50 % or above 130 %. Previous studies [55, 57] were able to show that, when geraniol is taken into account as released from geraniol acetate, transfer rates varied between 38 % and 62 %. As the geraniol concentrations in the dry hopped beers (Table 9) were not able to be explained by high transfer rates and no geraniol acetate was detected in the analyzed hop varieties, it is feasible that other hop-derived precursors such as neryl acetate, neryl butyrate and methyl geranate (see Table 7) as well as glycosylated volatiles [59] may be involved in the formation of geraniol during beer production.

4 Conclusions

In the hop samples, especially the concentrations of unsaturated esters, monoterpene and sesquiterpene hydrocarbons and oxygenated sesquiterpenoids showed great differences. In general, the varieties *Challenger*, *Cluster*, *Columbus*, *Palisade* and *Warrior* showed the lowest concentrations of the mentioned compound classes. Varieties *Cascade*, *Centennial*, *Citra*, *Galena*, *Magnum* and *Nugget* featured medium concentrations and the highest concentrations were found for the varieties *Saaz*, *Simcoe*, *Sorachi Ace* and *Tettnanger*. Results indicated furthermore a direct correlation of the concentrations of linalool and geraniol as well as for the concentrations of β-myrcene and β-pinene in the hop samples. Yet, these characteristic patterns were not carried over to the late hopped or dry hopped beers.

Total concentrations of higher alcohols and esters in beers were mainly influenced by the fermentation process and were found to be independent of the hop variety used for additional dry hopping, as similar concentrations were found for all late and dry hopped beers. However, the total concentrations of esters greatly differed before the fermentation and were found to be variety-dependent. Furthermore, it was observed that the occurrence as well as the concentration of volatiles is influenced by the hop variety, even after the fermentation.

The levels and the compositions of the “floral” (the oxygenated fraction of hop essential oil comprising monoterpene alcohols, esters, ketones and aldehydes) and the sesquiterpenoid hop oil fractions significantly changed along the brewing process. The fermentation was identified as the crucial process step, as indicated previously [49, 60]. Late and dry hopping showed a high impact on the levels

but not on the qualitative composition of the hop-derived volatile fractions in worts and beers.

As the qualitative profiles of the analyzed beers showed only small differences, mainly quantitative changes were noticed. Therefore, it is likely that the interaction of several volatile compounds affect the perceived aroma impressions. Especially the samples derived from the varieties *Saaz*, *Simcoe*, *Sorachi Ace* and *Tettnanger* behaved clearly different from all other samples. For example, high concentrations of monoterpene hydrocarbons and oxygenated sesquiterpenoids are increased in the pitching worts as well as in the beer samples, whereas only small concentrations were detected in the hop samples. In order to investigate possible interactions, it is essential to perform sensorial reconstitution and omission experiments to investigate the interactions of single compounds and compound classes. The present study demonstrates that dry hopping boosts individual volatiles (e.g., linalool and geraniol) with a floral aroma and oxygenated β-caryophyllene and α-humulene derivates for spicy, herbal and woody aroma perceptions [61, 62] for beers derived from the hop varieties *Galena*, *Simcoe* and *Sorachi Ace*.

Calculations of the transfer rates of the most predominant hop-derived essential oil compounds revealed that linalool and geraniol are the only compounds for which significant transfer rates were found for the pitching wort, the late hopped beers and the dry hopped beers. The determined transfer rates of linalool and geraniol indicated a strong varietal impact on final product concentrations for the dry hopped beers. Regarding synergistic effects, this may indicate that interactions with other volatiles could account for differently perceived aromas of dry hopped beers. As already observed by Forster et al. [32, 33, 56, 57], the occurrence of geraniol could not entirely be explained by hop-derived free geraniol, but must be released or intrinsically formed from precursors such as esters [57] or glycosides [58].

From a practical point of view, the results of this investigation, combined with further insights that will be obtained from following projects, will help to improve hop utilization and will open up new perspectives in view of enhanced quality control of hop aromatic character and the prediction of varietal fresh hoppy flavors in beers.

Notes

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