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Characterization of Novel Varietal Floral Hop Aromas by Headspace Solid Phase Microextraction and Gas Chromatography−Mass Spectrometry/Olfactometry

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ABSTRACT: In this study, headspace solid phase microextraction (HS-SPME) and gas chromatography−mass spectrometry (GC-MS) were optimized and implemented to investigate the volatile composition of novel floral hop essences prepared from four German aroma hop varieties. In total, 91 different constituents were assigned, which were further grouped into monoterpene hydrocarbons, esters, ketones, aldehydes, furans, and oxygenated and nonoxygenated sesquiterpenes. Most volatiles belong to the ester group, whereas the monoterpene hydrocarbon β-myrcene appears to be the predominant compound in all hop oil preparations investigated. Furthermore, as demonstrated by principal component analysis, varietal floral hop essences are clearly discriminated on the basis of their characteristic volatile composition. Via GC−olfactometry on the floral essence variety Spalter Select, β-myrcene and 2-undecanone were identified as the most potent odorants. Several hop oil constituents were reported for the first time as impact odorants of hop aroma.

KEYWORDS: *hop aroma, hop essences, headspace solid phase microextraction, gas chromatography−mass spectrometry, gas chromatography−olfactometry*

INTRODUCTION

A pleasant beer flavor is the result of a fine and subtle balance between numerous volatile and nonvolatile chemical compounds originating or derived from the brewing raw materials. A key role for beer flavor and consumers' appreciation of the final product is attributed to the use of hops (*Humulus lupulus* L.). Indeed, although hops or hop products represent only a minor ingredient compared to brewing water or malt, hops have a determining impact on the organoleptic properties of beer by imparting typical beer bitterness and hoppy aroma.

The typical and pleasant aroma characteristics of fresh hops are assigned to the composition of hop essential oil present in the lupulin glands of the female hop flowers. Hop essential oil is quantitatively a relatively small and volatile fraction representing 0.5−3.0% (v/w) of dried hop cones. Its composition is however enormously complex, and more than 400 different chemical compounds have been identified.[1] It has even been suggested that hop oil comprises over 1000 different volatiles.[2] The amount and chemical composition of the essential oil of hops mainly depends on the hop variety, although the composition may be influenced by agronomic factors such as the place of growth or seasonal aspects.[3−5]

Gas chromatography−mass spectrometry (GC-MS) is successfully applied for the analysis of essential oils in general[6] and for the identification and quantification of hop oil constituents in particular. For sample preparation, vacuum distillation or steam distillation−solvent extraction,[7−10] extraction using organic solvents[11] or supercritical carbon dioxide[12,13] and headspace sampling[14,15] have been applied to isolate and characterize hop essential oil. In the past decade, fast and automated isolation techniques yielding increased sample concentrations were developed for the characterization of essential oils, including the volatile fraction from hops, for example, direct thermal desorption[16] (DTD) and headspace solid phase microextraction[17,18] (HS-SPME). Because differences between hop varieties can be observed with respect to their sensory characteristics and essential oil composition, many studies focused on varietal recognition through chromatographic fingerprinting of hop oil.[7,8,14,16,17,19−26]

Gas chromatography−olfactometry (GC-O) has been applied extensively in studies regarding the sensory activity of individual components of the odors of various alcoholic beverages, including beer.[27] Already in 1983, Fukuoka and Kowaka[28] published an early paper on GC-O analysis of beer aroma, aiming at identification of hop-derived volatiles imparting herbal flavor notes. Since then, several authors have reported the use of GC-O in the field of beer flavor research in general and hop aroma (the odor/aroma of hops as such) and hoppy aroma (the odor/aroma derived from hops in final beer) in particular. As a result, a relatively high number of character impact compounds of hop aroma and hoppy aroma of beer have been proposed in the literature.[29] For example, the monoterpene alcohols linalool and geraniol are associated with floral impressions in hops and beer.[30,31] Esters are known for their fruity notes,[32−36] whereas oxidation products of the main sesquiterpene hydrocarbons are supposed to be associated with the spicy/herbal hop character of beer.[11,12,34,37] Some hop-derived sulfur compounds may also play an important role in the unique flavor palette of special types of beers.[38−42] Nevertheless, due to the enormous chemical complexity, our

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Table 1. Selected Marker Constituents of Floral Hop Essence for Evaluation of the Extraction Efficiency of Different SPME Fiber Coatings, Relative Composition of Floral Hop Essence cv. Hallertau Tradition upon Using Different SPME Extraction Times (SPME: PDMS (100 μm), 40 °C) or Direct Injection of the Essence, and Coefficients of Variation, Regression Coefficients, and Estimated Limits of Detection for the Marker Constituents

					SPME (PDMS, 100 μm) extraction time at 40 °C						liq inj[a]			
compd	RI[b]	MW[c]	compd class[d]	ident[e]	5 min	15 min	30 min	40 min	50 min	60 min	1 μL	CV[f] (%)	R^{2g}	LOD[h] (μg/L)
β-pinene	972	136	MH	MS, RI, RC	0.7[i]	0.6	0.5	0.5	0.5	0.5	0.6	3.2	0.997	0.06
β-myrcene	988	136	MH	MS, RI, RC	86.4	78.5	72.8	69.7	68.0	67.2	73.7	2.8	0.998	0.08
2-methylbutyl 2-methylpropanoate	1003	158	BE	MS, RI	0.9	0.8	0.8	0.8	0.8	0.8	0.6	3.0	nd[j]	nd
perillene	1089	150	MF	MS, RI	1.0	0.9	0.8	0.8	0.7	0.7	0.8	4.5	nd	nd
methyl octanoate	1108	158	ME	MS, RI, RC	0.4	0.6	0.7	0.7	0.7	0.7	0.8	3.1	0.999	0.24
2-methylbutyl 2-methylbutanoate	1091	172	BE	MS, RI, RC	1.0	1.3	1.5	1.6	1.6	1.6	1.6	2.7	0.999	0.06
2-decanone	1174	156	K	MS, RI, RC	0.3	0.4	0.5	0.6	0.6	0.7	0.5	2.4	0.998	0.20
methyl nonanoate	1208	172	ME	MS, RI, RC	0.8	1.1	1.3	1.4	1.5	1.4	1.4	3.1	0.999	0.05
2-undecanone	1276	170	K	MS, RI, RC	3.7	6.7	8.6	9.6	10.2	10.4	8.0	1.8	0.997	0.08
methyl *trans*-4-decenoate	1292	184	UE	MS, RI	3.3	5.9	7.7	8.5	8.9	9.2	7.3	2.3	nd	nd
2-dodecanone	1377	184	K	MS, RI, RC	0.8	1.6	2.3	2.7	3.1	3.1	2.2	1.7	0.996	0.06
2-tridecanone	1479	196	K	MS, RI, RC	0.7	1.6	2.5	3.1	3.4	3.7	2.5	2.1	0.997	0.05
sum relative areas (%)					100	100	100	100	100	100	100			

[a]Liquid injection of the floral essence; relative composition calculated on the basis of absolute peak areas; five liquid injections (coefficient of variation for all compounds <2.6%). [b]Calculated retention index (RTX-1 capillary column, 40 m × 0.18 mm i.d. × 0.20 μm film thickness). [c]Molecular weight. [d]Chemical compound class of the identified constituent: MH, monoterpene hydrocarbon; BE, branched ester; MF, monoterpenoid furan; ME, methyl ester; K, ketone; UE, unsaturated ester. [e]Identification based on mass spectrum (MS), literature retention index (RI), and reference compound (RC). [f]Coefficient of variation based on peak areas determined via HS-SPME-GC-MS analyses (5 times, extraction time: 30 min, extraction temperature: 40°C, PDMS 100 μm). [g]Regression coefficient via linear regression. [h]limit of detection: concentration at which signal to noise ratio (S/N) is 3. [i]Relative composition is calculated on the basis of absolute peak areas; fve SPME extractions at each extraction time. [j]Not determined, reference compound not available.

current understanding of the sensory attributes of hop aroma and especially hoppy aroma is far from complete.

During the past decades, knowledge collected through chemical and sensory analyses of hop essential oils and hopped wort and beer has led to the development of commercially available, so-called advanced hop oil products. It has been argued that advanced beer aromatization using such hop oil preparations offers high potential in brewing practice in view of the enhanced reproducibility in the intensity and quality of hoppy aroma and the development of new beers with pleasant and distinct flavor attributes. However, the spectrum of hop oil products currently available is rather limited, and in particular the typical varietal nature of hoppy aroma is not fully exploited as most commercial hop essences are generic preparations. Therefore, we developed a new hop aroma methodology based on supercritical fluid extraction (SFE) with carbon dioxide for the production of novel single-variety total hop essential oils and hop oil essences with floral, citrus, and spicy sensory attributes. The flavoring potential and varietal character of the novel hop oil preparations were clearly demonstrated in our previous papers.[43,44]

The present study aims at detailed characterization of the volatile composition of novel floral hop essences prepared from four German aroma varieties and at determination of the odor-active constituents in the essences. For that purpose, an analytical procedure based on HS-SPME is developed and olfactometric methods are performed on the GC effluent of SPME extracts from the floral essence cv. Spalter Select. Results from GC-O are combined with GC-MS analysis in an attempt to identify and allocate 'floral' hop aroma impact compounds.

MATERIALS AND METHODS

Chemicals. All reference compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were of analytical grade: α-humulene (≥98.0%), α-pinene (98.0%), α-terpinene (≥95.0%), β-caryophyllene (98.5%), β-myrcene (≥95.0%), β-pinene (99.0%), camphene (95.0%), caryophyllene oxide (≥99.0%), decanal (≥98.0%), 2-decanone (99.5%), 2,6-dimethyl-2,4,6-octatriene (≥80.0%), dodecanal (≥95.0%), 2-dodecanone (97.0%), ethyl nonanoate (≥98.0%), γ-terpinene (≥97.0%), hexyl 2-methylbutanoate (≥98.0%), hexyl 2-methylpropanoate (98.0%), limonene (97.0%), linalool (98.5%) 2-methylbutyl hexanoate (≥98.0%), 2-methylbutyl 2-methylbutanoate (90.0%), 3-methylbutyl 2-methylbutanoate (≥98.0%), 3-methylbutyl 2-methylpropanoate (98.0%), methyl decanoate (99.5%), methyl heptanoate (99.0%), methyl 2-methyl-propanoate (99%), methyl nonanoate (99.8%), methyl 3-nonenoate (≥96.0%), methyl octanoate (99.8%), nonanal (95.0%), 2-nonanone (99.5%), ocimene (≥90.0%, mixture of isomers), octyl propanoate (≥99.0%), *p*-cymene (99.0%), terpinolene (≥90.0%), 2-tridecanone (97.0%), undecanal (97.0%), and 2-undecanone (99.0%).

Carbon dioxide (≥99.998%) was purchased from Air Liquide Benelux (Luik, Belgium); 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).

Plant Material. Single-variety floral hop essences were prepared from hop pellets T90 (crop year 2007) from the varieties Hallertau Tradition, Saphir, Spalter Select, and Tettnanger (HVG, Wolnzach, Germany). Pellets (250 g) were stored under recommended conditions (cold storage at 0 °C, packaged under vacuum in metallized polyethylene laminates)[45] to prevent oxidative transformations of the brewing principles. Prior to extraction, the hop material (50 g) was disrupted using a mortar and pestle to facilitate subsequent extraction. Stainless steel extraction cells (10 mL) were filled with ground hop

pellets (6 g), and triplicate extractions using supercritical carbon dioxide were performed as described below.

Preparation of Single-Variety Floral Hop Oil Essences. Single-variety floral essences were prepared according to our hop aroma extraction technology, based on density programmed supercritical fluid extraction (SFE) using carbon dioxide and subsequent solid phase extraction (SPE) using ethanol/water mixtures for further fractionation of SFE extracts.

Ground hop pellets were extracted using a Dionex SFE-703 supercritical fluid extractor (Dionex, Sunnyvale, CA, USA). A carbon dioxide density of 0.29 g/mL was applied, the extracted volatiles were collected in ethanol, and further fractionation of the SFE extracts was performed via solid phase extraction. Varian Bond Elut C18 cartridges (500 mg) (Varian, Palo Alto, CA, USA) were employed for this purpose. For more details on the extraction/fractionation procedure, reference is made to our paper on the production of novel varietal hop aromas by supercritical fluid extraction of hop pellets.[44]

Headspace Solid Phase Microextraction (HS-SPME) for Isolation of Hop Oil Volatiles. Headspace solid phase microextractions of hop oil preparations were automated using a CombiPal autosampler (CTC Analytics, Zwingen, Switzerland). Preliminary experiments were performed to obtain GC-MS profiles from floral hop essences that provide maximum qualitative information while maintaining good chromatographic separation of the extracted volatiles. In conclusion, floral essences were diluted prior to extraction of the headspace volatiles by pipetting a volume of 50 μL into 5 mL of Milli-Q water in a carbon dioxide purged extraction vial (20 mL). Next, the extraction vial was immediately closed with a magnetic bimetal crimp cap containing a silicone/Teflon septum (Interscience, Louvain-la-Neuve, Belgium).

Comparison of Different Fiber Coatings for Isolation of Hop Oil Volatiles from Floral Essences. For isolation of the volatiles, SPME fibers with different coatings, that is, polydimethylsiloxane (PDMS, 100 μm), polydimethylsiloxane−divinylbenzene (PDMS-DVB, 65 μm), divinylbenzene−carboxen−polydimethylsiloxane (DVB-CAR-PDMS, 85 μm), and polyacrylate (PA, 85 μm), were tested (Supelco, Bellefonte, PA, USA). The extraction fiber was exposed into the headspace of the vial (25 mm); extraction time and temperature were set at 30 min and 40 °C for extraction. Before the actual extraction was begun, samples were preincubated at the respective temperature for 5 min. During preincubation and extraction, samples were stirred at 500 rpm.

From a qualitative point of view, the resulting GC-MS profiles were very similar for the different fibers. On the basis of the extraction efficiency of 12 marker compounds (for selected markers see Table 1) and the lowest contamination originating from the fibers in the analytical profiles, PDMS-coated fibers (100 μm) were selected for further analyses on the volatile composition of floral essences (data not shown).

Evaluation of SPME Extraction Time (PDMS, 100 μm). Different SPME extraction times were evaluated by comparison of relative peak areas of the 12 selected marker compounds extracted from floral hop essence cv. Hallertau Tradition as a function of extraction time (see Table 1). The extraction time at which equilibrium is reached depends on the particular compound. Longer SPME extraction times give rise to an increased extracted level of less volatile compounds and, consequently, the relative composition of the extracted essence depends on the applied extraction time (see Table 1). An extraction time of 30 min is chosen as a compromise for HS-SPME of floral hop essences as the relative composition of the SPME fraction is most similar to the relative composition of the essence as determined via direct liquid injection.

GC-MS Conditions for Separation and Detection of the Extracted Volatiles. Gas chromatographic operating conditions were as follows. Extracted volatiles were thermally desorbed in the heated inlet (split/splitless injector, 250 °C) of the Ultra Trace gas chromatograph (Interscience, Louvain-la-Neuve, Belgium) for 3 min. Helium (Alphagaz 2, Air Liquide, Luik, Belgium) was used as a carrier gas at a constant flow 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 Corp., 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 (1 min isotherm).

Mass spectrometric detection of volatiles was performed by a dual stage quadrupole MS (DSQ I, Interscience) 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. Retention indices were determined by using a homologous series of normal alkanes (C8−C18; 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 MS match factor <750 and literature RI significantly different from the calculated RI were considered as "unknown".

Determination of Coefficients of Variation (CV), Regression Coefficients, and Detection Limits. CV for marker compounds were determined by analyzing a floral essence (five times) according to the optimized experimental HS-SPME-GC-MS conditions as described above.

Regression coefficients, detection limits, and quantitative data on marker constituents were determined via 12-point calibration curves. For that purpose, 5 mL ethanol/water (5% ethanol, v/v) solutions spiked with authentic reference compounds (concentration range 0.05−200 μg/L) were extracted via SPME. Thirty microliters of dodecane was added as an internal standard (1.66 μg C12/mL ethanol). All calibration curves were determined in triplicate. Regression coefficients (R^2) were determined by plotting the ratio of peak area of the marker component to the area of internal standard as a function of compound concentration. Detection limits (LOD) for the marker constituents were estimated by determining the signal-to-noise ratio (S/N) for the marker constituents via the Signal to Noise Calculator, v1.1.0.13 (Interscience). The detection limit was the concentration when S/N equals 3.

Table 1 shows CVs, R^2 values, and LODs for representative markers of floral hop essence. Clearly, the developed SPME method (PDMS, 100 μm; extraction time = 30 min; extraction temperature = 40 °C) affords good linearity (R^2 > 0.996) within a wide range of concentrations (0.05−200 ppb), low CVs (CV ≤ 4.5%), and low LODs, ranging from 0.05 μg/L (methyl nonanoate) to 0.24 μg/L (methyl octanoate).

Sensory Analysis. *Odor Characteristics of Varietal Floral Hop Essences.* Odor characteristics of varietal floral essences (cv. Hallertau Tradition, cv. Saphir, cv. Spalter Select, and cv. Tettnang Tettnanger) were evaluated via descriptive sensory analysis by a trained taste panel (12 panelists). Ethanol/water solutions (5% ethanol, v/v) were spiked with floral essences (level of addition 20 ppb), and the odor was evaluated by carefully sniffing glass vials containing the respective hop essences. Panelists were asked to assign odor descriptors ('fresh hops', 'floral', 'citrus', 'fruity', 'green/grassy') and to score the intensity of the selected descriptors on a scale ranging from 0 (not perceptible) to 8 (very intense odor). Sensory analysis of the four varietal hop essences was performed in one session. The session was repeated three times, and the mean score for each descriptor was further used in principal component analysis (PCA).

Gas Chromatography−Olfactometry. For olfactory assessment, a Sniffer 9000 system (Brechbüchler Inc., Schlieren, Switzerland) was coupled to the GC-MS. The effluent was split to the mass spectrometer (50%) and the sniffing port (50%). The transfer line connecting the GC to the olfactory port, as well as the heated block of the sniffing device, was maintained at 280 °C. Volatiles eluting at the sniffing port were presented to the assessors in a stream of humidified

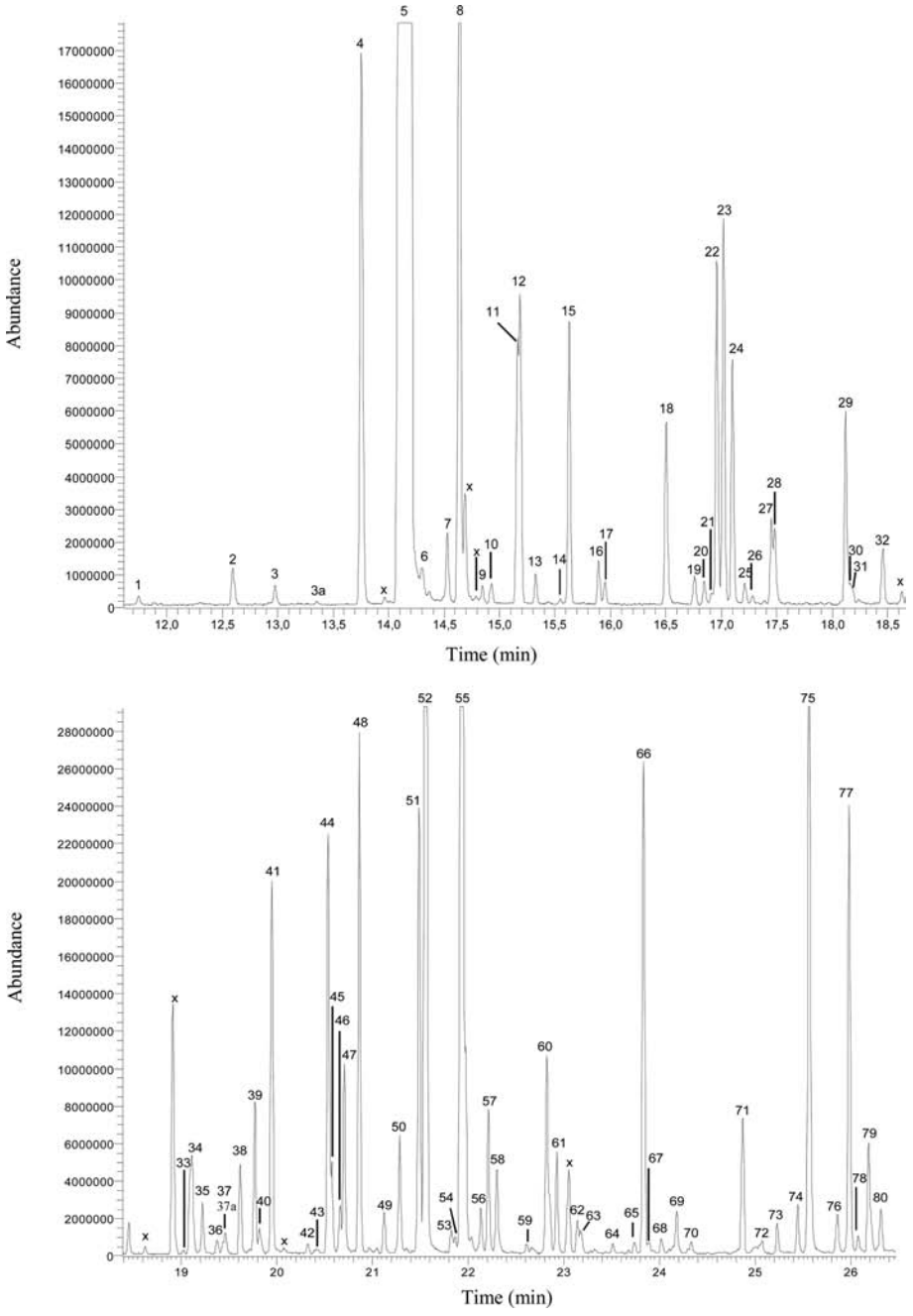

Figure 1. HS-SPME-GC-MS profile (TIC) of floral SFE/SPE hop essence cv. Hallertau Tradition. (Peak numbering in accordance with numbering in Table 2; x, column or septum bleed.)

air. Assessors were asked to describe the odor of eluting compounds and to record the duration of odor perception by using a hand-held control unit with cursor wheel for signal generation. Assessors were thoroughly trained for odor detection and description using reference compounds (linalool, β-myrcene, 2-nonanone, 2-undecanone, methyl octanoate, nonanal, α-humulene, β-caryophyllene, caryophyllene oxide) and total hop essential oil prepared by SFE.[43]

GC-O on Floral Hop Essence cv. Spalter Select. Prior to SPME extraction and subsequent GC-O, the floral hop essence was diluted 50 times. Dilution of the essence was required because preliminary tests showed that sniffing the undiluted extract resulted in overwhelming odor impressions at the sniffing port; that is, a constant green-floral background odor was noted during the whole time of the analysis. Sniffing a 50-fold dilution of floral hop essence appeared to be the best compromise for olfactory assessment.

Both a detection frequency method (olfactory global analysis, OGA) and aroma extract dilution analysis (AEDA) were applied in different sessions.

OGA was carried out by 10 trained assessors sniffing the effluent of splitless injected SPME extract of 50-fold diluted floral hop essence. Analyses were repeated three times by each assessor. Assessors were asked to describe the sensory attributes of eluting odorants as well as to generate a signal by the cursor wheel of the olfactometric unit when odorants were perceived ("on" signal) until the odor changed in character or vanished ("off" signal).

In AEDA, serial dilution of the SPME extract was applied by changing the GC injection conditions, that is, splitless injection (dilution 1) and split injection (split ratio 1/10, dilution 10; split ratio 1/20, dilution 20; split ratio 1/40, dilution 40, respectively) of SPME extract of 50-fold diluted floral hop essence. Sniffing sessions for

Table 2. Volatile Composition of Single-Variety Floral Hop Essences cv. Hallertau Tradition (HAL), cv. Saphir (SAP), cv. Spalter Select (SPA), and cv. Tettnanger (TET)

compd	peak[b]	RI[c]	relative composition[a] (%) HAL	SAP	SPA	TET	odor description[d]	ident[e]
ESTERS								
SATURATED ESTERS								
propanoates								
2-methylbutyl propanoate	3a	958	0.005	nd[f]	nd	nd	sweet, fruity, apple, melon (A)	MS, RI
heptyl propanoate	37a	1188	0.023	nd	nd	nd	rose, apricot (C)	MS, RI
octyl propanoate	53	1287	0.072	0.048	0.022	0.106	waxy, myrtle berries, pineapple (C)	MS, RI, RC
butanoates								
heptyl butanoate	45	1234	0.188	0.124	0.042	0.030	fruity, herbaceous (A)	
2-methylpropanoates								
methyl 2-methylpropanoate	1	902	0.010	0.005	nd	nd	pineapple (C)	MS, RI, RC
3-methylbutyl 2-methylpropanoate	7	999	0.053	0.033	0.031	nd	spicy (D)	MS, RI, RC
2-methylbutyl 2-methylpropanoate	8	1003	0.611	0.413	0.269	0.044	fruity (A)	MS, RI
pentyl 2-methylpropanoate	14	1037	0.003	0.005	0.005	nd	apricot (C)	MS, RI
hexyl 2-methylpropanoate	29	1135	0.198	0.134	0.141	0.023	green, fruity, apple, pear (C)	MS, RI, RC
heptyl 2-methylpropanoate	44	1233	1.13	0.583	0.462	0.207	apricot, cherry, apple, green (C)	MS, RI
octyl 2-methylpropanoate	60	1332	1.08	0.432	0.301	0.105	earthy, fatty, green, woody (C)	MS, RI
2-methylbutanoates								
2-methylpropyl 2-methylbutanoate	6	991	0.079	nd	nd	nd	sweet, fruity (A)	MS, RI
3-methylbutyl 2-methylbutanoate	21	1087	0.011	nd	nd	nd	fruity (A), citrus (C)	MS, RI, RC
2-methylbutyl 2-methylbutanoate	23	1091	0.358	0.175	0.147	0.020	fruity, apple (A)	MS, RI, RC
hexyl 2-methylbutanoate	42	1224	0.026	0.011	0.018	nd	fresh, green, fruity (A)	MS, RI, RC
heptyl 2-methylbutanoate	59	1322	0.040	0.013	0.030	nd	apple (A)	MS, RI
3-methylbutanoates								
2-methylbutyl 3-methylbutanoate	24	1094	0.242	0.094	0.067	0.016	apple, fatty, minty, herbaceous (C)	MS, RI
hexyl 3-methylbutanoate	43	1228	0.015	nd	nd	nd	unripe fruit, apple, strawberry (C)	MS, RI
hexanoates								
2-methylpropyl hexanoate[g]	30	1136	0.025	0.039	0.046	0.014	apple, pineapple, fruity (C)	MS, RI
methylbutyl hexanoate	40	1202	0.075	nd	nd	nd		MS
2-methylbutyl hexanoate	46	1238	0.113	0.087	0.033	0.086	ethereal (C)	MS, RI, RC
heptanoates								
methyl heptanoate	8a	1007	nd	0.019	nd	nd	fruity (C)	MS, RI, RC
2-methylbutyl heptanoate	61	1335	0.445	0.237	0.085	0.060		MS, RI
octanoates								
methyl octanoate	27	1108	0.062	0.241	0.024	0.023	waxy, orange (H), fruity, green (A)	MS, RI, RC
3-methylbutyl octanoate	72	1435	0.067	0.035	nd	nd	fruity, green, soapy, pineapple (C)	MS, RI
methylheptanoates								
methyl 2-methylheptanoate	16	1050	0.032	0.034	0.019	0.035	pineapple (C)	MS, RI
methyl 6-methylheptanoate	18	1072	0.127	0.228	0.113	0.094		MS, RI
nonanoates								
methyl nonanoate	41	1208	0.757	0.994	0.180	0.335	citrus (B)	MS, RI, RC
ethyl nonanoate	48	1247	1.41	0.378	0.606	1.60	fruity, rose, nutty, oily (C)	MS, RI, RC
methyl octanoates								
methyl 4-methyloctanoate	35	1178	0.097	0.037	0.051	0.018		MS
dimethylheptanoates								
methyl 2,6-dimethylheptanoate	28	1109	0.057	0.044	0.032	0.039		MS, RI
decanoates								
methyl decanoate	58	1308	0.310	0.503	0.070	0.279	oily, fruity (A)	MS, RI, RC
dimethyloctanoates								
methyl 4,6-dimethyloctanoate	50	1264	0.356	0.224	0.200	0.525		MS
UNSATURATED ESTERS								
methyl 3-nonenoate	38	1194	0.144	0.172	0.102	nd	fruity, green, pear-like, melon(C)	MS, RI, RC
methyl *trans*-4-decenoate	55	1292	7.02	5.75	3.16	5.76	fruity (G)	MS, RI
methyl *cis*-4-decenoate	56	1300	0.127	0.046	0.070	0.155		MS, RI
methyl geranate	57	1304	0.368	0.917	0.435	0.819	floral (A)	MS, RI
ethyl *cis*-4-decenoate	64	1363	0.037	0.025	0.035	0.052	green, fruity, waxy, leathery (C)	MS, RI
methyl 10-undecenoate	67	1385	0.068	0.014	0.036	0.029	banana, honey, rose, earthy (C)	MS
methyl 3,6-dodecadienoate	69	1392	0.149	0.070	0.074	0.357	floral (B)	MS

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Table 2. continued

compd	peak[b]	RI[c]	relative composition[a] (%) HAL	SAP	SPA	TET	odor description[d]	ident[e]
methyl 10-undecenoate (isomer, RI = 1385)	70	1400	nd	0.014	nd	nd	banana, honey, rose, earthy (C)	
methyl *trans*-3-dodecenoate	78	1483	0.081	0.034	0.039	0.047		MS
methyl 3,6-dodecadienoate (isomer, RI = 1392)	79	1488	0.534	0.388	0.224	1.26	floral (B)	MS
UNIDENTIFIED ESTERS								
m/z 88, 101	25	1099	0.026	0.004	0.018	nd		
m/z 88, 101	32	1148	0.060	0.022	0.038	0.052		
m/z 74, 87, 143	51	1273	1.41	0.512	0.542	0.651		
m/z 88, 101, 143	62	1346	0.116	0.028	0.051	0.229		
m/z 88, 101, 166	63	1347	0.107	0.014	0.087	0.080		
m/z 74, 87, 157	65	1373	0.065	0.020	0.038	0.037		
m/z 74, 87, 143	66a	1379	0.033	nd	nd	nd		
TERPENES								
MONOTERPENES								
α-pinene	2	932	0.048	0.057	0.045	0.050	fruity, green (A); piney, resinous (B)	MS, RI, RC
camphene	3	945	0.029	0.019	0.024	0.022	camphoraceous, oily (B)	MS, RI, RC
β-pinene	4	972	0.824	1.07	0.920	0.858	resinous, dry, woody (B)	MS, RI, RC
β-myrcene	5	988	67.6	66.7	73.6	62.6	geranium-like, lemon, woody (A, D)	MS, RI, RC
cis-dihydroocimene	6a	997	nd	0.010	0.025	nd		MS
α-terpinene	9	1011	0.016	0.022	0.021	0.026	herbaceous, citrus, woody, spicy (C)	MS, RI, RC
p-cymene	10	1014	0.030	0.037	0.030	0.024	fruity, sweet, lemon, spicy (A)	MS, RI, RC
β-phellandrene	11	1022	0.507[h]	0.608[h]	0.634[h]	0.601[h]	terpenic, fruity, spicy (A)	MS, RI
limonene	12	1023					green, citrus, fruity (A)	MS, RI, RC
cis-*β*-ocimene	13	1028	0.038	0.071	0.042	0.034	citrus, terpene, woody, green (C)	MS, RI, RC
trans-*β*-ocimene	15	1040	0.312	1.272	0.322	0.248	citrus, terpene (C)	MS, RI, RC
γ-terpinene	17	1051	0.020	0.019	0.027	0.027	citrus, terpene, spicy (A)	MS, RI, RC
terpinolene	19	1082	0.028	0.031	0.041	0.035	woody, fruity, piney (A)	MS, RI, RC
unknown (*m/z* 93, 136)	28a	1121	nd	0.011	nd	nd		
SESQUITERPENES								
nonoxygenated								
β-caryophyllene	71	1426	0.031	0.510	0.036	0.029	green, spicy, woody, terpene (A)	MS, RI, RC
α-humulene	75	1459	0.093	1.432	0.086	0.103	oily, green, woody (A)	MS, RI, RC
γ-cadinene	81	1521	0.010	nd	nd	nd	thyme, herbal, woody (A)	MS, RI
oxygenated								
caryophyllene oxide	82	1581	0.127	0.159	0.178	0.155	cedar, lime, floral, cosmetic (E, F)	MS, RI, RC
humulene epoxide II	83	1605	0.189	0.111	0.211	0.273	moldy, cedar, lime (E, F)	MS, RI
humulene epoxide III	84	1628	0.043	0.019	0.052	0.064	cedar (E)	MS, RI
τ-cadinol	85	1634	0.004	0.005	0.004	0.003		MS, RI
KETONES								
unidentified (*m/z* 58)[j]	31	1137	tr[i]	tr	tr	tr		
2-decanone	34	1174	0.275	0.534	0.394	0.550	citrus (A, B)	MS,RI, RC
unidentified (*m/z* 58)	47	1240	0.480	0.625	1.45	1.82		
5-undecen-2-one	49	1257	0.090	0.145	0.277	0.494		MS
2-undecanone	52	1276	5.58	7.38	6.70	11.1	fruity, citrus (A)	MS, RI, RC
unidentified (*m/z* 58)	73	1443	0.164	0.147	0.445	0.318		
cis-5-tridecen-2-one	74	1453	0.201	0.142	0.395	0.708		MS
2-dodecanone	66	1377	1.60	1.99	1.52	2.74	fruity, citrus, orange (C)	MS, RI, RC
2-tridecanone	77	1479	2.20	2.70	3.15	3.42	fatty, herbal (C)	MS, RI, RC
MISCELLANEOUS								
ALDEHYDES								
nonanal	20	1084	0.020	0.006	0.047	0.030	green, fruity, floral, wax (A)	MS, RI, RC
decanal	37	1187	0.012	nd	nd	nd	green, wax, floral, fruity (A)	MS, RI, RC
undecanal	54	1289	tr	tr	tr	tr	fruity, green, wax (A)	MS, RI, RC
dodecanal	68	1390	0.007	nd	nd	nd		MS, RI, RC
FURANS								
perillene	22	1089	0.480	0.620	0.774	0.363	woody, citrus (A)	MS, RI
2-hexyl-5-methylfuran	33	1170	0.010	0.004	0.022	0.023		MS

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Table 2. continued

compd	peak[b]	RI[c]	relative composition[a] (%) HAL	SAP	SPA	TET	odor description[d]	ident[e]
OTHERS								
2,6-dimethyl-2,4,6-octatriene	28b	1132	nd	0.025	nd	nd		MS, RI, RC
unidentified compd (*m/z* 81, 99, 117)	26	1101	0.001	nd	nd	nd		
unidentified compd (*m/z* 71, 81, 96, 170)	36	1184	0.033	0.012	0.043	nd		
unidentified compd (*m/z* 69, 93, 121)	76	1473	0.207	0.127	0.227	0.035		
unidentified compd (*m/z* 69, 93, 121)	80	1495	0.197	0.156	0.254	0.053		

[a]Relative peak areas represent the mean of triplicate HS-SPME-GC-MS analyses. [b]Peak number in accordance with the peak numbering in Figure 1. [c]Calculated retention index (RTX-1 capillary column, 40 m × 0.18 mm i.d. × 0.20 μm film thickness). [d]Odor descriptors found in the literature: (A) El Sayed;[50] (B) database of aroma descriptors. Citrus research and education center, Color and flavor chemistry group, University of Florida (http://www.crec.ifas.ufl.edu./crec_websites/Rouseff/Website 2002/Subpages/database_b_Frameset.htm (accessed Feb 7, 2010); (C) http://www.thegoodscentscompany.com (accessed Feb 7, 2010); (D) Lermusieau and Collin;[48] (E) Deinzer and Yang;[51] (F) Fukuoka and Kowaka;[28] (G) Pino et al.;[52] (H) Du et al.[53] [e]Compounds identified on the basis of (i) mass spectral comparison with the reference data bases (MS), (ii) comparison of retention index (RI), and (iii) comparison with authentic reference compounds (RC). [f]Not detected. [g]Co-elution with peak 31. [h] Co-elution of peaks 11 and 12. [i]Traces. [j]Co-elution with with peak 30.

AEDA were performed by three assessors. Analyses were repeated three times by each assessor.

Multivariate Data Analysis by PCA. PCA was performed to enhance data analysis and for interpretation of the results. In this study, PCA was used for discrimination of single-variety hop essences on the basis of their volatile composition. PCA was performed by using the multivariate data analysis software package The Unscrambler v9.2 (CAMO, Oslo, Norway).

RESULTS AND DISCUSSION

HS-SPME-GC-MS of Single-Variety Floral Hop Oil Essences. Single-variety floral hop essences were prepared via supercritical fluid extraction/solid phase extraction (SFE/SPE) from hop pellets T90 cv. Hallertau Tradition, cv. Saphir, cv. Spalter Select, and cv. Tettnanger. Next, the volatile composition of the varietal SFE/SPE essences was determined via the optimized HS-SPME-GC-MS procedure. As an example, Figure 1 shows the GC-MS chromatogram (TIC) of floral hop essence cv. Hallertau Tradition.

In total, 91 volatile constituents were assigned in the single-variety floral hop essences (see Table 2). Eighty-six volatiles were classified into five different chemical compound classes, that is, esters (50), terpenes (21), ketones (9), aldehydes (4), and furanes (2). Seventy-six compounds were (tentatively) identified. The identity of 37 compounds was determined by combination of mass spectral information and the retention index (RI) of authentic reference compounds, whereas 26 volatiles were tentatively identified via mass spectral comparison and literature RI.

Monoterpene Hydrocarbons. The monoterpene hydrocarbon group represents the compound class with the highest area percentage in all essences, ranging between 64.6% (cv. Tettnanger) and 75.7% (cv. Spalter Select) (see Figure 2). Fourteen different monoterpene hydrocarbons were detected, among which β-myrcene is the predominant compound in all essences, accounting for >95% of the monoterpene hydrocarbon group (Table 2). β-Myrcene has been proposed as a key character impact compound of hop aroma, showing geranium-like, lemon, and woody odor characteristics[32,36] (see also Table 2). The content of β-myrcene in the floral essences depends on the hop variety and ranges from 142 to 927 μg/mL (see Table 3). Because the odor threshold value of β-myrcene has been reported to be 13 μg/L in water,[46] we propose β-myrcene as a potent contributor to the odor character of the novel floral hop essences. The concentrations of the other monoterpene hydrocarbons in the essences are always much lower than the β-myrcene content (e.g., α-pinene (0.28−1.10 μg/mL) and β-pinene (1.64−12.4 μg/mL), see Table 3).

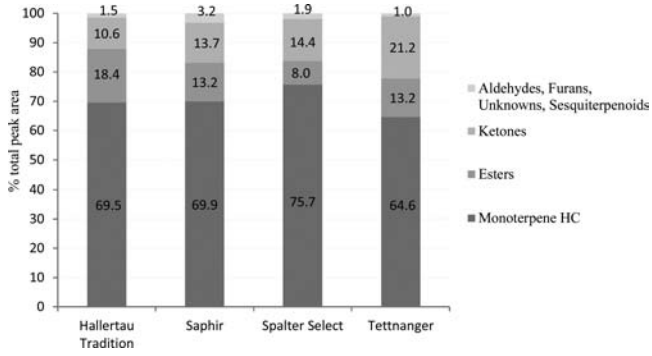

Figure 2. Relative composition (% of total peak area) of single-variety floral hop essences based on classification of the extracted volatiles in four groups: monoterpene hydrocarbons (monoterpene HC), esters, ketones, and a group comprising aldehydes, furans, unknowns, and sesquiterpenoids. Results are based on triplicate analyses.

Esters. A high number (50) of different esters (saturated, unsaturated, branched, unbranched) was detected in the four varietal floral essences (see Table 2). Forty-three esters were identified on the basis of mass spectral information, comparison with literature RI, and authentic reference compounds or tentatively identified (mass spectral information, comparison with literature RI), whereas the identities of 7 compounds remain unknown. The odor characteristics of most esters are described as 'fruity', 'green', and 'floral' (see Table 2). Figure 2 points to clear differences between varietal hop essences when considering the relative proportion of the total ester group to the total floral essence (relative proportion ranges between 8.0% (cv. Spalter Select) and 18.4% (cv. Hallertau Tradition)). In all essences, methyl *trans*-4-decenoate is found to be the major constituent of the ester group (3.2−7.0% of total essence, see Table 2), its concentration ranging between 2.8 and 14.4 μg/L (see Table 3). In the literature, methyl 4-decenoate has been reported as an analytical marker for distinguishing non-European and European bitter hops from aromatic cultivars[7] and as a potent odorant (odor threshold = 3 μg/L in water) in the oxygenated hop oil fraction of Brewers Gold hops.[46]

As can be derived from Table 2, particular esters allow us to discriminate between varietal floral essences. For instance, 2-

Table 3. Contents of Selected Volatiles in Single-Variety Floral Hop Essences[a]

compd	HAL (μg/mL)	SAP (μg/mL)	SPA (μg/mL)	TET (μg/mL)
α-pinene	0.28 ± 0.02	1.10 ± 0.04	0.32 ± 0.02	0.31 ± 0.01
β-pinene	4.28 ± 0.20	12.4 ± 0.5	4.92 ± 0.23	1.64 ± 0.06
β-myrcene	424 ± 12	927 ± 23	424 ± 15	142 ± 6.1
methyl *trans*-4-decenoate[b]	9.76 ± 0.27	14.4 ± 0.5	2.77 ± 0.11	2.92 ± 0.10
2-methylbutyl 2-methylbutanoate	2.35 ± 0.09	2.55 ± 1.11	0.90 ± 0.04	0.05 ± 0.001
methyl octanoate	0.82 ± 0.03	7.05 ± 0.20	0.29 ± 0.01	0.11 ± 0.01
hexyl 2-methylpropanoate	0.75 ± 0.03	1.13 ± 0.04	0.50 ± 0.02	0.03 ± 0.001
methyl nonanoate	2.27 ± 0.08	6.61 ± 0.20	0.50 ± 0.02	0.37 ± 0.02
heptyl 2-methylpropanoate	1.52 ± 0.06	1.74 ± 0.07	0.58 ± 0.02	0.10 ± 0.01
2-undecanone	16.3 ± 0.4	47.7 ± 1.1	18.3 ± 0.6	12.0 ± 0.4
2-dodecanone	4.15 ± 0.23	10.8 ± 0.35	3.85 ± 0.17	2.67 ± 0.07
2-tridecanone	5.75 ± 0.28	15.5 ± 0.11	7.23 ± 0.21	3.56 ± 0.13

[a]HAL, cv. Hallertau Tradition; SAP, cv. Saphir; SPA, cv. Spalter Select; TET, cv. Tettnang Tettnanger. Mean of three determinations ± standard deviation. [b]Reference compound not available; content calculated via calibration curve for methyl decanoate.

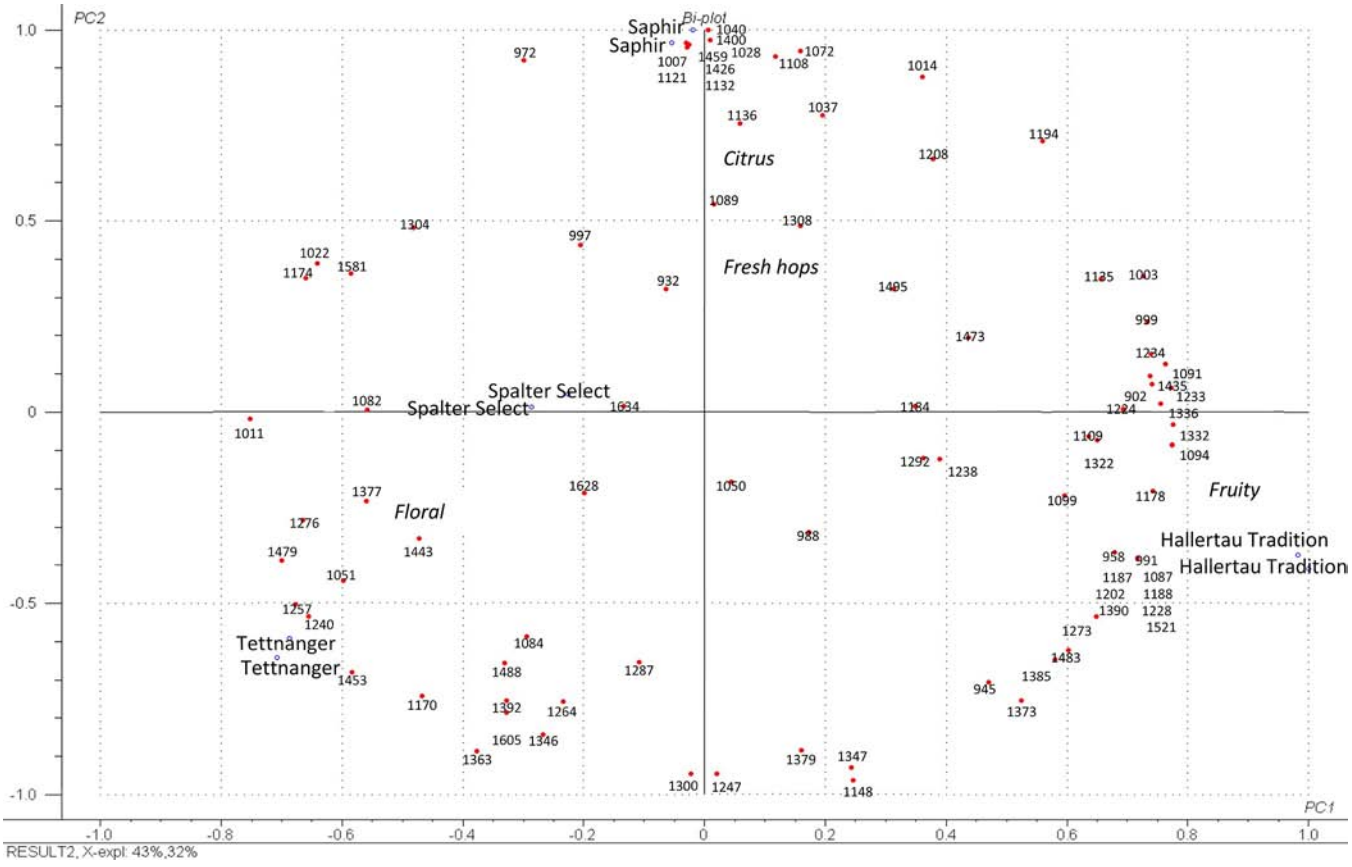

Figure 3. Biplot of principal component analysis on single-variety floral hop essences prepared via SFE/SPE extraction/fractionation of pellets T90 cv. Hallertau Tradition, cv. Saphir, cv. Spalter Select, and cv. Tettnang Tettnanger, respectively. Hop essences are represented as scores, and volatiles determined by HS-SPME-GC-MS and sensory descriptors as loadings. Variables are represented by their retention index and are in accordance with Table 2.

methylbutyl propanoate, heptyl propanoate, 2-methylpropyl 2-methylbutanoate, 3-methylbutyl 2-methylbutanoate, hexyl 3-methylbutanoate, methylbutyl hexanoate, and an unidentified ester at RI = 1379 are detected only in the analytical profile of floral hop essence cv. Hallertau Tradition, whereas methyl heptanoate and methyl 10-undecenoate (RI = 1400) are typical for the floral hop essence cv. Saphir. Moreover, quantitative data on selected ester volatiles as presented in Table 3 clearly show pronounced differences in their content among the varietal floral hop essences.

Ketones. The ketone group accounts for 10.6% (cv. Hallertau Tradition) to 21.2% (cv. Tettnanger) of the total volatiles in the floral hop essences (see Figure 2) and comprises nine different constituents, which are present in all essences investigated (see Table 2). Reported odor descriptors for the detected ketones are usually 'citrus/fruity'. Clearly, 2-undecanone is the predominant compound of the ketone fraction, representing 5.6−11.1% of the total peak area (Table 2), its concentration ranging from 12.0 μg/mL (cv. Tettnanger) to 47.7 μg/mL (cv. Saphir) in floral hop essence (Table 3).

According to Perpète et al.,[7] 2-undecanone is a useful analytical marker in hop variety classification.

Miscellaneous Compounds. Aldehydes, furans, oxygenated and nonoxygenated sesquiterpenes, and several unidentified constituents represent a minor fraction of the varietal floral hop essences, accounting for only 1.0−3.2% of the total composition (see Figure 2).

PCA on Single-Variety Floral Hop Essences. To further explore and gain insight into the large data set in Table 2, PCA was performed on a data matrix comprising eight floral SFE/SPE hop essences (four varieties, duplicate extractions) as objects and all assigned volatiles as variables. Figure 3 displays the result of this analysis by plotting the first two principal components (PC1 and PC2), which together explain 75% of the total variance.

Obviously, the single-variety floral essences are discriminated via PCA on account of their volatile composition. Floral essences prepared from hop pellets T90 cv. Hallertau Tradition are differentiated from the other varietal essences by specific estery compounds, for example, 2-methylbutyl propanoate (RI = 958), 2-methylpropyl 2-methylbutanoate (RI = 991), 3-methylbutyl 2-methylbutanoate (RI = 1087), methylbutyl hexanoate (RI = 1202), heptyl propanoate (RI = 1188), and hexyl 3-methylbutanoate (RI = 1228). Floral essences of cv. Saphir contain a number of volatiles that are detected only in this variety, that is, methyl heptanoate (RI = 1007), unknown monoterpene (RI = 1121), 2,6-dimethyl-2,4,6-octatriene (RI = 1132), and methyl 10-undecenoate (RI = 1400). Differences in the relative proportions of all other volatiles, present in all essences, led to further discrimination between the essences. For example, several ketones (unidentified ketone (RI = 1240), 5-undecen-2-one (RI = 1257), 2-undecanone (RI = 1276), 2-dodecanone (RI = 1377), 2-tridecanone (RI = 1479), *cis*-5-tridecen-2-one (RI = 1453)) were found to be more characteristic for floral essence cv. Tettnanger because of their high relative content in this particular essence (see also Table 2).

Next to the clear differentiation between varietal preparations, good reproducibility of the applied SFE/SPE and HS-SPME-GC-MS procedures for preparation and analysis of single-variety floral hop essences, respectively, is proven by close clustering of the floral essences from the same variety on the PCA biplot.

Besides their differentiation on analytical grounds, the varietal typicality of floral hop essences is also reflected in their sensory properties, as shown by the position of the sensory descriptors, i.e. 'floral', 'fresh hops', 'citrus', and 'fruity', in the PCA biplot. Figure 3 clearly displays the relationship between hop essences cv. Tettnang Tettnanger and Spalter Select and the descriptor 'floral', whereas the 'citrus' and 'fruity' odor descriptors are more linked with the essences prepared from cv. Saphir and cv. Hallertau Tradition, respectively.

Determination of Odor-Active Constituents in Floral Hop Essence cv. Spalter Select. HS-SPME-GC-O/MS analysis of undiluted floral hop essence cv. Spalter Select resulted in a nearly constant and overwhelming green-floral odor at the sniffing port during the time of the analysis. Consequently, it was impossible for the analysts to generate aromagrams in this way. Subsequent preliminary sniffing sessions on diluted samples pointed out that diluting the floral essence 50 times prior to extraction and analysis is the best compromise to perform significant GC-O.

The odor-active compounds of floral hop essence cv. Spalter Select were determined by a detection frequency method (OGA) and AEDA, respectively. Panelists were asked to describe the aromatic character of eluting odorants and to scrupulously record the duration of the odor perceptions. The results of OGA on the SPME extract from floral hop essence cv. Spalter Select are shown in Figure 4. In total, 13 odor-active

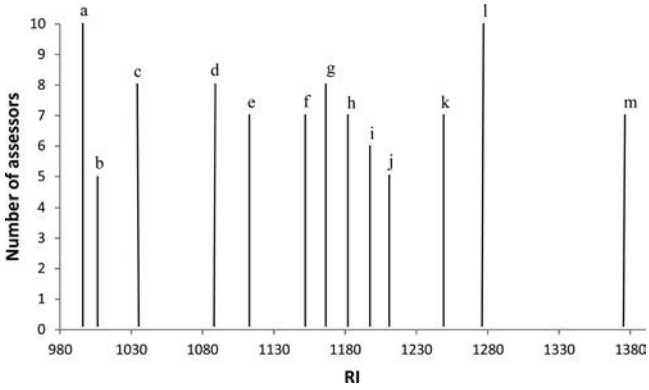

Figure 4. Detection frequency of odorants in floral hop essence cv. Spalter Select (RI, calculated retention index on RTX-1 capillary column (40 m × 0.18 mm i.d. × 0.20 μm film thickness)). Assignment of odorants corresponds to Table 4.

regions with various detection frequencies were recorded in the aromagram. Table 4 provides a summary of all data obtained through GC-O/MS of the floral hop essence, including compound identifications in the odorous regions, odor descriptions of volatiles, and dilution detection levels as determined by AEDA. Clearly, the odor-active compounds belong to different chemical classes; that is, seven esters, two ketones, two monoterpene hydrocarbons, one monoterpenoid furan, and one aldehyde were (tentatively) identified. Twelve compounds were (tentatively) identified on the basis of their MS/EI spectrum, the RI indices, and (when available) the use of authentic references. Two compounds remain unknown (see Table 4, peaks f and g. Peak f presumably belongs to the group of esters as is apparent from the MS/EI spectrum (typical fragmentation pattern showing intense fragment ions at m/z 88 (base peak, McLafferty rearrangement) and m/z 101 (γ-cleavage), whereas the mass spectral signal for peak g was too weak to provide any structural information.

β-Myrcene (a) and 2-undecanone (l) were detected by all assessors (see Figure 4), suggesting that these compounds are high character impact compounds of the floral hop essence. The odor character perceived at the outlet of the sniffing port was described as 'fresh hops' and 'floral/citrus' for β-myrcene and 2-undecanone, respectively (Table 4). Furthermore, according to sensory evaluation via the OGA method, major contributors to the odor of this essence are *cis*-β-ocimene (c; green, floral), nonanal/perillene (d; citrus), and an unknown compound (g; RI = 1162; fruity) because these volatiles were detected by eight panelists (see Figure 4). Remarkably, the citrus odor-active region d in the aromagram at RI = 1084−1087 (see Table 4) comprises two compounds, that is, nonanal and perillene. To find out which of these volatiles actually contributes to the perceived citrus odor, additional GC-O experiments were performed using a capillary column with a polar stationary phase (CP-Wax-57CB; 50 m × 0.25 mm i.d. × 0.20 μm film thickness). In these experiments, full separation

Table 4. Odor-Active Compounds Detected by GC−Olfactometry in Floral Hop Essence cv. Spalter Select

peak[a]	RI[b]	odorant[c]	odor descriptor[d]	dilution[e]	identification[f]
a	988	*β*-myrcene[g]	fresh hops (10)	40	MS, RI, RC
b	999	*3-methylbutyl 2-methylpropanoate*	fruity (5)	1	MS, RI
c	1028	*cis-β-ocimene*	green (5), floral (3)	10	MS, RI
d	1084	nonanal[g]/perillene	citrus (8)	20	MS, RI, RC
e	1108	methyl octanoate[g]	fruity (7)	20	MS, RI, RC
f	1148	*unidentified ester*	citrus (5), fruity (2)	10	MS
g	1162	*unknown*	fruity (8)	10	
h	1178	*methyl 4-methyloctanoate*	citrus (7)	10	MS
i	1194	methyl 3-nonenoate[g]	floral (3), citrus (2), green (1)	20	MS, RI, RC
j	1208	methyl nonanoate[g]	floral (3), fruity (2)	1	MS, RI, RC
k	1247	ethyl nonanoate[g]	fruity (7)	10	MS, RI, RC
l	1276	2-undecanone[g]	floral (7), citrus (3)	40	MS, RI, RC
m	1377	2-dodecanone[g]	citrus (7)	20	MS, RI, RC

[a]Peak assignment corresponds to Figure 4. [b]Calculated retention index (RTX-1 capillary column; 40 m × 0.18 mm i.d. × 0.20 μm film thickness). [c]Compounds identified in odor-active regions (tentative identifications and unknowns in italics). [d]Odor descriptions given by the assessors upon sniffing of the effluent of splitless injected SPME extract (numbers in parentheses show the number of assessors (10 in total) using the indicated descriptor). [e]Highest dilution at which odor activity is perceived (serial dilution was obtained by adjusting the split ratio (1, 1/10, 1/20, 1/40) at the GC inlet). [f]Compounds were identified on the basis of (i) mass spectral comparison using the reference libraries (MS), (ii) comparison of retention index (RI), and (iii) comparison of retention time, RI, and mass spectrum with authentic reference compound (RC). [g]Odor contribution confirmed by GC-O analysis of authentic reference compound.

between nonanal (RI = 1396) and perillene (RI = 1304) was achieved, and sniffing analysis provided clear evidence on the odor activity of both nonanal (citrus) and perillene (citrus/lemon) (data not shown).

On the basis of detection frequencies (see Figure 4), other character impact compounds of floral hop essence cv. Spalter Select are methyl octanoate (e; fruity), an unidentified ester (f; citrus/fruity), methyl 4-methyloctanoate (h; citrus), ethyl nonanoate (k; fruity), 2-dodecanone (m; citrus), and methyl 3-nonenoate (i; floral/citrus/green). 3-Methylbutyl 2-methylpropanoate (b; fruity), and methyl nonanoate (j; floral/fruity) may have a lower impact on the odor of the essence as these compounds were perceived by only 5 of the 10 panelists.

The above findings (OGA method) are largely comparable with the results obtained from AEDA of the essence. In particular, the importance of *β*-myrcene (a) and 2-undecanone (l) for the 'fresh hop' and 'floral/citrus' characters of this essence is proven by AEDA because these compounds were still detectable upon olfactometric analysis of the most diluted extract (see Table 4). Furthermore, 3-methylbutyl 2-methylpropanoate (b) and methyl nonanoate (j) were detected only during the analysis of the splitless injected SPME extract (see also Table 4), confirming their lower impact as observed from OGA.

Among the 14 most odor-active compounds in floral hop essence cv. Spalter Select, *β*-myrcene, nonanal, methyl nonanoate, and 3-methylbutyl 2-methylpropanoate have been reported in the literature as key odorants in the volatile fraction of fresh hop cones, dried hop cones, or hop pellets.[32,36,47,48] However, in this work, the hop oil constituents perillene, *cis-β*-ocimene, 2-undecanone, 2-dodecanone, and several esters (methyl octanoate, methyl 3-nonenoate, methyl 4-methyloctanoate, and ethyl nonanoate) are reported for the first time as impact odorants of hop aroma, in particular as important contributors to the floral, fruity, and citrusy aspects of hop aroma. Interestingly, components such as linalool, geraniol, and geranyl isobutyrate, commonly associated with the floral bouquet of hops and hop-derived floral aroma in beer,[30,34,49] are not detected in our SFE/SPE fractionated floral hop oil essences, pointing to the very specific chemical composition and, following the results of this study, the characteristic flavoring potential of this novel type of hop oil preparation.

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Notes

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