Exploring the multisensory perception of terpene alcohol and sesquiterpene rich hop extracts in lager style beer

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Abstract

Understanding the contribution of hop essential oil to the multisensory profile of beer is known to be challenging because of its chemical and sensory complexity. Limited research has been conducted investigating hop-derived volatiles' role in the modulation of taste and mouthfeel sensations. Supercritical CO_2 can be used to extract specific fractions from hop oil, thereby enabling the localisation of compounds responsible for different sensory impressions. Terpene alcohol and sesquiterpene fractions were extracted from a Magnum hop oil and further fractionated into seven sub-fractions and individual compounds. All extracts were evaluated in lager (4.5% v/v) by a trained panel (n=10) using a newly developed attribute lexicon and following a sensory descriptive analysis approach. The sensory data was analysed using ANOVA, followed by Tukey's test (HSD) and correlated with chemical profile data obtained by gas chromatography-mass spectrometry (GC-MS) by Principal Component Analysis. The study revealed evidence for hop extracts to impart multisensory characteristics to beer due to sensory interactions within and across modalities. The monoterpene alcohols-rich fractions and particularly geraniol, added fruity- and floral aromas and flavours, modified the sweetness and induced a smooth bitterness in the beer matrix. Flavouring the beer with sesquiterpene fractions resulted in a harsh bitterness sensation. Contrary to previous findings, the humulene epoxides fraction appeared to have limited effects on lingering bitterness and astringency, illustrating the need for temporal sensory assessments in future studies. This research shows that splitting hop oil into fractions and sub-fractions provides a source of natural, sustainable flavouring preparations with distinct sensory characteristics.

Keywords: Hop oil fractions; sensory descriptive analysis; sensory interactions; bitterness; sweetness

Abbreviations: ABV, Alcohol by volume; AEDA, Aroma extract dilution analysis; ANOVA, Analysis of variance; C, Compound; CATA, Check-all-that-apply; GC-MS, Gas chromatography - mass spectrometry; HSD, Honestly significant difference; ISTD, Internal standard; log *P*, logarithm of the octanol-water partition coefficient; OAV, Odour activity value; PCA, Principal component analysis, PLS, Partial least squares; QDA, Quantitative Descriptive Analysis; R^2, R-squared/ goodness-of-fit; RI, Retention index; RMSE, Root mean square errors; VIP; Variable importance in projection

1. Introduction

Historically, hops (*Humulus lupulus* L.) have been added to beer to provide microbial protection and as a source of bitterness and aroma. With the craft beer sector's growth and changing consumer preferences, hop products have become key in the brewing process adding aroma, flavour, taste, and mouthfeel (Dietz, Cook, Huismann, Wilson, & Ford, 2020; MarketDataForecast, 2020). The composition of hop essential oil is complex. Around 1000 volatile compounds are suggested to be present in hops, mainly comprising hydrocarbons, terpene alcohols, sesquiterpenoids, esters, ketones, aldehydes, and sulphur-containing compounds, with potentially less than half of these identified so far (Roberts, Dufour & Lewis; 2004). Research has shown that complex mixtures of volatile hop compounds contribute to the sensory, 'hoppy' profiles of beer (Dietz et al., 2020).

Meilgaard (1975a) hypothesised that half of the flavour intensity in beer could be attributed to sensory interactions between volatile and non-volatile fractions. Non-volatile fractions in the beer matrix affect the physical release and concentration of volatiles in the headspace eventually determining the perceived intensity and quality of aroma-active compounds (Poinot, Arvisenet, Ledauphin, Gaillard, & Prost, 2013). Depending on the relative concentrations of two or more volatiles, sensory characteristics can be increased due to additive- or synergistic-type behaviours, suppressed or masked due to antagonistic-type behaviour or even eliminated (Meilgaard, 1982). Moreover, sensory interactions can occur across modalities (cross-modal interactions). Oladokun et al. (2017) investigated the impact of a Hersbrucker hop aroma extract on perceived bitterness qualities in beer and found significant effects but also suggested a taste-trigeminal interaction responsible for some of the bitterness quality changes. Kaltner and Mitter (2006) attributed the modification of bitterness perception to different concentrations of linalool and terpene hydrocarbons. Interestingly, ratings for "bitterness harmony" increased for the beer with the highest linalool concentration. Beers with terpene hydrocarbons and a low concentration of linalool resulted in high ratings for "harmonious, but increasing bitter taste" and significantly lowered ratings for "mild bitterness" (Kaltner & Mitter, 2006). Sensory interactions particularly occur in heterogeneous mixtures depending on compound combinations, ratios, and threshold concentrations of compounds for aroma, flavour, taste, and/or mouthfeel. Overall, there has been limited research studying the role of sensory interactions related to the perception of hop volatiles.

In a preceding study, five hop oil fractions were extracted from a Magnum hop oil using supercritical CO_2 (Dietz, Cook, Wilson, Marriott, & Ford, 2020). The total oil and the fractions were applied at 800 µg/L in an ethanol-water solution (4% ABV) and evaluated by external sensory panellists following a Quantitative Descriptive Analysis (QDA) approach to determine their sensory characteristics. Correlation of sensory and compositional data suggested that the terpene alcohol fraction added taste and trigeminal-type sensations, including sweetness, lingering bitterness, and a "peppery tingling" mouthfeel. This fraction also induced pronounced fruity and floral aroma and flavour sensations to the model solution. Correlation analysis suggested that the monoterpene alcohols, linalool and geraniol were key compounds responsible for the aroma and flavour characteristics in this fraction, whilst sesquiterpene alcohols (humulenol II, humulol) might have caused taste and mouthfeel sensations. However, there is a high probability that additional compounds present at lower concentrations might have contributed to these sensations rather than the measurable key volatiles as such. Also, cross-modal interactions between ortho- and retronasal smell and taste and mouthfeel might have resulted in the perceived multisensory profile induced by terpene alcohols making it difficult to specify key compounds responsible for either aroma and flavour or taste and mouthfeel (Dietz et al., 2020).

This study aims to understand the multisensory profile perceived when drinking beer flavoured with specific hop oil extracts and sensory interactions causing this multisensory experience. Based on the preceding study's outcome, the current research investigates whether it is possible to separate the hop compounds driving floral and fruity aroma and flavours from those adding sweetness, a "peppery tingling" mouthfeel or modifying bitterness.

2. Materials & Methods

2.1 Hop oil extracts

Supercritical CO_2 hop oil fractions and compounds were extracted from hop oil obtained by distillation from Magnum variety hop pellets following the extraction method described by Marriott (2019). For the set of hop extracts, specific fractions, sub-fractions and individual volatile compounds were extracted from the Magnum hop oil (total oil), namely extracts enriched in sesquiterpenes, terpene alcohols, humulene epoxides, monoterpene alcohols, sesquiterpene alcohols, humulol + humulenol II, linalool, geraniol, and caryophyllene oxide (Fig. 1, Fig. 2). Aliquots of the extracts were flushed with nitrogen, hermetically sealed and stored at 4°C until further use, within the expire date of 6 months.

2.2 Sensory evaluation

Ethics approval was granted by the Faculty of Medicine & Health Sciences Research Ethics Committee of the University of Nottingham (Ethics Reference No. 88-1707). Prior to sensory screening, informed consent was obtained from all candidates.

2.2.1 Preparation of samples

Stock solutions were prepared by diluting the hop extract aliquots in food-grade ethanol (96%, ferm, fa, F200481, Haymankimia, UK) and stored at 4°C for the period of the study. A commercial pale lager beer (4.5% ABV, 10 BU, pH 4.35, brewed from barley malt with rice adjunct) was purchased and flavoured with the hop extracts at different concentrations to obtain an equiflavour intensity achieved by conducting bench tests followed by Rank-Rating tests with the panel. All beers were sourced from the same batch to prevent batch-to-batch variation. The diluted hop extracts were dosed volumetrically into 300 mL lager bottles to obtain the following concentrations: total oil and fractions - 1500 µg/L, sub-fractions - 1000 µg/L, enriched fractions - 300 µg/L, fractions enriched in single compounds - 100 µg/L (linalool and geraniol fractions) or 300 µg/L (caryophyllene oxide fraction). Additions were made in a cold room (4°C) to minimise CO_2 breakout with bottles immediately recapped, inverted three times, and allowed to equilibrate overnight (21 h) at 4°C prior to each session. The non-flavoured control lager was treated in the same way. For presentation to the panel, samples (30 mL) were poured into 60 mL tempered (4°C) amber glass bottles labelled with randomly assigned 3-digit codes, immediately closed with screw-top caps 30 min prior to testing sessions to limit decarbonation and volatilisation of hop compounds.

2.2.2 Sensory panel

Sensory characteristics of control and flavoured beers were evaluated by external sensory panel (n=10, 7 female, 3 male, mean age 55.5 years) following a Quantitative Descriptive Analysis approach (Stone, Sidel, Oliver, Woolsey, & Singleton, 2008). The previously screened and trained panelists were re-screened to ensure they met specific criteria for this study following the approach described by Dietz et al. (2020) to evaluate their sensory abilities, including basic smell and taste detection, ability to detect the main compounds and to confirm advanced descriptive and discriminative abilities.

2.2.3 Panel training

Following screening, selected candidates were invited to participate in sensory training sessions. An attribute lexicon was generated where panelists were asked to individually generate aroma, flavour, taste, and mouthfeel attributes (tactile sensations during and after swallowing) by comparing and describing the flavoured beers (with hop extracts added at different concentrations). Three sessions were used for attribute consolidation by conducting Check-All-That-Apply (CATA) tests and group discussions moderated by the panel leader to select the most descriptive and discriminating attributes (Delarue, Lawlor, & Rogeaux, 2014). Attribute descriptions were compiled in further group discussions aided by reference materials at different concentrations for each attribute. Attribute intensities were quantified using a 10 cm unstructured line scale anchored at the extremes by "no sensation" and "very strong". Quantities of reference materials listed in the attribute lexicon (Table 1) refer to "very strong" intensities of the sensory characteristics in the beers. The attribute order, assessment protocols, and palate-cleansing materials and protocols were developed and defined based on panelists' comments during training. In total, 14 training sessions and one mock evaluation session (120 min each) were conducted to achieve panel consensus i. e. sufficient discriminative ability and reproducibility, as confirmed by the panel performance data.

2.2.4 Sensory descriptive analysis

For the final evaluation, the 12 samples were evaluated in triplicate by all panelists (n=10) over nine evaluation sessions of 100-120 min. The sensory evaluation was performed in sensory testing booths according to the guidelines and conditions described in ISO 8589-2007 (ISO, 2007). Each panelist consumed less than one UK alcohol unit (8 g/L) per session, and a maximum of two sessions were conducted per week. First-order and carryover effects were limited by monadically presenting the samples in a randomised and counterbalanced order (Latin Square Design) (Stone et al., 2008). The panelists received a fresh sample (8^{o}C; with replenished headspace) after each attribute set (1-4 attributes) to maximise the opportunity to evaluate subtle sensory characteristics. The scales for all attribute sets were displayed with Compusense®Cloud (Compusense Inc., Guelph, Canada) on a computer. Breaks were scheduled to prevent carryover effects and fatigue and panelists were asked to close the bottles and neutralise their senses where they smelled the back of their hands to neutralise their nasal cavity, ate a piece of honeydew melon and consumed some water to wash away residues.

2.3 Gas chromatography-mass spectrometry

The volatile composition of the hop extracts was analysed (n=3) by GC-MS. A Thermo Scientific system (TRACE^{TM} 1300; Massachusetts, USA) was equipped with a Zebron ZB-5MS capillary column (30 m x 0.25 mm ID x df = 0.25 µm; Phenomenex, Torrance, USA) coupled to a single quadrupole mass spectrometer (ISQ QD Thermo Scientitic Inc.; Massachusetts, USA) and operated in positive electron ionisation mode. A Zebron ZB-WAX capillary column (30 m x 0.25 mm ID x df = 0.25 µm; Phenomenex, Macclesfield, UK) was used to obtain additional retention indices on a polar column. The hop extracts (10 µL) were diluted into 1 mL iso-octane (≥99%; Thermo Fisher Scientific, Loughborough, UK), and aliquots (1 µL) of the dilution were analysed with helium as a carrier gas (1 mL/min flow rate) operating in split mode (1:50). The temperature of the injector, ion source, interface, and detector were 250°C, 240°C, 250°C, and 250°C, respectively. The oven temperature increased at 5°C/min from 60°C to 240°C. Hop extracts were spiked with 1 µL of 1050 mg/L benzyl acetate (≥99%; Sigma Aldrich, UK) as an internal standard (ISTD) after checking its absense in the extracts and separate elution from other compounds. Peak identification was based on mass spectra, retention indices (RI), and reference compounds (where available), including: *endo*-borneol (≥97%), caryophyllene oxide (≥99.0%), geraniol (≥99%), geranyl isobutyrate (≥97%), geranyl propionate (≥95%), linalool (≥97.0%), *R*-(+)-limonene (≥97%), methyl decanoate (≥99%), methyl geranate (≥94.0%), methyl octanoate (≥99%), α-humulene (≥ 96%), *β*-caryophyllene (≥98.5%), *α*-terpineol (≥97), *β*-myrcene (≥90.0%), *β*-pinene (≥99%), 2-dodecanone (≥97%), 2-nonanone (≥99%), 2-tridecanone (≥97%), and 2-undecanone (≥98.0%) (Sigma Aldrich (UK)). RIs under experimental conditions were determined using a homologous series of n-alkanes (C6-C30; Sigma-Aldrich, St. Louis, MO). NIST Mass Spectral Library (NIST08) and Wiley7n.1 (Hewlett-Packard, US) databases were used for library matching. Further compound verification was conducted by comparing mass spectra and RIs published in databases (Flavornet, Pherobase, Pubchem) or studies using columns with similar stationary phases. Peaks were assigned to compounds if the MS fit factor was ≥ 800 (reverse/forward) and the calculated RI closely matched literature values. Otherwise, compounds were specified as "unknown".

2.4 Statistical analysis

Sensory and analytical datasets were analysed using XLSTAT (2020.5.1, Addinsoft, US). Three-factor Mixed Model Analysis of Variance (ANOVA) (panelist, sample, replicate) with interactions was conducted on the 24 sensory attributes to examine panel performance (sample*panelist and sample*replicate interactions). After confirmation of satisfactory performance, a two-way ANOVA (sample as fixed factor, panelist as random factor) followed by Tukey's Honest Significant Difference (HSD) test was performed for multiple pairwise comparisons to study significant differences ($p <$ 0.05; CI 95%) between the samples for each attribute. Panelists' averaged attribute scores were further analysed by Principal Component Analysis (PCA) on the covariance matrix to study the main relationships between samples and attributes in a sensory-perceptual space. Pearson correlation analysis was conducted to calculate linear correlations between attributes. Semi-quantification was used for the non-targeted analysis of hop compounds and performed by normalising the integrated peak areas of the hop compounds relative to the ISTD ion peak area. Sensory and GC-MS datasets were standardised (1/standard deviation) and analysed by PCA. Standardisation was conducted to allow all variables to have equal influence in the PCA model despite differences in their numerical range. While PCA outcomes reveal few linear combinations of variables best explaining correlations between datasets of X and Y matrices without losing too much information, Partial Least Squares (PLS) regression is capable of dealing with strongly collinear, noisy data including numerous X-variables capturing more correlation information between the matrices (Maitra & Yan, 2008). Therefore, PLS regression models were developed to identify correlations between hop compound concentrations (X-matrix) and sensory attribute scores for the beers (Y-matrix) using PLS1 algorithms for single, and PLS2 for multiple attributes (all and within modalities). Jack-knife uncertainty tests were performed to obtain estimated regression coefficients. Confidence intervals were set at 95%. Logarithmic transformation of GC-MS data was applied to improve the goodness-of-fit (R^2) since the sample comprised many volatiles at different concentrations having different sensory threshold levels (Lykomitros, Fogliano, & Capuano, 2016). Sensory data is inherently 'noisy'; therefore, PLS models with $R^2>0.700$ were considered as having good predictive ability (Schmidtke, Blackman, Clark, & Grant-Preece, 2013). Standardised coefficients of compounds (>0.05 for clarity) were plotted to visualise their relative weights in the models.

3. Results and discussion

3.1 Sensory evaluation

3.1.1 Attribute generation and consolidation

250 attributes were generated by the panelists and consolidated to a list of 39 attributes using a Check-All-That-Apply (Delarue et al., 2014) approach to exclude attributes that could not be reliably identified in the samples or adequately describe or discriminate differences. Table 1 lists the final 24 attributes, their descriptions, and associated reference materials in order of their evaluation. Where aromas were perceived both through the nose (orthonasally) and mouth (retronasally), they were selected to represent either an aroma or a flavour (where the highest intensity was recorded), to avoid attribute replication for both modalities.

3.1.2 Panel performance

Panel performance was evaluated by conducting three-factor ANOVA with interactions (panelist, sample, replicate) on all attributes (Table 2). The dataset of one panelist was excluded because of lack of reproducibility across replicates and evaluation sessions. Sample*panelist interactions were reported for 10 attributes indicating disagreement regarding sample rankings or scale use effects

(Stone et al., 2008). Interrogation of interaction plots and other significant factors (replicate, panelist) concluded minor variations of scale use with no impact on the data's interpretation for half of these attributes. However, five attributes ("sweetcorn", "dark fruits", "biscuity", "sour", "peppery tingling") were excluded from further discussions due to inadequate panel performance as indicated from the ANOVA. Twelve attributes ("lemon", "crushed grass, sap", "resinous", "earthy", "musty", "soapy", "rose water", "orange fruit", "grapefruit", "sweet", "smooth bitterness", "harsh bitterness") and overall aroma and flavour intensities significantly differed across all samples (Table 2) and were of adequate quality to be interpreted and discussed.

3.1.3 Sensory descriptive analysis

Table 3 shows the mean intensity scores for the attributes and significant differences between the samples. The two experimental replicates (total oil) were not significantly different from each other indicating panel reliability. Six aroma attributes and "overall aroma intensity" differed significantly between the samples, whilst "pine wood" showed a trend (p = 0.092) towards higher scores for the geraniol-flavoured and terpene alcohol fraction-flavoured beers compared to the control and caryophyllene oxide fraction-flavoured beer. The assessment of gustatory perception revealed three flavours and "overall flavour intensity" and three taste attributes to significantly discriminate between the samples. No mouthfeel attributes were found to be significant.

PCA was performed to reduce the data's complexity and visually represent the samples in a sensory space (Fig. 4). The first two principal components explained the majority of the total variance (86.39%) with the main discriminating dimension PC1 explaining 61.85% and PC2 explaining 24.53%. PC1 was positively loaded with the attributes "rose water", "orange fruit", "grapefruit", "lemon", "pine wood", "soapy" and "sweet". PC2 was positively loaded with the primary distinguishing aroma attributes "crushed grass, sap", "resinous", "musty" and "earthy". PC3 only accounted for 5.31% of the variance in the sample set and was positively loaded with "smooth bitterness" (r = 0.527) and negatively loaded with "harsh bitterness" (r = -0.566), "lingering bitterness" (r = -0.559), and "astringent" (r = -0.672) indicating that both aroma and flavour, as well as taste and mouthfeel attributes, are differentiating between the flavoured beers. Nevertheless olfactory characteristics clearly were the key discriminators.

Overall aroma and flavour intensity. All flavoured beers were designed to be equi-intense. However, inspection of the ANOVA outcome indicated a significant effect of "overall flavour intensity". Tukey's HSD tests revealed that the flavour intensity was higher for the geraniol- and terpene alcohol fraction-flavoured beers and lower for the caryophyllene oxide- and humulene epoxide-fraction flavoured beers compared to the other samples. The latter two showed only slightly increased flavour intensities compared to the control beer. Caryophyllene oxide is known to impart little aroma to beer (Lafontaine & Shellhammer, 2018). Flavour descriptors or threshold concentrations of caryophyllene oxide in beer have not yet been published. It should be noted that caryophyllene oxide is prone to oxidation, hydrolysis and isomerisation reactions, and measures have been taken to reduce volatile loss to a minimum, but could not be completely ruled out (Yang, Lederer, McDaniel, & Deinzer, 1993). The findings that geraniol- and terpene alcohol fraction-flavoured beers obtained significantly higher scores for "overall flavour intensity" might be explained by differences in volatility or aroma and flavour threshold levels of the compound mixture in the extracts.

Evaluation of the base beer. Inspection of the control beer scores indicated that it was characterised by attributes intrinsic to standard lager such as "malty" which was not significantly higher than those in the flavoured beers suggesting that the base maltiness was not significantly impacted by the any of the hop extracts used.

Sensory characteristics induced by total Magnum hop oil. The beer flavoured with total oil could be distinguished from the other flavoured beers by the highest scores for “crushed grass, sap”, “resinous”, “earthy”, and “musty” aromas. The main compounds accounting for up to 80% in Magnum hop oil are *β*-myrcene, *β*-caryophyllene, and *α*-humulene, with the most abundant compound *β*-myrcene being described as “spicy” and “resinous” at 200 µg/L (Sharpe, 1988), “metallic” and “geranium-like” at 860 µg/L (Schnaitter et al., 2016) or “geranium-leaf”-like at 6.65-15.0 µg/L (Neiens & Steinhaus, 2018). Depending on the beer matrix assessed, the sensory characteristics of *β*-caryophyllene and *α*-humulene in beer have hardly been defined. *β*-caryophyllene and *α*-humulene have been described as “rubber-like”, “mouldy” (Zhai & Granvogl, 2019) and “woody”, “spicy” (Navarro-Martínez et al., 2019). Similar aroma characteristics could also be found in the sesquiterpene fraction-flavoured beer which was described by “crushed grass, sap” and “pine wood” aromas, but at comparably low intensities compared to the total oil-flavoured beer. This may indicate that the total oil’s composition increases the aroma intensity of these characteristics.

Impact of hop extracts on beer bitterness and mouthfeel. Interestingly, the sesquiterpene fraction-flavoured beer obtained the highest score for “harsh bitterness”, which was significantly higher than the score for the geraniol-flavoured beer. Instead, this beer stood out with the highest score for “smooth bitterness”, indicating opposing bitterness qualities in these hop extracts. Panelists’ descriptors of the two attributes (“irritating, spiky”, “soft, pleasant”) suggest these bitterness qualities have trigeminal-type dimensions. Interestingly, the sesquiterpene fraction-flavoured and geraniol-flavoured beers also showed opposing scores for “lingering bitterness” with a higher score obtained for the latter, although not significant. Oxygenated sesquiterpenes including caryophyllene oxide (Goiris et al., 2014; Praet et al., 2015) and linalool (Kaltner & Mitter, 2006; Praet et al., 2015) have been suggested to affect bitterness intensity, duration and quality, although the majority of effects have not been assessed using a systematic sensory analysis approach. Effects on bitterness have not yet been reported for a geraniol extract individually applied in beer and limited studies have been conducted to study the impact of sesquiterpene extracts on bitterness qualities and decline.

The beer flavoured with the humulene epoxide enriched fraction only received a slightly higher score for “astringent” than the other beers. Caryophyllene oxide has been suspected to be part of a compound mixture in the sesquiterpenoid fraction enhancing spicy hop flavour, fullness, mouthfeel, and bitterness of beer (Goiris et al., 2014; Praet et al., 2015). Based on previous research, humulene epoxides and sesquiterpene alcohols including caryophyllene oxide were expected to add bitterness and a “peppery tingling” mouthfeel to the beer that was described as an irritating sensation, suggesting a trigeminal effect (Dietz et al., 2020). However, the preceding study's test matrix was non-carbonated and the carbonation might have masked this mouthfeel and impeded its recognition. Both beer astringency and bitterness can linger for several minutes (Kaneda, Takashio, Shinotsuka, & Okahata, 2001; McLaughlin, Lederer, & Shellhammer, 2008); therefore, temporal sensory methods may be more appropriate for discriminating these attributes.

Impact of hop extracts on beer aroma, flavour and sweetness. In agreement with previous findings, the geraniol-flavoured beer was characterised by citrusy (“lemon”, “orange”, “grapefruit”) and “rose water” aromas and flavours (Eyres, Marriott, & Dufour, 2007; Kishimoto, Wanikawa, Kono, & Shibata, 2006). The geraniol-fraction flavoured beer was also significantly sweeter than the sesquiterpene- and humulol enriched-fraction flavoured beers and slightly sweeter compared to the control and total oil-flavoured beers. Pearson correlation revealed significant correlations between “sweet” and “lemon” (r = 0.899), and “orange fruit” (r= 0.812), “rose water” (r = 0.820), and “grapefruit” (r = 0.764) indicating that the aroma and flavour profiles of the geraniol, and terpene alcohol fractions (all containing geraniol) increase the perceived sweetness intensity in beer. Sweetness was also significantly, positively correlated with “smooth bitterness” (r = 0.801) and negatively with “harsh bitterness” (r = 0.943) suggesting a sensory interaction effect between sweetness and bitterness qualities, where one is pivotal for the other.

The terpene alcohol-, monoterpene alcohol-, linalool- and geraniol fraction-flavoured beers were characterised by “lemon”, “pine wood”, and “soapy” aromas and “rose water”, “orange fruit”, and “grapefruit” flavours. The geraniol- and terpene alcohol fraction-flavoured beers were perceived to be significantly higher for “rose water” flavour compared to the other beers. The terpene alcohol fraction induced significantly increased “rose water” flavour compared to the monoterpene alcohol sub-fraction. This suggests that the terpene alcohol fraction contained volatiles besides the two key compounds linalool and geraniol inducing both floral and fruity notes due to additive- or synergistic-type behaviour. It is interesting to note that the linalool fraction-flavoured beer was not strongly characterised by any aroma and flavour attribute supporting the suggestion that linalool primarily acts as an aroma/flavour enhancing molecule in certain volatile mixtures as opposed to having a major impact on the sensory profile of beer when applied individually (Kaltner & Mitter, 2009; Takoi et al., 2010b).

3.2 Effect of fraction composition on sensory characteristics

Table 4 lists the 49 volatiles identified in the hop extracts including a range of monoterpene and sesquiterpene hydrocarbons, oxygenated sesquiterpenoids, monoterpene alcohols and smaller fractions of esters, ketones and unknowns (Fig. 3). Table 5 shows the average proportion (%) of the compounds in the corresponding hop extract. The fractions enriched in the single compounds linalool, geraniol and caryophyllene oxide contained only minor proportions of other compounds. Sample carryover between GC-MS runs was excluded as a possible cause of trace compounds by running ‘blanks’, suggesting these were naturally present as a result of the fractionation process. The chemical profiles of other extracts, however, showed significant overlaps, suggesting that a clear separation of sub-fractions was not achieved.

Trace components could potentially have contributed to the sensory profiles of the flavoured beers even if present at sub-threshold concentrations. Sensory threshold data of the compounds applied in comparable beer matrices was gathered from the available literature and compared with the relative concentrations applied in the samples (Table 5). Sensory detection thresholds in water are not shown since these are usually much lower than those in complex matrices such as beer. To date, no taste and mouthfeel threshold data has been published for the compounds identified. Several aroma and flavour threshold concentrations could be sourced and comparison with the applied concentrations showed that several compounds were added supra-threshold such as β-myrcene, α-humulene, β-caryophyllene, linalool, geraniol, humulene epoxide II, and humulenol II.

PCA was conducted to visualise the relationships between the samples, their sensory profiles and the volatile compositions. The plot in Fig. 5 shows the significant principal components PC1 (38.22%) and PC2 (26.43%) explaining 64.65% of the variance. The majority of volatile compounds loaded positively on PC1, together with either “crushed grass, sap” and “resinous” aromas or fruity aromas/flavours that could be assigned to volatiles in the hop extracts (among others C7 – linalool, C17 – geraniol, C16 - nerol, C19 - 2-undecanone, C25 – 2-dodecanone). PC2 is foremost positively loaded with “earthy”, “musty”, “harsh bitterness” and “lingering bitterness” and negatively loaded with “sweet” and “smooth bitterness”. This component is predominantly loaded with oxygenated sesquiterpenes and sesquiterpene hydrocarbons such as β-pinene (C1), β-myrcene (C2), *cis*-β-ocimene (C4), β-caryophyllene (C26), γ-muurolene (C30), β-eudesmene (C31), and humulene epoxide I (II (C42) and III (C45).

Hop compounds related to beer bitterness. β-caryophyllene (C26), α-humulene (C28) and humulene epoxides I and III (C40, C45) significantly positively correlated with “harsh bitterness” and “lingering bitterness”. In contrast, β-caryophyllene (C26), α-humulene (C28), humulene epoxides I (C40) and caryophyllene oxide (C39) significantly negatively correlated with “smooth bitterness”.

Caryophyllene oxide (C39) had no significant effect on the beer's taste and mouthfeel properties. The compound might rather act with a mix of oxygenated sesquiterpene to modify beer bitterness due to synergistic-type behaviour. Also of interest was that β-pinene (C1), *D*-limonene (C3), *cis*-β-ocimene (C4), and β-eudesmene (C31; or β-selinene) positively correlated with "harsh bitterness", which has not yet been reported elsewhere. The majority of the compounds related to modified bitterness qualities were therefore mainly present in the total oil and the sesquiterpene and humulene epoxide enriched fractions, the latter agreeing with the work of others (Goiris et al., 2002; Oladokun et al., 2016) who found a change in bitterness perception with oxygenated sesquiterpene fractions. Oladokun et al. (2016) also investigated the temporal profile of perceived beer bitterness at different concentrations with a Hersbrucker hop extract and found it induced a prolonged bitterness, although specific compounds or fractions were not attributed to this sensation. Mikyška et al., 2018) suggested increased concentrations of β-caryophyllene, α-humulene, and α-caryophyllene epoxide to be responsible for higher "harsh" bitterness scores in kettle+dry hopped beers. Also, Kaltner and Mitter (2006) reported a modified beer bitterness perception at different concentrations of linalool and terpene hydrocarbons added (Kaltner & Mitter, 2006).

Another compound that impacted beer bitterness was geraniol (C17) with the "smooth bitterness" score being significantly increased in the geraniol fraction-flavoured beer, particularly compared to the sesquiterpene fraction-flavoured beer. However, no significant, positive correlation was detected to explain the relationship between geraniol and the increased "smooth bitterness" intensity. It was concluded the bitterness quality was influenced by the perceived aromas and flavours, causing sensory interactions within (taste) and across (aroma/flavour) modalities. Limited research has been conducted in the field of hop volatiles and their effect on temporal and qualitative dimensions of bitterness and other taste sensations. Moreover, the hop extracts used might be too complex to draw reliable conclusions on concentration-dependent effects.

Hop compounds related to beer sweetness. In line with the preceding study (Dietz et al., 2020), beers flavoured with geraniol-containing fractions were mainly differentiated from the other beer by higher scores for "sweet", "rose water", "orange fruit", "grapefruit" and "lemon". Geraniol significantly correlated with several aroma and flavour attributes; particularly with "rose water" (r = 0.725), "orange fruit" (r = 0.753), and "grapefruit" (r = 0.858). It was concluded that beer sweetness was mainly added with 'fruity/floral' aromas perceived ortho- and retronasally, suggesting the sweetness to be increased through a sensory interaction between aroma and taste.

Hop compounds related to mouthfeel sensations. A "spicy" sensation in beer has previously been assigned to oxygenated sesquiterpenoids, humulene epoxides and oxidation products of β-caryophyllene, mostly describing a flavour or a mouthfeel sensation (Goiris et al., 2002; Praet et al., 2015). The sesquiterpene alcohol and humulol enriched fractions had limited effects on the beer's sensory profile, although results of previous studies indicated that the sub-fraction containing humulol (C41) and humulenol II (C46) could be responsible for the spicy/"peppery tingling" sensation (Deinzer & Yang, 1994; Goiris et al., 2002). The extracts contained ~351 μg/L and ~50 μg/L humulol and 123 μg/L and 36 μg/L humulenol II, respectively. Aroma threshold concentrations of these compounds in beer were determined to be 150-2500 μg/L for humulenol II (aroma, flavor) and 2000 μg/L for humulol (Table 5). Goiris et al. (2002) applied 20 μg/L of a sesquiterpenoid preparation that contained much lower concentrations of humulenol II (1.5 μg/L) in beer and observed effects on "spicy", "mouthfeel", and "fullness". It should be considered that results of previous studies are contradictory. Also, the relationship between "spicy" characters and sesquiterpenoids including humulene epoxides and humulenol II has not always been confirmed (Kishimoto, Wanikawa, Kagami, & Kawatsura, 2005). The studies applied different sensory approaches and beer matrices to assess the sensory properties of hop extracts. For the current study, it has to be noted that the sub-fractions contained other compounds at flavour-active concentrations (geraniol) and unknowns at trace levels. Further fractionation or purification should be conducted to obtain a better separation between sesquiterpene alcohols, monoterpene alcohols and compounds of other chemical classes. The concentrations of humulene epoxides were estimated to range between ~2 µg/L and ~697 µg/L, respectively, partly exceeding aroma threshold levels without affecting the "peppery tingling" sensation due to the aforementioned reasons. The same applied for the astringency, which positively correlated with α-humulene (C28; r = 0.630) and humulene epoxide I (C40; r = 0.758). Since a significant effect could not be reported, further research is required to investigate this potential cause-effect relationship.

Role of linalool in relation to aroma and flavor characteristics. Linalool (C7) as such, hardly modified the beer's aroma profile and only slightly increased the "rose water" flavour. Other research groups previously suggested linalool as a key contributor to floral (rose, lavender) and several citrus characters which acts synergistically with other monoterpene alcohols to increase fruity and floral aroma and flavour intensities (Takoi et al., 2010a; Takoi et al., 2010b). The concentration of linalool was significantly higher in the monoterpene alcohol than in the terpene alcohol fractions (~276 µg/L vs ~55 µg/L), while the opposite was the case for geraniol (~84 µg/L vs ~149 µg/L) and thus could have caused this effect on the citrusy/"rose water" aromas/flavours in the terpene alcohol fraction-flavoured beer. Linalool (C7) also significantly correlated with the "grapefruit" flavour (r = 0.605), which adds to the hypothesis that it may act synergistically in a mixture with other hop volatiles.

3.3 Prediction of sensory scores from GC-MS data

PLS regression analyses were conducted to explore the correlation between the 49 volatile hop compounds (Table 5) and the 12 sensory attributes found to be significant, plus the one approaching significance ("pine wood"; Table 3). PLS1 model performances resulted in relatively good fits of the data (Table 6), whilst PLS2 algorithm results were not satisfactory (data not included). The results are generally in agreement with the PCA's outcome, and the compound-attribute relationships seemed coherent with previous results (Dietz et al., 2020). It was difficult to identify clear causal relationships between hop compounds and one sensory sensation and vice versa. Most models could explain a moderate to high percentage of the original variance. However, the models also required between 10 and 25 variables, with the model for "earthy" being the most complex. This indicates the complexity of the sensory profiles of hop extracts and the difficulty in understanding their molecular basis. Positive and negative correlations were broadly balanced, suggesting compounds positively or negatively affect the perception of sensory characteristics.

The strongest models were built for "earthy", "musty", "crushed grass, sap", "resinous", "grapefruit", and "harsh bitterness". Moderate models were built for "lemon", "soapy", "orange fruit", "rose water", "sweet", and "smooth bitterness", and unsatisfactory model performance was found for "pine wood". Compounds with high regression coefficients (>0.05) and variable importance in projection (VIP) criteria (>1.00) were considered as impactful compounds. Several compounds correlated with the sweetness and smooth bitterness in the flavoured beers. Fig. 6. shows the standardised regression coefficients map with compounds found to be important for each corresponding sensory attribute. Compounds with standardised coefficients lower than 0.05 are not included. In line with previous findings, geraniol appeared to be the most important compound for "sweet" and "smooth bitterness" while α-humulene, β-caryophyllene, δ-cadinene and caryophyllene oxide had the largest negative coefficients and negatively correlate with these taste characteristics. Interestingly, geranyl and octyl isobutyrate and some other esters also negatively correlated with these attributes, but this might be because these compounds were mainly present in the total oil and the sesquiterpene fraction. The model structures for "sweet" and "smooth bitterness" were distinct from the model for "harsh bitterness", the latter featuring important contributions from α-humulene, δ-cadinene, β-caryophyllene, β-myrcene, and caryophyllene oxide. The humulene epoxides (I-III) seemed not to play a significant role for the model of "harsh bitterness" indicating that a combination of sesquiterpenes were mainly driving of this bitterness sensation.

The terpene alcohols terpinen-4-ol, myrtenol, perillol, and *endo*-borneol all negatively correlated with "crushed grass, sap", "resinous", "earthy", and "musty", which is surprising because they were expected to positively contribute to one or more of these sensations. However, negative correlations can also occur if strong aroma compounds overpower weaker ones or if compound concentrations are significantly lower than those of other compounds contributing to the same sensation. The same reasons were considered for the standardised coefficients recorded for linalool oxide and methyl octanoate, which were either absent in the extracts or present at relatively low concentrations.

Linalool played an important role in the models for "lemon", "grapefruit" and "rose water" and negatively correlated with "musty", once again indicating its importance as a synergist and an antagonist in the perception of the aromas and flavours. This was one of the main differences between the outcomes of the PCA and PLS studies. PCA is focused on demonstrating causality between compounds and attributes in a multisensory space, just by virtue of the compounds being present. Conversely, PLS aims to detect correlative connections between compounds and individual attributes, including mixture-dependent perceptual effects. In turn, correlation does not necessarily imply causation. Results should be seen as tentative and need to be validated, for instance, by performing recombination studies. PLS models can only display sensory interaction effects to a certain extent. Consequently, including threshold concentrations (aroma/flavour/taste/mouthfeel) and further sensory and analytical inputs (temporal sensory data, odour activity (OAV), Charm values, physico-chemical, physiological) into Multi-Block PLS regressions would likely improve the model performance and simplify the selection of components for supervised developments of algorithms.

It should be noted that, due to the limits of detection with the analytical approach used, compounds at very low concentrations or trace levels (sulphur compounds), were not incorporated, but could still have contributed to the sensory profiles of the flavoured beers. It should also be taken into account that the hop oil extracts were solely tested in a lager type beer. The fractions and compounds could potentially be perceived in a slightly different way if applied in a different beer style due to matrix-dependent effects. Moreover, threshold concentrations were only retrieved from previous publications but not measured in the current study. Measuring these and considering further parameters such as OAV (ratio of a compounds' concentration to odour threshold concentration in the same matrix) assessed using aroma extract dilution analyses (AEDA) in combination with GC-Olfactometry (GC-O) and GC-MS (Dunkel et al.; 2014), will provide further insights to understand the contribution of the applied volatile hop compound and compound combinations to the aroma perceived in beer.

4. Conclusions

The approach to break hop oil fractions into its constituents and study the sensory profiles of individual compound and compound groups revealed important insights into the sensory differences between the hop extracts and several compounds involved in sensory interactions and thereby modifying beer flavour and taste. Nevertheless, a certain chemical complexity seems to be required to trigger sensory interactions and induce multisensory effects. Understanding these mechanisms presents challenges but will help to characterise the diverse sensory properties in hop oil fractions and guide further investigations into potential commercial versions thereof. These flavouring preparations are developed to be added post-fermentation to increase the transfer of volatile compounds into beer, reduce the volume of hops required to achieve desired sensory characteristics and decrease the environmental impact of hops in the brewing process. Moreover, hop harvests and supply to the brewing industry are subjected to crop seasonality and different conversion of oil and aroma active functionals on a year to year basis. Since the industry aims to maintain beer brand identities, this research may also provide the basis for further standardisation of sustainable hop materials.

Author Contribution

Christina Dietz: PhD student. Conducted all research and formal analysis in this manuscript. Writing – original draft.

David Cook: Funding acquisition, supervision of PhD, conceptualisation and input to design of GC-MS analytical study and writing – review and editing of manuscript.

Colin Wilson: Conceptualisation and input to design of investigation. Writing – review and editing of manuscript.

Rebecca Ford: Supervision of PhD, conceptualisation and input to design of sensory studies and writing – review and editing of manuscript.

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Conflict of interest

The authors declare there are no conflicts of interest.

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Tables & figures

Table 1
Overview of sensory attributes (in order of presentation), definitions, and training reference standards.

Modality	Sensory attribute	Definition	Training reference standard
Aroma	Sweetcorn	Sweetcorn aroma as when smelling canned, cooked sweetcorn, the dimethyl sulphide reference solution or cooked vegetable gone off	10 mL 150 µg/L dimethyl sulphide (DMS; Aroxa, UK) – water solution (deionised water), 10 g of canned, cooked sweetcorn (with dripping water)
	Soapy	Soapy aroma as when smelling an unscented bar of soap	5 g unscented bar of soap (Sainsbury's Supermarkets Ltd., UK)
	Pine wood	Pine wood aroma as when smelling pine shavings or the pine wood reference solution	10 g pine shavings (Sainsbury's Supermarkets Ltd., UK); 5 mL 6 mg/L (1*R*)-(+)-*α*-Pinene (food grade; Sigma Aldrich, UK) in deionised water
	Crushed grass, sap	Crushed grass, sap aroma as when smelling crushed grass, sap, tomato leaf, or carrot leaf	20 g crushed cut grass and sap that has been left in the closed sample bottles for 2 days; 10 g fresh tomato leaf or carrot leaf
	Dark fruits	Dark fruits aroma as when smelling raisins, prunes	10 g chopped raisins and prunes (Sainsbury's Supermarkets Ltd., UK)
	Pear	Pear aroma as when smelling a pear fruit (peel, flesh)	5 g freshly chopped pear pieces with peel
	Lemon	Lemon aroma as when smelling a lemon fruit or artificial lemon aroma, e.g. in citrus wet wipes	5 g freshly chopped lemon and lime; 1 citrus wet wipe (Dettol, UK)
	Resinous	Resinous aroma as when smelling the wood resin reference	10 g pine resin and 10 g myrrh resin (Indigo Herbs, UK)
	Earthy	Earthy aroma as when smelling wet earth or soil	10 g fresh wet earth, soil
	Musty	Musty aroma as when smelling mildew or mould, stale damp cellar, mouldy damp cardboard, or an old, dirty, dried sponge or dish cloths	20 g damp cardboard soaked in deionised water for 24h in the closed sample bottles; damp, used sponge that has been left for 24h in the closed sample bottle
	Overall aroma intensity	Overall aroma intensity in the sample	No physical reference
Flavour	Rose water	Rose water flavour as when eating a piece of Turkish delight or having a sip of geranium oil solution	½ piece (5 g) Turkish delight (Sainsbury's Supermarkets Ltd., UK); 5 mL 0.6% (w/v) geranium essential oil (Ecodrop, UK) in deionised water
	Malty	Malty flavour as when eating malt extract or a piece of fruitless malt loaf or Shreddies	10 g malt extract (Holland & Barrett, UK); 10 g Soreen malt loaf; 3 pieces Shreddies (Nestlé, UK)
	Orange fruit	Orange fruit flavour as when eating a piece of orange, mandarin, tangerine	5 g freshly cut orange and mandarin (flesh, peel)
	Biscuity	Biscuity flavour as when eating Digestive biscuits	¼ piece Digestive biscuit (McVitie's, UK)
	Grapefruit	Grapefruit flavour as when eating a piece of grapefruit or drinking a sip of grapefruit juice	5 g fresh cut grapefruit; 10 mL pink grapefruit juice (Tropicana, UK)
	Overall flavour intensity	Overall flavour intensity in the sample	No physical reference
Taste	Sweet	Sweet taste; immediate sensation after swallowing	10 mL 1% sucrose (Sainsbury's Supermarkets Ltd., UK); 10 mL 4% (v/v) EtOH (96%, ferm., FG; Haymankimia, UK) in deionised water
	Sour	Sour taste; immediate sensation after swallowing	10 mL 0.2% (v/v) citric acid (Sigma Aldrich, UK) ; 10 mL 4% (v/v) EtOH

Modality	Sensory attribute	Definition	Training reference standard
			(96%, ferm., FG; Haymankimia, UK) in deionised water
	Smooth bitterness	Soft, pleasant bitterness intensity; immediate sensation after swallowing	10 mL 3 mg/L HopAlpha® Iso30% (TNS Ltd., UK) in deionised water
	Harsh bitterness	Irritating, spiky bitterness intensity; immediate sensation after swallowing	0.3% (v/v) caffeine in deionised water (food grade; Sigma Aldrich, UK)
	Lingering bitterness	Persistence of the overall bitterness in the mouth; 20 seconds after swallowing	10 mL 3 mg/L HopAlpha® Iso30% (TNS Ltd., UK) in deionised water
Mouthfeel	Astringent	Mouth drying, rough sensation, shrinking/tightening in the mouth, as when chewing banana peel or taking a sip of the reference solution; 30 seconds after swallowing	10 mL 1% (w/v) tannic acid (Alfa Aesar, US) in deionised water; 5 g banana peel
	Peppery tingling	Peppery tingling sensation as when eating chilli, fresh ginger, horseradish/radish; tingling mouthfeel, irritating, itching; immediate sensation after swallowing	No physical reference

Table 2
Analysis of variance (ANOVA) F-ratios and for sensory attributes rated for the hop oil extracts applied in lager.

Modality	Sensory attribute	Sam	Pan	Rep	Sam x Pan[b]	Sam x Rep[b]	Rep x Pan[b]
Aroma	Sweetcorn	1.24	4.86***	0.58	1.58*	1.32	1.14
	Pear	1.45	21.39***	0.25	0.96	0.56	0.90
	Dark fruits	0.79	11.58***	1.11	1.71*	1.46	1.55*
	Lemon	5.61***	5.65***	0.75	1.59*	0.94	1.28
	Pine wood	1.68	9.82***	0.60	1.72*	1.27	1.42
	Crushed grass, sap	3.43**	11.02***	0.49	1.00	1.03	1.07
	Resinous	2.06*	7.47***	0.76	1.69*	1.42	1.19
	Earthy	2.18*	4.99***	1.31	1.53*	0.90	1.05
	Musty	1.98*	1.83*	1.67	1.20	0.95	1.12
	Soapy	2.57**	14.80***	1.67	0.98	0.60	0.64
	Overall aroma intensity	4.01***	15.88***	0.37	1.20	0.79	0.93
Flavour	Rose water	8.49***	3.40**	0.58	1.95**	0.96	0.92
	Malty	0.50	6.24***	1.09	1.48	1.47	1.25
	Biscuity	0.52	7.68***	2.71*	2.04**	1.86**	1.51
	Orange fruit	4.82***	8.81***	0.79	1.69*	1.05	1.18
	Grapefruit	4.74***	7.03***	0.31	1.45	1.02	1.18
	Overall flavour intensity	6.37***	7.56***	0.74	1.72*	1.11	1.26
Taste	Sweet	3.16**	6.54***	0.63	1.13	0.93	1.21
	Sour	1.17	9.96***	1.26	1.15	1.37	1.46
	Smooth bitterness	2.09*	5.67**	1.61	0.87	0.90	0.95
	Harsh bitterness	1.49**	6.80**	1.07	0.70	0.81	1.03
	Lingering bitterness	0.90	15.45***	0.67	0.79	0.80	1.16
Mouthfeel	Peppery tingling	0.61	10.97***	0.69	1.08	1.06	1.16
	Astringent	1.06	10.63***	0.81	1.06	0.97	1.09

*, **, *** indicating a significant effect at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, from three-factor ANOVA with interactions (Sample (Sam), Panelist (Panel), Replicate (Rep)).

Table 3
Mean sensory intensities (n = 9, triplicates) for the control beer, the flavoured beer samples, and an experimental replicate.

Modality	Sensory attribute	C	TO	TO (repl)	SQ	HUM EPOX	TA	MTA	LIN	GER	SQA	HUM	CAR
Aroma	Sweetcorn	2.51^{a}	1.72^{a}	2.05^{a}	1.39^{a}	1.95^{a}	1.39^{a}	1.96^{a}	2.29^{a}	1.59^{a}	2.43^{a}	2.41^{a}	2.07^{a}
	Pear	1.27^{ab}	3.00^{a}	2.30^{ab}	1.80^{ab}	1.15^{ab}	2.16^{ab}	2.61^{ab}	0.91^{b}	2.63^{ab}	1.91^{ab}	1.49^{ab}	1.36^{ab}
	Dark fruits	2.01^{a}	2.27^{a}	2.07^{a}	2.28^{a}	1.67^{a}	2.50^{a}	2.41^{a}	2.19^{a}	2.10^{a}	2.48^{a}	3.18^{a}	2.02^{a}
	Lemon	0.83^{bc}	1.61^{bc}	1.58^{bc}	0.90^{bc}	0.68^{c}	3.00^{ab}	2.55^{abc}	1.79^{abc}	3.81^{a}	1.07^{bc}	1.34^{bc}	0.73^{c}
	Pine wood	2.04^{a}	4.03^{a}	3.57^{a}	2.99^{a}	2.53^{a}	4.11^{a}	3.47^{a}	2.50^{a}	4.11^{a}	2.87^{a}	2.79^{a}	2.01^{a}
	Crushed grass, sap	1.00^{b}	4.47^{a}	4.42^{a}	2.86^{ab}	1.86^{b}	2.74^{ab}	2.78^{ab}	1.34^{b}	2.57^{ab}	2.13^{b}	2.33^{ab}	1.66^{b}
	Resinous	1.15^{c}	4.43^{a}	3.57^{ab}	2.06^{bc}	1.41^{bc}	2.20^{bc}	2.11^{bc}	1.69^{bc}	2.72^{abc}	2.04^{bc}	1.99^{bc}	1.70^{bc}
	Earthy	1.33^{abc}	2.81^{a}	2.54^{ab}	1.98^{abc}	1.59^{abc}	1.18^{abc}	0.94^{bc}	1.19^{abc}	0.63^{c}	1.91^{abc}	1.26^{abc}	1.00^{bc}
	Musty	1.76^{ab}	3.15^{a}	3.10^{a}	2.14^{ab}	2.43^{ab}	1.73^{ab}	1.49^{ab}	1.34^{ab}	0.82^{b}	2.25^{ab}	1.43^{ab}	1.71^{ab}
	Soapy	1.32^{b}	3.52^{a}	3.15^{ab}	1.99^{ab}	1.55^{ab}	3.26^{ab}	3.01^{ab}	2.11^{ab}	3.17^{ab}	1.53^{ab}	2.24^{ab}	1.66^{ab}
	Overall aroma intensity	3.19^{c}	5.75^{a}	5.27^{ab}	4.20^{abc}	3.65^{bc}	4.61^{abc}	4.51^{abc}	4.12^{abc}	5.10^{ab}	3.74^{bc}	3.93^{bc}	3.29^{c}
Flavour	Rose water	0.40^{d}	2.97^{bcd}	2.49^{cd}	1.66^{cd}	0.94^{cd}	5.68^{ab}	3.64^{bc}	2.50^{cd}	6.89^{a}	1.36^{cd}	2.37^{cd}	1.85^{cd}
	Malty	3.99^{a}	2.76^{a}	2.96^{a}	2.87^{a}	3.73^{a}	3.04^{a}	3.21^{a}	3.77^{a}	2.41^{a}	3.12^{a}	3.03^{a}	3.47^{a}
	Biscuity	2.07^{a}	1.40^{a}	1.79^{a}	1.73^{a}	1.55^{a}	1.94^{a}	1.66^{a}	1.96^{a}	1.49^{a}	2.09^{a}	1.70^{a}	1.99^{a}
	Orange fruit	1.25^{d}	2.64^{abcd}	2.16^{abcd}	1.80^{bcd}	2.3^{abcd}	4.36^{a}	3.74^{abc}	1.93^{bcd}	3.92^{ab}	1.56^{cd}	2.16^{abcd}	1.54^{cd}
	Grapefruit	1.57^{d}	2.87^{abcd}	2.21^{bcd}	2.19^{bcd}	1.71^{d}	4.60^{a}	4.07^{ab}	2.19^{bcd}	3.91^{abc}	2.00^{bcd}	2.57^{abcd}	1.80^{cd}
	Overall flavour intensity	3.96^{d}	5.39^{abcd}	5.17^{bcd}	4.80^{bcd}	4.50^{cd}	6.52^{ab}	5.90^{abc}	5.13^{bcd}	7.10^{a}	4.96^{bcd}	5.24^{bcd}	4.15^{cd}
Taste	Sweet	2.45^{abc}	2.58^{abc}	2.22^{bc}	1.84^{c}	2.75^{abc}	3.42^{ab}	3.43^{ab}	2.79^{abc}	3.99^{a}	2.53^{abc}	2.37^{bc}	2.61^{abc}
	Sour	2.42^{a}	2.97^{a}	2.70^{a}	3.03^{a}	2.43^{a}	3.23^{a}	2.99^{a}	3.38^{a}	2.55^{a}	2.36^{a}	3.29^{a}	2.70^{a}
	Smooth bitterness	2.67^{ab}	2.42^{ab}	1.93^{b}	1.57^{b}	2.57^{ab}	2.44^{ab}	3.45^{ab}	3.07^{ab}	4.32^{a}	2.90^{ab}	2.93^{ab}	2.12^{b}
	Harsh bitterness	2.89^{ab}	3.64^{ab}	3.92^{a}	4.12^{a}	3.27^{ab}	3.68^{ab}	2.33^{ab}	2.67^{ab}	1.89^{b}	2.71^{ab}	2.96^{ab}	3.25^{ab}
	Lingering bitterness	3.60^{a}	4.60^{a}	4.73^{a}	4.58^{a}	4.57^{a}	4.54^{a}	4.36^{a}	4.06^{a}	3.60^{a}	3.91^{a}	4.59^{a}	4.54^{a}
Mouthfeel	Peppery tingling	3.78^{a}	4.68^{a}	4.39^{a}	4.44^{a}	4.51^{a}	4.56^{a}	4.50^{a}	4.40^{a}	4.22^{a}	4.05^{a}	4.28^{a}	4.30^{a}
	Astringent	4.55^{a}	5.19^{a}	5.10^{a}	5.15^{a}	5.40^{a}	5.26^{a}	4.76^{a}	4.92^{a}	4.44^{a}	4.39^{a}	4.33^{a}	5.22^{a}

Superscripts of different letters within an attribute indicate a significant difference between means of samples of an attribute by Tukey's Honest Significant Difference (HSD) test at $p < 0.05$. repl, experimental replicate; TO, Total oil; SQ, Sesquiterpene fraction; HUM EPOX, Humulene epoxides enriched fraction; TA, Terpene alcohol fraction; MTA, Monoterpene alcohol fraction; LIN, Linalool fraction; GER, Geraniol fraction; SQA, Sesquiterpene alcohol fraction; HUM, Humulol enriched fraction; CAR, Caryophyllene oxide fraction

Table 4
Volatile compounds (tentatively) identified (n=49) in the nine hop extracts using library/database matching (>80%) and authentic standards (*). Identification confirmed by calculated retention indices (RI).

#	Compound	CAS	RI		Literature RI	
			5MS	WAX	5MS	WAX
1	*β*-Pinene*	127-91-3	985	1130	980-990[a,b]	1113-1124[a,b]
2	*β*-Myrcene*	123-35-3	990	1153	991-994[a,b]	1145-1176[a,b]
3	*R*-(+)/D-Limonene*	5989-27-5	1027	1190	1030-1039[a,b]	1201-1234[a,b]
4	*cis*-*β*-Ocimene	3338-55-4	1036	1240	1038-1043[a,b]	1242-1245[a,b]
5	*cis*-Linalool oxide	1365-19-1	1072	1417	1070-1074[a,b]	11420[b]
6	2-Nonanone*	821-55-6	1088	1395	1093[b]	1388[b]
7	Linalool*	78-70-6	1098	1526	1098-1112[a,b]	1537[b]
8	*exo*-*β*-Fenchol	470-08-6	1115	1550	1117[a]	1576[n]
9	Myrcenol	543-39-5	1123	1561	1118[a]	n/a
10	Methyl octanoate*	111-11-5	1135	1391	1127[c]	1389[b]
/	Benzyl acetate (ISTD)	140-11-4	1162	1737	1162-1164[a,b]	1735[o]
11	*endo*-Borneol*	464-45-9	1163	1680	1162-1165[a,b]	1642-1677[a,b]
12	Terpinen-4-ol	562-74-3	1175	1614	1177-1182[a,b]	1591-1616[a,b]
13	*trans*-3(10)-Caren-2-ol	93905-79-4	1176	1698	1175[d]	1700[p]
14	*α*-Terpineol*	8000-41-7	1187	1686	1185-1207[a,b]	1688-1720[a,b]
15	Myrtenol	19894-97-4	1197	1756	1196[e]	1757[q]
16	Nerol	106-25-2	1224	1773	1228-1233[a,b]	1753-1770[a,b]
17	Geraniol*	106-24-1	1276	1826	1255-1276[a,b]	1788-1862[a,b]
18	Methyl 8-methylnonanoate	5129-54-4	1290	1527	1287[f]	1520[r]
19	2-Undecanone*	112-12-9	1292	1595	1296[b]	1596[s]
20	Perillol (Perillyl alcohol)	7644-38-4	1292	1983	1295[c]	1985[t]
21	2-Undecanol	1653-30-1	1307	1710	1301[g]	1719[b]
22	Methyl (*E*)-4-decenoate	93979-14-7	1314	1612	1311[h]	1608[s]
23	Methyl geranate*	2349-14-6	1319	1677	1323[h]	1678[s]
24	Octyl Isobutyrate	109-15-9	1328	1538	1326 [i]	1535[r]
25	2-Dodecanone*	6175-49-1	1381	1662	1379 [j]	1673[r]
26	*β*-Caryophyllene*	87-44-5	1418	1592	1418-1467[a,b]	1594-1618[a,b]
27	*α*-Bergamotene	17699-05-7	1433	1759	1430-1434[a,b]	1779[b]
28	*α*-Humulene*	6753-98-6	1452	1671	1467[b]	1663[b]
29	Geranyl propionate*	105-90-8	1472	1826	1475[a]	1830[u]
30	*γ*-Muurolene	30021-74-0	1474	1671	1477-1475[a,b]	1681-1684[a,b]
31	*β*-Eudesmene	515-17-3	1489	1717	1485[a]	1711[b]
32	2-Tridecanone*	593-08-8	1491	1814	1496[h]	1817[s]
33	Methyl 3,6-dodecadienoate	16106-01-7	1493	1872	1488[j]	1857[r]
34	Geranyl isobutyrate*	2345-26-8	1515	1773	1514[a]	1777[s]
35	*δ*-Cadinene	483-76-1	1530	1774	1519-1539[a,b]	1788[v]
36	*trans*-*Z*-α-Bisabolene epoxide	n/a	1533	NF	1531[k]	NF
37	Nerolidol	7212-44-4	1539	2021	1534-1565[a,b]	2009-2054[a,b]
38	Caryophyllenyl alcohol	56747-96-7	1568	2025	1556-1568[a,b]	n/a
39	Caryophyllene oxide*	1139-30-6	1577	1974	1573-1606[a,b]	1982[w]
40	Humulene epoxide I	19888-34-7	1578	2012	1578[l]	2000[x]
41	Humulol	28446-26-6	1581	2122	1582[l]	n/a
42	Humulene epoxide II	19888-34-7	1592	2010	1593[j]	2022[r]
43	Widdrol	6892-80-4	1598	NF	1597[b]	NF
44	Epicubenol	19912-67-5	1608	2054	1613-1645[a,b]	n/a

#	Compound	CAS	RI		Literature RI	
			5MS	WAX	5MS	WAX
45	Humulene epoxide III	21624-36-2	1612	2075	1611[l]	2055[y]
46	Humulenol II	19888-00-7	1619	2230	1613[l]	n/a
47	11,11-Dimethyl-4,8-dimethylene bicyclo[7.2.0]undecan-3-ol	79580-01-1	1636	NF	1639[m]	NF
48	*τ*-Cadinol	5937-11-1	1638	2135	1640[a]	n/a
49	*δ*-Cadinol	36564-42-8	1641	2164	1635-1674[a,b]	2167[b]

ISTD, Internal standard; NF, not found

[a] Pherobase; [b] Flavornet; [c] Nance and Setzer (2011); [d] Kang, Zhang, Du, and Wang (2010); [e] Maggi et al. (2009); [f] Ilic-Tomic et al. (2015); [g] Zhang et al. (2017); [h] Pistelli et al. (2018); [i] Venkatachallam, Pattekhan, Divakar, and Kadimi (2010); [j] Jackson and Linskens (2002); [k] Al-Reza, Rahman, Sattar, Rahman, and Fida (2010); [l] Tatiana Praet et al. (2016); [m] Zeng, Zhang, Luo, and Zhu (2011); [n] Pino, Marbot, and Bello (2002); [o] Perry, Wang, and Lin (2009); [p] Palá-Paúl et al. (2005); [q] Giuseppe, Manuela, Marta, and Vincenzo (2005); [r] Yan et al. (2018); [s] Liu, Wang, and Liu (2018); [t] Minh Tu et al. (2002); [u] Choi and Sawamura (2000); [v] Stashenko et al. (2010); [w] Richter, Eyres, Silcock, and Bremer (2017); [x] Hofmann, Fritz, Nitz, Kollmannsberger, and Drawert (1992); [y] Miyazawa, Kawauchi, and Matsuda (2010)

Table 5
Semi-quantified volatile composition of the nine hop extracts (average relative peak area %), Log *P* (logarithm of the octanol-water partition coefficient) used as an indicator for the hydrophobicity, solubility in water, and sensory detection thresholds of volatile compounds in beer (where available), labelled in bold if the relative concentration of a compound added to the base beer potentially exceeded its sensory threshold concentration.

#	Compound	TO	SQ	HUM EPOX	TA	MTA	LIN	GER	SQA	HUM	CAR	Log *P**	Solubility [mg/L]*	Sensory detection threshold [µg/L]**
1	*β*-Pinene	0.37	0.01	-	-	-	-	-	-	-	-	4.16	7.06	n/a
2	*β*-Myrcene	**34.74**	**4.04**	0.04	-	0.07	0.26	-	0.02	0.04	-	4.88	6.92	A: 9-1000[a]; F: 40[b]
3	*R*-(+)/D-Limonene	0.29	0.03	-	-	-	-	-	-	0.01	-	4.57	4.58	n/a
4	*cis*-*β*-Ocimene	0.07	0.03	0.05	-	-	-	-	-	-	-	4.67	2.01	n/a
5	*cis*-Linalool oxide	0.01	-	-	-	-	-	-	-	0.1	-	2.08	3353.00	n/a
6	2-Nonanone	0.19	-	-	0.07	0.54	-	-	-	1.01	-	3.14	170.60	F: 2000[c]
7	Linalool	**0.42**	-	-	**4.22**	**31.68**	**98.94**	-	-	**5.29**	-	2.97	683.70	A: 2-80[a]; F: 27-80[c,d]
8	*exo*-*β*-Fenchol	-	-	-	1.01	-	-	-	-	-	-	2.85	461.40	n/a
9	Myrcenol	0.04	-	-	0.26	2.08	-	-	-	0.58	-	3.46	260.90	n/a
10	Methyl octanoate	0.45	-	-	-	0.06	-	-	-	0.03	-	3.46	101.90	n/a
11	*endo*-Borneol	0.05	-	-	1.65	7.04	-	-	0.02	1.91	-	2.69	260.90	n/a
12	Terpinen-4-ol	0.01	-	-	0.2	2.21	-	-	-	0.26	-	3.26	386.60	n/a
13	*trans*-3(10)-Caren-2-ol	0.05	-	-	0.1	0.74	-	-	-	0.61	-	1.97	489.00	n/a
14	*α*-Terpineol	0.19	-	-	4.28	13.7	-	-	0.12	3.85	-	2.98	371.70	A: 330[a]; F: 2000[c]
15	Myrtenol	0.01	-	-	0.05	0.02	-	-	-	0.05	-	2.98	426.90	n/a
16	Nerol	0.14	0.03	0.03	1.38	1	-	-	0.17	0.99	-	4.70	39.90	A: 80-500[a]
17	Geraniol	0.05	-	-	**11.33**	**9.6**	-	**99.32**	**4.14**	**16.19**	-	3.47	255.80	A: 4-300[a]; F: 36[e]
18	Methyl 8-methyl-nonanoate	0.25	0.13	0.13	0.01	0.06	-	-	-	0.03	-	4.40	12.56	n/a
19	2-Undecanone	1.8	0.27	0.42	2.04	9.55	-	-	0.5	10.29	-	3.69	19.71	F: 400[c]
20	Perillol	0.02	-	-	0.19	0.17	-	-	0.14	0.46	-	3.17	471.00	n/a
21	2-Undecanol	0.09	-	-	0.49	0.39	-	-	0.41	0.31	-	4.21	49.73	F: 70[c]
22	Methyl (*E*)-4-decenoate	1.4	0.05	1.07	-	0.4	-	-	-	0.25	-	4.09	16.67	n/a
23	Methyl geranate	0.73	0.44	0.57	-	0.28	-	-	-	0.91	-	3.98	21.24	F: 21.5[f]
24	Octyl Isobutyrate	0.15	0.16	0.13	-	0.02	-	-	0.01	0.06	-	4.71	4.06	n/a
25	2-Dodecanone	0.3	-	0.15	0.96	1.57	-	-	1.29	2.76	-	4.18	13.99	F: 250[c]
26	*β*-Caryophyllene	8.76	**19.05**	0.21	0.12	0.18	-	-	-	0.09	-	6.30	0.05	A: 160-420[a]
27	*α*-Bergamotene	0.02	0.27	0.03	-	0.04	-	-	-	0.01	-	6.57	0.03	n/a

#	Compound	TO	SQ	HUM EPOX	TA	MTA	LIN	GER	SQA	HUM	CAR	Log *P**	Solubility [mg/L]*	Sensory detection threshold [μg/L]**
28	*α*-Humulene	**36.39**	**55.4**	7.02	0.48	0.51	-	-	-	0	-	6.95	0.01	A: 120-747[a,g]
29	Geranyl propionate	0.02	0.02	-	0.29	0.02	-	-	0.06	0.13	-	3.64	2.22	n/a
30	γ-Muurolene	1	3.12	3.13	-	0.06	-	-	-	0.04	-	6.27	0.05	n/a
31	*β*-Eudesmene	0.46	1.63	1.09	-	0.01	-	-	-	0.04	-	6.38	0.04	n/a
32	2-Tridecanone	0.05	0.21	1.21	2.85	3.41	-	-	0.81	0.35	-	4.68	4.53	F: 100[c]
33	Methyl 3,6-dodecadienoate	0.23	-	1	-	-	-	-	0.02	0.02	-	4.10	2.77	n/a
34	Geranyl isobutyrate	1.5	4.55	3.16	-	0.03	-	-	0.21	0.24	-	4.77	0.82	A: 450[a]; F: 450[e]
35	*δ*-Cadinene	2.3	9.42	-	-	0.02	-	-	0.06	0.25	-	6.64	0.05	n/a
36	*trans*-*Z*-*α*-Bisabolene epoxide	0.04	0.01	1.37	-	-	-	-	0.01	0.01	-	4.86	7.27	n/a
37	Nerolidol	0.1	-	-	**2.12**	0.25	-	-	**2.75**	1.38	-	5.68	1.53	F: 21.44[f]
38	Caryophyllenyl alcohol	0.18	-	-	11.08	1.09	-	-	14.76	5.65	-	4.20	9.13	n/a
39	Caryophyllene oxide	0.55	1.63	15.04	0.53	0.09	-	-	0.95	0.19	99.74	3.60	2.21	n/a
40	Humulene epoxide I	0.04	0.04	**2.55**	**0.95**	-	-	-	-	-	-	4.56	0.62	A: >10[a;] F: 100[h]
41	Humulol	0.67	-	-	30.58	2.08	-	-	39.72	18.9	-	3.80	44.17	A: 2000[i]
42	Humulene epoxide II	1.11	2.23	**78.63**	-	0.24	-	-	1.16	-	-	4.51	5.43	A: 450[a]
43	Widdrol	0.03	-	-	0.7	0.47	-	-	1.73	0.29	-	4.10	7.93	n/a
44	Epicubenol	0.04	-	-	1.46	0.18	-	-	2.02	0.94	-	3.69	9.13	n/a
45	Humulene epoxide III	0.04	0.03	1.16	-	-	-	-	-	-	-	4.45	0.51	F: 450[e]
46	Humulenol II	0.1	-	-	**12.06**	1.52	-	-	13.39	13.04	-	3.50	2.26	A: 150-2500[a]; F: 2500[e]
47	11,11-Dimethyl-4,8- dimethylenebicyclo[7.2.0]undecan-3-ol	0.03	-	-	1.59	0.08	-	-	2.25	-	-	3.70	8.12	n/a
48	*τ*-Cadinol	0.1	-	-	4.96	0.21	-	-	4.86	1.64	-	4.90	9.13	n/a
49	*δ*-Cadinol	0.03	-	-	1.34	-	-	-	2.12	0.59	-	4.95	9.13	n/a

"-" compound not detected; TO, Total oil; SQ, Sesquiterpene fraction; HUM EPOX, Humulene epoxides enriched fraction; TA, Terpene alcohol fraction; MTA, Monoterpene alcohol fraction; LIN, Linalool fraction; GER, Geraniol fraction; SQA, Sesquiterpene alcohol fraction; HUM, Humulol enriched fraction; CAR, Caryophyllene oxide fraction

* Log *P* and solubility in water estimated using EPI Suite™ v.4.1 software (U.S. Environmental Protection Agency)

** Aroma (A) and/or flavour (F) threshold concentrations. Taste and mouthfeel threshold concentration have not yet been determined for the compounds identified in the hop extracts used in this study.

[a] Schönberger et al. (2015); [b] M. Meilgaard, Civille, and Carr (1999); [c] Morton C Meilgaard (1975b); [d] Hanke (2009); [e] Peacock and Deinzer (1981); [f] Jiang et al. (2017); [g] Bordiga and Nollet (2019); [h] Shimazu, Hashimoto, and Kuroiwa (1975); [i] Irwin (1989)

Table 6
Sensory scores mean ranges and PLS regression model performances (PLS1) for prediction of the sensory attributes (significant in the sensory study) among hop extracts based on their volatile compositions (Table 5).

	Sensory scores				PLS model performance[a]		
Attribute	Min	Max	Mean	SD	R^2	RMSE	n *X*
Lemon	0.68	3.81	1.66	1.04	0.661	0.583	17
Pine wood[b]	0.68	3.81	1.66	1.04	0.537	0.452	10
Crushed grass, sap	1.00	4.42	2.34	0.92	0.873	0.289	19
Resinous	1.15	3.57	2.06	0.65	0.791	0.264	21
Earthy	0.63	2.54	1.41	0.55	0.933	0.142	25
Musty	0.81	3.10	1.84	0.62	0.908	0.188	22
Soapy	1.31	3.26	2.27	0.75	0.682	0.381	15
Rose water	0.40	6.89	2.71	1.99	0.668	0.157	20
Orange fruit	1.25	4.36	2.43	1.07	0.661	0.579	15
Grapefruit	1.57	4.60	2.62	1.06	0.787	0.462	16
Sweet	1.84	3.99	2.76	0.62	0.635	0.370	11
Smooth bitterness	1.57	4.32	2.72	0.76	0.637	0.455	13
Harsh bitterness	1.89	4.11	3.06	0.67	0.805	0.296	16

[a]PLS1 algorithm for univariate sensory attributes applied with logarithmic transformed GC-MS data
[b]Pine wood was included because it an approached significant effect in the sensory study.
RMSE, Root mean square error; R^2; R-squared, goodness-of-fit; n *X*, number of *X* variables integrated in the model

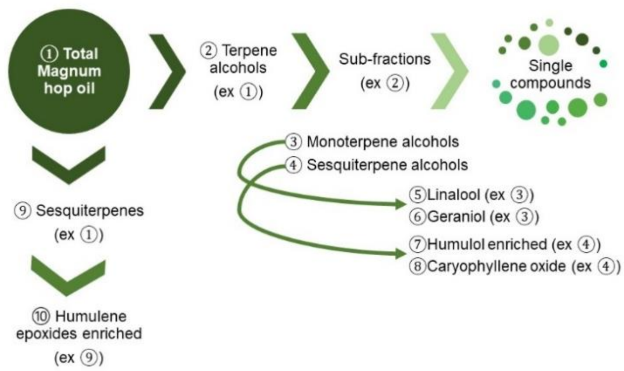

Fig. 1. Fractions, sub-fractions and single compounds extracted (ex) from the total Magnum hop oil included in the sample set.

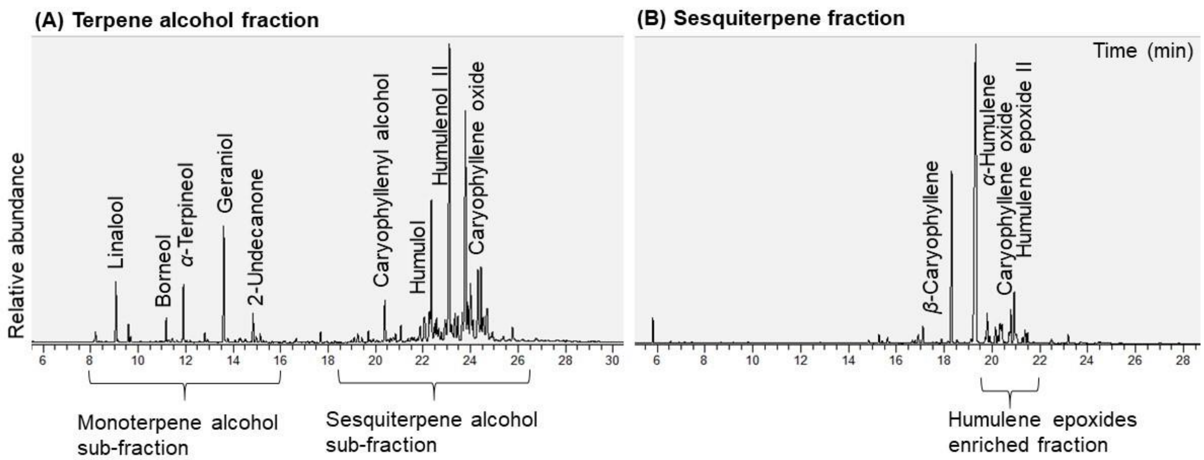

Fig. 2. Total ion chromatograms (TIC) of the terpene alcohol and sesquiterpene fractions showing the distribution of the sub-fractions and the main volatile compounds.

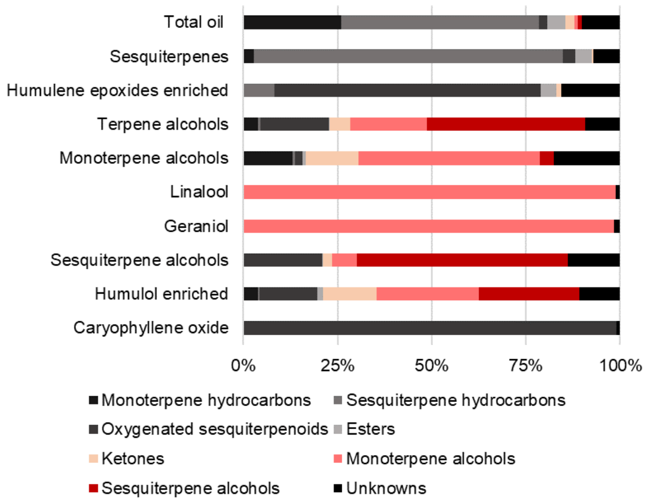

Fig. 3. Chemical class profiles (%mean of the total normalised integrated peak area in the GC-MS chromatograms) of the hop extracts applied in the base beer.

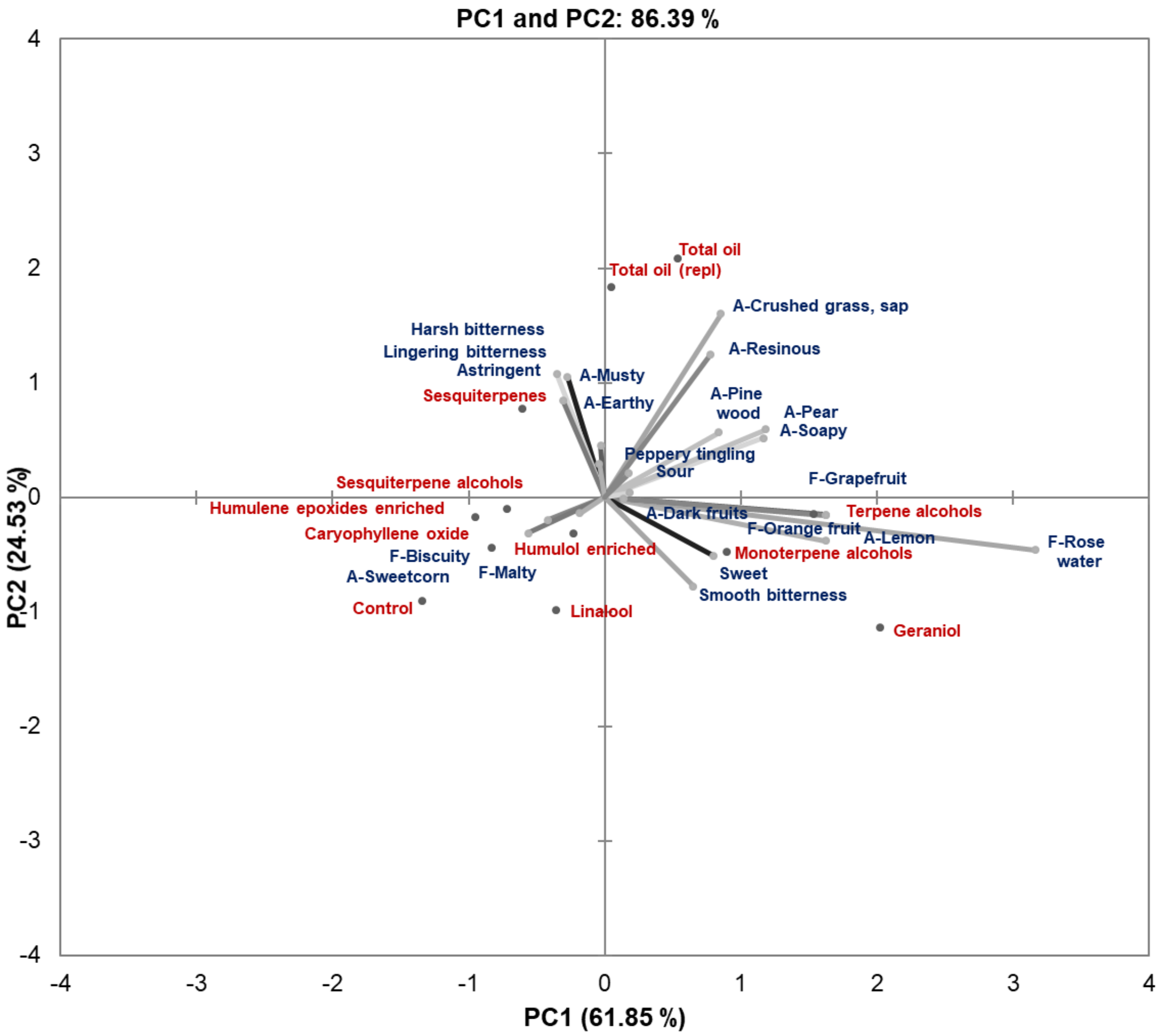

Fig. 4. Principle Component Analysis (PCA) biplot of sensory attributes present on principal component 1 (PC1) and 2 (PC2) by the covariance matrix of mean attribute intensity rating across the hop extracts. Sensory attributes in **blue**, samples in **red**; repl, experimental replicate; A, aroma attribute, F, flavour attribute.

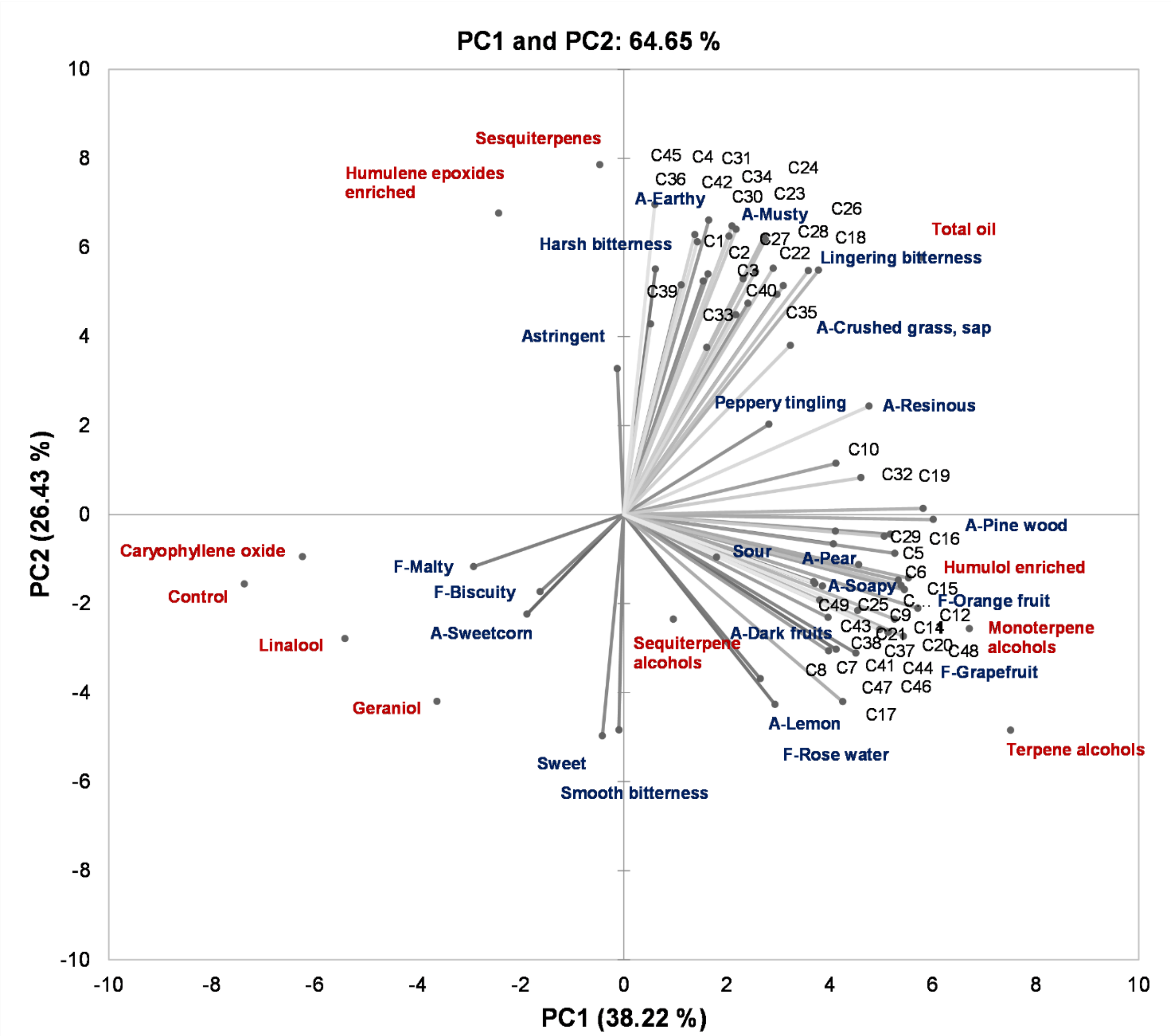

Fig. 5. Principle Component Analysis (PCA) biplot of standardised sensory attribute means and compounds' relative concentrations as applied in the base beer showing the correlation between the two variables principal component 1 (PC1) and 2 (PC2). Volatile hop compounds (C) numbered in black, sensory attributes in **blue**, samples in **red**; A, aroma attribute, F, flavour attribute.

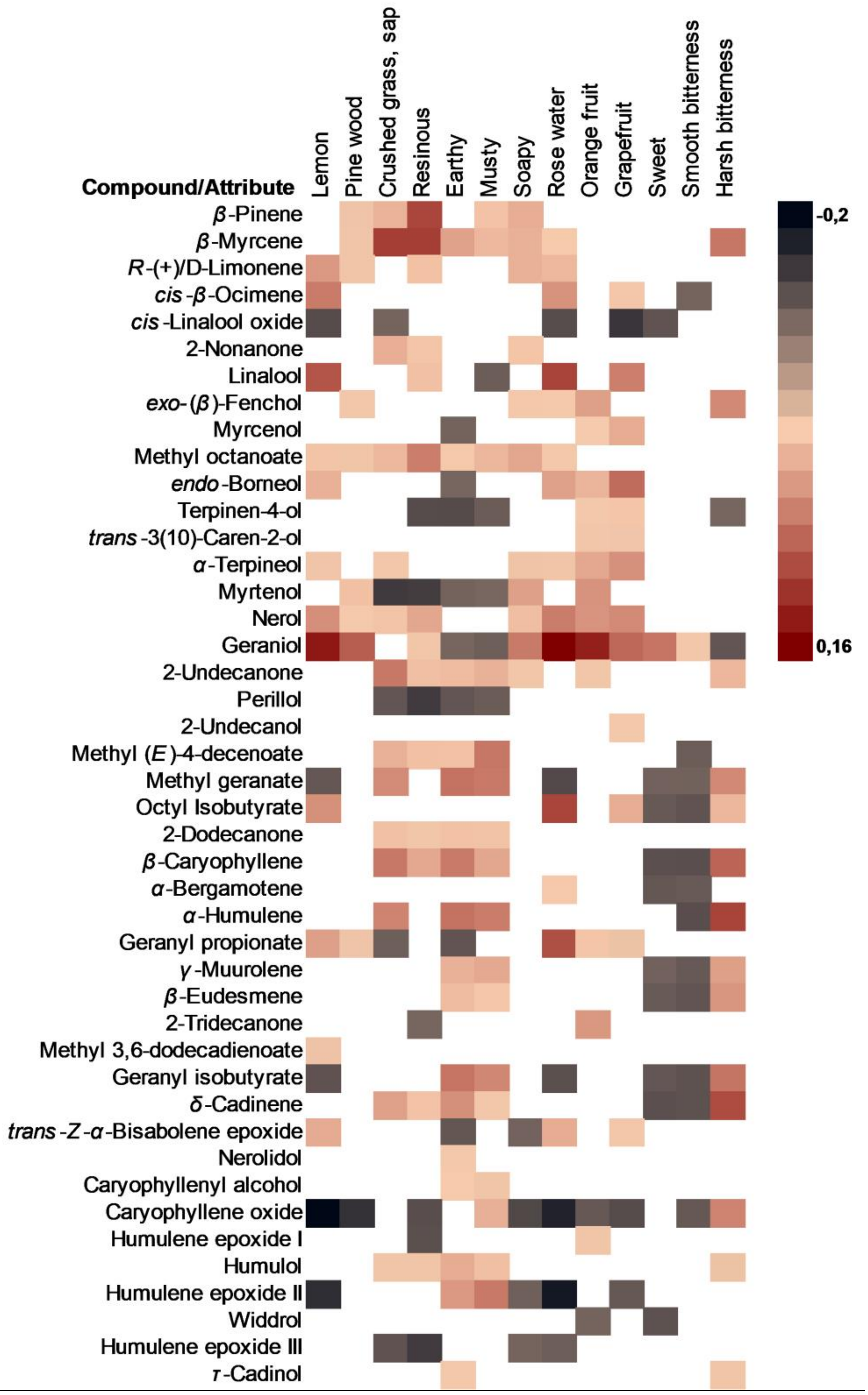

Fig. 6. Standardised regression coefficient map with the *X*-variables (volatile compounds) included in the models explaining the main weight into the *Y*-variables (sensory attributes). Only coefficients larger than 0.05 are shown.