Research article

Received: 31 October 2019 Revised: 20 April 2020 Accepted: 21 April 2020 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/jib.612

Sensory properties of supercritical CO_2 fractions extracted from Magnum hop essential oil

Christina Dietz,[1,2] David Cook,[2] Colin Wilson,[3] Ray Marriott[4] and Rebecca Ford[1]*

Hop oil fractions with unique sensory characteristics can be extracted from hop essential oil using green solvents such as supercritical (sc) CO_2. These extracts meet clean label requirements and can be used to manage fluctuations in volatile composition caused by global warming. A sensory descriptive analysis approach was applied to assess the sensory profiles of Magnum hop oil and five $scCO_2$ fractions. Ten sensory panellists were trained and used to establish an attribute lexicon. All samples, a control, and an experimental replicate were evaluated at 800 μg/L in ethanol (4% abv) in triplicate. Data were analysed by three-factor Analysis of Variance (ANOVA) and Tukey's test (HSD). Volatile compounds were determined using gas chromatography-mass spectrometry (GC-MS). Relationships between the volatile compounds and sensory profiles were analysed using Principal Component Analysis (PCA) and Partial Least Squares (PLS) regression. In contrast to the majority of fractions, the total oil (the most complex sample) and the sesquiterpene fraction (as the largest chemical group in the total oil) were not described by any key sensory attributes. This illustrates the advantage of hop oil fractionation to pull out specific sensory characteristics. The β-myrcene in the myrcene fraction induced an intense 'crushed grass, sap' aroma while the fractions containing several geranyl and methyl esters and ketones were characterised by fruity- and floral-type aroma and flavour attributes. Interestingly, the most polar fraction comprising of terpene alcohols delivered a complex sensory experience by adding sweetness. Moreover, a trigeminal 'peppery tingling' sensation was detected, which is likely to be caused by sensory interactions. © 2020 The Authors. Journal of the Institute of Brewing published by John Wiley & Sons Ltd on behalf of The Institute of Brewing & Distilling

Keywords: Hop aroma; hop oil fractions; sensory descriptive analysis; sensory interactions

Introduction

Volatile compounds in hops and hop essential oil are recognised as one of the major contributing components that determine the sensory perception of beer *(1)*. Hop essential oil has been suggested to be the most complex essential oil in plants *(2)* and is mainly composed of hydrocarbons (mono- and sesquiterpenes), esters, ketones, aldehydes, terpene alcohols, and sulphur containing compounds. Mono- and sesquiterpenes including the most abundant compounds β-myrcene, α-humulene, and β-caryophyllene account for around 80% of hop essential oil depending on the hop variety. The remaining volatiles are present at up to 1000x lower concentrations compared to the terpene hydrocarbons *(1,3,4)*. It has been proposed that more than 1000 volatile compounds are present in hops, including a large number of compounds at trace levels *(5)*. Some of these compounds are likely to be present at sub-odour threshold levels, but might still contribute to the overall aroma, flavour intensity and quality depending on the co presence of other volatile and non-volatile compounds and on sensory interactions between these *(6,7)*.

Hop oil products have been added to beer for decades and the time point of addition in the brewing process determines the final composition of the volatiles or hop aroma compounds, which in turn contributes to the perception of the sensory profile *(8)*. However, the perception is also affected by physicochemical properties of the matrix in which the hop oil products are applied, such as interaction with the components of the matrix. These properties determine the retention and release of the volatiles *(9)*. Different research approaches have been applied to understand the aroma and flavour contribution of hops in beer which mainly included the correlation of quantitative and descriptive data obtained by gas chromatography-olfactometric (GC-O), different mass spectrometry (MS) and flavour threshold determining techniques. The focus of hop oil analysis has largely been on instrumental profiling whilst somewhat neglecting the sensory evaluation of the volatiles in a realistic composition as naturally present in hop essential oil. Studies have since shown the importance of sensory descriptive

Correspondence: Rebecca Ford, Division of Food, Nutrition and Dietetics, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Leicestershire LE12 5RD, UK. Email: sbzrac@exmail.nottingham.ac.uk

[1] Sensory Science Centre, Division of Food, Nutrition and Dietetics, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Leicestershire LE12 5RD, UK

[2] International Centre for Brewing Science, Division of Microbiology, Brewing and Biotechnology, The University of Nottingham, Sutton Bonington Campus, Leicestershire LE12 5RD, UK

[3] Totally Natural Solutions Ltd., Paddock Wood, Kent TN12 6BU, UK

[4] BioComposites Centre, Bangor University, Bangor LL57 2UW, UK

analysis to understand aroma and flavour sensations of the hop oil compounds in different beer matrices *(10,11)*. Multivariate statistical methods including principal component analysis (PCA) and partial least square (PLS) regression are used to explore the relationship between sensory data and the chemical composition of mixtures of volatile compounds *(12)*. However, the correlation between volatiles in hop oil or hop oil fractions and attributes describing sensory characteristics of hop oil fractions by PLS regression has not yet been conducted.

Due to current and future challenges, arising from global warming and climate fluctuations *(13)*, human interventions causing competition for agricultural areas *(14)*, legislation *(15)*, and consumer demands *(16)*, hop oil products are gaining more interest and are challenging traditional hop products and material intensive hopping techniques. It has been shown that warm climate and decreased levels of rain significantly affect hop harvest yields as well as essential oil content and composition in the hops *(17)*. Hop varieties that are usually used for bittering purposes because of their high α-acids concentrations (referred to as 'alpha cultivars' or 'bitter hop varieties') have been found to be more resistant to changing weather conditions compared to 'aroma hop varieties' containing higher concentrations of aroma active compounds *(17)*. The use of hop oil fractions extracted from resistant hop varieties may be desirable to balance out inconsistencies in hop oil composition and to standardise hop flavour profiles in beer.

Advanced methods have been developed to produce natural, 'clean label' hop products for the brewing and beverage industry that have standardised and novel hop flavour profiles. Supercritical (sc) CO_2 is used as it is a green, non-polar solvent and reduces or replaces conventional organic solvents that are regulated as volatile organic compounds (VOC). This is highly desirable as maximum residual levels of VOC are defined by EU legislation *(18,19)* and the solvents have to be disposed of in an environmentally safe manner, which is expensive and involves considerable effort. VOC solvents can also cause environmental problems such as atmospheric and land toxicity. CO_2 is considered an organically certified non-polar solvent that enables the production of clean label products. By separating hop oil compounds from the bittering substances and selectively extracting hop oil fractions based on their molecular polarity, it is possible to obtain different volatile mixtures *(20)*. However, to date, only a few publications have focused on the different profiles of volatile aroma compounds in hop essential oil and hop oil fractions extracted using $scCO_2$, and only limited attention has been given to the sensory sensations in hop oil fractions other than those describing aroma and flavour *(11,21)*.

It was hypothesised that by fractionating hop essential oil it may be possible to create hop oil fractions with novel or moderated aroma and flavour properties. Therefore, the aim of this study was to define the sensory characteristics of Magnum hop oil and five $scCO_2$ fractions in ethanol (4% abv) using a sensory descriptive analysis approach to determine olfactory, gustatory and trigeminal differences among the hop oil and its fractions. A sensory attribute lexicon was developed to describe the different sensory sensations in the samples. In addition, volatile compounds in the different hop oil samples were identified and semi-quantified using GC-MS, and were characterised regarding their molecular polarity. Finally, PLS regression analysis was not only used to investigate which hop oil compounds may be involved in different sensory sensations in the samples but also to evaluate the predictability of certain sensory characteristics and sensory interactions in complex hop oil fractions since this has not yet been explored.

Materials and methods

Fractionation of Magnum hop essential oil

Hop oil was obtained by distillation from hop pellets *(20)* from a Magnum hop variety cultivated in the Hallertau region in Germany. The hop oil was fractionated using CO_2 in liquid and supercritical form as the non-polar solvent and ethanol as the polar co-solvent, as described by Marriott *(20)*. The hop oil was coated onto an inert support for sequential extraction at 10-20 % (m/m). By applying increasing temperature-pressure combinations ranging between 70-300 bar and 5-45°C, five fractions were extracted mainly comprising of 1) myrcene, 2) sesquiterpenes, 3) esters, 4) ketones, and 5) terpene alcohols. The total hop oil and the fractions were flushed with nitrogen and stored at 4°C. The myrcene fraction was stored at -20°C.

Sensory evaluation

Prior to the start of the sensory evaluation, ethics approval was sought and granted by the Faculty of Medicine & Health Sciences Research Ethics Committee at the University of Nottingham (Ethics Reference No. 88-1707). Informed consent was obtained from all panellists to confirm their awareness of the presence of alcohol in the solutions and their willingness to take part. Information on the nature of the study was kept to a minimum in order to reduce potential bias.

Preparation of samples

Stock solutions of the hop oil/fractions were prepared in food grade ethanol (96%, ferm, fa, F200481, Haymankimia, UK). All stock solutions were stored at 4°C for the period of the study. Samples for sensory evaluation (total oil/fractions in $EtOH/H_2O$) were prepared by dissolving the stock solutions in $EtOH/H_2O$ (purified water; 18.2 MΩ cm, 22°C) to obtain solutions containing 800 μg/L hop oil/fraction and 4% abv. All samples were evaluated at 800 μg/L in order to achieve a general understanding of the sensory characteristics of the fractions at equi-concentration. This was also the concentration at which the panellists were able to provide sufficiently detailed descriptions to the attributes especially those describing subtle sensations. The solutions were mixed on a roller bed for 30 min after preparation. New solutions for screening, training, and evaluation were prepared 48 h prior to the sensory sessions and were stored at 4°C overnight before use. The solutions were taken out of the fridge 4 h prior to the sessions and then mixed for 30 min on a roller bed. 30 mL aliquots were transferred into 60 mL amber glass bottles with screw top caps labelled with randomly assigned 3-digit codes and were kept at room temperature (22 ± 2°C) prior to testing. All solutions and bottled samples were prepared in a fume hood in a food safe environment.

Sensory panel

The sensory characteristics of the hop oil/fractions were identified and quantified by an external sensory panel following a modified Quantitative Descriptive Analysis approach *(22)*. The panel consisted of ten panellists (5 female and 5 male, mean age 49.3 years, age range 29-64 years). Recruitment and selection of the panellists

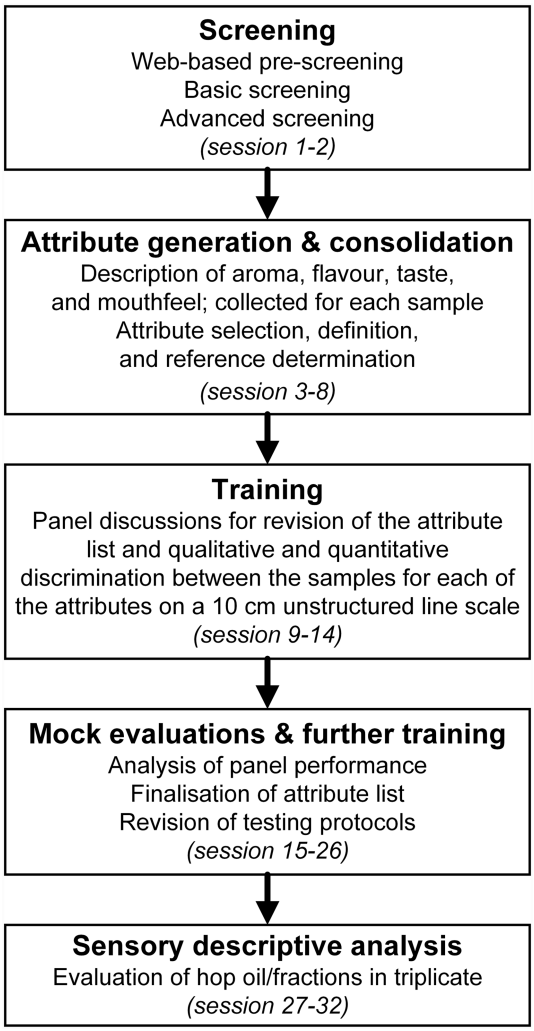

Figure 1. Flowchart describing the path to establish a sensory attribute lexicon for the evaluation of sensory profiles of hop essential oil and hop oil fractions using a sensory descriptive analysis approach. Each session lasted approximately 2 h.

was based on a three stage screening procedure (see Figure 1) and included a web based pre-screening to request information on demographics, general health, allergies, intolerances, medication, pregnancy, smoking, average beer consumption, native language, and availability. A basic screening session following the principles of the ISO standard 8586:2012 *(23)* was conducted in order to select candidates with good sensory abilities including basic smell and taste detection, descriptive, and discriminative abilities. A second, advanced screening session was conducted to check for specific anosmia's to compounds in the hop oil fractions, for the ability to communicate sensory descriptions of these compounds in ethanol solutions (4% abv), and to express and discuss the identified differences between sensory characteristics in a group discussion. The screening took place in the sensory training facilities in the Sensory Science Centre at the University of Nottingham. The panellists were asked not to eat or drink any food or liquids other than water at least 1 h prior to each sensory session.

Panel training

As shown in Figure 1, after recruitment of the sensory panel, the next steps included the establishment of an attribute lexicon and the training of the panellists on the identification and quantification of the sensory characteristics in the samples. 24 training sessions of 120 min each were required for attribute generation and consolidation (6 sessions). Subsequently, training was performed for discriminative ability and reproducibility (18 sessions) including two mock evaluation sessions to analyse the performance of the sensory panel. First, the panellists were asked to freely generate a list of aroma (orthonasal only), flavour (retronasal flavour), taste (five basic tastes), and mouthfeel attributes (tactile sensations during and after swallowing) by comparing and describing all hop oil/fraction solutions at different concentrations as well as a control solution (pure EtOH/H_2O). The aim was to collect those attributes that the panellists were able to identify on their own. In the second step, Check-all-that-apply (CATA) tests *(24)* using all samples at 800 μg/L in ethanol (4% abv), were performed to consolidate the list of attributes and identify those that were overlapping and most describing and discriminating between the samples *(24)*. Attribute descriptions were compiled in group discussions in which the panellists were provided with several reference materials for each of the attributes. Furthermore, reference materials and hop oil/fraction solutions at different concentrations were provided to aid the understanding of the attributes, to clarify the meaning of the attribute definitions, and to facilitate the evaluation of the perceived intensities *(25)*. Quantities of reference materials that were selected for the attribute lexicon refer to 'very strong' intensities of the attributes in the hop oil/fraction samples and the control sample. The overall aroma intensity had no physical reference and the meaning and quantification were discussed until consensus was achieved across the panel. Panellists were trained on the evaluation of the attributes on a 10 cm unstructured line scale anchored at the extremes by 'no sensation' and 'very strong'. In order to improve their discriminative abilities and to detect subtle differences between the samples, several rank rating tests were performed, and the outcome was discussed in group discussions moderated by the panel leader. In view of the final evaluation of the samples, an attribute order was defined by the panel following the chronological order in which the sensations were perceived resulting in eight attribute sets (Table 1). Smelling, tasting and palate cleansing protocols were developed based on panellists' comments and performance. Training continued until the outcome of the rank rating tests and the mock evaluation sessions confirmed adequate discriminative abilities and reproducibility confirmed by assessing intra- and inter-panellist variability. Performance was assessed using PanelCheck (v1.4.2) software and with Mixed Model Analysis of Variance (ANOVA) on the attribute ratings using the Excel Add-on XlStat (v.19.01; Addinsoft, US) as described in Kemp et al *(26)* and Tomic et al *(27)*.

Sensory descriptive analysis

Sensory evaluation was carried out according to the guidelines and conditions detailed in ISO 8589-2007 *(28)*. The total hop oil, five hop oil fractions, control sample and an experimental replicate (total oil) were analysed in triplicate on a 10 cm unstructured line scale by all panellists (n = 10) over four sessions of approximately 90-100 min each. Each panellist evaluated six samples per session in order to comply with the ethical considerations regarding alcohol intake (less than 1 UK alcohol unit per session) and to prevent fatigue. Samples were presented monadically in a randomised and counterbalanced order (Latin Square Design) to reduce first order

Table 1. Attribute sets and order for the sensory evaluation and time points of sample provision.

Fresh samples provided	Attribute set	Attributes in order of sensory evaluation
1	1	Soapy
		Musty
		Pine wood
	2	Resinous
		Orange citrus fruit
		Artificial lemon
2	3	Earthy
		Crushed grass, sap
		Fresh lemon
		Grapefruit zest
	4	Overall aroma intensity
3	5	Astringent
	6	Rose water
		Alcohol
		Bitter
4	7	Lingering bitterness
	8	Peppery tingling
		Sweet
		Sour

and carryover effects *(22)*. All samples were presented at room temperature (22 ± 2°C) to avoid temperature changes which could affect the perception of different sensations. Four bottles of each sample were provided, and the panellists were asked to use a fresh sample for certain sets of attributes to ensure that they could evaluate subtle aroma sensations before the aroma active compounds volatilised (Table 1). The scales for all attribute sets were simultaneously displayed with CompusenseCloud on a screen together with the corresponding attribute descriptions. Breaks of 40 s after each attribute set, 120 s before provision of the next bottle, and a 10 min comfort break after the third sample was enforced to avoid carryover effects and fatigue. During the breaks, the panellists closed the bottles, and followed the neutralisation or palate cleansing protocols where they smelled the back of their hands or a glass of water or cleansed their palate with water, a piece of honeydew melon and more water. All palate cleansing materials were served at room temperature.

Gas chromatography-mass spectrometry

Volatile compounds in the total hop oil and five hop oil fractions were analysed using a gas chromatography-mass spectrometry (GC-MS) method. A Thermo Scientific system (TRACE^{TM} 1300; Massachusetts, USA) 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) was used which was operated in a positive electron ionisation mode. The analysis was carried out using helium as a carrier gas at 1 mL/min flow rate operating in split mode (1:50). The temperature of the injector, ion source and interface were 250°C, 240°C, and 250°C, respectively. The oven temperature was programmed from 60°C at an increasing rate of 5°C/min to 240°C. The detector temperatures were held at 250°C. Hop oil/fractions (10 μL) were diluted into 1 mL iso-octane (≥99%; Thermo Fisher Scientific, Loughborough, UK) and 1 μL of the aliquot was directly injected using an autosampler. Peak identification was conducted by comparing peak areas and mass spectra of external standards to those in the samples, where available including: endo-borneol (≥97%), caryophyllene oxide (≥99.0%), geraniol (≥99%), geranyl acetate (≥99%), geranyl isobutyrate (≥97%), geranyl propionate (≥95%), linalool (≥97.0%), 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%), all purchased from Sigma Aldrich (UK). Retention indices (RI) of the volatiles were determined by using a homologous series of n-alkanes (C6-C30; Sigma-Aldrich, St. Louis, MO). In addition to compound identification with authentic standards, volatiles were identified by library matching using the NIST Mass Spectral library (NIST08) and Wiley7n.1 (Hewlett-Packard, US) databases. Only those compounds are included, which have a MS fit factor ≥ 800) and literature RI similar to the calculated RI.

Data processing and statistical analysis

For the sensory data, three-factor Mixed Model ANOVA (panellist, sample, replicate) and two-way ANOVA (panellist, sample) including the corresponding two-way interactions as explanatory variables were conducted on all sensory attributes to examine the panel performance. Significant effects of samples, and non-significant effects of sample x panellist and sample x replicate interactions indicate satisfactory panel performance. Analysis of sensory data was conducted by two-way ANOVA (sample as fixed factor and panellist as random factor) followed by Tukey's Honest Significant Difference (HSD) test for pairwise multiple comparisons at 95% confidence interval to determine significant differences between samples at $p = 0.05$ for each attribute. PCA was conducted on the average scores of the attributes to detect relationships between the samples and the attributes in a sensory perceptual space. Average peak areas of the volatile compounds in the hop oil samples detected in the GC-MS analysis were calculated from the three replicate injections. Relative percentages of the compounds were obtained by peak area normalisation (PAN) relative to the total area for all peaks in the chromatogram. The sensory (scores) and instrumental datasets (areas) were standardised (1/standard deviation) and analysed by PCA. Standardisation was conducted to allow for all variables to have equal influence in the PCA model despite differences in their numerical range. The logarithm of the octanol/water partition coefficient (Log*P*) was used as an indicator for the polarity or hydrophilicity of the compounds and was predicted using the EPIWEB 4.1 software (EPI Suite TM, US). The sample Log*P* was calculated on the basis of the relative contribution of the individual compounds in the total oil/fractions. PLS regression was performed with the relative peak areas of the volatile compounds obtained from the GC-MS analysis as the independent variable (*X*-matrix) and the average sensory scores and samples as the dependent variables (*Y*-matrix) to model the relation between these two variables. PLS1 was applied for the correlation between individual sensory attributes and volatile compounds. PLS2 was performed to illustrate correlations among the GC-MS data, the hop oil samples and the complete sensory attribute list of the attribute lexicon. Estimated regression coefficients were derived from jack-knife uncertainty

tests. Data analyses were performed using XlStat 2017 (v.19.01; Addinsoft, US).

Results and discussion

Sensory evaluation

Attribute generation and validation. More than 290 attributes were initially generated by the panellists which were consolidated down to 35 aroma and flavour attributes, four taste and three mouthfeel attributes. The list also included attributes that were generated for more than one modality i.e. to describe both aroma and flavour sensations. Based on the outcome of the CATA tests, 13 attributes were excluded as panellists could not anymore identify the attributes in the samples. A number of attributes was further removed in subsequent training sessions which did not adequately describe or discriminate differences between the samples *(29)*. The final attribute list, their descriptions, and reference materials are listed in Table 2. The majority of aroma sensations were perceived through the orthonasal and retronasal pathways as aroma and flavour sensations. Therefore, it was decided to select attributes representing aroma or flavour that showed the highest intensities during either orthonasal or retronasal perception rather than replicating such attributes for both aroma and flavour.

Panel performance evaluation. The evaluation of panel performance was conducted in order to identify intra- and inter-panellist variation following the approach of Kemp et al *(26)*. Three-factor ANOVA with interaction (panellist, sample, replicate) was conducted on all 18 attributes and 'overall aroma intensity' (see Table 3). Significant panellist (Panel) variation ($p < 0.05$) and sample x panellist (Sam x Panel) interactions were reported for several attributes. However, interrogation of the interaction plots showed that the source of variation for the majority of attributes was minor variations in scale use, which did not impact interpretation of resulting data and showed adequate discrimination ability between samples *(29)*. Interaction effects for 'alcohol', 'sour', 'bitter', and 'astringent' were explained by a lack of sample discrimination using these attributes. In total, 12 of the 18 attributes and the 'overall aroma intensity' significantly differed ($p < 0.05$) across all samples.

Sensory descriptive analysis. Three-factor ANOVA (sample, panellists, replicate) with interactions was applied to all samples and sensory scores for the 18 attributes and the overall aroma intensity. Table 4 shows the mean sensory scores and significant differences between the samples. No significant differences were observed between the total oil and the experimental replicate indicating panel reliability. It was noticed that more panellists used lower scores to rate the attribute intensities using the lower end of the scale while fewer panellists used high scores. This is not displayed in the mean sensory scores. There were significant differences ($p < 0.05$) among the samples for all aroma attributes as well as for 'rose water' flavour, 'sweet' taste and the overall aroma intensity. No significant differences ($p > 0.05$) were reported for 'alcohol' flavour, 'sour' and 'bitter' taste and 'astringent' mouthfeel, indicating that the panellists could not significantly discriminate between the samples for these attributes. Tukey's (HSD) post-hoc tests were conducted for pairwise multiple comparison of the samples for each attribute where a significant difference could be detected or which showed a trend towards a significant difference ($p < 0.07$) in the outcome of the ANOVA. The attributes 'peppery tingling' mouthfeel and 'lingering bitterness' were not found to be significant but approached a significant effect ($p = 0.053$; $p = 0.067$) due to higher attribute scores for the terpene alcohol and ester fractions compared to the other samples.

As shown in Table 4, the control sample (ethanol, 4% abv) was mainly described by taste and mouthfeel attributes and an 'alcohol' flavour suggesting that the total oil and the fractions added diverse aroma and flavour notes to the control solution and were able to significantly ($p < 0.05$) potentiate taste and mouthfeel attributes as explained in the following sections.

The total oil was characterised by the fewest number of key attributes i.e. this sample was not characterised by specific aroma, flavour, taste or mouthfeel sensations. Interestingly, the total oil, the ester fraction, and particularly the terpene alcohol fraction added sweetness in comparison to the control sample. This is likely due to an aroma-taste interaction, however, the cause of this interaction is not clear from the sensory data alone and further work is required to identify the source of the perception of the sweet taste.

The sensory profile of the sesquiterpene fraction was not described by specific key attributes and compared to the total oil and the other fractions, it exhibited the lowest score for the 'overall aroma intensity'. The spider plots in Figure 2 illustrate the differences between the sesquiterpene fraction with the lowest sensory potential and the terpene alcohol fraction as one of the hop oil fractions that induced several sensory sensations in the test solution. Both plots were overlaid with attribute scores of the total oil and the control samples to show the similarity or differences between these samples and the sesquiterpene fraction or terpene alcohol fraction, respectively. Overall, the panel could only perceive low intensities of 'crushed grass, sap', 'pine wood', and 'orange citrus fruit' aromas in the sesquiterpene fraction. This is in agreement with the literature, although so far, rather general sensory terms have been used to describe the aromas of sesquiterpene hydrocarbons such as 'green' *(30)*, 'herbal', 'woody', 'earthy', and 'citrusy' *(31,32)*. Precise terms were used in this study to highlight different sensory potentials among the hop oil fractions and the total hop oil and to facilitate conclusions about cause-effect relationships between volatile compounds and sensory characteristics.

The myrcene fraction was described by 'crushed grass, sap', 'musty', and 'resinous' aromas and a high overall aroma intensity. Similarities between the aroma profiles of the myrcene fraction and the total oil and sesquiterpene fraction could be observed. In contrast to the other fractions, the myrcene fraction was highly enriched in one compound and, due to the aim to evaluate all hop oil samples at equi-concentration, the myrcene was present far above its odour detection threshold concentration *(33)*. Myrcene is commonly found to significantly contribute to the aroma profile of hop oil accounting for up to 58% of the total aroma *(34)*. Previous studies investigating the sensory characteristics of myrcene in beer observed spicy and resinous flavour notes at 200 μg/L *(35)* with metallic and geranium-like aroma notes at around 860 μg/L *(36)*. Recently, Neiens and Steinhaus *(37)* determined the odour threshold of myrcene in an aqueous solution to be 1.2 μg/kg. Brendel et al *(38)* found the odour of myrcene to be detected in oil at 1800 μg/kg. The perception of myrcene appears to be concentration and matrix-dependent.

The ester, ketone and terpene alcohol fractions were described by a number of key attributes. The ester fraction was characterised by 'soapy', 'pine wood', 'orange citrus fruit', and 'fresh lemon' aroma, and 'rose water' flavour, and 'peppery tingling' mouthfeel sensations. These attributes obtained significantly higher scores compared to the control and the total oil sample while the

Table 2. Overview of sensory attributes, definitions, and training reference standards.

Category	Sensory attribute	Definition	Training reference standard
Aroma	Soapy	Aroma of an unscented bar of soap	30 g unscented bar of soap (Tesco Stores Ltd., UK)
	Musty	Mildew/mouldy aroma or musty aroma associated with damp cardboard	20 g damp cardboard soaked in deionised water for 24 h; damp, old sponge
	Pine wood	Aroma of pine shavings or scented wood	20 g pine shavings (Sainsbury's Supermarkets Ltd., UK); 5 mL 5.9 mg/L (1R)-(+)-α-pinene (FG; Sigma Aldrich, UK) in deionised water
	Earthy	Aroma of wet earth or soil	40 g fresh wet earth, soil
	Resinous	Aroma of wood resin	25 g pine resin and 25 g myrrh resin (Indigo Herbs, UK)
	Crushed grass, sap	Aroma of crushed cut grass, sap or fresh tomato leaf or carrot leaf	30 g crushed cut grass and sap that has been left for two days; 10 g fresh tomato leaf/carrot leaf
	Orange citrus fruit	Round aroma of orange, mandarin or tangerine	5 g freshly cut flesh and peel
	Grapefruit zest	Aroma of grapefruit zest; aroma peak at the beginning and flattens off gradually	5 g freshly cut grapefruit zest
	Fresh lemon	Aroma of lemon or lime fruits; sharp citrus aroma peak at the beginning, which quickly flattens off after a few seconds	30 g freshly chopped lemon and lime
	Artificial lemon	Aroma of citrus wet wipe or cheap lemon squash; flat but sharp, pungent citrus aroma	1 citrus wet wipe (Dettol, UK)
	Overall aroma intensity	Overall aroma intensity in the sample	No physical reference
Flavour	Rose water	Rose water flavour as in Turkish delight or diluted geranium essential oil	½ piece Turkish delight (Sainsbury's Supermarkets Ltd., UK); 0.6% (w/v) geranium essential oil (Ecodrop, UK) in deionised water
	Alcohol	Alcohol flavour as in the alcohol/water sample	1% (v/v) EtOH (96%, ferm., FG; Haymankimia, UK) in deionised water
Taste	Sweet	Sweet taste as in the alcohol/water sample	10 mL 1% (v/v) sucrose (Sainsbury's Supermarkets Ltd., UK) or 10 mL 4% (v/v) EtOH (96%, ferm., FG; Haymankimia, UK) in deionised water
	Sour	Sour taste as in citrus fruits, in the citrusy reference and the alcohol/water solution	10 mL 0.2% (v/v) citric acid (Sigma Aldrich, UK) or 10 mL 4% (v/v) EtOH (96%, ferm., FG; Haymankimia, UK) in deionised water
	Bitter	Pleasant, smooth bitterness as in the bitter reference solution	10 mL 2 mg/L HopAlpha® Iso30% (TNS Ltd., UK) in deionised water
	Lingering bitterness	Persistence of the bitterness in the mouth as in the bitter reference solution; perceived 20 s after swallowing	10 mL 2 mg/L HopAlpha® Iso30% (TNS Ltd., UK) in deionised water
Mouthfeel	Peppery tingling	Peppery tingling sensation when eating chili, fresh ginger, horse radish/radish; tingling mouthfeel on the front half of the tongue	Chili, fresh ginger, horse radish/radish
	Astringent	Mouth drying, rough, puckering sensation as in the astringent reference solution; perceived 20 s after swallowing	10 mL 1% (w/v) tannic acid (Alfa Aesar, US) in deionised water

'peppery tingling' mouthfeel sensation was increased compared to the control solutions. The ketone fraction was mainly described by 'soapy', 'pine wood', 'artificial lemon', 'resinous', 'orange citrus fruit', and 'grapefruit zest' aroma notes, all of these being significantly increased compared to the control sample and the latter three compared to the total oil sample. Various fruity aroma and flavour notes have been reported for esters and ketones in hop essential oil. Particularly, short chain esters (up to C6) added soft fruit,

Table 3. Analysis of variance (ANOVA) *F*-ratios for sensory attributes rated for Magnum hop oil and five hop oil fractions. NS indicating no significant effects and *, **, *** indicating a significant effect at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, from three-factor ANOVA with interactions (Sample (Sam), Panellist (Panel), Replicate (Rep)).

Modality	Attribute	Sam	Panel	Rep	Sam x Panel[a]	Sam x Rep[a]	Rep x Panel[a]
Aroma	Soapy	4.38**	2.43*	NS	1.96**	NS	NS
	Musty	3.67**	7.19***	3.41*	1.48*	NS	NS
	Pine wood	4.65***	6.26***	6.49**	2.32***	1.92*	NS
	Earthy	4.09**	3.11**	NS	2.08**	NS	NS
	Resinous	3.09**	22.65***	NS	NS	NS	NS
	Crushed grass, sap	5.91***	4.13**	NS	3.18***	NS	2.13**
	Orange citrus fruit	4.55***	NS	NS	NS	NS	NS
	Grapefruit zest	3.92**	3.27**	NS	2.23***	1.78*	2.25**
	Fresh lemon	5.70***	NS	NS	2.27***	NS	2.52**
	Artificial lemon	5.11***	NS	NS	1.89**	NS	1.65*
	Overall aroma intensity	14.31***	NS	NS	2.27***	NS	1.93*
Flavour	Rose water	5.75***	7.82***	NS	3.09***	NS	NS
	Alcohol	NS	17.49***	3.17*	2.09**	2.43**	NS
Taste	Sweet	3.38**	9.93***	NS	1.73**	NS	1.80*
	Sour	NS	17.07***	NS	NS	NS	NS
	Bitter	NS	17.60***	NS	NS	NS	NS
	Lingering bitterness	NS	10.53***	NS	1.60*	NS	2.38**
Mouthfeel	Peppery tingling	NS	10.23***	NS	1.53*	NS	NS
	Astringent	NS	23.94***	NS	NS	NS	NS

[a] Sam x Panel, Rep x Panel and Sam x Rep represent the interaction between oil/fraction samples and panellists, replication and panellists and oil/fraction samples and replications, respectively.

Table 4. Mean sensory intensities ($n = 10$, triplicates) for Magnum total oil, five hop oil fractions and an experimental replicate at 800 μg/L in ethanol (4% abv) and for a control sample (pure ethanol, 4% abv). 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 $p < 0.05$.

Modality	Attribute	Total oil	Total oil (repl)	Myrcene fraction	Sesquiterpene fraction	Ester fraction	Ketone fraction	Terpene alcohol fraction	Control
Aroma	Soapy	1.67^{cd}	2.10^{bcd}	1.00^{d}	1.23^{d}	3.93^{a}	3.65^{ab}	2.96^{abc}	0.78^{d}
	Musty	1.39^{b}	1.95^{ab}	3.41^{a}	1.51^{b}	1.35^{b}	0.91^{b}	1.20^{b}	0.44^{b}
	Pine wood	2.49^{cd}	2.81^{bcd}	3.40^{abc}	2.37^{cd}	4.38^{ab}	4.54^{a}	3.60^{abc}	1.11^{d}
	Earthy	1.51^{ab}	0.53^{c}	1.96^{a}	0.84^{bc}	0.42^{c}	0.49^{c}	0.49^{c}	0.33^{c}
	Resinous	1.98^{abc}	2.04^{abc}	2.96^{a}	1.46^{bc}	2.11^{abc}	2.83^{ab}	2.53^{ab}	0.74^{c}
	Crushed grass, sap	1.94^{b}	2.73^{b}	5.37^{a}	2.18^{b}	1.80^{b}	2.21^{b}	2.38^{b}	0.23^{c}
	Orange citrus fruit	1.57^{cd}	1.82^{cd}	1.43^{cd}	2.21^{bcd}	3.81^{ab}	2.91^{abc}	4.04^{a}	0.70^{d}
	Grapefruit zest	1.43^{bc}	1.63^{abc}	1.50^{bc}	1.21^{bc}	2.35^{ab}	3.04^{a}	3.13^{a}	0.20^{c}
	Fresh lemon	1.16^{cd}	1.79^{bcd}	1.30^{cd}	0.91^{d}	3.24^{ab}	2.68^{abc}	3.39^{a}	0.33^{d}
	Artificial lemon	1.32^{bc}	1.39^{bc}	0.41^{c}	0.32^{c}	1.98^{ab}	2.76^{a}	2.23^{ab}	0.27^{c}
	Overall aroma intensity	4.17^{b}	4.32^{b}	6.65^{a}	3.60^{b}	5.79^{a}	5.70^{a}	6.14^{a}	1.34^{c}
Flavour	Rose water	1.34^{cd}	1.83^{c}	1.31^{cd}	1.04^{cd}	4.02^{ab}	3.71^{b}	5.45^{a}	0.12^{d}
	Alcohol	3.21^{a}	2.99^{a}	2.83^{a}	3.27^{a}	3.50^{a}	3.42^{a}	2.92^{a}	3.11^{a}
Taste	Sweet	2.03^{a}	1.50^{a}	1.24^{ab}	1.09^{ab}	2.16^{a}	1.31^{ab}	2.28^{a}	0.28^{b}
	Sour	2.04^{a}	1.66^{a}	1.16^{a}	1.66^{a}	2.01^{a}	1.88^{a}	1.93^{a}	1.57^{a}
	Bitter	3.51^{a}	2.94^{a}	2.71^{a}	2.93^{a}	3.16^{a}	3.21^{a}	3.59^{a}	2.36^{a}
	Lingering bitterness	2.93^{ab}	3.46^{ab}	2.46^{b}	3.11^{ab}	3.09^{ab}	2.93^{ab}	4.22^{a}	2.54^{b}
Mouthfeel	Peppery tingling	1.98^{ab}	2.19^{ab}	2.15^{ab}	2.06^{ab}	2.90^{a}	1.61^{ab}	2.59^{ab}	1.19^{b}
	Astringent	4.30^{a}	4.29^{a}	4.09^{a}	3.60^{a}	4.79^{a}	3.76^{a}	4.29^{a}	3.46^{a}

repl, experimental replicate

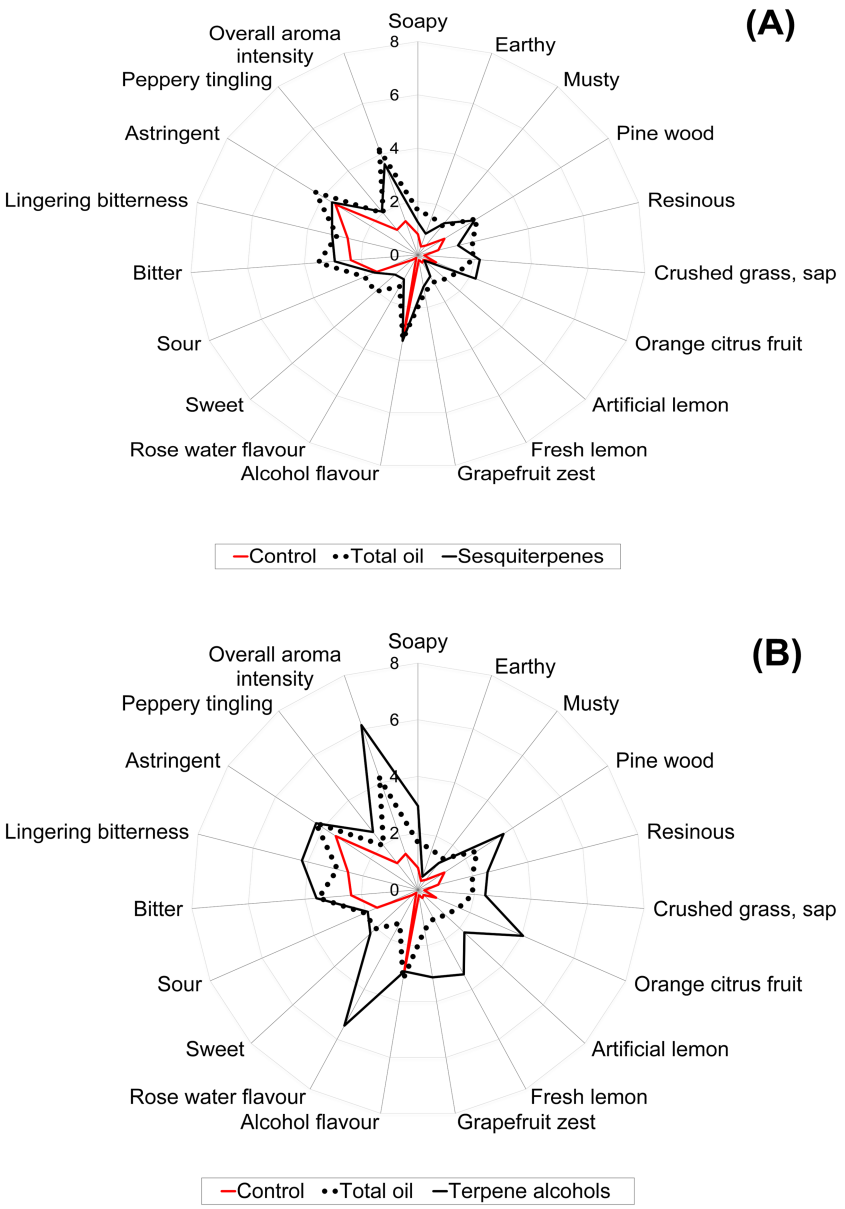

Figure 2. Spider plots of mean attribute intensities for the sesquiterpene fraction (A) and the terpene alcohol fraction (B) plotted with the total Magnum hop oil and control samples. [Colour figure can be viewed at wileyonlinelibrary.com]

citrusy, pear/apple, as well as tropical fruit-like aromas to beer while medium chain esters (C8–C12) have been found to induce soapy aroma notes *(36,39)*. As observed in the present study, ketones have mostly been suggested to contribute to the citrus/fruity and floral characters in beer *(40,41)*.

In comparison to the total oil and the other fractions, the terpene alcohol fraction was described by diverse aroma, flavour, taste, and mouthfeel sensations at higher intensities. This fraction exhibited stronger 'orange citrus fruit', 'fresh lemon', and 'grapefruit zest' aroma notes, 'rose water' flavour, 'sweet' and 'lingering bitterness', and a 'peppery tingling' mouthfeel sensations compared to the control and the total oil samples with the aroma and flavour attributes as well as sweetness showing a significant effect. The scores for the attributes 'peppery tingling' and 'lingering bitterness' were only slightly increased and approached the significance level. The attribute 'peppery tingling' refers to a trigeminal-type sensation, which is a similar sensation imparted by compounds in terpene alcohol or oxygenated sesquiterpeneoid fractions observed in previous studies that have been referred to as 'spicy' essences *(42,43)*. In past studies *(21,44,45)*, the polar oxygenated sesquiterpenoid fractions from different hop varieties have been observed to increase the perception of fullness and to induce a 'spicy' mouthfeel in beer, the latter sensation has been described as a coating effect on the tongue and in the throat indicating the occurrence of a trigeminal-type sensation. Trigeminal stimuli are those that can induce a sensation of temperature (cooling, warming), pain or irritation (spicy, pungent) such as high carbonation levels in beer being perceived as a sparkling, tingly, and irritating sensation in the oral cavity (induced by bursting bubbles of CO_2 on the tongue *(46)* and conversion of CO_2 to carbonic acid *(47)*). In addition to the perceived 'fullness' and the 'spicy' sensation, Goiris et al *(11)* found an oxygenated sesquiterpene fraction (*ex* Hersbrucker hop oil) to increase the perceived bitterness in pilsner beer. It was suggested

that a synergistic compound interaction occurred between the bitter substances and the hop volatiles causing the modulation of the perceived bitterness. However, the effect was not attributed to individual compounds or compound groups in the hop fraction and requires further work to identify the cause. The slightly increased 'lingering bitterness' intensity in the terpene alcohol solution in this study could also not be assigned to specific compounds. Therefore, the lingering bitterness sensations might indeed have been the result of a sensory interaction within or across modalities caused by sesquiterpene alcohols. However, further research is required to confirm whether this sensory interaction effect was induced by compounds in the sesquiterpene alcohol subfraction alone or whether other compounds in the monoterpene alcohol subfraction or other mechanisms such as the stimulation of bitter taste receptors might be involved.

PCA was conducted to reduce the complexity of the data and visually represent the samples in a sensory space (Figure 3 (A). The analysis was based on the covariance matrix, which is chosen for sensory evaluations conducted by a trained panel that used the same scale for all attributes *(29)*. The first two principal components (PC) explained the majority of the total variance (86.38%) with PC1 explaining 69.87% and PC2 explaining 16.52%. The main discriminating dimension (PC1) was loaded with the aroma attributes 'soapy', 'pine wood', 'orange citrus fruit', 'fresh lemon', 'artificial lemon', 'grapefruit zest' and with 'rose water' flavour. PC2 was loaded with the main distinguishing aroma attributes being 'musty', 'earthy', and 'crushed grass, sap'. As could be shown from the outcome of the ANOVA, the myrcene fraction was related to high intensities of 'crushed grass sap', 'musty' and 'earthy' aroma notes which is why it is positively correlated with PC2. The total oil and the sesquiterpene fraction are plotted close to the centre of the PCA biplot showing that fewer attributes dominated their sensory profiles which is not surprising as the total oil is comprised of a complex mixture of compounds. However, other fractions comprised of fewer compounds, which was particularly the case for the monoterpene alcohols in the terpene alcohol fraction and the myrcene in the myrcene fraction, and therefore obtained high scores on specific aroma attributes. The ester, ketone, and terpene alcohol fractions were related to high intensities in the fruity aroma notes, 'soapy' and 'pine wood' aroma, and 'rose water' flavour. The taste and mouthfeel attributes 'sweet' (r = -0.436), 'lingering bitterness' (r = -0.638), and 'peppery tingling' (r = -0.638) were loaded on PC3 that only contained 6.29% of the variation (Figure 3 (B)) indicating that both aroma and flavour as well as taste and mouthfeel attributes are differentiating between the hop oil samples.

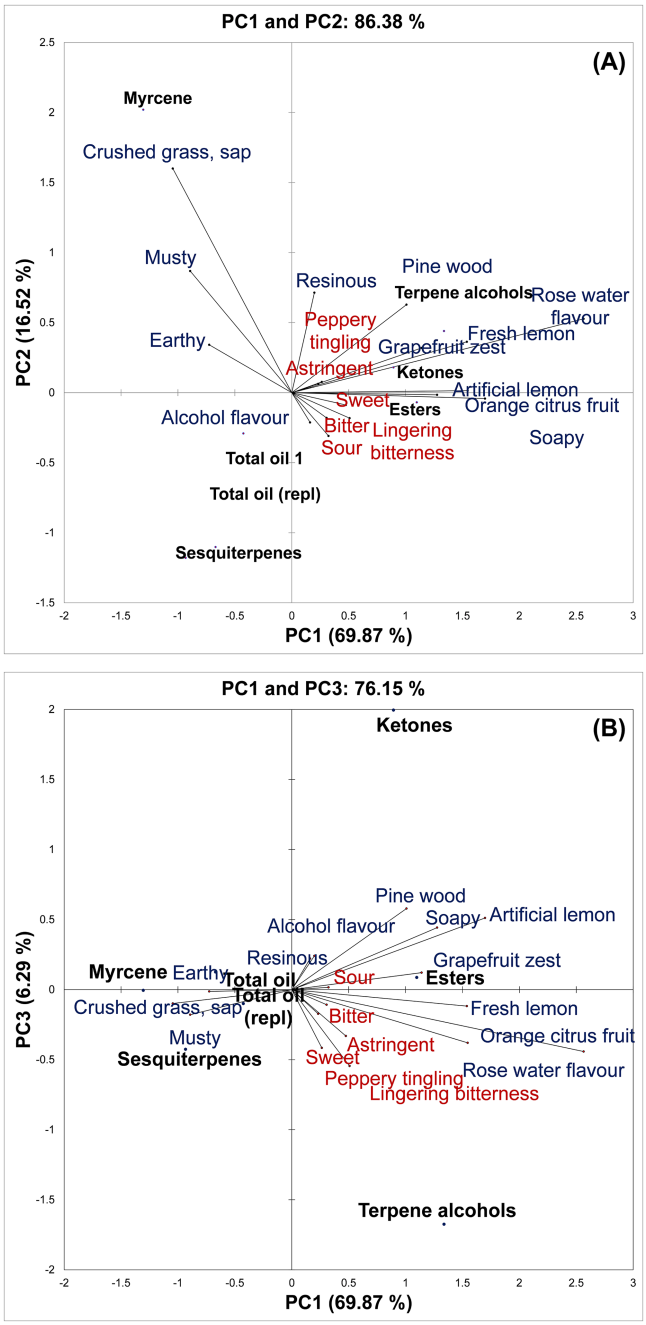

Figure 3. Principle Component Analysis (PCA) biplot of sensory attributes present on **(A)** principal component 1 (PC1) and 2 (PC2) and **(B)** PC1 and PC3 by the covariance matrix of mean attribute intensity rating across the total hop oil and five hop oil fractions. Aroma and flavour attributes in **blue**, taste and mouthfeel attributes in **red**; repl, experimental replicate [Colour figure can be viewed at wileyonlinelibrary.com]

Effect of compositional and physicochemical characteristics on sensory scores

Relationship between sensory scores and main volatile compounds. GC-MS was used to obtain a general overview of the main volatile compounds present in the Magnum hop essential oil and its fractions. In total, 66 compounds could be identified. The total ion chromatogram (TIC) in Figure 4 illustrates the distribution of the fractions in the total oil sample. Table 5 displays all compounds successfully identified using NIST database searches and authentic reference compounds run under identical instrumental conditions. The relative contributions (% derived from peak area normalisation based on the relative peak areas) of the compounds in the total oil/fractions obtained are provided in Table 5. Generally, it was found that several compounds were detected in more than one fraction. Quantitative differences were recorded between these compounds and trace levels were found if no clear separation of the hop oil fractions was possible in the fractionation process. It is considered that these compounds could still contribute to the overall sensory profile depending on the threshold concentration in the individual volatile mixtures.

PCA was conducted to visualise the relationship between the samples, sensory attribute scores and the volatile compositions (displayed as [**numbers**] listed in Table 5). Figure 5 shows the plot with the significant principal components PC1 (40%) and PC2

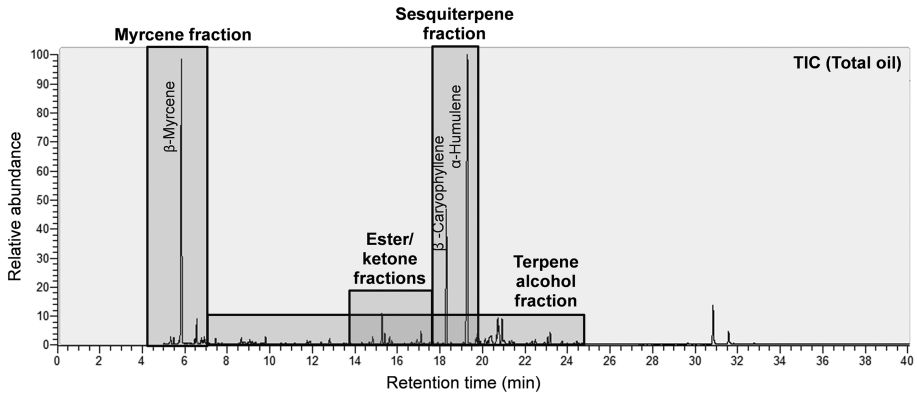

Figure 4. Total ion chromatogram (TIC) of the Magnum hop essential oil (total oil) showing the distribution of the five hop oil fractions.

(37%) explaining 77% of the variance. The biplot displays the different and overlapping sensory characteristics of the five fractions with the total oil again plotted the closest to the plot centre because it was not described by any key attribute and contained many volatile compounds at much lower concentrations compared to the fractions including compounds that were present below detection level. On the right side of the plot, the terpene alcohol fraction stood out in terms of sensory characteristics, including the majority of taste and mouthfeel sensations. Also, aroma and flavour attributes were scored higher in this fraction compared to the ester and ketone fractions which is demonstrated by their position in the biplot closer to the terpene alcohols. However, while the mono- and sesquiterpenes could be assigned to some extent to certain aroma sensations, there has been no clear correlation between the sensory attribute and volatile compounds in the ester, ketone and terpene alcohol fractions. The reasons for this are explained in the following sections.

The terpene hydrocarbons β-myrcene [**3**], β-caryophyllene [**41**], and α-humulene [**42**] constituted the largest chemical group in the total oil, the myrcene and sesquiterpene fraction (Figure 4). These hydrocarbons are most abundant in the majority of hop essential oils, but are suggested to be evaporated during wort boiling, discarded with spent hops, lost during wort filtration or fermentation, or transformed to oxygenated terpenes and sesquiterpenes. This is why hops or hop oil extracts are usually added post-fermentation *(11,48)*. It was found that the myrcene fraction contained a few compounds at trace levels such as α-humulene [**42**] and β-pinene [**1**] which may have contributed to the 'crushed grass, sap', 'earthy', and 'musty' aroma *(32,33)*.

The ester fraction mainly comprised of geranyl isobutyrate [**49**], methyl 4-decenoate [**31**], and methyl geranate [**33**] as well as α-humulene [**42**] (also contained in the ketone fraction). The α-humulene [**42**] might have contributed to the 'crushed grass, sap' and 'pine wood' aroma background notes in the two fractions *(33,49)*. 2-tridecanone [**46**] was found in both ester and ketone fractions and has been suggested to impart green and woody aromas in Hallertau Tradition, Spalter Select, and Tettnanger hops *(42)*. In addition, the ketone 2-undecanone [**27**] was present in the ester and the ketone fraction, which is one of the most abundant methyl ketones in hop essential oil, known to impart floral *(50)* and citrusy *(51)* aroma notes and therefore might have contributed to the 'fresh lemon' or 'artificial lemon' aroma and the 'rose water' flavour in these two fractions.

Geranyl isobutyrate was identified as one of the key flavour compounds in beers hopped with Cascade and Cluster varieties and added floral flavour, although present well below its sensory threshold concentration *(52,53)*. It has been suggested to add to the complexity of the floral flavour together with monoterpene alcohols linalool and geraniol rather than being solely responsible for this flavour sensation in the beers *(52,53)*. The fact that linalool [**8**] and geraniol [**25**] were not detected in the ester fraction suggests that geranyl isobutyrate [**49**] added to the 'rose water' flavour note in the sensory evaluation, either independently or together with other compounds (e.g. methyl esters). Methyl 4-decenoate [**31**], methyl geranate [**33**] as well as other methyl esters such as methyl 4,8-decadienoate [**32**] are frequently identified in different hop varieties *(54–56)*, however, their contribution to sensory profiles of the hop volatile mixtures has not yet been specified.

Apart from 2-undecanone [**27**], the main ketone in the ketone fraction was found to be 2-dodecanone [**39**] which is suggested to be one of the main contributing compounds to the 'orange citrus fruit', 'fresh lemon', and 'rose water' aroma and flavours. This is in agreement with a previous study where 2-dodecanone induced fruity, citrus, and orange aroma notes *(42)*. The ketone fraction also contained a considerable amount of geranyl isobutyrate [**49**] and methyl 4-decenoate [**31**]. The similarity between the composition of the ester and the ketone fractions explains the similar sensory profiles of these fractions and a clearer separation of the two compound groups might have resulted in more sensory differences between them.

The main compounds in the terpene alcohol fraction could be categorised into monoterpene alcohols (mainly geraniol [**25**], linalool [**8**], α-terpineol [**20**]), sesquiterpene alcohols (humulenol II [**63**], humulol [**58**], caryophyllenyl alcohol [**54**]), and caryophyllene oxide [**55**]. The aroma of monoterpene alcohols is known to be perceived at low compound concentrations. The most abundant compounds geraniol and linalool were found to contribute to fruity, citrus, and rose-like aroma notes in beer *(40,50)*. In addition, previous studies have shown that linalool, at sub- and supra-threshold concentrations, acts as a synergist by significantly increasing the intensities of those sensory characteristics induced by geraniol (floral, rose-like aroma) or oxygenated sesquiterpenoids (spicy/herbal, floral/fruity flavour, bitterness) *(7,49,57)*. In the current study, linalool [**8**] was mainly associated with the 'grapefruit zest', and 'fresh lemon' aroma as well as the 'rose water' flavour. Further work is required to investigate if linalool has a role in the slightly increased 'lingering bitterness' intensity perceived in

Table 5. Volatile compounds (n=68) identified in the total Magnum hop oil (TO) and the sesquiterpene (SQ), myrcene (MYR), ester (EST), ketone (KET), and terpene alcohol (TALC) fractions. Identification using external standard compounds (*), linear retention indices (LRI), and library matching (MS Mass Spectral library (MS08) and Wiley7n.1 (Hewlett-Packard, US) databases). Relative (%) chemical composition obtained by peak area normalisation (PAN). Log*P* used as an indicator for the polarity of the identified volatile compounds. 'Sample Log*P*' estimated based on the relative contribution of the compounds' Log*P* to the polarity of the hop oil or fraction.

No	RT (min)	LRI[a]	LRI[b]	Compound	TO	SQ	MYR	EST	KET	TALC	Log*P*[a]	Compound class
1	5.48	975	970	β-Pinene *	0.01	-	3.12	-	-	-	4.16	Terpene hydrocarbon
2	5.67	988	984	6-Methyl-5-heptene-2-one	0.01	-	-	0.07	0.09	0.05	2.05	Ketone
3	5.82	990	991	β-Myrcene *	37.3	2.29	91.7	-	-	-	4.88	Terpene hydrocarbon
4	7.45	1049	1049	Cis-β-Ocimene	0.09	0.02	-	-	0.01	-	4.67	Terpene hydrocarbon
5	8.15	1070	1072	Cis-Linalool oxide	0.01	-	-	-	-	0.14	2.08	Monoterpene alcohol derivate
6	8.66	1085	1087	Methyl 6-methyl heptanoate	0.67	-	-	0.71	0.35	-	3.40	Ester
7	8.78	1090	1092	2-Nonanone *	0.24	-	-	0.95	1.42	0.63	3.14	Ketone
8	9.06	1099	1100	Linalool *	0.39	-	-	-	0.50	5.58	2.97	Monoterpene alcohol
9	9.17	1101	1099	3-Methylbutyl 2-methylbutanoate	0.18	-	-	0.17	0.03	-	3.56	Ester
10	9.34	1107	1107	2-Methylbutyl 3-methylbutyrate	0.13	-	-	0.18	0.04	-	3.66	Ester
11	9.59	1115	1114	Fenchol	0.07	-	-	-	0.03	0.96	3.17	Monoterpene alcohol
12	9.68	1128	1126	Myrcenol	0.04	-	-	-	0.03	0.36	3.46	Monoterpene alcohol
13	9.79	1135	1138	Methyl octanoate *	0.69	-	-	1.62	0.83	-	3.46	Ester
14	10.53	1151	1151	Hexyl isobutyrate	0.03	-	-	0.06	-	0.03	3.28	Ester
15	10.75	1156	1155	5-Decanone	0.12	-	-	0.73	1.08	0.45	3.20	Ketone
16	11.18	1167	1167	Endo-Borneol *	0.08	-	-	-	-	1.54	2.69	Monoterpene alcohol
17	11.45	1178	1177	Terpinen-4-ol	0.02	-	0.01	0.01	0.03	0.32	3.26	Monoterpene alcohol
18	11.63	1187	1188	trans-3(10)-Caren-2-ol	0.04	-	0.04	0.15	0.26	0.37	1.97	Monoterpene alcohol
19	11.75	1193	1193	Methyl 6-methyloctanoate	0.32	0.06	0.19	1.49	0.72	-	3.32	Ester
20	11.90	1194	1197	α-Terpineol *	0.21	-	-	-	-	4.02	2.98	Monoterpene alcohol
21	12.04	1195	1194	Myrtenol	0.01	-	-	-	-	0.05	2.98	Monoterpene alcohol
22	12.18	1201	1202	2-Decanol	0.03	-	-	-	-	0.13	3.71	Monoterpene alcohol
23	12.41	1225	1225	Methyl (4E)-4-nonenoate	0.11	0.01	0.06	0.64	0.34	0.01	2.90	Ester
24	12.82	1228	1225	Nerol	0.09	0.02	0.05	0.54	0.30	1.15	4.70	Monoterpene alcohol
25	13.63	1253	1255	Geraniol *	0.08	-	-	-	-	17.8	3.47	Monoterpene alcohol
26	14.69	1285	1287	Methyl 8-methyl-nonanoate	0.24	0.07	0.06	2.66	1.50	0.02	4.40	Ester
27	14.85	1295	1294	2-Undecanone *	1.15	-	-	13.8	22.0	12.3	3.69	Ketone
28	14.95	1296	1297	Perillol	0.02	-	-	-	-	0.28	3.17	Monoterpene alcohol
29	15.16	1307	1307	2-Undecanol	0.04	-	-	0.37	0.26	0.58	4.21	Aliphatic alcohol
30	15.16	1308	1302	Octyl propionate	0.05	-	-	0.49	0.35	0.19	4.35	Ester
31	15.31	1311	1311	Methyl 4-decenoate	1.91	-	-	15.8	10.3	-	4.09	Ester
32	15.42	1314	1314	Methyl-4,8 decadienoate	0.27	0.05	0.06	2.65	2.12	0.09	3.87	Ester
33	15.66	1322	1322	Methyl geranate *	0.71	-	-	12.3	9.84	-	3.98	Ester
34	15.74	1324	1324	Methyl decanoate	0.07	-	-	0.92	0.58	-	4.41	Ester
35	16.33	1325	1326	Octyl Isobutyrate	0.13	0.09	0.02	1.93	0.97	-	4.71	Ester
36	17.07	1371	n/a	Methyl 2-decenoate	0.07	-	-	0.11	0.23	-	3.97	Ester

(*Continues*)

Table 5. *(Continued)*

No	RT (min)	LRI[a]	LRI[b]	Compound	TO	SQ	MYR	EST	KET	TALC	Log*P*[a]	Compound class
37	17.25	1372	1372	Geranyl acetate *	0.12	-	-	1.43	1.85	-	3.98	Ester
38	17.52	1373	1375	Methyl undecanoate	0.10	0.03	0.01	1.46	0.96	0.01	4.86	Ester
39	17.69	1379	1377	2-Dodecanone *	0.27	-	-	3.88	6.51	3.66	4.18	Ketone
40	17.87	1395	1396	Methyl undecenoate	0.01	-	-	0.12	0.11	-	4.79	Ester
41	18.29	1419	1418	β-Caryophyllene *	8.57	19.0	-	2.12	2.55	-	6.30	Sesquiterpene hydrocarbon
42	19.38	1453	1455	α-Humulene *	37.0	69.0	4.67	9.46	12.7	0.89	6.95	Sesquiterpene hydrocarbon
43	19.42	1474	1475	Geranyl propionate	0.01	-	-	0.14	0.24	-	3.64	Ester
44	20.10	1475	1475	Neryl isobutyrate	0.20	-	-	0.94	0.50	-	3.45	Ester
45	20.14	1487	1486	β-Eudesmene	0.32	1.05	-	-	-	-	4.58	Sesquiterpene hydrocarbon
46	20.39	1494	1495	2-Tridecanone *	0.06	0.06	-	0.60	1.02	-	4.68	Ketone
47	20.47	1495	n/a	Cis-5-Dodecenoic acid, methyl ester	0.10	-	-	1.54	1.46	-	4.00	Ester
48	20.66	1496	1497	Methyl 3,6-dodecadienoate	0.20	-	-	0.90	0.95	-	4.10	Ester
49	20.74	1516	1516	Geranyl isobutyrate *	1.84	-	-	17.0	12.02	-	4.77	Ester
50	21.07	1526	1527	δ-Cadinene	2.76	7.79	-	0.77	0.86	-	6.64	Sesquiterpene hydrocarbon
51	21.14	1532	-	*Unknown*	0.03	-	-	0.21	0.18	-	-	-
52	21.62	1532	1531	trans-Z-α-Bisabolene epoxide	0.01	-	-	-	-	0.10	4.86	Oxygenated sesquiterpene
53	22.08	1534	1535	(E)-Nerolidol	0.09	-	-	0.85	1.57	2.14	5.68	Sesquiterpene alcohol
54	22.32	1570	1572	Caryophyllenyl alcohol	0.20	-	-	-	-	5.24	4.20	Sesquiterpene alcohol
55	22.50	1574	1579	Caryophyllene oxide *	0.27	-	-	-	0.43	0.27	3.60	Oxygenated sesquiterpene
56	22.65	1575	-	*Unknown*	0.02	-	-	-	-	0.85	-	-
57	22.95	1576	1572	Humulene epoxide I	0.01	-	-	-	-	-	4.56	Oxygenated sesquiterpene
58	23.08	1580	1577	Humulol	0.68	-	-	-	1.00	15.5	3.80	Sesquiterpene alcohol
59	23.19	1591	1589	Humulene epoxide II	1.07	0.40	-	-	-	-	4.51	Oxygenated sesquiterpene
60	23.45	1602	1606	Widdrol	0.04	-	-	-	-	1.31	4.10	Sesquiterpene alcohol
61	23.64	1602	1609	1-Epicubenol	0.04	-	-	-	-	1.80	3.69	Sesquiterpene alcohol
62	23.76	1604	1604	Humulene epoxide III	0.01	-	-	-	-	-	4.45	Oxygenated sesquiterpene
63	23.78	1605	1605	2-Humulenol	0.15	-	-	-	-	12.5	3.50	Sesquiterpene alcohol
64	23.87	1636	1639	11,11-Dimethyl-4,8-dimethylenebicyclo[7.2.0]undecan-3-ol	0.02	-	-	-	-	2.66	3.70	Aliphatic alcohol
65	24.00	1638	1640	tau-Cadinol	0.09	-	-	0.08	0.33	3.13	4.90	Sesquiterpene alcohol
66	24.31	1639	1638	δ-Cadinol	0.02	-	-	-	-	1.02	4.95	Sesquiterpene alcohol
67	25.38	1700	1697	2-Pentadecanone	0.02	-	-	0.21	0.47	0.38	5.66	Ketone
68	25.76	1714	1713	(Z,E)-Farnesol	0.02	-	-	-	-	1.47	5.77	Sesquiterpene alcohol
				Estimated sample (fraction/total oil) log*P*:	**5.69**	**6.71**	**4.95**	**4.51**	**4.47**	**3.77**		

[a] Calculated retention indices
[b] Retention indices published in the literature or NIST Chemistry WebBook [Online]
'-' Compound was not detected.

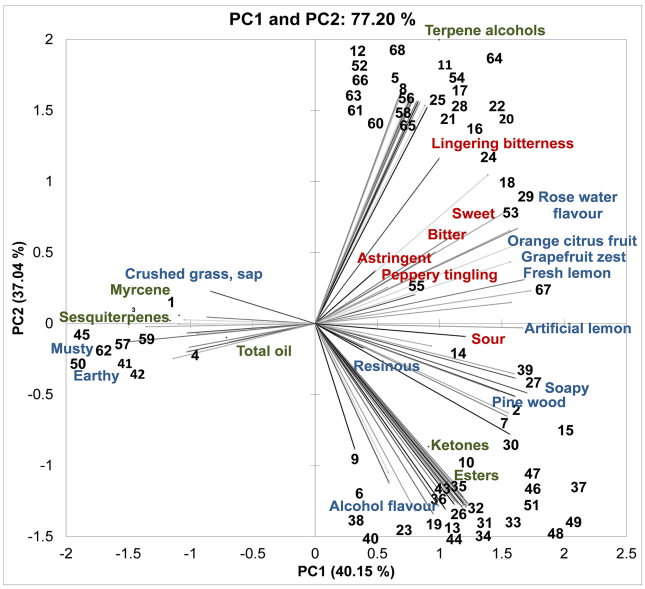

Figure 5. Principle Component Analysis (PCA) biplot of normalised sensory and GC-MS data presented on principal component 1 (PC1) and 2 (PC2). Numbered volatile compounds in the total oil and hop oil fractions in black (numbers see Table 5), aroma and flavour attributes in **blue**, taste and mouthfeel attributes in **red**, samples in **green** [Colour figure can be viewed at wileyonlinelibrary.com]

the terpene alcohol fraction. α-terpineol [**20**] has been detected in Hallertau and Spalt hop varieties and added lilac or pine-like flavour to beer *(58)* suggesting that it might have been involved in more than one sensory sensation.

Previous studies indicate that some sesquiterpenoids in hop oil that have also been identified in the terpene alcohol fraction in the current study are involved in sensory interactions. Caryophyllene oxide and humulenol II have been detected in spicy essences prepared from different hop varieties and were suggested to be two of the compounds inducing spicy aroma, flavour and herbal aroma notes in beer *(11,59)*. Humulenol II was also suggested to contribute to woody and green aromas *(43)*. Interestingly, Van Opstaele et al *(42)* found that humulol and humulenol II could not be sensorially detected at the sniffing port in an olfactometric analysis, although present at reasonable concentrations in all tested hop varieties. However, whilst caryophyllenyl alcohol has previously been detected in hops, its aroma or flavour profile has not been specified *(58)*. Overall, this indicates that sesquiterpene alcohols are contributing to flavour, mouthfeel and trigeminal-type sensations rather than to aroma, and the exact sensations elicited are foremost dependent on the matrix in which these compounds are applied. Further research is required in order to confirm the occurrence of the suggested sensory interactions and the role of the monoterpene and sesquiterpene alcohols. Furthermore, it is acknowledged that a GC coupled to a single quadrupole MS was used to identify and semi-quantify the volatile compounds in the total oil and fractions. Due to the limits of detection with this approach, compounds at very low concentrations, such as trace sulphur compounds, were not analysed, but could still have contributed to the sensory profiles of the samples.

Relationship between compound polarities and the sensory perception of hop oil fractions. The release of aroma and flavour depends on various factors including intrinsic chemical properties of the volatile compounds (polarity or hydrophobicity/hydrophilicity), the composition of the matrix in which they are applied, and environmental conditions such as temperature or pH *(60)*. The latter two factors were consistent in all samples, however the chemical properties differed. In Table 5, the Log*P*, the logarithm of the octanol/water partition coefficient, is listed for each volatile compound as an index of their polarity *(61)*. The sample Log*P* for the total oil and the fractions was calculated on the basis of the relative contribution of individual compounds in the oil/fraction. It was found that the total oil and the fractions differ considerably with respect to their polarity, with the total oil and the sesquiterpene fraction comprising of nonpolar compounds. In contrast, the terpene alcohol fraction contained several polar compounds that readily dissolve in water.

It was hypothesised that the differences in polarity among the hop oil fractions (and compounds) might have an impact on the perception of the orthonasal aroma intensity due to different degrees of volatile retention in the ethanol solution and the partitioning and release of the volatiles into the headspace *(62,63)*. In contrast to the terpene alcohol and the myrcene fractions, the total oil and sesquiterpene samples obtained comparably low scores for the 'overall aroma intensity'. Polar volatiles present in high concentrations in the terpene alcohol fraction are more soluble in water and thus sustain headspace concentrations more effectively in a dynamic headspace situation such as that when sniffing an opened jar. However, as previously mentioned, sensory and compound interactions and differences in odour threshold concentrations should also be considered when evaluating the intensity of the aroma of a complex volatile matrix. The myrcene and the terpene alcohol fractions obtained the highest overall aroma intensity scores. This was probably due to the fact that the myrcene fraction was enriched in β-myrcene and the terpene alcohol fraction contained relatively high amounts of linalool and geraniol. All compounds were present at concentrations considerably in excess of their aroma threshold levels. The ranges of aroma threshold concentration of β-myrcene, linalool and geraniol in beer have been suggested to be 30-1000 µg/L *(3)*, 2.2-5 µg/L and 6-7 µg/L *(7,64)*, respectively, depending on the composition of the beer matrix and the method of threshold determination.

Based on the data generated in the sensory training sessions and the mock evaluation, it was decided whether an attribute should be selected to describe aroma or flavour. 'Alcohol' and 'rose water' were selected to describe flavour sensations in the samples. Recently, Piombino et al *(65)* suggested that the release of polar volatiles from wine was increased in retronasal conditions while the release of nonpolar volatiles diminished. More polar compounds have been found to be retained in the oral and nasal cavities through retention by the nasal mucosa and are released at higher concentrations in exhaled breath *(66)*. The attribute 'rose water' flavour appears to be mainly induced by polar monoterpene alcohols (linalool, geraniol) in the terpene alcohol fraction. Overall, the perception of hop oil compounds appears to be highly complex and it is important to take the composition of compound mixtures and their physicochemical properties into account in order to fully understand the sensory profile that is obtained.

Prediction of sensory scores from GC-MS peak areas. PLS regression methods can be used to analyse data that is strongly collinear, noisy, and has numerous *X*-variables whilst simultaneously modelling response variables *(67)*. This method has been used in previous studies to predict sensory qualities e.g. of wine based on GC-MS data *(68)*. PLS regression analyses were conducted to verify the correlation between 68 different hop oil compounds

Table 6. Mean range of sensory scores and PLS regression model performance (PLS1, PLS2) for prediction of the sensory attributes using the normalised peak areas of principal hop oil compounds in the hop oil/fraction samples (Table 5).

Modality	Attribute	Sensory scores				PLS2 model performance[a]		PLS1 model performance[b]	
		Min	Max	Mean	SD	R^2	RMSE	R^2	RMSE
Aroma	Soapy	1.52	4.10	2.72	1.18	0.929	0.287	0.995	0.075
	Musty	1.26	3.35	1.87	0.79	0.587	0.461	0.984	0.091
	Pine wood	2.66	4.64	3.68	0.85	0.704	0.422	0.962	0.151
	Earthy	0.67	2.21	1.25	0.62	0.713	0.305	0.982	0.077
	Resinous	1.93	3.15	2.57	0.48	0.049	0.429	0.927	0.119
	Crushed grass, sap	2.19	5.44	2.94	1.24	0.227	0.994	0.961	0.224
	Orange citrus fruit	1.95	4.26	2.97	1.00	0.776	0.432	0.964	0.174
	Grapefruit zest	1.59	3.29	2.37	0.79	0.892	0.237	0.997	0.037
	Fresh lemon	1.38	3.71	2.50	1.06	0.951	0.214	0.998	0.041
	Artificial lemon	0.81	2.89	1.75	0.87	0.883	0.270	0.972	0.133
Flavour	Rose water	1.20	5.46	2.94	1.73	0.880	0.549	0.995	0.113
	Alcohol	3.06	3.51	2.94	0.16	0.213	0.131	0.956	0.031
Taste	Sweet	1.35	2.37	1.85	0.40	0.423	0.281	0.948	0.084
	Sour	1.47	2.37	1.85	0.38	0.467	0.255	0.983	0.046
	Bitter	2.97	3.82	3.46	0.34	0.292	0.262	0.968	0.056
	Lingering bitterness	2.71	4.18	3.29	0.51	0.309	0.387	0.886	0.157
Mouthfeel	Peppery tingling	1.94	3.17	2.53	0.41	0.438	0.358	0.956	0.079
	Astringent	3.70	4.79	4.28	0.41	0.447	0.365	0.972	0.064

[a] PLS2 algorithms for multivariate sensory attributes
[b] PLS1 algorithms for univariate sensory attributes
RMSE = root mean square error; R^2 = R-squared, goodness-of-fit

(*X*-matrix) listed in Table 6 and 18 sensory qualities of the six hop oil samples (*Y*-matrix) listed in Table 4. PLS1 and PLS2 were conducted for univariate and multiple sensory attributes, respectively. PLS2 is used to provide a global impression of the sensory profiles. PLS1 models provided a clearer fit of the data compared to PLS2 for multiple attributes as shown in the model performance data presented in Table 6. R^2 or the goodness-of-fit indicates how close the data are to the fitted regression line. The Root Mean Square Error (RSME) is the standard deviation of the residuals or predication errors. The closer the RSME to 0, the less prediction errors have been found. The advantage of PLS2 is that only one set of PLS factors exists for all analytes, which simplifies the interpretation and allows for graphical inspection. However, if aiming for the best predictive accuracy PLS1 should be used *(67)*.

For the PLS1, the best models could be obtained for the attributes 'soapy', 'earthy', 'orange citrus fruit', 'grapefruit zest', 'artificial lemon', 'fresh lemon' aroma and 'rose water' flavour. Many attributes obtained large ranges of scores among the samples in the sensory evaluation and helped to define the sensory characteristics of the total oil and the five fractions. For PLS1, good models were obtained for all attributes. However, after evaluation of the model by checking the RSME, degrees of freedom, and standardised coefficients plots for the predictors (95% confidence interval), it was concluded that the model overfits the data in view of the taste and mouthfeel attributes suggesting that the model obtained by PLS1 should not be used. Overall, it appears to be difficult to identify linear relationships between compounds and one sensory sensation. Based on the measurement errors in the data, one might assume that the robustness of both models might have been dependent on the uncertainty in the sensory scores and to a lesser extent on the analytical data that was obtained using GC-MS analysis (PAN data). However, as concluded in the previous sections, more than one compound is likely to be involved in the perception of a sensory sensation due to sensory interactions (synergistic, additive) between compounds and within or across sensory modalities. This was expected to be the main reason for the weak prediction of taste and mouthfeel attributes. The goodness-of-fit was lowest for all of these attributes which is explained by the fact that sensations are to a certain extent the result of sensory interactions as previously discussed . For instance, the fruity aroma – sweet taste interactions are suggested to be induced by methyl esters, ketones and/or monoterpene alcohols and a cross-modal interaction might have been induced by compounds in the terpene alcohol fraction causing a slightly increased 'peppery tingling' mouthfeel sensation.

Overall, it was concluded that the sensory scores were not entirely predictable based on GC-MS data, but PLS2 models give a good overview of important compound groups that are involved in different sensations of the multi-sensory profiles of the hop oil fractions. PLS models might help to identify the occurrence of sensory interactions that contribute to the sensory characteristics of hop essential oil. The outcome of the PLS regression analysis in this study shows once more that when evaluating the sensory contribution of volatile compounds in hop essential oil or hop oil fractions to a 'hoppy' flavour sensation, simple cause-effect-relationships between sensations and compounds are only able to explain half of the story.

Conclusions

This is the first study to establish a sensory attribute lexicon and to investigate the sensory characteristics of a hop essential oil and five $scCO_2$ fractions extracted thereof in ethanol (4% abv). The study provides significant insight into the sensory differences

between the hop oil fractions and suggests a relationship between the perception and intensities of the analysed sensory characteristics and the physicochemical nature of the fractions. While the total oil and the sesquiterpene fractions obtained moderate to low sensory scores for all sensory attributes - likely due to the nonpolar character of the compounds, compound concentrations and sensory threshold levels - the myrcene, ketone, ester and terpene alcohol fractions showed comparatively high sensory potentials by inducing different grassy, musty, fruity, floral aromas and flavours. In case of the latter two fractions, the aroma and flavour sensations occurred in combination with increased taste and mouthfeel characteristics. As hypothesised, due to sensory interactions, single compounds could not be assigned to specific sensory sensations (and vice versa) even in a simple ethanol-water system. However, few single compounds in the monoterpene alcohol fraction (linalool, geraniol) and compound groups in the ketone and ester fractions (methyl and geranyl esters) positively correlated with the 'rose water' flavour sensation whereas the 'crushed, grass sap' aroma could be clearly assigned to the presence of β-myrcene. Whilst, increased or added taste and mouthfeel sensations could not be assigned to any compound suggesting that these were perceived as a result of sensory interactions within (e.g. 'sweet' taste) or across (e.g. 'peppery tingling' mouthfeel) sensory modalities. This explains why the PLS models could not successfully predict the sensory scores for these taste and mouthfeel attributes based on the analytical data. It is recommended to consider temporal sensory profiling methods for future studies to investigate the sensory characteristics of hop oil fractions. The lack of significant effects for the lingering taste and mouthfeel attributes assessed in the current study may have been caused by only one time point being selected for their assessment. Temporal sensory methods such as Progressive Profiling or Time-Intensity where the intensity of attributes is continuously assessed over a defined period of time may be more appropriate to obtain a dynamic sensory profile of these sensations.

Considering the volatile composition of the highly polar monoterpene alcohol and the less polar sesquiterpene alcohol subfractions, it remains to be investigated which role the polarities of compounds or fractions have in the multi-sensory profile presented by the terpene alcohol fraction. Omission or addition studies appear to be suitable to identify compounds that could be involved in these interactions. Further research is required into the chemical composition of Magnum hop essential oil and its fractions to detect those compounds that were present at subdetection threshold in the current study. Moreover, the hop extracts used here were produced to be added post-fermentation. In this way, volatile losses due to biotransformation reactions or evaporation can be limited to a minimum. The addition of hops is simple and less time consuming, and the hop flavourings are less prone to deterioration compared to traditional hop materials, thereby improving efficiency and sustainability of the hopping procedure. Considering that the hop extracts were exclusively tested in an ethanol-water base in order to obtain a general understanding of their sensory characteristics, the next essential step will be to investigate their sensory impact in a beer matrix. Conversely, it is required to study the effect of other components in the beer matrix on the perception of the hop oil fractions. The investigation of mutual influences may help to understand the potential of these fractions as flavouring materials in various beer styles. It should be taken into account that the current study solely focused on the Magnum hop variety and a number of fractions applied at one concentration. The results should not be generalised, but this research could provide the basis for future studies investigating other hop varieties using modified experimental designs.

Overall, it has been shown that the fractionation of Magnum hop essential oil can be applied to obtain distinct and sustainably produced flavouring preparations. These may be used in isolation or combination in order to achieve distinct aroma, flavour, taste, and mouthfeel sensations. The findings of this study, together with the potential impacts of global warming and climate change on oil yield and composition of hop varieties, suggest that more attention should be given in future to the sensory properties of 'bitter hop varieties'.

Authors Contributions

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.

Ray Marriott: Conceptualisation and input to design of investigation. PhD supervisor at industry partner.

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

This research was funded by Totally Natural Solutions Ltd.

Acknowledgements

We thank Dr Qian Yang for her advisory support during the sensory study, Dr Robert Linforth for technical support with the volatile profile analysis, and the University of Nottingham Sensory Beer Panel for conscientiously assessing numerous hop oil samples and for their commitment.

Conflict of interest

The authors declare there are no conflicts of interest.

References

1. Rettberg N, Biendl M, Garbe L-A. 2018. Hop aroma and hoppy beer flavor: chemical backgrounds and analytical tools—a review. *J Am Soc Brew Chem 76*:1-20. https://doi.org/10.1080/03610470.2017.1402574
2. King AJ, Dickinson JR. 2003. Biotransformation of hop aroma terpenoids by ale and lager yeasts. *FEMS Yeast Res 3*:53-62. https://doi.org/10.1111/j.1567-1364.2003.tb00138.x
3. Schönberger C, Kostelecky T. 2011. 125th anniversary review: the role of hops in brewing. *J Inst Brew 117*:259-267. https://doi.org/10.1002/j.2050-0416.2011.tb00471.x
4. Almaguer C, Schönberger C, Gastl M, Arendt EK, Becker T. 2014. *Humulus lupulus* – a story that begs to be told. A review. *J Inst Brew 120*:289-314. https://doi.org/10.1002/jib.160
5. Eyres G, Dufour J-P. 2009. Hop essential oil: Analysis, chemical composition and odor characteristics, p 239-254. In Preedy VR (ed), *Beer in health and disease prevention*, Academic Press, London, UK. http://doi.org/10.1016/B978-0-12-373891-2.00022-5
6. Hanke S, Ditz V, Herrmann M, Back W, Krottenthaler M, Becker T. 2010. Influence of ethyl acetate, isoamyl acetate and linalool on off-flavour perception in beer. *Brew Sci*, *63*:94-99.
7. Takoi K, Itoga Y, Koie K, Kosugi T, Shimase M, Katayama Y, Nakayama Y, Watari J. 2010. The contribution of geraniol metabolism to the citrus flavour of beer: synergy of geraniol and β-citronellol under coexistence with excess linalool. *J Inst Brew 116*:251-60. https://doi.org/10.1002/j.2050-0416.2010.tb00428.x

8. Howard GA, Slater CA. 1957. Evaluation of hops VII. Composition of the essential oil of hops. *J Inst Brew 63*:491-506. https://doi.org/10.1002/j.2050-0416.1957.tb06290.x
9. Taylor AJ. 2002. Release and transport of flavors in vivo: physicochemical, physiological, and perceptual considerations. *Compr Rev Food Sci Food Saf 1*:45-57. https://doi.org/10.1111/j.1541-4337.2002.tb00006.x
10. Lafontaine SR, Shellhammer TH. 2018. Sensory directed mixture study of beers dry-hopped with Cascade, Centennial, and Chinook. *J Am Soc Brew Chem 76*:199-208. https://doi.org/10.1080/03610470.2018.1487747
11. Goiris K, De Ridder M, De Rouck G, Boeykens A, Van Opstaele F, Aerts G, De Cooman L, De Keukeleire D. 2002. The oxygenated sesquiterpenoid fraction of hops in relation to the spicy hop character of beer. *J Inst Brew 108*:86-93. https://doi.org/10.1002/j.2050-0416.2002.tb00129.x
12. Yu P, Low MY, Zhou W. 2018. Design of experiments and regression modelling in food flavour and sensory analysis: A review. *Trends Food Sci Technol 71*:202-15. https://doi.org/10.1016/j.tifs.2017.11.013
13. Ray DK, Gerber JS, MacDonald GK, West PC. 2015. Climate variation explains a third of global crop yield variability. *Nat Commun 6*:5989. http://doi.org/10.1038/ncomms6989
14. Harvey M, Pilgrim S. 2011. The new competition for land: Food, energy, and climate change. *Food policy 36*:40-51. https://doi.org/10.1016/j.foodpol.2010.11.009
15. Demyttenaere JC. 2018. Natural or synthetic? The legal framework in the EU for the production of natural flavouring ingredients, p 281-305. In Schwab W, Lange BM, Wüst M (eds), *Biotechnology of Natural Products*, Springer Nature, Cham, Switzerland. https://doi.org/10.1007/978-3-319-67903-7_11
16. Roman S, Sánchez-Siles LM, Siegrist M. 2017. The importance of food naturalness for consumers: Results of a systematic review. *Trends Food Sci Technol 67*:44-57. https://doi.org/10.1016/j.tifs.2017.06.010
17. Gahr A, Forster A, Schuell F. 2018. A comparison of the hop varieties Callista and Ariana in years of different climate conditions 2015 and 2016. *Hopfen-Rundschau International - Technical Publications* 48-55.
18. Directive 2010/59/EC. 2010. Commission Directive amending Directive 2009/32/EC of the European Parliament and of the Council on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients, p 10-12, *Official Journal of the European Communities*, L225. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32010L0059
19. Directive 2009/324/EC. 2009. Commission Decision concerning the non-inclusion of certain substances in Annex I, IA or IB to Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market, p 3-11, *Official Journal of the European Communities*, L141. https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32009D0324
20. Marriott R. 2016. U.S. Fractionation of hop oils using liquid and supercritical carbon dioxide. Patent Application No. 15/028,534 https://patents.google.com/patent/US20160298064
21. Van Opstaele F, Rouck G, Clippeleer J, Aerts G, Cooman L. 2010. Analytical and sensory assessment of hoppy aroma and bitterness of conventionally hopped and advanced hopped pilsner beers. *J Inst Brew 116*:445-458. https://doi.org/10.1002/j.2050-0416.2010.tb00796.x
22. Stone H, Sidel J, Oliver S, Woolsey A, Singleton RC. 2008. Sensory evaluation by quantitative descriptive analysis, p 23-34. In Gacula MC (ed), *Descriptive Sensory Analysis in Practice*, Food & Nutrition Press, Trumbull, Connecticut, USA. https://doi.org/10.1002/9780470385036.ch1c
23. International Organization for Standardization (ISO) 2008. 8586-2 2008. Sensory analysis – general guidance for the selection, training and monitoring of assessors – part 2: expert sensory assessors, International Organization for Standardization, Geneva, Switzerland. https://www.sis.se/api/document/preview/909851/
24. Delarue J, Lawlor B, Rogeaux M. 2014. *Rapid sensory profiling techniques: applications in new product development and consumer research*, Woodhead Publishing, Cambridge, England. http://doi.org/10.1016/B978-1-78242-248-8.50025-3
25. Civille GV, Lyon BG. 1996. Aroma and flavor lexicon for sensory evaluation: terms, definitions, references, and examples. ASTM Data Series Publication DS 66, American Society for Testing and Materials, West Conshohocken Pennsylvania, USA.
26. Kemp S, Hollowood T, Hort J. 2011. *Sensory evaluation: a practical handbook*, John Wiley & Sons, Oxford, England. http://doi.org/10.1002/9781118688076
27. Tomic O, Luciano G, Nilsen A, Hyldig G, Lorensen K, Næs T. 2010. Analysing sensory panel performance in a proficiency test using the PanelCheck software. *Eur Food Res Technol 230*:497. https://doi.org/10.1007/s00217-009-1185-y
28. International Organization for Standardization (ISO) 2007. 8589: 2007. Sensory analysis -- General guidance for the design of test rooms, International Organization for Standardization, Geneva, Switzerland. https://www.sis.se/api/document/preview/909357/
29. Lawless HT, Heymann H. 2010. *Sensory evaluation of food: principles and practices*, Springer Science & Business Media, New York, USA. https://doi.org/10.1007/978-1-4419-6488-5
30. Lermusieau G, Bulens M, Collin S. 2001. Use of GC-olfactometry to identify the hop aromatic compounds in beer. *J Agric Food Chem 49*:3867-3874. https://doi.org/10.1021/jf0101509
31. Nance MR, Setzer WN. 2011. Volatile components of aroma hops (*Humulus lupulus* L.) commonly used in beer brewing. *J Brew Distil 2*:16-22.
32. Whittock SP, Koutoulis A. 2010. The aroma potential of Australian hops. In Blomley G, (ed), *Institute of Brewing and Distilling Asia Pacific Section 31st Asia Pacific Convention*, Surfers Paradise, Australia.
33. Peltz M, Shellhammer T. 2017. Ethanol content has little effect on the sensory orthonasal detection threshold of hop compounds in beer. *J Am Soc Brew Chem 75*:221-7. https://doi.org/10.1094/ASBCJ-2017-3994-01
34. Guadagni D, Buttery RG, Harris J. 1966. Odour intensities of hop oil components. *J Sci Food Agric 17*:142-144. https://doi.org/10.1002/jsfa.2740170311
35. Sharpe FR. 1988. Assessment and control of beer flavour. *J Inst Brew 94*:301-305. https://doi.org/10.1002/j.2050-0416.1988.tb04586.x
36. Schnaitter M, Kell A, Kollmannsberger H, Schüll F, Gastl M, Becker T. 2016. Scale-up of dry hopping trials: importance of scale for aroma and taste perceptions. *Chem Ing Tech 88*:1955-65. https://doi.org/10.1002/cite.201600040
37. Neiens SD, Steinhaus M. 2018. Investigations on the impact of the special flavor hop variety Huell Melon on the odor-active compounds in late hopped and dry hopped beers. *J Agric Food Chem 67*:364-371. https://doi.org/10.1021/acs.jafc.8b05663
38. Brendel S, Hofmann T, Granvogl M. 2019. Characterization of key aroma compounds in pellets of different hop varieties (*Humulus Lupulus* L.) by means of the Sensomics approach. *J Agric Food Chem 67*:12044-12053. https://doi.org/10.1021/acs.jafc.9b05174
39. Tokita K, Takazumi K, Oshima T, Shigyo T. 2014. A new method for analyzing the characteristic flavor of beer using selectable one-dimensional or two-dimensional gas chromatography-olfactometry/mass spectrometry. *J Am Soc Brew Chem 72*:154-161. https://doi.org/10.1094/ASBCJ-2014-0402-01
40. Kishimoto T, Wanikawa A, Kono K, Shibata K. 2006. Comparison of the odor-active compounds in unhopped beer and beers hopped with different hop varieties. *J Sci Food Agric 54*:8855-8861. https://doi.org/10.1021/jf061342c
41. Van Opstaele F, De Causmaecker B, Aerts G, De Cooman L. 2012. Characterization of novel varietal floral hop aromas by headspace solid phase microextraction and gas chromatography–mass spectrometry/olfactometry. *J Agric Food Chem 60*:12270-81. https://doi.org/10.1021/jf304421d
42. Van Opstaele F, Praet T, Aerts G, De Cooman L. 2013. Characterization of novel single-variety oxygenated sesquiterpenoid hop oil fractions via headspace solid-phase microextraction and gas chromatography–mass spectrometry/olfactometry. *J Agric Food Chem 61*:10555-64. https://doi.org/10.1021/jf402496t
43. Praet T, Van Opstaele F, Steenackers B, De Vos D, Aerts G, De Cooman L. 2016. Flavor activity of sesquiterpene oxidation products, formed upon lab-scale boiling of a hop essential oil–derived sesquiterpene hydrocarbon fraction (cv. Saaz). *J Am Soc Brew Chem 74*:65-76. https://doi.org/10.1094/ASBCJ-2016-1205-01
44. Lawless H, Rozin P, Shenker J. 1985. Effects of oral capsaicin on gustatory, olfactory and irritant sensations and flavor identification in humans who regularly or rarely consume chili pepper. *Chem Senses 10*:579-89. https://doi.org/10.1093/chemse/10.4.579
45. Van Opstaele F, Goiris K, De Rouck G, Aerts G, De Cooman L. 2012. Production of novel varietal hop aromas by supercritical fluid extraction of hop pellets—Part 2: Preparation of single variety floral, citrus, and spicy hop oil essences by density programmed supercritical fluid extraction. *J Supercrit Fluids 71*:147-61. https://doi.org/10.1016/j.supflu.2012.06.004

46. Yau NN, McDaniel MR. 1990. The power function of carbonation. *J Sens Stud 5*:117-28. https://doi.org/10.1111/j.1745-459X.1990.tb00484.x
47. McEvoy S. 1998. Sensory evaluation of carbonated beverages utilizing a hyperbaric chamber or what would soda taste like if you could get inside the can. Chemical senses day XIV abstracts. Santa Rosa, CA.
48. Dresel M, Van Opstaele F, Praet T, Jaskula-Goiris B, Van Holle A, Naudts D, De Keukeleire D, De Cooman L, Aerts G. 2013. Investigation of the impact of the hop variety and the hopping technology on the analytical volatile profile of single-hopped worts and beers. *Brew Sci 66*:162.
49. Praet T, Van Opstaele F, De Causmaecker B, Bellaio G, De Rouck G, Aerts G, De Cooman L. 2015. De novo formation of sesquiterpene oxidation products during wort boiling and impact of the kettle hopping regime on sensory characteristics of pilot-scale lager beers. *Brew Sci 68*:130-145.
50. Eyres GT, Marriott PJ, Dufour JP. 2007. Comparison of odor-active compounds in the spicy fraction of hop (*Humulus lupulus* L.) essential oil from four different varieties. *J Agric Food Chem 55*:6252-61. https://doi.org/10.1021/jf070739t
51. Gros J, Peeters F, Collin S. 2012. Occurrence of odorant polyfunctional thiols in beers hopped with different cultivars. First evidence of an S-cysteine conjugate in hop (*Humulus lupulus* L.). *J Agric Food Chem 60*:7805-16. https://doi.org/10.1021/jf301478m
52. Peacock VE, Deinzer ML, Likens ST, Nickerson GB, McGill LA. 1981. Floral hop aroma in beer. *J Agric Food Chem 29*:1265-9. https://doi.org/10.1021/jf00108a041
53. Lam KC, Foster RT, Deinzer ML. 1986. Aging of hops and their contribution to beer flavor. *J Agric Food Chem 34*:763-70. https://doi.org/10.1021/jf00070a043
54. Forster A, Gahr A. 2013. On the fate of certain hop substances during dry hopping. *Brew Sci 66*:94-103.
55. Lafontaine SR, Shellhammer TH. 2018. Impact of static dry-hopping rate on the sensory and analytical profiles of beer. *J Inst Brew 124*:434-442. https://doi.org/10.1002/jib.517
56. Van Opstaele F, Goiris K, De Rouck G, Aerts G, De Cooman L. 2013. Production of novel varietal hop aromas by supercritical fluid extraction of hop pellets. Part 1: Preparation of single variety total hop essential oils and polar hop essences. *Cerevisia 37*:97-108. https://doi.org/10.1016/j.cervis.2012.12.002
57. Bailey B, Schönberger C, Drexler G, Gahr A, Newman R, Pöschl M, Geiger E. 2009. The influence of hop harvest date on hop aroma in dry-hopped beers. *Tech Q Master Brew Assoc Am 46*:1-7. http://doi.org/10.1094/TQ-46-2-0409-01
58. Roberts MT, Dufour J-P, Lewis AC. 2004. Application of comprehensive multidimensional gas chromatography combined with time-of-flight mass spectrometry (GC× GC-TOFMS) for high resolution analysis of hop essential oil. *J Sep Sci 27*:473-4788. https://doi.org/10.1002/jssc.200301669
59. Praet T, Van Opstaele F, Baert J, Aerts G, De Cooman L. 2014 Comprehensive characterisation of the hop-derived sesquiterpenoid fingerprint of American kettle hopped lager beers. *Brew Sci 67*:183-194.
60. Ammari A, Schroen K. 2018. Flavor retention and release from beverages: A kinetic and thermodynamic perspective. *J Agric Food Chem 66*:9869-9881. https://doi.org/10.1021/acs.jafc.8b04459
61. Moriguchi I, Hirondo S, Liu Q, Nakagome I, Matsushita Y. 1992. Simple method of calculating octanol/water partition coefficient. *Chem Pharm Bull 40*:127-30. https://doi.org/10.1248/cpb.40.127
62. Goubet I, Le Quere JL, Voilley AJ. 1998. Retention of aroma compounds by carbohydrates: influence of their physicochemical characteristics and of their physical state. A review. *J Agric Food Chem 46*:1981-1990. https://doi.org/10.1021/jf970709y
63. Clark R, Linforth R, Bealin-Kelly F, Hort J. 2011. Effects of ethanol, carbonation and hop acids on volatile delivery in a model beer system. *J Inst Brew 117*:74-81. https://doi.org/10.1002/j.2050-0416.2011.tb00446.x
64. Kaltner D, Mitter W. 2009. Changes in hop derived compounds during beer production and ageing, p 37-47. In Shellhammer TH (ed), *Hop flavor and aroma – Proceedings of the 1st International Brewers Symposium*, Master Brewers Association of the Americas: St. Paul, Minnesota, USA.
65. Piombino P, Moio L, Genovese A. 2019. Orthonasal vs. retronasal: Studying how volatiles' hydrophobicity and matrix composition modulate the release of wine odorants in simulated conditions. *Food Res Int 116*:548-58. https://doi.org/10.1016/j.foodres.2018.08.072
66. Sánchez-López JA, Ziere A, Martins SI, Zimmermann R, Yeretzian C. 2016. Persistence of aroma volatiles in the oral and nasal cavities: real-time monitoring of decay rate in air exhaled through the nose and mouth. *J Breath Res 10*:036005. https://doi.org/10.1088/1752-7155/10/3/036005
67. Wold S, Ruhe A, Wold H, Dunn, III WJ. 1984. The collinearity problem in linear regression. The partial least squares (PLS) approach to generalized inverses. *SIAM J Sci Comput 5*:735-743. https://doi.org/10.1137/0905052
68. Schmidtke LM, Blackman JW, Clark AC, Grant-Preece P. 2013. Wine metabolomics: objective measures of sensory properties of semillon from GC-MS profiles. *J Agric Food Chem 61*:11957-67. https://doi.org/10.1021/jf403504p