Review article

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The multisensory perception of hop essential oil: a review

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Hops are a key ingredient to add bitterness, aroma and flavour to beer, one of the most consumed beverages worldwide. Essential oils from different hop varieties are characterised by similar classes of chemical compounds and complexity, but their contribution to sensory characteristics in beer differs considerably. Volatiles in hop oil are categorised into several chemical classes. These induce diverse aroma and flavour sensations in beer being described as 'floral', 'fruity' (e.g. contributed by alcohols, esters, sulphur-containing compounds), 'spicy', 'woody', 'herbal' (sesquiterpenes, oxygenated sesquiterpenoids), and 'green' (aldehydes). The perception of hop volatiles depends on their concentrations and combinations, but also on threshold levels in different beer matrices or model systems. Several studies attributed modified taste and mouthfeel sensations to the presence of hop volatiles contributing to a multisensory perception of hop flavour. Linalool is frequently observed to show additive and synergistic-type behaviour and to affect aroma perception if combined with geraniol. Linalool has also been found to be involved in aroma-taste interactions, modifying the perception of bitterness qualities in beer. Particularly oxygenated sesquiterpenoids are suggested to be responsible for an irritating, tingling sensation indicating the activation of trigeminal receptors. The majority of these sensory interactions have been discovered almost by accident and a systematic research approach is required to gain a broad understanding of these complex phenomena. This review provides an overview of factors affecting the perception of hop derived volatiles involved in different sensory characteristics of beer, while illustrating the latest advances and highlighting research gaps from a sensory science perspective. © 2020 The Authors. Journal of the Institute of Brewing published by John Wiley & Sons Ltd on behalf of The Institute of Brewing & Distilling

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Keywords: Hop essential oil; hop oil fractions; multimodal perception; sensory interaction effects; bitterness; trigeminal sensations

Introduction

Hops contain both volatile and non-volatile fractions that contribute to the sensory quality of beer. While volatile compounds in hop essential oil add aroma and flavour, non-volatile components such as carboxylic acids, hop resins, amino acids, carbohydrates, and polyphenols are known to affect the taste and mouthfeel characteristics of the final beer product *(1, 2)*. After more than half a century of research in hop flavour chemistry, it is commonly agreed that the overall sensory sensation that is experienced when drinking beer, is not a sum of individual sensations *(3)*. Meilgaard *(4)* already hypothesised in 1975 that approximately half of the flavour intensity in beer can be attributed to sensory interactions between the volatile and the non-volatile fractions.

Hop oil is one of the most complex essential oils known in plants *(5)*. To date, approximately 200 studies have been published investigating the composition of hop oil. Since the early 1960s the number of identified volatile compounds in hop oils has increased steadily. Based on the number of peaks that are reported in studies using advanced chromatographic techniques and taking into account that there is still a need for more sensitive methods in order to capture compounds at trace levels, it is thought that more than 1000 volatile compounds are present in hop oil *(6)*. Beer contains many hop derived volatiles at subthreshold-level, nevertheless, these are expected to contribute to the overall aroma and flavour profile depending on the co-presence of other volatile compounds and components in the beer, such as bittering substances, ethanol and carbon dioxide *(7–13)*. Therefore, one of the factors that complicate the understanding of 'hoppy' aroma and flavour is the occurrence of sensory interactions between hop oil compounds as well as between volatile and non-volatile beer components. Interactions occur at specific compound combinations, ratios, and below and above certain sensory threshold concentrations, particularly in heterogeneous mixtures. The types of interactions between hop oil compounds have been described as synergistic, antagonistic, additive or masking *(14)*. Many attempts have been made to exploit the sensory potential of hop oil compounds, but little attention has been paid to the role of sensory interactions between hop volatiles.

To date, reviews published in the area of hops have focused on the chemical composition of hop oil, the transfer of hop derived

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volatiles into the final beer as a result of different hopping techniques, hop oil analysis techniques and the odour characteristics of single hop oil compounds *(6, 15–17)*. Recently, Rettberg et al. *(2)* published a comprehensive review examining the current status of methodology used in hop research including isolation, separation, detection, identification, and quantification techniques for the investigation of volatile compounds in hop material and in beer. All reviews briefly summarise the contribution of hop oil compounds to the aroma and flavour profile in beer, however sensory characteristics such as somatosensory sensations are not discussed. Sensory interactions between hop oil volatiles and components of the beer matrix, resulting for instance in modified flavour intensities and qualities, have largely been neglected. Being aware of the source of these effects facilitates not only the assessment of the actual sensory potential but also of the targeted application of advanced and complex hop oil products in beverages.

This review aims to outline the current state of scientific knowledge by examining the sensory impact of volatile compounds of hop oil in beer and model matrices often used in this research field. Interactions between hop oil compounds and other beer components (ethanol, carbonation, hop acids) and the resulting effects on the sensory profile of the final beer remain to be elucidated. Moreover, research gaps from a sensory perspective are highlighted and future research is proposed.

Factors determining the hop oil composition in hops and beer

On average dried hop cones contain between 0.5-3% of hop oil comprising of aroma and flavour-active compounds that belong to several chemical classes such as terpenes, alcohols, esters, aldehydes, and ketones. Both quantity and composition of hop essential oil are largely dependent on genetic factors, hop plant or rootstock age, growing conditions including soil, pH, carbon, nitrogen and moisture content, microbial mass, etc., but also on climatic conditions (temperature, humidity, sunshine hours), and time of harvest *(18–20)*. In addition, the quantity of essential oil and the proportion of individual fractions varies across hop varieties *(6)*. For example, the amount and composition of oxides, epoxides and alcohols in the sesquiterpene fraction differ markedly between hop varieties, with Hallertauer Mittelfrueh and Hersbrucker hops comprising of a large proportion of oxygenated or sesquiterpene derivates compared to other varieties *(14, 21, 22)*. The concentrations of single compounds in specific hop oil fractions also differ across hop varieties and geraniol is a prime example being a varietal specific compound that cannot be found in every hop variety at detectable concentrations *(23, 24)*.

Depending on the brewing process and hopping technique, physical, chemical and biochemical changes take place in the volatile fractions of hops that have been found to impact flavour perception. Traditional hopping techniques are kettle, late, and dry hopping. For kettle hopping, the hops are added during wort boiling to ensure that hop α-acids are isomerised to iso-α-acids, which are mainly responsible for the bitterness character of beer. However, up to 85% of the hop oil compounds, particularly hydrocarbons including the most abundant terpene hydrocarbons myrcene, humulene, and caryophyllene, are suggested to be evaporated from the kettle, discarded with the spent hops, lost during wort filtration or fermentation, or transformed to oxygenated terpenes and sesquiterpenes when applying this hopping technique. Oxidation products are more likely to survive the brewing process due to their water solubility *(25)*. Apart from some hydrophobic hop volatiles, the majority of hop oil compounds are not found in the wort in their native form and only few are found unchanged in the beer. The degree of hydrolysis and biotransformation of compounds depends on several factors and matrix effects, including contact time, temperature, pH, and exposure to yeast making it difficult to predict the final volatile composition in beer *(26, 27)*.

Evaporation of hop volatiles can be limited when hops are added towards the end of the boiling process by applying a late hopping technique. The reduced thermal exposure favours the retention of polar oxygenated compounds, terpene derivatives, free alcohols, carbonyls, ketones, and cyclic esters *(28, 29)*. However, the later the addition of hops, the lower the conversion of α-acids to iso-α-acids. Consequently, the intensity of bitterness in the beer decreases and the bitterness quality is modified. The addition of hops to the fermentation vessel or after fermentation during lagering and before filtration or centrifugation, is described as dry hopping *(30)*. In the latter case, the hops are added to the stored cold beer. The final beer contains unmodified hop oil compounds including some hydrocarbons. If added to primary or secondary fermentation, yeasts can still convert hop derived compounds. Lager and ale yeasts have also been found to transform geraniol into β-citronellol or linalool and nerol into α-terpineol via yeast metabolism *(5)*. In addition to transformation reactions, yeasts may adsorb hop oil compounds as observed for several monoterpene alcohols (linalool, geraniol) *(31, 32)*. However, it should not be forgotten that yeast strains can also induce *de novo* synthesis of monoterpene alcohols. This has been found for instance for geraniol and linalool, and to a lesser extent for β-citronellol, α-terpineol, and nerol *(33)*.

Overall, it is still not clear which hop volatiles are directly transferred to the beer without undergoing any biochemical transformations by yeast such as esterification or enzymatic cleavage. A comprehensive review on the molecular biology of fruity and floral volatiles (higher alcohols, esters) derived from hops or formed by yeast during the fermentation process has recently been published by Holt et al. *(34)*.

Cross-modal and multisensory interactions

Before defining the impact of volatiles in hop essential oil on specific sensory characteristics of beer and their role in cross-modal and multisensory interactions, it is necessary to understand the basic sensory sensations known to be involved. **Odour or aroma** sensations are perceived when orthonasally smelling the volatile fraction of beer prior to consumption. Hop derived volatiles are detected by the olfactory system, which comprises around 390 odourant receptor proteins located in the human nose *(35)*. Volatile compounds reach the olfactory epithelium via the orthonasal (via nostril) or the retronasal pathway (via nasopharynx) while the orthonasal pathway is exclusively related to aroma sensations. Volatiles that are delivered through the retronasal pathway are part of flavour sensations *(36)*. **Flavour** sensations perceived when drinking beer are a combination of retronasally delivered aroma together with in-mouth sensations including taste, mouthfeel and trigeminal sensations *(37)*. **Taste** sensations include the perception of bitterness, sweetness, sourness, saltiness, umami, and a number of potential other tastes such as fatty *(38)* and metallic *(39)* that are not fully understood. If using nose-clips, it is possible to split the taste and mouthfeel of a beer from its aroma sensations, thereby limiting the perception of flavour *(40, 41)*. **Trigeminal** stimuli are those that can induce a sensation of irritation (spicy, pungent), pain, or temperature (cooling, warming). High carbonation levels in beer are perceived as a sparkling, tingly, slightly irritating sensation in the oral cavity induced by bursting CO_2 bubbles on the tongue. The bursting bubbles activate the mechanoreceptors in the mouth and, at the same time, the CO_2 is converted to carbonic acid, which induces the tingling response *(42)*. Moreover, carbonation has also been found to impact flavour perception in beer *(43)*. **Mouthfeel** characters are considered as the tactile perception of stimuli such as hop derived polyphenols, which are known to induce astringency in beer. Astringency is driven by inhibited lubrication in the oral cavity and is described as a drying, roughing, and puckering sensation *(44, 45)*.

Hop oil compounds might activate more than one sensory modality or cause interactions such as '**cross-modal interactions**', thereby contributing to the **multisensory perception** of beer. The modulation of one sensation by the perception of another is not easily examined due to the fact that effects of cross-modal interactions can be the result of different mechanisms (physico-chemical, psychological, physiological) and occur at different levels (cognitive, receptor, neural) *(44, 46, 47)*. In case of physico-chemical mechanisms, non-volatile fractions in the beer matrix affect the partitioning of volatiles, their molar concentration, water activity coefficient, or diffusion through the beer matrix. These factors impact on the physical release and concentration of volatiles in the headspace, which in turn can have a major effect on the perceived intensity and quality of aroma-active compounds. Physiological mechanisms which influence flavour release and perception include food/beverage matrix breakdown in the mouth, saliva composition, saliva production and flow, temperature, and swallowing behaviour. These factors, for instance affect the time point of volatile release and delivery through the retronasal pathway *(48)*.

Sensory or cross-modal interactions can cause additive (increasing), synergistic (enhancing/potentiating), antagonistic (suppressing, masking) or eliminating (cancelling/extinguishing) effects *(47)*. Figure 1 illustrates these effects and clarifies the difference between additive and synergistic mechanisms, which are often confused. Synergistic mechanisms are the result of sensory sensations delivering a greater response than the sum of individual compound effects *(47)*. Sensory interaction effects might even result in the perception of a novel sensory sensation, known as 'configural processing' i.e. two compounds that would separately induce a similar (or a different) aroma give a completely new aroma sensation if mixed together *(46)*. It should also be taken into account that volatiles and non-volatiles not only have a threshold concentration for the detection or recognition of aroma, flavour, taste, or mouthfeel but also have an interaction threshold describing a concentration or combination range at which the sensory interactions occur (Figure 1).

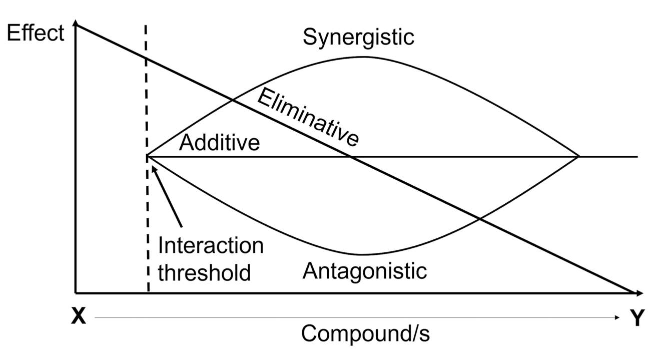

Figure 1. Graphical illustration of sensory interaction effects induced by the combination of two or more compounds causing a modification of sensory characteristics (based on Guichard et al. *(46)* and Langeveld et al. *(172)*).

Meilgaard *(47)* was the first to address sensory interactions between flavour constituents in beer and calculated the degree of interaction based on the assessed flavour intensity of a mixture and the sum of flavour intensities of all volatile compounds present. By comparing the factor to a weak or unflavoured (null) beer, a conclusion regarding the type of interaction could be drawn. The weaker the base flavour in the null beer, the more likely it is that volatiles of interest are not masked, and that sub- and supra-threshold interactions can be identified *(49)*. In general, it should be considered that both threshold concentrations and sensory characteristics of volatile and non-volatile compounds differ between studies if non-identical test matrices have been used since compositional differences can potentially affect the perception of single compounds. Ideally, the concentration of a compound in a matrix and its threshold concentrations of interest (i.e. aroma, flavour, taste or mouthfeel threshold concentrations) in the same test matrix (e.g. water or beer) should be known if aiming to determine the contribution of a compound to the sensory profile.

The scheme in Figure 2 illustrates an example of the complexity of a sensory profile for a test matrix containing a range of compounds (A-L) that contribute in different ways to different sensory characteristics. Each of the compounds can be present at a different concentration range. In addition, each of the compounds has a threshold concentration range at which they are sensorially detected and add one or more sensory characteristics to the matrix. It should be noted that the threshold concentration range does not include subthreshold concentrations that might be important in view of sensory interactions such as additive or synergistic type behaviours. It is likely that one compound is involved in more than a single sensory sensation and for instance contributes to a flavour and a mouthfeel sensation. Whether single sensations or sensory interactions in a matrix with complex volatile mixtures occur and whether these take place at sub- or at supra-threshold level can only be explored by excluding the compound of interest or by

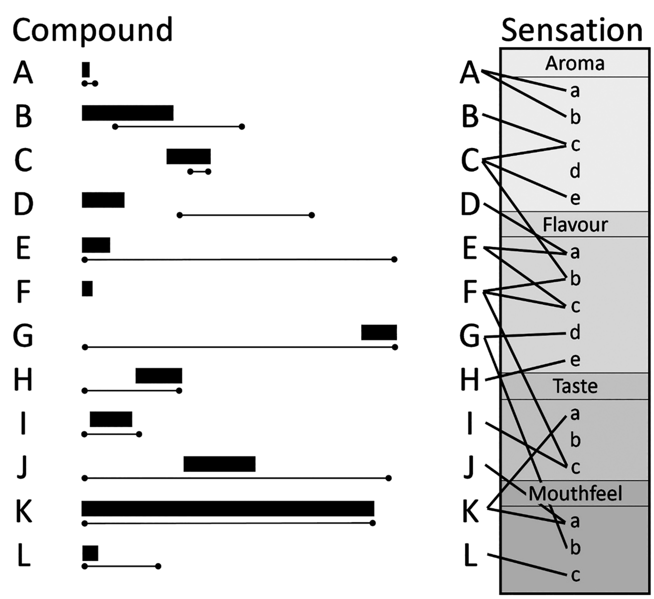

Figure 2. Exemplary illustration of the relationship between sensory sensations and chemical substances (based on Siebert *(60)*). Volatile and non-volatile compounds (A-L) are detected at different concentrations and have different threshold concentrations at which their effect on aroma, flavour, taste, and mouthfeel sensations can be perceived. Some of the compounds are contributing to more than one sensory sensation. ▬ Concentration range detected in beer; •–• threshold concentration range

varying its concentration while keeping the concentrations of the other compounds in the volatile mixture unchanged.

Sensory characterisation of single hop volatiles and compound mixtures in hop essential oil

Combining sensory with instrumental techniques

Different sensory and instrumental techniques can be combined in order to evaluate the relative flavour importance of beer constituents. Peacock et al. *(50)* were one of the first research groups to combine sensory evaluation using triangle tests with instrumental analysis by gas chromatography-mass spectrometry (GC-MS). The determination of threshold concentrations of volatiles has been proven to be challenging since concentrations can vary by a factor of more than 100 across different sensory panels and by a factor up to 100,000 between individual assessors *(51)*. This is mainly due to individuals' genetics and physical conditions determining the sensitivity variation among assessors *(52)*. In addition, experience or exposure plays an important role. Threshold concentrations can change after a certain number of exposures due to training effects *(53)*. Another factor that limits the approach of Peacock et al. *(50)* is the fact that several volatiles present at subthreshold level may still play an important role in the perception of hop aromas and flavours in beer. In addition, several volatiles are likely to remain undetected if hop oil is exclusively analysed using basic chromatographic techniques. This is particularly the case for low level sulphur compounds *(6)*.

A second approach to investigate the relationship between the chemical composition of hop oil and its sensory characteristics is to couple GC-MS analysis with olfactometry (GC-O) based techniques (e.g. aroma extract dilution analysis (AEDA), combined hedonic aroma response measurement (CHARM), OSME (focusing only on one concentration of an extract; named after the Greek word for odour, οσμη)). In this way, hop volatiles can be separated, located, identified, quantified and sensorially characterised in isolation *(28)*. GC-O analysis is used to identify the aroma-active compounds from the bulk of non-active compounds as these are suggested to remain undetected by the human olfactory system *(54)*. AEDA is one of the most frequently applied dilution methods used to determine the highest sample dilution factor at which an odour of a volatile compound is still detectable. A limitation of this method is that it can lead researchers to focus only on the most odour-active volatiles in hops or beer *(23, 54)* and thereby ignore the potential for sensory interactions involving compounds present at lower flavour potencies. It is now well established that these could significantly contribute to sensory characteristics, for instance due to additive- or synergistic-type behaviours. In addition, sample preparation techniques (distillation, concentration) for AEDA experiments have been found to cause volatile losses and consequently the underestimation of flavour contributions *(55, 56)*. It is therefore recommended to use methods that are able to analyse the sensory potential of complex mixtures containing compounds that are contributing to the sensory volatile profile as such or as part of a compound group due to sensory interactions.

Another successful example of combining sensory with instrumental techniques is a study of Sanchez et al. *(57)* who combined sensory descriptive analysis with GC-O OSME. Their study may be the first good example of adequate sensory work in hop flavour research including the correlation of sensory and compositional data. Moreover, the authors established a comprehensive attribute lexicon comprising of sensory attributes, their descriptions, and details of reference materials. GC-O OSME is a dynamic GC-O technique for which assessors are asked to continuously record the intensity and name the description of aroma sensations that are perceived at the sniffing port *(58)*. In GC-O studies, assessors only receive aroma sensations of a single volatile compound at a time (subject to chromatographic separation), thus sensory interactions are neglected *(14, 59, 60)*. Therefore, Sanchez et al. *(57)* trained sensory assessors who evaluated beer samples and subsequently a mixture of standards based on the hop volatile concentrations in the beers that were previously quantified using GC-MS. In this way, the authors could conclude on the volatile compounds present at varying concentrations that contributed to the sensory properties of the different test beers. This study demonstrates the importance of combining GC-O techniques with sensory descriptive analysis when examining the contribution of single volatiles in hop volatile mixtures to beer flavour.

Whenever interpreting GC-O/MS data, it should be taken into account that compounds can co-elute, particularly if the number of compounds present exceeds the resolving power of the chromatographic method. This is particularly difficult to identify when many trace odourants are present *(6)*. Co-elution can lead to misinterpretation regarding volatile compounds and associated sensory sensations *(60)*. GC-MS is a frequently used method for the analysis of hop essential oils. At this time, it may be impossible to separate all hop oil components solely by one- or two-dimensional GC-analysis. This applies particularly to terpenes since their empirical chemical formulae are often identical and mass isomers may follow very similar fragmentation patterns *(61)*. Advanced chromatographic techniques are therefore essential to obtain the best possible outcome. Such approaches include GC-MS in single ion monitoring (SIM), multidimensional and high resolution GC (MDGC, HRGC) combined with time-of-flight MS (TOFMS), and the use of automated selective devices for enrichment of volatiles such as solid phase micro-extraction (SPME). In particular, headspace (HS) traps have been found to be a powerful tool for the gentle enrichment of volatiles from headspace systems prior to their quantification.

Misidentification can also occur if compounds have very similar mass spectra and if literature and libraries lack retention indices and reference mass spectra for compounds of interest. This has often been observed in hop oil analysis *(62, 63)*. In order to avoid misidentification, Van Opstaele et al. *(63)* suggested authentic reference compounds by chemical transformation to be used for the verification of analytical data and to include structure elucidation of compounds of interest by state-of-the-art spectroscopic techniques. Comprehensive reviews on the chemical analysis of hop essential oil have been published by Rettberg et al. *(2)*, Eyres et al. *(6)*, Plutowska et al. *(64)*, and Andrés-Iglesias et al. *(65)*.

The quantification of hop derived volatiles is important for the understanding of hop aroma and flavour, but a high compound concentration does not necessarily mean that it will be one of the main contributors to hoppy aromas and flavours in beer. Therefore, sensory panels are required to evaluate compound mixtures rather than single hop oil compounds and training should be designed to maximise their ability to do so.

Omission and reconstitution experiments for sensory analysis

Two decades ago, Siebert *(60)* suggested that the effect of flavour-active hop compounds in beer can only be fully understood if fractionating a hoppy beer, i.e. extracting and analysing the volatile fractions that have been suggested to be responsible for the hoppy flavour, and then adding step-wise these fractions back to the beer for sensory descriptive analysis. Langos et al. *(66)* and Intelmann et al. *(67)* conducted so-called 'Sensomics' studies that followed the principle of this approach. In the first step, the volatile fraction is extracted and separated from the non-volatile fraction followed by localisation, identification, and quantification of the most aroma-, flavour-, or taste-active compounds. These are recombined at the concentrations present in the original product and evaluated using sensory descriptive analysis as well as methods considering time-dependent perception. In this way, it is possible to identify and quantify those compounds that are responsible for the overall sensory properties in the beer while determining those compounds that are playing a minor role, which may not change the overall beer flavour if, for instance, recipes or processing conditions are modified *(66, 67)*.

Goiris et al. *(68)* fractionated hop oil to obtain fractions of decreasing numbers of compounds and to successively lower the complexity for subsequent sensory evaluation of these fractions in a beer base. Also, fractions derived from the extraction of different hop oils could be compared since these are usually expected to differ in view of volatile composition and concentration *(68)*. However, when fractionating hop oil, it should be considered that some extraction techniques, such as steam distillation, can induce thermal or hydrolytic reactions in hop oil and thus change the oil composition. In particular, thermolabile compounds are easily decomposed, and therefore extraction techniques at low temperatures, such as solvent based supercritical fluid chromatography, are preferred *(69, 70)*. By using a solvent or solvent combination (liquid/supercritical CO_2, ethanol), and controlling temperature, pressure and flow rates for sequential extraction and fractionation, it is possible to separate a wide range of hop oil compounds for subsequent instrumental and sensory analysis *(71)*. However, to date, this type of approach has rarely been applied.

Temporal measurement of sensory perception

Sensory descriptive analysis has been proven to be a valuable tool to investigate the sensory profiles of hop oil compounds in different matrices or to identify aroma-related interactions if combined with instrumental measurements. However, this is a static descriptive method and can only provide a snapshot of sensory profiles. To date, temporal physico-chemical changes that the beer matrix undergoes during consumption are largely neglected. Time-intensity (TI) or temporal dominance of sensations (TDS) analysis are used to monitor the intensity of a single descriptor over time or to assess dominant attributes perceived during consumption *(48)*. Another method that can be used to assess the temporal perception of hop volatiles in beer is the Temporal Check-All-That-Apply (TCATA) method. For this method, the assessors are asked to continuously check the terms that describe the sensory sensations when they are perceived and uncheck them when they are no longer apparent, at each moment of the evaluation for a defined period. It has to be taken into account that the data does not present the attributes that dominate the sensory profile but only when they are apparent and then fade *(72)*. However, according to Ares et al. *(73)*, TCATA tends to be more discriminating across samples compared to TDS since more attributes are usually selected in the TCATA approach. This appears to be relevant for the sensory evaluation of hop oil extracts since these are complex flavour mixtures.

Sensory perception of hop derived volatiles and their combinations

Native hop oil consists of several chemical classes in different proportions and with different compositions depending on the hop variety. The three main classes in hop oil are hydrocarbons, oxygenated compounds and sulphur-containing compounds, which can be further sub-classified as illustrated in Figure 3. The most abundant compounds in the hydrocarbon fraction, which can account for up to 80% of hop oil, are the monoterpene myrcene and the sesquiterpene humulene. These can account for up to 30-40% of their individual subclass. On average, 30-65% of the hop oil consists of oxygenated compounds comprising a complex mixture of oxygenated sesquiterpenoids, alcohols, aldehydes, acids, ketones, epoxides, and esters *(62, 63, 68)*. Sulphur-containing compounds are only present at trace or undetectable levels but are amongst the most flavour active naturally occurring substances. As previously mentioned, not all of the volatile compounds in hop oil can be found in the final beer *(25)*. The following sections summarise important findings that contribute to the understanding of different sensory sensations and interactions induced by hop derived compounds.

Table S1 (see supplementary information) provides an overview of hop oil and hop derived compounds that were investigated in publications using both sensory and quantitative instrumental analysis. Hop volatiles were quantified in beer and sensorially evaluated by sensory assessors (in the same study). Individual compounds were attributed to, or at least associated with, specific sensory sensations.

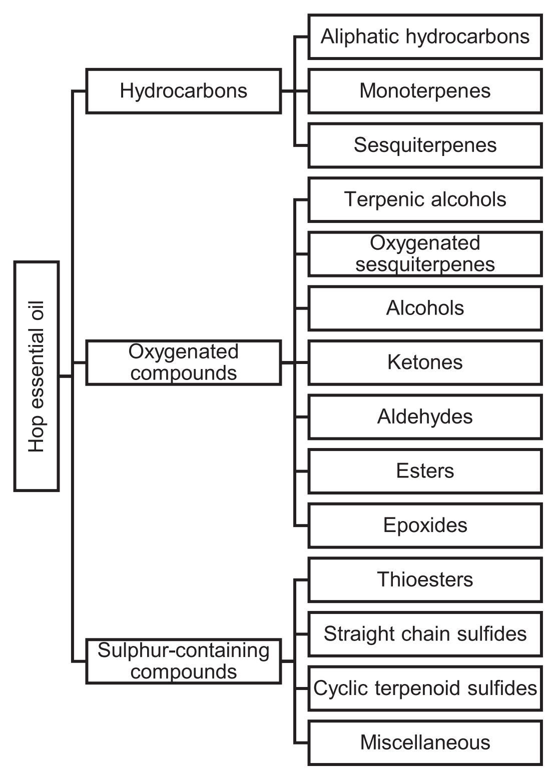

Figure 3. Main and sub-chemical classes in hop essential oil (based on Almaguer et al. *(16)*).

Terpene hydrocarbons

Monoterpene and sesquiterpene hydrocarbons account for the major portion of hop oil. However, when using traditional hopping techniques and in contrast to the oxygenated compounds, they are transferred to beer at trace levels due to their nonpolar character and are therefore often suggested to only contribute to a minor extent to the hop aroma and flavour sensations in the final beer. Instead, they play an important role as precursor compounds that are transformed into oxidation products, thereby adding to 'noble hop' or 'kettle hop' aroma and flavour of beer *(74)*. For instance, it has been suggested that boiling β-myrcene in water in the presence of oxygen, might result in the formation of perillene, a compound that induces citrusy/lemony aroma notes *(15, 75, 76)*, as well as to linalool and geraniol, two of the most impactful odourants derived from hop essential oil *(77)*.

When dry hopping beer, some compounds of the hydrocarbon fraction, including myrcene, humulene and caryophyllene, have been observed to survive the brewing process at reduced levels. Myrcene, the dominant monoterpene in the hydrocarbon fraction accounts for up to 75% of total hop oil *(15, 78)*. However, β-myrcene was found to be adsorbed to the non-polar surface of yeast cells or to be transported to the surface of the fermenting beer by carbon dioxide bubbles and stripped with the fermentation gases. Another cause for significant β-myrcene losses during fermentation are higher temperatures resulting in increased release of the compound *(79)*. However, since the threshold concentrations of hydrocarbons are usually low, they might still contribute to the aroma and flavour of beer *(75, 80)*. This has recently been confirmed by Neiens et al. *(81)* who conducted a spiking experiment to investigate the contribution of several Huell Melon hop derived volatiles to the overall aroma intensity of top and bottom fermented beers. Trained panelists completed several Alternative Forced Choice (3-AFC) tests and compared nonspiked control beer with a beer spiked with hop volatiles at concentrations present in the original test beers. Myrcene was found to significantly contribute to the overall aroma intensity at concentrations between 6.65 and 15.0 μg/L in all test beers apart from the top fermented dry hopped beer which was present at 8.20 μg/L *(81)*. It is interesting that no significant effect was detected at this intermediate concentration and it might be that other components in the matrix masked the aroma. However, matrix effects were not discussed in this study. Neiens and Steinhaus *(81)* determined the odour threshold of myrcene in an aqueous solution to be 1.2 μg/kg and suggested that myrcene was present above its threshold level in all test beers. Previous studies investigated the sensory characteristics of myrcene in beer and observed spicy and resinous flavour notes at 200 μg/L *(82)* together with metallic and geranium-like aromas at around 860 μg/L *(83)*. Moreover, it was stated that the threshold concentrations of myrcene can deviate by up to 1 mg/L depending on the matrix in which it is tested, suggesting that the perception of this compound is concentration- and matrix-dependent.

The importance of hydrocarbons for the overall aroma profile of beer have previously been highlighted by a study of Guadagni et al. *(80)* who determined the relative contribution of individual hop oil compounds and fractions extracted from a Brewers Gold hop oil to the overall aroma sensation in beer. The relative contribution was calculated by dividing the number of odour units of the fraction or compound by the total number of odour units in the whole oil. The odour units were derived from the threshold concentrations of the compounds and fractions in water. The hydrocarbon fraction contained high concentrations of myrcene, humulene and caryophyllene and further terpenes at trace levels. While the hydrocarbon fraction accounted for 86%, the myrcene fraction accounted for 58% of the total odour. This observation was explained by the lower threshold concentrations of the two hop oil fractions compared to those of the other fractions and demonstrates the sensory potential of hydrocarbons at low concentrations.

Myrcene has often been suggested to be involved in sensory interactions with other compounds. Kishimoto et al. *(84)* suggested the resinous character in beer hopped with Saaz hops to be mainly caused by β-myrcene, although its concentration was far below sensory threshold level. Since there was no further key compounds detected that could have added resinous aroma, it was suggested that further compounds below their detection level might have contributed to this aroma sensation. This suggests that an additive-, synergistic- or configural processing-type behaviour has caused the formation and perception of the resinous aroma character.

Further interesting sensory effects of β-myrcene in beer have been observed by Schnaitter et al. *(83)* who found the compound to impart a 'rawhop-like/green-grassy' aroma. In the same study, the compound was also suggested to induce fruity aromas in a different beer sample suggesting β-myrcene might also have interacted with other components in the beer, which in turn could have influenced its aroma character. Moreover, it was observed that high concentrations of β-myrcene might result in negative i.e. antagonistic or masking effects on perceived fruity aromas and these effects were expected to be matrix-dependent *(83)*. The fact that β-myrcene has been observed to impart diverse aroma notes including lime *(85)*, peppery, terpene, balsam, plastic *(86)*, metallic, geranium-like *(23)*, and spicy *(82, 86)*, supports this suggestion. Sensory descriptive analysis in a controlled beer matrix and an extended attribute list could be used to investigate concentration and matrix dependent effects on the sensory profile of β-myrcene in beer. In order to simplify the localisation of other volatiles or components that could potentially be involved, the sensory attributes used should be specific (e.g. 'geranium', 'lime'), rather than generic (flowery, fruity, etc.).

Apart from β-myrcene, other hydrocarbons are mostly of a monocyclic (e.g. limonene, β-cymene, α- and β-phellandrene) or bicyclic nature (e.g. α- and β-pinene) *(16)* and have been found to impart citrus/fruity and woody aroma notes *(82, 87)*. Sharp et al. *(87)* identified α- and β-pinene and limonene in beers hopped with Citra and Simcoe varieties using stir-bar sorptive extraction (SBSE) and GC-MS. A trained sensory panel generated a lexicon of 18 terms for the description of hop aroma notes in different beer samples. Correlations between sensory scores and GC-MS data showed that these compounds added guava-, fruit cocktail-, and onion/garlic-like flavour notes to the beer *(87)*. Unfortunately, any possible effects of the hop volatiles on taste and mouthfeel or sensory interactions contributing to the flavour sensations were not discussed in this study.

It appears that compounds of the hydrocarbon fractions impart diverse aroma and flavour sensations in beer ranging from fruity-type to woody- and vegetative-type characteristics. Sensory characterisation has mainly focused on myrcene, which has been suggested to interact with other hop derived compounds or components in beer, which determines its sensory perception. Limited research has been conducted to investigate sensory interaction or matrix-dependent effects between other hydrocarbons in beer.

Sesquiterpenoids

Sesquiterpene hydrocarbons and their derived oxygenated sesquiterpenoids have been found to be responsible for the herbal, spicy and woody kettle hop aroma notes in beer *(68, 88, 89)*. Several compounds in the sesquiterpene fraction are transformed during the brewing process and only low concentrations in the range of 10 to 100 μg/L have been detected in the final beer *(63, 88)*. The majority of aroma and flavour characteristics have been attributed to sesquiterpene oxidation and hydrolysis products, such as caryophyllene and humulene mono- and diepoxides and their derivates, which are significantly more soluble than their precursor molecules. The amount of sesquiterpenes in the beer present in their original form depends highly on the hopping technique *(68)*.

However, Goiris et al. *(68)* and Praet et al. *(88)* found that the spicy and herbal hop aroma characters in beer are induced by several compounds in the sesquiterpenoid fraction such as caryophyllene oxide, humulene epoxides (I, II), humulol, and humulenol II that are present in the raw hop essential oil. The sesquiterpenoid fraction was added post-fermentation showing that these compounds have not had to be transformed during wort boiling to achieve the spicy and herbal characteristics in beer. In line with their research, Van Opstaele et al. *(24)* determined humulene epoxides (I,II), humulenol II and caryophyllene oxide to be key compounds in spicy essences prepared from Tettnang Tettnanger, Perle and Hersbrucker Spaet hops and to induce the spicy flavour sensations in a pilot-scale lager. It was suggested that these compounds might sensorially interact and that their synergistic-type behaviour causes the spicy sensation in the beers *(68, 90–92)*. It appeared to be difficult to assign specific compounds of the oxygenated sesquiterpene fraction to the 'spiciness' in the beer samples.

In contrast, Kishimoto et al. *(84)* could not confirm the relationship between the 'spicy' character and a mixture of sesquiterpenoids including humulene epoxides and humulenol II in a beer produced with Saaz, Tettnang, and Hersbrucker hops. No relationship was found between frequently selected 'spicy' attributes and the mixture of identified higher threshold substances. This indicated that the mixture of sesquiterpenoids was not sufficient to induce 'spicy' aroma characters as previously suggested by Goiris et al. *(68)* and Praet et al. *(88)* due to additive or synergistic interaction effects between these compounds. Van Opstaele et al. *(63)* also observed that these compounds as well as other humulene and caryophyllene oxidation products (humuladienone, 14-hydroxy-β-caryophyllene, caryophylla-3,8-(13)-dien-5-β-ol), could not be orthonasally detected at a GC-O sniffing port, although present at concentrations above aroma threshold in all tested hop varieties. The findings of both studies confirm what Eyres et al. *(62)* had already hypothesised, that sesquiterpenoids are predominantly contributing to flavour, mouthfeel and trigeminal sensations rather than to aroma sensations, potentially due to matrix-dependent or cross-modal interaction effects.

In addition, it seems that the term 'spicy' has been used to describe very different sensory characteristics including olfactory, gustatory and trigeminal sensations or as a term covering multimodal interactions between the senses *(93, 94)*. For instance, the oxygenated sesquiterpenoid fraction was found to affect mouthfeel and fullness perception of beers at low concentrations of 20 μg/L. At higher concentrations (50 and 100 μg/L) the mouthfeel and fullness sensation occurred in synchrony with a 'spicy' flavour *(68)*. However, the mechanism behind this multisensory perception was not further investigated. In another study, the fraction extracted from Hersbrucker Spaet, Saaz and East Kent Golding hops has been found to not only increase the mouthfeel and fullness but also the bitterness intensity in a pilot-scale lager. The mouthfeel was further described as a 'spicy' sensation referring to a coating effect on the tongue and in the throat suggesting that the oxygenated sesquiterpenoid fraction added a sensation similar to astringency to the beer *(24, 95)*. These findings highlight the importance of including objective descriptors for sensory terms.

Unfortunately, very few studies have been conducted to investigate the activation of human receptors by hop oil compounds in beer in order to explain the trigeminal sensations that are induced by hop oil fractions. To date, only the effect of eudesmol, a sesquiterpenoid alcohol, has been investigated *(96)*. The compound was found to activate the human transient receptor potential ankyrin 1 channel (hTRPA1). This receptor is a calciumpermeable non-selective cation channel that is activated by noxious or irritating compounds *(97)*. Eudesmol activated the receptor although its concentration (1 μm) was below the actual effective concentration required for channel activation. Therefore, the authors suggested that there might have been a synergistic effect between the compound and other chemicals in the beer that caused the channel activation *(96)* and therefore, this mechanism should be considered for other compounds present below threshold levels. Moreover, Ohara et al. *(96)* observed eudesmol to activate hTRPV3, a warmth sensitive Ca^{2+}permeable cation channel. It was suggested that eudesmol might be able to produce warm and pungent sensations on the tongue (96), indicating trigeminal-type sensations *(46)*. The compounds α-, β-, and γ-eudesmol have frequently been detected in hop oil, but their concentrations appear to be varietydependent and, as with other cadinols, they have hardly been detected post wort boiling *(84, 92, 98, 99)*.

Only few hop derived sesquiterpenoids could be assigned to aroma notes in beer. The most potent odourant appeared to be 14-hydroxy-β-caryophyllene which was reported to impart a strong woody/cedar wood odour. However, it was suggested that there might be more compounds that have yet to be identified such as minor compounds partly responsible for cedar wood aroma characters due to additive or synergistic interaction effects *(62)*. Praet et al. *(92)* identified caryophylla-3, 8-(13)-diene-5β-ol, caryophylla-4*(12)*, 8(13)-diene-5-α/β-ol, and 14-hydroxy-β-caryophyllene as well as humulene epoxides and humulenol II as potent woody, green, and hoppy (and spicy) odour impact compounds.

Overall, sesquiterpenoids, their oxidation products and further derivatives including a number of epoxides appear to contribute to woody, herbal and green aromas, but to be mainly responsible for mouthfeel and trigeminal sensations in beer. These sensations occur at different concentrations and vary between test matrixes. Further, they have been linked to the frequently reported so-called 'spicy' flavour sensation, which is also used to describe a variety of other sensations and often lacks a clear description. This indicates that it might be difficult to describe the sensation since it might be the result of a complex multimodal interaction effect.

Monoterpene alcohols

The flavour activity of the alcohol fraction in hop oil, consisting of terpene, sesquiterpene, and aliphatic/aromatic alcohols, was discovered in 1983 *(100)*. Monoterpene alcohols such as linalool, geraniol, citronellol, and nerol have been found to contribute to different fruity and floral dimensions of hoppy aroma and flavour in beer as discussed in the following sections.

Depending on the variety, hop oil contains around 1% linalool by weight *(14)* but it has been found that the concentration rapidly decreases during wort boiling *(29, 84)* and high concentrations of linalool are only achieved by late hop additions *(101)*. Since linalool is transferred at high concentrations (present at up to 8 times of its sensory threshold in beer), it is considered to be one of the major aroma-active compounds in dry and late hopped beers *(5, 102, 103)*. The threshold concentration of (*R*)-linalool is 2.2 μg/L while the (*S*)-enantiomer is detected at 180 μg/L (in beer) *(104)*. Up to 92-94% of linalool in beer is present in its (*R*)-isomeric form *(54, 105)* and so it has been concluded that only the (*R*)-linalool is important for the overall hop aroma in beer. Furthermore, linalool appears to be one of the volatiles that are omnipresent across the majority of hop varieties, and its amount in hop oil does not vary as much as is the case for other terpene alcohols such as geraniol. Therefore, it is considered as a marker compound responsible for aroma and flavour characteristics in the majority of hops *(29, 54, 95)*.

Particularly during the last decade, several findings have been reported in studies that systematically combined sensory and instrumental measures, which provide evidence for numerous additive or synergistic interaction effects between compounds of the monoterpene alcohol fraction. Sanchez et al. *(57)* used GC-O (OSME) analysis and a trained panel to investigate the sensory profile of beers brewed with Hallertauer Mittelfrueh, USDA 21455, and USDA 21459, observing that linalool and nerol contribute to the overall aroma of beers. However, nerol was also suggested to additively interact with geraniol thereby imparting increased flowery aromas to beer *(101)*. Linalool, geraniol, and nerol all are known to add fresh, fruity, citrus-, and rose-like aroma notes to beer *(28, 57, 62, 86)* suggesting that compounds of the same chemical class with similar aroma characteristics are likely to show additive- or synergistic interaction-type behaviour, and less likely to result in new flavour sensations due to configural processing.

Likewise, the existence of linalool and geraniol in combination with β-citronellol has been found to cause sensory interaction effects in a simple model system. Takoi et al. *(32)* found that a trained sensory panel could distinguish between linalool, geraniol and β-citronellol combinations and their individual application in a carbonated 5% ethanol/water solution. Linalool was suggested to be the key contributor to floral ('lavender') and citrus characters. Whereas the aroma sensations attributed to geraniol ('floral', 'rose-like') and β-citronellol ('lemon, lime'), individually, in combination, and at different concentrations in the model solution, were found to be enhanced if coexisting with linalool at the threshold level (3 μg/L) *(32)*, but also at much higher concentrations at 70 and 1000 μg/L *(31)*. However, it should also be taken into account that geraniol is known to have very different thresholds in different matrices *(106–108)*. Meilgaard *(107)* reported a bimodal distribution in sensory threshold concentrations for geraniol, whereby 35% of the panel perceived geraniol at 18 μg/L, while for the other panellists, the concentration had to be increased up to 350 μg/L. Recently, Neiens et al. *(81)* reported an odour threshold concentration of 1.1 μg/kg geraniol in an aqueous solution and a concentration of 31.2 μg/L for a significant contribution to the overall aroma intensity in a beer matrix *(81)*. This research highlights that sensory interaction effects should be investigated at different concentrations in order to determine true threshold ranges.

Other researchers observed β-citronellol to induce 'rose bud', 'floral', and 'citrus' aroma notes *(86, 109)*, which are aromas comparable to the characteristics reported for linalool and geraniol *(28, 57, 62, 86)* and may therefore be describing an interaction effect in combination with these compounds. It would be interesting to test further monoterpene combinations at different concentrations to determine whether these sensory interactions are concentration-dependent. It must be mentioned that Takoi et al. *(32)* used a commercial racemic mixture of β-citronellol and linalool for sensory evaluation and found additive effects, but it is not known whether these effects would hold true if the *R*/*S* ratio was changed for linalool, as the (*R*)-linalool is more flavour active than the (*S*)-enantiomer *(104)*.

Linalool and geraniol have also been found to interact with compounds of other chemical classes or hop oil fractions such as with fermentation by-products 2-phenylethanol and 2- and 3-butylacetate to increase floral ('flowery', 'rose-like') aroma characteristics *(28, 101)*. Further research provides evidence for sensory interaction effects caused by a combination of terpene alcohols and carboxylic acids. Using a triangle test, Sanekata et al. *(111)* found 399 μg/L geranic acid significantly increased the flavour of linalool at 210 μg/L and geraniol at 49 μg/L in a pilsner by adding 'green', 'woody', and 'lemon'-like flavour notes. Geranic acid is usually present at low concentrations (1μg/L *(111)*, 133-178 μg/L *(110)*) in beer and far below its olfactory threshold level (2.2 mg/L in a 0.1% v/v EtOH model carbonated solution *(110)*). Interestingly, the odour of geranic acid could not be detected using a 2-dimensional GC-O technique and thus the flavour threshold concentration was not determined. Furthermore, it should be taken into account that no quantitative data was collected to confirm the synergistic effect that was suggested by the authors.

In another experiment, Sanekata et al. *(110)* added geranic acid (178 μg/L) and neric acid (51 μg/L) to a model beer that contained geraniol (98 μg/L) and linalool (97 μg/L) as the main hop volatiles together with a range of hop-dervived alcohols (α-terpineol, β-citronellol), aldehydes (geraniol, neral), esters (e.g. methyl geranate), and hydrocarbons (e.g. myrcene). The two carboxylic acids could significantly increase the sensory scores for 'flowery' and 'lemon' attributes given by a trained panel in a descriptive analysis study indicating a sensory effect of geranic acid at sub-threshold level on the flavour characteristics of hop derived terpenoids *(110)*. Further research is required to investigate whether geranic acid principally has an effect on monoterpene alcohols or whether further chemical groups in hop essential oil may be involved in sensory interaction effects.

Sensory interactions between oxygenated sesquiterpenoids and monoterpene alcohols were reported by Praet et al. *(89)*. Based on sensory descriptive analysis and the volatiles quantified in lager beers hopped at different time points, it was suggested that, depending on the linalool/oxygenated sesquiterpenoid ratio, the floral-type aroma attributed to linalool might mask the 'spicy/herbal' aroma attributed to oxygenated sesquiterpenoids (such as humulene epoxide III, humulenol II, caryophylla-4*(12)*,8(13)-diene-5-ol, 3Z-caryophylla-3,8(13)-diene-5α-ol, 14-hydroxy-β-caryophyllene, and 3Z-caryophylla-3,8(13)-diene-5β-ol) *(89)*. This is important to know if aiming to target a specific hop aroma profile in beer. However, this should not be generalised, and the masking effect might not only depend on the ratio but also on other aroma-active compounds present, depending on the hop variety.

In conclusion, it can be said that (*R*)-linalool and geraniol are by far the most potent compounds in the monoterpene alcohol fraction contributing to the sensory properties in hops and beer – individually and by eliciting sensory interactions with other volatile compounds. Besides contributing to floral (mainly rose-like) and fruity (mainly citrus-like) aroma characteristics, these compounds are prone to interactive behaviours with other compounds, in particular those of the monoterpene alcohol fraction such as nerol and β-citronellol, but also with other compound groups such as terpene hydrocarbons or carboxylic acids. (*R*)-linalool appears to act as a trigger for additive or synergistic interaction effects resulting in pronounced aroma sensations. The majority of these findings have only been discovered coincidentally and therefore further systematic research is required to confirm and explain these effects at different concentrations and in different beer matrices.

Esters

Meilgaard *(47)* suggested that esters are secondary flavour constituents in beer and present between 0.5 and 2 Flavour Units (FU) which is defined as the concentration of a compound divided by its threshold *(112)*. Thus, only minor changes are caused if they are removed from the beer matrix. A significant amount of hop oil esters are either hydrolysed by yeast or transesterified, while esters of conjugated acids, such as methyl geranate, have been found to resist hydrolysis and are transferred to the final beer in their original form *(113, 114)*. If targeting a specific sensory profile by using an ester hop oil fraction, this has to be taken into account.

It was found that methyl esters in particular, contribute to the hop aroma and flavour in beer due to their low threshold concentrations *(6, 115)*. For instance, ethyl-2-methylbutanoate, ethyl 2-methylpropanoate, ethyl-4-methylpentanoate, methyl 2-methylbutanoate, and derivates of geraniol and linalool, such as linalool oxide, and geranyl acetate, have been found to impart fruity, green, floral, but also waxy aroma notes in beer and model systems *(14, 28, 60, 81, 106, 113)*. The majority of these are transferred to the beer base above their odour threshold concentrations at ng/L level *(81)*. Both the chain length and the degree of branching appear to have an impact on the aroma profile. Short chain esters add aroma notes to beer such as soft fruit (apple, plum), citrusy, pear/apple, and tropical fruit-like aromas *(116, 117)*. In general, short chain esters have higher flavour thresholds compared to long-chain esters (C-7 to C-10) resulting in different odour activities *(118)*.

Odour activity values (OAV) are frequently used to determine the odour activity or potency of a compound to address the influence of a matrix on the volatility of a given odourant *(8)*. OAVs are equivalent to FUs and express the ratio of the concentration to the odour threshold. At OAVs higher than 2-3 times the compounds' threshold, the compound is likely to contribute to the overall aroma of the matrix. Compounds having an OAV close to 1 do not significantly affect the intensity or the aroma profile unless synergistic effects occur between these compounds *(119)*.

The OAV approach was applied by Schieberle *(120)* and Fritsch et al. *(54)* who investigated key aroma compounds in Bavarian pilsner-type and pale lager beer in a GC-O (AEDA) study. High OAVs were reported for ethyl 2-methyl-propanoate, ethyl 4-methylpentanoate, (*S*)-ethyl 2-methylbutaonate, ethyl butanoate, and ethyl hexanoate suggesting these compounds to be key contributors to the fruity characters and to the overall aroma of the beers. It should be taken into account that the aroma profiles of the esters have been assessed individually *(54, 120)*. There is no evidence as to whether these compounds contribute individually to the fruity and the overall aroma of the beers or as part of a compound mixture featuring additive- or synergistic-type behaviours.

In order to address this problem and enable the detection of individual contributors or additive or synergistic behaviours, Charm (combined hedonic aroma response measurement) analysis can be used. This method has been applied in combination with sensory evaluation and GC-MS analysis to investigate the odour-active compounds in strongly hopped beers. Charm values are used to indicate odour activity or the potential relative contribution of a flavour-active compound to the overall flavour of the matrix (air, water, beer) in which the compound is tested *(121)*. Basically, Charm analysis combines the sniffing of the GC effluent with the measurement of retention indices. In this way, the odour intensity of the extracted components is measured in units of Charm over the ranges of the retention indices and gives the ratio of the concentration of the volatile compound to its detection threshold at the sniffing port *(122)*. Kishimoto et al. *(28)* applied this approach and recorded high aroma values of >1 and 'Charm' values of >1000 for ethyl 3-methylbutanoate ('citrus, sweet, apple like'), ($\pm$)-ethyl 2-methylbutanoate ('citrus, apple like'), ethyl 2-methylpropanoate ('citrus, pineapple, sweet') and ethyl 4-methylpentanoate ('citrus, pineapple'). Combined with linalool, 3MH, 4-(4-hydroxyphenyl)-2-butanone, and another unknown compound, these esters have significantly contributed to the citrus characteristics of the beers hopped with Cascade and Saaz hops. Interestingly, the sensory score for citrus aroma was higher for the beer brewed with Cascade hops than expected from the Charm values, therefore it was concluded that the compounds synergistically interacted with each other. Further unknown components below detection level might have been involved in this sensation and a recombination/omission study is suggested to confirm these hypotheses *(28)* rather than investigating the volatile compounds in isolation.

Xu et al. *(123)* investigated the flavour contribution of esters in lager beers using HS-SPME-GC-O/MS. Twenty esters could be detected and identified while only eleven esters could be identified at the sniffing port. Unfortunately, the authors did not investigate to which extent the other esters contributed to the flavour profile. Six esters were further investigated, namely isobutyl acetate, ethyl octanoate, ethyl butyrate, phenyl ethyl acetate, ethyl benzoate, and ethyl 3-phenylpropionate. Based on their concentrations and detection at the sniffing port, these compounds were suggested to be the main contributors to the aroma and flavour of the lager beer. Determination of flavour thresholds of these esters revealed concentrations in a range of 0.14 mg/L and 1.29 mg/L. Interestingly, flavour characteristics of esters with lower threshold concentrations, such as ethyl octanoate and ethyl butyrate, were perceived as being 'unpleasant', 'solvent-like' or 'cheesy' if present at higher concentrations approximately 3-fold of their respective threshold levels *(123)*. In another experiment, Xu et al. *(123)* tested different combinations of ethyl octanoate, isobutyl acetate and phenylethyl acetate in order to identify sensory interactions. Interaction effects were suggested based on the finding that 0.26 mg/L ethyl octanoate, 1.53 mg/L isobutyl acetate, and 0.64 mg/L phenylethyl acetate obtained the highest score from a trained sensory panel compared to a number of other combinations tested in this study *(123)* indicating additive- or synergistic-type behaviour between the compounds.

However, esters are not only interacting with each other, they are also affected by other components in the beer matrix. Recently, Hotchko et al. *(124)* investigated the influence of ethyl esters, terpenes, and aliphatic γ- and δ-lactones on the fruity aroma in beer.

Lactones are formed during fermentation when yeasts transform fatty acids into cyclic esters. Since lactones are mostly present at subthreshold levels, they are expected to increase fruity aromas of other esters rather than having a large impact on the final overall aroma profile of beer in their own right. From the outcome of the sensory descriptive analysis, the authors concluded that lactones (30 μg/L γ-nonalactone, 2 μg/L γ-decalactone, 3 μg/L δ-decalactone) at low or subthreshold levels support the fruity aroma sensations of ethyl 2- and ethyl 3-methylbutanoate (6 μg/L each) as well as of linalool (100 μg/L) and of β-damascenone (3 μg/L), all added at realistic concentrations to a 5.6% ABV unhopped and uncarbonated pale ale. Interestingly, the lactones combined with ester compounds increased the 'stone fruit-/peach-like' aroma. Moreover, the combination of lactones+terpenes and lactones+esters+terpenes increased the intensity of the 'berry' and the overall fruity aroma *(124)*. Further investigation with a wider variety of compounds is required to explore additive and synergstic effects of lactones on 'fruity' hop volatiles since only a limited number of compounds were tested in this study.

Other synergistic effects have been observed on the flavour profile of lager beer if produced with particular yeast strains (TUM 34/70, TUM 193) and dry hopped with Mandarina Bavaria, Hersbrucker, and Hallertauer Magnum hop varieties *(33)*. Trained panellists conducted a descriptive tasting following the DLG (Deutsche Landwirtschafts-Gesellschaft) scheme and Pearson correlation of the sensory data revealed a significant effect between the yeast strains and the citrus flavour intensity that was assigned to the content of geraniol, nerol, and isobutyl isobutyrate in the beers. However, a direct cause-effect relationship could not be determined since the citrus flavour intensity in the two affected test beers was not significantly higher than in the other test beers. It was suggested that other flavour-active compounds could have contributed to the citrus flavour as well and further research is required to investigate the combinatory effect between hop- and yeast derived volatiles on the flavour profile of beer produced with different yeast strains. In order to understand the role of isobutyl isobutyrate in the citrus flavour perception, Haslbeck et al. *(33)* used model solutions (1% EtOH/H_2O) containing geraniol (20 μg/L), linalool (20 μg/L), and β-citronellol (2 μg/L) at concentrations as present in the test beer dry hopped with Mandarina Bavaria, which had the highest citrus intensity among all test beers. Isobutyl isobutyrate was added at different concentrations below, equal to, or above its odour threshold concentration. It should be noted that the concentrations were based on the odour rather than the flavour threshold level. As emphasised previously, these should not be confused because threshold concentrations highly depend on the test matrix that is used. Interestingly, the sensory data indicated that the addition of 10 μg/L isobutyl isobutyrate to the flavoured model solution resulted in a minor increase in the citrus flavour intensity while the addition of 30 μg/L and 80 μg/L appeared to lower the intensity, indicating suppressing or masking effects. As suggested by the authors, this outcome might suggest a concentration-dependent interaction effect between the compounds and requires further research.

It can be concluded that compounds of the ester fraction play an important role in the fruity, floral and green aroma notes in beer. Further, there appears to be sufficient evidence regarding aroma and flavour enhancing effects between certain methyl esters causing pronounced fruity/citrus aroma characters in different beer matrices. In addition, esters appear to interact with compounds of other chemical classes such as lactones and terpenes. Further research should be conducted to investigate sensory interaction between esters and other compound groups and to evaluate differences between esters with different chain lengths. Moreover, limited research has been published on sensory interactions with other beer components.

Ketones

The well known representatives of the ketone fraction in hop oil are β-damascenone, β-ionone, 2-dodecanone, and 2-undecanone. These compounds have been suggested to impart citrus/fruity and floral characters in beer *(28, 62, 75, 85)*. The most abundant methyl ketone appears to be 2-undecanone. The sensory profiles of ketones have been found to highly depend on their concentration and molecular weight. The higher the molecular weight, the more the fruity aroma character is transformed into a floral aroma character. For instance, β-ionone and 2-undecanone are known to impart floral *(28, 62, 82)*, but also fruity (berry-like *(28)*, citrusy *(85)*) aroma notes at different concentrations. Since these compounds have been found in beer above their sensory threshold levels, they are expected to contribute to the hop aroma and flavour in beer *(113)*. Nevertheless, lowmolecularweight ketones should not be neglected since these may still contribute to the overall aroma sensation due to sensory interaction effects *(25)*.

β-ionone belongs to the group of so-called 'rose ketones' and has been identified in beer brewed with Saaz hops to impart a 'floral-violet' aroma *(62)*. Low odour threshold values ranging between 0.008 and 0.170 μg/L in water, 10 μg/L in beer, and high Charm values of $>$1000 in beer have been reported for β-ionone illustrating the aroma potential of this compound *(28, 47)*, which is usually found in beer at concentrations between 1-3 μg/L *(47)*. Nevertheless, it should be taken into account that 50% of the population is expected to have an anosmia for β-ionone *(125)*. This should be considered if recruiting a sensory panel for hop aroma or flavour analysis. Kishimoto et al. *(28)* observed β-ionone to add 'floral', 'violet-like', and 'berry' aroma notes to beer and suggested that other beer components or hop compounds such as 2-phenylethyl 3-methylbutanoate had either synergistic or antagonistic effects on the floral characteristics of β-ionone. 2-phenylethyl 3-meythylbutanoate was found to exhibit a 'floral' and 'minty' aroma *(28)*. However, the findings and the underlying mechanism were not further investigated. A follow-up study would be required to confirm these findings, for instance by using sensory profiling of aroma combinations with and without β-ionone in a controlled base beer. Independent from the method of choice, panellists should be checked for β-ionone anosmia, particularly if performing GC-O analysis, which can be performed with as few as two assessors *(62)*.

Another hop derived ketone that is frequently identified in beer at concentrations between 1-30 μg/L *(126)* and is also only perceived by 50% of the population is β-damascenone *(6, 125)*. Due to its high OAV and low flavour dilution (FD) factors, Fritsch et al. *(54)* and Schieberle *(120)* suggested (*E*)-β-damascenone, a ketone that appears to be mostly present in Saaz hops *(62)*, to be one of the key aroma compounds imparting 'fruity' and 'honey'-like aroma in Bavarian pale lager and pilsner-type beer, respectively. FD factors express the ratio of an odourant concentration in the initial extract to the concentration in the most dilute extract at which the odour is still detectable using GC-O. The greater the dilution factor at which the compound is detected, the greater the probability of contributing to the overall aroma *(122)*.

In addition to the previously mentioned aroma notes, β-damascenone was also perceived as 'cooked apple', 'apple sauce', 'sweet tobacco' *(62)*, 'cooked fruit' *(127)*, 'citrus' *(28)*, 'apple/peach-like' *(128)*, and 'rhubarb, red fruit, and strawberry-like' *(56)*. Since different aroma notes were attributed to β-damascenone in different beer matrices, this suggests that the aroma profile of β-damascenone changes due to other components present in the beers. However, this was not investigated in these studies. Moreover, variations in the aroma quality of β-damascenone at different concentration ranges might explain why diverse sensory descriptors were obtained for this compound.

Aldehydes

The majority of hop derived aldehydes in beer have been detected at low or subthreshold concentrations depending on the hop variety and hopping technique *(129)*. They have also been found to be reduced to their corresponding alcohols by yeast during primary fermentation, dry hopping, or conditioning of the beer. For instance, geranial is reduced to geraniol and β-citronellol *(31, 130)*. Aldehydes such as *(E)*-2-hexenal, *(Z)*-3-hexenal, 3-ethylbutanal, benzaldehyde, 2-phenylacetalaldehyde, geranial, and neral are well known to add different green/grassy and floral aroma notes to beer *(129, 131, 132)*. Citrusy and fruity flavours are characteristic of aldehydes having shorter chain lengths, while with increasing chain length odours become 'unpleasant' and are then described as 'rancid', 'fat-' and 'cardboard-' or metallic-like *(117)*. Marker compounds for these 'unpleasant' odours are for instance (E,E)-2,4-nondienal and trans-4,5-epoxy-(*E*)-2-decenal *(6, 55)*.

Using sensory evaluation and GC analysis, Kishimoto et al. *(28)* found the short chain aldehydes 1-hexanal and (*Z*)-3-hexenal and the long chain aldehyde (*E,Z*)-2,6-nonadienal to be key compounds with regard to 'green' aroma characteristics in beers hopped with Hersbrucker, Saaz, and Cascade hops. The concentrations of the two former compounds were detected at subthreshold levels suggesting that the combination of these compounds was responsible for the perception of the 'green' aroma notes in the three beers indicating additive or synergistic interactions. However, this hypothesis requires confirmation, for instance by conducting a recombination or omission study, such as GC-GOOD (global olfactometry omission detection) *(133)*, or GC-R (recombination) *(134)*. In general, limited research has been conducted to investigate sensory interactions between hop derived aldehydes in beer, therefore, this requires further investigation.

Sulphur-containing compounds

Hop oil contains potentially flavour-active organo-sulphur volatiles (thioesters, sulphides, and other sulphur-containing compounds), such as dimethyl sulphide (DMS), dimethyl disulphide, dimethyl trisulfide, diethyl disulphide and 2-methyl-3-furanethiol, that have been found to contribute to the hoppy aroma in beer *(128)*. The determination of the actual flavour contribution of sulphur-containing compounds has proven to be difficult. These compounds are present in small quantities in hops and in beer at ng/L level or lower. The most considerable progress in quantitative determination of sulphur-containing compounds has been shown after the introduction of sulphur-specific flame photometric detectors for GC. This has enabled the identification of many, but still not all, sulphur-containing compounds at trace levels *(87, 135)*.

Sulphur-containing compounds induce aroma and flavour characteristics in beer and are also observed to change the perception of other hop aroma compounds. For instance, Schnaitter et al. *(83)* used HS-SPME-GC-MS-O to identify hop oil volatiles in beer and found 2,3,5-trithiahexane, *S*-methylthiomethyl 2-methylpropanethioate, and *S*-methylthiomethyl 2-methylbutanethioate to impart respectively 'leek-like', 'onion-like' and 'green' aromas. These three compounds were also suggested to suppress the 'citrus/fruity' aromas induced by citronellol, linalool, and geraniol. Sulphur-containing compounds have low aroma thresholds and, even when present at trace levels, have the potential to overpower other aromas such as fruity notes.

Thiols such as 4-mercapto-4-methylpentan-2-one (4MMP) and 3-mercaptohexan-1-ol (3MH) detected in Nelson Sauvin, Cascade, Saaz, Tomahawk, and Nugget hops have been observed to impart intense 'black-currant', 'citrus/grapefruit', 'tropical fruit' and 'nutmeg'-like aroma notes at trace concentrations due to their extremely low odour threshold levels. However, these compounds are also known to impart 'cat urine' aroma notes *(28, 85, 136)* due to the interplay with components in the beer matrix and the receptor the compounds interact with.

In another study, 4MMP was observed to increase the overall hop aroma intensity and to add 'black current-like' aroma characteristic to beers brewed with US-Simcoe, US-Summit, and US-Apollo. Due to its low threshold value in beer (1500 ng/L), Kishimoto et al. *(136)* concluded that 4MMP might be even more important for the overall hop aroma than β-myrcene, linalool, geraniol, and ethyl 4-methylpentanoate. However, the authors could not detect 4MMP in the same varieties grown in European countries. Copper ions in the copper sulphate that is used for protection against mildew can conjugate with the sulphanyl group in thiols, which might have caused the decrease in 4MMP concentration.

As with 4MMP and 3MH, a number of other volatile hop thiols (such as 3-mercapto-4-methylpentan-2-one, 3-sulfanyl-4-methylpentan-1-ol (3S4MP), and 3-sulfanyl-4-methylpentyl acetate (3S4MPA) have low threshold concentrations between 0.8 and 120 ng/L *(137, 138)*. These compounds have been observed to impart among others 'grapefruit' (3S4MP, 3S4MPA), 'rhubarb' (3S4MP), and 'blackcurrant-like' (4MMP) aroma notes in beers brewed with Nelson Sauvin hops *(23, 85, 110, 136, 139)*.

Interestingly, Takoi et al. *(110)* found 3S4MP and 3S4MPA but also 2-methylbutyl isobutyrate (2MIB) derived from Nelson Sauvin hops to interact synergistically with each other. Using sensory triangle tests, the compounds were added in a carbonated 5% ethanol solution and the addition of 3S4MP ('grapefruit, rhubarb-like') was found to increase the flavour intensity of 3S4MPA ('grapefruit, peach-like') and 2MIB ('apple, apricot-like') at concentrations below their threshold levels. In addition, the flavour intensity of linalool ('lavender') and geraniol ('rose-like') flavours were also increased. Therefore, the researchers suggested that 3S4MP acts as a flavour enhancer for other compound classes, such as isobutyric esters and further terpene alcohols, by increasing 'floral' and decreasing 'green' and 'smoky' flavours *(110, 139)*. These compounds might act collaboratively and thereby inducing the characteristic flavour impression found in beer brewed with Nelson Sauvin hops. In view of the synergistic effects investigated in this study, it has to be noted that only one concentration combination was tested (40 ng/L, 3S4MP with 20 ng/L 3S4MPA and/or 5 μg/L 2MIB) and this effect might be concentration dependent *(110)*. Therefore, further concentrations should be tested.

Besides the aforementioned effects on fruity and floral aroma and flavour characteristics, sulphur-containing compounds are also known to impart 'unpleasant' aromas in beer. For instance, Lermusieau et al. *(128)* found DMS and dimethyltrisulphide to add 'cheesy/glue' and 'onion'-like aromas to beer produced with

Challenger hops *(128)*. DMS is usually not associated with hops, although it is found at trace levels in hop essential oil. DMS is well known as being produced during kilning and wort boiling because of thermal cleavage of *S*-methylmethione from malt. Its presence in beer indicates insufficient removal or evaporation of malt-derived precursors, which are produced during wort boiling. The concentration of DMS increases in aged beer depending on the pH level *(56, 87)*. Interestingly, Hanke et al. *(13)* found linalool to decrease the perceived intensity of the 'cabbage-like' off-flavour of DMS at 15 μg/L by increasing the flavour threshold from 129 μg/L to 176 μg/L when added to a commercial German lager beer. However, it increased the perceived intensity or decreased the flavour threshold (to 102 μg/L) when added at a concentration of 60 μg/L. This is also remarkable because it was suggested that linalool showed the suppressive effect at a concentration near to, but below, its flavour threshold level (27 μg/L in the same beer). Unfortunately, the mechanism behind this effect could not be explained and requires further research. Furthermore, the authors found that the esters, isoamyl acetate (0.75 μg/L) and ethyl acetate (4 and 7 mg/L) decreased the flavour threshold of DMS. The suppressive effect of isoamyl acetate was only recorded at the highest concentration that was tested and the authors suggested a masking effect due to its overpowering 'banana' and 'apple'-like flavour *(13)*. This research not only shows that sensory interactions are concentration-dependent but also that interaction effects depend on different mechanisms.

In conclusion, sulphur-containing compounds have been found to contribute to the overall hop aroma and flavour of beer, even when present in trace amounts, due to their extremely low threshold concentrations. Several compounds of this chemical class are suggested to interact in additive- or synergistic-type behaviour, thereby imparting intense and diverse aroma sensations ranging from undesired (e.g. onion, garlic) to in vogue, fruity-type aroma characteristics (e.g. blackcurrent, tropical fruit, whitewine) in beer. Further research is required to investigate whether these sensory interactions are concentration-dependent and whether hop derived sulphur-containing compounds are involved in cross-modal interactions, for instance by modifying taste or mouthfeel sensations, since this has not been investigated. The concentration of sulphur-containing compounds in hop oil is highly variety-dependent, but this fraction could be combined with other hop oil fractions of different hop varieties to investigate the interactions between different compound classes.

Interactions between hop oil compounds and other beer components

As has been discussed in the previous sections, the perception of hop derived volatiles is affected by the beer matrix in which they are consumed, due to the impact on the diffusion, partitioning, and release of the volatiles. Factors such as pH, temperature, ethanol level, protein, starch, and phenolic compounds can all impact upon the partitioning and release of aroma compounds *(46)*. Sensory interactions between hop volatiles and beer components, including ethanol, carbon dioxide (carbonation), and bittering substances (hop acids, polyphenols), are likewise important for the perception of hoppy aroma and flavour in beer.

Ethanol

In contrast to water, ethanol decreases the polarity of a solution, which influences retention, partitioning, threshold concentration, and perception of volatile compounds *(140)*. Limited research has been conducted to investigate the solvating properties of ethanol on hop oil compounds, particularly on compounds in the more polar oxidised fraction *(108)*. For instance, due to the presence of oxygen in the chemical structure of monoterpene and sesquiterpene alcohols, these compounds are more polar and soluble in water and in alcoholic solutions compared to compounds in the hydrocarbon fraction *(30)*. As for other alcoholic beverages, it is difficult to explain the effect of ethanol on hop derived volatiles and further investigations are required *(108, 141, 142)*. Moreover, according to Peltz et al. *(108)*, the majority of studies have only investigated the aroma activity of hop oil compounds in pale adjunct lagers of 5% ABV or less, and other beer types and ethanol concentrations have been neglected.

In MS-Nose studies, ethanol has been found to promote the delivery of volatiles during the consumption of beverages. Due to its surface activity, surface generation abilities, and physico-chemical modification of aroma partitioning, ethanol can modify the sensory perception of volatiles *(43)*. This was observed in a study of Perpète et al. *(142)* who investigated the influence of ethanol at concentrations between 0 and 5% on the flavour perception of a typical lager beer using GC-FID analysis and sensory triangle tests. A concentration of 0.5% ethanol was sufficient to cause a slight modification in aldehyde retention while $>$5% ethanol resulted in increased aldehyde retention, particularly of 3-methylthiopropionaldehyde. It was concluded that ethanol could have major effects on partitioning of odourants by retaining the volatiles in the beer medium, thereby modifying threshold levels and the perception of aroma sensations as imparted by aldehydes. Consequently, the perception of these compounds might be higher in low-alcohol beers *(142)*. Other researchers suggested that the aroma intensity of odourants is generally lower in alcohol-free beer and that the presence of ethanol, as one of the primary odourants in beer, has a significant effect on its overall aroma and flavour sensations *(120)*.

Peltz et al. *(108)* investigated the effects of 5 and 10% ABV on the orthonasal detection thresholds of 10 hop oil compounds in unhopped pale ale. The compounds represented a range of chemical classes and included (-)-β-caryophyllene, (±)-β-citronellol, β-damascenone, geraniol, geranyl acetate, α-humulene, (±)-β-linalool, β-myrcene, nerol, and 4MMP. In order to achieve 5 and 10% ABV in the production beer, 95% ABV food grade ethanol and Milli-Q water were added while maintaining equivalent residual extract concentrations. Hydrocarbons were suggested to be retained in high ethanol rather than in lowethanol beer, which affected their threshold levels in the different beer matrices. Increasing ethanol concentration from 5% to 10% resulted in a significantly decreased threshold concentration for β-damascenone (~2.5-fold). The opposite was the case for some terpene alcohols. The threshold concentrations of linalool and geraniol increased by 166 μg/L and 122 μg/L, respectively, but the actual impact of the threshold difference on the sensory perception of these compounds in beer was questioned. The authors concluded that, since linalool and geraniol are more hydrophilic than hydrocarbons, they might largely be retained in higher ethanol systems whilst myrcene was suggested to be retained to a lesser degree in the higher ethanol base and to escape into the air phase.

Overall, ethanol at increased concentrations has a low potential to suppress the odour activity of terpene alcohols *(108)*.

In conclusion, the focus of previous studies was to investigate the effect of ethanol on a limited number of single chemical compounds. It would be interesting to study the effect of a broader range of ethanol concentrations (equivalent to no, low, high, ultra-high alcohol beers) on the delivery of compounds to the nasal cavity using the MS nose, and on the perception of hop oil compound mixtures using sensory evaluation.

Carbonation

Carbonation in beverages is perceived as a sparkling, tingling, and sometimes astringent sensation in the oral cavity. It was also found to stimulate salivary production and to affect taste perception *(143)*. Harrison *(144)* observed flavour threshold concentrations of some esters and alcohols to be reduced by approximately half in degassed beer compared to carbonated beer. Therefore, different carbonation levels will bias flavour perception during sensory evaluation. Thus, for sensory descriptive analysis, it is necessary to control the carbonation level *(124)*.

Using MS-Nose analysis, Clark et al. *(43)* observed that the carbonation level (~3.6 volumes) present in a model system increased the release of isoamyl alcohol and ethyl acetate into the breath. The carbonation increased the delivery of the two high partitioning compounds in the first exhalation after the consumption of the model beer by around 86% proposed to be due to an increase in interfacial surface area for release. Based on the finding that only the release of high partitioning compounds was increased, a relationship between the volatile air-water partition coefficient (K_{aw}) of individual compounds and their delivery in the breath has been suggested *(43)*. However, sensory analysis did not find an increase in aroma or flavour perception due to increasing carbonation levels *(43)*.

To date, understanding of the effect of the carbonation level on the perception of hop derived volatiles in beer is limited. It would be interesting to test the effect of different carbonation levels on the release of hop derived volatile mixtures (oil/fractions) in a controlled beer matrix and the resulting effect of potential sensory interactions on taste or mouthfeel sensations or the activation of trigeminal neurons, since this has not been investigated.

Hop acids and polyphenols

Iso-α-acids (isohumulones), the isomerisation products of α-acids (humulones), are formed during wort boiling, and are mainly responsible for the bitter taste of beer *(16)*. Considering the low threshold concentration of iso-α-acids (6 mg/L in water), they are readily perceived. However, the concentration can vary considerably up to 100 mg/L depending on the hop materials or products added in the brewing process. Moreover, the utilisation of iso-α-acids during wort boiling varies due to the polarity of the compounds *(145)*.

The perception of beer bitterness is complex since several hop derived compounds appear to be involved *(1)* including polyphenols, which represent approximately 4-6% of the hop dry weight *(146)*. It was found that the addition of 200 mg/L polyphenols induced a higher bitterness intensity compared to 10 mg/L iso-α-acids alone in the same beer. In addition, polyphenols were found to increase perceived 'fullness' *(147–149)*, lingering bitterness and astringency in beer *(150–152)*, whilst high concentrations caused 'unpleasant', 'harsh' bitterness and 'medicinal' or 'metallic' tastes *(147, 151)*. For instance, Goiris et al. *(153)* found a hop polyphenol extract to increase the perception of the 'fullness' in a pilsner-type beer when combined with a polar floral hop essence or a dry hop essence containing oxygenates. However, this was not the case when the polyphenols were applied together with a spicy hop essence enriched in oxygenated sesquiterpenes. Furthermore, the bitterness intensity was increased when flavonol glycosides were added, but not when prenylated flavonoids were applied. In contrast, astringency levels only increased when the total polyphenols or prenylated flavonoids were added. These findings highlight that the different chemical classes in hop polyphenols have different effects on the sensory profile of beer, as is the case for the different hop oil fractions. To date, limited research has been conducted to investigate the impact of polyphenol fractions on the perception of hop volatiles in beer and vice versa. The majority of studies have focused on the investigation of hop acids and their impact on beer bitterness, but not on other sensory characteristics.

Daoud et al. *(154)* evaluated taste and aroma profiles of beers bittered with liquid CO_2 and ethanol extracts derived from fresh and deteriorated hops. The beers brewed with extracts from undeteriorated or 46% deteriorated pellets showed different sensory profiles in view of hoppiness aroma intensity compared to the control beers, which were brewed with extracts of undeteriorated pellets. A sensory panel perceived the aroma of the beer brewed with extracts of 46% deteriorated pellets containing a significantly lower concentration of iso-α-acids and uncharacterised resins, as less 'hoppy', 'estery', 'fruity', 'floral', and 'sweet' compared to the control beer and a beer brewed with extract of 28% deteriorated pellets. Thus, the composition of the bittering substances and the quantity of iso-α-acids appeared to have significant effects on different sensory characteristics of the beers, which may be due to cross-modal interactions. However, this was not further investigated in this study. A major limitation of this study is that the concentration and the composition of the hop oil possibly varied between the samples to an extent that no reliable conclusion can be drawn in regard to the relationship between the chemical composition and the sensory characteristics.

Despite the limited number of studies, it has been suggested that cross-modal interactions occur between hop acids and hop derived volatiles, which affect the perception of hop aroma and flavour sensations in beer. This might depend on the bitterness level and the composition of bittering substances present in the beer matrix. A factorial design including hop oil compounds at different concentrations and combinations for evaluation with sensory descriptive analysis should be used in order to confirm these hypotheses and to identify the sources of sensory interaction effects that might have caused the observed modifications in aroma, flavour, taste, and mouthfeel characteristics.

Hop derived volatiles and perceived bitterness

In the previous section, it was suggested that bittering hop compounds modify the perception of hop derived volatiles. Further sensory interactions have been observed driven by hop oil compounds affecting bitterness intensity and quality.

Oladokun et al. *(155)* investigated the impact of the hop variety on perceived bitterness qualities in beer. A trained sensory panel evaluated the bitterness profile of different beers individually hopped with East Kent Golding, Zeus, and Hallertauer Hersbrucker T90 hop pellets using Check-all-that-apply (CATA) and rank-rating sensory tests. CATA is a rapid sensory profiling technique that can be used for product characterisation with a trained panel or with consumers, who are asked to check or uncheck all sensory attributes that describe the sensory profile of the samples *(156)*. Hersbrucker hop aroma extract was added post-bottling and was found to cause an increase in CATA frequency of 'harsh' and 'metallic' bitterness in the East Kent Golding beer and an increase of 'citric' and 'progressive/lingering' bitterness in the Hersbrucker and Zeus beers. In a rank rating study, each of the three base beers with added Hersbrucker aroma extract was perceived as being significantly 'harsher' in bitterness than the Hersbrucker bittered base beer, indicating a 'tingly, rasping, and irritating' sensation. A taste-trigeminal sensation effect was suggested to be promoted by hop oil compounds. Interestingly, the frequency of the 'artificial bitterness' character was reduced for all beers compared to the control beer suggesting a masking effect of 'artificial bitterness' by hop aroma sensations. After spiking the beers with Hersbrucker hop aroma extract, an increased bitterness intensity, lingering bitterness and astringency was found in the Hersbrucker beer compared to the East Kent Golding and Zeus beers. The analytical profiles of bittering substances were found to be similar for all beers and only the polyphenols concentration was slightly higher in the Hersbrucker beer (290 vs 216 and 207 mg/L) *(155)*. The contribution of the higher concentration of polyphenols and enriched oxygenated sesquiterpenes compounds derived from the Hersbucker hops might have caused the pronounced bitterness and astringent sensations. Since the volatile composition and the sensory aroma profiles of the beers were not published in this study, it would be interesting to explore these to understand the suggested sensory interaction effects.

Overall, several volatile fractions in hop oil are considered to modify bitterness intensity as well as bitterness qualities. The hop oil fractions that were applied mainly comprised of hydrocarbons, terpene alcohols (linalool), and sesquiterpenoids. The effects on bitterness intensity and quality were mainly attributed to the occurrence of cross-modal interactions induced by the perception of the hop oil fractions. Volatile compounds in these fractions have also been suggested to add trigeminal-type and mouthfeel sensations to beer and to be susceptible to sensory interactions with other beer components.

Linalool

Several researchers found linalool to have an effect on lingering bitterness and bitterness quality. Kaltner et al. *(157)* attributed the modification of the bitterness perception to different concentrations of linalool and terpene hydrocarbons (myrcene, caryophyllene, humulene). Ratings on 'bitterness harmony' increased for the beer with the highest linalool concentration. In contrast, the lowest linalool concentration resulted in the highest rating for 'mild bitterness'. Scores for 'long-lasting taste of bitterness' and 'bitterness harmony' decreased if the linalool concentration increased above 51 μg/L. The addition of hop oil products containing terpene hydrocarbons and a low concentration of linalool to the beer resulted in the highest ratings for 'harmonious but increasing bitter taste' (102 μg/L) and significantly lower ratings for 'mild bitterness' (13 μg/L). It was concluded that the addition of terpene hydrocarbons decreased the mildness of the bitterness and increased the bitter taste at low linalool concentrations indicating concentration- and matrix-dependent effects *(157)*. These results suggest cross-modal interactions, however, in order to fully understand the factors that are determining these findings, it would be important to observe the increase/decrease of other compounds present in the added hop oil products, but this information was not provided.

Like Kaltner et al. *(157)*, Praet et al. *(89)* observed an effect of linalool combined with further hop derived volatiles on bitterness profiles. Praet et al. *(89)* hopped lager beers at different time points in order to investigate *de novo* formation of sesquiterpene oxidation products. The beer containing the highest concentration of oxygenated sesquiterpenoids and linalool obtained the highest scores for 'spicy/herbal', 'floral/fruity', and 'bitterness quality' in a sensory descriptive evaluation confirming the findings of Kaltner et al. *(157)* and suggested linalool to be one of the impactful hop oil compounds to have an effect on bitterness qualities in beer. However, the attribute 'bitterness quality' was not further described. Accordingly, it would be interesting to investigate the different effects of linalool in combination with hop oil fractions on defined bitterness qualities in beer.

Further interesting findings were reported by Bailey et al. *(158)*, who investigated the impact of the harvest date of Hallertauer Mittelfrueh hops on the sensory properties of a dry hopped beer. Hop oil and α-acid concentrations were found to be 30% higher in hops harvested 24 days later than hops harvested at an earlier stage. In order to identify effects on bitterness perception, the dry hopped beers were evaluated using a flavour profiling test and triangle tests. The results suggest that the later the hops were harvested (or the higher the hop oil and α-acids content was reported), the higher the linalool concentration and the scores on 'spicy' aroma notes, 'bitterness intensity' and 'bitterness balance', while the intensity of 'fruity' aroma notes decreased *(158)*. However, further research is required focusing on these correlations and systematically assessing the relationship and sensory interactions between linalool, α-acids, hop aroma sensations, and bitterness intensity and quality to confirm this hypothesis.

Sesquiterpenoids

Further effects on bitterness qualities have been observed when hop extracts comprising of sesquiterpenoids were used for brewing. Goiris et al. *(68)* added hop aroma essences - containing all the main oxygenated sesquiterpenes including humulene epoxides - post-fermentation to a non-aromatised pilot pilsner beer which was bittered with isomerised hop extract. The hop essence (20 μg/L) not only introduced a 'spicy' hop flavour, but also resulted in an enhanced 'mouthfeel', 'fullness', and perception of 'bitterness'. It was suggested that synergistic-type interactions occurred between the bitter extract and hop oil compounds and caused the modulation of bitterness perception. In order to investigate this suggested mechanism, sensory descriptive analysis could be used, which should involve the establishment of a detailed attribute lexicon including bitterness quality, mouthfeel terms and the corresponding reference materials. In this way, hop oil compounds involved in cross-modal interactions could be identified.

Similarly, Opstaele et al. *(95)* found a spicy oxygenated sesquiterpenoid and a polar hop essence to increase 'bitterness' intensity and 'fullness' perception in beer. In contrast, a floral hop essence decreased the bitterness intensity. In a follow-up study, Van Opstaele et al. *(24)* added different hop oil essences to non-aromatised pilot-scale lager and observed the spicy essence to increase 'bitterness' intensity, 'mouthfeel' and 'fullness'. Therefore, it appears that interactions between beer bitterness and hop oil compounds are highly dependent on the composition and polarity of the aroma fractions. However, to date, this has not been further investigated.

Oladokun et al. *(159)* provided evidence for the modification of bitterness intensity and quality induced by volatiles in a Hersbrucker Spaet hop extract rich in oxygenated sesquiterpenes. Different levels of hop extract (0, 245, 490 mg/L) were added to beers bittered with iso-α-acids (13, 25 or 42 IBU). Perceived overall bitterness intensity and the intensities of the bitterness characters 'harsh' ('tingly, painful, irritating, raspy bitterness') and 'rounded' ('pleasant, smooth, lingering bitterness') were evaluated using rank-rating tests. At each bitterness level, addition of the Hersbrucker aroma extract caused an increase in mean bitterness intensity ratings, which was statistically significant at the 13 and 25 IBU levels. Nose clips were used to decouple olfactory from gustatory stimuli and mouthfeel sensations that could be related to the beer bitterness. This removed any statistically significant impacts of hop oil addition on perceived bitterness intensity, clearly indicating that the olfactory stimulus was required for the noted enhancement of bitterness intensity. At the high bitterness level, with the panel wearing nose clips, differences in bitterness intensity were again non-significant; however, the panel on average scored higher bitterness intensity for samples with Hersbrucker aroma addition and could reliably differentiate the samples in-mouth. This suggested the stimulation of trigeminal receptors by the hop volatiles *(159)*. High bitterness levels combined with trigeminal sensations might have caused a taste-trigeminal sensation and the perception of increased bitterness intensity and modified bitterness character. Furthermore, it was suggested that the addition of hop oil compounds modulated different bitterness characters depending on the bitterness level in the beers. A 'round' bitterness was perceived in low bitterness beers and a 'harsh' bitterness in high bitterness beers. It appears that the impact of hop volatiles on the bitterness qualities depends on the IBU level in the beer *(159)*. The increase of bitterness intensity and the occurrence of trigeminal sensations were not attributed to specific compounds, but as observed in previous studies, the oxygenated sesquiterpene fraction contributed to sensory interactions.

Oladokun et al. *(159)* also investigated the temporal profile of perceived beer bitterness at different concentrations of a Hersbrucker hop extract rich in polar oxygenated sesquiterpenes. TI analysis was used to assess the time course of bitterness intensity for a period of 60 seconds. Aroma sensations induced by hop oil compounds perceived through the retronasal pathway were suggested to have an effect on the temporal bitterness profile of the beers. This was already observed at low iso-α-acid concentrations *(159)*. The results suggest that the hop volatiles induced a prolonged bitterness, although specific compounds or fractions were not attributed to this sensation in this study. It would be interesting to conduct this analysis using different hop oil fractions or compounds in order to investigate the effect of aroma compound polarity on the temporal perception of bitterness. This is the only study identified in this review that systematically investigated the effect of a hop aroma extract on temporal perception of bitterness, hence further research is required to understand the mechanism behind the temporal effect.

Recently, Mikyška et al. *(160)* investigated the impact of kettle hopping and kettle + dry hopping on the volatile composition and sensory profile of beers. Aroma and flavour characteristics of the beers and the effect on the bitterness profiles and lingering bitterness was analysed by a trained sensory panel. The lingering bitterness sensation was rated at 10-second intervals for 120 seconds. Interestingly, the rate of bitterness decay was found to be slower for the majority of kettle + dry hopped beers. Based on this finding, it was suggested that higher concentrations of hop oil compounds, bitter acids, oxidative products of α- and β-acids, and polyphenols might have caused this effect, which are expected to be extracted at higher levels when dry hopping beer. In addition, GC-MS analysis revealed that kettle + dry hopped beers contained higher concentrations of hydrocarbons (myrcene, β-pinene), terpene alcohols (linalool, α-terpineol), and slightly increased concentrations of sesquiterpenoids (α-humulene, β-caryophyllene, β-caryophyllene epoxide) independent of the hop variety *(160)*. Therefore, increased concentrations of β-caryophyllene, α-humulene, and α-caryophyllene epoxide were suggested to be responsible for higher scores for the 'harsher' bitterness in the kettle + dry hopped beers. In conclusion, several factors could have caused the effect on bitterness qualities and further investigations are required to identify those components that are involved in the mechanism behind this in beer. Since the mechanism appears to be complex and to involve several components, as a first step, a model beer could be created that contains all components that are expected to be involved, for instance by following a 'Sensomics' type approach. In a second step, an omission experiment could be performed by step-wise excluding components from the model beer, and subsequently evaluating the resulting sensorial impact from this omission.

Further investigations are required to explore the relationship between different chemical classes in hop oil and the occurrence of cross-modal sensations resulting in diverse bitterness characters. Moreover, limited research has been conducted to investigate the impact of the sesquiterpenoid fraction or single sesquiterpenoids on the lingering bitterness sensation or bitterness qualities to identify the key compounds which confer these sensations.

Reconstitution of beer flavour in model beer systems

Reconstitution or recombination studies usually comprise of four steps: 1) analysis of the volatile composition in a matrix using GC-MS, 2) identification and selection of key volatile compounds based on OAVs in water or concentrations in beer, 3) comparison of chemical reference compounds and the original compounds in the matrix using GC-O, and 4) evaluation of the recombinate in a model matrix using sensory analysis. In this way, it is possible to determine key volatiles that are responsible for the overall aroma sensations in a matrix, to identify aroma sensations that are driven by volatiles at low concentrations or subthreshold level, to detect sensory interactions (e.g. between volatiles at sub- and suprathreshold levels), but also to evaluate the impact of other (non-volatile) components in the matrix on the perception of the mixture of volatiles. To date, only a few recombination studies have been conducted that investigated hop volatiles responsible for hop aroma and flavour in beer *(11, 54, 66)*.

Fritsch et al. *(54)* conducted a recombination study to test whether it is possible to mimic the aroma profile of a pilsner-type beer (4% ABV) by applying a mixture of 22 chemical reference compounds in carbonated water. Volatiles were selected as reference compounds if their OAV was greater than 1. All compounds were dissolved at concentrations as found in a pilsner-type beer. Key compounds with the highest OAVs were ethanol, (*E*)-β-damascenone, (*R*)-linalool, acetaldehyde, and ethyl butanoate followed by ethyl 2-methylpropanoate and ethyl 4-

methylpentanoate. The reference compounds were checked and compared with the original compounds detected in the pilsner beer regarding similarity of retention indices and odour qualities using GC-MS and GC-O. A sensory panel evaluated the orthonasal perception of the pilsner beer and the model system and found them to be very similar. The authors suggested that the origin of compounds, the alcohol concentration, and bitter substances had no significant effect on the overall aroma quality and aroma intensity of the beer. This conflicts with several studies which considered these parameters that have been discussed previously in this review. Equally surprising is that the sensory training was conducted on attributes describing aroma sensations of single reference compounds but not on aroma combinations. Therefore, this suggests that sensory interactions did not significantly contribute to the aroma of the model system or the actual beer. However, the descriptive profile test was conducted by using six general aroma terms on a scale from 0 (no similarity) to 3 (very good similarity) *(54)*. Similarity testing or sensory quantitative descriptive analysis (QDA) using a more specific list of terms might result in a different outcome and the disclosure of sensory interaction effects.

Langos et al. *(66)* adopted a Sensomics approach by preparing an aroma recombinate using predetermined key volatiles in Bavarian wheat beer. As in the study of Fritsch et al. *(54)*, compounds with OAVs lower than 1 were suggested not to contribute to the overall aroma of the beer and were excluded. Subsequently, 27 purified chemicals were evaluated at 4% ABV in acidified, carbonated tap water to simulate a wheat beer. Compound concentrations for the recombinate were determined based on their OAVs in isolation. A trained sensory panel evaluated the samples using a pre-defined attribute list to describe different aroma sensations. The recombinate was found to successfully mimic the aroma sensations of a wheat beer and (*E*)-β-damascenone, 3-methylbutyl acetate, ethyl methylpropanoate, and ethyl butanoate were determined to be the most potent contributors for the aroma characteristics. The non-volatile fraction was suggested to have little influence on the overall aroma and on aroma release *(66)*. As reported in the preceding sections, bittering substances are likely to affect aroma and flavour sensations due to cross-modal interactions with hop volatiles. Since the non-volatile fraction included no bittering substances, the addition of different bitter acids and/or polyphenols may have resulted in a different outcome.

In contrast to previous studies, Tokita et al. *(11)* used compound concentrations as the selection criteria for key volatiles. Aiming to reconstitute the characteristic odour sensations of a fruity flavoured pilsner-type beer, a list of 30 key volatiles was determined by comparing the chemical profiles of a pilsner-type control beer and a fruity flavoured pilsner-type beer. The key volatile mixture mainly consisted of esters and alcohols including ethyl acetate, 3-methylbutanol, phenethyl alcohol, 2-methylbutanol, 3-methylbutyl acetate, and 2-methylpropanpol. The reference compounds were dissolved in the base beer at concentrations equal to the odourants in the original fruity flavoured beer. The outcome of the sensory study showed that the application of the recombinate in the base beer could reconstitute the majority of odour characteristics ('caramel, roast', 'cereal', 'chemical', 'green', 'floral'), but not the 'fruity', and 'sweet' odour notes. The findings of this study demonstrate that an authentic matrix is required if aiming to match specific odour characteristics in beer. As in previous studies, the reason why the fruity and sweet odour profiles could not be matched may have been that mainly general descriptors were used. Even if it is the aim to work with general attributes, the panel should be trained on detailed attribute descriptions and these should be provided to clarify differences between the attributes, for the investigator, the panellists and the reader.

Overall, reconstitution studies are a promising technique to identify key volatile compounds. Nevertheless, the fact that up to 30 reference compounds were required for aroma and flavour recombinates reiterates the complexity of aroma and flavour characteristics in beer. Only compounds having an OAV higher than one were included, further compounds are expected to contribute to the overall aroma, due to synergistic effects occurring between the volatiles. The aroma recombinates reviewed in the studies were applied in water and showed no difference compared to the reference beers indicating that the non-volatile fraction in beer might be more important for cross-modal interactions than for modification of volatile release, but the latter effect should not be neglected. It appears to be questionable whether flavour recombinates are equally successful as aroma recombinates since several different receptor-types are involved, volatiles are released through different pathways, and sensory interactions are likely to occur at different levels due to other components present in the beer matrix, as is recognised in this review.

Prediction of the hop flavour intensity in beer

Partial Least Squares (PLS) regression analysis is frequently used to study relationships between sensory and physico-chemical characteristics in foods and beverages. Briefly explained, PLS is used to build regression models between independent and dependent variables by extracting linear combinations of one set of variables to predict the variation in another set of variables expressed as mathematical functions *(161)*. This approach enables for instance the modelling of flavour profiles, i.e. prediction of flavour intensities or scores, based on the quantified volatile composition in the sample matrix. To date, only one study has been published that investigated the predictability of the 'hoppy' flavour while focusing on the 'fruity-citrus' intensity of beers that were dryhopped with Mandarina Bavaria *(162)*. Machado Jr et al. *(162)* proposed an equation for the estimation of the sensory perception (i.e. the intensity score) of 'total hoppy', 'citrus', 'green fruit', and 'sweet fruit' flavours in the two different beer samples. The equation was based on data obtained from a trained sensory panel that assessed the beer samples on a scale from 0 to 5 following a QDA approach and obtained from HS-SPME-GC-MS analysis conducted to quantify 24 selected volatiles during a 15 days dryhopping period. The volatile compounds were selected based on previous research where these volatiles were most frequently associated with 'hoppy' flavour, but also to cover the main chemical classes described for the Mandarina Bavaria hop. For instance, the intensity of the 'total hoppy' flavour could be estimated by an equation including the compounds myrcene, 2-methylbutyl-2-methylpropanoate (2MB2MP), linalool, and α-humulene, and perfectly demonstrates the complexity of the volatile group behind a single flavour sensation associated with the 'fruity-citrus' flavour dimension associated with the overall 'hoppy' flavour in beer. The researchers indicate that the majority of volatiles were present at concentrations above their threshold levels. However, this was not the case for α-humulene although it was suggested as a key contributor compound in the model. Surprisingly, other compounds present at supra-threshold concentration such as geraniol were not important to the model. As stated by the researchers, model data should not be used to identify direct cause and effect relationships but implies associations between volatile groups and sensory characteristics *(162)*. Moreover, as discussed previously, differences in physico-chemical parameters between matrices and the occurrence of sensory interactions should be taken into account when evaluating the explanatory power of regression models. These factors can cause pronounced nonlinearity in the data and weaken the model *(161)*. It has to be noted that, due to the dryhopping design, replicates of the beer samples have not been assessed in this study and it is not clear whether or not the panel performance data was taken into account when building the regression models *(162)*. Further research is required to explore different types of regression techniques as a tool to predict single or multiple sensory dimensions associated with multi-sensory perception of 'hoppy' flavour in beer.

'Sensory best practice' for the sensory analysis of hop essential oil

In contrast to the instrumental analysis methodologies reported in the reviewed publications, which are often highly detailed, papers in the brewing literature are surprisingly limited with regard to sensory evaluation protocols and methodologies. The importance of adequate panel training and panel management has frequently been highlighted (see e.g. Bamforth et al. *(163)*, Rogers *(164)*), but is often overlooked in the field of hop or brewing research, with only a few studies providing data regarding panel training and performance.

Internal and external panellists should be sufficiently trained prior to the sensory evaluation. However, the level of training can be deemed as void if the panellists have not been tested regarding their sensory abilities, and potential anosmia for key compounds. Even if anosmics cannot be identified, it should still be taken into account that the majority of individuals show high sensitivity for certain compounds and low sensitivity for others *(126)* as discussed above (e.g. β-ionone). In order to check the suitability of potential assessors, they should undergo a screening based on their general health, sensory, discriminative, and descriptive abilities *(164)*.

Another part of the experimental design of sensory studies that often lacks information is the attribute list or sensory attribute lexicon used in the research studies. When establishing an attribute lexicon for sensory descriptive analysis, there are clear advantages in including specific attributes and descriptions, detailed description of references, their preparation, and presentation. Detailed information facilitates the interpretation of the study outcome, but also the reproducibility of the study in view of follow-up research. Overall, it has been found that it is easier for the panellists to recognise and remember flavourings and foodstuffs rather than chemical compounds in clear solution *(143)*. Where chemically isolated compounds are used as references, they should be obtained, purchased or produced to the highest possible purity and the purity should be reported *(126)*. If applying volatile combinations (as in hop oil fractions), and assuming the occurrence of compound or sensory interactions that might result in newly formed sensory characteristics (configural processing), it is recommended to develop the attribute list together with the sensory panellists rather than pre-defining the terms and training with chemical references.

In order to ensure that assessors are testing and evaluating all samples in the same manner and that reliable, meaningful data is obtained, concise smelling and tasting protocols should be developed and practiced during the training period. Considering that hop oil compounds are highly volatile, small differences can cause large deviations in the results. Tasting protocols are particularly important for the evaluation of lingering sensations in a defined time span. If tongue movement, mouth closure, periods between taking the sips and swallowing, and the number of sips have not been predetermined, it is likely that the volatiles are released and perceived at different time points and intensities, which will have a significant effect on the sensory data *(72, 159)*. Panel training on the attributes, scale usage, and evaluation protocols should be conducted until sufficient consensus is obtained. Panel performance can be examined during the training period by conducting mock evaluations that follow the protocol of an actual evaluation session.

Panel performance monitoring still plays an important role after completion of the sensory evaluation. By obtaining and providing performance data on the evaluation results, the reliability of the data can be established, the study can be replicated, but also (and most importantly), the outcome of a study can be fully interpreted and understood. In some publications, previous experience in a related field or the number of training hours was mentioned to justify the suitability of the individuals as sensory assessors. However, for the reasons set out above, this should not be seen as justification or evidence for the quality of data. The robustness of sensory data highly depends on the effectiveness of every single panellist and should be evaluated for all attributes separately *(164)*. As reported by Sharp et al. *(27)* and Vollmer et al. *(165)*, interactions between panellists and panellist x replicate interactions can be obtained by analysis of variance (ANOVA) using a mixed model on the descriptive analysis results. Additionally, interaction plots should be interrogated to graphically illustrate the performance of the panel for specific attributes and to highlight significant interaction effects.

Another factor that affects the robustness of sensory data is the number of replicates. The evaluation of samples in duplicate or ideally in triplicate by each assessor is essential in order to generate robust data from a statistical point of view *(166)*. Also important in this regard is the panel size, which should include 8-10 assessors for sensory descriptive quantitative analysis, while other sensory methods require different numbers of assessors in order to reach significance *(166, 167)*. Some of the reviewed studies mentioned that not all assessors attended each evaluation session suggesting that each of the assessors evaluated not all samples indicating that an incomplete block design was applied. This has to be taken into account if interpreting the data based on the experimental design that was used. Complete or incomplete balanced block designs are used if multiple products are compared where all panellists evaluate all samples or all levels of treatment variables within each block. The complete balanced block design approach should be preferred since an efficient and powerful partitioning of panellist variance can be achieved *(168)*. Incomplete designs are sometimes used if the number of samples is too large to be tested by each assessor in one block. In this case, each assessor only evaluates a subset of the samples (in each block) and the subsets change for each assessor. Balanced incomplete block designs should be used to ensure that all samples and all sample-pairs appear the same number of times to avoid the introduction of experimental bias and order effects which could negatively affect the statistical robustness of the dataset *(169)*.

Finally, the experimental design of the actual sensory evaluation should be carefully planned. Many factors can influence how the trained sensory panel perceives and evaluates samples containing highly volatile compounds. The most important factors are briefly summarised in Figure 4. For instance, depending on the study aim, samples are evaluated at different temperatures. Beer samples are evaluated at cooler temperatures in the majority of published

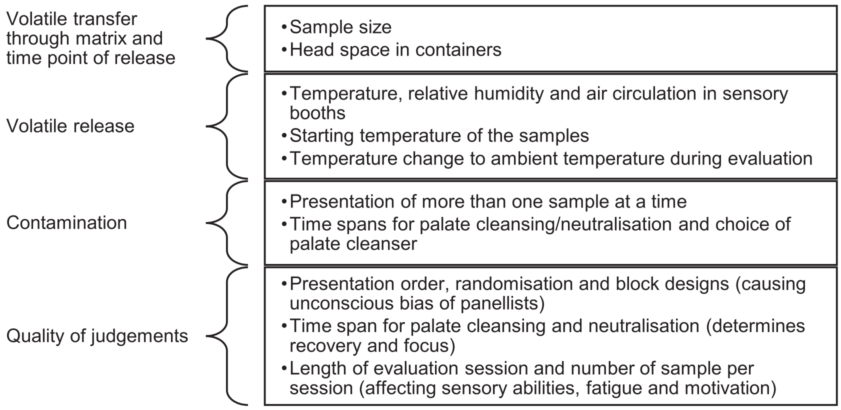

Figure 4. Factors influencing the perception of volatiles during sensory evaluation (based on Taylor *(173)* and Taylor and Roberts *(40)*).

studies while samples that are evaluated to characterise single hop oil compounds or fractions in model systems (usually with trained panels) are mostly evaluated at ambient temperatures to avoid temperature changes during the testing period *(108)* and ensure that aroma sensations are maximised *(130)*. At lower temperature, compounds volatilise less readily above the tongue before the sample is swallowed, causing reduced flavour sensations *(3)*. In either case, one should be aware of temperature changes, which might influence the perception of the volatiles.

Summary

Compounds in hop essential oil have long been suspected to contribute to a multisensory experience perceived when drinking a beer. To date, it appears that less than half of the compounds in hop oil have been identified and quantified; those quantified include the majority of compounds present at higher concentrations. Several compounds in the sesquiterpenoid, alcohol, ester, ketone, and aldehyde fractions, as well as sulphur-containing compounds have been identified as marker volatiles for certain hop varieties and associated with specific aroma and flavour sensations. Nevertheless, the full sensory potential of hop oil volatiles can only be understood if going a step beyond quantitative and qualitative analysis of hop derived volatiles in isolation. Sensory analysis has largely been neglected and only during the last two decades have researchers attempted to systematically combine sensory and instrumental methods. Recent advances in our knowledge of the concentration- and matrix-dependent perception of hop derived volatiles and sensory interactions between hop volatiles and with other beer components have been made using dynamic headspace techniques, temporal sensory methods, and reconstitution studies. It was found that ethanol and carbonation levels affect polarity and volatile retention or partitioning and consequently the delivery of volatiles in the breath. In addition, hop acids have been found to modify perceived aroma and flavour characteristics and intensities of the sensations imparted by hop oil volatiles. In turn, hop oil compounds also affect the perception of bitterness intensity, quality, and persistence. Moreover, the co-existence of hop derived volatiles and bitter extracts at specific ratios caused the perception of mouthfeel and trigeminal-type sensations. Since the majority of such findings were incidental discoveries, much more remains to be explored in order to systematically understand the sensory properties of hop derived volatiles in beer, beyond the scope of hoppy aroma and flavour.

Future perspective

It has frequently been found that the perception of hop derived volatiles cannot exclusively be explained based on their concentrations in a matrix or their threshold concentrations. Sensory interactions involving compounds below detection or threshold levels (e.g. sulphur containing compounds, oxygenates, terpene hydrocarbons) complicate the association of single volatiles in a complex mixture with specific sensory sensations. It appears to be more important to unravel the sensory characteristics induced by volatile compound mixtures rather than to identify a set of isolated 'key' compounds that are assumed to contribute to a sensory sensation.

The investigation of hop volatiles or fractions in simplified model solutions appears to be a suitable first approach to unveil multisensory interactions. Experimental designs should also pay attention to physico-chemical processes occurring in the test matrix as well as to dynamic sensory analysis. Subsequent investigation of the perception of hop volatiles in 'real' beer matrices should back up the data of studies evaluating simple model systems.

The outcome of instrumental methods gains more meaning when combined with sensory analysis. Novel approaches combining instrumental and sensory analysis, such as GC-O (AEDA)-OASIS (Original Aroma Simultaneously Input to the Sniffing port) *(170)*, Olfactoscan (GC-O coupled with a multi-channel dynamic dilution olfactometer) *(171)*, GC-GOOD (global olfactometry omission detection) *(133)* or GC-R (recomposition) *(134)* should be considered for the identification of key volatile mixtures. These methods have already been applied to identify key odourants in different food matrices, but not as yet in the field of hop research. *In vivo* data (nose space measured during consumption) can be collected while drinking a beer to quantify the delivery of volatiles through the retronasal pathway experienced during consumption. Different components of the beer matrix can have significant effects on the partitioning of volatiles under dynamic conditions as during consumption *(43)*. By combining sensory and instrumental techniques that enable the analysis of volatiles in static and dynamic conditions, it might also be possible to identify matrix-dependent sensory interactions between hop derived volatiles and beer components.

Author Contributions

Christina Dietz: PhD student. Writing – original draft.

David Cook: Funding acquisition, supervision of PhD, writing – review and editing of manuscript.

Colin Wilson: Funding provision, industry supervisor of PhD, review and editing of manuscript.

Margaux Huismann: Funding provision, industry supervisor of PhD, review and editing of manuscript.

Rebecca Ford: Supervision of PhD, writing – review and editing of manuscript.

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Conflict of interest

The authors declare there are no conflicts of interest.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

