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Hop Essential Oil: Analysis, Chemical Composition and Odor Characteristics

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Abstract

The essential oil of hops (*Humulus lupulus* L.) imparts odor and aroma characteristics to beer. Hops can influence beer aroma in terms of floral, spicy, herbal, woody and fruity characters. There are a large number of hop varieties commercially available with distinct odor characteristics, which can be attributed to the different composition of their essential oils. This composition is complex, potentially containing up to 1,000 compounds from a wide range of chemical classes. Fresh essential oil is dominated by terpene hydrocarbons, predominantly myrcene, α-humulene and β-caryophyllene. The composition varies depending on: intrinsic and extrinsic factors during growth, processing conditions, and the extraction method used to isolate the essential oil. In addition, oxidation and hydrolysis reactions occurring during storage alter the composition and further increase the chemical complexity.

Despite more than 50 years of research, not all character-impact odorants in hop essential oil have been identified. Due to its abundance, myrcene is important for the odor of fresh hop essential oil. Linalool and geraniol have been determined to be important odorants contributing to the floral character of hop essential oil and beer. Other compounds such as β-ionone, β-damascenone, geranial, neral, *trans*-4,5-epoxy-(*E*)-2-decenal, 1,3(*E*),5(*Z*)-undecatriene, 1,3(*E*),5(*Z*), 9-undecatetrene, ethyl 2-methylpropanoate, methyl 2-methylbutanoate, propyl 2-methylbutanoate, (*Z*)-1,5-octadien-3-one, nonanal and isovaleric acid have been implicated as potent odorants in hop essential oil.

Hoppy aroma in beer is still not completely understood due to the physical, biochemical and chemical changes that occur during brewing and fermentation. Hop-derived odorants identified in beer but not present in hop essential oil include citronellol, γ-nonalactone, humuladienone, geranyl acetate and ethyl cinnamate. Oxidation and hydrolysis products of sesquiterpenes (e.g. humulene epoxides) have commonly been associated with "noble" hop characters in beer; however, the importance of these compounds remains controversial. The complexity of hop aroma in beer has led to increasing trends to add fractionated hop oils with specific odor characteristics to beer post-fermentation.

List of Abbreviations

AU	Aroma Units
^{1}D	First column
^{2}D	Second column
DMS	Dimethyl sulfide
DMTS	Dimethyl trisulfide
FID	Flame ionization detector
GC × GC	Comprehensive two-dimensional gas chromatography
GC–O	Gas chromatography–olfactometry
HACP	Hop aroma component profile
HPLC	High performance liquid chromatography
MDGC	Multidimensional gas chromatography
MS	Mass spectrometry
SDE	Simultaneous distillation extraction
SPE	Solid-phase extraction
Syn.	Synonym
TOFMS	Time-of-flight mass spectrometry

Introduction

Hops (*Humulus lupulus* L.) are added to beer to impart bitterness, odor and aroma. Both hop resins and essential oil are found in the lupulin glands of the female flower cone. The essential oil is comprised of the components that are volatile in steam, usually isolated by distillation (Lawrence, 2002). The iso-α-acids originating from hop resins are predominantly responsible for bitterness, whereas a number of compounds in the essential oil are responsible for imparting hoppy odor and aroma to beer. Essential oil makes up between 0.5% and 3% of the gross composition of the

Beer in Health and Disease Prevention
ISBN: 978-0-12-373891-2

dried hop cone, varying between varieties (Benitez *et al.*, 1997; Briggs *et al.*, 2004).

Hops are typically added to wort during the boil (kettle hopping) to extract the bitterness and allow the chemical isomerization of the α-acids to the more bitter iso-α-acids. To minimize evaporation of essential oil and retain aroma compounds, premium aroma hops are added at the end of boiling (late hopping) or even to the whirlpool (Benitez *et al.*, 1997; Fritsch and Schieberle, 2003).

There are a large number of hop varieties commercially available with varying α-acids contents, essential oil levels and odor profiles (Benitez *et al.*, 1997; Briggs *et al.*, 2004). It is well known that different hop varieties produce beers with distinct aroma characteristics. Differences in the odor profiles between hop varieties can be attributed to the composition of their essential oil (Gardner, 1994). Brewers often use several varieties in a single beer to achieve the desired balance of bitterness, odor and aroma. This is usually based on α-acids content (which does not directly contribute to aroma), past experience, and trial and error. Varieties of hops are typically classified into bitter varieties with high α-acids, aroma varieties with desirable odor characteristics and dual purpose varieties that meet both criteria.

The terms odor, aroma and flavor are often used synonymously, but in this chapter a distinction will be made between them. Odor and aroma both result from a perception of volatile compounds at the olfactory epithelium in the nose. *Odor* can be defined as an orthonasal perception, where volatiles are breathed in directly through the nose. In comparison, *aroma* is a retronasal perception, where volatiles reach the olfactory epithelium via the mouth during consumption (Acree, 1993; Blank, 2002). Finally, *flavor* is a complex and integrated perception consisting of odor, aroma, taste, texture or mouthfeel, and any other trigeminal sensations such as irritation, cooling or heat (Lawless and Heymann, 1998).

This chapter discusses the chemical composition of hop essential oil and the factors that influence its composition. Methods of extraction, methods of analysis and the fractionation of essential oil are also considered. The odor characteristics of hop essential oil and the current understanding of the compounds responsible are presented with an inference to hop aroma in beer.

Variation and Changes in Composition

Much like other plant essential oils, the composition of hop oil depends on a number of intrinsic and extrinsic factors during growth. The most important of these are genetic differences where the composition varies markedly between hops of different varieties. Minor genetic variation may also occur between hops of the same variety from different growing regions. Hops are typically grown without male plants to reduce the amount of seeds, but this may reduce yields (kg/ha) (Benitez *et al.*, 1997). However, seedless hops typically produce more essential oil (Briggs *et al.*, 2004). An alternative method is the production of seedless triploid varieties, which have three sets of chromosomes instead of the normal two (diploid), and produce normal harvest yields (Beatson *et al.*, 2003).

Geographical location, climate and agronomical factors also affect the oil composition, potentially creating different profiles for hop samples with the same genetic material. Good yields depend on adequate soil nutrients and nitrogen levels, thus fertilizer is typically applied (Benitez *et al.*, 1997). Variation also occurs between harvest years due to different climatic conditions such as rainfall, temperature and sunshine. Irrigation is also required in arid conditions to ensure a good yield of hops and α-acids." As the flower cone develops, not only does the amount of essential oil increase but the proportions of compounds change. For example, oxygenated compounds are synthesized first, followed by sesquiterpenes (predominantly α-humulene (**1**) (Figure 22.1) and β-caryophyllene (**2**)), and the monoterpenes (primarily myrcene (**3**)) are produced last as the flowers ripen (Briggs *et al.*, 2004). Therefore, the harvest time will impact upon the composition of the essential oil and myrcene concentration can be used as a measure of hop ripeness. However, the humulene:caryophyllene ratio remains constant and is a varietal characteristic.

Infection from viruses and diseases, such as downy mildew, powdery mildew and verticillium wilt, and attack from pests, such as the damson hop aphid, the red spider mite and the two-spotted spider mite, can also change the composition and yield of essential oil as the plant becomes stressed (Benitez *et al.*, 1997; Hysert *et al.*, 1998). This has implications on the quality of the hops and the consistency of the oil composition. These pests and diseases are controlled by selective breeding for natural resistance, application of pesticides and fungicides (Briggs *et al.*, 2004), or alternatively via biological control using predatory mites (Barber *et al.*, 2003).

The composition of the essential oil changes during post-harvest processing, storage and transport. Compounds are partially lost through evaporation to varying degrees depending on their volatility. Furthermore, the composition of the essential oil continues to change due to oxidative degradation. The degree of change during storage depends on: the extent of physical damage to the lupulin glands withstood during harvesting, baling and kiln drying; the processing and packaging method; and the subsequent storage temperature. Larger bale sizes and density increase the damage to the lupulin glands (Forster, 2001). Baled hop cones waiting processing are often stored at ambient temperature, which allows significant changes to occur. It is recommended that temperatures during kiln drying do not exceed 60°C to prevent major losses of essential oil and oxidative degradation (Forster, 2001).

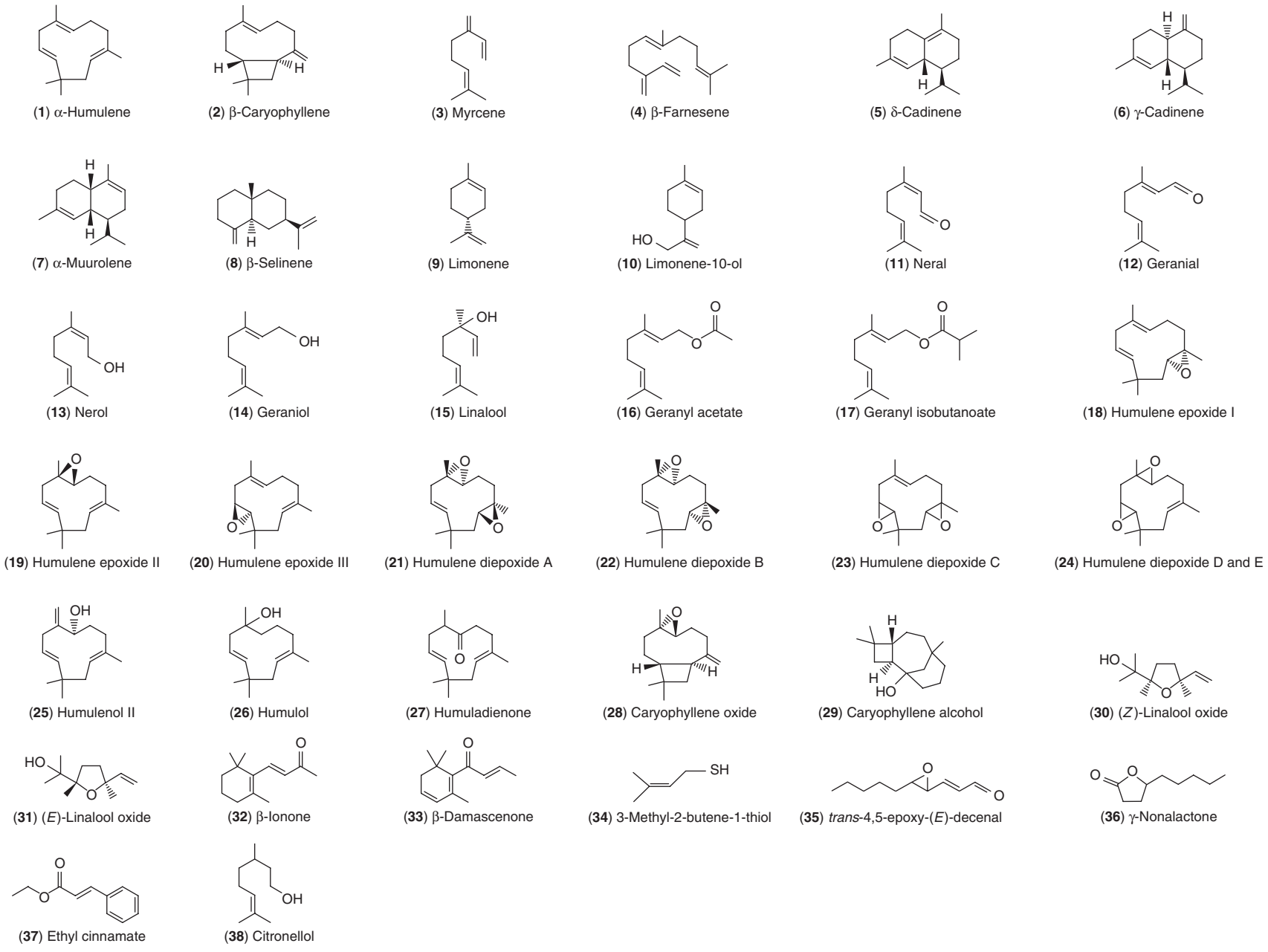

Figure 22.1 Chemical structures of major compounds and potential odorants in hop essential oil and beer.

Whole hops are the most susceptible to oxidation during storage, with significant losses of resins and essential oil occurring. Between 50% and 70% of the essential oil can be lost during 6 months storage at 20°C, mainly due to loss of myrcene (Beatson *et al.*, 2003). Therefore, hop bales should ideally be stored at refrigeration temperatures (0–5°C) (Forster, 2001).

During pelletization, hops are exposed to temperatures up to 65°C due to friction in the pelleting die, which causes the resins and essential oil that are released from the crushed lupulin glands to be susceptible to rapid oxidation. The EBC Manual of Good Practice for Hops and Hop Products (Benitez *et al.*, 1997) advocates a maximum pelleting temperature of 55°C followed by immediate cooling and vacuum packaging under an inert atmosphere (CO_2 or N_2). Packaged pellets should ideally be stored at refrigeration temperatures, but where this is impractical, keeping the temperature below 15°C should be sufficient to maintain freshness and quality (Benitez *et al.*, 1997; Forster, 2001). Extracts of resins and essential oil (see section "Methods of Extraction") are considerably more stable than pellets or whole hops, and therefore a maximum storage temperature of 20°C is acceptable (Forster, 2001).

Essential oil composition also depends on the method of isolation, as different techniques vary in their selectivity for different compound classes. This will be discussed further below (see section "Methods of Extraction").

Analysis and Characterization of Hop Essential Oil

The first characteristic of interest for a hop variety is the percentage yield of essential oil from the dried hop cones. This is determined by measuring the volume of oil recovered from steam distillation of dried hop cones and expressed in ml/g (Analytica-EBC, 2005; ASBC Methods of Analysis, 2006). The ratio of oil to α-acids is also important as brewers will primarily add hops to the kettle based on the content of α-acids to achieve a desired bitterness. Subsequently, the variable volume of essential oil added concurrently with the resins will impact upon the consistency of hop aroma in beer.

Routine analysis to determine the composition of hop essential oil is performed by gas chromatography with either flame ionization detection (GC–FID) or mass spectrometry (GC–MS). The hop essential oil is usually isolated prior to analysis (see section "Methods of Extraction"), although headspace analysis of hop cones or pellets is also performed. Headspace analysis can be achieved by either static or dynamic sampling, and the volatiles may also be concentrated by trapping on adsorbents. A convenient and popular technique for rapid characterization of hop volatiles is solid-phase microextraction (Kenny *et al.*, 2000; Steinhaus *et al.*, 2003).

Common criteria used to characterize a hop variety are the ratios of various sesquiterpene hydrocarbons, the most important being the humulene:caryophyllene ratio. A high humulene:caryophyllene ratio is typically associated with European aroma hops (Deinzer and Yang, 1994). The ratio of these sesquiterpenes is characteristic of a variety, independent of ripeness or storage, and have therefore been used to discriminate between varieties (Kralj *et al.*, 1991; Moir, 1994). However, while these ratios may be useful markers, they cannot be used to predict or explain differences in odor characteristics between varieties.

Consistent bitterness is achieved in beer by hop addition based on α-acids content. However, controlling hop aroma is more difficult as there is no single compound to measure to determine hopping rate. Addition based on total essential oil is not satisfactory due to variable composition, varietal differences and changes during storage. To address this, Foster and Nickerson (1985) proposed the "hoppiness potential" concept to control hopping rates based on the quantitative analysis of 24 compounds per gram of α-acids. This concept was further developed by Nickerson and Van Engel (1992) who refined the list of compounds and renamed it the "hop aroma component profile" (HACP) (Table 22.1). The compounds were classified into three categories: humulene and caryophyllene oxidation products; floral–estery compounds and citrus–piney compounds. These authors defined Aroma Units (AU) as the quantitative sum of the 22 HACP compounds per gram of hops (nl/g). HACP has subsequently been commonly used as a criterion for characterizing the essential oil of different hop varieties. It was envisaged that the compounds comprising HACP would evolve as new odorants were identified and their impact on beer aroma

Table 22.1 Classification of compounds comprising the hop aroma component profile[a]

Oxidation products[b]	*Floral–estery compounds*	*Citrus–piney compounds*
Humulene epoxide I (**18**)	Geraniol (**14**)	δ-Cadinene (**5**)
Humulene epoxide II (**19**)	Linalool (**15**)	γ-Cadinene (**6**)
Humulene epoxide III (**20**)	Geranyl acetate (**16**)	α-Muurolene (**7**)
Humulene diepoxide A (**21**)	Geranyl isobutanoate (**17**)	β-Selinene (**8**)
Humulene diepoxide B (**22**)		Limonene (**9**)
Humulene diepoxide C (**23**)		Limonene-10-ol (**10**)
Humulenol II (**25**)		Citral (neral + geranial)[c]
Humulol (**26**)		Nerol (**13**)
Caryophyllene oxide (**28**)		
Caryophyllene alcohol (**29**)		

[a] Adapted from Nickerson and van Engel (1992). Numbers in parentheses refer to the chemical structure depicted in Figure 22.1.
[b] Oxidation products of humulene and caryophyllene.
[c] Citral is a mixture of the two isomers – neral (**11**) and geranial (**12**).

elucidated. This has not really eventuated and an updated review of HACP is required for its potential to be realized.

The problem with analysis of hop essential oil using conventional GC is that the maximum number of compounds that can be resolved on a single 50 m column is limited to only 260 peaks (Bartle, 2002). In addition, peaks are neither evenly nor randomly distributed in a chromatogram because compounds demonstrate related chemical properties (Marriott, 2002). Because the number of compounds present in hop oil exceeds this peak capacity, severe co-elution occurs in conventional GC. This makes identification and quantification of compounds challenging, particularly for trace odorants co-eluting with larger odor inactive peaks. A solution is to use GC–MS in single ion monitoring (SIM) mode to quantify known compounds by a unique mass. This may be performed in conjunction with stable isotope dilution assay, which uses the deuterated target compound as an internal standard (Blank *et al.*, 1999; Steinhaus *et al.*, 2003). However, identification of unknown compounds remains difficult, particularly for trace odorants.

A potential solution to improve resolution is multidimensional gas chromatography (MDGC), which uses two columns with different stationary phases to create two independent separations. Compounds that co-elute on a first column may be resolved on a second column. For example, two compounds with similar boiling points that co-elute on a non-polar column may be resolved on a polar second column if they differ in their polarity. Traditional MDGC (Figure 22.2a) uses either a mechanical or pneumatic valve (V) to selectively transfer discrete regions, known as "heart-cuts," from the first column (^{1}D) to a second column (^{2}D) (Marriott, 2002). Regions that are not heart-cut to the ^{2}D column are diverted to FID 1, which monitors the separation on the ^{1}D column. The second detector is often another FID, but a mass selective detector may also be used to identify the compounds eluting from the ^{2}D column. The limitation of the heart-cut technique is that there must be sufficient time between sequential heart-cuts to prevent compounds from the two separate cuts overlapping on the ^{2}D column. Therefore, only a certain number of regions can be transferred and only a portion of the sample can be separated in two dimensions.

Another technique known as comprehensive two-dimensional gas chromatography (GC × GC) separates the *entire* sample in two dimensions in a single analysis. This is achieved using two columns connected in series with a cryogenic modulator (M) at the interface (Figure 22.2b). The modulator sequentially traps and pulses zones from the first column to the second column (e.g. every 5 s) creating two independent separation dimensions based on different compound properties. A short (0.5–2 m), narrow diameter (0.1 mm) and thin-film (0.1 μm) ^{2}D column is typically used to create a fast, efficient separation in the second dimension so that peaks from sequential pulses do not overlap (wraparound). GC × GC results are typically converted to a matrix and plotted as a contour plot analogous to a topographical map (Figure 22.3b). Retention time on the ^{2}D column is plotted against retention time on the ^{1}D column with detector signal plotted on the *z*-axis with shaded or colored contour levels used to denote peak height and compound abundance.

The greater peak capacity, resolution and sensitivity of GC × GC provide superior analyses compared with conventional GC analysis. Figure 22.3a presents a section of a single column separation of a sample of Cascade hop essential oil and exhibits considerable co-elution in the complex region of oxygenated sesquiterpenoid compounds. Figure 22.3b demonstrates the superior resolution obtained using GC × GC and illustrates the complexity of the region.

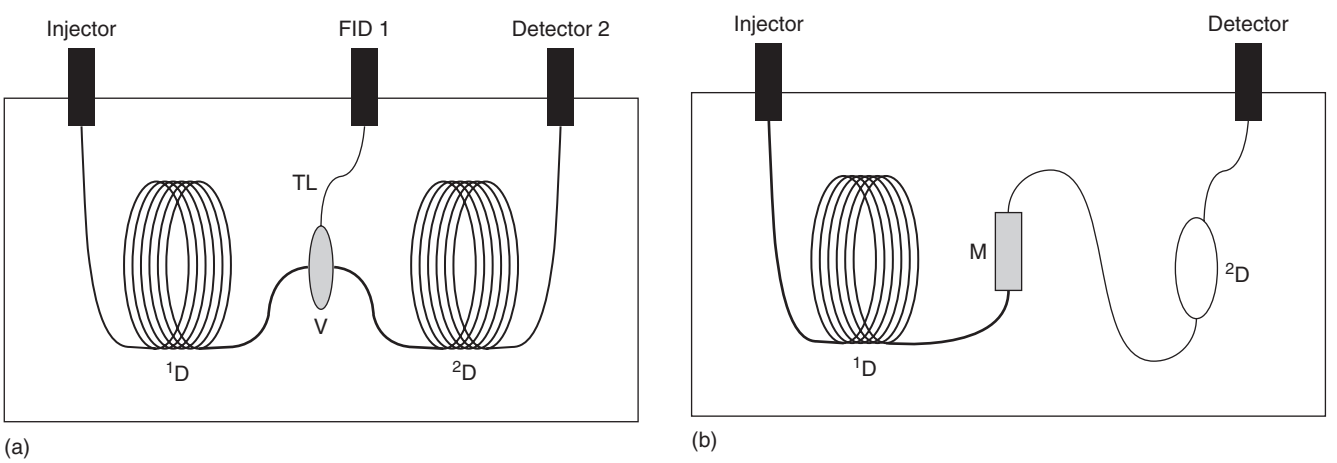

Figure 22.2 Schematic diagrams of (a) a traditional heart-cut MDGC system and (b) a comprehensive GC × GC system. ^{1}D, first column; ^{2}D, second column; V, valve; TL, transfer line to FID 1; M, cryogenic modulator. Detector 2 in the MDGC system could either be a FID or a mass selective detector. The detector in the GC × GC system could either be a FID or a TOFMS.

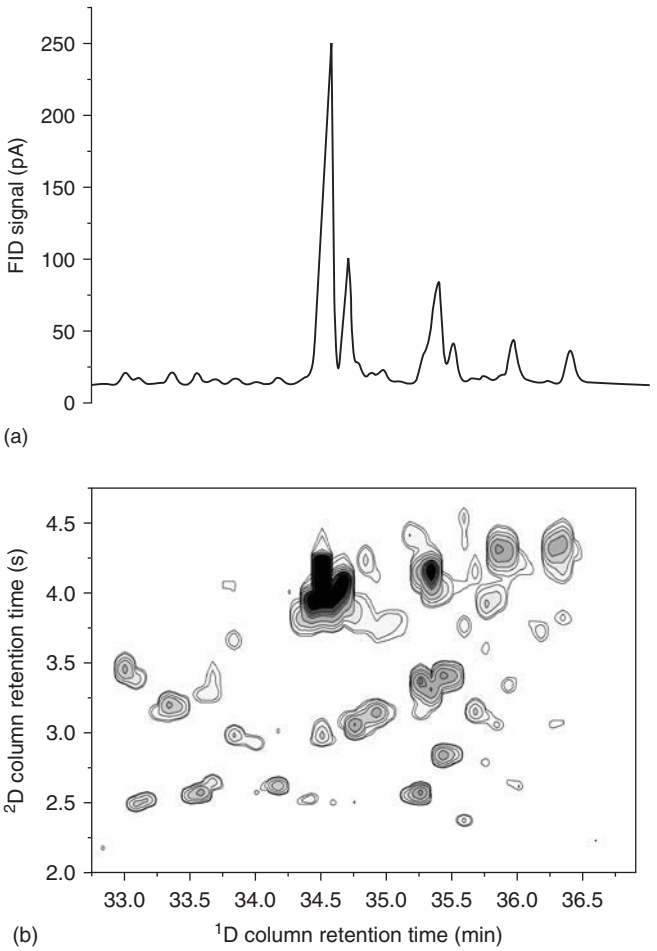

Figure 22.3 Separation of the oxygenated sesquiterpenoid region of Cascade hop essential oil sample using (a) conventional GC–FID and (b) GC × GC–FID. Note the severe co-elution of compounds for conventional GC and the superior resolution achieved using GC × GC. For the GC × GC plot, retention time on the ^{2}D column (polar) (*y*-axis) is plotted against retention time on the ^{1}D column (non-polar) (*x*-axis). Detector signal is plotted in the *z*-axis with abundance indicated by the contour levels and increasing shading.

Combining GC × GC to time-of-flight mass spectrometry (TOFMS) results in a very powerful identification tool (Roberts *et al.*, 2004).

Another advantage of GC × GC is that it generates a structured chromatogram which aids peak identification. Different compound classes elute in specific regions and clusters of the chromatogram depending on their interaction with the two stationary phases. For example, using a polar ^{2}D column, early eluting hydrocarbons are at the bottom of the GC × GC plot whereas alcohols are at the top (Figure 22.4). In addition, homologous series of compounds form linear or logarithmic relationships in the separation plane which helps to discriminate between isomeric compounds (Roberts *et al.*, 2004; Eyres *et al.*, 2005) (Figure 22.5).

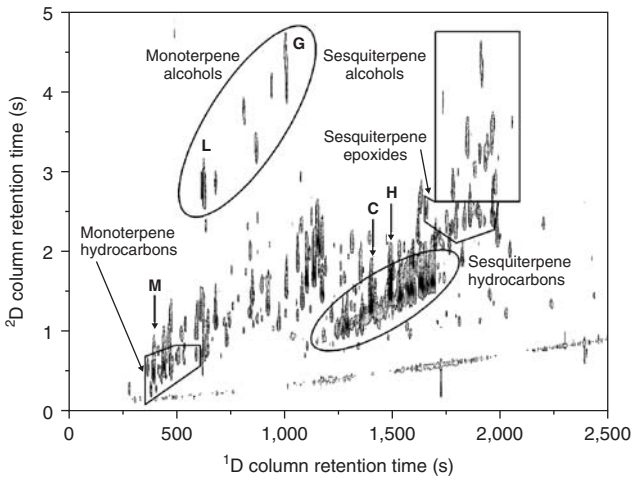

Figure 22.4 Contour plot of GC × GC separation of Target hop essential oil. Outlined regions correspond to regions where classes of terpenoid compounds elute. M, myrcene; C, β-caryophyllene; H, α-humulene; L, linalool; G, geraniol.

At present, these multidimensional techniques are generally used for research purposes rather than routine analysis, but their potential for the discovery of new compounds is immense. For more information on the development and operation of GC × GC and TOFMS the reader is directed toward two comprehensive reviews (Phillips and Beens, 1999; Marriott, 2002).

Composition of Hop Essential Oil

The composition of hop essential oil is complex. Nijssen *et al.* (1996) compiled a comprehensive list of 425 compounds reported in hop essential from 75 references. During the last decade, a further 60 compounds have been identified and reported bringing the total to 485 (Roberts *et al.*, 2004). However, recent research suggests that up to 1,000 compounds may actually be present (Roberts *et al.*, 2004). This leaves great scope for further identification and discovery of important odor active compounds. Hop essential oil contains a wide range of aliphatic, aromatic and terpenoid compound classes. Figure 22.4 presents a GC × GC separation of a sample of Target hop essential oil demonstrating the complexity of the chemical composition.

Terpenoid compounds

Figure 22.4 shows the main classes of terpenoid compounds present in hop essential oil, namely monoterpene hydrocarbons, monoterpene alcohols, sesquiterpene hydrocarbons, sesquiterpene epoxides and sesquiterpene alcohols. The composition of fresh hop essential oil is dominated by terpene hydrocarbons, primarily the monoterpene

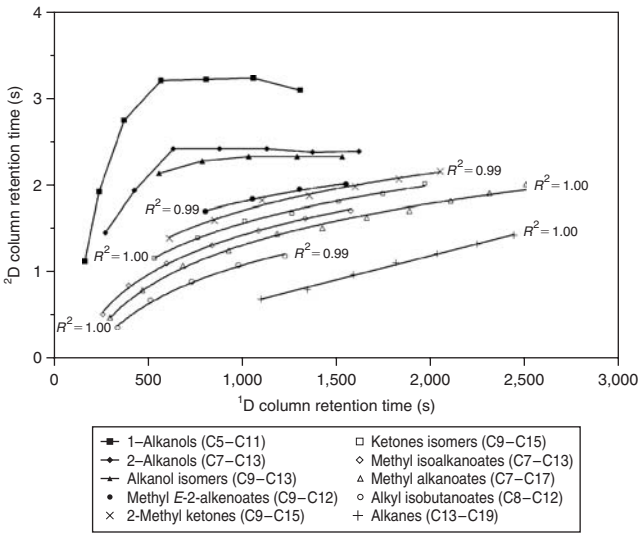

Figure 22.5 Homologous series of compounds identified in a GC × GC separation of Target hop essential oil. The apex of each peak is plotted with ^{2}D retention time (*y*-axis) plotted against ^{1}D retention time (*x*-axis). The trendline for the alkane series was fitted using a linear function. Ester and ketone series were fitted with a logarithmic function. Trendlines could not be fitted to the alcohol series. The numbers in parentheses in the key refer to the range of total number of carbons in each series.

myrcene (**3**) and sesquiterpenes α-humulene (**1**), β-caryophyllene (**2**) and β-farnesene (**4**). Other monoterpenes include α- and β-pinene, sabinene, δ-3-carene, camphene, *p*-cymene, (*Z*)- and (*E*)-β-ocimene, α- and γ-terpinene, α-terpinolene and limonene (**9**). There is a bewildering array of over 40 acyclic, monocyclic, bicyclic and tricyclic sesquiterpene hydrocarbons including (*Z*)- and (*E*)-α-bergomotene, cadinenes (e.g. **5**, **6**), muurolenes (e.g. **7**), selinenes (e.g. **8**), ylangenes, copaenes, germacrenes, selinadienes and many others (Moir, 2000). Sesquiterpenoids are notoriously difficult to resolve and identify because they have the same molecular formulae and therefore interact with column stationary phases in the same manner and exhibit very similar mass spectra.

Autoxidation and subsequent hydrolysis and rearrangement of sesquiterpene hydrocarbons lead to a large number of reaction products that increase during storage of hops (Deinzer and Yang, 1994). These include the epoxides, with the most abundant being humulene epoxide II (**19**) and caryophyllene oxide (**28**). Humulene epoxides I (**18**) and III (**20**) are typically present at lower levels. However, humulene epoxide I is more resistant to hydrolysis than humulene epoxide II and III, and therefore persists during storage and is found at greater concentrations in beer (Deinzer and Yang, 1994). Further oxidation results in the formation of five humulene diepoxide isomers (A–E) (**21**–**24**), although humulene diepoxide A (**21**) predominates in hop oil and beer (Deinzer and Yang, 1994). Sesquiterpene epoxides and diepoxides undergo further hydrolysis and rearrangements to form various ketones and alcohols (see below).

Monoterpene alcohols are generally biosynthetic products related to the biosynthesis of myrcene. These include geraniol (**14**) and linalool (**15**), which are particularly important as floral odorants in hop essential oil. Other monoterpene alcohols present are nerol (**13**), α-terpineol, borneol, fenchol, myrtenol and limonene-10-ol (**10**). Sesquiterpene alcohols are typically oxidation degradation products and among many others include humulenol II (**25**), humulol (**26**), caryophyllene alcohol (syn. caryolan-1-ol) (**29**), caryophyllenol, nerolidol, farnesol isomers, cadinol isomers and eudesmol isomers. Biosynthetic alcohols tend to decrease during storage, whereas oxidation-derived alcohols tend to increase (Moir, 1994).

The monoterpene aldehydes neral (**11**) and geranial (**12**) have been identified in freshly distilled hop essential oil but these compounds will be rapidly reduced to their corresponding alcohols during storage or fermentation (Sanchez *et al.*, 1992a). The terpenoid ketones β-ionone (**32**) and β-damascenone (**33**) are found at trace levels in hop essential oil resulting from the degradation of β-carotene (Sell, 2003). The ketones humulenone and humuladienone (**27**) result from the oxidation reactions of humulene.

A number of esters of the terpene alcohols are also present including methyl geranate, methyl nerolate, geranyl propanoate, neryl propanoate, geranyl isobutanoate (**17**), neryl isobutanoate, geranyl acetate (**16**), neryl acetate and linalyl acetate.

Oxygen heterocyclic compounds in hop essential oil include (*Z*)- and (*E*)-linalool oxide (**30**, **31**), rose oxide and cyclic ethers such as hop ether and karahana ether (Moir, 1994).

Non-terpenoid compounds

The composition of hop essential oil includes many homologous series of aliphatic compounds (Figure 22.5). Aliphatic hydrocarbons are present at low levels and are represented by a series of linear alkanes, a number of branched alkanes and several trace alkenes. Hop essential oil contains a number of isomeric series of straight chain and branched ketones. The foremost series is the methyl ketones with the most abundant compound being 2-undecanone (syn. methyl nonyl ketone). Levels of aldehydes are generally low in hop essential oil and are mainly lost during kiln drying. Aldehydes identified include linear alkanals (e.g. nonanal), *E*-2-hexenal, *Z*-3-hexenal, *E*-2-nonenal, benzaldehyde and phenylacetaldehyde (Nijssen *et al.*, 1996).

Hop essential oil is rich in a large variety of aliphatic esters. Many exist as homologous series including: linear methyl alkanoates (e.g. methyl decanoate); branched methyl alkanoates such as methyl 2-methyl-alkanoates

and the methyl isoalkanoates (e.g. methyl 6-methyl-heptanoate); unsaturated methyl alkenoates (e.g. methyl *E*-2-decenoate); alkyl propanoates (e.g. pentyl propanoate); alkyl isobutanoates (e.g. hexyl 2-methyl-propanoate) and unsaturated alkenyl acetates with unconfirmed stereochemistry (e.g. octenyl acetate). Important esters that do not exist in homologous series include methyl *Z*-4-decenoate, 2-methylpropyl 2-methyl-propanoate (syn. 2-methyl-propyl isobutanoate), 2-methylbutyl 2-methyl-propanoate (syn. 2-methylbutyl isobutanoate), 3-methylbutyl 3-methyl-butanoate (syn. isoamyl isovalerate) and 2-methylbutyl 3-methyl-butanoate (syn. 2-methylbutyl isovalerate). The homologous series of straight chain methyl esters most likely originate from fatty acid biosynthesis, whereas the branched chain esters (e.g. 2-methylbutyl isobutanoate) are derived from amino acid biosynthesis (Briggs *et al.*, 2004).

Alcohols are represented by straight chain alcohols, such as 1- and 2-alkanols, and branched chain alcohols, such as 2-methyl-3-buten-2-ol, which is formed by cleavage of the isoprenyl side chains of the α- and β-acids (Briggs *et al.*, 2004). Acids are also present in hop essential oil and are usually associated with aged hops as degradation products of the α- and β-acids. Cleavage of the acyl side chains yields 3-methylbutanoic acid (syn. isovaleric acid) from humulone and lupulone, 2-methylbutanoic acid from adhumulone and adlupulone, and 2-methylpropanoic acid (syn. isobutyric acid) from cohumulone and colupulone. These acids are responsible for the cheesy aroma of aged hops (Benitez *et al.*, 1997; Briggs *et al.*, 2004). Photo-oxidation of the ring structure of the α- and β-acids also produces 4-methyl-3-pentenoic acid. Other acids include decanoic acid and *Z*-4-decenoic acid. The degree of acids found in essential oil also varies depending on the sample preparation and extraction or distillation method used to isolate the oil.

Sulfur compounds

Lermusieau and Collin (2003) recently reviewed the occurrence and origins of sulfur compounds in hops and beer. Sulfur compounds are present at trace levels but can have very low odor thresholds and so impact upon the odor of essential oil and beer (Lermusieau *et al.*, 2001). Methyl thioesters have been commonly identified in hop essential oil and Lermusieau and Collin (2003) assert that these are not artifacts of steam distillation because they are also present in cold solvent extracts. The authors suggested a possible biosynthetic pathway from methionine degradation. The concentration of thioesters depends on variety and local growing conditions, and increase considerably upon kiln drying, independent of sulfur dioxide (SO_2) application.

Other sulfur containing compounds include thiophenes, sulfur adducts of myrcene and humulene, and episulfides of sesquiterpenes. These compounds result from reactions with elemental sulfur either applied in the field to control powdery mildew (Briggs *et al.*, 2004) or from burning sulfur during kilning (Benitez *et al.*, 1997). In the presence of light or heat, sesquiterpenes react with the residual elemental sulfur to generate episulfides. These compounds have the same structure as the corresponding epoxides except oxygen is substituted by sulfur. A number of sulfur adducts of myrcene and humulene also form due to reaction with elemental sulfur. These compounds are also formed during steam distillation as a result of the high temperature applied, and can be thought of as artifacts of sample preparation. In contrast, vacuum distillation and CO_2 extracts have much lower levels of sulfur compounds (Briggs *et al.*, 2004). However, these compounds could also form in the kettle during boiling and be introduced into the beer, particularly upon late hopping where limited evaporation occurs (Lermusieau and Collin, 2003).

Dimethyl sulfide (DMS) is also generated during steam distillation and wort boiling by thermal degradation of *S*-methylcysteine sulfoxide. The levels of DMS and polysulfides (e.g. dimethyl trisulfide, DMTS) also increase with levels of elemental sulfur and when kilning is performed without SO_2. These sulfide compounds have characteristic cooked vegetable, onion, rubbery and sulfury odors that may impact on beer aroma. The compound responsible for the skunky aroma of lightstruck beer, 3-methyl-2-butene-1-thiol (**34**), has also been found in hop pellets being derived from the isoprenyl side chains of α- and β-acids (Lermusieau *et al.*, 2001).

Odor Characteristics of Hop Essential Oil

Although there maybe several hundred compounds present in hop essential oil, only a certain number will be present at a concentration above their detection threshold and contribute to the odor of the oil (Guadagni *et al.*, 1966; Buttery, 1999). Compounds that are responsible for, or significantly contribute to, a sample's distinctive odor profile are known as *character-impact odorants*.

Instrumental analysis of character-impact odorants

The characterization of essential oils is usually based on chemical composition determined by GC–MS or GC–FID. However, odor detection thresholds of volatile compounds can differ by many orders of magnitude (e.g. parts per trillion up to odorless compounds) (Buttery, 1999). The relationship between concentration and odor intensity may also vary considerably between compounds. Therefore, the response of a chemical detector is not representative of odor activity. For example, the most abundant compound in a chromatogram may not be the most important odorant (Eyres *et al.*, 2005). Consequently, the impact of

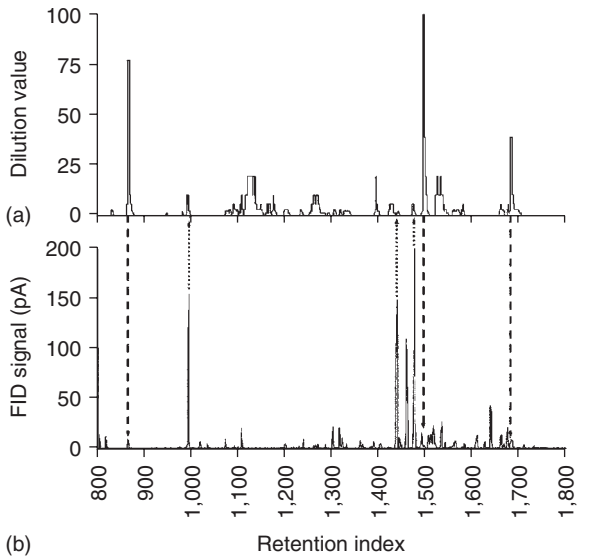

Figure 22.6 Comparison of (a) the GC–O "aromagram" with (b) the FID chromatogram for Saaz hop essential oil. Retention index (*x*-axis) is plotted against (a) dilution value for GC–O and (b) FID signal. Peak areas in the GC–O aromagram are proportional to odor potency.

a compound on the odor of a sample must be evaluated using human assessors. A valuable tool for identifying character-impact odorants is gas chromatography–olfactometry (GC–O), where human "sniffers" are used to detect and evaluate the odor of compounds as they elute from a GC separation (Delahunty *et al.*, 2006). Figure 22.6 demonstrates that the odor profile generated by GC–O is rather different than the FID chromatogram for a hop essential oil sample. Several odor peaks correspond to major FID peaks but conversely, other important odorants do not correspond to any noteworthy FID peaks, only being present at trace concentrations. The advantage of GC–O is that it specifically measures odor activity and can therefore be used to locate character-impact odorants. Once odorants have been identified and their odor activity confirmed, their concentrations can be routinely measured using a conventional detector.

There are a number of issues that should be taken into account when interpreting GC–O data. Various GC–O methodologies use different properties to rate odorant importance, including odor potency by dilution analysis (concentration/threshold; synonymous to odor activity values or odor units), detection frequency of a panel and direct odor intensity (Delahunty *et al.*, 2006). Two methods may produce different results because the relationship between concentration and odor intensity differs considerably between compounds (Petersen *et al.*, 2003). The objective of GC–O is to assess the odor activity of compounds individually without co-elution. This allows identification of the odorants that are potentially important but does not take into account possible interactions that occur in the mixture such as synergistic, antagonistic (suppression) or additive effects (Delahunty *et al.*, 2006). For these reasons, assessment of hops using sensory evaluation is indispensable.

Sensory evaluation of odor characteristics

There have been relatively few sensory evaluations of raw hop cones or essential oils. Sanchez *et al.* (1992b) used a descriptive sensory panel and GC–O to evaluate oxygenated fractions of three hop varieties. Stucky and McDaniel (1997) used free-choice profiling to discriminate 15 varieties, and correlated the sensory results with the concentration of 21 compounds. Myrcene and linalool demonstrated a strong association with the combined sensory characters of fruity, floral, pine and sage in principal components analysis (PCA).

Odorants identified in hop essential oil

The odor characteristics of compounds that are potential odorants in hop essential oil and beer are summarized in Table 22.2. At harvest, the most potent odorant in green hops is *Z*-3-hexenal (green, cut grass), but this is mostly lost during kiln drying (Steinhaus and Schieberle, 2000). This odorant is common in plants and herbs as a secondary metabolite of linoleic acid. Myrcene is typically the most abundant compound in fresh hop essential oil. Its odor threshold in water has been determined to range between 13 and 36 ppb (Guadagni *et al.*, 1966; Ahmed *et al.*, 1978; Masanetz and Grosch, 1998), and so is expected to exert a large impact on the odor profile of the essential oil. This has been supported in studies using GC–O (Steinhaus and Schieberle, 2000). It has odor descriptors of resinous, herbaceous, balsamic and geranium-like (Masanetz and Grosch, 1998; Steinhaus and Schieberle, 2000).

Odorants that contribute to floral characteristics of hop essential oil include linalool (floral – citrusy), geraniol (floral – rose, geranium) and β-ionone (floral – violet). The importance of linalool in hop essential oil has been confirmed by various authors using GC–O (Sanchez *et al.*, 1992b; Steinhaus and Schieberle, 2000; Lermusieau *et al.*, 2001). Geraniol has been determined to be a potent odorant in hop essential oil (Peacock and Deinzer, 1981; Lam *et al.*, 1986; Eyres *et al.*, 2006) but was not deemed important by Sanchez *et al.* (1992b) because only one of four assessors detected it during GC–O. It was also not detected by Steinhaus and Schieberle (2000). These results could be influenced by varietal differences (Peacock *et al.*, 1981) or the age of the hops, as geraniol concentration increases during storage. The monoterpene aldehydes geranial and neral have also been implicated for the floral odor of essential oil (Nickerson and Van Engel, 1992). Sanchez *et al.* (1992b) reported that neral contributed a citrus-spicy odor during GC–O analysis. However, these compounds

Table 22.2 Odor characteristics of hop-derived compounds that potentially contribute to the odor of the essential oil and beer

Compound[a]	*Odor descriptors*	*References*[b]
α-Humulene (**1**)	Balsamic	1
β-Caryophyllene (**2**)	Cloves, turpentine	2
Myrcene (**3**)	Resinous, herbaceous, balsamic, geranium-like	1, 2
Z-3-hexenal	Green, cut grass, leafy	1, 2
Neral (**11**)	Citrus, spicy, lemon	2, 3
Geranial (**12**)	Citrus, lemon	2
Nerol (**13**)	Floral – rose	2
Geraniol (**14**)	Floral – rose, geranium	2, 4
Linalool (**15**)	Floral – citrus, coriander seed	2, 4
Geranyl acetate (**16**)	Floral – lavender, perfumed pine	2, 5
Geranyl isobutanoate (**17**)	Floral – rose	2
Humulene epoxide I (**18**)	Hay-like	6
Humulene epoxide II (**19**)	Moldy, cedar	6, 7
Humulene epoxide III (**20**)	Cedar	7
Humulene epoxides I, II, III	Musty, floral, spicy	3
Humulene diepoxide A, B (**21**, **22**)	No odor	3
Humulenol II (**25**)	Sage-brush, pineapple	6, 7
Humulol (**26**)	Hay-like	6
Humuladienone[c] (**27**)	Flowery, fresh	5
Caryophyllene oxide (**28**)	Musty, floral, spicy, cedar	3, 7
β-Ionone (**32**)	Floral – violet	2, 4
β-Damascenone (**33**)	Cooked apple, tobacco, prunes	3, 4
3-Methyl-2-butene-1-thiol (**34**)	Sulfurous, skunky, mercaptan	2
Dimethyl disulfide	Cheesy, glue	5
Dimethyl trisulfide	Onion, soup	2, 5
trans-4,5-epoxy-(*E*)-2-decenal (**35**)	Metallic	1
γ-Nonalactone[c] (**36**)	Coconut, fruity, sweet	2, 5
Ethyl cinnamate[c] (**37**)	Cinnamon-like, honey-like strawberry, sweet	2, 5
Citronellol[c] (**38**)	Floral – rose, fruity, apple, citrus	2, 8
Isovaleric acid	Rancid, sweaty, cheesy	2, 4
Ethyl 2-methylpropanoate	Sweet, fruity	1, 2
Methyl 2-methylbutanoate	Sweet, fruity, apple-like	1, 2
Propyl 2-methylbutanoate	Sweet, fruity	1
1,3(*E*),5(*Z*)-undecatriene	Fresh, balsamic	1
1,3(*E*),5(*Z*),9-undecatetrene	Fresh, balsamic	1
(*Z*)-1,5-octadien-3-one	Geranium-like	1
Nonanal	Citrus, soapy, fatty	1, 2

[a] Numbers in parentheses refer to the chemical structure depicted in Figure 22.1.
[b] References: 1: Steinhaus and Schieberle (2000); 2: Burdock (2002); 3: Sanchez *et al*. (1992b); 4: Eyres *et al*. (2006); 5: Lermusieau *et al*. (2001); 6: Fukuoka and Kowaka (1983); 7: Deinzer and Yang (1994); 8: Sanchez *et al*. (1992a).
[c] Hop-derived compounds in beer not found in hop essential oil.

will be rapidly reduced to their corresponding alcohols during storage (Briggs *et al*., 2004).

β-ionone and β-damascenone (cooked apple) have previously been suggested to be important odorants due to their low odor thresholds (Tressl *et al*., 1978), which range between 0.008–0.17 ppb and 0.002–0.009 ppb in water, respectively (Plotto *et al*., 2006). Their importance as odorants has also been supported by various GC–O investigations for β-ionone (Eyres *et al*., 2006) and β-damascenone (Sanchez *et al*., 1992b; Lermusieau *et al*., 2001; Murakami *et al*., 2003), respectively. However, it is estimated that approximately 1/3 of the population have a specific anosmia for β-ionone and therefore cannot detect it (Brenna *et al*., 2002; Plotto *et al*., 2006). Recent research indicates that β-damascenone may also be partially affected by anosmia (Plotto *et al*., 2006) and that it has low odor intensity even at high concentrations above threshold (Petersen *et al*., 2003). Therefore, the overall impact of these two compounds is still unconfirmed.

Steinhaus and Schieberle (2000) found *trans*-4,5-epoxy-(*E*)-2-decenal (**35**), a fatty acid oxidation product with a metallic odor, to be the most potent odorant in an extract of dried hop cones using GC–O. However, it was not present in a headspace sample and has not been reported in hops since, so its importance is still unverified. The same study also identified 1,3(*E*),5(*Z*)-undecatriene and 1,3(*E*),5(*Z*),9-undecatetrene as important odorants in both the extract and headspace samples contributing a fresh, balsamic odor. Other potent odorants identified were ethyl 2-methylpropanoate (sweet, fruity), methyl 2-methylbutanoate (sweet,

fruity), propyl 2-methylbutanoate (sweet, fruity), (*Z*)-1,5-octadien-3-one (geranium-like), nonanal (citrus, soapy), and 2- and 3-methylbutanoic (isovaleric) acid (cheesy).

A panel of four assessors used the terms musty, floral and spicy to describe the odors perceived during GC–O for humulene epoxide I, II and III and caryophyllene oxide in oxygenated fractions of hop oil (Sanchez *et al.*, 1992b). This was confirmed using a synthesized mixture of the three humulene epoxides. In contrast, no odors were detected for humulene diepoxide A or B, even for a synthesized sample at high concentration. Steinhaus and Schieberle (2000) did not detect any humulene oxidation products by GC–O, but the oil may have been too fresh for oxidation to have occurred. Deinzer and Yang (1994) reported that almost all humulene and caryophyllene oxidation products exhibited cedar-like aromas during sensory evaluations. Fukuoka and Kowaka (1983) evaluated the aroma of several synthesized oxidation products. Humulene epoxide I and humulol had a hay-like odor, humulene epoxide II had a moldy odor and humulenol II had a sage-brush odor. The authors concluded that these compounds were not responsible for the odor of a concentrated high performance liquid chromatography (HPLC) fraction exhibiting a strong herbal, spicy character. The two herbal odorants actually responsible were not identified, but were reported to have an oxygenated sesquiterpenoid structure ($C_{15}H_{24}O$ and $C_{15}H_{26}O$). One of these compounds was also found in a sample of commercial Japanese beer.

Guadagni *et al.* (1966) and Tressl *et al.* (1978) suggested that hop ether, karahana ether, methyl-4-decenoate and methyl thiohexanoate were important odorants based on their odor activity values (concentration/threshold). The detection threshold and the odor impact of the two ethers in beer were re-evaluated by Lam and Deinzer (1986) who determined that neither compound was a major contributor to hop aroma.

Hop Aroma in Beer

Because all of the compounds responsible for hop aroma in beer have not been completely identified, sensory evaluation is still the method of choice for product development and quality control of beer. It is well established that the hoppy aroma in beer is due to the perception of complex mixtures of volatiles rather than single compounds. Hop aroma in beer is usually complex, and accurately describing the specific characteristics can be challenging. In addition, it is often difficult to differentiate hop-derived aroma from aroma compounds produced during fermentation. Hops can impact on beer aroma in terms of floral, spicy, herbal, woody and fruity (particularly citrus and tropical fruit) characters. However, the official beer flavor wheel does not adequately reflect this complexity of hop aroma, only using "hoppy" as a specific first-tier term (Meilgaard *et al.*, 1979). This is subdivided into three second-tier terms which are "kettle hop," "dry hop" and "hop oil." Use of the term "noble hop aroma" is common in the literature and is usually associated with traditional aroma hop varieties from Europe such as Hallertauer mittelfrüh, Hallertauer Hersbrucker, Saaz, Spalter and Tettnanger (Sanchez *et al.*, 1992b; Deinzer and Yang, 1994). However, the actual aroma description of this character is poorly defined, but is often described as herbal or spicy (Sanchez *et al.*, 1992b).

Physical, chemical and biochemical changes that occur during wort production and fermentation complicate the analysis of hop-derived compounds in beer. Thereby, not all compounds present in hop essential oil are found in kettle-hopped beer, and conversely not all hop-derived compounds in beer are found in hop essential oil itself. Hydrocarbons are not typically detected in beer except when dry hopping is used. Conversely, oxygenated compounds are much more likely to dissolve into wort and survive the boiling and fermentation processes.

Correlating sensory characteristics of hopped beer with instrumental composition may elucidate associations to aid understanding of hop aroma in beer. For example, Peppard *et al.* (1989) used multivariate statistics to correlate sensory characteristics for beer brewed with 8 different hop varieties with the concentration of 36 hop-derived compounds. Linalool oxide, and to a lesser extent caryophyllene alcohol and humulol, were correlated with "European hop aroma." A large number of compounds were associated with the spicy character including spiroacetal, dihydrospiroacetal, humulene epoxide I, humulenol II and humulene diepoxides. However, a good correlation does not prove a cause and effect relationship, so the impact of the compounds reported must still be directly confirmed (Peppard *et al.*, 1989).

Peacock *et al.* (1981) concluded that geraniol and linalool were responsible for most of the floral aroma in a beer brewed with Cascade hops. Geranyl isobutanoate was present below threshold, but could be hydrolyzed by yeast to yield free geraniol and contribute to the aroma. Linalool in particular has been implicated as being important in overall hoppy aroma and the noble hop aroma in beer (Steinhaus and Schieberle, 2000; Steinhaus *et al.*, 2003; Fritsch *et al.*, 2005). These compounds would be expected to produce a floral hop aroma in beer when added post-fermentation but are also expected to survive fermentation (Irwin, 1989).

There is also a difference in sensory threshold and odor character between linalool enantiomers. In hop essential oil, 92–95% of linalool is present as the more active (*R*)-enantiomer, which has an odor threshold approximately 80 times lower than (*S*)-linalool (Kaltner *et al.*, 2003; Steinhaus *et al.*, 2003). It has been shown that interconversion between the enantiomers occurs during wort boiling so that (*S*)-linalool may actually constitute 30% in beer, potentially decreasing the overall odor impact of linalool

(Fritsch and Schieberle, 2003). The extent of this conversion appears to be dependent on the wort pH (Marriott *et al.*, 2006).

Lermusieau *et al.* (2001) assessed amberlite resin (XAD-2) extracts of beer using GC–O. The authors compared the odorants present in unhopped beer with those in two beers late-hopped with Saaz and Challenger, respectively. Potent hop odorants were linalool, β-damascenone, dimethyl disulfide (cheesy, glue), DMTS (onion, soup) and an unidentified spicy, hoppy odorant eluting with a retention index of 810 on an apolar stationary phase. Hop-derived odorants that were detected in hopped beer but not in steam distilled hop essential oil were γ-nonalactone (fruity, sweet) (**36**), humuladienone (flowery, fresh), geranyl acetate (perfumed pine) and ethyl cinnamate (strawberry, sweet) (**37**). Sanchez *et al.* (1992a, 1992b) reported 9-methyl-2-decanone (musty, vinyl, rancid) as a possible odorant in hop oil and beer extracts.

Citronellol (floral – citrus, fruity, apple) (**38**) has also been identified as a hop-derived compound in beer by GC–MS and GC–O and is implicated in contributing to hop aroma (Lam *et al.*, 1986; Sanchez *et al.*, 1992a). It has been shown that citronellol can be transformed from geraniol by yeast during fermentation (King and Dickinson, 2000, 2003). It has also been suggested that it is formed by reduction of geranial and neral by yeast (Lam *et al.*, 1986; Sanchez *et al.*, 1992a).

β-damascenone has been identified as a potent odorant in beer by various authors (Schieberle, 1991; Sanchez *et al.*, 1992a; Lermusieau *et al.*, 2001; Chevance *et al.*, 2002; Fritsch *et al.*, 2005). Its concentration has been shown to increase during wort boiling (Kishimoto *et al.*, 2005), decrease during fermentation due to reduction or adsorption by yeast, and then increase again upon storage (Chevance *et al.*, 2002). β-damascenone is likely to contribute to the odor of beer because of its low threshold. However, its sensory impact on overall beer flavor still needs to be confirmed using sensory evaluation to assess its odor intensity and interaction with other aroma compounds. It should also be noted that β-damascenone only partially originates from hops, also being present in unhopped wort and beer (Lermusieau *et al.*, 2001; Chevance *et al.*, 2002; Fritsch and Schieberle, 2003).

Oxidation and hydrolysis products of sesquiterpenes have been associated with the noble and spicy hop characters in beer (Peacock and Deinzer, 1981; Lam *et al.*, 1986; Deinzer and Yang, 1994; Goiris *et al.*, 2002). Good correlations between increasing concentrations of humulene epoxides and these hop characters have been demonstrated (Kowaka *et al.*, 1983; Peppard *et al.*, 1989). As a result, so-called noble hop varieties are often purposefully stored prior to brewing to increase the levels of oxygenated compounds (Deinzer and Yang, 1994; Briggs *et al.*, 2004). However, a good correlation does not prove a cause and effect relationship (Peppard *et al.*, 1989), and the importance of these oxidation compounds for imparting hoppy aroma remains controversial (Fukuoka and Kowaka, 1983; Irwin, 1989; Goiris *et al.*, 2002). The compounds so far identified have exhibited concentrations below their detection thresholds and their aroma characteristics do not correspond to the desired spicy or noble hop aroma (Deinzer and Yang, 1994).

Yang *et al.* (1993) found that a hydrolysis reaction mixture from humulene epoxide I and II contributed a cedar, lime, spicy character to beer, but with a relatively high sensory threshold of 2.3 ppm. This concentration was exceeded in pilot beers, but not in any commercial brands tested. In the study by Sanchez *et al.* (1992a), only one out of four assessors detected the odors associated with humulene oxidation products in beer extracts using GC–O, despite being detected in hop oil and identified in the beer extracts by GC–MS. It was concluded that the compounds were not present at high enough concentration. This may indicate that humulene epoxides may contribute to hop aroma but are not essential to it (Deinzer and Yang, 1994).

Goiris *et al.* (2002) found that adding 20 ppb of an oxygenated sesquiterpene fraction isolated by supercritical CO_2 extraction and solid-phase extraction (SPE) to a bland pilot beer produced a desirable spicy or herbal aroma reminiscent of noble hop aroma. The authors concluded that this was due to unidentified compounds present in this fraction, associated with humulene oxidation products.

There is growing evidence for the release of glycosidically bound hop aroma compounds during wort boiling, fermentation or ageing (Goldstein *et al.*, 1999; Chevance *et al.*, 2002; Fritsch *et al.*, 2005; Kishimoto *et al.*, 2005). Examples that are implicated in hop aroma are geraniol, linalool and β-damascenone. These glycosidically bound compounds are not isolated with the essential oil but may affect hop aroma in kettle-hopped beer as they are released by acid catalyzed hydrolysis during boiling (Chevance *et al.*, 2002).

Methods of Extraction

One should distinguish between methods used to isolate hop essential oils for analysis or for the manufacture of commercial products. The simplest method for isolation of essential oil is either steam distillation or hydro-distillation. A method solely used for analytical sample preparation are many adaptations of the Likens–Nickerson simultaneous steam distillation-solvent extraction (SDE) (Likens and Nickerson, 1964). Distillation methods involve the application of heat and therefore there is the possibility to produce artifacts by thermal degradation. Composition will differ depending on whether the distillation is performed at atmospheric or reduced pressure due to the temperature that is applied (Briggs *et al.*, 2004). Steam distillation at atmospheric pressure is known to cause a number of degradative changes so that the odor of the resultant oil

is not representative of the original sample (Moyler, 1993; Gardner, 1994). As a result, early attempts at using these oils for dry hopping were unsuccessful (Gardner, 1994).

Solvent extraction is another method of obtaining hop volatiles, although according to Lawrence (2002) these extracts cannot strictly be called essential oils and are more accurately described as "volatile concentrates." However, for all intents and purposes the final result is very similar – an isolated volatile oil. Various solvents have been used commercially including hexane, ethanol, methanol, trichloroethylene and methylene chloride (Gardner, 1993). However, only hexane and ethanol are still in use and even these are in decline (Briggs *et al.*, 2004). These solvent extracts are known to decrease the yield and alter the composition and odor characteristics of hop essential oil due to the loss of volatile compounds during evaporation of the solvent (Gardner, 1993; Benitez *et al.*, 1997). The most volatile compounds, which are responsible for top notes in the odor profile, are most severely affected. There are also safety and regulation concerns regarding solvent residues remaining in the extracts.

Currently the method of choice to extract hop essential oil is extraction using liquid or supercritical CO_2. Liquid CO_2 extraction is typically carried out at 5–15°C and 60–65 bar whereas supercritical CO_2 requires greater temperature (40–60°C) and pressure (200–250 bar) (Benitez *et al.*, 1997). Composition of the two extracts is likely to be extremely similar, except that supercritical CO_2 extracts contain more hard resins, polar bitter substances and pigments (e.g. chlorophyll), the latter giving a dark green color to supercritical extracts (Gardner, 1993; Benitez *et al.*, 1997). The extraction efficiency and flexibility of supercritical CO_2 are greater, because the solvent properties can be altered by varying the temperature and pressure. In comparison, the properties of liquid CO_2 can only be altered by small changes in temperature (Gardner, 1993; Benitez *et al.*, 1997). The impact on trace odor compounds has not been thoroughly investigated, with liquid CO_2 extraction theoretically giving a milder extraction and a more representative extract (Moyler, 1993). In practice, liquid CO_2 is used when the extract is further processed for essential oil and aroma products due to its greater selectivity and lower temperature (Gardner, 1993).

Oil enriched extracts (~26 ml oil per 100 g extract) may be produced using either partial extraction with liquid CO_2 or by two-step fractionation using supercritical CO_2, where the resins are initially precipitated by reducing the pressure to 100–120 bar before recovering the essential oil in an evaporator (Benitez *et al.*, 1997). However, it is more practical to make a total extract comprising both hop resins and essential oils and isolate the essential oil using molecular distillation under high vacuum (1.33×10^{-6} bar) (Gardner, 1994; Benitez *et al.*, 1997; Briggs *et al.*, 2004). For essential oils, the great advantage of CO_2 extraction is that the aroma compounds are obtained quantitatively without the creation of artifacts. Therefore, the odor profile is much more representative of the original sample than steam distillation or other solvent extraction methods (Moyler, 1993; Gardner, 1994). Separating the essential oil from the resins allows hop aroma and bitterness to be controlled independently in the brewing process (Gardner, 1993).

Essential Oil Fractionation

Hop essential oils are often fractionated by physical and chemical properties in an attempt to improve the resolution of compounds for the chemical analysis of hop essential oils. Historically, hop essential oils were separated into a hydrocarbon fraction and an oxygenated fraction by elution from a silica gel column with light petroleum and ether, respectively. More recently, pre-analytical fractionation has been achieved by HPLC (Fukuoka and Kowaka, 1983; Deinzer and Yang, 1994) and SPE (Irwin, 1989; Goiris *et al.*, 2002).

A great deal of research has been invested into developing commercially fractionated hop oil products with specific aroma qualities that may be added either pre- or post-fermentation (Haley and Peppard, 1983; Westwood and Daoud, 1985; Westwood, 1987; Gardner, 1994; Marriott, 2001; Goiris *et al.*, 2002). Isolated hop oil was originally dosed into wort and beer as aqueous emulsions or entrained in a liquid CO_2 stream (Westwood, 1987). However, post-fermentation products must be soluble in beer to prevent problems with haze. A soluble Dry Hop Essence was subsequently developed by removing the insoluble monoterpene and sesquiterpene hydrocarbons by liquid–liquid extraction (Westwood and Daoud, 1985; Marriott, 2001).

Further fractionation by functional groups using a combination of fractional distillation and column chromatography gave rise to four Late Hop Essences with specific aroma characteristics (Westwood and Daoud, 1985; Gardner, 1994; Marriott, 2001). These fractions became known as: the Spicy fraction, rich in monoterpene and sesquiterpene alcohols; the Floral fraction, containing ketones, epoxides and esters; the Ester fraction, predominantly made up of branched and straight chain fatty acid methyl esters; and the Citrusy fraction, composed of a mixture of terpene alcohols, short chain aliphatic alcohols and ketones (Marriott, 2001). Post-fermentation products are currently sold as Pure Hop Aroma and also now include Herbal (herbaceous, green, vetivert odor) and Sylvan (woody, earthy, resinous, pine odor) fractions (Marriott and Parker, 2004). They are supplied dissolved in food grade ethanol and are used at typical dose rates of 50–100 ppb (Marriott, 2001). The impact on hop aroma in beer will greatly depend on interactions with the aroma compounds present in the base beer and must therefore be evaluated in each case (Gardner, 1994; Marriott and Parker, 2004). Hop fractions obtained from different hop varieties also retain distinct aroma profiles due to differences in their chemical composition (Gardner,

1994; Marriott, 2001). Post-fermentation products allow great flexibility in new product development and allow the introduction of specific hop aroma without the changes that occur during wort boiling and fermentation (Marriott, 2001).

Concluding Remarks

Despite more than 50 years of research, the compounds responsible for important odorants in hop essential oil and hop aroma in beer are still not completely understood (Moir, 2000). More research is required to identify character-impact odorants in hop essential oil, determine their sensory impact on beer aroma and ascertain their fate during the brewing process. Identification of important odorants will allow hop breeders to select for varieties containing these compounds. In addition, knowledge of the important odorants for "hoppy" aroma in beer will allow for better quality control and development of new products. The authors contend that MDGC techniques in combination with GC–O are essential to improve our understanding of hop aroma in beer. The impact of identified character-impact odorants must then be confirmed using sensory evaluation.

Summary Points

- Hop essential oil is a complex mixture of volatile compounds from a wide range of compound classes.
- Differences in odor characteristics of hop varieties can be attributed to differences in the composition of the essential oil.
- The composition of the essential oil varies considerably with genetics, geographical location, growth conditions, infection from diseases and attack from pests. The composition alters during storage increasing the complexity due to oxidation, hydrolysis and rearrangements.
- Composition also depends on how the essential oil is isolated prior to analysis.
- Routine analysis of composition is performed by conventional GC but multidimensional techniques using two columns are often required to resolve and identify co-eluting compounds.
- The odor of hop essential oil and hop aroma in beer is due to a complex mixture of contributing volatile compounds.
- Not all character-impact odorants in hops have been identified and hoppy aroma in beer is still not completely understood.
- Hop aroma in beer is complex and complicated by physical, biochemical and chemical changes occurring during brewing and fermentation. This has led to increasing trends to add fractionated hop oils with specific odor characteristics to beer post-fermentation.

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