# Introduction to aroma compounds in foods

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### 1.1 Introduction to aroma

The aroma of food has several functions, not only conveying the essential character of the food and providing variety and interest to what we consume, but also alerting us to rancid and unsafe food, stimulating the appetite as well as providing an emotional link to past experiences. The compounds that are responsible for aroma are the highly volatile, low molecular weight compounds that are present in foods at low levels. Whereas we perceive colour as combinations of three primary colours, and taste as a function of five (or six) basic tastes, we have the ability to detect and recognise thousands of different aromas, each being made up of potentially hundreds of volatile compounds. In the case of complex cooked foods, such as meat and coffee, the number of volatile compounds identified reaches well beyond 1000. The aim of this chapter is to provide an overview of the types of aroma compounds typically identified in food products and to put these into context, highlighting character impact compounds in some of the major food categories.

## 1.2 Sensomics and some definitions

One of the challenges facing flavour chemists is determining which of the many thousands of volatile compounds, that have been identified by increasingly powerful analytical instrumentation, actually contribute to the aroma profile of the food. This cannot be achieved with instruments alone, and methods that employ the human nose as an extremely sensitive detector have been devised by the research groups of Schieberle and Hofmann at the Technische Universität München. This involves the combination of gas chromatography-olfactometry (GC-O), sensory analysis and accurate quantification methods that are discussed in more detail in Chapters 2 and 3. In this chapter, we will define some of the key elements of the sensomics approach as used for the determination of odour-active components in a complex food extract.

## 1.2.1 Gas chromatography-olfactometry

GC-O is essential in determining which aroma compounds are likely to contribute to the aroma of a food, and is the basis for the sensomics approach. After appropriate extraction of the aroma compounds (see Chapter 2), the compounds in the extract are separated by gas chromatography and, as they elute from the column, they are assessed by trained individuals who describe the aroma and estimate its intensity (see Section 3.4, Chapter 3). A very practical review of GC-O has also been published by Delahunty et al. (2006).

## 1.2.2 Aroma extract dilution analysis

Aroma extract dilution analysis (AEDA) involves assessing an aroma extract by GC-O in order to identify all odour-active compounds present in the extract and then repeating the GC-O on a set of serial dilutions until only the most potent aroma compounds are detected. The compounds persisting through the final dilutions are deemed to be those that contribute to the aroma of the food.

#### 1.2.3 Flavour dilution factors

The relative flavour dilution (FD) factor is defined as the number of times the original extract can be diluted before the aroma is lost by GC-O. For example, in a series of dilutions where the original extract is serially diluted on a 1:1 basis, those compounds that lose their aroma after the first dilution have a FD factor of 2, those that lose their aroma after the second dilution have a FD factor of 4 and those that remain for one more dilution have a FD factor of 8. This obviously depends on the concentration of the original extract, but FD factors of 1024 or 2048 are not uncommon, and serial dilutions based on a 1:2 dilution can be used to decrease the number of GC-O assessments required (FD factors become 3, 9, 27, etc.). In practice, most authors use just one or two trained assessors for AEDA, but Ferreira et al. (2002) considered the statistics involved in using several assessors to estimate average FD factors. They recommended that, where a broad range of odorants is present, better efficiency (i.e. minimising the standard deviation with the minimum number of GC-O runs) is obtained by using more assessors and fewer dilutions. Dilutions of 1:10 and a larger pool of assessors is recommended.

## 1.2.4 Stable isotope dilution analysis

The compounds with the highest FD factors are the compounds of interest. However, this does depend on how the extract was obtained. Exhaustive methods such as solvent extraction, or SAFE (see Chapter 2), reflect the composition of the food, but are subject to losses of the most volatile components during work-up and do not account for the relative volatility of the individual components, thus the role of high molecular weight compounds may be exaggerated. For this reason, often the next step is to accurately quantify the most important compounds present (usually  $\sim 10$ –15).

Quantification is discussed in more detail in Chapter 3, but the technique most commonly employed in sensomics is stable isotope dilution analysis. In this case, extraction (whether it be by SAFE or by headspace methods) is carried out after addition of known amounts of isotopically labelled standards of all compounds of interest (where possible), which act as a known reference, against which the compound of interest can be measured. This can often involve some organic synthesis, since only a limited number of isotopically labelled standards are available.

#### 1.2.5 Odour thresholds

A threshold concentration is defined as the concentration at which an individual first perceives the stimulus. For aroma, this can be either a detection threshold – the point at which the individual can sense an aroma, or a recognition threshold – the point at which an individual can recognise the aroma. Because individual aroma thresholds can vary by several orders of magnitude, odour thresholds are normally determined for a number of assessors, and the value quoted is the concentration at which half the assessors can perceive the aroma. Many odour thresholds are quoted in the literature, but care must be taken to ascertain whether these have been determined in oil, water or air, or by GC-O.

## 1.2.6 Odour-activity values

Once the odour threshold of a compound and its concentration in the extract have been determined, this allows calculation of the odour-activity value (OAV). (Note that these two concentrations must be in the same units.) The OAV is defined as the concentration of the aroma compound divided by its odour threshold, so an OAV > 1 indicates that the compound is present above its threshold value and is likely to contribute to the aroma profile. However, OAVs can be misleading since, for many aroma compounds, the perceived intensity is not proportional to the concentration (Doty, 1991), and there is a decelerating relationship as the concentration increases (see Chapter 12). In other words, a doubling of an OAV that is several orders of magnitude above the threshold, may have little impact on the perceived aroma whereas, a doubling of the OAV around threshold could have a major impact.

#### 1.2.7 Recombinates

The FD factor and the odour-activity value (OAV) give different, but complementary, information. However, the only way to ascertain the role of individual compounds in a food is to reconstruct the aroma in a bland but representative base to produce a recombinate. The aroma of the recombinate is usually assessed by a sensory panel and compared to the original extract. (The sensory techniques used specifically for this procedure are discussed in detail in Chapter 12.) If a good match is obtained, this is a good indication that all of the compounds contributing to the aroma have been identified. If not, the hunt continues for the missing components.

#### 1.2.8 Omission tests

By systematic removal of each compound from the recombinate, those compounds that truly have an impact on the aroma profile can be determined. Likewise, the impact of fractional changes in concentration can be assessed to give an idea of the tolerance of the aroma to small changes in the concentration of each volatile. In practical terms, this exercise indicates which of the volatile components are worth targeting for flavour optimisation.

## 1.2.9 Character impact compounds

The volatile profile of most foods contains many odour-active compounds but very few of these actually give character to the food. For example, cooked meat contains hundreds of odour-active compounds (Cerny, 2012), many of which impart generic savoury, roasted, toasted or fried notes that are important for meat aroma, but are also present in snacks, fries, nuts and so on. Others impart seemingly unrelated aromas such as green, rose, mushroom and candy floss (cotton candy). There are only a few compounds that impart a characteristic meaty aroma and the most common examples are 2-methyl-3-furanthiol (62) and bis-(2-methyl-3-furan) disulfide. These are called the 'character impact compounds' of meat because without these, the food would be unrecognisable. Interestingly, the 2-methyl-3-furanthiol gives a generic meaty note to all meats, and species character is given by other character impact compounds. It is also worth noting, however, that not all foods have character impact compounds; their unique character may be attributed to a combination of aroma compounds. There is no character impact compound for wine, for example, although individual wines do contain some very recognisable aromas. Table 1.1 shows a list of character impact compounds in common foods; their structures are discussed in the following sections.

## 1.3 Structure, aroma and occurrence of compounds containing carbon, hydrogen and oxygen

The primary aim of this chapter is to serve as an introduction to the range of volatile aroma compounds that have been identified to date, many using the sensomics approach, and many of which are discussed in more detail in subsequent chapters. This section is dedicated to those compounds that contain just carbon, hydrogen and oxygen. Selected structures are shown in Figure 1.1.

## 1.3.1 Aldehydes

Aldehydes are extremely common components of any food or flavouring and many have a low odour threshold. The straight-chain unbranched aldehydes are ubiquitous. Acetaldehyde is a crucial component of many fruit flavourings, imparting fruity ether notes, whereas the C3–C5 aldehydes (propanal, butanal and pentanal) tend to have a rather chemical/malty/green note to them that is hard to define. The branched chain

Table 1.1 Character impact compounds in common foods

Compound	Characteristic aroma
Vegetables	
Methional (55) 2-Methoxy-3-isobutylpyrazine Hexanal ( <i>E,Z</i> )-2,6-Nonadienal 1-Octen-3-ol (6) 5-Methylthiopentanenitrile (76) 2,4-Dithiapentane (57)	Potato Bell pepper Green beans Cucumber Mushroom Broccoli, cabbage Truffle oil
Fruits	
4-(4-Hydroxyphenyl)butan-2-one (raspberry ketone) (11) 3-Methylbutyl acetate (15) Allyl hexanoate γ-Decalactone (4-decanolide) (17)	Raspberry Pear drops Pineapple Peach
Spices and herbs	
Thymol Eugenol L-Carvone (E)-2-Undecenal (—)-Menthol (27)	Thyme Clove Caraway Coriander Mint
Cereals/grains	
2-Acetyl-1-pyrroline (46) 6-Acetyl-1,2,3,4-tetrahydropyridine (48) 2-Methylimino-3-butanone (43)	Cooked rice, popcorn Bread crust Corn tortilla
Meat	
2-Methyl-3-furanthiol (62) Bis-(2-methyl-3-furan) disulfide ( <i>E,E</i> )-2,4-Decadienal (3) 12-Methyltridecanal 4-Methyloctanoic acid (22)	Cooked meat Aged prime rib of beef Roast chicken Beef tallow Sheep meat
Sweet	
4-Hydroxy-3-methoxybenzaldehyde (vanillin) (41) Dimethyl-4-hydroxy-3[2 <i>H</i> ]furanone (furaneol) (32) ( <i>E</i> )-5-Methyl-2-hepten-4-one (filbertone) Phenylacetaldehyde 2,3-Butanedione (diacetyl) (10)	Vanilla Candy floss, burnt sugar Hazelnuts Rose Buttery, creamy, butterscotch

C5 aldehydes, namely, 2- and 3-methylbutanal (1), have low odour thresholds and are found in most cooked foods as well as in fresh fruits and vegetables. They can impart a very chemical note, particularly when assessed by GC-O but, on dilution, provide malty, bitter cocoa notes that are essential for many malty and chocolate aromas.

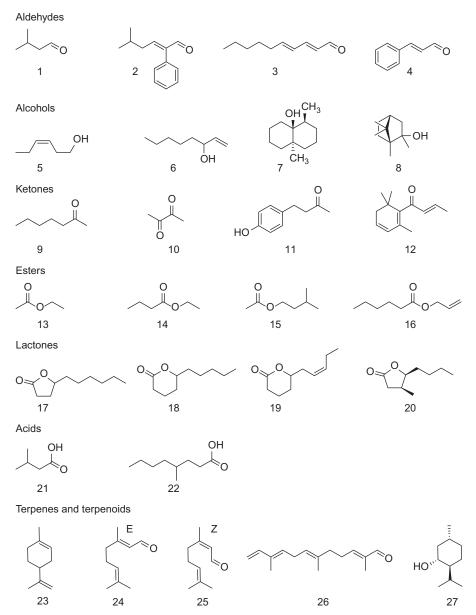


Figure 1.1 Examples of odour-active compounds containing carbon, hydrogen and oxygen.

At C6, the aldehydes become characteristically green. Hexanal imparts a greenbean and cut-grass character when assessed alone, and contributes to the fresh green aroma of green beans and green apples for example, as well as the leafy and less ripe notes in many fruit aroma profiles. It has been shown, for example, that hexanal and related C6 aldehydes decrease as nectarine fruit matures on the plant (Engel et al., 1988b). The use of AEDA has shown hexanal amongst the compounds with the highest FD factors found in tomatoes (Mayer et al., 2003), pomegranate (Cadwallader et al., 2010), Pontianak oranges (Fischer et al., 2008) and apples (Fuhrmann and Grosch, 2002) and undoubtedly this is the case for many other fruits and vegetables. Hexanal plays a major role in sweet cherries (Sun et al., 2010) where it, and the other C6 aldehydes and alcohols, comprise the major components of the volatile profile. Since it is also formed during thermal processing, it is often detected by GC-O in cooked foods. Indeed, there are few instances in the literature where hexanal is not detected by GC-O, having been reported with high FD factors in many food products, including soy milk (Kaneko et al., 2011), Turkish olive oil (Kesen et al., 2013), wild mushroom (Miyazawa et al., 2010) and Beijing roast duck (Chen et al., 2009).

As the chain length increases beyond C6, the aldehydes take on a dual character and have both fruity/floral and fatty descriptors, depending on the concentration present and, of course, the individual who is perceiving them. Octanal still has a fruity note with a fatty character, and decanal is characteristically orange with fatty undertones. However, as the chain gets longer, the character becomes, without doubt, fatty (e.g. dodecanal). Aldehydes are very common in all types of fruits, and the longer chain aldehydes, being lipid-derived, are abundant in meats, fish and fried snacks. Isobranched and ante-isobranched aldehydes, for example, 12-methyltridecanal, have been identified as character impact components of boiled beef (Gasser and Grosch, 1988) and were identified as one of the compounds with the highest OAV in stewed beef (Guth and Grosch, 1994). However, in a recombinate, the addition of this compound had little effect on the aroma.

The unsaturated aldehydes, which exist as both E- and Z-isomers, tend to have lower odour thresholds and are often character impact compounds, with the shorter chain analogues providing green aromas. Cis-3-hexenal has an odour threshold of 0.25 µg/kg in water (Buttery et al., 1971) and gives a particularly fresh note to tomatoes, but is also important in pomegranate, oranges and apples (as above) as well as freshly picked coriander (Cadwallader et al., 1999) and bay (Kilic et al., 2004). It is readily isomerised to trans-2-hexenal (Chapter 15, Figure 15.2), which is also described as green, but secondary descriptors such as bitter and stink-bug indicate a less clean fresh green aroma, and the odour threshold is about 100 times higher than for the cis-3-isomer. The decline in the perceived freshness of fresh fruit during storage is often attributed to this interconversion. At C7, the aroma starts to become fatty with 2-heptenal being described as green and fatty. The far more powerful isomer, cis-4-heptenal, is described as potatoey and also is likened to linseed oil and is very typical of lamb fat. It is odour-active in many food products, particularly meat, but seems to give the characteristic fishy aroma to fish and seafood, including hake (Triqui, 2006), turbot (Prost et al., 1998), grey mullet (Cayhan and Selli, 2011), mussels (Le Guen et al., 2000) and salt-fermented anchovy (Cha et al., 1999). The C9 (Z)-6-nonenal gives a cucumber note to immature Charentais melons (Lignou et al., 2014), whereas from C10 (2-decenal) to C14 (2-tetradecenal), the (E)-2-alkenals are abundant in coriander and give the typical aroma of coriander, particularly (E)-2-undecenal.

Branched 2-alkenals are often formed as a result of the aldol condensation between two aldehydes (see Section 8.2.1.3, Chapter 8); for example, 5-methyl-2-isopropyl-2-hexenal (woody, lavender) or 5-methyl-2-phenyl-2-hexenal (2), which is otherwise known as cocoa hexenal and is very important in chocolate aroma.

Many aldehydes with two double bonds (i.e. alkadienals) have low odour thresholds. The 2,4-alkadienals are particularly important in fried aromas and have a characteristic fried chip note when assessed by GC-O. (E,E)-2,4-Decadienal (3) imparts a characteristic fried note, although some assessors describe the same note as lemon or citrus. The aroma threshold in water is 0.2  $\mu$ g/kg (Belitz et al., 2004) and it is also reported to provide the species character in fried chicken (Gasser and Grosch, 1990). The C9 analogue, 2,4-nonadienal, imparts a similar fried note, but a shift in the position of the double bond to (E,Z)-2,6-nonadienal gives the character impact compound responsible for the aroma of cucumber. Lipid-derived aldehydes are discussed in more detail in Chapters 8 and 9.

Related closely to the 2,4-alkadienals is a series of *trans*-4,5-epoxy-(E)-2-alkenals, many of which have been found in food. They all have a metallic odour, but the most potent of these is *trans*-4,5-epoxy-(E)-2-decenal, which is often reported to be amongst those compounds with high FD factors for example in soy milk (Kaneko et al., 2011), potato chips (Kasuga et al., 2008) and in black tea (Kumazawa et al., 2008) where both *cis* and *trans* isomers were reported to impart sweet juicy notes to the black tea infusion. The odour threshold, which is incredibly low (6 x  $10^{-7}$  µg/kg), was determined by GC-O by Buettner and Schieberle (2001).

Aldehydes containing an aromatic ring such as benzaldehyde (cherry, almond), phenylacetaldehyde (rose, honey) and cinnamaldehyde (4) (cinnamon) are important components of foods and flavourings. The most ubiquitous of all aroma compounds, vanillin, is an aldehyde with the chemical name 4-hydroxy-3-methoxybenzaldehyde (41).

Aldehydes very readily react with alcoholic solvents (e.g. ethanol and polyethylene glycol) to form acetals (Elmore et al., 2014). This happens readily at room temperatures, and acetals are abundant in alcoholic beverages, as well as being formed in many alcohol-based flavourings. The aroma of the acetal is often similar to that of the corresponding aldehydes, but they tend to be less potent. However, the reaction is reversible and the equilibrium can be shifted toward the aldehyde in dilute solution.

#### 1.3.2 Alcohols

Alcohols are also abundant in foods and flavourings, but their contribution to aroma tends to be less than for the aldehydes. The straight-chain alcohols are abundant in fruits, often increasing with maturity. 2-Methyl-1-propanol can be associated with brown apples and bruised fruit, whereas the longer chain alcohols can be very soapy (e.g. 1-nonanol). Introducing a double bond into the chain renders them more interesting from an organoleptic point of view, with *cis*-3-hexen-1-ol (5) imparting a characteristic green leaf note, 1-octen-3-ol (6) being a character impact compound in mushrooms. Geosmin (7) and 2-methylisoborneol (8) – both bicyclic alcohols – impart earthy, musty notes and have very low odour thresholds. Geosmin gives a characteristic note to beetroot (Murray et al., 1975) and baby corn (Mottram et al., 2011) where its presence is attributed to the action of actinomycetes in the soil in which the baby corn was grown. These compounds are often implicated as taints in drinking water, for example.

#### 1.3.3 Ketones

The straight-chain methylketones, containing one carbonyl group in the 2-position, e.g. 2-heptanone (9), impart both a blue cheese and a fruity pear aroma, whereas 3-octanone produces earthy, mushroom notes. The  $\alpha$ -dicarbonyl compounds such as 2,3-butanedione (10) (diacetyl) and 2,3-pentanedione have far lower thresholds and provide buttery, creamy notes in many cooked foods. Some of the more structurally complex ketones have a key role in aroma: For example, (*E*)-5-methyl-2-hepten4-one (filbertone) is a character impact compound of hazelnuts (Matsui et al., 1998), 6,10-dimethylundeca-5,9-dien-2-one (geranyl acetone) is present in many fruits and imparts a floral rose aroma and 4-(4-hydroxyphenyl)butan-2-one (11) (raspberry ketone) has been isolated from raspberries and imparts a characteristic sweet, raspberry milkshake aroma (Larsen and Poll, 1990).

The carotenoid-derived ketones such as  $\beta$ -ionone (also important in raspberries) and  $\beta$ -damascenone (12) provide, respectively, a pippy note in orchard fruits and deep juicy notes in, for example, berries, tomatoes and apples. Both were found to give woody notes to red peppers (Jun and Kim, 2002) and carrots (Buttery and Takeoka, 2013) where linden ether (3,6-dimethyl-2,4,5,7a-tetrahydrobenzofuran) was found to have the highest FD factor.

#### 1.3.4 Esters

Esters are fundamental to the aroma of most fruits, comprising the major proportion of the volatile compounds in, for example, melons, apples, pineapple and strawberries. The most abundant is ethyl acetate (13), present in most ripe or ripening fruits. For example, as strawberries ripen, there is a marked increase in the abundance of acetates, promoted by an increase in the alcohol acyl transferase that brings about the esterification of acyl CoAs and alcohols (Gonzalez et al., 2009). Ethyl esters are major components of fruit aroma, particularly ripe fruit where the production of ethanol has boosted their formation. Ethyl butanoate (14) resembles strawberry aroma whilst ethyl hexanoate is characteristic of fresh pineapple and more tropical fruits. The longer chain ethyl esters become soapy, cheesy and waxy. Some esters can be quite characteristic of specific fruits: 3-methylbutyl acetate (15) is characteristic of pear or pear drops, allyl hexanoate (16) is typically pineapple, *cis*-3-hexenyl butanoate imparts the green leafy aroma of the parent alcohol, and the C9 esters are important for melon aroma.

Esters also contribute to the more delicate aromas found in cured ham (Theron et al., 2010) and some cheeses. Ethyl butanoate (14) and ethyl hexanoate are key odorants in Parmigiano Reggiano (Qian and Reineccius, 2003) and blue cheese (Qian et al., 2002).

#### 1.3.5 Lactones

Lactones are cyclic (or intramolecular) esters that are potent aroma compounds formed from the corresponding hydroxy acid. Those based on a furan ring are  $\gamma$ -lactones (e.g.  $\gamma$ -octalactone (or 4-octanolide) and  $\gamma$ -decalactone (17) (4-decanolide)) and tend to

impart peachy, creamy and coconut aromas. Consequently, they are very popular in tropical flavours; for example,  $\gamma$ -decalactone (17) is the major lactone in both peaches and nectarines (Engel et al., 1988a) and has a threshold of 11 µg/kg in water. The odour thresholds of lactones decrease significantly as the number of constituent carbons increases.

The  $\delta$ -lactones, which are based on a pyran ring, are less odour-active than their furanyl isomers. Several lactones were identified in an extract of sweet cream butter, of which  $\delta$ -decalactone (18) had the highest OAV and was believed to contribute to the sweet cream aroma.  $\gamma$ -Nonalactone,  $\delta$ -decalactone and the two unsaturated lactones (5-hydroxyoct-2-enoic acid lactone and 5-hydroxydec-2-enoic acid lactone) were found to have relatively high OAVs in milk chocolate (Schnermann and Schieberle, 1997), and all but the 5-hydroxydec-2-enoic acid lactone were found in the cocoa that had been used in the production of the chocolate. Jasmine lactone (19) provides a floral petal-like aroma to green tea (Katsuno et al., 2014), and lactones also make a significant contribution to the volatile profile of Bourbon whisky, with  $\delta$ -nonalactone having a FD factor of 2048, and *cis*-3-methyl-4-octanolide (20) (which is also known as whisky lactone) and  $\gamma$ -decalactone (17) also contributing to the aroma.

## 1.3.6 Carboxylic acids

Short-chain carboxylic acids are pungent compounds. The aroma of 3-methylbutanoic acid (21), for example, can be described as Parmesan cheese, but is also very reminiscent of vomit. Short-chain acids are present in many food products and give characteristic notes to balsamic vinegar (Ugliano et al., 2003) and cheese (Qian and Reineccius, 2003). Acetic, butanoic, hexanoic, octanoic and decanoic acids all had high FD factors in Parmigiano Reggiano (Qian and Reineccius, 2003). The longer chain acids are less intense, imparting creamy or, in the case of octanoic acid, a blue cheese note. However, with a methyl or ethyl substituent, 4-methyloctanoic (22) and 4-methylnonanoic acids are believed to give character species to sheep meat (Sutherland and Ames, 1996) and also to goat cheeses (Le Quere et al., 1996).

## 1.3.7 Terpenes and terpenoids

Terpenes, terpenoids and sesquiterpenes are major components of essential oils and are responsible for the characteristic aroma profile of many fruits (particularly citrus), herbs and spices. They are biosynthesised in plants from units of isoprene ( $C_5H_{10}$ ) and can be linear, cyclic or polycyclic; however, those that are responsible for odour tend to contain two or three isoprene units (monoterpenes and sesquiterpenes, respectively). One of the most ubiquitous is limonene (23), which has a weak orangey citrus peel aroma but it is not a powerful odorant. It is more commonly used as a solvent, a cleaning agent or as a starting material for the production of other natural flavour compounds. Other less abundant terpenes such as  $\alpha$ -thujene (woody) and sabinene (citrus) are present in fruits and spices, and the sesquiterpenes provide more interesting aromas with  $\alpha$ -valencene imparting classic citrus notes and both farnesene and

humulene a woody spicy note. Pinene, myrcene and ocimene are major components of basil aroma.

Many terpenes can be readily oxygenated, and these are technically terpenoids, rather than terpenes. The terpenoid alcohols such as nerol and geraniol (which are cis/trans isomers of each other, respectively) and citronellol and linalool (both existing as two isomers) provide delicate lemon, rose and violet aromas, which are abundant in herbs, spices and fruits and are essential to many flavourings. The closely related terpene aldehydes are also extremely important in fruit flavouring. Citral is popular in the flavour industry and exists as a mixture of the (E)- and (Z)-isomers, which are called geranial (24) and neral (25), respectively. Perillaldehyde has a herbal spicy, cumin, citrus aroma and is often incorporated in flavourings whilst sinensal (26) gives a characteristic orange aroma.

Terpineol exists as four different isomers, but the most abundant is  $\alpha$ -terpineol. It is present in many fruits, herbs and spices and has been shown to contribute to the aroma profiles of orange (Chen et al., 2012), but being the oxidation product of limonene, it increases during storage (Perez et al., 2004) and can be indicative of flavour deterioration.

(–)-Menthol (27) is the most familiar of terpenoids, not only providing the classic minty note, but also activating the cold-sensitive receptors in the oral cavity to produce a cooling effect (Eccles, 1994). L-Carvone is of particular interest because it exists in two enantiomeric forms with different aroma properties. *R*-(–)-carvone smells of spearmint (and is extracted from *Mentha spicata* L.) whilst the *S* enantiomer resembles caraway, accounting for 50% of the essential oil in caraway seeds.

## 1.4 Structure, aroma and occurrence of oxygen heterocycles and phenols

This section is dedicated to cyclic compounds containing oxygen. Selected structures are given in Figure 1.2.

#### 1.4.1 Furans and furanoids

Most furans are formed during the thermal processing of ingredients. Those that are found in fresh produce tend to be furanoid terpenes, such as linalool oxide (28) which imparts a floral herby note but tends to appear during storage and is indicative of oxidation. Theaspirane is another bicyclic furanoid, derived from carotenoids, which has been found in tea, grapes, wine and sherry (Collin et al., 2011). It exists as four diastereoisomers, each with slightly different aroma properties: the 2R,5R and the 2S,5S isomers have camphoraceous notes, whilst the 2R,5S is blackcurrant (29) and the 2S,5R is more like naphthalene (Collin et al., 2011). Eucalyptol (30) (6-membered pyran rather than a furan) has a camphor-like aroma and is the major aroma component of the bay leaf, along with eugenol, cis-3-hexenal, (E)-isoeugenol and linalool (Kilic et al., 2004).

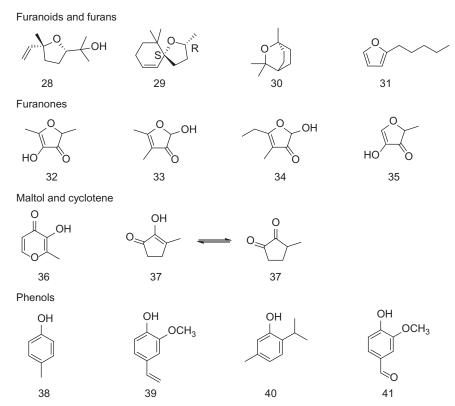


Figure 1.2 Examples of odour-active oxygen heterocycles and phenols.

Furans derived from thermal processing, can be derived from lipid oxidation or the Maillard reaction. The former tend to be derived from unsaturated lipids (Chapter 9) and their aromas are fairly powerful, but uncharacteristic, having been described with a multitude of different terms. 2-Ethylfuran and 2-pentylfuran (31) are common examples of alkylfurans, being indicative of the oxidation of  $\omega$ -3 and  $\omega$ -6 fatty acids, respectively.

#### 1.4.2 Furanones

Furans derived from the Maillard reaction tend to be oxygenated and some have low odour thresholds, for example 2-acetylfuran, which imparts sweet, nutty notes. However, there is a series of furanones that impart sweet aromas and have very low thresholds. 2,5-Dimethyl-4-hydroxy-3[2*H*]furanone (32) (furaneol) is perhaps the most ubiquitous of these, being found in both raw and cooked foods. It has an odour threshold in water of 60 µg/kg (Buttery et al., 1994) and a threshold in air of 1–4 µg/kg (Schieberle and Grosch, 1994) and provides sweet caramel notes to strawberry (Schieberle and Hofmann, 1997), where its methoxy analogue (mesifuran) also plays

a key role in flavour. Furaneol also makes an important contribution to the flavour of tomatoes (Mayer et al., 2008), pineapple (Tokitomo et al., 2005), tea (Schieberle and Schuh, 2006), pet food (Didzbalis et al., 2007) and beer (Fritsch and Schieberle, 2005). The isomeric 3-hydroxy-4,5-dimethyl-2(5H)-furanone (33) (sotolone) also imparts a powerful spicy note, which becomes maple-like on dilution with a recognition threshold in water of 1 µg/kg (Zeller and Rychlik, 2006). Using AEDA, it has been found to contribute to the aroma of many intensely savoury foods such as beef and pork gravies (Christlbauer and Schieberle, 2009), pan-fried mushrooms (Grosshauser and Schieberle, 2013), miso paste (Kumazawa et al., 2013), soy sauce (Kaneko et al., 2012; Steinhaus and Schieberle, 2007) and also Dornfelder wine (Frank et al., 2011). The ethyl analogue of sotolone (34) (5-ethyl-3-hydroxy-4-methyl-5H-furan-2-one, maple furanone or abhexon) has a powerful sweet caramellic and maple aroma and was found to be important in coffee (Sanz et al., 2002), whereas the ethyl analogue of furaneol was found to contribute to the aroma of emmentaler cheese (Preininger and Grosch, 1994) and soy sauce (Steinhaus and Schieberle, 2007). Norfuraneol (35) (2methyl-4-hydroxy-3(2H)-furanone) possesses one less carbon than furaneol and imparts similar sweet notes, but has an odour threshold of 23,000 µg/kg (Buttery et al., 1994).

### 1.4.3 Pyranones

Closely related to the furanones are the pyranones, derived from a similar route and imparting powerful sweet aromas. Maltol (36) (3-hydroxy-2-methyl-4(*H*)-pyran-4-one) is the best known of these, imparting a sweet fruity note, but it has a relatively high odour threshold (similar to norfuraneol). 2-Hydroxy-3-methyl-2-cyclopenten-1-one (37) (cyclotene) (not a furan, but organoleptically classed in the same category as the furanones and pyranones) has a strong caramellic note, is found to be important in soy sauce (Kaneko et al., 2012) and is often used in commercial flavourings, particularly toffee and caramel. It exists as a number of tautomeric forms of which two are shown.

#### 1.4.4 Phenols

Many phenols are particularly odour-active compounds. The methylphenols, for example *p*-cresol (38), are particularly phenolic and smoky and 2-ethylphenol is reminiscent of manure. However, the more complex phenols have more desirable aroma characteristics. The guaiacols (methoxyphenols) are described with many terms; for example, 4-methylguaiacol is described as sweet, candy, vanilla, leather, spicy and smoky, whereas 4-ethylguaiacol and 4-vinylguaiacol (39) have a similar range of descriptors but also include a meaty bacon character. The terpene-derived thymol (40) and eugenol are examples of phenols providing character impact notes to thyme and clove, respectively. Vanillin (41), the most popular of all flavouring substances and the key constituent of vanilla (*Vanilla planifolia* L.), is a phenol with that characteristic sweet vanilla aroma with smoky undertones. The most odour-active phenols

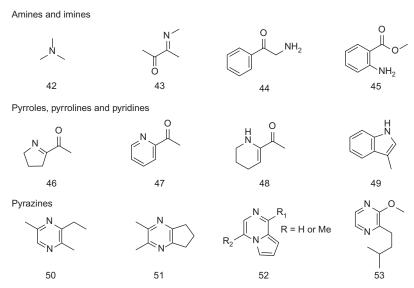
are the chlorophenols and bromophenols (and related anisoles) which are often implicated as taints. Examples are shown in Table 4.2 in Chapter 4 where they are discussed in more detail.

## 1.5 Structure, aroma and occurrence of nitrogen compounds

Nitrogen-containing aroma compounds are relatively few rare in nature, but abundant in cooked foods where the Maillard reaction is responsible for generating series of nitrogen heterocycles. Selected examples are shown in Figure 1.3.

#### 1.5.1 Amines

The simplest nitrogen-containing compounds are the amines, which are typically fishy and often impart an unpleasant ammoniacal note. This holds for all the short-chain amines, of which trimethylamine (42) has the lowest odour threshold (Angelino, 1991), as well as for phenylethylamine. 2-Methylimino-3-butanone (43) (Karahadian and Johnson, 1993) and 2-aminoacetophenone (44) (Buttery and Ling, 1995) were reported to be character impact compounds of corn tortilla chips, and 2-aminoacetophenone (44) has since been reported to be odour-active in many foods. Methyl anthranilate (45) is one of few amino compounds that has a desirable fruity character. The heterocyclic nitrogen compounds are far more interesting from an organoleptic point of view.



**Figure 1.3** Examples of odour-active nitrogen compounds.

## 1.5.2 Pyrroles, pyrrolines and pyridines

Pyrroles are typically associated with roasted, cooked and burnt notes generated in the Maillard reaction and are found in most cooked foods. 2-Acetyl-1-pyrroline (46) is the character impact compound in cooked fragrant rice (such as Basmati and Jasmine) and is also important in fresh bread and popcorn, imparting a popcorn and cracker-like aroma with a threshold in water as low as 0.007 µg/kg (Buttery et al., 1988). It has a high FD factor in many cooked foods and has recently been shown to give the characteristic toasty notes found in roasted duck liver (Strasser and Schieberle, 2014) as well as contributing to the aroma of pan-fried mushrooms (Grosshauser and Schieberle, 2013) and pistachios (Acena et al., 2011). The 2-propionyl analogue is similarly odour-active, but extending the side chain by one or more carbon atoms pushes the odour threshold up by several orders of magnitude. The related 2-acetylpyrrole has a caramel, nutty aroma but an odour threshold in water several orders of magnitude higher than its more saturated counterpart (170,000 µg/kg; Buttery et al., 1988). 2-Acetylpyridine (47) also imparts a bready, popcorn-like note, and 6-acetyl-1,2,3,4-tetrahydropyridine (48) imparts a bread-crust aroma and was found in rye bread crust (Schieberle and Grosch, 1987). These 'extraordinary' Maillard flavour compounds are reviewed by Adams and De Kimpe (2006).

Indole is a powerful nitrogen-containing compound that, to some individuals, imparts a pleasant floral note. It has a high FD factor in green tea (Hattori et al., 2003; Katsuno et al., 2014) and pomelo (Cheong et al., 2011) where its contribution is a fragrant, floral or green note. However, it also imparts an unpleasant faecal note that is often associated with boar taint. The related 3-methylindole (49) (or skatole) is present in the aroma of lilies (Smith and Meeuse, 1966), which for some people is perceived as pleasant and floral but for others is objectionable. In conjunction with  $5\alpha$ -androst-16-en-3-one, it is partly responsible for the highly objectionable boar taint found in meat from uncastrated male pigs (Chen et al., 2006). It also contributes to the aroma of pasture-fed sheep (as opposed to grain-fed sheep), which is often seen as a negative attribute (Young et al., 2003). It was also found to contribute a faecal note to stored non-fat dry milk (Karaguel-Yueceer et al., 2002). These indoles are products of the microbial degradation of amino acids, one example being the increase during cold storage of mussels. After four days, the mussels become unpalatable (Erkan, 2005).

## 1.5.3 Pyrazines

Most pyrazines are generated during thermal processing of foods at temperatures  $>100\,^{\circ}$ C. The simple unsubstituted or monosubstituted pyrazines have a roasted, biscuity aroma and relatively high aroma thresholds, but as the substitution increases, the odour threshold decreases. 2-Ethyl-3,6-dimethylpyrazine (50), for example, has a potatoey, woody, earthy aroma and a threshold of 0.4  $\mu$ g/kg (Karahadian and Johnson, 1993), whereas the 2-ethyl-3,5-dimethylpyrazine has a sweeter, more chocolate character (1  $\mu$ g/kg) and 2,3-diethyl-5-methylpyrazine has a roasty potato note. 2-Ethyl-3,6-dimethylpyrazine (50) (earthy) and 2,3-diethyl-5-methylpyrazine (potato

chip-like) both have high FD factors in cocoa mass (Liu et al., 2013) but are also important in meat (Cerny and Grosch, 1994). Acetylpyrazines tend to impart a nutty note, whereas the more complex pyrazines, for instance, 6,7-dihydro-2,3-dimethyl-5(*H*)-cyclopentapyrazine (51) and the pyrrolopyrazines (52), have been found in grilled, roasted and burnt meat, imparting the characteristic roast note to their aroma profile (Flament et al., 1978).

The pyrazines found in uncooked potatoes and vegetables are methoxy-substituted, and powerful odorants. For example, 2-methoxy-3-isobutylpyrazine (53) is the character impact compound in green bell pepper and is identified as the most potent odorant in raw French beans (Hinterholzer et al., 1998). The homologous 2-isopropyl-3-methoxypyrazine is known as bean pyrazine because it imparts earthy, pea and beany notes to soy milk (Kaneko et al., 2011), earthy notes to potato (Buttery and Ling, 1973) and was also found to be odour-active in parsley leaves (Jung et al., 1992) and gravies containing vegetables (Christlbauer and Schieberle, 2009). The thresholds for these powerful odorants are 0.002 µg/kg (Seifert et al., 1970).

## 1.6 Structure, aroma and occurrence of sulfur compounds

Sulfur compounds are incredibly important as a group of flavour compounds, so much so, that Chapter 5 is dedicated to their thermal generation pathways and more examples are shown in Tables 5.1–5.3 in Chapter 5. A comprehensive review of volatile sulfur compounds in plants has been published by Iranshahi (2012). Sulfur compounds are exceptionally odour-active and are often present at levels below the analytical detection thresholds yet, still perceived by the nose. They are responsible for tropical fruity notes, as well as meaty, coffee and vegetable aromas and are diverse in their chemical nature. Selected structures are shown in Figure 1.4.

#### 1.6.1 Sulfides

The simple sulfides (dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide (54)) and the parent thiol (methanethiol) make an important contribution to cooked aromas, despite the fact that individually their aromas are fairly objectionable and sulfurous. Dimethyl sulfide is important for fruit flavours, but also at certain concentrations gives the smell of the sea (Haas, 1935; Web, 2007) as well as sweet corn and asparagus aromas. Dimethyl trisulfide is the major cause of off-flavour in overcooked *Brassica* vegetables. Thermal degradation of the naturally occurring *S*-containing amino acid, *S*-methyl-L-cysteine, and its sulfoxide were shown to make a substantial contribution to the aroma of cooked *Brassica* and *Allium* vegetables, however, the degradation pathways of the two precursors were different. Dimethyl disulfide was the predominant volatile generated from both precursors, whereas dimethyl trisulfide, dimethyl thiosulfinate, dimethyl thiosulfonate and 2-methylthiopyridine were identified as

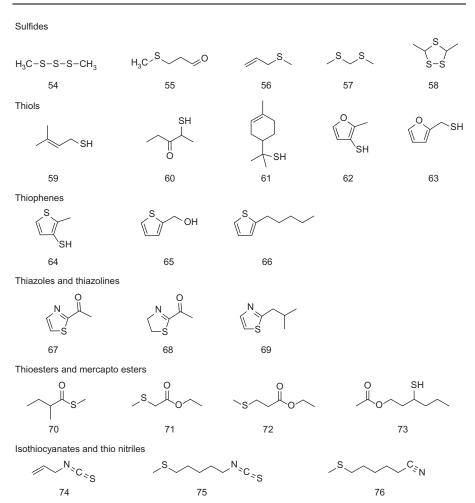


Figure 1.4 Examples of odour-active sulfur compounds.

odour-active breakdown products arising from the *S*-methylcysteine sulfoxide (Kubec et al., 1998).

3-Methylthiopropanal (55) (methional) is a very potent baked or boiled potato character impact compound found in potatoes but also shown to have a high FD factor in many other foods. Allyl and 1-propenal sulfides, and the respective di- and tri-sulfides, are present in large amounts in garlic and onion, respectively and are discussed in Chapter 5. One key example is allyl methyl sulfide (56), which is important in garlic aroma, whereas 2,4-dithiapentane (57) is the character impact compound of truffle oil (Bellesia et al., 1996). Various cyclic sulfides are often found in cooked meat, including 3,5-dimethyl-1,2,4-trithiolane (58) in beef (Werkhoff et al., 1993) and goat meat (Madruga et al., 2009), and the 3-methylbutyl derivatives (3,5-di-isobutyl-1,2,4-trithiolane and 5,6-dihydro-2,4,6-tri-isobutyl-4H-1,3,5-dithiazine) have been

synthesised and shown to impart a typical bacon note (Shu et al., 1985). Meat aroma, reviewed recently by Parker (in press), contains many sulfide structures based on the furanthiol (62) and discussed in more detail below.

#### 1.6.2 Thiols

Alkylthiols such as methanethiol, ethanethiol and propanethiol have low odour thresholds and sulfurous vegetable-like aromas. Recently, 3-methyl-2-butene-1-thiol (59), which has long been known to be a sweaty, skunky aroma compound usually associated with the off-flavour of light-struck off-beer (Gros and Collin, 2012), was found to make an important contribution to the aroma of wine (San-Juan et al., 2012). It was also found to be one of the most odour-active compounds in pan-fried sesame (Tamura et al., 2011), in this instance being described as meaty and sulfurous.

As a series, the mercaptoketones are significant odour-active compounds. Mercaptopropanone imparts a pickled, meaty note (Guentert et al., 1990), whereas 2-mercapto-3-pentanone (60) and particularly 4-methyl-4-merapto-2-pentanone impart a strong blackcurrant or cat's pee type aroma. Both 3-mercaptohexanol and 3-mercaptohexyl acetate have been shown to give a strong blackcurrant aroma to red wine (Rigou et al., 2014). A tropical mango note is obtained from the cyclic thiol 2,7,7-trimethylbicyclo[3.1.1]heptane-2-thiol (mercaptopinane) and intense grapefruit notes are obtained from both enantiomers of *p*-menth-1-en-8-thiol (61), which have odour thresholds in water of < 0.0001  $\mu$ g/kg (Demole et al., 1982). Recently, the odour-active compounds in durian fruit have been reported (Li et al., 2012) and many of these were thiols, dithiohemiacetals or dithioacetals based on 1,1-dithioethane, which imparted roasted onion aromas characteristic of the durian fruit.

Two furanthiols are discussed in detail in Chapter 5: 2-methyl-3-furanthiol (62) and 2-furanmethanethiol (63). The former is a character impact compound of meat with an odour threshold determined by GC-O of 0.005 μg/kg (Gasser and Grosch, 1988) whereas the latter, which is also important in meat aroma, has more roasted, toasted notes, and can have a coffee-like character. These thiols are reported as odour-active in many food products, including oat flakes (Klensporf and Jelen, 2008), coffee (Sanz et al., 2002) and sesame (Schieberle, 1996), and 2-methyl-3-furanthiol also contributes to the flavour changes caused by heating orange juice (Kumazawa et al., 2007; Ruiz Perez-Cacho et al., 2007). Upon oxidation, these thiols form potent disulfides, which are also extremely odour-active, including bis(2-methyl-3-furan) disulfide with a threshold of 0.00002 μg/kg (Buttery et al., 1984) and 2-methyl-3-furan methyl disulfide. Many other potent sulfur compounds are formed when thiols and disulfides interact with each other. Thirty such compounds were reported by Mottram et al. (1995), imparting meaty, oniony and roasted notes.

## 1.6.3 Thiophenes

These sulfur-heterocycles are formed during thermal processing, particularly when cysteine levels are high, providing sulfur for their formation. The aroma of the simple thiophenes is unremarkable, often described as sulfurous and is generally not very

potent. One exception is 2-methyl-3-thiophenethiol (64), which has a roasted meat character and has been found in pan-fried sesame seeds (Tamura et al., 2011). It is the thiophene analogue of the highly potent 2-methyl-3-furanthiol (62). Coffee contains many sulfur compounds. Kahweofuran is a bicyclic thiophene found in coffee, imparting a roasty, smoky and sulfurous aroma, whereas thiophenemethanol (65) imparts a coffee-like aroma. The longer chain alkylthiophenes, such as 2-pentylthiophene (66) and alkylthiazoles, have relatively little aroma and seem to act as a sink for excess hydrogen sulfide (Elmore et al., 2000).

#### 1.6.4 Thiazoles and thiazolines

Also formed during the Maillard reaction, thiazoles tend to give cooked, roasted and toasty notes. Many substituted thiazoles have been identified in cooked food and potatoes (Buttery and Ling, 1974), but the thiazole that appears most frequently is 2-acetylthiazole (67). It imparts a nutty, roasted, popcorn aroma, and the related compound, 2-acetyl-2-thiazoline (68), has a lower threshold and the aroma of freshly baked bread. 2-Isobutylthiazole (69) is present in raw tomatoes and gives the characteristic green and viney note to tomatoes (Krumbein and Auerswald, 1999).

### 1.6.5 Thioesters and mercapto esters

These esters are intense and objectionable in concentrated form, but when diluted sufficiently, give the characteristic fruity and tropical aromas to many tropical fruits. S-Methyl 2-methylbutanethioate (70), ethyl (methylthio)acetate (71) and ethyl 3-(methylthio)propanoate (72) were shown to be odour-active in ripe Charentais melons (Lignou et al., 2014) and seemed to contribute to the ripe aroma of the melon. 3-Mercaptohexyl acetate (73) has been found to be important in guava fruit (Sinuco et al., 2010), and low levels of many sulfur compounds are important in wine (San-Juan et al., 2010).

## 1.6.6 Isothiocyanates

Isothiocyanates are hydrolysis products of glucosinolates, secondary plant metabolites that are found in high concentration in *Brassica* vegetables. The glucosinolate compounds have bitter taste characteristics. Once hydrolysed by the myrosinase enzymes that co-exist in the plant, they generate a range of bioactive compounds as well as a pungent aroma (Ghawi et al., 2012). The breakdown products can include isothiocyanates and thiocyanates as well as nitriles and epithionitriles, depending on the reaction conditions. It is the isothiocyanates that are known to give both the pungent aromas and the beneficial bioactive effects. In cooked cauliflower, allyl isothiocyanate (74) was found to be a key odorant, contributing pungent, black mustard-like notes (Engel et al., 2002). Methyl thiocyanate, butyl isothiocyanate, 2-methylbutyl isothiocyanate and sulfides have been found to be important in broccoli aroma (Jacobsson et al., 2004). In salad rocket (*Eruca sativa*) two isothiocyanates and a thionitrile were

noted to be abundant odour-active compounds (4-methylthiobutyl isothiocyanate, 5-methylthiopentyl isothiocyanate (75) and 5-methylthiopentanenitrile (76)) (Jirovetz et al., 2002).

### 1.7 The future of flavour research

This chapter covers only a fraction of the thousands of aroma compounds that have been isolated and identified in food during the last 60 years. After the development of capillary GC–MS in the 1950s, there was an exponential increase in the number of novel odour-active compounds discovered. Isolation of new compounds will continue as instrument sensitivity continues to improve but, these days, the discovery and authentication of new compounds is a less common achievement. However, there is still much to be achieved in understanding the role of these compounds in our perception of flavour, particularly how mixtures of aroma compounds behave. This approach has been pioneered in wine (Ferreira, 2012).

The sensomics approach is the best method to date to identify those compounds which play an active role in aroma perception and will continue to elucidate new compounds. The approach also lends itself to isolation of novel taste components (see Chapter 15), which is a rapidly expanding area as much less is known about tastants. However, taste and aroma do not function independently, and there is increasing evidence of multimodal interaction where the presence of a tastant can influence the perception of the aroma and vice versa (see Chapters 12 and 13).

Although we have identified the key aroma compounds responsible for many foods and beverages, there is still a great deal to be discovered as to how they are formed in complex food matrices, how they behave in mixtures, how they behave in the mouth and how our brain interprets such complex signals to give us the overall perception of flavour.

## 1.8 Further reading

This chapter can serve only as an introduction to the range of volatile compounds that have been identified to date, and for detailed information, readers are directed to comprehensive standard reference books. A systematic and exhaustive list of all compounds found up until about the year 2000 has been compiled in a large volume entitled *Volatile Compounds in Foods* (BACIS, Service and Research, 1996–2000), which is also available online. It contains more than 8000 individual compounds and covers more than 700 food products. These include, for example, 15 different varieties of apple, 25 different citrus oils, or juices as well as the more exotic cloudberries, cupuaçu and durian fruit to name only a few. It covers 110 different herbs and spices, as well as various types of cheeses, roasted, boiled, grilled, fried and canned meats as well as a range of tea, coffee and alcoholic drinks and liqueurs. *Fenaroli's Handbook of Flavor Ingredients* (Burdock, 2010) is a valuable reference book, listing natural occurrences, syntheses, regulatory statuses and reported usage of several thousand

flavour ingredients and botanicals. *Volatile Compounds in Foods and Beverages* by Maarse is another useful text containing individual chapters on 19 different categories of food and beverages. Each chapter covers the key aroma compounds, their sensory properties and their formation pathways.

Information is also available online, with particularly useful sites being found at:

- www.leffingwell.com/odorthre.htm where odour thresholds (with references) are listed by FEMA No.
- www.odour.org.uk provides odour descriptors and odour thresholds with references and LRI values in a searchable database
- · www.flavornet.org/flavornet.html with odour descriptors and LRIs on several columns
- www.pherobase.com/database/kovats/kovats-index.php although based on pheromones, also provides Kovats indices for many flavour compounds
- www.thegoodscentscompany.com contains a wealth of information (structure, synonyms, organoleptic properties) on its commercially available aroma compounds and extracts

In the general area of flavour chemistry, there are a number of key sources of information. Proceedings from the major international flavour conferences such as the Weurman Flavour Research Symposium and the Wartburg Symposium as well as annual ACS conferences are a source of internationally recognised and peer-reviewed research articles in the area of flavour science. The *Journal of Food and Agricultural Science*, *Food Chemistry*, *Flavour and Fragrance Journal* and the online journal *Flavour* are the main sources of articles on flavour chemistry. FlavourHorizons.com is a new, quarterly, electronic bulletin providing expert interpretation, analysis and insight into flavour technologies and regulatory issues for senior managers, technologists and innovators in the food, beverage and flavour industries.

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