Wine flavor: chemistry in a glass

Pavla Polášková, Julian Herszage and Susan E. Ebeler*

Received 13th May 2008
First published as an Advance Article on the web 12th August 2008
DOI: 10.1039/b714455p

Although hundreds of chemical compounds have been identified in grapes and wines, only a few compounds actually contribute to sensory perception of wine flavor. This *critical review* focuses on volatile compounds that contribute to wine aroma and provides an overview of recent developments in analytical techniques for volatiles analysis, including methods used to identify the compounds that make the greatest contributions to the overall aroma. Knowledge of volatile composition alone is not enough to completely understand the overall wine aroma, however, due to complex interactions of odorants with each other and with other nonvolatile matrix components. These interactions and their impact on aroma volatility are the focus of much current research and are also reviewed here. Finally, the sequencing of the grapevine and yeast genomes in the past ~10 years provides the opportunity for exciting multidisciplinary studies aimed at understanding the influences of multiple genetic and environmental factors on grape and wine flavor biochemistry and metabolism (147 references).

Introduction

From Pasteur's discoveries of the role of microorganisms in fermentation and his studies on the analytical separations of chiral organic acids in grape juice[1,2] to Kepler's development of early calculus theories to measure wine barrel volumes,[3] grapes and wines have provided a rich basis for many discoveries that have had fundamental impacts on mathematics, microbiology, and chemistry over the past several centuries. The chemistry of grape and wine flavor, in particular, has been the focus of much research due to the complexity of the volatile aromas that contribute to flavor and the nuanced variations that arise from different grape varieties, growing regions, and vintage years. In the 19th and early part of the 20th centuries, much of the focus of wine flavor chemistry research was on measuring the major components that contribute to taste and aroma (ethanol, organic acids, sugars), the compounds associated with protecting wine quality,[4] and on those compounds associated with "defects" or undesirable aromas such as acetic acid (which results in a vinegar aroma). As fermentation technology improved, the incidence of defects decreased, and in the mid-1900s flavor chemists turned their focus toward understanding the chemical components that contribute to specific sensory attributes associated with different grapes and wines and different wine styles (*e.g.*, table wines, port, Sauternes-style wines, *etc.*). These studies were enabled by important advances in the development of gas chromatography (GC) in the 1950s and the introduction of commercial capillary GC columns in the 1980s. In this review we will first summarize the components that contribute to wine flavor, focusing on aroma components, then we present an overview of more recent developments in analytical techniques for the analysis of wine volatiles, methods for relating chemical composition to sensory perception of aroma, and the emerging role of genomics and proteomics for understanding aroma development in grapes.

Department of Viticulture and Enology, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA. E-mail: seebeler@ucdavis.edu; Fax: +1 530-752-0382; Tel: +1 530-752-0696

Pavla Polášková earned her PhD degree in Analytical Chemistry in 2003 from the Masaryk University Brno in the Czech Republik. Later she worked for two years as a postdoctoral scholar in the Chemistry Department at the University of California Davis. Three years ago she joined the Viticulture and Enology Department in UC Davis as a postdoctoral scholar, where she is focusing on aroma compounds and flavor interactions in wine.

In 2001 Julian Herszage earned his PhD degree in Chemistry from the Universidad de Buenos Aires in Argentina. Following his degree he worked for two years as a postdoctoral scholar at the Scripps Institution of Oceanography in San Diego, California. He joined the Viticulture and Enology Department in UC Davis as a postdoctoral scholar in 2006. His research focuses on detection and quantification of volatile sulfur compounds in wine and understanding the factors that influence formation of these important wine aroma compounds.

Susan Ebeler is a professor in the department of Viticulture and Enology at UC Davis. Her research is focused on the development and application of analytical chemistry techniques to study grape and wine flavor chemistry, understanding the physico-chemical interactions of flavors with nonvolatile food and beverage components, and elucidating the chemical mechanisms for observed health effects of grape and wine components. She teaches undergraduate and graduate classes on Grape and Wine Analysis and Flavor Chemistry of Foods and Beverages.

Chemical components contributing to flavor

Grape and wine flavor is complex and many different sensory modalities and chemical compounds influence flavor perception (Table 1).[5] However, aroma (smell) is the major contributor to overall flavor perception and this review will focus largely on the volatile aroma compounds that contribute to grape and wine flavor.

The basic processes for producing red and white wines are shown in Fig. 1, with the main distinction being that red wines are fermented with the skins present so that more chemical components from the skins (*e.g.*, anthocyanins, polyphenols, flavor compounds) are extracted into the juice/wine during the fermentation. The complex aromas of the final wine are therefore derived from the grape, the yeast fermentation (typically *Saccharomyces cerevisiae*), any secondary microbial fermentations that occur, and the aging/storage conditions.

There are clear sensory differences in the aromas of most grape varieties, however the overall volatile composition of most varieties is similar, with the varietal aroma deriving largely from differences in relative ratios of many volatile compounds, as further discussed below. In only a few cases have individual character impact compounds (see Fig. 2) been identified and associated with specific varietal aroma attributes (Table 2) (an impact compound is a single compound that conveys the named flavor[6]). Most of the impact compounds that have been identified are present at low concentrations in grapes and wines, however because of their very low (ng L^{-1}) sensory thresholds they can have a large impact on the overall grape/wine aroma.

Table 1 Sensory modalities and selected chemical components contributing to grape and wine flavor

Sensory modality	Attribute	Example chemical compounds in wine
Taste	Sweet	Glucose, fructose, glycerol, ethanol
	Sour	Tartaric acid
	Salty	Sodium chloride, potassium chloride
	Bitter	Catechin
Smell/aroma	Floral, lily-of-the valley aroma	Linalool
	Banana-like aroma	Isoamyl acetate
Chemesthesis	Mouth-warming/heat	Ethanol
Tactile	Viscosity	Glycerol, polysaccharides
	Astringency	Tannins
Vision	Red	Malvidin-3-glucoside

In general, the fermentation-derived volatiles make up the largest percentage of the total aroma composition of wine. Fermentation by *Saccharomyces cerevisiae* leads to formation of many alcohols (predominantly ethanol and the C_3–C_5 straight chain and branched *n*-alcohols, and 2-phenylethanol)

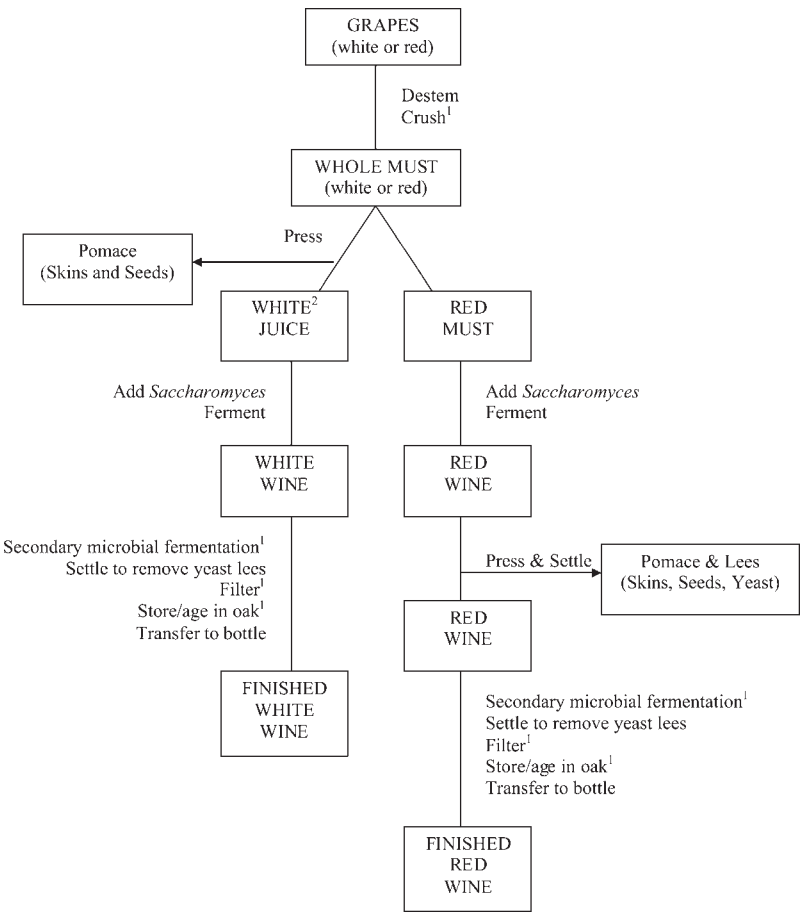

Fig. 1 White and red wine production. [1]Indicates steps that are optional and/or not done on every variety or wine style. [2]If skins are removed from red grape must, a blush or rosé juice is obtained; color is dependant on grape varietal and contact time with skins.

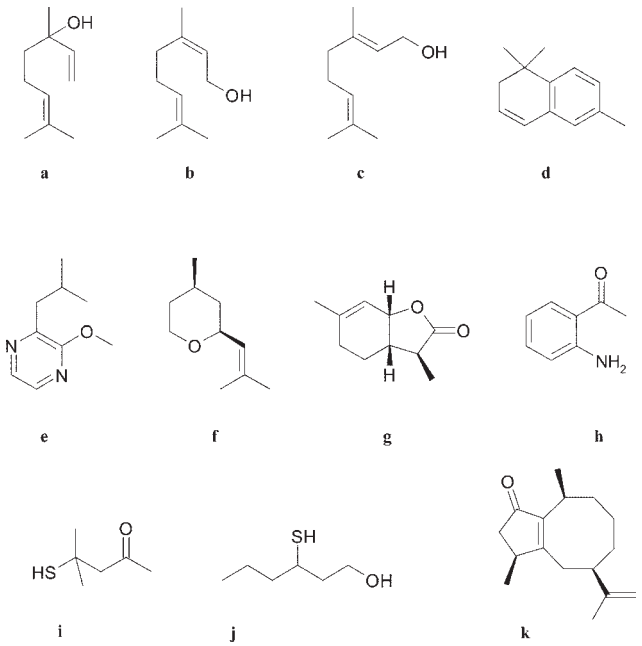

Fig. 2 Structures of compounds from Table 2: (**a**) linalool, (**b**) geraniol, (**c**) nerol, (**d**) IBMP, (**e**) *cis*-Rose oxide, (**f**) Wine lactone, (**g**) oaminoacetophenone, (**h**) 4-methyl-4-mercaptopentan-2-one, (**i**) 4-methyl-4-mercaptopentan-2-one, (**j**) 3-mercapto-1-hexanol, (**k**) rotundone.

and esters (predominantly ethyl acetate and isoamyl acetate). The ester 3-methylbutyl acetate appears to be an important aroma component of many varieties[18,27] however, in general, most of the fermentation-derived compounds have relatively high sensory thresholds and therefore do not individually contribute significantly to the aroma of wines. Combined, however, their impact may be important as shown in model systems.[28]

In addition to the primary yeast fermentation, some wines (*e.g.*, Chardonnay in the US) undergo a secondary microbial fermentation with *Oenococcus oeni* (also called malolactic fermentation) and as a result may contain high concentrations of diacetyl (2,3-butanedione), which contributes a buttery aroma to these wines. The effects of fermentation conditions and reviews of the biochemical processes involved in formation of the fermentation-derived aromas have been reviewed by others.[29–31]

Finally, changes in concentrations of many aroma compounds occur during storage and wine aging. Many wines are stored or fermented in oak barrels and one of the most important volatiles extracted from the wood is β-methyl-γ-octalactone (known as oak- or whiskey-lactone) which contributes a woody, oaky, coconut-like aroma to the wine. This compound occurs as two isomers, *cis*- and *trans*-, and like many isomeric compounds, the sensory properties are dependent on the isomeric structure. As reviewed by Waterhouse and Towey,[32] the *cis*-oak lactone isomer has an aroma threshold reported as 92 μg L^{-1}, compared to 460 μg L^{-1} for the *trans*-isomer and the ratio of the two isomers varies with oak species and origin. Interestingly, several studies have shown that the wood can also adsorb some aroma compounds (2-phenylethanol, ethyl decanoate)[33–35] changing their concentration in solution. These adsorption reactions appear to be a function of the ratio of wood surface area/solution volume and are driven by acid–base and polar characteristics of the wood rather than solubility and hydrophobicity of the studied aroma compounds.[33] Wines can also be fermented and aged in stainless steel tanks leading to wines that have simpler sensory properties mostly due to the lack of the compounds found in wine aged in oak barrels such as lactones and some phenolic compounds.[36,37]

In addition to extraction of flavor compounds from oak, chemical and microbial (*e.g.*, *Acetobacter*) oxidative reactions can make significant contributions to the flavor of aged wines as a result of formation of compounds such as acetaldehyde (nutty, sherry-like aroma) and acetic acid (vinegar aroma). While acetaldehyde can contribute desirable characteristic aromas to aged wines and Sherries, if oxidative reactions are uncontrolled they can lead to very high concentrations of acetaldehyde and acetic acid and the overall sensory impact is undesirable.

Table 2 Impact odorants contributing to varietal aromas of selected wines

Variety[a]	Characteristic odorants	Odor quality	Sensory threshold	Ref.
Muscat	Linalool,	Floral	170 ng L^{-1} (in water)	7,8
	Terpenols, *e.g.* geraniol, nerol	Citrus, floral		
Riesling	TDN (1,1,6-trimethyl-1,2-dihydronaphthalene)	Kerosene, bottle age	20 μg L^{-1}	9,10
Cabernet Sauvignon, Sauvignon blanc, Cabernet franc, Merlot, Carmenere	3-Isobutyl-2-methoxypyrazines (IBMP)	Bell pepper	2 ng L^{-1} (in water)	11–14
Gewürztraminer	*cis*-Rose oxide	Geranium oil, carrot leaves	200 ng L^{-1}	15–20
	Wine lactone	Coconut, woody, sweet	0.02 pg L^{-1} (in air)	
Vitis labrusca, *Vitis rotundifolia*	*o*-Aminoacetophenone	Foxy, sweet	400 ng L^{-1}	21,22
Sauvignon blanc, Scheurebe	4-Methyl-4-mercaptopentan-2-one	Blackcurrant	0.6 ng L^{-1} in water–ethanol (90 : 10, w/w)	16,18
Grenache rosé, Sauvignon blanc, Semillon	3-Mercapto-1-hexanol	Grapefruit/citrus peel (*R* isomer)	50 ng L^{-1}	23
		Passion fruit (*S* isomer)	60 ng L^{-1}	24
Shiraz	Rotundone	Black pepper	16 ng L^{-1} (in wine)	25,26

[a] All varieties are *Vitis vinifera* except where indicated.

Analytical tools for analysis of grape and wine volatiles

Sample preparation

Over 1000 volatile compounds comprising numerous chemical classes are present in wines, including esters, alcohols, terpenes, C13-norisoprenoids, and sulfur compounds and the concentrations of the individual components range from several mg L^{-1} (*e.g.*, ethyl acetate) to less than a few ng L^{-1} (*e.g.*, 3-isobutyl-2-methoxypyrazine, IBMP).[27,29,38–45] This can result in significant analytical challenges in completely characterizing the chemical components involved in wine aroma. Because the analytes are volatile, gas chromatography is most widely used for the analyses and, as is true for most analyses, sample preparation is critical. Historically, wine volatiles have been isolated using distillation or solvent extraction techniques.[29] These traditional methods are typically time- and labor-intensive and involve multi-step procedures, which can lead to analyte losses and a reduction in sensitivity. Also the use of solvents can be hazardous to the user's health and damaging to the environment. However in the past 10 years, the emphasis has been on developing rapid and sensitive methods for isolating volatiles of interest that also minimize the use of toxic solvents. A common approach is headspace analysis by using either a gas-tight syringe to take an aliquot of the gas in equilibrium above the solution (static headspace) or by sweeping the headspace (with an inert gas or by pulling a vacuum) towards a sorbent that traps and preconcentrates the volatiles (dynamic headspace). Following the sampling step, the headspace aliquot is directly injected into the GC for static headspace analysis or, for dynamic headspace analysis the analytes are desorbed from the sorbent trap using solvent or heat and transferred onto the GC. These headspace techniques have been widely reviewed.[29,46–49]

An alternative sampling technique, solid-phase microextraction (SPME), was developed in the 1990s by Pawliszyn[50] to provide a quick and solventless technique for the isolation of analytes in a sample matrix. SPME can be used with liquid, gaseous or even solid samples, eliminating the need for solvent extraction. The technique has been extensively used for the analyses of aromas in many types of foods and beverages including wine, beer and spirits.[50–53]

The SPME assembly consists of a needle with a retractable fiber coated with a polymeric sorbent material that is pierced through the septum of a vial containing the sample. The fiber is exposed to the sample headspace and allowed to concentrate the volatiles on the polymer. The assembly is transferred to the heated GC injector where the fiber is exposed to the carrier gas and the volatiles are desorbed. The sampling can be done manually or with an autosampler.

A variety of SPME fibers coated with different polymeric materials aimed at sorbing different classes of volatiles are commercially available (*e.g.*, polydimethylsiloxane, polyethylene glycol, polyacrylate, divinylbenzene, carboxen); therefore the choice of SPME polymer for a given application will influence the selectivity of the extraction. In addition to the choice of fiber sorbent chemistry, some of the variables influencing the effectiveness and accuracy of the SPME technique are: time of fiber exposure, sample temperature and in the case of liquid samples, the pH, ionic strength and type of solvent or matrix composition (*i.e.*, water and ethanol solvents in the case of grape wines) that may be present.[54,55] SPME fibers should also be calibrated regularly to ensure the integrity of the fiber. With some fiber types, competition for sorptive sites on the fiber can occur so that small changes in the composition of the matrix can significantly alter the quantitative extraction of the analytes of interest.[56] All of these variables should be optimized to enhance the sensitivity, accuracy, and reproducibility of the method for each analyte and matrix type.

An effective way to avoid matrix effects during the HS-SPME analysis of grapes and wines is to use stable isotope dilution analysis (SIDA). In this technique, wine samples are spiked with stable, isotopically labeled (typically ^{2}H or ^{13}C) internal standards (IS) that are matched to the analyte(s) of interest; therefore due to the similarity in chemical structure, any interactions with the matrix will be comparable for both the analyte and the IS. The spiked samples can be analyzed by SPME-GC coupled to a mass spectrometer detector (MSD) and the response ratio of analyte to internal standard used to quantify the analyte concentrations in the sample using previously determined calibration curves for each compound of interest. This technique has been effectively used by Chapman *et al.* to quantify IBMP (Table 2) in Cabernet sauvignon wines at ng L^{-1} levels and to relate effects of viticultural practices on concentrations of IBMP in wines.[57,58] Similarly, HS-SPME-GC MS combined with SIDA has been used to measure ng L^{-1} levels of 2,4,6-trichloroanisole (low ng L^{-1} can contribute a musty/corky off aroma to some wines[59]) and diacetyl in wines.[60,61] These methods give comparable reproducibility and limits of detection as traditional solvent extraction procedures but the SPME extractions can typically be completed in 30 min or less, compared to several hours for solvent extractions.[60] The approach is also valuable for monitoring multiple analytes in a single analysis. For example, Siebert *et al.*[62] used SPME-GC MS combined with SIDA to successfully quantify 31 different fermentation derived compounds in wine (fatty acids, alcohols, acetates and ethyl esters) using 29 different deuterated compounds as internal standards. Using the SIDA technique, the precision was excellent ($<5\%$) for all compounds.

Recently Cox *et al.*[63] used a HS-SPME SIDA method to obtain information about levels of a newly identified C13-norisoprenoid (apocarotenoid) aroma compound (*E*)-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB) in wines. Because the sensory threshold of this compound is $\sim$40 ng L^{-1}, an analytical method capable of quantifying concentrations at this level or lower was needed. Using a [$^{2}H_{6}$]-TPB analogue as an IS, TPB was quantified using HS-SPME GC MS with a detection limit of 10 ng L^{-1}, a concentration about five times lower than that obtained using liquid–liquid solvent extractions. TPB is thought to contribute important aromas described as "green" or "cut-grass" to several white wine varieties (*e.g.*, Semillon, Chardonnay, Riesling), while when present at higher concentrations, less favorable descriptors such as "pungent" or "chemical" have been used. By enabling the relatively rapid quantitation of this compound at low

levels, the authors were able to compare levels in different varieties, as well as to monitor chemical changes that occurred during aging and processing.

The number of applications using SPME with or without SIDA for analysis of volatiles in grapes and wines is rapidly increasing with over 200 papers published in 2007 alone (BIOSIS Previews® database search for [(solid phase microextraction) AND (grape/s OR wine/s) AND (flavor)]). It is expected that this technique will continue to be widely used in the future enabling rapid analysis of many compounds that have previously not been widely studied in grapes and wines. Interpretation of results from SPME analyses will always require careful consideration of matrix effects and extraction conditions, however.

An interesting alternative to SPME, Stir Bar Sorptive Extraction (SBSE), has been recently developed.[64–66] In this technique a magnetic stir bar coated with a polymeric sorbent (polymethylsiloxane, PDMS), is placed in the sample and stirred for a defined time to extract nonpolar analytes from the sample into the polymeric coating. After extraction, the stir bar is placed in a thermal desorption unit coupled online to a GC usually equipped with an MS detector. The apparent advantage of SBSE is the relatively high content of polymeric sorbent (about 50 to 250 times the amount present on a SPME fiber) available for extraction of analytes, making it about 50 to 250 times more sensitive than SPME.[67] However, as pointed out by Demyttenaere *et al*.,[68] the higher recovery frequently can lead to overloaded chromatograms with broad or distorted peaks which may require further optimization of GC inlet conditions (split flow, inlet temperature, *etc*.).

As with SPME, several parameters must be optimized during method development including sorption time and temperature, ionic strength and pH of the sample.[64] The octanol–water partition coefficient of the analyte(s) provides a good estimate of the relative partitioning between the liquid sample and the stir bar. The effects of variable matrix conditions on efficiency and selectivity of analyte extraction appear to be less well studied with the SBSE technique (compared to SPME) and future studies are still needed comparing this method with SPME and other traditional extraction procedures.

Fang and Qian[69] used SBSE GC-MS to study changes in volatile composition of Pinot noir wines as a function of the maturity of the grapes used to make the wines. They monitored volatiles that had previously been shown to be important contributors to the aroma of Pinot noir wines.[70] Using the SBSE GC-MS technique, the concentrations of 28 compounds, including terpene alcohols, phenols, C13-norisoprenoids, short chain fatty acids and aromatic esters were quantified. The largest differences were observed in concentrations of terpene alcohols, phenols, and C13-norisoprenoids which increased with grape maturity. On the other hand, concentrations of several esters (ethyl 2-methylpropanoate, ethyl 3-methylbutanoate, ethyl cinnamate, ethyl dihydroxycinnamate, ethyl anthranilate) decreased in the wines as grape maturity increased. The authors concluded that SBSE coupled with GC-MS enabled the accurate and rapid quantitation of many key aroma compounds present at low levels in the Pinot noir wines.

In an interesting application showing the potential of SBSE for monitoring aroma composition of complex mixtures, Alves *et al*.[67] was able to distinguish the aroma profiles of 33 Portuguese Madeira wines made from five grape varieties (Sercial, Verdelho, Boal, Malvasia and Tinta Negra Mole) with differing levels of sugar and aging times. The fermentation of Madeira wines is stopped by the addition of natural grape spirits, therefore the resulting wines can be classified by level of sugar remaining at the point when the fermentation is stopped (*e.g*., dry, medium dry, medium sweet, and sweet corresponding to <1.5 mass% to ≥ 3.5 mass% residual sugar). Madeira wines can also be classified according to their age and length of time stored in oak barrels ranging from 3 to more than 20 years, therefore, these processing variables ultimately will also impact the final volatile composition. In this study, Alves *et al*. compared both HS-SPME and SBSE procedures. While HS-SPME analysis revealed few differences in the aroma composition across the whole set of 33 wines, by using SBSE differences in concentrations of trace- and ultra-trace compounds were apparent (*e.g*., *cis*- and *trans*-oak lactone). Using multivariate statistical analysis tools (principal component analysis, PCA) on a subset of 12 of the wines, the authors were able to use the volatile chemical composition, as quantified by the SBSE method, to classify the Madeira wines by age and sweetness. For the 12 wines in the set, the concentrations of ethyl octanoate were important for discriminating among Madeiras with different residual sugar levels, while diethyl succinate and *cis*-oak lactone concentrations were used to differentiate the wines as a function of age. Further study with a larger group of wines is needed however, to confirm the predictive ability of these compounds for classifying Madeira wines.

Separation and detection of analytes

While new sample preparation techniques have improved the ability to rapidly and sensitively sample volatiles from complex mixtures, co-elution of compounds during the chromatographic separation step remains a common problem limiting the ability to accurately identify and quantify many components. However, recent developments in bidimensional gas chromatography (GC×GC) show great potential for improved separation of highly complex mixtures such as those encountered with grape and wine samples.

GC×GC uses two ''orthogonal'' columns to create a bidimensional plane of separation based on two different compound properties[71,72] such as volatility and polarity. Although GC×GC techniques have been used for over 20 years (ref. 73, reviewed in ref. 74), they did not receive widespread acceptance until recently when developments in commercial instrumentation have improved the ease-of-use of this technique. Because the peaks eluting from the second GC column can be extremely narrow ($\sim$1 s), the use of scanning mass spectrometers as detectors has also limited the widespread use of multidimensional GC analyses. However, time-of-flight mass spectrometers (TOFMS) offer fast scanning rates sufficient for GC×GC requirements. In addition, TOFMS can be highly sensitive, provide full mass spectral acquisition, and the deconvolution software offers

improvements in the ability to distinguish co-eluted peaks. For these reasons, the combination of GC×GC with TOFMS is emerging as a powerful tool for the efficient separation of complex mixtures of volatiles such as occur with grapes and wines.

In a recent application, Ryan *et al.*[71] quantified methoxypyrazines, including 3-isobutyl-2-methoxypyrazine (IBMP) in Sauvignon blanc wines using GC×GC TOFMS combined with isotope dilution for accurate quantitation. Because of the important sensory properties of IBMP (Table 2), there is much interest in developing accurate quantitation methods that will allow for improved study of the effects of environmental conditions (*e.g.*, temperature, soil characteristics) and vineyard practices (*e.g.*, pruning treatments and light exposure to grape clusters, irrigation effects) on the levels of IBMP in the berries. However, typical levels of IBMP range from $<$2–80 ng L^{-1} in grapes and wines requiring a very sensitive analytical method, and interferences in the analytical separation have been a common problem limiting the analysis.[71] Using GC×GC TOFMS for IBMP analysis Ryan *et al.*[71] concluded that the increase in separation efficiency of GC×GC allowed for shorter extraction times and less sample pre-treatment prior to HS-SPME as compared to single dimension GC analysis, while providing comparable detection limits and reproducibility. The TOFMS detector also provided advantages due to the deconvolution capabilities which enabled mass resolution of [$^2H_3$]-IBMP, IBMP, and the isomer *sec*-butylmethoxypyrazine which were not resolved by GC×GC alone.

The high resolving power of GC×GC TOFMS was also highlighted in a recent report by Rocha *et al.*[75] where 20 new monoterpene aroma compounds were identified in the headspace of *Vitis vinifera* L. cv. Fernao-Pires white grapes. Many of the newly identified compounds have low sensory thresholds and may make important contributions to the overall aroma of this grape variety. The GC×GC TOFMS procedure detected approximately two times more analytes than GC combined with a quadrupole mass spectrometric detector.

Finally, in another approach, Setkova *et al.*[76,77] used single dimension GC separation combined with TOFMS for the very rapid analysis ($<$5 min) of 201 compounds in the headspace of ice wine samples. A set of 130 different ice wine samples were classified according to their origin, grape variety, and fermentation/aging conditions (oak barrels or stainless steel tanks) by comparing the volatiles in the headspace using multidimensional statistical tools.

In summary, these developments in analytical separations and detection show much promise for identifying new compounds that may contribute to grape and wine aroma. In addition, these methods provide the opportunity for rapid, comprehensive, non-target analysis of volatile profiles that when combined with multivariate statistical tools may be used for sample classification and other sample comparisons.[67,78,79]

Gas chromatography–olfactometry

Although analytical tools have enabled the identification and quantitation of hundreds of volatiles in grapes and wines, typically only a small number (10–20) contribute directly to the aroma as has been observed with most other foods and beverages.[80] Therefore, the development of analytical methods that relate the chemical composition to sensory perception of aroma are currently of extensive interest. While volatile compounds can be identified based on comparisons of the mass spectra and GC retention indices (RI) to synthesized standards, the importance of the analyte to the overall aroma can be recognized when the gas chromatographic effluent is coupled to a sniffing port. In this technique, referred to as GC–olfactometry (GC-O), a human assessor sniffs the effluent as it emerges from the GC column and the aroma quality, the time at which the aroma is sensed, and in some cases, the aroma intensity are recorded. Using GC-O, odorants which may be present at trace levels (and may not even be detected by common GC detection methods) can be detected in a food or beverage extract if their sensory impact is large, while compounds that may be present in high concentrations may be found to not contribute significantly to the overall aroma. GC-O is now widely accepted as an objective method for the evaluation of the odorant profile of foods and beverages.

Several different GC-O methods have been developed over time. The techniques that are most widely used to screen for impact odorants and measure their potency are Charm® analysis and Aroma Extract Dilution Analysis (AEDA).[81,82] In both of these techniques, aroma extracts (typically prepared by solvent extraction of the sample) are sequentially diluted until no odor is perceived at the sniffing port. For each dilution, the presence or absence of a particular odorant is recorded.

In the AEDA method the aroma dilution factor is plotted against the retention indices (RI) of the odorants and the potency is defined as the last dilution where a given aroma is detected. While Charm® chromatograms are very similar to AEDA plots, Charm chromatograms also take into account the length of time the odor is perceived as well as the final dilution at which the compound is detected.[83] Ideally the peak heights on both types of aromagrams should be the same; however, Charm® values are obtained from the areas of the peaks in the chromatogram and it is assumed that they represent the ratio of the amount of odorant present in the sample to its odor threshold.

For both methods, the odorants that are still perceived, even at high dilution, are considered to contribute significantly to the overall aroma of the original sample. However, additional sensory analyses (*e.g.*, recombination studies, to be discussed in a later section) are still needed in order to find the odorants that truly contribute to the overall aroma.

Another group of GC-O methods that are useful for determining the contribution of a particular compound to the aroma of the sample, are the time-intensity (TI) methods.[84–86] With the TI methods, the undiluted extract is injected in the GC and the perceived odor intensity of the compounds eluting from the chromatographic column is recorded using an odor-specific magnitude estimation (OSME) method, *e.g.*, with a variable resister that is moved as the aroma intensity changes.[84] A related intensity based procedure, known as the posterior intensity method,[85,86] is quite similar to OSME except the perceived odor intensity of each odorant is rated

Table 3 Important odorants in several varietal wines identified using GC-O techniques as reported in selected literature sources

Variety	Most important odorants identified by Various GC-O methods	Ref.
Scheurebe	4-Mercapto-4-methylpentan-2-one, ethyl 2-methylbutyrate, 3-methylbutanol, 2-phenylethanol, 3-ethylphenol, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone and wine lactone	16
Gewürztraminer	*cis*-Rose oxide, ethyl 2-methylbutyrate, 3-methylbutanol, 2-phenylethanol, 3-ethylphenol, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone and wine lactone	16
Grenache rosé	3-Mercapto-1-hexanol, furaneol, homofuraneol	23
Chardonnay	Ethyl butanoate, octanoic acid, 2-phenylacetaldehyde, 4-vinyphenol, δ-decalactone, 2-methyltetrahydrothiophen-3-one, 3-methylbutyl acetate, decanoic acid, 4-vinyl-2-methoxyphenol and linalool	94,95
Spanish Rioja (blend of Tempranillo, Grenache and Graciano grape varieties)	4-Ethylguaiacol, (*E*)-whiskey lactone, 4-ethylphenol, *β*-damascenone, fusel alcohols, isovaleric and hexanoic acids, eugenol, fatty acid ethyl esters, ethyl esters of isoacids, furaneol, 2-phenylacetic acid and (*E*)-2-hexenal	96
Zalema	Mainly fatty acids and their ethyl esters, β-damascenone and β-ionone, isoamyl alcohol and 2-phenylethanol, 4-mercapto-4-methyl-2-pentanone, 3-mercaptohexyl acetate, 3-mercapto-1-hexanol, acetaldehyde and 2-phenylacetaldehyde	97
Castañal	β-Ionone, 3-methyl-1-butanol, benzyl alcohol, 2-phenylethanol, ethyl acetate, isoamyl acetate, ethyl lactate, ethyl butyrate, ethyl hexanoate and ethyl octanoate	98
Pinot Noir	2-Phenylethanol, 3-methyl-1-butanol, 2-methylpropanoate, ethyl butanoate, 3-methylbutyl acetate, ethyl hexanoate, benzaldehyde,	70
Cabernet Sauvignon and Merlot from Bordeaux	Methylbutanols, 2-phenylethanol, 2-methyl-3-sulfanylfuran, acetic acid, 3-(methylsulfanyl)propanal, methylbutanoic acids, β-damascenone, 3-sulfanylhexan-1-ol, furaneol, homofuraneol	99
Cabernet Sauvignon and Merlot from USA and Australia	3-Methyl-1-butanol, 3-hydroxy-2-butanone, octanal, ethyl hexanoate, ethyl 2-methylbutanoate, β-damascenone, 2-methoxyphenol, 4-ethenyl-2-methoxyphenol, ethyl 3-methylbutanoate, acetic acid and 2-phenylethanol	100
Madeira (Malvazia, Boal, Verdelho and Sercial varieties)	Sotolon, 2-phenylacetaldehyde, (*Z*)-whiskey lactone	101
Riesling (from Croatia)	2-Phenylethanol, 3-methyl-1-butanol, 3-(methylthio)-1-propanol, ethyl propanoate, ethyl butanoate, ethyl 3-methylbutanoate, 3-methyl-1-butanol acetate, ethyl hexanoate, ethyl octanoate, ethyl 3-hydroxybutanoate, 2-phenylethyl acetate, hexanoic acid, 3-methylbutanoic acid, butanoic acid, β-damascenone, γ-undecalactone and 4-vinylguaiacol	102
Riesling (from US)	β-Damascenone, 2-phenylethanol, linalool, fatty acids, ethyl 2-methyl butyrate, *trans*-2-hexenol, *cis*-3-hexenol, geraniol, ethyl butyrate, carvone, ethyl hexanoate, isoamyl acetate	103
Seyval blanc	*o*-Aminoacetophenone, β-damascenone, C_4 fatty acids, linalool, 1-octen-3-ol, vanillin	103
Vidal blanc	β-Damascenone, 2-phenylethanol, methyl anthranilate, vanillin	103
Cayuga White	β-Damascenone, vanillin, 2-phenylethanol, geraniol, hexanal	103

on a point interval scale as it elutes from the GC column. An advantage of the TI methods relative to other GC-O methods is that more sniffers/assessors can evaluate the same sample in a given time span since intensity is measured during a single GC run while the dilution methods require multiple analyses of the same sample over several dilutions to calculate the aroma dilution or Charm® values. In addition, the intensity ratings from multiples assessors can be statistically evaluated using TI methods.

Another final variation, the Detection Frequency Method, is based on the frequency of odorant detection by a panel of 8–12 persons who separately sniff the GC eluent of the nondiluted extract. The individual aromagrams are recorded and the odor's intensity is estimated based on the number of panelists who detect the odor (detection frequency).[87–89]

Limitations to the GC-O methods have been reviewed.[90] Most importantly, the GC-O techniques are based on separation of mixtures into individual components, while human sensory perception of the overall aroma of a wine or other food sample is integrative and takes into account the combined sensations of all components, including any additive or masking effects that may occur when the aroma of complex mixtures is smelled. In addition, odor quality of some compounds can change with changing concentration so that perceptual differences may occur as peaks elute from the GC column and as odorant concentrations in solution change. If peaks are poorly resolved, odor perception may be dependent on relative concentrations of the unresolved odorants as they elute. Finally significant differences in individual sensitivities to odorants occur, requiring careful training and standardization of GC-O protocols.[91–93] However, GC-O remains a powerful tool for identifying important odorants that contribute to grape and wine aroma and for relating the contributions of individual odorants to the differences among different wines samples (Table 3).

Reconstitution and omission tests

As discussed previously, one of the principal drawbacks of GC-O approaches for identifying important odorants, is that they consider only the impact of isolated aroma compounds and they overlook the additive (or masking) effects of aroma compounds in a mixture. Therefore, once a set of potentially important odorants are identified by GC-O, additional reconstitution tests are often performed by mixing together these odorants at the concentrations at which they are present in the

original sample and in a matrix similar to that of the original sample.[104] The reconstituted mixtures are then evaluated by sensory descriptive analysis or simple sensory difference tests and the odor quality of the reconstituted mixture is compared with that of the original sample. If there is not a good match between the original sample and the reconstituted mixture, additional compounds may need to be identified and incorporated into the mixture. In additional evaluations, omission tests are also commonly used to verify the importance of an individual component to the overall aroma character of the reconstituted mixture. In these omission tests, odor quality of the reconstituted mixtures is evaluated and compared after removing one or more individual compounds—if a compound that contributes significantly to the overall aroma is omitted, the overall aroma quality of the mixture will change.

Ferreira's group has extensively used GC-O, reconstitution, and omission methods to understand the contributions of individual odorants to the aroma of a wide range of wine varieties and styles.[23,96,97,105] Their work has identified compounds that act synergistically when present together in mixtures.[23] For example, furaneol and homofuraneol individually have aromas described as cotton candy. However, when present together in a mixture of compounds designed to reconstitute the aroma of Grenache rosé wine, furaneol and homofuraneol act synergistically to significantly impact the overall fruit and caramel aroma notes of the mixture. On the other hand, omission of some compounds that have low sensory thresholds does not always significantly impact the overall aroma. For example removal of β-damascenone with a low aroma threshold of 50 ng L^{-1} only caused a slight change in the aroma of the Grenache rosé reconstitution mixture; this may because of the unique psychophysical curve for β-damascenone whereby large concentration changes are required in order to significantly impact the aroma intensity.[106] Escudero *et al.*[105] further hypothesize that compounds with highly specific and unique aroma notes (*e.g.*, 4-methyl-4-mercaptopentan-2-one, Fig. 2), highly impact the odor of the wine by ''rupturing the aroma equilibrium'' or ''aromatic buffer'' created by volatiles with similar aroma properties. On the other hand when several compounds have similar aroma properties (ethyl esters, fusel alcohols), removing these compounds individually from the reconstitution mixtures has little impact on the overall odor of the mixture, even if the individual compound may be present at a concentration above its odor threshold. These results point to the need for additional studies using reconstitution and omission methods in order to fully understand the role of individual compounds on the perception of complex aroma mixtures.

Flavor interactions and effects on odorant volatility and perception

The studies discussed in the previous section emphasize the fact that knowledge of volatile composition and concentration alone is not enough to completely understand the flavor of a sample. Interactions among odorants, interactions between sense modalities[107–111] and matrix effects can all impact the odorant volatility, flavor release, and overall perceived flavor (or aroma) intensity and quality.

In recent studies on aroma interactions in complex mixtures, Hein[112] has observed that vegetal/bell pepper aromas of Cabernet wines may be masked by the presence of fruity aromas. Escudero *et al.*[113] has observed that fruity aroma notes in red wines can be enhanced by the presence of C13-norisoprenoids and dimethyl sulfide and suppressed by the presence of ethanol. These studies are important not only in understanding interactions of odorants in wines but they point to the need for improved analytical methods that will allow as many compounds as possible to be measured rapidly and with high sensitivity in order to more fully relate the effects of compositional changes on flavor and aroma properties.

Advances in the studies of olfactory receptor proteins are increasing our knowledge of the mechanisms of odor perception as well as providing exciting insights into the qualitative changes in odor perception that occur as compounds are mixed together.[114,115] In addition, some studies have indicated that the changes in sensory perception that occur in binary mixtures of odorants can be described mathematically as the result of qualitative (odor quality) or quantitative (odor intensity) interactions between the odorants in the mixture.[116,117] So far these models have been tested only on simple binary mixtures and apparently there is not one universal model to describe most of the combinations. More work is needed on model development and data quality.

In addition to interactions of odorants with each other, interactions of odorants with nonvolatile matrix components can change the odorant volatility and concentration in the headspace above the solution. These changes in headspace concentration can then lead to differences in perceived aroma intensity. The extent of odorant-matrix interactions can be quantified by analyzing the concentration of the analyte in the headspace above the solution, typically by using gas chromatographic procedures.

In wine, odorants can interact with macromolecules such as proteins, polysaccharides and lipids.[118,119] However, polyphenols and tannins make up a significant portion of the nonvolatile matrix composition of red wines; therefore, recent studies have focused on the influence of odorant/polyphenol interactions on odorant release and volatility. Dufour and Bayonove[120] investigated interactions between wine polyphenols and selected aroma compounds by means of an exponential dilution technique and ^{1}H NMR spectroscopy. They found that ethyl hexanoate, isoamyl acetate, and benzaldehyde, but not limonene, weakly interacted with catechin (a monomeric polyphenol) in solution; however when a polymeric wine tannin fraction was present, the volatility of the two esters was not influenced by presence of the tannin, benzaldehyde interacted with the tannin resulting in decreased volatility, and limonene was salted out of solution (*i.e.*, headspace concentration increased). These authors hypothesized that hydrophobic interactions were important in determining the extent of the interactions and used NMR tools to calculate thermodynamic data for odorant–polyphenol complex formation. However, they did not extensively study further mechanisms that may be involved in complex formation and stability. Jung *et al.*[121] used one- and two-dimensional NMR to study the nature of the polyphenol–odorant interactions using

ethyl benzoate, 2-methylpyrazine and vanillin as model odorants and naringin and gallic acid as model polyphenols. This study showed a structure dependence of the interactions and the presence of specific π–π stacking, stabilized by hydrogen bonds between the galloyl ring of phenolic compounds and the aromatic ring of the odorant, influenced the strength of the interaction.

The NMR results were confirmed by means of headspace GC-MS and sensory analysis where gallic acid significantly decreased the volatility of 2-methylpyrazine, while naringin had less of an effect on the headspace volatility of this odorant. 2-Methylpyrazine aroma intensity was the most affected by polyphenols while ethyl benzoate had the least interaction with both polyphenols.[122]

Jung *et al.* also used advanced diffusion based NMR methods to investigate interactions of odorant mixtures with other macromolecules. These methods are based on the difference of the diffusion coefficient of the aroma compound alone and in the complex with a macromolecule. Benzaldehyde and vanillin were found to bind more selectively than 2-phenylethanol with bovine serum albumin, a model for wine proteins. Ethyl benzoate had stronger binding affinity to polymeric epicatechin units of cacao bean extract than did benzaldehyde and 2-phenylethanol.[123]

In summary, the non-volatile matrix composition can significantly impact aroma volatility and perception.[118,119,124] In particular, vineyard or winemaking practices that influence the concentrations of polyphenols/tannins, proteins, ethanol or other matrix components may influence aroma perception even if no other changes in odorant concentrations occur. In addition, analytical measurements using GC and NMR allow an improved understanding of the mechanisms of the odorant–matrix interactions and may provide the opportunity to better predict or optimize volatiles release and perception in grapes and wine.

Genomics and biochemistry of grape and wine flavor

The past 10 years have seen rapid growth in our understanding of grape, yeast and human genetics—and these advances in genomics can now be translated into an improved understanding of grape and wine flavor and aroma composition and perception.[125] Further, by combining genomic and proteomic approaches with high throughput analytical methods that profile a large number of flavor metabolites, as discussed previously,[76,77] there is now the opportunity for extensive studies on the influences of a multitude of genetic and environmental factors on grape and wine flavor biochemistry and metabolism.

Although the biochemical and regulatory pathways involved in flavor and aroma development in grapes have previously not been well understood, the recent sequencing of the grapevine genome[126,127] ushers in an exciting new era in the understanding of flavor development in grapes and wines. While terpene biosynthesis is among the best understood of the various classes of aroma compounds (see also review by Trapp and Croteau[128] and Pichersky *et al.*[129]) to date only a few enzymes and genes involved in grape terpene biosynthesis have been identified and characterized.[130–133] However, analysis of the Pinot noir genome indicates that at least 89 functional genes and 27 pseudogenes in the terpene synthase (TPS) family are present in this variety—more than twice as many as the family of TPS genes in *Arabidopsis*, rice, and poplar. This observation attests to the selective amplification of these important flavor enhancing genes in the grape genome, even in a variety where terpenes are not thought to contribute significantly to the overall varietal aroma (Table 3). Identification of these genes will now allow comparison of genetic variation among other grape cultivars and studies of differential gene and protein expression associated with terpene production in a variety of grape tissues and as a function of developmental stages or growing practices.

Another class of genes and enzymes receiving extensive interest are the carotenoid cleavage dioxygenase (CCD) enzymes. In a recent study, CCD enzymes from tomato and *Arabidosis* were shown to cleave a number of different carotenoids with broad substrate specificity resulting in formation of a variety of aroma volatiles, including the important C13-norisoprenoid, β-ionone.[134] Mathieu *et al.*[135] monitored expression of a CCD gene during grape ripening, however, there was a lag between gene expression and accumulation of the C13-norisoprenoids indicating that further study is needed to further relate gene expression and protein accumulation and activity to aroma development in grapes.

Finally, although not yet studied in grape, Klee's group has recently identified genes in tomatoes that are involved in formation of 2-phenylethanol and 2-phenylacetaldehye *via* decarboxylation and deamination of phenylalanine.[136] 2-Phenylethanol and 2-phenylacetaldehye have important floral/rose-like aromas, they have been identified in many grape varieties, and their concentrations increase during fermentation, as discussed previously. By identifying genes related to formation of these compounds in grapes (and yeast) it may be possible to better control and optimize formation of these important aroma compounds during grape ripening and fermentation. In addition, the molecular and biochemical tools used in these studies may also provide valuable insight toward understanding the external and internal mechanisms that control synthesis of these and other important grape volatiles.[129]

While the grapevine genome was only recently sequenced, the yeast genome was sequenced over 10 years ago[137] and a wide range of genomic and proteomic techniques are now employed to understand the factors that influence aroma formation and metabolism during wine fermentations.[125] Swiegers *et al.*[138] have provided an extensive review of many of the genes involved in aroma formation by *Saccharomyces* and Bisson *et al.*[139] have reviewed the role that functional genomics techniques have played in understanding transcript, protein and metabolic profile differences among yeast strains. These studies are providing increasing understanding of how yeast strain effects and fermentation conditions can influence aroma formation in wines. Ultimately this knowledge may be used to select and identify commercial yeast strains that will produce distinctive wine flavor and aroma profiles under typical fermentation conditions.

Finally, with the sequencing of the human genome, we now have the opportunity to better understand the genetic factors that influence individual differences in flavor perception. Both taste and olfactory receptors have now been identified[140–143] and

differences in specific genes encoding for these receptors have been associated with food intake patterns, food preferences, and alcohol preferences.[144–147] An increased understanding of variability in the human genome may provide an improved ability to tailor wine styles to different population groups with different taste and olfactory acuities and preferences.

Summary

When we enjoy a glass of wine with a meal or to toast a special occasion, all of our senses are stimulated and the chemical compounds that evoke those sensory responses are highly variable in structure acting over a wide range of concentrations. While past research has shed much light on the chemistry of the volatile aroma compounds contributing to wine flavor, there is still much to be learned about the specific compounds involved, their sensory impact both alone and in mixtures, and about the biochemical and chemical changes that occur in the berry, during fermentation, and during wine storage. Future understanding of the chemistry in a glass of wine will be advanced through (1) development of improved and high throughput analytical methods that will allow monitoring of a large number of volatiles including those present at low concentrations; (2) improved understanding of the relationships between chemical composition and sensory perception including an emphasis on the mechanisms of how odorants and matrix components interact chemically to impact odorant volatility and overall flavor perception of wines; and (3) multidisciplinary studies using genomic and proteomic techniques to understand flavor and aroma formation in the grape and during fermentation. As has been true for centuries, the chemistry in a glass of wine will continue to provide scientists with many new discoveries in the years to come.

Acknowledgements

We thank Dr. John Thorngate for his careful review of this manuscript.

References

1 L. Pasteur, *Comptes Rendus*, 1861, **52**, 1260. On-line: http://web.lemoyne.edu/~GIUNTA/pasteur.html.
2 L. Pasteur, *Alembic Club Reprints*, 1861, **14**. On-line: http://web.lemoyne.edu/~GIUNTA/pasteur60.html.
3 Encyclopedia Britannica On-line: http://www.britannica.com/eb/article-8445/Johannes-Kepler.
4 P. Winterhalter, in *Authentication of Food and Wine*, ed. S. E. Ebeler, G. R. Takeoka and P. Winterhalter, ACS Symposium Series No. 952, American Chemical Society, Washington, DC, 2007, p. 2.
5 G. M. Shepherd, *Nature*, 2006, **444**, 316.
6 G. Reineccius, *Flavor Chemistry and Technology*, Taylor and Francis Group, New York, 2nd edn, 2006.
7 K. L. Stevens, J. Bomben, A. Lee and W. H. McFadden, *J. Agric. Food Chem.*, 1966, **14**, 249.
8 K. W. O. Wenzel and M. J. de Vries, *S. Afr. J. Agric. Sci.*, 1968, **11**, 273.
9 G. Ohloff, *Perfum. Flavor.*, 1978, **3**, 11.
10 R. F. Simpson, *Chem. Ind.*, 1978, 37.
11 M. J. Lacey, M. S. Allen, R. L. N. Harris and W. V. Brown, *Am. J. Enol. Vitic.*, 1991, **42**, 103.
12 M. S. Allen, M. J. Lacey, R. L. N. Harris and W. V. Brown, *Am. J. Enol. Vitic.*, 1991, **42**, 109.
13 D. Roujou de Boubée, C. Van Leeuwen and D. J. Dubourdieu, *J. Agric. Food Chem.*, 2000, **48**, 4830.
14 A. Belancic and E. Agosin, *Am. J. Enol. Vitic.*, 2007, **58**, 462.
15 K. Bauer, D. Garbe and H. Surburg, in *Common Fragrance and Flavor Materials*, ed. H. Surburg and J. Panten, Wiley–VCH, Weinheim, Germany, 1997.
16 H. Guth, *J. Agric. Food Chem.*, 1997, **45**, 3022.
17 H. Guth, *J. Agric. Food Chem.*, 1997, **45**, 3027.
18 H. Guth, in *Chemistry of Wine Flavor*, ed. A. L. Waterhouse and S. E. Ebeler, American Chemical Society, Washington, DC, 1998, p. 39.
19 H. Guth, *Lebensmittelchemie*, 1995, **49**, 107.
20 H. Guth, *Helv. Chim. Acta*, 1996, **79**, 1559.
21 T. E. Acree and E. H. Lavin, in *Flavour Science and Technology*, ed. Y. Bessière and A. F. Thomas, Wiley, Chichester, England, 1990, p. 49.
22 H. H. Baek, K. R. Cadwallader, E. Marroquin and J. L. Silva, *J. Food Sci.*, 1997, **62**, 249.
23 V. Ferreira, N. Ortín, A. Escudero, R. López and J. Cacho, *J. Agric. Food Chem.*, 2002, **50**, 4048.
24 T. Tominaga, Y. Niclass, E. Frérot and D. Dubourdieu, *J. Agric. Food Chem.*, 2006, **54**, 7251.
25 T. E. Siebert, C. Wood, G. M. Elsey and A. P. Pollnitz, *J. Agric. Food Chem.*, 2008, **56**, 3745.
26 C. Wood, T. E. Siebert, M. Parker, D. L. Capone, G. M. Elsey, A. P. Pollnitz, M. Eggers, M. Meier, T. Vössing, S. Widder, G. Krammer, M. A. Sefton and M. J. Herderich, *J. Agric. Food Chem.*, 2008, **56**, 3738.
27 P. X. Etiévant, in *Volatile Compounds in Foods and Beverages*, ed. H. Maarse, Marcel Dekker, Inc., New York, 1991, 483.
28 R. G. Buttery, in *Flavor Chemistry: 30 Years of Progress*, ed. R. Teranishi, E. Wick and I. Hornstein, Kluwer Academic, New York, 1999, p. 353.
29 S. E. Ebeler, *Food Rev. Int.*, 2001, **17**, 45.
30 J. H. Swiegers, E. J. Bartowsky, P. A. Henschke and I. S. Pretorius, *Aust. J. Grape Wine Res.*, 2005, **11**, 139.
31 E. J. Bartowsky, *Aust. J. Grape Wine Res.*, 2005, **11**, 174.
32 A. L. Waterhouse and J. P. Towey, *J. Agric. Food Chem.*, 1994, **42**, 1971.
33 G. Ramirez Ramirez, S. Lubbers, C. Charpentier, M. Feuillat, A. Voilley and D. Chassagne, *J. Agric. Food Chem.*, 2001, **49**, 3893.
34 V. D. Barrera-Garcia, R. D. Gougeon, A. Voilley and D. Chassagne, *J. Agric. Food Chem.*, 2006, **54**, 3982.
35 J. L. Puech, F. Feuillat and J. R. Mosedale, *Am. J. Enol. Vitic.*, 1999, **50**, 469.
36 I. Jarauta, J. Cacho and V. Ferreira, *J. Agric. Food Chem.*, 2005, **53**, 4166.
37 M. Ibern-Gomez, C. Andres-Lacueva, R. M. Lamuela-Raventos, C. Lao-Luque, S. Buxaderas and M. C. de la Torre-Boronat, *Am. J. Enol. Vitic.*, 2001, **52**, 159.
38 P. Schreier, *CRC Crit. Rev. Food Sci. Nutr.*, 1979, **12**, 59.
39 L. Nykänen and H. Suomaleinen, in *Aroma of Beer, Wine, and Distilled Alcoholic Beverages*, D. Reidel Publishing Co., Boston, 1983, p. 413.
40 L. Nykänen, *Am. J. Enol. Vitic.*, 1986, **37**, 84.
41 A. Rapp, in *Wine Analysis*, ed. H. F. Linskens and J. F. Jackson, Springer-Verlag, Berlin, 1988, p. 29.
42 A. Rapp and P. J. Pretorius, in *Flavors and Off Flavors*, ed. G. Charalambous, Elsevier, Amsterdam, 1990, p. 1.
43 A. C. Noble, in *Understanding Natural Flavors*, ed. J. R. Piggott and A. Paterson, Chapman & Hall, New York, 1994, p. 228.
44 V. C. Cole and A. C. Noble, in *Fermented Beverage Production*, ed. A. G. H. Lea and J. R. Piggott, Chapman and Hall, New York, 1994, p. 361.
45 A. L. Waterhouse and S. E. Ebeler, *Chemistry of Wine Flavor*, American Chemical Society, Washington, DC, 1998.
46 G. Charalambous, *Analysis of Foods and Beverages: Headspace Techniques*, Academic Press, New York, 1978.
47 W. G. Jennings and A. Rapp, *Sample Preparation for Gas Chromatographic Analysis*, Hüthig, Heidelberg, 1983.
48 R. Marsili, *Techniques for Analyzing Food Aroma*, Marcel Dekker, Inc., New York, 1997.
49 C. J. Mussinan and M. J. Morello, *Flavor Analysis. Development in Isolation and Characterization*, American Chemical Society, Washington, DC, 1998.

50 J. Pawliszyn, *Solid-Phase Microextraction: Theory and Practice*, VCH, New York, NY, 1997.
51 A. D. Harmon, in *Techniques for Analyzing Food Aroma*, ed. R. Marsili, Marcel Dekker, Inc., 1997, p. 81.
52 J. Pawliszyn and S. Pedersen-Bjergaard, *J. Chromatogr. Sci.*, 2006, **44**, 291.
53 J. Pawliszyn, *J. Chromatogr. Sci.*, 2006, **44**, 87 (Special Issue: Microextraction, Part I).
54 K. L. Howard, J. H. Mike and R. Riesen, *Am. J. Enol. Vitic.*, 2005, **56**, 37.
55 M. Boyce and E.E. Spicket, *Food Aust.*, 2002, **54**, 350.
56 R. A. Murray, *Anal. Chem.*, 2001, **73**, 1646.
57 D. M. Chapman, J. H. Thorngate, M. A. Matthews, J.-X. Guinard and S. E. Ebeler, *J. Agric. Food Chem.*, 2004, **52**, 5431.
58 D. M. Chapman, G. Roby, S. E. Ebeler, J.-X. Guinard and M. A. Matthews, *Aust. J. Grape Wine Res.*, 2005, **11**, 339.
59 C. E. Butzke, T. J. Evans and S. E. Ebeler, in *Chemistry of Wine Flavor*, ed. A. L. Waterhouse and S. E. Ebeler, American Chemical Society, Washington, DC, 1998, p. 208.
60 T. J. Evans, C. E. Butzke and S. E. Ebeler, *J. Chromatogr., A*, 1997, **786**, 293.
61 Y. Hayasaka and E. J. Bartowsky, *J. Agric. Food Chem.*, 1999, **47**, 612.
62 T. E. Siebert, H. E. Smyth, D. L. Capone, C. Neuwöhner, K. H. Pardon, G. K. Skouroumounis, M. J. Herderich, M. A. Sefton and A. P. Pollnitz, *Anal. Bioanal. Chem.*, 2005, **381**, 937.
63 A. Cox, D. Capone, G. M. Elsey, M. V. Perkins and M. A. Sefton, *J. Agric. Food Chem.*, 2005, **53**, 3584.
64 E. Baltussen, P. Sandra, F. David and C. Cramers, *J. Microcolumn Sep.*, 1999, **11**, 737.
65 P. Sandra, B. Tienport, J. Vercammen, A. Tredoux, T. Sandra and F. David, *J. Chromatogr., A*, 2001, **928**, 117.
66 A. Zalacain, J. Marin, G. L. Alonso and M. R. Salinas, *Talanta*, 2007, **71**, 1610.
67 R. F. Alves, A. M. D. Nascimento and J. M. F. Nogueira, *Anal. Chim. Acta*, 2005, **546**, 11.
68 J. C. R. Demyttenaere, J. I. Sánchez Martínez, R. Verhé, P. Sandra and N. de Kimpe, *J. Chromatogr., A*, 2003, **985**, 221.
69 Y. Fang and M. C. Qian, *J. Agric. Food Chem.*, 2006, **54**, 8567.
70 Y. Fang and M. Qian, *Flavour Fragrance J.*, 2005, **20**, 22.
71 D. Ryan, P. Watkins, J. Smith, M. Allen and P. Marriott, *J. Sep. Sci.*, 2005, **28**, 1075.
72 P. J. Marriott, in *Multidimensional Chromatography*, ed. L. Mondello, A. C. Lewis and K. D. Bartle, John Wiley & Sons Ltd., Chichester, UK, 2002, p. 77.
73 W. Jennings, E. Mittlefehldt and P. Stremple, *Analytical Gas Chromatography*, Academic Press, San Diego, CA, 2nd edn, 1997.
74 K. M. Pierce, J. C. Hoggard, R. E. Mohler and R. E. Synovec, *J. Chromatogr., A*, 2008, **1184**, 341.
75 S. Rocha, E. Coelho, J. Zrostlíková, I. Delgadillo and M. A. Coimbra, *J. Chromatogr., A*, 2007, **1161**, 292.
76 L. Setkova, S. Risticevis and J. Pawliszyn, *J. Chromatogr., A*, 2007, **1147**, 213.
77 L. Setkova, S. Risticevic and J. Pawliszyn, *J. Chromatogr., A*, 2007, **1147**, 224.
78 H. T. Lawless and H. Heymann, *Sensory Evaluation of Food*, Aspen Publishers, 1998.
79 A. C. Noble and S. E. Ebeler, *Food Rev. Int.*, 2002, **18**, 1.
80 P. Schieberle, in *Characterization of Food: Emerging Methods*, ed. A. Gaonkar, Elsevier, Amsterdam, 1995, p. 403.
81 T. E. Acree, J. Barnard and D. G. Cunningham, *Food Chem.*, 1984, **14**, 273.
82 F. Ulrich and W. Grosch, *Z. Lebensm.–Unters.–Forsch.*, 1987, **184**, 272.
83 T. E. Acree, *Anal. Chem.*, 1997, **69**, 170A.
84 M. R. McDaniel, R. Miranda-Lopez, B. T. Watson, N. J. Michaels and L. M. Libbey, in *Flavors and Off-flavors*, ed. G. Charalambous, Elsevier Science, Amsterdam, 1990, p. 23.
85 M. A. Petersen, L. Poll and L. M. Larsen, *Food Chem.*, 1998, **61**, 461.
86 D. Tønder, M. A. Petersen, L. Poll and C. E. Olsen, *Food Chem.*, 1998, **61**, 223.
87 J. P. H. Linssen, J. L. G. M. Janssens, J. P. Roozen and M. A. Posthumus, *Food Chem.*, 1993, **46**, 367.
88 S. M. van Ruth, J. P. Roozen and J. L. Cozijnsen, *Food Chem.*, 1996, **56**, 343.
89 P. Pollien, A. Ott, F. Montigon, M. Baumgartner, R. Muñoz-Box and A. Chaintreau, *J. Agric. Food Chem.*, 1997, **45**, 2630.
90 S. Mistry, T. Reineccius and L. K. Olson, in *Techniques for Analyzing Food Aroma*, ed. R. Marsili, Marcel Dekker, Inc., New York, 1997, p. 265.
91 A. B. Marin, T. E. Acree and J. Barnard, *Chem. Senses*, 1988, **13**, 435.
92 J. E. Friedrich, T. E. Acree and E. H. Lavin, in *Gas Chromatography–Olfactometry*, ed. J. V. Leland, P. Schieberle, A. Buettner and T. E. Acree, American Chemical Society, Washington, DC, 2001, p. 148.
93 T. E. Acree, K. D. Deibler and K. M. Kittel, in *Handbook of Flavor Characterization*, ed. K. D. Deibler and J. Delwiche, Marcel Dekker, Inc., New York, 2004, p. 83.
94 J. Ballester, C. Dacremont, Y. Le Fur and P. Etievant, *Food Qual. Pref.*, 2005, **16**, 351.
95 B. Lorrain, J. Ballester, T. Thomas-Danguin, J. Blanquet, J. M. Meunier and Y. Le Fur, *J. Agric. Food Chem.*, 2006, **54**, 3973.
96 M. Aznar, R. Lopez, J. F. Cacho and V. Ferreira, *J. Agric. Food Chem.*, 2001, **49**, 2924.
97 M. J. Gómez-Míguez, J. F. Cacho, V. Ferreira, I. M. Vicario and F. J. Heredia, *Food Chem.*, 2007, **100**, 1464.
98 M. Vilanova and C. Martínez, *Eur. Food Res. Technol.*, 2007, **224**, 431.
99 Y. Kotseridis and R. Baumes, *J. Agric. Food Chem.*, 2000, **48**, 400.
100 O. Gürbürz, J. M. Rouseff and R. L. Rouseff, *J. Agric. Food Chem.*, 2006, **54**, 3990.
101 E. Campo, V. Ferreira, A. Escudero, J. C. Marques and J. Cacho, *Anal. Chim. Acta*, 2006, **563**, 180.
102 D. Komes, D. Ulrich and T. Lovric, *Eur. Food Res. Technol.*, 2006, **222**, 1.
103 M. G. Chisholm, L. A. Guiher, T. M. Vonah and J. L. Beaumont, *Am. J. Enol. Vitic.*, 1994, **45**, 201.
104 W. Grosch, *Chem. Senses*, 2001, **26**, 533.
105 A. Escudero, B. Gogorza, M. A. Melus, N. Ortín, J. Cacho and V. Ferreira, *J. Agric. Food Chem.*, 2004, **52**, 3516.
106 V. Ferraira, J. Pet'ka, M. Aznar and J. Cacho, *J. Chromatogr., A*, 2003, **1002**, 169.
107 P. Dalton, N. Doolittle, H. Nagata and P. A. S. Breslin, *Nat. Neurosci.*, 2000, **3**, 431.
108 J. F. Delwiche, *Food Qual. Prefer.*, 2003, **15**, 137.
109 J. F. Delwiche and A. Heffelfinger, *J. Sens. Stud.*, 2005, **20**, 525.
110 J. C. Pfeiffer, T. A. Hollowood, J. Hort and A. J. Taylor, *Chem. Senses*, 2005, **30**, 539.
111 D. Labbe, A. Rytz, C. Morgenegg, S. Ali and N. Martin, *Chem. Senses*, 2007, **32**, 205.
112 K. A. Hein, M. S. Thesis, University of California, Davis, 2005.
113 A. Escudero, E. Campo, L. Fariña, J. Cacho and V. Ferreira, *J. Agric. Food Chem.*, 2007, **55**, 4501.
114 B. Atanasova, T. Thomas-Danguin, C. Chabanet, D. Langlois, S. Nicklaus and P. Etiévant, *Chem. Senses*, 2005, **30**, 209.
115 Z. Zou and L. B. Buck, *Science*, 2006, **311**, 1477.
116 D. G. Laing, H. Panhuber, M. E. Willcox and E. A. Pittman, *Physiol. Behav.*, 1984, **33**, 309.
117 M. J. Olsson, *Percept. Psychophys.*, 1994, **55**, 363.
118 A. Voilley, C. Lamer, P. Dubois and M. Feuillat, *J. Agric. Food Chem.*, 1990, **38**, 248.
119 A. Voilley, C. Beghin, C. Charpentier and D. Peyron, *Lebensmittel-Wissenschaft und-Technologie*, 1991, **24**, 469.
120 C. Dufour and C. L. Bayonove, *J. Agric. Food Chem.*, 1999, **47**, 678.
121 D. M. Jung, J. S. de Ropp and S. E. Ebeler, *J. Agric. Food Chem.*, 2000, **48**, 407.
122 J. Aronson and S. E. Ebeler, *Am. J. Enol. Vitic.*, 2004, **55**, 13.
123 D. M. Jung, J. S. de Ropp and S. E. Ebeler, *J. Agric. Food Chem.*, 2002, **50**, 4262.
124 H. Guth and R. Fritzler, *Chem. Biodiversity*, 2004, **1**, 2001.
125 R. J. Siezen, *Microbial Biotechnol.*, 2008, **1**, 97.
126 O. Jaillon, J. M. Aury, B. Noel, A. Policriti, C. Clepet, A. Casagrande, N. Choisne, S. Aubourg, N. Vitulo, C. Jubin, A. Vezzi, F. Legeai, P. Hugueney, C. Dasilva, D. Horner, E. Mica, D. Jublot, J. Poulain, C. Bruyère, A. Billault, B. Segurens, M. Gouyvenoux, E. Ugarte, F. Cattonaro, V. Anthouard, V. Vico,

C. Del Fabbro, M. Alaux, G. Di Gaspero, V. Dumas, N. Felice, S. Paillard, I. Juman, M. Moroldo, S. Scalabrin, A. Canaguier, I. Le Clainche, G. Malacrida, E. Durand, G. Pesole, V. Laucou, P. Chatelet, D. Merdinoglu, M. Delledonne, M. Pezzotti, A. Lecharny, C. Scarpelli, F. Artiguenave, M. E. Pè, G. Valle, M. Morgante, M. Caboche, A.-F. Adam-Blondon, J. Weissenbach, F. Quétier and P. Wincker for The French–Italian Public Consortium for Grapevine Genome Characterization, *Nature*, 2007, **449**, 463.
127 R. Velasco, A. Zharkikh, M. Troggio, D. A. Cartwright, A. Cestaro, D. Pruss, M. Pindo, L. M. FitzGerald, S. Vezzulli, J. Reid, G. Malacarne, D. Iliev, G. Coppola, B. Wardell, D. Micheletti, T. Macalma, M. Facci, J. T. Mitchell, M. Perazzolli, G. Eldredge, P. Gatto, R. Oyzerski, M. Moretto, N. Gutin, M. Stefanini, Y. Chen, C. Segala, C. Davenport, L. Demattè, A. Mraz, J. Battilana, K. Stormo, F. Costa, Q. Tao, A. Si-Ammour, T. Harkins, A. Lackey, C. Perbost, B. Taillon, A. Stella, V. Solovyev, J. A. Fawcett, L. Sterck, K. Vandepoele, S. M. Grando, S. Toppo, C. Moser, J. Lanchbury, R. Bogden, M. Skolnick, V. Sgaramella, S. K. Bhatnagar, P. Fontana, A. Gutin, Y. Van de Peer, F. Salamini and R. Viola, *PLoS ONE*, 2007 19;2(12):e1326.
128 S. C. Trapp and R. B. Croteau, *Genetics*, 2001, **158**, 811.
129 E. Pichersky, J. P. Noel and N. Dudareva, *Science*, 2006, **311**, 808.
130 B. G. Coombe, *Am. J. Enol. Vitic.*, 1992, **43**, 101.
131 M. Clastre, B. Bantignies, G. Feron, E. Soler and C. Ambid, *Plant Physiol.*, 1993, **102**, 205.
132 D. Martin and J. Bohlmann, *Phytochemistry*, 2004, **65**, 1223.
133 J. Lücker, P. Bowen and J. Bohlmann, *Phytochemistry*, 2004, **65**, 2649.
134 J. T. Vogel, B. C. Tan, D. R. McCarty and H. J. Klee, *J. Biol. Chem.*, 2008, **283**, 11364.
135 S. Mathieu, N. Terrier, J. Procureur, F. Bigey and Z. Günata, *J. Exp. Bot.*, 2005, **56**, 2721.
136 D. Tieman, M. Taylor, N. Schauer, A. R. Fernie, A. D. Hanson and H. J. Klee, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 8287.
137 A. Goffeau, B. G. Barrell, H. Bussey, R. W. Davis, B. Dujon, H. Feldmann, F. Galibert, J. D. Hoheisel, C. Jacq, M. Johnston, E. J. Louis, H. W. Mewes, Y. Murakami, P. Philippsen, H. Tettelin and S. G. Oliver, *Science*, 1996, **274**, 546.
138 J. H. Swiegers, E. J. Bartowsky, P. A. Henschke and I. S. Pretorius, *Aust. J. Grape Wine Res.*, 2005, **11**, 139.
139 L. F. Bisson, J. E. Karpel, V. Ramakrishnan and L. Joseph, *Adv. Food Nutr. Res.*, 2007, **53**, 65.
140 R. Axel, in *Les Prix Nobel. The Nobel Prizes 2004*, ed. Tore Frängsmyr, Nobel Foundation, Stockholm, 2005.
141 L. B. Buck, in *Les Prix Nobel. The Nobel Prizes 2004*, ed. Tore Frängsmyr, Nobel Foundation, Stockholm, 2005.
142 J. Chandrashekar, M. A. Hoon, N. J. P. Ryba and C. S. Zuker, *Nature*, 2006, **444**, 288.
143 G. M. Shepherd, *Nature*, 2006, **444**, 316.
144 M. E. Dinehart, J. E. Hayes, L. M. Bartoshuk, S. L. Lanier and V. B. Duffy, *Phys. Behav.*, 2006, **87**, 304.
145 L. M. Bartoshuk, V. B. Duffy, J. E. Hayes, H. R. Moskowitz and D. J. Snyder, *Philos. Trans. R. Soc. London, Ser. B*, 2006, **361**, 1137.
146 E. T. Rolls, *Philos. Trans. R. Soc. London, Ser. B*, 2006, **361**, 1123.
147 D. R. Reed, T. Tanaka and A. H. McDaniel, *Phys. Behav.*, 2006, **88**, 215.