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### Review Article

# Origins of Grape and Wine Aroma. Part 2. Chemical and Sensory Analysis

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**Abstract:** Part 1 of this review summarized the current state of knowledge with respect to the chemical compounds contributing to grape and wine aroma. Much of our understanding of the chemistry of grape and wine composition comes from advances in analytical and sensory methods for identifying and quantifying the compounds that contribute to flavor. Therefore, Part 2 of this review provides an overview of the chemical and sensory analysis approaches that have been used to deconstruct wine flavor into its component parts with an aim toward relating the chemical composition to the unique sensory properties that are associated with different wine varieties and styles.

**Key words:** gas chromatography-mass spectrometry, multidimensional separations, sample preparation, sample extraction, GC-olfactometry, sensory descriptive analysis

## Introduction

Part 1 of this review provided an overview of the chemical components in grapes and wines and the viticultural, winemaking, and storage practices that influence their formation and concentrations. In Part 2 we provide a summary of the analytical chemistry and sensory approaches for assessing wine flavor. As noted previously, we have attempted to be as complete

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as possible, however, given the numerous publications in these areas, not all research can be covered (e.g., a Google Scholar search for the phrase "analysis of wine flavor" returns >6,600 publications from the years 2012–2013; the phrase "sensory analysis of wine flavor" returns >5,600 publications over the same period). Therefore, we have chosen to focus on selected recent applications to demonstrate the power and types of information that can be obtained with current analytical and sensory approaches. The reader is also referred to several reviews for more detailed discussions of selected topics (Francis and Newton 2005, Lesschaeve 2007, Plutowska and Wardencki 2008, Polášková et al. 2008, Ebeler and Thorngate 2009, Muñoz-González et al. 2011, Ebeler 2012).

## Chemical Analysis of Aroma and Flavor Compounds

Essential to understanding the various influences on wine flavor is the ability to purify, identify, and quantify the compounds responsible for the sensory attributes experienced by a wine consumer. Multiple techniques have been used for characterizing the composition of wine, including flame atomic absorption spectrophotometry and flame atomic emission spectrophotometry (Frías et al. 2003), inductively coupled plasma mass spectrometry (Baxter et al. 1997), liquid chromatography (LC) (Bellomarino et al. 2009), gas chromatography (GC) (Marengo et al. 2002), UV, visible, near-infrared, and mid-infrared spectroscopy (Liu et al. 2006, Cozzolino et al. 2010), nuclear magnetic resonance spectroscopy (Brescia et al. 2002), and electronic nose (Cynkar et al. 2010). Because GC can separate the volatile compounds in a complex mixture, providing quantitative information as well as the ability to tentatively identify compounds based on their retention times (which reflect the boiling point and polarity of the analytes), the majority of studies assessing volatile compounds that contribute to aroma have used GC methods.

## Gas Chromatography and GC-Mass Spectrometry

Gas-liquid chromatography (GC) was developed in 1952 (James and Martin 1952). Initial GC separations used relatively short (3–10 m length, ~2 mm i.d.), packed columns that separated only a few compounds in complex mixtures. For example, an early wine application separated 10 fusel alcohols in wine distillates (Webb and Kepner 1961). The introduction of fused-silica capillary columns in 1979 (Dandeneau and Zerenner 1979) provided increased chemical inertness and the ability to reproducibly produce very long (30–100 m) and very narrow diameter (0.25–0.32 mm) columns. This resulted in significantly improved efficiencies and the ability to reliably separate hundreds of compounds, many present at trace levels.

Numerous GC detectors are available, including flame ionization, which detect all molecules containing reduced carbon (i.e., -CH-); nitrogen phosphorous, which specifically detect only molecules containing N or P; sulfur chemiluminescence or flame photometric, which specifically detect only molecules containing S; and mass spectrometer (MS), which detect ionized molecules based on the ratio of their mass to charge. GC-MS has significant advantages for compound identification relative to other GC detectors and analytical techniques due to the availability of extensive mass spectral and retention index databases (Stein 1999, Babushok 2007). This, in addition to the fact that the costs of bench-top and user-friendly mass spectrometers have become more affordable for the majority of users, has made GC-MS analysis among the most widely used methods for analysis of wine flavors.

Mass spectrometry was discovered by J.J. Thomson in the early 20th century and was developed by F.W. Aston in 1919, who demonstrated the existence of isotopes in non-radioactive elements (Aston 1919). Modern commercial GC-MS instrumentation combines compound ionization, resulting in unique mass spectral fragmentation patterns, with high-resolution separation of the resulting ions, and selective and sensitive mass detection. Numerous reviews discuss various aspects of wine composition with an emphasis on the role that GC-MS analysis has played in contributing to current knowledge in the field of wine and grape chemistry (Schreier et al. 1976, Ebeler 2001, 2012, Ebeler and Thorngate 2009, Hayasaka et al. 2005, Polášková et al. 2008).

Contemporary MS detectors include various instrument configurations and types of mass analyzers, including transmission quadrupole, ion trap, and time of flight. Tandem MS (MS/MS) detectors also offer distinct advantages for sensitive and targeted analysis of known compounds.

Transmission quadrupole mass analyzers use four parallel, hyperbolic rods (electrodes) (Murray et al. 2013). Opposing pairs are connected to each other and a radio frequency and direct current voltage are applied alternately across the rods. Ions oscillate in the field with a frequency dependent on the ratio of their mass to charge (*m/z*). As the radio frequency voltage is varied, only ions of a specific *m/z* will have a stable trajectory through the quadrupoles to the detector. Quadrupole ion traps (or Paul ion traps) are similar, with two ring-shaped electrodes (rather than cylindrical rods) and two end-cap electrodes (Murray et al. 2013). A radio frequency field is applied and ions are spatially confined within the electrodes with a cyclic motion. By scanning the radio frequency field, ions of a given *m/z* are excited, ejected through a small aperture in one end of the cap, and detected. Most quadrupole analyzers provide the ability to separate ions based on a mass difference of 1 amu. With time-of-flight (TOF) mass analyzers, ions leaving the GC interface and source are accelerated to the same kinetic energy. The ions then travel through a flight tube with a velocity that is proportional to the *m/z* of the ion: that is, ions with different *m/z* will travel at different speeds. TOF-MS instruments may provide unit mass resolving powers similar to those of quadrupole instruments; however, high-resolution TOF-MS instruments are also available that provide the ability to distinguish the mass of ions with much greater accuracy (e.g., ethyl acetate *m/z* 88.1051 and 1-pentanol *m/z* 88.1482). This can significantly aid compound identification. Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometers offer even greater mass accuracy. With these instruments, ions leaving the source are captured in a magnetic field where they rotate with an angular frequency that is proportional to the magnetic field and inversely proportional to the ion mass (Murray et al. 2013). All ions are detected simultaneously and ions with different cyclotron frequencies (i.e., different *m/z*) are extracted mathematically through a Fourier-transform calculation.

The majority of wine flavor research applications have used unit mass resolution instruments (transmission quadrupoles, ion traps, or low-resolution TOF-MS). However high-resolution GC-TOF-MS instruments are now commercially available and the number of published applications should increase over the next several years. The high cost of FT-ICR-MS has limited their application for wine flavor research, although several recent articles have emphasized the potential of this technique for identification of novel compounds, nontargeted profiling, and authentication (Cooper and Marshall 2001, Gougeon et al. 2009, Liger-Belair et al. 2009, Cuadros-Inostroza et al. 2010, Marchal et al. 2011).

Tandem mass spectrometry (MS/MS) uses more than one mass separation/analysis step, with mass separation occurring sequentially either in space or in time (e.g., ion trap instruments). In MS/MS analysis, ions from the first mass analyzer (MS1) enter into a reaction cell where they are bombarded with a reaction gas resulting in fragmentation of the ions (referred to as collision induced dissociation, CID, or collision-activated dissociation, CAD). The second mass analyzer (MS2) is then set to analyze selected fragments resulting from the fragmentation in the reaction cell. Several types of experiments can be performed depending on whether MS1 and MS2 are set to scan a range of *m/z* or to filter and detect only ions of a given *m/z* (see review by de Hoffmann 1996). Because each mass analyzer can be set to selectively analyze only peaks of a given *m/z*, effectively filtering out noise and nontarget analytes, MS/MS can be a highly sensitive and selective method for targeted analysis and quantification of trace compounds.

GC-MS/MS analysis is increasingly used for targeted analysis of aroma compounds in wines with particular emphasis on analysis of cork-taint related haloanisoles and volatile phenols produced by *Brettanomyces* (Pizarro et al. 2011a, 2011b, Hjelmeland et al. 2012, Collins et al. 2012). In other selected applications, MS/MS has also been used for trace analysis of odor-active polyfunctional thiols, quantification of rotundone as a function of grape maturity and winemaking practices, characterization of stereoisomers of wine lactone, analysis of furaneol and homofuraneol, and screening of aroma-active aldehydes (Schneider et al. 2003, Luan et al. 2006, Schmarr et al. 2008a, Masson and Schneider 2009, Caputi et al. 2011, Mattivi et al. 2011). MS/MS in combination with high-performance liquid chromatography (HPLC) has also been used to monitor the nonvolatile glycosidically bound aroma precursors in a number of studies (Fedrizzi et al. 2009, Hayasaka et al. 2010b, Capone et al. 2010, 2011, Kobayashi et al. 2011, Wilkinson et al. 2011). In an interesting application, HPLC-MS/MS proved invaluable in the characterization of guaiacol conjugates in grapevine leaves and berries following exposure to stable isotope-labeled guaiacol ($d_3$-guaiacol), simulating smoke contamination of the vine (Hayasaka et al. 2010a). The MS/MS profile of the isotopic doublets associated with the deuterated guaiacol conjugates enabled identification of seven different glycoside conjugates. In addition, translocation of the guaiacol conjugates between leaves and berries was observed. While not a comprehensive list of applications, these examples demonstrate the use of MS/MS for a wide range of flavor characterization studies.

## Multidimensional Separations

While fused-silica, open-tubular capillary columns revolutionized GC separations, significantly improving column efficiencies and resolution, coelution of peaks in compound mixtures still frequently occurs. High-resolution accurate MS detectors and deconvolution software that can distinguish closely eluting peaks based on statistical differences in their mass spectra (Tikunov et al. 2005, Jiang et al. 2010, Furbo and Christensen 2012, Yang et al. 2013) can aid in peak identifications. However, improvements in chromatographic resolution through multidimensional separations have also received much attention. In early multidimensional separations, a chromatographic peak from a first separation column was passed onto a second column with a different stationary phase, via a switching or splitting device. Peaks that coelute on the first column are separated in the second dimension due to differences in interactions with the stationary phase on the second column. Often called "heart-cutting," this technique has been frequently used in wine applications to separate chiral analytes by using a chiral column as the second analytical column (Bouchilloux et al. 2000, Darriet et al. 2001, Fernandes et al. 2003, Barba et al. 2010).

More recently, the development of comprehensive two-dimensional gas chromatography (GC×GC) (Liu and Phillips 1991) has received significant interest for analysis of highly complex samples, including food, beverage, environmental, biological, and petrochemical (Adahchour et al. 2008). In GC×GC analysis, the column effluent of the first analytical column is trapped in small pulses and then quantitatively transferred to the second column. In this way, the entire set of analytes eluting from the first column is separated in the second dimension. Pulse times must be carefully selected to minimize peak splitting and to optimize transfer to the second column. GC×GC offers enhanced separation efficiency, reliability in qualitative and quantitative analysis, capability to detect low quantities, and information on the whole sample and its components, as recently reviewed (Dallüge et al. 2003, Górecki et al. 2004, Ong and Marriott 2002).

GC×GC coupled with headspace analysis using solid-phase microextraction (HS-SPME) has been used to isolate volatile compounds from the sample matrix in a range of foods and beverages, including honey (Čajka et al. 2007), coffee (Ryan et al. 2004), cachaça (Cardeal et al. 2008), pepper (Cardeal et al. 2006), ginger (Shao et al. 2003), and grapes and wine (Perestrelo et al. 2010, Rocha et al. 2007, Ryan et al. 2005, Ryona et al. 2008, 2009, 2010, Schmarr et al. 2010). The majority of these studies have used the method for targeted analysis where analytes are selected prior to analysis, such as ethyl carbamate (Perestrelo et al. 2010), methoxypyrazines (Ryan et al. 2005, Ryona et al. 2008, 2009, 201,) and aroma-active aldehydes (Schmarr 2008b).

Only a few publications have used the technique for nontargeted volatile profiling of grapes and wines (Robinson et al. 2011a, 2011b, 2011c, Rocha et al. 2007, Schmarr et al. 2010, Vestner et al. 2011, Weldegergis et al. 2011a, 2011b). In nontargeted profiling, the overall MS fingerprint, or pattern, is used to distinguish among samples and chromatographic peaks need not be identified before (or after) the analysis. The improved resolution of GC×GC separations enhances the ability to obtain detailed fingerprints of complex samples since individual peaks are more likely to correspond to unique analytes, ultimately aiding in compound identification.

In a recent example, GC×GC was used to analyze monoterpenes in grapes and 56 monoterpenes were identified in the Fernão-Pires variety, of which 20 were reported for the first time in grapes (Rocha et al 2007). This highlighted the advantage that structured chromatographic separation can provide for compound classification and confirmation of compound identity. There continues to be new aroma compound discoveries in the grape and wine research field, including (*E*)-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB) (Cox et al. 2005) and 3,4,5,6,7,8-hexahydro-3,8-dimethyl-5-(1-methylethenyl)azulene-1(*2H*)-one, or (-)-rotundone (Wood et al. 2008). Therefore, it is anticipated that GC×GC will provide significant advantages in the identification of new and novel compounds, which were previously unresolved using traditional one-dimensional chromatography. Finally, GC×GC TOF-MS data are highly data intensive, and improved methods for peak alignment, deconvolution, and compound identification for large data sets are needed (Yang et al. 2013).

## Analysis of Glycoconjugates

Because glycosidically bound aroma compounds are nonvolatile, the most common method of glycoconjugate analysis has been to measure the aglycone and/or the sugar moiety separately following controlled acid or enzyme hydrolysis (Williams 1993, Günata et al. 1993). In these studies, GC-MS is most commonly used to analyze, identify, and quantify the aglycones, such as terpenes, benzenoids, and aliphatic residues. The released sugar moieties, such as mono- or di-glucoside, arabinofuranoside, and rhamnopyranoside (Williams 1993), can be analyzed by HPLC or other chromatographic approaches.

There are a limited number of studies where the glycoconjugates are measured directly. Typically in these studies, the glycoconjugates are initially isolated and fractionated from the matrix using low- (or atmospheric) pressure liquid chromatography, HPLC, counter-current chromatography (CCC), and/or supercritical fluid extraction (SFE) (Strauss et al. 1987, Winterhalter et al. 1990, Bonnländer et al. 1998, Palma et al. 2000). The isolated glycoconjugate fraction is then analyzed directly by fast atom bombardment tandem MS (FAB-MS) (Marinos et al. 1994), HPLC-MS and/or MS/MS (Hayasaka et al. 2010a), and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS (Nasi et al. 2008). There are opportunities to use other analytical techniques, including high-resolution TOF-MS and FT-ICR MS. However, these techniques have not yet been used for the analysis of glycoconjugated aroma compounds.

A rapid form of glycoconjugate analysis in wine is possible through application of the so-called glycosyl-glucose assay (Williams et al. 1995). In this approach, the glycosides are hydrolyzed and the released glucose and fructose are measured enzymatically. The assay is rapid, accurate, and precise (Williams et al. 1995) and has been used for determination of aroma potential in grapes (Zoecklein et al. 1998, Escalona et al. 1999). The determination of glycosyl-glucose provides a measure of the amount of conjugated compounds present but provides no qualitative information about the speciation of either the sugars or the aroma compounds. In addition, since grapes also contain significant amounts of glycosidically bound polyphenols (e.g., malvidin-3-glucoside and rutin), the glycosyl-glucose assay will also measure sugars released by hydrolysis of these compounds. Therefore, sample pretreatment with polyvinylpolypyrrolidone (PVPP) may be used to remove the phenolic glycosides prior to analysis of aroma potential (Zoecklein et al. 2000).

## Sample Preservation and Preparation

Sample preparation is a critical step in any chemical analysis, and the information obtained from chromatographic or MS analyses is highly dependent on the sample preparation method chosen. Techniques that effectively isolate volatile aroma compounds or their precursors from nonvolatile matrix components are typically of greatest interest for analysis of flavor compounds and will be the main focus of this discussion. At any stage of sample preparation it is important to preserve the compounds of interest; control of temperature, oxygen, and enzyme activity are keys to reducing formation of oxidative or other chemical artifacts. As noted in Part 1 of this review, many aroma compounds are present as glycoconjugates (or cysteinyl- and glutathionyl conjugates), and preservation of conjugated compounds may be important in determining aroma potential. Therefore, cold storage prior to analysis is preferable to minimize the acid hydrolysis of glycoconjugates that can slowly occur at juice pH and ambient temperature (Skouroumounis and Sefton 2000). Further, enzyme hydrolysis by most native enzymes is inhibited in juice environments (Günata et al. 1993), but enzyme inhibitors can be added to the extract as a precaution (Razungles et al. 1993).

**Liquid extraction.** In many studies, volatile and glycosylated aroma precursors are isolated from plant extracts, fruit juice, dealcoholized wine, and other liquid media either by selective retention on Amberlite XAD resins (typically XAD-2) (Günata et al. 1985), on $C_{18}$ reversed-phase silica adsorbent (Williams et al. 1982), or by simple liquid/liquid extraction. These techniques allow the isolation of aroma and aroma precursor compounds free of sugars and organic acids (Günata et al. 1985). The compounds of interest are selectively eluted from the resins/adsorbents or extracted with organic solvents of varied polarity (Mateo et al. 1997, Guyot-Declerck et al. 2000). The organic eluates are collected, dried, and concentrated for analysis. These are simple and effective methods. However, there is little scope for automation, limiting sample throughput, and the methods involve contact with potentially hazardous organic solvents, which must be disposed of.

There has been recent interest in liquid microextraction techniques to minimize use of organic solvents. These use very small amounts of solvents to extract analytes and include (1) single-drop microextraction (extraction solvent flows past a drop of liquid sample); (2) membrane extraction (sample and solvent are separated by a porous polymeric membrane and solutes partition into the organic phase based on their partition coefficients); and (3) dispersive liquid-liquid microextraction (extracting solvent is dispersed as fine droplets in the liquid sample; analytes partition into the solvent which is then separated by density from the liquid sample). Principles and applications of these techniques for wine analysis have been described (Kloskowski et al. 2007, Pena-Pereira et al. 2009, Ebeler 2012). In particular, dispersive liquid-liquid microextraction has been used for analysis of grape and wine flavor compounds, including polyfunctional thiol aroma compounds, halophenols, geosmin, and methyl isoborneol (Campillo et al. 2010, Fontana et al. 2010, Jofré et al. 2010, Pizarro et al. 2010, 2011a, Cortada et al. 2011).

The methods are rapid and sensitive; however, matrix interferences can be significant, particularly with red wines (Montes et al. 2009).

**Static and dynamic HS extraction.** The greatest advantage of static and dynamic HS extraction methodologies is that they directly sample the volatile composition of the sample headspace, which can then be directly related to the aroma of the sample. Static HS extraction involves sampling the headspace, at equilibrium, typically using a syringe and injecting the headspace gas directly into the GC inlet, often with cryofocusing to sharpen the early eluting peaks. Dynamic HS extraction involves flushing the headspace of the sample vial with inert gas over a defined time period. The liberated volatiles are usually captured in a cold trap or adsorbent such as Tenax prior to injection onto a GC (Rosillo et al. 1999). Static HS extraction has been used effectively to determine partition coefficients of analytes in aqueous ethanol solutions (Conner et al. 1994, 1998, Athès et al. 2004). These methods are simple but have poor reproducibility, are biased toward extraction of highly volatile and semivolatile compounds, have limitations in detecting trace analytes, and as a result are often unrepresentative of the sample composition (Ortega-Heras et al. 2002). Static and dynamic HS extractions have been shown to be less sensitive and less selective methods for headspace analysis when compared to SPME (Kataoka et al. 2000). However, in some cases, particularly for highly volatile compounds, static HS methods can be highly sensitive, as when detection limits of 0.1 to 3 ug/L for low molecular weight sulfur compounds in wines were obtained (e.g., $H_2S$ and DMS) (Rauhut et al. 1998), similar to those reported using other approaches, including HS-SPME-GC (Herszage and Ebeler 2011).

**HS-SPME.** Solid-phase microextraction (SPME) has been increasingly used in volatile flavor analysis since its introduction in the 1990s (Arthur and Pawliszyn 1990, Arthur et al. 1992, Zhang and Pawliszyn 1993, Pan et al. 1995, Steffen and Pawliszyn 1996). The primary advantage of this technique is that it combines analyte extraction and preconcentration in a single step, without significant sample preparation. Sampling with the SPME fiber can occur either from the headspace (HS-SPME) or from the liquid phase (direct immersion or DI-SPME), although most applications for analysis of aroma volatiles sample from the headspace. A number of grape and wine profiling studies have used HS-SPME to better understand the role of various compounds in differentiating varieties, regions, and wine vintage (Marengo et al. 2002, Câmara et al. 2007, Setkova et al. 2007b, Robinson et al. 2011c), and the technique has been repeatedly documented as a sensitive, reproducible, automated method for preconcentration of wine volatiles prior to analysis (Howard et al. 2005, Câmara et al. 2006, Setkova et al. 2007a). Various parameters are routinely optimized in the development of HS-SPME techniques for the analysis in wine of ethyl esters, acetates, acids, and alcohols (Siebert et al. 2005), monoterpenes and norisoprenoids (Câmara et al. 2006), methoxypyrazines (Hartmann et al. 2002, Ryona et al. 2009), thiols, sulfides, and disulfides (Mestres et al. 1999a, 1999b), and furfural derivatives, phenolic aldehydes, volatile phenols, and oak lactones (Carrillo et al. 2006). Most methods described within the literature explore parameters such as fiber type, sample temperature, salt concentration, agitation speed, and extraction time as part of method development and optimization (Sala et al. 2000, Rocha et al. 2001, Silva Ferreira and Guedes de Pinho 2003, Howard et al. 2005, Câmara et al. 2006, Carrillo et al. 2006, Setkova et al. 2007a, Robinson et al. 2011b). This agrees with a recently published protocol for SPME method development (Risticevic et al. 2010a). A recent review provides an extensive summary of the applications of SPME for grape and wine analysis (Jelen et al. 2012). The following sections address the relevant parameters that should be considered for wine sample preparation for HS-SPME volatile analyses.

*SPME fiber type.* SPME fibers are coated with a variety of single or mixed polymers that vary in polarity, thickness, and length. The mechanisms of extraction differ: single and/or liquid phases absorb analytes into the entire fiber coating and mixed and/or solid phases adsorb analytes to the surface of the fiber coating. This has implications with respect to sensitivity and time to reach extraction equilibrium (Risticevic et al. 2010a) and to influence the potential for matrix components to compete with analytes for fiber adsorption/binding sites, as can occur with carboxen-based polymers (Murray 2001). The polarity of the fiber coating also allows the user to target specific compounds based on their affinity for the fiber, that is, based on the principle that "like dissolves like" (Risticevic et al. 2010a). Common SPME sorptive phases or mixtures of phases that are used for the analysis of volatile compounds in wine include polydimethylsiloxane (PDMS), polyacrylate (PA), divinylbenzene (DVB), and carboxen (CAR) (Rocha et al. 2001, Howard et al. 2005, Setkova et al. 2007a, Risticevic et al. 2010a).

*Sample temperature.* Increasing the sample temperature during extraction can increase analyte partitioning into the headspace and thus increase the amount extracted improving the sensitivity of the HS-SPME method (Risticevic et al. 2010a). However, desorption from the fiber may also increase at higher temperatures, decreasing the overall analyte partitioning into the SPME fiber phase (Fischer and Fischer 1997). In addition, it has been observed that elevated extraction temperatures can modify monoterpenes (Varming et al. 2004, 2006, Perez-Cacho and Rouseff 2008), esters, and acetates (Marais and Pool 1980, Ramey and Ough 1980, Pérez-Coello et al. 2003) and release volatiles from glycosylated aroma precursors (Silva Ferreira and Guedes de Pinho 2004). While no studies to date have thoroughly addressed the role of temperature in modifying volatiles in a wine sample during SPME sampling at elevated temperatures, it has been noted that different volatile profiles were observed from honey samples conditioned at temperatures >60°C, compared to those kept at room temperature (Čajka et al. 2007). It is possible that similar results could occur if wine or grape juice samples were also heated. This aspect of HS-SPME method development should be more fully studied. However, when trying to relate HS-SPME results to data obtained by sensory panels, it would seem sensible to use temperatures either between 20 and 25°C, corresponding to room temperature tasting conditions, or between 30 and 40°C, simulating mouth temperatures and aroma release in the mouth.

*Salting-out.* The salting-out, or Setschenow effect, describes how a nonelectrolyte, in this case an organic volatile compound, decreases in solubility following the addition of an electrolyte such as sodium chloride to the solution (Mazo 2006). The addition of salt to wine samples can increase the partitioning of aroma compounds into the headspace and increase their extraction/partitioning onto the SPME fiber. Given that the Setschenow effect is related to the preferential association of electrolytes with the solvent with respect to the nonelectrolyte solute, it can be assumed that once the electrolyte reaches saturation the further addition of electrolyte will not cause a greater effect. By extrapolating from the raw data presented in one study (Farelo et al. 2004), it is observed that in a 13% ethanol solution at 30°C sodium chloride reaches saturation at ~274 g/L. Dry white and red table wines are characterized by an alcohol content ranging from 10 to 15% ethanol by volume, where sodium chloride is saturated at 292 and 262 g/L, respectively. Thus, sodium chloride concentrations between 250 and 300 g/L will generally accommodate the alcohol content of wine products at or around ambient temperatures.

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Interestingly, this falls within the frequently reported 100 and 350 g/L of added salt determined experimentally for optimal extractions of wine volatiles by HS-SPME (De La Calle García et al. 1998, Rocha et al. 2001, Azenha and Vasconcelos 2002, Rodríguez-Bencomo et al. 2002, Castro Mejías et al. 2003, Demyttenaere et al. 2003, Castro et al. 2004, Siebert et al. 2005, Câmara et al. 2006, Setkova et al. 2007a, Robinson et al. 2011c).

*Sample agitation.* Mechanical agitation plays an important role in accelerating mass transfer of molecules from the liquid into the headspace. Agitation increases mixing of molecules within the liquid, creating a relatively homogenous mixture at any point in time (Zhang and Pawliszyn 1993). Agitation also increases the rotational velocity of the liquid, forcing the liquid toward the sides of the container and thus increasing the liquid-gas interface surface area. The effects of sample agitation on HS-SPME analyses have been reviewed (Zhang and Pawliszyn 1993). The rate of analyte diffusion (i.e., diffusive flux) from the liquid to the headspace is described by Fick's first law (Equation 1):

$$J = -D\frac{\Delta\phi}{\Delta x} \qquad \text{Eq. 1}$$

where $J$ is the diffusive flux, $D$ is the diffusion coefficient, $\phi$ is the concentration, and $x$ is the position. The diffusive flux of a compound is dependent on the concentration gradient and is directly proportional to the area of the gas-liquid interface. As agitation speeds increase, the surface area is maximized such that it is approximately equal to the ratio of the internal circumference of the vessel to the height (Equation 2):

$$\Delta A \approx \frac{H}{0.5r} \qquad \text{Eq. 2}$$

where $\Delta A$ is the change in surface area, $H$ is the height of the vessel, and $r$ is the internal radius of the vial. As a consequence, the greater the agitation speed, the greater the effective surface area for the transfer of volatiles across the gas-liquid interface. In addition, a higher level of agitation will result in a constant concentration of volatiles at the gas-liquid interface due to continuous effective mixing.

*Extraction time.* The SPME extraction time chosen will depend on the method objectives. If sampling of only volatiles in the headspace is desired, without influencing the partitioning of the volatiles from the liquid phase into the headspace, then short sampling times are used (~1 to 5 min) (Lloyd et al. 2011). Sampling under these conditions is similar to a static HS extraction (Jung and Ebeler 2003). However, in most cases, SPME extraction is optimized to maximize extraction from the liquid phase and sampling may be long enough to establish an equilibrium partitioning of the volatile analytes among the liquid phase, vapor phase, and fiber phase.

When choosing and optimizing extraction times, there are three scenarios that are generally considered (Risticevic et al. 2010a). First, high-throughput methods require that the extraction time be proportional to the separation and detection time requirements: for example, if the user intends to complete a chromatographic run in 5 minutes (Setkova et al. 2007a), then the extraction time is only likely to be as long or slightly longer than 5 minutes (taking into account cool-down time). Short extraction times are usually pre-equilibrium conditions and are subject to errors associated with slight variations in extraction times; thus, automation control is essential for maximum reproducibility of the analysis (Risticevic et al. 2010a). Second, high-sensitivity methods may require longer extraction times to establish equilibrium between the SPME fiber and the headspace. In some instances, HS-SPME extraction times of 120 minutes have been used for wine volatile analysis to maximize extraction efficiency (Câmara et al. 2006). Third, good reproducibility is paramount in quantitative and semiquantitative analysis; thus to minimize variability in the amount of analyte extracted, equilibrium conditions should be used since these reduce timing-related errors. The exception is when precise automation is available and the user can demonstrate that the errors incurred due to timing are minimal (Risticevic et al. 2010a).

The optimal extraction time is dependent on the chemical and physical properties of the analytes. Optimal extraction times for a given analyte can be estimated from Graham's law of diffusion (Equation 3):

$$K_m = \frac{1}{2} . m_m . v_m^2 \qquad \text{Eq. 3}$$

where $K_m$ is the kinetic energy of the analyte molecule, $m_m$ is the mass of the molecule, and $v_m$ is the velocity of diffusion of the molecule. Assuming that the kinetic energy of any molecule is constant at any given temperature and pressure, Equation 3 can be simplified (Equation 4):

$$v_m \propto \sqrt{\frac{1}{m_m}} \qquad \text{Eq 4}$$

This demonstrates that the diffusion of the molecule in the gaseous phase toward the SPME fiber is dependent on the molecular mass of the molecule. Consequently, it is expected that the analysis of higher molecular weight compounds would require a longer extraction time compared to low molecular weight compounds, which have faster diffusion rates in the headspace comparatively.

Thus, the optimum extraction time depends on the nature of the analysis and the analytes being measured. High-throughput analysis necessitates shorter extraction times but may forego the benefits of increased sensitivity for particular compounds; longer extraction times will have a greater likelihood of the samples reaching equilibrium between the liquid, vapor, and fiber phases, which will result in greater sensitivity and improved precision.

*Desorption conditions.* After extraction, the SPME fiber is inserted into the heated GC inlet and the volatile analytes are desorbed and swept into the GC column by the carrier gas. Desorption time and temperatures should be selected to (1) rapidly desorb the analytes within the first ~30 seconds after injection; (2) quantitatively and completely transfer the analytes to the column, minimizing carry-over on the fiber; and (3) minimize any thermal reactions that may occur in the inlet. Artifact formation in the inlet has been observed in analysis of volatile sulfur compounds using SPME sample preparation (Lestremau et al. 2004). High inlet temperatures used for SPME desorption increased the oxidation of thiols; carboxen-based fibers also appeared to increase the extent of oxidation during desorption in the inlet, with the carboxen possibly acting as a catalyst (Lestremau et al. 2004). The formation of Maillard products following the immediate thermal desorption of a liquid SPME sample from strawberry and apple fruit has been documented (Verhoeven et al. 1997), and the study identified the importance of washing the SPME fiber prior to introduction in the inlet when conducting liquid SPME analysis of samples high in carbohydrates and/or amines.

**Stir-bar sorptive extraction and HS sorptive extraction.** Stir-bar sorptive extraction (SBSE), or Twister, was developed in 1999 (Baltussen et al. 1999) and works on the same basic principal as SPME where the analyte of interest partitions between the sample matrix and a polymeric extraction phase. However, rather than coating the extracting polymer on a short (1 to 2 cm) narrow-diameter (~0.56 to 0.64 mm) fused silica needle, the polymer is coated onto the outside of a glass magnetic stir bar (10 to 20 mm length). With SBSE, sampling occurs by placing the stir bar directly into the liquid phase for extraction, while HS-sorptive extraction (HSSE) samples from the headspace/vapor phase. The major advantage of SBSE and HSSE over SPME is that the stir bar is coated with 25 to 125 µL of sorptive phase (PDMS) compared with only ~0.5 µL of sorptive phase on a SPME fiber; the increased mass of the SBSE sorptive phase allows for a substantial increase in sensitivity (Lancas et al. 2009). However, the SBSE/HSSE coatings are currently limited with respect to sorptive phase types: PDMS and PDMS/ethylene glycol co-polymer are the only commercially available phases. In addition, specialized thermal desorption and cryofocusing inlets and sampling stations are required on the GC-MS instruments. An early application of SBSE for wine analysis was for the sensitive measurement of 2,4,6-trichloroanisole (TCA) contamination, and more recent applications for grape and wine flavor analysis have recently been reviewed (Sponholz et al. 2001, Jelen et al. 2012).

## Quantification of Volatiles

All sample preparation techniques will result in some selectivity and/or analyte losses during sample extraction/isolation (Flath 1977, Jennings and Filsoof 1977, Risticevic et al. 2010a). In addition, GC detector responses are often dependent on analyte chemical structure. While GC-MS detectors are often considered universal detectors responding to all chemical masses (within the defined mass range of the instrument), differences in analyte ionization and fragmentation efficiencies can result in different detector response ratios. For this reason, careful calibration is needed for accurate analyte quantification, depending on experimental objectives.

General reviews of external standard, internal standard, and standard addition calibration methods are widely available in most analytical chemistry textbooks (e.g., Christian 2007, Harris 2003), and the choice of calibration method will depend on the analyte, the matrix, the analysis throughput needs, and the amount of sample available. Internal standards are widely used for

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correcting for matrix effects, sample preparation losses, and/or injection variability. Surrogate internal standards are added at the beginning of the sample preparation or extraction steps to monitor the entire analytical process. Using a surrogate internal standard, average losses of ~50% were observed during preparation of samples for HS-SPME GC-MS profiling of volatiles in Cabernet Sauvignon skins; by monitoring these losses with the internal standard, analyte recoveries could be estimated for accurate quantification (Canuti et al. 2009). Internal standards may also be added immediately before injection/analysis to account for injection variability or variability in HS-SPME extraction. A HS-SPME approach where the internal standard is loaded onto the fiber coating before sample extraction (rather than adding the internal standard separately to each sample) has recently been proposed for the high throughput and reproducible analysis of volatile, semivolatile, and nonvolatile analytes (Risticevic et al. 2010b).

Internal standards labeled with stable isotopes (e.g., $^{2}$H, $^{13}$C, and $^{15}$N) that match the analyte/s of interest are widely used for accurate GC-MS quantification of aroma volatiles in foods and beverages, including grapes and wines (Polášková et al. 2008, Ebeler 2012, Schieberle and Molyneux 2012). Multiple internal standards are often used, as demonstrated in a recent study where 29 stable isotope-matched internal standards were used to accurately quantify 31 different wine components with HS-SPME GC-MS analysis (Siebert et al. 2005). Availability of stable isotope standards is often limited, however, and require chemical synthesis if commercial standards are not available. A recent approach used a single uniformly labeled precursor (e.g., [U-$^{13}$C]-α-linolenic acid) to produce a suite of labeled products that were subsequently characterized using reverse-isotope dilution procedures and then used for stable isotope dilution analysis (SIDA) in volatile profiling studies (Gomez-Cortez et al. 2012). Such an approach may be significantly more time and cost effective than synthesis of multiple labeled internal standards but has not yet been applied to analysis of grape and wine volatiles.

## GC-Olfactometry

GC-Olfactometry (GC-O), originally proposed in 1964 (Fuller et al. 1964), uses the human nose as a detector for the compounds eluting from a chromatographic column (Acree et al. 1984). The method has been promoted as a useful tool in determining the sensory character of some flavor compounds (Deibler et al. 1999, Kotseridis and Baumes 2000, Aznar et al. 2001,

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Grosch 2001, Friedrich and Acree 2002, Plutowska and Wardencki 2008). In addition, compounds with the most impact on the overall aroma can be defined through sequential dilutions of an aroma extract—those compounds having the greatest impact on the aroma are those that still can be detected sensorially by GC-O after numerous serial dilutions. Using this process, a sample containing hundreds of aroma compounds can typically be reduced to a subset of ~10 to 20 compounds that are most important to the overall aroma of the sample (Grosch 2001).

Although useful in characterizing aroma compounds and for initial investigations, GC-O may not allow for the extrapolation of the sensory contribution of an aroma compound to the actual wine sample (Barbe et al. 2008). This can be attributed to the fact that GC-O analysis essentially evaluates the aroma of the compounds individually and separate from the sample matrix. GC-O does not take into account the interaction effects that occur when aroma compound interact with the nonvolatile matrix (Pineau et al. 2007, Robinson et al. 2009, Sáenz-Navajas et al. 2010, 2012) and with other volatile compounds (Atanasova et al. 2005b, Escudero et al. 2007, Pineau et al. 2009). As discussed later, these interactions may result in variations in the sensory character of the mixture due to enhancement and suppression effects. GC-O also tends to focus on potent aroma compounds, which chromatograph well at low concentrations, while abundant compounds tend to overload the chromatographic column, resulting in broad peaks that elute in the effluent over a long period of time and presenting only a fraction of the compound to the operator to smell.

A recent modification of GC-O allows individual peaks or groups of peaks to be selectively recombined as they elute off the column and smelled as a mixture (Johnson et al. 2012). The GC-recomposition olfactometry (GC-R) technique allows the creation of aroma reconstitutions without the need for chemical standards or reductive aroma models. This technique provides a holistic approach for understanding the aroma of complex mixtures and allows for additive, masking, and synergistic effects of mixtures to be readily evaluated. When applied to wine samples, this approach may make it possible to more fully characterize grape and wine varietal characters, which are the result of interactions of many aroma compounds acting together to produce the overall sensory perception.

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Odor activity values (OAVs) are also widely used for selecting compounds in a mixture that have the most impact on perceived aroma (Grosch 2001). In this approach, the concentration of all aroma volatiles in the sample is determined and then combined with sensory threshold information determined separately using the pure chemicals. The OAV is then determined as a ratio of the measured analyte concentration in the sample to the analyte sensory threshold. Those compounds with high OAVs would be expected to most directly impact the overall aroma of the sample (Grosch 2001). This technique requires that analytes be accurately quantified and that pure compounds be available to determine sensory thresholds, which, in many cases, may not be possible. In addition, this approach does not account for the complex interactions that occur in perception of mixtures. Since sensory thresholds are determined statistically using a large number of panelists, they are dependent on a large number of variables, including the sample matrix, and as such they may not reflect the range of concentrations that may actually be perceived in a given food matrix by an individual. Finally, the relationship between perceived intensity and concentration at suprathreshold concentrations cannot be predicted from the threshold concentration (i.e., the slope of a plot of perceived intensity vs. concentration is different for different compounds). Because of these limitations, OAVs are not always good indicators of the contribution of individual odorants to the perceived intensity of a mixture (Audouin et al. 2011), as further demonstrated with the white wine variety Maccabeo using reconstitution studies with a dearomatized white wine matrix and pure aroma compounds (Escudero et al. 2004). Selected compounds such as fusel alcohols, acids, and esters, even when present at concentrations above their sensory thresholds, did not have a significant impact on the overall wine aroma. On the other hand, a group of compounds with odor activities less than one were important to reconstructing the aroma of the original Maccabeo wine.

The analytical approaches described in the above sections are focused on fully characterizing the chemical composition of grapes and wines. GC-O approaches attempt to link composition directly to the sensory impact of individual compounds. However, as discussed above with GC-recomposition olfactometry analysis, holistic measurements of sensory perception are also necessary to fully characterize wine flavor. Such approaches for sensory evaluation of wine are discussed in the following section.

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## Sensory Evaluation of Wine

Sensory evaluation stems from the experimental psychology field of psychophysics, which explores how human responses are elicited by chemical and physical stimuli. Early work in this field by researchers including Ernst Weber and Gustav Fechner investigated the mathematical relationship between the physical and perceptual magnitude of stimuli and is still the subject of much cognitive research (Dehaene 2003). Where psychophysics might focus experimental research on understanding how humans respond to stimuli, sensory evaluation focuses on using human subjects to explore the sensory properties of stimuli. Sensory evaluation has been defined as a scientific method used to evoke, measure, analyze, and interpret those responses to products as perceived through the senses of sight, smell, touch, taste, and hearing (Lawless and Heymann 2010). Sensory evaluation in the food, beverage, and fragrance industries was historically conducted by product experts such as the cheesemaker, winemaker, or perfumer, who had training and extensive knowledge of how raw materials and processing affected the finished product quality (Sidel and Stone 1993). Although these product experts are still commonly used in industry today, the use of specialized sensory panels for conducting discriminative, descriptive, and affective evaluations of products has many more advantages. It is generally recognized that the judgments of a panel are generally more reliable than the judgments of an individual, there is less potential risk that the single expert might be ill/retire/die or otherwise be unavailable to make decisions, and, most importantly, the opinions of the expert may or may not reflect what consumers want in a product (Sidel and Stone 1993).

Several types of sensory tests are possible, including discrimination, descriptive, and consumer. Discrimination tests determine whether two products are perceptibly different from one another, for example, due to a modification in the production process or the identification of a defect. These tests are commonly fast and easy to conduct and require little training. However, they provide little additional detail about why the differences exist or the relative impact of the difference (Peryam and Swartz 1950, Lawless and Heymann 2010). Descriptive tests are used to obtain a more detailed description of the sensory attributes of a product. These tests assist in identifying which attributes vary due to a modification to the product or to compare attributes between/among products. Descriptive tests typically require additional time and panel training compared to difference tests (Murray et al. 2001, Lawless and Heymann 2010). Consumer (hedonic) tests are used to determine if a consumer likes a product, prefers it to another product, or finds the product acceptable based on its sensory characteristics. These tests are different from market research where the extrinsic factors that can affect wine choice are also considered, such as brand, region, price, and awards (Lockshin et al. 2006, Lawless and Heymann 2010).

Wine is a highly diversified food product made from numerous grape varieties, grown in a diverse range of environments worldwide, and produced using a multitude of permutations in viticultural management and winemaking techniques that have been developed over the course of centuries. As such, the sensory characteristics of wines are highly varied. Use of standardized terminology can aid in the communication of sensory attributes of wine products among winemakers, marketers, consumers, and researchers (Noble et al. 1984, 1987, Gawel 1998, Gawel et al. 2000). However, standardized terminology is not always used by winemakers, wine writers, and consumers, who frequently use everyday language to infer relationships between the wine product and the sensory properties of other common food products and smells. This was demonstrated in a study of vegetal aromas in Cabernet Sauvignon wines using expert winemakers and trained sensory panelists (Preston et al. 2008). Experts used a variety of terms to group and describe vegetal characters in the wines, and in some cases vegetal and sulfur attributes were often grouped similarly. However, trained panelists reliably distinguished between related vegetal terms such as a fresh, bell pepper-like aroma, a cooked vegetal aroma (a mixture of canned potatoes corn, green been, and asparagus), a green/black olive aroma, and a eucalyptus aroma. Each of these aroma attributes would arise from different chemical components. Therefore, in order to minimize or optimize specific aroma characteristics in a wine, precise and well-defined terminology for sensory attributes, as is obtained with sensory descriptive analyses, is needed when communicating about wine sensory properties.

**Descriptive analysis of wine and linking sensory and chemical data.** To quote a succinct summary of the importance of descriptive sensory analyses, “Without sensory evaluation, even precise information about the volatile composition in the nasal passages cannot predict the flavor of the system as perceived by humans” (Noble and Ebeler 2002). Descriptive sensory analysis is one of the most powerful tools for conducting product comparisons and for determining relationships between sensory properties of foods and beverages and their composition or consumer liking (Murray et al. 2001, Lawless and Heymann 2010). There are a number of different methods for conducting descriptive analysis, including the flavor profile method, texture profile method, Quantitative Descriptive Analysis™, the Spectrum™ method, quantitative flavor profiling, and free-choice profiling, and their comparable advantages and disadvantages have been previously discussed in a review of the field (Murray et al. 2001). Descriptive sensory analysis has been used extensively in the wine industry over the last 30 years following pioneering work conducted by Ann Noble and coworkers at the University of California, Davis, in the late 1970s and early 1980s (Arnold and Noble 1979, Schmidt and Noble 1983, Aiken and Noble 1984, Heymann and Noble 1987, Noble and Shannon 1987).

Numerous studies have used descriptive sensory analysis to explore differences in the sensory characteristics of single variety wines, including Cabernet Sauvignon (Heymann and Noble 1987), Chardonnay (Arrhenius et al. 1996), Pinot noir (Guinard and Cliff 1987), and Zinfandel (Noble and Shannon 1987) from California, Seyval blanc from Missouri (Andrews et al. 1990), Cabernet franc from the Niagara region (Hakimi Rezaei and Reynolds 2010), Cabernet Sauvignon from Australia (Robinson et al. 2011a), Riesling from Germany (Fischer et al. 1999), Malbec from Argentina (Goldner and Zamora 2007), Albariño from Spain (Vilanova and Vilariño 2006), Touriga Nacional and Tinta Roriz from Portugal (Falqué et al. 2004), and Sauvignon blanc from New Zealand and other countries (Lund et al. 2009). In addition, numerous studies have predominantly used descriptive sensory analysis to explore the sensory impacts of various viticultural and enological treatments (Lesschaeve 2007, Robinson et al. 2011b), such as the influence of oak (Francis et al. 1992, Reynolds et al. 2001, Cano-López et al. 2008), fermentation with different yeast strains and temperatures (Eglinton et al. 2000, Soden et al. 2000), wine storage temperature conditions (Francis et al. 1994, De La Presa-Owens and Noble 1997, Robinson et al. 2010, Hopfer et al. 2012b, 2013), closure types (Godden et al. 2001, Skouroumounis et al. 2005a, 2005b, Hopfer et al. 2012b, 2013), grapevine diseases including *Botrytis cinerea* and *Uncinula necator* (powdery mildew) (Stummer et al. 2003, 2005, Sivertsen et al. 2005), and grapevine water status, crop yield, and canopy management (Reynolds et al. 1996, 2013, Chapman et al. 2004, 2005).

Although many studies have explored the sensory differences among imposed treatments, there is much current interest in exploring the relationships between these sensory differences and wine composition and/or the sensory aspects of wine associated with consumer preferences

(Francis and Newton 2005, Lesschaeve 2007). Recent examples include identification of sensory attributes that drive consumer and expert acceptance of Shiraz and Cabernet Sauvignon wines (Lattey et al. 2010), assessment of the relationship between sensory and chemical data for oak-derived compounds found in French and Spanish wines (Prida and Chatonnet 2010), modeling of wine mouthfeel attributes using metabolomic data (Skogerson et al. 2009), comparisons between vine vigor status with tannin and sensory data (Cortell et al. 2008), assessment of the relationships between red wine textural characteristics and the chemical composition of Shiraz wines (Gawel et al. 2007), comparisons of volatile components of sweet Fiano wines and sensory data (Genovese et al. 2007), and comparisons between volatile compositional data and sensory data of Chardonnay, Cabernet Sauvignon, Malbec, and Sauvignon blanc wines made in different styles and/or from different regions (Lee and Noble 2006, Robinson et al. 2011a, 2011b, Benkwitz et al. 2012, Hjelmeland et al. 2013, King et al. 2014). Future work may also consider aspects of wine and food interactions, given that an understanding of how people enjoy pairing wine with food combinations is commonly discussed in the popular literature (Madrigal-Galan and Heymann 2006).

In a recent study both sensory descriptive analysis and chemical analysis were used to understand the influences of blending on chemical and sensory profiles of Cabernet Sauvignon, Merlot, and Cabernet franc monovarietal wines and their blends (Hopfer et al. 2012a). Blending resulted in changes to both sensory and chemical characteristics relative to the original monovarietal wines. The measured chemical composition reflected the average composition of each mono-varietal wine in the blend. However, the sensory profiles exhibited examples of suppressing or amplifying effects, and the overall sensory profile was not a simple average of the sensory profile of the monovarietal base wines. Interestingly, similar sensory profiles could be obtained with very different blending mixtures. This study demonstrates the value of using both sensory and chemical analyses to describe wine aroma and flavor, but also points to the difficulties in fully linking compositional information to sensory properties due to complex aroma interactions. These aroma interactions are further discussed below.

In all cases, descriptive sensory analysis produces multivariate data in relation to a single sample set. In the above examples, multivariate statistical techniques have also been critical in exploring relationships between descriptive sensory data and compositional and/or consumer sensory data, such as multivariate analysis of variance (MANOVA), principal component analysis (PCA), canonical variate analysis (CVA), generalized Procrustes analysis (GPA), and partial least squares (PLS) regression. In general, the use of multivariate statistics for relating sensory and chemical data has been well described (Noble and Ebeler 2002, Lawless and Heymann 2010, Naes et al. 2011).

There is still need for improved methods for handling the large data sets involved in correlating analytical and sensory data. Standard workflows, particularly for analysis of chromatographic data, are time-consuming and involve many steps where automated data processing is still limited, as discussed previously. In addition, many of the statistical approaches currently used have limitations when the number of samples or treatments is small with respect to the number of variables (as frequently happens with hyphenated GC data such as GC×GC and high-resolution MS where hundreds of peaks may be present in a sample). Further, relationships among analyte concentrations, sensory responses, and treatment variables may not be linear, making it difficult to interpret results from statistical approaches based on linear relationships. Finally, in cases where predictive models are developed (e.g., PLS analysis relating analyte concentrations to sensory attribute intensities), methods of cross-validation and model testing must be clearly defined and the variability in responses observed within the training sets must represent the variability in the samples tested. An excellent overview of these issues has recently been presented (Smilde et al. 2013). An understanding of these limitations and new approaches for analysis of complex multivariate data sets may provide new opportunities for linking chemical and sensory data to fully understand the flavor of complex chemical mixtures.

**Interaction effects on wine flavor.** As previously discussed, much wine sensory research has focused on correlating descriptive sensory and quantitative analytical data in order to successfully identify odor compounds that contribute to the overall aroma perception of wine (Guth 1997a, 1997b, 1998, López et al. 1999, 2003, Kotseridis and Baumes 2000, Ferreira et al. 2001, 2002, Lee and Noble 2003, Escudero et al. 2004, Campo et al. 2005, Bailly et al. 2006, Benkwitz et al. 2012). The use of sensory evaluation to elucidate the impact of complex aroma compound interactions, including masking and enhancing effects, is likely to improve our understanding of the perceived aroma of wine (Atanasova et al. 2005a). For example, using descriptive analysis, it has been shown that fruity aromas in wines can significantly mask vegetal characters in the wines (Hein et al. 2009). Similarly, at low concentrations, ß-damascenone has the ability to mask the herbaceous aroma associated with 3-isobutyl-2-methoxypyrazine (Pineau et al. 2007) while enhancing the berry fruit aromas in red wines (Escudero et al. 2007, Pineau et al. 2007). As noted in the above section, the aromas of wine blends exhibited suppressive and amplifying effects when the intensity of specific aroma attributes of the blends was compared to the original aroma of the monovarietal wines (Hopfer et al. 2012a). These results indicate that aroma interactions in the complex wine blend mixtures can impact the overall aroma in a nonadditive manner (Hopfer et al. 2012a).

The matrix components can also impact aroma volatility, release, and perception. Recent sensory research has shown that ethanol suppresses the fruity notes in model wine solutions (Grosch 2001, Escudero et al. 2007, Le Berre et al. 2007, King et al. 2013) due to the increased solubility of the volatiles when ethanol is present (Le Berre et al. 2007, Robinson et al. 2009) and to the inhibition of volatile compound odor activity by ethanol (Grosch 2001). For example, ß-damascenone is recognized universally as a potent wine aroma compound (Skouroumounis and Sefton 2002, Pineau et al. 2007, Sefton et al. 2011); however, its reported aroma threshold varies from 2 to 13 ng/L in water (Buttery et al. 1990, Czerny et al. 2008), to 50 ng/L (Guth 1997a) in 10% aqueous ethanol, to 7000 ng/L in a red wine matrix (Pineau et al. 2007).

These results indicate that in addition to ethanol, other wine components also impact aroma perception, therefore understanding the factors that influence the release of volatiles from the complete wine matrix is of major importance to understanding wine aroma perception (Plug and Haring 1994). Several studies have shown that polyphenols, tannins, polysaccharides, and proteins can interact with aroma compounds and affect their volatility and release from solution (Dufour and Bayonove 1999a, 1999b, Voilley and Lubbers 1999, Jung et al. 2000, 2002, Jung and Ebeler 2003, Aronson and Ebeler 2004, Jones et al. 2008, Saenz-Navajas et al. 2010, 2012, Villamor and Ross 2013). A recent study clearly demonstrated that nonvolatile tastants (e.g., polyphenols, tannins/high molecular weight components, glycerol, and organic acids) can significantly impact the perception of wine aroma compounds, enhancing the intensity of some attributes and decreasing the perceived intensity of others (Frank et al. 2011). The mechanisms for the interactions are not entirely clear but may be due to changes in solubility or noncovalent interactions/associations of the odorants with the solute components in solution (Connor et al. 1998, Jung et al. 2000, 2002).

The existence of interaction effects clearly supports the use of holistic approaches, such as descriptive analysis, in the sensory assessment of wine products where physical, chemical, and perceptual enhancement and suppression effects complicate the assessment of volatile components in isolation.

## Summary

Advances in analytical, sensory, and statistical analysis have been critical for understanding the relationships between grape and wine composition and sensory perception. However, it is still not possible to fully predict aroma quality based on chemical composition alone, due, in part, to the potential presence of trace compounds that may be difficult to quantify and identify and to the complex interactions of aroma compounds with each other and with the wine matrix that impact aroma volatility, release, and perception. As outlined in this review, future discoveries will continue to be driven by development of improved and high-throughput analytical methods that will allow monitoring of a large number of volatiles, including those present at low concentrations. Sensory and statistical approaches that allow for handling of large multivariate data sets are also needed. Ultimately, multidisciplinary studies using genomic, proteomic, metabolomics, and sensory techniques to understand flavor and aroma formation in the grape and during fermentation will generate essential knowledge about the role that production processes—from the vineyard to the cellar—have on the final product presented to a consumer.

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