

Wine Chemistry and Flavor: Looking into the Crystal Glass

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Over the past century, advances in analytical chemistry have played a significant role in understanding wine chemistry and flavor. Whereas the focus in the 19th and early 20th centuries was on determining major components (ethanol, organic acids, sugars) and detecting fraud, more recently the emphasis has been on quantifying trace compounds including those that may be related to varietal flavors. In addition, over the past 15 years, applications of combined analytical and sensory techniques (e.g., gas chromatography–olfactometry) have improved the ability to relate chemical composition to sensory properties, whether identifying impact compounds or elucidating matrix effects. Many challenges remain, however. This paper discusses some of the recent research aimed at understanding how viticultural and enological practices influence grape and wine volatiles. In addition, the challenges in linking composition to sensory properties will also be reviewed. Finally, future advances in linking grape, yeast, and human genomics to wine chemistry and flavor will be briefly discussed.

KEYWORDS: Wine; flavor; volatiles; viticulture; genomics; analysis

“When the chemist looked for employment in the wine industry, he was naturally asked what he could do for the benefit of the industry. On replying that he could make an analysis of the wine, he was met with this answer: “Well, after I know the composition of the wine, what good does it do me? Can you tell me whether the wine is good, bad or indifferent? An expert taster can tell all these things” (1).

It is appropriate that wine chemistry be included as a topic in the Division of Agricultural and Food Chemistry’s 100th anniversary symposium, as the development of this sub-branch of food chemistry has paralleled that of the Division itself. The Division dates from 1908 (2, 3); seven years later Charles Ash published an article in the *Journal of Engineering and Industrial Chemistry* titled “Contributions of the Chemist to the Wine Industry” (1). Ash notes that trying to introduce chemistry proper to the wine industry 15 years earlier was an “entirely thankless task,” although the importance of chemistry to characterizing wines had already been recognized (4). What follows is a brief history of wine and wine chemistry and an attempt to gaze into the “crystal glass” at what challenges and opportunities wine chemists will encounter in the future.

Divining the paleohistory of wine has been a task highly dependent upon the analytical chemist; traditional archeological evidence in the form of pottery and written or pictographic evidence has traced winemaking to the Neolithic period (8500–4000 B.C.) in the Trans-Caucasus and ancient Near East (5). More direct evidence, however, has been acquired by analyzing for tartaric acid residues (6, 7); few fruits other than grapes accumulate appreciable concentrations of this organic acid (8).

From what is modern Iran wine production technology spread throughout the Fertile Crescent and was further developed and refined by the ancient Mesopotamian, Egyptian, Greek, and Roman civilizations. However, once the basic technology was developed, there was little progress in wine processing and certainly none in understanding the underlying chemistry. Interestingly, the distillation of wine following the creation of the alembic still (attributed to Jābir ibn Hayyān) proved to be essential to alchemical investigations (9); nevertheless, it would take alchemy’s own transmutation into modern chemistry under Dalton and the subsequent investigations of Pasteur to mold wine chemistry into a recognizable discipline.

Thus, in the late 1800s with the discovery of the role of microorganisms in fermentation and improved understanding of organic chemistry pioneered by Pasteur, the focus began to shift to the analysis of the major components known to contribute to wine quality, including ethanol, sugars, and organic acids. An early instrument used to measure ethanol concentrations, the ebulliometer, is based on the boiling point depression of aqueous solutions as the percent ethanol in the solution increases, an effect discovered by the Abbé Brossard-Vidal (10). Ebulliometers can still be found in many wineries today due to their low cost and relative ease of use. Methods of analysis for ethanol and the other components, including the “odoriferous constituents”, were outlined by Mulder in his 1857 text, one of the earliest treatises to specifically focus on wine chemistry as opposed to wine production. It is fascinating that, with respect to the aroma compounds, Mulder despairingly noted that “The attempt has often been made to separate the actual peculiar odoriferous ingredient out of aromatic wine. Since, however, we know that of the most important by weight of these odoriferous ingredients 1/40000th only appears in wine, this plan may be given up ...” (11).

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Much of this early interest in, and development of, wine chemistry and compositional analysis was actually driven by issues of adulteration and fraud. Such issues have existed since the earliest days of food commerce; ensuring authenticity and safety was dependent upon the development of “modern” analytical tools and techniques (12). C. R. Fresenius, who many consider a “father” of analytical chemistry, devoted much of the analyses in his laboratory to wine analysis, and many of the first written food/beverage analysis procedures authored by Fresenius and his co-workers were for the analysis of wine components (13).

This was essentially the state of the discipline in the second half of the 19th century; however, different pressures were being brought to bear on wine science in the United States. Using *Vitis vinifera* grape varieties and winemaking techniques developed in Europe, the rapidly growing American industry was faced with adapting these varieties and processes to the very different conditions that often existed in the United States, particularly in California. In 1879 Eugene Hilgard, the second Professor of Agriculture in the University of California, reported to the State Legislature on the problems facing the grape and wine industries. This led to an 1880 act mandating that the University establish instructional programs in viticulture and enology; originally located in Berkeley, the programs were shifted to the University Farm in Davis, eventually evolving into the Department of Viticulture and Enology (14). Hilgard’s vision ushered in a new age in the United States for the scientific understanding of wine.

Much of the early focus was on matching varieties to the unique growing conditions within a particular region with an analytical focus on optimizing sugar and acid levels in the grapes as parameters for determining ripeness/quality. Early pioneers, including Hilgard and Frederic Bioletti, published seminal papers focused on maximizing sugar and acid ratios in the grapes to achieve optimal wine acidities after fermentation. In an extensive comparison of 67 varieties over a period of five years in Vineland, NJ, Caldwell (15) was one of the first to show a clear linkage between environmental conditions (temperature, sun exposure, and annual rainfall) on chemical composition of the grapes (sugars, acids, tannins). In particular, “the amount of sunlight received by the plant during the formation of the crop” was positively correlated with the amount of sugar and negatively correlated with acid and astringent constituents. Interestingly, the author also observed that the “soil and cultural conditions appear to affect varietal composition more profoundly than do variations in environmental conditions encountered over a series of years in one location”. Early forays into grape flavor chemistry were made by Frederick Power at the Phytochemical Laboratory of the USDA’s Bureau of Chemistry; Power and his associate Victor Chestnut studied the occurrence of methyl anthranilate in grape juices (16–18).

During the period of Prohibition in the United States (1920–1933) much of the nascent scientific knowledge learned during the previous years was lost or forgotten; with the repeal of prohibition a renaissance of wine science began. Analytical chemistry was still focused, of necessity, on major components, but now the focus was on those compounds associated with defects, largely acetic acid, and on identifying winemaking conditions that could prevent oxidative spoilage. The scientific study of winemaking conditions was enhanced by a plethora of studies at University of California—Davis using small-scale fermentations with controlled conditions to evaluate individual winemaking variables (cataloged by ref 19). Dissemination of this scientific knowledge obtained a boost with the establishment of the Fermentation Subdivision of the American Chemical Society in 1946 (3) and the subsequent formation of the American Society of Enologists

(the current American Society for Enology and Viticulture) in 1950.

As these major defects became less prevalent, the focus again began to shift toward those analytes that contributed to the positive quality attributes of grapes and wines, particularly those components contributing to flavors and aromas. Advances in two scientific areas, (1) chromatography (20, 21), which allowed complex mixtures of compounds, including those present at trace levels, to be separated, identified, and quantified in wines and other alcoholic beverages; and (2) analytical sensory analysis (22), which allowed the perceptual impact of these compounds to be objectively determined, greatly advanced enological knowledge. These advances inaugurated the “revolution” in linking compositional analyses to quantitative assessments of sensory attributes determined using robust statistical analyses.

The components contributing to wine flavor arise from the grapes and the *Saccharomyces* fermentation; other microbes (*Brettanomyces*, lactic acid bacteria) and other processes (oak barrels and oak adjuncts, bottle aging) also contribute but, due to space considerations, will not be addressed here. Furthermore, although wine flavor perception involves all of the senses (reviewed by ref 23) the following sections will focus largely on the volatile aroma compounds that can be sensed by the olfactory receptors either orthonasally or retronasally.

VOLATILE AROMAS AND IMPACT COMPOUNDS

Alcohols and Esters. In the 1950s, the newly available commercial gas chromatographs (GC) became indispensable tools for aroma analysis, making it possible to begin to identify the trace “odiferous components” described by Mulder nearly 100 years earlier (24, 25). The focus of early GC studies was typically on the analysis of esters and alcohols, formed during fermentation, and which quantitatively constituted the majority of the volatile components in wines (typically present in approximately mg/L concentrations in wines). In addition, much of the early analytical chemistry focus was on identifying stationary phase materials suitable for separating the complex mixtures in alcoholic beverages—one report even used Tide detergent for separation of amyl alcohols (26). Sample preparation and potential for artifact formation during the analysis were also the focus of much research. For example, water as a major “contaminant” hampered many early analyses (27, 28) often requiring distillation, solvent extraction, concentration, and/or other cleanup steps such as passage through Celite or calcium sulfate columns.

Numerous esters are formed during fermentation including the fatty acid ethyl esters and the acetate esters, both of which contribute important fruity notes to wines. Ethyl acetate (with a solvent, nail polish aroma) and isoamyl acetate (banana-like aroma) typically predominate with concentrations of 50–150 and 0.5–10 mg/L, respectively (29).

In an early GC analysis of alcohols in wines and alcoholic beverages using flame ionization detection (GC-FID), Webb and Kepner recognized the difficulty in separating the four primary alcohols, *n*-propyl, isobutyl, active amyl, and isoamyl alcohols (30). Using a 10 ft diglycerol column, they successfully separated these compounds as well as isopropyl, ethyl, *sec*-butyl, *n*-butyl, *n*-amyl, and *n*-hexyl alcohols. Interestingly, this paper was also among the first to analytically demonstrate that formation of these compounds was dependent on the yeast strain used to ferment the Sauvignon blanc grape juice; previous work had relied on either sensory analysis (see, e.g., ref 31) or analysis on model solutions (see, e.g., ref 32).

These advances in the analysis of fermentation volatiles were also augmented by improved knowledge of biochemical pathways

involved in alcohol and ester synthesis (33), reviewed by ref 34. In addition to yeast strain effects discussed above, other fermentation conditions, particularly temperature and availability of yeast nutrients, have an important impact on the formation of alcohols and esters during fermentation (35, 36). Currently, there is a general understanding that genetic variations among yeast species and strains influence the expression of the genes responsible for ester formation and hydrolysis (see, e.g., ref 37), and it is thought that fermentation conditions such as temperature and availability of yeast nutrients impact gene expression and ester synthesis. This remains an active area of research (reviewed by refs 38 and 39).

Although quantitatively important to the volatile composition of wines, there was early recognition, however, that many of the esters and alcohols formed during fermentation did not make significant contributions to overall flavor and aroma perception. To quote Webb and Kepner (30): "These four (fusel) alcohols have different odors and one would expect the aromas of different wines and brandies to reflect variations in the proportions of these alcohols, even though other, trace components are of greater importance in defining the aroma." In addition, early chemical and gas chromatographic analyses indicated that compositional differences contributed to important varietal characters independent of formation of fermentation volatiles (40–42). Therefore, there was increasing interest in identifying "impact" compounds, many of which were present at trace microgram or nanogram per liter levels and most of which were derived from the grapes. Identification and analysis of these trace impact compounds were enabled by developments in capillary columns, new detectors such as mass spectrometers, and improved GC electronics for detecting the narrow peaks associated with high-resolution GC. In the next sections we will focus on these important impact volatiles that contribute to the overall aromas of grapes and wines. Comprehensive treatments of each class of aroma compound are beyond the scope of this paper; we have merely attempted to provide an overview of the historically important advances with respect to these odorants.

Terpenes. The monoterpenes are a diverse class of natural products that contribute important floral and citrus characters to wines. These terpenes are present in the grapes (largely in the skins); their overall levels increase during grape maturation (43), and they are extracted into the wine from the grapes during fermentation. Although monoterpenes are present in most grapes and wines, they are particularly prevalent in varieties of the Muscat and Riesling families, as shown in early studies by Cordonnier (44) and Usseglio-Tomasset (45, 46). Improvements in separations obtained with high-resolution capillary GC columns in the 1970s and 1980s enabled the identification of over 50 monoterpenes in grapes and wines, and the terpene composition is widely used for varietal characterization (reviewed by refs 42 and 47–49).

In the early 1980s the presence of numerous terpene glycosides in grapes was reported (50–53). The terpene glycosides are hydrolyzed during fermentation by yeast glycosidase enzymes and by the acidic fermentation conditions (pH ~3.5) to form the free volatile terpene aroma compounds. These hydrolysis reactions may be particularly important during the aging of wines, and Williams et al. (54) proposed a simple assay that could estimate or predict the "aroma potential" of wines from the glycosyl-glucose (G-G) concentrations of grapes or musts. These G-G analyses are complicated by the fact that many nonvolatile polyphenols, including anthocyanins, are also glycosylated in the grape, so that the total amount of glycosylated components is greater than just those contributing to aroma. A modified assay that measures the "red-free" (i.e., anthocyanin-free) glycosides was also proposed (55), and both assays have been shown in some

cases to correlate with aroma intensity of selected wine attributes (56, 57). Prediction of aroma potential from grape terpene composition is also complicated by the fact that acidic conditions present during fermentation and storage can catalyze many terpene rearrangements, yielding new compounds with different aroma qualities and intensities. For example, linalool, an important floral aroma component of Muscat and Riesling varieties, can be transformed to α -terpineol, hydroxylinalool, geraniol, or nerol under aqueous, acidic conditions (42).

Terpene composition of grapes can be influenced by climate and viticultural conditions (58–60). In general, cool climates and shade decrease terpene concentrations by mechanisms that are not well understood. This is partially due to the fact that the biochemistry of terpene synthesis, although well-studied in other plants, has not been well-characterized in grapes; only a few enzymes and genes involved in grape terpene biosynthesis have been identified and characterized (61–68). In an interesting paper, Gholami and co-workers (69) grafted Muscat grape clusters onto the vines of Shiraz or Sultana (which have only very low terpene concentrations in the grapes) and vice versa. High terpene levels were observed in the Muscat berries even when grafted onto Shiraz or Sultana vines, whereas Shiraz and Sultana clusters did not have high terpene levels when grafted onto the Muscat vines. The results indicate that the synthesis of these important aroma compounds is genetically determined and occurs in the berries (as opposed to synthesis in leaves or other parts of the vine followed by translocation). Recently, Jaillon et al. (70) observed that a significant portion of the Pinot Noir genome was dedicated to terpene synthesis genes (89 functional genes and 27 pseudogenes). Although this grape variety is not characterized by high levels of terpenes, the prevalence of these genes may point to an important evolutionary role of terpenes in grape development and/or pest and disease resistance. Further information relating effects of grape variety, environmental conditions, and grape maturity on gene expression, terpene concentrations, and aroma properties is needed.

Methoxy-pyrazines. In the late 1960s Buttery and co-workers identified 3-isobutyl-2-methoxypyrazine (IBMP) as the main impact compound responsible for the aroma of bell peppers (71). Using vacuum steam distillation followed by capillary GC and mass spectrometric detection (GC-MS), Buttery identified this compound and determined that it had a sensory threshold of 2 ng/L in water, making it one of the most potent odorants known at that time. On the basis of this groundbreaking work, wine flavor chemists hypothesized that the bell pepper character associated with Cabernet Sauvignon and Sauvignon Blanc grape varieties could be due to IBMP (72, 73); however, it was not until 1987 with developments in mass spectrometry (i.e., application of GC-MS with selected ion monitoring and chemical ionization) and improved extraction techniques (distillation, ion exchange at pH 10, solvent extraction, and concentration) that Allen and co-workers were able to identify IBMP in these grape varieties and link the IBMP concentration to sensory perception of vegetal characters in the final wines (74, 75). Quantitation at levels of 0.3 ng/L (and detection limits as low as 0.1 ng/L) was also enabled by the use of stable isotope labeled internal standards. In addition to IBMP, 2-*sec*-butyl-3-methoxypyrazine (SBMP) and 3-isopropyl-2-methoxypyrazine (IPMP) have also been identified in grapes and wines; IBMP levels range from 4 to 30 ng/L (depending on variety, maturity, and growing conditions) and are typically at least 8 times greater than the other two pyrazines (reviewed by ref 76). High levels (> 15 ng/L in white wines, > 25 ng/L in red wines) contribute an "undesirable" herbaceous aromas to wines (76), and each of the pyrazines has slightly different aroma qualities; IBMP is described as bell pepper/green gooseberry,

whereas IPMP is described as asparagus/green bean and SBMP as pea/bell pepper (77). It is important to note, however, that not all vegetal aromas can be related to methoxyppyrazines (78).

Heymann et al. (79) reported that IBMP was readily degraded with light exposure, and studies of Cabernet Sauvignon in the vineyard showed that high levels of light in the fruiting zone yielded wines with lower IBMP concentrations compared to shaded clusters (80). IBMP levels are also thought to be influenced by grape maturity (levels decrease with maturation), temperature of the grape cluster, microclimate (cooler climates have higher levels), pruning (i.e., manipulation of buds per vine), and vine water potential (75, 76, 81–89).

No biosynthetic pathways for IBMP have been identified, although leucine has been proposed as a precursor and source for the isobutyl side chain (90), similar to biosynthesis of isopropylmethoxyppyrazine by bacteria (76, 91, 92). IBMP has been identified in only a few varieties, mainly Cabernet Sauvignon, Sauvignon Blanc, Cabernet Franc, Merlot, and Carmenere (93–96). Interestingly, Bowers and Meredith (97) showed that Cabernet Sauvignon grapes are derived from a cross between Sauvignon Blanc and Cabernet Franc, indicating that there may be a yet unidentified genetic basis for methoxyppyrazine formation in these varieties.

C₁₃-Norisoprenoids. C₁₃-Norisoprenoids are a diverse group of aroma compounds derived from grape carotenoids. Although present at only trace levels, sensory thresholds of most norisoprenoids are very low (e.g., 700 ng/L for β -ionone and 200 ng/L for β -damascenone) (98); therefore, these compounds can contribute important aroma properties to many red and white wine varieties, including Chardonnay, Chenin Blanc, Semillon, Sauvignon Blanc, Cabernet Sauvignon, and Syrah (reviewed by refs 57 and 99). As with other volatile compounds, many new norisoprenoids were identified in grapes and wines during the 1970s and 1980s concomitant with advances in gas chromatographic analyses (98). Climate, sunlight exposure, and grape maturity have significant effects on levels of norisoprenoids in grapes (99–108).

Similar to the monoterpenes, many norisoprenoids occur in grapes as nonvolatile glycoside precursors. Hydrolysis during fermentation and storage releases the free aroma compounds (57), which then make important contributions to the wine aroma. For example, acid hydrolysis of glycoside precursors during storage leads to the formation of 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), which gives a kerosene aroma to aged Riesling wines (109–111). Recently, Janusz et al. (112) observed that the potent odorant (*E*)-1-(2,3,6-trimethylphenyl)buta-1,3-diene (TPB) is derived from a glycoside precursor and may contribute a strong green or cut-grass aroma to white wines, particularly Semillon. In addition, Sefton et al. (113) and Francis et al. (57) have shown that glycosidic precursors of norisoprenoids can contribute to the honey, tea, and lime attributes of Chardonnay and Cabernet Sauvignon wines.

Photochemical and thermally induced degradation of carotenoids is known to produce many norisoprenoids (114–116). However, there is growing evidence that enzymes are involved in the regioselective cleavage of the carotenoid chain. The exact reaction mechanism through which these enzymes operate (i.e., via a mono- or a dioxygenase mechanism) has still not entirely been clarified, although experiments in *Arabidopsis* strongly point to a dioxygenase mechanism (117). These so-called “carotenoid cleavage dioxygenase” (CCD) enzymes cleave carotenoids at the 9, 10 (and 9', 10'), and other positions (118). cDNAs encoding CCDs have been isolated and characterized from numerous plant species including grapes, and their involvement in the biosynthesis of various norisoprenoids has been suggested (119–124). This

is currently an active area of research, and much remains to be discovered with respect to the genetic and environmental factors influencing norisoprenoid production in grapes.

Volatile Sulfur Compounds (VSC). The study of VSC in wines originally focused on their contributions to “off-odors” as simple sulfides (e.g., dimethyl sulfide), disulfides (e.g., dimethyl disulfide), and thiols (e.g., hydrogen sulfide, benzyl mercaptan) are responsible for alliaceous and rubbery aromas (125, 126). In certain cases specific yeast strains are known to overproduce H₂S (127), and thus controlling yeasts' formation of VSC is a highly desired outcome (128). In other cases there is actually formation of VSC through light activation—the so-called “sun-struck” phenomenon (129). There is extensive literature in the beer and dairy industries on these light-activated flavors (see, e.g., refs 130–132). In each case there are three necessary components: (1) light at wavelengths <450 nm, (2) sulfur-containing amino acids or isohumulones, and (3) riboflavin (133, 134).

However, not all sulfur-containing compounds are deleterious to wine quality; a number of organic VSC have been found to make important contributions to wine aroma (135–137). Dubourdieu and co-workers extracted aromatic thiols from Sauvignon Blanc using *p*-hydroxymercuribenzoic acid and identified 4-mercapto-4-methylpentan-2-one as an impact odorant in this cultivar (138). The same group then identified 3-mercaptohexyl acetate (139) and 3-mercaptohexan-1-ol (140) as other important impact volatiles in Sauvignon Blanc. VSC have also been found in red cultivars; 3-mercapto-2-methylpropanol, 3-mercaptohexan-1-ol, and 3-mercaptohexyl acetate were isolated from Merlot and Cabernet Sauvignon wines (141).

Interestingly, these compounds also have nonvolatile precursors, but as opposed to the monoterpenes and norisoprenoids, these particular VSC are bound as their cysteine conjugates (142). Indeed, the volatile thiols are almost devoid in must (143) and were supposed to be released during fermentation by the C–S β -lyase activity of *Saccharomyces cerevisiae*, leading to the creation of a method to assess grapes for their VSC aroma potential (143). The cysteine conjugates themselves were postulated to be breakdown products of glutathionyl conjugates, commonly formed as detoxification products (144), and indeed Dubourdieu's group identified *S*-3-(hexan-1-ol)-glutathione in Sauvignon Blanc musts (145). Recently Subileau and co-workers demonstrated that it was the glutathionyl conjugate, and not the cysteine conjugate, which was the major precursor for 3-mercaptohexan-1-ol formation (146).

The instrumental analysis of VSC in wines was comprehensively reviewed by Mestres et al. (147).

LINKING VOLATILE CHEMISTRY TO HUMAN PERCEPTION

Although advances in gas chromatography and its corresponding detectors led to the identification and quantification of an increasing number of volatile compounds, it soon became clear that many of the compounds that could be identified analytically (and therefore were present in relatively high concentrations) had little impact on the overall sensory properties.

Gas Chromatography–Olfactometry (GC–O). Following the development of GC by James and Martin (148), it was hardly unexpected that flavor researchers would sniff the column effluent in an effort to sensorially characterize the separated volatiles. However, such efforts, although routine, remained informal and outside the scientific literature. In 1964, though, Fuller and co-workers published an article describing such an approach (149). Their original apparatus resembled a rather Dali-esque space helmet; as the authors rather wryly stated “several disadvantages appeared with this system. It was inconvenient for the perfumer in that he had to maintain a continuously stationary position to

prevent burning his nose on the heated tubing, and while in this position, he could not conveniently write or dictate his odor impressions." Their next design, the so-called "bird cage" was a marked improvement, and, with the exception of a humidified air source (as described by ref 150), is similar to modern designs (see, e.g., ref 151). As GC-O techniques have recently been exhaustively reviewed (152) this section will be limited to notable wine flavor applications.

In some of the first applications of GC-O to wines, Nelson and Acree studied the aroma profiles of Concord and Catawba wines and identified the major volatile components (153, 154). Noble et al. (155) were able to distinguish aroma profiles of Riesling, Chardonnay, and French Colombard by multivariate classification methods utilizing only those headspace volatiles ascertained to be of perceptual importance by GC-O. McDaniel's group studied the aroma profiles of Pinot Noir wines produced from grapes harvested at different degrees Brix (156).

Seminal work by Guth utilizing GC-O focused on the aroma profiles of Scheurebe and Gewürztraminer wines. He first identified some ~40 odor-active compounds in extracts of those wines (157). He then performed reconstitution and omission studies in which he found that six compounds with high odor activity values could be differentially combined to closely reproduce the aromas of those wines (158). The impact of compounds with low odor activity values has also been recognized; Ferreira's group has demonstrated this in an elegant series of wine studies (see, e.g., refs 159–161). Clearly GC-O techniques are now widely used to identify important odor active compounds—most recently identification of rotundone in Syrah grapes and wines (162). GC-O is also a powerful tool for identifying those compounds contributing to deleterious odors (42, 49).

Matrix Effects. Understanding volatile composition alone is not enough to fully understand or predict grape and wine aroma. This is due to the interactions that can occur between the odorants and other nonvolatile matrix components, including proteins, polysaccharides, lipids, and polyphenols. Beginning in the 1980s flavor chemists became increasingly interested in how interactions with matrix components affect odorant release, volatility, and overall sensory perception of many foods and beverages, including wines. For example, as reviewed by Voilley and Lubbers (163), volatile aroma compounds in juice and wine can interact (or bind) with yeast polysaccharides, proteins, and lipids, changing the headspace concentration of the aroma compound during fermentation and processing. In general, the extent of the interaction increases with increasing odorant hydrophobicity (particularly for protein–odorant and lipid–odorant interactions); however, the interaction is complex and dependent on the structural properties of both the odorant and the macromolecule.

Polyphenols and tannins comprise a significant portion of the nonvolatile matrix components of grapes and wines. The polyphenols and tannins contribute important taste (bitter), mouth-feel (astringent), and color properties to wines. However, several studies have also shown that polyphenols can interact with odorant compounds in solution, altering the odorant volatility and aroma perception (164–169). As recently reviewed (23), hydrophobic interactions again play a significant role in modulating the extent of the interactions; however, hydrogen bonding interactions can also stabilize the odorant–polyphenol complex that is formed in solution. Studies at the molecular level (e.g., using NMR) can be used to understand the mechanisms of the interactions and predict effects on odorant volatility that are observed by GC–headspace analysis and sensory analysis.

Ethanol also has a significant effect on altering the volatility of other aroma components in solution. In general, ethanol

increases odorant solubility and decreases odorant volatility (170). In model systems, Guth (171) showed that increasing amounts of ethanol decreased the overall fruity perception of a mixture of odorants, consistent with studies by Fischer et al. (172) and reviewed by Ebeler (173) and Escudero et al. (174). In recent studies Tsachaki et al. (175, 176) have shown that under dynamic conditions (as opposed to the equilibrium systems typically evaluated) the presence of ethanol (> 50 mL/L) increased the absolute concentration of a range of volatile compounds in the headspace above the solution. This effect was attributed to increased mass transfer in the presence of ethanol and may have impacts on aroma perception under nonequilibrium situations such as those that occur during sniffing and tasting of alcoholic beverages.

Finally, odorant–odorant interactions also occur and can affect perception; Escudero et al. (174), for instance, found that norisoprenoids and dimethyl sulfide enhanced perception of fruitiness. These interactions may be based on physical/chemical interaction mechanisms but are also perceptual in nature. For example, Buttery (177) has shown an additive effect on the perception of a mixture of compounds in which each compound is present in subthreshold concentrations. Hein et al. (178) has shown that masking effects may be important in influencing the perception of fruity and vegetal flavors in wines. In addition, qualitative changes in aromas can also occur when odorants are presented in mixtures (e.g., a distinct butterscotch aroma arises when ethyl butyrate, which by itself has a bubble-gum/fruity character, is combined with diacetyl, which has a buttery aroma; mixtures of eugenol with a clove aroma and 2-phenylethyl alcohol with a rose-like aroma yield a unique carnation-like aroma). Current developments in neurobiology and increased knowledge of taste and olfactory receptor processes are leading to an improved understanding of how these additive, masking, and perceptual changes may occur (179–183) and may in the future make it easier to predict aroma properties of complex mixtures from compositional information.

NEW ANALYTICAL TECHNOLOGIES

As discussed previously, developments in analytical methods have been closely linked to improved understanding of grape and wine flavor chemistry. Currently, there is much interest in rapid, high-throughput methods for quantifying volatile components and monitoring qualitative changes in volatile composition as a result of viticultural practices, winemaking techniques, or storage processes. A promising approach combines GC with a time-of-flight (TOF) mass spectrometric detector for rapid analysis of complex matrices such as wines. In recent studies, Setkova et al. (184, 185) used GC-TOF to identify and quantify ~201 components in ice wine samples with a GC run time of < 5 min. The method was highly reproducible (RSD < 9.0%). When combined with multivariate statistical analysis, the GC-TOF volatile profile was able to classify over 130 Canadian and Czech ice wines according to their origin, grape variety, and fermentation/aging conditions (oak or stainless steel storage).

Two-dimensional techniques, especially the development of comprehensive GC × GC (reviewed by ref 186), have also greatly improved the analysis of wine volatiles. Heartcut techniques were used by Darriet et al. (187) to identify impact odorants from grapes contaminated by powdery mildew. Ferreira and co-workers have used similar techniques to identify three strawberry-smelling esters, 2-, 3-, and 4-methylpentanoate (188), and the cooked meat-smelling 2-methyl-3-(methylthio)furan (189) in wine. Comprehensive GC × GC-TOF has been applied to methoxy-pyrazine analysis in grapes (190, 191).

In addition, the role of improved chemometric software cannot be understated. It has made a definitive difference in the success of comprehensive GC \times GC (reviewed by ref 192) and is critical to wine aroma classification and discrimination (see, e.g., refs 193–195). The central role of chemometrics in food chromatographic analysis has recently been discussed (196).

Chemometrics also underpins nonspecific sensor systems (so-called “electronic noses” or “electronic tongues”). As extensively reviewed by Röck et al. (197) these systems typically rely on pattern recognition and chemometrics to distinguish subtle differences in complex spectra or instrument responses produced by different samples. For example, Moreneno i Codinachs et al. (198) recently demonstrated a promising multisensor array system that was able to classify wines by the grape variety and vintage year. Berna et al. (199) compared two different electronic nose designs to predict *Brettanomyces* spoilage in red wines. An inexpensive vapor-sensing array composed of chemoresponsive dyes has been used by Suslick and co-workers (200–202) to classify commercial beverages including lager and ale beers. Adulteration of the beers by watering could also be identified. While not applied to grape or wine analyses, the chemoresponsive dye sensors may provide a rapid and simple tool for monitoring changes in volatile composition in the vineyard and during processing.

Most sensor systems developed to date, however, have limited sensitivity for the many aroma-active compounds that are present in grape and wine samples at low concentrations. In addition, specificity of a particular instrument’s response is limited by the physical and chemical properties of the sensors used. Many of the sensor arrays are influenced by humidity (moisture content), pH, or other matrix variables independent of the analytes of interest, and therefore application of these systems requires large sample sets for training and validation to ensure statistical robustness. The high concentration of ethanol in particular has limited the applicability of most electronic noses to alcoholic beverage analysis (203). Röck et al. (197) have provided a more detailed review of the limitations, advantages, and applications of sensor systems in current use.

LOOKING INTO THE CRYSTAL GLASS

Although Charles Ash, in 1915, lamented the fact that the contributions of chemists to understanding wine flavor were not fully appreciated, it is clear that over the past 100+ years, chemists, and analytical chemists in particular, have played critical roles in increasing our knowledge of the many variables that affect grape and wine flavor. As we look to the next 100 years, it is clear that chemistry and analytical chemists will continue to be integral to any future advances that occur in our understanding of grape and wine flavor. These advances will come in many areas, but exciting opportunities exist particularly for the development of novel analytical approaches that can be used to link metabolic profiles to gene and protein expression in plants, microorganisms, and animals. To date, many of these metabolomic studies have focused on obtaining broad chemical profiles of the nonvolatiles involved in primary metabolism, for example, sugars, amino acids, and organic acids (204–206). Few studies have focused on the secondary metabolites, particularly the volatile components, that affect fruit flavor; however, a recent GC-MS-based metabolomic profiling study of over 322 different volatiles in tomatoes (207) demonstrates the exciting potential for these comprehensive analytical tools. Although not yet widely applied to grapes, it is clear that these novel “-omics” approaches can yield valuable metabolic information and increase our understanding of the regulatory mechanisms involved in the biochemical production of volatiles, both in grapes during berry

development and by yeast during fermentation. As a result of these many advances, the contributions of the wine chemist are clear, and when combined with improved understanding of human perception of odorants and odorant mixtures, it will become increasingly possible to control viticultural and wine-making practices to optimize grape and wine flavor.

ABBREVIATIONS USED

CCD, carotenoid cleavage dioxygenase; DMS, dimethyl sulfide; DMDS, dimethyl disulfide; GC, gas chromatography; GD-FID, gas chromatography–flame ionization detector; GC-O, gas chromatography–olfactometry; GC-TOF, gas chromatography–time of flight; G-G, glycosyl-glucose; IBMP, 3-isobutyl-2-methoxypyrazine; IPMP, 3-isopropyl-2-methoxypyrazine; NMR, nuclear magnetic resonance; SBMP, 2-*s*-butyl-3-methoxypyrazine; TDN, 1,1,6-trimethyl-1,2-dihydronaphthalene; TPB, (*E*)-1-(2,3,6-trimethylphenyl)buta-1,3-diene; TOF, time of flight; VSC, volatile sulfur compounds.

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LITERATURE CITED

- (1) Ash, C. S. Contributions of the chemist to the wine industry. *J. Ind. Eng. Chem.* **1915**, *7*, 273–274.
- (2) Clifcorn, L. E. The ACS Division of Agricultural and Food Chemistry. *J. Agric. Food Chem.* **1959**, *7*, 544–546.
- (3) Pattee, H. E. Commemorative history of the Agricultural and Food Chemistry Division. *J. Agric. Food Chem.* **2002**, *50*, 3–6.
- (4) Krug, W. H. Some characteristics of California wines. *J. Am. Chem. Soc.* **1894**, *16*, 597–617.
- (5) Michel, R. H.; McGovern, P. E.; Badler, V. R. The first wine and beer: chemical detection of ancient fermented beverages. *Anal. Chem.* **1993**, *65*, 408A–413A.
- (6) McGovern, P. E.; Hartung, U.; Badler, V. R.; Glusker, D. L.; Exner, L. J. The beginnings of winemaking and viniculture in the ancient near east and Egypt. *Expedition* **1997**, *39*, 3–21.
- (7) McGovern, P. E.; Zhang, J.; Tang, J.; Zhang, Z.; Hall, G. R.; Moreau, R. A.; Nuñez, A.; Butrym, E. D.; Richards, M. P.; Wang, C.-S.; Cheng, G.; Zhao, Z.; Wang, C. Fermented beverages of pre- and proto-historic China. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 17593–17598.
- (8) Jackson, R. S. *Wine Science: Principals and Applications*; Academic Press: Burlington, MA, 2008; p 281.
- (9) Maxwell-Stuart, P. G. *The Chemical Choir: A History of Alchemy*; Continuum Books: New York, 2008; pp 47–50.
- (10) Hassall, A. H. *Food and its Adulterations, Comprising the Reports of the Analytical Sanitary Commission of “The Lancet”*; Longman, Brown, Green and Longmans: London, U.K., 1855; p 639.
- (11) Mulder, G. J. *The Chemistry of Wine*; Jones, H. B., Ed.; Churchill: London, U.K., 1857; p 294.
- (12) Winterhalter, P. Authentication of food and wine. In *Authentication of Food and Wine*; Ebeler, S. E., Takeoka, G. R., Winterhalter, P., Eds.; ACS Symposium Series 952; American Chemical Society: Washington, DC, 2007; pp 2–12.
- (13) Linskens, H. F.; Jackson, J. F. Wine analysis. In *Wine Analysis*; Linskens, H. F., Jackson, J. F., Eds.; Springer-Verlag: Berlin, Germany, 1988; pp 1–8.
- (14) Amerine, M. A. Foreward. In *Proceedings of the University of California, Davis Grape and Wine Centennial Symposium*; Webb, A. D., Ed.; University of California: Davis, CA, 1982; pp III–IX.
- (15) Caldwell, J. S. Some effects of seasonal conditions upon the chemical composition of American grape juices. *J. Agric. Res.* **1925**, *30*, 1133–1176.

- (16) Power, F. B. The detection of methyl anthranilate in fruit juices. *J. Am. Chem. Soc.* **1921**, *43*, 377–381.
- (17) Power, F. B.; Chestnut, V. K. The occurrence of methyl anthranilate in grape juice. *J. Am. Chem. Soc.* **1921**, *43*, 1741–1742.
- (18) Power, F. B.; Chestnut, V. K. Examination of authentic grape juices for methyl anthranilate. *J. Agric. Res.* **1923**, *23*, 47–53.
- (19) Amerine, M. A.; Phaff, H. *Bibliography of Publications by the Faculty, Staff, and Students of the University of California, 1876–1980*, on Grapes, Wines, and Related Subjects; University of California Press: Berkeley, CA, 1986.
- (20) Tswett, M. S. Physical chemical studies on chlorophyll adsorptions. *Ber. Dtsch. Bot. Ges.* **1906**, *24*, 316–323; translated and excerpted in Source Book in Chemistry 1900–1950; Leicester, H. M., Ed.; Harvard University Press: Cambridge, MA, 1968; pp 23–28.
- (21) Tswett, M. S. Adsorption analysis and chromatographic method. Application to the chemistry of chlorophyll. *Ber. Dtsch. Bot. Ges.* **1906**, *24*, 385–393. Translated and excerpted in *A Documentary History of Biochemistry, 1770–1940*; Teich, M., Needham, D. M., Eds.; Leicester University Press: Leicester, U.K., 1992; pp 92–93.
- (22) Amerine, M. A.; Pangborn, R. M.; Roessler, E. B. *Principles of Sensory Evaluation of Food*; Academic Press: New York, 1965.
- (23) Polášková, P.; Herszage, J.; Ebeler, S. E. Wine flavor: chemistry in a glass. *Chem. Soc. Rev.* **2008**, *37*, 2478–2489.
- (24) Bayer, E. Anwendung Chromatographischer Methoden zur Qualitätsbeurteilung von Weinen und Mosten. *Vitis* **1958**, *1*, 298–312.
- (25) Bayer, E.; Bässler, L. Systematische Identifizierung von Estern im Weinroma. II. Mitteilung zur systematischen Identifizierung verdampfbarer organischer Substanzen. *Z. Anal. Chem.* **1961**, *181*, 418–424.
- (26) Porcaro, P. J.; Johnston, V. D. Primary amyl alcohols determined by gas chromatography. *Anal. Chem.* **1961**, *33*, 361–362.
- (27) Zarembo, J. E.; Lysyj, I. Use of a new stationary phase in gas chromatography determination of alcohols in the presence of large amounts of water. *Anal. Chem.* **1959**, *31*, 1833–1834.
- (28) Austin, F. L.; Boruff, C. S. Concentration of congeners of grain spirits and their analysis by gas chromatography. *J. Assoc. Off. Agric. Chem.* **1960**, *43*, 675–679.
- (29) Ough, C. S.; Amerine, M. A. *Methods for Analysis of Musts and Wines*; Wiley: New York, 1988; pp 159–171.
- (30) Webb, A. D.; Kepner, R. E. Fusel oil analysis by means of gas-liquid partition chromatography. *Am. J. Enol. Vitic.* **1961**, *12*, 51–59.
- (31) Tritton, S. M. The influence of yeast on the flavour and stability of wine. *Am. J. Enol.* **1952**, *3*, 161–166.
- (32) Castor, J. G. B. Fermentation products and flavor profiles of yeasts. *Wines Vines* **1954**, *35* (8), 29–31.
- (33) Nordstrom, K. Formation of ethyl acetate in fermentation with brewer's yeast. III. Participation of coenzyme A. *J. Inst. Brew.* **1962**, *68*, 398–407.
- (34) Nykänen, L. Formation and occurrence of flavor compounds in wine and distilled alcoholic beverages. *Am. J. Enol. Vitic.* **1986**, *37*, 84–96.
- (35) Ough, C. S.; Amerine, M. A. Studies with controlled fermentation X. Effect of fermentation temperature on some volatile compounds in wine. *Am. J. Enol. Vitic.* **1967**, *18*, 157–164.
- (36) Hernández-Orte, P.; Cacho, J. F.; Ferreira, V. Relationship between varietal amino acid profile of grapes and wine aromatic composition. Experiments with model solutions and chemometric study. *J. Agric. Food Chem.* **2002**, *50*, 2891–2899.
- (37) Verstrepen, K. J.; Van Laere, S. D. M.; Vanderhaegen, B. M. P.; Derdelinckx, G.; Dufour, J.; Pretorius, I. S.; Winderickx, J.; Thevelein, J. M.; Delvaux, F. R. Expression levels of the yeast alcohol acetyltransferase genes ATF1, Lg-ATF1, and ATF2 control the formation of a broad range of volatile esters. *Appl. Environ. Microbiol.* **2003**, *69*, 5228–5237.
- (38) Swiegers, J. H.; Bartowsky, E. J.; Henschke, P. A.; Pretorius, I. S. Yeast and bacterial modulation of wine aroma and flavour. *Aust. J. Grape Wine Res.* **2005**, *11*, 139–173.
- (39) Miller, A. C.; Wolff, S. R.; Bisson, L. F.; Ebeler, S. E. Yeast strain and nitrogen supplementation: dynamics of volatile ester production in Chardonnay juice fermentations. *Am. J. Enol. Vitic.* **2007**, *58*, 470–483.
- (40) Kepner, R. L.; Webb, A. D. Volatile aroma constituents of *Vitis rotundifolia* grapes. *Am. J. Enol.* **1956**, *7*, 8–18.
- (41) Webb, A. D.; Kepner, R. E. Some volatile aroma constituents of *Vitis vinifera* var. Muscat of Alexandria. *Food Res.* **1957**, *22*, 384–395.
- (42) Rapp, A. Wine aroma substances from gas chromatographic analysis. In *Wine Analysis*; Linskens, H. F., Jackson, J. F., Eds.; Springer-Verlag: Berlin, Germany, 1988; pp 29–66.
- (43) Park, S. K.; Morrison, J. C.; Adams, D. O.; Noble, A. C. Distribution of free and glycosidically bound monoterpenes in skin and mesocarp of Muscat of Alexandria grapes during development. *J. Agric. Food Chem.* **1991**, *39*, 514–518.
- (44) Cordonnier, R. Recherches sur l'aromatisation et le parfum des vins doux naturels et des vins de liqueur. *Ann. Inst. Natl. Recherches Agron., Ser. E., Ann. Technol. Agric.* **1956**, *5*, 75–110.
- (45) Usseglio-Tomasset, L. L'aroma di moscato nelle uve e nei vini. *Ind. Agrar.* **1966**, *4* (5), 216–227.
- (46) Usseglio-Tomasset, L.; Astegiano, V.; Matta, M. Il linalool composto responsabile dell'aroma delle uve e dei vini aromatici. *Ind. Agrar.* **1966**, *4* (12), 583–584.
- (47) Schreier, P. Flavor compositions of wines: a review. *CRC Crit. Rev. Food Sci. Nutr.* **1979**, *12*, 59–111.
- (48) Marais, J. Terpenes in the aroma of grapes and wines: a review. *S. Afr. J. Enol. Vitic.* **1983**, *4*, 49–60.
- (49) Rapp, A. Volatile flavour of wine: Correlation between instrumental analysis and sensory perception. *Nahrung* **1998**, *42*, 351–363.
- (50) Williams, P. J.; Strauss, C. R.; Wilson, B.; Massy-Westropp, P. A. Novel monoterpene disaccharide glycosides of *Vitis vinifera* grapes and wines. *Phytochemistry* **1982**, *21*, 2013–2020.
- (51) Williams, P. J.; Strauss, C. R.; Wilson, B.; Massy-Westropp, P. A. Use of C18-reversed phase liquid chromatography for the isolation of monoterpene glycosides and norisoprenoid precursors from grape and wines. *J. Chromatogr., A* **1982**, *235*, 471–480.
- (52) Dimitriadis, E.; Williams, P. J. The development and use of a rapid analytical technique for estimation of free and potentially volatile monoterpene flavorants of grapes. *Am. J. Enol. Vitic.* **1984**, *35*, 66–71.
- (53) Günata, Y. Z.; Bayonove, C. L.; Baumes, R. L.; Cordonnier, R. E. The aroma of grapes. I. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. *J. Chromatogr., A* **1985**, *331*, 83–90.
- (54) Williams, P. J.; Cynkar, W.; Francis, I. L.; Gray, J. D.; Iland, P. G.; Coombe, B. G. Quantification of glycosides in grapes, juices, and wines through a determination of glycosyl glucose. *J. Agric. Food Chem.* **1995**, *43*, 121–128.
- (55) Iland, P. G.; Cynkar, W.; Francis, I. L.; Williams, P. J.; Coombe, B. G. Optimisation of methods for the determination of total and red-free glycosyl glucose in black grape berries of *Vitis vinifera*. *Aust. J. Grape Wine Res.* **1996**, *2*, 171–178.
- (56) Francis, I. L.; Tate, M. E.; Williams, P. J. The effect of hydrolysis conditions on the aroma released from Semillon grape glycosides. *Aust. J. Grape Wine Res.* **1996**, *2*, 70–76.
- (57) Francis, I. L.; Kassara, S.; Noble, A. C.; Williams, P. J. The contribution of glycoside precursors to Cabernet Sauvignon and Merlot aroma. In *Chemistry of Wine Flavor*; Waterhouse, A. L., Ebeler, S. E., Eds.; ACS Symposium Series 714; American Chemical Society: Washington, DC, 1999; pp 13–30.
- (58) Reynolds, A. G.; Wardle, D. A. Influence of fruit microclimate on monoterpene levels of Gewürztraminer. *Am. J. Enol. Vitic.* **1989**, *40*, 149–154.
- (59) Belancic, A.; Agosin, E.; Ibacache, A.; Bordeu, E.; Baumes, R.; Razungles, A.; Bayonove, C. Influence of sun exposure on the aromatic composition of Chilean Muscat grape cultivars Moscatel de Alejandría, and Moscatel rosada. *Am. J. Enol. Vitic.* **1997**, *48*, 181–186.
- (60) Marais, J.; Hunter, J. J.; Haasbroek, P. D. Effect of canopy microclimate, season and region on Sauvignon blanc grape composition and wine quality. *S. Afr. J. Enol. Vitic.* **1999**, *29*, 19–30.
- (61) Coombe, B. Research on development and ripening of the grape berry. *Am. J. Enol. Vitic.* **1992**, *43*, 101–110.

- (62) Clastre, M.; Bantignies, B.; Feron, G.; Soler, E.; Ambid, C. Purification and characterization of geranyl diphosphate synthase from *Vitis vinifera* L. cv Muscat de Frontignan cell cultures. *Plant Physiol.* **1993**, *102*, 205–211.
- (63) Luan, F.; Wüst, M. Differential incorporation of 1-deoxy-xylulose into (3S)-linalool and geraniol in grape berry exocarp and mesocarp. *Phytochemistry* **2002**, *60*, 451–459.
- (64) Martin, D.; Bohlmann, J. Identification of *Vitis vinifera* (–)- α -terpineol synthase by in silico screening of full-length cDNA ESTs and functional characterization of recombinant terpene synthase. *Phytochemistry* **2004**, *65*, 1223–1229.
- (65) Lückner, J.; Bowen, P.; Bohlmann, J. *Vitis vinifera* terpenoid cyclases: functional identification of two sesquiterpene synthase cDNAs encoding (+)-valencene synthase and (–)-germacrene D synthase and expression of mono- and sesquiterpene synthases in grapevine flowers and berries. *Phytochemistry* **2004**, *65*, 2649–2659.
- (66) Luan, F.; Mosandl, A.; Münch, A.; Wüst, M. Metabolism of geraniol in grape berry mesocarp of *Vitis vinifera* L. cv. Scheurebe: demonstration of stereoselective reduction, E/Z-isomerization, oxidation and glycosylation. *Phytochemistry* **2005**, *66*, 295–303.
- (67) Doligez, A.; Audiot, E.; Baumes, R.; This, P. QTLs for muscat flavor and monoterpenic odorant content in grapevine (*Vitis vinifera* L.). *Mol. Breed.* **2006**, *18*, 109–125.
- (68) Battilana, J.; Costantini, L.; Emanuelli, F.; Sevini, F.; Segala, C.; Moser, S.; Velasco, R.; Versini, G.; Grando, M. S. The 1-deoxy-D-xylulose 5-phosphate synthase gene co-localizes with a major QTL affecting monoterpenic content in grapevine. *Theor. Appl. Genet.* **2009**, *118*, 653–669.
- (69) Gholami, M.; Hayasaka, Y.; Coombe, B. G.; Jackson, J. F.; Robinson, S. P.; Williams, P. J. Biosynthesis of flavour compounds in *Muscat Gordo Blanco* grape berries. *Aust. J. Grape Wine Res.* **1995**, *1*, 19–24.
- (70) Jaillon, O.; Aury, J. M.; Noel, B.; Policriti, A.; Clepet, C.; Casagrande, A.; Choisne, N.; Aubourg, S.; Vitulo, N.; Jubin, C.; Vezzi, A.; Legeai, F.; Huguency, P.; Dasilva, C.; Horner, D.; Mica, E.; Jublot, D.; Poulain, J.; Bruyere, C.; Billault, A.; Segurens, B.; Gouyvenoux, M.; Ugarte, E.; Cattonaro, F.; Anhouard, V.; Vico, V.; Del Fabbro, C.; Alaux, M.; Di Gasparo, G.; Dumas, V.; Felice, N.; Paillard, S.; Juman, I.; Moroldo, M.; Scalabrin, S.; Canaguier, A.; Le Clainche, I.; Malacrida, G.; Durand, E.; Pesole, G.; Lauacou, V.; Chatelet, P.; Merdinoglu, D.; Delledonne, M.; Pezzotti, M.; Lecharny, A.; Scarpelli, C.; Artiguenave, F.; Pe, M. E.; Valle, G.; Morgante, M.; Caboche, M.; Adam-Blondon, A. F.; Weissenbach, J.; Quetier, F.; Wincker, P. French–Italian Public Consortium for Grapevine Genome Characterization. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature* **2007**, *449*, 463–467.
- (71) Buttery, R. G.; Seifert, R. M.; Guadagni, D. G.; Ling, L. C. Characterization of some volatile constituents of bell peppers. *J. Agric. Food Chem.* **1969**, *17*, 1322–1327.
- (72) Bayonove, C. R.; Cordonnier, R.; Dubois, P. Etude d'une fractions caractéristique de l'arôme du raisin de la variété Cabernet-Sauvignon; mise en évidence de la 2-méthoxy-3-isobutylpyrazine. *C. R. Acad. Sci. Paris. Ser. D* **1975**, *281*, 75–78.
- (73) Augustyn, O. P. H.; Rapp, A.; Van Wyk, C. J. Some volatile aroma components of *Vitis vinifera* L. cv. Sauvignon blanc. *S. Afr. J. Enol. Vitic.* **1982**, *3*, 53–60.
- (74) Harris, R. L. N.; Lacey, M. J.; Brown, W. V.; Allen, M. S. Determination of 2-methoxy-3-alkylpyrazines in wine by gas chromatography/mass spectrometry. *Vitis* **1987**, *26*, 201–207.
- (75) Lacey, M. J.; Allen, M. S.; Harris, R. L. N.; Brown, W. V. Methoxy-pyrazines in Sauvignon blanc grapes and wines. *Am. J. Enol. Vitic.* **1991**, *42*, 103–108.
- (76) Allen, M. S.; Lacey, M. J. Methoxy-pyrazines of grapes and wines. In *Chemistry of Wine Flavor*; Waterhouse, A. L., Ebeler, S. E., Eds.; ACS Symposium Series 714; American Chemical Society: Washington, DC, 1999; pp 31–38.
- (77) Sala, C.; Busto, O.; Guasch, J.; Zamora, F. Factors affecting the presence of 3-alkyl-2-methoxy-pyrazines in grapes and wines. A review. In *3-Alquil-2-metoxipirazines en mostos i vins: determinació analítica i estudi de la influència d'alguns factors vitivinícoles*; C. Sala, Doctoral Dissertation, Universitat Rovira i Virgili, **2004**; pp 53–58.
- (78) Preston, L. D.; Block, D. E.; Heymann, H.; Soleas, G.; Noble, A. C.; Ebeler, S. E. Defining vegetal aromas in Cabernet Sauvignon using sensory and chemical evaluations. *Am. J. Enol. Vitic.* **2008**, *59*, 137–145.
- (79) Heymann, H.; Noble, A. C.; Boulton, R. B. Analysis of methoxy-pyrazines in wines. 1. Development of a quantitative procedure. *J. Agric. Food Chem.* **1986**, *34*, 268–271.
- (80) Noble, A. C.; Elliot-Fisk, D. L. Vegetative flavor and methoxy-pyrazines in Cabernet Sauvignon. In *Fruit Flavors: Biogenesis, Characterization and Authentication*; Rouseff, R. L., Leahy, M. M., Eds.; ACS Symposium Series 596; American Chemical Society: Washington, DC, 1995; pp 226–233.
- (81) Allen, M. S.; Lacey, M. J.; Harris, R. L. N.; Brown, W. V. Viticultural influences in methoxy-pyrazines in Sauvignon blanc. *Aust. N. Z. Wine Ind. J.* **1990**, *5*, 44–46.
- (82) Arnold, R. A.; Bledsoe, A. M. The effect of various leaf removal treatments on the aroma and flavor of Sauvignon blanc wine. *Am. J. Enol. Vitic.* **1990**, *41*, 74–76.
- (83) Morrison, J. C.; Noble, A. C. The effects of leaf and cluster shading on the composition of Cabernet Sauvignon grapes and on fruit and wine sensory properties. *Am. J. Enol. Vitic.* **1990**, *41*, 193–200.
- (84) Allen, M. S.; Lacey, M. J.; Boyd, S. Determination of methoxy-pyrazines in red wines by stable isotope dilution gas chromatography–mass spectrometry. *J. Agric. Food Chem.* **1994**, *42*, 1734–1738.
- (85) Hashizume, K.; Samuta, T. Grape maturity and light exposure affect berry methoxy-pyrazine concentration. *Am. J. Enol. Vitic.* **1999**, *50*, 194–198.
- (86) Roujou de Boubée, D.; Van Leeuwen, C.; Dubourdieu, D. Organoleptic impact of 2-methoxy-3-isobutylpyrazine on red Bordeaux and Loire wines. Effect of environmental conditions on concentrations in grapes during ripening. *J. Agric. Food Chem.* **2000**, *48*, 4830–4834.
- (87) Sala, C.; Mestres, M.; Marti, M. P.; Busto, O.; Guasch, J. Head-space solid-phase microextraction method for determining 3-alkyl-2-methoxy-pyrazines in musts by means of polydimethylsiloxane–divinylbenzene fibres. *J. Chromatogr., A* **2000**, *880*, 93–99.
- (88) Chapman, D. M.; Thorngate, J. H.; Matthews, M. A.; Guinard, J.-X.; Ebeler, S. E. Yield effects on 2-methoxy-3-isobutylpyrazine concentration in Cabernet Sauvignon using a solid phase microextraction gas chromatography/mass spectrometry method. *J. Agric. Food Chem.* **2004**, *52*, 5431–5435.
- (89) Chapman, D. M.; Roby, G.; Ebeler, S. E.; Guinard, J.-X.; Matthews, M. A. Sensory attributes of Cabernet Sauvignon wines made from vines with different water status. *Austr. J. Grape Wine Res.* **2005**, *11*, 339–347.
- (90) Murray, K. E.; Whitfield, F. B. The occurrence of 3-alkyl-2-methoxy-pyrazines in raw vegetables. *J. Sci. Food Agric.* **1975**, *26*, 973–986.
- (91) Gallois, A.; Kergomard, A.; Adda, J. Study of the biosynthesis of 3-isopropyl-2-methoxy-pyrazine produced by *Pseudomonas taetrolens*. *Food Chem.* **1988**, *28*, 299–309.
- (92) Cheng, T. B.; Reineccius, G. A.; Bjorklund, J. A.; Leete, E. Biosynthesis of 2-methoxy-3-isopropylpyrazine in *Pseudomonas perolens*. *J. Agric. Food Chem.* **1991**, *39*, 1009–1012.
- (93) Kotseridis, Y.; Beloqui, A. A.; Bertrand, A.; Doazan, J. P. An analytical method for studying the volatile compounds of Merlot noir clone wines. *Am. J. Enol. Vitic.* **1998**, *49*, 44–48.
- (94) Kotseridis, Y.; Baumes, R. L.; Bertrand, A.; Skouroumounis, G. K. Quantitative determination of 2-methoxy-3-isobutylpyrazine in red wines and grapes of Bordeaux using a stable isotope dilution assay. *J. Chromatogr., A* **1999**, *841*, 229–237.
- (95) Kotseridis, Y.; Razungles, A.; Bertrand, A.; Baumes, R. Differentiation of the aromas of Merlot and Cabernet Sauvignon wines using sensory and instrumental analysis. *J. Agric. Food Chem.* **2000**, *48*, 5383–5388.
- (96) Belancic, A.; Agosin, E. Methoxy-pyrazines in grapes and wines of *Vitis vinifera* cv. Carmenera. *Am. J. Enol. Vitic.* **2007**, *58*, 462–469.
- (97) Bowers, J. E.; Meredith, C. P. The parentage of a classic wine grape, Cabernet Sauvignon. *Nat. Genet.* **1997**, *16*, 84–87.

- (98) Winterhalter, P.; Rouseff, R. Carotenoid-derived aroma compounds: an introduction. In *Carotenoid-Derived Aroma Compounds*; Winterhalter, P., Rouseff, R. L., Eds.; ACS Symposium Series 802; American Chemical Society: Washington, DC, 2002; pp 1–19.
- (99) Gerdes, S. M.; Winterhalter, P.; Ebeler, S. E. Effect of sunlight exposure on norisoprenoid formation in White Riesling grapes. In *Carotenoid-Derived Aroma Compounds*; Winterhalter, P., Rouseff, R. L., Eds.; ACS Symposium Series 802; American Chemical Society: Washington, DC, 2002; pp 262–272.
- (100) Strauss, C. R.; Wilson, B.; Anderson, R.; Williams, P. J. Development of precursors of C₁₃ nor-isoprenoid flavorants in Riesling grapes. *Am. J. Enol. Vitic.* **1987**, *38*, 23–27.
- (101) Razungles, A.; Bayonove, C. L.; Cordonnier, R. E.; Sapis, J. C. Grape carotenoids: changes during the maturation period and localization in mature berries. *Am. J. Enol. Vitic.* **1988**, *39*, 44–48.
- (102) Razungles, A.; Bayonove C. Les caroténoïdes du raisin et leur potentialité aromatique. In *La Viticulture à L'aube du IIIe Millénaire*; Bouard, J., Guimberteau, G., Eds.; Vigne et Vin Publications Internationales: Martillac, France, 1996; pp 85–88.
- (103) Razungles, A. J.; Baumes, R. L.; Dufour, C.; Sznaper, C. N.; Bayonove, C. L. Effect of sun exposure on carotenoids and C₁₃-norisoprenoid glycosides in Syrah berries (*Vitis vinifera* L.). *Sci. Aliments* **1998**, *18*, 361–373.
- (104) Marais, J.; van Wyk, C. J.; Rapp, A. Effect of sunlight and shade on norisoprenoid levels in maturing Weisser Riesling and Chenin blanc grapes and Weisser Riesling wines. *S. Afr. J. Enol. Vitic.* **1992**, *13*, 23–32.
- (105) Marias, J.; van Wyk, C. J.; Rapp, A. Effect of storage time, temperature and region on the levels of 1,1,6-trimethyl-1,2-dihydronaphthalene and other volatiles, and on quality of Weisser Riesling wines. *S. Afr. J. Enol. Vitic.* **1992**, *13*, 33–44.
- (106) Marais, J.; Versini, G.; van Wyk, C. J.; Rapp, A. Effect of region on free and bound monoterpene and C₁₃-norisoprenoid concentrations in Weisser Riesling wines. *S. Afr. J. Enol. Vitic.* **1992**, *13*, 71–77.
- (107) Lee, S.-H.; Seo, M.-J.; Riu, M.; Cotta, J. P.; Block, D. E.; Dokoozlian, N. K.; Ebeler, S. E. Vine microclimate and norisoprenoid concentration in Cabernet Sauvignon grapes and wines. *Am. J. Enol. Vitic.* **2007**, *58*, 291–301.
- (108) Stevens, S. M. G.; Ebeler, S. E. C₁₃-Norisoprenoid concentrations in grapes as affect by sunlight and shading. In *Food Flavor: Chemistry, Sensory Evaluation and Biological Activity*; Tamura, H., Ebeler, S. E., Kubota, K., Takeoka, G. R., Eds.; ACS Symposium Series 988; American Chemical Society: Washington, DC, 2008; pp 68–77.
- (109) Winterhalter, P. 1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN) formation in wine. 1. Studies on the hydrolysis of 2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-ene-2,8-diol rationalizing the origin of TDN and related C₁₃ norisoprenoids in Riesling wine. *J. Agric. Food Chem.* **1991**, *39*, 1825–1829.
- (110) Winterhalter, P.; Sefton, M. A.; Williams, P. J. Volatile C₁₃-norisoprenoid compounds in Riesling wine are generated from multiple precursors. *Am. J. Enol. Vitic.* **1990**, *41*, 277–283.
- (111) Versini, G.; Carlin, S.; Dalla Serra A.; Nicolini, G.; Rapp, A. Formation of 1,1,6-trimethyl-1,2-dihydronaphthalene and other norisoprenoids in wine: considerations of the kinetics. In *Carotenoid-Derived Aroma Compounds*; Winterhalter, P., Rouseff, R. L., Eds.; ACS Symposium Series 802; American Chemical Society: Washington, DC, 2002; pp 285–299.
- (112) Janusz, A.; Capone, D. L.; Puglisi, C. J.; Perkins, M. V.; Elsey, G. M.; Sefton, M. A. (*E*)-1-(2,3,6-Trimethylphenyl)buta-1,3-diene: a potent grape-derived odorant in wine. *J. Agric. Food Chem.* **2003**, *51*, 7759–7763.
- (113) Sefton, M. A.; Francis, I. L.; Williams, P. J. The volatile composition of Chardonnay juices: a study by flavor precursor analysis. *Am. J. Enol. Vitic.* **1993**, *44*, 359–370.
- (114) Enzell, C. R.; Wahlberg, I.; Aasen, A. J. Isoprenoids and alkaloids of tobacco. *Prog. Chem. Org. Nat. Prod.* **1977**, *34*, 1–79.
- (115) Ohloff, G. Importance of minor components in flavors and fragrances. *Perfum. Flavor.* **1978**, *3*, 11–22.
- (116) Ohloff, G.; Flament, I.; Pickenhagen, W. Flavor chemistry. *Food Rev. Int.* **1985**, *1*, 99–148.
- (117) Schmidt, H.; Kurtzer, R.; Eisenreich, W.; Schwab, W. The carotenase AtCCD1 from *Arabidopsis thaliana* is a dioxygenase. *J. Biol. Chem.* **2006**, *281*, 9845–9851.
- (118) Bouvier, F.; Isner, J. C.; Dogbo, O.; Camara, B. Oxidative tailoring of carotenoids: a prospect towards novel functions in plants. *Trends Plant Sci.* **2005**, *10*, 187–194.
- (119) Schwartz, S. H.; Qin, X. Q.; Zeevaert, J. A. D. Characterization of a novel carotenoid cleavage dioxygenase from plants. *J. Biol. Chem.* **2001**, *276*, 25208–25211.
- (120) Bouvier, F.; Suire, C.; Mutterer, J.; Camara, B. Oxidative remodeling of chromoplast carotenoids: identification of the carotenoid dioxygenase CsCCD and CsZCD genes involved in crocus secondary metabolite biogenesis. *Plant Cell* **2003**, *15*, 47–62.
- (121) Simkin, A. J.; Schwartz, S. H.; Aldridge, M.; Taylor, M. G.; Klee, H. J. The tomato carotenoid cleavage dioxygenase 1 genes contribute to the formation of the flavor volatiles β -ionone, pseudoionone, and geranylacetone. *Plant J.* **2004**, *40*, 882–892.
- (122) Simkin, A. J.; Underwood, B. A.; Aldridge, M.; Loucas, H. M.; Shibuya, K.; Schmelz, E.; Clark, D. G.; Klee, H. J. Circadian regulation of the PhCCD1 carotenoid cleavage dioxygenase controls emission of β -ionone, a fragrance volatile of petunia flowers. *Plant Physiol.* **2004**, *136*, 3504–3514.
- (123) Mathieu, S.; Terrier, N.; Procureur, J.; Bigey, F.; Günata, Z. A carotenoid cleavage dioxygenase from *Vitis vinifera* L.: functional characterization and expression during grape berry development in relation to C-13-norisoprenoid accumulation. *J. Exp. Bot.* **2005**, *56*, 2721–2731.
- (124) Ibdah, M.; Azulay, Y.; Portnoy, V.; Wasserman, B.; Bar, E.; Meir, A.; Burger, Y.; Hirschberg, J.; Schaffer, A. A.; Katzir, N.; Tadmor, Y.; Lewinsohn, E. Functional characterization of CmCCD1, a carotenoid cleavage dioxygenase from melon. *Phytochemistry* **2006**, *67*, 1579–1589.
- (125) Spedding, D. J.; Raut, P. The influence of dimethyl sulphide and carbon disulphide in the bouquet of wines. *Vitis* **1982**, *21*, 240–246.
- (126) Goniak, O. J.; Noble, A. C. Sensory study of selected volatile sulfur compounds in white wine. *Am. J. Enol. Vitic.* **1987**, *38*, 223–227.
- (127) Rankine, B. C. Hydrogen sulphide production by yeasts. *J. Sci. Food Agric.* **1964**, *15*, 872–876.
- (128) Sweigers, J. H.; Pretorius, I. S. Modulation of volatile sulfur compounds by wine yeast. *Appl. Microbiol. Biotechnol.* **2007**, *74*, 954–960.
- (129) Dozon, N.; Noble, A. C. Sensory study of the effect of fluorescent light on a sparkling wine and its base wine. *Am. J. Enol. Vitic.* **1989**, *40*, 265–271.
- (130) Kuroiwa, Y.; Hashimoto, N. Composition of sunstruck flavor substance and mechanism of its evolution. *Proc. Am. Soc. Brew. Chem.* **1961**, 28–36.
- (131) Kuroiwa, Y.; Hashimoto, N.; Hashimoto, H.; Kokubo, E.; Nakagawa, K. Factors essential for the evolution of sunstruck flavor. *Proc. Am. Soc. Brew. Chem.* **1963**, 181–193.
- (132) White, C. H.; Bulthaus, M. Light activated flavor in milk. *J. Dairy Sci.* **1982**, *65*, 489–494.
- (133) Mattivi, F.; Monetti, A.; Vrhovšek, U.; Tonon, D.; Andrés-Lacueva, C. High-performance liquid chromatographic determination of the riboflavin concentration in white wines for predicting their resistance to light. *J. Chromatogr., A* **2000**, *888*, 121–127.
- (134) Sakuma, S.; Rikimaru, Y.; Kobayashi, K.; Kowaka, M. Sunstruck flavor formation in beer. *J. Am. Soc. Brew. Chem.* **1991**, *49*, 162–165.
- (135) Segurel, M. A.; Razungles, A. J.; Riou, C.; Salles, M.; Baumes, R. L. Contribution of dimethyl sulfide to the aroma of Syrah and Grenache noir wines and estimation of its potential in grapes of these varieties. *J. Agric. Food Chem.* **2004**, *52*, 7084–7093.
- (136) Fedrizzi, B.; Magno, F.; Badocco, D.; Nicolini, G.; Versini, G. Aging effects and grape variety dependence on the content of sulfur volatiles in wine. *J. Agric. Food Chem.* **2007**, *55*, 10880–10887.
- (137) Starkenmann, C.; Troccaz, M.; Howell, K. The role of cysteine and cysteine-S conjugates as odour precursors in the flavour and fragrance industry. *Flavour Fragrance J.* **2008**, *23*, 369–381.
- (138) Darriet, P.; Tominaga, T.; Lavigne, V.; Boidron, J.-N.; Dubourdiou, D. Identification of a powerful aromatic component of *Vitis*

- vinifera* L. var. Sauvignon wines: 4-mercapto-4-methylpentan-2-one. *Flavour Fragrance J.* **1995**, *10*, 385–392.
- (139) Tominaga, T.; Darriet, P.; Dubourdieu, D. Identification de l'acétate de 3-mercaptopentanol, composé à forte odeur de buis, intervenant dans l'arôme des vins de Sauvignon. *Vitis* **1996**, *35*, 207–210.
- (140) Tominaga, T.; Furrer, A.; Henry, R.; Dubourdieu, D. Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon blanc wines. *Flavour Fragrance J.* **1998**, *13*, 159–162.
- (141) Bouchilloux, P.; Darriet, P.; Henry, R.; Lavigne-Cruège, V.; Dubourdieu, D. Identification of volatile and powerful odorous thiols in Bordeaux red wine varieties. *J. Agric. Food Chem.* **1998**, *46*, 3095–3099.
- (142) Tominaga, T.; Peyrot des Gachons, C.; Dubourdieu, D. A new type of flavor precursors in *Vitis vinifera* L. cv. Sauvignon blanc: S-cysteine conjugates. *J. Agric. Food Chem.* **1998**, *46*, 5215–5219.
- (143) Peyrot des Gachons, C.; Tominaga, T.; Dubourdieu, D. Measuring the aromatic potential of *Vitis vinifera* L. cv. Sauvignon blanc grapes by assaying S-cysteine conjugates, precursors of the volatile thiols responsible for their varietal aroma. *J. Agric. Food Chem.* **2000**, *48*, 3387–3391.
- (144) Blum, R.; Beck, A.; Korte, A.; Stengel, A.; Letzel, T.; Lenzian, K.; Grill, E. Function of phytochelatin synthase in catabolism of glutathione-conjugates. *Plant J.* **2007**, *49*, 740–749.
- (145) Peyrot des Gachons, C.; Tominaga, T.; Dubourdieu, D. Sulfur aroma precursor present in S-glutathione conjugate form: identification of S-3-(hexan-1-ol)-glutathione in must from *Vitis vinifera* L. cv. Sauvignon blanc. *J. Agric. Food Chem.* **2002**, *50*, 4076–4079.
- (146) Subileau, M.; Schneider, R.; Salmon, J.-M.; DeGryse, E. New insights on 3-mercaptopentanol (3MH) biogenesis in Sauvignon blanc wines: Cys-3MH and (E)-hexen-2-al are not the major precursors. *J. Agric. Food Chem.* **2008**, *56*, 9230–9235.
- (147) Mestres, M.; Busto, O.; Guasch, J. Analysis of organic sulfur compounds in wine aroma. *J. Chromatogr., A* **2000**, *881*, 569–581.
- (148) James, A. T.; Martin, A. J. P. Gas-liquid partition chromatography: the separation and microestimation of volatile fatty acids from formic acid to dodecanoic acid. *Biochem. J.* **1952**, *50*, 679–690.
- (149) Fuller, G. H.; Steltenkamp, R.; Tisserand, G. A. The gas chromatograph with human sensor: perfumer model. *Ann. N.Y. Acad. Sci.* **1964**, *116*, 711–724.
- (150) Dravnieks, A.; O'Donnell, A. Principles and some techniques of high-resolution headspace analysis. *J. Agric. Food Chem.* **1971**, *19*, 1049–1056.
- (151) Acree, T. E.; Butts, R. M.; Nelson, R. R.; Lee, C. Y. Sniffer to determine the odor of gas chromatographic effluents. *Anal. Chem.* **1976**, *48*, 1821–1822.
- (152) d'Acampora Zellner, B.; Dugo, P.; Dugo, G.; Mondello, L. Gas chromatography-olfactometry in food flavour analysis. *J. Chromatogr., A* **2008**, *1186*, 123–143.
- (153) Nelson, R. R.; Acree, T. E. Concord wine composition as affected by maturity and processing technique. *Am. J. Enol. Vitic.* **1978**, *29*, 83–86.
- (154) Nelson, R. R.; Acree, T. E.; Butts, R. M. Isolation and identification of volatiles from Catawba wines. *J. Agric. Food Chem.* **1978**, *26*, 1188–1190.
- (155) Noble, A. C.; Flath, R. A.; Forrey, R. R. Wine headspace analysis. Reproducibility and application to varietal classification. *J. Agric. Food Chem.* **1980**, *28*, 346–353.
- (156) Miranda-Lopez, R.; Libbey, L. M.; Watson, B. T.; McDaniel, M. R. Odor analysis of Pinot noir wines from grapes of different maturities by a gas chromatography-olfactometry technique (Osme). *J. Food Sci.* **1992**, *57*, 985–993, 1019.
- (157) Guth, H. Identification of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3022–3026.
- (158) Guth, H. Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3027–3032.
- (159) Ferreira, V.; López, R.; Escudero, A.; Cacho, J. F. The aroma of Grenache red wine: hierarchy and nature of its main odorants. *J. Sci. Food Agric.* **1998**, *77*, 259–267.
- (160) Ferreira, V.; Ortín, N.; Escudero, A.; López, R.; Cacho, J. Chemical characterization of the aroma of Grenache rosé wines: aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. *J. Agric. Food Chem.* **2002**, *50*, 4048–4054.
- (161) Escudero, A.; Gogorza, B.; Melús, M. A.; Ortín, N.; Cacho, J.; Ferreira, V. Characterization of the aroma of a wine from Macabeo. Key role played by compounds with low odor activity values. *J. Agric. Food Chem.* **2004**, *52*, 3516–3524.
- (162) Wood, C.; Siebert, T. E.; Parker, M.; Capone, D. L.; Elsey, G. M.; Pollnitz, A. P.; Eggers, M.; Meier, M.; Vössing, T.; Widder, S.; Krammer, G.; Sefton, M. A.; Herderich, M. J. From wine to pepper: rotundone, an obscure sesquiterpene, is a potent spicy aroma compound. *J. Agric. Food Chem.* **2008**, *56*, 3738–3744.
- (163) Voilley, A.; Lubbers, S. Flavor-matrix interactions in wine. In *Chemistry of Wine Flavor*; Waterhouse, A. L., Ebeler, S. E., Eds.; ACS Symposium Series 714; American Chemical Society, Washington, DC, 1999; pp 217–229.
- (164) Dufour, C.; Bayonove, C. L. Interactions between wine polyphenols and aroma substances. An insight at the molecular level. *J. Agric. Food Chem.* **1999**, *47*, 678–684.
- (165) Dufour, C.; Sauvaitre, I. Interactions between anthocyanins and aroma substances in a model system. Effect on the flavor of grape-derived beverages. *J. Agric. Food Chem.* **2000**, *48*, 1784–1788.
- (166) Jung, D.-M.; de Ropp, J. S.; Ebeler, S. E. Study of interactions between food phenolics and aromatic flavors using one- and two-dimensional ¹H NMR spectroscopy. *J. Agric. Food Chem.* **2000**, *48*, 407–412.
- (167) Jung, D.-M.; de Ropp, J. S.; Ebeler, S. E. Application of pulsed field gradient NMR techniques for investigating binding of flavor compounds to macromolecules. *J. Agric. Food Chem.* **2002**, *51*, 4262–4269.
- (168) Jung, D.-M.; Ebeler, S. E. Headspace solid-phase microextraction method for the study of the volatility of selected flavor compounds. *J. Agric. Food Chem.* **2003**, *51*, 200–205.
- (169) Aronson, J.; Ebeler, S. E. Effect of polyphenol compounds on the headspace volatility of flavors. *Am. J. Enol. Vitic.* **2004**, *55*, 13–21.
- (170) Williams, A. A.; Rosser, P. R. Aroma enhancing effects of ethanol. *Chem. Senses* **1981**, *6*, 149–153.
- (171) Guth, H. Comparison of different white wine varieties in odor profiles by instrumental analysis and sensory studies. In *Chemistry of Wine Flavor*; Waterhouse, A. L., Ebeler, S. E., Eds.; ACS Symposium Series 714; American Chemical Society: Washington, DC, 1999; pp 39–52.
- (172) Fischer, U.; Berger, R. G.; Håkansson, Å.; Noble, A. C. The impact of dealcoholization on the flavour of wine—relating concentration of aroma compounds to sensory data using PLS analysis. In *Flavour Science. Recent Developments. Proceedings of the Eighth Weurman Flavour Research Symposium*, Reading, U.K., July 23–26, 1996; Taylor, A. J., Mottram, D. S., Eds.; Royal Society of Chemistry: Cambridge, U.K., 1996; pp 335–338.
- (173) Ebeler, S. E. Linking flavor chemistry to sensory analysis of wine. In *Flavor Chemistry: 30 Years of Progress*; Teranishi, R., Wick, E., Hornstein, I., Eds.; Kluwer Academic: New York, 1999; pp 409–421.
- (174) Escudero, A.; Campo, E.; Farina, L.; Cacho, J.; Ferreira, V. Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. *J. Agric. Food Chem.* **2007**, *55*, 4501–4510.
- (175) Tsachaki, M.; Linforth, R. S. T.; Taylor, A. J. Dynamic headspace analysis of the release of volatile organic compounds from ethanolic systems by direct APCI-MS. *J. Agric. Food Chem.* **2005**, *53*, 8328–8333.
- (176) Tsachaki, M.; Gady, A.-L.; Kalopesas, M.; Linforth, R. S. T.; Athes, V.; Marin, M.; Taylor, A. J. Effect of ethanol, temperature, and gas flow rate on volatile release from aqueous solutions under dynamic headspace dilution conditions. *J. Agric. Food Chem.* **2008**, *56*, 5308–5315.
- (177) Buttery, R. G. Flavor chemistry and odor thresholds. In *Flavor Chemistry: 30 Years of Progress*; Teranishi, R., Wick, E., Hornstein, I., Eds.; Kluwer Academic: New York, 1999; pp 353–365.
- (178) Hein, K. A.; Ebeler, S. E.; Heymann, H. Perception of fruity and vegetative aromas in red wine. *J. Sens. Stud.* **2008**, *24*, 441–455.

- (179) Axel, R. Scents and sensibility: A molecular logic of olfactory perception. Nobel Lecture, Dec 8, 2004. In *Les Prix Nobel. The Nobel Prizes 2004*; Frångsmyr, T., Ed.; Noble Foundation: Stockholm, Sweden, 2005; http://nobelprize.org/nobel_prizes/medicine/laureates/2004/axel-lecture.html.
- (180) Buck, L. B. Unraveling the sense of smell. Nobel Lecture, Dec 8, 2004. In *Les Prix Nobel. The Nobel Prizes 2004*; Frångsmyr, T., Ed.; Noble Foundation: Stockholm, Sweden, 2005; http://nobelprize.org/nobel_prizes/medicine/laureates/2004/buck-lecture.html.
- (181) Chandrashekar, J.; Hoon, M. A.; Ryba, N. J. P.; Zuker, C. S. The receptors and cells for mammalian taste. *Nature* **2006**, *444*, 288–294.
- (182) Shepherd, G. M. Smell images and the flavour system in the human brain. *Nature* **2006**, *444*, 316–321.
- (183) Zou, Z.; Buck, L. B. Combinatorial effects of odorant mixes in olfactory cortex. *Science* **2006**, *311*, 1477–1481.
- (184) Setkova, L.; Risticovic, S.; Pawliszyn, J. Rapid headspace solid-phase microextraction–gas chromatographic–time-of-flight mass spectrometric method for qualitative profiling of ice wine volatile fraction I. Method development and optimization. *J. Chromatogr., A* **2007**, *1147*, 213–223.
- (185) Setkova, L.; Risticovic, S.; Pawliszyn, J. Rapid headspace solid-phase microextraction–gas chromatographic–time-of-flight mass spectrometric method for qualitative profiling of ice wine volatile fraction II: Classification of Canadian and Czech ice wines using statistical evaluation of the data. *J. Chromatogr., A* **2007**, *1147*, 224–240.
- (186) Górecki, T.; Harynyuk, J.; Panić, O. The evolution of comprehensive two-dimensional gas chromatography (GC × GC). *J. Sep. Sci.* **2004**, *27*, 359–379.
- (187) Darriet, P.; Pons, M.; Henry, R.; Dumont, O.; Findeling, V.; Cartolaro, P.; Calonne, A.; Dubourdieu, D. Impact odorants contributing to the fungus type aroma from grape berries contaminated by powdery mildew (*Uncinula necator*); incidence of enzymatic activities of the yeast *Saccharomyces cerevisiae*. *J. Agric. Food Chem.* **2002**, *50*, 3277–3282.
- (188) Campo, E.; Ferreira, V.; López, R.; Escudero, A.; Cacho, J. Identification of three novel compounds in wine by means of a laboratory-constructed multidimensional gas chromatographic system. *J. Chromatogr., A* **2006**, *1122*, 202–208.
- (189) Culleré, L.; Escudero, A.; Pérez-Trujillo, J. P.; Cacho, J.; Ferreira, V. 2-Methyl-3-(methylthio)furan: A new odorant identified in different monovarietal red wines from the Canary Islands and aromatic profile of these wines. *J. Food Compos. Anal.* **2008**, *21*, 708–715.
- (190) Ryona, I.; Pan, B. S.; Intrigliolo, D. S.; Lakso, A. N.; Sacks, G. L. Effects of cluster light exposure on 3-isobutyl-2-methoxypyrazine accumulation and degradation patterns in red wine grapes (*Vitis vinifera* L. cv. Cabernet Franc). *J. Agric. Food Chem.* **2008**, *56*, 10838–10846.
- (191) Ryan, D.; Watkins, P.; Smith, J.; Allen, M.; Marriott, P. Analysis of methoxypyrazines in wine using headspace solid phase microextraction with isotope dilution and comprehensive two-dimensional gas chromatography. *J. Sep. Sci.* **2005**, *28*, 1075–1082.
- (192) Pierce, K. M.; Hoggard, J. C.; Mohler, R. E.; Synovec, R. E. Recent advancements in comprehensive two-dimensional separations with chemometrics. *J. Chromatogr., A* **2008**, *1184*, 341–352.
- (193) Giraudel, J. L.; Setkova, L.; Pawliszyn, J.; Montury, M. Rapid headspace solid-phase microextraction–gas chromatographic–time-of-flight mass spectrometric method for qualitative profiling of ice wine volatile fraction III. Relative characterization of Canadian and Czech ice wines using self-organizing maps. *J. Chromatogr., A* **2007**, *1147*, 241–253.
- (194) Cozzolino, D.; Smyth, H. E.; Cynkar, W.; Janik, L.; Damberg, R. G.; Gishen, M. Use of direct headspace-mass spectrometry coupled with chemometrics to predict aroma properties in Australian Riesling wine. *Anal. Chim. Acta* **2008**, *621*, 2–7.
- (195) Kruzlicova, D.; Mocak, J.; Balla, B.; Petka, J.; Farkova, M.; Havel, J. Classification of Slovak white wines using artificial neural networks and discriminant techniques. *Food Chem.* **2009**, *112*, 1046–1052.
- (196) López-Feria, S.; Cárdenas, S.; Valcárcel, M. Simplifying chromatographic analysis of the volatile fraction of foods. *Trends Anal. Chem.* **2008**, *27*, 794–803.
- (197) Röck, F.; Barsan, N.; Weimar, U. Electronic nose: current status and future trends. *Chem. Rev.* **2008**, *108*, 705–725.
- (198) Moreno i Codinachs, L.; Kloock, J. P.; Schöning, M. J.; Baldi, A.; Ipatov, A.; Bratov, A.; Jiménez-Jorquera Electronic integrated multisensor tongue applied to grape juice and wine analysis. *Analyst* **2008**, *133*, 1440–1448.
- (199) Berna, A. Z.; Trowell, S.; Cynkar, W.; Cozzolino, D. Comparison of metal oxide-based electronic nose and mass spectrometry-based electronic nose for the prediction of red wine spoilage. *J. Agric. Food Chem.* **2008**, *56*, 3238–3244.
- (200) Zhang, C.; Bailey, D. P.; Suslick, K. S. Colorimetric sensor arrays for the analysis of beers: a feasibility study. *J. Agric. Food Chem.* **2006**, *54*, 4925–4931.
- (201) Zhang, C.; Suslick, K. S. Colorimetric sensor array for soft drink analysis. *J. Agric. Food Chem.* **2007**, *55*, 237–242.
- (202) Janzen, M. C.; Ponder, J. B.; Bailey, D. P.; Ingison, C. K.; Suslick, K. S. Colorimetric sensor arrays for volatile organic compounds. *Anal. Chem.* **2006**, *78*, 3591–3600.
- (203) Martí, M. P.; Boqué, R.; Busto, O.; Guasch, J. Electronic noses in the quality control of alcoholic beverages. *Trends Anal. Chem.* **2005**, *24*, 57–66.
- (204) Fiehn, O.; Kopka, J.; Dörmann, P.; Altmann, T.; Trethewey, R. N.; Willmitzer, L. Metabolite profiling for plant functional genomics. *Nat. Biotechnol.* **2000**, *18*, 1157–1161.
- (205) Roessner, U.; Luedermann, A.; Brust, D.; Fiehn, O.; Linke, T.; Willmitzer, L.; Fernie, A. R. Metabolic profiling allows comprehensive phenotyping of genetically or environmentally modified plant systems. *Plant Cell* **2001**, *13*, 11–29.
- (206) Fiehn, O. Metabolomics—the link between genotypes and phenotypes. *Plant Mol. Biol.* **2002**, *48*, 155–171.
- (207) Tikunov, Y.; Lommen, A.; de Vos, C. H. R.; Verhoeven, H. A.; Bino, R. J.; Hall, R. D.; Bovy, A. G. A novel approach for nontargeted data analysis for metabolomics. Large-scale profiling of tomato fruit volatiles. *Plant Physiol.* **2005**, *139*, 1125–1137.

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