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Chemical Markers for Aroma of *Vitis vinifera* Var. Chardonnay Regional Wines

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Since objective measures of appellations are needed for wine, California *Vitis vinifera* Var. Chardonnay wines (n = 48) were surveyed for several aroma compounds including esters, norisoprenoids, and terpenes. For the first time concentrations of volatile fragrances were directly correlated with descriptive analysis scores that statistical analysis of the sensory data showed were associated with regional uniqueness and distinctness. Sensory scores for 10 terms used by industry quality experts to describe wines from four regions were compared to the concentrations of over 30 wine chemical compounds. Frequency of use of individual grape-based aroma terms were significantly correlated ($p < 0.05$) with linalool, 1,1,6-trimethyldihydronaphthalene, 3-methylbutyl acetate, ethyl 2-hydroxypropanoate, and 2-phenylethanol. Linalool concentrations were also correlated with α-terpineol. Also, 4-terpineol, geraniol, nerol, and linalool oxide (furan) were found in Chardonnay wines from all of the regions of California.

Keywords: *Aroma; Chardonnay; descriptive analysis; flavorants; norisoprenoids; sensory analysis; terpenoids; terroir; Vitis vinifera; wine*

INTRODUCTION

Appellations have been important in European wine-growing regions for a century (Jackson and Lombard, 1993; Kramer, 1992; Larousse, 1991; Moio et al., 1993; Morlat, 1989; Peynaud, 1984; Seguin, 1986). As a result, U.S. wine growers encouraged the U.S. Bureau of Alcohol, Firearms, and Tobacco (BATF) to increase regulatory controls in 1978, resulting in the establishment of American Viticultural Areas (AVA) (Kramer, 1992). In retrospect, wine producers have asked whether different regions actually produce different bottled wines. Therefore, the objective determination of appellations is one of the great challenges for wine industry professionals.

Chemical analyses have been used by wineries to manage the production steps that sensory analyses have shown may cause bottled wine differences (Stone and Sidel, 1993). Since the 1970s the volatile (Schreier et al., 1974) and nonvolatile derivatives (Cordonnier and Bayonove, 1974) of grape fragrances have been investigated in several grape varietals. Identification of secondary chemical compounds has been one strategy employed for investigating flavor compounds (Marais, 1987; Marais et al., 1991; Simpson, 1978), distinguishing different varietals (Herrick and Nagel, 1985; Rapp, 1988; Wilson et al., 1986). As a result most *Vitis vinifera* Var. Chardonnay flavorants have been unequivocally identified. Theoretically, the flavor chemistry may now be linked to the entire wine-making process from grape (biosynthesis) to the regional typicalness of bottled wines found in the marketplace. However, no studies have shown that regional U.S. white grape chemistry is unique or distinct in bottled wines (clearly different and not overlapping).

Traditional sensory analysis provides empirical evidence suggesting that the differences among bottled wine products are associated with appellations. In France the traditional concepts of the *cru* and *terroir* describe tasters' detection of regionality and place. Terroir has been defined as a distinct region with an environment that produces an original quality agricultural product (Larousse, 1991). Since terroir is a powerful model, it has remained the basis of the regulatory controls instituted to protect French regional wines and is a focal point of new enological research (Moio et al., 1993; Morlat, 1989; Seguin, 1986; Larousse, 1991). Recently "the modern terroir" has been described as "the unique ecotypic expression of regional grapes which causes differences in the secondary chemical flavorants detected in bottled wines which are defect free" (McCloskey, et al., 1996b).

Modern sensory analysis is potentially useful for selecting regional white and red wines for chemical studies of appellations (Guiniard and Cliff, 1993; McCloskey et al., 1996a; Moio et al., 1993; Noble et al., 1984). Clustering in the statistical analysis of the sensory data has been considered an objective test of appellations (Guiniard and Cliff, 1983; McCloskey et al., 1996a). However, wine industry professionals devising appellations must reconcile their traditional methods with the modern processes that have been developed since World War II for large food companies (Lawless and Classen, 1993; McCloskey et al., 1996a,b). Moio et al. (1993) encountered several problems that made it difficult to demonstrate the uniqueness of Burgundy wines. Most wine professionals use traditional quality assessment (TQA) and do not use the modern methods (Lawless and Classen, 1993; McCloskey et al., 1995, 1996a; Peynaud, 1984, 1987). In contrast, two major schools of descriptive analysis have been developed for wine, which seldom include TQA. These are (i) a consumer-based quantitative descriptive analysis process (QDA), which has been used very successfully with consumer panels to subsequently forecast consumer acceptance of wines for large producers and has been correlated with chemistry (Stone and Sidel, 1993), and (ii) descriptive analysis developed atthe University

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of California at Davis (UCD DA) to educate enology students and in teaching the "Standardized System of Wine Aroma Terminology" (Amerine and Roessler, 1976; Noble et al., 1987; Noble, 1988). The UCD DA processes derive from flavor profiling (FP) methods developed by the Arthur D. Little Co. (Caul, 1957), which are based on the work of Sjostrom and Cairncross (1954). FP processes use a standard language, which poses well-known problems for research which includes panels comprised entirely of wine professionals who already have a strong sensory language (Stone and Sidel, 1993; Williams and Stevens, 1984). As a result free-choice profiling (FCP) was developed for wine to overcome the problems related to the FP-type methods (Williams and Stevens, 1984). Since UCD DA scores are not widely correlated with instrumental analysis, a new method that integrates several FCP and QDA strategies was used in the present study since results have suggested sensory scores may be correlated with wine chemistry (McCloskey et al., 1996a).

The goal of this study was to determine whether chemical markers in grapes were related in a quantitative way to aroma scores from a new descriptive analysis process used to validate appellation. Regional wines were analyzed to determine which of several chemical compounds reported in white wines (Marais, 1987; Marais et al., 1992; Rapp, 1988; Webster et al., 1993; Williams et al., 1982, 1992) were linked to the descriptive analysis of U.S. appellations (McCloskey et al., 1996a).

EXPERIMENTAL PROCEDURES

Wine Products. California producers (n = 54) supplied vintage 1991 Chardonnay wines. A survey by questionnaire showed that the wine-making and viticultural practices were similar. Wines were made from vineyards planted with similar scion wood such as the Wente selections or clones (UCD 3 and 4). Six wines were rejected because they were made with less than 100% regional grapes and for major defects (volatile acidity) and taints ("corked"). A final set of wines (n = 48) was comprised of equal numbers of wines (n = 12) from the four regions (Carneros AVA, Central Coast, south of San Luis Obispo; Napa and Sonoma.

Judges and Perceived Wine Quality. Because judges (n = 26) were also wine industry quality experts who testified in oral discussions that they had perceived quality biases, the wines were sorted into three equal size groups (n = 16) based on large, neutral, and low perceived quality groups A, B, and C. This was done with a multiwine preference (MWP) test used by wine industry professionals (McCloskey et al., 1995, 1996a,b). During this process judges provided terms that they used in their professional work to describe the 48 wines.

Chemical Analysis, Gas Chromatography, and Mass Spectrometry. Methods used to analyze the primary chemistry are described in Amerine and Ough (1980) and Zoecklin et al. (1990) and include free sulfur dioxide, titratable acidity, volatile acidity, calcium, potassium, pH, citric, malic, and lactic acids, glucose, fructose, glycerol, ethanol, and fusel oils. The methods used for extraction of volatile aromatic compounds were taken from the methods of Gunata et al. (1985), Webster et al. (1993), and Williams et al. (1982, 1992). Samples for GLC analysis were extracted using SPE cartridges packed with C_{18} absorbent, and organic solvents were used to elute extracts which were stored at 0 °C until analysis. Extracts were analyzed with a GC–MS Finnigan 450 equipped with a DB-5 MS fused silica column (60 m × 0.25 mm i.d. × 0.25 μm film thickness). Because terpenes and norisoprenoids were present in low concentrations (<150 μg/L), analysis was done separately on 22 wines with a HP5890 series II GC interfaced to an HP 5971 mass selective detector (Hewlett-Packard Co., Palo Alto, CA). GC–MS and retention times using reference compounds were used to identify wine aroma compounds. Internal standards were used to quantify concentrations of individual compounds. Reference compounds were all available commercially except norisoprenoids, which were donated by P. J. Williams (Australian Wine Research Institute).

Multiwine Descriptive Analysis (MWDA). The descriptive analysis has been described (McCloskey et al., 1996a). First, it was determined by frequency analysis, including Poisson fit, that judges routinely used one to four terms to describe wines. Next, the 10 most frequently used terms were selected for inclusion in the scorecard. Judges were instructed to describe wines by selecting 2–5 of the 10 terms provided on the scorecard. The raw descriptive analysis score (β) for each wine was the number of times a term was selected by a panel of judges. Whereas the individual scores, β, were used in the regression analysis with chemical compounds, the variation of β about the mean was used in the principal component analysis of the sensory data.

The terms in the MWDA scorecard were selected by analyzing the entire judge language recorded in the MWP scorecards used prior to the MWDA. In over 1100 wine × judge MWP interactions, judges used free-choice to profile wines. All terms used by judges were accounted for in a spreadsheet and regression analysis was used to combine redundant terms for subsequent MWDA. For example, "oaky", "woody", and "vanilla" were combined into the single term "oaky/woody/vanilla" in the MWDA scorecard. Those terms for which some ambiguity existed in the judges understanding, such as herbal, were clarified by oral discussion and using C_{18} extracts of wines or grape vine tissue.

Calculations and Statistics. Statistical analyses of the attribute scores (β) were performed using the SAS program (Statistical Analysis Systems, SAS Institute, Inc. Cary, NC) SysSTAT Student (Abacus Systems, Berkeley, CA), Lotus 123 (Lotus Development, Cambridge, MA) and Delta-Graph V.3.5 (Monterey, CA). Interjudge consistency was analyzed using Spearman's Rho and a similarity index (Amerine and Roessler, 1983). Judges were eliminated from the analysis on the basis of criteria suggested. The statistical analysis of the sensory data included principal components analysis (PCA) of the sensory attribute scores, ANOVA, and polar plots. Multiple regression and correlation analysis was used in the analysis of the chemical and tasting data.

RESULTS AND DISCUSSION

This is the largest survey of California Chardonnay to date which includes chemical and sensory analysis of bottled wines. It reports for the first time that concentrations of several chemical compounds correlate with aroma attribute scores distinguishing appellations. Chemical markers selected (i) were significantly correlated with grape-based sensory attributes, (ii) have been extensively investigated in several grape varietals (Marais, 1987; Marais et al., 1992; Rapp, 1988; Webster et al., 1993; Williams et al., 1982, 1992), (iii) may be under genetic or environmental controls in other plant systems (Harborne, 1988; Langenheim, 1994), and (iv) may be easily analyzed using simple GLC methods.The correlations between frequency of use of grape-based aroma terms and grape chemistry suggest that ecotypic expression of *V. vinifera* chemistry may be the basis of differences between Carneros, Central Coast, and Napa Chardonnay bottled wine aromas.

Sensory Analysis. Groups of 16 wines were each analyzed separately, and each group represented a level of perceived quality determined by the judges. Since the sensory analysis processes accommodated the industry professionals' biases for perceived quality, several interesting results were found related to quality. First, analysis of the group A wines, those for which the judges had no large negative quality bias, showed three of the four regions were clearly different and not overlapping. However, the detection of regionality was clearly linked to the perceived quality bias. As quality

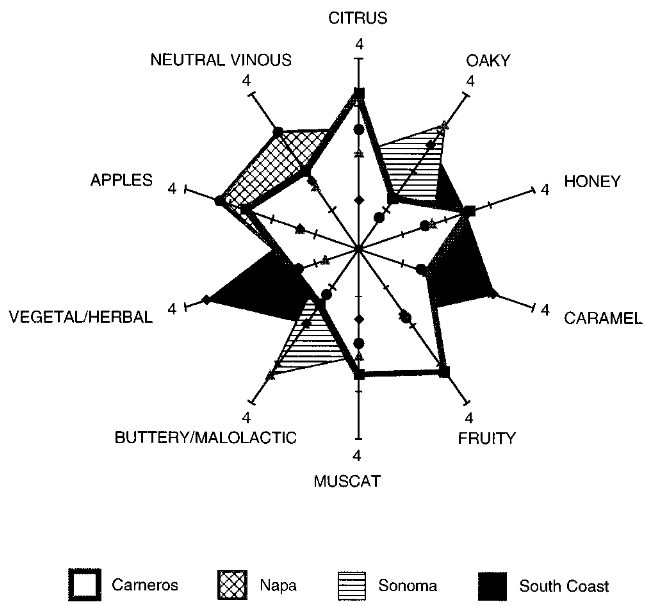

Figure 1. Polar plot analysis showing regional variation in use of 10 aroma attributes for group A (high-quality wines, variation about the mean).

dropped to the midrange, only two regions were unique. As quality dropped further, it was not possible to determine regional uniqueness. This finding confirms the traditional model of terroir. The most important sensory results came from the PCA of the regional wines.

Group A wines from three regions, Carneros, Central Coast, and Napa, clustered and separated in the PCA of the sensory data. Sonoma wines did not cluster. The Carneros AVA, Central Coast, and Napa clusters separated along principal components (PC2 × PC1) comprised of a linear combination of positively weighted aroma terms including citrus, fruity, muscat (PC1) and green-apples/pears (PC2). Negatively weighted terms included caramel-pumpkin and herbal/leafy vegetal (PC1).

Group B wines from two regions, Carneros and Central Coast, clustered in the PCA of the sensory data, which duplicated the first sensory results. Carneros AVA wines clustered tightly and separated from the other wines along the second and third principal components. The two PCs accounted for 57% of the variance. Group B wines were assessed a second time, and the results were compared with the results of the initial group B tasting. Carneros wines again clustered in the PC3 × PC2 plane. Important terms included muscat, honey, and fruity and the negatively weighted attributes of herbal/leafy vegetal. Wines separated along PC3 based on positive weights for oaky and green-apples/pears and the negatively weighted herbal/leafy vegetal.

The low-quality (group C) wines did not cluster, suggesting that concentrations of flavorants were lower in group C or that the judges perceptions were confounded by low quality. Because the ranges of concentrations of compounds were the same in group A and B wines, it is likely that quality affected judge perception.

To determine which grape and wine attributes contributed to regional typicity of Carneros wines, we analyzed the data using a polar plot of the 10 terms (Figure 1). citrus, fruity, muscat and green-apples/pears were 40−75% above the mean for Carneros wines. Equally important in the description of the Carneros Chardonnay wines was the infrequent use of caramel-pumpkin and herbal-leafy vegetal (~30% below the mean). This was exemplified by the highly significant negative correlation between citrus and caramel-pumpkin squash attributes ($r = -0.77$; $p < 0.001$). This result explained why Carneros wines are distinct from Central Coast wines. The cluster formed by group B Carneros wines in the PCA duplicated the first results. The citrus and green-apples/pears attributes were used to describe both high- and mid-range-quality Carneros wines. Honey, caramel/pumpkin squash and herbal/leafy vegetal attributes were used infrequently with Carneros group B wines. In summary, sensory results found six important grape-based aromas including caramel-pumpkin squash, citrus, herbal-leafy vegetal, honey, and muscat.

Chemical Analysis of the Regional Wines. Whereas regional uniqueness was related to quality in the analysis of the sensory data, the range of chemical concentrations did not vary with quality (groups A and B). However, chemical concentrations were related to the individual sensory scores in the regression analysis. Both grape- and wine-making-based attributes were linked to chemistry, but a distinction was evident. Grape-based sensory attributes were related to grape derived compounds, whereas wine-making-based attributes were related to microbial metabolism in the wine-making process. Within the high-quality group A the aroma attribute scores important in defining regions were significantly correlated ($p < 0.05$) with concentrations of specific compounds (Table 1). Several of these correlations were replicated at a 10% level of significance or less including as quality dropped to the midrange (group B wines). While wines were assayed for sugars, fermentation byproducts, inorganic salts, and preservatives (see Experimental Procedures), only secondary compounds were found to be correlated with the sensory analysis scores.

A significant correlation of 3-methylbutyl acetate concentration with frequency of use of the green-apples/pears term was found for group A and B wines. This result was repeated in a duplicate tasting of group B wines using a different group of judges. 3-Methylbutyl acetate was a useful marker for an attribute important in establishing the uniqueness of the Carneros and Napa wine-growing regions and may represent one element of a suite of compounds that are responsible for the green-apples/pears aroma. This is supported by the finding for fresh apples for which the ratio of hexyl acetate to 2- and 3-methylbutyl acetate was reported to be related to fruitiness (Loyaux et al., 1981). Since this study we have found 3-methylbutyl acetate concentrations of 300 μg/L in California Chardonnay wines.

Although the buttery/malolactic attribute was not a factor in the clustering of group A and B wines, there were several interesting correlations with products of malolactic fermentation. First, an inverse correlation was found between malate and the buttery/malolactic attribute. As malic acid is converted to lactic acid, ethyl 2-hydroxypropanoate is formed, and a very highly significant negative correlation ($p < 0.001$) was found between ethyl 2-hydroxypropanoate and malic acid in group A wines. Finally, ethyl 2-hydroxypropanoate was also positively correlated with the buttery/malolactic attribute (Table 1).

Table 1. Correlation Matrix among the Chemical Compounds and Aroma Attributes (n = 16) for Group A wines[a]

	range	citrus	oaky	honey	caramel	fruity	muscat	buttery	vegetal	apple	neutral
malate	0.83−2.71 g/L	−0.12	−0.29	−0.29	0	−0.13	−0.23	**−0.54***	0.45	0.24	0.30
ethyl 2-hydroxypropanoate	0.07−3.50 mg/L	0	0.38	0	−0.30	0	0.37	**0.63****	−0.29	−0.38	**−0.55***
2-phenylethanol	0.6−4.8 mg/L	0	0.28	**−0.54***	0	−0.17	−0.40	−0.15	0	−0.14	0.34
3-methylbutyl acetate	0.2−1.6 mg/L	0.38	−0.31	−0.14	−0.28	0.20	0.16	−0.33	−0.25	**0.69****	0.11

[a] *, **, significant at $p < 0.05$ and $p < 0.01$, respectively.

Table 2. Corelation Matrix among the Terpenoid and Norisoprenoid Compounds and Aroma Attributes (n = 22)[a]

	range	aroma attributes from the sensory analysis								
	μg/L	citrus	oaky	honey	caramel	fruity	muscat	buttery	vegetal	apple
				Norisoprenoids						
total norisoprenoids	10−90	0	−0.20	0.30	0	0.14	0.14	0.14	0.17	0
TDN-I	5−30	−0.36	−0.14	0.58**	0.37	−0.24	−0.17	0.20	0.17	−0.36
				Terpenes						
total terpenoids	5−580	0.14	−0.36	0	0	−0.14	0.14	−0.24	0.24	0
linalool oxide	20−60	0.24	−0.14	−0.26	−0.10	−0.14	−0.17	−0.10	0.20	0.14
linalool	5−50	0.63**	−0.28	−0.24	−0.31	0.31	0.10	−0.28	−0.14	0.36
α-terpineol	0−500	0	−0.36	0	0	−0.10	−0.14	−0.24	0.24	0

[a] *, **, significant at $p < 0.05$ and $p < 0.01$, respectively.

Terpenes are widespread throughout the plant kingdom (Harborne, 1988; Langenheim, 1994) and contribute significantly to the varietal character of *V. vinifera* Var. Gewurztraminer, Muscat, Riesling, and Viognier. Although terpenes have not been considered to contribute to Chardonnay aroma due to their low concentrations, we detected α-terpineol, 4-terpineol, linalool, geraniol, nerol, and linalool oxide (furan) in wines from all of the regions analyzed. Linalool concentrations were highly significantly correlated with the frequency of use of the citrus attribute ($p < 0.01$) (Table 2), important in assessing the uniqueness of the Carneros region. Flavor thresholds of linalool (100 μg/L for linalool) are higher than the concentrations found in the Chardonnay wines studied here (10−80 μg/L). Thus, linalool may be a marker for a group of free terpenes, terpene oxidation products, and other compounds contributing to the citrus aroma of regional Chardonnay wines. This is supported by the finding of a correlation between linalool and α-terpineol. Although α-terpineol was not significantly correlated with the sensory analysis, α-terpineol concentrations (10−500 μg/L) were very highly correlated with linalool concentrations ($p < 0.001$). α-Terpineol, which may not be found in the grape, can be formed in wine from linalool by the acid-catalyzed cyclization reactions (Rapp, 1988).

The norisoprenoids, methyldihydronaphthalenes (TDNs), free and glycosidically bound, have been widely reported in grapes and white wines (Strauss et al., 1987). They are important grape-derived wine flavorants, beneficial at low concentrations, and are responsible for the undesirable "kerosene-like" odor of aged white Riesling when present in high concentrations (Strauss et al., 1987; Williams et al., 1992). Williams (1994) has also reported norisoprenoids in association with "honey" aromas of Australian Chardonnay wines. In Weisser Riesling and Chenin blanc grapes concentrations of glycosides of C_{13} norisoprenoids, including 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN-I) and damascenone, were higher in sun-exposed compared to shaded grapes and increased with ripeness (Marais et al., 1992). They may be degradation products of carotenoids in the grape (Williams et al., 1992) which reflect the geographic origin of wines since they are found in higher concentrations in grapes from hot compared to cold regions (Marais, 1991).

Low levels of TDN-I, below sensory threshold, were reported in Chardonnay juice (Sefton et al., 1993). In the present study the marker TDN-I was also found at low concentrations, ranging from 1 to 30 μg/L. The sensory threshold has been estimated at 20 μg/L in wine (Simpson and Miller, 1984). However, a highly significant statistical correlation ($p < 0.01$) was found for TDN-I with honey (Table 2). Notably, a highly significant negative correlation of TDN-I with citrus ($p < 0.01$) supported the observation of a very highly significant negative correlation of the term Citrus with the term caramel/pumpkin squash ($p < 0.001$).

Several Chardonnay chemical markers associated with grape aroma attributes were probably synthesized by both grapes and yeasts. While 2-phenylethanol is largely produced during yeast fermentation, glycosides of 2-phenylethanol have been found in *V. vinifera* var. Chardonnay, Muscat Alexandria, and Riesling grapes and may contribute to the total found in wines (Sefton et al., 1993; Williams et al., 1983). In this study 2-phenylethanol was significantly correlated with the honey attribute ($p < 0.05$) in group A wines (Table 1). Similarly, 3-methylbutyl acetate was correlated with green-apples/pears. It may also be formed both in vivo and in vino, for example by autoesterification of 3-methylbutyl alcohol formed by yeast.

Although it is unlikely that compounds present at subthreshold levels could exert a significant aroma influence individually, they may be representative of a group of compounds could act synergistically. Thus, the compounds reported here are markers, not flavorants, of the aroma attributes. Chemical markers have several uses including in solving problems not answered by sensory analysis and in developing quality control procedures related to appellations.

The determination of grape versus wine-making markers is a useful strategy for assessing *the modern terroir* (McCloskey et al., 1996b), which forecasts that certain differences detected by modern sensory analysis among bottled wines in the marketplace results from regional variations in grape flavorants. Markers may be useful to determine whether sensory variations are associated with regional physical environmental and genetic factors. In this study, significant environmental factors distinguished several of the wine-growing regions including photoperiod difference between the Carneros and Central Coast. Genetic variations are also important to flavorant concentrations, but the clones of Chardonnay studied are genetically relatively homogeneous. Many Carneros AVA and Central Coast vineyards are planted largely with related clones (UCD 4−7, 12, and 14 came from Wente selections), and from an ecological perspective the vineyards are monocultures. The analytical processes presented when used with *the modern terroir* model offer an integrated approach for validating U.S. wine-growing regions for the BATF such as the Carneros American Viticultural Area.

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