Volatile Profiling of U.S. Cabernet Sauvignon Wines Using HS-SPME and the Agilent 5975 Series GC/MSD System: Relating the Chemical Profile to Sensory Properties

Application Note

Food

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Abstract

A semiquantitative, automated headspace solid phase microextraction (HS-SPME) gas chromatography/mass spectrometry (GC/MS) method has been developed on the Agilent 5975 Series GC/MSD System using synchronous Selected Ion Monitoring (SIM)/scan detection to measure 61 volatile compounds in wine. The method was used to analyze 24 commercial U.S. Cabernet Sauvignon varietal and blended wines. The results were compared to a descriptive sensory analysis of the wines to develop a rapid, targeted profiling method that was able to predict a number of aroma sensory descriptors. This work will help the U.S. wine industry define current Cabernet Sauvignon wine styles and identify areas that require further market development.

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Introduction

There is increased interest in the use of chemical profiling methods to investigate wine sensory profiles. Time-of-flight (TOF) mass spectrometry, coupled to comprehensive gas chromatography, can resolve thousands of compounds. However, not all compounds will necessarily have a sensory impact in wine. Powerful and complex statistics are required to relate these complex chemical composition findings to sensory attributes.

Targeted profiling for specific compounds can provide a rapid alternative for assessing a broad range of volatile compounds in wine that can be associated with sensory descriptors. The use of synchronous SIM/scan acquisition on a single quadrupole mass selective detector (MSD) can quickly provide full scan spectra for analyte confirmation, as well as sensitive and selective quantitation and detection of the targeted compounds.

This application note describes a published study that used HS-SPME and the 5975 Series GC/MSD System to successfully develop a profiling method for U. S. Cabernet Sauvignon wines and blends that can predict several important sensory descriptors of the wines [1]. Synchronous SIM/scan has previously been used to profile wine volatiles [2,3] however, this was the first report of the use of synchronous SIM/scan to identify volatile compounds that may contribute to aroma in wines. In all, 55 volatile compounds were identified that were significantly different among the wines. Statistical analysis linked these compounds to seven aroma attributes and 12 taste and mouth feel attributes that were also significantly different among the wines.

Experimental

Wines

All wines included in the study were commercially available and made in the U.S. In total, 24 Cabernet Sauvignon varietal and blended wines (minimum 56% Cabernet Sauvignon) were included in the study, 19 from California and five from Washington.

Standards

Sixty-one compounds were targeted in this study, based on previous reports [4,5]. Of these, 58 were verified by analysis of reference compounds, as previously described [1]. The retention times and spectra of the authentic standards were matched to the compounds measured.

Instruments

This profiling method was developed using a 5975 Series GC/MSD System coupled to an Agilent 6890 GC equipped with a Gerstel MPS2 autosampler. The GC and MS run conditions are shown in Table 1. The ions used in the SIM parameters and the retention times for each compound were as previously described in the published study [1]. 2-Isobutyl-3-methoxyprazine (MIBP) was also analyzed using the same GC, column and autosampler, coupled to an Agilent 7000B Triple Quadrupole GC/MS, as described previously [1].

Sample preparation

Wine samples (10 mL, undiluted) were pipetted into 20-mL round-bottomed, amber glass headspace vials, each containing 3 g (± 0.02) NaCl. The vials were immediately placed on the instrument for analysis. A 2-cm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco), 23-gauge SPME fiber was used for sampling. Samples were warmed at 30 °C and agitated at 500 rpm for 5 minutes before exposing the fiber for 30 minutes at 30 °C with agitation at 250 rpm. All samples were analyzed in triplicate in a randomized order within 18 hours of loading on the instrument.

Table 1. Instrument Run Conditions

GC run conditions	
Analytical columns	Agilent J&W DB-Wax, 30 m × 0.25 mm, 0.25 µm (p/n 122-7032)
Injection mode	The SPME fiber was desorbed in split mode with a 20:1 spit ratio
Inlet temperature	240 °C
Carrier gas	Helium in constant flow mode, 1 mL/min
Oven temperature	40 °C for 5 minutes, 3 °C/min to 180 °C, 30 °C/min to 240 °C, hold for 10 minutes
Transfer line temperature	240 °C
MS run conditions	
Acquisition mode	Synchronous SIM/Scan, electron ionization, *m/z* 40 through 300
Scan rate	5.8 scans/s (15 scans over each peak)
HS-SPME conditions	
Fiber	2 cm × 23 gauge divinylbenzene/carboxin/polydimethylsiloxane (DVB/CAR/PDMS, Supelco)
Sampling	Samples were warmed at 30 °C and agitated at 500 rpm for 5 minutes before exposing the fiber for 30 minutes at 30 °C with agitation at 250 rpm.
Desorption	10 minutes at 240 °C

Sensory analysis

The wines were presented in a randomized and balanced order, and assessed in triplicate by 11 sensory assessors who were selected and trained as previously described [1]. Twenty aroma attributes and 14 taste and mouthfeel attributes were assessed. The intensity of each attribute was rated using an unstructured 15 cm line scale anchored by wordings of low and high, except for a few attributes, for which other opposite adjective pairs were used.

Data analysis

For the semiquantitative targeted profiling method, peaks were quantified relative to the internal standard (2-undecanone), using peak area of an extracted ion. For MIBP, measured using GC/MS/MS, the absolute concentration was determined by relative response of MIBP to the internal standard ($^2H_3$MIBP) using a standard curve.

The chemical data were analyzed using analysis of variance (ANOVA) measuring for the effects of wine and replicate using a pseudo-mixed model test, with mean square (wine*replicate) as the error. For the descriptive sensory analysis, an ANOVA measured the effects of wine, replicate and assessor, and all two-way interactions, using a pseudo-mixed model test, with mean square of interaction terms containing wine as the error. The chemical and sensory data were related to one another using multiple pairwise correlations and partial least squares regression (PLSR) relating the chemical data as x-variables (predictor variables) and sensory descriptive data as y-variables by PLS2 [1]. A further series of PLS1 models was generated using individual sensory attributes (y-variable) related to the chemical data (x-variables).

Results and Discussion

Relating chemical and sensory data

A partial least squares regression (PLSR) was performed on standardized significant chemical and sensory data for the 24 Cabernet Sauvignon varietal and blended wines included in the study [1], the results are shown in Figure 1. The first two principal components (PCs) explained 38% of the variance for the chemical data (x-variables), and 46% of the variance for the sensory data (y-variables). The third principal component explained an additional 7% and 10% of variance for the chemical and sensory data, respectively (data not shown).

In this model, 36 of the 56 compounds measured in the Cabernet Sauvignon wines and blends using the developed GC methods contributed significantly ($p < 0.05$) to the prediction of the sensory attributes (PLS2), as indicated by their positions towards the outside of the plot (Figure 1A). To further explore the compounds responsible for the sensory attributes in more depth, PLS1 regression analyses were performed for each sensory attribute.

Chemical composition and aroma

The volatile compounds measured could be associated with a number of the sensory descriptors. For example, diacetyl and acetoin were associated with butterscotch aroma (Figure 1A). Both of these compounds are known to contribute buttery characters to foods and wines. The compounds 4-ethylphenol and 4-ethylguaiacol are known to contribute leathery and horse saddle characters to wines and in this study, along with α-cedrene, they were associated with barnyard aroma (Figure 1A). Vegetal aroma was positively associated with the bell pepper impact compound 2-isobutyl-3-methoxypyrazine (MIBP). Several esters (for example, hexyl acetate, 2-phenylethylacetate), terpenes (for example, linalool, β-citronellol) and norisoprenoids (for example, β-damascenone, α-ionone) were associated with berry and fresh fruit aromas in the PLS2 analysis (Figure 1A).

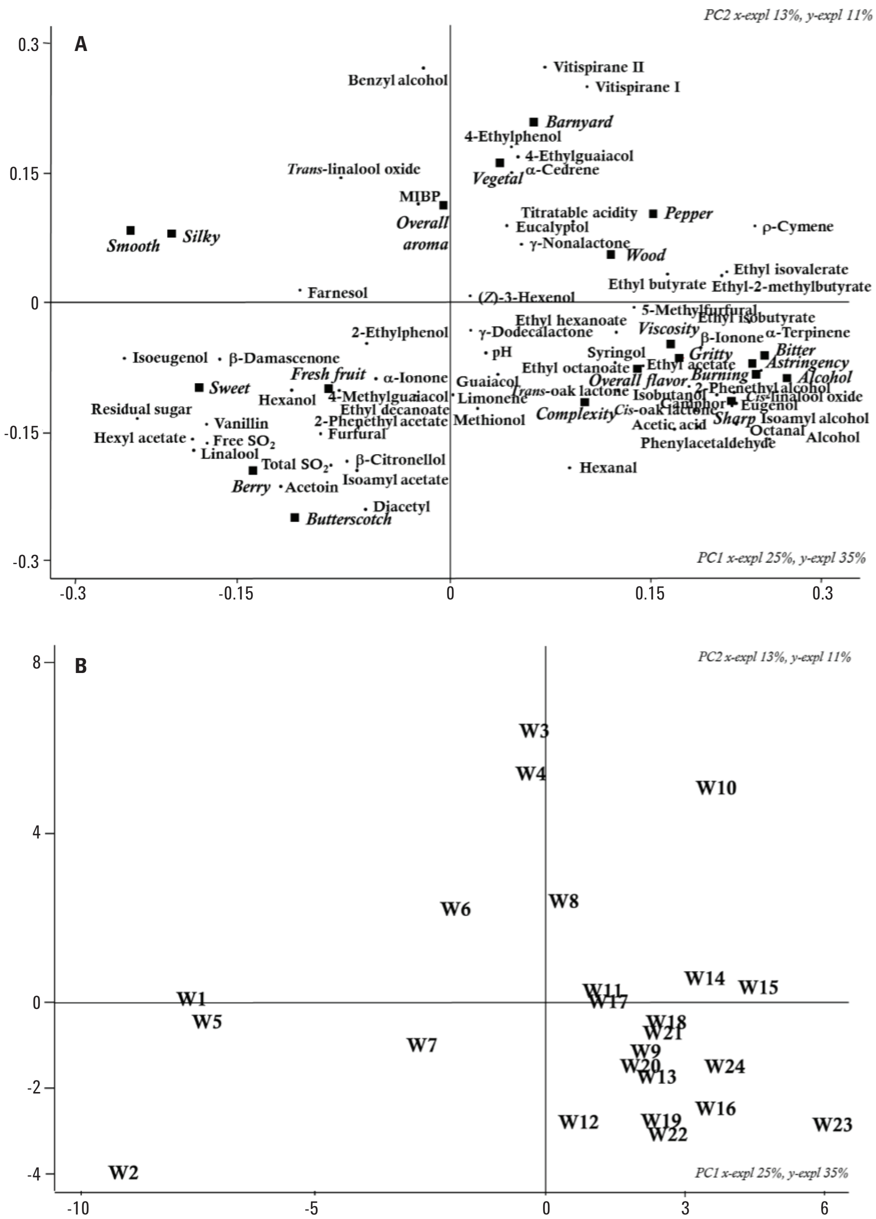

Figure 1. PLSR of A), standardized chemical compounds as x-variables (predictor variables) (small black circles) and standardized significant ($p < 0.07$) sensory attributes as y-variables (black squares) for B), 24 commercial U.S. Cabernet Sauvignon varietal and blended wines. The codes indicate increasing alcohol concentration from W1 = 12.4% v/v to W24 = 15.9% v/v. Source: Hjelmeland et al. [1].

The chemical data were found to significantly predict a number of sensory descriptors, as summarized in Tables 2 and 3. While many of the correlations are logical, as described above, positive correlations do not necessarily reflect causal relationships and most wine sensory attributes are the result of the perception of mixtures of several compounds.

Table 2. Significant associations of Volatile Compounds with Individual Aroma Attributes of U.S. Cabernet Sauvignon Wines and Blends from PLS1

	Aroma							
Compound	**Berry**	**Butterscotch**	**Fresh fruit**	**Sweet taste**	**Pepper**	**Vegetal**	**Wood**	**Barnyard**
Acetic acid								
Acetoin	+	+			–			
Camphor							+	
α-Cedrene	–	–						+
p-Cymene					+		+	
Diacetyl		+						
Ethyl butyrate					+			
4-Ethylguaiacol								+
Ethyl isobutyrate						–		
Ethyl isovalerate	–	–			+			
Ethyl-2-methylbutarate	–	–			+			
4-Ethylphenol								+
Eucalyptol						+		
Eugenol†					+		+	
Farnesol		+					–	
Guaiacol†							+	
Hexyl acetate	+		+		–			
β-ionone					+		+	
Isobutanol						–		
Isoeugenol					–	–	–	
Linalool	+	+			–			–
cis-Linalool oxide					+			
4-Methylguaiacol†							–	
MIBP (2-isobutyl-3-methoxypyrazine)						+		
trans-Oak lactone†							+	
Octanal					+			
2-Phenethyl alcohol						–		
Residual sugar	+	+	+	+	–	–	–	
SO_2 (free)	+	+			–			
α-Terpinene			–		+		+	
Vanillin†		+				–	–	
Vitispirane I		–						+
Vitispirane II								+

\+ Positive association

– Negative association

† Oak-derived compounds

Those compounds highlighted in yellow are associated with the denoted aroma attribute but are not statistically significant.

Table 3. Association of Chemical Composition with Taste and Mouthfeel Attributes of U.S. Cabernet Sauvignon Wines and Blends from PLS1

	Taste or mouthfeel								
Compounds	**Sweet**	**Bitter**	**Astringency**	**Alcohol flavor**	**Viscosity**	**Smooth and silky**	**Sharp**	**Gritty**	**Burning**
Acetic acid		+	+	+		–			+
Acetoin			–						
Alcohol (ethanol)		+	+	+	+	–	+	+	+
Camphor		+		+	+	–			+
p-Cymene		+		+	+	–		+	+
Diacetyl			–						
Ethyl acetate		+	+	+	+	–	+	+	+
4-Ethylguaiacol									
Ethyl butyrate		+		+					+
Ethyl isobutyrate		+		+		–		+	+
Ethyl isovalerate		+		+		–			+
Ethyl-2-methylbutarate		+		+		–			+
Ethyl octanoate		+		+					+
Eugenol†		+		+	+	–		+	+
Hexyl acetate									
β-ionone		+		+	+	–		+	+
Isoamyl alcohol		+		+		–		+	+
Isobutanol		+		+		–		+	+
Isoeugenol		–		–	-	+		–	–
Linalool				–					
cis-Linalool oxide		+		+	+	–		+	+
5-methylfurfural		+		+		–		+	+
cis-Oak lactone		+		+	+			+	+
trans-Oak lactone†				+	+	–			+
Octanal		+		+	+	–		+	+
Phenyl acetaldehyde		+		+	+	–			+
2-Phenethyl alcohol		+	+	+		–		+	+
Residual sugar	+	–		–	–	+		–	–
SO_2 (free)		–		–	–	+		–	–
SO_2 (total)			–				–		
pH			+						
Syringol		+	+	+		–	+		+
α-Terpinene		+	+	+	+	–		+	+
Vanillin†									
Vitispirane I									
Vitispirane II									

+ Positive association
– Negative association
† Oak-derived compounds

Compounds associated with taste and mouthfeel

A large number of volatile compounds were associated with taste and mouthfeel attributes in the model (Figure 1, Table 3). However, this suggests a correlative and not causal relationship. The Cabernet Sauvignon varietal and blended wines shown in the scores plot (Figure 1B) are identified by a code numbered from lowest measured alcohol concentration (W1 = 12.4% v/v) to highest alcohol concentration (W24 = 15.9% v/v). The wines are primarily differentiated in the model by alcohol concentration (Figure 1B), indicating that alcohol concentration may also be indirectly separating the compounds and sensory attributes in the loadings plot (Figure 1A). Thus, a number of the significant compounds driving the taste and mouthfeel attributes in the model, such as the esters, higher alcohols, monoterpenes, norisoprenoids, lactones, and phenols occur in higher levels in high alcohol wines, and are correlated to the sensory attributes that are also higher in high alcohol wines, such as bitter taste, astringency, viscosity, sharp and gritty mouthfeel, and burning sensation.

The importance of alcohol level

Wines with lower alcohol concentrations (denoted by lower code numbers) are positioned on the left side of the plot in Figure 1B, while wines with higher alcohol concentrations are positioned on the right side of the plot. PC1 displays the separation of samples primarily on the basis of fruity attributes and sweet taste on the left hand side of the plot, compared with alcohol flavor, bitter taste and astringency on the right (Figure 1A). Wines with lower alcohol levels, W1-7 (12.4–13.8% v/v) were generally sweeter, and higher in fruity and butterscotch aromas, and smooth and silky mouthfeel. On the contrary, the wines with higher alcohol levels, W8-24 (13.9–15.9% v/v) on the right side of the plot (Figure 2) were generally more bitter, astringent, and higher in wood aromas, overall flavor intensity, alcohol flavor, and complexity, with sharp and gritty mouthfeel, and burning sensation. One group of wines located in the bottom right quadrant, containing wines W9, W12-13, W16-22, and W24 were tightly clustered together, indicating that these wines had similar chemical and sensory profiles, despite alcohol concentrations ranging from 14.3–15.9% v/v.

Other sensory attributes

A number of sensory attributes were not well modeled by the PLSR, possibly because the compounds responsible were not measured using the GC methods developed. One such class of compounds, the sulfur-containing volatiles, was not measured. Due to high volatility, analysis of these compounds often requires special GC columns and/or selective detectors [1]. These compounds have been reported to contribute varietal character in Cabernet Sauvignon wines. Future work will involve expanding and adapting the HS-SPME/GC/MS method to include other important volatile compounds known to impact wine sensory profiles.

Conversely, a number of the compounds measured were not found to be important to any of the sensory attributes. This may be because they were below their sensory limit of detection, or they may be contributing to the sensory profiles of the wines without clear individual descriptors. This may also be due to mixture effects, where synergistic or masking interactions of volatile compounds make it difficult to determine individual sensory contributions.

Conclusions

A rapid, targeted profiling method has been developed for measuring a broad range of volatile compounds in red wine. It has the ability to predict a number of important sensory descriptors, without extensive sample preparation or the use of more expensive instrumentation (that is, multidimensional GC or TOF MS). This method used HS-SPME combined with GC/MS using synchronous SIM/scan detection to optimize sensitivity and selectivity for the targeted analytes. Using this method, commercial U. S. Cabernet Sauvignon varietal and blended wines were found to differ in their chemical and sensory profiles, in part, as a result of the direct and indirect influences of varying alcohol levels.

References

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