Stir Bar Sorptive Extraction Applied to Volatile Constituents Evolution during *Vitis vinifera* Ripening

MªRosario Salinas,*,[†] Amaya Zalacain,[†] Francisco Pardo,[§] and
Gonzalo Luis Alonso[†]

Cátedra de Química Agrícola, ETSI Agrónomos, Universidad Castilla-La Mancha, Campus
Universitario, 02071 Albacete, Spain, and Bodega San Isidro (BSI), Carretera de Murcia s/n,
30520 Jumilla (Murcia), Spain

The stir bar sorptive extraction (SBSE) technique is used to determine volatile constituents in Monastrell grapes. The method proposed involves a maceration step lasting 2 h and a sorption time of 6 h. The coefficients of variation obtained were <4% in the case of the volatile compounds and <2% for the internal standard. The behavior of the volatiles during grape ripening was studied, and 34 compounds were determined, among which geranyl acetone, geranyl butyrate, farnesol, and a bicycloterpene, tentatively identified as 6-methylene[3.1.0]byciclohexane, were recorded for the first time in this variety. The highest terpene and norisoprenoid contents together with the lowest values of C6 compounds were observed during the fifth week of ripening, meaning that this would be the optimal time for harvesting in terms of volatile compounds.

INTRODUCTION

The organic compounds of complex matrices, such as fruit juices, drinks, and food, are usually analyzed by gas chromatography coupled to mass spectrometry (GC-MS). This, however, requires a prior preparation step, during which these analytes must be separated from the matrix before they can be introduced into the chromatograph column. This fractionation step can be carried out by several means, and different methods may give varying results, depending on the volatile and the polarity of the compounds.

The most frequently used methods for the isolation and concentration of organic compounds involve extraction with organic solvents, which is time-consuming, and the excessive manipulation of the samples may lead to serious errors (*1−4*). For this reason, alternative analytical methods, which use an extractant as fractionating and preconcentrating agent, are increasingly used. Many extractants have been assayed, for example, Tenax (*5, 6*), but poly(dimethylsiloxane) (PDMS) is the most widely used extractant as part of the solid-phase microextraction (SPME), the most popular among such methods. In SPME, the extractant covers a capillary fiber while the analytes are partitioned between the matrix and the PDMS phase according to their partitioning coefficients. Next, the analytes are introduced into the gas chromatograph by a rapid thermal desorption step at a high temperature in a current of inert gas. SPME has been used to analyze food, grapes, and wine (*7−*

11). In 1999, Baltussen et al. (*12*) pointed out the advantages of using a magnetic stir bar covered by PDMS instead of the above-mentioned capillary fiber. This stirrer is sold under the name "Twister", whereas the technique became known as stir bar sorptive extraction (SBSE). The extraction theory of SBSE is the same as for SPME but with a higher phase ratio coating, therefore leading to an increase in sensitivity by a factor of >100 compared to SPME (*12, 13*). This method has been used with success in numerous situations including foodstuffs, for example, in the analysis of the aroma composition of tea extracts, orange juice, and apple juice (*14*), cork taint in wines (*13, 15*), and pesticides in wine and fruit (*16−18*). The results show that SBSE is a powerful technique for flavor profiling of different types of matrices because it combines ease of use, ruggedness, precision, and sensitivity. In addition, the absence of organic solvents in the sample preparation step makes this process environmentally friendly. However, we have found no reference of its application to grapes or of the possibility of using it to monitor fruit ripening, probably because it is still a relatively new technique.

Knowledge of the aroma composition of grapes destined for winemaking may provide useful information for predicting wine quality, although most studies have concentrated on aromatic or floral varieties, neutral or nonfloral grapes having received less attention (*19−22*). The reason for this is that the compounds responsible for aroma are found in very low concentrations, and samples have to be painstakingly prepared if the aromas are to be isolated and concentrated sufficiently for subsequent chromatographic analysis. The application of SBSE to the analysis of grape volatile constituents would be interesting in order to ascertain the extent to which environmental conditions and

* Author to whom correspondence should be addressed (telephone +34 967 599310; fax +34 967 599238; e-mail Rosario.Salinas@uclm.es).
† Universidad Castilla-La Manch.
§ Bodega San Isidro.

agricultural practices affect the aroma composition of grapes and, therefore, wine quality.

Among the nonfloral grapes, the red Monastrell variety (known in other countries as Mourvedre) is ranked third in Spanish cultivars and is increasingly popular worldwide. In southeastern Spain, several origin appellations elaborate their wines with this cultivar. In the Jumilla Origin Apellation area, for example, 88% of the total area dedicated to vines is given over to this variety.

The ripening period of grapes is a physiological event that begins at veraison and finishes ~40 days later, during which time the grapes develop the typical characteristics that will decide the wine quality. Substantial qualitative and quantitative differences in volatile compounds have been observed in grapes, according to their stage of ripening (*20*, *23*, *24*), and for this reason it is useful to monitor the evolution of these volatiles during the maturation process. This would permit growers to determine the best moment for harvesting, a time when a balanced sugar/acidity ratio should be accompanied by the maximum concentration of favorable volatiles.

The aims of this study are to apply SBSE to the determination of volatile constituents in Monastrell grapes and to determine the suitability of this method for deciding when grapes reach their optimal volatile composition and are ready for harvesting.

MATERIALS AND METHODS

Healthy Monastrell grapes were harvested weekly in the Jumilla area (Murcia, Spain) from the beginning of maturation (August 21, 2003) to their final maturation stage (September 25, 2003).

Classic and Color Grape Analysis. One hundred berries were weighed and crushed without breaking the seeds for 1 min in a Robot Coupe GT 550 at 1400 rpm. Analysis of sugars and titrable acidity were measured in the fresh homogenized grape must following the official methods (*25*). Color intensity was measured in this extract following Glories's method (*26*), which is the sum of the absorbances at 420, 520, and 620 nm. For this purpose a Unicam Helios UV−vis spectrophotometer (Cambridge, U.K.) was used to scan the extract between 380 and 780 nm at 5 nm intervals with quartz cells 1 mm thick.

Volatile Analysis. *Sample Preparation.* Two hundred grams of berries was crushed for 1 min in a Robot Coupe GT 550 at 1400 rpm. Skins and must were kept together for 2 h at 5 °C in hermetically closed recipients.

Stir Bar Sorptive Extraction (SBSE). Water was added to the grape mixture to 1 L. One hundred milliliters of a homogeneous fraction of the early grape extract was used, to which 2 μL of internal standard (methyl octanoate) solution at 6 μL/L in absolute ethanol was added. The aqueous grape extract was stirred with a PDMS-coated stir bar (0.5 mm film thickness, 10 mm length, Twister, Gerstel GmbH, Mülheim and der Ruhr, Germany) during 2, 4, and 6 h at room temperature at 700 rpm in hermetically closed recipients. The stir bar was then removed from the sample, rinsed with distilled water, dried with a cellulose tissue, and later transferred into a thermal desorption tube for GC-MS analysis. All analyses have been carried out in triplicate.

Thermal Desorption−Gas Chromatography−Mass Spectrophotometry Analysis. In the thermal desorption tube, the volatile compounds were desorbed from the stir bar at the following conditions: desorption temperature, 290 °C; desorption time, 4 min; cold trap temperature, −30 °C; helium inlet flow, 45 mL/min. The compounds were transferred into the Hewlett-Packard 6890 gas chromatograph coupled to an LC 3D mass detector HP (Palo Alto, CA) with a fused silica capillary column (BP21 stationary phase, 50 m length, 0.22 mm i.d., and 0.25 μm film thickness) (SGE, Ringwood, Australia). The chromatographic program was set at 50 °C, raised to 180 °C at 2.5 °C/min (held for 2

min) and to 200 °C (1 °C/min), and held for 10 min. The mass range varied from 35 to 500 u, and the detector temperature was at 150 °C.

Compound identification was carried out using the NIST library and with data from authentic compounds. The chemical standards were supplied by Aldrich (Gillingham, U.K.), Sigma (St. Louis, MO), and Chemservice (West Chester, PA) except for 6-methylenebyciclo[3.1.0]-hexane, 3-oxo-α-ionol, vomifoliol, and 4-hexen-1-ol acetate, which have been identified by EI mass spectra. Kovats index (KI) was calculated in relation to a homologous series of *n*-alkenes, with those reported in the literature. Quantification was carried out in the scan mode and was determined if the peak height was 3 times the baseline. The results were expressed as microgram equivalents of internal standard per kilogram of grapes.

Significant differences among sampling times were assessed with a one-way analysis of variance (ANOVA) using SPSS version 10 statistical package for Windows (SPSS, Chicago, IL). Statistical differences among the means were evaluated using Duncan's test at the $p = 0.05$ level.

RESULTS AND DISCUSSION

Stir Bar Sorptive Extraction Method. This paper describes for the first time the application of SBSE to the analysis of volatile constituents in grapes, taking as reference the analytical parameters studied in other types of samples (*13*, *15−18*). Although these studies introduce the stir bar directly into the samples, we have considered it necessary to previously macerate the grapes because a substantial number of grape volatiles are located in the skin (*20*, *24*, *27*, *28*) and are transferred to the juice during this process. A maceration time of 2 h and a temperature of 5 °C were used to help prevent fermentation from occurring.

Sorption times of 2, 4, and 6 h were assayed, although other authors have used 90 min for wines (*13*), 2 h for orange juice (*17*), and between 1.5 and 16 h for brewed herbal tea (*14*). No significant differences were found between the chromatographic peak areas obtained at 2 and 4 h, whereas those obtained after 6 h increased substantially, which is why this last time was used for subsequent analyses. The method provided coefficients of variation of <4% for the compounds studied [for example, 1.74% geranyl acetone; 3.76% geraniol; 4.1% 1-hexanol; 2.62% (*Z*)-3-hexenol; 2.6% ethyl acetate; 2.8% hexyl acetate; 3% hexanoic acid; 3.9% nonanoic acid; 4% (*E*)-2-hexenal; 2.2% decanal; 1.1% β-ionone] and <2% for the internal standard used (methyl octanoate). Similar coefficients were obtained during the analysis of chlorophenols and chloroanisoles in previous studies carried out in wines using SBSE (*15*). Similarly good values having been obtained when the method was used to analyze organic compounds in other matrices such as tea, wine, and apple, orange, or pear juice (*14*, *16−18*).

Volatile Constituents during Ripening. Because most volatile flavor compounds are produced after veraison (*24*, *29*), we began our study 1 week after the berries began to change from green to red and continued for 6 weeks. **Tables 1** and **2** show the volatile composition of the grapes during this ripening stage together with the absolute retention times and mass fragment ratio (*m/z*) of each compound. The results are expressed in micrograms of methyl octanoate (IS) per kilogram of berries.

A total of 34 compounds were identified and quantified, most of them well-known in grape musts and wines. Some of these (such as terpenes and norisoprenoids) have their origin in the grapes themselves; some, such as the aldehydes and alcohol with six carbons (C6), arise from endogenous enzymatic action, activated by oxygen on certain fatty acids. Some, such as ethyl

Grape Volatile Analysis by SBSE

J. Agric. Food Chem., Vol. 52, No. 15, 2004 **4823**

Table 1. Volatile Compounds Content (Microgram Equivalents of Internal Standard per Kilogram of Grapes) in Monastrell Grapes (*n* = 3)[a]

| | | weeks after veraison | | | | | | |
compound	KI	1	2	3	4	5	6	OT
terpenes								
terpene 1*	1196	0.60f	0.32c	0.25a	0.28b	0.54e	0.42d	unknown
geranyl butyrate	1798	nd	nd	nd	nd	0.25a	0.33b	13 (*57*)
nerol	1810	nd	nd	nd	nd	1.08a	1.03b	0.3 (*55*)
geraniol	1860	0.21a	0.40c	0.26b	0.59e	0.46d	0.25b	4–75 (*58*)
geranyl acetone	1867	0.53b	0.94d	0.29a	0.82c	0.86c	0.83c	9 (*57*)
farnesol	2467	nd	nd	nd	nd	nd	0.94	20 (*58*)
total		1.34b	1.65c	0.80a	1.69c	3.19d	3.81e	
alcohols								
2-phenylethanol	1905	nd	nd	nd	nd	nd	0.31	1.80 (*55*)
1-hexanol	1368	6.56e	5.40d	4.83c	5.57d	2.73a	3.62b	1.1 (*55*)
(*Z*)-3-hexenol	1382	0.12d	0.13e	0.10bc	0.10c	0.09ab	0.09a	13 (*55*)
2-hexanol	1410	1.23d	1.23d	1.04c	1.45e	0.69b	0.45a	15 (*55*)
total		7.91e	6.76d	5.97c	7.11d	3.51a	4.46b	
esters								
ethyl acetate	885	2.70b	1.97ab	1.30a	1.81a	14.85c	29.42d	12.3 (*55*)
isopentyl acetate	1125	0.48b	0.10a	0.09a	0.16a	5.87d	5.07c	0.16 (*55*)
pentyl acetate	1180	nd	nd	nd	nd	0.32b	0.22a	0.18 (*55*)
hexyl acetate	1275	16.22ab	15.65ab	13.03a	17.22b	83.93c	83.43c	2 (*57*)
4-hexen-1-ol acetate*	1326	1.06c	0.24b	0.08a	0.16ab	3.13e	1.48d	unknown
3-hexen-1-ol acetate	1337	1.17d	0.45b	0.17a	0.22a	1.17d	0.87c	0.21 (*56*)
2-hexen-1-ol acetate	1352	4.70c	3.11b	0.94a	3.84bc	32.16e	17.64d	0.32 (*56*)
octyl acetate	1439	nd	nd	nd	nd	0.68b	0.84b	0.80 (*55*)
nonyl acetate	1610	nd	nd	nd	nd	2.16a	3.74b	0.80 (*55*)
2-phenylethyl acetate	1814	nd	nd	nd	nd	1.67b	1.03a	1.80 (*55*)
total		26.32b	21.53b	15.62a	23.41b	145.94c	143.73c	

[a] KI, Kovats index; OT, olfactory threshold in mg/L except for geraniol and geranyl acetone, which are in μg/L; nd, not detected; *, compounds tentatively identified on the basis of their EI mass spectra (terpene 1 as 6-methylene[3.1.0]bicyclohexane, *m/z* 79/94; 4-hexen-1-ol acetate, *m/z* 43/67/82); different letters between columns indicate significant differences at the 0.05% level.

Table 2. Volatile Compounds Content (Microgram Equivalents of Internal Standard per Kilogram of Grapes) in Monastrell Grapes (*n* = 3)[a]

| | | weeks after veraison | | | | | | |
compound	KI	1	2	3	4	5	6	OT
acids								
acetic	1435	4.86c	4.49b	4.22ab	5.49d	4.02a	5.23d	<100 (*55*)
hexanoic	1845	0.58b	0.72d	0.65c	0.87e	1.02f	0.48a	8.2 (*55*)
heptanoic	2004	0.94c	1.00d	0.74a	0.90c	0.74a	0.83b	3 (*57*)
octanoic	2072	2.21b	1.82a	1.82a	1.96a	1.95a	2.85c	8.8 (*55*)
nonanoic	2134	2.57c	3.56f	2.04b	2.91d	3.12e	1.87a	30 (*57*)
decanoic	2261	3.60e	2.67cd	2.25b	2.57c	1.67a	2.82d	15 (*55*)
dodecanoic	2499	5.42d	4.25c	3.03a	4.02bc	3.89b	5.82e	10 (*57*)
total		20.18d	18.50c	14.74a	18.73c	16.41b	19.90d	
aldehydes								
hexanal	1084	0.68b	3.47f	2.85e	1.5dc	1.22c	0.12a	0.35 (*55*)
(*E*)-2-hexenal	1207	8.48b	16.27e	15.34d	12.92c	2.11a	1.55a	0.6 (*55*)
nonanal	1401	1.49b	2.25c	2.94c	0.80a	6.89c	0.70a	1.0 (*57*)
decanal	1457	2.04a	2.36a	3.97c	2.10a	8.81d	2.17a	0.1–2 (*57*)
total		12.69b	24.35e	25.10e	17.40c	19.02d	4.53a	
norisoprenoids								
vomifoliol*	1680	nd	nd	0.20a	0.81b	0.87c	0.96d	unknown
3-oxo-α-ionol*	1817	nd	nd	nd	nd	0.91b	0.28a	unknown
h$\iota\nu\delta$;1gβ-ionone	1952	0.93d	0.97d	0.61c	0.63c	0.52b	0.33a	0.09 (*55*)
total		0.93b	0.97b	0.81a	1.44c	2.31e	1.57d	

[a] KI, Kovats index; OT, olfactory threshold in mg/L except for β-ionone, which is in μg/L; nd, not detected; *, compounds tentatively identified on the basis of their EI mass spectra (vomifoliol, *m/z* 67/82/95/124; 3-oxo-α-ionol, *m/z* 91/108/150); different letters between columns indicate significant differences at 0.05% level.

acetate and the acids, stem from fermentation processes, whereas others, such as 2-phenylethanol and the other esters, have both a varietal and fermentative origin.

Although esters were the most abundant compounds in most samplings, aldehydes were the most abundant during the second and third weeks after veraison. The total ester content increased significantly during the fifth week and remained constant until the end of the maturation period. All of the esters found were acetates, the most abundant being derivates from C6 alcohols, especially hexyl acetate, a compound that alone accounted for

>50% of the total esters. Of note is the fact that pentyl, octyl, nonyl, and 2-phenylethyl acetates appeared only in the fifth and sixth weeks following veraison. Acetates have been widely studied in wines, where they are responsible for imparting the pleasant fruity flavor because they are present in quantities above the olfactory threshold. These compounds are synthesized in substantial quantities by yeasts during alcoholic fermentation, so the contribution of such acetates of varietal origin to the wine aroma is relatively insignificant. However, Bravdo (*7*), using SPME, pointed to the importance of hexyl acetate and five other

compounds for differentiating fertigation treatments provided to Cabernet Sauvignon grapes.

(*E*)-2-Hexenal was the most abundant aldehyde. It has a "green" or "grassy" aroma and, if present in wine, can be perceived easily because its olfactory threshold is very low (*30*). Its levels and those of hexanal, which has a similar aroma, fell significantly toward the end of ripening. Nonanal and decanal have a pleasant aroma, but both behaved erratically during the ripening period.

Acetic acid was the most abundant acid, with its concentration showing no significant variation during ripening. At the end of this period, there was a significant fall in the levels of hexanoic and nonanoic acids and a significant increase in those of octanoic, decanoic, and dodecanoic acids. The total level of acids remained constant during the three last samplings. Hexanoic acid was another of the compounds able to differentiate among fertigation treatments in Cabernet sauvignon grapes (*7*).

Among the alcohols, those with six carbon atoms predominated, the most abundant being 1-hexanol, although its levels fell significantly as ripening progressed. At the last sampling time, it was possible to detect very low concentrations of 2-phenylethanol, which has a pleasant "rose" aroma, whereas the other alcohols are more herbaceous. The bibliography indicates that 2-phenylethanol is very abundant in wines; however, it arises mainly from fermentation, with 2-phenylalanine being its precursor (*31*). Very little of this alcohol in wine comes from the grape itself, in which it is found mainly as a nonodor precursor (*32*).

Terpenes and norisoprenoids are volatile compounds that have been widely studied because they are involved in the sensorial differentiation of wines, depending on the grape variety used in winemaking (*19*). These compounds have a very pleasant aroma and very low olfactory thresholds, so that they can be perceived during wine tasting even in low concentrations (*33*). In general, winemaking grapes are not very aromatic fruits, and only some, denominated floral (e.g., Muscats), have a particular typicity that is derived from their high terpene content (*19*). Other grape varieties, those known as nonfloral, despite having neutral berries, produce wines with a characteristic varietal aroma, which can be attributed to the terpenes and norisoprenoids that are manifested during the winemaking process and aging (*21, 34, 35*). It is known that grapes contain nonodorous precursors of these compounds, most of them in the form of glycosides, which are transformed into odorous compounds during the different winemaking stages. The study we describe here shows that Monastrell has a low terpene and norisprenoid content because both groups together constitute the least abundant of all the compounds determined. It must be remembered that SBSE is capable of determining compounds in lower quantities than is possible with other methods of analysis (*37*), although even SBSE can determine only those compounds capable of being absorbed by the PDMS directly from the matrix. Future studies must look into the nonodorous aromatic precursors of this grape variety.

Six terpenes were determined in Monastrell grapes, among which terpene 1 (tentatively identified as 6-methylene[3.1.0]-byciclohexane), geraniol, and geranyl acetate were found throughout the sampling period. The others gradually appeared as ripening progressed, so a significant increase of total terpenes was observed in the last stage. Other studies of Monastrell grapes, using analytical techniques other than SBSE, have also demonstrated the existence of geraniol (*23, 37*) but none of the other terpenes depicted in **Table 1**. Furthermore, for the first

time in grapes, we have identified a bicycloterpene (terpene 1), the nature of which must be definitively confirmed in future studies, because it may be suitable for characterizing the Monastrell variety. It is known that Monastrell wines possess an organoleptic descriptor defined as "sweet", which is perceived only retronasally (*38*). As geraniol and geranyl acetone, both present throughout ripening, and geranyl butyrate, which was found in only the last two samplings, possess an organoleptic descriptor defined as "sweet" (*36*), so these compounds may have some effect on this characteristic note. No bibliographic references have been found concerning the direct application of SBSE to grapes, so the results presented here should be treated with caution. However, although it is clear that all natural terpenes have gerenylpyrophosphate as precursor, it has also been demonstrated that the thermal degradation of carotenoids may give rise to terpenic derivates (*39*), and this technique implies the use of thermal desorption.

Norisoprenoids in grapes arise from the transformation of carotenoids (*22, 40, 41*). We found three norisoprenoids, vomifoliol, oxo-α-ionol, and β-ionone, only the last of which was present throughout ripening. The compound tentatively identified as vomifoliol was observed from the third week onward, whereas oxo-α-ionol was seen only after week five. β-Ionone and oxo-α-ionol fell as ripening progressed, whereas vomifoliol increased. These norisoprenoids have been amply documented in the bibliography as components of the aroma fraction of grapes and wines, although they are more common in the form of glycosides (*42, 43*). These compounds were also quantified in Monastrell grapes (*42*), whereas vomifoliol was tentatively identified by searching for specific ions, 95 and 124, in the full-scan mass spectra (*34*).

Volatile Composition and Harvest Relation. During ripening, substantial changes take place in the composition of grapes. Therefore, it is important to choose the correct moment for their harvest because grape quality will determine the quality of the resulting wine. Traditional parameters for evaluating grape quality, such as berry weight, sugar content, and acidity, have been seen to be insufficient because they do not provide information on the phenolic and aromatic contents, both of which greatly influence wine quality. For this reason, the optimal harvesting time must take into consideration both the evolution during ripening of the traditional parameters and the polyphenols and volatile compounds present at various times. Two of the most widely used ways of evaluating grape quality involve measuring the polyphenol content (*45−47*) or color (*48*). With regard to the evaluation of grape aromatic quality, analytical methods by gas chromatography have been proposed that involve the prior isolation and concentration of volatiles using organic solvents, which are time-consuming steps (*49−51*). More rapid methods for measuring the aromatic quality of grapes are based on spectrophotometric measurements of the glycoside content (*52*), which provide an index of the aromatic potential of the grapes, because a large number of the aromatic compounds are in the form of glycosidic precursors (*21, 43*). However, SBSE is not as labor-intensive as the above methods and provides better reproducibility. Below, we shall apply the results of this technique to determine the aromatic quality of grapes and the optimal harvesting time.

Table 3 shows some grape characteristics during ripening. Maximum berry weight is reached 2 weeks after veraison, whereas the maximum sugar content and lowest acidity level are reached after 5 weeks. Color intensity was also used to ascertain grape quality because this parameter is closely related to phenol maturity and provides additional information in regard

Grape Volatile Analysis by SBSE

J. Agric. Food Chem., Vol. 52, No. 15, 2004 **4825**

Table 3. Some Characteristics of the Monastrell Grapes during Ripening

weeks after veraison	wt of 100 berries (g)	sugar content (g/L)	total acidity (g/L of tartaric acid)	color density	ratio of sugar to wt
1 (Aug 21, 2003)	122.6	197.6	6.32	1.32	1.61
2 (Aug 28, 2003)	140.5	207.9	6.22	2.02	1.48
3 (Aug 04, 2003)	126.2	239.3	6.0	5.39	1.89
4 (Sept 11, 2003)	124.2	240.1	5.25	3.93	1.93
5 (Sept 18, 2003)	128.7	245.2	4.35	2.86	1.90
6 (Sept 25, 2003)	127.2	232.3	4.55	2.71	1.83
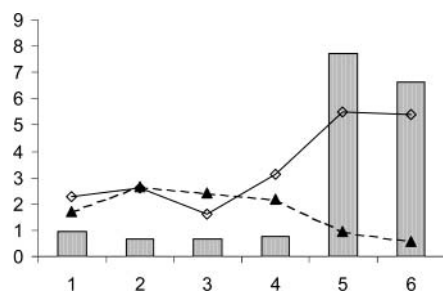
Figure 1. Evolution during Monastrell ripening of favorable/unfavorable volatile compounds ratio (bars), terpenes + norisoprenoids (◇), and C6 compounds (▲).

to wine color (*53*). From these results, it may be concluded that maximum grape quality is reached 4 weeks after veraison; this, therefore, would be the optimal harvesting time. The greatest sugar/weight ratio is reached at this time, acidity is not too low, and color density is high.

Among the grape volatile constituents, there is an interesting group denominated C6 compounds because they influence wine quality by implementing a characteristic green odor, which is considered to be deleterious to wine quality. These C6 alcohols and aldehydes arise from the consecutive action of the lipoxygenase and alcohol deshydrogenase enzymes on polyunsaturated fatty acids (*30*). The total C_6 compounds (1-hexanol, (*Z*)-3-hexenol, 2-hexanol, hexanal, and (*E*)-2-hexenal) content during the six weeks of ripening were 17.1, 26.5, 24.2, 21.6, 9.6, and 5.2 μg/kg, respectively (**Figure 1**). They reached their maximum levels during the second week after veraison, probably because lipoxygenase enzyme activity was greatest at this time (*54*). Their total content decreased considerably at the end of the ripening period. The most abundant was (*E*)-2 hexenal, although the concentration of 1-hexanol was higher at the end of ripening. From a sensorial point of view, this is beneficial for grape quality because the olfactory threshold of C6 saturates, such as 1-hexanol, is higher than that of the unsaturates, such as 2-hexenal, so that the "vegetal" aroma diminishes as ripening progresses. The C6 alcohol/C6 aldehyde ratio increased from 0.33 during the third week to 2.5 in the last week of ripening.

To obtain information concerning the optimum harvest date, in terms of volatile compounds, they have been grouped according to their organoleptic descriptor in wine. On the one hand, we have added those compounds with pleasant aroma (denominated "favorable" compounds) and, on the other, those with unpleasant aroma (denominated "unfavorable" compounds). The first group comprises acetates, terpenes, norisoprenoids, 2-phenylethanol, nonanal, and decanal, whereas the second group contains the C6 compounds and the acids. The favorable/unfavorable ratio can be seen in **Figure 1**, the maximum value occurring at week five of ripening. The total terpene and norisoprenoid contents (the only exclusively varietal compounds) can also be appreciated in this figure. These reach their

maximum at the same time, although, in this case, there is no significant difference from the results obtained for the following sampling time. These findings suggest that, even after the date considered to be optimal for harvesting according to the ratio of sugar to weight and color, grapes continue to synthesize compounds that have a positive influence on the varietal aroma of a wine.

Conclusion. The use of SBSE, which introduces a stir bar directly into an aqueous extract of crushed and macerated grapes, makes it possible to determine the volatile constituents of grapes with a high degree of reproducibility. The method identified the presence of certain compounds in Monastrell grapes for the first time and demonstrated its usefulness for determining, in terms of volatile compounds, the optimal harvesting time for winemaking.

LITERATURE CITED

(1) Blanch, G. P.; Tabera, J.; Herráiz, M.; Reglero, G. Preconcentration of volatile components of foods: optimization of the steam distillation−solvent extraction at normal pressure. *J. Chromatogr. A* **1993**, *628*, 261−268.

(2) Ferreira, V.; Rapp, A.; Cacho, J.; Hastrich, H.; Yavas, I. Fast and quantitative determination of wine flavor compounds using microextraction with Freon 113. *J. Agric. Food Chem.* **1993**, *41*, 1412−1420.

(3) Huerta, M. D.; Salinas, M. R.; Masoud, T. Revisión sobre los métodos de análisis de la fracción volátil de los alimentos. *Boll. Chim. Farm.* **1997**, *136*, 500−510.

(4) Leahy, M. M.; Reineccius, G. A. Comparison of methods for the isolation of volatile compounds from aqueous model system. In *Analysis of Volatiles. Methods and Applications*; Schreier, P., Ed.; de Gruyter: Berlin, Germany, 1984; pp 19−47.

(5) Salinas, M. R.; Alonso, G. L.; Esteban-Infantes, F. J. Adsorption−thermal desorption−gas chromatography applied to the determination of wine aromas. *J. Agric. Food Chem.* **1994**, *42*, 1328−1331.

(6) Salinas, M. R.; Alonso, G. L. Adsorption−thermal desorption−gas chromatography applied to the determination of wine aromas. In *Modern Methods of Analysis, Vol. 19, Plant Volatile Analysis*; Linskens, H. F., Jackson, J. F., Eds.; Springer-Verlag: Berlin, Germany, 1997.

(7) Bravdo, B. A. Effect of cultural practices and environmental factors on fruit and wine quality. *Agric. Conspec. Sci.* **2001**, *66*, 13−20.

(8) Sides, S.; Robards, K.; Helliwell, S. Developments in extraction techniques and their application to analysis of volatiles in foods. *Trends Anal. Chem.* **2000**, *19*, 322−329.

(9) Azodanlou, R.; Darbellay, C.; Luisier, J.; Villettaz, J.; Amode, R. A. new concept for the measurement of total volatile compounds of food. *Z. Lebensm. Unters. Forsch. A* **1998**, *2008*, 254−258.

(10) De la Calle, D.; Séller, K.; Reichbacher, M.; Danzer, K.; Hurlbeck, C.; Bartzech, C. Analysis of wine bouquet components using headspace SPME capillary gas chromatography. *J. High Resolut. Chromatogr.* **1998** *21*, 368−373.

(11) Ong, P.; Acrees, T. Similarities in the aroma chemistry of Gewurztraminer variety of wines and Lychee (*Litchi chinensis Sonn.*) fruit. *J. Agric. Food Chem.* **1999**, *47*, 665−670.

(12) Baltussen, E.; Sandra, P.; David, F.; Cramers, C. A. Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: theory and principles. *J. Microcolumn Sep.* **1999**, *11*, 737−747.

(13) Hayasaka, Y.; MacNamara, K.; Baldock, G. A.; Taylor, R. L.; Pollnitz, A. P. Application of stir bar sorptive extraction for wine analysis. *Anal. Bioanal. Chem.* **2003**, *375*, 948−955.

(14) Hoffmann, A.; Heiden, A.; Pfannkoch, E. Flavor profiling of beverage by stir bar sorptive extraction (SBSE) and thermal desorption GC/MS/PFPD. *Appl. Note Gerstel* **2000**, *4*, 1−16.

(15) Zalacain, M.; Alonso, G. L.; Lorenzo, C.; Iñiguez, M.; Salinas, M. R. Stir bar sorptive extraction (SBSE) for the analysis of wine cork taint. *J. Chromatogr. A* **2004**, *1033*, 173−178.

(16) Sandra, P.; Tienpont, B.; Vercammen, J.; Tredoux, T.; David, F. Stir bar sorptive extraction to the determination of dicarbox-imide fungicides in wine. *J. Chromatogr. A* **2001**, *928*, 117−126.

(17) Blasco, C.; Font, G.; Picó, Y. Comparison of microextraction procedures to determine pesticides in oranges by liquid chromatography−mass spectrometry. *J. Chromatogr. A* **2002**, *970*, 201−212.

(18) Bicchi, C.; Cordero, C.; Rubiolo, P.; Sandra, P. Stir bar sorptive extraction (SBSE) in sample preparation from heterogeneous matrices: determination of pesticide residues in pear pulp at ppb (ng/g) level. *Eur. Food Res. Technol.* **2003**, *216*, 449−456.

(19) Bayonove, C. El aroma Varietal. El potencial aromático de la uva. In *Enología: Fundamentos Científicos y Tecnológicos*; Flanzy, C., Ed.; AMV Ediciones, Mundi-Prensa: Madrid, Spain, 2003; pp 137−146.

(20) Günata, Y.; Bayonove, C.; Baumes, R.; Cordonnier, R. The aroma of grapes. Localization and evolution of free and bound fractions of some grape aroma components cv. Muscat during first development and maturation. *J. Sci. Food Agric.* **1985**, *36*, 857−862.

(21) Razungles, A.; Günata, A.; Pinatel, S.; Baumes, R.; Bayonove, C. Étude quantitative de composés terpéniques, norisoprénoides et leurs précurseurs dans diverses variétés de raisins. *Sci. Aliment.* **1993**, *13*, 59−72.

(22) Winterhalter, P. The generation of C13-norisoprenoids volatiles in Riesling wine. In *Connaissance Aromatique des Cépages et Qualité des Vins*; Bayonove, C., Crouzet, J., Flanzy, J. C., Martin, J. C., Sapis, J. C., Eds.; Montpellier, France, 1993; pp 65−73.

(23) Gómez, E.; Martínez, A.; Laencina, J. Changes in volatile compounds during maturation of some grape varieties. *J. Sci. Food Agric.* **1995**, *67*, 229−233.

(24) Park, S. K.; Morrison, J. C.; Adams, D. O.; Noble, A. C. Distribution of free and glycosidic bound monoterpenes in the skin and mesocarp of Muscat of Alexandria grapes during development. *J. Agric. Food Chem.* **1991**, *39*, 514−518.

(25) OIV. *Récueil des Méthodes Internationales d'Analyse des Vins et des Moûts*; Office International de la Vigne et du Vin: Paris, France, 1990.

(26) Glories, Y. La couleur des vins rouges. Les equilibres des anthocyanes et des tanins. *Connaiss. Vigne Vin* **1984**, *18* (3), 195−217.

(27) Wilson, B.; Strauss, C. R.; Williams, P. J. The distribution of free and glycosidically bound monoterpenes among skin, juice and pulp fractions of some white grape varieties. *Am. J. Enol. Vitic.* **1986**, *37*, 107−111.

(28) Gómez, E.; Martínez, A.; Laencina, J. Localization of free and bound aromatic compounds among skin, juice and pulp fractions of some grape varieties. *Vitis* **1994**, *33*, 1−4.

(29) Marais, J. Sauvignon blanc cultivar aroma. A review. *S. Afr. J. Enol. Vitic.* **1994**, *15*, 41−45.

(30) Crouzet, J. Los constituyentes volátiles de la etapa preferment-tativa. In *Enología: Fundamentos Científicos y Tecnológicos*; Flanzy, C., Ed.; AMV Ediciones, Mundi-Prensa: Madrid, Spain, 2003; pp 146−147.

(31) Laminkanra, O.; Grimm, C. C.; Inyang, I. D. Formation and occurrence of flavor components in Noble muscadine wine. *Food Chem.* **1996**, *56*, 373−376.

(32) Winterhalter, P.; Baderschneider, B.; Bonnländer, B. Analysis, structure and reactivity of labile terpenoid aroma precursors in Riesling wine. In *Chemistry of Wine Flavour*; Waterhouse, A. L., Ebeler, S. E., Eds.; ACS Symposium Series 719; American Chemical Society: Washington, DC, 1999; pp 1−12.

(33) Winterhalter, P.; Rouseff, R. Carotenoid-derived aroma compounds: An introduction. In *Carotenoid-Derived Aroma Compounds*; Winterhalter, P., Rouseff, R., Eds.; ACS Symposium Series 802; American Chemical Society: Washington, DC, 2002; pp 1−79.

(34) Boido, E.; Lloret, A.; Medina, K.; Fariña, L.; Carrau, F.; Versini, G.; Dellacassa, E. Aroma composition of *Vitis vinifera* Cv. Tanat: the typical red wines from Uruguay. *J. Agric. Food Chem.* **2003**, *51*, 5408−5413.

(35) Sefton, M. A.; Francis, L.; Williams, P. J. Free and volatile secondary metabolites of *Vitis vinifera* grape cv. Sauvignon Blanc. *J. Food Sci.* **1994**, *59*, 142−147.

(36) Sigma-Aldrich. *Flavors and Fragrances*, Int. Ed.; 2003−2004.

(37) Rosillo, L.; Salinas, M. R.; Garijo, J.; Alonso, G. L. Study of volatiles in grapes by dynamic headspace analysis. Application to the differentiation of some *Vitis vinifera* varieties. *J. Chromatogr A* **1999**, *847*, 155−159.

(38) Pardo, F. *Jumilla: Viñas, Bodegas y Vinos*; Pardo: Murcia, Spain, 1996.

(39) Crouzet, J.; Kanasawud, P.; Sakho, M. Thermal generation of carotenoid-derived compounds. In *Carotenoid-Derived Aroma Compounds*; Winterhalter, P., Rouseff, R., Eds.; ACS Symposium Series 802; American Chemical Society: Washington, DC, 2001; pp 115−129.

(40) Baumes, R.; Wirth, J.; Bureau, S.; Gunata, Z.; Razungles, A. Biogeneration of C13-norisoprenoid compounds: experiments supportive for an apo-carotenoid pathaway in grapevines. *Anal. Chim. Acta* **2002**, *458*, 3−14.

(41) Britton, G. Overview of carotenoids biosynthesis. In *Carotenoids. Biosynthesis and Metabolism*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser Verlag: Basel, Switzerland, 1998; Vol. 3, pp 13−147.

(42) Strauss, C. R.; Wilson, B.; Anderson, R.; Williams, P. J. Development of precursors of C13 norisoprenoid flavorants in Riesling grapes. *Am. J. Enol. Vitic.* **1987**, *38*, 23−27.

(43) Williams, P. J.; Sefton, M. A.; Marinos, V. A. Hydrolytic flavor release from non volatile precursors in fruits, wines and some other plant derived foods. In *Proceedings of the 3rd International Hearman and Reiner Symposium*; Hoppak, M. R., Ed.; VCH: Weinheim, Germany, 1993; pp 283−290.

(44) Salinas, M. R.; Alonso, G. L.; Pardo, F.; Bayonove, Cl. Free and bound volatiles of Monastrell wines. *Sci. Aliments.* **1998**, *18*, 223−231.

(45) Lamadon, F. Protocole pour l'evaluation de la richesse polyphé-nolique des raisins. *Rev. Fr. Oenol.* **1995**, *76*, 37−38.

(46) Pardo, F.; Garijo, J.; Alonso, G. L.; Salinas, M. R. Valoración del potencial fenólico en uvas Monastrell. Predicción del contenido polifenólico de los vinos obtenidos. *Vitic. Enol. Prof.* **1999**, *61*, 57−66.

(47) Saint-Cricq de Gaulejac, N.; Vivas, N.; Glories, Y. Maturité phénolique: définition et contrôle. *Rev. Fr. Oenol.* **1998**, *173*, 22−25.

(48) Franco, E.; Iñiguez, M. Estudio de la relación entre el color de la uva tinta y el color del vino. *Vitic. Enol. Prof.* **1999**, *63*, 23−34.

(49) 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.

(50) Rapp, A.; Hastrich, H.; Engel, L.; Knipser, W. Caractérisation des cépages par les substances aromatiques des raisins. *Bull. O.I.V.* **1980**, *588*, 92−111.

Grape Volatile Analysis by SBSE

J. Agric. Food Chem., Vol. 52, No. 15, 2004 **4827**

(51) Ferrari, G.; Broussous, P. Evaluation rapide du potential aromatique des cepages blancs meridionaux. In *Connaissance Aromatique des Cépages et Qualité des Vins*; Bayonove, C., Crouzet, J., Flanzy, J. C., Martin, J. C., Sapis, J. C., Eds.; Montpellier, France, 1993; pp 395−398.

(52) Williams, P. J.; Cynkar, W.; Francis, I. L.; Gray, J. D.; Iland, P. G.; Cooambe, B. G. Quantification of glycosides in grapes, juices and wines through a determination of glycosyl glucose. *J. Agric. Food Chem.* **1995**, *43*, 121−128.

(53) Glories, Y. La maturitá fenólica delle uve: primo parámetro da controllare per una correta vinificazione in rosso. *Vigne Vin* **1999**, *3*, 46−50.

(54) Crouzet, J. Les enzymes et l'arôme des vins. *Rev. Fr. Oenol.* **1986**, *102*, 42−49.

(55) Etiévant, P. X. Wine. In *Volatile Compounds in Foods and Beverages*; Marse, H., Ed.; Dekker: New York, 1991; pp 483−587.

(56) King, B. M. Sensory evaluation of some matrices made from *E* and *Z* isomers of 3-hexenyl of *E* and *Z* isomers of 3-hexyl-1-acetate. *Profile Flavour Res.* **1985**, 67−80.

(57) Fazzalari, F. A. *Compilation of Odor and Taste Threshold Data*; ASTM Data Series DS; ASTM: Philadelphia, PA, 1978; 48A.

(58) Ohloff, G. Importance of minor components in flavors and fragrances. *Perfum. Flavor.* **1978**, *1* (3), 11−22.