Research Article

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Characterization of volatile compounds and olfactory profile of red minority varietal wines from La Rioja

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Abstract

BACKGROUND: The aim of this work was to study for the first time the volatile compounds and olfactory profile of La Rioja red wines made with the local varieties *Vitis vinifera* cv. Monastel and Maturana Tinta de Navarrete, using Tempranillo as a reference variety. The impact of vintage on these compounds was also evaluated, and chemometric techniques were applied to achieve a possible differentiation of the wines.

RESULTS: A clear classification of wines according to grape variety and vintage was obtained. Volatile compounds that differentiated wines by grape variety were varietal aromas whereas vintage was mainly differentiated by compounds formed during the alcoholic fermentation and extracted from wood during the elaboration process in wooden barrels. Sensory analysis also allowed differentiation of wines by grape variety. Tempranillo wines were characterized by liquorice notes, whereas Maturana Tinta de Navarrete wines were the least fruity and showed herbaceous and pepper notes. The sensory profile of Monastel varied between vintages.

CONCLUSION: These minor grape varieties could provide a good alternative to the most widespread variety in La Rioja: Tempranillo. The use of these varieties produced wines with their own personality and different aromatic profile from other wines on the market.

Keywords: volatile compounds; grape variety; red minority varietal wines; vintage; sensory profile

INTRODUCTION

Wine aroma is one of the most important properties when it comes to consumer preference, and it is mainly determined by the volatile compounds. These volatile compounds are produced through metabolic pathways during ripening and harvesting of grapes, during their fermentation and/or during the storage of wines.[1]

Although the overall composition of most grape varieties is very similar, there are clear and distinct aroma and flavour differences among most cultivars.[2] Varietal aromas are some of these compounds which allow differentiating wines by their variety. However, several studies support the contribution of other volatile compounds such as fusel alcohols, esters and fatty acids to varietal differentiation.[3–5]

The phenomenon of replacement of local grape varieties with widely spread international cultivars is coming to a standstill. In addition, wine consumers' taste and preferences have changed during the last few years, since there are other values and motivations apart from aroma and taste for drinking wines such as marketing attributes and new wine styles. Having in mind these new tendencies, several Denominations of Origin are starting to promote varieties linked to specific locations which produce original and high-quality wines. Minor varieties, perfectly adapted to the local environmental conditions, may represent a good option. In this sense, in La Rioja (Spain) – an autonomous community with a large vitiviniculture tradition – has increased the need to preserve and characterize its minority grape varieties in order to maintain the authenticity and differentiation of its wines. Previous studies of local red varieties from this region[6,7] highlighted the vine-growing interest of Monastel and Maturana Tinta de Navarrete grape varieties, which could be a good complement to the most widespread variety of the area – Tempranillo – which implies 85% of the surface of red grapes cultivated in La Rioja. Therefore, studies on the sensory properties and phenolic composition of varietal wines made with these varieties have recently been carried out.[8] However there is no published information about the aromatic profile of these wines. Moreover, it is important to highlight that the mere knowledge of the volatile composition of a wine, without sensory evaluation, is

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inadequate to predict the flavour of the whole system as perceived by a trained sensory judge. In fact, interactions among odorants and interactions between the odorant and different elements of the wine's non-volatile matrix can affect the odorant volatility, flavour release and overall perceived flavour, intensity and quality.[9]

Considering all the previous comments and studies, the aim of this work was to identify the aroma characteristics of red varietal wines made from the minor varieties Monastel and Maturana Tinta de Navarrete, using Tempranillo as a reference variety. Wines were elaborated in a real winery during three consecutive vintages, and both the sensory olfactory profile and the volatile composition were studied. Multivariate techniques of data analysis were employed in order to establish differentiation criteria among the wines as a function of the grape variety or the vintage.

MATERIALS AND METHODS

Chemicals

Volatile standards were of analytical quality. Ethyl butyrate, ethyl isovalerate, ethyl hexanoate, ethyl octanoate, β-phenylethyl acetate, isobutanol, benzyl alcohol, 2-methyl-1-butanol, 3-methyl-1-butanol, β-phenylethanol, 1-hexanol, *cis*-3-hexenol, hexanoic acid, octanoic acid, decanoic acid, guaiacol, γ-butyrolactone and citronellol were purchased from Fluka (Buchs, Switzerland); ethyl 2-methylbutyrate, ethyl decanoate, isoamyl acetate, *trans*-3-hexenol, 2,6-dimethoxyphenol, γ-nonalactone, acetovanillone, linalool, β-ionone, ethyl cinnamate and methyl octanoate were obtained from Sigma-Aldrich (Steiheim, Germany); and finally methyl vanillate, ethyl vanillate, 4-ethylphenol, 4-vinylphenol, 4-vinylguaiacol and 3,4-dimethylphenol were purchased from Lancaster (Strasbourg, France). Dichloromethane (HPLC grade) was supplied by Merck (Darmstadt, Germany).

Vinifications and samples

Vinifications were carried out in the wine cellar *Juan Carlos Sancha SL* (Baños de Río Tobia, La Rioja, Spain) using the grape varieties *Vitis vinífera* L. cv. Tempranillo (T), Monastel (O) and Maturana Tinta de Navarrete (V) harvested in 2009, 2010 and 2011. All grape varieties were harvested in good health conditions at their optimal stage of ripeness, with sugar concentrations ranging between 22.2 and 24.9 °Brix, and total acidity of 4.80–7.15 g L^{-1} tartaric acid. All grapes were vinified under the same controlled winemaking techniques.

Grapes were destemmed and distributed into 500 L French oak barrels/containers, sulfited with 3 g hL^{-1} SO_2 and inoculated with 25 g hL^{-1} *Saccharomyces cerevisiae* yeast strain (Uvaferm VRB, Lallemand Inc., Spain). The fermentation–maceration process was carried out at a maximum temperature of $28 \pm 2\,^{\circ}C$ and lasted around 10 days. Wines were then run off and introduced again into the same 500 L French oak containers, where they were maintained at controlled wine cellar temperature. After spontaneous malolactic fermentation, which lasted from 1 to 2 months, wines were racked, clarified and bottled. In 2009, two batches for each grape variety were studied. In 2010 and 2011 vintages, four batches for each variety were collected. Samples after bottling were analysed for aromatic compounds and tasted. A total amount of 30 samples were taken for this study from the three vintages; 10 corresponded to Tempranillo, 10 to Monastel and 10 to Maturana Tinta de Navarrete wines. The same barrels (*Quercus petraea*, fine grain, medium toasting, thickness of stave 27 mm) were used in the three vintages; vintage 2009 was their fourth use, 2010 their fifth use and 2011 their sixth use. Thus both the year of harvest and the time of use of the barrel are included in the term *vintage*.

Analysis of volatile compounds

Volatile compounds were extracted by liquid–liquid extraction following the method developed by Rodríguez-Bencomo *et al.*[10] Chromatographic analyses were performed with an HP-6890 N GC (Agilent Technologies, Waldbronn, Germany) coupled to a HP-5973 inert MS detector equipped with a Quadrex 007CWBTR capillary column (60 m length, 0.25 mm i.d. and 0.25 µm film thickness), following the chromatographic conditions established by the method.

Quantification was carried out following the internal standard quantification method. Quantitative data of the relative areas (absolute areas/internal standard area) were subsequently interpolated in the calibration graphs built from results of pure reference compounds. Forty-four volatile compounds were identified and quantified in the red wines that were classified into ten groups: ethyl esters, alcohols, alcohol acetates, acids, terpenes, lactones, volatile phenols, oak compounds, fusel alcohols and isoamilic alcohols. All analyses were performed in duplicate.

Sensory analysis

A panel of 12 tasters, wine professionals from the DOCa Rioja, was formed. All wine tasters had participated in previous wine-tasting panels. Wines were presented at 18 °C in coded standard wine-tasting glasses according to standard 3591 (ISO 3591, 1997). The tasting sessions took place in a standard sensory analysis chamber (ISO 8589, 1998) equipped with separate booths. In a first session, the panellists were asked to describe the olfactory attributes in their own words. Descriptive terms and their definitions were debated among the assessors, and a common consensus vocabulary was then compiled and discussed further with panellists. Tasters selected eight attributes for vintage 2009 and 2010, and 11 for vintage 2011, which were agreed upon as best for describing the olfactory characteristics of the wines. All the generated terms were usual wine terms for describing red wines. In the following sessions, assessors used the consensus vocabulary, scoring the intensity of each attribute on an interval scale with five levels of intensity (0 = no aroma; 1 = weak aroma; 5 = strong aroma; intermediate values did not bear description). One wine was replicated in order to ascertain judges' consistency.

Statistical analysis

Significant differences between analytical determinations were analysed by a two-way analysis of variance (ANOVA) taking in account variety and vintage. Tukey HSD tests were performed to determinate the statistically significant effect of the parameters with a value of $P < 0.005$. Principal component analyses (PCA) were carried out with the data of the volatile compounds. Only factors with eigenvalues greater than unity were selected. Stepwise discriminant analysis (SDA) following the forward method was used to select the variables most useful for differentiating wines according to grape variety and vintage. The *F*-statistical function was used as the criterion for variable selection. Generalized Procrustes analysis (GPA) was applied on the full data for sensory olfactory attributes, and a permutation test was also made to explain that the results obtained were significant (83.07%).

ANOVA evaluations were performed using the Statistica 8.0 program for Microsoft Windows (Statsoft Inc., Tulsa, OK, USA).

PCA and GPA were carried out using the Senstools Version 3.3.2. Program (Utrecht, Netherlands). Discriminant analysis was carried out using the Statgraphics Plus 5.0 statistical package.

RESULTS AND DISCUSSION

Table 1 presents the concentrations of the 44 volatile compounds quantified by variety and vintage. Data in the table have been arranged into nine chemical families (ethyl esters, acetates, acids, C_6 alcohols, terpenes, lactones, volatile phenols, oak compounds and fusel alcohols). Alcohols were the major group of volatile compounds in all the wines, followed by ester compounds. In the three vintages, Maturana Tinta de Navarrete wines were characterized by the highest concentration of wine volatile compounds because they reached the highest values in ethyl esters and C_6 alcohols (hexanol and *cis*- and *trans*-3-hexen-ol). Also, they reached the highest values in 4-ethyl phenol. The high values found in ethyl esters were due to the high values of ethyl lactate. Ethyl lactate concentrations agreed with those found in other red varietal wines that underwent malolactic fermentation.[11] C_6 alcohol contents allowed differentiating red varietal wines.[4,12,13] Although the ratio *cis*-3-hexen-1-ol/*trans*-3-hexen-1-ol has been also used as a varietal marker,[13] our results indicated that these compounds were also influenced by the vintage. The low values of vinyl and ethyl phenols, which are frequently found in wines aged in barrels, were as expected for young red wines.[14] Monastel wines stood out by a high content in γ-nonalactone and decanoic acid, with similar values to those found in other red varietal wines.[11,14] However, Tempranillo wines showed the lowest contents in lactones, isoamyl alcohols, ethyl-2-methylbutyrate and ethyl isovalerate in the three vintages. It should be pointed out that the aroma of the three varietal wines did not seem to be terpene dependent, as all of them showed terpene values below their odour thresholds.

Wines from the 2009 vintage showed the lowest values in C_6 alcohols and the highest in oak compounds. In spite of the higher relation area/volume of the barrels and taking into account that they had 4 years of use, wines from the 2009 vintage showed more compounds from wood than the rest of the wines. Wines from 2010 showed the lowest concentrations of linear ethyl esters, fusel alcohols and lactones but they were the richest in acetates. Wines from 2011 stood out by the highest levels of volatile phenols. The content of acids and C_6 alcohols were similar in the last two vintages.

Table 2 shows the significance of the ANOVA results for the factor variety and vintage. It is worth mentioning that all the volatile compounds analysed, except eugenol and *trans* whisky lactone, varied significantly among samples with respect to the variety factor. Results of the ANOVA showed that the effect of the vintage on volatiles was also important as significant differences among vintages were found in 39 of the 44 volatiles analysed. It was remarkable that three of the five volatiles with no significance when the factor vintage was analysed corresponded to C_6 compounds (hexanoic acid, 1-hexanol and *trans*-3-hexen-1-ol). Finally, a total of 31 volatile compounds presented a significant interaction between the two factors variety and vintage. Interaction variety $\times$ vintage was not significant for most of the fusel alcohols, lactones, octanoic acid, 1-hexanol, methyl vanillate, ethyl vanillate, 2,6-dimethoxyphenol and siringaldehyde, indicating that these compounds showed the same behaviour for all the varieties in the three vintages of study.

PCA was used in order to clarify data and highlight those variables that better explained the compositional differences among varietal wines and vintage. The PCA selected seven factors with an eigenvalue greater than 1, which explained 93% of the total variance. However, the variables associated into five factors were enough to explain more than 87% of total variability. Table 3 shows the loadings for each variable on the selected factor, as well as the eigenvalue and the cumulative variance. The variables with higher loading values contributed most significantly to the explanatory meaning of the factors (marked in bold). The first factor (PC1) explained 33% of data variability and it was strongly correlated with acetates, acids (except hexanoic and octanoic acid), ethyl esters, oak compounds, fusel alcohols, γ-butyrolactone, acetovanillone and vanillin. Except for oak compounds that are extracted from wood, the rest of the compounds are formed during the winemaking process.[2,15]

PC2 was positively correlated with C6 alcohols (except *cis*-3-hexen-1-ol), hexanoic acid, octanoic acid, α-terpineol and methyl vanillate, and negatively with citronellol. C6 alcohols which are produced from unsaturated linoleic and linolenic acids by grape enzymes during destemming and crushing[16] have been related to vegetal and herbaceous aromas of wines.[17] Terpene and benzene compounds have been also associated with the grape variety,[15] but hexanoic and octanoic acids are related to the fermentation process.[15] PC3 was positively correlated with *cis*-3-hexen-1-ol, the varietal terpene geraniol, and most of the volatile phenols (with the exception of vanillin, methyl vanillate and acetovanillone), and negatively correlated with β-phenylethyl alcohol. PC4 was positively correlated with the varietal volatile compounds linalool and ethyl vanillate, and with γ-nonalactone, which derives from precursors present in grapes[14] and it is associated with the fermentation process.

Figure 1(1a) shows the distribution of the different wines in the plane defined by the first two components, which explained 53% of the total variance. Variables associated with PC2 allowed separation of varietal wines. Thus Maturana Tinta de Navarrete wines were mainly characterized by higher concentrations of C6 alcohols, hexanoic acid, octanoic acid, linalol and methyl vanillate, whereas Tempranillo wines showed the opposite behaviour. Monastel wines were located between Maturana Tinta de Navarrete and Tempranillo wines. These results were in agreement with data of Table 1. Conversely, variables associated with PC1 were less suitable for differentiating varietal wines.

Figure 1(1b) shows the distribution of wines according to variety taking into account PC3 and PC4. A good separation was only achieved for Monastel wines, placed in the second quadrant. Monastel wines were characterized for the lowest contents in *cis*-3-hexen-1-ol, 4-ethyl-2-methoxyphenol, 4-ethyl phenol, 2-metoxy-4-vinylphenol guaiacol and eugenol and 2,6 dimethoxyphenol and the highest contents in γ-nonalactone and ethyl vanillate, in agreement with the data of Table 1.

Regarding the factorial analysis for vintage, Fig. 1(2a, 2b) shows the distribution of wines in the plane defined by PC1 and PC2, and PC3 and PC4, respectively. A good separation by vintage was achieved in both figures. In Fig. 1(2a), factor 1 allowed separation of wines by vintage. Wines from 2009 showed the highest values of this factor and 2010 wines the lowest. Differences among the three vintages could be explained by different reasons. Firstly, it is important to highlight that important families of compounds derived from yeast amino acid metabolism, i.e. isoacids, fusel alcohols, ethyl esters of isoacids and fusel alcohol acetates,[5] were associated with this factor, and it is well known that the

Table 1. Concentration (mg L^{-1}) of wine volatile compounds by variety and vintage

	Vintage 2009			Vintage 2010			Vintage 2011		
	T	O	V	T	O	V	T	O	V
Ethyl esters									
Ethyl butyrate (13.72)	0.131 ± 0.011a	0.136 ± 0.001a	0.262 ± 0.004cde	0.227 ± 0.035bcd	0.319 ± 0.023de	0.222 ± 0.014bcd	0.196 ± 0.013abc	0.266 ± 0.015cd	0.188 ± 0.021ab
Ethyl 2-methylbutyrate (14.54)	0.011 ± 0.001ab	0.025 ± 0.002f	0.022 ± 0.000ef	0.010 ± 0.000a	0.016 ± 0.001cd	0.016 ± 0.000cd	0.014 ± 0.000bc	0.020 ± 0.002e	0.018 ± 0.002de
Ethyl isovalerate (15.52)	0.017 ± 0.002a	0.038 ± 0.002d	0.036 ± 0.001d	0.015 ± 0.000a	0.028 ± 0.000c	0.024 ± 0.000bc	0.023 ± 0.003b	0.034 ± 0.002d	0.028 ± 0.003c
Ethyl hexanoate (26.96)	0.268 ± 0.019a	0.295 ± 0.003ab	0.430 ± 0.001cd	0.486 ± 0.019de	0.536 ± 0.023e	0.542 ± 0.017e	0.400 ± 0.032c	0.476 ± 0.008d	0.373 ± 0.037bc
Ethyl lactate (34.42)	73.49 ± 10.53ab	61.15 ± 1.81ab	108.66 ± 12.74b	49.01 ± 2.356a	49.85 ± 6.22a	70.96 ± 9.35ab	85.40 ± 23.40ab	79.43 ± 12.46ab	104 ± 28b
Ethyl octanoate (40.45)	0.221 ± 0.015a	0.256 ± 0.002ab	0.327 ± 0.002bc	0.466 ± 0.015ef	0.518 ± 0.016f	0.464 ± 0.012ef	0.383 ± 0.030cd	0.450 ± 0.012de	0.295 ± 0.060ab
Ethyl decanoate (53.15)	0.045 ± 0.006a	0.058 ± 0.005a	0.051 ± 0.000a	0.169 ± 0.005c	0.221 ± 0.005d	0.145 ± 0.002bc	0.172 ± 0.018c	0.216 ± 0.016d	0.120 ± 0.024b
Acetates									
Isoamyl acetate (19.27)	0.546 ±0.021abc	0.501 ± 0.002ab	0.637 ± 0.022abc	1.40 ± 0.24f	1.18 ± 0.06ef	1.034 ± 0.113de	0.745 ± 0.048bc	0.868 ± 0.015cd	0.460 ± 0.016a
Hexyl acetate (29.72)	ND1	ND1	0.004 ± 0.000a	0.023 ± 0.006c	0.015 ± 0.001b	0.012 ± 0.005ab	0.006 ± 0.000a	0.009 ± 0.000ab	ND1
β-Phenylethyl acetate (62.76)	0.032 ± 0.004a	0.041 ± 0.001a	0.033 ± 0.000a	0.115 ± 0.006d	0.086 ± 0.002c	0.083 ± 0.009bc	0.073 ± 0.005bc	0.070 ± 0.002b	0.043 ± 0.009a
Acids									
Isovaleric acid (55.41)	1.57 ± 0.12ab	2.18 ± 0.15c	2.49 ± 0.11c	1.13 ± 0.03a	1.49 ± 0.07ab	1.28 ± 0.06ab	1.15 ± 0.11a	1.44 ± 0.08ab	1.58 ± 0.40b
Hexanoic acid (64.78)	1.67 ± 0.05a	1.89 ± 0.01ab	3.63 ± 0.04c	1.92 ± 0.04ab	2.41 ± 0.15ab	2.46 ± 0.10ab	2.13 ± 0.08ab	2.39 ± 0.06ab	2.76 ± 0.61b
Octanoic acid (75.82)	2.19 ± 0.11a	2.54 ± 0.16ab	2.99 ± 0.32abc	3.03 ± 0.09bc	3.40 ± 0.17c	3.31 ± 0.19bc	2.99 ± 0.24abc	3.37 ± 0.40bc	3.12 ± 0.27bc
Decanoic acid (85.35)	0.18 ± 0.02a	0.23 ± 0.00ab	0.21 ± 0.00ab	0.450 ± 0.01de	0.487 ± 0.008de	0.417 ± 0.012cd	0.379 ± 0.052c	0.434 ± 0.016cde	0.283 ± 0.035b
C6 alcohols									
1-Hexanol (35.07)	1.60 ± 0.06ab	1.51 ± 0.04ab	2.33 ± 0.03ab	1.92 ± 0.063ab	1.68 ± 0.10a	2.36 ± 0.10ab	1.88 ± 0.14ab	1.61 ± 0.06a	2.57 ± 0.91b
trans-3-hexen-1-ol (35.64)	0.057 ± 0.002ab	0.055 ± 0.001ab	0.107 ± 0.000cd	0.054 ± 0.002a	0.075 ± 0.004abc	0.085 ± 0.003bcd	0.058 ± 0.001a	0.071 ± 0.008ab	0.101 ± 0.025d
cis-3-Hexen-1-ol (36.96)	0.200 ± 0.005bc	0.032 ± 0.000a	0.085 ± 0.003a	0.274 ± 0.007c	0.133 ± 0.009ab	0.388 ± 0.011d	0.287 ± 0.005c	0.129 ± 0.011ab	0.412 ± 0.077d
Benzyl alcohol (65.89)	0.218 ± 0.004a	0.53 ± 0.01bc	0.65 ± 0.03cd	0.270 ± 0.019a	0.688 ± 0.046de	0.661 ± 0.013cd	0.444 ± 0.016b	0.836 ± 0.048f	0.784 ± 0.090ef
Terpenes									
Linalool (47.61)	0.006 ± 0.001abc	0.007 ± 0.000bcd	0.005 ± 0.000ab	0.006 ± 0.000ab	0.007 ± 0.000cd	0.005 ± 0.000a	0.008 ± 0.001d	0.008 ± 0.000cd	0.008 ± 0.001d
α-Terpineol (56.06)	0.003 ± 0.000ab	0.005 ±0.000abc	0.004 ± 0.000abc	0.003 ± 0.000a	0.004 ± 0.000bc	0.010 ± 0.000e	0.006 ± 0.001c	0.008 ± 0.000d	0.013 ± 0.001f
Citronellol (60.11)	0.010 ± 0.001e	0.010 ± 0.001de	0.007 ± 0.000abc	0.007 ± 0.000bc	0.008 ± 0.000cd	0.005 ± 0.000a	0.008 ± 0.001bc	0.008 ± 0.000bc	0.007 ± 0.001b
Geraniol (64.56)	0.006 ± 0.001a	0.003 ± 0.000a	0.003 ± 0.000a	0.006 ± 0.001a	0.005 ± 0.000a	0.006 ± 0.000a	0.011 ± 0.002b	0.006 ± 0.000a	0.006 ± 0.002a
Lactones									
γ-Butyrolactone (52.16)	17.37 ± 1.62abc	26.48 ±3.03bc	26.59 ± 3.50bc	8.31 ± 0.913a	12.77 ± 1.06ab	14.62 ± 1.62ab	12.11 ± 1.92ab	17.07 ± 2.32ab	29.86 ± 10.57c
γ-Nonalactone (73.21)	0.013 ± 0.002ab	0.016 ± 0.001ab	0.011 ± 0.000a	0.013 ± 0.001a	0.019 ± 0.001b	0.014 ± 0.000a	0.014 ± 0.001a	0.019 ± 0.001b	0.015 ± 0.004ab

Table 1. (*Continued*)

	Vintage 2009			Vintage 2010			Vintage 2011		
	T	O	V	T	O	V	T	O	V
Volatile phenols									
Vanillin (96.78)	0.102 ± 0.003c	0.098 ± 0.000bc	0.024 ± 0.001a	0.044 ± 0.007a	0.038 ± 0.006a	0.045 ± 0.008a	0.071 ± 0.011bc	0.070 ± 0.008b	0.078 ± 0.016bc
Methyl vanillate (98.25)	0.008 ± 0.000a	0.015 ± 0.000ab	0.018 ± 0.000b	0.021 ± 0.002bc	0.026 ± 0.002cd	0.029 ± 0.002d	0.019 ± 0.001b	0.026 ± 0.001cd	0.026 ± 0.003cd
Ethyl vanillate (99.36)	0.164 ± 0.021a	0.188 ± 0.018a	0.137 ± 0.015a	0.138 ± 0.030a	0.157 ± 0.008a	0.145 ± 0.008a	0.146 ± 0.015a	0.173 ± 0.008a	0.159 ± 0.014a
Acetovanillone (99.61)	0.041 ± 0.001abc	0.045 ± 0.000bcd	0.030 ± 0.001a	0.068 ± 0.002f	0.053 ± 0.003cd	0.055 ± 0.003de	0.063 ± 0.007ef	0.056 ± 0.005de	0.043 ± 0.006ab
4-Ethyl-2-methoxyphenol (73.45)	0.006 ± 0.000c	0.004 ± 0.000abc	0.004 ± 0.000abc	0.004 ± 0.001bc	0.001 ± 0.000a	0.014 ± 0.000d	0.005 ± 0.000bc	0.003 ± 0.000ab	0.014 ± 0.002d
4-Ethyl phenol (80.45)	0.024 ± 0.001c	0.007 ± 0.000ab	0.020 ± 0.000c	0.009 ± 0.002b	0.002 ± 0.001a	0.069 ± 0.002d	0.020 ± 0.001c	0.008 ± 0.000b	0.072 ± 0.002d
2-Methoxy-4-vinylphenol (81.26)	0.048 ± 0.002a	0.090 ± 0.001abc	0.042 ± 0.002a	0.161 ± 0.012abc	0.122 ± 0.010ab	0.272 ± 0.020de	0.216 ± 0.023cd	0.190 ± 0.043bcd	0.327 ± 0.081e
Guaiacol (65.02)	0.005 ± 0.000bcd	0.004 ± 0.000bc	0.004 ± 0.000a	0.005 ± 0.000cd	0.003 ± 0.000a	0.005 ± 0.000d	0.007 ± 0.000e	0.004 ± 0.000b	0.007 ± 0.000e
4-Propylguaiacol (77.22)	0.000 ± 0.000abc	0.000 ± 0.000abc	0.000 ± 0.000bcde	0.000 ± 0.000cd	0.000 ± 0.000a	0.001 ± 0.000de	0.001 ± 0.000e	0.000 ± 0.000ab	0.001 ± 0.000de
Eugenol (79.89)	0.011 ± 0.001a	0.014 ± 0.001ab	0.012 ± 0.000a	0.013 ± 0.001a	0.011 ± 0.000a	0.013 ± 0.002a	0.020 ± 0.001b	0.014 ± 0.002a	0.015 ± 0.004a
2,6-Dimethoxyphenol (84.39)	0.017 ± 0.001de	0.014 ± 0.000bcd	0.016 ± 0.000de	0.012 ± 0.000b	0.009 ± 0.000a	0.012 ± 0.001bc	0.020 ± 0.000e	0.015 ± 0.001cd	0.018 ± 0.002e
Oak compounds									
Furfural (42.36)	0.097 ± 0.003b	0.134 ± 0.005c	ND	ND	ND	ND	ND	ND	0.058 ± 0.016a
trans Whisky lactone (66.40)	0.072 ± 0.009bc	0.084 ± 0.001c	0.067 ± 0.001abc	0.041 ± 0.006a	0.036 ± 0.001a	0.048 ± 0.009ab	0.066 ± 0.010bc	0.052 ± 0.004ab	0.079 ± 0.018c
cis Whisky lactone (69.83)	0.068 ± 0.009ab	0.110 ± 0.001c	0.082 ± 0.002bc	0.047 ± 0.007a	0.050 ± 0.001a	0.048 ± 0.006a	0.086 ± 0.010bc	0.077 ± 0.005b	0.079 ± 0.016b
Siringaldehyde (111.99)	0.116 ± 0.015b	0.061 ± 0.004ab	0.111 ± 0.003b	ND	ND	0.004 ± 0.000a	0.032 ± 0.031a	0.004 ± 0.000a	0.029 ± 0.011a
Fusel alcohols									
β-Phenylethyl alcohol (67.82)	36.4 ± 0.6ab	57.4 ± 5.4bc	59.34 ± 0.95c	37.2 ± 1.2abc	41.3 ± 4.9abc	41.5 ± 1.3abc	34.65 ± 3.92a	37.12 ± 3.50abc	42.58 ± 15.40abc
1-Propanol	32.8 ± 1.2b	22.77 ± 0.5a	21.97 ± 0.62a	26.18 ± 0.81ab	26.26 ± 0.45ab	21.11 ± 1.65a	26.29 ± 7.16ab	24.41 ± 1.63ab	23.57 ± 0.16ab
Isobutanol	56.23 ± 2.1a	59.82 ± 2.1a	49.37 ± 2.03a	43.32 ± 4.59a	52.20 ± 3.83a	40.87 ± 6.94a	43.78 ± 12.42a	53.73 ± 2.05a	50.96 ± 0.24a
2-Methyl-1-butanol	46.11 ± 1.0ab	76.00 ± 2.2d	75.15 ± 1.91cd	44.75 ± 4.18a	69.09 ± 7.64cd	58.87 ± 6.23bcd	57.86 ± 4.25bc	60.09 ± 2.75bcd	70.76 ± 6.23d
3-Methyl-1-butanol	201.57 ± 3.71ab	261.8 ± 5.1abc	290.6 ± 7.0c	195.7 ± 16.1a	221.3 ± 12.8abc	218.5 ± 26.9abc	205.1 ± 43.7a	224.4 ± 16.2abc	260.8 ± 1.6bc
Isoamyl alcohols	247.68 ± 5.3a	337.8 ± 6.7bcd	365.7 ± 9.1d	240.4 ± 20.2a	290.4 ± 5.2abcd	277.4 ± 33.2abc	262.9 ± 39.5ab	284.5 ± 18.9abc	331.6 ± 4.6cd

ND, not detectable; values are means ± standard deviations. Different letters in the same row indicate that means significantly differ at $P < 0.05$.

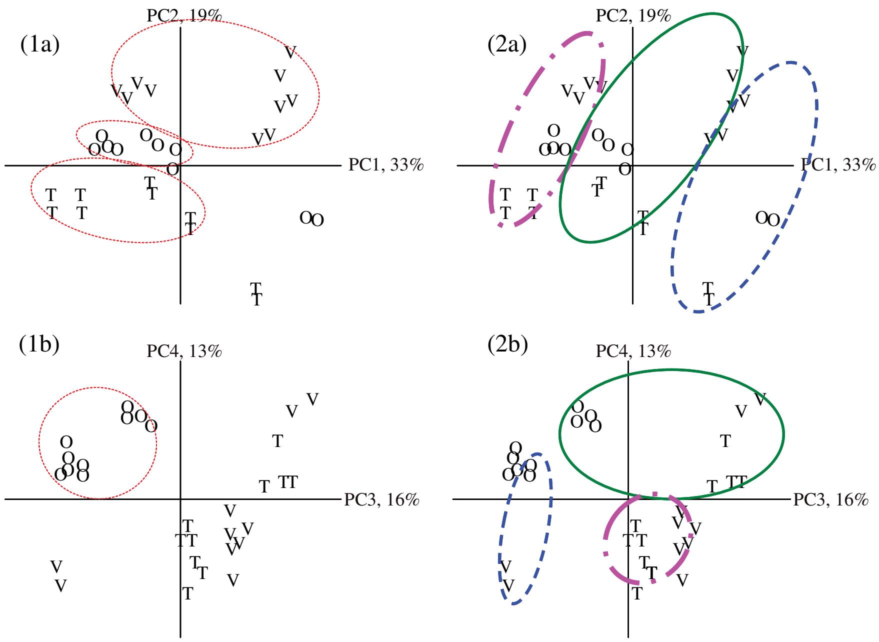

Figure 1. Distribution of the wines in the plane defined by variety: (1a) factors 1 and 2 and (1b) factors 3 and 4; and by vintage: (2a) factors 1 and 2 and (2b) factors 3 and 4. T, Tempranillo wines; V, Maturana Tinta de Navarrete wines; O, Monastel wines. ----, vintage 2009; ----, vintage 2010; ----, vintage 2011.

concentration of amino acids in grape depends on the climatic conditions of each year. Secondly, changes during the alcoholic and malolactic fermentation (temperature, aeration, etc.) could have been occurred among vintages. Finally, oak compounds were also associated with this factor. The number of times the barrels were used could have determined the release of oak wood compounds into wine as their quantity and rate of extraction generally diminish with the utilization of the barrel over successive years. Therefore, this could explain why 2009 wines had higher concentrations of furfural, *cis* and *trans* whisky lactones and siringaldehyde than 2010 wines. However, 2011 wines were richer in these compounds than 2010 wines, which could be due to differences between years in the microbial activity in extractable compounds of the wood. In this way, Hernández-Orte *et al.*[18] found a significant decrease of furfural and 5-methylfurfural during the malolactic fermentation. Figure 1(2b) shows that the variables associated with PC3, mainly related to volatile phenols, also permitted differentiating wines by vintage.

Stepwise linear discriminant analysis was applied as a supervised classification technique in order to determine the volatile compounds most useful for differentiating wines according to grape variety and vintage. The final model by grape variety selected six volatile compounds: linalool, octanoic acid, 4-ethyl phenol, guaiacol, ethyl isovalerate and methyl vanillate (with *F*-values between 41 and 7). Linalool is a varietal terpene characteristic of aromatic varieties.[15] Octanoic acid and ethyl isovalerate are mainly formed during alcoholic fermentation due to yeast metabolism. Many researchers have found the importance of some esters in the differentiation of red varietal wines, resulting in a fruity character of the final wines.[1,15] Guaiacol and 4-ethylpenol can be extracted from wood[1] but they can also be released from non-aromatic precursors present in wine through the fermentation process.[19] Guaiacol and 4-ethyl phenol have also been found in young wines without wood contact, and they may arise from degradation of the lignin of the herbaceous part of the cluster or from the release of their glycosidic precursors.[20] Also, it is important to take into

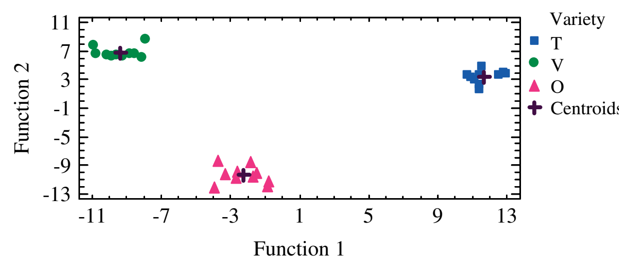

Figure 2. Distribution of wines in the plane defined by the two first discriminate functions by grape variety.

account that 4-ethyl phenol may be formed from ferulic acid,[21] whose levels show important variability among grape varieties. The relationship between grape variety and the latter compound has previously been described in the literature.[22] Methyl vanillate is a varietal compound originating from the precursors present in grapes.[14] It is important to highlight that these results contrasted with those obtained in the PCA, where some C6 alcohols, hexanoic and octanoic acid, and α-terpineol and linalool were able to differentiate wines according to variety, especially Maturana Tinta de Navarrete wines. However, these results agreed with those found by Ortega-Heras *et al.*,[23] who observed that not all wines have the same capacity to extract volatile compounds from the oak wood.

The distribution of wines in the plane defined by the first two discriminant functions is shown in Fig. 2. Applying discriminant analysis, an accurate classification of wines by grape variety was obtained. Taking into account that the distance between centroids is proportional to the similarity between groups, Maturana Tinta de Navarrete wines differed most from the rest of the varieties studied, since they were situated on the left part of the plane.

When stepwise forward discriminant analysis was applied to discriminate wines by vintage, the final model selected nine variables: β-phenylethyl alcohol, furfural, eugenol, ethyl

Table 2. Significance of ANOVA for the factors variety and vintage

	Significance		
	Variety	Vintage	Variety × Vintage
Ethyl esters			
Ethyl butyrate	***	***	***
Ethyl 2-methylbutyrate	***	***	***
Ethyl isovalerate	***	***	***
Ethyl hexanoate	***	***	***
Ethyl lactate	***	***	ns
Ethyl octanoate	***	***	***
Ethyl decanoate	***	***	***
Acetates			
Isoamyl acetate	**	***	***
Hexyl acetate	*	*	*
β-Phenylethyl acetate	***	***	***
Acids			
Isovaleric acid	***	***	*
Hexanoic acid	***	ns	***
Octanoic acid	**	***	ns
Decanoic acid	***	***	**
C6 alcohols			
1-Hexanol	***	ns	ns
trans-3-Hexen-1-ol	***	ns	*
cis-3-Hexen-1-ol	***	***	***
Benzyl alcohol	***	***	*
Terpenes			
Linalool	***	***	***
α-Terpineol	***	***	***
Citronellol	***	***	***
Geraniol	***	***	**
Lactones			
γ-Butyrolactone	***	***	ns
γ-Nonalactone	***	*	ns
Volatile phenols			
Vanillin	***	***	***
Methyl vanillate	***	***	ns
Ethyl vanillate	**	ns	ns
Acetovanillone	***	***	***
4-Ethyl-2-methoxyphenol	***	***	***
4-Ethyl phenol	***	***	***
2-Methoxy-4-vinylphenol	***	***	**
Guaiacol	***	***	***
4-Propylguaiacol	***	***	***
Eugenol	ns	***	*
2,6-Dimethoxyphenol	***	***	ns
Oak compounds			
Furfural	*	*	*
trans Whisky lactone	ns	***	*
cis Whisky lactone	*	***	***
Siringaldehyde	*	***	ns
Fusel alcohols			
β-Phenylethyl alcohol	**	**	ns
1-Propanol	***	ns	ns
Isobutanol	*	*	ns
2-Methyl-1-butanol	***	**	***
3-Methyl-1-butanol	***	**	ns
Isoamyl alcohols	***	***	*

Asterisks indicate significance at
* $P < 0.05$,
** $P < 0.01$,
*** $P < 0.001$; ns indicates no significant difference.

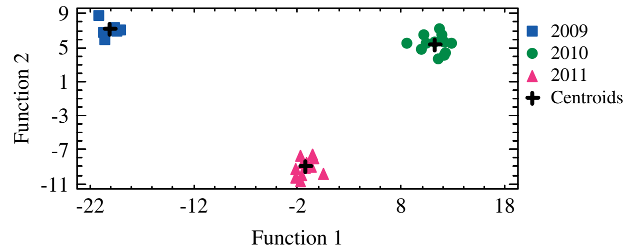

Figure 3. Distribution of wines in the plane defined by the two first discriminate functions by vintage.

2-methylbutyrate, hexyl acetate, siringaldehyde, 4-propylguaiacol, isoamyl alcohol and 1-propanol. (with *F*-values between 43 and 6). Furfural, eugenol, siringaldehyde and 4-propylguaiacol are compounds extracted from the oak wood.[23] Differences between 2009 and 2010 vintages in siringaldehyde and furfural could be explained as the extraction of these compounds decreases due to the depletion of the oak barrel with the years of use. Eugenol showed an irregular behaviour between vintages. Besides the fact that it can be extracted from wood, eugenol is also a varietal aroma belonging to benzene compounds, whose identification in wines is related to a sweet, spicy aroma, especially clove.[15] Isoamyl alcohol and 1-propanol are fusel alcohols and they are correlated with the initial amino acid content in grapes,[1] and thus the ripeness stage and climatic factors can affect the amount of these compounds. Hexyl acetate can change among vintages due to differences in the factors that affect the development of the alcoholic and malolactic fermentation.[24] β-phenylethyl alcohol shows three origins: variety and fermentation (fusel alcohol), and it can also be extracted in small concentrations from oak wood.[23] These results showed that the selected variables to discriminate wines by vintage were strongly dependent on the initial must characteristics, which are strongly dependent on the climatic factors but also on the compounds than can be extracted from the oak wood. Three groups representing each vintage were clearly differentiated in the discriminant analysis by vintage (Fig. 3). It is noteworthy that this distribution matched those obtained in the PCA (Fig. 1(2b)).

Both models, discriminating analysis by grape variety and vintage, were satisfactory, with a global classification of 100% of the wines. However, the mere knowledge of the volatile composition of a wine without a sensory evaluation is inadequate to predict the flavour of the whole system as it is perceived by a trained sensory judge. For that reason, a sensory analysis of the different varietal wines in each vintage was carried out.

Figure 4 provides a GPA consensus configuration of the relationship of the wines in each vintage as determined from their olfactory perceptions. GPA was applied to sensory data to ascertain consistency among the 12 tasters. Before that, the within-judges reproducibility was evaluated by mean of two replicated wines in the tasting session and replications were demonstrated not to be a source of variation.

In the olfactory GPA space of wines from the 2009 vintage (Fig. 4a), wines were properly located in the vectorial dimension defined by the two factors, which accounted for 93.2% of the total variance. The resulting plot showed the wines quite spread, indicating a marked difference among wines. Tempranillo showed a higher correlation with liquorice aromas. Monastel was more correlated with spicy, dairy, coffee and toasted aromas, whereas Maturana Tinta de Navarrete was more correlated with pepper

Table 3. Factor loadings of the wines

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
Eigenvalue	14.56	8.57	7.09	5.66	2.25	1.62	1.27
Cumulative variance (%)	33	53	69	82	87	90	93
Ethyl esters							
Ethyl butyrate	**−0.54**	0.45	−0.53		0.29		
Ethyl 2-methylbutyrate	**0.60**	0.34	−0.42	0.45		−0.27	
Ethyl isovalerate	**0.54**	0.35	−0.45	0.51			
Ethyl hexanoate	**−0.79**	0.51					
Ethyl lactate	**0.59**	0.42	0.27		0.35		−0.38
Ethyl octanoate	**−0.89**	0.26		0.25			
Ethyl decanoate	**−0.76**			0.56			
Acetates							
Isoamyl acetate	**−0.92**						
Hexyl acetate	**−0.88**						
β-Phenylethyl acetate	**−0.94**						
Acids							
Isovaleric acid	**0.71**		−0.58				
Hexanoic acid	0.32	**0.79**			0.38		
Octanoic acid	−0.47	**0.65**		0.33			
Decanoic acid	**−0.91**			0.34			
C6 alcohols							
1-Hexanol		**0.68**	0.32	−0.53			
trans-3-Hexen-1-ol	0.37	**0.84**					
cis-3-Hexen-1-ol		0.38	**0.83**	−0.27			
Benzyl alcohol		**0.78**		0.47	−0.34		
Terpenes							
Linalool	0.28			**0.81**			
α-Terpineol	0.32	**0.63**	0.53	0.35	−0.27		
Citronellol	0.29	**−0.79**	−0.32	0.28			
Geraniol		−0.29	**0.65**	0.41	0.43		
Lactones							
γ-Butyrolactone	**0.82**	0.41					
γ-Nonalactone				**0.91**			
Volatile phenols							
Vanillin	**0.55**	−0.52	0.34	0.34	−0.27		
Methyl vanillate	−0.43	**0.74**		0.41			
Ethyl vanillate	0.34			**0.58**	−0.40		
Acetovanillone	**−0.81**		0.30				
4-Ethyl-2-methoxyphenol	0.27	0.44	**0.68**		−0.41		
4-Ethyl phenol	0.29	0.56	**0.65**		−0.32		
2-Methoxy-4-vinylphenol		0.46	**0.76**	0.36			
Guaiacol			**0.92**				
4-Propylguaiacol							
Eugenol			**0.60**	0.45	0.46	−0.28	
2,6-dimethoxyphenol	0.55		**0.60**		0.45		
Oak compounds							
Furfural	**0.72**	−0.40			−0.50		
trans Whisky lactone	**0.83**		0.33				0.26
cis Whisky lactone	**0.74**	−0.26		0.43	0.27		
Siringaldehyde	**0.74**	−0.39		−0.31			
Fusel alcohols							
β-Phenylethyl alcohol	**0.49**	0.36	**−0.49**		−0.25		
1-Propanol		−0.67				**0.71**	
Isobutanol	**0.53**	−0.26	−0.44	0.33		0.49	
2-Methyl-1-butanol	**0.52**	0.45	−0.30	0.41			
3-Methyl-1-butanol	**0.71**	0.36	−0.26				0.42
Isoamyl alcohols	**0.71**	0.41	−0.29	0.26			0.37

Loadings lower than absolute values of 0.250 are not shown. Bold numbers indicate the higher weight of each compound in each factor.

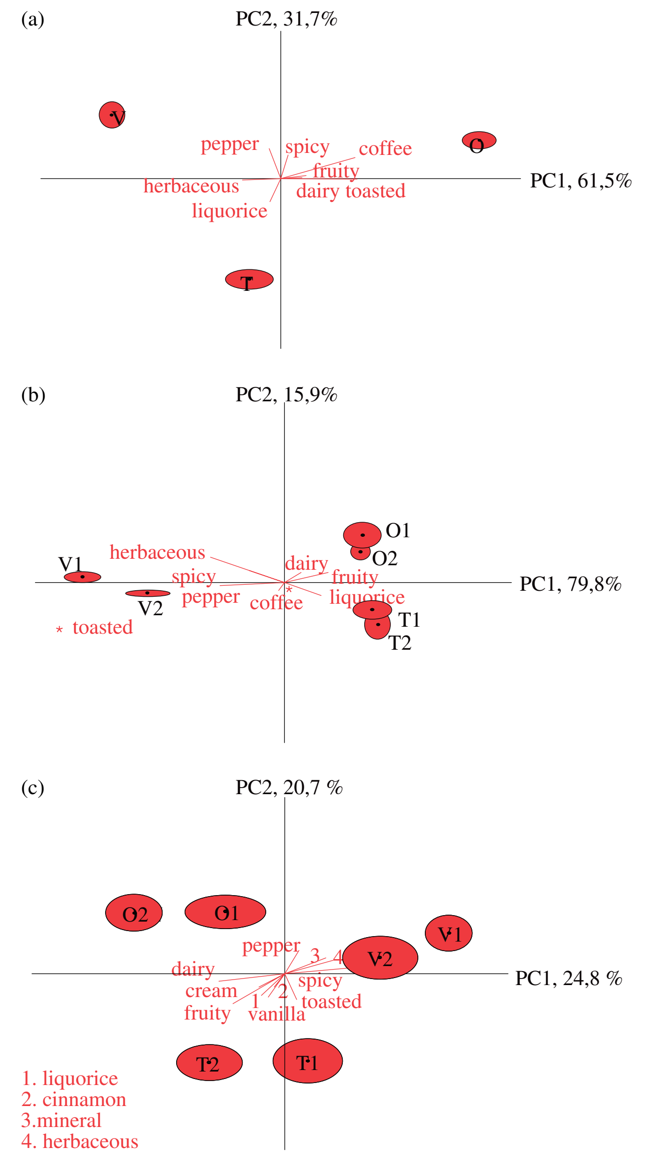

Figure 4. GPA on the mean ratings for olfactory attributes with individual variances of wines in (a) vintage 2009, (b) vintage 2010 and (c) vintage 2011. T, T1, T2: Tempranillo wines; V, V1, V2: Maturana Tinta de Navarrete wines; O, O1, O2: Monastel wines.

and herbaceous odours. In the GPA space of wines from the 2010 vintage (Fig. 4b), 95.7% of the total variance was explained. A good differentiation among varietal wines was also achieved. Maturana Tinta de Navarrete wines were the least fruity, probably due to its low values in acetates, and they were more correlated with herbaceous, pepper and spicy aromas, as well as with C6 alcohols, in good agreement with the results found in Table 1 and Fig. 1(1a). Monastel wines were mainly characterized by fruity and dairy aromas, whereas Tempranillo wines were correlated with liquorice aromas. Furthermore, all tasters gave low scores to coffee and toasted attributes in all wines, which could be related to non-detectable amounts of furfural and other oak volatile compounds in the samples. These results also agreed with those obtained in Table 1 and Fig. 1-2a), where wines of vintage 2010 were poor in volatile compounds extracted from wood. Finally, GPA space of wines from the 2011 vintage only explained 45.5% of the total variance. A higher variability in the wines from the same grape variety was found. As in the 2010 vintage, Maturana Tinta de Navarrete wines were again more related to herbaceous, pepper and spicy attributes and less to fruity notes. Tempranillo wines were characterized by liquorice, fruity and vanilla aromas, whereas Monastel wines were the least aromatic in that year.

CONCLUSIONS

A clear differentiation of the wines according to grape variety and harvesting year was achieved both with PCA and stepwise linear discriminant analysis. The volatile compounds that allowed differentiating wines by grape variety were mainly varietal aromas, whereas vintages were mainly differentiated by volatile compounds formed during the alcoholic fermentation and/or extracted from wood during the elaboration process in wooden barrels. The sensory analysis also allowed differentiating wines by grape variety. Tempranillo wines were characterized by liquorice notes, whereas the Maturana Tinta de Navarrete wines were the least fruity and showed high herbaceous and pepper notes. The sensory profile of Monastel varied between vintages.

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