



Volatile compounds as markers of ageing in Tempranillo red wines from *La Mancha* D.O. stored in oak wood barrels

Lucía Castro-Vázquez^{a,*}, M. Elena Alañón^a, Elena Calvo^a, M. Jesús Cejudo^a, M. Consuelo Díaz-Maroto^b, M. Soledad Pérez-Coello^a

^a Food Technology Area, Faculty of Chemistry, University of Castilla-La Mancha, Campus Universitario, s/n, 13071 Ciudad Real, Spain

^b IRICA (Instituto Regional de Investigación Científica Aplicada), University of Castilla-La Mancha, Campus Universitario, s/n, 13071 Ciudad Real, Spain

ARTICLE INFO

Article history:

Available online 28 December 2010

Keywords:

Wine
Ageing
Aroma compounds
Multiple linear regression

ABSTRACT

Solid-phase extraction cartridges (SPE)–GC/MS method was used to analyse red wines aromas. The matrix effect was studied with chemicals standard prepared in synthetic wines with water/alcohol solutions (12% ethanol, v/v) following the procedure proposed. The method offers good reproducibility since the relative standard deviations (RSD%) for the volatile compounds levels were less than 9%. This method was used to differentiate the aroma of one hundred mono-varietal *young*, *crianza*, *reserva* and *gran reserva* *La Mancha* D.O. wines (cv. Tempranillo) on the basis of oak barrel contact period. Samples were checked at ten time points over 36 months. Sixty important wine odorants, such as volatile phenols, vanillin derivatives, lactones, norisoprenoids, benzene compounds, esters and terpenols, can be quantitatively determined in a single run. Results showed significant quantitative differences in the volatile profiles of wines depending on the length of time in contact with wood, even in wines belonging to the same commercial category. Stepwise multiple linear regression (SMLR) was used to obtain a model that predicted the time of barrel ageing to which wines were submitted in relation with the wine volatile composition. A successful function based on eight compounds with a mean deviation of 0.37 months in the predictions, was obtained.

Published by Elsevier B.V.

1. Introduction

The ageing of wines is a technological procedure commonly used in winemaking which helps to improve stability, spontaneous clarification and a complex aroma. Oak barrels have commonly been used in wine ageing improving the quality of the wine. Barrel contact modifies the wine composition due to the compounds that are extracted from wood, such as tannins, phenolic acids, and volatile compounds.

Extraction of volatile compounds from oak barrels depends on several factors, including the botanical and geographic origin of the wood [1–3] and the degree of oak toasting [4,5]. Moreover, the quantity of volatile compounds that are potentially extractable is influenced by the rate of release of these compounds from the wood and the contact time between wine and wood. This last factor has been the least studied despite its importance.

The aroma of wines aged in oak barrels depends not only on the volatile compounds such as lactones, furanic compounds, vanillin derivatives or phenol derivatives that could migrate from oak wood during the ageing process; but also on simultaneous phenomena

such as the formation of new volatile compounds, oxidations [6,7], microbiological formation of ethyl phenols [8], sorption of wine aroma compounds by oak wood [9] and hydrolysis-esterification equilibria [10–12]. The aroma composition, the sensory properties and the overall aroma [13] of aged wines are influenced by the variability of all these factors.

In recent years there have been papers on the development of wine aroma after several weeks of accelerated ageing [10,14]. Some works just control ten or fifteen compounds during the oxidative maturation of wines [15]. There have also been other studies of wood-aged-wines aromas after 12–24 months [11,16–18] but, there are no data available up to 24 months. Furthermore, studies of aged wine aromas have generally used hydroalcoholic solutions which have been kept in barrels for limited periods [19–22]. We therefore believe that further studies of wine volatile composition after long-term of wood ageing would be useful.

In Spain, aged red wines are defined according to the time of storage in wood and in bottle. Thus, the term “*crianza*” is used in D.O. La Mancha to describe wines that have been kept for at least 6 months in wood barrels and a further 12 months in bottles. The term “*reserva*” applies to wines that have been kept for at least 12 months in wooden barrels and 24 months in bottles. Finally, “*gran reserva*” refers to wines that have been kept for at least 24 months in wood and 36 months in bottles. So, a wide margin for time in

* Corresponding author. Tel.: +34 926 295300; fax: +34 926 295318.
E-mail address: Lucialsabel.Castro@uclm.es (L. Castro-Vázquez).

contact with oak wood for the three categories is afforded by Spanish legislation. For instance, *reserva* refers to wines that have been kept for 12–18 months in oak barrels before marketing under that category. The number of months of cask ageing normally affects the price since this process entails a significant outlay that must be recovered in the final cost of the aged wine. By checking the volatile profiles of aged wines at different points could be certified within the broad intervals of the commercial categories for the purpose of identifying possible frauds. But in spite of this, there has been no study of oxidation-driven development of wine aroma after several time points through a long-term exposure due to the difficulty of obtaining wine samples from the producers with an exact and well known ageing time period.

This study proposes first to assess the changes in the volatile profile of *crianza*, *reserva* and *gran reserva* Tempranillo varietal red wines from *La Mancha* D.O. aged in oak barrels at 10 time points over 36 months. And second to determine a model, based on the study of a small number of volatile compounds, whereby we may verify the actual months of contact with the barrel indicated on the label and related to the price of wine.

2. Experimental

2.1. Analytical reagents and standards

The chemical standards were supplied by Aldrich (Gillingham, U.K.), Fluka (Buchs, Switzerland) and Sigma (St. Louis, MO). LiChrolut EN resins, prepacked in 500 mg cartridges were obtained from Merck (Darmstadt, Germany). Dichloromethane and methanol, GC-quality, were purchased from Scharlau (Barcelona, Spain). Pure water was obtained from a Milli-Q purification system (Millipore, U.S.A.).

2.2. Wine samples

This study was carried out with one hundred red mono-varietal wines from *vitis vinifera* Tempranillo from *La Mancha D.O* (Spain). Wines were produced on an industrial scale following standard winemaking practices. Tempranillo wines were aged in oak barrel during 6, 7, 8, 9, 12, 14, 16, 18, 24 and 36 months. Samples were obtained from different wineries sheltered by the Origin Denomination “*La Mancha*”.

2.3. Isolation of volatile compounds

Volatile compounds were extracted by solid phase extraction with polypropylene–divinylbenzene cartridges (Lichrolut EN), Merck, 0.5 g of phase, using the method developed by Sanchez-Palomo et al. [23].

2.3.1. Cartridge conditioning

Cartridge was conditioned with 10 mL of dichloromethane, 5 mL of methanol and finally with 10 mL ethanol/distilled water (90:10, v/v) at a flow rate of 2 mL/min.

2.3.2. Sample loading

One hundred millilitres of wine, with 40 μ L of 4-nonanol as internal standard (1 g/L), were passed through the SPE cartridge at 1 mL/min. The resin was washed with 100 mL of pure water to eliminate sugars and other low-molecular weight polar compounds.

2.3.3. Elution

Analytes of interest were recovered by elution with 10 mL of dichloromethane at a flow rate of 1 mL/min. Extracts were concentrated under nitrogenous stream to a volume of 200 μ L. Then, they

were hermetically capped and stored at -18°C until the GC–MS analysis.

2.4. Method validation

The reproducibility of the SPE–GC method was determined by replicate analysis of one *young* wine and one *crianza* wine on different days. The existence of matrix effect was taking into account using water/alcohol solutions (12% ethanol, v/v) with 5 g/L of tartaric acid and pH adjusted to 3.6. Hydroalcoholic solutions were spiked with known amounts of the volatile compounds of interest in analogous concentrations that the red wines. They were submitted to the complete procedure with the purpose of determined the response factors after the SPE extraction–GC/MS analysis.

2.5. GC–MS analysis of wines

An Agilent 6890 N gas chromatograph, coupled to a 5973 Inert 121 mass selective detector was used. One microliter of extracts was injected in splitless mode (0.6 min) on a polyethylene glycol capillary column BP-21 (50 m \times 0.32 mm \times 0.25 μ m of film thickness). Oven temperature was programmed to remain at 60°C for 3 min and then increased $2^{\circ}\text{C}/\text{min}$ to 200°C and held for 30 min. Helium was used as carried gas at a flow rate of 0.8 mL/min. Injector and transfer line temperatures were 250°C and 280°C , respectively. Ionization was performed by electron impact mode at 70 eV. Mass spectrum acquisition was performed in scan mode (40–450 m/z range).

Peak identifications were based on comparison of their mass spectra with those of pure standards from Sigma–Aldrich and/or with those reported by the NBS75K and Wiley A commercial libraries. The quantitative analysis was performed by total ion using the response factors calculated for each compounds in hydroalcoholic solution (12%, v/v) like a model wine. For compounds which commercial standards were not available, the response factors of compounds with similar chemical structures were used. All the samples were injected in duplicate.

2.6. Statistical analysis

Data were treated statistically by the analysis of variance (ANOVA). Unsupervised techniques such as principal component analysis (PCA) were also carried out. Stepwise multiple linear regression (SMLR) was applied to the dataset to predict the wine storage period in oak barrels, using an equation of the form:

$$t_i = b_0 + \sum_{j=1}^m b_j \cdot x_{ji}$$

where t_i was the i th wine storage time; b_0 was the y-intercept and b_j was the regression coefficients for the j th prediction parameters (x_{ji}) in the model. The root mean square error of prediction (RMSEP) obtained by cross-validation was used as a measure of the abilities of the three models to furnish accurate predictions. The value was calculated using the equation:

$$\text{RMSEP} = \left(\sum_{i=1}^n (t_i - t(i))^2 / n \right)^{1/2}$$

where t_i is the real storage time for the i th sample of the standard wines, $t(i)$ is the predicted storage time obtained with the model constructed without the i th sample, and “ n ” is the number of standard wines used in the calibration model ($n = 100$). Statistical processing was carried out by using the SPSS 17.0 for Windows statistical package.

3. Results and discussion

3.1. Method validation

Among all the possible sorbents for the solid phase extraction (SPE), Lichrolut-EN resins have been selected because in previous studies carried out in our laboratory, they have shown to have an excellent ability for the extraction of neutral compounds from must and wine [23].

The response factors of volatile compounds determined using water/alcohol solutions (12% ethanol, v/v) with 5 g/L tartaric acid and pH adjusted to 3.6, can be seen in Table 1. Ethyl and diethyl esters, together with some benzene compounds such as benzyl alcohol, 2-phenylethyl alcohol and 2-phenylethyl acetate showed the highest values near one. On the contrary, response factor for linear alcohols, C₆ alcohols and ethyl lactate were very low. In the cases of terpene and volatile phenols values range were determined between 0.63 and 0.89.

The reproducibility of the method, expressed in RSD (%) is given in Table 1. Those values have been obtained by the replicate analysis of two different days. Relative standard deviations (RSDs) for the concentration of wine volatile compounds were less than 9.0%, and in nearly half were below 6%, showing a good reproducibility. The proposed method showed a quite satisfactory precision, both in young and aged wines and can be considered adequate for the purposes of the analysis.

3.2. Analysis of wines

3.2.1. Volatile composition of aged wines

The proposed method was applied to the analysis of one hundred Tempranillo red wines from *La-Mancha* D.O. One of the most important contributions of this paper is the large number of wines and aged period processed. The aroma profiles coming from *young*, *crianza*, *reserva* and *gran reserva* wines was determined after 11 time points over 36 months of ageing in woods barrels. The concentrations of sixty volatile compounds, grouped in chemical families, are shown in Table 2. A Student–Newman–Keuls multiple-range test was used to determine significant differences depending on the wood contact time. Many compounds were present in the young wines before ageing in oak wood barrels. However, there were appreciable changes in the wine volatile composition as a result of oxidative storage period.

Average alcohol content, mainly 1-hexanol, decreased in mayor proportion in *reserva* wines aged over 12 months. The C₆ alcohols concentration in *reserva* wines matured 18 months was around 50% lower compared with that wines matured 12 months. The tendency observed in isopropanol, 1-heptanol and 1-octanol, was quite similar, although the diminishment was smaller.

Significant quantitative differences in the behaviour of esters during storage in oak barrels were detected. Fatty acids ethyl esters such as ethyl hexanoate or ethyl octanoate together with acetates like isoamyl acetate or 2-phenylethyl acetate gradually reduced their concentrations from 6 to 30 months. On the contrary, levels of ethyl lactate and diethyl succinate increased progressively as consequence of hydrolysis-esterification equilibria reported by other authors [10,24]. Esters levels revealed the importance of wood contact time even to distinguish wines in the same commercial category. Although, there is no variable that, taken alone, could be used to designate the age of the wine, data show that isoamyl acetate and ethyl lactate levels can be used to differentiate *crianza* wines with differing ages.

Acids contents showed significant decreases mainly in *reserva* wines over a period of 12–18 months (Table 2). The clearest case in this category was octanoic acid, although hexanoic and decanoic acids also behave similarly. This fact may be related to the disap-

pearance of compounds in the wood-aged wines as a consequence of sorption processes, according to Jaurauta et al. [11].

Similarly, significant reductions in nerol and α -terpineol levels could be useful to differentiate among *crianza* wines stored for 6, 7, 8 and 9 months in terms of volatile profiles. Hotrienol and geraniol were not detected after 6 months of ageing. These results suggest that terpene levels in Tempranillo aged wines are hardly affected by the casks storage.

Benzenic compounds levels underwent considerable changes during ageing. Concentrations of benzaldehyde, phenylacetaldehyde and benzyl alcohol were lower in young wines than in aged wines, although their levels increased gradually up to sixth month. Significant quantitative differences between wines aged 24 and 36 months suggests that the cited compounds can be used to discriminate between *gran reserva* wines with different wood contact periods. Phenylacetaldehyde levels absent in *young* and *crianza* Tempranillo wines and linked to the wood-oxidation character of red wines [25], can be also considered to differentiate among the analysed *gran reserva* wines.

In the same way, stronger decreases of furfural concentrations after periods equal or superior to 24 months, due of its transformation in furfury alcohol, allow distinguish between *gran reserva* wines.

One of the most affected compounds by the months of storage in oak casks was β -methyl- γ -octalactone isomers, according to previous studies [3,11,15–18,26]. Both levels underwent positive exponential increased from 6 to 36 months providing a direct relation with the length of ageing period of *crianza*, *reserva* and *gran reserva* wines. They can be considered important markers for differentiating between wines of the same category.

Volatile phenols such as guaiacol, eugenol, syringol and derivatives showed a direct relationship between the period of wood ageing and the wines volatile composition. Eugenol and guaiacol were extracted constantly and almost linearly in wines matured from 6 to 18 months according to other studies [11,15–18,27–29]. The accumulation of these compounds was much greater after 24–36 months of ageing, indicating the importance of wood contact time. In the case of 4-ethylphenol related to *Brettanomyces/Dekkera* [30,31] complex evolution (reflected in high RSDs) had been observed.

Vanillin derivatives such as butyrovanihone and methylvanillyl ether are wood-extractable compounds which were mainly extracted in the first nine months of ageing, corresponding to *crianza* wines. After that, both levels remained more or less constant or decreased slightly. The most important compound of this group in terms of its contribution to wine aroma is vanillin. Significant quantitative increases in concentrations of vanillin, ethyl vanillate and propiovanillone were registered at all sampling times over the whole ageing period from 6 to 36 months, making it possible to discriminate among samples belonging to the same category.

To obtain more detailed information on the volatile compounds involved in the differentiation of Tempranillo aged wines with different wood contact period, factorial principal component analysis was applied to the contents of the sixty wine compounds. Young wines were not included in the whole data matrix.

The two-dimensional projection of variables is presented in Fig. 1. Results indicated several compounds which were particularly useful for differentiation of Tempranillo red wines from La Mancha region. The first component axis explained 69.1% of the total variation and clearly separates *gran reserva* wines stored for 24 and 36 months, grouped in the positive area of PC-1. *Reserva* wines aged for 16 and 18 months were therefore also differentiated separately. PC-1 displayed a strong positive correlation with benzene compounds (phenylacetaldehyde, benzaldehyde and benzyl alcohol), oak lactone isomers, vanillin, ethyl vanillate, propiovanillone and some volatile phenols (eugenol, guaiacol, syringol and allyl syringol) all

Table 1

Response factors on water/alcohol solutions (12% ethanol, v/v) after the Lichrolut-EN cartridges extraction. Mean concentration ($\mu\text{g/L}$) and Coefficient of Variations of *young* and *crianza* Tempranillo wines.

Compounds	t_R (min)	Response factor (water/alcohol solutions 12%, v/v)	Reproducibility ^a			
			Young wine		Crianza wine	
			Mean ($\mu\text{g/L}$)	RSD (%)	Mean ($\mu\text{g/L}$)	RSD (%)
Isopropanol	6.8	0.38	352.6	2.4	302.8	4.6
Isoamyl acetate	7.8	0.48	1559.0	8.7	1094.8	8.1
Ethyl hexanoate	12.1	1.02	470.5	1.7	366.8	0.5
Ethyl lactate	18.7	0.04	1790.3	8.5	2698.1	6.5
1-Hexanol	19.1	0.59	1553.4	2.4	1418.3	1.5
(E)-3-Hexen-1-ol	19.8	0.48	64.4	4.2	57.7	6.0
(Z)-3-Hexen-1-ol	21.4	0.48	179.1	3.0	157.3	3.6
Ethyl octanoate	25.9	1.22	808.2	7.7	534.9	7.5
Acetic acid	26.9	0.39	17.4	4.2	35.4	5.6
1-Heptanol	27.4	0.61	30.9	3.0	17.3	3.4
Furfural	28.89	1.01	4.3	8.7	13.9	7.5
Ethyl 3-hydroxybutanoate	33.4	–	71.1	5.5	76.7	6.6
Benzaldehyde	34.1	0.7	7.9	3.2	10.4	3.0
Linalool	37.2	0.89	7.7	4.2	1.6	3.2
1-Octanol	38.3	0.76	22.4	7.6	18.7	6.1
Isobutyric acid	39.1	0.06	33.1	4.9	35.3	4.9
Isoamyl lactate	39.4	–	34.1	4.5	32.1	6.6
Hotrienol	45.1	–	0.3	3.1	n.d.	–
γ -Butyrolactone	46.5	0.01	5.3	2.9	3.7	7.5
Butyric acid	46.5	0.21	151.5	5.7	88.4	9.0
Ethyl decanoate	48.4	1.06	174.3	2.7	177.8	4.8
Phenylacetaldehyde	48.5	0.33	Tr	–	Tr	–
Diethyl succinate	52.4	0.91	4891.3	6.4	10178.3	4.3
α -Terpineol	53.2	0.77	17.6	7.1	8.2	3.8
3-Methylthio-1-propanol	54.9	–	54.3	5.3	38.4	4.0
Succinate derivative I	58.8	–	22.5	7.4	22.9	8.8
Nerol	60.2	0.89	13.9	3.3	8.6	4.0
β -Damascenone	61.5	0.92	1.3	5.1	2.2	4.3
2-Phenylethyl acetate	62.2	0.99	166.9	7.7	101.1	7.0
Hexanoic acid	63.9	0.44	1923.5	7.2	1591.7	8.1
Geraniol	64.8	0.77	2.7	3.1	n.d.	–
Guaiacol	65.1	0.63	19.1	4.8	26.8	4.7
Benzyl alcohol	66.1	0.92	180.3	5.8	198.4	8.7
<i>trans</i> - β -Methyl- γ -octalactone	66.5	1.01	n.d.	–	17.5	6.3
Phenylethyl alcohol	68.4	0.98	16968.4	4.6	14874.2	6.8
<i>cis</i> - β -Methyl- γ -octalactone	70.6	0.89	n.d.	–	53.3	4.3
(E)-3-Hexenoic acid	71.1	–	33.6	6.4	33.1	4.9
Phenol	73.4	–	24.6	4.5	37.5	3.3
4-Ethylguaiacol	74.8	0.72	n.d.	–	21.3	4.1
Octanoic acid	76.1	0.61	2236.5	7.1	1870.7	8.4
δ -Nonalactone	74.35	0.81	12.2	8.7	26.3	7.1
<i>p</i> -Cresol	77.8	0.91	n.d.	–	2.3	6.2
Eugenol	81.6	0.93	4.2	4.4	16.6	7.2
4-Ethyl phenol	82.02	–	12.5	7.6	315.4	8.7
4-Vinylguaiacol	83.09	–	193.4	6.0	172.7	7.2
Decanoic Acid	86.2	0.68	406.2	8.0	331.8	7.8
Syringol	86.4	0.89	138.2	2.6	165.6	2.1
Isoeugenol	89.9	0.91	n.d.	–	8.2	7.3
Diethyl monosuccinate	91.35	–	11214.1	6.0	14302.9	7.3
Benzoic acid	93.01	–	43.7	8.4	79.7	6.7
Vanillin	100.06	0.83	12.4	8.6	37.7	8.7
Ethyl vanillate	104.3	0.83	137.2	4.6	170.3	4.0
3-Oxo- α -ionol	104.48	1.07	42.7	7.0	106.2	8.2
Acetovanillone	104.77	1.15	15.4	6.0	47.5	5.1
Propiovanillone	109.45	–	n.d.	–	22.0	6.8
Allyl syringol	112.7	0.84	Tr	–	6.5	7.0
Zingerone	118.88	–	4.7	8.8	11.6	7.6
Butyrovaniillone	120.11	–	15.2	5.8	52.6	4.1
Methylvanillyl ether	123.9	–	32.5	3.2	54.5	4.7

^a Reproducibility data for two Tempranillo wines. The extraction procedure was repeated three times. Tr: Traces. Concentration values ranged from 0.05 to 0.09. n.d.: (m/z) spectra were not detected.

typically associated with aged red wine aromas. *Reserva* wines with 12 and 14 months of barrel contact were independently grouped in the left part of the plot with negative scores in the first component, correlated with higher levels of (Z)-3-hexen-1-ol, octanoic acids and ethyl hexanoate.

The second Principal Component axis (PC-2) explains 14.1% of the total variation. PC-2 shows that *crianza* wines aged for 6, 7, 8

and 9 months, which were plotted in the top left part of the graph (Fig. 1), could be distinguished separately. A small percentage of wines stored for 6 and 7 months were plotted together due to the proximity of the wood contact periods. The compounds that correlated most strongly with this axis were α -terpineol, linalool, 2-phenylethyl acetate and isoamyl acetate with positive scores, and acetovanillone with negative score. As the PCA indicated, wines

Table 2 (Continued)

	Young wine (n = 32)	Crianza				Reserva				Gran reserva	
		6 months (n = 12)	7 months (n = 6)	8 months (n = 8)	9 months (n = 7)	12 months (n = 13)	14 months (n = 4)	16 months (n = 3)	18 months (n = 4)	24 months (n = 8)	36 months (n = 3)
Phenol	23.2 ^a ± 5.3	31.6 ^b ± 4.2	32.0 ^{bc} ± 3.2	36.9 ^{bc} ± 3.1	39.7 ^c ± 1.8	47.8 ^d ± 4.4	53.3 ^{de} ± 3.9	62.1 ^e ± 3.4	61.8 ^e ± 2.9	60.5 ^e ± 4.9	58.1 ^e ± 2.2
4-Ethylguaiaicol	n.d.	21.0 ^a ± 3.9	24.6 ^{ab} ± 5.0	34.1 ^b ± 4.8	37.8 ^b ± 3.5	49.8 ^c ± 5.0	54.2 ^{cd} ± 4.8	57.0 ^d ± 4.2	57.7 ^d ± 6.2	66.6 ^e ± 3.0	68.7 ^a ± 8.7
p-Cresol	n.d.	2.1 ^a ± 0.6	2.2 ^a ± 0.5	2.6 ^a ± 0.4	3.3 ^b ± 0.2	4.9 ^c ± 0.6	5.6 ^c ± 0.4	6.2 ^d ± 0.5	6.4 ^d ± 0.5	10.0 ^e ± 1.8	11.4 ^e ± 1.3
Eugenol	8.5 ^a ± 0.9	14.7 ^b ± 1.2	21.0 ^c ± 1.3	29.8 ^d ± 1.6	37.7 ^e ± 2.8	48.9 ^f ± 3.0	58.5 ^g ± 2.8	65.1 ^h ± 1.1	72.0 ⁱ ± 3.0	84.0 ^j ± 3.6	114.3 ^k ± 5.9
4-Ethyl phenol	20.4 ^a ± 5.7	185.2 ^b ± 89.2	179.7 ^b ± 78.2	162.0 ^b ± 89.5	157.2 ^b ± 97.3	137.7 ^b ± 93.7	138.6 ^b ± 84.3	128.8 ^b ± 67.7	134.8 ^b ± 82.3	244.0 ^c ± 83.6	150.7 ^b ± 93.7
4-Vinylguaiaicol	148.9 ^a ± 16.7	162.6 ^b ± 16.3	168.6 ^b ± 7.0	161.8 ^b ± 9.1	139.6 ^c ± 16.0	118.0 ^d ± 10.2	111.3 ^d ± 12.1	100.8 ^e ± 15.0	97.8 ^e ± 8.0	87.1 ^f ± 11.4	80.2 ^f ± 13.6
Syringol	129.8 ^a ± 14.6	165.5 ^b ± 10.8	168.1 ^a ± 9.4	174.5 ^b ± 9.2	209.8 ^c ± 20.3	264.1 ^d ± 18.1	279.6 ^e ± 29.6	300.9 ^f ± 12.7	332.0 ^g ± 7.3	368.3 ^h ± 24.6	378.7 ^h ± 23.1
Isoeugenol	n.d.	9.2 ^a ± 1.6	11.8 ^{ab} ± 1.4	13.9 ^b ± 0.9	18.1 ^c ± 2.2	26.2 ^d ± 2.6	29.0 ^d ± 1.3	35.4 ^e ± 2.2	35.4 ^e ± 4.1	31.5 ^{de} ± 3.4	28.7 ^d ± 3.6
Allyl syringol	Tr	3.3 ^a ± 0.9	7.7 ^b ± 1.0	12.0 ^c ± 1.2	17.5 ^d ± 1.8	24.3 ^e ± 2.5	31.6 ^f ± 1.5	39.2 ^g ± 2.9	47.5 ^h ± 2.7	56.3 ⁱ ± 1.6	64.3 ^j ± 3.8
C ₁₃ -norisoprenoids											
β-Damascenone	1.0 ^a ± 0.4	1.8 ^{bc} ± 0.5	1.9 ^{bc} ± 0.3	1.9 ^{bc} ± 0.6	2.3 ^b ± 0.3	1.4 ^c ± 0.5	1.2 ^{ac} ± 0.3	1.2 ^{ac} ± 0.3	0.7 ± 0.2	n.d.	n.d.
3-Oxo-α-ionol	49.6 ^a ± 13.7	110.0 ^b ± 11.0	116.7 ^b ± 11.8	119.7 ^b ± 3.3	133.2 ^c ± 5.4	148.0 ^d ± 7.6	153.1 ^e ± 8.2	156.8 ^e ± 3.4	158.3 ^e ± 5.2	149.4 ^d ± 8.8	135.9 ^c ± 8.4
Vanillin derivatives											
Vanillin	12.1 ^a ± 1.3	34.4 ^b ± 3.1	42.7 ^c ± 2.2	50.2 ^d ± 2.2	57.7 ^e ± 3.9	70.9 ^f ± 2.1	78.4 ^g ± 3.5	86.3 ^h ± 3.6	96.7 ⁱ ± 3.4	112.5 ^j ± 4.9	161.5 ^k ± 10.2
Ethyl vanillate	56.3 ^a ± 5.5	155.7 ^b ± 15.2	175.1 ^c ± 5.2	187.5 ^d ± 3.4	196.2 ^e ± 4.7	220.3 ^f ± 7.7	232.9 ^g ± 4.6	243.6 ^h ± 6.2	259.9 ⁱ ± 8.6	278.0 ^j ± 8.2	334.2 ^k ± 12.2
Acetovanillone	9.3 ^a ± 3.1	16.3 ^b ± 2.7	23.8 ^b ± 3.0	31.4 ^c ± 3.4	41.6 ^d ± 5.9	66.5 ^e ± 7.1	75.3 ^f ± 4.4	88.7 ^g ± 6.7	91.0 ^g ± 7.3	88.8 ^g ± 9.9	85.6 ^g ± 5.0
Propiovanillone	n.d.	11.9 ^a ± 1.2	18.4 ^b ± 2.3	26.2 ^c ± 1.5	33.5 ^d ± 2.5	45.6 ^e ± 3.1	53.9 ^f ± 3.6	65.0 ^g ± 4.4	73.7 ^h ± 3.4	84.1 ⁱ ± 2.8	93.7 ^j ± 4.1
Zingerone	5.3 ^a ± 2.3	11.6 ^b ± 2.7	14.2 ^b ± 2.4	14.8 ^b ± 3.0	21.6 ^c ± 1.6	25.2 ^{cd} ± 3.3	27.2 ^{cd} ± 5.2	32.6 ^d ± 4.3	32.3 ^d ± 3.1	27.6 ^{cd} ± 2.3	24.9 ^{cd} ± 3.4
Butyrovanillone	14.5 ^a ± 3.3	47.6 ^b ± 7.2	59.3 ^c ± 2.1	61.5 ^c ± 4.3	61.9 ^c ± 5.6	64.8 ^c ± 5.6	63.8 ^c ± 3.9	59.8 ^c ± 5.7	58.5 ^c ± 3.6	50.1 ^b ± 4.2	46.8 ^b ± 2.8
Methylvanillyl ether	31.8 ^a ± 6.6	55.2 ^b ± 7.5	57.0 ^b ± 7.7	67.6 ^c ± 2.8	69.0 ^c ± 11.8	66.1 ^{cd} ± 6.6	60.8 ^d ± 7.9	57.5 ^b ± 4.5	58.3 ^b ± 3.9	54.8 ^b ± 3.8	44.4 ^e ± 4.3
Miscellaneous											
Furfural	9.4 ^a ± 0.6	14.3 ^b ± 1.3	21.2 ^c ± 2.0	28.1 ^d ± 2.3	34.9 ^e ± 2.4	42.0 ^f ± 3.1	54.6 ^g ± 6.0	67.8 ^h ± 2.4	82.1 ⁱ ± 6.1	69.8 ^h ± 4.0	57.9 ^g ± 3.7
Succinate derivative	19.8 ^a ± 3.7	24.8 ^b ± 4.6	25.0 ^b ± 4.3	27.1 ^b ± 3.3	32.2 ^{bc} ± 3.4	33.2 ^{bc} ± 9.2	33.6 ^{bc} ± 6.5	36.3 ^c ± 4.8	36.4 ^c ± 4.7	40.8 ^{cd} ± 9.1	48.4 ^d ± 6.4
3-Methylthio-1-propanol	61.5 ^a ± 7.0	46.3 ^b ± 7.2	43.4 ^b ± 6.5	41.3 ^b ± 6.7	36.1 ^{bc} ± 4.3	32.2 ^c ± 7.3	31.2 ^c ± 3.5	25.4 ^{cd} ± 3.6	22.7 ^d ± 2.2	12.4 ^e ± 2.7	12.1 ^e ± 2.5

a–k: Different letters in the same row indicate statistical differences at $P < 0.05$ level according to the Student–Newman–Keuls test. Tr: Traces. Concentration values ranged from 0.05 to 0.09 n.d.: (m/z) spectra were not detected.

* Results expressed as mg/L.

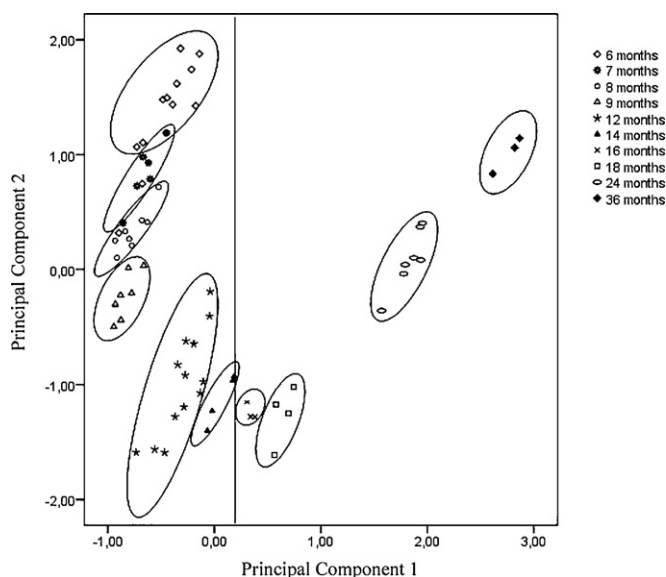


Fig. 1. Principal component analysis performed considering *crianza*, *reserva* and *gran reserva* Tempranillo wines from La-Mancha (D.O) aged for 10 time points among 6–36 months in woods barrels.

were classified according to the volatile profile linked to the ageing times, confirming that the method is effective for the purposes of classification.

3.2.2. Prediction of ageing time

Finally, stepwise multiple linear regression (SMLR) procedure was carried out in order to find usefully regression models to predict the ageing months for the 100 calibration Tempranillo wines using the volatile compounds. A model was obtained with eight independent variables that explain 98.96% of the variability in age with an r^2 of 0.989, a standard deviation of residuals of 0.343, and a mean error in prediction by cross validation (*RMSEP*) of 0.372.

Vanillin, eugenol, ethylvanillate, allyl syringol, furfural, α -terpineol, propiovanillone, and *trans*- β -methyl- γ -octalactone, was used as a criterion to predict the wood ageing months of La Mancha Tempranillo wines. When the model was applied to the set of samples it was found that the mean deviation of the predicted values was less than 0.40 months. Table 3 summarizes the results of the regression coefficients obtained using the whole data set of the standard aged wines using SMLR regression. The function obtained is consistent with the oxidative ageing to which the wines were subjected. The model establishes a strong relationship between the age and: (1) a cumulative linear increase of the wood extractable compounds in wines matured in oak barrels; (2) degradation of terpene as a consequence of long-term wood storage. Fig. 2 shows the graph of predicted versus observed age, in which the success of the

Table 3

Variables selected and regression coefficients (b_j) in the equation calculated for the prediction of wood ageing period of the standard Tempranillo wines using SMLR: $[t_i = b_0 + \sum_{j=1}^{m=8} b_j x_{ji}]$.

Variables	Regression coefficients (b_j)
Vanillin	0.100
Eugenol	0.092
Propiovanillone	0.062
<i>trans</i> - β -Methyl- γ -octalactone	0.062
Allyl syringol	0.050
Ethyl vanillate	0.012
Furfural	-0.098
α -Terpineol	0.240
Intercept (b_0)	-5.370

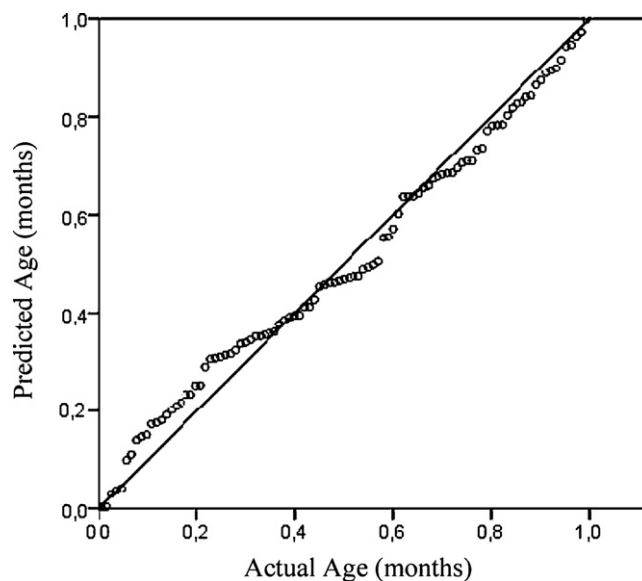


Fig. 2. Regression line drawn against the prediction obtained in the calibration model using SMLR (cross-validation).

model can be appreciated. These results constitute a great advance since they allow discriminate between Tempranillo aged wines belonging to the same market category processing exclusively a small number of volatile compounds.

4. Conclusions

Solid-phase extraction column-GC/MS method is suitable for identifying and quantifying a wide range of volatile compounds in aged wines present at minor and trace concentration. The proposed method offers good reproducibility and can be considered adequate for the purposes of the study of wine aroma.

When the method was applied to the analysis of one hundred varietal Tempranillo red wines significant quantitative differences among volatile profiles of *crianza*, *reserva* and *gran reserva* wines depending on the duration of wood ageing were observed. Results also discriminated among aged wines belonging to the same commercial category.

Using stepwise multiple linear regression (SMLR) procedure it was possible to predict the number of months of wood ageing of Tempranillo wines over periods ranging from 6 to 36 months using a function based on eight compounds which is consistent with the oxidative ageing to which the wines were subjected.

Acknowledgements

The authors thank to the Junta de Comunidades de Castilla La-Mancha (Consejería de Ciencia y Tecnología) for the financial support under the project P II 2109-0245-6646.

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