

Determination of Odorants in Varietal Wines from International Grape Cultivars (*Vitis vinifera*) Grown in NW Spain

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This work was carried out to investigate the odorants found in ten varietal wines from different international grape cultivars (Merlot, Cabernet Sauvignon, Pinot noir, Tempranillo, Sauvignon blanc, Riesling, Chardonnay, Pinot gris, Pinot blanc and Gewürztraminer) grown in northwest Spain. Monoterpenes, alcohols, fatty acids, ethyl esters, acetates and volatile phenols were determined by gas chromatography-mass spectrometry (GC-MS). The results showed that Gewürztraminer white wines had the highest concentration of volatile compounds (35.7 mg/L). Monoterpenes, linalool, terpineol, citronellol and nerol were detected only in Riesling and Gewürztraminer white wines. In the red wines, Cabernet Sauvignon followed by Merlot wines showed the highest concentration of total volatile composition (55.60 mg/L and 50.90 mg/L respectively), characterised by a higher concentration of alcohols. Based on the individual odour threshold, white Gewürztraminer and red Pinot noir wines showed the highest total OAV value. ANOVA has shown significant differences among wines. Principal component analysis performed a grouping of the monovarietal wines – *Sauvignon blanc-Pinot blanc-Riesling* and *Pinot gris gris-Gewürztraminer* in the white wines, and *Cabernet Sauvignon-Tempranillo* in the red wines.

INTRODUCTION

Flavour composition plays a significant role in the quality of a wine. The flavour of wine is composed of volatile compounds, which are responsible especially for the aroma (Schreier, 1979). More than 800 volatile compounds have been identified in wines, with a concentration range varying from hundreds of mg/L to the µg/L or ng/L level (Li, 2006). It is well known that the chemical compounds responsible for wine aroma are mainly alcohols, esters, volatile fatty acids, aldehydes and ketones, of which esters are particularly important (Rapp & Mandery, 1986). In addition, these mentioned compounds, *e.g.* terpenols and C₁₃ norisoprenoids, typically contribute to the character of wines.

Many authors have studied the volatile composition of different grapes and wines (Gunata *et al.*, 1985; Rapp & Mandery, 1986; Etievant, 1991; Ferreira *et al.*, 2000; Oliveira *et al.*, 2004; Swiegers & Pretorius, 2005; Vilanova & Sieiro, 2006, 2007). The amount of wine volatile compounds can be influenced by several factors, such as grape variety, the degree of ripeness, growing climate, fermentation conditions, and winemaking and ageing practices (Gomez *et al.*, 1994; Rapp, 1998; Bueno *et al.*, 2003; Oliveira *et al.*, 2006). International varieties, considered the most cultivated

varieties in the world (Robinson, 2006), have been studied in relation to different aspects during the last number of years. Recently, several authors studied wine volatile compounds from international cultivars grown in different geographic areas, namely Cabernet Sauvignon from China, California and Brazil (Falcao *et al.*, 2008; Preston *et al.*, 2008; Tao *et al.*, 2008; Jiang & Zhang, 2010), Sauvignon blanc from New Zealand, France and Australia (Berna *et al.*, 2009; Lund *et al.*, 2009), Chardonnay from Croatia and China (Hejavec *et al.*, 2007; Preston *et al.*, 2008; Falcao *et al.*, 2008; Berna *et al.*, 2009; Jiang & Zhang 2010), Tempranillo, Cabernet Sauvignon and Merlot from Spain (Ferreira *et al.*, 2000), and others. These studies have attempted to characterise the different wines from different *terroir* to show the influence of geographic area on wine composition, thus showing the versatility of these grape varieties.

The aim of this work was to define the major volatile compounds of young wines produced from international cultivars (Merlot, Cabernet Sauvignon, Tempranillo, Pinot noir, Sauvignon blanc, Riesling, Chardonnay, Pinot gris, Pinot blanc and Gewürztraminer) in Galicia (NW Spain) and to identify the most odour-active compounds.

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MATERIALS AND METHODS

Grape samples

Ten international *Vitis vinifera* grape cultivars – four red (Merlot, Cabernet Sauvignon, Tempranillo and Pinot noir) and six white (Sauvignon blanc, Riesling, Chardonnay, Pinot gris, Pinot blanc and Gewürztraminer) from NW Spain were considered in this study. The different cultivars, from the 2008 vintage, were grown in the same conditions in the vineyards of the Station of Viticulture and Enology (EVEGA) in Galicia. The must composition of the different cultivars is shown in Table 1. Total acidity, pH, density, reducing sugar and potential alcohol were analysed in the musts before fermentation according to official OIV (1990) methods.

Must fermentation

The wines analysed in this study were elaborated in the EVEGA winery. White and red wines were produced in 16 L glass vessels, in triplicate. Before fermentation, sulphur dioxide (4 g/hL) and ascorbic acid (5 g/hL) were added to the musts. The wines were elaborated using standard winemaking practices. The fermentation of white cultivars was conducted by a yeast strain from the Institut Œnologique de Champagne, *Saccharomyces cerevisiae* var. *bayanus*, IOC-18-2007 (20 g/hL) at 18°C. *Saccharomyces cerevisiae* var. *cerevisiae* 71 B from Lalvin, INRA (30 g/hL) at 28°C was used to ferment musts from the red cultivars. Malolactic fermentation was performed with the red wines. After fermentation the wines were filtered and transferred to 0.75 L bottles. The bottles were stopped with cork and stored at 16°C until analysis.

Total acidity, density, ethanol, tartaric, acetic and malic acids, and the Folin-Ciocalteu index were determined after fermentation. All analyses were performed in triplicate according to official OIV (1990) methods.

Volatile compound extraction and analysis

All solvents were analytical grade and further purified. In a 10 mL culture tube (Pyrex, ref. 1636/26MP), 8 mL of wine, 2.4 µg of internal standard (4-nonanol, Merck ref. 818773, Darmstadt, Germany) and a magnetic stir bar (22.2 mm x 4.8 mm) were added. Extraction of volatiles was done by

stirring the sample with 400 µL of dichloromethane (Merck, ref. 1.06054; Darmstadt, Germany), according to the method of Oliveira *et al.* (2006). After cooling at 0°C for 10 min, the magnetic stir bar was removed and the organic phase was detached by centrifugation (RCF = 5 118, 5 min, 4°C) and the extract was recovered into a vial, using a Pasteur pipette. The aromatic extract (200 µg/L) was dried with anhydrous sodium sulphate (Merck, ref. 1.06649; Darmstadt, Germany) and placed in a new vial. Extractions of volatile compounds from each of the respective wines were performed in triplicate.

A Chrompack CP-9000 gas chromatograph equipped with a split/splitless injector and a flame ionisation detector (FID) with a capillary column, coated with CP-Wax 52 CB (50 m x 0.25 mm i.d., 0.2 µm film thickness, Chrompack), was used. The temperature of the injector and detector were both set to 250°C. The oven temperature was held at 40°C for 5 min, then programmed to rise from 40°C to 235°C, at 3°C min⁻¹, and then finally programmed from 235°C to 255°C, at 5°C min⁻¹. The carrier gas was helium 55 (Praxair; Teruel, Spain) at 103 kPa, and the split vent was set to 13 mL/min. Each 3 µL extract was injected in the splitless mode (vent time 15 s). Quantification of volatiles, as 4-nonanol equivalents, was performed with Varian MS Workstation version 6.6 by comparing the retention indices with those of pure standard compounds and confirming these by GC-MS (Oliveira *et al.*, 2008).

Odour activity value

The odour activity value (OAV) was determined to evaluate the contribution of a chemical compound to the aroma of a wine. OAV is a measure of the importance of a specific compound to the odour of a sample. OAV > 1 indicates possible contribution to the wine aroma. This was calculated as the ratio between the concentration of the individual compound and the perception threshold found in the literature (Etievant, 1991; Ferreira *et al.*, 2000; Francis & Newton, 2005; Vilanova *et al.*, 2009).

Statistical analyses

The data analyses were performed using XLstat-Pro (Addinsoft). The data were subjected to one-way analysis of

TABLE 1
General chemical parameters of red and white musts from international cultivars

Musts	Chemical parameters				
	Total acidity/(g/L)	pH	Density/(g/mL)	Reducing sugar/(g/L)	Probably degree/(% v/v)
Red varieties					
Cabernet-sauvignon	5.02	3.57	1.089	212.0	12.47
Pinot noir	5.62	3.75	1.100	238.7	14.04
Tempranillo	4.50	3.58	1.087	206.7	12.16
Merlot	4.50	3.69	1.097	232.4	13.67
White varieties					
Chardonnay	4.15	3.29	1.098	235.8	13.87
Pinot blanc	5.47	3.44	1.080	187.4	11.02
Pinot gris	4.50	3.61	1.100	238.5	14.03
Riesling	6.67	3.15	1.076	177.3	10.43
Sauvignon blanc	7.20	3.30	1.094	225.6	13.27
Gewürztraminer	2.84	3.90	1.097	231.4	13.61

variance (ANOVA). The mean differences between cultivars were calculated according to the least significant difference from Fisher's test (LSD), with a confidence interval of 95%. Principal component analysis (PCA) was applied to the wine volatile compounds to study the possible grouping of the varietal wines to investigate correlations between cultivars and volatile compounds.

RESULTS AND DISCUSSION

Classical parameters

Table 1 shows the chemical composition of the must from international cultivars. In the red cultivars, Pinot noir must showed the highest values for the chemical parameters analysed. In the white cultivars, Pinot gris and Chardonnay musts showed the highest sugar content, thus high technology maturity was presented by these two cultivars. The total acidity was higher for Sauvignon blanc and Riesling musts, while the lowest total acidity was found for Gewürztraminer (2.84 g/L).

The general composition of the wines is showed in Table 2. In the red wines, Pinot noir and Merlot presented the highest values of ethanol (13.5% v/v and 13.1% v/v respectively) and the lowest values of tartaric acid. However, Tempranillo and Merlot showed the lowest total acidity values. Similar values for the Folin-Ciocalteu index were found among the wines, with the highest concentrations found in Cabernet Sauvignon (45.7) and Merlot (45.8). In the white wines, Pinot gris, Gewürztraminer and Chardonnay showed the highest ethanol concentration (14.6% v/v, 14.3% v/v and 14.1% v/v respectively). In terms of total acidity and tartaric acid, the highest values were found in Riesling wines (6.50 g/L and 3.8 g/L respectively) and in Sauvignon blanc (6.70 g/L and 3.3 g/L respectively). Sauvignon blanc also showed the highest value for malic acid (2.0 g/L).

Volatile composition of wines

Tables 3 and 4 show the mean concentration and standard deviation of the volatile compounds determined in the white and red wines. In the white wines (Table 3), 28 volatile compounds were identified – four monoterpenes, three C₆

compounds, four alcohols, eight ethyl esters and acetates, seven volatile fatty acids and two volatile phenols. Many of these volatile compounds are commonly found in wines and are derived from the grapes and the vinification process (Cliff *et al.*, 2002). Gewürztraminer wines showed the highest concentration of volatile compounds (35.7 mg/L), represented by the highest levels of monoterpenic alcohols (0.83%), ethyl esters and acetates (24%), volatile fatty acids (25.7%) and volatile phenols (0.2%). *Vitis vinifera* L. cv. Gewürztraminer is an aroma-rich grape cultivar in which terpene constituents are mainly responsible for the flavour attributes (Marais, 1987).

The results obtained from the ANOVA indicate that, of the 28 compounds quantified in the white wines, only 1-hexanol and ethyl 3-methylbutyrate did not show significant differences between the wines tested. Regarding the red wines, 22 volatile compounds were identified and quantified – three C₆ compounds, three alcohols, nine ethyl esters and acetates, and seven volatile fatty acids (Table 4). Significant differences among wines were found for all the volatile compounds. Cabernet Sauvignon, followed by Merlot wines, showed the highest concentration of total volatile composition (55.60 mg/L and 50.90 mg/L respectively).

Alcohols

Alcohols are produced mainly during yeast fermentation of sugars and yeast metabolism of amino acids, and their aroma contribution to wine is not considered to be particularly pleasant, with the exception of 2-phenylethanol (rose-like aroma) (Simpson, 1979; Etievant, 1991). The alcohol chemical family was the largest volatile group in the white wines, showing values between 62.8% for Chardonnay and 49% for Gewürztraminer wine. 2-Phenylethanol was the compound that was present at the highest concentration in all white wines. This compound can be present in the grapes, but it is largely produced by wine yeast during fermentation (Quian *et al.*, 2009). In the red wines studied, alcohols were also the largest group, showing the highest concentration in the Cabernet Sauvignon and Merlot wines, accounting for 88% and 89% of the total volatile concentration respectively.

TABLE 2

General chemical composition of wines from international red and white varieties.

Wines	Chemical parameters						
	Total acidity/ (g/L)	Volatile acidity/ (g/L)	Density (g/mL)	Ethanol/ (% v/v)	Malic acid/ (g/L)	Tartaric acid/ (g/L)	Folin index
Red varieties							
Cabernet-sauvignon	5.00	0.30	0.993	11.8	< 0.3	1.9	45.7
Pinot noir	4.60	0.40	0.992	13.5	1.3	1.3	41.3
Tempranillo	3.60	0.40	0.992	11.6	< 0.3	1.9	42.5
Merlot	3.90	0.30	0.992	13.1	< 0.3	1.8	45.8
White varieties							
Chardonnay	5.80	0.40	0.990	14.1	1.8	2.0	-
Pinot blanc	5.00	0.30	0.990	11.6	1.8	2.4	-
Pinot gris	4.40	0.50	0.988	14.6	1.6	1.3	-
Riesling	6.50	0.40	0.991	11.0	1.2	3.8	-
Sauvignon blanc	6.70	0.40	0.989	13.6	2.0	3.3	-
Gewürztraminer	2.90	0.40	0.987	14.3	0.6	1.4	-

TABLE 3
Volatile composition ($\mu\text{g/L}$) of white wines from international cultivars from NW Spain

Compounds	Riesling		Pinot Blanc		Pinot gris		Gewürztraminer		Chardonnay		Sauvignon blanc		Sig.
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Monoterpenic alcohols													
Linalool	16.41b	0.89	0.81c	1.40	NDc	ND	189.50a	18.97	NDc	ND	NDc	ND	***
α -Terpineol	45.54a	1.48	8.39d	0.46	13.42c	2.98	37.42b	2.75	NDe	ND	NDe	ND	***
Citronellol	2.46b	0.29	NDc	ND	NDc	ND	30.61a	3.22	NDc	ND	NDc	ND	***
Nerol	0.18b	0.31	1.02b	1.76	NDb	ND	82.42a	2.74	NDb	ND	NDb	ND	***
C₆-compounds													
1-hexanol	195.06	14.98	201.53	143.88	243.60	24.78	308.24	44.39	274.59	99.22	390.41	37.65	ns
(E)-2-hexen-1-ol	5.37b	0.65	6.48b	0.89	NDc	ND	15.55a	2.87	NDc	ND	17.91a	3.27	***
(Z)-2-hexen-1-ol	3.21c	1.12	11.57b	1.10	NDd	ND	NDd	ND	NDd	ND	15.19a	2.35	***
Alcohols													
1-Butanol	8.96b	1.23	16.13b	1.32	56.20a	10.06	76.98a	9.13	67.58a	35.22	59.34ba	1.78	***
3-Methyl-1-pentanol	44.02b	3.35	28.11b	0.97	55.19b	13.60	48.57b	7.31	110.02a	42.07	28.63b	5.35	**
Methionol	23.89b	2.32	20.98c	2.16	30.68bc	3.68	44.52a	7.90	44.85a	9.29	NDd	ND	***
2-Phenylethanol	10271.83b	864.32	8647.40b	296.94	9468.81b	1060.72	17990.16a	2236.10	18660.54a	1025.62	6140.79c	981.77	***
Ethyl esters and acetates													
Ethyl 2-methylbutyrate	11.33b	0.55	5.25c	0.25	13.06b	1.20	19.86a	2.66	0.00d	0.00	11.07b	0.66	***
Ethyl 3-methylbutyrate	21.97	1.07	8.44	0.41	15.76	0.85	8.22	0.87	29.78	17.26	21.98	3.63	ns
Isoamyl acetate	110.73d	5.09	693.20c	13.07	1763.67b	90.13	2604.88a	287.00	659.08c	247.37	1479.70b	83.05	***
Ethyl hexanoate	631.97d	31.19	795.65c	18.76	1215.17b	77.09	1538.00a	206.36	935.27c	197.04	868.77c	49.66	***
Hexyl acetate	7.29d	0.48	31.57b	0.74	39.67a	1.24	20.55c	2.98	0.00e	0.00	40.93a	4.27	***
Ethyl octanoate	946.93cd	35.23	1093.58bc	27.12	1125.93b	113.10	1473.8a3	108.19	234.52e	135.01	916.58d	74.22	***
Ethyl decanoate	173.12d	7.15	185.52cd	16.64	218.81b	28.53	273.78a	13.20	18.44e	5.57	213.51bc	18.89	***
2-Phenylethyl acetate	33.76c	2.30	72.70c	2.79	162.48b	61.49	358.36a	21.47	147.16b	1.47	130.94b	13.33	***
Volatile fatty acids													
2 + 3-methylbutyric acid	56.18b	4.59	43.52b	2.46	122.73a	11.69	123.47a	26.45	122.47a	15.45	64.55b	13.56	***
Butyric acid	371.23c	36.30	345.04c	16.35	1004.20a	125.44	615.12b	139.94	973.63a	178.30	673.75b	106.24	***
Hexanoic acid	1356.86c	118.49	1455.08bc	85.07	1904.41ab	171.99	2327.73a	606.32	1489.38bc	80.27	1102.92c	267.45	**
Octanoic acid	5594.06ab	242.51	5263.31bc	261.17	4604.96c	241.97	6061.82a	802.64	3783.14d	371.23	3293.69d	463.89	***
Decanoic acid	1110.79b	31.70	1176.11b	73.69	1211.79ab	70.89	1331.82a	89.40	839.94c	103.63	1159.21b	86.91	***
Dodecanoic acid	21.45c	2.63	29.10c	8.30	NDc	ND	90.13b	16.00	NDc	ND	199.47a	75.28	***
Volatile phenols													
Guaiacol	2.00a	1.16	ND	ND	NDb	ND	NDb	ND	NDb	ND	NDb	ND	*
4-Vinylguaiacol	16.89b	1.38	ND	ND	NDc	ND	85.01a	10.24	NDc	ND	NDc	ND	***

Notes: *, ** and *** indicate significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively. ns indicates no significant difference. Values with different Roman letters (a to d) in the same row are significantly different according to Fisher's test ($p < 0.05$)

TABLE 4
Volatile composition ($\mu\text{g/L}$) of red wines from international cultivars in NW Spain.

Compounds	Tempranillo		Pinot noir		Merlot		Cabernet Sauvignon		Sig.
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
C₆-compounds									
1-Hexanol	1025.62b	81.39	265.15d	5.85	724.63c	25.06	1337.97a	48.52	***
(E)-3-hexen-1-ol	9.91a	5.85	6.48a	0.89	11.90a	2.30	NDb	ND	*
(Z)-3-hexen-1-ol	196.18a	18.97	11.57b	1.10	3.31b	3.46	32.50b	18.78	***
Alcohols									
3-Methyl-1-pentanol	336.78a	26.40	28.11c	0.97	273.40b	7.17	302.71b	17.11	***
Methionol	461.67c	41.97	20.98d	2.16	890.34a	14.23	659.09ab	67.02	***
2-Phenylethanol	41183.79b	2927.39	8647.40c	296.94	44284.54ab	2849.82	48357.01ab	3421.43	***
Ethyl esters and acetates									
Ethyl butyrate	587.08b	29.02	411.53c	45.84	751.03ab	20.90	858.52a	165.22	**
Ethyl 2-methylbutyrate	7.52b	4.35	5.25b	0.25	27.17a	0.87	27.27ab	15.74	*
Ethyl 3-methylbutyrate	6.81b	3.98	8.37b	0.32	28.38a	0.94	25.75ab	14.92	*
Isoamyl acetate	1221.05a	14.02	693.20c	13.07	1027.29b	16.26	1186.32a	51.52	***
Ethyl hexanoate	250.84b	3.61	795.66a	18.76	167.55d	1.05	195.42c	8.72	***
Hexyl acetate	NDc	ND	31.57b	0.74	45.66a	0.86	NDc	ND	***
Ethyl octanoate	212.72b	10.20	1093.58a	27.12	116.25d	1.28	162.24c	14.50	***
Ethyl decanoate	25.89b	3.21	185.52a	16.64	15.35b	1.19	24.33b	14.05	***
2-Phenylethyl acetate	181.08b	16.77	72.70d	2.79	120.64c	5.03	216.88a	14.99	***
Volatile fatty acids									
2 + 3 Methylbutyric acid	144.34c	20.08	43.52d	2.46	338.39a	9.15	223.49b	75.99	***
Butyric acid	601.04b	60.11	231.60c	198.91	978.88a	10.44	710.14b	78.67	***
Hexanoic acid	558.95b	49.11	1455.08a	85.07	337.21c	4.21	304.06d	84.26	***
Octanoic acid	1121.83b	59.81	5358.25a	173.75	644.56c	5.03	792.23c	154.09	***
Decanoic acid	159.13b	16.14	1175.83a	73.20	120.66b	12.90	189.41b	72.32	***
Dodecanoic acid	NDb	ND	37.15a	7.83	NDb	ND	NDb	ND	***

Notes: *, ** and *** indicate significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively.
Values with different roman letters (a to d) in the same row are significantly different according to Fisher's test ($p < 0.05$)

Studies performed by Tao *et al.* (2008) reported high values for higher alcohols and ethyl esters in Cabernet Sauvignon wines from China. 2-Phenylethanol was the compound that presented the highest concentration in all of the red wines, with the highest concentration found in Cabernet Sauvignon wines (48.36 mg/L), followed by Merlot (44.28 mg/L). Methionol was also present at a high concentration in the Merlot and Cabernet Sauvignon wines.

C₆ compounds

C₆ compounds showed the highest levels in Sauvignon blanc wine (423.51 µg/L), with the highest value for 1-hexanol (390.41 µg/L). This compound is known as “leaf” alcohol and has a “grassy” flavour (Berna *et al.*, 2009). In the red wines, 1-hexanol showed high values in Cabernet Sauvignon (1370.49 µg/L) and Tempranillo (1231.71 µg/L) wines; however, 1-hexanol was only present in trace amounts in the Cabernet Sauvignon from the Loess Plateau region of China (Jiang & Zhang, 2010). C₆ compounds are partly responsible for the green and herbaceous aroma of grapes and wines (Gómez *et al.*, 1995). High water status, especially during the later stages of ripening, could have negative effects on wine aroma. Too much water results in wine with more vegetal, bell pepper and herbaceous aroma (Quián *et al.*, 2009). In our study, the rainfall in late September and October (the last weeks of maturity) was high, above 500 mm, thus affecting the herbaceous character in all the red cultivars, especially the Cabernet Sauvignon wine. Cabernet Sauvignon has its origin in the Bordeaux region of France, but now is planted all over the world. Research shows that the aroma profiles of Merlot and Cabernet Sauvignon wines in Bordeaux are very close (Allen *et al.*, 1994). The aroma of these wines is often described as fruity or floral, with roasted, wood-smoke, cooked meat nuances, and often as herbaceous, especially the wines of Cabernet Sauvignon (Allen *et al.*, 1994). At low levels, vegetative aromas such as bell pepper or asparagus contribute to the distinctive varietal aromas of Cabernet Sauvignon and Merlot wines, and are associated with different volatile compounds (Preston *et al.*, 2008). Other authors have also indicated that young red wines of Cabernet Sauvignon and Merlot have similar aromatic characteristics, and they concluded that differences between these varieties are quantitative rather than qualitative (Gómez *et al.*, 1994; López *et al.*, 1999).

Monoterpenes

Terpene compounds belong to the secondary plant constituents, of which the biosynthesis begins with acetyl-coenzyme A (CoA) (Manitto, 1980). Monoterpenic alcohols like linalool, geraniol, nerol, citronellol and α -terpineol are known to be important compounds in grape berries, and are responsible for floral notes in the aromatic grape varieties Muscat, Riesling, Viognier, Loureiro and Gewürztraminer, as well as other varieties not usually considered to be floral, including Pinot gris and Chardonnay (Arrhenius *et al.*, 1996; Swiegers & Pretorius, 2005; Luan *et al.*, 2006; Oliveira *et al.*, 2008). In our study, the Gewürztraminer wines showed the highest concentrations of linalool, citronellol and nerol. Citronellol was only present in Riesling and Gewürztraminer white wines. Linalool is considered a very

important compound, in both the free and bound form, in Gewürztraminer wines from the Maule Valley (Chile), which is classified as having a Mediterranean climate (Agosin *et al.*, 1994). Gewürztraminer produced in the Alsace region showed differences in the levels of the three monoterpenes (*cis*-rose oxide, linalool, and geraniol) when compared to those from New York State, which could be attributed to differences in viticultural and oenological practices between these regions (Peter *et al.*, 1999). Linalool, geraniol and citronellol have often been considered important contributors to the aroma of Gewürztraminer (Marais, 1987). These authors suggested that these terpenes were important to the typical character and quality of Gewürztraminer wines. α -Terpineol showed the highest value for Riesling wines; however, terpenes have not been identified in Chardonnay and Sauvignon blanc wines and low values of these compounds were found in Pinot blanc (10.22 µg/L) and Pinot gris (13.42 µg/L) wines. Another study determined that terpenes are not considered to contribute to Chardonnay wines, or else their concentration was very low (Arrhenius *et al.*, 1996).

Ethyl esters and acetates

Acetates and esters are formed by the reaction of acetyl-CoA with higher alcohols formed by the degradation of amino acids or carbohydrates (Etievant, 1991). Esters in wine mainly originate from yeast metabolism during fermentation, but some esters are also found in small amounts in the grape berry (Perestrelo *et al.*, 2006). The yeast strain chosen for fermentation and the fermentation conditions will influence the concentration and types of esters formed (Rapp & Mandery, 1986). Only a few esters, occurring in small quantities in grapes, contribute to the aroma of *Vitis vinifera* varieties. They are mainly acetates of short-chain alcohols (Swiegers & Pretorius, 2005). The present research found significant differences in esters between among cultivars, with the exception of ethyl 3-methylbutyrate. Gewürztraminer wines showed the highest concentrations of these compounds (6297.49 µg/L), with the exception of hexyl acetate, followed by Pinot gris (4554.55 µg/L). Hexyl acetate and ethyl 2-methylbutyrate were absent from Chardonnay wines. Research on Californian Chardonnay wines showed that the fruity and floral terms were associated with 2-phenylethyl acetate (Lee & Noble, 2003). In the red wines, ethyl butyrate, ethyl 2-methylbutyrate, ethyl 3-methylbutyrate, isoamyl acetate, ethyl hexanoate and ethyl octanoate made an important contribution to the fruity character of the wines (Meilgaard, 1975; Escudero *et al.*, 2004; Li *et al.*, 2008). In our study, Pinot noir showed the highest concentration of ethyl esters and acetates, with ethyl octanoate and ethyl hexanoate showing the major concentrations in this cultivar. Isoamyl acetate showed the highest concentration in Tempranillo wine. This result is in concordance with studies undertaken by Ferreira *et al.* (2000) on Tempranillo wine elaborated with grapes grown in a Mediterranean climate. Hexyl acetate was absent from Tempranillo and Cabernet Sauvignon.

Volatile fatty acids

Although there are many different types of acid in wine, the fatty acids are considered to be the most likely of this class

of compounds to contribute to the aroma of wine (Etievant, 1991). Fatty acids are believed to originate primarily from yeast and bacteria biosynthesis during the fermentation stage of winemaking (Etievant, 1991). Volatile fatty acids showed the highest values for Gewürztraminer wines (10550.09 µg/L), followed by Pinot gris (8848.10 µg/L), but with the exception of butyric acid and dodecanoic acid. Dodecanoic acid was absent from Pinot gris and Chardonnay. The major compound in all white wines was octanoic acid, showing the highest concentration in Gewürztraminer wines. In the red wines, Pinot noir was characterised by high values of the volatile fatty acid family, reaching 40% of total volatile composition and characterised by high values of octanoic, decanoic and hexanoic acids. Pinot noir originated from the Burgundy region of France and has become popular in the United States of America, especially in Oregon. It exhibits distinct red fruity aromas, showing particularly odours of small-stone fruits (plum and cherry). However, the grape requires a long, cool growing season to develop the right flavour attributes (Fang & Quian, 2006). Dodecanoic acid was absent from all wines with the exception of Pinot noir.

Volatile phenols

Volatile phenols bearing a vinyl radical and an ethyl radical are abundant in white and red wine respectively. Volatile phenols are formed from phenolcarboxylic acids (ferulic and coumaric) by sequential decarboxylation and reduction of the vinyl group to the ethyl group. Wine yeasts are incapable of reducing the vinyl group actively (Shimizu *et al.*, 1982).

Concerning the volatile phenols, only Riesling wines showed clear signs of guaiacol (2 µg/L). Similar concentrations of guaiacol have been found in Australian Riesling wines (0.2 to 2 µg/L) (Smyth *et al.*, 2008). 4-Vinylguaiacol was only present in the Riesling and Gewürztraminer wines (16.89 and 85.01 µg/L respectively). 4-Vinylguaiacol has been detected as an active odorant in Rhine Riesling wine (Komes *et al.*, 2006), Niagara Peninsula (Bowen & Reynolds, 2012) and Australian Riesling wines (Smyth *et al.*, 2008). This compound is responsible for the spice and smoke notes in wines from the Gewürztraminer cultivar (Versini *et al.*, 1999). Volatile phenols were not detected in the red wines.

Principal component analysis (PCA)

The principal component analyses (PCA) performed on the wine volatile concentration is shown in Fig. 1. PCA provides a visual representation of the relationship between the wines based on their volatile composition.

With regard to the white wines (Fig. 1A), the first two principal components (PC1 and PC2) accounted for 69.19% of the variance (42.26% and 26.93% respectively). The first component (PC1) was characterised by a higher concentration of nerol, linalool, citronellol, ethyl 2-methylbutyrate, isoamyl acetate, ethyl hexanoate, 2-phenylethyl acetate, ethyl hexanoate, hexanoic acid and 4-vinylguaiacol in the positive loading. For the second principal component (PC2), the volatile compounds 3-methyl-1-pentanol, methionol and 2-phenylethanol showed positive loading. The resulting graphs illustrate relationships between wines. A good separation among four wine groups was observed

in the PCA. The first group, situated on the negative side of PC1, is formed by the Riesling, Pinot blanc and Sauvignon blanc wines. A second group is formed by the Pinot gris and Gewürztraminer wines, located on the positive side of PC1 and PC2. Finally, a third, isolated group of Chardonnay wines is located at high and positive values in PC2.

The PCA performed on the red wine volatile compounds is shown in Fig. 1B. The two principal components (PC1 and PC2) accounted for 90.14% of the variance (72.35% and 17.78% respectively). The first principal component (PC1) was characterised by higher concentrations of ethyl hexanoate, ethyl octanoate, hexanoic acid, decanoic acid and dodecanoic acid in the positive loading of PC1, and 1-hexanol, 3-methyl-1-pentanol, methionol, 2-phenylethanol, ethyl butyrate, isoamyl acetate, 2+3 methylbutyric acid and butyric acid in the negative loading of the same axis. The second principal component (PC2) showed hexyl acetate in the positive loading and (*Z*)-3-hexen-1-ol in the negative loading of the same axis. Three groups can be visualised in the PCA: Cabernet Sauvignon and Tempranillo formed one group on the negative side of PC1; a second group, formed by Merlot wines, is also located on the negative side of the same axis in PC1. The third group was formed by Pinot noir, on the positive side of the PC1 and PC2 axes.

Odour activity value (OAV)

In order to assess the influence of the compounds studied on overall wine aroma, the odour activity value (OAV) was calculated by dividing the concentration of each compound by its perception threshold. Only the compounds with an OAV greater than 1 contribute individually to the wine aroma (Guth, 1997). When the OAV of a particular compound is less than 1, however, it might contribute to the aroma of a wine because of the additive effect of similar compounds (similar structure or odour) (Francis & Newton, 2005).

The odour descriptor, OAV and threshold for each compound analysed is listed in Table 5. Fifteen volatile compounds (54%) identified in the white wines and fourteen in the red wines (64%) presented an OAV > 1, contributing to the aroma of the wines analysed. Gómez-Míguez *et al.* (2007) found, for Zalema wines, that only 32% of the quantified volatile components were present at concentrations higher than their corresponding threshold values (OAVs > 1). Other authors (López *et al.*, 1999; Escudero *et al.*, 2004; Jiang & Zhang, 2010) have shown similar results in other young white and rosé wines. Ethyl-3-methylbutyrate, isoamyl acetate, ethyl hexanoate, butyric acid, hexanoic acid and octanoic acid contributed to the wine aroma of all the white cultivars studied. The Pinot gris and Sauvignon blanc wines had the same odorants with OAVs above 1, and Gewürztraminer contained the most odour-active compounds, suggesting it to be the white wine with the most complex flavour. The floral character was represented by only two compounds with an OAV > 1. Linalool only contributed to the floral character of the Gewürztraminer wines, and 2-phenylethanol only seemed to contribute to the same character in the Chardonnay, Riesling and Gewürztraminer wines, because their concentrations were above the odour threshold.

In the red wines, Merlot and Cabernet Sauvignon showed the same odorants with OAVs > 1, and the highest OAV for

both wines was ethyl butyrate. The most intense odorants in Merlot and Cabernet Sauvignon wines produced in California and Australia are 3-methyl-1-butanol, 3-hydroxy-2-butanone, octanal, ethyl hexanoate, 2-methoxyphenol, 4-ethenyl-2-methoxy-phenol, ethyl 3-methylbutanoate, ethyl 3-methylbutanoate and 2-phenylethanol (Gürbüz *et al.*, 2006; Tao *et al.*, 2008). Other authors have shown that the aroma profiles obtained by sensory analysis of Merlot and Cabernet Sauvignon wines from the Bordeaux region were very similar, characterised by high fruity, caramel, green and earthy aroma totals (Kortseridis *et al.*, 2000; Gürbüz *et al.*, 2006). The highest OAV was shown

in Pinot noir (ethyl octanoate, 218.72), followed by that in Tempranillo (ethyl octanoate, 42.54), and only the caramel descriptor showed a difference in intensity between the wines. Octanoic acid also made an important contribution to the aroma of all monovarietal wines. However, hexanoic acid only contributed to the aroma of Tempranillo and Pinot noir wines. Hexanoic and octanoic acids are important wine odorants, but they are mostly formed as by products of yeast metabolism, and the contribution of grape precursors is residual from a quantitative point of view (López *et al.*, 2004).

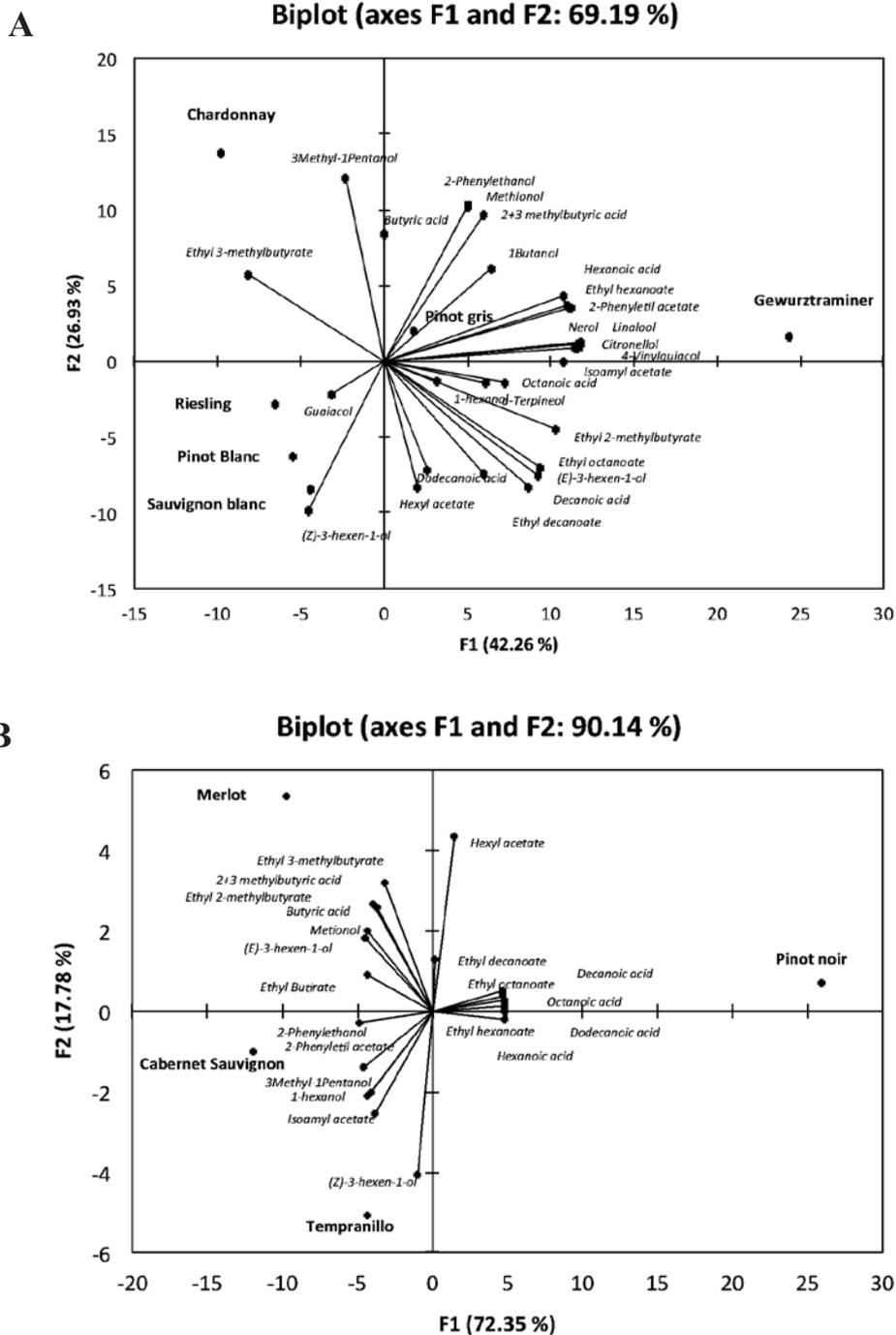


FIGURE 1
Principal component analysis (PCA) based on the volatile composition of A) white wines and B) red wines.

TABLE 5
 Odour activity values for white and red varietal wines. OAV > 1 indicated in bold

Volatile compounds	Descriptor	Threshold $\mu\text{g/L}$	OAV in white wines										OAV in red wines			
			Riesling	Pinot Blanc	Pinot gris	Gewürztraminer	Chardonnay	Sauvignon blanc	Tempranillo	Pinot noir	Merlot	Cabernet Sauvignon				
Linalool	Floral, citrus	25	0.66	0.03	0.00	7.58	0.00	0.00	-	-	-	-	-	-	-	
2-phenylethanol	Rose, sweetish	10000	1.03	0.86	0.95	1.80	1.87	0.61	4.12	0.86	4.43	4.84				
Ethyl 2-methylbutyrate	Fruity	18	0.63	0.29	0.73	1.10	0.00	0.61	0.42	0.29	1.51	1.51				
Ethyl 3-methylbutyrate	Fruity, apple	3	7.32	2.81	5.25	2.74	9.93	7.33	2.27	2.79	9.46	8.58				
Isoamyl acetate	Banana	30	3.69	23.11	58.79	86.83	21.97	49.32	40.70	23.11	34.24	39.54				
Ethyl hexanoate	Fruity, strawberry	65	9.72	12.24	18.69	23.66	14.39	13.37	17.92	56.83	11.97	13.96				
Ethyl octanoate	Soap	580	1.63	1.89	1.94	2.54	0.40	1.58	42.54	218.72	23.25	32.45				
Ethyl decanoate	Grape	200	0.87	0.93	1.09	1.37	0.09	1.07	0.13	0.93	0.08	0.12				
2-phenylethyl acetate	Roses	250	0.14	0.29	0.65	1.43	0.59	0.52	0.72	0.29	0.48	0.87				
Butyric acid	Cheese	33	11.25	10.46	30.43	18.64	29.50	20.42	3.47	1.34	5.66	4.10				
Hexanoic acid	Cheese	420	3.23	3.46	4.53	5.54	3.55	2.63	1.33	3.46	0.80	0.72				
Octanoic acid	Rancid	500	11.19	10.53	9.21	12.12	7.57	6.59	2.24	10.72	1.29	1.58				
Decanoic acid	Rancid	1000	1.11	1.18	1.21	1.33	0.84	1.16	0.16	1.18	0.12	0.19				
4-vinylguaiacol	Clove, curry	40	0.42	0.00	0.00	2.13	0.00	0.00	-	-	-	-				
Ethyl butyrate	Papaya, butter	20	-	-	-	-	-	-	29.35	20.58	37.55	42.93				
2+3 methylbutyric acid	Vomit, cheese	173	0.32	0.25	0.71	0.71	0.71	0.37	4.25	1.28	9.95	6.57				

Descriptors and flavour thresholds of volatile compounds found in the literature (Etievant, 1991; Ferreira et al., 2000; Francis & Newton, 2005; Vilanova et al., 2009).

CONCLUSIONS

The formation of volatile compounds in wines is a complex process and depends on grape composition and the fermentation process. This work provides better knowledge of the volatile composition of wines made from international red and white cultivars (Merlot, Cabernet Sauvignon, Pinot noir, Tempranillo, Sauvignon blanc, Riesling, Chardonnay, Pinot gris, Pinot blanc and Gewürztraminer) grown in NW Spain.

The results obtained show that international cultivars can be characterised and grouped by their volatile composition. Most of the volatiles studied are minor compounds, present in low concentrations. Gewürztraminer showed the highest concentration of volatile compounds. In the red wines the highest concentration of volatile compounds was found in Cabernet Sauvignon, followed by Merlot wines. PCA showed high similarities for the volatile composition of Sauvignon blanc-Pinot blanc-Riesling and Pinot gris gris-Gewürztraminer in the white wines. Red wines like Cabernet Sauvignon-Tempranillo showed a very similar volatile composition, characterised by alcohols and volatile phenols. According to the OAV, white wines from Gewürztraminer and red wines from Pinot noir showed the highest total value.

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