Study of the Volatile and Glycosidically Bound Compounds of Minority *Vitis vinifera* Red Cultivars from NW Spain

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ABSTRACT

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Aroma composition was analyzed in grape musts from five *Vitis vinifera* red cultivars (*Pedral, Sousón, Caíño Redondo, Espadeiro* and *Mencía*) grown in Galicia (NW Spain) with the aim to know the potential profile of these varieties. All cultivars were collected from Coto Redondo Winery vineyard (A.O.C. Rías Baixas) and grown under the same conditions. All cultivars (with a total of 96 aroma compounds, 35 free volatile and 61 glycosidically bound compounds) were identified and quantified by GC-MS. The results showed that *Caíño Redondo* and *Pedral* had the highest concentrations of volatile compounds. The free fraction showed the highest concentration for all cultivars studied with the exception of *Pedral*. High concentrations of free C_6-compounds and free alcohols were found for all red cultivars studied, especially for *Caíño Redondo*. *Sousón* and *Mencía* presented similar characteristics in the free fraction. *Pedral* showed an important contribution of glycosidically bound compounds. This cultivar showed the highest concentration of bound alcohols, terpenoids, volatile phenols, and C_{13}-norisoprenoids. With terpenoids, higher levels were found in the bound fraction than in the free fraction. Regarding C_{13}-norisoprenoids, *Pedral* also showed an important contribution of these varietal compounds. The principal component analysis (PCA) showed a good separation of the different grape cultivars, so they could be discriminated based on their potential volatile composition. The oenological potential of ancient and minority grape varieties from NW Spain, with the aim to provide genetic diversity, is illustrated with this work.

Key words: aroma compounds, Caíño Redondo, Espadeiro, Mencía, minority cultivars, Pedral, Sousón, Spain.

INTRODUCTION

Wine aroma depends on numerous factors, with special importance being given to the variety of grape, vinification, maturation, and aging. It is well known that the secondary metabolites of grapes are responsible for the principal aroma compounds in grape must and provide the basis of varietal character[27]. Several studies have recognized a relationship between wine character and grape/must volatile composition[38]. Wine aroma is determined by a complex balance of several volatiles[23]. The volatile compounds responsible for the primary or "varietal" aroma of wines are of great importance in the current effort to differentiate the latter on the basis of the grape variety used in production areas[1]. These volatile compounds are mainly terpenols and C_{13}-norisoprenoids, which supply fruity or floral notes to the aroma[3,22]. Some of these volatile compounds are modified during yeast fermentation[15]. Among aroma compounds present in the grapes, a significant part is assumed to come from specific odourless precursors, especially glycosidically linked forms (bound fraction), that during the winemaking process are transformed into odour-active forms (free fraction). Some authors were able to demonstrate that terpenols released from grape precursors play an important role in the aroma of *Muscat* wines[7,24,37] or in the formation of some characteristic off-flavours in Riesling wines[28,39,40]. Both the free and bound fraction give rise to the wine's aromatic profile.

Galicia, a region of northwestern Spain, has a long tradition of winemaking, however although this area has a wide range of traditional varieties of *Vitis vinifera*, few have been thoroughly studied. Such investigations have focused mainly on varieties such as *Albariño, Treixadura* and *Godello*, which form the majority of grapevines in Galician vineyards[5,6,33,35,36].

Nowadays, however, there is a major interest in the study of minor cultivars that may have potential uses as complementary varieties. The study of such varieties is necessary since they could provide a good source of diversification and, consequently, a market expansion of wines from Galicia[5]. Several minority Galician cultivars, such as *Castañal*[32], *Caíño Tinto, Caíño Bravo, Caíño Longo*[34], *Blanco Lexítimo, Agudelo* and *Serradelo*[35], have been previously studied by our group. Other red *Vitis vinifera* cultivars *Sousón*, *Pedral*, *Caíño Redondo*, *Mencía* and *Espadeiro* are traditionally grown in Rías Baixas Apellation of Origin. As far as we know, the potential aroma composition in grapes from these cultivars has not yet been studied. Some of these, whose surfaces have continually diminished in recent decades, may soon be on the verge of extinction, and are already affected by an important loss of genetic diversity.

For this reason, the aim of this study was to know the potential aroma and to identify and quantify the principal

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volatile compounds in free and bound fractions of *Vitis vinifera* red cultivars *Sousón*, *Pedral*, *Caíño Redondo*, *Mencía* and *Espadeiro* grown in Galicia. The second purpose of this study was the varietal differentiation of red cultivars according to their aromatic composition and the oenological potential of ancient and minority grape varieties from NW Spain with the aim of providing genetic diversity.

MATERIALS AND METHODS

Samples and reagents

Five minority grape cultivars (*Mencía, Sousón, Pedral, Espadeiro* and *Caíño redondo*) grown in Galicia during the 2009 vintage were considered in this study. The different cultivars were collected in triplicate from the Coto Redondo Winery vineyard (Appellation of Origin Rías Baixas), where all cultivars studied were grown under the same conditions (same vineyard). Grape berry samples were crushed in a blender (turbo blender, Moulinex, position 4, 7 sec) and frozen at –20°C until sample preparation.

Methanol, distillated azeotropic pentane-dichloromethane (2:1 v/v), ethyl acetate and ethanol were analytical grade and purchased from Merck (Darmstadt, Germany). Citrate-phosphate buffer (100 mmol/L) pH 5.0 and sodium sulphate anhydrous were made with analytical grade reagents from Riedel de Haën (Seelze, Germany). Internal standard (4-nonanol) was supplied by Merck. A solution of 235 mg/L was prepared in ethanol to spike must samples.

Extraction of free and bound fractions

About 500 g of frozen grape berries were crushed and centrifuged at 4°C during 30 min at 8,000 rpm. The supernatant was filtered with glass wool. To 75 mL of juice, 2.35 µg of 4-nonanol (Merck, ref. 818773) was added and passed through a LiChrolut EN cartridge (Merck, 500 mg, 40–120 µm) according to Ibarz et al.[14] and Oliveira et al.[21] The resin was previously pre-conditioned with 10 mL of dichloromethane, 5 mL of methanol and 10 mL of hydroalcoholic solution (10%, v/v). Free and bound fractions were eluted successively with 5 mL of azeotrope pentane–dichloromethane and 7 mL of ethyl acetate respectively. The pentane–dichloromethane eluate was dried over anhydrous sodium sulphate and concentrated to 200 µL by solvent evaporation at 34°C through a Vigreux and then a Dufton column, prior to analysis. The ethyl acetate eluate was concentrated to dryness in vacuum (40°C) and redissolved in 200 µL of 100 mmol/L citrate–phosphate buffer (pH 5.0). Residual free compounds were extracted five times with the azeotropic mixture and discarded. Fourteen milligrams of enzyme AR2000 (Gist-Brocades) was added to the glycosidic extract and the mixture was incubated at 40°C, for 12 h. Released aglycons were extracted with the azeotrope pentane-dichloromethane, after addition of 2.35 µg of 4-nonanol, as an internal standard. The organic phase was concentrated to 200 µL through a Dufton column. The extractions were performed in triplicate.

Chromatographic analysis

Must extracts were analysed by gas chromatography-mass spectrometry (GC-MS). The employed system consisted of a Varian 3800 gas chromatograph (Walnut Creek, CA, USA) equipped with a 1079 Programmable Temperature Vaporizing Injector (PTV) and connected to an ion-trap mass spectrometer (Varian Saturn 4000). Separations were carried out using a Factor Four VF-Wax MS capillary column (60 m × 0.25 mm i.d., df: 0.25 µm, Varian). Helium (99.999%) was used as carrier gas at constant flow of 1 mL/min. One microlitre of organic extract was injected in splitless mode during 2 min, and then the split ratio was fixed to 15. The temperature of the injector was set initially to 40°C, then raised to 250°C, at 180°C/min (held for 60 min). The GC oven was programmed as follows: 2 min at 50°C, 3°C/min to 250°C (held for 22 min). The GC-MS interface and the ion trap temperature were set at 250°C and 220°C, respectively. Mass spectra, in the electron impact mode (70 eV), were obtained in the range from 29 m/z to 260 m/z units.

Identification and quantification of volatile compounds

Identification was performed using the software Saturn, version 6.6 (Varian), by comparing the mass spectra and retention index with those of pure standard compounds. In some cases, the identification was achieved by comparing retention index and mass spectra with those of published data or spectra library. All the compounds were quantified as 4-nonanol equivalents.

Statistical analysis

The data were analyzed using XLstat-Pro (Addinsoft, 2009, Paris, France). ANOVA was performed on volatile compounds analysis data. The mean differences between cultivars were calculated using the Least Significant Difference Fishers' test (LSD). For interpreting the results, Principal Component Analysis (PCA) on volatile compounds was applied. PCA is used as a tool for screening, extracting and compressing data. PCA employs a mathematical procedure that transforms a set of possibly correlated response variables into a new set of noncorrelated variables called principal components[8].

RESULTS AND DISCUSSION

Must volatile composition

Study of the different families of compounds found in the grapes was used to describe varietal composition of five minority cultivars from Northwest of Spain. Figure 1 shows the total concentration of volatile compounds in free and bound fractions, identified and quantified in grape cultivars. The total concentration was obtained as the sum of the individual concentration of all compounds detected in the free and bound fractions of musts. *Caíño Redondo* and *Pedral* cultivars showed an elevated concentration of total volatile compounds (1.65 mg/L and 1.77 mg/L respectively) where *Pedral* was an important contribution of the glysosidic fraction (0.93 mg/L). Wine volatile compounds from different *Caíño* cultivars were studied. The highest concentrations of free volatile compounds were found for *Caíño longo* followed by the *Caíño tinto* wines[34], while *Espadeiro* showed the lowest volatile concentrations (total concentration 0.69 mg/L;

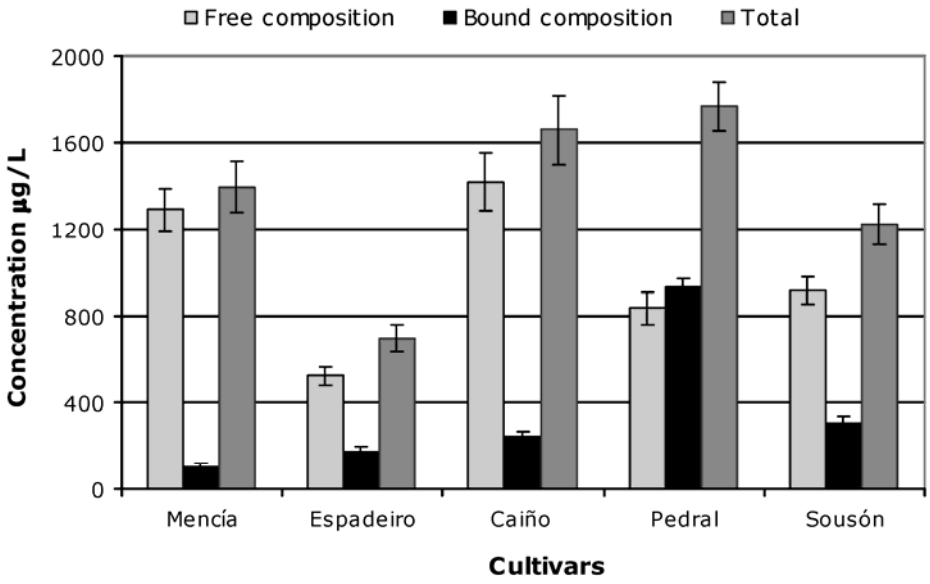

Fig. 1. Total, free and bound concentration (µg/L) from Mencía, Espadeiro, Caíño Redondo, Pedral and Sousón *Vitis vinifera* cultivars.

free concentration 0.52 mg/L; bound concentration 0.17 mg/L). *Mencía* was previously studied in different subzones from Galicia. Sensorial analysis showed different profiles depending on geographical area and weather, predominating red fruit aroma in all cases[33,35].

Tables I and II show the free and bound volatile concentrations of individual compounds grouped by families. An elevated number of compounds were detected (C_6-compounds, alcohols, terpenoids, volatile phenols, fatty acids, carbonyl compounds and C_{13}-norisoprenoids). The total concentration of volatile phenols and terpenoids was ten times higher in the bound fraction than in the free fraction, whereas other compounds such as C_6-compounds were one hundred times lower in the bound fraction.

Free varietal volatile fraction

The free fraction showed the highest concentration for all cultivars studied with the exception of the *Pedral* cultivar. Table I shows the mean concentration, standard deviation and LSD of individual free volatile compounds quantified in *Mencía, Espadeiro, Caíño Redondo, Pedral* and *Sousón* cultivars from Galicia. A total of 35 volatile compounds were identified, 7 C_6-compounds, 13 alcohols, 5 terpenoids, 4 volatile phenols, 4 carbonyl compounds and 2 fatty acids (19 volatile compounds are shown in Fig. 2). ANOVA showed that only six compounds (hexanal, 2-methyl-1-butanol + 3-methyl-1-butanol, 3-methyl-3-buten-1-ol + 1-pentanol and 2-ethyl-1-hexanol) did not show significant differences among the cultivars.

High concentrations of C_6-compounds and alcohols were found for all red cultivars studied. *Caíño redondo* showed the highest levels of total C_6-compounds, alcohols, carbonyl compounds and fatty acids, while *Espadeiro* had the lowest levels of the same families.

C_6-compounds supply "vegetal" and "herbaceous" nuances to the wine; however, it could be a defect depending of their concentration[14]. Several studies showed that C_6-compounds were used to distinguish grape varieties[20]. The compounds 1-hexanol and *E*-2-hexen-1-ol showed the highest values of volatile compounds found in the free fraction of all varieties. Moreover, high levels of hexanal were found in *Caíño redondo* and *Mencía* must cultivars.

Alcohols were also identified and quantified in high concentrations. Benzyl alcohol and 2-phenylethanol were present in high concentrations compared to other alcohols. The presence of these compounds contributes to flowery-sweet (benzyl alcohol) and flowery/rose/honey (2-phenylethanol) notes[2]. These results were correlated with previous studies from different wine varieties of *Caíño* and *Mencía* from different subzones of Galicia. In the free fraction, 2-phenylethanol was identified as the most abundant volatile alcohol in *Caíño Tinto*, *Caíño Longo* and *Caíño Bravo*[34], and *Mencía*[6]. On the other hand, benzyl alcohol was the most abundant alcohol found in *Pedral.*

Regarding terpenoids, they contribute at low concentrations to the aroma profile of these cultivars. They provide floral notes to the must at low concentrations[9]. The presence of the two pyran-linalool oxide isomers and geraniol was detected in *Mencía, Espadeiro* and *Pedral* cultivars. Citronellol, a varietal compound with fresh and rosy-notes, was only found at low concentration (0.6 µg/L) in *Mencía* must. Previous works reported levels of citronellol in Spanish and Galician red wines[16], in special *Mencía* wine from two different Appellation of Origin Controlled (AOC)[6] and *Serradelo*[35]. *Caíño Redondo*, in opposition to previous studies with *Caíño* cultivars, did not show levels of citronellol[34].

Volatile phenols and carbonyl compounds were found in free fraction at low concentrations, with the exception of benzaldehyde and phenylethanal, especially in *Caíño Redondo, Sousón* and *Pedral*. In general, the levels of volatile phenols from Galician red cultivars were lower than other cultivars from Mediterranean region[16]. In *Mencía* cultivar, eugenol and vanillin were identified at levels near the odour threshold in several previous studies[6]. In fruit wines, 2-phenoxyethanol in low concentra-

Table I. Mean values and standard deviation (SD) of free volatile compounds (μg/L) of cultivars from Rías Baixas A.O.C[a].

Compound (μg/L)	RI[b]	Mencía		Espadeiro		Caíño redondo		Pedral		Sousón		Sig[c]
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
C_6-compounds												
Hexanal	1084	234.5	136.9	94.1	13.0	276.6	30.2	88.4	16.5	93.9	0.3	ns
E-2-hexenal	1220	50.3^{2}	21.7	75.4^{2}	11.0	181.7^{1}	13.6	72.4^{2}	2.8	56.8^{2}	4.1	***
1-hexanol	1349	363.7^{2}	48.6	164.5^{4}	0.4	452.3^{1}	32.3	249.3^{3}	25.1	358.9^{2}	27.0	***
E-3-hexen-1-ol	1360	2.4^{3}	0.6	1.2^{4}	0.1	3.3^{2}	0.2	$2.0^{3,4}$	0.4	5.7^{1}	0.1	***
Z-3-hexen-1-ol	1381	6.0^{3}	1.1	11.2^{2}	0.7	14.5^{2}	1.6	26.5^{1}	2.8	6.1^{3}	0.6	***
E-2-hexen-1-ol	1403	128.7^{1}	17.7	95.2^{2}	1.6	128.6^{1}	9.5	140.6^{1}	12.4	151.8^{1}	9.2	*
Z-2-hexen-1-ol	1412	0.8^{4}	0.1	0.6^{4}	0.1	1.1^{3}	0.1	1.8^{1}	0.1	1.3^{2}	<0.1	***
Total		786.4		442.2		1058.1		581.0		674.5		
Alcohols												
2-pentanol	1111	ND^{3}	ND	ND^{3}	ND	ND^{3}	ND	0.4^{2}	0.1	0.6^{1}	0.1	***
1-butanol	1137	2.6^{2}	3.2	ND^{2}	ND	5.0^{2}	0.5	3.3^{2}	0.9	14.7^{1}	3.1	**
2-methyl-1-butanol + 3-methyl-1-butanol	1201	86.4	64.0	15.0	3.5	133.1	21.1	24.2	5.6	49.7	5.2	ns
3-methyl-3-buten-1-ol + 1-pentanol	1245	3.7	1.9	2.7	0.4	5.6	1.5	4.1	0.5	6.8	0.2	ns
Z-2-penten-1-ol + 3-methyl-2-buten-1-ol	1316	$3.3^{2,3}$	1.3	2.4^{3}	0.4	2.4^{3}	0.1	4.6^{2}	0.8	6.5^{1}	0.4	**
1-octen-3-ol	1447	1.5^{2}	0.2	0.8^{3}	0.1	3.7^{1}	0.6	0.7^{3}	<0.1	$1.3^{2,3}$	0.1	***
2-ethyl-1-hexanol	1487	1.9	0.4	1.9	0.3	2.9	0.7	1.9	0.4	2.0	0.1	ns
1-octanol	1555	0.7^{1}	0.1	0.4^{2}	0.1	ND^{3}	ND	0.4^{2}	0.1	0.6^{1}	0.1	***
Benzyl alcohol	1872	36.5^{2}	3.7	12.6^{4}	3.3	25.6^{3}	4.2	102.5^{1}	1.7	36.2^{2}	4.6	***
2-phenylethanol	1907	63.4^{3}	9.9	36.8^{3}	7.9	111.8^{1}	14.7	45.9^{2}	0.8	68.7^{2}	6.3	**
Total		200.0		72.6		290.1		188.0		187.1		
Terpenoids												
Linalool	1544	0.1^{2}	<0.1	0.2^{1}	<0.1	ND^{3}	ND	0.2^{1}	0.1	ND^{3}	ND	***
trans-Pyran linalool oxide	1735[d]	0.2^{2}	<0.1	0.8^{1}	0.1	ND^{3}	ND	ND^{3}	ND	0.3^{2}	<0.1	***
cis-Pyran linalool oxide	1757[d]	ND^{2}	ND	0.4^{1}	<0.1	ND^{2}	ND	ND^{2}	ND	ND^{2}	ND	***
Citronellol	1762	0.6^{1}	0.1	ND^{2}	ND	ND^{2}	ND	ND^{2}	ND	ND^{2}	ND	***
Geraniol	1842	2.1^{1}	0.5	1.7^{1}	0.3	ND^{3}	ND	2.3^{1}	0.2	0.7^{2}	0.1	***
Total		3.0		3.1		ND		2.5		1.0		
Volatile phenols												
Methyl salicylate	1778	0.3^{2}	<0.1	0.4^{2}	<0.1	1.6^{1}	0.1	0.4^{2}	<0.1	1.7^{1}	0.1	***
2-phenoxyethanol	2137	0.2^{2}	<0.1	0.4^{1}	0.1	ND^{3}	ND	ND^{3}	ND	ND^{3}	ND	***
Eugenol	2163	0.1^{2}	<0.1	0.5^{2}	0.5	ND^{2}	ND	2.0^{1}	0.2	0.5^{2}	0.1	**
Vanillin	2558	0.6^{3}	0.4	0.6^{3}	0.1	1.3^{2}	0.1	1.9^{1}	0.1	1.9^{1}	0.1	**
Total		1.2		1.9		2.9		4.3		4.1		
Carbonyl compounds												
Heptanal	1186	0.5^{2}	0.2	ND^{3}	ND	0.8^{1}	0.1	0.5^{2}	<0.1	$0.6^{1,2}$	0.1	**
Octanal	1290	0.3^{1}	<0.1	ND^{2}	ND	0.4^{1}	0.1	0.3^{1}	<0.1	0.4^{1}	<0.1	***
Benzaldehyde	1524[d]	7.4^{3}	1.0	1.5^{4}	<0.1	21.0^{1}	1.2	$12.2^{2,3}$	2.1	15.3^{2}	0.9	***
Phenylethanal	1644	2.3^{3}	0.3	1.8^{3}	0.1	12.3^{2}	0.6	17.9^{1}	0.5	1.7^{3}	0.3	***
Total		10.5		3.3		34.5		30.9		18.0		
Fatty acids												
Hexanoic acid	1846	2.5^{2}	0.8	ND^{3}	ND	8.6^{1}	0.6	1.3^{2}	0.2	1.9^{2}	0.3	***
Nonanoic acid	2165	ND^{3}	ND	ND^{3}	ND	1.1^{1}	0.2	ND^{3}	ND	0.7^{2}	0.1	***
Total		2.5		ND		9.7		1.3		2.6		

[a] LSD were explained with numbers in the average concentration. ND: Not detected.
[b] Linear retention index.
[c] *: p<0.05, **: p<0.01, ***: p<0.001, ns: not significant.
[d] Identified by comparing retention index and mass spectra published data (Oliveira et al. 2008).

tions was identified with a fruity note[10]. Benzaldehyde, with a bitter almond aroma, could appear in musts or wines with a *Botrytis cinerea* infection. Several studies demonstrated that the fungus oxidizes benzyl alcohol into benzaldehyde[18]. In fact, a relationship between quantities of benzyl alcohol and benzaldehyde was shown in both the free and bound fractions. The compounds 2-phenylethanol and benzaldehyde could be correlated according to Fabre et al.[11]

Principal Component Analysis (PCA) was applied to free volatile compounds with significant differences among the red cultivars (Fig. 3). The two first components (PC1 and PC2) represented 72.9% of the initial variance, and with three components the initial variance was 90% (data not shown). PC1 (46.4%) was characterized by 1-hexanol, *E*-3-hexen-1-ol, several alcohols (1-butanol, 1-octen-3-ol and 2-phenylethanol), terpenoids except citronellol, fatty acids and carbonyl compounds except 2-phenylethanal. PC2 (26.6%) was represented by *Z*-2-hexen-1-ol, 2-pentanol, *Z*-2-penten-1-ol, 3-methyl-2-buten-1-ol, benzyl alcohol, eugenol and vanillin.

Four different groups have been obtained with the distribution in the new axes. *Espadeiro* and *Mencía*, situated in the negative axes of PC1 and PC2, had similar properties with an important contribution of pyran linalool oxides and 2-phenoxyethanol. *Pedral* was characterized for an important contribution of PC2. Eugenol and, especially geraniol were found in the largest levels with respect to

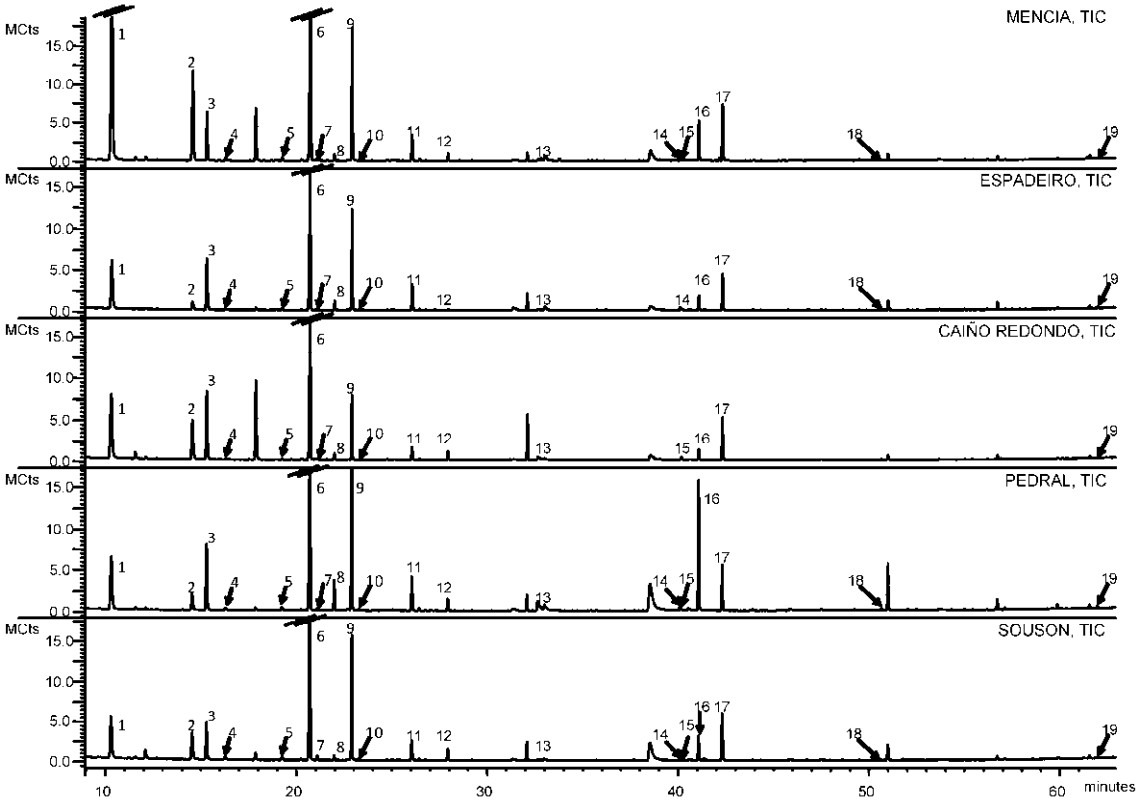

Fig. 2. Chromatograms of free volatile compounds of different cultivars grown in Galicia. (1) hexanal, (2) 3-methyl-1-butanol + 2-methyl-1-butanol, (3) E-2-hexenal, (4) 3-methyl-3-buten-1-ol +1-pentanol, (5) Z-2-penten-1-ol + 3-methyl-2-buten-1-ol, (6) 1-hexanol, (7) E-3-hexen-1-ol, (8) Z-3-hexen-1-ol, (9) E-2-hexen-1-ol, (10) Z-2-hexen-1-ol, (11) 4-nonanol, (12) benzaldehyde, (13) phenylethanal, (14) geraniol, (15) hexanoic acid, (16) benzyl alcohol, (17) 2-phenylethanol, (18) eugenol, (19) vanillin.

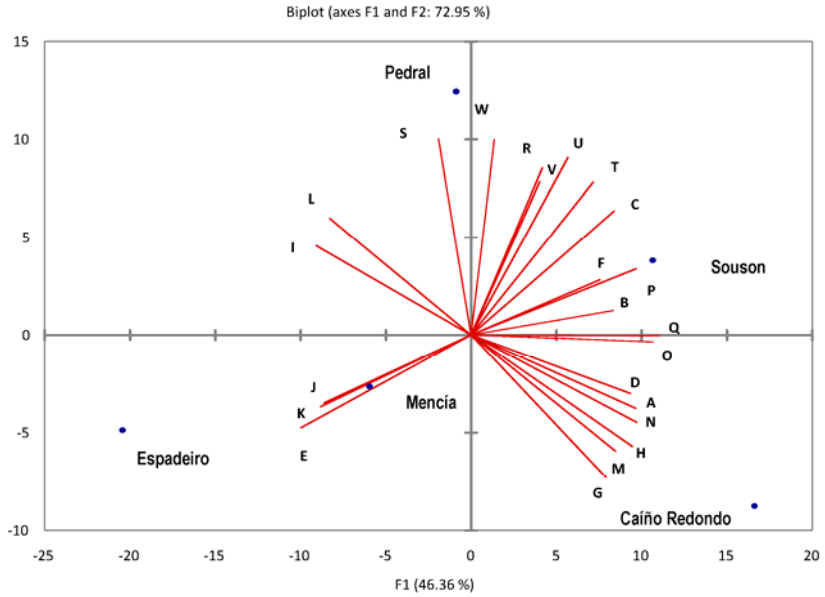

Fig. 3. Principal component analysis (PCA) of free volatile compounds from Mencía, Espadeiro, Caíño Redondo, Pedral and Sousón cultivars. A: 1-hexanol; B: e-3-hexen-1-ol; C: e-2-hexen-1-ol; D: methyl salicylate; E: 2-phenoxyethanol; F: 1-butanol; G: 1-octen-3-ol; H: 2-phenylethanol; I: linalool; J: *trans*-pyran linalool oxide; K: *cis*-pyran linalool oxide; L: geraniol; M: hexanoic acid; N: nonanoic acid; O: heptanal; P: octanal; Q: benzaldehyde; R: z-2-hexen-1-ol; S: eugenol; T: vanillin; U: 2-pentanol; V: z-2-penten-1-ol + 3-methy-2-buten-1-ol; W: benzyl alcohol.

the other varieties. On the other hand, *Caíño redondo* was characterized for low levels of terpenoids in the must, several alcohols (1-octen-3-ol and phenylethanol) and carbonilic compounds (heptanal and benzaldehyde). Finally, *Sousón* was identified with high quantities of benzaldehyde, 1-hexanol, *E*-2-hexen-1-ol and *E*-3-hexen-1-ol.

Bound volatile fraction

A total of 61 volatile compounds were identified and quantified in the bound fraction, 7 C_6-compounds, 15 alcohols, 10 terpenoids, 11 volatile phenols, 1 carbonyl compound, 7 fatty acids and 10 C_{13}-norisoprenoids. Table II shows the mean, standard deviation and LSD of the individual bound compounds identified. ANOVA showed significant differences among cultivars with the exception for three alcohols (2-methyl-1-butanol+3-methyl-1-butanol and 1-hexanol), one volatile phenol (3,4,5-trimethoxyphenol) and one fatty acid (hexanoic acid).

Pedral shows an important contribution of glycosidically bound compounds. This cultivar showed the highest concentration of alcohols, terpenoids, volatile phenols, and C_{13}-norisoprenoids. C_6-compounds were identified in lower concentrations in the bound fraction than in the free fraction. The compound 1-hexanol was the most abundant in all cases. The *Sousón* cultivar presented the higher contribution of this family of compounds.

Alcohols were also detected in minor concentrations with respect to the free fraction, with the exception of benzyl alcohol in *Pedral*. The compounds 2-phenylethanol and benzyl alcohol were found as the most abundant alcohols in all cultivars.

With respect to the terpenoids, the levels in the bound fraction were higher than those in the free fraction. The compound α-terpineol was identified in the four cultivars and the highest level was found in the *Pedral* cultivar. Both isomers of pyran and furan linalool oxides were identified in low concentrations for all varieties. Linalool was only identified in the *Pedral* and *Sousón* cultivars. Linalool was the most abundant compound in *Alvarinho* and *Loureiro* white cultivars from the *Vinhos Verdes* Region (Portugal)[12]. Monoterpenic compounds are particularly abundant in aromatic white grape varieties such as *Muscat, Riesling, Loureiro, Alvarinho, Arinto* and *Gewürztraminer*[12,19,21,31], but they are lower in red wines, such as *Merlot, Shiraz* or *Cabernet-sauvignon*[17].

In the same way, volatile phenols were in high quantities with respect to the free fraction, especially in the *Pedral* cultivar. The compounds 4-vinylguaiacol and methyl 2,5-dihydroxybenzoate were the most abundant compounds found in this cultivar, with levels between 60 μg/L and 80 μg/L. In the other cultivars, the levels were ten times lower.

Carbonyl compounds and fatty acids were also identified at low levels. In all cases, benzaldehyde was detected, but acetic acid was only identified in *Pedral* (2.8 μg/L) and *Sousón* (1.0 μg/L). In all varieties, 2-methyl and 3-methylbutanoic acids and hexanoic acid were identified, with major concentrations in the *Caiño redondo* and *Pedral* musts.

Regarding the C_{13}-norisoprenoids, *Pedral* showed an important contribution of these varietal compounds (Table II and Fig. 4), with a total concentration of 136.8 μg/L. The highest levels of the C_{13}-norisoprenoids in the Pedral cultivar were found as 3-oxo-α-ionol, 3-hydroxy-β-damascone, 3-hydroxy-7,8-dihydro-β-ionol, 3-hydroxy-7,8-dehydro-β-ionol and vomifoliol. With the other cultivars, especially *Mencía*, the levels were lower. While 3-oxo-α-ionol was a major compound found in *Espadeiro* and *Sousón*, 3-hydroxy-β-damascone was major compound in *Caíño redondo*. C_{13}-norisoprenoids are considered grape-derived compounds that arise from carotenoids and are present in the bound fraction and are considered to be important to the aroma of wine[4,29,30]. In general, the presence of norisoprenoids is a quality factor, typical from each cultivar, and supplies an agreeable scent of tobacco, fruit or tea[26]. They contribute to the characteristic profile of *Merlot, Chardonnay* and *Syrah* wines[17].

Principal component analysis (PCA) was performed with the bound composition of red cultivars (Fig. 5). The PC1 (60.0%) was characterized by the volatile phenols (except 3,4,5-trimethoxybenzyl alcohol), C_{13}-norisoprenoids (except 3-hydroxy-7,8-dehydro-α-ionol), carbonyl compounds, fatty acids, alcohols and *Z*-3-hexen-1-ol on the positive side. Furfuryl, 2-methyl butanoic acid + 3-methyl butanoic acid were described on the negative side of PC1. PC2 (21.3%) showed the contribution of several C_6-compounds (1-hexanol, *E*-3-hexen-1-ol, *E*-2-hexen-1-ol, *Z*-2-hexen-1-ol), 3,4,5-trimethoxyphenol and nonanoic acid on the positive side.

Four different groups were obtained according to the bound composition (Fig. 5). *Caíño Redondo* and *Mencía*, situated on the negative axis of PC1, showed similar properties. They showed higher concentrations of furfuryl alcohol (0.2 μg/L to 0.3 μg/L) and 2-methyl and 3-methyl butanoic acid (0.7 μg/L to 1.6 μg/L) than the other musts. Moreover, they showed low levels of terpenoids and C_{13}-norisoprenoids.

On the positive axis of PC2, *Sousón* was characterized by the highest concentration of C_6-compounds (except hexanal and *E*-2-hexanol) and 3,4,5-trimethoxyphenol. Nonanoic acid (0.2 μg/L) was only identified in this cultivar.

Pedral had an important contribution of PC1 on the positive side, and lower of PC2. Bound benzyl alcohol (378.7 μg/L) and 2-phenylethanol (97.9 μg/L), terpenoids and C_{13}-norisoprenoids were important in this variety. Terpenoid compounds provide a floral odour with a low perception threshold[13,25].

Finally, *Espadeiro* had an important contribution of PC1 and PC2 on the negative axes. It was characterized by high levels of both pyran linalool oxides, 3,7-dimethyl-1,5-octadien-3,7-diol (3.3 μg/L), furfuryl (0.2 μg/L). The compound 2-ethyl-1-hexanol (0.1 μg/L) only showed in this cultivar.

A correlation matrix performed on the bound composition of red cultivars showed that *E*-3-hexen-1-ol, *Z*-2-hexen-1-ol, nonanoic acid and 3,4,5-trimethoxybenzyl alcohol showed a r = 1, therefore they were redundant in the model. These results also showed a correlation between several compounds, confirming that some compounds could be the precursor of other compounds, as is the case of 2-phenylethanol, phenylethanal and benzaldehyde.

Table II. Mean values and standard deviation (SD) of bound volatile composition (μg/L) of cultivars from Rías Baixas A.O.C[a].

Compound (μg/L)	RI[b]	Mencía		Espadeiro		Caíño redondo		Pedral		Sousón		Sig[c]
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
C_6-compounds												
Hexanal	1084	3.0[1]	0.2	ND[4]	ND	ND[4]	ND	1.7[2]	0.2	1.3[3]	<0.1	***
E-2-hexenal	1220	2.0[1]	0.8	1.5[1,2]	0.2	0.7[2]	<0.1	2.1[1]	0.1	0.9[2]	0.1	*
1-hexanol	1349	4.5[3]	2.5	3.6[3]	0.4	6.1[3]	0.4	10.2[2]	0.1	17.9[1]	0.7	***
E-3-hexen-1-ol	1360	ND[2]	ND	ND[2]	ND	ND[2]	ND	ND[2]	ND	0.3[1]	<0.1	***
Z-3-hexen-1-ol	1381	0.9[3]	0.2	0.9[3]	0.1	0.4[4]	<0.1	1.9[1]	<0.1	1.3[2]	<0.1	***
E-2-hexen-1-ol	1403	1.9[2]	0.6	0.7[3]	0.2	0.7[3]	<0.1	2.0[2]	0.1	11.1[1]	0.6	***
Z-2-hexen-1-ol	1412	ND[2]	ND	ND[2]	ND	ND[2]	ND	ND[2]	ND	0.1[1]	<0.1	***
Total		12.3		6.7		7.9		17.9		32.9		
Alcohols												
3-pentanol	1102	ND[4]	ND	ND[4]	ND	0.4[3]	0.1	0.6[1]	<0.1	0.5[2]	0.1	***
2-pentanol	1111	ND[3]	ND	1.0[2]	0.2	0.8[2]	0.1	1.8[1]	0.2	1.1[2]	0.1	***
1-butanol	1137	3.5[2,3]	2.1	1.8[3]	0.4	2.1[3]	<0.1	7.5[1]	0.1	5.4[1,2]	0.5	**
2-methyl-1-butanol + 3-methyl-1-butanol	1201	8.6	4.8	2.2	0.5	5.8	0.1	4.3	<0.1	5.8	0.2	ns
3-methyl-3-buten-1-ol + 1-pentanol	1245	1.6[2]	0.8	0.7[2]	0.1	1.2[2]	0.1	4.6[1]	0.3	1.6[2]	0.2	***
2-heptanol	1316	0.9[2,3]	0.6	0.5[3]	0.1	0.6[2,3]	<0.1	2.6[1]	0.1	1.4[2]	0.1	**
1-octen-3-ol	1447	0.1[4]	0.1	ND[4]	ND	1.0[1]	<0.1	0.6[2]	0.1	0.4[3]	<0.1	***
1-heptanol	1453	2.2	2.7	ND	ND	ND	ND	0.4	0.1	ND	ND	ns
2-ethyl-1-hexanol	1487	ND[2]	ND	0.1[1]	<0.1	ND[2]	ND	ND[2]	ND	ND[2]	ND	***
1-octanol	1555	0.3[3]	0.1	ND[4]	ND	ND[4]	ND	0.7[1]	<0.1	0.4[2]	<0.1	***
Furfuryl alcohol	1655	0.3[1]	<0.1	0.2[1]	<0.1	0.2[1]	<0.1	ND[2]	ND	ND[2]	ND	***
Benzyl alcohol	1872	31.0[3]	16.6	25.3[3]	1.1	80.7[2]	6.3	378.7[1]	8.9	68.7[2]	5.0	***
2-phenylethanol	1907	35.0[4]	8.6	19.2[4]	0.5	60.1[3]	1.6	97.9[1]	2.5	66.2[2]	4.3	***
Total		83.5		51.0		152.9		499.7		151.5		
Terpenoids												
trans-Furan linalool oxide	1442	0.6[3]	0.4	1.9[2]	0.3	0.9[3]	<0.1	2.5[1]	0.1	0.8[3]	<0.1	***
cis-Furan linalool oxide	1470	1.0[5]	0.5	8.6[2]	0.7	3.1[4]	<0.1	18.1[1]	0.4	5.9[3]	0.1	***
Linalool	1544	ND[3]	ND	ND[3]	ND	ND[3]	ND	0.4[1]	<0.1	0.3[2]	<0.1	***
α-terpineol	1695	0.3[3]	0.1	4.3[2]	0.3	5.2[2]	0.9	17.3[1]	0.2	ND[3]	ND	***
trans-Pyran linalool oxide	1735[d]	0.8[2]	0.5	1.9[1]	0.2	0.5[2]	0.1	2.3[1]	0.2	0.6[2]	<0.1	**
cis-Pyran linalool oxide	1757[d]	0.5[2]	0.4	2.8[1]	0.6	0.6[2]	0.2	2.5[1]	<0.1	1.0[2]	0.2	**
Geraniol	1842	1.2[2]	0.5	1.5[2]	<0.1	ND[3]	ND	3.6[1]	0.2	1.0[2]	0.3	***
3,7-dimethyl-1,5-octadien-3,7-diol	1935[d]	ND[3]	ND	3.3[1]	0.1	ND[3]	ND	1.0[2]	<0.1	ND[3]	ND	***
Z-8-hydroxylinalool	2297[d]	3.1[2]	2.1	ND[3]	ND	ND[3]	ND	9.2[1]	0.4	1.7[2,3]	0.2	***
Geranic acid	2336	ND[3]	ND	2.0[2]	<0.1	ND[3]	ND	7.0[1]	0.6	ND[3]	ND	***
Total		7.5		26.3		10.3		63.9		11.3		
Volatile phenols												
Methyl salicylate	1778	0.3[4]	0.1	0.6[4]	0.1	1.1[3]	0.1	2.5[2]	0.2	2.9[1]	0.2	***
Guaiacol	1857	ND[3]	ND	ND[3]	ND	ND[3]	ND	8.4[1]	0.3	0.6[2]	0.1	***
Phenol	2001	ND[4]	ND	ND[4]	ND	0.5[3]	0.1	2.9[1]	0.2	0.8[2]	0.2	***
Eugenol	2163	0.4[3]	0.2	2.5[2]	0.1	ND[4]	ND	5.3[1]	0.1	0.7[3]	0.1	***
4-Vinylguaicacol	2191	0.4[3]	0.2	8.6[2]	0.9	7.7[2]	1.2	82.5[1]	8.5	12.1[2]	3.9	***
Vanillin	2558	1.1[2]	0.7	1.0[2]	0.1	1.1[2]	0.2	3.5[1]	0.2	4.2[1]	1.0	**
Methyl vanillate	2596	ND[4]	ND	10.4[2]	0.7	2.4[3]	0.5	12.9[1]	0.2	1.4[3]	0.3	***
Zingerone	2788	2.3[2,3]	1.6	ND[3]	ND	ND[3]	ND	6.6[1]	1.4	3.4[2]	0.5	**
3,4,5-Trimethoxybenzyl alcohol	2870	ND[2]	ND	ND[2]	ND	ND[2]	ND	ND[2]	ND	1.0[1]	0.2	***
Methyl 2,5-dihydroxybenzoate	3000	ND[3]	ND	3.6[2,3]	1.0	4.2[2,3]	3.7	57.4[1]	5.7	11.0[2]	2.4	***
3,4,5-4 Trimethoxyphenol	3046	2.8	4.0	3.3	1.0	ND	ND	ND	ND	7.0	0.8	ns
Total		7.3		30.0		17.0		182.0		45.1		
Carbonyl compounds												
Benzaldehyde	1524[d]	0.5[3]	0.2	0.4[3]	0.1	1.0[2]	0.2	1.6[1]	<0.1	0.7[2]	<0.1	***
Total		0.5		0.4		1.0		1.6		0.7		
Fatty acids												
Acetic acid	1454	ND[3]	ND	ND[3]	ND	ND[3]	ND	2.8[1]	0.1	1.0[2]	0.1	***
2-Methylbutanoic acid + 3-methylbutanoic acid	1673	1.6[1]	0.5	0.2[2]	<0.1	0.7[2]	0.2	ND[2]	ND	0.6[2]	0.1	**
Hexanoic acid	1846	2.9	1.6	4.6	3.9	10.1	2.9	7.2	0.3	5.3	2.3	ns
E-2-hexenoic acid	1971	ND[4]	ND	ND[4]	ND	7.3[2]	1.2	9.9[1]	1.0	4.2[3]	0.4	***
Octanoic acid	2059	ND[4]	ND	ND[4]	ND	0.5[3]	0.1	0.8[1]	<0.1	0.6[2]	<0.1	***
Nonanoic acid	2165	ND[2]	ND	ND[2]	ND	ND[2]	ND	ND[2]	ND	0.2[1]	<0.1	***
Total		4.5		4.8		18.6		20.7		11.9		

(continued on next page)

[a] LSD were explained with numbers in the average concentration. ND: not detected.
[b] Linear retention index.
[c] *: p<0.05, **: p<0.01, ***: p<0.001, ns: not significant.
[d] Identified by comparing retention index and mass spectra published data (Oliveira et al. 2008).

Table II. (continued from previous page).

Compound (μg/L)	RI[b]	Mencía		Espadeiro		Caíño redondo		Pedral		Sousón		Sig[c]
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
C_{13}-norisoprenoids												
3,4-Dihydro-3-oxo-actinidol I	2416[d]	1.9[2]	1.1	1.4[2]	0.2	1.6[2]	0.3	5.5[1]	0.6	1.7[2]	0.1	**
3,4-Dihydro-3-oxo-actinidol II + 3,4-dihydro-3-oxo-actinidol III	2463[d]	2.5[2]	1.4	4.1[2]	0.1	2.2[3]	0.3	7.0[1]	<0.1	3.0[2]	0.5	**
3-Hydroxy-β-damascone	2525[d]	4.8[3]	3.0	5.7[3]	1.0	10.9[2]	1.4	31.9[1]	1.1	12.3[2]	0.4	***
3-Oxo-α-ionol	2627[d]	5.2[4]	4.0	27.9[2]	2.8	9.4[3]	2.4	38.7[1]	0.3	15.7[3]	0.9	***
3-Hydroxy-7,8-dihydro-β-ionol	2649[d]	ND[4]	ND	ND[4]	ND	2.3[3]	0.4	19.6[1]	0.6	5.0[2]	0.6	***
4-Oxo-7,8-dihydro-β-ionol	2670[d]	ND[4]	ND	6.2[2]	0.5	ND[4]	ND	9.0[1]	1.8	3.8[3]	0.3	***
3-Oxo-7,8-dihydro-α-ionol	2701[d]	ND[2]	ND	3.9[1]	0.8	3.8[1]	1.1	ND[2]	ND	ND[2]	ND	**
3-Hydroxy-7,8-dehydro-β-ionol	2735[d]	2.5[2]	1.5	2.7[2]	0.1	4.1[2]	0.9	11.6[1]	1.6	4.5[2]	0.2	**
Vomifoliol	3139[d]	2.4[3]	3.4	3.3[2,3]	0.8	ND[3]	ND	13.5[1]	0.5	7.0[2]	1.2	**
Total		19.3		55.2		34.3		136.8		53.0		

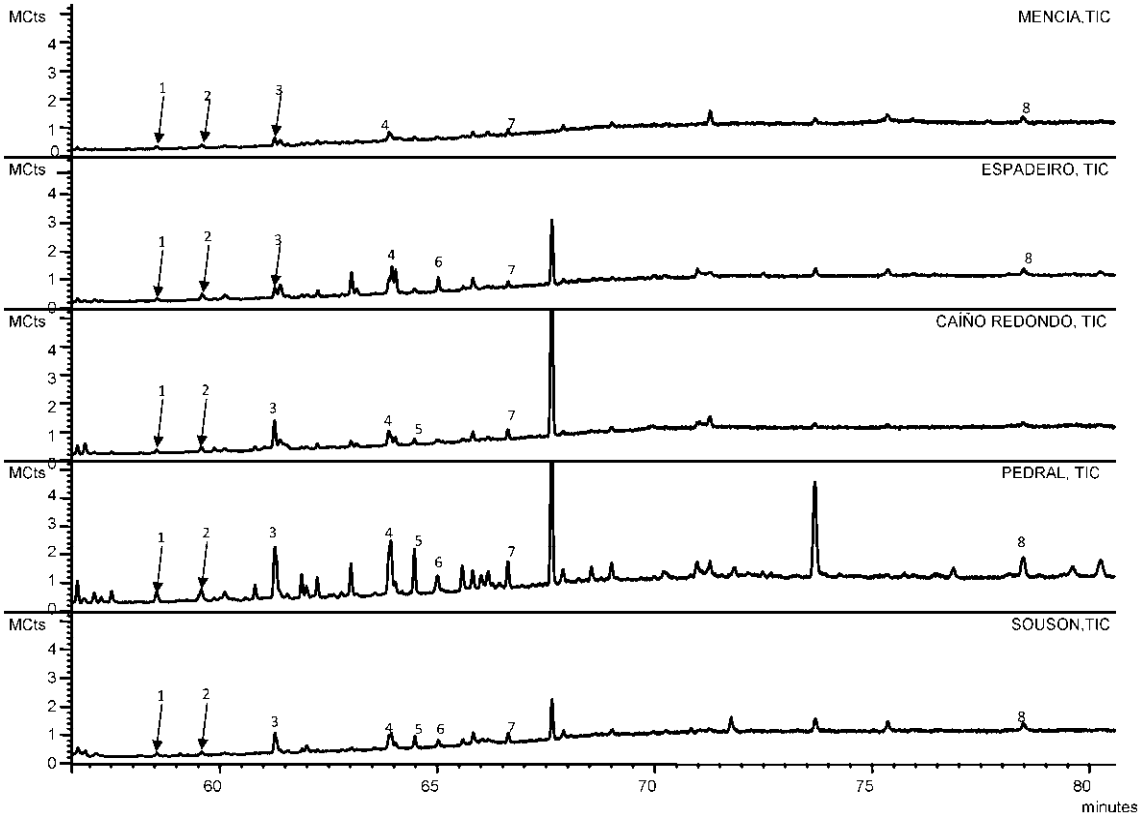

Fig. 4. Chromatograms of bound norisoprenoids in different cultivars grown in Galicia. (1) 3,4-dihydro-3-oxo-actinidol I, (2) 3,4-dihydro-3-oxo-actinidol II & III, (3) 3-hydroxy-β-damascone, (4) 3-oxo-α-ionol, (5) 3-hydroxy-7,8-dihydro-β-ionol, (6) 4-oxo-7,8-dihydro-β-ionol, (7) 3-hydroxy-7,8-dihydro-β-ionol, (8) Vomifoliol.

CONCLUSIONS

In summary, the volatile composition of grape must from the red cultivars *Pedral, Sousón, Mencía, Caíño Redondo* and *Espadeiro* grown in NW Spain, was analyzed in this study. The potential aroma profile was described in the base of the volatile composition of the musts. Different families of compounds were identified in the free and bound fractions. The results obtained showed the possibility of discrimination among the five red cultivars. Some of these results are in agreement with previous studies of the red *Mencía* cultivar from *Galicia. Pedral* was classified on the basis of these results as a potential aromatic red wine, due to its high levels of free and especially the bound aromas. Nevertheless, additional studies, with a larger number of grape samples and different geographic areas must be conducted in order to confirm these conclusions.

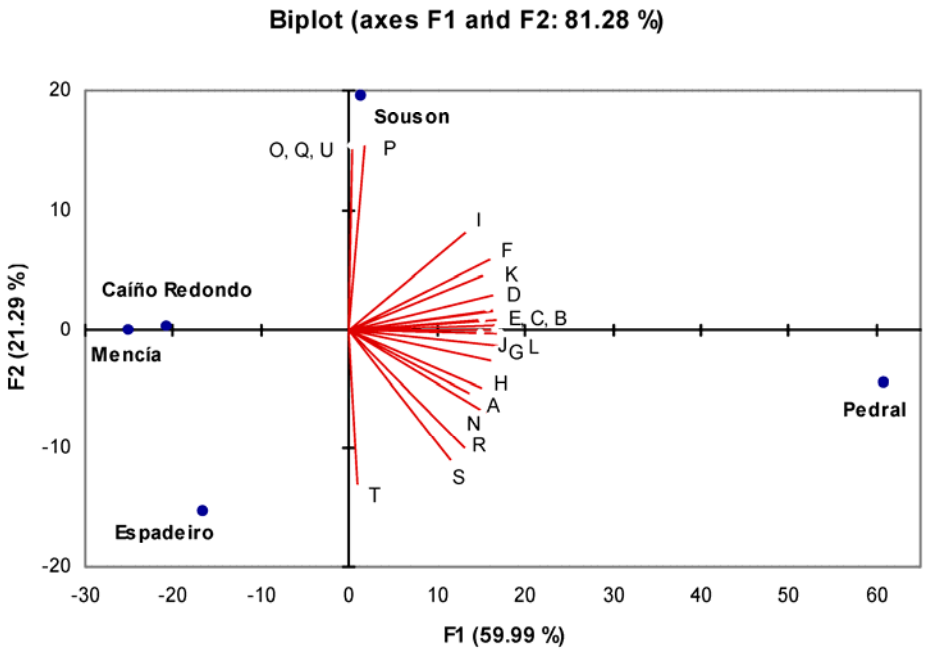

Fig. 5. Principal component analysis (PCA) of bound volatile compounds from Mencía, Espadeiro, Caíño Redondo, Pedral and Sousón cultivars. A: 3,4-dihydro-3-oxo-actinidol II & III; B: 3-hydroxy-β-damascone; C: 3-hydroxy-7,8-dehydro-β-ionol; D: vomifoliol; E: 3-methyl-3-buten-1-ol + 1-pentanol; F: 2-heptanol; G: benzyl alcohol; H: *cis*-furan linalool oxide; I: linalool; J: (z)-8-hydroxylinalool; K: acetic acid; L: guaiacol; M: phenol; N: geranic acid; O: e-3-hexen-1-ol; P: e-2-hexen-1-ol; Q: z-2-hexen-1-ol; R: *trans*-pyran linalool oxide; S: *cis*-pyran linalool oxide; T: 3,7-dimethyl-1,5-octadien-3,7-diol; U: 3,4,5-trimethoxybenzyl alcohol.

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