Monoterpenic Characterization of White Cultivars from *Vinhos Verdes* Appellation of Origin (North Portugal)

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ABSTRACT

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The monoterpenic composition of *Alvarinho*, *Arinto*, *Avesso*, *Azal*, *Batoca*, *Loureiro* and *Trajadura Vitis vinifera* cultivars from the *Vinhos Verdes* region (Portugal), in respect to free volatile compounds as well as glycosidically bound aroma precursors, was determined by GC-MS after adsorption on XAD-2 resin. Eighteen monoterpenic compounds were identified and quantified in the free form and twenty-two compounds in the glycosidically bound form. In the free fraction, the *Loureiro* variety could be easily differentiated from the other six varieties by the important levels of linalool, above the odor perception threshold. This was in contrast to *Alvarinho, Avesso*, *Arinto, Azal* and *Trajadura*, where geraniol prevailed. The *Batoca* variety showed a very poor monoterpenic profile in the free form. On the other hand, *Alvarinho* was the richest variety in respect to the glycosidically bound form, followed by *Loureiro*. In this fraction, linalool and 3,7-dimethylocta-1,5-dien-3,7-diol were the most abundant compounds in the *Loureiro* grape cultivar; additionally, the isomer (*E*)-8-hydroxylinalool was in a higher concentration than (*Z*)-8-hydroxylinalool (sub-region of Sousa), while for the rest of the varieties the (*Z*) isomer prevailed.

Key words: grape variety, monoterpenic profile, volatile compounds, *Vinhos Verdes*.

INTRODUCTION

The appellation of origin *Vinhos Verdes*, in the Northwest of Portugal is divided into 9 sub-regions: Amarante, Ave, Baião, Basto, Cávado, Lima, Monção and Melgaço, Paiva, and Sousa. In this appellation, there are seven white grape varieties (*Alvarinho, Arinto, Avesso, Azal, Batoca, Loureiro* and *Trajadura*) and eight black grape varieties (*Amaral, Borraçal, Brancelho, Espadeiro, Padeiro de Basto, Pedral, Rabo de Ovelha* and *Vinhão*) recommended for wine production.

Monovarietal wines play an important role in the economy of the *Vinhos Verdes* region, since it is frequent to find them from the white varieties *Alvarinho*, *Arinto*, *Azal*, *Loureiro* and *Trajadura*, as well as from the red cultivars *Espadeiro* and *Vinhão*. Among the white varieties, *Alvarinho* and *Loureiro* are employed for high quality monovarietal wine production and these are characterized by freshness and floral and fruity flavours[19]. The designation *Vinho Verde Alvarinho* is only used for white wines made exclusively from *Alvarinho* grapes, which are cultivated specifically in the sub-region of Monção and Melgaço. The white varieties *Loureiro* and *Alvarinho* are also well known in Galicia (NW Spain) by the names of *Loureira* and *Albariño*, respectively; *Arinto* is also recognized as *Pedernã*[15].

According to the origin and considering the biotechnological sequence of winemaking, wine flavor can be classified into four different groups[1,4]: I) varietal aroma, typical of grape variety that directly passes to the must; II) pre-fermentative aroma, originates during grape processing and the subsequent operations (the varietal and pre-fermentative aromas are also called the primary aroma); III) fermentative aroma (the secondary aroma), produced by yeast during alcoholic fermentation and by lactic acid bacteria during malolactic fermentation; and IV) post-fermentative aroma (the tertiary aroma), resulting from the transformations that occur during the conservation and aging of wine.

The "floral varieties" such as the *Muscats*, produce odoriferous musts with aromas similar to those of the resulting wines[6], although the must of a lot of varieties with "simple flavor" are practically odorless. In spite of this, they may produce wines with specific aromatic characteristics related to the grape variety from which they are made. *Merlot, Cabernet-Sauvignon, Chardonnay, Syrah, Pinot Noir*, etc., are within this type of grape. The odorless substances that can contribute to the varietal aroma of a wine are recognized as precursors of aroma[1,4].

The presence and level of odoriferous compounds in grapes, free volatile and/or precursors depend on the grape variety, with the climate and soil playing a decisive role in the quality and regional character of the wines[20,23]. Certain compounds are often used for varietal discrimination between grapes[12,14,17,18].

The terpenic compounds are one of the most important volatile constituents in grapes and wines. Their composition and concentration permit the differentiation of grape varieties and are responsible for peculiarities in aroma[17,27]. In this group, the monoterpenes are the most important compounds from an oenological point of view, being responsible for the varietal character of grapes[8,11]. Regarding the total concentration of monoterpenes (bound and

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free fractions), the *Vitis vinifera* varieties were classified into three groups, Muscat-type, aromatic and neutral[25]; and the German grape varieties were divided on *Riesling*, *Muscat* and *Sylvaner*[21]. Among these, *Riesling* wines from different regions were also discriminated based on the monoterpenic compounds present[13]. Camara et al.[3] differentiated grape varieties used for Madeira wine production on the basis of varietal aromas. Glycosidically bound compounds (a group of aroma precursors) have also been used for discrimination of grape cultivars[9,14,24].

Several studies[2,5,17,18,29] have been published about the varietal composition of common white grape cultivars from Portugal and Galicia (NW Spain), but a complete study of recommended *Vinhos Verdes* grape varieties, was not performed up till now.

The aim of the present work was to perform a characterization of seven white grape varieties recommended for the *Vinhos Verdes* (Portugal), in terms of monoterpenic compounds (free and glycosidically bound).

MATERIALS AND METHODS

Grape samples

Alvarinho, Arinto, Avesso, Azal, Batoca, Loureiro and *Trajadura Vitis vinifera* varieties were manually harvested in 2005 vintage in two different geographic areas (*Vinhos Verdes* appellations sub-regions) from Portugal, Sousa and Lima, which have similar soil characteristic with granitic origin. Different rootstocks were used: 196-17 Castel (sub-region of Sousa cultivars) and 161-49 Couderc (sub-region of Lima). The training system of all vines was a single cordon.

Berries from 5 grape vines (5 kg) were manually picked, randomly selected, frozen in liquid nitrogen and stored at –20°C. The different varieties were harvested on different dates in order to have similar degrees of maturation. The following codes were attributed to the samples: ALV-L and ALV-S *(Alvarinho),* ARI-L and ARI-S *(Arinto),* AVE-L and AVE-S (*Avesso),* AZA-L and AZA-S (*Azal),* BAT-L and BAT-S (*Batoca),* LOU-L and LOU-S (*Loureiro),* TRA-L and TRA-S (*Trajadura),* from sub-region of Lima (L) and sub-region of Sousa (S), respectively.

Solvents

All solvents were analytical grade and further purified. Diethyl ether (*Merck*) was distilled on iron (II) sulphate (*Merck*). Dichloromethane (*Merck*) was washed with de-ionized water, and then distilled. Pentane (*Riedel de Haën*) was washed with H_2SO_4 (*Merck*), $KMnO_4$ (*Carlo Erba*) and de-ionized water, and then distilled on potassium hydroxide (*Merck*). Azeotrope pentane-dichloromethane was distilled after combination of pentane and dichloromethane (2:1, v/v), being redistilled whenever necessary.

Extraction of volatiles and glycoconjugates

About 550 g of berries were defrosted at 4°C overnight and then crushed in a blender (turbo blender, Moulinex, position 4, 7 s). To each 150 mL of previously centrifuged (25 min, *RCF* = 9660, 4°C) must, 6.075 µg of 4-nonanol (*Merck*) was added. The solution was passed through an Amberlite XAD-2 resin (20–60 mesh, Supelco) column according to Günata et al.[10], modified by Oliveira[16]. Free and glycosidically bound fractions were successively eluted with 50 mL of azeotrope pentane-dichloromethane and 50 mL of ethyl acetate. Pentane-dichloromethane eluate was dried over anhydrous sodium sulphate and concentrated to about 200 µL by solvent evaporation at 34°C through a Vigreux column, prior the analysis. The ethyl acetate eluate was concentrated to dryness under vacuum (40°C) and the extracts were dissolved in 100 µL of citrate-phosphate buffer (pH 5). Residual free compounds were extracted five times with the azeotropic mixture and discarded. The enzyme AR2000 (Gist-Brocades), prepared in citrate-phosphate buffer in a ratio of 70 mg/mL, was added to the glycosidic extract and the mixture was incubated at 40°C for 12 h. Released aglycons were extracted with pentane-dichloromethane and 6.075 µg of 4-nonanol (as internal standard) was added to the organic phase and concentrated to 200 µL, through a Dufton column. Extractions of free and glycosidically bound fractions were made in triplicate.

Gas-chromatography-mass spectrometry (GC-MS)

Gas chromatographic analysis of volatile compounds was performed using a GC-MS system composed of a Varian 3400 Chromatograph and an ion-trap mass spectrometer Varian Saturn II. Injection of the final extracts (1 µL) was made in a CP-Wax 52 CB capillary column (50 m × 0.25 mm i.d., 0.2 µm film thickness, Chrompack). The temperature of the injector (SPI – septum-equipped programmable temperature) was programmed from 20°C to 250°C, at 180°C/min. The temperature of the oven was held at 60°C, for 5 min, then programmed from 60°C to 250°C at 3°C/min, subsequently held at 250°C for 20 min, and finally programmed from 250°C to 255°C at 1°C/min. The carrier gas was helium N60 (Air Liquide), at 103 kPa. The detector was set to electronic impact mode (70 eV), with an acquisition range from 29 to 360 (m/z), and an acquisition frequency of 610 ms.

Identification and quantification of volatile compounds

Identification was performed using the software Saturn, version 5.2 (Varian), by comparing mass spectra and retention indices with those of pure standard compounds. All of the compounds were quantified as 4-nonanol equivalents.

Statistical analysis

Principal Component Analysis and a Hierarchical Tree Plot of Cluster were performed to verify similarities between grape varieties, with respect to specific volatile compounds. SAS Enterprise Guide® 3 for Windows was the software used.

RESULTS AND DISCUSSION

Free and glycosidically bound monoterpenes were determined in different varieties from two geographic areas of the *Vinhos Verdes* appellation of origin (Portugal). The varietal profiles from each variety were determined in this study and are discussed below.

Free fraction

Eighteen monoterpenic compounds were identified and quantified in the free form of aroma in the studied varieties (Fig. 1). Table I shows the mean concentration and confidence limits ($p = 0.05$) obtained for each compound in the seven studied varieties from two sub-regions of the *Vinhos Verdes* appellation of origin. *Loureiro* was the richest variety in regard to total concentrations of free monoterpenic compounds, followed by *Alvarinho* and *Arinto*, while *Batoca* presented the lowest concentrations. *Loureiro* was the only variety in this study containing Ho-trienol; and linalool represented 98% of the total monoterpenic alcohols in this grape variety. Linalool, HO-trienol and α-

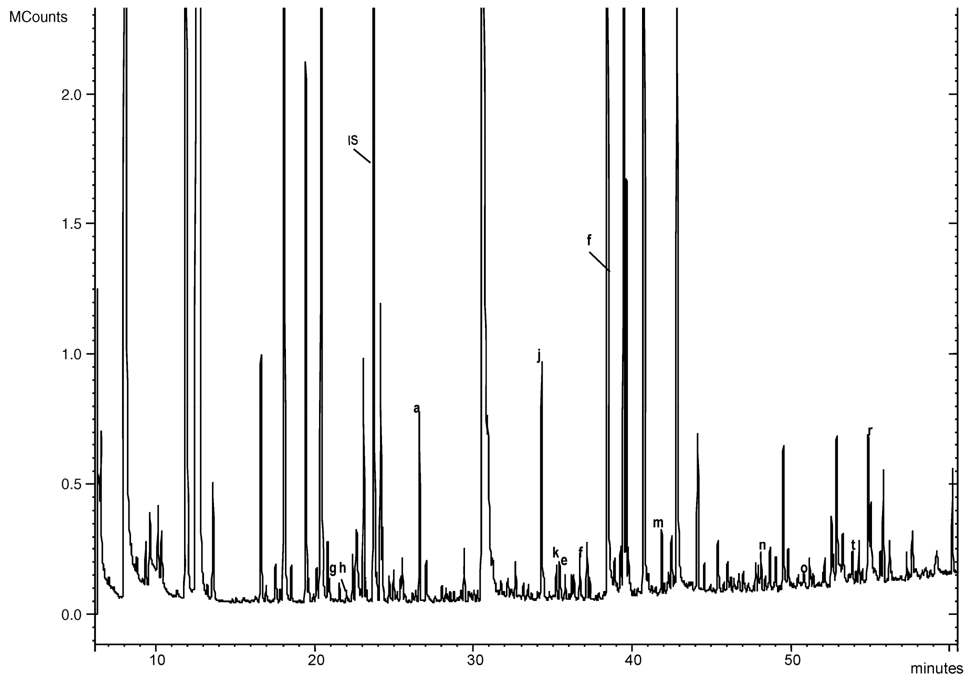

Fig. 1. Representative section of a GC-MS chromatogram for *Alvarinho* grape variety (ALV-S), for the monoterpenic compounds found in the free fraction (peak identification may be assessed on Table I). IS – internal standard.

Table 1. Mean levels (μg/L) ± confidence limits ($p = 0.05$), for the monoterpenic compounds found in the free fraction of the seven varieties studied.

		RI*	ALV-L	ALV-S	ARI-L	ARI-S	AVE-L	AVE-S
Monoterpenic alcohols (MA)								
linalool	a	1541	12.5 ± 2.5	5.7 ± 0.4	0.8 ± 0.3	0.2 ± 0.0	--**	--
HO-trienol	b	1605	--	--	--	--	--	--
α-terpineol	c	1691	--	--	--	--	0.8 ± 0.1	0.9 ± 0.7
citronellol	d	1760	1.5 ± 0.5	1.6 ± 0.3	4.3 ± 1.1	7.1 ± 2.5	--	1.6 ± 0.3
nerol	e	1793	1.6 ± 1.3	1.7 ± 0.2	1.2 ± 0.6	2.8 ± 1.0	0.2 ± 0.2	0.7 ± 0.2
geraniol	f	1847	47.0 ± 4.1	58.0 ± 7.4	56.2 ± 6.7	82.9 ± 38.1	13.4 ± 3.8	23.4 ± 11.1
Total MA			62.6	67.0	62.5	93.0	14.4	26.6
Monoterpenic oxides and diols (MOD)								
α-tujone	g	1426	1.9 ± 2.0	0.7 ± 0.2	1.5 ± 0.3	--	1.9 ± 0.9	1.1 ± 0.7
trans-furan linalool oxide	h	1436	0.8 ± 0.7	0.4 ± 0.1	--	--	--	--
cis-furan linalool oxide	i	1464	0.8 ± 0.8	0.6 ± 0.2	--	--	--	--
trans-pyran linalool oxide	j	1732	7.8 ± 2.7	7.2 ± 2.1	--	--	--	--
cis-pyran linalool oxide	k	1756	0.9 ± 0.8	1.5 ± 0.3	0.4 ± 0.4	--	--	--
exo-2-hydroxy-1,8-cineole	l	1857	--	--	2.6 ± 1.3	--	1.6 ± 0.5	0.9 ± 0.6
3,7-dimehtylocta-1,5-dien-3,7-diol	m	1935	1.9 ± 0.3	2.4 ± 0.6	0.3 ± 0.1	0.8 ± 0.4	--	--
3,7-dimethylocta-1,7-dien-3,6-diol	n	2121	1.3 ± 0.2	1.6 ± 0.9	--	--	--	--
8-hydroxy-6,7-dihydro-linalool	o	2197	--	0.3 ± 0.5	--	--	--	--
(*E*)-8-hydroxylinalool	p	2265	--	--	--	--	--	--
(*Z*)-8-hydroxylinalool	q	2302	0.9 ± 0.8	1.8 ± 0.8	--	--	--	--
geranic acid	r	2347	3.1 ± 4.2	4.9 ± 4.4	5.2 ± 0.2	15.6 ± 9.8	--	0.4 ± 0.4
Total MOD			19.4	21.4	10.0	16.4	3.5	3.4
Total monoterpenes			82.0	88.4	72.5	109.4	17.9	29.0

(continued on next page)

* Linear retention index on column CP-Wax 52 CB.
** Not detected.

terpineol are varietal compounds with a particular influence in the aroma of wines, bringing fruity and floral notes[19]. Other studies with *Vinhos Verdes* varieties[17] have shown that *Loureiro* is an aromatic grape variety where linalool is found in amounts above its olfactory perception threshold[17,18] of 25 µg/L[7]. In addition, the concentration of this compound in *Loureiro* grapes is much higher than those found for other Portuguese white varieties such as *Boal*, *Malvasia*, *Sercial* and *Verdelho*[3]. In the present work, the total concentration of the monoterpenic alcohols in *Loureiro* was higher than in other grape varieties such as *Syrah*, *Semillon*, *Sauvignon Blanc* and *Cabernet Sauvignon*[22]. The Italian variety *Fiano*[26] showed a total monoterpenic compound concentration similar to *Alvarinho* and *Arinto*, but lower than in *Loureiro*.

Geraniol was the most abundant monoterpenic compound present in the varieties *Alvarinho*, *Arinto*, *Avesso*, *Azal* and *Trajadura*, representing between 61% (*Alvarinho*) and 97% (*Azal*) of the total monoterpenic alcohols. In addition, geraniol was found in concentrations above its olfactory perception threshold (36 µg/L[7]) in *Alvarinho* and *Arinto* from the Sousa and Lima sub-region,

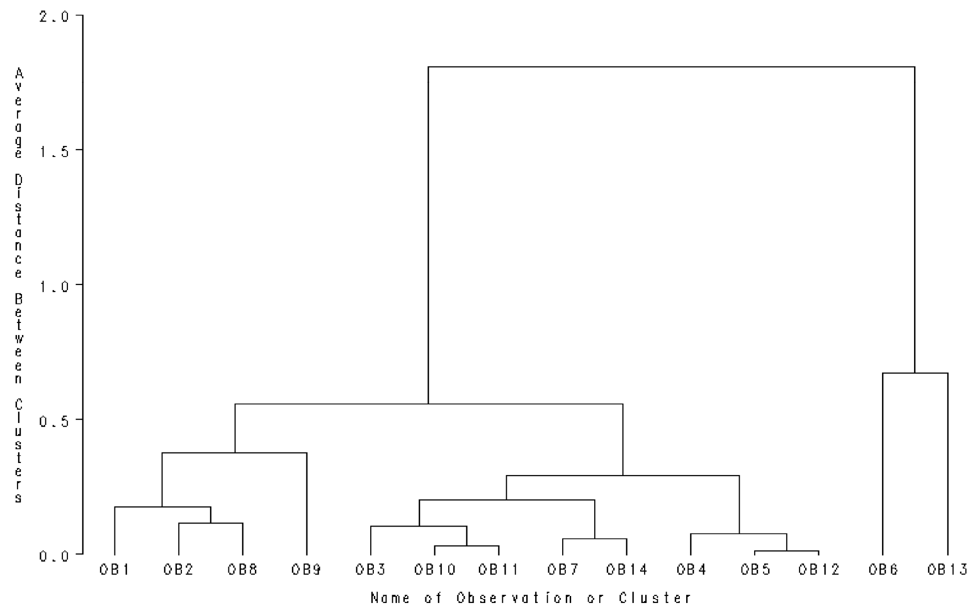

Fig. 2. Dendogram of the monoterpenic compounds of the free fraction for the seven cultivars (OB1 – ALV-L; OB2 – ARI-L; OB3 – AVE-L; OB4 – AZA-L; OB5 – BAT-L; OB6 – LOU-L; OB7 – TRA-L; OB8 – ALV-S; OB9 – ARI-S; OB10 – AVE-S; OB11 – AZA-S; OB12 – BAT-S; OB13 – LOU-S; OB14 – TRA-S).

Table 1. Mean levels (µg/L) ± confidence limits (*p* = 0.05), for the monoterpenic compounds found in the free fraction of the seven varieties studied.
(continued from previous page)

	AZA-L	AZA-S	BAT-L	BAT-S	LOU-L	LOU-S	TRA-L	TRA-S
Monoterpenic alcohols (MA)								
linalool	--	0.1 ± 0.0	--	--	114.6 ± 6.3	161.0 ± 25.8	--	--
HO-trienol	--	--	--	--	0.2 ± 0.1	0.9 ± 0.6	--	--
α-terpineol	--	--	0.7 ± 0.0	--	1.5 ± 0.2	2.4 ± 1.4	--	--
citronellol	0.1 ± 0.0	0.6 ± 0.0	--	--	--	--	0.7 ± 0.8	1.8 ± 0.2
nerol	0.1 ± 0.2	--	--	--	0.3 ± 0.1	--	0.8 ± 0.6	2.1 ± 1.1
geraniol	6.5 ± 0.1	21.5 ± 39.1	--	--	0.9 ± 0.4	--	34.5 ± 23.8	38.7 ± 14.9
Total MA	6.7	22.2	0.7	0.0	117.5	164.3	36.0	42.6
Monoterpenic oxides and diols (MOD)								
α-tujone	--	0.4 ± 0.4	--	0.7 ± 0.4	--	--	0.9 ± 0.6	--
trans-furan linalool oxide	--	--	--	--	4.7 ± 0.5	6.3 ± 4.4	--	--
cis-furan linalool oxide	--	--	--	--	1.6 ± 0.4	8.0 ± 2.9	--	--
trans-pyran linalool oxide	--	--	--	--	51.5 ± 12.6	80.3 ± 39.5	--	--
cis-pyran linalool oxide	--	--	--	--	14.6 ± 5.7	31.0 ± 17.6	--	--
exo-2-hydroxy-1,8-cineole	--	--	--	--	2.2 ± 1.0	3.0 ± 2.8	--	--
3,7-dimehtylocta-1,5-dien-3,7-diol	--	0.2 ± 0.1	--	--	4.2 ± 1.5	14.0 ± 17.5	--	--
3,7-dimethylocta-1,7-dien-3,6-diol	--	--	--	--	3.7 ± 1.8	17.9 ± 23.2	--	--
8-hydroxy-6,7-dihydro-linalool	--	--	--	--	--	1.2 ± 1.0	0.3 ± 0.3	--
(*E*)-8-hydroxylinalool	--	--	--	--	--	--	0.6 ± 0.3	--
(*Z*)-8-hydroxylinalool	--	--	--	--	--	0.3 ± 1.4	--	--
geranic acid	0.6 ± 1.1	0.4 ± 0.1	--	--	0.5 ± 0.7	0.2 ± 0.4	1.8 ± 1.9	--
Total MOD	0.6	1.0	0.0	0.7	83.0	162.3	3.6	0.0
Total monoterpenes	7.3	23.2	0.7	0.7	200.5	326.6	39.6	42.6

and *Trajadura* only from the Sousa sub-region. In contrast, other authors have reported that nerol was the most abundant free monoterpene in the *Albariño* variety from Galicia (NW Spain) and that the overall aroma of the *Albariño* could be attributed to the fruity aroma followed by the floral aroma[28,29]. Pyran and furan oxides of linalool were only identified in *Alvarinho* and *Loureiro* varieties. Such compounds normally have their concentration increased from grapes to wine[19].

The hierarchical tree plot of cluster presented in Fig. 2 shows a division of the seven studied grape varieties in three groups: The first group being formed by the cultivars *Alvarinho* (OB1 and OB8) and *Arinto* (OB2 and OB9); the second group including only *Loureiro* (OB6 and OB13); the third group consisting of *Avesso* (OB3 and OB10), *Azal* (OB4 and OB11), *Batoca* (OB5 and OB12) and *Trajadura* (OB7 and OB14). Inside of each group, the grape samples have an affinity greater than 75%.

Principal component analysis (PCA) was used to identify the free volatile compounds that best discriminated between the varieties from the two geographic areas, Lima (Fig. 3A) and Sousa (Fig. 3B). In Fig. 3A, the first two principal components accounted for 75.19% of the total variance (51.11% and 24.08%, respectively); while in Fig. 3B, they accounted for 87.36% of the total variance (67.76% and 19.60%, respectively). For both sub-regions, the first principal component Prin 1 discriminated *Loureiro* from the other six varieties. However, Prin 2 in sub-region of Lima divided the remaining six grape varieties into two groups, while the sub-region of Sousa was divided in three groups. Nevertheless, it was obvious that *Avesso, Azal* and *Batoca* were very close among them and differed from *Alvarinho*, *Arinto* and *Trajadura.* The results for *Loureiro*, *Alvarinho* and *Arinto* varieties were similar to others previously published[2,17,18].

In general, the two statistical analyses showed comparable results, with *Loureiro* being easily differentiated from the rest of the studied varieties. *Alvarinho* and *Arinto* are two similar varieties, while *Trajadura* has an intermediate position in both L (Lima) and S (Sousa) samples.

Glycosidically bound fraction

Twenty-two monoterpenic compounds were identified and quantified in the glycosidically bound form in the

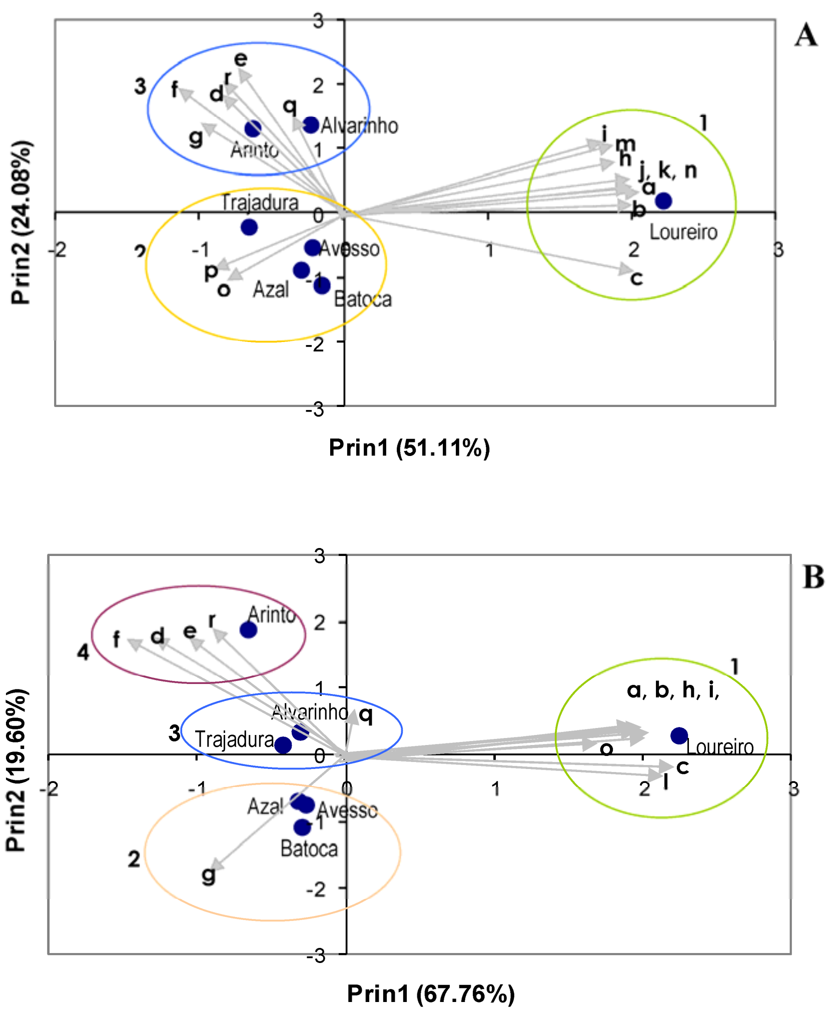

Fig. 3. Principal component analysis applied on the monoterpenic compounds of the free fraction of the seven cultivars: A – sub-region Lima; B – sub-region Sousa (peak identification may be assessed on Table I).

seven *Vinhos Verdes* varieties from two geographic areas (Sousa and Lima) of the *Vinhos Verdes* appellation of origin (Fig. 4). Table II shows the mean concentration levels and the confidence limits (p = 0.05), obtained for each compound. Among the varieties, *Alvarinho* was the richest in monoterpenic compounds, followed by *Loureiro*. The total amount of monoterpenic alcohols found in *Loureiro* was minor compared to those found in the variety *Syrah*[22]. Studies performed by Ugliano and Moio[26] revealed an elevated concentration of monoterpenic com-

Table II. Mean levels (μg/L) ± confidence limits (p = 0.05), for the monoterpenic aglycons found in the glycosidically bound fraction of the seven varieties studied.

		RI*	ALV-L	ALV-S	ARI-L	ARI-S	AVE-L	AVE-S
Monoterpenic alcohols (MA)								
linalool	a	1541	19.5 ± 3.5	58.4 ± 10.4	17.5 ± 13.0	2.5 ± 1.3	--**	0.1 ± 0.1
4-terpineol	b	1597	--	--	--	--	--	--
HO-trienol	c	1605	0.5 ± 0.5	1.1 ± 0.6	--	--	--	--
α-terpineol	d	1691	0.5 ± 0.6	1.4 ± 1.1	0.6 ± 0.2	0.4 ± 0.5	0.4 ± 0.5	1.8 ± 0.9
citronellol	e	1760	0.2 ± 0.3	0.2 ± 0.3	0.7 ± 0.6	0.8 ± 0.4	0.1 ± 0.2	0.8 ± 0.7
nerol	f	1793	1.5 ± 1.0	2.2 ± 0.8	3.3 ± 0.5	1.6 ± 0.9	0.1 ± 0.1	0.9 ± 0.5
geraniol	g	1847	9.0 ± 5.6	9.8 ± 1.2	10.5 ± 1.9	11.5 ± 10.4	1.6 ± 0.2	4.9 ± 2.3
Total MA			31.3	73.1	32.5	16.8	2.3	8.5
Monoterpenic oxides and diols (MOD)								
geranial	h	1735	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.3	--	--	--
trans-furan linalool oxide	i	1436	5.0 ± 0.0	21.7 ± 8.5	1.2 ± 0.6	0.7 ± 0.4	0.2 ± 0.3	--
cis-furan linalool oxide	j	1467	4.5 ± 3.1	10 ± 3.6	0.8 ± 0.5	0.9 ± 0.3	0.8 ± 1.0	1.0 ± 0.2
trans-pyran linalool oxide	k	1732	3.8 ± 3.2	12.7 ± 5.1	0.4 ± 0.1	--	0.5 ± 0.1	--
cis-pyran linalool oxide	l	1756	0.5 ± 0.5	2.8 ± 1.4	0.2 ± 0.2	--	0.1 ± 0.1	--
exo-2-hydroxy-1,8-cineole	m	1857	0.4 ± 0.5	1.4 ± 0.9	0.5 ± 0.7	--	0.5 ± 0.5	1.9 ± 1.4
3,7-dimehtylocta-1,5-dien-3,7-diol	n	1935	18.5 ± 11.7	43.0 ± 27.7	2.4 ± 0.9	--	--	--
Linaloolhydrate	o	1967	--	--	--	--	--	--
3,7-dimethylocta-1,7-dien-3,6-diol	p	2121	4.2 ± 2.7	10.5 ± 6.3	--	--	--	--
citronellol hydrate	q	2196	--	1.4 ± 1.0	--	--	--	--
8-hydroxy-6,7-dihydro-linalool	r	2197	0.7 ± 0.2	2.0 ± 1.3	4.0 ± 1.5	1.5 ± 1.9	0.8 ± 0.4	--
(*E*)-8-hydroxylinalool	s	2265	7.8 ± 3.5	19.1 ± 8.7	3.1 ± 1.0	--	0.9 ± 0.6	--
(*Z*)-8-hydroxylinalool	t	2302	38.2 ± 7.4	119.1 ± 59.1	40.4 ± 16.5	11.2 ± 16.4	2.1 ± 1.8	2.9 ± 2.3
geranic acid	u	2347	4.6 ± 2.3	10.4 ± 10.0	10.3 ± 9.8	8.2 ± 6.2	0.7 ± 1.2	1.1 ± 0.9
p-1-menhten-7,8-diol	v	2517	--	0.1 ± 0.1	--	--	0.9 ± 0.7	0.3 ± 1.1
Total MOD			95.4	254.1	63.5	22.5	7.4	7.2
Total Monoterpenes			119.5	327.5	96.1	39.3	9.7	15.6

(continued)

* Linear retention index on column CP-Wax 52 CB.
** Not detected.

Table II. Mean levels (μg/L) ± confidence limits (p = 0.05), for the monoterpenic aglycons found in the glycosidically bound fraction of the seven varieties studied. *(continued)*

	AZA-L	AZA-S	BAT-L	BAT-S	LOU-L	LOU-S	TRA-L	TRA-S
Monoterpenic alcohols (MA)								
linalool	1.4 ± 2.2	1.7 ± 1.2	--	0.1 ± 0.1	7.2 ± 4.2	75.2 ± 49.6	0.2 ± 0.2	0.2 ± 0.2
4-terpineol	--	--	--	--	--	0.1 ± 0.1	--	--
HO-trienol	--	--	--	--	--	1.4 ± 1.5	--	--
α-terpineol	0.4 ± 0.5	0.9 ± 0.6	--	0.1 ± 0.1	0.5 ± 0.3	8.0 ± 5.5	0.5 ± 0.4	0.6 ± 0.6
citronellol	--	0.4 ± 0.2	--	0.2 ± 0.1	--	--	--	0.2 ± 0.1
nerol	--	0.3 ± 0.2	--	0.6 ± 0.4	--	0.9 ± 0.7	1.1 ± 0.5	0.6 ± 0.4
geraniol	1.3 ± 2.0	8.9 ± 2.6	1.4 ± 1.2	2.9 ± 2.3	0.2 ± 0.3	0.8 ± 0.5	3.6 ± 3.3	6.1 ± 4.0
Total MA	3.1	12.2	1.4	3.9	7.9	86.5	5.4	7.8
Monoterpenic oxides and diols (MOD)								
geranial	--	0.1 ± 0.0	--	0.1 ± 0.0	--	--	--	--
trans-furan linalool oxide	0.1 ± 0.1	0.5 ± 0.4	--	0.4 ± 0.4	1.9 ± 1.0	25.2 ± 23.2	0.1 ± 0.1	--
cis-furan linalool oxide	0.8 ± 1.2	1.2 ± 0.3	--	0.5 ± 0.2	0.2 ± 0.0	3.7 ± 3.2	0.1 ± 0.1	--
trans-pyran linalool oxide	0.3 ± 0.3	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.5	0.5 ± 0.3	4.4 ± 2.8	--	--
cis-pyran linalool oxide	0.2 ± 0.4	0.2 ± 0.0	0.1 ± 0.0	0.3 ± 0.2	--	1.1 ± 0.9	--	--
exo-2-hydroxy-1,8-cineole	0.2 ± 0.2	0.4 ± 0.3	--	--	0.2 ± 0.1	1.1 ± 0.8	0.3 ± 0.2	1.3 ± 0.8
3,7-dimehtylocta-1,5-dien-3,7-diol	0.3 ± 0.4	0.4 ± 0.0	--	--	10.0 ± 10.9	42.6 ± 38.8	--	--
Linaloolhydrate	--	--	--	--	--	2.1 ± 1.7	--	--
3,7-dimethylocta-1,7-dien-3,6-diol	--	--	--	--	1.8 ± 2.4	19.4 ± 17.9	--	--
citronellol hydrate	--	--	--	--	--	--	--	1.4 ± 0.4
8-hydroxy-6,7-dihydro-linalool	--	0.5 ± 0.4	0.5 ± 0.2	1.3 ± 1.5	--	0.8 ± 1.4	0.5 ± 0.6	0.5 ± 0.5
(*E*)-8-hydroxylinalool	0.5 ± 0.7	1.4 ± 0.6	0.2 ± 0.1	0.9 ± 0.5	--	9.2 ± 7.5	0.3 ± 0.4	--
(*Z*)-8-hydroxylinalool	7.8 ± 11.3	18.0 ± 14.8	0.3 ± 0.0	2.1 ± 1.4	0.6 ± 1.1	6.0 ± 6.2	3.7 ± 3.0	3.6 ± 2.5
geranic acid	1.6 ± 2.8	3.7 ± 3.9	4.3 ± 3.6	11.3 ± 6.5	0.2 ± 0.4	1.1 ± 0.8	1.6 ± 2.0	2.0 ± 2.7
p-1-menhten-7,8-diol	0.2 ± 0.3	1.3 ± 0.8	--	1.3 ± 1.1	0.5 ± 0.6	5.1 ± 7.1	0.4 ± 0.4	--
Total MOD	11.9	27.9	5.5	18.4	15.9	121.9	7.0	8.9
Total monoterpenes	15.0	40.1	6.9	22.3	23.8	208.4	12.4	16.7

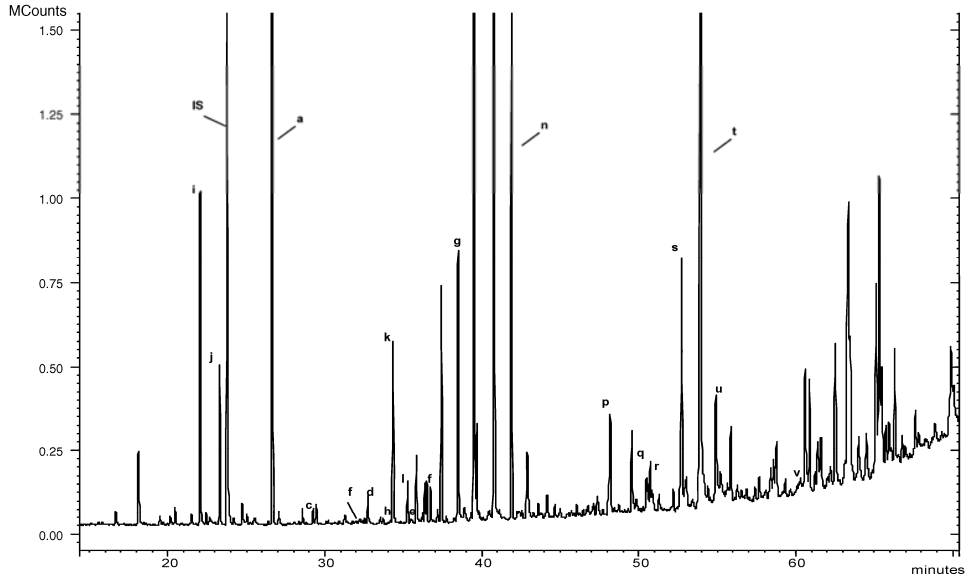

Fig. 4. Representative section of a GC-MS chromatogram for the *Alvarinho* grape variety ALV-S, for the monoterpenic compounds found in the glycosidically bound fraction (peak identification may be assessed on Table II). IS – internal standard.

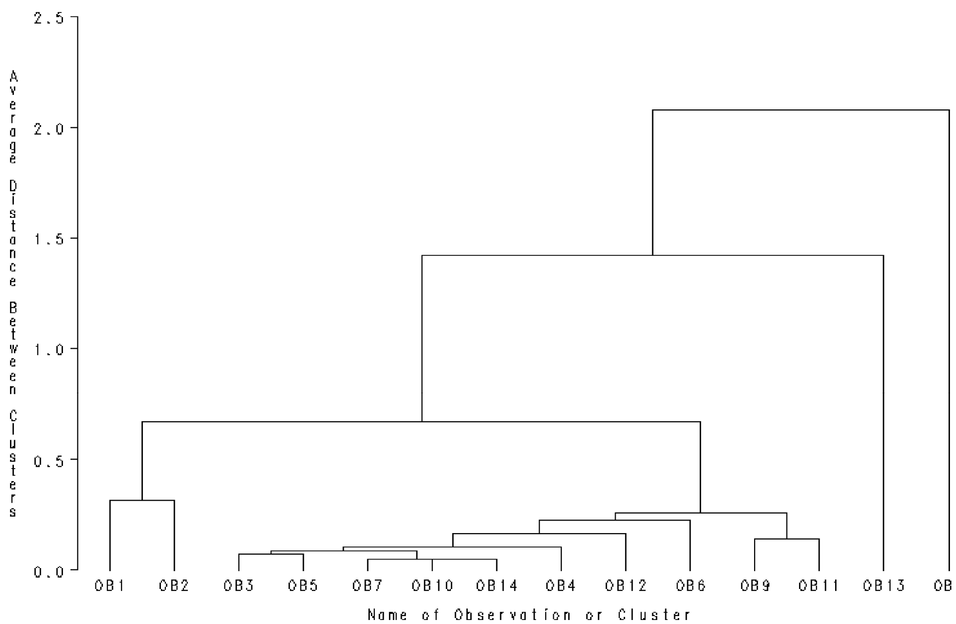

Fig. 5. Dendogram of the monoterpenic compounds of the glycosidically bound fraction for the seven cultivars (OB1 – ALV-L; OB2 – ARI-L; OB3 – AVE-L; OB4 – AZA-L; OB5 – BAT-L; OB6 – LOU-L; OB7 – TRA-L; OB8 – ALV-S; OB9 – ARI-S; OB10 – AVE-S; OB11 – AZA-S; OB12 – BAT-S; OB13 – LOU-S; OB14 – TRA-S).

pounds in *Fiano* and these concentrations were higher than those observed for the varieties studied in this paper.

Linalool was the most abundant monoterpenic compound in *Alvarinho*, in contrast with the free fraction of aroma, where geraniol was found in higher amounts. Zamuz and Vilanova[28] reported that linalool and limonene were the most important terpenols identified in the *Albariño* variety from Galicia (Spain). Diéguez et al.[5] demonstrated a high content of bound forms in *Albariño*, where linalool, geraniol, benzyl alcohol and 2-phenylethanol predominated. Linalool and 3,7-dimethylocta-1,5-dien-3,7-diol were also the most abundant compounds in the *Loureiro* variety, with linalool representing 89% of the total monoterpenic alcohols. Among the monoterpenic oxides, (*Z*)-8-hydroxylinalool was found in much higher concentrations than the isomer (*E*)-8-hydroxylinalool in the *Alvarinho* variety. *Loureiro* (sub-region of Sousa) was the only variety where the concentration of the (*E*) isomer

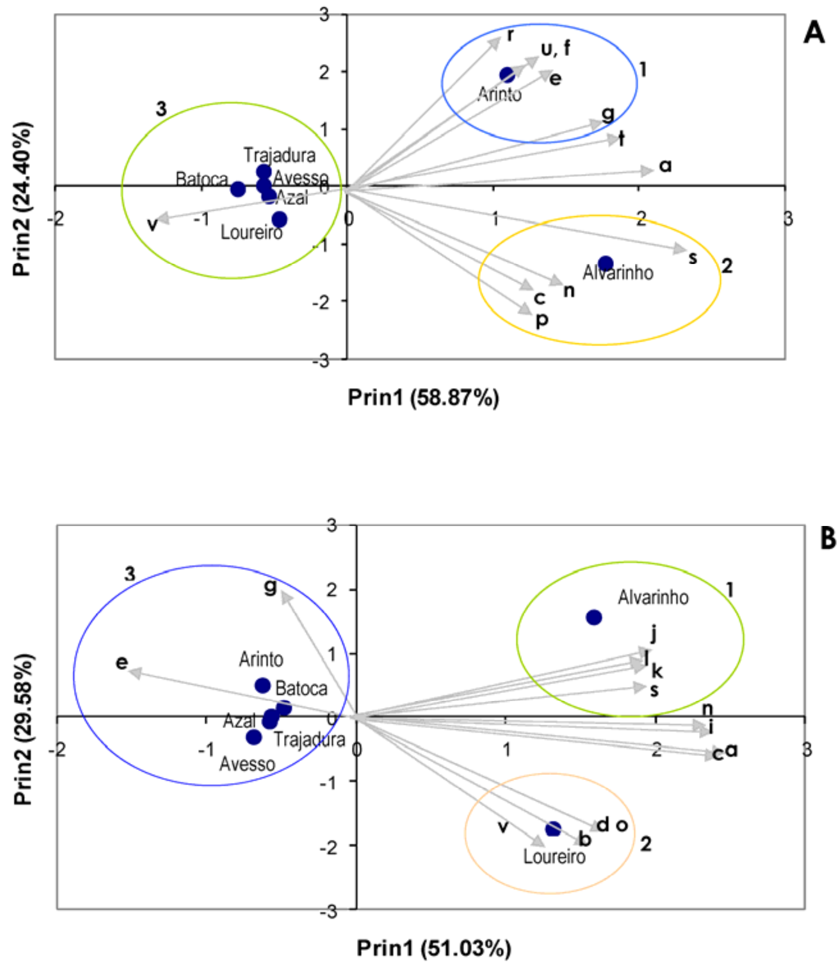

Fig. 6. Principal component analysis applied on the monoterpenic compounds of the glycosidically bound fraction of the seven cultivars: A – sub-region Lima; B – sub-region Sousa (peak identification may be assessed on Table II).

was higher than that of the (*Z*) isomer, as already reported by Oliveira[16]. In *Alvarinho* and *Loureiro* varieties from the sub-region of Sousa, linalool was found in a concentration level above of its olfactory perception threshold of 25 μg/L[7].

The hierarchical tree plot of cluster, based on bound compounds from the Sousa and Lima sub-region, revealed four groups in the studied cultivars (Fig. 5): the first one, formed by *Alvarinho* (OB1) and *Arinto* (OB2) from the sub-region Lima; the second one included *Avesso* (OB3 and OB10), *Batoca* (OB5 and OB12), *Trajadura* (OB7 and OB14), *Azal* (OB4 and OB11), *Loureiro* from the Lima sub-region (OB6), and *Arinto* from the Sousa sub-region (OB9); the third one was composed of *Alvarinho* (OB8) from the sub-region Sousa; the fourth one was composed of *Loureiro* (OB13) from the sub-region Sousa. Major similarities were found between the first two groups.

Principal component analysis (PCA) was used to identify the volatile compounds that best discriminated the varieties from the two geographic areas, Lima (Fig. 6A) and Sousa (Fig. 6B). In Fig. 6A, the first two principal components accounted for 84.27% of the total variance (58.87% and 24.40%, respectively). The PCA also showed three groups: *Arinto* (1), *Alvarinho* (2) and *Avesso, Azal, Batoca, Loureiro* and *Trajadura* (3). In Fig. 6B, the first two principal components accounted for 80.61% of the total variance (51.03% and 29.58%, respectively) and three groups formed were *Alvarinho* (1), *Loureiro* (2) and *Arinto*, *Avesso*, *Azal*, *Batoca* and *Trajadura* (3).

The hierarchical tree plot of cluster and the PCA showed similar results, with *Alvarinho* variety L and S being differentiated from the others varieties. *Arinto* Lima and *Loureiro* Sousa samples were also different from the remaining samples.

CONCLUSIONS

The results showed that it was possible to differentiate the seven recommended white varieties for the production of *Vinhos Verdes*, regarding free and bound monoterpene composition. Cultivars *Loureiro*, *Arinto* and *Alvarinho* were clearly distinct from the other studied varieties, while *Trajadura* showed an intermediate position. Linalool and its oxides contributed to the discrimination of

Loureiro. *Loureiro* had a typical aromatic profile, and linalool was the most abundant compound for both free and bound fractions of the varietal aroma. In contrast, *Alvarinho* showed a different aromatic profile in the free and bound fractions, with linalool being the most abundant compound in the glycosidically bound fraction (as for *Loureiro*), while geraniol prevailed in the free fraction. *Arinto*, *Avesso*, *Azal* and *Trajadura* had a more equilibrated profile in terms of aroma compounds in both fractions. The *Arinto* variety showed a high potential of aroma compounds in the bound fraction. *Batoca* revealed a specific aromatic profile, i.e., few monoterpenic compounds in the free fraction. Based on the obtained results and on previous studies, in spite of some differences between samples from different sub-regions, the monoterpenic profile of each variety stayed unchanged.

This study showed the possibility to differentiate grapes of the seven cultivars recommended for white *Vinhos Verdes* production. Additionally, it could serve as a tool for the technological valorization of the raw-materials used to produce high quality wines.

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