

Free and Bound Aromatic Components of *Loureiro* and *Alvarinho* Grape Varieties from the *Vinhos Verdes* Region

Flüchtige und Gebundene Aromakomponenten der Sorten *Loureiro* und *Alvarinho* aus dem Anbaugebiet *Vinhos Verdes*

Potentiel Aromatique des Cépages *Loureiro* et *Alvarinho* de la Région des *Vinhos Verdes*

José M. Oliveira¹*, M. Odete Maia¹, Raymond L. Baumes², Claude L. Bayonove²

¹ Centro de Engenharia Biológica, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

² Institut Supérieure de la Vigne et du Vin, IPV-ENSAM-INRA, Laboratoire des Arômes et Substances Naturelles, 2 Place P. Viala, 34060 Montpellier Cedex 01, France

* corresponding author: Email: jmoliveira@deb.uminho.pt

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Abstract

Free and glycosidically bound volatiles were quantified in five grape samples grown in 1996 from two of the most important *Vitis vinifera* varieties of *Vinhos Verdes* Region, *Loureiro* and *Alvarinho*. The most recommended sub-region for monovarietal wine production and an alternative sub-region were chosen. A third sample of *Alvarinho* was selected from a different and particular pebble soil.

Forty compounds in the free fraction and sixty-four in the glycosidically bound fraction including alcohols, monoterpenic compounds, C₁₃ norisoprenoids, phenols, C₆ compounds and carbonyl compounds were quantified in the samples of the two varieties.

The results showed that these two Portuguese varieties were quite different regarding the varietal flavour compounds, even grown in the different sub-regions. Samples of *Loureiro* variety showed higher content of free monoterpenic compounds than *Alvarinho*, mainly linalool, pyran linalool oxides and 3,7-dimethylocta-1,7-dien-3,6-diol. Free linalool seemed to be characteristic of *Loureiro* variety, with levels higher than its perception threshold. On the contrary, *Alvarinho* samples contained only traces of free linalool but contained higher amounts of alcohols, mainly benzyl alcohol and 2-phenylethanol, of geraniol and 2-phenylethanal, this last one above its perception threshold. Both cultivars contained important levels of bound monoterpenic compounds and C₁₃ norisoprenoids, bound linalool occurring in high levels in both varieties. Nevertheless, *Alvarinho* variety contained higher amounts of bound geraniol and monoterpenic oxides and diols, mainly *trans*-pyran linalool oxide, geranic acid and (*Z*)-8-hydroxylinalool, while *Loureiro* variety was richer in bound α -terpineol and p-1-menthen-7,8-diol.

Key-Words: varietal flavour, *Loureiro*, *Alvarinho*, *Vinhos Verdes*

1. Introduction

The *Vinhos Verdes* region, in the Northwest of Portugal, is geographically divided into 6 sub-regions: *Monção*, *Lima*, *Braga*, *Basto*, *Amarante* and *Penafiel*. For white wine production with Appellation of Origin *Vinho Verde*, there are 7 recommended and 18 authorized varieties; the latter must represent less than 25 % of the vineyard. *Loureiro*, *Alvarinho*, *Trajadura*, *Pedernã*, *Azal-branco*, *Avesso* and *Batoca* are the recommended varieties. *Loureiro* is spread throughout the region but is not recommended for *Basto* and *Amarante* sub-regions. *Alvarinho* variety is only recommended for *Monção* sub-region and authorized in the *Lima* sub-region. These two grape varieties can give rise to varietal wines with interesting sensorial characteristics (1, 2).

Vinhos Verdes wines have an alcoholic degree between 8.5 and 11.5 % vol., except those that are made from *Alvarinho* variety which must have 11.5 % vol. minimum acquired and 13 % vol. maximum. They are slightly acidic and are characterised by some effervescence. They are pale yellow, citrine, or straw-coloured and the flavour is delicate, fresh and fruity. They must be consumed young (1-2 years) except *Alvarinho* wines, which keep their sensorial characteristics during a longer period.

Only a few works were made about the aroma characterisation of *Loureiro* and *Alvarinho* varieties. The results refer mainly to aromatic compounds identified not in grapes, but in wines. In this context, Guedes de Pinho (3) and Rogerson et al. (4) quantified the five principal terpenols in wines of the two varieties. Versini et al. (5) made an exhaustive characterisation of the free and bound compounds in Galician *Loureira* and *Albariño* wines. Recently, López (6) quantified five terpenols in wines of the same Galician varieties. This paper describes a qualitative and quantitative analytical study of the volatile and bound constituents of *Alvarinho* and *Loureiro* grapes grown in three sub-regions of the Portuguese *Vinhos Verdes* Region.

2. Material and Methods

Grape samples

Grapes from *Loureiro* and *Alvarinho* varieties were manually harvested in 1996 in two different vineyards; both soils are from granitic origin. The most recommended sub-regions (*Lima* and *Monção*) for the monovarietal wine production and an alternative sub-region (*Braga* and *Lima*) inside the *Vinhos Verdes* Region were chosen. For the *Alvarinho* variety an additional vineyard cultivated

in a pebble soil was chosen. The codes attributed to the samples were the following:

- A_{SS}: *Alvarinho* – *Solar de Serrade*, sub-region of Monção
 A_{CR}: *Alvarinho* – *Lagoa Verde*, (“*Calhau Rolado*” - pebble), sub-region of Monção
 A_{AV}: *Alvarinho* – *Estação Vitivinícola Amândio Galhano*, sub-region of Lima
 L_{AV}: *Loureiro* – *Estação Vitivinícola Amândio Galhano*, sub-region of Lima
 L_{CT}: *Loureiro* – *Casa da Tapada*, sub-region of Braga

For each vineyard, three vines were selected and the grapes were picked at random at technological maturity (A_{CR} and A_{SS} - September 23th; L_{CT} - 24th; L_{AV} and A_{AV} - 25th).

The berries were manually picked from grape clusters, selected, frozen in liquid nitrogen and stored at -20 °C. For each sample the average berry weight, total acidity, pH and sugar content were determined using standard procedures.

Solvents

All solvents used were analytical grade and further purified. Ethyl acetate (Merck) and diethyl ether (Merck) were distilled, the last one on iron (II) sulphate. Dichloromethane was washed with de-ionised water, then distilled. Pentane (Carlo Erba) was washed with H₂SO₄, KMnO₄ and de-ionised water then distilled on potassium hydroxide.

Extraction of free and bound fractions

About 550 g of frozen berries were thawed at 4 °C overnight, then crushed (*turmix*, *Sofraca*), centrifuged (RCF = 6544, 25 min, 4 °C) and filtrated. The yield of juice production was determined (table 1). To 100 mL juice, 32.15 µg of 4-nonanol (Merck) were added and passed through an Amberlite XAD-2 resin (20-50 mesh, Fluka) column according to Gunata et al. (7). Free and bound fractions were eluted successively with 50 mL of pentane-dichloromethane (2:1) and 50 mL of ethyl acetate. Pentane-dichloromethane eluate was dried over anhydrous sodium sulfate and concentrated to 200 µL by distillation through a Vigreux, then a Dufton column prior to analysis. The ethyl acetate eluate was concentrated to dryness in vacuum (40 °C) and dissolved in 0.2 mL of 100 mM citrate-phosphate buffer (pH 5.0). Residual free compounds were extracted five times with pentane-dichloromethane and discarded. 14 mg 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 pentane-dichloromethane. 21.43 µg of 4-nonanol as standard was added to the organic phase and concentrated to 200 µL through a Dufton column. Analyses were made in triplicate.

Chromatographic analysis

Gas chromatographic analysis of free and released volatile compounds was performed using a Varian 3400 Chromatograph equipped with a fused capillary column coated with DB-Wax (30 m x 0.32 mm i.d., 0.5 mm film thickness, J&W). The injection was on-column and the temperature of the injector was programmed from 20 °C to 250 °C at 180 °C/min. The temperature of the oven was held at 60 °C for 3 min, then programmed from 60 to 245 °C at 3 °C/min, then held 20 min at 250 °C. The FID temperature was 250 °C. The carrier gas was H₂ at 3.1 psi.

Qualitative analysis was made by GC-MS, using a HP 5890 II chromatograph coupled to a HP 5989A Mass Spectrometer. The chromatographic column and oven temperatures were the same as above. The carrier gas was helium 60 at 1.35 mL/min. The injection was on-column, programmed from 35 °C to 250 °C at 180 °C/min,

then held 82 min at 250 °C. Identifications were carried out by comparing retention times and EI mass spectra with published data or with authentic compounds.

2. Results and Discussion

1. General analysis

pH, total acidity, sugar content, berry mean weight and juice yield for the five samples studied are summarised in table 1. Sugar content and pH are higher for the *Alvarinho* variety and the berry mean weight and juice efficiency are higher for the *Loureiro* variety. Total acidity is quite high for both varieties. These results are in agreement with some published data (8, 9, 10).

Tab. 1: General analysis of berries and juices.

Tab. 1: Analysen von Beeren und Mosten.

Tab. 1: Analyse classique des raisins et des jus.

	L _{CT}	L _{AV}	A _{SS}	A _{CR}	A _{AV}
pH	-	2.88	2.94	3.10	3.15
Acidity as tart. ac., mg/L	-	8.6	9.0	7.9	10.5
Sugar content, g/L	175.1	188.4	195.1	208.6	210.9
Berry mean weight, g*	1.34 ⁽¹⁴¹⁶⁾	1.63 ⁽¹¹⁰⁵⁾	1.24 ⁽¹⁴⁵⁴⁾	1.11 ⁽¹⁶³²⁾	1.16 ⁽¹⁶³⁷⁾
Juice efficiency, mL/kg**	714	735	674	654	652
* n° of berries weighted ** efficiency of juice extraction from grapes					

3. Free and bound constituents of *Loureiro* and *Alvarinho* grapes

3.1. Free compounds

GC-MS analysis allowed to identify in the free fraction 40 volatile compounds including 5 C₆-compounds, 7 alcohols, 17 monoterpene compounds, 1 C₁₃ norisoprenoid compound, 8 volatile phenols and 2 carbonyl compounds. Table 2 shows the mean level and the coefficient of variation obtained for each compound in the five samples studied. These levels were determined as 4-nonanol equivalents.

Tab. 2: Mean levels of the volatile compounds in the free fraction of *Loureiro* and *Alvarinho* grape samples.

Tab. 2: Mittelwerte der flüchtigen Verbindungen in den freien Fraktionen aus Beeren der Sorten *Loureiro* und *Alvarinho*.

Tab. 2: Teneurs moyennes en composés volatils de la fraction libre des échantillons de raisins *Loureiro* et *Alvarinho*.

	L_{CT}		L_{AV}		A_{SS}		A_{CR}		A_{AV}	
	C ($\mu\text{g/L}$)	C.V. (%)	C ($\mu\text{g/L}$)	C.V. (%)	C ($\mu\text{g/L}$)	C.V. (%)	C ($\mu\text{g/L}$)	C.V. (%)	C ($\mu\text{g/L}$)	C.V. (%)
C_6 Compounds										
hexanal	618.4	2.3	352.4	4.0	720.8	1.3	544.2	24.8	344.4	6.0
(E)-hex-2-enal	1290.0	1.5	883.1	4.1	1165.4	0.2	1031.7	3.1	1045.9	3.1
hexan-1-ol	10.9	8.4	9.5	7.9	22.3	5.1	16.4	11.1	18.8	3.7
(Z)-3-hexen-1-ol	2.8	7.5	2.5	2.5	8.5	5.7	7.4	43.6	9.9	17.9
(E)-2-hexen-1-ol	20.8	4.0	16.6	4.9	20.7	4.5	16.6	1.5	13.9	15.1
total	1942.9		1264.1		1937.7		1616.3		1432.9	
Alcohols										
3-methylbutan-1-ol	8.1	40.0	11.9	13.7	21.6	18.5	14.9	9.7	13.1	20.6
3-methylbut-3-en-1-ol	28.8	8.2	17.7	10.1	40.2	11.2	34.8	20.7	17.9	21.0
pentan-1-ol	21.1	8.8	12.5	18.8	26.1	12.9	28.3	17.2	11.4	0.5
octan-1-ol	tr		tr		1.0	11.0	1.9	45.9	2.5	31.5
benzyl alcohol	126.9	2.0	133.0	5.1	230.1	2.3	200.8	2.5	254.4	9.1
2-phenylethanol	61.7	2.5	41.8	1.9	112.5	2.8	89.4	4.2	166.3	9.4
2-phenoxyethanol	29.0	4.2	3.7	1.9	11.1	15.5	25.1	3.9	8.6	25.7
total	275.6		220.6		442.6		395.2		474.2	
Monoterpenic alcohols										
linalool	274.2	0.9	178.8	1.3	3.7	9.1	4.7	23.1	3.7	14.3
α -terpineol	1.1	41.7	1.3	32.6	tr		tr		tr	
citronellol	tr		tr		1.8	15.7	1.8	7.9	3.0	22.2
nerol	tr		tr		1.8	4.0	2.0	3.6	4.3	36.4
geraniol	0.8	15.1	0.9	43.1	15.0	4.9	13.6	5.75.7	21.4	12.7
total	276.1		181.0		22.3		22.1		32.4	
Monoterpenic oxides and diols										
trans-furan linalool oxide	4.1	8.3	3.6	9.2	2.6	11.2	1.9	10.6	2.8	19.6
cis-furan linalool oxide	1.5	11.1	2.2	10.0						
trans-pyran linalool oxide	48.6	5.5	64.2	3.6	17.9	2.5	11.5	4.5	20.7	19.5
cis-pyran linalool oxide	20.4	6.3	22.4	8.8	tr		tr		2.7	20.1
exo-2-hydroxy-1,8-cineole	1.3	14.3	0.5	13.9						
3,7-dimethylocta-1,5-dien-3,7-diol	54.6	6.0	44.0	32.9	43.0	12.2	50.6	3.4	20.8	32.8
3,7-dimethylocta-1,7-dien-3,6-diol	16.7	8.6	15.6	38.1	1.8	16.4	3.6	22.3	3.4	39.2
8-hydroxy-6,7-dihydro-linalool	0.7	19.2	1.1	22.4	1.9	33.3	3.2*	32.2	2.2*	4.5
(E)-8-hydroxy-linalool	3.2	5.2	2.4	12.3	tr		tr		tr	
(Z)-8-hydroxy-linalool	tr		tr		2.0	31.6	2.0	11.2	6.0	16.8
geranic acid	11.5	2.9	5.3	2.6	21.2	9.2	20.4	2.6	21.2	3.1
total	162.6		161.3		90.4		93.2		79.8	
C_{11} norisoprenoids										
3-hydroxy-5,6-epoxy- β -ionone	6.4	3.6	5.2	6.6	4.1	3.8	5.1	13.7	11.5	39.8
total	6.4		5.2		4.1		5.1		11.5	
Volatile phenols										
o-cresol + phenol	1.0	43.1	1.1	9.8	1.5	14.5	1.6	20.0	4.8	16.4
p-cresol	-		-		-		-		1.3	65.9
m-cresol	-		-		-		-		1.5	60.3
eugenol	1.8	22.9	1.7	51.2	1.5	26.0	2.8	18.5	1.6	5.1
vanillin	14.9	9.4	10.9	9.6	21.1	0.9	26.1	6.7	15.2	5.7
methyl vanillate	-		-		2.4	12.0	2.8	7.2	4.5	19.1
2-(4'-gaiacyl)ethanol	2.6	25.3	2.4	21.2	-		-		-	
total	20.3		16.1		26.5		33.3		28.9	
Carbonyl compounds										
benzaldehyde	3.4	17.2	10.1	9.4	6.9	6.3	5.2	14.4	7.8	18.9
phenylethanal	17.8	2.1	23.8	5.4	65.2	1.6	71.5	3.0	44.2	8.3
total	21.2		33.9		72.1		76.7		52.0	
Total	2705.1		1882.2		2595.7		2241.9		2111.7	

tr = traces; - = not detected; * - citronellol hydrate

The six-carbon compounds are the most abundant group, representing about 70 % (67.2-71.8 % for *Loureiro*; 67.9-74.7 % for *Alvarinho*) of the total level of the free compounds. These compounds have an herbaceous flavour (11, 12); they arise mainly under the action of grape enzymes during sample crushing, in presence of oxygen, from grape lipids (13, 14, 15). The total levels of these C_6 compounds are very dependent on the maturity of grapes; thus the sample with the lowest maturity for each cultivar (L_{CT} and A_{SS}) has the highest total level. The C_6 aldehydes, which make up about 98 % of the total level, and the unsaturated C_6 alcohols are for their most part reduced by

yeast during wine making; therefore, the differences in the unsaturated C_6 alcohols observed by Versini et al. (5) between *Loureira* and *Albariño* Galician wines were not retrieved.

Other than these statements, it appears from the C_6 compounds levels, that hexanal and (E)-hex-2-enal were the only contributors to the green aroma of the 5 juice samples analysed, as their odour thresholds in water were respectively 19 and 17 $\mu\text{g/L}$ (16). But this is not typical of these varieties.

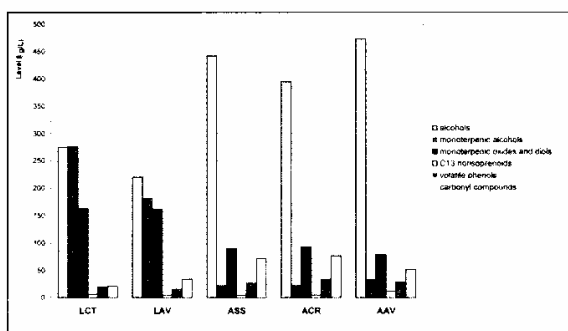


Fig. 1: Profile of the free fraction (except C_6 compounds) of the grape samples analysed.

Fig. 1: Profil der freien Fraktion (außer C_6 -Verbindungen) der analysierten Traubenproben.

Fig. 1: Profil de la fraction libre (excepté les composés en C_6) des échantillons de raisin analysés.

The other chemical groups amount to about 30 % of the volatiles. They are not identical for the two grape varieties (figure 1). The *Loureiro* variety contains about 16-18 % monoterpene compounds, 10-12 % alcohols, and in lower amounts volatile phenols (0.8-0.9 %), carbonyl compounds (0.8-1.8 %) and C_{13} norisoprenoids (0.2-0.3 %). The *Alvarinho* variety has lesser content of monoterpenes (4.3-5.3 %), higher amount of alcohols (17.1-22.5 %) and about the same levels of phenols (1.0-1.5 %), C_{13} norisoprenoids (0.2-0.5 %) and carbonyl compounds (2.5-3.4 %).

As regards alcohols, *Alvarinho* samples are about 2 folds more richer than those from *Loureiro* variety, mainly due to benzyl alcohol (200.8-254.4 $\mu\text{g/L}$ *Alvarinho*; 126.9-133.0 $\mu\text{g/L}$ *Loureiro*) and 2-phenylethanol (89.4-166.3 $\mu\text{g/L}$ *Alvarinho*; 41.8-61.7 $\mu\text{g/L}$ *Loureiro*). The grapes of these two Portuguese varieties show considerable contents in these two compounds, higher than other varieties, including Muscat varieties, *Riesling* and *Gewürztraminer* (7). However their levels are much lower than their odour threshold (17); therefore they have no sensory importance in these samples, as well as the other non-terpeneic alcohols.

The monoterpene compounds differentiate the two varieties. Indeed the *Loureiro* cultivar has total levels of monoterpenols much higher than those of the *Alvarinho* cultivar. Apart from their levels, their profiles are also very different. Linalool makes up more than 98 % of the monoterpenols at the same oxidation state (and lower for citronellol) in *Loureiro*, while their distribution is more even in *Alvarinho*, geraniol being the major one. The levels of linalool in the *Loureiro* variety (178.8 and 274.2 mg/L), although poorer than the amounts occurring in some Muscat varieties (18,19,20) are higher than those in some other aromatic varieties like *Müller-Thurgau* and *Gewürztraminer* (20,21,22) and in the so-called neutral varieties like *Chardonnay* and *Emir* (23, 24). These levels are higher than its odour perception threshold (25), which allows to classify this cultivar among the aromatic varieties. On the contrary, the level of each monoterpenol in *Alvarinho*, as well as their total level, are below their perception threshold.

Loureiro samples also exhibits higher amounts of monoterpene oxides and diols, mainly pyranic linalool oxides and 3,7-dimethylocta-1,7-dien-3,6-diol while *Alvarinho* contains a slightly higher content of geranic acid. It must be noted that only in *Loureiro* samples were identified cis-furanic linalool oxide and exo-2-hydroxy-1,8-cineole, although as traces. All the linalool oxides have odour perception thresholds much higher than the levels found in these samples (25).

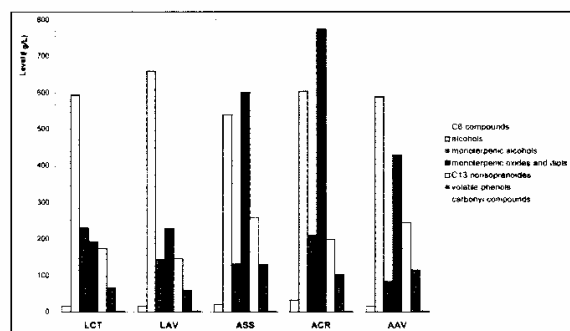


Fig. 2: Profile of the aglycons from the bound fraction of the five grape samples studied.

Fig. 2: Profil der Aglycone der gebundenen Fraktionen aller fünf untersuchten Traubenproben.

Fig. 2: Profil des aglycones de la fraction liée des cinq échantillons de raisin étudiés.

Furthermore neither any monoterpene diol nor geranic acid are known to be more odorant than these linalool oxides (17). Therefore, these compounds have no direct impact on the aroma of these juice samples.

Only one C_{13} norisoprenoid compound was identified in these samples, which is in agreement with the occurrence of these compounds in grapes almost exclusively as glycosides (24, 26).

Another relevant fact is that phenylethanal is two-fold to three-fold more abundant in *Alvarinho* samples. Its levels in the five samples are higher than its odour perception threshold in water (4 $\mu\text{g/L}$) (16). Thus its influence to the aroma of the juices of these grapes would be more important for *Alvarinho* than for *Loureiro*, particularly as the aroma of *Alvarinho* juice is not influenced by any monoterpeneol.

3.2. Bound compounds

60 aglycons from the bound fraction were identified and quantitatively determined as 4-nonanol equivalents: 3 C_6 compounds, 8 alcohols, 20 monoterpene compounds, 14 C_{13} norisoprenoids, 14 phenols and 1 carbonyl compound (table 3). 4 unknowns, presumably 3 volatile phenols and 1 C_{13} norisoprenoid from their mass spectra, were also quantitatively determined.

Alcohols (46.5-52.4 % *Loureiro*; 31.4-39.7 % *Alvarinho*), monoterpene compounds (29.6-33.1 % *Loureiro*; 34.6-51.1 % *Alvarinho*) and C_{13} norisoprenoid compounds (11.6-13.6 % *Loureiro*; 10.3-16.5 % *Alvarinho*) are the most abundant compounds in the bound fraction (figure 2). The levels of the other two chemical groups, phenols (4.7-7.8 %), C_6 compounds (1.1-1.7 %) are much lower; traces of benzaldehyde were also detected in this fraction, but its occurrence as a glycoconjugate in grape is not yet proved.

The occurrence of bound C_6 compounds shows that the corresponding aglycons can be biosynthesised in the plant. They do not significantly differentiate the two varieties, as reported previously by Versini et al. (5) in wines.

Tab. 3: Mean levels of the aglycons found in the bound fraction of the *Loureiro* and *Alvarinho* samples.

Tab. 3: Mittelwerte der Aglycongehalte der gebundenen Fraktionen aus Beeren der Sorten *Loureiro* und *Alvarinho*.

Tab. 3: Teneurs moyennes des aglycones de la fraction liée des échantillons de raisins *Loureiro* et *Alvarinho*.

	C ^{L_{CT}} (µg/L)	C.V. (%)	C ^{L_{AV}} (µg/L)	C.V. (%)	C ^{A_{SS}} (µg/L)	C.V. (%)	C ^{A_{CR}} (µg/L)	C.V. (%)	C ^{A_{AV}} (µg/L)	C.V. (%)
C₆ Compounds										
hexan-1-ol	7.5	5.2	9.8	12.4	15.2	12.0	24.9	7.2	11.6	15.8
(Z)-hex-3-en-1-ol	1.2	6.1	3.0	36.9	3.5	20.2	3.6	13.7	2.5	19.1
(E)-hex-2-en-1-ol	8.3	10.9	4.2	10.6	2.4	34.9	4.3	16.9	2.5	23.0
total	17.0		17.0		21.1		32.8		16.6	
Alcohols										
butan-1-ol	2.0	7.1	4.1	32.9	12.7	3.8	19.3	15.3	13.7	32.1
3-methylbutan-1-ol	4.4	23.0	8.8	16.4	11.9	17.8	15.1	9.1	9.3	22.2
3-methylbut-3-en-1-ol	11.5	18.7	17.4	8.1	13.5	16.3	16.0	9.8	11.3	21.4
3-methylbut-2-en-1-ol	4.2	6.2	4.7	16.4	8.2	14.4	9.9	9.9	5.5	36.5
octan-1-ol	4.8	13.7	3.4	26.0	3.7	8.6	8.4	10.9	3.2	18.8
1-phenylethanol	3.2	9.4	3.4	5.2	2.6	12.3	2.7	9.5	1.4	9.1
benzyl alcohol	384.4	9.1	380.9	2.7	307.5	9.4	344.7	4.2	336.4	10.7
2-phenylethanol	179.3	8.7	237.3	3.8	179.6	7.3	188.5	5.5	207.7	10.3
total	593.8		660.0		539.7		604.6		588.5	
Monoterpene alcohols										
linalool	194.8	2.8	126.0	4.0	76.4	7.0	157.1	10.0	37.3	24.5
α-terpineol	31.0	2.7	13.5	5.5	1.9	20.5	3.8	9.8	1.1	10.6
citronellol	0.1	16.9	0.2	29.0	0.7	23.9	0.4	14.9	0.3	33.6
nerol	2.0	29.0	1.9	19.7	5.5	5.8	6.4	3.4	6.2	12.6
geraniol	2.7	33.1	2.6	14.1	47.4	6.2	41.9	7.6	38.1	20.0
total	230.6		144.2		131.9		209.6		83.0	
Monoterpene oxides and diols										
trans-furan linalool oxide	29.6	2.0	45.3	3.5	54.9	10.9	64.9	7.0	38.3	10.7
cis-furan linalool oxide	1.6	8.9	3.4	7.1	8.3	13.2	8.2	11.8	5.7	10.7
HO-trienol	2.4	19.0	3.6	13.7	17.0	15.6	21.5	5.2	15.5	34.1
trans-pyran linalool oxide	5.6	9.2	9.1	9.2	48.1	8.3	60.6	4.4	30.8	10.4
cis-pyran linalool oxide	0.8	21.8	1.5	22.1	6.0	13.3	5.4	3.7	5.1	12.5
exo-2-hydroxy-1,8-cineole	3.4	39.0	3.8	3.8	0.3	0.0	0.4	20.2	0.4	35.4
3,7-dimethylocta-1,5-dien-3,7-diol	40.9	12.1	44.7	31.9	80.5	7.0	72.2	10.2	39.8	66.3
linalool hydrate	8.2	9.6	7.8	6.2	5.4	14.4	5.4	4.8	2.7	8.9
3,7-dimethylocta-1,7-dien-3,6-diol	12.3	7.1	11.0	5.4	5.9	6.8	14.6	2.1	3.8	30.1
8-hydroxy-6,7-dihydrolinalool + + citronellol hydrate*	5.3*	18.0	5.8	12.1	10.0*	0.9	13.0*	1.9	9.5*	22.5
(E)-8-hydroxy-linalool	33.5	5.5	34.1	10.5	44.0	5.9	62.0	1.5	31.5	38.1
(Z)-8-hydroxy-linalool	15.3	4.2	26.6	8.9	272.9	6.8	387.7	2.9	204.3	14.1
geranic acid	14.4	11.2	13.5	7.0	45.7	4.0	55.7	10.5	39.0	25.8
p-1-menthen-7,8-diol	19.0	3.5	19.0	12.5	1.8	8.6	2.5	6.2	2.4	20.3
total	192.3		229.2		600.8		774.1		428.8	
C₁₁ norisoprenoids										
3,4-dihydro-3-oxo-actinidol I	2.9	18.0	2.1	45.8	3.3	7.8	2.4	24.5	6.2	34.9
3,4-dihydro-3-oxo-actinidol II - III	4.9	46.6	4.0	34.0	8.0	7.8	7.1	6.7	7.4	17.2
3-hydroxy-β-damascone +										
3,4-dihydro-3-oxo-actinidol IV	12.3	1.0	11.9	18.0	28.5	5.6	20.3	5.6	22.4	3.2
3-hydroxy-7,8-dihydro-β-ionone	1.7	8.3	3.8	7.9	3.5	21.6	2.1	40.4	2.5	17.0
unknown C ₁₁ (I)	4.9	10.7	8.1	0.2	17.3	11.9	11.5	25.8	27.8	4.6
3-oxo-α-ionol	35.1	3.0	28.3	2.5	63.7	2.3	54.1	8.1	51.5	8.2
3-hydroxy-7,8-dihydro-β-ionol	18.4	3.2	15.3	12.4	6.7	4.7	8.8	10.1	7.6	21.8
4-oxo-7,8-dihydro-β-ionol +										
3-hydroxy-β-ionone (tr)	10.6	27.0	6.0	45.6	5.2	33.8	2.4	29.5	5.7	2.5
3-oxo-7,8-dihydro-α-ionol	14.8	29.5	8.4	20.8	35.0	3.0	30.9	30.9	30.9	26.1
3-oxo-α-retroionol	1.9	55.1	1.3	25.1	0.7	13.4	1.6	39.3	2.3	20.5
3-hydroxy-7,8-dehydro-β-ionol	14.1	9.9	12.0	6.9	39.6	1.6	15.2	17.7	35.3	13.0
vomifolol	51.6	19.9	45.4	20.8	46.7	5.2	42.8	5.6	44.8	15.5
total	173.2		146.6		258.2		199.2		244.4	
Phenols										
methyl salicylate	0.2	39.7	1.0	25.5	2.5	17.1	2.3	6.0	1.9	19.9
o-cresol + phenol	1.5	11.7	1.9	1.8	1.4	14.0	1.8	3.8	3.4	37.3
p-cresol	1.7	0.5	1.3	7.6	2.0	12.6	2.0	12.7	1.2	34.6
m-cresol	1.5	12.6	0.9	15.7	1.5	17.1	1.7	14.1	0.6	38.6
eugenol	4.5	20.2	2.9	58.0	13.2	3.5	14.9	10.0	15.0	23.5
4-vinylguaiaicol	1.2	6.5	0.6	40.4	1.8	5.9	1.3	7.8	1.6	32.8
4-vinylphenol	2.0	17.7	0.9	15.7	2.1	3.4	1.3	17.0	1.5	47.1
vanillin	0.7	30.1	1.6	44.8	1.4	25.3	2.7	42.8	2.3	12.3
methyl vanillate	6.0	7.7	1.8	20.8	37.2	1.1	21.4	11.7	33.0	0.9
acetovanillone	15.1	3.0	12.1	2.5	15.9	2.2	18.0	8.1	17.2	8.2
3,4-dimethoxyphenol	1.8	20.2	1.8	60.6	5.0	2.8	3.5	16.2	7.0	26.3
zingeronol	2.7	6.4	3.2	25.1	3.9	18.0	3.5	12.1	4.1	29.0
tyrosol analogue	0.8	70.7	3.3	23.0	4.3	30.8	5.8	6.8	2.0	14.5
2-(4'-gaiacyl)ethanol	11.9	21.9	6.7	50.3	3.7	5.8	2.5	24.7	6.1	20.1
unknown phenol (I)	3.7	51.9	2.3	29.5	6.6	29.1	5.1	12.6	4.8	65.6
unknown phenol (II)	10.6	22.7	17.3	21.0	26.8	9.2	15.3	11.0	14.0	18.2
total	65.9		59.6		129.3		103.1		115.7	
Carbonyl compounds										
benzaldehyde	3.0	10.7	3.3	2.6	1.9	19.7	2.0	9.6	3.9	9.0
total	3.0		3.3		1.9		2.0		3.9	
Total	1275.8		1259.9		1682.9		1925.4		1480.9	

Benzyl alcohol and 2-phenylethanol are the most abundant bound alcohols occurring in both Portuguese varieties at similar levels contrary to what was observed in the free fraction. These levels, much more higher than the levels of the corresponding free compounds, are similar to those in *Muscat Ottonel* and *Riesling*, the richest varieties mentioned by Gunata et al. (7). However, none of these aglycons exhibits levels that could influence the aroma of the corresponding wines, even if totally liberated from the bound forms. The total levels found for glycosylated monoterpenes are much higher than those published for other non-Muscat *Vitis vinifera* varieties, like *Müller-Thurgau* and *Chardonnay* (22, 24). Bayonove et al. (20) reported, in *Gewürztraminer* grapes, levels of monoterpenic glycosides similar to those found in *Loureiro* but lower to those found in *Alvarinho*. The same authors observed in *Muscat Ottonel* grapes similar contents to those found in *Alvarinho*.

As regards bound monoterpenic alcohols, the distribution observed in *Loureiro* grapes was similar to that observed in the free fraction, excepted that the levels of α -terpineol were more abundant. However, the levels of bound linalool were slightly lower than in the free fraction, although reaching values above its detection threshold. Therefore the *Loureiro* bound fraction makes up aroma reserve that could be used in the corresponding wines.

On the contrary, bound monoterpenic alcohols in *Alvarinho* grapes were different from their free fraction. First their total levels were much higher, particularly in A_{CR} and A_{SS} , i.e. in samples from the recommended sub-region Monção. In these two samples the total levels of these compounds reached the odour threshold reported for similar mixture (25) and thus could also make up an aroma reserve in wines. The distribution was also different, with high levels of linalool, higher than those of geraniol, although the latter were higher than in the free fraction.

Thus the important differences observed in the free fraction for these monoterpenic alcohols between the two cultivars were subtler in the bound fraction, although geraniol was still much more abundant in *Alvarinho* and α -terpineol in *Loureiro*. Furthermore while the *Loureiro* samples from the two sub-regions were equally differentiated by the free and bound monoterpenic alcohols, the *Alvarinho* samples from the three vineyards in the two sub-regions were only clearly differentiated by the bound monoterpenic alcohols.

As regards the monoterpenic oxides and diols in the bound fraction of the two cultivars, the differences observed between the free fractions are completely reversed: the levels of these compounds in *Alvarinho* grapes being much higher than in *Loureiro* grapes. Furthermore the *Alvarinho* samples from the three vineyards are clearly differentiated contrary to what was observed for the free fraction or for the *Loureiro* samples. When comparing the bound forms to the free forms, both bound monoterpenic oxides and diols are increased in *Alvarinho* samples (the most dramatic increases are observed for the levels of 8-hydroxylinalool isomers), while in the *Loureiro* samples, the oxides levels are lower and the diols levels are slightly increased. Strauss et al. (27) reported, in *Muscat de Frontignan* juices, contents in (Z)-8-hydroxylinalool similar to those found in *Alvarinho* grapes. The same authors indicated lower contents in *Riesling* and *Gewürztraminer*, as well as Nicolini et al. (22) and Aubert et al. (18) in *Müller-Thurgau* and *Muscat of Alexandria* varieties.

However none of these compounds reached its odour perception threshold, neither in the *Loureiro* nor in the *Alvarinho* samples, but they could generate at the low pH of the corresponding wines, more odorous monoterpenes such as roseoxide, neroloxide, HO-trienol,...

Bound C_{13} norisoprenoids are more abundant in the *Alvarinho* samples, particularly 3-oxo- α -ionol, 3-oxo-7,8-dihydro- α -ionol, 3-hydroxy-7,8-dehydro-7,8- β -ionol, 3-hydroxy- β -damascone and the unknown compound 1, but the *Loureiro* samples contain higher levels of 3-hydroxy-7,8-dihydro- β -ionol. The total levels, 146.6-173.2 $\mu\text{g/L}$ in *Loureiro* and 199.2-258.2 $\mu\text{g/L}$ in *Alvarinho*, are lower than those reported by Sefton et al. (24) in *Chardonnay*, a C_{13} norisoprenoid dependent variety, but higher than those reported in wines from *Muscat Ottonel*, *Gewürztraminer* and *Emir* varieties (20, 23), and in Moscatel Rosada grapes (19), and similar to those reported in *Muscat of Alexandria* grapes (19). However, 3-hydroxy- β -damascone exhibits comparatively lower levels (18, 20, 23).

As reported previously, these compounds are able to generate in the corresponding wines during the ageing process much more odorous compounds, as β -damascenone, 1,1,6-trimethyl-1,2-dihydronaphthalene, 3-oxo-megastigma-4,6,8-trienes, ... (28, 29).

Finally, bound phenols are much more abundant than the free ones in both cultivars, but none has levels reaching its threshold level, excepted eugenol. However, sensory data about any synergism between these related compounds are lacking to conclude on their sensory relevance, particularly in wines in which 4-vinylphenol and 4-vinylguaiacol are biogenerated by yeast.

3.3. Global characterisation of the two varieties

The *Loureiro* variety contains similar levels of monoterpenes in both free and bound fractions, while the *Alvarinho* is much richer in bound fraction. The ratio between bound monoterpenes and free monoterpenes was about 1 for the *Loureiro* samples and between 4.6 and 8.5 for the *Alvarinho* samples (figure 3). The monoterpene total amounts are 716-862 $\mu\text{g/L}$ (49-51 % free) in the *Loureiro* samples and 624-1099 $\mu\text{g/L}$ (10-18 % free) in the *Alvarinho* samples. It must be noted that linalool makes up 52.2-62.5 % of free monoterpenes and 9.5-10.1 % of free volatiles in the *Loureiro* samples, but only 3.3-4.1 % and 0.1-0.2 % respectively in the *Alvarinho* samples.

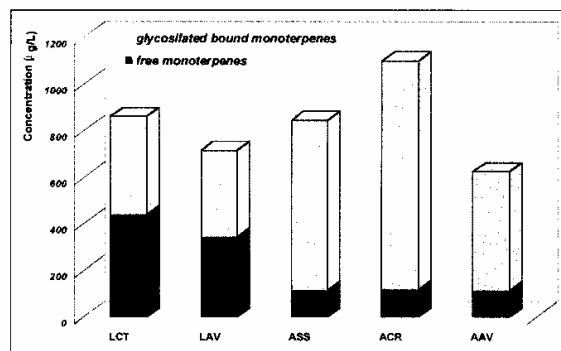


Fig. 3: Total free and bound monoterpenes.

Fig. 3: Gesamte frei und gebundene Monoterpene.

Fig. 3: Monoterpènes libres et liés totaux.

Grapes of the same variety even grown in different sub-regions and soils have similar aromatic «fingerprints», but the two varieties are quite different (figures 1 and 2).

As regards free monoterpene profile similarities, *Loureiro* and *Alvarinho* grapes can be considered close to the *Rhine Riesling* typology as observed on the corresponding wines from Spain by Versini et al. (5). However the *Loureiro* and *Alvarinho* profiles are more different from the *Rhine Riesling* typology than those of the corresponding wines from Spain. Indeed in *Loureiro* grapes, the

linalool dominance is extreme, as its content is even higher than that of HO-diendiol (I) (i.e. 3,7-dimethylocta-1,5-dien-3,7-diol). On the contrary, in the *Alvarinho* grapes only traces of linalool were detected while the contents of the other monoterpenols were very low, as observed for neutral varieties. However the profile of the monoterpene oxides and diols can be related to the *Rhine Riesling* typology.

As regards the bound forms, *Alvarinho* grapes can be considered very close to the *Rhine Riesling* typology, with dominance in the decreasing order of (Z)-8-hydroxylinalool, linalool, HO-diendiol (I), (E)-8-hydroxylinalool; furthermore their contents are very close to those reported by Versini et al. (30) for this typology. However linalool and furan linalool oxides contents are slightly different.

On the contrary, *Loureiro* grapes have a bound monoterpene profile related to the *Muscat fleur d'oranger*-type, with dominance of (E)-8-hydroxylinalool, linalool, HO-diendiol (I), trans-furan linalool oxide, HO-diendiol (II) (3,7-dimethylocta-1,7-dien-3,6-diol) and (Z)-8-hydroxylinalool (30), but the amount of bound linalool is relatively much higher than that of the other bound monoterpenols. Furthermore the amounts of these compounds in *Loureiro* grapes are much more lower than in *Muscat fleur d'oranger*.

4. Conclusion

The studied varieties were differentiated by profiles of their free and bound compounds, even when they were grown in different sub-regions/soils. These results are consistent with the knowledge of the aroma of the wines made from these two varieties: Loureiro possesses a floral character while *Alvarinho* is characterised by an intense mouth aroma, equilibrate and with a more complex profile (1, 2).

Nevertheless, the geographical area/soil influenced their aroma potential, which were higher in samples grown in the most recommended sub-regions. However these conclusions should be validated by further work on more samples for different vintages, as well as on the corresponding wines.

Zusammenfassung

Es wurden freie und glykosidisch gebundene flüchtige Verbindungen quantifiziert in fünf Traubenproben des Jahrganges 1996 der beiden bedeutendsten *Vitis vinifera*-Sorten des Vinhos Verdes Gebiets, *Loureiro* und *Alvarinho*. Die Proben stammten aus der Subregion, die als bestgeeignetste für die Erzeugung von Wein aus einer Sorte gilt, sowie aus einer Vergleichssubregion. Außerdem wurde eine dritte *Alvarinho*-Probe von einem speziellen Kiesboden untersucht.

Vierzig Stoffe aus den freien Fraktionen und vierundsechzig der glykosidisch gebundenen wurden in den Proben beider Sorten quantifiziert, darunter Alkohole, Monoterpene, C₁₃ norisoprenoide, Phenole, C₆-verbindungen und Carbonyle.

Die Resultate zeigen einen deutlichen Unterschied zwischen beiden portugiesischen Sorten in bezug auf die Geschmacksstoffe selbst bei verschiedenen geografischen Herkünften. Die *Loureiro*-Proben wiesen einen höheren Gehalt an freien Monoterpenen auf als *Alvarinho*, vor allem an Linalool, Pyran-linalooloxid und 3,7-dimethylocta-1,7-dien-3,6-diol. Freies Linalool schien für die Sorte *Loureiro* charakteristisch zu sein, wobei die Gehalte über dem Geruchsschwellenwert lagen. Dahingegen enthielten Proben von *Alvarinho* freies Linalool nur in Spuren, dafür aber höhere Gehalte an Alkoholen, vor allem Benzylalkohol und 2-phenylethanol, sowie an Geraniol und 2-phenylethanal, letzteres über dem Geruchsschwellenwert. Bei beiden Sorten wurden bedeutende Konzentrationen gebundener Mono-

terpene und C₁₃ norisoprenoide gefunden, vor allem die Gehalte an gebundenem Linalool waren hoch. Außerdem enthielt die Sorte *Alvarinho* größere Mengen an gebundenem Geraniol, Monoterpenoxiden und Diolen, vor allem trans-pyran-linalooloxid, Geraniumsäure und (Z)-8-hydroxylinalool, die Sorte *Loureiro* dagegen mehr gebundenes α -terpineol und p-1-menthen-7,8-diol.

Résumé

Les composés libres et glycoconjugués de cinq échantillons de raisin du millésime 1996 et issus de deux des plus importantes variétés de *Vitis Vinifera* de la région des Vinhos Verdes, le *Loureiro* et l'*Alvarinho*, ont été quantifiés. La sub-région la plus favorable à la production de chacun des vins monocépages correspondants ainsi qu'une autre zone de production ont été sélectionnées. Dans le cas de l'*Alvarinho*, un terroir graveleux particulier de la zone de référence a également été retenu.

Quarante composés dans la fraction libre et soixante-quatre aglycones dans la fraction glycosidique, dont des alcools, des monoterpènes, des norisoprénoides en C₁₃, des phénols, des composés en C₆ et carbonylés ont été quantifiés dans les échantillons des deux variétés. Les résultats montrent que ces deux variétés portugaises sont très différentes en ce qui concerne ces composés variétaux, même pour des zones de production différentes. Les échantillons de *Loureiro* ont des teneurs en dérivés terpéniques libres plus élevés que ceux d'*Alvarinho*, surtout en linalol et en ses oxydes pyraniques de même qu'en 3,7-diméthyl-octa-1,7-dièn-3,6-diol. Le linalol libre paraît être caractéristique de la variété *Loureiro*, ses teneurs dépassant son seuil de perception olfactive. Au contraire, les échantillons d'*Alvarinho* ne contiennent que des traces de linalol libre, mais leurs teneurs en alcools, principalement l'alcool benzyle et le 2-phényléthanol, en geraniol et en phényléthanal sont plus élevées, ce dernier atteignant son seuil de perception olfactive.

Les deux variétés ont des teneurs élevées en composés norisoprénoides en C₁₃ liés et en dérivés monoterpéniques liés, particulièrement en linalool. Néanmoins l'*Alvarinho* contient plus de geraniol lié et plus d'oxydes et diols monoterpéniques liés, surtout l'oxyde pyranique de linalol trans, l'acide géranique et le (Z)-8-hydroxylinalool, tandis que le *Loureiro* est plus riche en α -terpinéol lié et en p-menth-1-èn-7,8-diol lié.

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