VOLATILE AND SENSORY CHARACTERIZATION OF WHITE WINES FROM THREE MINORITY PORTUGUESE GRAPEVINE VARIETIES

CARACTERIZAÇÃO VOLÁTIL E SENSORIAL DE VINHOS BRANCOS DE TRÊS CASTAS PORTUGUESAS MINORITÁRIAS

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SUMMARY

This work focused on the characterization of the volatile compounds and sensory profile of white wines produced from three minority grapevine varieties of Portugal namely 'Malvasia' (Colares), 'Verdelho' and 'Galego Dourado'. The characterization took place using sensory and gas chromatography analysis. Furthermore, the data obtained were analysed through the use of multivariate analysis, which made it possible to evaluate the similarities and dissimilarities between the varieties. The results obtained show a differentiation of the wines produced from each grapevine variety but above all a differentiation of the two vintages was verified. The results obtained, both from a sensory and a chemical point of view, show an interesting oenological potential of these varieties, but still require further studies, in order to evaluate the influence of climatic effects on the profile of volatile compounds and also on the sensory profile.

RESUMO

Este trabalho centrou-se na caracterização sensorial e da composição volátil de vinhos brancos produzidos a partir de três castas minoritárias, designadamente 'Malvasia' (Colares), 'Verdelho' e 'Galego Dourado'. A caracterização ocorreu por meio de análise sensorial e por cromatografia gás líquido de alta resolução, e os resultados obtidos foram analisados através de análise multivariada, que permitiu avaliar as semelhanças e as diferenças entre as castas. Os resultados obtidos mostram uma diferenciação dos vinhos produzidos a partir de cada casta, mas acima de tudo uma diferenciação das duas colheitas. Os resultados obtidos, tanto do ponto de vista sensorial como na composição química, mostram um potencial enológico interessante destas castas, embora sejam necessários mais estudos para avaliar a influência dos efeitos climáticos no perfil de compostos voláteis e também no perfil sensorial.

Key words: Malvasia, Verdelho, Galego Dourado, white wine, sensory profile, volatile compounds.
Palavras-chave: Malvasia, Verdelho, Galego Dourado, vinho branco, perfil sensorial, compostos voláteis.

INTRODUCTION

Wine aromas were common drivers of consumer preferences and they are mainly determined by volatile compounds. The aroma compounds (volatile compounds) contribute to all those sensations perceived at the olfactory-gustative level during the tasting of the wines, together with the other chemical compounds present in the wine such as acids, sugars, polyphenols, mineral substances and therefore play a role on the quality and degree of appreciation of a wine. The volatile compounds are small hydrophobic

molecules with a molecular weight ranging from 30 g/mol to 300 g/mol (Morrot and Brochet, 2000) and with a concentration varying in wine from several mg/L to a few ng/L, or even less (Vilanova and Oliveira, 2012).

The wine aroma can be classified in different ways according to origin and biotechnological conditions (Bayonove *et al.*, 1998): varietal, pre-fermentative, fermentative aroma and post-fermentative aroma.

The varietal aroma, which originates during the development process of the berry, is closely linked to the climatic conditions, the soil, the phytosanitary conditions and the degree of ripeness of the grapes (Cordonnier and Bayonove, 1979). The compounds that contribute to the formation of the varietal aroma are synthesized and then stored in the exocarp, vacuoles and the smallest part are stored in the pulp (Lichtenthaler *et al.,* 1997). The compounds that contribute to the formation of the varietal aroma are part of large chemical families such as terpenes, norisoprenoids and benzenoid compounds like aromatic alcohols, volatile phenols and phenolic aldehydes. In addition, it can be found as varietal aroma compounds, some linear alcohols, fatty acids, methoxypyrazines and sulfur compounds (Oliveira *et al.,* 2000; Ribéreau-Gayon *et al.*, 2006).

The pre-fermentative aroma is developed during the phases prior to fermentation, from the harvest, transport, storage, destemming, crushing, sorting, pressing and maceration as the result of several enzymatic activities where distinct compounds may be produced and released into the must (Cordonnier and Bayonove, 1981). The fermentative aroma, composed of several compounds from different families, such as esters, aldehydes, cetones, alcohols, volatile acids and volatile phenols, originate through microorganisms present in the medium during alcoholic and malolactic fermentation (Rapp and Mandery, 1986; Riberéau- Gayon *et al*., 2006). Currently, there are many strains of yeast in commerce, with various metabolic capacities more or less accentuated, thanks to the precursors present in the grapes, which are able to develop new compounds and through the selection of different strains, the winemaker is able to differentiate the final product and to develop a wine that complies with the needs of the market (Molina *et al*., 2009; Vilanova *et al*., 2012). Finally, the post-fermentative aroma originates after fermentation due to several chemical reactions, which may occur during the wine conservation and ageing (Marais and Pool, 1980; Usseglio-Tomasset, 1983; Vilanova and Oliveira, 2012).

Despite great knowledge about the volatile composition of wines of the most cultivated white grape varieties in the world, such as 'Sauvignon Blanc', 'Semillon', 'Riesling', 'Gewurztraminer' and 'Muscat' (Styger *et al*., 2011), there is little information on the volatile composition of wines produced from other grape varieties, namely minority grapevine varieties.

The wine world is constantly evolving, which forces many historical countries such as Italy, France, Spain and Portugal to face new market needs, while maintaining its historical identity. Europe, and particularly Portugal, presents a unique and enormous genetic patrimony, with around 230 varieties considered autochthonous to Portugal or the Iberian Peninsula listed in Portaria nº 380/2012 of 22 November, which establishes the 343 grapevine varieties suitable to wine production in Portugal (Eiras-Dias *et al*., 2016). Thus, the conservation and enhancement of minor varieties should be the goal of the historic winegrowing countries, to diversify and implement production and meet new market needs (Alifragkis *et al.,* 2015).

In this work, three grapevine minor varieties ('Malvasia', 'Galego Dourado' and 'Verdelho') were studied, that despite the possibility of having propagating material over the entire surface area of Portugal, they are under the threshold of 1 % of the total vineyard area (IVV, 2017).

'Verdelho' is one of the main white grapevine varieties used to produce fortified wines in the Madeira wine region (Portugal). This variety is also used to produce table wines in Madeira and in other winegrowing regions such as continental Portugal, Açores (Portugal), the Canary Islands (Spain), Australia and South Africa. Some information on the aromatic characteristics of this variety can be obtained owing to the studies of Câmara *et al.* (2004) and Gaspar *et al.* (2016), which show a high concentration of terpenoids in free form, and the wines presented sweet fruity and floral notes. Also, in the study conducted by Ferreira (2011), thiol characters have been found. 'Malvasia', commonly known as 'Malvasia de Colares', is a grapevine variety cultivated in the region of Colares (Portugal), located on the south-western coast of the Atlantic Ocean. Finally, 'Galego Dourado' is a white grapevine variety widely used to produce fortified wines (McCallum *et al.*, 2019) from the Carcavelos wine region (Portugal).

According to our knowledge there is no published data about the volatile composition of wines produced from 'Malvasia' or 'Galego Dourado'. Thus, this work aimed to characterize the volatile and sensory profile of white wines produced from these three

grapevine varieties: 'Malvasia', 'Galego Dourado' and 'Verdelho', during two vintages.

MATERIAL AND METHODS

Vineyard and wine experiment

The grapes were harvested on the Portuguese National Ampelographic Collection located at Dois Portos, Portugal. The vineyard was grafted on SO4, located on the plain, with a 2.3 m x 1 m training system in the counter and with a spurred cordon system (Eiras-Dias, 2003). There are seven grapevines for each variety in this collection and the grapes from all these plants were harvested and used in the wine experiment.

During two vintages (2017 and 2018), wines were produced from the three varieties – 'Malvasia', 'Galego Dourado' and 'Verdelho'. Grapes from each variety were harvested by hand and processed in the experimental winery of INIAV, at Dois Portos. In 2017, it was harvested 22 Kg, 21 Kg and 53 Kg of grapes respectively of 'Galego Dourado', 'Malvasia' and 'Verdelho', while in 2018 it was harvested 46 Kg, 27 Kg and 74 Kg of the grapes of the same varieties. The microvinifications were performed using the usual procedures for white wine vinifications. The grapes were crushed and pressed and the grape juice was added with 15 g/hL of a 70% solution of potassium metabisulfite and 30% of ascorbic acid (Oxyless, Proenol, Portugal). The musts were clarified at 4 °C during 48 hours, inside a small stainless-steel tank (50 L). Then they were transferred to another similar tank and inoculated with selected yeasts from the Lalvin company (QA23, 25 g/hL).

The fermentation was carried out at a controlled temperature (16 °C), and the temperature and density were checked daily. At the end of the fermentation, the wines were transferred into glass containers (20 L), and 15 g/hL of a solution of metabisulfite and ascorbic acid (Oxyless-Proenol) was added.

In December, the wines were racked and 9 g/hL of Oxyless was added. After three months in the winery at a temperature of 14 ℃, the wines were cold stabilized, with a temperature of about 4 °C for about two weeks. Subsequently, the wines were bottled (bottles of 750 mL with cork stoppers) without any promoted clarification, and three bottles of each wine variety were taken for chemical and sensory analysis.

Given the low quantity of some grape varieties, it was chosen to perform only one vinification of each one (trying to be more representative of the industrial process). Consequently, the results of the experimental design were only evaluated by multivariate analysis.

Climatic conditions on 2017 and 2018

Given the influence of climatic conditions on grapevine growing, some climate data collected by the INIAV, at Dois Portos, was also presented (Figure 1). The 2017 vintage was characterized by a dry year with a total rainfall of around 435 mm, but with a more homogeneous trend compared to the year 2018, in which heavy rainfall events were concentrated in the period between February and April and then occuring between October and November with a total rainfall of about 650 mm.
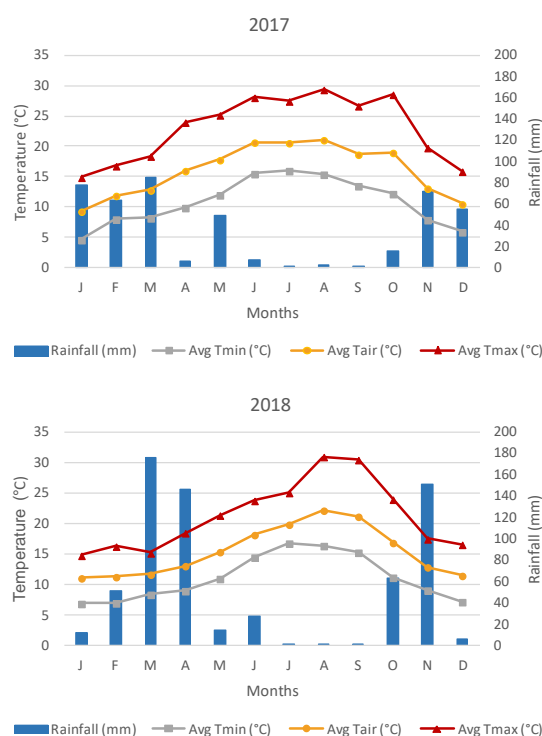

Figure 1. Temperature and rainfall 2017-2018 at Dois Portos. Rainfall: rainfall; Avg Tmin: average minimum temperature; Avg Tair: average air temperature; Avg Tmax: average maximum temperature.

Temperatura e precipitação em 2017 e 2018 em Dois Portos. Rainfall: precipitação; Avg Tmin: média da temperatura mínima; Avg Tair: temperatura média do ar; Avg Tmax: média da temperatura máxima.

It is interesting, as can be seen in Figure 1, that the period of maturation of the grapes between July and September in the year 2017 was cooler and rainier than the year 2018.

Reagents

Dichloromethane and anhydrous sodium sulphate, both of analytical grade, were purchased from Merck (Darmstadt, Germany). Dichloromethane was bidistilled before use.

Musts and wine analyses

The pH, total acidity, soluble solids and potential alcoholic strength were determined in the musts according to the official methods (OIV, 2014).

In the wines, the following chemical analyses were performed: pH, total acidity, volatile acidity, density, free sulphur dioxide, total sulphur dioxide and reducing substances (OIV, 2014). All the analyses were performed in duplicate.

Wine volatile compounds analysis

The extraction of volatiles followed the method proposed by Cocito *et al.* (1995) using the conditions described by Botelho (2008). A volume of 50 mL of each wine was added of 400 μL of 2-octanol (internal standard, 81.9 mg/L in 50 % ethanol solution). After, the extraction was done by the liquid-liquid ultrasonic technique in discontinuous mode with redistilled dichloromethane, dried on anhydrous sodium sulphate. Then, the sample was concentrated and a volume of about 0.30 mL is recovered with a glass graduated pipette.

The extraction of the compounds was carried out in duplicate and each extract was then stored at -20 °C until the analysis by high-resolution gas-liquid chromatography coupled with a flame ionization detector (GC-FID) and up to high-resolution gas-liquid chromatography coupled with mass spectrometer (GC-MS).

Quantification and analysis of volatile compounds by GC-FID

The obtained extracts were analyzed by high-resolution gas-liquid chromatography coupled with a flame ionization detector (GC-FID) and each extract was injected (~0.6 μL) in triplicate.

An Agilent Technologies 6890N chromatograph equipped with a flame ionization detector (FID) (260 °C), injector (260 °C) was used in split mode and with a 30 m x 0.32 mm x 0.25 mm capillary column of polyethylene glycol silica (INNOWax, J&W Scientific Technologies, Agilent, USA). The carrier gas was hydrogen (2.4 mL/min) and the split ratio will be 1:3. The samples were injected (~0.8 μL) manually. The thermal gradient program in the chromatograph was: 35 °C (6 min), 3.5 °C /min at 55 °C, 7.5 °C/min at 130 °C, 5 °C/min at 210 °C (30 min).

The quantification was performed with the internal standard method and the results have been expressed as 2-octanol (internal standard).

Identification of compounds by GC-MS

The identification of the compounds was performed on a GC-MS (Finnigan Mat Magnum) equipment. The GC-MS system was equipped with a 30 m x 0.25 mm x 0.25 μm polyethylene glycol silica capillary column (INNOWax from J&W). Conditions of analysis: injector and transfer line at 250 °C; helium gas (12 psi of internal pressure and division ratio of 1:60), 0.2-0.4 μL of injection volume. The mass spectrometer worked in electron impact mode at 70 eV, evaluating an m/z range of 40-340 amu. The identification was performed by comparing the mass spectrum with those of the spectra libraries (NIST and WILEY) and when possible, confirmed with the analysis of the standard substances. The temperature program used is similar to that for GC-FID.

The compounds were uniquely identified, by calculating the retention index of Kovats (KI) and the MS fragmentation pattern with those of reference compounds or with mass spectra in the NIST and Wiley libraries. The Kovats retention indices (RI) of compounds were calculated by linear interpolation (Philips, 1989) after injecting a sample with a homologous series of alkanes (C9-C30).

Sensory analysis

The wine tests were carried out in the INIAV tasting room, in Dois Portos, with individual workstations, equipped with lights, sinks, with white surfaces as required by the ISO 8589 standard. The test tulip glasses were used as required by the ISO 3591 standard, with a volume of wine per sample of approximately 50 mL.

In the sensory sessions, which were made in the morning (11 a.m.), the samples were provided at temperature of 14 °C ± 1 °C. It was supplied water to the tasters for rinsing their mouth between samples.

The descriptive sensory analysis of wines was carried out by a trained jury composed eight of judges. All the judges were trained in accordance to the international standards (ISO 8586) including the detection and identification of odor and tastes, and also the use of scales. The training sessions comprised of the assessment of several flavour standards (apple extract, banana extract, strawberry extract, lemon extract, rock-rose extract, straw extract, nuts extract, raisin extract, 1-hexanol, *cis*-3-hexenol, ethanol, ethyl acetate, ethyl butyrate, 2-phenylethanol, acetaldehyde, geraniol, isoamyl acetate, linalool, vanillin, glucose, fructose, tartaric

acid, quinine sulphate, acetic acid, lactic acid, citric acid, malic acid, and glycerol), the finding of odor defects in spiked wines, as well as the evaluation of several samples of commercial white wines.

The evaluation of the wines was focused on the colour, aroma and taste and the tasters were asked to evaluate the intensity of several attributes, with a structured scale from 0 to 10.

The evaluation form was made starting from the work of Odello *et al.* (2007) and the descriptors used were chosen starting from the works of Vilanova *et al.* (2008, 2013). The score sheet is composed of attributes on colour, aroma and flavour. Intensity, yellow, green and limpidity were evaluated for the visual attributes. The aroma attributes contained aroma intensity, floral, white fruit, nuts, tropical fruits, citric, herbaceous, terpenic-muscat and persistence. The gustatory attributes included sweet, sour, bitter, softness, balance, alcohol and body.

The wine samples were presented anonymously to the tasters with a 3-digit identification code for each sample following a balanced order with the purpose of eliminate first-order carryover effects (MacFie *et al.*, 1989).

Statistical analysis

Multivariate analysis of data (Abdi and Williams, 2010) was applied to the sensory and volatile results in order to extract information from the data matrix. Principal component analysis (PCA) and hierarchal clustering analysis (HCA) were performed by using Statistica software (Version7).

RESULTS AND DISCUSSION

Chemical analysis of musts and wines

The chemical composition of the musts and corresponding wines from different varieties of the two vintages are presented in Tables I and II.

Table I

Chemical composition (average values and standard deviation) of musts from the two vintages (2017 and 2018)

Composição química (valores médios e desvios padrão) dos mostos das duas vindimas (2017 e 2018)

Variety	Vintage	Soluble solids (%m/m)	Potential alcohol strength (% v/v)*	pH	Total acidity (g tartaric acid/L)
'Galego Dourado'	2017	21.6 ± 0.2	12.4 ± 0.1	3.30 ± 0.02	6.4 ± 0.1
	2018	21.8 ± 0.1	12.6 ± 0.2	3.13 ± 0.01	6.8 ± 0.2
'Malvasia'	2017	20.6 ± 0.2	11.7 ± 0.1	3.01 ± 0.01	7.4 ± 0.2
	2018	20.5 ± 0.2	11.7 ± 0.1	3.21 ± 0.01	7.5 ± 0.1
'Verdelho'	2017	24.0 ± 0.1	14.0 ± 0.2	3.21 ± 0.02	6.7 ± 0.1
	2018	21.6 ± 0.1	12.4 ± 0.1	3.06 ± 0.01	6.4 ± 0.1

* Potential alcoholic strength was calculated from the soluble solids results.

Table II

Chemical composition of wines (average values and standard deviation) of different grapevine varieties from the two vintages (2017 and 2018)

Composição química dos vinhos (valores médios e desvios-padrão) de diferentes castas nas duas vindimas (2017 e 2018)

Wine samples	Vintage	TAV (% v/v)	Total acidity (g tartaric acid/L)	Volatile acidity (g acetic acid/L)	pH	Free sulfur dioxide (mg/L)	Reducing substances (g/L)
'Galego Dourado'	2017	13.7 ± 0.1	4.2 ± 0.1	0.66 ± 0.02	3.49 ± 0.1	13 ± 0.1	1.8 ± 0.1
	2018	13.3 ± 0.2	6.0 ± 0.1	0.28 ± 0.01	3.37 ± 0.1	38 ± 0.5	1.5 ± 0.2
'Malvasia'	2017	12.9 ± 0.2	5.7 ± 0.2	0.48 ± 0.02	3.13 ± 0.1	13 ± 0.1	2.8 ± 0.1
	2018	12.7 ± 0.1	7.9 ± 0.1	0.23 ± 0.02	3.03 ± 0.1	41 ± 0.2	1.4 ± 0.1
Verdelho	2017	15.1 ± 0.1	6.1 ± 0.2	0.29 ± 0.01	3.20 ± 0.2	22 ± 0.1	6.9 ± 0.2
	2018	13.3 ± 0.1	7.8 ± 0.1	0.40 ± 0.01	3.09 ± 0.2	34 ± 0.5	1.0 ± 0.1

Analyzing the results of the musts (Table I), all the samples showed potential alcoholic strength ranging between 11.7% v/v and 14% v/v. The total acidity in these varieties was very high, showing values between 6.5 and 7.5, as well as the pH ranging from 3.01 until 3.30.

The results of the chemical analysis of wines, obtained before the sensory analysis session (Table II), showed the wines from the 2017 vintage had a tendency to present a higher residual sugar content, estimated by the reducing substances, than those from the 2018 vintage. Also, the wines from the 2017 vintage tended to present higher alcohol content than the corresponding wines produced in 2018, but all presented optimal acidity values in spite of low values in the vintage of 2017.

Sensory profile

The wine tasting was done with a panel of expert tasters as described previously. The average results from two vintages are shown in Figure 2, and the wines had a tendency to present different characteristics at each vintage. 'Malvasia' wine, from the 2017 vintage, was characterized by the intensity of colour, with light floral and white fruit notes; notes of dried fruit prevailed with aromatic persistence and harmony. In the 2018 vintage, 'Malvasia' wine had a sensory profile with a prevalence of white fruit notes, light notes of dried fruit and citric aromas, and also with high aroma persistence, body and acidity of the wine.
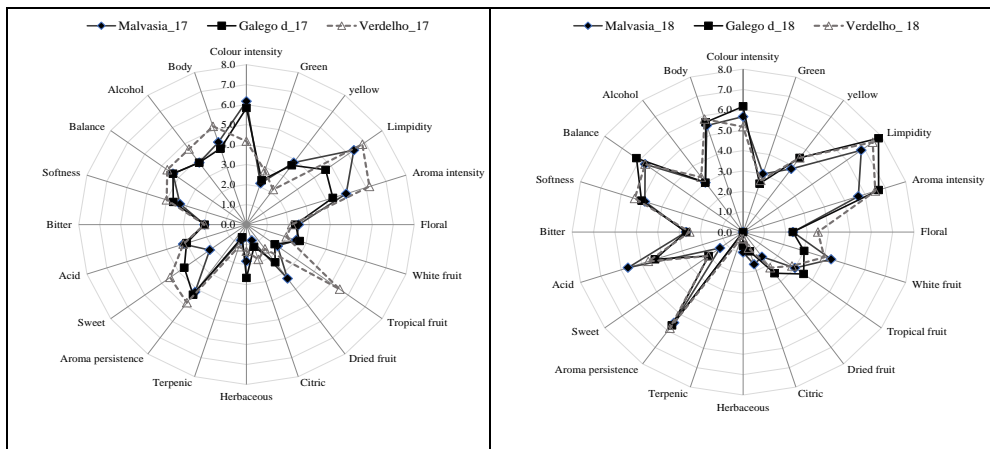

Figure 2. Averaged sensory analysis results of descriptive evaluation of wines in the two vintages.

Média dos resultados sensoriais da análise descritiva realizada aos vinhos nas duas vindimas.

The 'Galego Dourado' wine from 2017 was characterized by a high intensity of colour and aroma, with low floral notes, white fruit, dried fruit and citric aromas with a presence of an herbaceous note. Moreover, the wine has a medium balance and persistence. The 'Galego Dourado' wine produced in the 2018 vintage was characterized by high intensity and persistence of aroma, with notes of white fruit, dried fruit and tropical fruit notes. The wine also presented high acidity and balance.

'Verdelho' wine from 2017 showed a high aroma intensity and persistence with light floral notes and high intensity of tropical fruit attribute, and medium values for body and balance attributes. In 2018, the 'Verdelho' wine also showed high aroma intensity, with light floral notes, dried fruit and predominantly white fruit notes. Moreover, it showed a trend for a greater acidity and balance than the previous year.

The data of the sensory results were submitted to the hierarchical cluster analysis (HCA) and to the principal components analysis (PCA) using the results for the 20 descriptors of each wine. The matrix for the analysis was composed of the average intensity of the judges, for each descriptor and for each wine.

The PCA analysis shows a cumulative variance of 81.8% for the first two components with 54.2 % to component 1 and 27.6 % to component 2 as showed in Figure 3. The variables that showed the greatest relevance in component 1 were bitter, persistence, balance, softness, body, acid, white fruit, herbaceous, terpenic, alcohol and sweet. For component 2 the variables with more importance were tropical fruit, citric aroma, dried fruit and colour intensity.

From Figure 3, it is clear that there was a separation of the wines from the two years. Actually, all the wines from 2018 were positioned on the positive side of the component 1, well related with attributes such as bitter, persistence, balance, softness, body, acid and white fruit. The wines produced in 2017 were located on the negative side of component 1, showing a separation of the 'Verdelho' wine from the other two wine varieties, across component 2, closer to the sensory attributes of tropical and citric aromas. On the contrary, 'Galego Dourado' and 'Malvasia' wines were more related to herbaceous and dried fruit attributes.
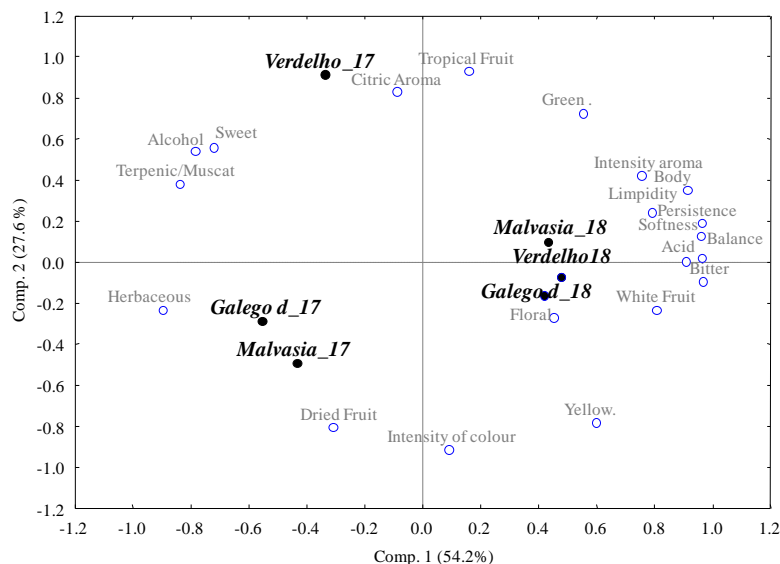

Figure 3. Projection of wine samples of three grapevine varieties (vintage 2017 and vintage 2018) and sensorial descriptors in the plane defined by the two components of the standardized PCA.

Projeção das amostras de vinho das três castas (vindima 2017 e 2018) e dos atributos da análise sensorial no plano definido pelos dois componentes da PCA padronizada.

This differentiation was also highlighted by the HCA analysis. The dendrogram of wines (Figure 4) exhibits the wine clustering based on the vintages but in case of 2017 vintage it was verified the separation of 'Verdelho' wine.

Figure 4. Dendrogram obtained using sensory data results of wines.

Dendrograma obtido com os resultados da análise sensorial dos vinhos.

A second PCA (data not shown) was done only with aroma attributes, showing a similar distribution of the samples across the two components, which explained 75% of variation.

Volatile compounds in wines

The chromatographic analysis (GC-FID and GC-MS) allowed to detect several compounds, and thirty nine compounds were identified (Figure 5) and quantified (Table III). Figure 5 shows a chromatogram of the 'Malvasia' 2017 wine sample, which is representative for all the varieties, since in general all the varieties presented a similar chromatographic profile. The concentrations of the various compounds had tendency to present differences in the studied wines.
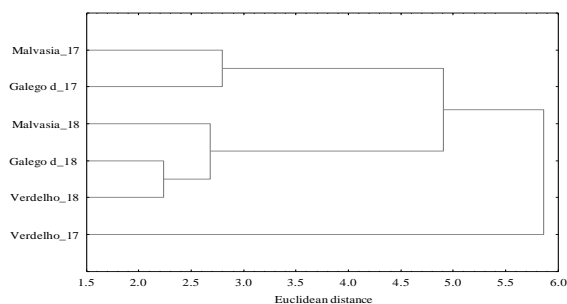
The quantitative data of volatile compounds found in these mono-varietal wines are shown in the Table III. In addition, the code assigned to each identified compound, the Kovats index and the sensory attribute and detection sensory threshold from the literature were also shown.
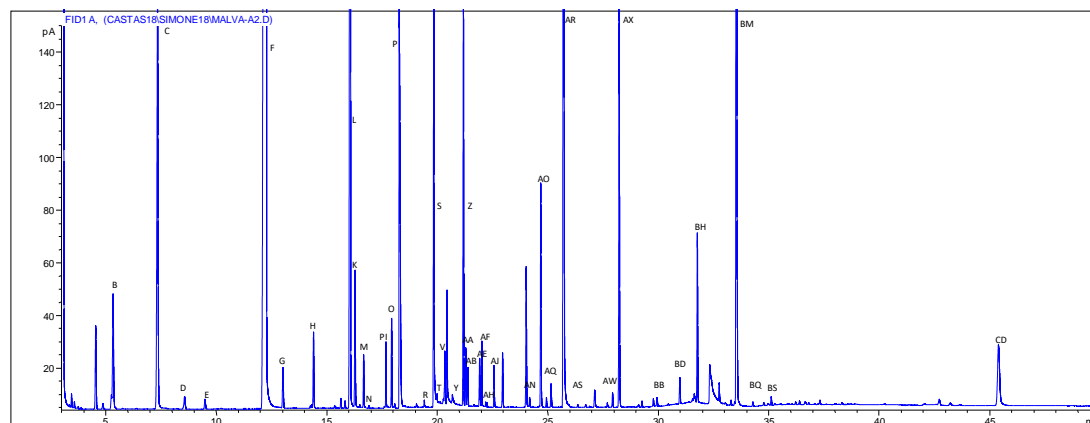
Figure 5. Chromatogram of dichloromethane extract from wine 'Malvasia'. Peak identification in Table III.

Cromatograma de um extrato de diclorometano, obtido a partir de um vinho Malvasia. Identificação dos picos na Tabela III.

Table III

Volatile compounds concentrations (average values and standard deviation) in the wines from the three grapevine varieties (mg of 2-octanol/L)

Teores de compostos voláteis (valores médios e desvios-padrão) nos vinhos das três castas (mg de 2-octanol/L)

Peak code	Compounds	Odour descriptor (threshold; mg/L)	KI	2017 vintage			2018 vintage		
				'Malvasia'	'Verdelho'	'Galego dourado'	'Malvasia'	'Verdelho'	'Galego Doiurado'
	Alcohols								
B	1-Propanol	ripe fruit, alcohol (830)[a]	1034	1.413 ± 1.046	2.924 ± 0.838	1.239 ± 0.752	2.606 ± 0.333	2.272 ± 1.380	3.821 ± 0.392
C	Isobutyl alcohol	oily, bitter, green (40)[a]	1094	5.538 ± 3.634	6.467 ± 1.119	2.668 ± 1.377	7.780 ± 0.972	7.589 ± 2.136	9.449 ± 0.700
E	1-Butanol	medicine, fruit[c]	1146	0.093 ± 0.052	0.342 ± 0.038	0.091 ± 0.013	0.207 ± 0.017	0.202 ± 0.034	0.294 ± 0.006
F	Isoamyl alcohols	burnt, alcohol, fusel[a,d] (30)[a]	1215	96.430 ± 39.019	107.910 ± 8.563	46.035 ± 6.510	167.628 ± 3.123	151.004 ± 17.082	168.256 ± 0.706
K	1-Hexanol	flower, green, cut grass (8)[f]	1358	1.451 ± 0.202	0.876 ± 0.033	0.779 ± 0.110	1.614 ± 0.033	1.519 ± 0.030	1.583 ± 0.015
M	3-Ethoxy-1-propanol	ripe pear (0.1)[g]	1376	0.582 ± 0.019	1.525 ± 0.048	0.528 ± 0.074	0.525 ± 0.055	0.392 ± 0.087	0.659 ± 0.080
N	*cis*-3-Hexenol	cutted grass (0.4)[f]	1386	nd	nd	0.194 ± 0.027	0.013 ± 0.002	0.073 ± 0.018	0.687 ± 0.010
S	2,3-Butanediol	caramel, sweet[c] (0.035)[k]	1540	7.212 ± 0.528	21.630 ± 1.203	7.872 ± 1.113	4.779 ± 1.256	6.385 ± 0.622	6.606 ± 0.746
AJ	3-Methylthiopropanol	cooked vegetable (1)[f]	1715	0.519 ± 0.067	0.162 ± 0.001	0.259 ± 0.036	0.606 ± 0.028	0.503 ± 0.078	0.640 ± 0.080
AQ	Benzyl alcohol	fruity blackberry (0.9)[o]	1876	0.391 ± 0.051	0.277 ± 0.012	0.191 ± 0.027	0.143 ± 0.010	0.122 ± 0.028	0.200 ± 0.014
AR	Phenylethyl alcohol	floral, roses (10)[f]	1909	42.772 ± 6.410	16.634 ± 0.10	16.683 ± 2.359	47.700 ± 2.229	41.252 ± 4.644	29.163 ± 5.065

Table III

(continued)

Peak code	Compounds	Odour descriptor (threshold; mg/L)	KI	2017 vintage			2018 vintage		
				'Malvasia'	'Verdelho'	'Galego dourado'	'Malvasia'	'Verdelho'	'Galego Doiurado'
	Esters								
D	Isoamyl acetate	banana (0.03)[b]	1117	0.185 ± 0.097	4.862 ± 0.234	0.345 ± 0.487	1.795 ± 0.066	1.498 ± 0.153	2.520 ± 0.030
G	Ethyl hexanoate	green apple (0.014)[a]	1240	0.502 ± 0.128	1.428 ± 0.037	0.293 ± 0.041	1.075 ± 0.078	0.817 ± 0.126	0.998 ± 0.022
L	Ethyl lactate	strawberry, acid, medicine (150)[d,e]	1350	32.908 ± 6.573	2.453 ± 0.005	9.810 ± 1.387	4.141 ± 0.025	3.456 ± 0.032	2.330 ± 0.040
O	Ethyl octanoate	fruity, sweet (0.005)[a]	1437	0.969 ± 0.056	1.814 ± 0.069	0.566 ± 0.079	1.472 ± 0.080	1.243 ± 0.123	1.618 ± 0.024
R	3-Hydroxy ethyl butanoate	frutado (1.2)[i]	1518	0.081 ± 0.009	0.159 ± 0.003	0.070 ± 0.010	0.112 ± 0.009	0.088 ± 0.011	0.138 ± 0.002
AB	Ethyl decanoate	sweet/fruity (0.2)[b]	1638	0.502 ± 0.054	0.052 ± 0.007	0.105 ± 0.014	nd	nd	nd
AF	Ethyl succinate	ripe melon (1000)[g]	1680	0.693 ± 0.075	0.634 ± 0.010	0.242 ± 0.034	0.427 ± 0.045	0.544 ± 0.060	0.297 ± 0.007
AN	2-Phenylethyl acetate	floral (0.25)[f]	1815	0.129 ± 0.020	0.448 ± 0.009	0.104 ± 0.015	0.288 ± 0.038	0.234 ± 0.057	0.250 ± 0.004
AW	Diethyl malate	over-ripe, peach, prune (760)[p]	2042	0.222 ± 0.081	1.121 ± 0.015	0.097 ± 0.014	0.760 ± 0.044	0.854 ± 0.022	0.381 ± 0.023
BM	Monomethyl succinate	caramel, coffee (1000)[s]	2383	19.743 ± 6.290	12.887 ± 0.322	7.379 ± 1.043	9.884 ± 1.711	12.819 ± 0.277	6.375 ± 0.419
	Terpenes								
T	Linalool	floral (0.01)[f]	1551	0.057 ± 0.004	nd	nd	0.013 ± 0.002	nd	nd
Y	Hotrienol	floral, citrus (0.015)[m]	1613	nd	nd	nd	0.005 ± 0.007	0.064 ± 0.001	nd
AH	α-Terpineol	lilac (0.25)[m]	1695	0.055 ± 0.009	nd	nd	0.000 ± 0.000	nd	nd
AS	2,6-Dimethyl-3,7-octadiene-2,6-diol	-	1948	0.033 ± 0.003	nd	nd	0.025 ± 0.004	nd	nd
	Cetones								
H	Acetoin	butter/cream (150)[e]	1283	0.841 ± 0.316	1.349 ± 0.070	0.654 ± 0.093	nd	nd	nd
	Lactones								
Z	Butyrolactone	caramel, sweet[c,k] (0.035)[k]	1625	4.668 ± 0.179	4.637 ± 0.132	1.535 ± 0.217	3.907 ± 0.553	4.766 ± 0.504	3.049 ± 0.190
BD	γ-Undecalactone	spice, lactone-like[r]	2225	0.408 ± 0.103	0.380 ± 0.014	0.165 ± 0.023	0.439 ± 0.072	0.382 ± 0.081	0.284 ± 0.015
	Acids								
P	Acetic acid	vinegar (26)[h]	1465	6.573 ± 2.250	6.107 ± 0.451	5.125 ± 0.725	3.910 ± 1.383	5.909 ± 0.866	4.818 ± 0.335
V	Isobutanoic acid	rancid, butter, cheese (2.3)[a]	1572	0.644 ± 0.019	0.273 ± 0.011	0.204 ± 0.021	0.307 ± 0.049	0.386 ± 0.041	0.297 ± 0.010
AA	Butanoic acid	rancid, cheese, sweat (0.173)[a]	1631	0.615 ± 0.191	0.984 ± 0.048	0.267 ± 0.038	0.479 ± 0.037	0.393 ± 0.044	0.462 ± 0.030
AE	Isovaleric acid	sweet, acid, rancid (0.033)[b]	1672	0.592 ± 0.017	0.309 ± 0.005	0.273 ± 0.038	0.626 ± 0.020	0.487 ± 0.236	0.641 ± 0.030
AO	Hexanoic acid	sweat (0.42)[a]	1846	2.934 ± 0.347	5.589 ± 0.181	1.745 ± 0.246	4.331 ± 0.405	3.357 ± 0.547	3.942 ± 0.119
AX	Octanoic acid	sweat, cheese [a,k] (10)[a]	2060	5.972 ± 1.083	8.266 ± 0.108	3.795 ± 0.537	8.602 ± 0.872	6.490 ± 1.380	8.554 ± 0.271
BH	Decanoic acid	fat, rancid [a,k] (6)[a]	2273	2.679 ± 0.675	2.477 ± 0.086	1.575 ± 0.222	2.537 ± 0.235	2.051 ± 0.458	2.896 ± 0.071
BQ	Benzoic acid	chemical (1)[a]	2431	0.072 ± 0.017	0.061 ± 0.002	0.031 ± 0.004	0.010 ± 0.001	0.021 ± 0.004	nd
BS	Dodecanoic acid	dry, metallic, laurel oil (1)[t]	2485	0.174 ± 0.058	0.119 ± 0.011	0.104 ± 0.015	0.132 ± 0.033	0.069 ± 0.035	0.148 ± 0.010
	Phenols								
BB	Eugenol	clove, cinnamon (0.005)	2161	0.140 ± 0.033	0.168 ± 0.021	0.061 ± 0.009	0.145 ± 0.005	0.131 ± 0.022	0.050 ± 0.021
CD	Tyrosol	-	2995	2.691 ± 1.190	1.335 ± 0.133	1.774 ± 0.251	1.790 ± 0.010	1.836 ± 0.321	1.530 ± 0.015

nd – not detected. a) Etiévant (1991); b) Ferreira *et al.* (2000); c) Acree and Heinric (2004); d) López *et al.* (2003); e) Bartowsky and Pretorius (2009); f) Guth (1997); g) Mestre *et al.* (2019); h) Salo *et al.* (1972); i) Pineau *et al.* (2009); j) Hongku *et al.* (2011); k) Sánchez-Palomo *et al.* (2010); m) Waterhouse *et al.* (2016); n) Marcon *et al.*(2019); o) Amores-Arrocha *et al.* (2018); p) García-Carpintero *et al.* (2012); q) Moyano *et al.* (2002); r) López *et al.* (2004); s) Sánchez-Palomo *et al.* (2012); t) Li *et al.* (2008).

The compounds identified were from different chemical families, including 11 alcohols, 10 esters, 9 acids, 4 terpenes, 2 lactones, 2 phenolic compounds and 1 cetone. Most of the volatile compounds identified result from yeast metabolism (Styger *et al.*, 2011) namely the alcohols, esters, acids and acetoin. It was also identified the 1-hexanol and *cis*-3-hexenol usually formed during the pre-fermentative steps due to enzymatic activities (Cordonnier and Bayonove, 1981). The eugenol, usually related with ageing or fermentation in wood (Herrero *et al.*, 2016), has also been identified by other researchers in the unaged white wines (González-Álvarez *et al.,* 2011). Regarding the lactones, the butyrolactone seems to be formed during the fermentation (Clarke and Bakker, 2004), and the δ-undecalactone has been reported in aged champagne (Escudero *et al.*, 2000) and in different wines (Ferreira *et al.*, 2004). The tyrosol, also identified by other authors in red wine and white wines (Selli *et al.*, 2004, 2006), is a phenolic compound formed from tyrosine by yeast during fermentation, which seems to play an important role on the white wine mouthfeel (Gawel *et al.*, 2018). Four terpenic alcohols were also identified namely in 'Malvasia' wines and in 'Verdelho' wine from 2018 vintage. These compounds are normally considered as varietal compounds since they are present in the grapes, and they were used to classify the grape varieties in Muscat varieties in which the concentration of free terpenes is higher than 6 mg/L, non-Muscat, but aromatic varieties, with a concentration around 1-4 mg/L, and neutral varieties, such as 'Chardonnay' (Cañas *et al.,* 2018), in which the aromatic profile does not depend on the concentration of free terpenes present (Mateo and Jimenez, 2000). Concerning the low level of free terpenes in all the wines (Table III), it appeared that 'Malvasia', 'Verdelho' and 'Galego Dourado' could be considered as neutral varieties. Given that these compounds are normally associated with floral notes; these results could explain that floral attribute is a variable with a low contribution for variability explanation in the PCA of the sensory results (Figure 3) as well as the low intensity of terpenic attribute of all the wines at Figure 2. Taking into account the volatile amounts in the wines (Table III) and the corresponding sensory thresholds found in the scientific literature, it was expected that the compounds with higher impact in the aroma of these wines would be three esters (isoamyl acetate, ethyl hexanoate and ethyl octanoate), some alcohols (isoamyl alcohols, 3-ethoxy-1-propanol, 2-phenylethyl alcohol and 2,3-butanediol), two acids (isovaleric and hexanoic acid), butyrolactone and eugenol. Additionally, the linalool could have importance in 'Malvasia' wine of 2017 and the hotrienol could impact the aroma of 'Verdelho' from 2018 vintage. However, as sensory thresholds are influenced by additive, synergic and antagonistic effects in the wine matrix, further research is needed about the odorant compounds of these wines.

The results in Table III show different contents of various compounds in different wines. For example, 'Malvasia' wine from 2017 compared to the sample of wine 'Malvasia' from 2018 seems to presented higher concentration of terpene compounds but in both years a terpene alcohol (3,7-dimethyl-1,5-octadien-3,7-diol) was found, which was also identified by Di Stefano (1982) and Liberatore *et al.* (2010) in white wines. However, it is not possible to assess the significance of these differences taking into account the type of experimental design used in this work.

A PCA analysis was performed based on the volatile compounds quantified in the all wine from the three grapevine varieties at the two vintages (Table III) to verify the relevant compounds for both years (Figure 6). A first PCA (data not shown) analysis was performed starting with all compounds of Table III. The analysis indicated that the first two principal components explained only 60.7 % of the total variance among the samples studied. A comparison of scores and loadings for the components allowed to identify the compounds having lower influence for the ranking of different wines. Thus, a second PCA was done after excluding the compounds with a lower contribution for the explained variability, namely AS, BH, BQ, BS, M, N, P, T, V and Y. The analysis of the main components showed 68.6 % of cumulative variance for the two first components: 43.1 % in component 1 (PC1) and 25.2 % in component 2 (PC2). The plot of the wine samples and the volatile compounds, in the planed defined by the components in the PCA (Figure 6), exhibits results in accordance with PCA of sensory results. Indeed, the wines produced in 2018 were much closer, while the wines of the same varieties produced in 2017 were well separated. As noted for the sensory results, it seems that the vintage imparted more dissimilarity than the grape variety, probably due to the strong differences in the climatic conditions verified in the two vintages.

The variables with high loadings for the positive side of component 1 were mainly esters: 3-hydrox ethyl butanoate (R), isoamyl acetate (D), 2-phenylethylacetate (AN), ethyl octanoate (O), ethyl hexanoate (G) diethylmalate (AW) and also alcohols (isobutanol-C, 1-propanol-B, 1-butanol-E) and acids (octanoic acid-AX, hexanoic acid-AO), which seemed well related with wine of 'Verdelho' from 2017 vintage. The 'Malvasia' wine from 2017 was

located in the opposite side of the component, closer to the tyrosol (CD) and the ethyl lactate (L) compounds. Taking into account the fruity notes of the majority of the esters (Table III), this may explain the sensory results where the 'Verdelho' wine of 2017 were related to citric and tropical fruits.
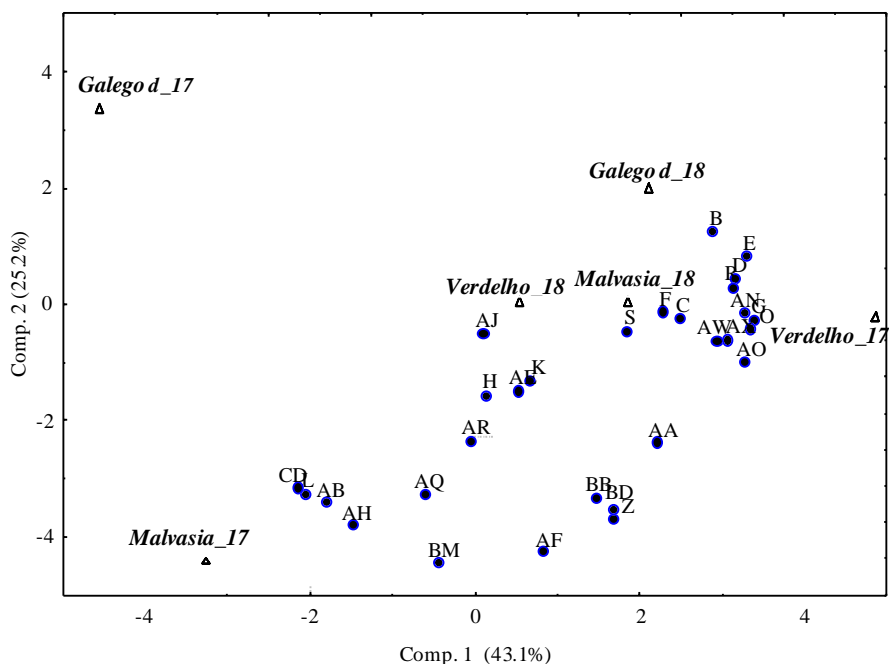

Figure 6. Projection of wine samples of the three grapevine varieties (vintage 2017 and vintage 2018) and volatile compounds quantified in the plane defined by the two components of PCA. Compounds identification in the Table III.

Projeção das amostras de vinho das três castas (vindima 2017 e 2018) e dos compostos voláteis quantificados no plano definido pelas duas componentes da PCA. Identificação dos compostos na Tabela III.

Legend - B: 1-Propanol; C: Isobutyl alcohol; D: Isoamyl acetate; E: 1-Butanol; F: Isoamyl alcohols; G: Ethyl hexanoate; H: Acetoin; L: Ethyl lactate; K: 1-Hexanol; O: Ethyl octanoate; P: Acetic acid; R: 3-Hydroxy ethyl butanoate; S: 2,3-Butanediol; Z: Butyrolactone; AA: Butanoic acid; AB: Ethyl decanoate; AE: Isovaleric acid; AF: Ethyl succinate; AH: α-Terpineol; AJ: 3-Methylthiopropanol; AN: 2-Phenylethyl acetate; AO: Hexanoic acid; AQ: Benzyl alcohol; AR: Phenylethyl Alcohol; AW: Diethyl malate; AX: Octanoic acid; BB: Eugenol; BD: γ-Undecalactone; BM: Monomethyl succinate; CD: Tyrosol.

In the component 2, there are several compounds with high loadings in the negative side, namely α-terpineol (AH), lactones (butyrolactone-Z, δ-undecalactone-BD), esters (monoethylsuccinate-BM, ethylsuccinate-AF, ethyl decanoate-AB, ethyl lactate-L) and phenolic compounds (benzyl alcohol-AQ, tyrosol-CD, eugenol-BB). Only the 'Malvasia' wine from the 2017 vintage seemed to be related to high amounts of these compounds. Taking into account the sweet notes of several of those compounds, this may explain the relation of this wine with dried fruit attribute in the sensory results. The 'Galego Dourado' wine produced in 2017 is located in the opposite side of the component 2. All the wine samples of vintage 2018 presented an intermediate location, but in the positive side of PC1 and PC2, indicating intermediate amounts of compounds with positive loadings in the component 1 and low amounts in the compounds with high loading in the negative side of component 2.

The multidimensional analysis of sensory and volatile results suggested a similar discrimination of the white wines samples of the three grapevines varieties 'Malvasia', 'Galego Dourado' and 'Verdelho'. The samples separation in the PCA plots appeared more related to the vintage year than to the grapevine variety. Therefore, further research is needed to re-evaluate these varietal wines and to study the variety and the year as factors that may impart significant differences on their volatile compounds and sensory profile. Indeed, other researchers also found a significant effect of the vintage in several volatile compounds in white and red wines (Selli *et al.*, 2004; Vilanova *et al.*, 2013, Sánchez-Palomo *et al.*, 2019) and also in white must (Rocha *et al.*, 2010).

CONCLUSIONS

All the wines produced with the different grapevines – 'Malvasia', 'Verdelho' and 'Galego Dourado' - showed a balanced sensory profile with medium white fruity, tropical fruit and dried fruit notes. The wines also presented high aroma intensity and persistence and medium intensity in the body and balance attributes. The sensory attributes that presented high contribution to the explained variability of the wine samples were bitter, persistence, balance, softness, body, acid, white fruit, herbaceous, terpenic, alcohol, sweet, tropical fruit, citric aroma, dried fruit and colour intensity.

The multidimensional analysis of the sensory properties and volatile compounds results showed a similar discrimination of the wine samples. In both analyses, the separation of the wines samples in the PCA plots seemed more related to the vintage than to the grapevine variety.

Regarding the volatile composition, the volatile compounds with higher contribution to the samples variability were esters (3-hydroxiethyl butanoate, isoamyl acetate, 2-phenylethylacetate, ethyl octanoate, ethyl hexanoate, diethylmalate, monoethylsuccinate, ethylsuccinate, ethyl decanoate, ethyl lactate), alcohols (isobutanol, 1-propanol, 1-butanol), acids (octanoic acid, hexanoic acid), lactones (butyrolactone, δ-undecalactone), phenolic compounds (benzyl alcohol, tyrosol, eugenol) and a terpenic compound, the α-terpineol.

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