

Volatile and sensory characterization of white wines from minority grapes varieties

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To my dad ...

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Resumo

Este trabalho surgiu da necessidade do setor vitivinícola português de aumentar a biodiversidade na produção e cultivo da videira, já que, ao longo dos anos, o mercado teve uma homogeneização com conseqüente empobrecimento do património genético, como também afirma García-Munozet *et al.* (2011).

O estudo de variedades minoritárias poderá trazer respostas para as novas necessidades do mercado.

Assim, o presente estudo centrou-se na caracterização de compostos voláteis de cinco vinhos produzidos por castas minoritárias portuguesas, tais como: Malvasia (de Colares), Verdelho, Galego Dourado, Moscatel Nunes e Boal Espinho.

Os resultados obtidos são muito interessantes, pois mostram uma diferenciação de variedades, mas acima de tudo, uma diferenciação entre as duas colheitas tidas em consideração, provavelmente devido ao efeito “vintage”. Quer do ponto de vista sensorial, quer do ponto de vista químico, as variedades estudadas mostram um potencial enológico interessante. No entanto, são necessários mais estudos acerca da influência dos efeitos climáticos sobre o perfil de compostos voláteis.

Palavras chave: Castas Minoritárias, Vinhos Brancos, Compostos Voláteis, Cromatografia Gás-líquido, Análise Sensorial

Abstract

This work was born from the need of the Portuguese wine sector to increase biodiversity in the production and cultivation of the vine; since over the years, the market has had a homogenization with consequent impoverishment of genetic heritage, as also claimed by García-Munozet *et al.* (2011).

The study of minority varieties could bring answers to new market needs.

This thesis work focused on the characterization of the volatile compounds of five wines produced by minority varieties of Portugal such as: Malvasia (Colares), Verdelho, Galego Dourado, Moscatel Nunes and Boal Espinho.

The characterization took place using sensory analysis and chromatographic analysis; furthermore, the data obtained were analysed through the use of multivariate analysis, which allowed knowing the similarities and divergences of the varieties.

The results obtained show a differentiation of the variety but above all a differentiation of the two vintages taken into consideration, probably due to the vintage effect.

The results obtained, both from a sensory and a chemical point of view, show an interesting oenological potential, but still require further studies, in order to evaluate the influence of climatic effects on the profile of volatile compounds.

Keywords: Minority grapes varieties, White wines, Volatile compounds, Gas-liquid Chromatography, Sensory analysis

Resumo alargado

Este trabalho surgiu da necessidade do setor vitivinícola português de aumentar a biodiversidade na produção e cultivo da videira, já que, ao longo dos anos, o mercado teve uma homogeneização com conseqüente empobrecimento do património genético, como também afirma García-Munozet *et al.* (2011).

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A caracterização das castas foi feita usando análise sensorial e análise cromatográfica dos vinhos. Os resultados obtidos foram analisados recorrendo a análise multivariada, o que permitiu conhecer as semelhanças e divergências das variedades.

Todos os vinhos foram muito apreciados no que respeita à análise sensorial, em particular, na colheita de 2017, destacaram-se três vinhos, Verdelho com notas de frutas tropicais, Malvasia e Moscatel Nunes, com notas florais-terpénicas.

Os resultados obtidos ao nível sensorial foram confirmados, em parte, por análises cromatográficas.

Em geral, todos os vinhos apresentam um perfil cromatográfico muito semelhante, com a prevalência de compostos provenientes da fermentação, com diferenças de concentração. No entanto, Moscatel Nunes e Malvasia diferem na presença de compostos terpênicos de carácter varietal como linalol, alpha-terpineol, hotrienol e citronelol.

Além das diferenças varietais, a partir de análises cromatográficas e sensoriais, existem diferenças entre as colheitas, o que muitos autores descrevem como um efeito "vintage" dos vinhos.

As condições climáticas nos dois anos considerados, 2017 e 2018, podem estar na origem das diferenças encontradas entre as colheitas diferentes. Em 2017, o ano foi considerado seco com precipitação total de cerca de 435 mm, mas com chuvas mais homogêneas e em 2018 a precipitação total foi 650 mm, com chuvas concentradas entre fevereiro-abril e outubro-novembro, no entanto, durante o período de maturação da uva, fizeram-se sentir temperaturas mais altas e menos chuvas do que no ano anterior.

Seria interessante prolongar o estudo destas variedades, ver as diferenças dos compostos voláteis devido ao efeito climático e talvez modificar os métodos de extração, utilizando técnicas mais sensíveis aos compostos varietais; avaliando também os precursores aromáticos e avaliando os compostos voláteis através da cromatografia gas-liquido com deteção olfatométrica.

Palavras chave: Castas Minoritárias, Vinhos Brancos, Compostos Voláteis, Cromatografia Gás-liquido, Análise Sensorial

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Abbreviations

INIAV: Instituto Nacional de Investigação Agrária e Veterinária

CNVV: National Catalogue of Vine Varieties

IPP: Isopentenyl diphosphate

DMAPP: Dimethylallyl diphosphate

MVA: Mevalonic Acid

MEP: 2-methyl-D-erythriol-4-phosphate

TCH: 2,2,6-trimethylcyclohexanone

TDN: 1,1,6-trimethyl-1,2-dihydronaphthalene

MP: Methoxypyrazine

3MH: 3-mercaptohexanol

A3MH: 3-mercaptohexanol Acetate

4MMPOH: 4-Mercapto-4-methyl-2-pentanone

MCFA: Medium chain fatty acid

CAN: Portuguese National Ampelographic Collection

ISO: International Organization for Standardization

QDA: Descriptive Quantitative Analysis

PCA: Principal Components Analysis

HCA: Hierarchial Cluster Analysis

PC: Principal Components

GC-FID: Gas Chromatography-Flame Ionization Detector

GC-MS: Gas Chromatography-Mass Spectrometry

NIST: National Institute of Standards and Technology

DNA: Deoxyribonucleic Acid

OAV: Odour Activity Value

GPB: Glyceraldehyde 3-phosphate

GPP: Geranyl diphosphate

FPP: Farnesyl diphosphate

GGPP: Geranyl Geranyl diphosphate

1. Introduction

The wine's 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 in the case of Portugal presents a unique and enormous genetic patrimony, with around 230 varieties considered autochthonous to Portugal or Iberian Peninsula listed in the ordinance n. 380/2012 of 22 November, that establish the 343 grapevine varieties suitable to wine production in Portugal (Eiras-Dias *et al.*, 2016).

In the yearbook of 2017, issued by the Instituto da Vinha e do Vinho, Portugal's total area of vines, is 191633 hectares including the autonomous regions of Madeira and Açores.

The regions with the largest vineyard area are: the Douro region (42023 hectares), the Alentejo (23879 hectares), the Minho (21307 hectares) and Lisbon (18641 hectares).

In Portugal, 343 vine varieties are authorized for wine production of which 34 are the most used; 17 red berry varieties including Aragonez, Touriga Franca and Touriga Nacional and 17 white berry varieties such as Fernão Pires, Loureiro and Arinto (IVV, 2017).

In the list we also find the Malvasia (de Colares), studied in this work, with an area of 1488 hectares; one might suppose, that erroneously, other biotypes very similar to this have been recorded as Malvasia, but to be sure, a more accurate evaluation should be made through a morphologic and genetic analysis (Eiras-Dias, personal communication).

At the National Ampelographic Collection (reference code PRT051), belonging to the National Institute of Agricultural and Veterinary Research (INIAV), located in Dois Portos, there are still some unidentified vines, which have been collected in different vineyards scattered all over the country (Eiras-Dias *et al.*, 2016).

The conservation and enhancement of minor varieties should be the goal of the historic wine-growing countries, to diversify and implement production and meet new market needs.

It is important to clarify the definition of minor varieties, since the Portuguese legislative level considers a minor variety when it satisfies the following conditions cumulatively (DGAV, 2015):

- The variety is registered in the National Catalogue of Vine Varieties (CNVV);
- The variety does not have any vine mother of active multiplication in the certification system in Portugal.

In this work five grapevines varieties will be analyzed, among which two are registered (legally) as minority varieties such as Boal Espinho (PRT52017) and Moscatel Nunes (PRT53015), and three others that despite the possibility of having propagating material, over the entire surface

area of Portugal, they are under the threshold of 1% like total vine area, such as Malvasia (PRT52714), Galego Dourado (PRT52913) and Verdelho (PRT50317) (IVV, 2017).

This work will focus on the aromatic characteristics from the chemical point of view and from the sensory point of view, evaluating the oenological potentialities of the wines produced from the five mentioned varieties.

1.1. Grapevine varieties

Since this work focused on five minority varieties in Portugal such as Malvasia (de Colares), Verdelho, Galego Dourado, Boal Espinho and Moscatel Nunes, some information about each variety will be presented.

1.1.1. Verdelho

Verdelho is one of the main white grapevine varieties used to produce fortified wines in the Madeira region. This variety is also used to produce table wines in Madeira and in other wine-growing regions such as continental Portugal, Açores, Canary Islands (Spain) and Australia.

Some information on the aromatic characteristics of this variety can be obtained thanks 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 notes and notes of hone. Also, with the study conducted by Ferreira (2011), thiol characters were found.

Verdelho is a variety with a medium vigour, which produces bunches of small size, with oval berries with a high ratio between peel and skin, which can be hard if eaten (Eiras-Dias *et al.* 2011).

This variety is sensitive to spring frosts and mildew due to its conformation of the berry. The maturation is precocious, usually has a good acidity and high sugar content, and for this reason is used to produce liqueur wines (Eiras-Dias *et al.* 2011)

1.1.2. Malvasia (de Colares)

Malvasia, commonly known as Malvasia de Colares, is a variety cultivated in the region of Colares, located on the south-western coast of the Atlantic Ocean.

In 1908, the Colares region, thanks to its unique characteristics, a combination of climate, variety and soil, has been established as DOC region (Controlled Denomination of Origin). This region is characterized by temperate and humid climate in summer and sandy soil, which allows the cultivation of the vine without rootstock, giving to the final product unique characteristics of its kind. The proximity to the sea means that, the vineyards undergo during

the year, strong winds and for this reason traditionally are surrounded by cane structures (Robinson *et al.*, 2013; Eiras-Dias *et al.*, 2016).

The cluster turns out to be medium-sized, little compact, with medium-sized, round berries, with yellowish-green colouring with thick peel and soft pulp (Eiras-Dias *et al.*, 2016).

From the organoleptic point of view, the wines analysed at the INIAV show a pale straw yellow colour, with a frank and fruity aromas and medium intensity (Eiras-Dias *et al.*, 2016).

The grape of Malvasia derives from a natural crossing between the varieties Gibi (Mourisco-Branco) x Amaral (Eiras-Dias *et al.*, 2016).

1.1.3. Galego Dourado

Galego Dourado is a white grape variety widely used to produce wines from the Carcavelos region. The current surface of this variety is about 10 hectares but as reported by J. Carvalho at the V International Congress of Vine and Wine, held in Lisbon (Carvalho) in 1938; this variety was one of the main vines planted in the region of Carcavelos.

The cluster of this variety has a medium, cylindrical, medium compact size, with a rounded berry, a medium-thick skin, with yellowish colours that tend to have a golden colour when ripe. It is a vigorous variety, with early ripening; it is sensitive to diseases and putrefaction (Eiras-Dias *et al.*, 2011).

Wines produced with this variety normally have a high alcohol content and an aromatic profile with citric notes and cut grass with good persistence (Eiras-Dias *et al.*, 2011; INIAV, 2017).

1.1.4. Moscatel Nunes

Moscatel Nunes is often erroneously confused with Moscatel de Setubal or Muscat of Alexandria, but on a genetic level they are different; in fact, from the researches on the DNA it has emerged that the parents of the Moscatel Nunes are: Muscat of Alexandria and Heben, a Spanish variety (Maul *et al.*, 2019).

The bunch is medium-large, conical, medium compact, mostly wingless, with round berries, medium (18-20 mm), yellow-green; due to its compactness it is sensitive to rot (INIAV, 2019).

It is a variety with high fertility and production and from this variety it is possible to produce wines with low alcoholic content, mainly used to produce liqueur wines. The vinification experiments show floral aromas with short life (INIAV, 2019).

1.1.5. Boal Espinho

Boal Espinho is a variety grown in the Estremadura region, currently renamed Lisbon; and is one of the minor varieties of Portugal. The cluster is medium-sized, conical in shape with 1-2 wings, moderately compact. The grape has an elliptical shape, of medium size, neutral with a low potential alcoholic strength and a medium-sized acidity. This variety has a vigorous average and thanks to its compactness it is sensitive to the rot (Eiras-Dias *et al.*, 2011).

1.2. Aromas in the grape and in wine

The aromatic component, especially the 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).

For the study of volatile compounds, there are several methods, depending on the type and concentration of the molecule, moreover, the aromatic profile of a wine is influenced, not only by volatile molecules, but also by its matrix (chemical composition) (Meilgaard *et al.*, 1999).

In addition to knowing the volatile compounds it is important to know their perception threshold, which is the lowest concentration that the molecule can be detected by 50% of the tasters. Thanks to this preliminary knowledge, it is possible to know the "odor activity value" (OAV), which gives us the opportunity to estimate the contribution of each single compound to the aroma of the wine (Dubois, 1994; Meilgaard *et al.*, 1999).

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

- *varietal aroma*, which originates during the development process of the berry, closely linked to the climatic conditions, the soil, the phytosanitary conditions and the degree of ripeness of the grapes (Cordonnier and Bayonove, 1979);
- *pre-fermentative aroma*, which develops during the phases prior to fermentation during transport, maceration, pressing and clarification of the must (Cordonnier and Bayonove, 1979);
- *fermentative aroma*, originated by microorganisms present in the medium during alcoholic and malolactic fermentation (Rapp and Mandery, 1986)

- *post-fermentative aroma*, originates after fermentation thanks to chemical reactions, which develop during the conservation and aging of wine (Marais and Pool, 1980; Usseglio-Tomasset, 1983; Vilanova and Oliveira, 2012).

1.2.1. Varietal aromas

The compounds that contribute to the formation of the varietal aroma are synthesized and then stored in the exocarp, vacuoles and in the smallest part are stored in the pulp (Lichtenthaler *et al.*, 1997).

These molecules are important for the development of the plant, playing a fundamental role in the interaction between plant and environment (Lichtenthaler *et al.*, 1997; Tholl *et al.*, 2006).

Many molecules are synthesized in response to biotic or abiotic stress, but they also have an attractive role towards pollinators, for entomophilic pollinating plants, or even they can discourage herbivores or are able to inhibit microbial growth. Subsequently, these molecules play a key role in the oenological industry (Lichtenthaler *et al.*, 1997; Failla *et al.*, 2006; Evert and Eichhorn, 2013).

The compounds that contribute to the formation of the varietal aroma are part of large families of compounds: terpenes, norisoprenoids, benzenoid compounds, methoxypyrazines and sulphur compounds (Ribéreau-Gayon, *et al.*, 2006a).

In general, it is possible to classify the varieties based on the concentrations of total free terpenes and we can have: *muscat varieties* with a concentration of free terpenes higher than 6mg/L, *non-muscat*, but aromatic varieties, with a concentration around 1- 4 mg/L and *neutral variety*, where their aromatic profile does not depend on the concentration of free terpenes present (Mateo and Jimenez, 2000).

In the aromatic varieties, already in the must, you can find floral aromas, unlike the non-aromatic varieties, where there are sweet and herbaceous scents characteristic of musts during the pre-fermentative phases (Moio, 2016).

High concentrations of terpenes characterized the highly aromatic varieties, such as those of the Muscat group, Gewurztraminer, Malvasia of Sardinia (Günata *et al.*, 1985, Wilson *et al.*, 1986). On the contrary, intermediate levels can be found in semi-aromatic varieties such as Muller-Thurgau or Riesling (Mateo and Jimenez, 2000). The most representative molecules in the aromatic grape varieties are linalol and geraniol, commonly associated with floral aromas, such as the rose (Ribéreau-Gayon *et al.*, 2006a).

1.2.1.1. Terpenes

The terpenic compounds are very widespread in the realm of plants, and they can be divided into monoterpenes (composed of 10 carbon atoms) and sesquiterpene formed by 15 carbon atoms.

All terpenoids are biosynthesized from just two C5 precursors: *isopentenyl diphosphate (IPP)* and *dimethylallyl diphosphate (DMAPP)* (Ribereau-Gayon et al., 1975; Eisenreich et al., 2001).

The synthesis of terpenes occurs in two different metabolic pathways, that of *MVA (mevalonic acid)* in the cytosol and that of the *MEP (2-methyl-D-erythritol-4-phosphate)* which occurs at the plastid level (Eisenreich et al., 2001; Tholl, 2015).

In the MVA biosynthetic pathway, six different enzymatic reactions occur, such as condensation and reduction reactions leading to the formation of IPP.

The MVA pathway includes six enzymatic reactions, mediated by enzymes that allow the condensation of three acetyl CoA molecules, followed by a reduction, phosphorylation and decarboxylation; the final product starting from mevalonic acid is IPP (Tholl, 2015).

Seven enzymatic reactions occur in the MEP pathway, starting from a condensation of pyruvate and glyceraldehyde3-phosphate (G3P) (Tholl, 2015).

The biosynthetic process of terpenes can be divided into three phases:

- synthesis of the base units with 5 carbon atoms (isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP))
- prenyltransferase, which uses as a substrate IPP and DMAPP, to produce prenyl diphosphate precursors, geranyl diphosphate (GPP), farnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP)
- conversion in final products by: a large family of enzymes known as terpene synthase (cyclase) following oxidation, dehydrogenation, methylation, acylation and glycosylation reactions (Dudareva et al., 2013).

Monoterpenes come in the form of simple hydrocarbons (limonene, myrcene, etc.), aldehydes (linalals, geranial, etc.), alcohols (linalool, geraniol, etc.), acids (linalic and geranic acid, etc.), and esters (linalyl acetate, etc.) (Ribereau-Gayon et al., 2006a).

The terpenic compounds that are found in greater frequency, are geraniol α -terpinelol and linalool, as shown in table 1. The concentrations vary from $\mu\text{g/L}$ to the few mg/L , with floral scents, geranium, lavender and with very low perception thresholds ranging from 0.2 micrograms up to 300 $\mu\text{g/L}$.

The concentrations of terpene compounds, during maturation, undergo variations; it tends to increase the total concentration and concentration of the linked forms, this thanks to the increase in the concentration of sugars (Gunata *et al.*, 1993).

Table 1 - Concentrations, odour quality and thresholds of odour-active monoterpenes compounds found in wine white (adapted Francis and Newton, 2005).

	Odour quality	Concentration white wine (µg/L)	Odour threshold (µg/L)
<i>Linalool</i>	flower, lavender	4.75 – 307	25.2
<i>Geraniol</i>	rose, geranium	221	30
<i>cis-Rose oxide</i>		3 – 21	0.2

The presence of terpenic compounds, in the Muscat variety, is already known, but such compounds can be found in white minority varieties, such as Zalema, a Spanish variety. This author found a total concentration around 40 µg/L of terpene compounds in wines produced from this variety (Gómez-Míguez *et al.* 2007).

It is well known the existence of a non-volatile fraction of terpenes present in Moscato grapes (monoterpenoid, terpene polyols), as they are present in glycosidic form, with different types of sugars such as glucose, rhamnose and apiose. Unlike other plants, the vines have a lower concentration of diglucosides (Ribereau-Gayon *et al.*, 2006a).

The research conducted by Gunata *et al.* (1985) and Wilson *et al.* (1986) highlighted the differences in concentration of the precursor's aromas in the various parts of the berry, showing higher concentrations of linked forms in the juice and in the peels with respect to the pulp. The precursors forms are not olfactory perceptible and to perceive the aroma in the wine these forms must be freed from the sugar, but it is not simple because the presence of the pentose more hinders the release of the aglycone. The release of the aglycones (aromatic) is done by hydrolysis mechanisms with a specific glycosidasic action, splitting the sugars linked to the aromatic molecule (Delcroix *et al.*, 1994)

The cleavage capacity is given by endoglicosidases, but these are inhibited by non-optimal pH values (optimum values around pH 5), and they do not have the ability to hydrolyse the glycosides of tertiary alcohols (such as linalool). In addition to these reasons, endogenous enzymes are inhibited by must clarifying operations (Grossmann *et al.*, 1990).

The enzyme invertase sequentially detaches first the pentose and subsequently the glucose from the terpene, releasing it; moreover, they can be liberated chemically (must/wine acid environment). The acidity of the medium is able to hydrolyse the sugar linked to the terpen but

this reaction is very slow. This reaction is also one of the reasons for the natural evolution of aromatic wines (Williams *et al.*, 1980).

1.2.1.2. C13-Norisoprenoid derivates

Norisoprenoids are molecules with 13 carbon atoms, that can be formed by a direct degradation of carotenoids (such as β -carotene and neoxanthine) or it can find them as glycoconjugates, which then, can release their volatile aglycone during fermentation through enzymatic and acid hydrolysis processes (Mendes-Pinto, 2009).

Carotenoid synthesis takes place through the MVA pathway, which produces β -carotene and lutein with concentrations of around mg/kg of grapes, which represent about 85% of total carotenoids, the remaining carotenoids such as xanthophylls they have been found with concentrations around the $\mu\text{g}/\text{Kg}$; among the carotenoids that show the greatest interest in the formation of aromatic compounds are β -carotene and neoxanthin (Mendes-Pinto, 2009).

Carotenoids play a fundamental function in photosynthesis; avoid the excessive irradiation of chlorophyll, absorbing the irradiation at 400-500 nm (Hashimoto *et al.*, 2016).

During the maturation of the berry, the concentration of carotenoids tends to decrease and at the same time increases the concentration of norisoprenoids, due to the degradation of carotenoids and the formation of the new molecules, the norisoprenoids (Ribereau-Gayon *et al.*, 2006a).

Norisoprenoids can be divided into:

- **megastigmans** including the family of ionones and damascenone with oxygen in different positions, e.g. with a keto group in C (9) as in β -ionone or in C (7) as in β -damascenone;
- **not megastigmans**, compounds with megastigma structure but without oxygen in the side chain (Enzell, 1985; Mendes-Pinto, 2009).

There are numerous norisoprenoids that are found in both white and red wines with different concentrations. Table 2 shows the values referring to white wine and the most important compounds are listed below: **TCH** (2,2,6-trimethylcyclohexanone), **β -damascenone** [(2,2,6-trimethyl-1,3-cyclohexadien-1-yl) -2-buten-1-one], **β -ionone** [(2,2,6-trimethyl-1,3-cyclohexen-1-yl) -3-buten-2-one], **vitispirane** (2,10,10-trimethyl-6-methylene-1-oxaspiro [4.5] dec-7 -ene), actinidiol, **TDN** (1,1,6-trimethyl-1,2-dihydronaphthalene) and **riesling acetal** (2,2,6,8-tetramethyl-7,11-dioxatricyclo [6.2.1.0^{1,6}] undec- 4-ene). The only norisoprenoid of oenological interest with 9 carbon atoms is the TCH, with a " rock-rose-like "descriptor (*Cistus ladaniferus*), and it is found only in Port wines, occurring at 50-400 ng/L (Mendes-Pinto, 2009).

Table 2 - Concentrations, odour quality and thresholds of odour-active norisoprenoids compounds found in white wine (adapted Francis and Newton, 2005 and Saeks et al., 2012).

	Odour quality	Concentration white wine($\mu\text{g/L}$)	Odour threshold ($\mu\text{g/L}$)
β-Damascenone	apple, rose, honey	nd–9.4	0.05
β-Ionone	seaweed, violet, flower, raspberry	0.11	0.09
TDN	petrol	1.3 – 17.1	1-3

1.2.1.3. Methoxypyrazines

Methoxypyrazines are primary aromatic forms and they are a class of chemical compounds characterized by a nitrogenated heterocycle ring, a carbon 2 bond with an oxygen, and carbon 3 have groups: isopropyl, isobutyl (2-methoxy-3-isobutylpyrazine or 2-isobutyl-3-methoxypyrazine), and butyl which are the aromatic part (Ribéreau-Gayon *et al.*, 2006a).

The origin of these compounds is not yet completely clear, and they are present both in the free state and in a small part in the combined state (normally they are present in free form). They give a vegetal scent (pepper, leaf, asparagus) and they have low thresholds of perception (a few ng/L). In the wine it was found very few mg/L, characterizing some cultivars such as Cabernet, Merlot, Sauvignon blanc (Bayonove *et al.*, 1998, Ribéreau-Gayon *et al.*, 2006a)

Herbal smell was attributed to methoxypyrazine with extremely low sensory detection threshold in water of about 2 ng/L (Czerny *et al.*, 2008).

The detection of methoxypyrazines is extremely difficult due to the very low concentrations in both grapes and wine. Methoxypyrazines are found mainly in the solid parts of the berry in different concentrations. The peel has a higher percentage than in the pulp, with 63% of the total and in the pulp is only 1% of the total (De Boubée *et al.*, 2002).

Table 3 - Concentrations, odour quality and thresholds of odour-active methoxypyrazine compounds found in white wine (adapted Francis and Newton, 2005 and Lacey et al., 1991)

	Odour quality	Concentration white wine(ng/L)	Odour threshold ($\mu\text{g/L}$)
3-Isobutyl-2-methoxypyrazine	earth, spice, green pepper	0,6 - 38,1	0.002

In grapes, their concentration decreases during the ripening phase, especially in hot climates, reaching low concentrations, 0.2 - 0.3 ng/L; this value remains stable even during over-maturation (De Boubée *et al.*, 2002; Shidu *et al.*, 2015; Pons *et al.*, 2017). In white wines

normally, these compounds are not present or in any case have very low concentrations as shown in table 3, with concentrations between 0,6 and 38 ng/L (Lacey *et al.*, 1991).

The concentration of methoxypyrazines is influenced by numerous factors such as: the *exposure* to the light of the bunches, in pre-veraison, reduces the accumulation from 21 to 44% during the vegetative development; also, a removal of the pre-veraison leaves favours a decrease of the herbaceous notes (Allen *et al.*, 1988; Ryona *et al.*, 2008).

Crop yield: increase the herbaceous hints increase when increase the crop yield, on the contrary with a decrease there is an increase in fruity scents (Chapman *et al.*, 2004).

Temperature: influences the concentration of methoxypyrazine; in cold regions there are higher concentrations than the warmer ones (Kotseridis *et al.*, 1999).

Ripening degree of grapes: influences the final concentration; with a good maturation there are low concentrations, remaining constant subsequently (Hashizume and Umeda, 1996, Kotseridis *et al.*, 1999).

Depth of soil: associated with a high content of nutrients and water, there are concentrations of methoxypyrazine, with an increase in herbaceous hints (Kotseridis *et al.*, 1999).

-Ladybug (Harmonia Axyridis): it is an insect that develops during the harvest, infesting the bunches; these insects can be found in the maceration phase, releasing high percentages of methoxypyrazines (200-400 coccinellids/t leads to serious defects) (Sidhu *et al.*, 2014). It reduces the risk by clarifying the must before vinification or by cooling the mass without maceration (grapes in the cell), promote the death of the insect that about three days after its death does not cause defects in the wine. Clarify in addition to the effect on turbidity has a diminutive effect on the final concentration of methoxypyrazine (Sidhu *et al.*, 2014).

Ractions with other compounds: the methoxypyrazines can react with phenolic substances, making them less volatile and therefore less perceptible (Sidhu *et al.*, 2014); in other to, the methoxypyrazines, hydrophobic molecules, are released very quickly even with crushing and pressing (2-4%); after about 3 days of maceration, about 80% is released; (Lacey *et al.*, 1991).

Technology: - the *thermovinification* due to the high temperatures, promote a degradation of the methoxypyrazines, however the thermovinification could cause other types of defects, hints of cooked due to the caramelization of residual sugars; increases the likelihood of reduced hints (Sidhu *et al.*, 2014).

- *Plastic material*, in contact with wine such as caps and tetra pack packs, absorb approximately 50% of these compounds.
- Leaf and other green tissues present during maceration.

1.2.1.4. Thiols

Thiol compounds, the last class of aromas discovered around 90 owing present perception thresholds extremely low, of a few ng/L (0.8 - 50) and sometimes close to or above these thresholds as seen from the table 4 (Ribereau-Gayon *et al.*, 2006a; Roland *et al.*, 2011).

The thiols are involved in the aromatic character of different fruits such as grapefruit (Demole *et al.*, 1982), blackcurrant (Rigaud *et al.*, 1986), passion fruit (Engel and Tressel, 1991) and guava (Idstein and Schreier, 1985; Bassols and Demole, 1994) with characteristic herbaceous, fruity and empyreumatic nuances; aromas reminiscent of green pepper, boxwood, broom, eucalyptus, blackcurrant buds, rhubarb, tomato leaves, nettles, cat urine, grapefruit, passion fruit, white peaches, gooseberries and asparagus broth, as well as acacia wood and flowers. These compounds, in wines, can exceed a concentration of 100 mg/L, namely in the wines of Sauvignon Blanc, but this concentration tends to decrease during the aging of the wine, especially if it is in oxidative conditions, due for example to reactions of addition with o-quinones present in the wine (Ribereau-Gayon *et al.*, 2006a, Ugliano, 2013, Coetzee *et al.*, 2016).

These compounds were found in the 1990s in Sauvignon and later also found in Gewürztraminer, Riesling, Pinot Blanc, Semillon, Cabernet-Sauvignon, Merlot (Tominaga and Dubourdieu, 2000).

The thiol compounds are found in the grapes conjugated to the cysteine-S, which due to the enzymatic action can be released during the vinification. They are oxygenated, aliphatic and small compounds, hence the low reactivity except for those having the thiol group -SH.

Among the most important thiols compounds there are (table 4) (Tominaga *et al.*, 1996;1998):

- **3-mercaptohexanol (3MH)**; with aromas of passion fruit and grapefruit, it is stable over time;
- **3-mercaptohexanol acetate (A3MH)** resulting from an esterification of acetyl by S. cerevisiae; with aromas of passion fruit and boxwood, owing to the acetyl group is enriched by hints of boxwood; this compound turns out to be unstable, since it has hydrolysis of the ester, both at low pH values and at high temperatures, and goes to form the 3MH.
- **4-mercapto-4methyl-2pentane (4MMPOH)** with a 5 C chain where on carbon 4 there is a thiol and a methyl and in position 2 a ketone; with hints of boxwood and floral

These forms are not found free in the middle, but we find them linked with the glutathione and/or with the cysteine (S-3-(esan-1-olo)-L-cisteina, S-3-(esan-1-olo)-glutathione, through the oxygenated hydrocarbon with sulphur; the tri-peptide (glutathione) is composed of glutamic

acid and cysteine and glycine and sulphur acts as a bridge between the molecules; the sulphide bridge makes the molecule non-volatile but much more stable (Darriet, 1993; Tominaga *et al.*, 1996, 1998; Winter *et al.*, 2011).

Table 4 Concentrations, odour quality and thresholds of odour-active sulfur compounds found in white wine (adapted Francis and Newton, 2005)

	Odour quality	Concentration white wine (µg/L)	Odour threshold (µg/L)
3-Mercaptohexyl acetate	box tree (grapefruit, passionfruit, cat urine)	0.12 – 1.3	0.004
4-Mercaptomethyl pentan-2-one	box tree (passionfruit, cat urine)	<0.08 – 0.4	0.0008
3-Mercaptohexanol	sulfur (passionfruit, cat urine)	0.11 – 2.6	0.06

The liberation and release of free forms occurs due to the Liasic activity of *S. Cerevisiae* (at the cytoplasmic level), which separates the sulphur from the thiol free the aromatic molecule, consequently the molecule turns out to be perceptible (Winter *et al.*, 2011).

The synthesis of thiol precursors is influenced by various factors such as: climate and nitrogen fertilization (of peptide origin). If the plant is in a state of nitrogen stress and water stress during the spring growth phase and during maturation, the production of the precursor Glutathione is stimulated (Peyrot des Ganchos *et al.*, 2005; Choné *et al.*, 2006; Pons *et al.*, 2017).

1.2.2. Pre-fermentative aromas

The pre-fermentative aromas develop during the phases prior to fermentation, during the phases of harvest, collection, transport, storage, mashing, pressing and maceration as result of enzymatic activities (Coordonnier and Bayonove, 1981).

The compounds produced are hexanal, Z-3-hexenals, E-2-hexenals and their corresponding alcohols (Rapp *et al.*, 1976; Schreier *et al.*, 1976; Moio *et al.*, 2004). These compounds derive from four fundamental enzymatic activities:

- *acylhydrolase*, which releases fatty acids from the membrane's lipids;
- *lipxygenase*, which catalyzes the fixation of oxygen on unsaturated fatty acids C18;
- *hydro peroxidase lyase* which splits the peroxides obtained in aldehydes;
- *alcohol dehydrogenase*, which reduces part of the aldehydes in alcohol (Crouzet, 1986).

The alcohols released, present herbaceous aromas on the wine, as shown in table 5 and for this reason in the past they were attributed to the leaves that were collected by mechanical harvesting (Joslin and Ough, 1978; Ramey *et al.*, 1986).

Table 5 - Concentrations, odour quality and thresholds of odour-active alcohols C6 compounds found in white wine (adapted Francis and Newton, 2005)

	Odour quality	Concentration white wines(µg/L)	Odour threshold (µg/L)
1-Hexanol	resin, flower, green (cut grass)	–	8000
(Z)-3-Hexenol	green (cut grass)	606	400

In addition to these main chemical families it has been shown that other families may be present as varietal aromas such as (Oliveira *et al.*, 2000):

- aromatic alcohols
- linear alcohols
- volatile phenols
- phenol aldehydes
- fatty acids

1.2.3. Fermentative aromas

In young wines, the sensory profile is influenced more by secondary aromas developed during alcoholic fermentation and to a lesser extent during malolactic fermentation.

The secondary compounds that develop during fermentation starting from two precursors such as glucides and amino acids, are esters, alcohols, volatile acids and volatile phenols (Ribereau-Gayon *et al.*, 2006a).

During the fermentation, numerous reactions take place by micro-organisms, which during this phase, change the aromatic profile, owing to their metabolism. 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, are able to develop new compounds and through the selection of different strains, the oenologist is able to differentiate the final product and is able to develop a wine that may be able to develop new market needs (Molina *et al.*, 2009; Vilanova *et al.*, 2012).

1.2.3.1. Higher Alcohols

The composition of nitrogen and its concentration in the must, in addition to other variables such as temperature, pH, sugar concentration, vitamins and oxygen, influence the growth of yeast during fermentation, thus also influencing the composition of volatile compounds in wine (Ribereau-Gayon *et al.*, 2006a, b).

The higher alcohols are one of the aromatic classes among the most present in wine and has a strong impact on the sensory profile; the concentration of higher alcohols varies from about 70 g/L to 300 g/L (Bell and Henscke, 2005).

According to the authors Rapp and Versini (1991), if the concentration of these compounds remains below 300 mg/L, they contribute positively to the sensory profile.

The main alcohols present in wine are isoamyl alcohol, isobutanol, 2-phenylethanol and methionol.

The concentrations of these compounds, as shown in the table 6, are also found in minority varieties like Monastel and Maturana Tinta de Navarrete (Spain), found from a few µg/L to 350 mg/L (Martinez-Pinilla *et al.*, 2013).

However, only isobutyl alcohol, isoamyl alcohol, 2-phenylethanol and methionol seem to contribute to the aroma of wine (Ferreira *et al.*, 2000; Czerny *et al.*, 2008).

Table 6 - Concentrations, odour quality and thresholds of odours-active higher alcohols compounds found in white wine (adapted Francis and Newton, 2005).

	Odour quality	Concentration white wines (mg/L)	Odour threshold (mg/L)
<i>Isobutanol</i>	wine, solvent, bitter	32–108	40,0
<i>Isoamyl alcohol</i>	whiskey, malt, burnt	109–356	30,0
<i>2-Phenylethyl alcohol</i>	honey, spice, rose, lilac	14 –87	14 -10
<i>Methionol</i>	sweet, potato	0,57–12,6	1,0

There is a greater proportion of higher alcohols, which derive from sugars, when amino acids are absent, and ammonium is the only source of nitrogen, despite the higher alcohols being synthesized in smaller concentrations (Bell and Henscke, 2005). In the absence of alpha-amino acid nitrogen from the transamination, excess of keto-oxides is used, produced from the biosynthetic pathway of the sugars and reduced to alcohols. Otherwise, with enough concentrations of amino acids in the must-wine, a higher concentration of higher alcohols is formed, via the Ehrlich route (Bell and Henscke, 2005).

1.2.3.2. Esters

The esters are important in the food industry especially in the production of beer, wine and sake, as most of them give the product fruity aromas pleasing to the consumer.

For this reason, it is important to know the various biosynthetic ways to regulate their production; recently, different genes and different pathways responsible of their synthesis have been identified (Molina *et al.*, 2009; Vilanova *et al.*, 2012).

The concentrations of esters in wine are close to the thresholds of perception and vary according to different factors such as concentration of the two co-substrates (ethanol and acyl-A) and enzymatic activity (Saerens *et al.*, 2008).

In fermented products two main categories of esters can be distinguished as shown in the table 7: acetate esters and ethyl esters. The acetate ester that mostly influences white wines is *isoamyl acetate* with hints of banana; but other acetate esters are present as *ethyl acetate* (aroma like the solvent), *isobutyl acetate* (fruity aroma) and *phenyl ethyl acetate* (roses, honey). This group has been studied extensively in the past for high concentrations and ease of measurement (Saerens *et al.*, 2008).

Furthermore, during fermentation, *Saccharomyces cerevisiae* releases large amounts of fatty acids with medium chain (MCFA) and their relative ethyl esters, which are very important for the sensory profile; the main ones are: *ethylanoan* (anise, flavored apple) and *ethyl octanoate* (sour apple flavor) (Bardi *et al.*, 1998; Saerens *et al.*, 2008; Molina *et al.*, 2009; Vilanova *et al.*, 2012).

The reaction between alcoholic groups and carboxylic groups leads to the formation of esters. This reaction can be mediated by enzymes like lipase and esterase, produced by microorganisms or these reactions can be of a chemical nature occurring during wine aging due to the complex nature of wine, thanks to hydrolysis, esterification and oxidation phenomena (Ramey and Ough, 1980).

The formation of esters occurs during alcoholic fermentation but also during malolactic fermentation, for example with the production of ethyl 2-hydroxypropanate which is coupled to the formation of lactic acid. The formation of these compounds may be related to the malic acid dehydration (De Revel *et al.*, 1999; Sumbly *et al.*, 2013).

Table 7 - Concentration, odour quality and thresholds of odour-active esters compounds found in white wine (adapted Francis and Newton, 2005)

	Odour quality	Concentration white wines (µg/L)	Odour threshold (µg/L)
Ethyl esters			
<i>Ethyl isobutyrate</i>	sweet, rubber	33–480	15
<i>Ethyl 2-methylbutyrate</i>	apple	3.6–4.5	18, 16
<i>Ethyl isovalerate</i>	fruit	3–20	3
<i>Ethyl butyrate</i>	apple	184–700	206
<i>Ethyl hexanoate</i>	apple peel, fruit	280–1022	14, 5
<i>Ethyl octanoate</i>	fruit, fat	2706–8208	5, 2
<i>Ethyl decanoate</i>	grape	423	200
Acetates			
<i>Isoamyl acetate</i>	Banana	163–4740	30
<i>Phenylethyl acetate</i>	rose, honey, tobacco	89–475	250
<i>Ethyl acetate</i>	pineapple	22500–208000	12264
Cinnamic esters			
<i>Ethyl dihydro cinnamate</i>	flower	–	1.6
<i>trans-Ethyl cinnamate</i>	honey, cinnamon	0.1–3.1	1.1

1.2.3.3. Volatile fatty acids

Volatile fatty acids are normally present in wine, and the most important of these is acetic acid, the main component of volatile acidity.

Acetic acid is normally produced during alcoholic fermentation and during malolactic fermentation, but its concentration is limited to the legislative level, as it is a sign of deterioration of the wine. In addition to acetic acid in wine, other C3 acids can be found, such as propionic

acid and C4 such as butyric acid, which indicate a microbiological (bacterial) contamination (Ribereau-Gayon *et al.*, 2006a,b).

The synthesis of fatty acids in wine occurs from lipid anabolism, from the oxidative decarboxylation of alpha-keto acids and from the oxidation of aldehydes (Vilanova and Oliveira, 2011).

The main volatile fatty acids synthesized by alpha-ketoacids are propanoic acid, isobutyric acid, 2-methyl-1-butanoic acid, 3-methyl-1-butanoic acid (isovaleric acid, 3-methylbutyric acid) and phenylacetic acid (Vilanova and Oliveira, 2011).

Since lipid metabolism are synthesized: butanoic acid (butyric acid), hexanoic acid (caproic acid), octanoic acid (caprylic) and decanoic acid (capric) (Dubois, 1994).

The medium chain fatty acids (C6-C8-C10) produced by yeasts in wine, can be found in a concentration ranging from a few mg/L and already some mg/L and can cause fermentation stops, influencing membrane permeability cellular and hindering exchanges between the interior of the cell and the fermentation medium. In contrast, long-chain unsaturated fatty acids (C18, C20) are fermentation activators, especially under anaerobic conditions. Among these compounds the most important are oleic and linoleic acids (Ribereau-Gayon *et al.*, 2000a,b; Vilanova *et al.*, 2012).

The main volatile fatty acids are shown in the table 8 with concentrations, thresholds of perception and their own descriptors described by Francis and Newton (2005) in their work.

Table 8 - Concentration, odour quality and thresholds of odour-active acid compounds found in white wine (adapted Francis and Newton, 2005)

	Odour quality	Concentration white wine (mg/L)	Odour threshold (mg/L)
<i>Isobutyric acid</i>	rancid, butter, cheese	2,53	2,30
<i>Isovaleric acid</i>	sweat, acid, rancid	0,50–0,58	0,03
<i>Acetic acid</i>	sour (pungent, vinegar)	30–489,37	200
<i>Butyric acid</i>	rancid, cheese, sweat	1,34–3,97	0,17
<i>Propanoic acid</i>	pungent, rancid, soy	34,83–113,40	8,10
<i>Hexanoic acid</i>	sweat	2,47–9,24	0,42
<i>Octanoic acid</i>	sweat, cheese	4,98–130008	0,50
<i>Decanoic acid</i>	rancid, fat	0,73–2,10	1,00

1.2.4. Post-fermentative aromas

In this work only, aromas derived from reduced conditions have been considered, however for fine red wines and for some types of white wines, there are conditions of aging in oxidation. Under oxidation conditions the wine has a different evolution due to the numerous reactions, mediated by oxygen, leading to the formation of carbonyl compounds, such as ketones and aldehydes. Amongst these latter the major compounds (for their concentration) are acetaldehyde, acetic acid and ethyl acetate, produced starting from ethanol through oxidation and esterification reactions. (Ribereau-Gayon *et al.*, 2000a,b).

In addition to acetaldehyde, in wine, there are other trace aldehydes, which contribute to the sensorial profile of the wine.

Maturation and aging are the period between the end of fermentation and bottling; this period is variable, as it depends on the objective to be achieved but above all on the quality of the wine.

During the maturation phase, even in conditions of reduction (absence of oxygen), the aromatic profile changes, due to different chemical reactions, related to the composition of the wine, pH, temperature and time (Marais and Pool, 1980; Usseglio-Tomasset, 1983).

Volatile compounds (higher alcohols, esters, fatty acids ...) during this period tend to change composition and concentration (Vilanova and Oliveira, 2011).

During storage, most of the ethyl esters tend to increase, unlike the ethyl esters of fatty acids which tend to decrease due to hydrolysis.

These variations are accelerated by high temperatures and low pH and depend on the balance between the esters and the corresponding acids, (Diaz-Maroto *et al.*, 2005).

During the maturation/aging period of the wine there is also a modification of the terpenic profile, studied by the research group of R. di Stefano. In this study the disappearance or strong decline of the compounds initially present was highlighted, with a simultaneous formation of other terpenic compounds with a higher oxidation state due to temperature and pH (Di Stefano and Castino, 1983; Marais *et al.*, 1992).

During this period, thanks to the action of wine acidity, the aromatic precursors are released, splitting the glycosidic link between the aromatic precursor and the sugars (Diaz-Maroto *et al.*, 2005).

2. *Aim of work*

The purpose of this work is the characterization of five minority vines of Portugal. The study focuses on the volatile composition and sensory profile of the five mono varietal wines using GC-FID, GC-MS and a tasting panel.

The study was conducted on Galego Dourado, Malvasia (de Colares), Boal Espinho, Verdelho and Moscatel Nunes.

In 2017 all five varieties were studied and in 2018 only Malvasia (de Colares), Verdelho and Galego Dourado were studied.

3. **Materials and methods**

3.1. **Vineyard and wine experiment**

The thesis project focused on the study of five wines produced by minority varieties of Portugal, coming from the National Ampelographic Collection of Portugal (CAN), located in Dois Portos.

In particular way, the vineyard is located on the plain, with a 2.3 m x 1 m training system in the counter and with a spurred cordon system.

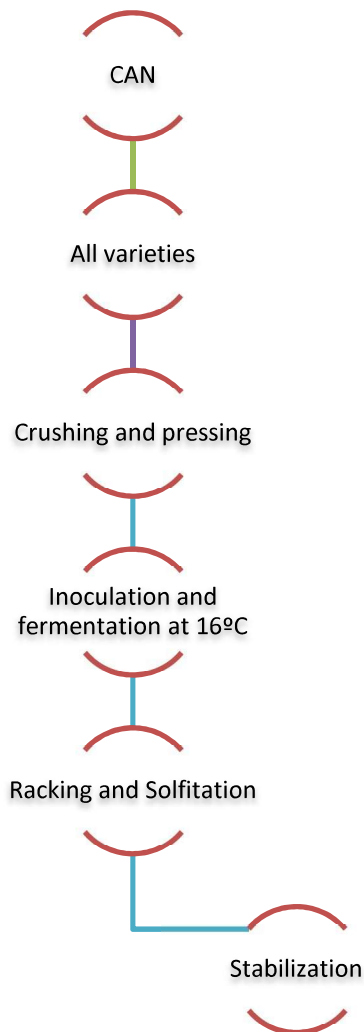


Figure 1 - Flowchart of vinification for both vintages

For each variety only one replica was produced due to the low productivity of the vineyard of the national ampelographic center; the three wines produced from three different varieties Malvasia, Verdelho and Galego Dourado, comparing two different vintages (2017 and 2018); furthermore, in 2017 it was possible to study two other varieties such as Moscatel Nunes and Boal Espinho. The wines were produced according to the scheme of figure 1.

The grapes were pressed and destemmed with the addition of 15 g/hl of a 70% solution of potassium metabisulfite and 30% of ascorbic acid (Oxyless, produced by Proenol). After pressing, the must was clarified at 4 °C for about 48 hours, inside a small stainless- steel tank (50 L).

Then they must be transferred to

another similar and later inoculated tank you, with selected yeasts from the Lalvin company (QA23, 25 g/hl).

The fermentation was carried out at a controlled temperature, around 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) were added.

In December, after a transfer, about 9 g/hl of Oxyless was added and then in March the wine

was cold stabilized, with a temperature of about 4°C for about two weeks.

In the period of March, the wines were bottled without the use of filters. Three samples of each wine variety were taken for chemical and sensory analysis.

All the wines were obtained according to the flow chart of figure 1, with the exception of the Moscatel Nunes, which were divided into two equal parts before fermentation. One part was added with tartaric acid at a dose of 1,5 g/L to increase acidity and the other was followed with any treatment to see the enological differences between the two wines. The wine samples will be identified as shown in the table 9.

Table 9 - Sample identification code table

Vintage	Verdelho	Malvasia	Galego Dourado	Moscatel Nunes	Boal Espinho
2017	Verdelho17	Malvasia17	Galego17	Moscatel_A_17 Moscatel_B_17*	Boal17
2018	Verdelho18	Malvasia18	Galego18	-	-

Table 9* the Moscatel_B_17 was acidified with tartaric acid

3.2. Climatic condition on 2017 and 2018

The study of only two years cannot lead to a study on the effect of climatic conditions and its influence on volatile compounds in wine; however, based on the data of the climate data provided by the INIAV viticulture section, it can be deduced, that the two years are very different.

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 month between February and April for then occur between October and November and with a total rainfall of about 650 mm.

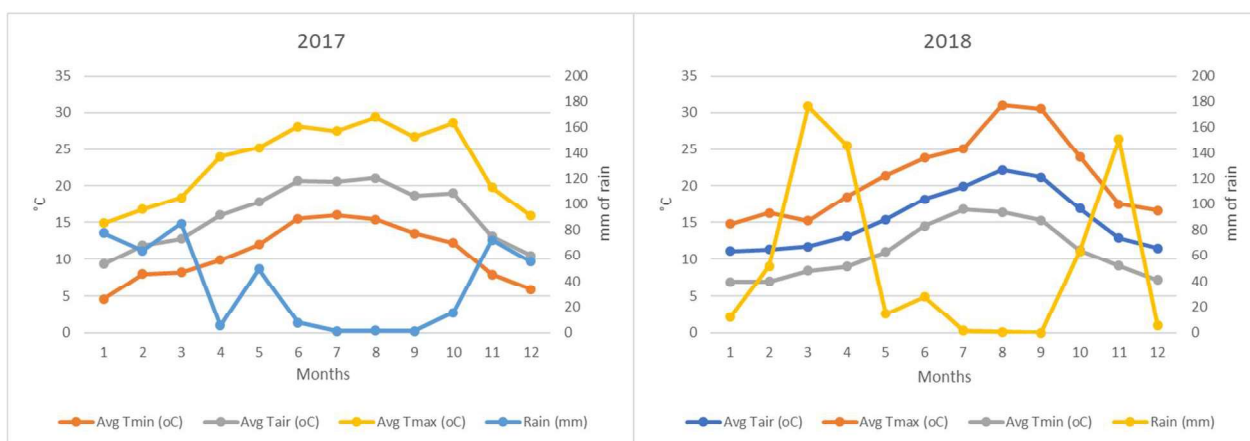


Figure 2 - Temperature and rainfall 2017-2018 - Avg Tair: average air temperature; Avg Tmax: average maximum temperature; Avg Tmin: average minimum temperature; Rain: mm of rain

It is interesting, as can be seen from the graph (figure 2) that in the period of maturation of the grapes between July and September in the year 2017 it was cooler and rainier than the year 2018.

3.3. Musts and wine analysis

Monovarietal white wines were analyzed using chemical analyzes as indicated in table 10; before fermentation the must was analyzed and before the sensory analysis the finished wine was analyzed.

The musts were analyzed by pH, total acidity, Brix and the table showed the values of sugars, Potential alcoholic strength by volume and bulk volume.

For the wines the following chemical analyzes were performed: pH, total acidity, volatile acidity, density, free SO₂, total SO₂ and reducing sugars.

All the analyzes were performed in the central INIAV laboratory (Dois-Portos), following the protocols recommended by the OIV as shown in the table and the internal protocol for reducing sugars.

Table 10 - Control Parameters and their OIV references or method.

Musts And Wines	Brix-sugar content	Total Acidity	Volatile Acidity	SO ₂ Free	SO ₂ Total	Density	Alcoholic strength	Reducing substances	pH
Method	OIV-MA-AS2-02-R2012	OIV-MA-AS313-01:2015	OIV-MA-AS313-02:2014	OIV-MA-AS323-04B:2014	OIV-MA-AS323-04B:2014	OIV-MA-AS2-01B:2014	OIV-MA-AS312-01B:2014	Internal method EVN	OIV-MA-AS-313-15

3.4. Volatile composition of the wines

3.4.1. Extraction of free volatile compounds

The extraction of volatiles followed the method proposed by Cocito et al. (1995) the conditions described by Botelho (2008). 50 ml of each wine was added of 400 microliters of 2-octanol (IS, 81.9 mg. L⁻¹, 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 and then concentrated at 0.30 ml. (Fig. 2).

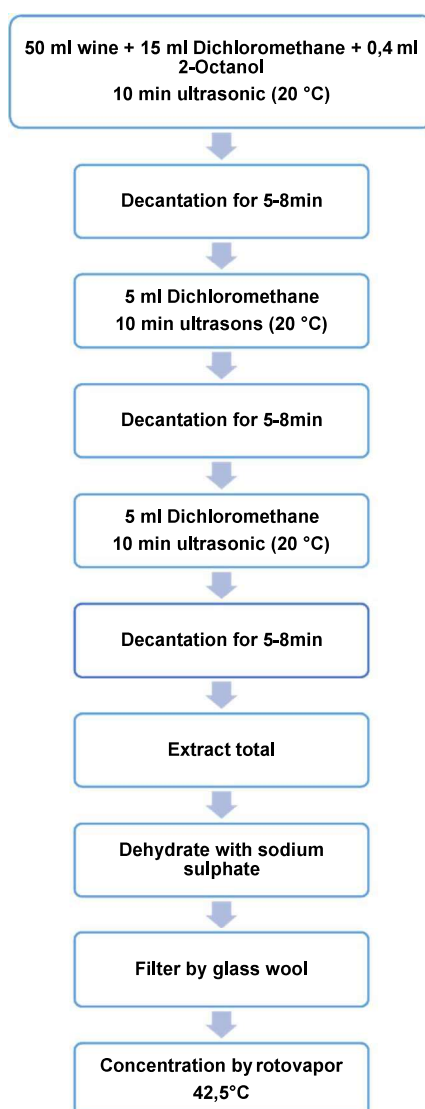


Figure 3 - Flowchart of extraction and concentration of volatiles

The extraction of the compounds was carried out in duplicate and each extract was then stored at -20 ° C until the analysis performed by high-resolution gas-liquid chromatography coupled

with a flame ionization detector and up to high-resolution gas-liquid chromatography coupled with mass spectrometer.

3.4.2. Reagents

The dichloromethane and the anhydrous sodium sulphate, both analytical, was purchased by Merck (Darmstadt, Germany). The dichloromethane was bidistilled.

3.4.3. Quantification and analysis of volatile compounds of GC-FID

The obtained extracts were analyzed by high-resolution gas-liquid chromatography and each extract was injected 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 capillary column of polyethylene glycol silica (INNOWax, J & W Scientific Technologies, Agilent, USA-30m x 0.32mm x 0.25mm). The carrier gas was used in hydrogen (2.4 cm³.min⁻¹) and the division ratio will be 1: 3. The volume of the injected sample is between 0.5 and 1.0 µl.

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 carried out following the internal standard quantification method. Therefore, 2-octanol was chosen as an internal standard (0.4 ml solution in ethanol of this internal standard was added to each sample)

3.4.4. 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 is equipped with a polyethylene glycol silica capillary column (INNOWax from J & W: 30 m x 0.25 mm x 0.25 µm). Conditions of analysis: injector and transfer line at 250 ° C; helium gas (12psi of internal pressure and division ratio of 1:60), 0.2-0.4µl of injection volume. The mass spectrometer works 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 that for GC-FID.

3.5. 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; moreover, during the test tulip glasses were used as required by the ISO 3591 standard, with a volume of wine per sample of approximately 50 ml.

The descriptive sensory analysis of wines was carried out by a jury composed of judges within the INIAV, adequately trained.

The evaluation of the wines was carried out on the color, smell and taste and the tasters were asked to evaluate the intensity of the attributes, with a structured scale from 0 to 10.

The evaluation form was made starting from the work of Otello *et al.* (2007) and the descriptors used were chosen starting from the works of Vilanova *et al.* (2008, 2013).

The card is composed of three aspects: attributes on color, aroma and taste aspect.

Intensity, yellow reflections, green reflections and clarity were evaluated for the color.

For the aroma attributes were selected intensity, floral, white fruit, nuts, tropical fruits, citric, herbaceous, terpenic-muscat and persistence.

For the section concerning the gustatory part, sweet, sour, bitter, softness, balance, alcohol and body were selected.

3.6. Statistical analysis

The statistical analysis was performed with the *Statistica* software (Version 0.7; 84-04), the main statistical analysis used for this study was the PCA analysis (Principal Components Analysis).

Multivariate analysis is one of the most widely used statistical analyses in the scientific field, as it allows us to analyse a data matrix, described by a series of dependent variables (Abdi and Williams, 2010).

The goal of principal component analysis (PCA) is that of extracting information from the data matrix and the result is given by a set of orthogonal variables called main components.

The PCA analysis also represents the model of similarity and not of the observations and variables, showing them as points in the maps.

The cluster analysis is part of the multivariate analysis, it allows to group homogeneous elements in a data set and allows to see the similarity of the groups or better the dissimilarity given by the multidimensional distance.

In this work, hierarchical clustering was used, in which a hierarchy of partitions is built, characterized by an increasing or decreasing number of groups, through a tree representation (dendrogram).

4. Results and discussion

4.1. Chemical analysis

The chemical composition of the must from the different varieties and from the two vintages are presented in the table 11 and in the table 12, are the results of chemical composition of corresponding wines.

Analyzing the results of the musts, in the table, all the samples showed a good sugar content for both years, with concentrations ranging between 190 g/dm³ and 240 g/dm³ and with an excellent potential alcoholic strength by variety white berry; in the year 2017 only the Verdelho and the Moscatel Nunes, showed higher values, with the potential alcoholic strength by volume around 14% v/v.

Table 11 - Chemical composition of must from the two vintages (2017 and 2018).

Sample-must	Vintage	Brix (%m/m)	Sugar* (g/dm³)	Density* (g/cm³)	TAVP* (% v/v)	pH	Ac.Total (Tartaric Acid g/dm³)
Galego Dourado	2017	21,6	210,9	1,092	12,4	3,30	6,4
Galego Dourado	2018	21,8	212,4	1,089	12,6	3,13	6,8
Malvasia	2017	20,6	199,6	1,087	11,7	3,01	7,4
Malvasia	2018	20,5	197,6	1,084	11,7	3,21	7,5
Verdelho	2017	24	238,2	1.103	14	3,21	6,7
Verdelho	2018	21,6	210,1	1,089	12,4	3,06	6,4
Boal Espinho	2017	20,4	197,4	1,086	11,6	3,01	6,5
Moscatel Nunes	2017	22,2	217,7	1,094	12,8	3,45	4,0

*Table 11 - The sugar content (g/dm³), density and potential alcoholic strength were calculated from brix results using a conversion table. TAVP- Potential alcohol strength.

The concentration of total acids in these varieties is very interesting, showing values between 6.5 and 7.5, except for the Moscatel variety in the 2017 vintage with a total acidity of 4 g/dm³ expressed as tartaric acid.

Analyzing the results of the wine analyzes, obtained before the sensory analysis session, it can be confirmed that the wines in 2017 have a slightly higher residual sugar content than the

2018 vintage, this can be due to slow fermentations, due to slow and difficult fermentations, which can be given by high sugar content, high temperatures, low nitrogen content for microbial development; however, thanks to optimal pH values, the risk of microbial contamination is low.

As can be seen from the table 12, the wines of 2017 have a higher alcohol content from the next year, but also supported by optimal acidity values; only the Moscatel_B_17 sample underwent acidification, bringing pH values from 3.47 to 3.28 and with a final acidity of 5.2 expressed as tartaric acid.

Table 12 - Chemical composition of wines of different varieties from the two vintages (2017 and 2018)

Wine samples	Vintage	TAV (%v/v)	Tot. Ac. (g/dm³ Tart ac.)	Vol. Ac. (g/dm³ Acetic acid)	pH	SO₂ free (mg/dm³)	Reducing substance (g/dm³) (FTIR)
<i>Galego D.</i>	2017	13,7	4,2	0,66	3,49	13	1,8
<i>Galego D.</i>	2018	13,3	6,0	0,28	3,37	38	1,5
<i>Malvasia</i>	2017	12,9	5,7	0,48	3,13	13	2,8
<i>Malvasia</i>	2018	12,7	7,9	0,23	3,03	41	1,4
<i>Verdelho</i>	2017	15,1	6,1	0,29	3,20	22	6,9
<i>Verdelho</i>	2018	13,3	7,8	0,40	3,09	34	1,0
<i>Boal E.</i>	2017	12,6	5,7	0,66	3,07	26	4,4
<i>Moscatel_A</i>	2017	14,0	4,5	0,45	3,47	10	4,0
<i>Moscatel_B</i>	2017	13,9	5,2	0,60	3,28	10	3,6

4.1. Sensory Analysis

The wine tasting was done with a panel of expert tasters as described in the chap. 3.5; the results were analyzed by PCA, HCA analysis and radar (figure 4-5-6) were also produced.

The wines were presented to the tasters anonymously, with a 3-digit identification code for each variety, following a presentation in a "latin framework".

From the results obtained during the wine tests, radar graphics were produced, using the averages of the values for each single variable; analyzing both vintages, the wines showed different characteristics, given the vintage effect as can be seen from figures 4-5.

Malvasia, in the 2017 vintage, is characterized by a good intensity of color, with light floral and white fruit notes; notes of dried fruit prevail, with good aromatic persistence and harmony.

In the 2018 vintage, Malvasia has a different sensory profile with a prevalence of white fruit notes, light notes of dried fruit and citric aromas; there is also an increase in the aromatic

persistence, structure and acidity of the wine.

The 2017 Galego sample is characterized by a good intensity of color and a good aromatic intensity, with light floral notes, white fruit, dried fruit and citric aromas with a prevalence of herbal notes; moreover the wine is characterized by a good harmony and aromatic persistence.

The Galego produced in the 2018 vintage is characterized by an increase in intensity and aromatic persistence, with notes of white fruit, dried fruit and with a prevalence of tropical fruit notes; also the galego in this vintage is characterized by an increase in acidity and harmony.

In 2017, during the sensory analysis, Verdelho showed an excellent aromatic intensity and persistence, with light floral notes and strong notes of tropical fruit; moreover, compared to the other varieties it showed a greater structure and harmony.

In 2018, the Verdelho sample showed a good aromatic intensity, with light floral notes, dried fruit and predominantly white fruit notes; moreover, it showed greater acidity and harmony than the previous year.

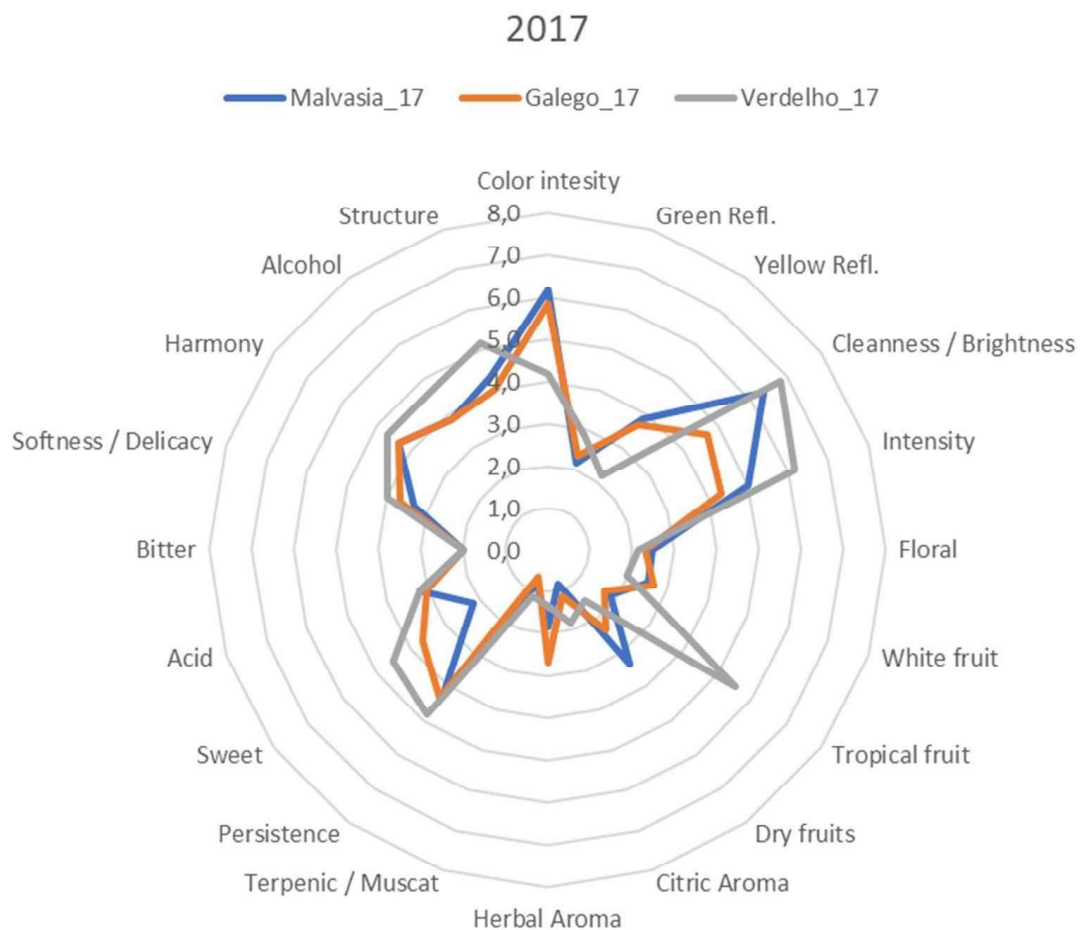


Figure 4 - Sensory analysis 2017

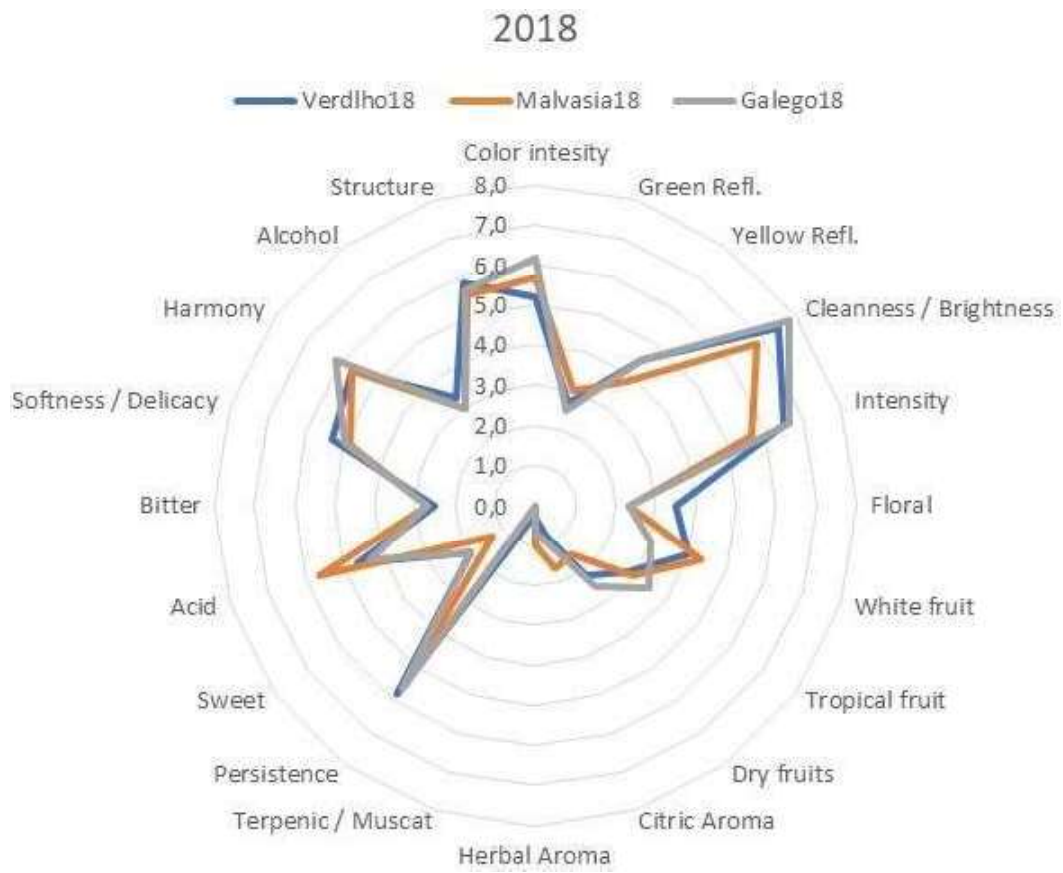


Figure 5 - Sensory analysis 2018

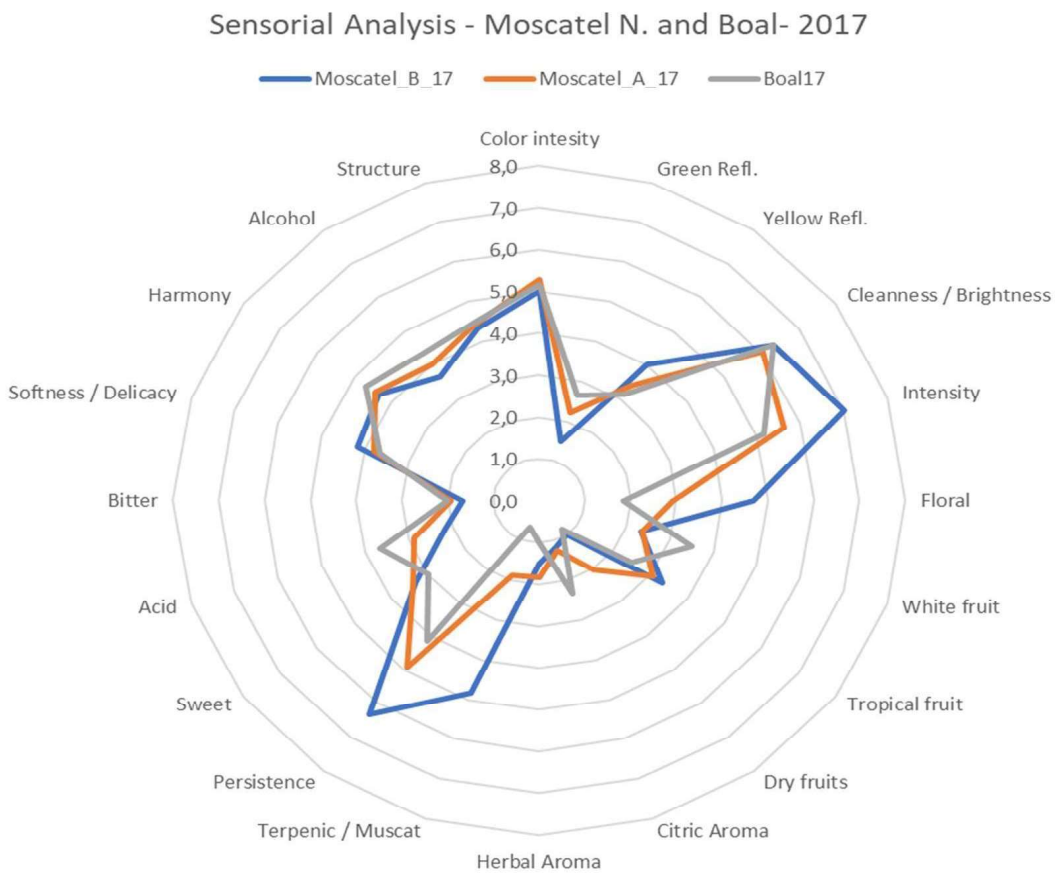


Figure 6 - Sensory analysis other varieties

Analyzing also the other varieties wine of the year 2017, the wine of Boal Espinho showed a good intensity and persistence, characterized by notes of white fruit, tropical fruit and citrus notes; moreover, the wine showed good acidity, delicacy and harmony.

Furthermore two samples of Moscatel Nunes were analyzed; sample 2 underwent acidification during the winemaking process. Sample B showed an excellent intensity and aromatic persistence with floral, terpenic and tropical fruit notes; besides it is characterized by a good harmony and delicacy. Sample A is characterized by a good intensity and aromatic persistence. Comparing the two samples the acidification of the sample B led to a greater persistence, aromatic intensity increasing the terpenic and tropical fruit aromas.

Furthermore, the data of the sensory results were analysed using the analysis of the main components (PCA) and the analysis of the hierarchical cluster (HCA) 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.

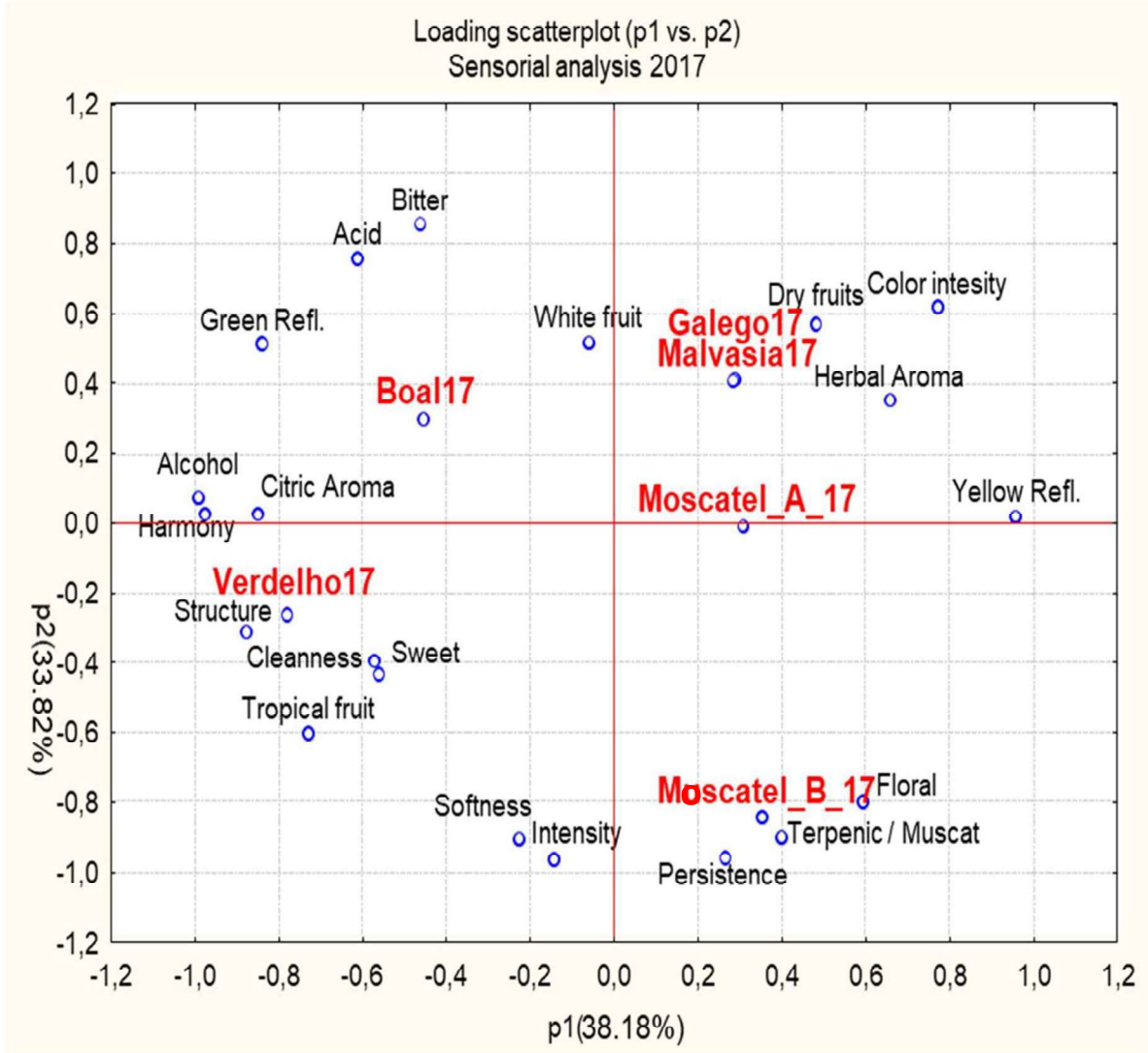


Figure 7 - Projection of wine samples (vintage 2017) and sensorial descriptors in the space defined by the two components of PCA.

The PCA and the HCA were performed to study the differences and/or similarities between the varieties.

The PCA analysis, done on the results from the wines produced in 2017, found 72% of the cumulative variance in the two first components: 38.18% in component 1 and 33.82% in component 2 (Fig. 7).

In PC1, the following variables showed the greatest relevance: yellow reflexes, color intensity, hints of dried fruit, herbaceous, floral and terpenic. The varieties closest to these variables were Galego, Malvasia and Moscatel. By observing the figure 7, it was noted the closeness between Malvasia and Galego, which showed a good proximity to the variables of white fruit and dried fruit. Moreover, from the graph it can notice a difference between the two Moscatel wines. During the production process, Moscatel B underwent a correction of titratable acidity which, as highlighted in the sensory analysis session, favoured a greater persistence of aromas, terpene-floral ones.

In PC2 the following variables showed greater relevance: bitter, acid, green reflexes, structure, clarity and tropical fruit. As it can see from the figure 6, wine Verdelho is the most differentiated sample, which shows a closeness to the variables of tropical, sweet fruit, structure and clarity.

The HCA analysis confirmed the distances between the various varieties as shown in the figure 8. The Verdelho and Moscatel-B samples are the wines that differ most from the others.

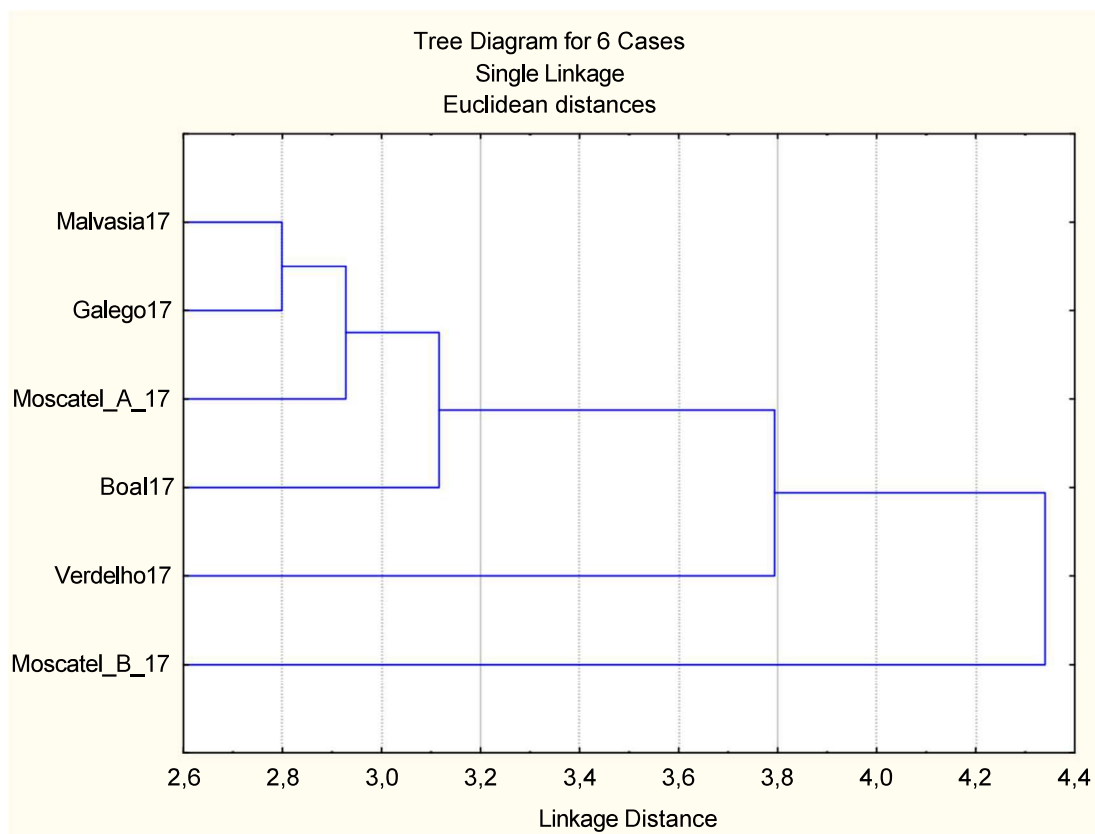


Figure 8 - Tree diagram by sensory analysis

Sensory analysis was also performed for the wines produced in the year 2018 from the Malvasia, Galego and Verdelho varieties; subsequently the data were analysed by PCA and HCA analysis in comparison with the previous year, using the same varieties.

The PCA analysis shows a cumulative variance of 72.18%, distributed with 46.33% to PC1 and with 25.85 to PC2 showed in the figure 9.

The variables that showed the greatest relevance in PC1 were bitters, persistence, harmony, softness, structure, acid, white fruit and floral.

In PC2 the variables were more important: tropical fruit, citric aroma, sweet, alcohol and terpenic aroma.

From the graph (figure 9) you can see the clear distinction of the two years. All the wines of 2018 were positioned on the right side of the graph, characterized by floral notes, white fruits, harmony and softness.

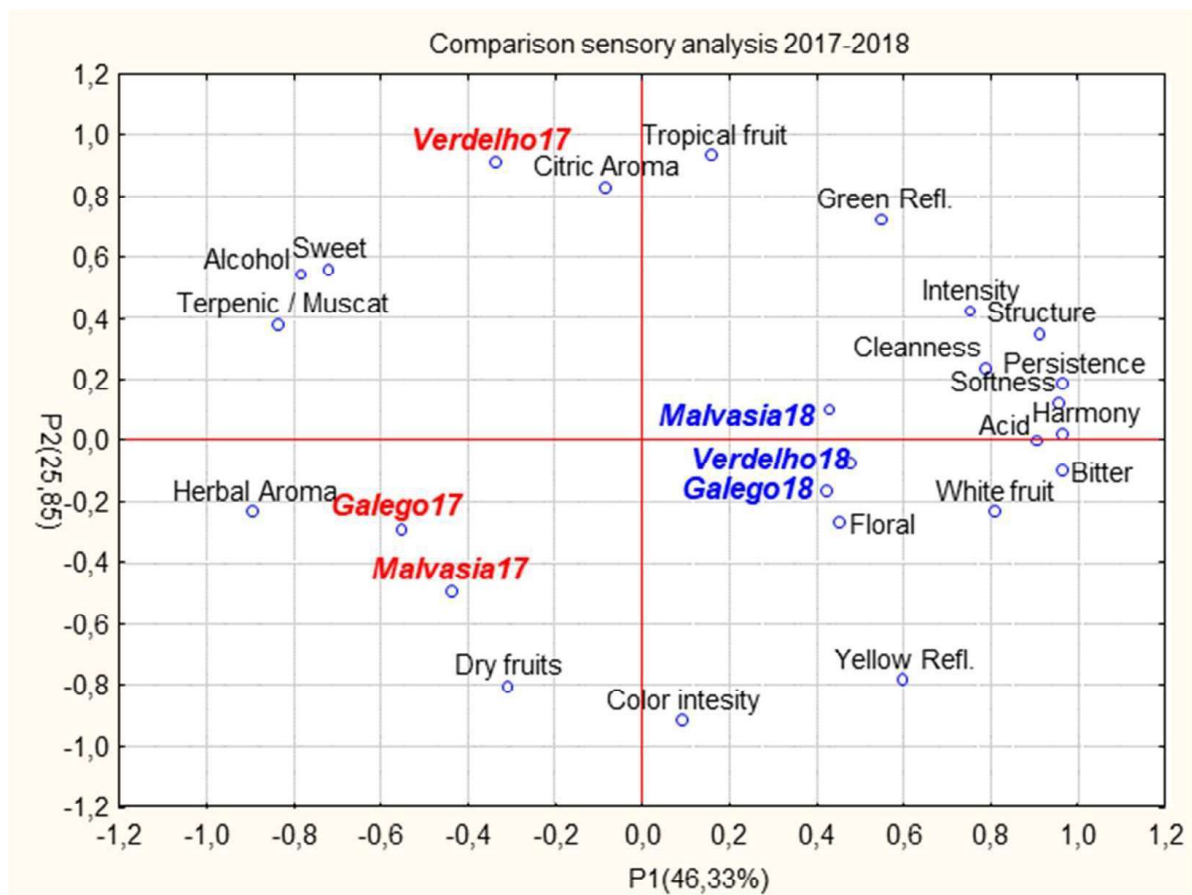


Figure 9 Projection of wine samples of three varieties (vintage 2017 and vintage 2018) and sensorial descriptors in the space defined by the two components of PCA

The wines produced in 2017, were located on the left side of the graph, showing a separation of the Verdelho wine from the other two wine varieties characterized by tropical and citric aromas, on the contrary Galego and Malvasia from vegetal and dried fruit notes.

This differentiation was also highlighted by the HCA analysis, showing the clear difference

between the two vintages and then the separation of Verdelho17 within his group showed in the figure 10.

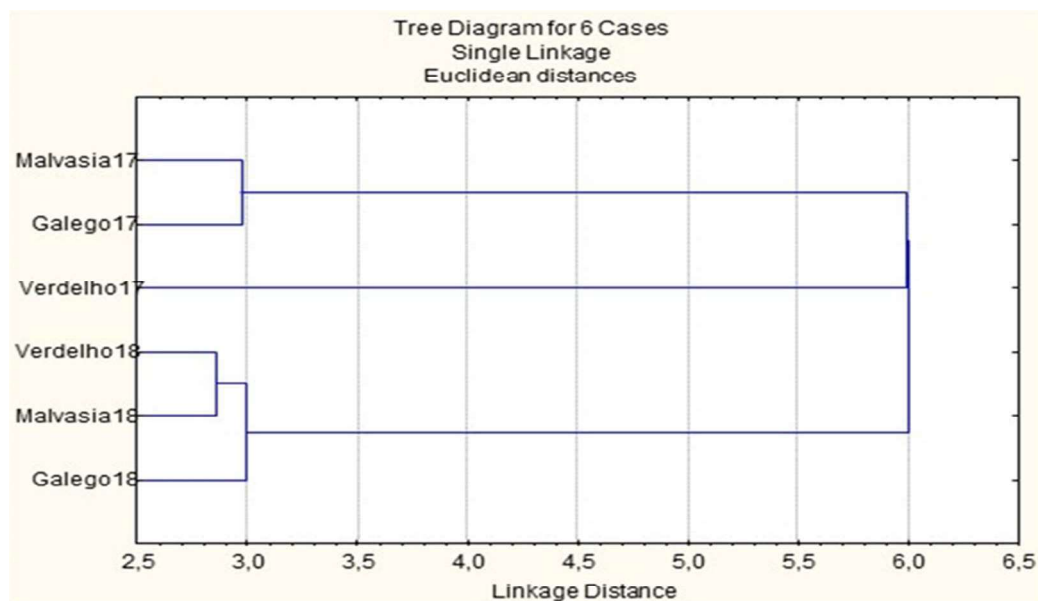


Figure 10 - Tree diagram by sensory analysis

4.3. Volatile compounds in wines

From the chromatographic analysis, numerous compounds were detected, about 60, but only 40 were identified.

In figure 11, it represents the chromatogram of the Malvasia 2017 sample, which is representative for all the varieties since in general all the varieties have the same chromatographic profile; the substantial differences were the concentrations of the various compounds and in particular in the sample Malvasia and Moscatel terpenic compounds were identified.

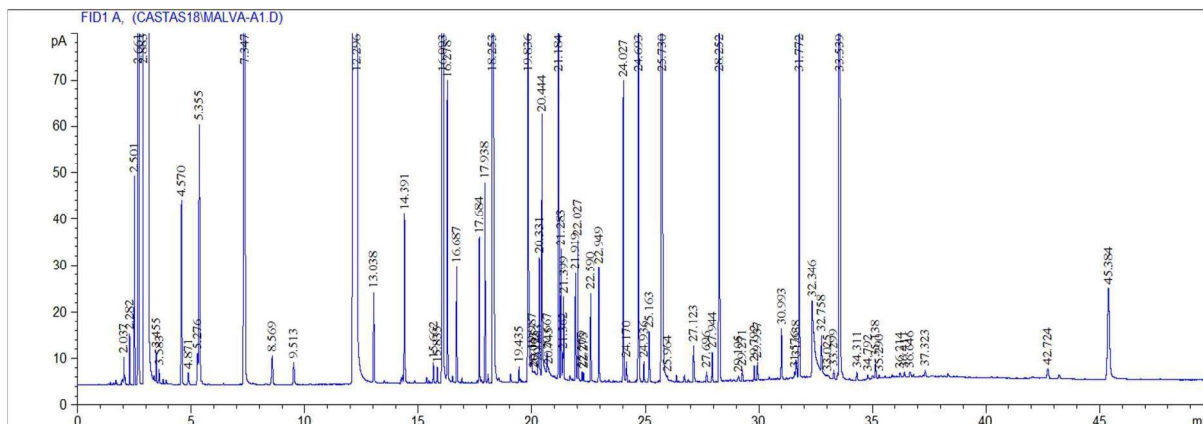


Figure 11 - Chromatogram of Malvasia sample, vintage 2017

*Figure 11: for peaks identification, please see table 13

The sample Malvasia17 compared to the sample Malvasia18, shows a higher concentration of terpene compounds, but in both years a terpene alcohol (3,7-dimethyl-1,5-octadien-3,7-diol) was found, which is it was also found by Di Stefano and reported in the 1982 publication and subsequently also by other authors, for example Liberatore *et al.* (2010), on chardonnay wines.

There are several studies on the influence of climate on the composition of the berry such as sugars, acid polyphenols (Coombe, 1987; Leeuwen and Darriet, 2016; Leewen and Destruct-Irvine, 2017), but there are few studies on the effect of climate on volatile composition.

Studies on the influence of climate on volatile compounds include studies on the Rotundone, IBMP, which decreases with temperatures and on the contrary, there is an increase in TDN with increasing temperatures; this compound gives the wine aromas of oil (Coombe, 1987; Leeuwen and Darriet, 2016; Leewen and Destruct-Irvine, 2017).

The quantitative data of volatile compounds found in mono-varietal wines are shown in the table 13; in which the data are expressed in mg/L expressed in 2-octanol (internal standard), given by the averages of the wines analyzed with duplicate extractions.

In addition to concentrations, the table 13 shows the identification code assigned to each identified compound, with CAS code and Kovats index.

The compounds were uniquely identified, calculating the retention index of Kovats and the MS fragmentation pattern with those of reference compounds or with mass spectra in the NIST and Wiley libraries.

Table 13 - Volatile compounds of wines, concentration expressed mg/L in 2-Octanol

IDENT. COD.	IDENT. GC-MS	CAS-REGISTRY NUMBER	RTIM FID	IRKOVAZPID	MALVASIA 17	GALEGO 17	VERDELHO17	GALEGO 18	VERDELHO 18	BOALL17	MOSCATELA 17	MOSCATEL B17
B	Ethyl butyrate	105-54-4	5,34	1034	1,413	1,239	2,924	3,821	2,272	0,807	1,649	1,381
C	Isobutyl alcohol	76-83-1	7,33	1094	5,538	2,668	6,467	9,449	7,589	6,370	5,017	4,597
D	Isoamyl acetate	123-92-2	8,31	1117	0,185	0,345	4,862	2,520	1,498	0,142	0,672	0,349
E	1-Butanol	71-36-3	6,99	1086	0,093	0,091	0,342	0,294	0,202	0,072	0,135	0,117
F	Isoamyl alcohol	123-51-3	12,25	1215	96,430	46,035	107,910	168,256	151,004	95,528	94,983	86,708
G	Ethyl hexanoate	123-56-0	13,04	1240	0,502	0,293	1,428	0,998	0,817	0,419	0,756	0,581
H	Acetoin	513-96-0	14,39	1283	0,841	0,654	1,349			0,703	0,274	0,277
L	Ethyl lactate	97-64-3	16,08	1350	32,908	9,810	2,463	2,330	3,456	30,530	1,239	1,843
K	1-Hexanol	111-27-3	16,27	1358	1,451	0,779	0,876	1,583	1,519	2,039	0,813	0,847
M	1-Propanol, 3-ethoxy-	111-35-3	16,68	1376	0,582	0,528	1,525	0,659	0,392	0,204	0,530	0,586
N	cis-3-Hexenol	928-96-1	16,92	1386	0,000	0,194	0,000	0,687	0,073	0,008	0,067	0,052
O	Ethyl octanoate	106-32-1	17,94	1437	0,969	0,566	1,814	1,618	1,243	0,668	1,391	1,167
P	Acetic acid	64-19-7	17,92	1437	6,573	5,125	6,107	4,818	5,909	9,190	5,822	6,318
Q	Furfural	98-01-1	18,54	1469	0,000	0,000	0,000			0,000	0,066	0,059
R	Butanoic acid, 3-hydroxy-, ethyl ester	5405-41-4	19,44	1518	0,081	0,070	0,159	0,138	0,088	0,054	0,114	0,114
S	2,3butanol diol		19,79	1540	7,212	7,872	21,630	6,606	6,385	5,378	6,146	6,338
T	Linalool	78-70-6	19,99	1551	0,057	0,000	0,000	0,000	0,000	0,000	0,193	0,222
V	Isobutanoic acid	78-31-2	20,33	1572	0,644	0,204	0,273	0,297	0,386	0,473	0,232	0,289
Y	Hotrienol	53834-70-1	21,00	1613	0,000	0,000	0,000			0,000	0,173	0,213
Z	Butyrolactone	96-48-0	21,18	1625	4,668	1,535	4,637	3,049	4,766	6,022	3,467	4,211

IDENT. COD.	IDENT. GC-MS	CAS-REGISTRY NUMBER	RTIM FID	IR KOVAZ FID	MALVASIA 17	GALEGO 17	VERDELHO17	GALEGO 18	VERDELHO 18	BOAL 17	MOSCATEL A 17	MOSCATEL B 17
AA	Butanoic acid	107-92-6	21,28	1631	0,615	0,267	0,984	0,462	0,393	0,412	0,506	0,486
AB	Ethyl decanoate	110-38-3	21,38	1638	0,502	0,105	0,052			0,143	0,147	0,170
AE	Isovaleric acid	503-74-2	21,92	1672	0,592	0,273	0,309	0,641	0,487	0,508	0,356	0,470
AF	Ethyl succinate	123-25-1	22,04	1680	0,693	0,242	0,634	0,297	0,544	1,619	0,294	0,650
AH	α -Terpineol	98-55-5	22,27	1695	0,055	0,000	0,000			0,000	0,093	0,131
AJ	3-Methylthiopropyl alcohol	505-10-2	22,59	1715	0,519	0,259	0,162	0,640	0,503	0,571	0,340	0,396
AL	Citronellol	106-22-9	23,40	1767	0,000	0,000	0,000			0,000	0,047	0,036
AN	2-Phenylethyl acetate	103-45-7	24,17	1815	0,129	0,104	0,448	0,250	0,234	0,093	0,565	0,330
AO	Hexanoic acid	142-62-1	24,69	1846	2,934	1,745	5,589	3,942	3,357	2,165	3,753	3,152
AQ	Benzyl alcohol	100-51-6	25,17	1876	0,391	0,191	0,277	0,200	0,122	0,176	0,257	0,242
AR	Phenylethyl Alcohol	60-12-8	25,72	1909	42,772	16,683	16,634	29,163	41,252	48,873	38,573	39,271
AS	3,7-Octadiene-2,6-diol, 2,6-dimethyl-	13741-21-4	26,37	1948	0,033	0,000	0,000	0,000	0,000	0,064	1,036	1,015
AW	Diethyl malate	626-11-9	27,94	2042	0,222	0,097	1,121	0,381	0,854	0,221	0,322	0,582
AX	Octanoic acid	124-07-2	28,25	2060	5,972	3,795	8,266	8,564	6,490	4,668	8,411	6,922
BB	Eugenol	97-53-0	29,94	2061	0,140	0,061	0,168	0,050	0,131	0,182	0,068	0,120
BD	γ -Caprolactone	695-06-7	30,99	2225	0,408	0,165	0,380	0,284	0,382	0,530	0,240	0,389
BH	Decanoic acid	334-48-5	31,77	2273	2,679	1,575	2,477	2,896	2,051	2,539	4,012	3,570
BI	Threitol, L-	2319-57-5	32,33	2307	0,633	1,514	0,000	1,100	0,997	3,165	1,225	0,000
BIM	Monomethyl succinate	3878-55-5	33,54	2383	19,743	7,379	12,887	6,375	12,819	43,762	10,990	16,202
BQ	Benzoic acid	65-85-0	34,31	2431	0,072	0,031	0,061	0,045	0,000	0,047	0,035	0,015
BS	Dodecanoic acid	143-07-7	35,14	2485	0,174	0,104	0,119	0,000	0,040	0,189	0,245	0,235
CD	Tyrosol	501-94-0	45,39	2995	2,691	1,774	1,335	1,530	1,836	3,148	2,470	2,720

The Kovats retention indices of compounds were calculated by injecting a sample with a homologous series of alkanes (C9-C30) by linear interpolation (Philips, 1989).

From the table it is possible to notice the differences in concentration of the different compounds among the varieties but above all we can note the differences in concentration due to the vintage effect. This effect was also noted for some volatile compounds in the study by Vilanova *et al.* (2013) on white wines from northern Spain.

In this work the major differences in the group of esters were found, including ethyl hexanoate, ethyl lactate and monomethyl succinate.

Another chemical group that showed greater differences in the two years was that of alcohols including C6 alcohols; isoamyl alcohol, phenylethyl alcohol, isobutyl alcohol and 1-hexanol.

According to Vilanova *et al.* (2013) terpene compounds, have no influences due to the "vintage" effect as they are closely related to variety; however, in this study it was found that there was a difference in the terpenic composition between the varieties, but a difference in concentration was found, in the two years of study, probably due to the vintage effect.

The data of table 13 were analyzed with the Statistica software, through the multivariate PCA analysis.

The multivariate analysis in the year 2017 showed 63.19% of cumulative variance in the two first components, with 35.49% in component 1 and 27.69% in component 2 as showed in the figure 12.

By analyzing the two components individually, it was noted the clear distinction of wine Verdelho, with a clear separation from the other wine varieties and the proximity to the compounds: ethyl butyrate, butanoic acid 3-hydroxy ethyl ester, 3-ethoxy-1-propanol, Hexanoic acid, Diethyl malate, Isoamyl acetate, butanoic acid, 2,3 butanol-diol, Ethyl hexanoate.

Analysing component 2, it was noted a positive correlation of the Moscatel Nunes and Galego Dourado varieties together with the compounds: Citronellol, 3-7-Octadiene-2-6-dio-2-6-dimethyl, Furfural, Hotrienol, Linalol, cis-3- Hexenol, α -terpineol, Dodecanoic acid and 2-Fenylethyl acetate.

In the graph (figure 12) it is possible to note the differences shown by the varieties, in particular the closeness between Galego and the Moscatel samples, from a very close group; furthermore, it is possible to see the proximity between the Malvasia and Boal E. samples and the complete separation of the Verdelho sample, which has a different behaviour from the other varieties.

In the table 13 it can see the differences in concentrations between the two different years and this shows the vintage effect as explained by Leeuwen and Darriet (2016) and Leeuwen and Destruct-Irvine (2017).

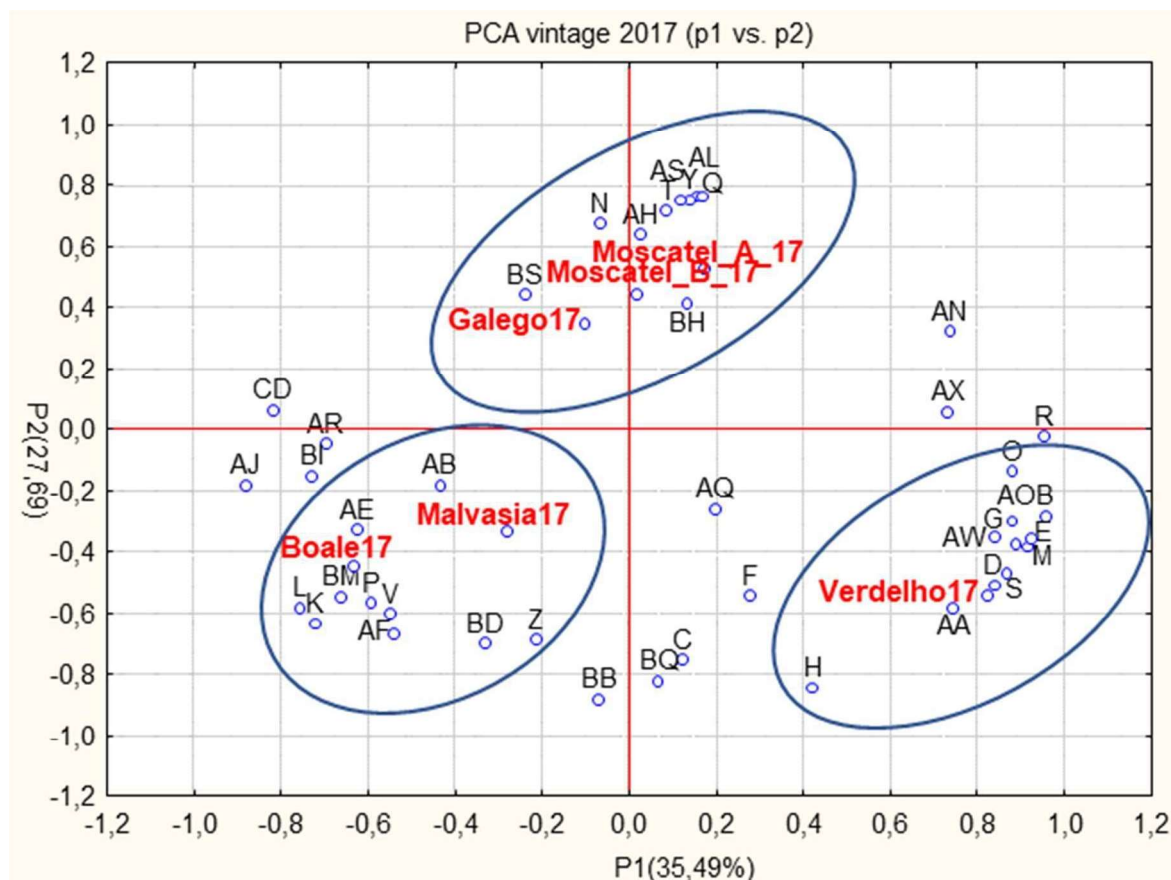


Figure 12 - Plot of varieties wine samples (vintage 2017) and volatile compounds on the plane of the two first components - B- Ethyl butyrate; C- Isobutyl alcohol, D- Isoamyl acetate, E- 1-Butanol, F- Isoamyl alcohol, G- Ethyl hexanoate, H- Acetoin, L- Ethyl lactate, K- 1-Hexanol, M- 1-Propanol, 3-ethoxy-, N- cis-3-Hexenol, O- Ethyl octanoate, P- Acetic acid, Q- Furfural, R- Butanoic acid, 3-hydroxy-, ethyl ester, S- 2,3butanol diol, T- Linalool, V- Isobutanoic acid, Y- Hotrienol, Z- Butyrolactone, AA- Butanoic acid, AB- Ethyl decanoate, AE- Isovaleric acid, AF- Ethyl succinate, AH- α -Terpineol, AJ- 3-Methylthiopropyl alcohol, AL- Citronellol, AN- 2-Phenylethyl acetate, AO- Hexanoic acid, AQ- Benzyl alcohol, AR- Phenylethyl Alcohol, AS- 3,7- Octadiene-2,6-diol, 2,6-dimethyl-, AW- Diethyl malate, AX- Octanoic acid, BB- Eugenol, BD- γ - Caprolactone, BH- Decanoic acid, BI- Threitol- L, BM- Monomethyl succinate, BQ- Benzoic acid, BS- Dodecanoic acid, CD- Tyrosol.

Verdelho in 2017 showed greater separation between the varieties this is given by the different concentrations of volatile compounds such as the two esters acetates, isoamyl acetate giving a banana-like aroma and 2-phenylethyl acetate giving fruity and flowery flavour with a honey note (Rapp and Mandery, 1986); moreover Verdelho stands out thanks to the higher concentrations of acid butanoic, hexanoic and decanoic acid which are the main fatty acids present in all the varieties described by fruity notes, cheese, fats and rancid (Gomez-Miguez *et al.*, 2007).

Verdelho, also in 2017, has the highest concentration of alcohols compared to other varieties and the most present alcohols were isoamyl alcohol with hints of whiskey, malt, burnt and phenylethyl alcohol with hints of honey, spice, roses, lilac (Francis, 2005).

In a previous study on Verdelho by Gaspar *et al.* (2016), showed a separation from the Gouveio variety, with high concentrations, as in this study of 2-3 butanediol and 2-phenylethyl acetate.

The higher alcohols are quantitatively the largest group of aromatic compounds in alcoholic beverages and the most significant alcohols are the isoamyl alcohol, isobutanol and the phenylethyl alcohol (Bertrand *et al.*, 1978; Amerine *et al.* 1980).

The Malvasia (de Colares) sample in 2017 was characterized by a high content of esters compared to other varieties, especially due to the presence of monomethyl succinate, ethyl lactate, ethyl succinate and ethyl decanoate with fruity scents.

Esters are the aromatic class of considerable importance, which makes a strong contribution to the aroma of wine; at low concentrations (≤ 100 mg/l) it gives the wine fruity scents, even at high concentrations it gives the wine an aroma like solvent / enamel (Sumbly *et al.*, 2010).

Furthermore, in the 2017 Malvasia sample, terpene compounds have been found, such as linalool and α -terpineol which generally present with high concentrations in wines from muscat grapes or related to it; terpenic compounds also in micro-concentrations influence the smell of wine and the different combinations of these characterize the muscat and related varieties.

Terpenic compounds give the wine floral, rose-like, green and herbaceous aromas but their concentrations are influenced by various factors such as for example the shading as demonstrated by Bureau *et al.* (2000) but the differences may also occur within the cultivar itself but cultivated in different areas.

Also, the Galego in the 2017 vintage differs from the other two varieties studied, it has a lower total concentration of volatile compounds, and for this reason it is positioned in the graph far from the other varieties taken into consideration; but if we consider also the auxiliary varieties to this study it is very close to the Moscato samples.

The 2017 Galego is characterized by a higher concentration of C-6 alcohols which give the wine vegetal and herbaceous scents (Joslin and Ough, 1978; Ramey *et al.*, 1986).

Analyzing also the auxiliary varieties, such as Moscatel Nunes and Boal Espinho, we can notice higher concentrations in terpene compounds in the Moscatel Nunes variety, where Linalool, Hotrienol, α -terpineol are present.

Boal E. is characterized by a high concentration of esters, especially ethyl lactate and monomethyl succinate, and a greater concentration of alcohols such as phenylethyl alcohol and isamyl alcohol, compared to Moscatel Nunes.

It was also performed the PCA analysis of the volatile compounds quantified in the same varieties at the two vintages, to verify the relevant compounds for both years as showed in the figure 13.

The analysis of the main components showed 62.15% of cumulative variance for the two first components: 34.52% in component 1 and 27.63% in component 2.

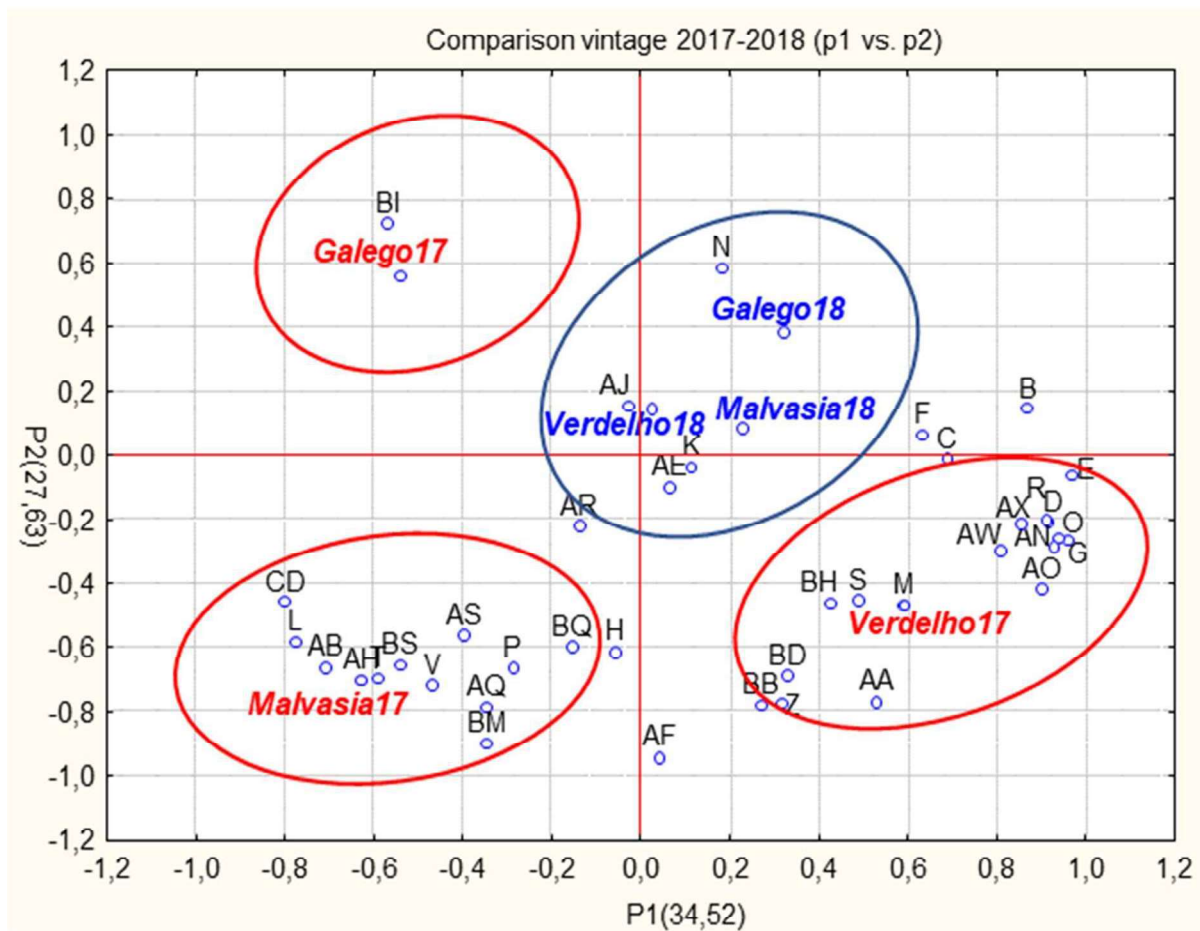


Figure 13 - Plot of three wine varieties (vintage 2017 and vintage 2018) and volatile compounds on the plane of the two first components - **B-** Ethyl butyrate; **C-** Isobutyl alcohol, **D-** Isoamyl acetate, **E-** 1-Butanol, **F-** Isoamyl alcohol, **G-** Ethyl hexanoate, **H-** Acetoin, **L-** Ethyl lactate, **K-** 1-Hexanol, **M-** 1-Propanol, 3-ethoxy-, **N-** cis-3-Hexenol, **O-** Ethyl octanoate, **P-** Acetic acid, **Q-** Furfural, **R-** Butanoic acid, 3-hydroxy-, ethyl ester, **S-** 2,3butanol diol, **T-** Linalool, **V-** Isobutanoic acid, **Y-** Hotrienol, **Z-** Butyrolactone, **AA-** Butanoic acid, **AB-** Ethyl decanoate, **AE-** Isovaleric acid, **AF-** Ethyl succinate, **AH-** α -Terpineol, **AJ-** 3-Methylthiopropyl alcohol, **AL-** Citronellol, **AN-** 2-Phenylethyl acetate, **AO-** Hexanoic acid, **AQ-** Benzyl alcohol, **AR-** Phenylethyl Alcohol, **AS-** 3,7- Octadiene-2,6-diol, 2,6-dimethyl-, **AW-** Diethyl malate, **AX-** Octanoic acid, **BB-** Eugenol, **BD-** γ - Caprolactone, **BH-** Decanoic acid, **BI-** Threitol- L, **BM-** Monomethyl succinate, **BQ-** Benzoic acid, **BS-** Dodecanoic acid, **CD-** Tyrosol.

In the PCA of the comparison of the two vintages in PC1 are present: ethyl hexanoate, butanol, ethyl octanoate, 2-phenylethyl acetate, isoamyl acetate, diethyl lactate, isobutyl alcohol, isoamyl alcohol, 3-ethoxy-1propanol, butanoic acid, 2,3 butanol diol, decanoic acid. There is a positive correlation of Verdelho17 with component 1 and with a certain proximity to wines produced in 2018, on the contrary, wines produced in 2017 show a negative correlation.

In component 2 the threitol and cis 3-Hexenol compounds show greater interest, showing a certain closeness the two wines produced by the Galego of the two different vintages.

On a graphical level it can note the main differences between the two different years; the 2018 vintage positioned on the right side of the graph in a compact way, while the 2017 vintage is positioned differently for each variety with a certain distance from each other.

The differences shown by the varieties are above all differences given by the different composition of volatile compounds, especially in terms of concentration in the two vintages.

The concentration of the compounds, in general, is greater than in the previous year but by analyzing the compounds, they result in lower quantities, esters and terpene compounds.

The compounds that characterize the 2018 vintage are 1-hexenol, cis-hexenol, phenylethyl alcohol, 3-methylpropyl alcohol and isovaleric acid, these compounds give the wine hints of sweat, acid, rancid, vegetable, floral, honey.

In the comparison of the vintages, the 2017 Verdelho is characterized by the compounds: hexanoic acid, decanoic acid, tyrosol, 1-propanol-3-ethoxy and 2 3-methylthiopropyl alcohol, which is positioned on the right side of the graph, showing greater difference to the inside of the same vintage.

5. Conclusion

The aim of this work was the characterization of the aromatic and sensorial profile of 5 minority vines of Portugal, through sensory analysis and chromatographic analysis of two vintages (2017 and 2018).

There are no studies on the aromatic profile of these varieties, except for the Verdelho, which was studied by Gaspar *et al.* (2016) compared to another Portuguese variety.

The study was based only on two vintage and allowed to see the main differences / similarities between the varieties, which was possible thanks to the multivariate analysis (PCA) produced starting from the data obtained from sensory and chromatographic analyses.

The graphs produced by the PCA and HCA analysis show a similarity between the sensory profile and the chromatographic one; from the obtained data emerged the behaviours of the three main varieties studied, which seem to be influenced by the climatic effects (vintage effect).

All the vines during the tasting sessions showed, in general, a good appreciation; in 2017, Verdelho was characterized by notes of tropical fruit, sweetness, good persistence and intensity, this reflects the chemical data, characterized by a good alcohol content, good acidity, with a high concentration of higher alcohols, esters and acetate esters, which confirm the sensory profile.

The profile of the Malvasia (de Colares) was also interesting, showing an interesting sensory profile, with floral notes, white fruit, good persistence and harmony; but the chemical results are also interesting.

This variety is characterized by a good alcohol content, acidity and a high concentration of

higher alcohols, especially isoamyl alcohol and phenylethyl alcohol, contributing positively to the fruity aroma; but of notable interest was the presence of terpenic compounds, which, however, have probably undergone a "vintage" effect.

The Galego Dourado, in 2017 showed a low aromatic profile, but in 2018, from the sensory analysis and from the aromatic profile it showed a greater impact; in 2018 it was characterized by notes of tropical fruit, white fruit and citric, this is confirmed by a general increase in the concentration of volatile compounds, especially for acetate esters and higher alcohols.

Among the other varieties studied, the Moscatel Nunes was of considerable interest, which showed sensory and floral scents from sensory analysis, classic in Muscat grapes, confirmed also by chromatographic analysis; in fact, the following terpenic compounds have been found: linalool, hotrienol, α -terpineol and citronellol, unfortunately the characterization with the following year could not be verified.

The Boal Espinho, from the sensory analysis showed a fruity aromatic profile, with hints of white fruit, tropical fruit and citric scents; the sensory profile was also confirmed by the chromatographic analysis with a high concentration of esters, higher alcohols and lactones which contributed positively to the sensory profile.

In general, all the chromatographic profiles show the same trend, only the Malvasia (de Colares) and Moscatel Nunes varieties differ due to the presence of terpenic compounds. However, it would be interesting to prolong the study of these varieties, to see the differences of the volatile compounds due to the climatic effect and perhaps modify the extraction methods, using techniques more sensitive to varietal compounds; evaluating also the aromatic precursors and evaluating the active volatile compounds through the olfactometric gas chromatography.

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INFORMATION

The student Simone Piras has introduced the minor corrections on the master thesis, under the subject ***Volatile and sensory characterization of white wines from minority grapes varieties***, which has been discussed at 18th of July.

The final version is in conditions, in my opinion, to be delivered at Technical University of Lisbon.

Dois Portos, August 16th, 2019

A handwritten signature in blue ink, reading "Ilda Caldeira", with a horizontal line underneath.

Ilda Caldeira, Ph D.

Assistant researcher of INIAV