Evolution of phenolic and sensorial characteristics of rosé wines aged with different alternative wood chips

Inês Filipa Santos Nunes

Dissertação para obtenção do Grau de Mestre em

Viticultura e Enologia

Orientador: Professor Doutor Jorge Manuel Rodrigues Ricardo da Silva
Co-orientador: Professor Doutor António Manuel Santos Tomás Jordão

Júri:

Presidente: Doutor Carlos Manuel Antunes Lopes, Professor Associado com Agregação do Instituto Superior de Agronomia da Universidade de Lisboa.

Vogais: Doutor António Manuel Santos Tomás Jordäo, Professor Adjunto da Escola Superior Agrária do Instituto Politécnico de Viseu;
Doutor Aníbal José Simões Coutinho, Especialista do grupo SONAE.

Lisboa, 2017

Agradecimentos

Após vários anos de altos e baixos na minha vida académica, sinto que devo os meus sucessos a todos os que dela foram parte, a todos os professores que me guiaram, inspiraram e desafiaram, e aos colegas que foram o apoio, ajuda e amizade que necessitei, especialmente o João e o Eduardo, que para além de amigos foram um grande suporte quando mais precisei nesta fase final.

Aos professores Jorge Ricardo da Silva e António Jordão, com quem entrei no que foi um grande desafio para mim, e que estiveram sempre prontos a dar apoio e a fazer todos os possíveis para me ajudar em todo este processo, com um agradecimento especial também à professora Ana Cristina Correia que foi uma ajuda indispensável e cedeu também o seu apoio e disponibilidade.

À Casa da Passarela e AEB Bioquímica que forneceram os vinhos e aparas de madeira para este estudo.

Devo um agradecimento enorme à Diana Faria, ao Daniel Duarte e à Juliane Oliveira, com quem passei todo o período experimental e quem me acompanhou e muito me ensinou no que foi um período de aprendizagem muito grande, por vezes cansativo, e todos os dias estiveram incondicionalmente disponíveis com a melhor disposição, que me ajudou a ser confortável e confiante num meio que me era ainda estranho.

Finalmente a todos os meus amigos e família, em especial aos meus pais que me trouxeram sempre apoio, amizade, compreensão, e tanto fizeram por mim para poder aqui chegar, assim como ao meu irmão que sempre me transmitiu força e determinação. Serão todos sempre uma grande inspiração.

Abstract

The aim of this study was evaluating the effects of cherry (*Prunus avium*) and oak (*Quercus petraea)* chips in the phenolic, volatile and sensorial profile of a Portuguese rosé wine, made from Touriga attempting to understand whether the application of cooperage, including alternative woods, is potentially enhancing.

For this purpose, several chemical analyses were carried out; for musts (at 0, 2, 6, 8,10 and 20 fermentation days), total phenols, non-flavonoids, chromatic characteristics, and colour due to copigmentation were assayed. For the wines (sampled at 40, 60 and 80 storage days), the same analysis took place, as well as HPLC determination of individual anthocyanins, polyamide column extraction and HPLC determination of proanthocyanidins, proanthocyanidin separation by degree of polymerization and sensory analysis.

Both woods improved colour intensity and pigment stability, which was significantly more relevant when using cherry wood, and phenolic content was consistently higher with this wood, in comparison with the control and oak wines, the latter even showing less total phenols than the control at one storage point. Both also had a considerably larger proportion of coloured anthocyanins than the control, more pronounced in the wines fermented and aged with chips, and with no discernible dominance of any wood type, and oak wood induced an increase of malvidin-3-glucoside in storage.

Cherry wood also showed a much higher concentration of monomeric procyanidins, namely (+)-catechins, with a possible effect of contact time.

From sensory analysis, wines aged and/or fermented with wood chips always scored highest in overall rating (CHFA at 40 storage days, CHF at 60 and OKFA at 80), significantly improving colour intensity and overall quality, as well as woody aromas, which developed more intensely in the wine fermented and aged with oak chips.

There were no definite differences between contact time when using wood, and results were sometimes irregular and with considerable associated error.

Keywords: Cherry, oak, phenolic composition, rosé wine, sensory analysis

Resumo

O objetivo do presente estudo foi avaliar os efeitos da aplicação de aparas de cerejeira (*Prunus avium*) e carvalho (*Quercus petraea*) a um vinho rosé (Touriga Nacional, região do Dão), no perfil fenólico, volátil e sensorial, durante a fermentação (20 dias) e conservação (80 dias), de modo a contribuir para a avaliação do uso de madeiras em rosés, assim como o uso de outras madeiras em vinificação, como uma alternativa viável.

Para este efeito, foram feitas várias análises químicas; para mostos (a 0, 2, 6, 8, 10 e 20 dias de fermentação) foram avaliados os fenóis totais e não flavonóides, características cromáticas e cor devido a copigmentação. No caso dos vinhos (a 40, 60 e 80 dias de conservação) foram feitas as mesmas análises mencionadas para os mostos, para além de poder tanante, antocianinas individuais por HPLC, extração por coluna de poliamida e determinação por HPLC de procianidinas, separação de procianidinas por nível de polimerização e análise sensorial.

Ambas as madeiras afetaram positivamente a intensidade e estabilidade da cor, muito mais significante com aparas de cerejeira, e a riqueza fenólica foi sempre maior com esta madeira, comparando com o controlo e as amostras com carvalho. As aparas induziram também maior proporção de antocianinas coradas, especialmente nos vinhos tanto fermentados como conservados na sua presença, sem que algum tipo de madeira se destaque neste parâmetro, e a madeira de carvalho teve um efeito de aumento de malvidina-3-glucósido na conservação.

O maior valor de procianidinas monoméricas foi atingido com cerejeira, aparentemente aumentando com o tempo de contacto.

Sensorialmente, as madeiras conferiram as melhores pontuações gerais aos vinhos, com mais intensidade da cor e qualidade, e mais aromas característicos de madeiras que se desenvolveram mais marcadamente no vinho que fermentou e estagiou com carvalho.

Não houveram diferenças significativas entre tempos de contacto no caso do uso de aparas, e os resultados estiveram muitas vezes sujeitos a erros padrão consideráveis.

Palavras-chave: Análise sensorial, cerejeira, carvalho, composição fenólica, vinho rosé

Resumo alargado

O setor vitivinícola é de grande importância económica para muitos países, e o seu crescimento contínuo faz com que seja de grande interesse dos produtores de aliarem conhecimentos novos e antigos de forma a produzirem vinhos da maior qualidade possível para os seus mercados-alvo.
Os vinhos rosé têm sofrido com estigmas negativos no passado por serem vinhos vistos como desinteressantes, de baixa qualidade e pouco sérios, no entanto a procura tem aumentado, e consequentemente a produção, e cada vez mais se vê vinhos rosés complexos e inovadores a entrarem no mercado. Uma das formas de inovar nestes vinhos, ainda pouco explorada, mas muito usada em brancos e tintos, é o uso de madeira na fermentação, conservação em ambas as etapas.
O uso de madeiras, nomeadamente barricas, data desde a antiguidade, e está hoje reservado a vinhos de elevada qualidade, visto que as barricas se traduzem num uso pouco eficiente de espaço, com custos consideráveis. A resposta a este problema foi de encontrar alternativas que permitam baixar os custos de produção, entrar no mercado mais cedo e conseguir vinhos com algumas características análogas ao uso de barricas. Assim se aprovou o uso de fragmentos de madeira que trazem mais superfície de contacto com a matriz líquida, acelerando o processo de transferência de compostos da madeira para o vinho. Mesmo sendo um processo mais rápido, não será completamente análogo ao uso de barricas, mas permite que se use em vinhos de gamas mais baixas, devido à redução de custos.
O contato de mostos /vinhos com madeira traz várias consequências, pela transferências de compostos que ocorrem entre os dois meios; o vinho absorve fenóis da madeira, lactonas, entre outros, que afetam consideravelmente a sensação de boca (mais amargor e adstringência) e torna mais dominantes os aromas característicos das madeiras (baunilha, especiarias, furânico, etc.). Esta contribuição está dependente de vários fatores, como a origem geográfica das madeiras, a sua composição natural, condições de armazenamento e manuseamento, presença de oxigénio e borras e o tipo de tratamentos feitos em tanoaria, nomeadamente a operação de tosta/queima da madeira.
Recentemente, vários estudos têm procurado estudar os efeitos de outras madeiras em vinificação, para além do carvalho e castanheiro, como sejam as madeiras de cerejeira, acácia e amoreira, mas estas não são ainda permitidas, o que acaba por justificar o interesse de estudar o seu potencial como alternativos ao carvalho, que tem uma procura quase exclusiva neste setor, tanto para barricas como para os alternativos, traduzindo-se num grande impacto ambiental para a espécie.

O presente estudo procurou estudar os potenciais efeitos de aparas de cerejeira *(Prunus avium*) e carvalho *(Quercus petraea)* no perfil fenólico e sensorial de um vinho rosé, obtido por prensagem direta de uvas da casta Touriga Nacional, cultivadas na região do Dão (Portugal), de forma a justificar se o uso de produtos de madeira traz melhoramentos num rosé, ao mesmo tempo testando a contribuição de uma madeira alternativa.
Para este efeito, foram feitas várias análises químicas; para mostos foram avaliados os teores de fenóis totais e não flavonóides, características cromáticas e cor devido a copigmentação. No caso dos vinhos, ao longo do tempo, foram feitas as mesmas análises mencionadas para os mostos, para além de poder tanante, antocianinas e procianidinas individuais por HPLC e ainda a quantificação das procianidinas

de acordo com o seu grau de polimerização, e, por último, a análise sensorial dos vinhos rosés elaborados.

O mosto obtido inicialmente foi dividido em 3 partes – Controlo, fermentação com aparas de cerejeira (1.5 g/L) e fermentação com aparas de carvalho (1.5g/L) - em cubas de 1000L, durante 20 dias de fermentação. Posteriormente, cada ensaio com madeira foi dobrado em dois; um conservado em contacto com as aparas e o outro conservado sem madeira, resultando num total de 5 ensaios durante a conservação dos vinhos.

O uso de aparas provocou o aumento da intensidade e estabilidade da cor, principalmente no caso da cerejeira que teve um efeito significativamente maior na intensidade, assim como nos pigmentos poliméricos, em ambas as fases de vinificação. A tonalidade é também um bom indicador da evolução química do vinho, e consequentemente da sua maior ou menor "jovialidade", no que toca à cor, já que o controlo teve uma maior perda de absorvência a 520 nm (cor vermelha) do que os restantes.

Tendo em conta o parâmetro de "idade química", este foi superior quando houve contacto com ambas as madeiras. Este está relacionado com a polimerização de pigmentos, e a sua estabilidade, e é também testemunha ao contributo da madeira na estabilização da cor. Nesta determinação, foram também verificado valores superiores com a utilização de madeira de cerejeira.

Ambas as madeiras promoveram a ionização de antocianinas, mostrando uma maior proporção das formas coradas ou ionizadas. Nos vinhos, tal facto poderá dever-se ao tempo de contacto, visto que os vinhos que fermentaram e depois estagiaram com aparas mostraram valores estatisticamente mais elevados.

Quanto às proantocianidinas, a concentração de catequina foi claramente superior nos vinhos estagiados e/ou fermentados com aparas de cerejeira, sem uma tendência identificável ao longo do tempo, enquanto o vinho controlo e os vinhos fermentados e estagiados com aparas de carvalho não tiveram diferenças muito significativas.

Sensorialmente, os vinhos com aparas mostraram melhor evolução de qualidade, e o vinho controlo manteve a sua avaliação de um modo geral e sempre com classificação abaixo dos restantes, portanto houve um aumento de qualidade, assim como de intensidade da cor que teve a significância estatística mais marcada. Os aromas característicos de madeiras tornaram-se percetíveis e a madeira de carvalho teve a maior evolução desse parâmetro, especialmente nos vinhos fermentados e estagiados com aparas de carvalho.

Este acabou por ser um estudo de evolução e comparação mais com o controlo, já que as madeiras mostraram entre si apenas as diferenças já mencionadas, e obtivemos pouca significância nas comparações entre tempo de contacto, que talvez tivessem sido mais identificáveis com uma maior dosagem de aparas. O estudo dos métodos propriamente ditos para o caso de rosés é de especial interesse, assim como a continuação de ensaios com madeiras nestes vinhos, e a procura de trazer novas madeiras de potencial interesse ao setor vinícola.

Index

Figure index

Table index

1. Introduction

1.1. Winemaking technologies of rosé wines

The winemaking sector is of the utmost cultural and economic importance to many countries throughout the world, and ever-evolving, thus forcing winemakers to constantly strive for innovation and ways to stand out in a growing global market.
Rosé wines are an increasingly popular style of wine, although lacking specific guidelines or laws to officially define it. A rosé is often considered to be intermediate between red and white wine, since it combines the freshness, fruitiness, acidity and young aromas and a typically lower alcohol content, with a certain amount of phenolic compounds lower than reds. (Fauvet and Guittard, 1998).

As mentioned by several authors (André and Aubert, 1970; Garcia Jares *et al.*, 1993; Ribéreau-Gayon *et al*., 2006), and according to EC documents, rosé wines lack a precise definition. The difficulty sits on the several varieties and winemaking techniques that can be used to produce them, as well as a wide range of analytical colour parameters which could contribute to characterize a rosé wine (Ribéreau-Gayon *et al.,* 2006). Due to such dubiety, the only parameter currently leading to identifying a wine as a rosé is its visible colour, generally sitting between that of a white and red wine, and ranging from more orange tints such as “onion skin” to deeper “cherry reds”, while appearing generally lighter than a red wine.
The colour range of rosé wines, as well as their general characteristics and quality, depend on many factors such as varieties used and winemaking techniques employed.

According to Fauvet and Guittard (1998), three main issues stand out while producing a rosé;

- Choosing the combination of grape varieties, as well as the appropriate harvest time.
- Extracting the most aromatic qualities possible, while also keeping the colour to a minimum, in a limited amount of time.
- Establishing the best storage conditions so the wine can keep its young characteristics.

Regarding grape varieties, rosés can be made with any red grapes, as well as a mixture of red and white varieties in some cases, but typically choosing red grapes that are likely to confer bright colours, fruity aromas and a moderate red pigment (Amerine and Singleton, 1977). The appropriate maturation achieved before harvest depends on the final product, and whether the goal is a crisp, light rosé or a fuller body; Ribéreau-Gayon *et al.* (2006) construe that grapes intended for the former case should not exceed ripeness or 12% of ethanol by volume, while also maintaining relatively high acidity, while grapes intended for the latter should have a slightly higher alcoholic content and lower acidity. A night-time harvest is sometimes advised to help preserve the fruity aromas in grapes.
Typically, fermentation of a rosé wine should be carried out between 15 and 20 °C, allowing the temperature to rise to 20-22 °C during the final stages (Fauvet and Guittard, 1998). Temperature is particularly important to the development of grape based aromas and the volatile by-products known as

"fermentation bouquet", formed by most strains of the *Saccharomyces* yeast when temperature is kept at 20 °C or below, which is desirable in young fruity wines such as a typical rosé, however these esters are so volatile they may hydrolyse within months of storage if temperatures are not kept low enough (Boulton *et al*., 1995). If fermentation temperatures are kept too high, the flavour profile will change as these esters give place to higher alcohols, ethanol may deplete, combination of SO_2 may be promoted, rendering it useless, and there is a more considerable risk of stopping fermentation altogether.

Allowing malolactic fermentation (MLF) to follow is considered optional in rosé winemaking, as it is important to preserve freshness and achieve the best alcohol-acidity balance. MLF may be allowed or induced if the main objective is to produce a bolder rosé, especially when there was prolonged maceration, as a lower acidity resulting from MLF will help soften the tannins (Ribéreau-Gayon *et al*., 2006). André *et al*. (1971) verified that the deacidification which occurs from MLF also causes anthocyanins to decrease in colour, due to being in a lower acidity medium, resulting in a general decrease of colour intensity in rosés.

The main winemaking methods for rosé wines are direct pressing, prolonged skin contact and *Saignée* (or drawing off). Carbonic maceration is not as common but may be used, however it often results in wines that are too deep in colour, ultimately having to be blended with lighter colour wines (Fauvet and Guittard, 1998). The use of wooden cooperage is not widely studied and documented.

1.1.1. Direct pressing

Direct pressing is a winemaking technique mostly associated with white wines, consisting in a direct pressing of the grapes as they enter the winery, and using free run juice and the resulting press juice for vinification. This allows for a short maceration time which occurs only during the pressing operation, which in turn allows for some extraction of phenolic compounds; higher pressure results in higher phenolic content (Ribéreau-Gayon *et al*., 2006). In direct pressing, the must is separated from every solid component, meaning all further stages occur in strictly liquid state, with no more components being extracted from the grapes.

Two separate musts can result from this process; free run juice and press juice. Free run juice is usually richer in sugar, acids and aromatic compounds, while lacking in total polyphenols in comparison with the later extracted musts, which have had a short period of contact with skins and thus some polyphenols have been extracted (Dias Cardoso, 2007). Depending on the desired characteristics of the final product, the different musts can be combined in different manners. Press juice should be selected and blended with free run juice in determined amounts if this controlled blending is desired, and the juice from the last pressing cycle may even be eliminated, due to having a more vegetal taste and supplying more tannins than anthocyanins (Ribéreau-Gayon *et al*., 2006).

After extraction, it is important to protect the must from oxidation through sulfiting, possibly associating this operation with the use of dry ice or inert gas injection in the press or vat (Dias Cardoso, 2007), also called inert gas blanketing. This minimises the oxygen pickup from the air to the juice during several stages, from harvesting to pressing, and typically using carbon dioxide or nitrogen (Boulton *et al*., 1995).

Clarification of the must follows, aiming to separate the gross lees from the must, preceding fermentation. This operation is optional, and it tends to refine the aroma and brighten colour as well as lower the susceptibility to oxidation (although due to anthocyanin fixation)(Ribéreau-Gayon *et al*., 2006). Fauvet and Guittard (1998) reference an ideal NTU (Nephelometric Turbidity Unit) level of 100-200 NTU for a rosé must. Excessive clarification may bring difficulties later on, in fermentation, due to the lack or heterogenous distribution of polysaccharides in the vat; clarification removes polysaccharides and lees, reducing the support effect necessary for optimal fermentability (Ribéreau-Gayon *et al*., 2006).
When working with non-aromatic varieties (most in Portugal's case), the secondary/fermentative aromas become predominant in a wine made by direct pressing, making it a "thin" wine, lacking persistence and varietal character. These aromas are highly unstable and modify noticeably after about one year (Dias Cardoso, 2007).

1.1.2. Pre-fermentative maceration

Rosé wines can also be produced while allowing for a more prolonged contact with grape skins before fermentation – pre-fermentative maceration – lasting from 2 to 20 hours of contact, depending on grape varieties and ripeness, as well as harvest temperature (Fauvet and Guittard, 1998).
During maceration, phenolic compounds, as well as amino acids, fatty acids and higher alcohols are extracted (Soufleros and Bertrand, 1988), which interfere in the aroma, structure and quality of wines, in an amount proportional to the contact time, creating bolder, rounder and more deeply coloured wines in comparison with direct pressing (figure 1). Excessive skin contact can result in wines that are too bitter or astringent, and possibly with too much colour, which challenges its identity as a rosé wine.

Figure 1 - Representation of a typical rosé colour range, as function of maceration time. (*Source: Wine Folly* at http://winefolly.com/review/what-is-rose-wine/)

A short maceration extracts less anthocyanins (Kelebek *et al*., 2007; Suriano *et al*., 2015), justifying a rosé's paler, pinkish hue, yet these still appear as important antioxidants and, consequently, preservatives of fruity aromas, which are also very characteristic of typical rosé wines. Because fewer

tannins are also extracted with shorter maceration times, colour stability is compromised, since much of the colour is ultimately due to free anthocyanins and their self-association and copigmentation processes. Suriano *et al*. (2015) found that maceration time was positively correlated with colour stability, but not favourable towards fruity and floral aromas, as shown by chromatography and sensory analysis, when considering maceration times of 3, 6 and 8 hours. The same authors also obtained results regarding the effect of skin maceration on colour, with less intensity and violet hues in the shortest maceration time (3h).

Besides contact time, other factors deeply affect maceration effectiveness, such as SO_2 levels in the must. According to Sudraud *et al*. (1968), the addition of SO_2 has shown to promote anthocyanin dissolution and enhancement of colour, lowering the tannin/anthocyanin ratio, and generally favouring phenolic extraction. The results of this study, conducted in rosé wines, comparing extraction with and without the presence of SO_2 and considering a maceration period of 12 hours, are shown in table 1.

Table 1 - Comparison of phenolic extraction in two rosé wine made from the same grapes, varying SO2 concentration during maceration (Sudraud *et al*.., 1968)

SO_2 concentration	**Total phenolic index** (a.u.)	**Total Anthocyanins** (mg/L)	**Tannins** (mg/L)	**Colour intensity** (a.u)	**Tannin/anth. ratio**
None	11	26	320	0.52	12.3
10 g/hL	16	100	760	1.53	7.6

These results support that sulfiting musts is a good practice when more extraction is needed during maceration, but also when aiming to protect the musts from spoilage and oxidation.

Regarding temperature, Jackson (2008) mentions maceration under 20 °C retards microbial action, and Murat (2005) found the top of this range to be preferable, due to more effective extraction of an important fragrance precursor present in the skins (S-3-hexan-1-ol-L-cysteine). This compound's conversion into 3-mercaptohexan-1-ol is responsible for fruity aromas. Crachereau (2009) recommends the temperature is kept within this low range, to enable a settling phase and slow down colour diffusion.

The presence of pectolytic enzymes will also affect the resulting musts. Salinas *et al*. (2003) have found that the addition of these enzymes during maceration to improve flavour development and colour stability, however it required about 12h of maceration to produce significant differences in the studied wine, and this time is even longer when lower temperatures are used in these preparations. Such issues have caused this to not be widespread practice in rosé winemaking, but several authors have studied the employment of a relatively new technique; the use of pulsed electric fields, or PEF, which consists in applying external electric fields that increase the membranes' permeability, enhancing the diffusion processes and promoting extraction of anthocyanins and varietal aromas, tested in both red and rosé winemaking (Puértolas *et al*., 2010), making this a promising alternative for improving pre-fermentative maceration especially at lower temperatures when the use of enzymes is hindered.

1.1.3. Drawing-off *(Saignée)*

Prolonged skin contact is generally aimed at making a bolder rosé wine, while drawing off is aimed at producing a more concentrated red wine, by draining some of the liquid from the vat, thus producing a rosé from the drawn off juice, as a by-product, and replacing the removed liquid with more crushed grapes, increasing the phenolic content and colour of the remaining red wine (Ribéreau-Gayon *et al.,* 2006).

In this case, the grapes are crushed, stemmed and placed in a closed vat in low temperature, for a period of 10 to 36 hours (Ribéreau-Gayon *et al.,* 2006), following which the liquid is separated by drawing off from the vat. Analogous to the previous method, the maceration time should be determined by the desired characteristics for the final product, taking into account its previously mentioned effects.

Fermentation will then proceed similarly to musts obtained from direct pressing, without the presence of solid parts, producing a rosé that is typically bolder and with a stronger red pigment than a direct pressed wine, due to the maceration that takes place before the drawing off (figure 2).

Figure 2 - Comparison of two rosés made from the same grapes, one produced by direct pressing (Left) and the other obtained from *Saignée* (Right). Source: Vinous at *http://vinous.com/articles/the-rose-roundup-of-2015-apr-2015*

1.1.4. Carbonic maceration

Carbonic maceration is an alternative skin contact method, in which the intact berries are provided a CO_2 saturated atmosphere to conduct partial fermentation by the grape's native glycolytic enzymes, in anaerobic conditions taking advantage of naturally occurring phenomena (Boulton *et al*., 1995). This could be achieved with the addition of nitrogen; however, carbon dioxide is typically preferred due to being more readily absorbed by cytoplasm (Yurgalevitch and Janes,1988) and having shown to induce synthesis of grape pectinases which are maceration enzymes, promoting extraction (Jackson, 2008).

According to Flanzy (1998) there are three independent phenomena in carbonic maceration:

- Intact berries' natural anaerobic metabolism;
- Exchange by diffusion;
- Fermentations (naturally released must).

The berry's anaerobic metabolism is naturally triggered when quickly transferred to an oxygen deprived atmosphere, through pre-existing enzymatic systems, mainly alcohol dehydrogenase enzymes, which plays a key role in providing energy in anaerobic conditions (Sauvage *et al*.,1991).
As grapes are put in a CO_2 medium, they switch from a respiratory metabolism to a fermentative one. Intracellular grape fermentation is similar to that performed by yeasts, producing mainly ethanol, although in smaller amounts than yeast fermentation (Flanzy *et al.*, 1995). According to Jackson (2008), grape synthesis of ethanol rarely exceeds 2%. During this fermentative state, grapes absorb CO_2 depending on variety and temperature, but after a point of saturation they also release CO_2, due partly to intracellular fermentation (Flanzy *et al*., 1995). Grapes stop producing CO_2 when cells begin to die due to ethanol toxicity or insufficient energy supply, at which point all control of membrane movements stops, and the grape cells begin the release of several compounds, most importantly phenols. Carbonic maceration also progressively weakens the berries due to pectin breakdown (Jackson, 2008).
Up to 15-60% of malic acid is also metabolised into other acids and ethanol during grape cell fermentation, depending on grape variety and temperature, causing a decrease in acidity (Flanzy *et al*., 1995).

Carbonic maceration should be carried out in a shallow vat, which allows for easier loading, oxygen removal and CO_2 release, and vat loading should be carried out with care to preserve berry integrity. Manual harvest is recommended for the same purpose, and harvesting in sunny warm conditions allows for quick-starting grape cell fermentation (Jackson, 2008). Alternatively, berries may also be heated.
Temperature is one of the most relevant factors; ideally, the process would start at about 30-35 °C (Flanzy *et al*., 1995), but this may be harder to control than in conventional vinification, seeing as the content of the vat is mostly in solid form. Typically, the entire process lasts 6-8 days, up to 2 weeks, and duration depends on the temperature as well. For a rosé, André *et al*. (1980) recommend, for example, 36 hours at 35 °C, or 48 hours at 25 °C, for the initial phase. The binomial of duration-temperature can be used to manage the intensity of the aromatic effects of carbonic maceration.
After completing carbonic maceration, free run juice is drawn off the vat, and the grapes are then pressed, and both juices may be fermented together or separately, but should be cooled at a temperature of 18-22 °C. Fermentation is very quick and could be complete within 48 hours (Jackson, 2008).
This method produces fruity, smooth wines and, depending on variety, may mask or enhance their varietal character (Jackson, 2008). In production of rosés, anthocyanin and tannin extraction are controlled by keeping the grapes from submerging.

1.1.5. Fermentation and storage in wood

Few studies have been carried out on the impact of wooden cooperage in rosé wines, be it fermentation and storage in barrels or in contact with wooden chips or staves. However, this is a known practice, which may be increasingly employed, since the rosé market is growing and entering new niches, sparking interest of new consumer types and distancing itself from "feminine" or "low status" descriptions, or considered "unworthy of critical examination" by critics (Fitzmaurice, 2007). Entering larger and more "serious" markets, wooden products may be a vessel for creating rosés that stand out, while creating more complexity, however this is a lacking subject in published literature.

Bragança (2013) carried out an assay using several types of cooperage in rosés, providing wooden contact during fermentation and ageing, either in oak barrels of three different species (*Q. petraea, Q.pyrenaica* and *Q.alba*) or using wooden staves from both *Q. alba* and *Q. petraea*, separately. The author concluded that after 3 months of storage, wines fermented and aged in oak barrels had a more positive appreciation, and were considered more complex by the tasting panel, without bitterness or astringency, which would be mostly negative in a rosé, but these showed less improvement in colour intensity than the control wine and wines fermented and aged with staves.

More recently, Santos (2017) made a similar comparison in rosé wines, studying the effect of a 20-day contact during storage, both prior to fining and after fining, with chips of two types of oaks, as well as cherry and acacia wood. Cherry wood aged wines were given a significantly better overall rating in tasting, followed by acacia wood and the control wine. In this study's conditions, no significant difference was found in colour intensity, when compared to the control, but wood showed an effect in total phenol content, as would be expected due to the extractability of phenolic components from wood.

1.2. Rosé wine composition and maturation

Rosé wine composition is very variable, depending on wine types, from the light and fruity pinkish wines, with low phenolic contents, to deeper red colours high in anthocyanins and tannins.

Rosé wines have much lower phenolic (Blouin and Peynaud, 2001; Li *et al*., 2009) and anthocyanin content than red wines (Blouin and Peynaud, 2001) (table 2), and can have several differences among themselves depending on vinification methods (table 3).

Table 2 – Comparative phenolic composition, between a rosé and red wines (Blouin and Peynaud, 2001)

	Total phenolic index (a.u.)	**Total Anthocyanins** (mg/L)
Rosé	8-18	20-50
Young red wine	10-30	90-250
Red wine with aging potential	>40	>350

Table 3 – Phenolic composition, anthocyanins and colour of several French rosé wines (Ribéreau-Gayon *et al*., 1976). Colour intensity and hue are expressed in absorbance units.

	TPI (a.u.)	**Total Anthocyanins** (mg/L)	**Tannins** (mg/L)	**Colour intensity** (a.u.)	**Hue**	**Tannin/Anthocyanin Ratio**
Anjou				0.10-2.00	0.50-1.80	
Béarn	7-14	14-74	150-430	0.76-1.18		4.3-10.4
Bourdeaux Rosé	7-11	35-41	440-850	0.69-1.67		10.0-21.1
Bordeaux clairet	10-14	115-160	720-800	1.05-1.50		5.3-6.3
Côtes de Provence (Direct pressing)	7-11	14-55	80-320	0.38-1.19	0.80-1.98	5.6-15.8
Côtes de Provence (Free run)	7-15	11-62	63-270	0.51-1.76	0.58-1.62	2.1-7.8
Midi (Direct pressing)	10-14	13-35	180-320	0.63-1.19	0.80-1.17	5.6-15.8

The fruity young aromas associated with rosé wines are mostly fermentative, from compounds released during fermentation (Marais, 1983; Baumes *et al*., 1986). Research has shown the most relevant precursors of a rosés fruitiness to include ethyl esters, higher alcohol acetates, furaneol and the thiols 3-MH (3-mercapto-1-hexanol) and 3-MHA (3-mercaptohexyl acetate), as well as the norisoprenoid β-damascenone (Murat, 2005; Ferreira *et al*., 2009; Masson and Schneider, 2009; Álvarez-Pérez *et al*., 2012; Darici *et al*., 2014). The 3-MH thiol has been highlighted, and associated with grapefruit and passionfruit aromas. Consequently, the aromatic profiles of rosés depend on grape varieties, winemaking operations, compound diffusion conditions (maceration, for example) and yeast strains.

Both aroma and colour are very volatile during storage, and a rosé wine must be able to keep its qualities over time. Colour and stability during ageing are heavily influenced by anthocyanins extracted from skins and the processes of association and copigmentation that occur (Boulton, 2001). As such, rosés subjected to prior maceration may be considered more viable for ageing, since pre-fermentative maceration is known to enhance the presence of volatile and non-volatile compounds (Arnold and Noble, 1979; Marais and Rapp, 1988; Suriano *et al*., 2015), which are mainly present in skins and contribute to colour and aromas. These are important protective compounds which are usually lower in rosé wines than reds, justifying more sensitive colour compounds, as observed by Stavek *et al*. (2012). This author also found results supporting the role of storage temperature; room temperature caused a reduction of anthocyanin concentration of about 50% when compared to storage at 3 °C.

Colour intensity tends to decrease, as anthocyanins gradually polymerize (Del Álamo and Dominguez, 2006) resulting in a decrease of red colour (Roman *et al*., 2013). These changes persist after bottling, according to experimental results by Hernández *et al*. (2011), in rosés from different denominations, finding a decrease in the red-green coordinate (parameter a* in the CIELAB method). Meanwhile, parameter b* (yellow) increased, especially in the first 6 months in bottle. In an attempt to classify typical colourations, the authors observed similar evolution in all samples, from a “raspberry” colour to “strawberry” in 3 to 4 months, followed by “redcurrant” 6-8 months later. Two of the samples reached a “salmon” colour after 16 months.

2. Wood in enology

2.1. Oak composition and its impact on wine

Oak wood is undoubtedly the most traditionally used wood type in winemaking. Its genus has around 300 known species, and differences are found not only between species but also due to the geographical origin of the wood.

White oak heartwood is the most used for barrel production, mainly *Q. robur* L. and *Q. sessilis* L. in Europe, and *Q. alba* L. in America. These species' main geographical distribution is represented below, in figure 3.

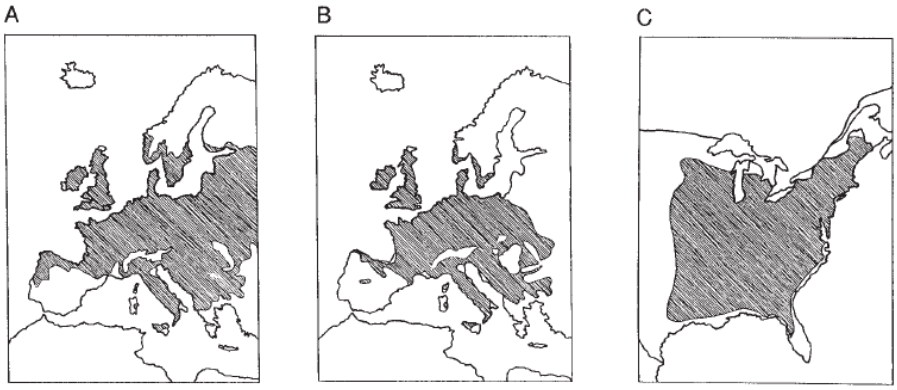

Figure 3 – Geographic distribution of (A) *Quercus sessilis,* (B) *Q.robur* and (C) *Q. alba* (in Jackson, 2008; Artwork by Herman Casteleyn.

The main components in oak wood are, as in other types of woods, cell wall constituents; cellulose, hemicellulose and lignin. Additionally, it contains other substances with lower molecular weight, extractible in hydroalcoholic solutions, such as benzoic and cinnamic compounds, hydrolysable tannins, gallic and ellagic acids and furanic compounds (Zhang *et al*., 2015), as well as lactones, the main volatile component in oak wood which slowly dissolves into wine (Jackson, 2008).These compounds strongly influence the aroma of wines, providing typical flavours of vanilla, spices, smoke and even berries, but also influence wine colour, contributing to its stabilisation due to the interactions between extracted ellagitannins and anthocyanins and flavanols (Barrera-Garcia *et al*., 2007).

More than 200 volatile compounds have been identified in oak, and even a small extraction can significantly affect the bouquet, unlike non-volatile compounds, which need to be absorbed in much larger portions to influence taste, appearance or mouth feel. According to Jackson (2008) wine absorbs about 30% of accessible tannins, which is enough to change the sensory characteristics.

The most relevant extractable substances in oak wood are ellagitannins, gallic and ellagic acid, aromatic compounds such as lactones, and aldehydes. In turn, oak wood adsorbs mostly water and alcohol, but

most importantly some wine aromatics (fruit esters) (Ramirez-Ramirez *et al*., 2004) reducing the fruity aromatic characteristic of young wines.

Regarding non-volatile phenols, oak tannins have an important antioxidant role, consequently promoting colour stability (Vivas and Glories, 1996). Oak wood is characterised by the presence of hydrolysable tannins, in majority ellagitannins (Chen,1970; Seikel *et al*., 1971) and gallotannins (Jackson, 2008), most of which correspond to vescalagin and castalagin (Jordão *et al*., 2016).

Ellagitannins have been found to significantly increase over wine ageing time (Navarro *et al*., 2016), however, this was not consistently observed in consecutive samples, possibly due to hydrolysation (Peng *et al*., 1991; Michel *et al*., 2011) and transformation (Jourdes *et al*., 2009) of these compounds, so the final considered results correspond to the equilibrium between the release from oak wood and its degradation/transformation, and that is where an overall increase was observed. Ellagitannins are intimately related to astringency (Glabasnia & Hoffman, 2006; Stark *et al*., 2010; Sáenz-Navajas, *et al*., 2012; Michel *et al*., 2013). A recent study by Chira and Teissedre (2014) found a 59% correlation between higher concentrations of this compound and astringent mouthfeel. Eight ellagitannins have been isolated in oak, most commonly vescalagin and castalagin, and these correspond to the majority of nonflavonoid compounds (Jackson, 2008). As the wood ages, polymerisation increases, although the process of toasting tends to hydrolyse the polymers on the contact surface, which is part of the reason only a small amount of ellagitannins is found in wines (Jackson, 2008).

The profile of extractible compounds in traditional oak woods for cooperage depends on several factors, namely the intrinsic composition of the wood, which varies between species, origins and silviculture parameters, and the processes of cooperage and winemaking. It is important to understand which compounds can be expected to be absorbed by and extracted from wood, when interacting with wines.

In comparison with European oak woods, American oak is typically richer in low molecular weight phenolic compounds, β-methyl-γ-octalactone and some volatile compounds, but poorer in extractible ellagitannins (Chatonnet and Dubourdieu, 1998).

Besides the inter-species variations in composition and mechanical properties, there is also an intra-species variation depending on both origin and even between trees, due to silviculture practices, climatic conditions and altitude. Alañon *et al*. (2011a) found significant correlation between higher altitudes and lower concentrations of volatile compounds. American oak is also denser and more resistant, with higher porosity. Results obtained by Doussot *et al.* (2002) support that oak cooperage choice should not be based on the wood characteristics or origin alone, but on a combination of both variables.

Q. pyrenaica has shown great results, showing higher aromatic intensity and complexity, as well as more woody, balsamic and cocoa aromas (Gallego *et al*., 2012) and spiciness (Bragança, 2013) than American or French oaks. A study by Fernández de Simón *et al*. (2009) compared volatile compositions of several woods, including Spanish, American and french oak (*Q. pyrenaica, Q. alba* and *Q.petrae* respectively), and found significantly higher levels of eugenol in Spanish oak.

2.2. Factors that affect wood contribution and interaction with wines

A few of the main factors which determine how wooden cooperage contributes to wine ageing have already been mentioned, such as origin of the wood, silviculture parameters, location, climate and the intrinsic composition and grain of the wood type and species, but additionally we can consider heating treatments, cooperage size, whether chips or barrels, oxygen intake, ageing time, the presence of lees and yeasts and proper care (barrels and cellar).

2.2.1 **Heating treatments –** Seasoning and toasting

Seasoning is the process through which the woods are dried, stabilizing their size, and also undergo a process of ageing due to rain leaching and microbial activity (Chattonet *et al*., 1994). This can be achieved naturally or artificially:

- Natural seasoning – Wood is placed in the open air, for 2-3 years, depending on stave size, undergoing intense dehydration for the first 10 months, followed by a maturation phase when the wood develops its organoleptic characteristics. The intensity and speed of seasoning varies depending on positioning in the wood pile (Doussot *et al*., 2002); the middle of the pile is less affected by rain or watering, and has a consistently lower humidity rate than the outer pile (Ribéreau-Gayon *et al*., 2006)
- Artificial seasoning – Wood cuttings are kept in a kiln, submitted to temperatures of 40-60 °C, during approximately one month (Ribéreau-Gayon et al., 2006)

Ribéreau-Gayon *et al*. (2006) suggest the wood's humidity should be in conformity with the surrounding atmosphere, approximately 14-18% in temperate regions, to ensure structural stability. Even though conditions are more difficult to control, natural seasoning is pointed out as the method providing the best quality to barrel aged wines, increasing the concentration of several aromatic compounds and reducing bitterness and astringency considerably, when compared to the artificial method, due to the hydrolysation of bitter glycosylated coumarins. The results of a comparison conducted by Chattonet (1995) found in Ribéreau-Gayon *et al*. (2006) are presented in the table below, for Limousin oak in a model wine solution (table 4).

Table 4 - Influence of seasoning method on oak composition a (adapted from Chatonnet, 1995 and Ribéreau-Gayon et al., 2006).

	Natural Seasoning	**Artificial Seasoning**
Total polyphenols (OD 280)	30.4	31.2
Flavanols (mg/L)	0.59	0.56
Ellagitannins (mg/L)	15.5	17.2
***cis*-β-methyl-octalactone**(µg/L)	12	0.85
***trans*-β-methyl-octalactone** (µg/L)	4.5	0.22
Eugenol (µg/L)	2	0.3
Vanillin (µg/L)	11	0.5

[a] Mean of 7 samples; compounds extracted in a dilute alcohol medium

These results, in conformance with those obtained by Spillman *et al*. (2004), support that natural seasoning has a significant difference in the extraction of aromatic compounds characteristic of wood ageing, previously mentioned, and also a higher proportion of the *cis* form of β-methyl-octalactone, which is the most odoriferous lactone (Ribéreau-Gayon et al., 2006). Literature is contradictory in this parameter; Maga (1989) obtained similar conclusions to Chatonnet (1995), represented in table 4. However, Sefton *et al*. (1993) found the same compounds to decrease. Oak is estimated to season about 10 mm per year, suffering intense dehydration in the first 10 months, followed by a maturing process that determines its quality. Rain leaching causes a decrease in ellagitannins (Vivas and Glories, 1996), which may be part of the reasons why artificially seasoned oaks have shown higher levels of this compound.

Toasting is the next step to conditioning wood before its use in cooperage. The staves cut from the seasoned wood are then used to assemble barrels, which are heated and toasted, interfering in the organoleptic contributions to wine. Temperatures on the inner surface of barrels typically reach around 200 °C after gradual rises for 20-30 minutes, during which the staves are bent into shape (heating phase), followed by toasting. Conditions vary between cooperages, but three types of toasting are considered: light, medium and heavy. Light toasting consists of subjecting the surface to temperatures between 120-180 °C for about 5 minutes; the wood appears spongy, but the cellulose structures remain intact. Medium toasting corresponds to a temperature of approximately 200 °C over 10 minutes, and heavy toasting has a duration of over 15 minutes, resulting in surface temperatures of up to 230 °C (Ribéreau-Gayon *et al*., 2006).

The general implications of toasting are based on processes of pyrolysis and thermohydrolisis, which affect the aromatic composition of wood. Depending on toasting level, these processes degrade wood components such as lignin (Sarni *et al*., 1990) hemicelluloses and ellagitannins, such as vescalagin and castalagin (Cadahia *et al*., 2001a), which are easily hydrolysed and first broken down into ellagic and then gallic acids. Desirably, toasting also degrades unsaturated aldehydes responsible for the saw-dust off-flavour found in wines aged in new barrels (Chatonnet and Dubourdieu, 1998). Toasting tends to reduce the amount of extractable ellagitannins, as observed by Navarro *et al*. (2016) who found wine samples aged for 12 months in light toasted oak to have a 3 times higher concentration of ellagitannins when compared to medium toasts, and 5 times higher than in heavy toasted oaks, when considering French oak. This difference was slightly smaller in American oak, at 2.5 and 4 times the concentration, respectively, confirming that there is heat degradation affecting extractable ellagitannins (Peng *et al*., 1991). Preceding the toasting process, ellagitannin loss can also occur due to rain leaching through the staves during natural seasoning (Vivas and Glories, 1996).

Light toasting is just enough to bend staves, leaving a natural woody aspect, and pyrolysis is limited, producing few by-products (Jackson, 2008).

Medium toasting generates some phenolic and furanilic aldehydes (Nishimura *et al*., 1983). This concedes toasty flavours due to hemicellulose breakdown and a vanilla roasted character from lignin derived phenolic.

Heavy toasting will begin a process of charring, halting or limiting the synthesis of phenolic and furanilic aldehydes, and instead producing volatile phenols, characterised by smoky spicy undertones, and progressively destroying compounds such as vanillin (Jackson, 2008). As previously mentioned, toasting will reduce extractable ellagitannins (Peng *et al*., 1991; Jordão *et al*., 2006; Navarro *et al*., 2016), as well as vanillin and syringaldehyde levels, which were found to increase during toasting, reaching maximum values between 195-215 °C, and then completely degrading when temperatures reached 250 °C (american oak) (Gimenez-Martinez *et al*., 1996).

According to Jackson (2008) this method is avoided in typical winemaking, since coal is responsible for discolouring red wines and stripping them of some desired aromas, but can be desired in the maturation of other spirits.

2.2.2. Barrel dimension

Wooden barrels come in many sizes and shapes. They are now mostly used for maturing wine with added complexity, however smaller sizes are also widely used for fermentation. The effect of size is intimately related with the contact surface area between the wine and the wooden staves, or chips, in fact, according to Singleton (1974), there is a logarithmic increase of surface area with the decrease of barrel size. Considering the use of wooden chips, surface area increases even more, which accelerates the process and influences all sorption behaviours from aromatic compounds, such as linalool and ethyl octanoate (Ramirez-Ramirez *et al*., 2004).

From an economic standpoint, larger barrels allow for a more efficient occupation in the winery, however, smaller sizes are favourable for handling and quicker maturation, due to more surface interaction between wood and wine. Using 200-250 litre barrels is most common, however many quality wines are matured in large cooperage of over 1000 litres.

2.2.3 Oxygen uptake

Oxidation is one of the main consequences of maturing wines in wooden barrels, which are generally protected from excessive oxidation, mainly occurring during winemaking operations such as racking, topping and sampling. In normal conditions, oxygen uptake during these operations has been estimated between 15 and 40 ml O_2 /litre/year. Tightly fitted bungs, preferably silicone, are important to ensure minimal exposure, however the minimal oxidation occurring during racking is often considered desirable, since it promotes the polymerisation of anthocyanins and tannins promoting colour stability (Jackson, 2008). Controlled oxidation affects the phenolic content of wines, contributing to more complex aromas, due to odoriferous substances extracted from the woods, as well as softer tannins.

Another important factor is the use of new or old barrels; a barrel that has been used 3 to 5 times will have reduced oxygen uptake, similar to that which occurs in stainless steel vats (Vivas, 1995).

2.2.4 Presence of lees and yeast

The presence of colloids, lees, yeast walls and other binding compounds is less mentioned in literature, but has an important role, in both maturation and fermentation stages in contact with wood.

When wines mature on lees, the released mannoproteins have the capacity of binding with phenolic compounds in wines and ellagic tannins from the wood, reducing their activity, thus softening astringency (Chatonnet *et al*., 1992).

When wines mature in wooden barrels, it is essential to keep contact with the lees, to serve as a reduction agent which helps keep oxidation in the desired levels, due to their own oxygen consumption (Feuillat, 1994) without developing oxidative off-flavours, preserving fruity younger aromas (Ribéreau-Gayon et al., 2006). According to Chatonnet *et al*. (1992) lees contribute to limiting wood aromas from overpowering the wines, supported by Jiménez Moreno *et al*. (2007) who also observed the binding of lees to oak volatile compounds, decreasing their concentration. The same authors also found eugenol, 4-propylguaiacol, 4-methylguaiacol, furfural and 5-methylfurfural to have the highest affinity with lees.

Mannoproteins are also thought to aid with protein and tartaric stability, by thermos-stabilizing the proteins responsible for turbidity (Moine-Ledoux and Dubourdieu, (1998), and they are also released by yeast autolysis, along with polysaccharides (Feuillat *et al*., 1989).

2.2.5 Cellar conditions

Wooden barrels are kept in cellars, with relatively low temperatures (16 °C) (Ribéreau-Gayon et al., 2006). When before or during fermentation, barrels should not be completely topped to avoid overflow, instead leaving about 10% headspace (Ribéreau-Gayon et al., 2006), and after fermentation is complete, consistent topping off is necessary as well as homogenisation of lees and yeast deposits – *bâtonnage*.

Cellar humidity is also an important factor, as well as ventilation. High relative humidity causes more ethanol to evaporate, consequently lowering alcoholic strength, while low humidity allows the natural evaporation of water to exceed that of ethanol, with an opposite effect. A relative humidity of 70% is a good reference (Margalit, 2012) where it would be safe to assume both water and ethanol evaporation are balanced, and the wine should maintain the expected alcoholic strength.

Ventilation in the cellar has a key role in the volume that does evaporate during winemaking, since air movement renews the micro-atmosphere around the barrel surface where evaporation occurred, creating a differential which promotes further losses.

If these precautions are not met and conditions are neglected, fermentation is going to be irregular between batches and quality will decrease, as well as productivity and profit.

2.3. Alternative wood products

In an effort to benefit from wood effects in wine ageing in a more economically viable manner, alternatives have been found and studied, namely wood dusts, staves and wood chips, previously approved by the OIV (Oeno Resolution 3/2005) and both authorized and regulated by the EC (EC Regulation 2165/2005 and 1507/2006, respectively.

Figure 4 - Oak wood alternatives; Small chips, large chips and dust. Fonte:www.theyachtcruwineguide.com/blog/2015/2/24/to-oak-or-not-to-oak

According to Singleton and Draper (1961), small oak chips (<1 mm diameter) allow for the removal of 90% of all extractable compounds within 1 week, which translates into quicker market entrance and less costly winery occupation. However, since the extraction is severely accelerated due to increased surface contact area, some aromatic compounds which typically result from slow ageing may take longer to appear (Jackson, 2008). Since inserting these fragments into containers will still lack the naturally occurring oxidation in barrel ageing, micro-oxygenation may be employed to better mimic the effects of the more traditional method (Pizarro *et al*., 2014; Oberholster *et al*., 2015). Without this process, using chips alone will not produce a wine with similar characteristics to long barrel ageing, but it still proves to be a viable and economic alternative to obtain young wines with slight olfactory and gustative woody notes, similar to those with short barrel ageing (3 months) (Ortega-Heras *et al*., 2010).

Del Álamo *et al*. (2008), when comparing red wines aged in barrels or contact with wooden alternatives of different oak types, found that generally, during the first six months of storage, those aged with staves acquired characteristics that were intermediate to the wines in barrels or with chips. As contact time increased, so did the discrepancies observed between barrel aged wines and vat aged wines (with chips and staves). This supports the viability of their use for shorter ageing periods, since these changes also grew during a 2 year bottled period, and the wines became completely distinguishable.

Franco Aladrén *et al*. (2007) found another interesting comparison factor, observing that wines aged in barrels had up to 5 times the concentration of whiskey-lactone, as well as eugenol and guaiacol, than

wines aged with chips, while the latter had higher levels of vanillin. Gutierrez-Afonso (2002) compared the sensory effects between a white wine ageing in barrels or with chips, and found that oak chips, regardless of species, had a greater impact on taste (astringency and bitterness) as well as wood aromas (coconut and vanilla).
Wood chips are typically added in enclosed netted polyester bags to facilitate their removal from the containers, although this limits compound extraction due to reducing the contact surface with wine.
Gallego *et al*. (2015) studied chip application in various stages, and obtained similar results. However these still suggested that performing this addition during malolactic fermentation leads to more pronounced effects. They are typically added during alcoholic fermentation, but may be placed in finished wines.

Using chips as alternatives to barrel ageing leads to reduced wine losses, eliminating the natural evaporation of the traditional method and allows for more efficient occupation of the winery and better temperature and hygiene control, while still conferring woody characteristics to wine, through short term ageing, such as oak, vanilla and spicy aromas, producing different wines with their own place in the market.

2.4. Potential alternative wood species for enology

Studying the potential of alternative woods for fermenting and ageing wine is a recent source of interest, considering mainly acacia (*Robinia pseudoacacia*), cherry (*Prunus avium)*, chestnut (*Castanea sativa*) and mulberry (*Morus alba* and *M.nigra*), among others, and aiming at weighing the differences relatively to oak wood. This helps assess whether these are viable alternatives, which may one day contribute to their widespread use, in turn bringing innovation to the wine market and contributing to lowering the oak demand and its ecological impact. Of these wood types, besides oak, only chestnut is allowed in winemaking.

2.4.1. Cherry *(Prunus avium)*

The heartwood of cherry wood can range from red to reddish brown in colouration, darkening with time and light exposure. Similarly to oak, it is a straight grain wood, characterised by medium resistance and density, as well as low stiffness. In comparison with oak wood, it is richer in lignin derivatives and poorer in lipids and carbohydrates (Fernández de Simón *et al*., 2014).
Cherry heartwood has been found to be more suitable for short term ageing, due to accelerated phenol oxidation (De Rosso *et al*., 2009a) and lower sensory scoring for wines aged 6 months and over in this type of wood (Fernández de Simón *et al*., 2014).

Regarding the phenolic composition, cherry wood has a wide variety of compounds, mainly considerable amounts of (+)-catechin, corresponding to about 90% of the quantified flavanols (Chinnici *et al*., 2015), condensed tannins of the procyanidin type and flavanones (most abundantly sakuranetin, naringenin and pinocembrin) (Chinnici *et al*., 2015; Jordão *et al*., 2016) which have been considered taxonomic markers for decades. However, another study has identified all of the flavanones mentioned above, except for sakuranetin (Sanz *et al*., 2012).

Although it remains a very abundant flavanol in this wood type, important comparisons regarding relative (+)-catechin values have been found. De Rosso *et al*. (2009a) found lowest values in cherry aged wines, especially after 3 months, in comparison with acacia, chestnut, mulberry and oak. Jordão *et al*. (2016) obtained other results, since in a comparison between cherry, acacia and 3 types of oak, (+)-catechins were even only quantifiable in cherry wood samples, although, in this case, the wine matrix was not a factor.
This compound has a remarkable deterioration over months of ageing, verified by Chinnici *et al*. (2015), who saw the value drop to nearly half from 2 to 4 months of ageing. In this same study, even at the 2-month mark, (+)-catechin values were always lower in cherry than oak.
Gortzi *et al*. (2013) reported the values correspondent to cherry aged wines were only surpassed by (+)-catechin values of white mulberry and apricot, which have a much less significant presence in literature, regarding cooperage, however also considering much lower ageing times (10 and 20 days). This compound is not only reduced by cherry wood's highly oxidative nature, but also by higher toasting levels (Sanz *et al*., 2010).

Toasting also causes a decrease in hydrolysable tannins, very characteristic in oak wood ageing, but with a very low presence in cherry wood, which combined with its lack of gallic and ellagic acids and ellagitannins establishes the very different composition between cherry and other cooperage woods, such as oak and chestnut, as it is mostly composed of flavanols, flavanones and condensed tannins (Alañón *et al*., 2011b). The presence of the aldehyde *p*-anisaldehyde both before and after toasting (Fernández de Simón *et al*., 2009; 2014), unlike in the other studied woods, has caused this to be suggested as a marker of cherry wood interaction with wines (Fernández de Simón *et al*., 2009). Since it has been found in other woods in small amounts, it cannot serve as an absolute marker.

Also in comparison with oak, cherry wood has resulted in faster colour stabilisation, higher colour density and generally better chromatic attributes (Chinnici *et al*., 2011) as well as less vanillin related aromas (vanilla, toasty, coffee) (Fernández de Simón *et al*., 2014), typically associated with wood ageing, due to relatively small amounts of this compound, in both seasoned and toasted woods (Fernández de Simón *et al*., 2009; Jordão *et al*., 2016). It is important to note that although colour may stabilise faster, the lack of ellagitannins and ellagic acid in cherry woods will cause faster browning, since these compounds are associated with reducing oxidation (Vivas and Glories, 1996), which also supports why, as previously mentioned and duly cited, cherry wood is considered a highly oxidative medium and poorly fitting for long term aging.

2.4.2. **Acacia** *(Robinia pseudoacacia)*

Native to North America, *Robinia pseudoacacia*, also known as false acacia, is characterised by a coarse grain, with strong irregular texture, which contrasts with oak and cherry (Carvalho, 1997), known for its durability, low porosity and resistance to insects (Roux and Paulus, 1962) and fungus, due to its heartwood's two dominant flavonoids; robinetin and dihydrorobinetin (Freudenberg and Hartmann, 1953). However, this varies with maturity, since the dihydrorobinetin flavonoid is more abundant in mature heartwood (Sergent *et al*., 2014).

The most abundant flavonoids in acacia are dihydrorobinetin and robinetin (Roux and Paulus, 1962;; Sanz *et al*., 2012b; Jordão *et al*., 2016) the latter being particularly present after toasting (Sanz *et al*., 2012a). These are considered characteristic to this wood type, since they were not detected in oaks, chestnut, cherry or mulberry (De Rosso *et al*., 2009a; Sanz *et al*., 2010; Jordão *et al*., 2016).
Regarding tannins, unlike oak and chestnut which show an abundance of ellagitannins, and cherry which mainly contains procyanidin types, tannins of the prorobinetin type are dominant in false acacia (Sanz *et al*., 2012b).

The presence of the phenolic aldehyde 2,4-dihydroxybenzadehyde is considered a marker of acacia interaction with wines over time (Fernández *et al*. 2009; 2013; 2014; Sanz *et al*., 2012b), along with their tannin profile. Sanz *et al*. (2012a) did not quantify this compound, but verified the presence of two aldehydes which could also be used as markers, since they were the only ones present even above medium toasting; gallic and β-resorcylic aldehyde. Phenolic markers will increase with ageing time, however, due to their concentration, it is possible to identify acacia aged wines as shortly as 2 months of ageing (Sanz *et al*., 2012b).
Vanillin is one of the most relevant compounds in wood aged wine, as it is responsible for its typical toasty and vanilla aromas. When compared to cherry, chestnut and oak woods, acacia has shown the lowest vanillin values (Sanz *et al*., 2012ab; Fernández *et al*., 2014) or second lowest, preceded by cherry (Fernández *et al*.,2009). Jordão *et al*. (2016) found lower values of vanillin in cherry and American oak than false acacia.

2.4.3. **Chestnut –** *(Castanea sativa)*

Chestnut wood was always widely available and used in barrels in the Mediterranean. Moreover, its interactions with wine have few focused studies. It has been found to be richer in volatile phenols such as isoeugenol, guaiacol and methylguaiacol, as well as vanillin and derivatives, when compared to traditional oak woods (Alañon *et al*., 2012), but it is also considered to be the most similar to oak when it comes to polyphenolic profile, being very rich in gallic acid and hydrolysable tannins (Salagoity-Auguste *et al*., 1987). Its larger content in low molecular weight phenols increases its antioxidant properties when compared to oak (Canas *et al*., 2008). When combined with its tannin content supports

why the main use of chestnut in winemaking is commercial tannin agents to protect musts from oxidation or improve astringency (Alañon *et al*., 2012).

De Rosso *et al*. (2009b) assess chestnut wood as a more oxidative ageing medium than oak, due to its higher porosity and, consequently, lower resistance to gaseous diffusion. Thus, it may be considered less appropriate for long term ageing. Alañon *et al*. (2013) also advise against long term ageing, due to undesirable concentrations of 4-ethylphenol and 4-ethylguaiacol, which may promote the appearance of off-flavours.

3. Objective of the study

The aim of this study was to evaluate the effects of wood chips, particularly oak and cherry, in the phenolic and sensorial profile of a rosé wine, throughout fermentation and storage. This should provide contribution to a lacking subject; the use of wood products in rosé winemaking and the viable use of alternative woods in order to innovate and preserve oak resources which are almost exclusively sought for this purpose.

To respond to this particular challenge, several essays were made in a semi-industrial scale, with a collaborative research work, using Touriga Nacional grapes provided by Casa da Passarella, where the production took place.

In this context, the alcoholic fermentation of red must from these grapes was carried out in contact with cherry and oak wood chips (separately), followed by an ageing process of 80 days, with and without contact with the wood chips.

4. Material and methods

4.1. Wine and wood materials

The wine used in this study is a rosé made in the Casa da Passarela winery in the Dão Region (Portugal), from Touriga Nacional grapes harvested in 2016, using direct pressing. Fermentation was carried out with no temperature control, in 1000L stainless steel vats, at cellar temperature (20 °C). After alcoholic fermentation, no fining treatments were made. Both the must and wine are included in this study.

The summary chemical analysis of this wine can be found below, in table 5.

Table 5 – General chemical analysis of the wine produced in this study Analysis carried out by IPViseu. MI methods are internal to the laboratory.

	Value	Units	Method
pH	3.04	—	OIV-MA-AS313-15:R2011
Total acidity	6.82	g tartaric acid /L	MI32-Edição 10 Rev. 1, 2014
Volatile acidity	0.27	g acetic acid /L	MI38-Edição 10 Rev.1, 2014
Glucose + fructose	< 0.3	g/L	MI07-Edição 10 Rev.3, 2016
Sucrose + glucose + fructose	0.3	g/L	MI08-Edição 10 Rev.3, 2016
Total SO_2	151	mg/L	MI37-Edição 10 Rev.1, 2014
Free SO_2	63	mg/L	MI35-Edição 10 Rev.1, 2014
Total dry extract	19.8	g/L	OIV-MA-AS2-03B:R2012
Alcoholic content	13.2	% vol	MI25-Edição 10 Rev.3, 2016
Copper	< 0.10	mg/L	MI02-Edição 10 Rev.3, 2016

The wood chips were purchased from AEB Bioquímica Portuguesa, SA (Viseu), both French oak (*Quercus petraea*) and cherry wood (*Prunus avium*), with a medium dimension (8 mm) and medium toast.

4.1.1. Experimental conditions

This experimental work was conducted on both must and wine, unfolding into 3 essays for musts, and 5 essays for wines. When applicable, the wood chip dosage was 1,5 g/L in 1000L vinification vats.

Alcoholic fermentation lasted 20 days, during which one must assay had no contact with wood chips (CONTROL), and two assays were in contact with cherry and oak wood chips (CH and OK respectively). Samples were collected in days 0, 2, 6, 8, 10 and 20 of fermentation.

Regarding the rosé wines produced, the control must remained as a control wine, having no contact with wood chips. The wood fermented musts were then split after alcoholic fermentation, into two different assays each, resulting in the following categories:

CONTROL	No contact with wood chips, prior to or post fermentation;
CHFA	Fermented and aged in contact with cherry chips;
CHF	Fermented with cherry chips; these were removed after AF and not present in ageing;
OKFA	Fermented and aged in contact with oak chips;
OKF	Fermented with oak chips; chips removed after AF and not present in ageing.

Wine samples were taken after 40, 60 and 80 ageing days. The diagram of the experimental model is shown in figure 5.

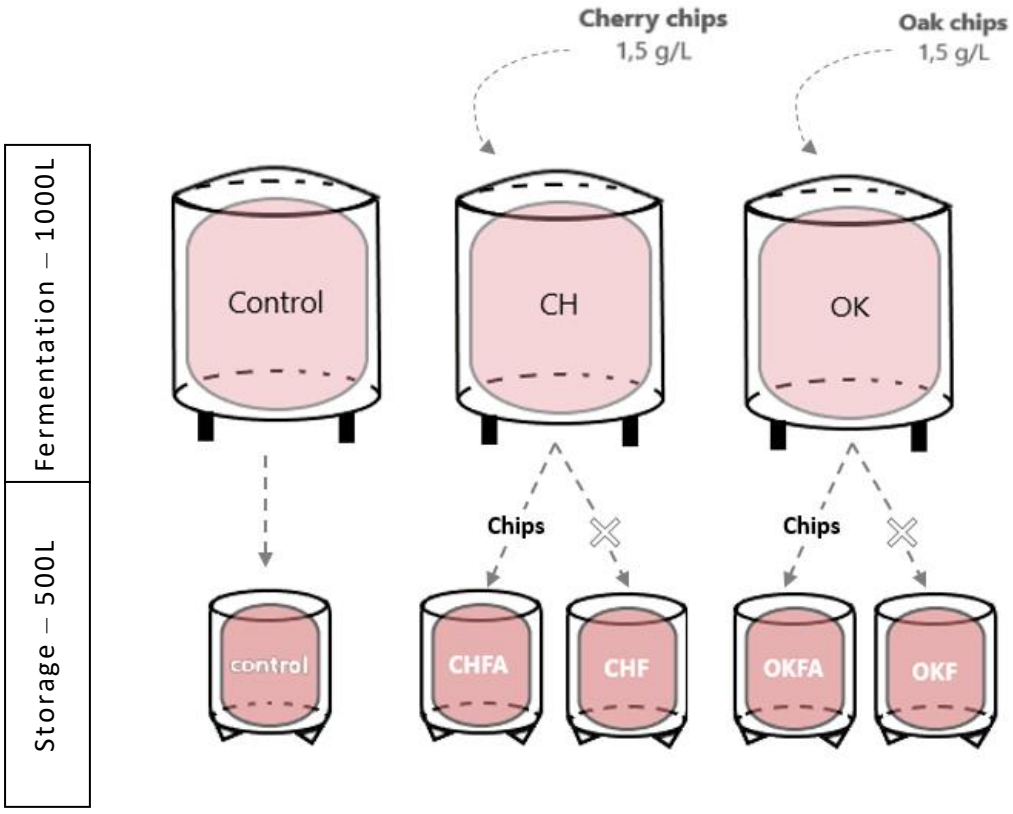

Figure 5 – Diagram representing the experimental conditions for this study. Legend: Control: control must and wine, CH: must fermented with cherry chips, OK: must fermented with oak chips, CHFA: wine aged with cherry chips, CHF: wine aged without cherry chips, OKFA: wine aged with oak chips, OKF: wine aged without oak chips

4.2. Chemical parameters

The analysis of chemical parameters was carried out in the Ferreira Lapa laboratory, in the Enology Sector of Instituto Superior de Agronomia. While certain parameters were analysed for both wines and musts, others were exclusive to wines – HPLC analysis of anthocyanins, catechins and proanthocyanidin and proanthocyanidin separation by degree of polymerization.

4.2.1. Total phenols; non-flavonoid and flavonoid phenols

The total phenol index (TPI) was measured as the absorbance at a 280 nm wavelength (A_{280}) of a centrifuged and diluted wine sample (Somers and Evans, 1977) (1:100 in wines and 1:50 in musts). Due to the previous dilution, the result is then multiplied by 100 or 50, in wines and musts respectively, and expressed in absorbance units, as the average of 3 repetitions.

$$TPI = A_{280} \times 100 \quad [Wines] \qquad \& \qquad TPI = A_{280} \times 50 \quad [Musts]$$

To calculate the amount of total phenols (TP) quantifiable from the absorbance, in mg of gallic acid per litre, a calibration curve was created, in order to correlate the absorbance value at 280 nm with the total phenol value expressed in mg of gallic acid per litre, obtaining the following:

$$TP = \frac{A_{280} + 0.0344}{0.038} \times 100 \qquad (mg\ gallic\ acid/L) \quad R^2 = 0.9962$$

The quantification of non-flavonoid phenols is based in that described by Kramling and Singleton (1969) and done in two phases, to both wine and must samples; first, the wine is mixed with a 1:4 HCl solution and formaldehyde at a 8 mg/ml concentration. The mixture is left to react in a dark environment, at room temperature, for 72 hours (red wine). Seeing as the studied wine is a rosé, the reaction was limited to approximately 48 hours. After this period, another dilution follows, with distilled water (1:10) and the respective absorbance is measured at 280 nm in the spectrophotometer. The value obtained is then multiplied by 10, due to the previous dilution. The value of non-flavonoid phenols is then calculated by subtracting the number obtained in this method from the number of total phenols. Three repetitions were carried out, for both musts and wines, and the value was expressed in absorbance units.

4.2.2. Colour intensity and hue

Colour intensity and hue were analysed through the OIV method MA-AS2-07B: R2009 (Type IV method) for chromatic characteristics, carried out for musts and wines. This method consists in measuring the absorbance at 420, 520 and 620 nm in a spectrophotometer, using a 1 cm optical cell, of the centrifuged samples of wine/must. Colour intensity is obtained by the sum of all three absorbance values, whereas colour hue is the ratio of absorbance at 420 nm to 520 nm.
Samples were analysed in triplicate, and the results are expressed in absorbance units (Abs).

$$Intensity = A_{420} + A_{520} + A_{620} \qquad (a.u.)$$

$$Hue = \frac{A_{420}}{A_{520}} \qquad (a.u.)$$

4.2.3. Anthocyanins and polymeric pigments

Spectrophotometry measurements in wines and musts

Total and ionised anthocyanins, as well as well as total and polymeric pigments, were determined according to the method described by Somers and Evans (1977), which consists in noting the absorbance of a clear wine sample at 420, 520 and 620 nm, in a 1 mm cell, and then adding 5 μL of sodium metabisulphite to the same cell, mixing by inversion, and note the absorbance at 520 nm after a 1-minute reaction time, allowing the decolourisation of anthocyanins. This value will be represented as $A_{SO^2,520}$.
Separately, another measurement is necessary, obtained from adding 100 μL of wine to 10 ml of HCl at 1M concentration, and allowing for a reaction time of 3 to 4 hours, assuring this occurs at room temperature, using a water bath at 25 °C. The absorbance is then measured at 520 nm in a 10 mm cell, against a reference cell of HCl. This result is represented as $A_{HCl,520}$ and corrected with a multiplication by 101.
Because the studied wine is a directly pressed rosé wine, and not a red wine, some changes were made; all measurements were made in a 1 cm cell, thus 50 μL of sodium metabisulphite were added in the first step instead. Three repetitions were carried out for each measurement.
According to the mentioned authors (Somers and Evans, 1977) the parameters can then be quantified through the following equation.

$$Total\ anthocyanins = 20\left(A_{520}^{HCl} \times 101\right) - \frac{5}{3} A_{520}^{SO^2} \qquad (mg/L)$$

In this case, since $A_{SO^2,520}$ was measured in a 1 cm cell, no correction factor is necessary. If a 1 mm cell is used, this value must be multiplied by 10.

$$Degree\ of\ ionised\ anthocyanins = \propto = \frac{A_{520} - A_{520}^{SO^2}}{A_{520}^{HCl} - \frac{5}{3} A_{520}^{SO^2}} \times 100 \qquad (\%)$$

$$Ionised\ anthocyanins = \frac{\propto}{100} \times Total\ anthocyanins \qquad (mg/L)$$

The degree of ionised anthocyanins and its respective concentration in the samples is an indicator of the percentage of total anthocyanins which are in their coloured form (flavylium). This varies remarkably with pH; higher pH values are associated with a lower degree of pigmented anthocyanins.

The method described above is also used to quantify total and ionised pigments, which are involved in the red colouration of wines, and consist of polymeric anthocyanins and polymers of anthocyanins and tannins (Boulton, 1999).

$$Total\ pigments = A_{520}^{HCl} \times 101 \qquad (a.u.)$$

$$Polymerization\ index = \frac{A_{520}^{SO^2}}{A_{520}^{HCl} \times 101} \times 100 \qquad (\%)$$

$$Polymeric\ pigments = A_{520}^{SO^2} \qquad (a.u.)$$

Individual anthocyanins analysis by HPLC

Monomeric anthocyanins in wine samples were quantified individually through an HPLC system - High performance liquid chromatography – Perkin Elmer Series 200LC- Pump, connector UV/VIS LC-95 and 7725i injector, at 520 nm absorbance, and the column was a Lichrocart 100, RP18 col. 5μm, 250mm * 4 mm. Solvent A was 40% formic acid, B was acetonitrile and C was bidistilled water. The program started at 25%A, 6% B and 69% C for 15 minutes, followed by a linear gradient to 25% A, 25.5% B and 49.5% C for 70 minutes. This methodology is based on a paper by Dallas and Laureano (1994).

Although other anthocyanins were present in some wine samples in residual amounts, the peaks considered quantifiable for this study were; delphinidin-3-O-monoglucoside, petunidin-3-O-monoglucoside, peonidin-3-O-monoglucoside, malvidin-3-O-monoglucoside, malvidin-3-O-monoglucoside-acetate and malvidin 3-O-monoglucoside-coumarate.

The injected sample volume was 20 μL and all samples were filtered and analysed in duplicate repetitions.

4.2.4. Chemical age

A wine's "chemical age", or polymeric pigment index, can be seen as an indicator of its ageing rate, which can be influenced by many variables in the winemaking process, altering its ageing characteristics. A higher value represents a wine that expresses more of the characteristics associated with ageing, namely the ratio between polymeric pigments and total pigments, which is used to quantify this parameter, as the extent to which polymeric pigments have become dominant and decreased the presence of anthocyanins, a natural occurrence during aging, as observed by Somers and Evans (1977). This feature is expressed in absorbance units.

$$Chemical\ age = \frac{A_{520}^{SO^2}}{A_{520}^{HCl}} \qquad (Abs)$$

4.2.5. Colour due to copigmentation

Copigmentation phenomena has proven to have a significant role in colouration, and first considered in enology in a study by Somers and Evans in 1974. It can be defined as the combination of anthocyanins with other components in a wine medium, such as flavonoid phenols. These associations can cause the pigments to express more colouration than expected, and can account for 30-50% of the colour in young wines, heavily influenced by the presence of non-coloured phenolic cofactors (Boulton, 2001). This parameter reflects the estimated increase in colour due to copigmentation associations, and is expressed as a percentage.

Colour due to copigmentation (CC) was determined by first adding 0.1 ml of acetaldehyde to 10 ml of wine, agitating the solution and allowing to react for 45 minutes, in order to release anthocyanins combined with SO_2, which is known to cause a bleaching effect. The absorbance of this solution is read at 520 nm after the reaction time, in a 1 mm cell representing the $A_{a,520}$ value. In this study, a 10 mm cell was used, thus this value does not require correction due to the dilution factor.

A second dilution is then carried out, at a 1:25 proportion with a hydroalcoholic solution, which will dissociate the anthocyanin-cofactor complexes, after a 45-minute reaction time. The absorbance is then read at 520 nm, in a 10 mm cell, thus obtaining the $A_{b,520}$ value, which must be multiplied by 25.

In this study, it was determined for both musts and wines, in triple repetitions for each sample.

$$CC = \frac{A_{520}^{a} - (A_{520}^{b} \times 25)}{A_{520}^{a}} \times 100 \qquad (\%)$$

4.2.6. Proanthocyanidins

Individual monomeric and small oligomeric proanthocyanidins

The separation of individual catechins and procyanidins was carried out using a polyamide column for each fraction, and then each compound was identified through HPLC, according to the method described by Ricardo da Silva *et al*. (1990). For each wine sample, 15 ml were directly fractioned through sequential elutions in the polyamide column; first using 80 ml of buffer solution (pH=7) to eliminate interferents such as phenolic acids, then 50 ml of acetonitrile to elute catechins, followed by 50 ml of acetone to elute oligomeric procyanidins. Elutions were occurring extremely slowly, thus the buffer solution and acetonitrile were sometimes pushed to leach through, using air compression, and the acetone was always left to leach overnight, which may have compromised the extraction.

Samples were then analysed by HPLC (Merck Hitachi Model L-7100 Pump with Waters 2487 dual absorbance detector) with an injection volume of 50 μL and a Lichrocart 100, RP18 col. (5μm, 250mm * 4 mm) column.

The solvents for catechins were (A) acetic acid/bidistilled water (2.5:97.5 v/v) and (B) acetonitrile/solvent A (80:20 v/v). The program ran with 93% of A and 7% of B for 26.1 minutes, followed by 88% of A and 22% of B for 90s and a "washing" period of 15 minutes running methanol/water (50:50 v/v).

For oligomeric procyanidins, solvents were (A) acetic acid/bidistilled water (10:90 v/v) and (B) bidistilled water, and the program started at 10% A and 90% and reaching 70% A and 30% B within the first 45 minutes, followed by another gradient from 90% A and 10% B during 25 minutes and the same washing period. All solvents were prepared at least weekly, and subjected to filtration through 45 μm filters and ultrasounds before being connected to the equipment.

The compounds included in this study, considered to have had quantifiable peaks, were (+)-catechins, (-)-epicatechin and procyanidins B1, B2, Trimer T2 and B-2,3-O-galate. Measurements were made only in rosé wines, with 4 repetitions in each wine sample, and expressed in mg/L of B1, except for B2 which is expressed as mg/L of B2.

Quantification of proanthocyanidins according to degree of polymerization

Proanthocyanidins were separated into 3 fractions; monomers (catechins), oligomers and polymers (F1, F2 and F3, respectively) using the method described by Sun *et al*. (1998). Fractioning was achieved with successive elutions trough C18 Sep Pak cartridges, and the assay was carried out by diluting each fraction in methanol and measuring the results of its reaction with vanillin.

Wine samples (30 ml) were centrifuged and evaporated in a rotative evaporator, under vacuum, mixed with 20 ml of buffer solution (pH=7) and added to the cartridges, which were preconditioned with a sequence of 3 elutions (10 ml of methanol, 20 ml of distilled water and 15 ml of buffer solution). After the wine sample leaches through the cartridges, these are left to dry with N_2 for approximately 1 hour.

The monomer and oligomer fractions are extracted with a 25 ml of ethyl acetate elution, and the polymers were eluted with 15 ml of methanol. To separate the monomeric and oligomeric fractions, the

elution is evaporated under vacuum, dissolved with 3 ml of buffer solution, redeposited into the cartridges and again left to dry in with N_2 for an hour, following which F1 and F2 are separated with an elution of 25 ml of diethyl ether and 15 ml of ethanol, respectively.

After all fractions are separated, they are evaporated in vacuum and diluted with 3 ml of methanol (F1 and F2) and 5 ml of methanol (F3), in preparation for the vanillin assay.

The monomeric catechin fraction (F1) reacted with vanillin at 30 °C for 15 minutes, followed by spectrophotometry reading of the absorbance at 500 nm, which is then used to determine the amount in mg/L, using the following equation (considering 30 ml as the initial sample volume and 3 ml the volume of methanol in which the dry extract was diluted)

$$F1 = \frac{3 \times A_{500}}{0.0081 \times 30} \qquad (mg/L)$$

The oligomer fraction (F2) is placed in the spectrophotometer immediately after vanillin is added, and allowed to react for about 40 minutes, during which the reaction curve is drawn. After the reaction time, the maximum absorbance achieved before stabilization will be the considered value in the following equation, taking into account 30 ml of initial wine sample and 3 ml of methanol dilution.

$$F2 = \frac{3 \times A_{500}}{0.0046 \times 30} \qquad (mg/L)$$

Finally, the polymer fraction (F3) is assayed with an analogous procedure to F2, however the reaction time is approximately 90 minutes and the methanol dilution was 5 ml.

$$F3 = \frac{5 \times A_{500}}{0.0037 \times 30} \qquad (mg/L)$$

All wine samples were assayed with 2 repetitions, however some samples required a third. Results represent an average, and were expressed in mg/L.

4.3. Sensory analysis

The sensory classification of wines was carried out by a panel of expert judges in the Polytechnic Institute of Viseu, in a sensory analysis room under room temperature conditions. Wine was served at random following a blind tasting principle.

Three separate tastings were carried out, for all three storage durations considered in this study (40, 60 and 80 days). In each tasting, panellists were asked to rate, on a scale of 1 to 5, several descriptors for visual aspects, aroma, taste and overall quality of the five different rosé wines; CONTROL, CHFA, CHF, OKFA and OKF. A list of all included descriptors is represented below (table 6).

Table 6 – Sensorial descriptors included in the tasting sheet used in this study

Visual	Colour intensity
	Clarity
Aroma	Intensity
	Persistence
	Quality
	Red Fruits
	Wood
	Floral
	Vegetal
	Balance
Taste	Acidity
	Sweetness
	Bitterness
	Persistence
	Astringency
	Balance
Overall	

4.4. Statistical analysis

The aim of this study, identifying the effects of different wood chip species during fermentation and ageing of musts and wines, required an analysis of variance (ANOVA) and Tukey tests, through which statistical significance between samples was identified.

The values presented for each parameter correspond to an average of its repetitions, and significant differences between means was assessed by Tukey's test at $p<0.05$. All statistical treatments were performed in Microsoft Excel and IBM SPSS version 24.

5. Results and discussion

5.1 Must analysis during alcoholic fermentation

5.1.1 Total phenols, non-flavonoid and flavonoid phenols

In musts (figure 6), total phenolic content showed close resemblance in all samples, decreasing slightly from 2 to 8 fermentation days, followed by an apparent stabilisation. Cherry chips (CH) were responsible for the highest values in every data point, which was especially significant at 0 and 20 days. In fact, considering start and finish of alcoholic fermentation, the rates at which total phenols increased and decreased were similar for all musts. This resulted in a significantly richer phenolic content for musts fermented in contact with wooden chips, especially cherry wood, relatively to the control. The same applies to total phenol in mg/L as this value is obtained from the TPI.

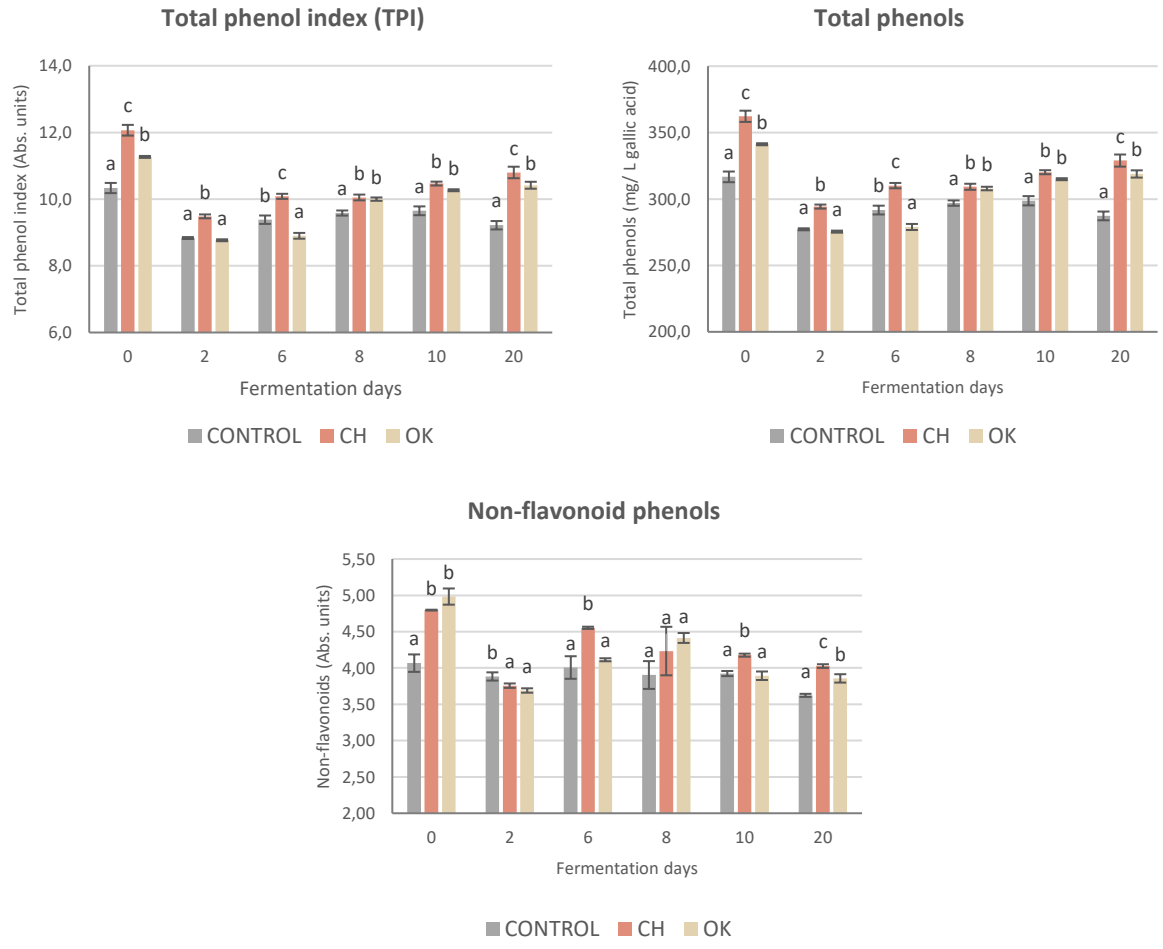

Figure 6 - Evolution of total phenols and non-flavonoids during alcoholic fermentation of a rosé must in contact with different wood chips. Legend: **CONTROL:** Control must; **CH:** must fermented in contact with cherry chips; **OK:** must fermented in contact with oak chips. Mean values with the same letter indicate no statistically significant differences (Tukey's test at $p<0.05$)

The increase of TPI is expected when wooden materials are used (Gortzi *et al.*, 2013), due to the polyphenol extraction which is known to occur between the contact surface with wood and the liquid

matrix. This effect has been verified by some authors in wines (Gonçalves e Jordão, 2009; Gortzi *et al*., 2013). However, generally, lower results are expected for cherry wood since it tends to have a lesser phenolic composition (De Rosso *et al*., 2009a; Sanz *et al*., 2010; Alanõn *et al*., 2011). The irregularity of the pattern is normal since polyphenol extraction from woods does not tend to follow a necessarily linear increase or decrease (Karvela *et al*., 2008). Also in figure 6, non-flavonoid evolution in musts is shown, expressed in absorbance units. In musts, similarly to TPI, there was a marked decrease within the first 2 days of fermentation. At 0 days, musts appeared to contain a higher index when in contact with wood chips. During fermentation, fluctuations occurred and at the end of the 20 fermentation days, the must in contact with cherry wood chips (CH), which has also shown a higher TPI, had a significantly superior value for non-flavonoid phenols, and appeared to maintain a higher proportion of non-flavonoids throughout the first stages of fermentation, relatively to the other samples.

5.1.2 Total anthocyanins and pigments

In musts, total anthocyanins were determined solely by spectrophotometry, along with their ionization degree, and the results are represented below in figure 7.

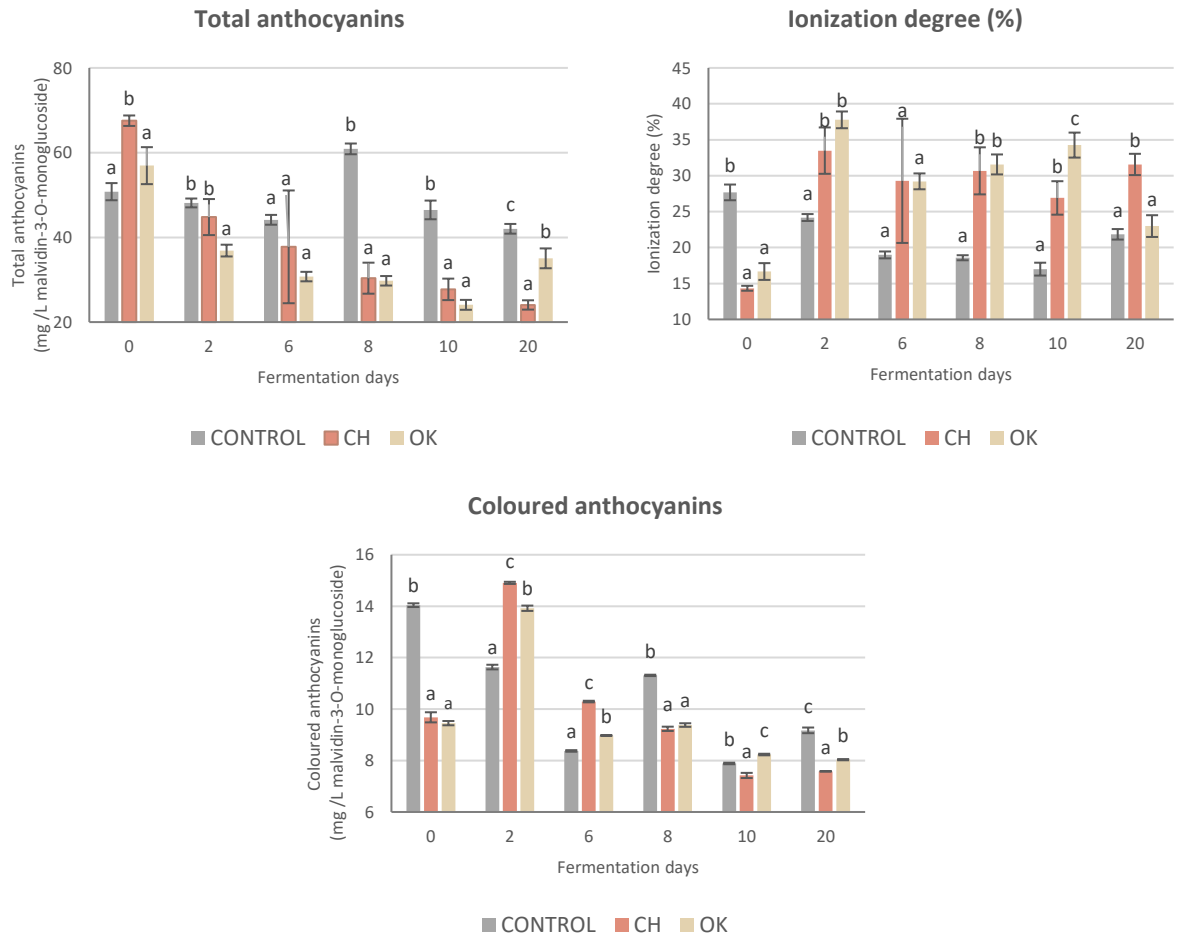

Figure 7 - Evolution of total and coloured anthocyanins during alcoholic fermentation of a rosé must in contact with different wood chips. Legend: **CONTROL:** Control must; **CH:** must fermented in contact with cherry chips**; OK:** must fermented in contact with oak chips. Mean values with the same letter indicate no statistically significant differences (Tukey's test at $p<0.05$)

Total anthocyanins decreased during the 20 days of alcoholic fermentation, achieving the lowest result of 24.059 mg/L for the must fermented with cherry chips, followed by the must in contact with oak chips (35.049 mg/L) and the control must (42.022 mg/L). These can be considered low levels of anthocyanins, however a must produced by direct pressing has very low contact time with skins for anthocyanin extraction, since they are mainly present in grape skins (Monagas and Bartolomé, 2009). The wood fermented musts tended towards a decrease, where the control must had less of an observable trend, and more fluctuations.

The ionization degree, or percentage of coloured anthocyanins (flavylium form) was very high for all musts; this usually happens in low pH conditions, but that parameter is not known. At the end of fermentation, the must fermented with cherry chips showed the highest percentage of coloured anthocyanins (31.6 %). However, the total value was so low it also had the lowest quantified amount of coloured anthocyanins (7.6 mg/L), followed by the must fermented with oak chips (8.033 mg/L) and the control must (9.173 mg/L).

According to Monagas and Bartolomé (2009), anthocyanin concentration reaches its maximum after a few days of fermentation, then decreasing due to adsorption, precipitation, condensation and degradation processes, and ellagic acid has shown to contribute to these processes, mainly through the reduction of malvidin-3-glucoside and other monomeric anthocyanins, in a study comparing the presence of oak wood with a standard wine (Jordão *et al*., 2016).

Total pigments (figure 8) decreased steadily over the 20 days for the must fermented with cherry chips, showed a decrease then slight increase between 10 and 20 days for French oak, and the control must maintained an overall stable value.

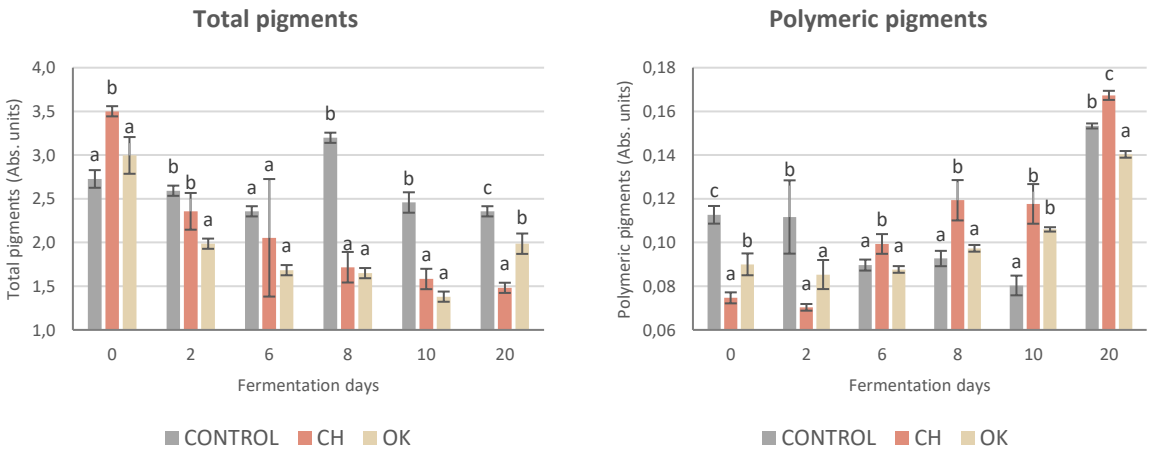

Figure 8 - Evolution of total and polymeric pigments during alcoholic fermentation of a rosé must in contact with different wood chips. Legend: **CONTROL:** Control must**; CH:** must fermented in contact with cherry chips; **OK:** must fermented in contact with oak chips. Mean values with the same letter indicate no statistically significant differences (Tukey's test at $p<0.05$)

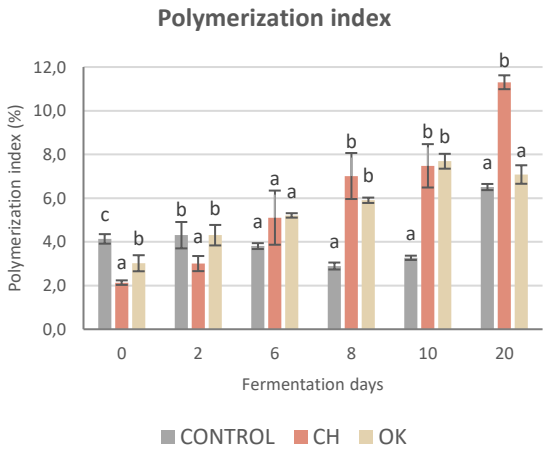

Figure 9 - Evolution of polymerization index during alcoholic fermentation of a rosé must in contact with different wood chips. Legend: **CONTROL:** Control must**; CH:** must fermented in contact with cherry chips; **OK:** must fermented in contact with oak chips. Mean values with the same letter indicate no statistically significant differences (Tukey's test at $p<0.05$)

After the 20 fermentation days, all musts were significantly different among themselves, with the control showing the highest amount of total pigments (2.36 a.u.), which was expected due to also having the higher value in total anthocyanins and flavonoids, included in this parameter, followed by the must fermented with oak chips (1.99 a.u.) and the must fermented with cherry chips (1.48 a.u.). However, the cherry chips induced the highest proportion (11.3%) (figure 9) and amount of polymeric pigments (0.167 a.u.), indicating better colour stability. This may be supported by Chinicci *et al*. (2015), who observed, in aged red wines, that although cherry wood is highly oxidative towards wine phenols, it also promotes a faster and more efficient stabilisation of pigments.

5.1.3 Colour parameters – intensity and hue

Considering figure 10, it is observed that colour intensity started off significantly higher in the control must, having suffered fluctuations that appear more pronounced than those of the musts fermented with wooden chips, which may be related to the effect wood has on colour stability, since according to Barrera-Garcia *et al*. (2007) this is due to the interactions between anthocyanins and flavanols and ellagitannins, which are more prominent in oak wood, and cherry has even shown a more efficient colour stabilization than oak (Chinnici *et al*., 2011). At the end of fermentation, the control must showed higher colour intensity, followed by cherry wood and oak wood. Regarding hue (figure 10), or shade, this parameter is related to the ratio between the absorbance at 420 nm and 520 nm, which means that from start to end of fermentation, all of the musts decreased their absorbance at 520 nm, which is dominant in red wines, and corresponds to the colour red, resulting in a less intense red hue, but this increase in hue was most remarkable in the cherry fermented musts.

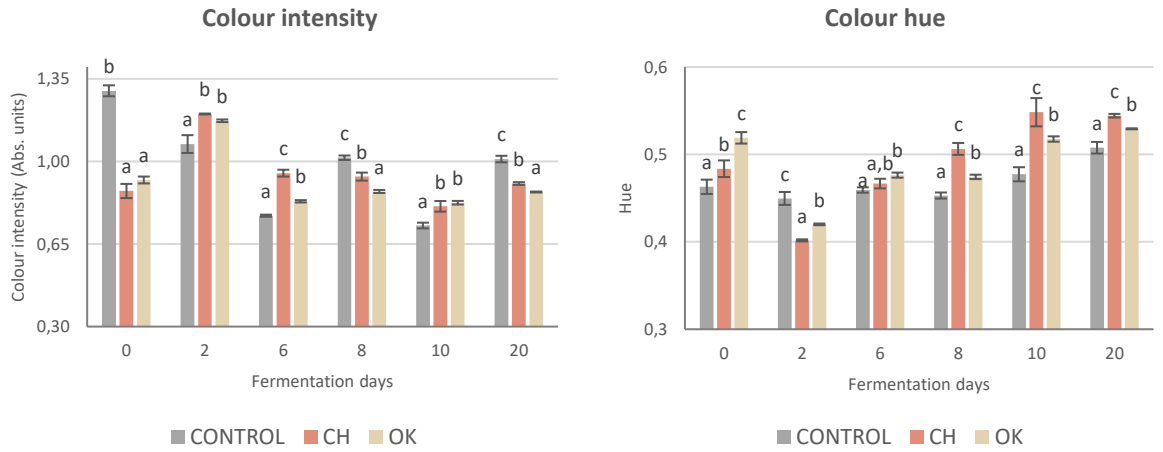

Figure 10 - Evolution of colour intensity and hue during alcoholic fermentation of a rosé must in contact with different wood chips. Legend: **CONTROL:** Control must; **CH:** must fermented in contact with cherry chips; **OK:** must fermented in contact with oak chips. Mean values with the same letter indicate no statistically significant differences (Tukey's test at $p<0.05$)

5.1.4 Chemical age

Chemical age (figure 11) is a feature which reflects how a certain wine or must has developed characteristics which are characteristic of wine ageing over time, or their ageing rate, and it is intimately related to the stabilization of pigments by polymerization. It was detected that cherry chips induced the highest value after 20 days (0.113 a.u.), as they also promoted pigment polymerization to a significant higher degree than oak chips and the control must, both of which showed no statistical significance among themselves in the last fermentation day. During the first 10 days, there is an observable increase for the must fermented with oak chips, followed by a slight decrease, however the dominant trend shows it accelerated the stabilization of the must initially, relatively to the control, only to have achieved similar results after all 20 days. These values are in conformity with Somers and Evans (1977) who observed this rate to be between 0.02 and 0.2 in red wines aged for about 8 months.

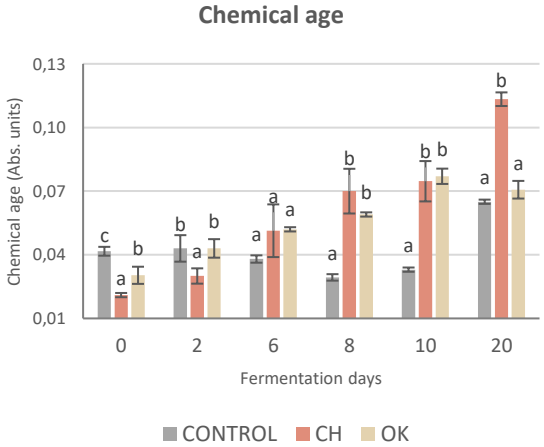

Figure 11 – Evolution of chemical age during alcoholic fermentation of a rosé must in contact with different wood chips. Legend: **CONTROL:** Control must; **CH:** must fermented in contact with cherry chips; **OK:** must fermented in contact with oak chips. Mean values with the same letter indicate no statistically significant differences (Tukey's test at $p<0.05$)

5.1.5 Colour due to copigmentation

The results of the assay of colour due to copigmentation can be found in figure 12, where it is observable that the process was extremely significant in must colouration. In wines, copigmentation has been reported to account for 30-50% of a young red wine's colour (Boulton, 2001). This reaction requires a certain anthocyanin concentration, as it involves both those compounds and copigments such as flavonoids extracted from skins and seeds. In presence of a must obtained by direct pressing, these are very high values. Copigmentation tended to decrease and, once again, the control mostly suffered fluctuations when compared to the musts fermented with wood chips. At the end of fermentation, the control showed the highest value (61%), followed by the must fermented with cherry chips (44%) and the must fermented with oak (33%), all significantly different.

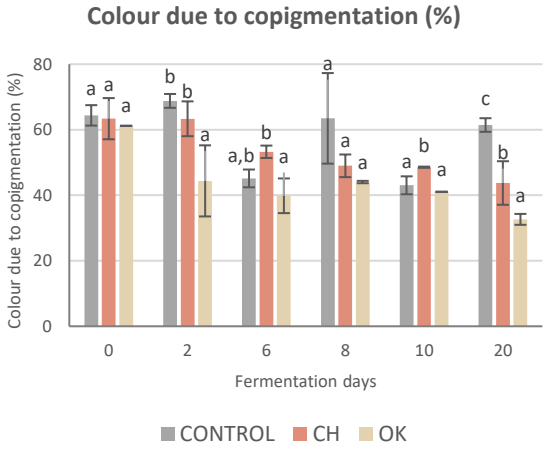

Figure 12 - Evolution of colour due to copigmentation during alcoholic fermentation of a rosé must in contact with different wood chips. Legend: **CONTROL:** Control must; **CH:** must fermented in contact with cherry chips; **OK:** must fermented in contact with oak chips. Mean values with the same letter indicate no statistically significant differences (Tukey's test at $p<0.05$)

5.2. Wines analysis during storage

5.2.1 Total phenols; non-flavonoid and flavonoid phenols

The cherry fermented/aged wines showed higher levels of total phenols throughout storage (11.7 to 12 TPI) , whereas the wines fermented and aged with oak chips were associated with the lowest values (around 10 TPI) and very little variation between sample dates, as observed below in figure 13, as well as always being statistically different from the other 3 wines.

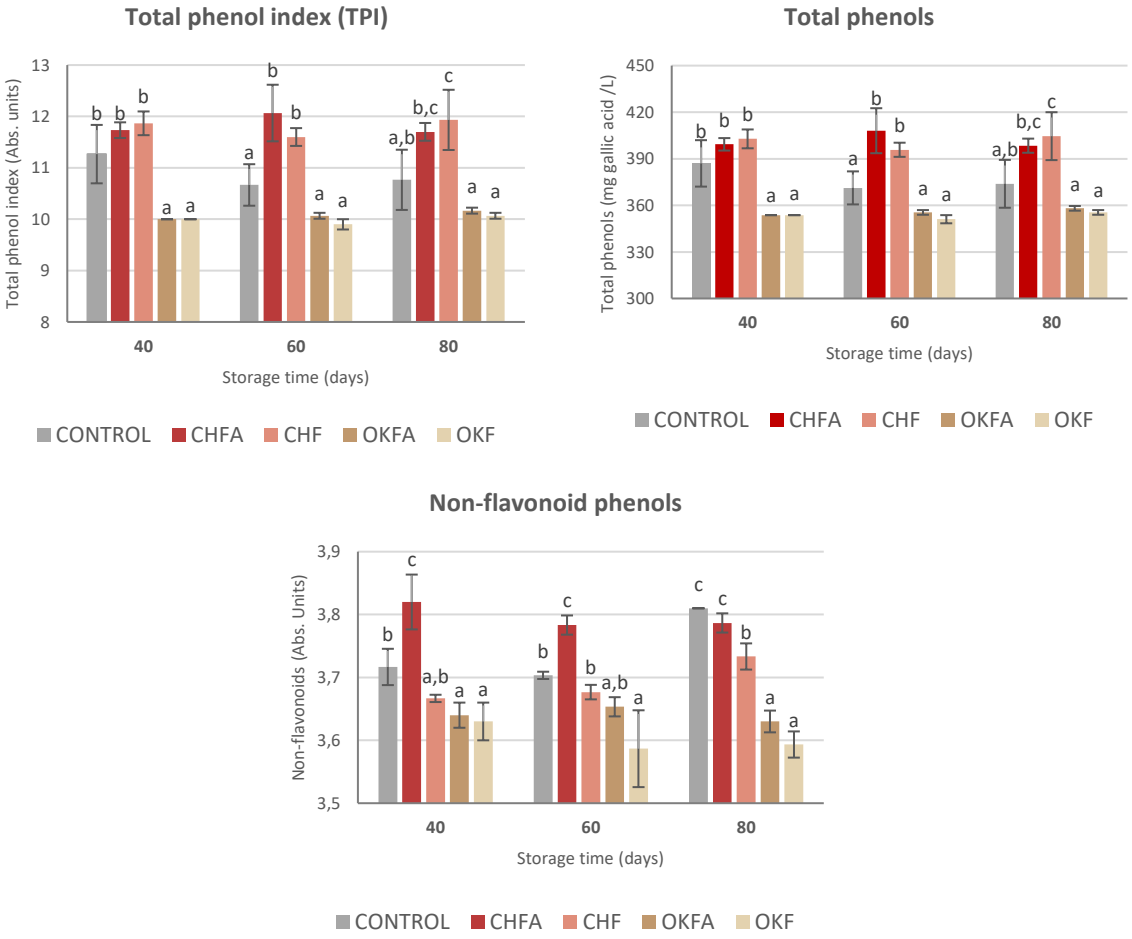

Figure 13 - Evolution of total and non-flavonoid phenols during storage of a rosé wine in current and/or previous contact with different wood chips. Legend: **CONTROL:** Control wine; **CHFA:** wine fermented and aged with cherry chips, **CHF:** wine fermented with and aged without cherry chips, **OKFA:** wine fermented and aged with oak chips, **OKF:** wine fermented with and aged without oak chips. Mean values with the same letter indicate no statistically significant differences (Tukey's test at $p<0.05$)

This is not expected since several studies have shown oak wood to have a higher phenolic content than cherry, in rosés and red wines (De Rosso *et al*., 2009a; Alañon *et al*., 2011b) however Santos (2017) found similar results to this study, with cherry wood promoting a significantly richer phenolic content than a standard wine and one aged with oak, at 20 days. Tavares (2015) found no significant differences,

suggesting that study conditions may have conditioned the results. It would be expected that wines in contact with wood chips would show higher values of total phenols than the control, and this was not the case in the present study.

These values can be considered within the norm for a rosé wine, taking into account the references suggested by Blouin and Peynaud (2001) and Ribéreau-Gayon *et al*. (1976) (8-18 and 7-15 TPI, respectively).

Regarding non-flavonoids, both wines in contact with oak chips again showed lower values throughout all storage periods, and cherry aged/fermented wines had significantly higher values until being statistically matched by the control wine after 80 days. Before that, from 40 to 60 days cherry always showed a significantly higher value.

It is important to note that non-flavonoid values were always higher in the wines fermented and aged with chips, when comparing to those only fermented with the same wood, suggesting an effect of contact time, however this only proved statistically significant for the comparison between CHFA and CHF (both cherry chip assays).

5.2.2 Total anthocyanins and pigments

The results obtained from the spectrophotometry method for total and coloured anthocyanins, as well as ionization degree, are shown in figure 14.

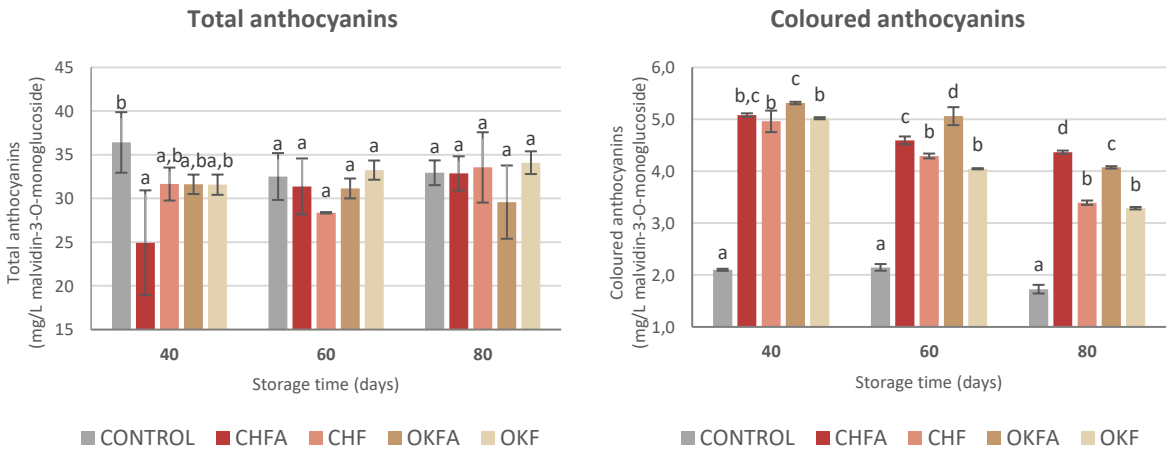

Figure 14 – Evolution of total and ionized anthocyanins during storage of a rosé wine in current and/or previous contact with different wood chips.. Legend: **CONTROL:** Control wine; **CHFA:** wine fermented and aged with cherry chips, **CHF:** wine fermented with and aged without cherry chips, **OKFA:** wine fermented and aged with oak chips, **OKF:** wine fermented with and aged without oak chips. Mean values with the same letter indicate no statistically significant differences (Tukey's test at $p<0.05$)

Total anthocyanin values varied from 24.9 mg/L (CHFA wine at 40 storage days) to 36.4 mg/L (CONTROL at 40 days). These are low values, considering a range of 20-50 mg/L (Blouin and Peynaud, 2001), however, Kelebek *et al*. (2007) verified that a longer maceration time translates to higher

anthocyanin concentrations, and since the wine assayed in the present study was obtained by direct pressing, low values were expected. Ribéreau-Gayon *et al*. (1976) found a range of 7-50 mg/L of total anthocyanins in this type of rosés. The variation of total anthocyanins was not particularly remarkable with the control wine or the other samples, which shows a lack of degradation or other hindering processes for these compounds, at least between the 40 and 80 days. There was no statistical difference between all samples at 60 and 80 storage days.

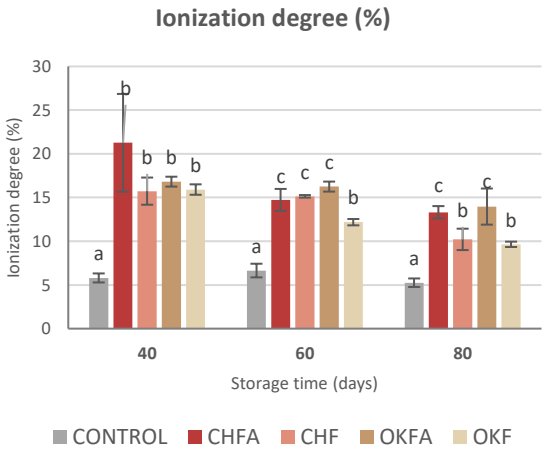

Figure 15 - Evolution of total and ionized anthocyanins during storage of a rosé wine in current and/or previous contact with different wood chips.. Legend: CONTROL: Control wine; CHFA: wine fermented and aged with cherry chips, CHF: wine fermented with and aged without cherry chips, OKFA: wine fermented and aged with oak chips, OKF: wine fermented with and aged without oak chips. Mean values with the same letter indicate no statistically significant differences (Tukey's test at $p<0.05$)

Ionized anthocyanins, or coloured forms, were always significantly less present in the control wine, again showing the effects of wood chips on the anthocyanin equilibrium, as well as of a very low pH (3.04) which contributed to the high ionization degrees observed in general (5% to 21%) (figure 15), when compared, for example, with the results obtained by Santos (2017). This author found a much lower value for non-fined wines (1.5% to 3%) in a rosé wine with a pH of 3.23, which also had a considerably higher amount of total anthocyanins (80 mg/L to approximately 140 mg/L), all assayed after 20 storage days, so a storage time factor may also be in place.

The chip contact time may have had some effect since at 80 days of storage, because the wines that fermented and aged with chips (CHFA and OKFA) had significantly higher values of coloured anthocyanins (4.37 and 4.07 mg/L, respectively).

Individual anthocyanins were assayed by HPLC as previously described, resulting in the distribution shown in figure 17.

As expected, malvidin-3-O-monoglucoside was, out of the quantified peaks, the most dominant anthocyanin, with no significant differences between samples, however the values varied between 3.9 mg/L (CHFA at 80 days) and 5.5 mg/L (OKF at 80 days). Considering the spectrophotometry showed total anthocyanins between 24.9 and 36.4 mg/L, it may seem like there is a discrepancy and the dominant anthocyanin should be in higher concentrations. Moreover, it is still the most predominant one among the quantified peaks (see figure 16), and the HPLC method considers only monomers, whereas spectrophotometry may also quantify dimers and the 5/3 correction is only empirical. These types of discrepancies have also been found for example in a study by Dallas (1998). In fact, the total quantified anthocyanins by HPLC was a far lower value than that observed by the first method, ranging from approximately 4.9 to 7.1 mg/L.

Literature shows that there is a decrease of monomeric anthocyanins during wine ageing (Somers, 1971), due to reacting with colourless phenols, as well as chemical and enzymatic degradation, and other precipitation and condensation processes (Dallas *et al*., 1996; Castellari *et al*., 2001; Matejícek *et al*., 2005; Jordão *et al*., 2006; Gonçalves and Jordão, 2009; Roman *et al*., 2013; Gallego *et al*., 2015), and this decrease may be especially noticeable when using oak woods (Gonçalves and Jordão, 2009; Gallego *et al*., 2015) and more pronounced as a drop of malvidin-3-O-monoglucoside in these cases. This did not happen in the present study, despite a robust report in others, however this study had a concentration of 1.5 g/L of chips whereas, for example, Gallego *et al*. (2015) used a concentration of 3-6 g/L. Wood chip concentration may be a factor, but even then, it was expected that monomeric anthocyanins would decrease during ageing; the dominant monomer, malvidin-3-O-monoglucoside, did tend to decrease if slightly in the case of the control wine and the wine fermented and aged with cherry chips (CHFA). However, oak seemed to tend to a very mild increase in both samples (OKFA and OKF). This is contrary to literature which showed that the decrease of monomeric anthocyanins was especially perceived in oak aged wines, mostly visible for malvidin-3-O-monoglucoside (De Conninck *et al*., 2006; Jordão *et al*., 2006) and malvidin-3-O-monoglucoside-coumarate (De Conninck *et al*., 2006).

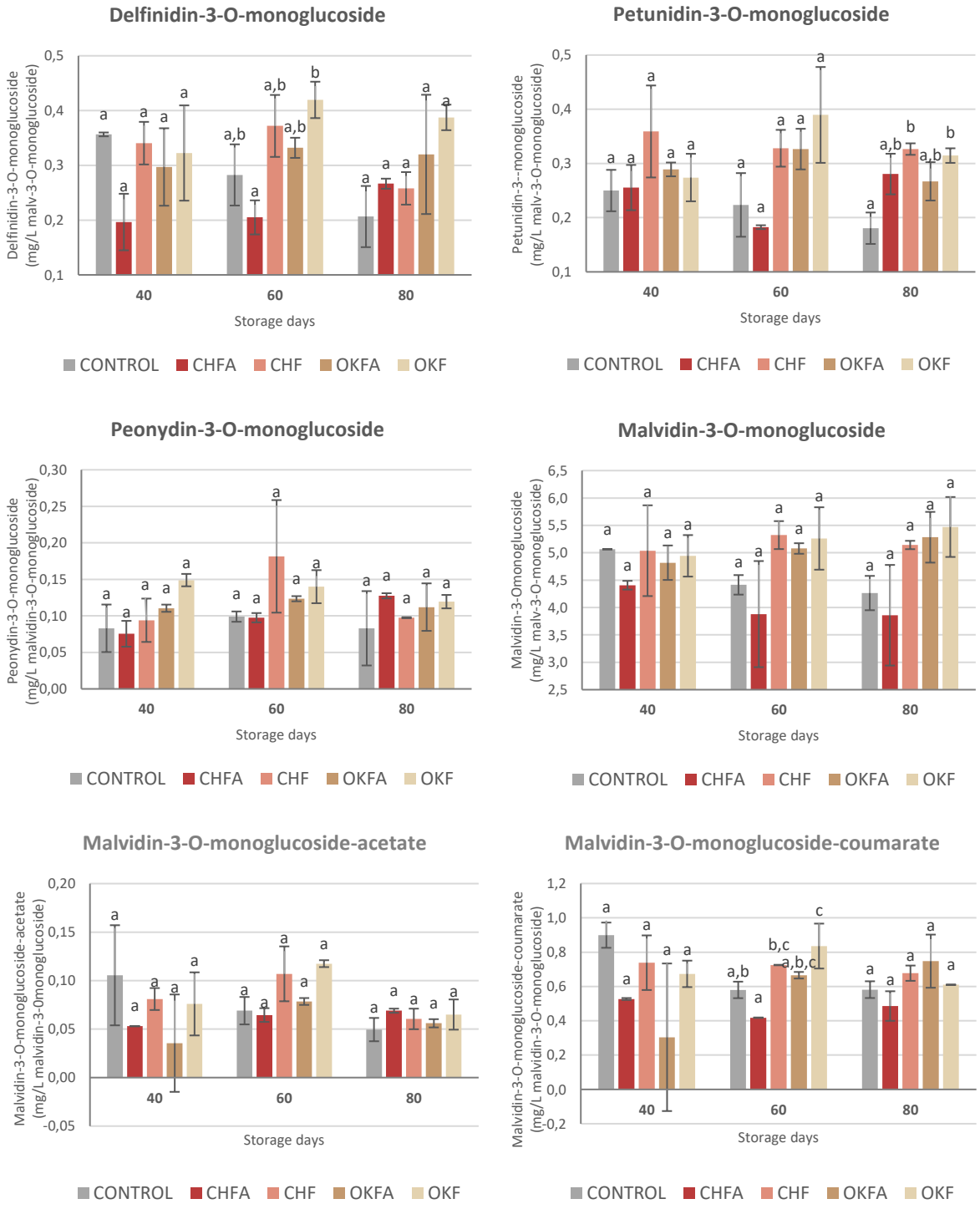

Figure 16 – Evolution of monomeric anthocyanins during storage of a rosé wine in current and/or previous contact with different wood chips, determined by HPLC. Legend: **CONTROL:** Control wine; **CHFA:** wine fermented and aged with cherry chips, **CHF:** wine fermented with and aged without cherry chips, **OKFA:** wine fermented and aged with oak chips, **OKF:** wine fermented with and aged without oak chips. Mean values with the same letter indicate no statistically significant differences (Tukey's test at $p<0.05$)

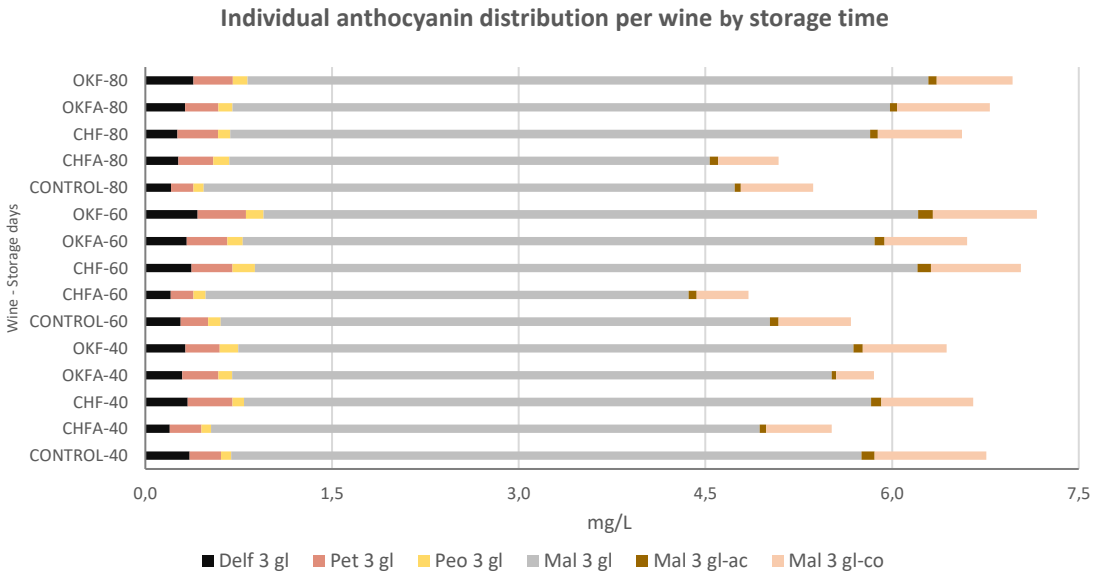

Figure 17 – Distribution of quantified individual anthocyanins, in mg/L, as determined by HPLC, during storage of a rosé wine. Legend: **CONTROL:** Control wine; **CHFA:** wine fermented and aged with cherry chips, **CHF:** wine fermented with and aged without cherry chips, **OKFA:** wine fermented and aged with oak chips, **OKF:** wine fermented with and aged without oak chips; **Delf 3 gl:** delfinidin-3-O-monoglucoside, **Pet 3 gl:** petunidin-3-O-monoglucoside, **Peo 3 gl:** peonidin-3-O-monoglucoside, **Mal 3 gl:** malvidin-3-O-monoglucoside, **Mal 3 gl-ac:** malvidin-3-O-monoglucoside-acetate, **Mal 3 gl-co:** malvidin-3-O-monoglucoside-coumarate. Wines are represented as (Assay-Storage period in days)

Total pigments and their polymerization was also assayed for wines, and the results are represented in figures 18 and 19.

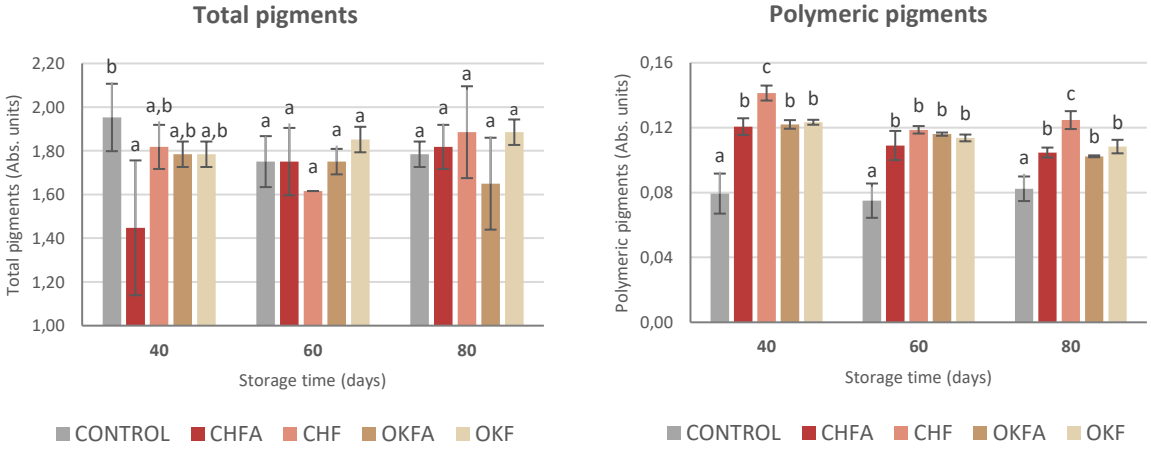

Figure 18 – Evolution of total and polymeric pigments during storage of a rosé wine in current and/or previous contact with different wood chips. Legend: **CONTROL:** Control wine; **CHFA:** wine fermented and aged with cherry chips, **CHF:** wine fermented with and aged without cherry chips, **OKFA:** wine fermented and aged with oak chips, **OKF:** wine fermented with and aged without oak chips. Mean values with the same letter indicate no statistically significant differences (Tukey's test at $p<0.05$)

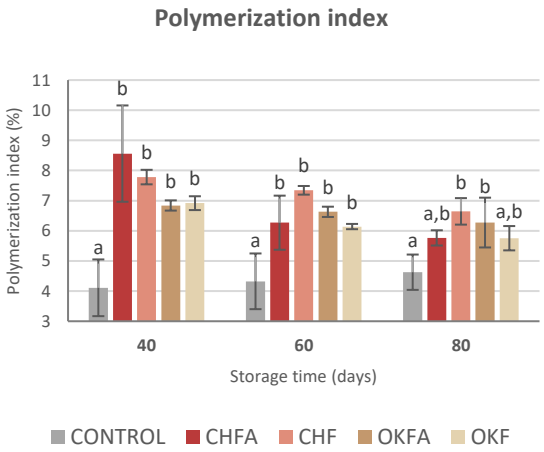

Figure 19 – Evolution of polymerization index during storage of a rosé wine in current and/or previous contact with different wood chips. Legend: **CONTROL:** Control wine; **CHFA:** wine fermented and aged with cherry chips, **CHF:** wine fermented with and aged without cherry chips, **OKFA:** wine fermented and aged with oak chips, **OKF:** wine fermented with and aged without oak chips. Mean values with the same letter indicate no statistically significant differences (Tukey's test at $p<0.05$)

At 60 and 80 storage days, there was no statistical difference between all wines in terms of total pigments. However, after the first 40 storage days, the only single wine that stood out as having a higher concentration was the control, with 1.95 a.u., which also had the highest value at the end of fermentation, although being statistically similar to other samples. As storage time progressed, the results did not show dramatic change and the wines remained very similar.

Unlike the results of musts, these show that there was no statistical difference between the effects of oak chips or cherry chips, as well as contact time, since all 4 assays showed statistical similarity in polymerization index, even though at 80 days CHF and OKFA stood out (6.65 and 6.27 a.u., respectively), but wood always showed to induce more polymerization than the control wine, which, again, is supported by the work of Chinnici *et al*. (2015), previously mentioned, regarding the impact of oxidative behaviour from woods in the stabilisation of colour and pigments, which is reflected by this parameter. These results are similar to those obtained by Santos (2017) who found that the control wine had significantly less polymerization, while finding little discrepancy between oak and cherry chips, however with very different values.

5.2.3 Colour intensity and hue

Typically, colour intensity will decrease during storage, due to the drop in red colour which is dominant (Del Álamo and Dominguez, 2006; Hernández *et al*., 2011;Roman *et al*., 2013) and also a decrease in monomeric and co-pigmented anthocyanins (Roman *et al*., 2013). Contrary to the most reported result, there is also indication that colour intensity should tend towards an increase during storage; a study by Ribéreau-Gayon *et al*. (1983), in red wines varying in aeration, showed that poorly aerated wines were the only ones that maintained or decreased their colour intensity.

Observing figure 20 it is clear that the contact with wood chips promoted colour intensity in the wines.

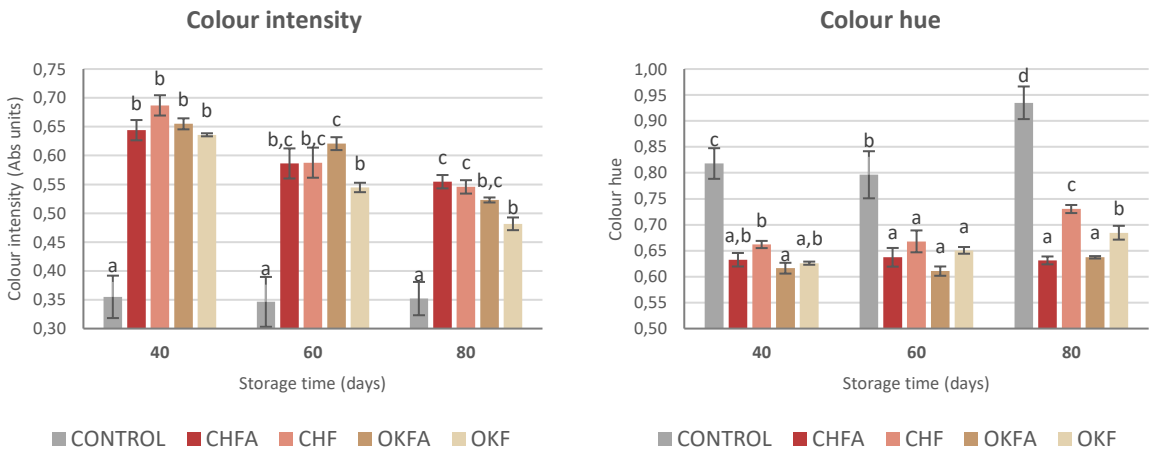

Figure 20 – Evolution of colour intensity and hue during storage of a rosé wine in current and/or previous contact with different wood chips. Legend: **CONTROL:** Control wine; **CHFA:** wine fermented and aged with cherry chips, **CHF:** wine fermented with and aged without cherry chips, **OKFA:** wine fermented and aged with oak chips, **OKF:** wine fermented with and aged without oak chips. Mean values with the same letter indicate no statistically significant differences (Tukey's test at $p<0.05$)

The control always showed significantly and visibly lower values, which can be deduced from a higher polyphenol content in wines which had absorbed polyphenols from woods, contributing to colour development through the polymerization of anthocyanins. During wine ageing, authors have verified that the values of absorbance at 520 nm tend to decrease, while those at 420 nm and 620 nm increase, which is caused by the same process (Del Álamo and Dominguez, 2006; Hernández *et al*., 2011;Roman *et al*., 2013). There was, in fact, a decrease in A_{520} for all wines, but, in this case, the other absorbances showed no absolute tendency to increase, leading to the conclusion that this was mostly due to a decline in the absorbance reflecting red colour, meaning these wines developed lighter colours in storage. In all storage periods, contact with wood chips showed a positive impact in this parameter, with no significant difference between the 4 samples at 40 days, but after 80 days the wines fermented and aged with cherry chips had the highest values, with statistical significance.

Regarding colour hue, the variations were not as marked as with musts. The decline of A_{520} should result in an increase of colour hue throughout the time, however A_{420} also decreased in a more pronounced manner in wines, translating into very small variations, except for the control wine, which showed the highest observed value (0.94 a.u. after 80 days), meaning it had a considerably lacking absorbance at 520 nm (red tone) comparing with the rest, and also it did increase over time in a more remarkable way thus suggesting a bigger loss of this colour component and less colour stability. This reinforces a positive effect of wood chips on wine colour, which translated into lower values of hue.

Considering only the wood aged/fermented wine samples, at 40 and 60 days the differences were not remarkable, but by the end of 80 days there was a significant difference between CHF wine (0.73 a.u.), OKF wine (0.69 a.u) and the other samples that kept contact with chips after fermentation, and showed lower values.

5.2.4 Chemical age

As previously mentioned, this feature is important to understand the ageing rate of wines, which is intimately related to the polymerization of pigments. This can be supported by observing figure 21 and establishing an analogy with the polymerization index graphic in figure 19. The control kept a younger character throughout storage, relatively to the wines fermented and aged with wood.

After the first 40 days, the difference was more marked especially in the wines fermented and aged with cherry chips, explained by this wood type's fast oxidative behaviour (Chinicci *et al*., 2015) which promotes polymerization. However, this showed no statistical difference from the other samples which aged and/or fermented with oak chips. In the next 20 days, there was a decrease of chemical age, and at 80 days these wines obtained with wood were approaching the value of the control wine (statistical marker a) especially CHFA and OKF (statistical marker a,b) whereas OKFA and CHF remained significantly superior (marker b). The control wine had a slight increase, contrarily to the other samples. Somers and Evans (1977) suggest a reference of chemical age feature between 0.02 and 0.2 in red wines aged for 8 months, so the values obtained in this study are included, and perhaps justified, since this is a direct press rosé with little extraction and a maximum storage of less than 3 months, placing in the lower half of this range. These results can be questioned due to the fact that even though a more aged profile was expected in samples which had contact with wood, it is not intuitive that chemical age would drop during storage, or present lower values than those of musts which indeed showed a rising pattern.

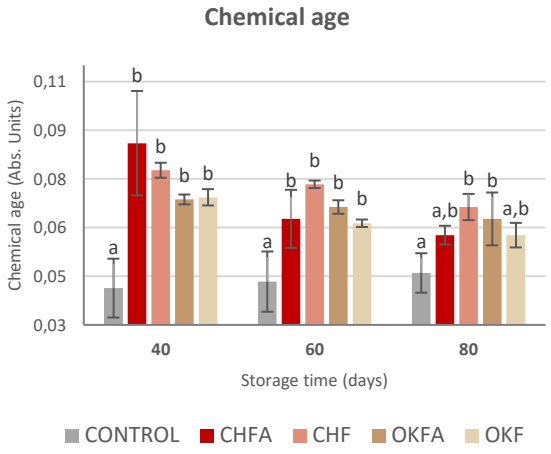

Figure 21 – Evolution of chemical age feature during storage of a rosé wine in current and/or previous contact with different wood chips. Legend: **CONTROL:** Control wine; **CHFA:** wine fermented and aged with cherry chips, **CHF:** wine fermented with and aged without cherry chips, **OKFA:** wine fermented and aged with oak chips, **OKF:** wine fermented with and aged without oak chips. Mean values with the same letter indicate no statistically significant differences (Tukey's test at $p<0.05$)

5.2.5. Colour due to copigmentation

The values obtained in this study, represented in figure 22 are extremely high, higher than often found in red wines, ranging from 35.5% (CHF at 40 days) to 43.7% (CONTROL at 60 days). Bragança (2013) also found very high levels in a rosé, ranging from 32 to 35%.

Boulton (2001) reports this parameter may be accountable for 30-50% of a young wine's red colour, but also defends the role of anthocyanin and copigment concentrations promoting the process, which were not high in this study. At 40 and 80 days, no statistical significance was found, but the CONTROL and CHFA showed higher values throughout storage, as OKFA caught up at 80 days.

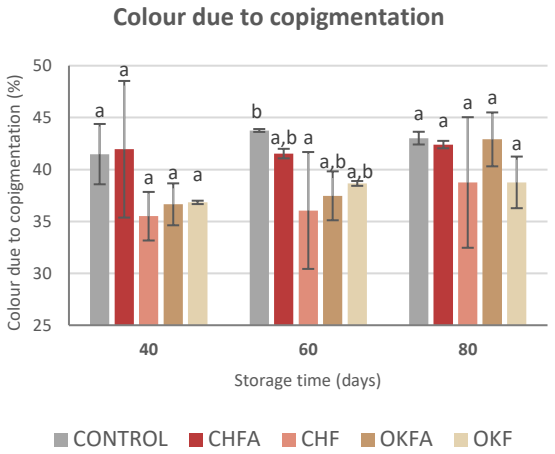

Figure 22 - Evolution of colour due to copigmentation feature during storage of a rosé wine in current and/or previous contact with different wood chips. Legend: **CONTROL:** Control wine; **CHFA:** wine fermented and aged with cherry chips, **CHF:** wine fermented with and aged without cherry chips, **OKFA:** wine fermented and aged with oak chips, **OKF:** wine fermented with and aged without oak chips. Mean values with the same letter indicate no statistically significant differences (Tukey's test at $p<0.05$)

5.2.6. Proanthocyanidins

Monomeric and small oligomeric proanthocyanidins in wines were assayed individually by polyamide column followed by HPLC analysis, and separated by degree of polymerization, as described in section 4.2.7 (A and B). The results regarding monomeric procyanidins (catechins) is shown in figure 23.

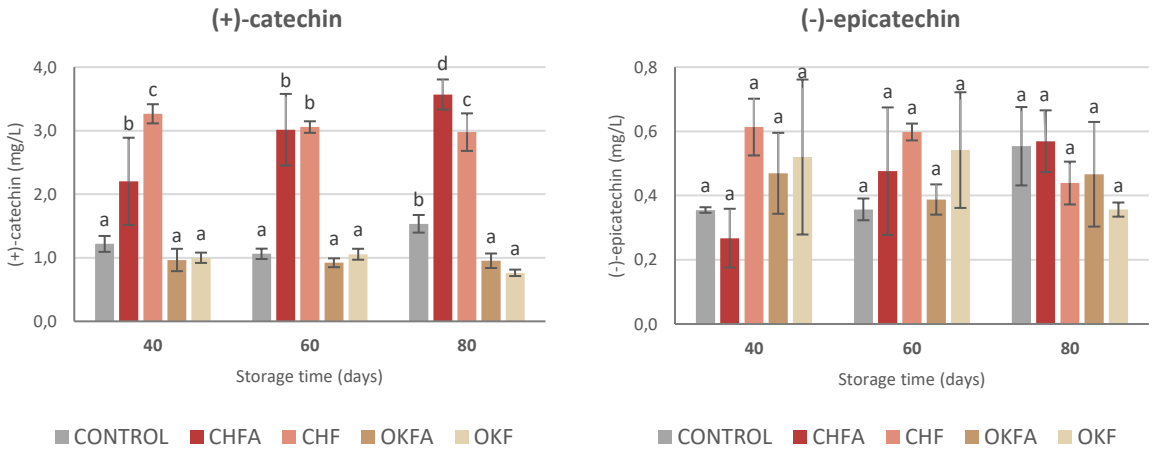

Figure 23 – Evolution of catechins and epicatechins during storage of a rosé wine in current and/or previous contact with different wood chips. Legend: **CONTROL:** Control wine; **CHFA:** wine fermented and aged with cherry chips, **CHF:** wine fermented with and aged without cherry chips, **OKFA:** wine fermented and aged with oak chips, **OKF:** wine fermented with and aged without oak chips. Mean values with the same letter indicate no statistically significant differences (Tukey's test at $p<0.05$)

Observing figure 23, there is no statistical significance at any sampling time for the (-)-epicatechin concentration, and no discernible tendency for both, but (+)-catechin concentrations presented remarkable results, considering the statistically and visibly significant difference between samples in contact with cherry wood and the other wines. Jordão *et al*. (2016), in a study comparing cherry, acacia and three oak types even found catechins to be quantifiable only in the cherry wood samples, and Gortzi *et al*. (2013), comparing those and less conventional wood types, found the catechin values of cherry wood to be only surpassed by mulberry and apricot woods, however in much lower storage time (10 and 20 days).

Other studies found contradictory results, for example finding the lowest values in 3-month aged wine in contact with cherry wood, when comparing to other woods including oak (De Rosso *et al*., 2009b) and a study which obtained similar patterns after 2 months of ageing and especially marked decreases for cherry wood after 4 months, while also finding oak to decrease (+)-catechin concentration (Chinnici *et al*., 2015) which suggests that wood ageing would promote this compound's degradation, but this study was carried out in 225L barriques, which may have different kinetics when it comes to proanthocyanidins aswell. Costa *et al*. (2016) obtained intermediate results, with oak chips showing the largest initial concentration of catechins, at 15 days, also decreasing over time, followed by cherry chips and the standard wine, but from 30 to 65 days, the oak chip wine had reduced its concentration to about half, whereas there was an increase in the cherry chips sample, so it is possible to see that results have been found all through the spectrum.

The variation was not discernible, even though literature indicated a decrease in catechins should be expected during storage, regardless of wood contact (Jordão *et al*., 2006; Barrera-Garcia *et al*., 2007; Chinnici *et al*., 2015).

Through HPLC analysis, only 4 other proanthocyanidins were found to show quantifiable peaks (figure 24). It is important to first note that due to some malpractice or poor maintenance of solvents, as well as the need to force the leaching through the polyamide columns, as previously mentioned, there was a considerable amount of error associated with this determination, as well as the flavanol extraction, and the resulting chromatography was mostly irregular and showed some interferents which hindered the results for this assay.

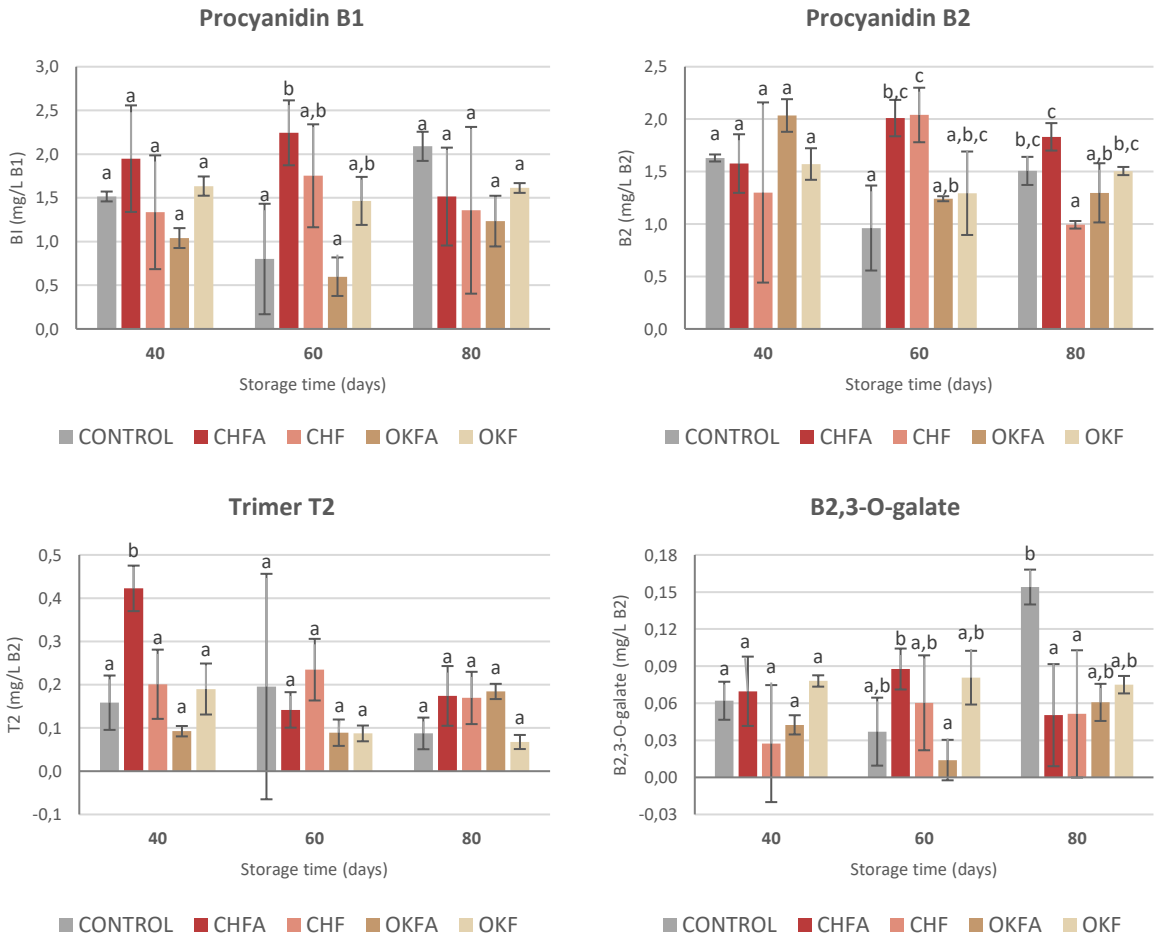

Figure 24 – Evolution of individual proanthocyanidins during storage of a rosé wine in current and/or previous contact with different wood chips. Legend: **CONTROL:** Control wine; **CHFA:** wine fermented and aged with cherry chips, **CHF:** wine fermented with and aged without cherry chips, **OKFA:** wine fermented and aged with oak chips, **OKF:** wine fermented with and aged without oak chips. Mean values with the same letter indicate no statistically significant differences (Tukey's test at $p<0.05$)

Procyanidins B1 and B2 were definitely dominant among the quantified oligomers and polymers, and for both there was no statistical difference between samples in the first 40 days of storage. At 60 days, the cherry chip samples (CHF and CHFA) showed significantly larger concentrations of both B1 and B2,

in comparison with the control, and generally larger values than the oak chip samples. As a side note, the behavioural pattern appears to be different, since the control and oak wines had an initial decrease followed by an increase of B1 and B2, and cherry samples had the opposite fluctuation, however there is little value to such observation, especially considering the associated error. The largest concentration of B1 and B2 (2.24 and 2.0 mg/L, respectively) was achieved using cherry wood after 40 days of storage. B2,3-O-galate, formed through a condensation reaction between gallic acid and procyanidin B2, was, despite oak wood's gallic acid concentration, not most present in these wines, as could be expected, and in fact especially not in the wine that had the longest contact time with oak chips (OKFA).

According to several authors (Bourzeix *et al*., 1986; Ricardo da Silva *et al*., 1992 ;Santos-Buelga *et al*., 1995; Fuleki and Ricardo da Silva, 1997; Jordão *et al*., 1998), B1 is the predominant oligomer in stems and skins, while B2 has its highest concentration in seeds. These wines were obtained from direct pressing, so extraction from skins, even if in a very short contact time, is more facilitated than from seeds, however both B1 and B2 were always in very balanced concentrations, as seen in figure 25, and it was not possible to determine any dominance.

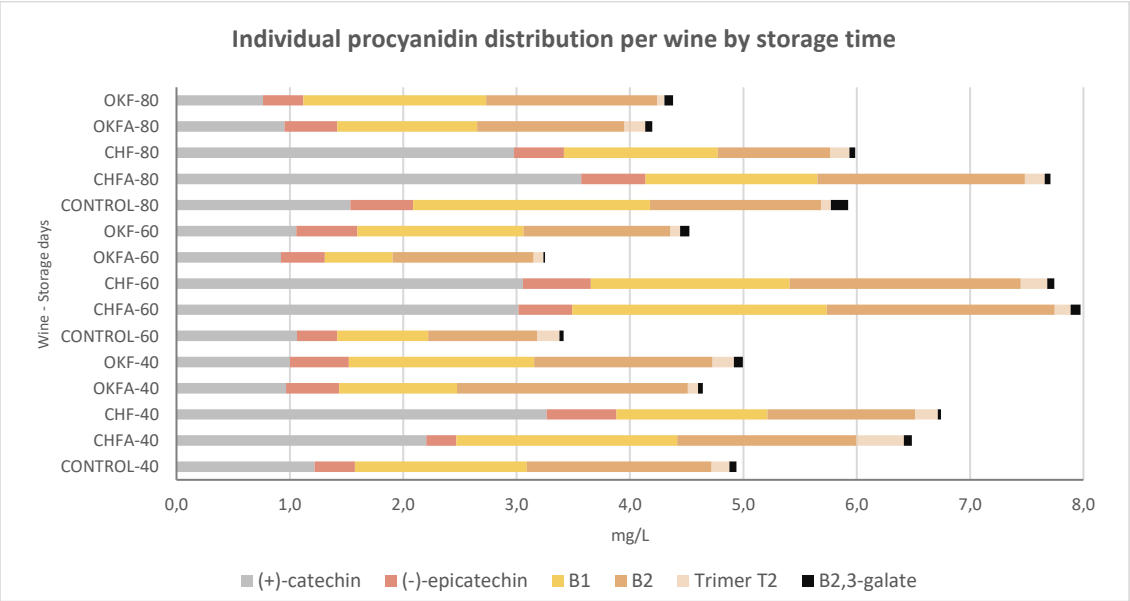

Figure 25 – Distribution of quantified individual procyanidins, in mg/L, as determined by HPLC, after 40, 60 and 80 storage days of a rosé wine. Legend: **CONTROL:** Control wine; **CHFA:** wine fermented and aged with cherry chips, **CHF:** wine fermented with and aged without cherry chips, **OKFA:** wine fermented and aged with oak chips, **OKF:** wine fermented with and aged without oak chips. Wines are represented as (Assay-Storage period in days)

Proanthocyanidins were also quantified according to degree of polymerization, into monomeric, oligomeric and polymeric fractions (F1, F2 and F3, respectively) (figures 26 and 27).

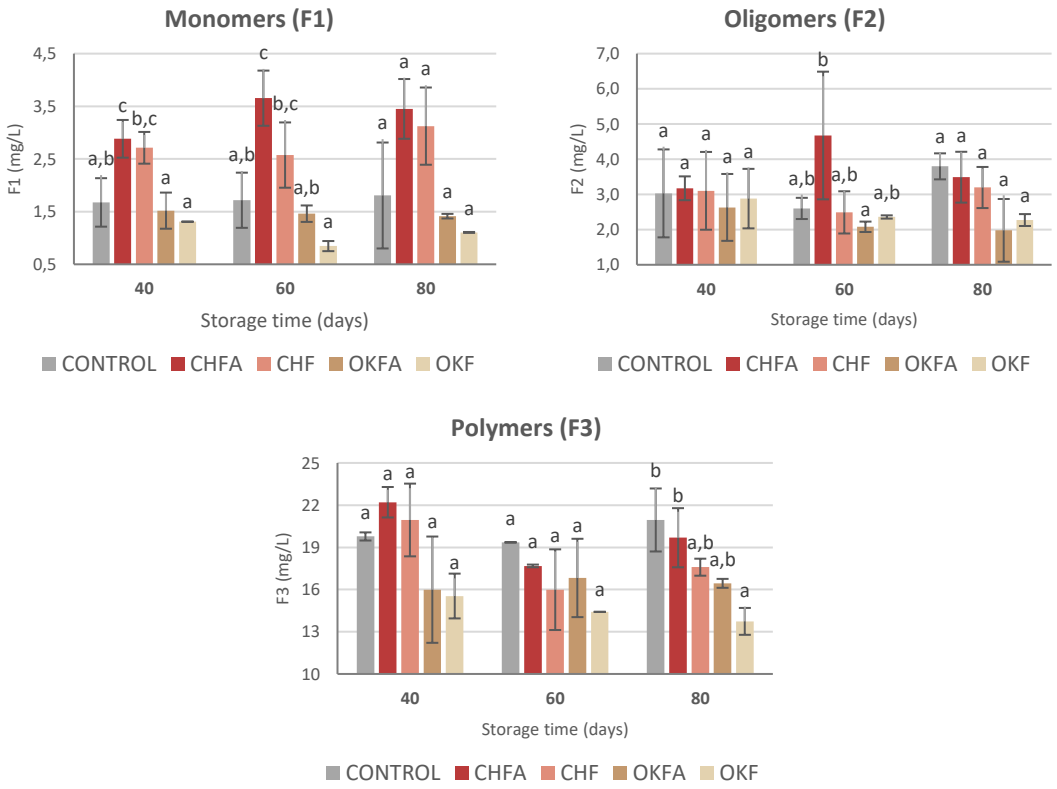

Figure 26 – Evolution of monomeric, oligomeric and polymeric proanthocyanidin fractions during storage of a rosé wine in current and/or previous contact with different wood chips. Legend: **CONTROL:** Control wine; **CHFA:** wine fermented and aged with cherry chips, **CHF:** wine fermented with and aged without cherry chips, **OKFA:** wine fermented and aged with oak chips, **OKF:** wine fermented with and aged without oak chips. Mean values with the same letter indicate no statistically significant differences (Tukey's test at $p<0.05$)

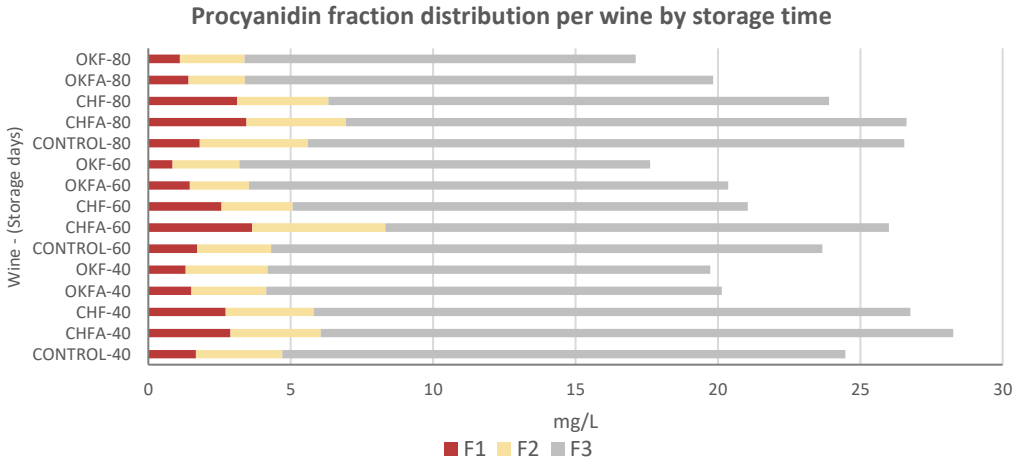

Figure 27 - Distribution of proanthocyanidins by degree of polymerization during storage of a rosé wine. Legend: **CONTROL:** Control wine; **CHFA:** wine fermented and aged with cherry chips, **CHF:** wine fermented with and aged without cherry chips, **OKFA:** wine fermented and aged with oak chips, **OKF:** wine fermented with and aged without oak chips, **F1:** monomeric proanthocyanidins, **F2:** oligomeric proanthocyanidins, **F3:** polymeric proanthocyanidins. Wines are represented as (Assay-Storage period in days).

The polymeric fraction was visibly dominant, which can be explained by the fact these are the most abundant in all grape parts (Sun *et al*., 1998), but it did not show significant differences between samples until 80 days of storage when the Control (20.9 mg/L) and CHFA (19.7 mg/L) showed the highest results. The lowest values of nearly all fractions corresponded to oak aged or fermented samples.

The oligomeric fraction, F2, showed little difference as well; only at 60 days did CHFA show a standout value of 4.7 mg/L .

The monomeric fraction had the most obvious differences: the cherry wood samples (CHFA and CHF) always showed statistically significant superiority in monomeric procyanidin concentration, explained by their superior levels of catechins, previously observed. Contact time may have had an effect since CHFA, which had the longest period of contact with the chips (fermentation and storage) presented higher values in all three storage periods, relatively to CHF which only fermented in contact with the wood.

6.8. Sensory analysis

Sensory analysis was carried out for all three analysed storage dates. Starting at 40 days (figure 28), only the parameters of “colour intensity” and “overall rating” presented statistical differences.

Regarding colour intensity, the difference was between the CONTROL wine (1.67 avg. rating) and the remaining wines, which scored 3.35 (wine fermented with cherry chips and wine fermented and aged with oak chips – CHF and OKFA), 3.75 (wine fermented and aged with cherry chips - CHFA) and 3.83 (wine fermented with oak chips - OKF). There is clearly an impact of wood chips, with contact time not necessarily affecting the increase in this case study.
Regarding average overall rating, the wines which had contact with wooden chips again stood out statistically, with the highest value being 3.21 (CHFA) and the lowest 2.21 (CONTROL) suggesting increased sensorial quality caused by the use of woods, especially cherry wood since CHFA stood out on its own. Even though only these two parameters showed statistical significance, it is possible to notice even if a slight increase on every parameter’s average rating.

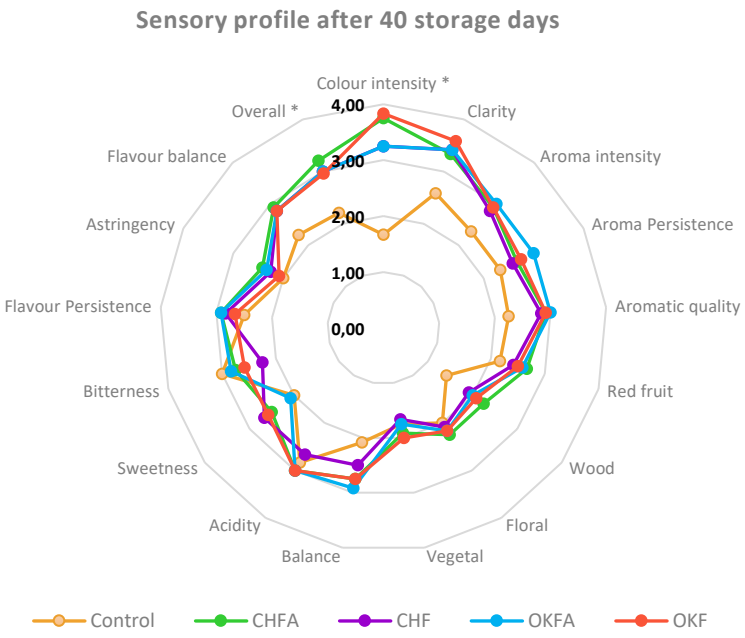

Figure 28 - Sensory analysis results for the studied wines, at 40 storage days. Legend: **CONTROL:** Control wine; **CHFA:** wine fermented and aged with cherry chips, **CHF:** wine fermented with and aged without cherry chips, **OKFA:** wine fermented and aged with oak chips, **OKF:** wine fermented with and aged without oak chips. An asterisk (*) represents significant differences between the wines (Tukey’s test, $p<0.05$)

At 60 days (figure 29) , colour intensity was again significantly different between samples; Control was, once more, awarded the lowest rating with 1.6, followed by OKF (2.56), CHF (3.44), and OKFA and CHFA (3.89) which again reflects a positive effect of wood cooperage in a wine's colour, and, in this case, since both OKFA and CHFA were statistically separated from the rest, perhaps suggests an increased effect with contact time, seeing as these were the wines that were kept in contact with wood chips after fermentation.

Flavour balance also showed interesting results, with the lowest results being given to the control wine (2.22) and the remaining scoring between 2.89 and 3.22. As a side note, the perception of red fruit aromas did show significant differences when an ANOVA test was carried out, however, through Tukey's test which was the chosen validation tool, these were not confirmed.

All of the flavour parameters were improved by wood contact, and regarding aromas, CHF was given the highest ratings for intensity, persistence, quality and red fruit and woody aromas, even though no significance was validated.

Overall quality was clearly superior in the wines which had contact with wood, especially CHF (3.67) and OKFA (3.33). It was observed throughout the study that wood seemed to unanimously improve the overall quality of wines.

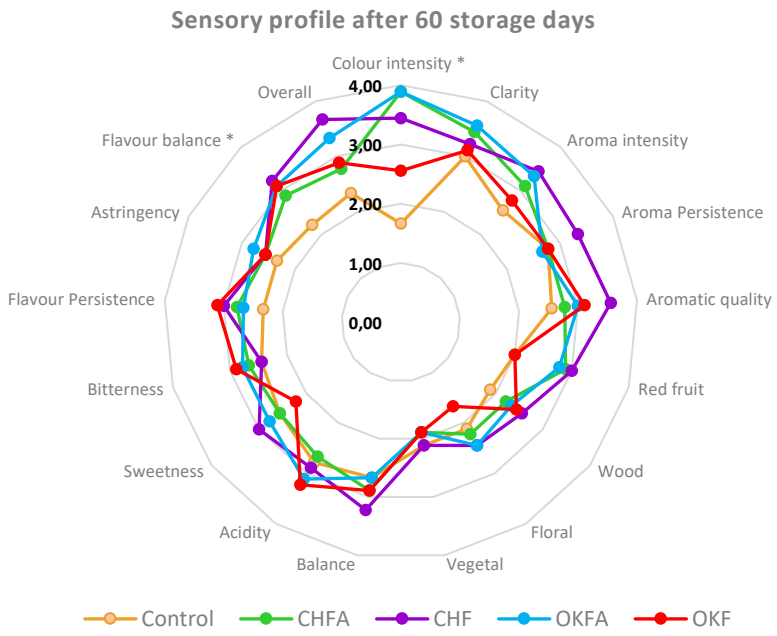

Figure 29 – Sensory analysis results for the studied wines, at 60 storage days. Legend: **CONTROL:** Control wine; **CHFA:** wine fermented and aged with cherry chips, **CHF:** wine fermented with and aged without cherry chips, **OKFA:** wine fermented and aged with oak chips, **OKF:** wine fermented with and aged without oak chips. An asterisk (*) represents significant differences between the wines (Tukey's test, $p<0.05$)

The wines that had been aged for 80 days (figure 30) showed differences in the parameters of colour intensity, as previously, and also aroma intensity. As seen in the previous sample groups, colour intensity was again lower in the control wine (1.8) than the wood aged and fermented wines (3.2 to 3.8) which is a remarkable difference, that clearly persisted throughout the ageing process, however, none of the wines had a very pronounced increase from 40 to 80 days of storage. The highest rating was given to the CHFA wine, once again, which was fermented and aged in contact with cherry chips, and, also, there was no statistically relevant effect to leaving the chips in contact with wines, even though there was an increase of 0.6 average rating between CHF and CHFA. Regarding aroma intensity, this was lowest in the OKF wine (2.4), which only had oak wood during fermentation, and highest in the control (3.4). In this moment, OKFA and OKF had the most perceived wood aromas, which developed over time. The highest overall rating was given to wood fermented and aged wines, both with cherry and oak (3.45) followed by OKF and CHF, thus again supporting that wood improved overall quality.

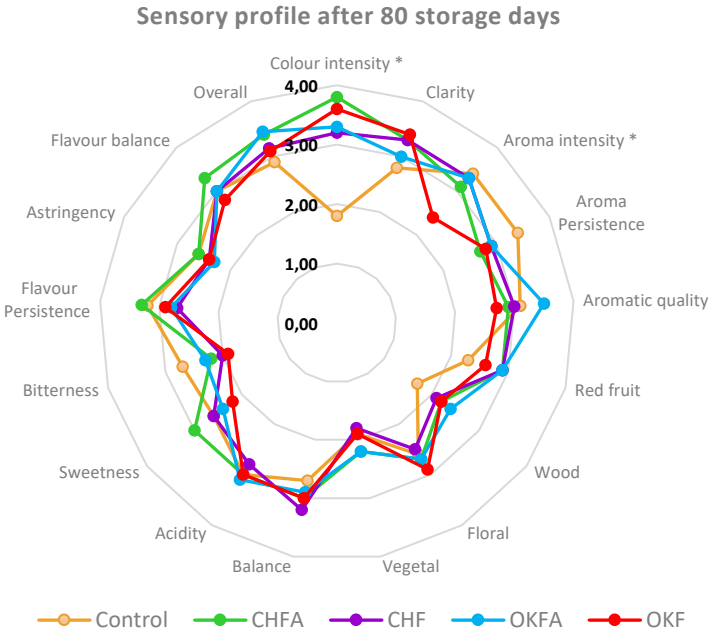

Figure 30 - Sensory analysis results for the studied wines, at 80 storage days. Legend: **CONTROL:** Control wine; **CHFA:** wine fermented and aged with cherry chips, **CHF:** wine fermented with and aged without cherry chips, **OKFA:** wine fermented and aged with oak chips, **OKF:** wine fermented with and aged without oak chips. An asterisk (*) represents significant differences between the wines (Tukey's test, $p<0.05$)

In regard to the evolution of the wines, from a sensory point of view, the colour intensity seemed to improve for the wood wines, whereas the control maintained its evaluation. The control wine was the worst rated overall, followed by OKF, and CHF obtained the highest overall rating at 60 storage days. All the wines had an overall improvement, even if slight, throughout the entire period.

The woody aromas developed significantly from 40 to 80 days, most remarkably for OKFA, which had the highest increase, and the wines generally became more balanced and with more aroma intensity and quality, but these are simply visual observations that can be made besides the statistical validation that took place.

6. Conclusions

This study aimed at pinpointing possible effects of ageing and/or fermenting rosé wines in oak wood and also cherry wood, as a potentially viable alternative. The phenolic impact was mostly obvious when using cherry wood, in both musts and wines, throughout both stages, with oak wood even showing less total phenols than the control wine at one point in ageing.

The use of wood chips had a clear result when it came to colour intensity and stability, mainly cherry wood, which enhanced intensity the most as well as pigment polymerization, all throughout winemaking. Hue is also a good indicator of stability, and in this parameter the control wine had a more pronounced loss of absorbance at 520 nm (red colour) than the others over storage time. Taking into account the chemical age parameter it was clear that both woods, with no particular standout, did confer more aged characteristics to the wine, which is based highly on pigment stability so all of these results suggest a more efficient colour stabilization when using cooperage, particularly cherry wood, in this case.

Both woods promoted anthocyanin ionization, showing a much higher proportion of coloured forms; in wines, there may have been a contact time effect since those which had fermented and aged with wood chips had higher results with statistical validation. The monomeric anthocyanins gave little information, the only interesting result being that malvidin-3-glucoside tended to decrease in the case of the control and one of the cherry samples, which is what would be expected from most literature, especially in the case of oak, but the latter actually increased its concentration of the dominant monomer.

In regard to monomeric procyanidins, (+)-catechin was remarkably more present in cherry wood wines, with no discernible tendency, whereas oak wood and the control did not have very significant differences, which was verified by both quantification methods (HPLC and separation by degree of polymerization considering the F1 fraction – monomers). There was no clear dominance between procyanidins B1 and B2, however both cherry wood samples of wines showed a higher amount of both at 60 storage days.

The sensory analysis showed all wines did become more balanced and the overall quality generally went up during storage, but the control did not vary too much its overall rating, whereas the wood fermented and aged wines were rated as higher quality wines. The most significant effect, from a sensory point of view, was an enhancement of colour intensity, which was very significant between the control and the other wines, but not very different between wood types.

The wood aromas developed significantly and became more perceivable in the wines that had contact with wood chips, most pronouncedly in the wine fermented and aged in oak wood, while cherry wood showed slightly higher values of the red fruit parameter, although, the latter was not statistically validated as significant.

There were not very obvious differences between the contact times of wood chips (fermentation or fermentation and storage).

7. References

Alañón, M. E., Castro-Vázquez, L., Díaz-Maroto, M. C., & Pérez-Coello, M. S. (2012). Aromatic potential of Castanea sativa Mill. Compared to Quercus species to be used in cooperage. *Food Chemistry*, *130*(4), 875–881.

Alañón, M. E., Pérez-Coello, M. S., Díaz-Maroto, I. J., Martín-Alvarez, P. J., Vila-Lameiro, P., & Díaz-Maroto, M. C. (2011a). Influence of geographical location, site and silvicultural parameters, on volatile composition of Quercus pyrenaica Willd. wood used in wine aging. *Forest Ecology and Management*, *262*(2), 124–130.

Alañón, M. E., Castro-Vázquez, L., Díaz-Maroto, M. C., Hermosín-Gutiérrez, I., Gordon, M. H., & Pérez-Coello, M. S. (2011b). Antioxidant capacity and phenolic composition of different woods used in cooperage. *Food Chemistry*, *129*(4), 1584–1590.

Alañón, M. E., Schumacher, R., Castro-Vázquez, L., Díaz-Maroto, M. C., Hermosín-Gutiérrez, I., & Pérez-Coello, M. S. (2013). Enological potential of chestnut wood for aging Tempranillo wines Part II: Phenolic compounds and chromatic characteristics. *Food Research International*, *51*(2), 536–543.

Álvarez-Pérez, J. M., Campo, E., San-Juan, F., Coque, J. J. R., Ferreira, V., & Hernández-Orte, P. (2012). Sensory and chemical characterisation of the aroma of Prieto Picudo rosé wines: The differential role of autochthonous yeast strains on aroma profiles. *Food Chemistry*, *133*(2), 284–292.

Amerine, M. A. & Singleton, V. L. (1977). *Wine: an introduction* (2nd ed.). Australia and New Zealand Book Co, Sydney.

André, P., Aubert, S. & Pelisse, C. (1970). Contribution aux études sur les vins rosés méridionaux – I. La couleur. Influence sur la degustation. *Annales de Technologie Agricole*, *19*, 323-340.

André, P., Aubert, S. & Pelisse, C. (1971). Contribution aux études sur les vins rosés meridionaux – III. Importance dum ode d'élaboration sur la qualité dês vins rosés. *Annales de Technologie Agricole*, *20*, 5-19.

André, P., Bénard, P., Bourzeix, M. & Flanzy, C. (1980). Vinification par maceration carbonique. Élaboration de vins rosés. *Annales de Technologie Agricole*, *29*(3): 497-508.

Arnold, R.A, & Noble, A.C. (1979). Effect of Pomace Contact on the Flavor of Chardonnay. *American Journal of Enology and Viticulture*, *30*(3), 179–181.

Barrera-García, V. D., Gougeon, R. D., Di Majo, D., De Aguirre, C., Voilley, A., & Chassagne, D. (2007). Different sorption behaviors for wine polyphenols in contact with oak wood. *Journal of Agricultural and Food Chemistry*, *55*(17), 7021–7027.

Baumes, R., Cordonnier, R., Nitz, S., & Drawert, F. (1986). Identification and determination of volatile constituents in wines from different vine cultivars. *Journal of the Science of Food and Agriculture*, *37*(9), 927–943.

Blouin J. and & Peynaud E. (2001). *Connaissance et Travail du Vin*, Dunod, Paris.

Boulton, R. (2001). The copigmentation of anthocyanins and its role in the color of red wine: A critical review. *American Journal of Enology and Viticulture 52*, 67–87.

Boulton, R.B., Singleton V.L. & Bisson L.F. (1995). *Principles and practices of winemaking* (1st ed.).New York: Chapman and Hall.

Bourzeix, M., Weyland, D., & Heredia, N. (1986). Etude des catéchines et des procyanidols de la grappe de raisin, du vin et d'autres dériveés de la vigne. *Bulletin de l'OIV*, *59*(669–670), 1171–1254.

Bragança, A.P. (2013). *Fermentação e estágio de vinho rosé em barrica* (Master's thesis). Instituto Superior de Agronomia, Lisboa.

Cadahia, E., Varea, S., & Mun, L. (2001a). Evolution of Ellagitannins in Spanish , French , and American Oak Woods during Natural Seasoning and Toasting. *Journal of Agricultural and Food Chemistry*, *49*(8), 3677–3684.

Canas, S., Casanova, V., & Pedro Belchior, A. (2008). Antioxidant activity and phenolic content of Portuguese wine aged brandies. *Journal of Food Composition and Analysis*, *21*(8), 626–633.

Carvalho A. (1997). *Madeiras Portuguesas. Estrutura anatómica, propriedades, utilizações*.II , 415 p. Direção Geral das Florestas, Lisboa.

Castellari, M., Piermattei, B., Arfelli, G., & Amati, A. (2001). Influence of aging conditions on the quality of red Sangiovese wine. *Journal of Agricultural and Food Chemistry*, *49*(8), 3672–3676.

Chatonnet P. (1995) Influence des procédés de tonnellerie et des conditions d'élevage sur la composition et la qualité des vins élevés en fûts de chêne. Thèse Doctorat, Université de Bordeaux II.

Chatonnet P., Boidron J.N., Dubourdieu D., Pons M. (1994). Évolution des composes polyphénoliques du bois de chêne au cours de son séchage. Premiers résultats. *Journal International des Sciences de la Vigne et du Vin, 28*, 337-357.

Chatonnet, P., & Dubourdieu, D. (1998). Comparative study of the characteristics of American white oak (Quercus alba) and European oak (Quercus petraea and Q. robur) for production of barrels used in barrel aging of wines. *American Journal of Enology and Viticulture*, *49*(1), 79–85.

Chatonnet, P., Dubourdieu, D., & Boidron, J.-N. (1992). Incidence des conditions de fermentation et d'élevage des vins blancs secs en barriques sur leur composition en substances cédées par le bois de chêne. *Sciences Des Aliments*, *12*(4), 665–685.

Chen, C.L. (1970). Constituents of Quercus alba heartwood. Phytochemistry, 9(5), 1149.

Chinnici, F., Natali, N., Bellachioma, A., Versari, A., & Riponi, C. (2015). Changes in phenolic composition of red wines aged in cherry wood. *LWT - Food Science and Technology*, *60*(2), 977–984.

Chinnici, F., Natali, N., Sonni, F., Bellachioma, A., & Riponi, C. (2011). Comparative changes in color features and pigment composition of red wines aged in oak and cherry wood casks. *Journal of Agricultural and Food Chemistry*, *59*(12), 6575–6582.

Chira, K., & Teissedre, P. L. (2014). Chemical and sensory evaluation of wine matured in oak barrel: effect of oak species involved and toasting process. *European Food Research and Technology*, *240*(3), 533–547.

Costa, A., Cosme, F., NUNES, F.M. & Jordão, A.M (2016) Evolução do perfil fenólico de um vinho tinto conservado com alternativos de madeira de acácia e cerejeira. In: *10º Simpósio de Vitivinicultura do Alentejo. Livro de Actas*, *Vol.1* (pp 231-238). Retrieved from https://repositorio.utad.pt/bitstream/10348/6608/1/LIVRO%20DE%20ACTAS%20VOLUME%201.pdf

Crachereau, J. (2009). La saignée : Point critique de l'élaboration des vins Rosés et clairets de Bordeaux. In Flanzy, C. (Ed) *Le Vin Rosé*. (p. 157). Editions Féret.

Dallas, C. (1998). Étude des transformations chimiques des anthocyanines et procyanidines dans les vins rouges (Doctorate dissertation), Instituto Superior de Agronomia, Lisboa.

Dallas, C., Ricardo-da-Silva, J. M., & Laureano, O. (1996). Products Formed in Model Wine Solutions Involving Anthocyanins, Procyanidin B_2, and Acetaldehyde. *Journal of Agricultural and Food Chemistry*, *44*(8), 2402–2407.

Darici, M., Cabaroglu, T., Ferreira, V., & Lopez, R. (2014). Chemical and sensory characterisation of the aroma of Çalkarası rosé wine. *Australian Journal of Grape and Wine Research, 20*(3), 340–346.

De Coninck, G. (2006). Evolution of Phenolic Composition and Sensory Properties in Red Wine Aged in Contact With Portuguese, 25–34.

De Freitas, V., & Mateus, N. (2001). Structural features of procyanidin interactions with salivary proteins. *Journal of Agricultural and Food Chemistry*, *49*(2), 940–945.

De Rosso, M., Panighel, A., Vedova, A. D., Stella, L., & Flamini, R. (2009a). Changes in chemical composition of a red wine aged in acacia, cherry, chestnut, mulberry, and oak wood barrels. *Journal of Agricultural and Food Chemistry*, *57*(5), 1915–1920.

De Rosso, M., Cancian, D., Panighel, A., Dalla Vedova, A., & Flamini, R. (2009b). Chemical compounds released from five different woods used to make barrels for aging wines and spirits: Volatile compounds and polyphenols. *Wood Science and Technology*, *43*(5–6), 375–385.

De Siḿon, B. F., Esteruelas, E., Muñoz, À. M., Cadaḥia, E., & Sanz, M. (2009). Volatile compounds in acacia, chestnut, cherry, ash, and oak woods, with a view to their use in cooperage. *Journal of Agricultural and Food Chemistry*, *57*(8), 3217–3227.

Del Alamo Sanza, M., & Nevares Domínguez, I. (2006). Wine aging in bottle from artificial systems (staves and chips) and oak woods: Anthocyanin composition. In *Analytica Chimica Acta* (Vol. 563, pp. 255–263).

Del Álamo, M., Nevares, I., Gallego, L., Martin, C., & Merino, S. (2008). Aging markers from bottled red wine aged with chips, staves and barrels. *Analytica Chimica Acta*, *621*(1), 86–99.

Dias Cardoso, A. (2007). O Vinho da uva à garrafa. Âncora Editora, Lisboa.

Doussot, F., De Jéso, B., Quideau, S., & Pardon, P. (2002). Extractives content in cooperage oak wood during natural seasoning and toasting; influence of tree species, geographic location, and single-tree effects. *Journal of Agricultural and Food Chemistry*, *50*(21), 5955–5961.

Dubourdieu, D., and Moine, V. (1998) Recent data on the benefits of aging wine on its lees. II. The effect of mannoproteins on wines tartrate stability. In: *The Barrel and the Wine, III (The Taste of Synergy)* (pp. 27–28). Seguin Moreau USA.

Fauvet, J., Guittard, A. (1998). La vinification en rosé. In: Flanzy, C (Ed). *Oenologie, fondements scientifiques et technologiques* (pp. 739-751). Ed. Tec & DOC.

Fernández de Simón, B. F., Sanz, M., Cadahía, E., Martínez, J., Esteruelas, E., & Muñoz, A. M. (2014). Polyphenolic compounds as chemical markers of wine ageing in contact with cherry, chestnut, false acacia, ash and oak wood. *Food Chemistry*, *143*, 66–76.

Fernández de Siḿon, Esteruelas, E., Muñoz, À. M., Cadaḥia, E., & Sanz, M. (2009). Volatile compounds in acacia, chestnut, cherry, ash, and oak woods, with a view to their use in cooperage. *Journal of Agricultural and Food Chemistry*, *57*(8), 3217–3227.

Ferreira, V., San Juan, F., Escudero, A., Culleré, L., Fernández-Zurbano, P., Sáenzenz-Navajas, M.-P., & Cacho, J. (2009). Modeling quality of premium Spanish red wines from gas chromatography-olfactometry data. *Journal of Agricultural and Food Chemistry, 57*(16), 7490–7498.

Feuillat, M. (1994). Fermentation dans le bois et élevage sur lies des vins blanc – Influence des macromolécules de levures sur les arômes. *Revue des Œnologues 71*, 19–21.

Feuillat, M., Freyssinet, M., & Charpentier, C. (1989). L'elevage sur lies des vins blancs de Bourgogne II. Evolution des macromolécules: polysaccharides et proteins. *VITIS - Journal of Grapevine Research*, *28*(3), 161.

Fitzmaurice, C. (2017). How rosé became high class: Categorical divestment and evaluation. *Poetics*, *61*, 1–13.

Flanzy, C. (1998). Vinification par maceration carbonique. In *Oenologie, fondements scientifiques et technologiques* (pp. 779-789). Ed. Tec & DOC

Flanzy, C., Flanzy, M., Bénard, P. (1995) *La vinification par maceration carbonique*. Inra Editions

Franco Aladrén E., Estella C., Haba Ejarque E., Martínez Gutiérrez J.A., Mendéz Sánchez J.V., Monzó García C., Navarro Blanco F. & Pérez Ruiz J. (2007). Vinos macerados com material de roble americano. Discriminación analítica y organoléptica de los vinos criados en barrica. *Enólogos. Investigación y Ciencia*, *49*: 32-39.

Freudenberg K., Hartmann L. (1953). Constituents from Robinia pseudoacacia. *Naturwiss 40*:413.
Fuleki, T., & Ricardo da Silva, J. M. (1997). Catechin and procyanidin composition of seeds from grape cultivars grown in Ontario. *Journal of Agricultural and Food Chemistry*, *45*(4), 1156–1160.

Gallego, L., Del Alamo, M., Nevares, I., Fernández, J., de Simón, B. F., & Cadahía, E. (2012). Phenolic compounds and sensorial characterization of wines aged with alternative to barrel products made of Spanish oak wood (*Quercus pyrenaica* Willd.). *Revista de Agaroquimica y Tecnologia de Alimentos*, *18*(2), 151–165.

Gallego,M.A, Sanchez-Palomo, E., Hermosin-Gutierrez, I. & Gonzalez Vinas M.A. (2015). Effect of oak chip addition at different winemaking stages on phenolic composition of Moravia Agria red wines. *South African Journal for Enology and Viticulture*, *36*(1), 21–31.

Garcia-Jares, C. M., Rozès, N., & Médina, B. (1993). Caractérisation et différenciation des vins rosés d'appellation d'origine et de table, Français Et Espagnols. *Journal international des sciences de la vigne et du vin*, *27*(1), 35–51.

Gimenez Martinez, R., Lopez Garcia De La Serrana, H., Villalon Mir, M., Quesada Granados, J., & Lopez Martinez, M. C. (1996). Influence of wood heat treatment, temperature and maceration time on vanillin, syringaldehyde, and gallic acid contents in oak wood and wine spirit mixtures. *American Journal of Enology and Viticulture*, *47*(4), 441–446.

Glabasnia, A., & Hofmann, T. (2006). Sensory-directed identification of taste-active ellagitannins in American (Quercus alba L.) and European oak wood (Quercus robur L.) and quantitative analysis in bourbon whiskey and oak-matured red wines. *Journal of Agricultural and Food Chemistry*, *54*(9), 3380–3390.

Gonçalves, F. J., & Jordão, A. M. (2009). Changes in antioxidant activity and the proanthocyanidin fraction of red wine aged in contact with portuguese (Quercus Pyrenaica Willd.) and American (Quercus Alba L.) oak wood chips. *Italian Journal of Food Science*, *21*(1), 51–64.

Gortzi, O., Metaxa, X., Mantanis, G., & Lalas, S. (2013). Effect of artificial ageing using different wood chips on the antioxidant activity, resveratrol and catechin concentration, sensory properties and colour of two Greek red wines. *Food Chemistry*, *141*(3), 2887–2895.

Gutiérrez Afonso, V. (2002) Sensory descriptive analysis between white wines fermented with oak chips and in barrels. *Journal of Food Science, 67*(6), 2415-2419.

Hernández, B., Sáenz, C., Alberdi, C., Alfonso, S., & Diñeiro, J. M. (2011). Colour evolution of rosé wines after bottling. *South African Journal of Enology and Viticulture*, *32*(1), 42–50.

Jackson, R.S. (2008). *Wine Science, Principles and Applications* (3rd ed.). Academic Press.

Jiménez Moreno, N., & Ancín Azpilicueta, C. (2007). Binding of oak volatile compounds by wine lees during simulation of wine ageing. *LWT - Food Science and Technology*, *40*(4), 619–624.

Jordão, A. M., Lozano, V., Correia, A. C., Ortega-Heras, M., & González-SanJosé, M. L. (2016). Comparative analysis of volatile and phenolic composition of alternative wood chips from cherry, acacia and oak for potential use in enology. *BIO Web of Conferences*, *7*, 02012.

Jordão, A. M., Ricardo-Da-Silva, J. M., & Laureano, O. (2006). Effect of oak constituents and oxygen on the evolution of malvidin-3-glucoside and (+)-catechin in model wine. *American Journal of Enology and Viticulture*, *57*(3), 377–381.

Jordão, A.M., Ricardo da Silva, J.M. & Laureano, O. (1998) Influência da rega na composição fenólica das uvas tintas da casta touriga francesa. *Ciencia y Tecnologia Alimentaria, 2*(2), 60-73.

Jourdes, M., Lefeuvre, D., & Quideau, S. (2009). C-glycosidic ellagitannins and their influence on wine chemistry. In S. (Ed.), Chemistry and Biology of Ellagitannins. An Underestimated Class of Bioactive Plant Polyphenols (pp. 320–365). World Scientific.

Karvela, E., Makris, D. P., Kefalas, P., & Moutounet, M. (2008). Extraction of phenolics in liquid model matrices containing oak chips: Kinetics, liquid chromatography-mass spectroscopy characterisation and association with in vitro antiradical activity. *Food Chemistry*, *110*(1), 263–272.

Kelebek, H., Canbas, A., & Selli, S. (2007). HPLC-DAD–MS Analysis of Anthocyanins in Rose Wine Made From cv. Öküzgözü Grapes, and Effect of Maceration Time on Anthocyanin Content. *Chromatographia*, *66*(3–4), 207–212.

.

Kramling, T. E., & Singleton, V. L. (1969). An estimate of non-flavonoid phenols in wines. *American Journal of Enology and Viticulture*, *20*(2), 86-92.

Li, H., Wang, X., Li, Y., Li, P., & Wang, H. (2009). Polyphenolic compounds and antioxidant properties of selected China wines. *Food Chemistry*, *112*(2), 454–460.

Maga, J. A. (1989). Formation and extraction of cis and trans β-methyl- γ-octalactone from Quercus alba. In: Pigott, J. R. & Paterson, A. (Eds.). *Distilled Beverages Flavour: Recent Developments* (pp.171-176). Ellis Horwood.

Marais J. & Rapp A. (1988). Effect of skin contact time and temperature on juice and wine composition and wine quality. *American Journal of Enology and Viticulture*, *9*(1): 22-30.

Marais, J. (1983). Terpenes in the Aroma of Grape and Wines: A Review. *South African Journal of Enology and Viticulture*, *4*, 49-58

Margalit, Y. (2012). Concepts in Wine Chemistry. *Wine Appreciation Guild*, 528, Board and Bench Publishing.

Masson, G., & Schneider, R. (2009). Key compounds of Provence rosé wine flavor. *American Journal of Enology and Viticulture, 60*(1), 116–122.

Matějíček, D., Mikeš, O., Klejdus, B., Štěrbová, D., & Kubáň, V. (2005). Changes in contents of phenolic compounds during maturing of barrique red wines. *Food Chemistry*, *90*(4), 791–800.

Michel, J., Jourdes, M., Le Floch, A., Giordanengo, T., Mourey, N., & Teissedre, P. L. (2013). Influence of wood barrels classified by NIRS on the ellagitannin content/composition and on the organoleptic properties of wine. *Journal of Agricultural and Food Chemistry*, *61*(46), 11109–11118.

Michel, J., Jourdes, M., Silva, M. A., Giordanengo, T., Mourey, N., & Teissedre, P. L. (2011). Impact of concentration of ellagitannins in oak wood on their levels and organoleptic influence in red wine. *Journal of Agricultural and Food Chemistry*, *59*(10), 5677–5683.

Monagas, M., & Bartolomé, B. (2009). Anthocyanins and anthocyanin-derived compounds. In *Wine Chemistry and Biochemistry* (pp. 439–462). Springer.

Murat, M.L. (2005). Recent findings on rosé wine aromas. Part 1: identifying aromas studying the aromatic potential of grapes and juice. *Australian and New Zealand Grapegrower & Winemaker* 497a, 64–65, 69,71, 73–74, 76.

Navarro, M., Kontoudakis, N., Gómez-Alonso, S., García-Romero, E., Canals, J. M., Hermosín-Gutíerrez, I., & Zamora, F. (2016). Influence of the botanical origin and toasting level on the ellagitannin content of wines aged in new and used oak barrels. *Food Research International*, *87*, 197–203.

Nishimura, K., Ohnishi, M., Masuda, M., Koga, K., & Matsuyama, R. (1983) Reactions of wood components during maturation. In: Piggott, J.R. (ed) *Flavour of Distilled Beverages: Origin and Development* (pp.241–255). Ellis Horwood.

Oberholster, A., Elmendorf, B. L., Lerno, L. A., King, E. S., Heymann, H., Brenneman, C. E., & Boulton, R. B. (2015). Barrel maturation, oak alternatives and micro-oxygenation: Influence on red wine aging and quality. *Food Chemistry*, *173*, 1250–1258.

Ortega-Heras, M., Pérez-Magariño, S., Cano-Mozo, E., & González-San José, M. L. (2010). Differences in the phenolic composition and sensory profile between red wines aged in oak barrels and wines aged with oak chips. *LWT - Food Science and Technology*, *43*(10), 1533–1541.

Peng, S., Scalbert, A., & Monties, B. (1991). Insoluble ellagitannins in Castanea sativa and Quercus petraea woods. *Phytochemistry*, *30*(3), 775–778.

Pizarro, C., Rodríguez-Tecedor, S., Esteban-Díez, I., Pérez-Del-Notario, N., & González-Sáiz, J. M. (2014). Experimental design approach to evaluate the impact of oak chips and micro-oxygenation on the volatile profile of red wines. *Food Chemistry*, *148*, 357–366.

Puértolas, E., Saldaña, G., Condón, S., Álvarez, I., & Raso, J. (2010). Evolution of polyphenolic compounds in red wine from Cabernet Sauvignon grapes processed by pulsed electric fields during aging in bottle. *Food Chemistry*, *119*(3), 1063–1070.

Ramirez-Ramirez, G., Chassagne, D., Feuillat, M., Voilley, A., & Charpentier, C. (2004). Effect of wine constituents on aroma compound sorption by oak wood in a model system. *American Journal of Enology and Viticulture*, *55*(1), 22–26.

Ribérau-Gayon, P. (1983). Interprétation chimique de la couleur des vins rouges. *VITIS - Journal of Grapevine Research, 12*(2), 119.

Ribéreau-Gayon J., Peynaud E., Ribéreau-Gayon P. & Sudraud P. (1976). *Sciences et Techniques du Vin*, Vol. III: *Vinifications - Transformations du vin*. Dunod, Paris.

Ribéreau-Gayon P., Glories Y., Maujean A. & Dubourdieu D. (2006). *Handbook of enology I: The microbiology of wine and vinifications* (2nd ed). John Wiley & Sons, Ltd.

Ricardo da Silva, J.M., Belchior, A.P., Spranger, M.I. & Bourzeix, M. (1992) Oligomeric procyanidins of three grapevine varieties and wines from Portugal. *Science des Aliments, 12,* 223-237.

Roman, L. A., Poiană, M. A., Dogaru, D. V., & Traşcă, T. I. (2013). Studies regarding the impact of aging time on color of red wine merlot obtained in recas vineyard. *Journal of Agroalimentary Processes and Technologies, 19*(3), 374-377

Roux D.G. and Paulus E. (1962). Condensed tannins. 13. Interrelationships of flavonoid components from the heartwood of Robiniapseudoacacia. *Biochemistry Journal, 82,* 324- 330.

Sáenz-Navajas, M. P., Fernández-Zurbano, P., & Ferreira, V. (2012). Contribution of Nonvolatile Composition to Wine Flavor. *Food Reviews International*, *28*(4), 389-411.

Salagoity-Auguste M. H., Tricard C., Marsal F. & Sudraud P. (1986). Preliminary investigation for the differentiation of enological tannins according to botanical origin: determination of gallic acid and its derivatives. *American Journal of Enology and Viticulture 37*:301–307.

Salinas, M. R., Garijo, J., Pardo, F., Zalacain, A., & Alonso, G. L. (2003). Color, polyphenol, and aroma compounds in rosé wines after prefermentative maceration and enzymatic treatments. *American Journal of Enology and Viticulture*, *54*(3), 195–202.

Santos, F. (2017). *Use of alternative wood chips from new botanical species. Their impact on phenolic composition and sensory properties of a rose wine from Touriga Nacional grape variety* (Master's thesis). Instituto Superior de Agronomia, Lisboa.

Santos-Buelga, C., Francia-Aricha, E. M., & Escribano-Bailón, M. T. (1995). Comparative flavan-3-ol composition of seeds from different grape varieties. *Food Chemistry*, *53*(2), 197–201.

Sanz, M., De Simón, B. F., Cadahía, E., Esteruelas, E., Muñoz, Á. M., Hernández, M. T., & Estrella, I. (2012a). Polyphenolic profile as a useful tool to identify the wood used in wine aging. *Analytica Chimica Acta*, *732*, 33–45.

Sanz, M., Fernández de Simón, B., Esteruelas, E., Muñoz, Á. M., Cadahía, E., Hernández, M. T., … Martinez, J. (2012b). Polyphenols in red wine aged in acacia (Robinia pseudoacacia) and oak (Quercus petraea) wood barrels. *Analytica Chimica Acta*, *732*, 83–90.

Sarni, F., Moutounet, M., Puech, J. L., & Rabier, P. (1990). Effect of Heat-Treatment of Oak Wood Extractable Compounds. *Holzforschung*, *44*(6), 461–466.

Sauvage, F. X., Romieu, C. G., Flanzy, C., & Robin, J. P. (1991). Aminotransferases in Grapes. Isolation and Characterization of Aspartate Aminotransferase. *American Journal of Enology and Viticulture*, *42*(3), 209–218.

Sefton M.A., Francis I.L., Pocock K.F. & Williams P.J. (1993). The influence of natural seasoning on the concentrations of eugenol, vanillin, and cis- and trans-β-methyl-Ɣ- octalactone extracted from French and American oakwood. *Science des Aliments, 13*(4), 629-643.

Seikel, M. K., Hostettler, F. D., & Niemann, G. J. (1971). Phenolics of Quercus rubra wood. *Phytochemistry*, *10*(9), 2249–2251.

Sergent, T., Kohnen, S., Jourez, B., Beauve, C., Schneider, Y. J., & Vincke, C. (2014). Characterization of black locust (Robinia pseudoacacia L.) heartwood extractives: Identification of resveratrol and piceatannol. *Wood Science and Technology*, *48*(5), 1005–1017.

Singleton, V. L. (1974). Some Aspects of the Wooden Container as a Factor in Wine Maturation. In *Chemistry of Winemaking, Adv. Chem. Ser.* (pp. 254–277). Washington.

Singleton, V. L., & Draper, D. E. (1961). Wood Chips and Wine Treatment; the Nature of Aqueous alcohol extracts. *American Journal of Enology and Viticulture*, *12*(4), 152–158.

Somers, T. C. (1971). The polymeric nature of wine pigments. *Phytochemistry*, *10*(9), 2175–2186.

Somers, T.C & Evans, M. E. (1977). Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO2, “chemical age.” *Journal of the Science of Food and Agriculture*, *28*(3), 279–287.

Soufleros, E.; Bertrand, A. (1988). Les acides gras libre du vin: observations sur leur origine. *Connaissance de la vigne et du vin*, *22*(4), 251-260.

Spillman, P. J., Sefton, M. A., & Gawel, R. (2004). The effect of oak wood source, location of seasoning and coopering on the composition of volatile compounds in oak-matured wines. *Australian Journal of Grape and Wine Research*, *10*(3), 216–226.

Stark, T., Wollmann, N., Wenker, K., Lösch, S., Glabasnia, A., & Hofmann, T. (2010). Matrix-calibrated LC-MS/MS quantitation and sensory evaluation of oak ellagitannins and their transformation products in red wines. *Journal of Agricultural and Food Chemistry*, *58*(10), 6360–6369.

Stavek, J., Papouskova, B., Balik, J., & Bednar, P. (2012). Effect of Storage Conditions on Various Parameters of Colour and the Anthocyanin Profile of Rose Wines. *International Journal of Food Properties*, *15*(5), 1133–1147.

Sudraud, P., Bar, M. & Martiniére, P. (1968). Essais de définition des vins blancs tachés et des vins rosés. *Connaissance de la Vigne et du Vin*, *2*, 349-357.

Sun, B., Leandro, C., Ricardo da Silva, J. M., & Spranger, I. (1998). Separation of Grape and Wine Proanthocyanidins According to Their Degree of Polymerization. *Journal of Agricultural and Food Chemistry*, *46*(4), 1390–1396.

Suriano, S., Basile, T., Tarricone, L., Gennaro, D. D., & Tamborra, P. (2015). Effects of skin maceration time on the phenolic and sensory characteristics of bombino nero rosé wines. *Italian Journal of Agronomy*, *10*(1), 21–29.

Tavares, M. (2015). *Impact of utilization of alternative wood products of less conventional species (cherry and acacia), on the phenolic composition and sensory profile evolution of a red wine* (Master’s thesis). Instituto Superior de Agronomia, Lisboa.

Vivas N. (1995). The notion of grain in cooperage . *Journal des Science et Techniques de la Tonnellerie*, *1*, 33-48.

Vivas, N., & Glories, Y. (1996). Role of Oak Wood Ellagitannins in the Oxidation Process of Red Wines During Aging. *American Journal of Enology and Viticulture*, *47*(1), 103–107.

Yurgalevitch, C. M., & Janes, H. W. (1988). Carbon dioxide enrichment of the root zone of tomato seedlings. *Journal of Horticultural Science*, *63*(2), 265–270.

Zhang, B., Cai, J., Duan, C. Q., Reeves, M. J., & He, F. (2015, April 1). A review of polyphenolics in oak woods. *International Journal of Molecular Sciences*, *16*(4), 6978–7014.