Impact of the use of different blends of wood chips from diverse botanical species on quality parameters of white wine from Encruzado variety.

A study on the effects of different kind of wood on a white wine with no contact with lees

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Summary

ABSTRACT

The aim of this study was to assess the influence of blends of wood from different botanical species in a white wine from Encruzado variety; the botanical species used in this study were French oak (Quercus peatraea), American oak (Quercus alba) and Cherry (Prunus avium) during a 60 days maceration period, with sampling done after 15, 30 and 60 days.

The blends were obtained by combining 2 woods species in a ratio of 1:1, obtaining 3 blends; that is, Cherry and American oak (Ch+Am), French and American oak (Fr+Am), and Cherry and American oak (Ch+Am). In addition to these treatments 3 Control treatments were created by adding single wood of Cherry (CCh), American oak (CAm) and French oak (CFr), moreover a treatment without wood was kept (C). The amount of wood used was the same for all the treatments, that is 0,5 g/l. on the different sampling days were run both sensory analysis, by a panel of judges, evaluating aroma and taste parameters, and chemical analysis, that is, total phenols, non flavonoids and flavonoids phenols, color evolution, maderization test and tanning power. At the end of the experiment statistical analysis showed relevant data in almost all the analysis, for the chemical analysis cherry showed the highest level of total phenols and flavonoids, which is reflected also in the blends with other woods, while the French oak and its blends showed significant levels of non flavonoids phenols. The browning potential showed an decrease in wines in contact with wood except for CCh, while tanning power showed an increase in all the wines in contact with wood with the blend of cherry and American being the highest tannicity. In the sensory analysis we can observe how the global appreciation of the wines decrease with increasing maceration period with the wood both for the taste and aroma parameters, with C being the most appreciated wine at the last sampling, while the sampling of 15 and 30 days shows the wine with cherry and with American oak showing the highest values.

KEY WORDS: wood, cherry, oak, chips, encruzado.

RESUMO

O objetivo deste estudo foi avaliar a influência de misturas de madeira de diferentes espécies botânicas num vinho branco da castaEncruzado. As espécies botânicas utilizadas neste estudo foram carvalho francês (*Quercus petraea*), carvalho americano (*Quercus alba*) e cerejeira (*Prunus avium*) durante um período de 60 dias de maceração, com amostragem feita após 15, 30 e 60 dias.

As misturas foram obtidas pela combinação de duas espécies de madeiras na proporção de 1:1, obtendo-se 3 misturas, ou seja, cerejeira e carvalho americano (Ch + Am), carvalho francês e americano (Fr + Am) e cerejeira e carvalho francês(Ch + Fr). Além desses tratamentos, 3 tratamentos de controlo foram criados, adicionando-se madeira única de cereja (CCh), carvalho americano (CAm) e carvalho francês (CFr) e além destes, foi mantido um tratamento sem madeira (C). A quantidade de madeira utilizada foi a mesma para todos os tratamentos: 0,5 g / l. Nos diferentes dias de amostragem foram realizadas análises sensoriais por um painel de juízes, avaliando-se os parâmetros aromáticos e gustativos, e análise química, ou seja, fenóis totais, não flavonóides e fenóis flavonóides, evolução de cor, teste de maderização e poder tanante. No final doensaio experimental, a análise estatística mostrou dados relevantes em quase todas as análises.Para a análise química, a cerejeira apresentou o maior teor de fenóis totais e flavonóides, o que se reflete também nas misturas com outras madeiras, enquanto que o carvalho francês e as suas misturas demonstrar am níveis significativos de fenóis não flavonóides. O potencial de oxidaçãoa presentou decréscimo nos vinhos em contacto com a madeira, com exceção do CCh, enquanto que o poder tanante apresentou aumento em todos os vinhos em contacto com a madeira, sendo a mistura de cerejeira e americano a de maior tanicidade. Na análise sensorial observou-sequea apreciação global dos vinhos diminui com o aumento do período de maceração com a madeira, quer para os parâmetros de sabor, quer de aroma, sendo o C o vinho mais apreciado na última amostragem, enquanto que o C+Am foi o mais apreciadonas amostragensaos 15 e 30 dias.

PALAVRAS-CHAVE: madeira, cerejeira, carvalho, encruzado, misturas.

RESUMO ALARGADO

O uso de madeira para o envelhecimento è reconhecido como uma ferramenta fundamental para a produção de vinho ha mais de 200 anos. De facto, , não só esta prática é cada vez mais importante para vinhos tintos, mas também para vinhos brancos, ajudando a aumentar a sua complexidade e estabilidados vinhos.

O envelhecimento do vinho em barris de madeira promove importantes modificações como clarificação espontânea, alterações de cor, adstringência, amargor, estrutura e, especialmente,modificações aromáticas.Tudo isto devido a os contributos que diferentes compostos extraídos da madeira dão à complexidade e estabilidade do vinho.

Contudo, o sistema tradicional de envelhecimento tem várias desvantagens, como os longos períodos de envelhecimento, os elevados custos de investimento e os requisitos de espaço na adega. Por estas razões, foram desenvolvidos recentemente métodos alternativos, como a adição de aparasde madeira em vinhos, de modo a simplificar e acelerar o processo de envelhecimento, bem como a utilização de novas espécies de madeira, mais disponíveis e económicas para as empresas.

Tradicionalmente, os vinhos são envelhecidos em barris de madeira feitos principalmente a partir de espécies de carvalho como *Quercus robur* (*Quercuspedunculata*) e *Quercus petraea* (*Quercus sessiliflora*), que são as mais comuns nas florestas francesas, e ainda *Quercus alba* (carvalho americano) do continente norte-americano.

Nos últimos anos, barris e aparasobtidos de espécies alternativas como a cerejeira (*Prunus avium*) também têm sido considerados como possíveis agentesde envelhecimento, originando vinhos com um perfil aromático único e mais complexo, mesmo queatualmente esta não seja uma prática enológica oficial.

O objetivo deste estudo foi avaliar as mudanças ocorridas, durante 60 dias, a nível químico e sensorial, de um vinho branco da castaEncruzado envelhecido em misturasde diferentes espécies botânicas, *i.e.* Cerejeira (*Prunus avium*), carvalho americano (*Quercus alba*) e carvalho francês (*Quercus petraea*), obtendo três diferentes misturas de 2 madeiras numa relação de 1:1, resultando em 3 tratamentos - cereja mais carvalho americano, carvalho francês maiscarvalho americano e carvalho francês maiscerejeira - com 3 vinhos de controlo de cada espécie botânica e um controlo sem madeira, para um total de 7 tratamentos.

Análise fenólica (fenóis totais, flavonóides e fenóis não flavonóides, aspectos cromáticos,suscetibilidade à oxidação epoder tanante) e uma avaliação sensorial foram realizadas para todas as amostras.

Os resultados mostraram diferenças estatisticamente significantes entre as amostras na maioria das análises fenólicas, permitindo notar diferenças entre o vinho de controlo e os vinhos envelhecidosemcontacto com as diferentesaparas de misturas de madeiras. A análise química mostrou resultados interessantes, destacando como os compostos extraídos da madeira mudaram de acordo com as espécies de madeira utilizadas e suas características. As aparas de madeira de cerejeira apresentaram a maior capacidade de extração para o perfil total de fenóis, com os vinhos envelhecidos com essas aparasa apresentarem uma concentração fenólica extrema. A análise para determinação dos flavonóides fenólicos confirmou o que foi observado para os fenóis totais, sendo os flavonóides uma das principais classes de compostos fenólicos (Ribéreau-Gayon, et al. 2006), com os vinhos com madeira de cerejeira a apresentarem o maior nível desse tipo de compostos. Decontrário, os ensaios com maior quantidade de fenóis não flavonóides foram os de carvalho francês, embora neste caso a mistura de carvalho francês/americano tenha sido a que apresentou o maior nível final (após 60 dias de maceração com madeira).

Os parâmetros de cores esperados mostraram um aumento na tonalidadeda cor em quase todos os vinhos, exceto para os tratamentos Controlo e carvalho francês/cerejeira, com a mistura cerejeira/carvalhoamericanoa demostrar o maior aumento de cor, destacando mais uma vez a ligação entre os fenóis e a cor no vinho branco.

O teste de madeirizaçãomostrou, em geral, um decréscimo no potencial de oxidaçãodos vinhos, com exceção do CCh, que confirma o estreito vínculo existente entre os fenóis totais e o potencial de oxidaçãode um vinho branco. É também de referir que todos os vinhos em contacto com a madeira apresentaram, eventualmente, um maior potencial de oxidaçãodo que o vinho C, como esperado e sugerido por váriosestudos.

Os resultados do poder tanante mostraram um grande aumento na tanicidade dos vinhos, especialmente entre as medições aos 30 e 60 dias, com as misturas de madeira a obterem os maiores valores, sendo Ch + Am significativamente maior do que os vinhos em madeira simples e o Controlo.

Considerando a baixa quantidade de aparasusada (0,5 g / l) - muito baixa se considerada a média de uso na produção de vinho branco (0,5-2 g / l) - os resultados mais marcantes eram esperados na análise sensorial.

Os vinhos commadeira, de facto, mostraram resultados interessantes ao níveldos parâmetros aromáticos e gustativos, com a madeira americana obtendoos melhores resultados logoapós 15 dias, apresentando uma maior qualidade aromática e notas mais frutadas e florais. Esta, juntamente com a madeira de cerejeira, foi mais apreciada do que as outras, embora os parâmetros de sabornão tenham mostrado significância nos 15 dias.

Depois de 30 dias, os vinhos mais apreciados ainda eram os que tinham madeira de cerejeira, mas também o Controlo, por causa do aumento das notas amadeiradas dos outros ensaios, que diminuíam a qualidade do aroma. O sabor também mostrou um aumento deequilíbriopara a madeira de cerejeira, enquanto que a perceção da acidez permaneceu a mais alta para o Controlo.

Na última amostragem, após 60 dias de maceração, as diferenças dos parâmetros aromáticos entre os vinhos commadeira tornaram-se cada vez menos acentuadas, enquanto que o vinho Controloapresentou diferenças significativas em relação ao resto dos vinhos, com maior qualidade aromática, maisfrutado e com notas florais.

Também a apreciação global mostrou o Controlocomo sendo o primeiro entre os vinhos, com a madeira de Cerejeira em segundo.

Assim, o uso de mistura de madeiras mostrou resultados interessantes a nível químico, com a cerejeira e as suas misturas dando mais ao perfil de flavanóides, enquanto que o carvalho francês e as suas misturas mais ao perfil de não flavanóides. Na análise sensorial os vinhos apresentaram os melhores resultados na apreciação global após 15 dias de contacto, diminuindo depois com o aumento dos dias de contacto entre o vinho e a madeira. Os ensaios com melhor feedback nesta fase foram o de cerejeira e o de carvalho americano, enquanto que aos 30 dias o vinho Controlo apresentou os melhores resultados, seguido da cerejeira.

Estes resultados preliminares mostram a complexidade das interações envolvidas e a necessidade de pesquisa no futuro sobre este assunto, incluindo análises químicas mais detalhadas sobre a composição e perfis voláteis aromáticos de vinhos brancos de alta qualidade envelhecidos em contacto com madeiras alternativas.

PALAVRAS-CHAVE: madeira, cerejeira, carvalho, encruzado, misturas, fenóis totais, análise sensorial.

LIST OF FIGURES

TABLES

1. INTRODUCTION

1.1.WOOD IN WINE AGING

The use of wood during the aging process of wine is a common practice in most of the wine producing regions having many positive effects on it, from color stabilization to aromatic molecular composition (Vivas, 2000; Nevares and Del-Alamo, 2008). These processes are influenced by numerous factors such as; the species of the wood, the level of toasting and the length of the aging process (Jordao et al. 2006).

The prevalent wine aging practice is the use of oak barrels, which has been proved by many studies to have beneficial effects on phenolic composition of the wine; increasing or adding into the wine, among others, polyphenols,coumarins, polysaccharides, volatile phenols (eugenol), cis- and trans-β-methyl-γ-octalactone, volatile phenol aldehyde, and furanic aldehydes(Sefton, 1991; Towey and Waterhouse, 1996; Flamini et al. 2009), but having beneficial effects also on spontaneous clarification (Del Alamo Sanza et al. 2004).

Moreover the pores present in the wood slowly convey oxygen to the wine inducing chemical changes between the wine phenols and the compounds extracted from the wood, eventually stabilizing the color, reducing the astringency of the wine and improving wine's quality and organoleptic characteristics (McRae et al., 2012; López-Roca & Gómez-Plaza, 2006; Cerdan and Ancin-Azpilicueta, 2006; Izquierdo-Canas et al., 2016*)*.

The barrel aging process gives structure and complexity to the wine inducing a higher persistence of the bouquet even though it cannot increase the quality of a wine, but rather it can enhance its intrinsic value, if rationally used (Frangipane et al., 2006).

On the other hand issues such as; the rising demand for barrels; the environmental issue linked to the decreased availability of the oak woodand the long period normally needed for aging in barrel (linked to higher maintenance costs), induced many producers to adopt alternatives to oak barrels (Jordão et al., 2017; Gonzalez-SanJose et al., 2016).

Chips and staves are a good example; theyare used in order to reduce the costs of productionand obtain an analogous extraction process, if compared to barrel aging, regarding the aromas and mouthfeel (Teissedre et al. 2011; Ancın-Azpilicueta et al., 2006).

Indeed even though a wine obtained through a chips maceration period does not show characteristics similar to abarrel-aged wine, it seems to slightly resembleas to the aroma profile, for 30 days of chips maceration, awine aged for about 3 months in a barrel (Wilker&Gallander, 1988; González-San José et al. 2010).

The use of this technology, can also have positive effects not present in the case of barrel-aged wines. In particular the oxidation aromas and color changes linked to the barrel aging in white wines could be avoided through the use of wood chips, which can also confer to the wine the typical wood aromas without sacrificing the fruity notes (Sanchez-Palomo et al., 2017).
Other alternatives to oak barrels could be the use of species other than oak, indeed even though wood chips addition practice appeared initially in the new wine world and became legal in the EU just in 2006 ((CE) N° 1507/2006), to this day only Oak (*Quercus* genus) and chestnut (*Castanea* genus) are recognized by the OIV as suited to be used in wine production (Kyraleou et al., 2015).
Nevertheless,the use of wood alternatives to oak could be an advantage for producers (besides for overcoming the previously mentioned problems), since the growing sensibility of the consumers on sustainability issues (Schäufele and Hamm, 2017).

This kind of approach would translate in a diversification in the market and in an increase of the range of products to offer to the consumers (Sanchez-Palomo et al., 2017). Using local species of wood rather than imported wood products can be seen in this optic, as well as linked to neweconomic possibilities that in some market sharecould be given by originality,rather than homogenization of the wine(Scozzafava et al., 2016).For these reasons the utilization of different wood species such as acacia, cherry, mulberry (Chinnici et al., 2011; Gortzi et al., 2013; De Rosso et al., 2009) has seen an increase in the last years both in barrels and in wood derivates (staves, chips, wood dust or oenological tannins from wood) utilization (Oberholster et al. 2015), due to an increase interest in wine typicality especially in Italy (Flamini et al. 2009).

1.2.OAK WOOD SPECIES AND COMPOSITION

Worldwide, hence, oak is the most spread wood used for the aging of the wine, both for the barrels and chips (Sanz et al., 2012). The species of wood from which a barrel is made of and its manufacturing, i.e. seasoning and toasting, are the main factor influencing the evolution of the wine, because of their influence on the extractable compounds (Dourtoglouet al., 2013; Kyraleou et al. 2015; Mosedale et al. 1996).

The most used oak species for barrel and chips production are:*Quercus alba* from North America also known as American oak and *Quercus peatraea* and *Quercus robur* from Europe normally referred to as French oak (Guchu et al., 2005; Spillman et al. 2004), but recently also *Quercus pyrenaica* has been subjected to many studies on wood chemical composition, and the effects on wine (Jordao et al., 2007; Fernandez de Simon et al., 2008).

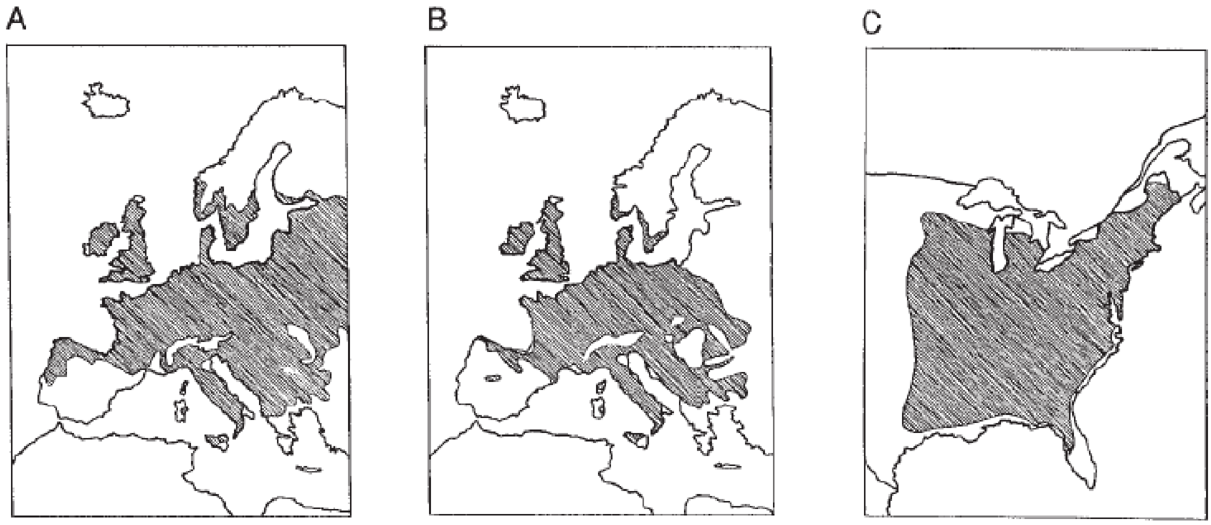


Figure 8- Distribution of A) QuercusSessil B) QuercusRobur and C) Quercus Alba (Jackson, 2008)

The vast use of oak for wine aging is to be searched in its aromatic nature, and into the various phenolic and volatile compounds that are transferred from the wood into the wine (Del Alamo Sanza et al., 2003*)*.

1.2.1. AROMATIC COMPOUNDS PRESENT IN OAK

Many aromatic compounds have been identified in oak wood but just few of them influence the sensory characteristic of the wine, which is to be attributed to the presence of β-methyl-γ-octalactone, also known as oak lactones or whisky lactones, and its isomers originated from oak lipids (Mosedale et al., 1999), specific to the *Quercus* genus and responsible for its typical aroma(Chatonnet, 1998; Baumes et al.1995). This compound has 4 isomers which can be found in the untreated wood, with different aromatic characteristics (coconut, spicy, leather), and whose concentration change in different oak species with Q. alba showing the highest concentration (Ribereau-Gayon et al., 2010).

Vanillin (*4-hydroxy-3- methoxybenzaldehyde*) is an aromatic aldehyde obtained from lignin and contributes to the typical vanilla aroma of all the oak-aged alcoholic bevarages including wine. It is present in high quantities, if compared to other aldehydes from lignin, with the exception of syringaldehyde, which is, though, less odorous (Marin et al., 2005; Singleton 1995). Such cited aldehydes are the most important under aromatic contribution and can be further oxidized in the correspondent phenolic acids (vanillic and syringic acid)resulting in a loss of flavour (Del Alamo Sanza et al., 2003;Singleton 1995).

Other volatile compounds which can pass from the wood to the wine are the volatile phenols which includes various forms ofeugenol,guaiacol and ethyl and vinyl phenolspresents in different quantities in untoasted wood according to the species and zone of origin and can be increased during the toasting process (Cadahia et al., 2003; Cadahia et al., 2001; Towey and Waterhouse, 1996).

Eugenol, which is the main volatile phenol according to Ribereau-Gayon(2010), and guaiacolare responsible respectively for the cloves and smoky aroma of the wine (Guchu et al., 2005*)*. While the 4-ethyl and 4-vynil forms of phenol and guaiacolare of importance because responsible for various off-flavorslike horse, animal and sweaty “saddle”-like notes if present in high concentrations. These unpleaseantflavours are mostly originated by *Brettanomyces* and not by the wood as initially thought; indeed, although being ubiquitous in red wines, are perceived just following the action of the mentioned microorganisms (Pollnitz et al., 2000;Doussot et al., 2002; Singleton, 1995).

The risk of developing these bacteria increase with the repeated use of the barrels as the latter become more and more impregnated with wine becoming more suited to the *Brettanomyces* and to the risk of wine spoilage, that is why a barrel is considered not anymore of use after five to six years (Towey and Waterhouse, 1996).

The last group of volatile phenols which can affect wine aromatic characteristics is the one of furans and its derivates. These compounds, differently from the previously mentioned, are originated in oak only after the toasting process, as visible in table 1,through the heating of pentose (Singleton, 1995)and confer to the wine a characteristic toasted aroma (Guchu et al., 2005).

The most common derivatives which are of interest for wine aroma are furanic aldehydes such as; *5-(hydroxymethyl)furfural*,*5-methylfurfural* and *furfural*whose chemical structure we can see in *figure 2*, those compounds areoriginated by the heating of the Glucose at 180 °C resulting from a complex series of reaction known as *Maillard reactions* (Cutzach et al., 1997).

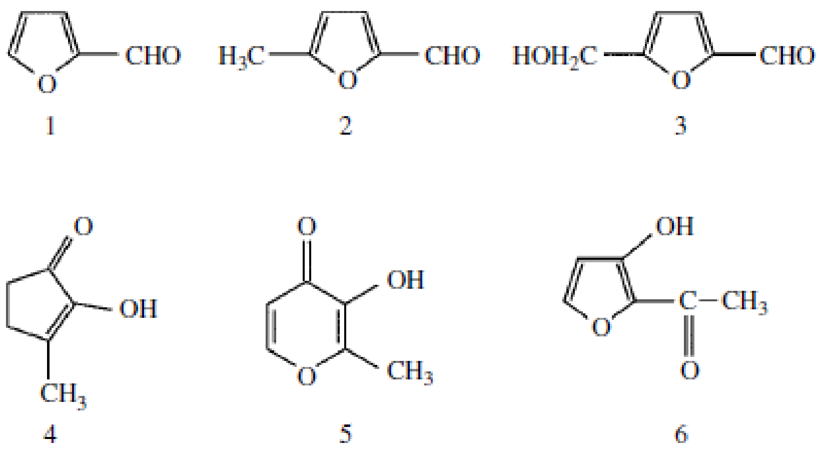


Figure 9- Furanic derivatives formed during barrel toasting: 1, furfural; 2, methyl- 5-furfural; 3, hydroxymethyl-5-furfural; 4, cyclotene; 5, maltol; 6, isomaltol (Ribereau-Gayon, 2010)

All the previously mentioned volatile compounds are present in very small or no quantities in untoasted wood, changing, as already mentioned, according to the species of oak, place of origin and single individuals (Jordao et al. 2006). The processes of staves seasoning is of extreme importance in order to let the wood dry (till 14-18 % of water), decreasing its level of water soluble compounds such as ellagitannins during a process of costantrehydratation in order to slow the process and preserve the wood integrity (Martínez et al., 2008). This phase can last from 2 to 3 years in case of natural seasoning, while for artificial seasoning this process is significantly shorter, but the wood such treated shows higher presence of bitter coumarins and astringent tannins (Martínez et al. 2008; Reynolds, 2010).

As to the volatile compounds, researches show different results; whisky lactones, eugenol and vanillin, indeed, seems to increase, decrease or remain stable during this process (Doussot et al., 2002). Another reason for seasoning it’s the action it shows to perform against possible wood shrinking and consequent leakage during wine aging in barrels (Spillman et al., 2004).

The following step is the toasting process, which can be made in different ways according to the desired characteristics of the woods; the heating method may change from various degrees of intensity by European coopers, to a more intense degree applied by American ones, with significant differences on the availability and concentration of the extractable compounds (Mosedale and Punch,1998). We can distinguish between light (120-180 °C for 5 minutes), medium (210 °C for 10 minutes) and heavy (230°C for 15 minutes) toasting (Ribereau-Gayon et al., 2010).

Among the various changes obtained in this process the most significant are the aldehydes which reach a maximum concentration with medium heat, volatile phenols which increase constantly with increasing heat level, whiskey lactones which lower as temperature increases, ellagitannins which are reduced, like during the seasoning process with increasing intensity for increasing temperatures (Bosso et al., 2008). In the case of oak lactones, has to be taken in account that even though the oak lactones are sensible to temperatures, exposure of wood to high temperatures (200 for less than 15 minutes) causes the formation of lactones isomers from lipids and fatty acids resulting in higher level of oak lactones, as visible in table 1(Ribereau-Gayon et al., 2010).

However even though many authors tried to categorize the level of toasting according to the T (as reported in table 1) it is difficult to control T variations during the toasting process, because of the fluctuations of the fire intensity and because of the convective movement of the air (Campbell et al., 2005).

	NON TOASTED	***LIGHT***	***MEDIUM***	***HEAVY***
Ellagitannins (mg/l)	333	267	197	101
β-methyl-*γ*-octalactone (mg/l)	0.8	0.7	1.5	1.7
Furfural (mg/l)	0	5.2	13.6	12.8
Guaiacol (µg/l)	1	5.2	27.7	30.3
Eugenol (µg/l)	20	17.7	71.7	44.3
Vanillin (mg/l)	<0.1	2.1	4.8	3.1
Syringaldehyde (mg/l)	0.2	5.6	12.9	12.2

Table 6- Concentration of different aromatic compounds according to the toasting level, for β-methyl-γ-octalactones the reported results shows the combined concentration of cis and trans isomers. Mean of 3 samples taken at depths of 1.2 and 3 mm; compounds extracted in a dilute alcohol medium, under standard conditions (Chatonnet, 1995)

1.2.2. TANNIC COMPOSITION

The other meaningful group of phenolic compounds released into the wine from the wood is the one of tannins (of which I already mentioned the ellagitannins); compounds known for their binding protein ability which makes them of high importance into the wine for their astringency-conferring capacity (Puech et al., 1999). We can distinguish between the hydrolizable (the only present in the oak wood) and non-hydrolizable, also known as condensed tannins or proantocyanidins tannins which are merely grape originated, but can be found in alternative kind of woods as we will see later on (Chinnici et al 2015; Ribereau-Gayon et al., 2010; Souquet et al. 1996).

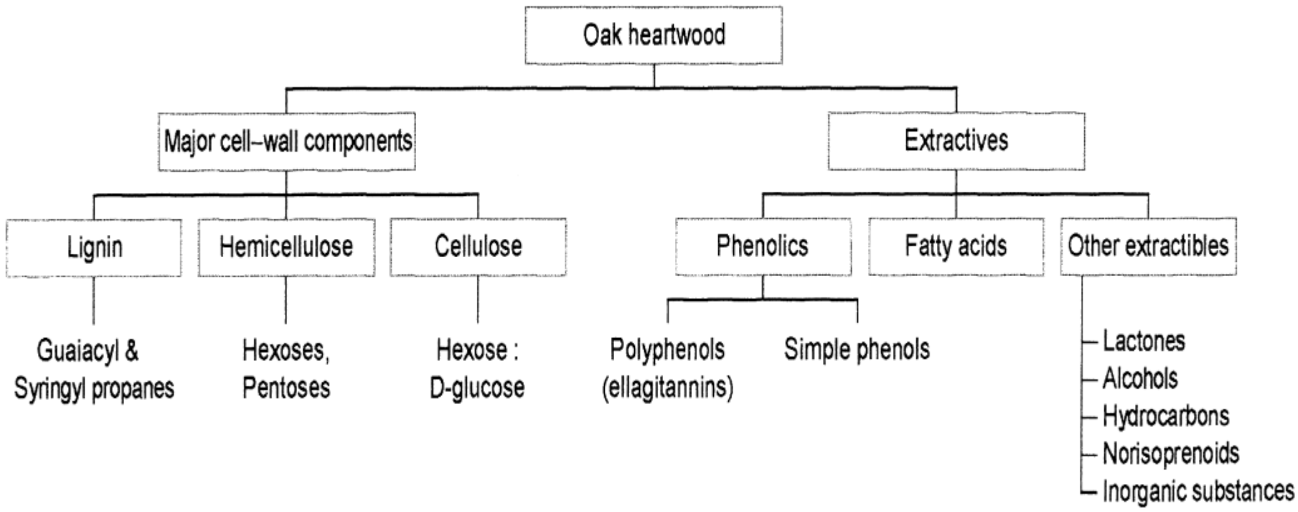


Figure10-Components of oak heartwood. (Mosedale and Puech, 1998)

The hydrolizable tannins are the only present in the oak wood and, as suggested by the name, are highly soluble and can be transferred to the wine (Viriot et al., 1993). The ellagitannins may make up to 10% of heartwood dry weight and are the main hydrolizable tannins present in the wood with castalagin and vescalagin being the most abundant ones (Puech et al., 1999). Those ellagitannins, once dissolved into the wine, are slowly but continuously converted into other compounds such as ethyl derivatives and flavano-ellagitannins through the combination with flavan-3-ols of grape origin (González-Centeno et al., 2016) which are found to be of highly importance in medical research against replication of HIV virus, but also to have antioxidant, anti-tumoral, anti-inflammatory,antibacterial properties (Sosic et al., 2015; Jourdes et al, 2011).

1.2.3. INFLUENCE OF OAK SPECIES ON COMPOSITION

To conclude this chapter is to be shortly mentioned the differences found on the heartwood composition of the different oak species already named. The quantities of potentially extractable compounds in oak barrels, as previously said, are determined by many factors, among which the species fromwhich the wood come from is one of themain, together with the zone of origin (Miller et al., 1992; Mosedale et al., 1996; Cadahia et al., 2001), with significant differences in both chemical composition and in flavor assessment.

	Quercuspaetrea	*Quercusrobur*	*Quercus alba*
Methyl-octalactone (µg/g)	77	16	158
Eugenol (µg/g)	8	2	4
Vanillin (µg/g)	8	6	11
Total extractables (mg/g)	90	140	57
Ellagitannins (mg/g)	8	15	6

Table 7- Differences in composition of the different oak species (Chatonnet, 1995)

American oak (*Quercusalba*) shows to have lower level of polyphenols but to be more aromatic because of higher presence of β-methyl-γ-octalactone (as evident in table 2) which is sometimes excessive and could be detrimental to wine quality (Chatonnet and Dubourdieu, 1998; Mosedale and Puech, 1998). About ellagitannins, we can state that their level independently from the origin of the oak see in the American a lower level and in French a higher one (Viriot et al., 1993; Jourdes et al, 2011). *Quercus robur* seems to have higher hydrolizable tannins than *Quercus paetrea*, which in turn shows higher levels of aromatic compounds, even though this seem to be influenced also by the growth area (Reynolds, 2010)

Moreover many authors have observed a high level ofscopoletin as a characteristic of American oak, even though in variable amounts (Puech and Moutounet, 1988). Another significant difference has been observed in the level of nor-isoprenoid, found in American wood but almost absent in European oak (Prida and Puech, 2006). Always according to Prida and Puech (2006) the species factor seems more correlated to whisky-lactones and ellagitannins, while for origin distinguishing is the eugenol level and aromatic aldehydes (mostly vanillin and syringaldehyde).

1.3.WOOD CHIPS: EFFECT ON WINE

Now that we have gone through the characteristics of the different oak and how the wood is treated in order to enhance wine quality, let’s take a look more in detail at the specific effect that wine chips have over a wine.

We have seen how the use of wood chips is a recent achievement (allowed since 2006 in Europe) and could be a valid alternative to wine aged in barrels being much cheaper and less time-consuming, resulting in a rather similar sensory profile for the treated wines, even though distinguishable due to, as suggested by Wilker and Gallander (1989), oxygen role in barrel aging. Moreover since the wine chips are traditionally added in wine stored in tanks or in old barrel with clogged pores; their use reduce wine loss due to evaporation with consequence economic advantages (Kyraleou et al., 2015).

The wood chips are obtained from wastes produced during barrel manufacturing and are treated with traditional methods in cooperage, such as boiling them in the water and toasting (Bozalongo et al., 2007; Navojska et al., 2012;Campbell et al., 2005).This leads to the perception that such products cannot yield the same quality results as those aged in barrels (Ribéreau-Gayon et al., 2010)

Wines treated with oak chips mature more quickly than barrel-aged wines showing deeper color at the end of the aging (Sanchez-Palomo et al, 2017; Gómez Gallego et al., 2014), even though has been observed a more rapid loss of anthocyanins of those when the wine is preserved in bottle (Bozalongo et al, 2007).

Jourdesin his study(2011) comparing levels of C-glucosidicellagitannins (Castalagin, vescalagin, roburin and β-1-O-ethylvescalagin) in red wine aged in oak barrels and with oak chips, showed how their concentration in wine, and hence, their extraction from wood, was always faster using oak chips, reaching a maximum in 2 months, but eventually being always higher in the wine from oak barrels, we can observe the level of extraction in Figure 4 and Figure 5.

This can be explained, according to Jourdes by the fact that the wood chips are surrounded by solution (wine) resulting in a deeper extraction by infiltrating the primary and secondary xylem vessels of the wood structure, while in wood barrels the vessels are not accessible from the barrel oak staves.

In the same study was discussed the effect of different toasting levels on extraction, stressing the relation between lower toasting level and higher ellagitannins content, because of their sensibility to thermal degradation; observation supported also by other studies (Garcia et al. 2012; Chira and Teissedre, 2012)

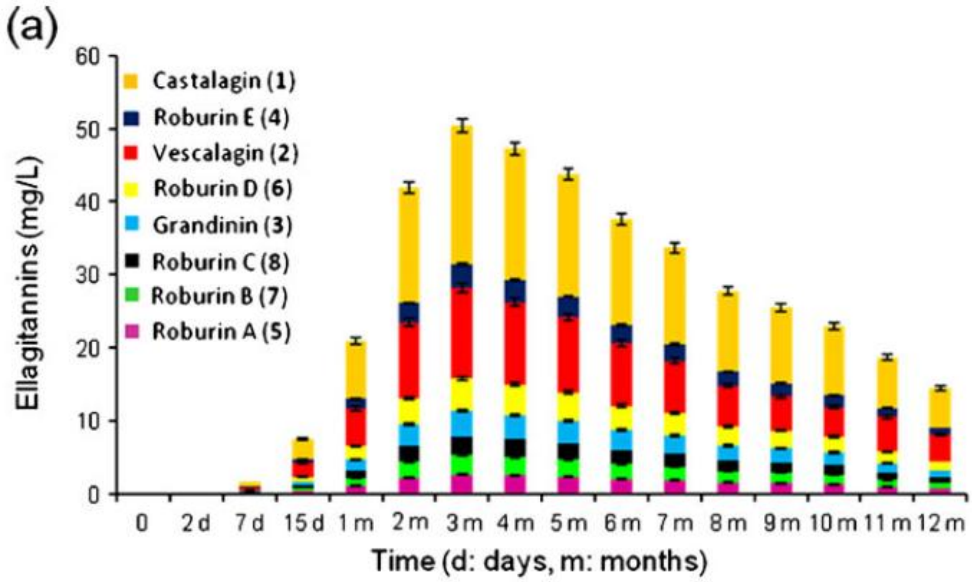


Figure 11- Graphic showing the extraction through time of different kinds of Ellagitannins in wine during aging in oak barrel as reported in Jourdes et al., 2011

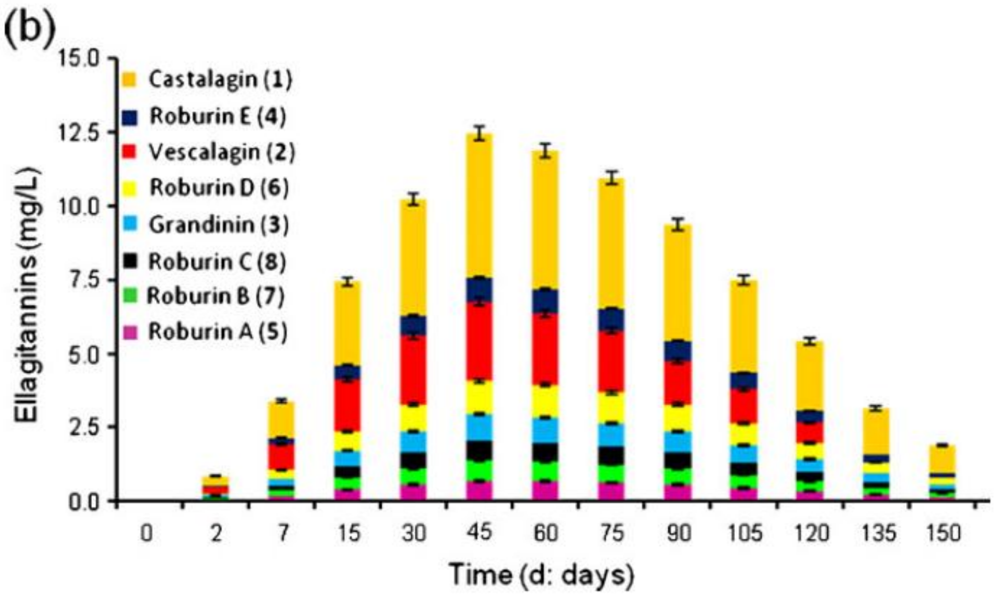


Figure 12- Graphic showing the extraction through time of different kinds of Ellagitannins in wine during aging with oak chips as reported in Jourdes et al., 2011

A different behavior has been instead observed for aromatic compounds previously mentioned; aldehydes such as syringaldehyde and vanillin. These compounds initially showsa trend similar to the one observed for the ellagitannins, accumulating in the wine in higher amounts, if compared to the barrel aged wines. Eventually, however, as opposed to the hydrolysable tannins the final amount of these aromatic compounds are on average higher in chip treated wines than in barrel aged winesespecially for furfural (more than double) and syringaldehyde,even though characterized by different extraction rates (Arapitsas et al, 2004).

A study by del Alamo Sanza(2004)also showed a higher extractionof low molecular weight phenolic compounds such as vanillin and gentisic acid in a wine treated with chips if compared to other aging techniques (staves or barrels), suggesting as reason the higher homogeneity and higher dispersion for the wine/chips system if compared to the others.

Gutiérrez Afonso, in his study comparing fermentation in barrels and with oak chips(2002) showed how oak chips in a white wine conferred even greater effect on the sensory profile of the resulting wine if compared to barrel fermentation, with more pronounced wood aromas such as coconut and vanilla and a greater taste impact on bitterness and astringency; those effects were more evident when American oak was used.

As to the other aromatic compounds we can state that the presence of whiskey lactones, together with eugenol and guaiacol, has been detected in higher amount in wines aged in wood barrels than in chips treated wine in Grenache wines (Franco Aladrén et al., 2007).

1.3.1. CHIPS ADDITION IN DIFFERENT WINEMAKING STAGES

It has be taken into account, however, that the toasting level,rather than the botanical species of the oak chips, shows to have relevance on the wine chemical composition and sensory characteristics, even though the differences in the variety of oak used were more noticeable in oak chips than in barrels (Bozalongo et al, 2007).

The chips' addition to the wine can produce different results on the final products according to the time of addition. Several studies shows how the addition of chips during fermentation can have a huge impact on wine composition as well as in the decrease of astringency and bitterness (Rodrıguez-Bencomo et al., 2009). In a study about Verdejo wines comparing different time of addition for chips has been observed how the wines fermenting with chips showa higher general level of benzenic compounds, such as aromatic alcohols, aldehydes and volatile phenols, ifcompared to post-fermentation addition, while lower quantity of eugenol, isoeugenol and vanillin as well as furfural and 5-methyl-furfural (Sanchez-Palomo et al, 2017). The lower presence of this compounds are due to the action of the yeasts which can convert them in their correspondent alcohol (vanillic and furfuryl) which has a much lower perception threshold (Perez-Coello et al, 2000; Gutierrez Afonso, 2002).

Compounds conferring the characteristic woody aroma to the wine such as cis-β-methyl-γ-octalactone, and considered detrimental if excessive in the wine, were not found in wines undergoing fermentation in presence of oak chips, while an higher presence of alcohols, acetates and certain esters in these wines has also been observed (Gómez García-Carpintero et al., 2012). These compounds are product of yeast metabolism and do not see an increase in wines after addition of chips following the alcoholic fermentation. The effect is explained by the higher fermentation yields obtained when the must is fermented in presence of the oak chips. Indeed the oak chips act as a carrier for the yeast cells exerting an effect similar to that of immobilized cells, as suggested by many authors (Perez-Coello et al, 2000;Rodrıguez-Bencomo et al., 2009; Sanchez-Palomo et al, 2017).

Studies about the addition of chips during malo-lactic fermentation, instead, are not numerous and in some cases giving contradictory results. However we can say that in the case of addition of chips during this phase we can observe a higher presence of the characteristic woody aromas like whiskey lactones vanillin and syringaldehyde and lower presence of fruity aromas due to the lower presence of esters of fatty acids (Gutierrez Afonso, 2003; Gomez García-Carpintero et al., 2011).

Maceration of chips in young wines, finally, showed results closer to the ones observed with chips added during Malolactic Fermentation than during Alcoholic Fermentation, with marked wood characteristics and presence of already mentioned whiskey lactones, vanillin and syringaldehyde, but in lower amount (Gómez García-Carpintero et al., 2012; Sanchez-Palomo et al, 2017).

1.3.2. INFLUENCE OF WOOD CHIPS SIZE

Another parameter which must be taken in account when using wood chips is their size. Indeed the chips' size influence the surface in contact with the wine, and hence, the capacity of extraction of the latter. Smaller size chips should import more flavor to the wine due to higher surface-to-volume ratio (Reynolds, 2010). Moreover we can observe how the process of toasting is also different according to the sizeof the wood fragments, changing its composition and effect on the wine.

That's why in using wood chips we should always be sure whether they are consistent in composition particle size and batch characteristics (Bowyer et al., 2007).

It has been observed that smaller size chips can absorb quicker the wine, which can penetrates and soaks the chips totally, facilitating the process of aroma compound diffusion from the wood to the wine (Morales et al., 2004).

Gimenez Martinez et al. (2001) studied the effect of toasting on chips composition according to the size, observing that for fragments with diameter between < 0.1 mm and 5 mm the concentration of aromatic compounds such as vanillin and syringaldehyde were proportional to the particle size, with more aldehydes extracted from the 3-5 mm diameter chips and highlighting the higher balance in composition of such fragments. However, it seems that when the size is <5 mm, losses due to evaporation may occur.

In particular guaiacol, as we can see in table 3, seems to be influenced by particles size, showing increasing concentration with higher particle size (Arapitsas et al., 2004).

Table 8- values of aromatic extractives from chips of different size, the amount of chips added to the wine were calculated in order to have the same surface exposed to the wine in both treatments. values expressed in mg/l of wine. The dimensions are 1 cm x 1 cm x 0.1 cm (SMALL) and 3.4 cm x cm x 1 cm (BIG) (adapted from Arapitsas et al. 2004)

CHIPS' DIMENSION	***SMALL***		***BIG***	
	1 WEEK	2 WEEKS	1 WEEK	2 WEEKS
Furfural	3,8	7,3	6,3	6,9
Guaiacol	0,05	0,07	0,125	0,151
Oak lactone	0,380	0,405	0,347	0,400
Eugenol	0,007	0,009	0,01	0,04
Vanillin	2,6	3,03	2,7	2,9

Concluding we can say that clearly the effects of chips on the wine differ from those given by barrel aging, even though being slightly similar; however, being the wine a highly diversified product, the use of this technological tool could provide several new and more economical solutions to winemakers, which thanks to the many possibilities of use of the chips can create products meeting the consumers demands and being economical advantageous for both the producer and the buyer.

1.3.3. WHITE WINE AND WOOD

In the last chapters we have seen the characteristic of the wood and in specific the effects of wood chips addition to the wine. Howsoever the utilization of wood in contact with wine is a technique used mainly with red wine, while for white wine, in order to maintain the freshness and fruitiness, is preferred to not use the wood, in order not to alter the aromatic profile with woody characteristics (Ribéreau-Gayon et al., 2010), but also to avoid oxidation to which the white wine is more sensible due to lower level of antioxidants (mainly phenolic compounds) (Waterhouse and Felipe Laurie, 2005).

In certain regions, though, the use of wood in the aging of white wine is a key technology in order to enhance the wine quality and obtain a product with unique characteristic. It is the case of Burgundy wine region in France where the link among its main white variety (Chardonnay) and wood barrels is well known. When for a white wine contact with wood is considered, special adjustment should be taken, in order to achieve the best quality; a main factor to be considered is contact with lees.

Indeed even though we know how the phenolic compounds are fundamental for wine quality (Hernández et al., 2006) some of them are also responsible for oxidation and consequent color change (browning) in white wines, while others act as antioxidant, with mechanisms involving both free-radical scavenging and metalchelation (Pérez-Serradilla and Luque de Castro, 2008). The importance of wine lees comes from the fact that they can adsorb phenolic compounds (Mazauric& Salmon, 2005) and release to wine both phenolic compounds and enzymes (after autolysis) that can modify the phenolic fraction (Ibern-Gomez et al., 2000). Besides the other beneficial effects of the wine lees, including prevention of tartrates precipitation (Vernhet et al., 1999) and protein haze formation (Waters et al., 1993), makes the use of wood strictly related with the aging on lees.

Anyway the aging on lees present some risks such as formation of unpleaseant aromas such as sulfur and animals odors (Delteil, 2002). In this optic could be interesting to study the effect of chips wood addition in white wines without contact with lees (as is the case of this study) in order to assess the effects on mouthfeel (modification of astringency and bitterness) due to the release of compounds from the chips into the wine, including polysaccharides of the wood (Rodrıguez-Bencomo et al., 2009).

In the case of a white wine where the lees were removed immediately or shortly after the end of fermentation the contact with wood is supposed to be more invasive on the aromatic profile (Pérez -Serradilla and Luque de Castro, 2008) and should consequently be limited to a shorter period.

1.4.ALTERNATIVE WOOD SPECIES

Hence the role of wood in winemaking is fundamental for the quality of the final product,releasing compounds in the wine and altering its composition and sensorial profile. Due to the high cost of oak wood new more economic alternatives are being considered, such as falseacacia (*Robinia pseudoacacia*), cherry (*Prunus avium*) and chestnut (C*astanea sativa*), and in minor extent also mulberry (*Morus alba* and *Morus nigra*) and ash (*Fraxinus excelsior*).

The extent of the extraction in the wine depends on the initial concentration of wood compounds, which in turn depends on the type of wood employed and its chemical composition (Alañon et al., 2011). The chemical composition of the different mentioned species can give different results ; acacia is characterized by significant contents in benzene aldehydes, chestnut by richness in polyphenols and cession of vanillin and eugenol to the wine, while cherry by release of methoxyphenols (Del Rosso et al., 2009).

Thus in order for the winemaker to decide which alternative is better for its winemaking purpose we need to analyze the characteristic of each wood.

1.4.1. CHERRY (*Prunus avium*)

The wild cherry is native of Europe being widespread in all the continent *(*Caudullo et al., 2016*)* due to this its use for winemaking purposes is increasing due to low costs and presence in traditional local production like balsamic vinegar and cider aging (Chinnici et al., 2014).

Extracts from cherry tree chips showed phenolic and furanic profiles, total phenols content, and antioxidant activities equivalent to commercial oaks (Rodríguez Madrera et al, 2010*)*. However, differently from other woods used in cooperage, mainly oak and chestnut, where gallic acid, hydrolizable tannins and ellagic acid prevail among all, cherry seasoned wood has been observed having high levels of flavanols and procyanidins (mainly catechin and its dimer constituting 90% of the flavanols concentration) with flavanones being the second chemical class by importance (Chinnici et al 2015). The ratio between hydrolizable tannins and procyanidins is more evident in seasoned wood being about 1:10, while less evident in toasted wood (Sanz et al. 2010).

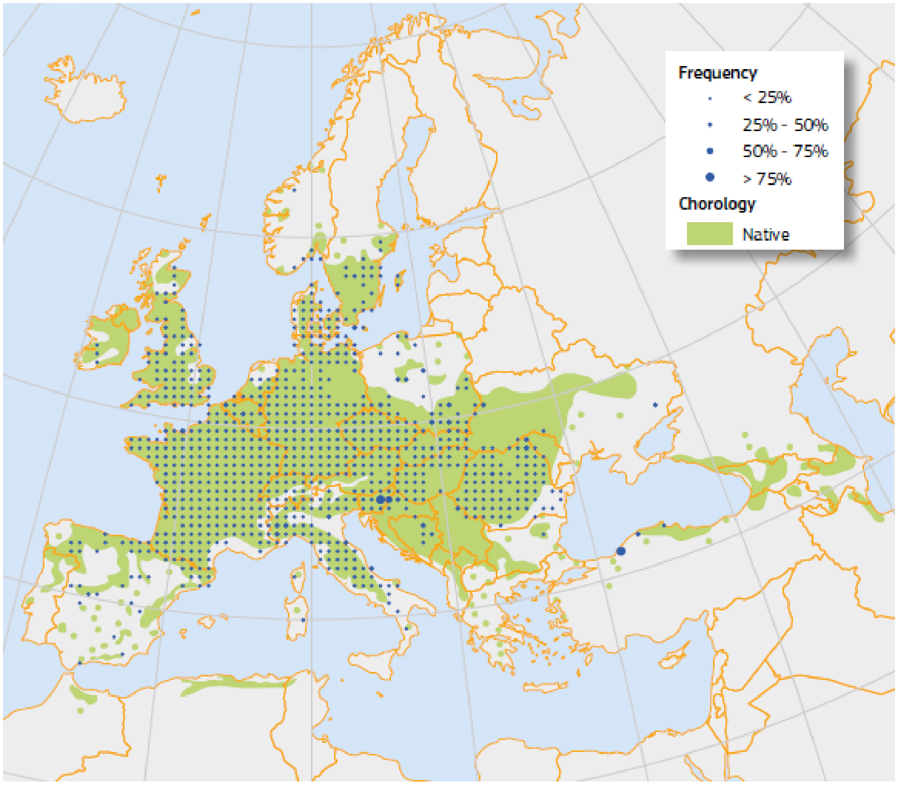


Figure13- native area and distribution of *Prunus avium* (Caudullo et al., 2016)

Trimethoxyphenol, methylsyringate, benzoic acid and methylphenol were also found abundant in cherry, with the latter conferring undesiderable ink or bitumen taints (Flamini et al, 2007). On the contrary phenyl aldehydes and phenyl ketones are found in lower concentration, with the exception of vanillin, while, after toasting, lignin derivatessuch as sinapaldehyde, syringaldehyde and coniferaldehyde were the most abundant. In toasted wood is also to be mentioned the lower presence of furfural compounds due to different structure of its polysaccharides, resulting in higher resistance to thermodegradation (Fernandez de Simon et al. 2009).

Further research is needed on cherry wood composition, however, even though wines aged from different wood specie present differences a general pattern of wine behavior cannot still be created, because during wine aging the presence in wine of constitutive phenols changes depending on a number of factors such as; wine type, initial phenolic composition, wood specie and permeability to oxygen (Chinnici et al., 2008).

Cerezo et al.(2008)substains that cherry wood positively contributed to red fruits notes and aromatic complexity of vinegars, other researchers reported this wood as the most oxidative between many species (De Rosso et al. 2009). In another recent study (Chinnici et al. 2011) was observed that if compared to oak, cherry could promote a faster pigment stabilization in 1000 l cask, maintaining at the same time the highest color density and the best chromatic attributes of wines.

The class of flavanolsin wines aged in cherry casks seems to have a fast decrease during barrel aging, this data is supported by Del Rosso et al.(2009)and Chinnici et al. (2015) but not by Fernandez de Simon (2014) where the level of catechin in wine are recorded to be higher.

The diminution of the said compounds could be due to oxidation or polymerization, however contrary to what observed for oak, where ellagitannins role in polymerization reactions have been understood, the action of wood components (flavanones, flavanonols and flavones) of cherry is still unknown, although there are reports (Chinnici et al. 2011) suggesting that in cherry aged wines,flavonols could be involved in acetaldehyde mediated condensation, enhancing pigment and thus color, stabilization.

Undoubtedly, however, cherry promoteflavonols decrease around 30% more than oak as reported by different studies (Chinnici et al 2015; De Rosso et al. 2009).

As we discussed in the chapter for chips, the effect on wine of barrel and chips from the same wood is different, not only for the different role of oxygen uptake (Bozalongo et al, 2007).

However in a study from Tavares et al. (2017) reported a similar behavior of the wine aged with cherry chips regarding the anthocyanins evolution pattern, showing a higher color fixation rate than control wines, suggesting that this effect is not solely due to oxygenation during barrel aging and for aroma descriptors, where fruity characters were enhanced.

In the same study was analyzed also the evolution of tanning power, which could be defined as the tendency of a wine’s tannins to react with salivary proteins giving an astringent effect. thewine showing the highest reactivity after 90 days was the cherry chips treated one , probably due to the high presence of procyanidins which are the most reactive among the tannins group (Hoffman et al, 2006).

1.4.2.ACACIA (*Robinia pseudoacacia*)

Robinia Pseudoacacia, known as black locust, is, among the alternative woods mentioned, the only one not original of Europe, eventhough nowadays is highly present due to its strong invasive nature (Huntley, 1990) making this specie available for cooperage use.

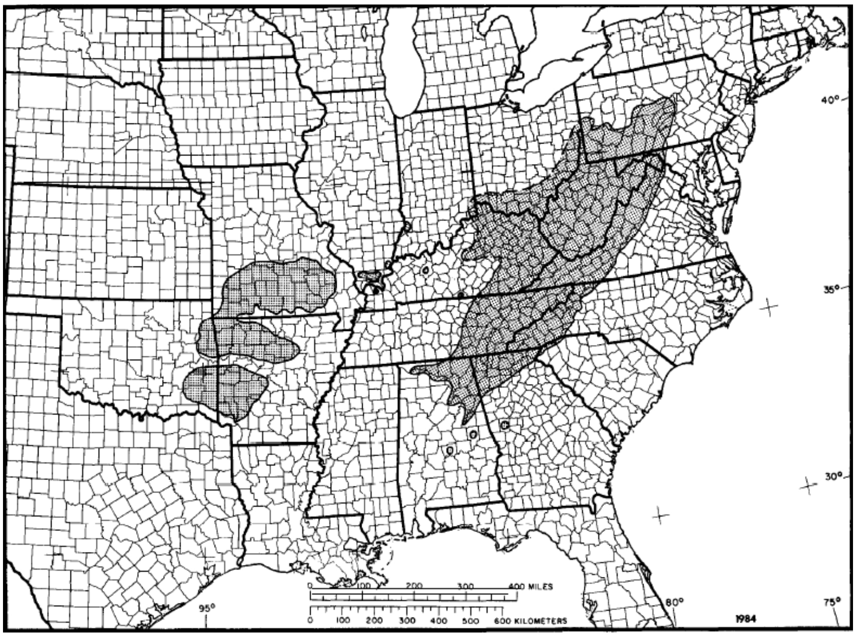


Figure 14-native area of RobiniaPseudoacacia (Huntley, 1990)

Acacia wood shows to have high concentration of flavonoids and low levels of non flavonoids compounds, with the main constituent being the dihydroflavonolsdihydrorobetin, fustin, theflavonolrobinetin and the flavanonesrobetin and butin (Roux and Paulus, 1962). The dihydrorobetin and robinetin flavonoids, the most abundant in acacia heartwood, seems to be characteristic of this specie, not being detected in other woods used for cooperage such as; oak, chestnut, cherry and mulberry (Sanz et al., 2010; Fernandez de Simon et al., 2009).Indeed the acacia wood is showing, among the mentioned ones, the highest number of distinctive non-anthocyanic phenolic compounds, i.e. compounds found only in its wood (15); making the acacia-aged winesextremely easy to detect through analysis (Fernández de Simón et al, 2014).

In particular dihydrorobetin and robinetin shows an antifungal activity and increase in acacia heartwood with maturity, as observed also for the phenolic compounds (Sergent et al., 2014; .Latorraca, Dünisch and Koch,2011).Regarding tannic composition, on the opposite of oak, rich in ellagitannins, and cherry, where procyanidins are mainly present, acacia heartwood seems to have no ellagitannins and low presence of condensed tannins, mainly as prorobinetin type(Tavares et al., 2017; Sanz et al., 2012).

The low molecular weight profile shows compounds with a β-resorcylic structure, gallic related compounds, protocatechuic aldehyde, and some hydroxycinnamic compounds as the main ones, but only a little gallic and ellagic acid; those compounds were found to increase proportionally to the intensity of toasting (Sanz et al., 2011).

Wines aged in acacia wood, therefore, presents the mentioned molecules in solution, in particular the dihydrorobinetin concentration is relevant, but also robinetin, fustin and butin; because of this, such compounds could be used as markers fro authenticity purposes (Sanz et al., 2012).

The acacia aged wine flavonoids level was slightly lower in acacia if compared to the effect of other wood specie, in a study from Tavares et al.(2017)was observed the lowest value in acacia wines second only to the French oak wine. The same report observed also an increase in color hue of the wine aged with acacia chips, which was maximum after 90 days.

1.4.3.CHESTNUT (*Castanea sativa*)

Chestnut wood was widely used in the past for winemaking, because of its availability and low price, and today, together with oak is the only wood allowed to be used by the OIV (Garcia et al., 2012).

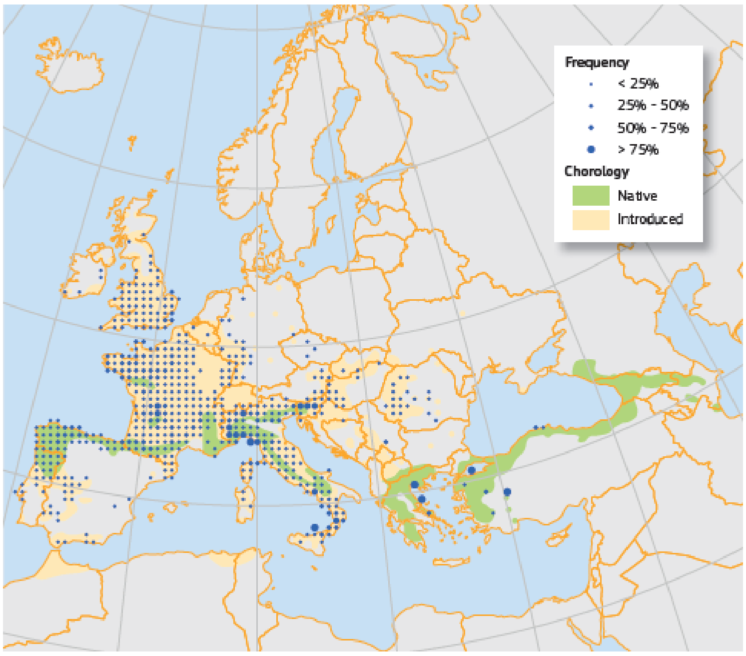


Figure 8- native area and distribution of Castanea Sativa (Caudullo et al 2016)

On a structural level is to mention the higher porosity observed in chestnut wood if compared to oak (De Rosso et al., 2009). Its heartwood shows the most similar polyphenols profile to oak although its LMW phenolic and tannic contents are higher, showing presence of hydrolysable tannins, condensed tannins and high level of gallic acid. On the contrary of oak, though where only ellagitannins are present both ellagitannins and gallotannins are found (Canas et al., 2000; De Vasconceloset al.2010).

Because of its richness in gallic acid and hydrolysable tannins, chestnut commercial tannin agents are used as enological tannins(Sanz et al, 2010). Higher levels of guaiacol and vanillin, if compared to oak, were also detected in this wood, as well as presence of furan compounds in untoasted wood (Alañón et al., 2013). When toasted, chestnut shows to be very rich in lignin derivatives, highlighting high concentration of sinapaldehyde, isopropiosyringone, syringaldehyde and vanillin, as to the Furfural and its derivatives it shows to have the highest concentration among the woods used for cooperage, like oak and its alternatives (Fernandez de Simon et al., 2009).

Due to its rich composition chestnut wood releases a huge amount of phenols into the wine contributing to alter the aromatic and organoleptic characteristic of the wine. Chestnut aged wine for a six months period shows an increased aromatic profile and excellent sensorial balance between wine and toasted chestnut wood scent, however undesirable concentration of 4-ethylphenol and 4-ethylguaiacol, originated by *Brettanomyces/Dekkera* species and responsible for off-flavours, were detected in red wine aged for longer period in chestnut barrels. This could be connected to the high micro oxygenation to which the wood is subjected, stimulating the action of such microorganisms(Alañón et al., 2013).

1.5. THE VARIETY

Encruzado variety finds its origin inDao region and is mostly grown in this region, where is the first cultivated variety with about 300 ha, representing the 0,1 % of the Portuguese vineyards, surface which seems low if compared to the enologic potential of the variety (Böhm, 2007). According to Mayson (2005) the origin of this variety are not totally clear, even though there are evidences that it was cultivated already in the XIX century under the name of Salgueirinho.

As to the morphology, the wines' leaves are small and pentagonal with five lobes, short and convex teeths , a particular of the leaf is the anthocyanic pigmentation of the main veins as visible in figure 8.

The bunch of grapes is small and compact, the grape berries are medium-sized, heterogeneous and slightly flattened and its epidermis is yellowish-greenish. It is a medium maturing variety, producing wines with strong citrus color good alcoholic content and good complexity, making them suitable for aging (Commissãovitivinicola regional do Dão; www.crvdao.it).

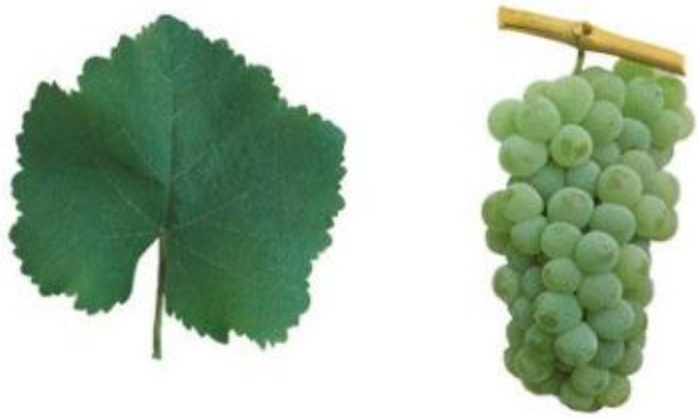


Figure 9- leaf and bunch at full maturity of Encruzado

The Encruzado variety shows a high vegetative vigour and a medium to high level in productive terms (2,5 kg/vine), the must obtained from its grape shows high alcoholic potential but high sensitivity to oxidation as well (Böhm, 2007). The acidity/sugar ratio is of great balance (Mayson, 2005) but the bunch seems to be prone to rot and unfavorable climatic conditions like wind and rain.

The wines made from this variety show good aromatic profile presenting good suitability for the aging (Loureiro, 2002), indeed many authors shows the potential of this wine, if properly managed, to resemble for elegance and body, a Burgundy-wise white (Böhm, 2007; Mayson, 2005; Loureiro, 2002). This make this variety particularly interesting for investigations on the interactions between the wood and the wine as testifies the increasing publications on the effects of wood contact with encruzado, both with chips or in barrel (Nunes et al., 2017; Jordao et al., 2017).

2. AIM OF THE STUDY

There is now increasing interest about the use of wood alternatives to oak for winemaking, in the previous chapters we have gone through some of them, both with barrels and chips. Many studies have stressed the effects and potentiality of chips from oak and alternatives species used in winemaking practices, mainly on red wines.

A previous study from Delia et al. (2017) studied the effects of Acacia and Cherry wood chips on the quality parameters of Encruzado wine, showing interesting results especially about the sensory profile.

The investigation reported in this work was conducted in Istituto Superior de Agronomia during 2018 and the Aim of our study was to detect the effect on the quality parameters of the wine, namely phenolic composition and sensorial profile, with the use of blends of wood from different species.

We used three kind of woods; American oak (*Quercusalba*), Cherry (*Prunusavium*) and French oak (*Quercuspaetreae)*. Each blend was composed of 2 species of the mentioned wood at 50% each.

The sperimentation started on February the 1st and samples were taken from each treatment at 15, 30 and 60 days from chips' addition. The amount added to each wine was 0,5 g/l in a 10 l bottle.

It is worth to remember that the wine we used was not prepared to be aged with wood, due to the fast removal of the lees. This was done in order to observe the evolution of a traditional white wine in contact with different kinds of wood.

3. MATERIAL AND METHODS

3.1. WINE AND WOOD MATERIALS

The wine used in this study is a white wine obtained in Istituto Superior de Agronomia from Encruzado grape variety of the 2017 harvest. Even though maceration of the grape with the skins was not used, it has to be taken into account that due to the fact that the harvest was done by volunteers during about 2:30 h the grapes collected in the press underwent a short period of premaceration. The winemaking process used was the one of classical white wine.

The grape harvested on 23/08/2017(3700 kg) was destemmed and crushed and the wine used in this studied was the one obtained by free run must from the press, corresponding to the 73% of the total must. Before alcoholic fermentation SO_2 was added in concentration of 25 mg/l, the first 24 hours the must was kept in the vat at 11 °C. The fermentation lasted from the 23rd of August till the 7th of August when density reached the value of 991. It was run under controlled temperature at 18 °C for 13 days. After Alcoholic fermentation racking was performed.

Right below is reported the summary of chemical analysis of the wine.

pH	**3,42**
total acidity (g/l of tartaric acid)	**5,25**
volatile acidity (mg/l of acetic acid)	**0,28**
total SO2 (mg/l)	**24**
free SO2 (mg/l)	**88**
alcohol content (%)	**14,6**

Table 9- results of the analysis of the Control wine on day 0

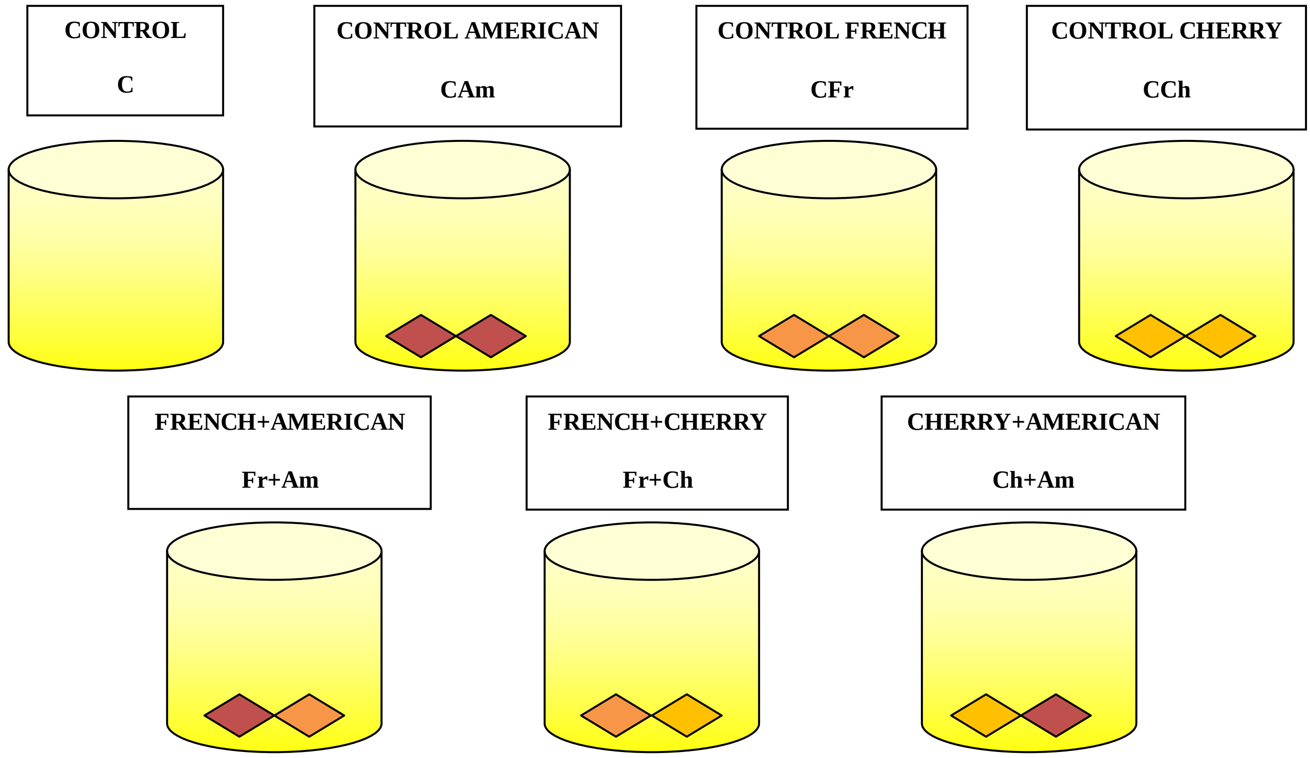
The wood chips material was obtained by AEB companywith medium toasting level, with a medium size; the chips are shown in figures 9-10-11.

Figures 10-11-12. The chips used in the study; A for American oak, B for French oak and C for Cherry

3.2. EXPERIMENTAL CONDITIONS

The experimental work was conducted on the wine starting from the 1st of February 5 month after fermentation. The wood chips dosage was 0,5 g/l in carboys of 10 l added in 6 of the 7 treatments. Samples were taken before the preparation of the carboys and the addition of the chips, 15, 30 and 60 days after the addition.

The assay created were 7 as reported below;



The 4 controls included 1 assay without any wood addition and 3 with one species each, that is American oak, French oak and Cherry. The remaining 3 treatments were a blend of the 3 species of wood in a 1:1 ratio, American and French oak, French oak and cherry, American oak and cherry.

The treatments were kept in the cellar of Tapada de Ajuda at a constant temperature (14-15 °C) and stirred twice a week. After the first samples were taken the bottle were saturated with inert gas (N_2), with this operation performed after every sampling.

3.3. CHEMICAL ANALYSIS

The chemical analysis, were performed at the laboratory Ferreira Lapa (Enology Sector) of Instituto Superior de Agronomia. The results obtained from the first sample showed in table 4 weranalysedfollowing the methods recommended by OIV.

3.3.1. TOTAL PHENOLS

The determination of total phenols was executed first through a dilution with distilled water (ratio 1:10), hence by absorbance reading at 280 nm at the spectrophotometer (Somers and Evans, 1977). To calculate the amount of total phenols (TP) quantifiable from the absorbency, in mg of gallic acid per litre, a calibration curve was created, in order to correlate the absorbency value at 280 nm with the total phenol value expressed in mg of gallic acid per litre, obtaining the following equation:

$$Total\ Phenols = \frac{\mathrm{A280} + 0{,}0344}{0{,}038} \mathrm{x}\ 10$$

the multiplication per ten is due to the dilution rate.

3.3.2. NON-FLAVONOIDS AND FLAVONOIDS

The quantification of non-flavonoids followed a more complex procedure, based on the procedure fromKramling and Singleton (1970) and done in two steps. Initially the wine is centrifuged in order to let the precipitates accumulate on the bottom of the cuvette, then the wine such obtained (10 ml) is mixed with a 1:4 HCl solution (10 ml) and formaldehyde at a 8 mg/ml concentration (5 ml).

The mixure is then left to rest in a dark environment for 24 hours after being saturated with inert gas (N_2). After this first phase the solution is centrifuged and a dilution in ratio 1:10 with water is followed and the respective absorbance is measured at 280 nm in the spectrophotometer. The obtained results are then multiplied by ten for the dilution as in the Total phenols.The concentration is also obtained in mg/l of gallic acid using the same equation previously mentioned for the total phenols.

As to the Flavonoids their concentration was obtained by subtracting from the total phenols the non-flavonoids concentration obtained through the previous method.

3.3.3. COLOR INTENSITY

The color intensity values are normally obtained following the OIV method MA-AS2-07B: R2009 (Type IV method) by measuring the absorbance of a wine sample (previously centrifuged) at 420, 520 and 620 nm using the already mentioned spectrophotometer.

In our case, though as we analyzed a red wine the value taken in account was just the 420 nm.

$$Intensity = A420$$

3.3.4.TANNING POWER

Tanning power is the parameter referring to when we want to measure the astringency of a wine, or its tannic ability to interact with proteins, such as those present in saliva, causing an astringent mouthfeel. This was determined according to the method described by De Freitas and Mateus (2001); a centrifuged wine sample is diluted (1:50) with a previously filtered hydroalcoholic solution (12% v/v, 5 g/L tartaric acid and pH=3.2), followed by turbidimeter reading, corresponding to the *d0* value.

A bovine serum albumin (ASB) solution (0.8 g/L concentration) is then added to the previous wine dilution, agitated in a vortex and allowed to react for 45 minutes, at room temperature and sheltered from light. After the reaction period, another turbidimeter reading follows, thus obtaining the *d* value, after precipitation of procyanidins has occurred.

Results are expressed in Nephelometric Turbidity Units (NTU)/ml using the following equation:

$$Tanning\ power = \frac{\mathrm{d} - \mathrm{d0}}{0{,}08} = \mathrm{NTU/l}$$

3.3.5. SUSCEPTIBILITY TO BROWNING (MADERIZATION TEST)

This kind of test measures, as suggested by the name, how much a wine is prone to color change through time, due to oxidation process. The test is conducted by leaving a wine in contact with the air for 5 days at a constant temperature of 55 °C and a sample from the same wine in contact with inert gas for the same period of time at same condition, as described by Singleton and Kramling (1976).

After this period of time absorbance is measured for both the samples at 420 nm in the spectrophotometer, a value indicating the susceptibility to browning is then obtained according the following equation.

$$Susceptibility\ to\ browning = Abs420(02) - Abs420(N2)$$

3.3.6. SENSORY ANALYSIS

The sensory evaluation of the collected wine samples was carried out in the Polytechnic Institute of Viseu, by a panel composed by seven expert judges trained in quantitative sensory descriptive analysis of wines on the samples collected on 15, 30 and 60 days from the chips' addition. The sensory analysis was performed at 18-20 °C (room T) in a room specific for sensory analysis with individual cabinet for each expert.

In the session about 30 ml of each sample were labeled in a three digit random code corresponding to a specific treatment and present to the audience. The order of presentation of the wine samples were random for each judge in order to don't influence the judges. The wines were evaluated using different descriptors showed in the table below.

VISUAL	Color
	Limpidity
AROMA	Fruity
	Floreal
	Vegetal
	Spicy
	Almonds
	Aroma intesity
	Aroma quality
TASTE	Oxidation
	Woody
	Body
	Astringency
	Balance
	Persistence
	Flavor quality
GLOBAL APPRECIATION	

Table 10- descriptors used for the sensory evaluation of the wines

The experts scored each sensory attribute, except global appreciation, on a 1 to 5 point scale (1=absence; 2=little intensity; 3=moderate intensity; 4=intense; 5=high intensity) for each characteristic according to their sensory knowledge, training and experience; whilst, the global appreciation was scored on a 0 to 20 point scale summing the single evaluation of every parameter.

3.3.7. STATISTICAL ANALYSIS

In order to understand the statistical significance of the data obtained an analysis of variance (ANOVA) and comparison of treatment means (Tukey's test) was performed using SPSS (25) program, both for chemical and sensory analysis. The results analyzed were the mean values of the 3 assays in all the chemical methods. Statistical significance (at $p<0,05$) of the differences between mean values was assessed by Tukey's test.

In order to facilitate the interpretation of the results the values compared were the ones among the same sampling date and not of the same wine with different maceration period.

4. RESULTS AND DISCUSSION

The treatments will be named as following in the figures; (**C**) Control without any wood chips, (**CAm**) Control American with chips from American oak (*Quercus alba*), (**CFr**) Control French with chips from French oak (*Quercus petreae*), (**CCh**) Control Cherry with chips from Cherry (*Prunus avium*), (**Fr+Ch**) blend of French oak and cherry wood, (**Ch+Am**) blend of American oak and cherry wood and finally (**Fr+Am**) for the French and American oak wood. The bars with different colors report the values for the different sampling as reported in the legend on the side of the figure. For the chemical analysis.

4.1. TOTAL PHENOLS

The phenolic profile of a wine has an high influence on its aroma, mouthfeel, but also color and antioxidant capacity (Dufour and Bayonove, 1999). In white wines where these compounds are less present due to short or no contact with the skin, the contact with wood is an interesting technique for increasing their levels and thus improving wine final quality.

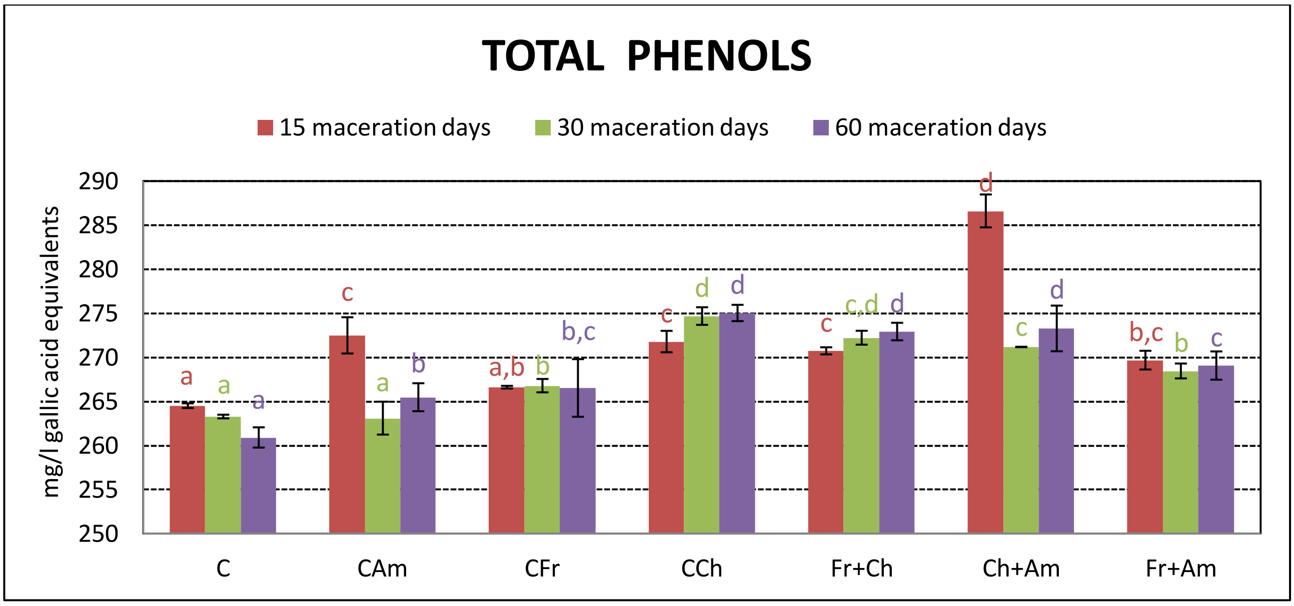


Figure 13- Evolution of total phenols for the 7 treatments during 60 days. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatments. The results with same letters are not significantly different. Legend: C: Control wine; CAm: wine aged with blend of cherry and American oak chips, Fr+Ch: wine aged with a blend of French oak and cherry chips, CCh: wine aged with cherry chips, Fr+Am wine aged with a blend of French and American oak chips: CFr wine aged with French oak chips; CAm wine aged with American oak chips.

We can observe how the essays shows highly variable pattern of extraction (Figure 12). The C wine has a steady decrease of Total phenols value, results expected and reported in different studies on white wines stored without any wood contact (Zafrilla et al., 2003; Recamales et al., 2006).

As was predictable, in the last sampling all the treatments with wood showed significant difference with the C. The highest increase in Total Phenols is reported in the wines with Cherry wood; that is CCh, which reaches a final value of 274 mg/l of gallic acid equivalent, followed by the the C+Am (273 mg/l) and F+CH (272 mg/l). This group of wines, after the statistical analysis,showed a significant difference with the other wines on the last sampling, as we can observe in Figure 12.

This result is in accordance with the study on Encruzado by Delia et al. (2017) where wine in contact with chips from cherry was the one with highest level of total phenols (after acacia chips, not present in our study), stressing out the relevance of cherry phenolic profile comparable to, if not higher than,the one of oak species (Rodríguez Madrera et al, 2010).

The statistical analysis for the American-Cherry group showed that the at the last sampling (60 days from the chips addition) the blend Ch+Am is significantly different from the CAm but not from CCh as visible in figure 13.

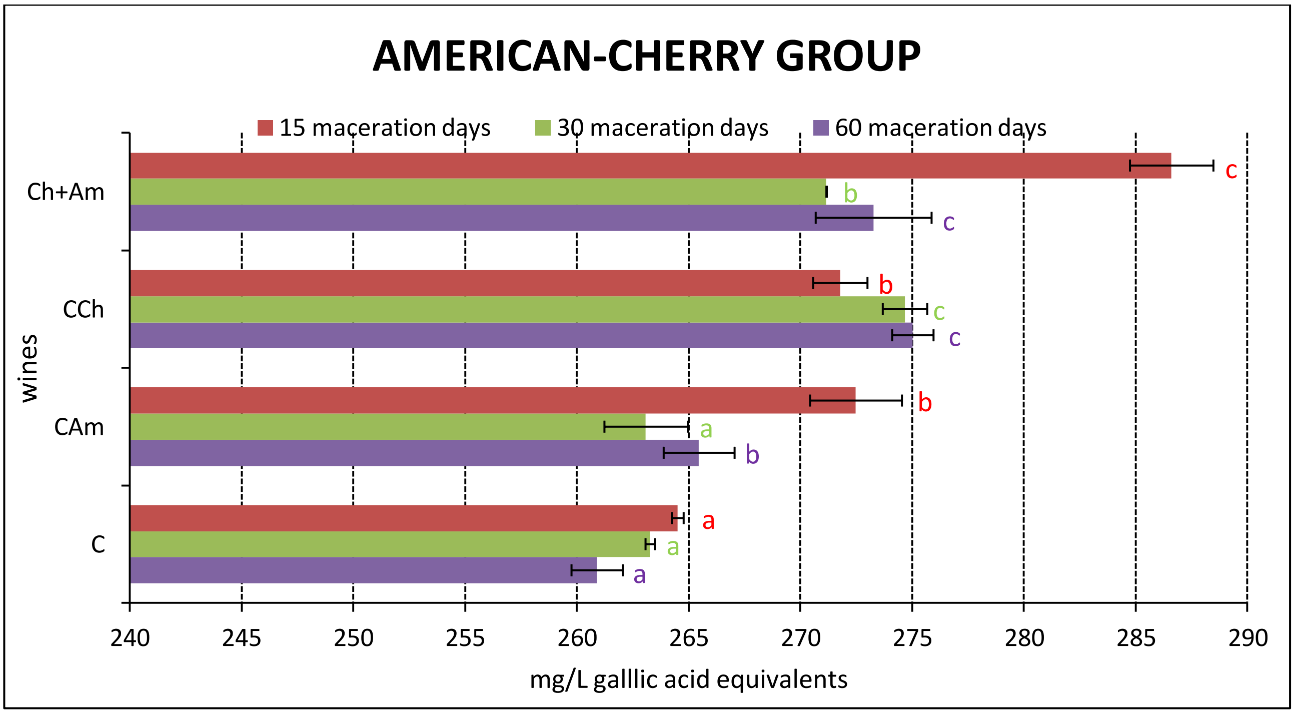


Figure 14- Evolution of Total phenols in the group with American oak and Cherry wood. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatments. The results with same letters are not significantly different. Legend: C: Control wine; C+Am: wine aged with blend of cherry and American oak chips, CCh: wine aged with cherry chips, CAm wine aged with American oak chips.

The CFr wine was the one showing almost no change during the samplings and a similar result was observed also for CAm with the difference of a higher result after 15 days, which is then mitigated in the following samplings. These results were also observed in the study from Delia et al. (2017) suggesting that as for French oak a short maceration time, of about 15-20 days is enough for an optimal phenolic extraction.

The statistical analysis of the French-American group showed that there is no significant difference among the wines with wood, but there is between those and the C as we would expect.

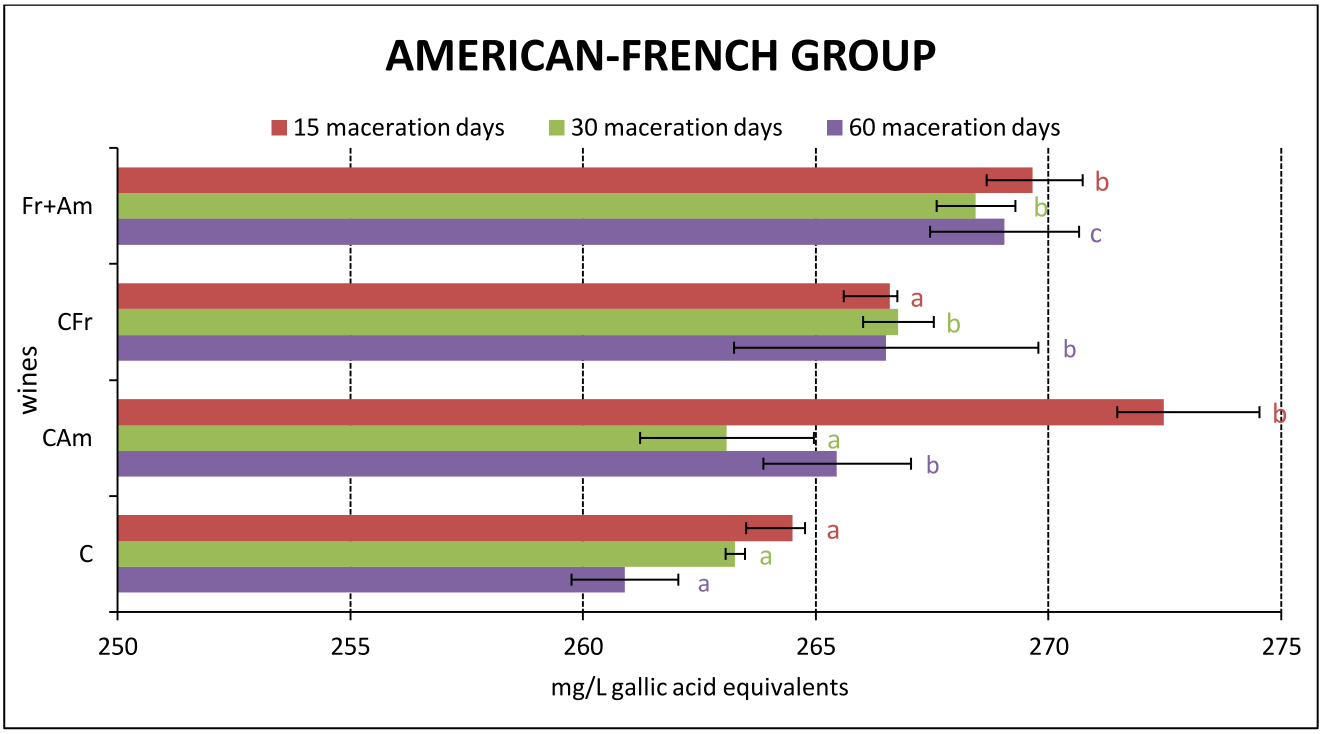


Figure 15- Evolution of Total phenols in the group with American oak and French oak wood. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatment. The results with same letters are not significantly different. Legend: C: Control wine; Fr+Am wine aged with a blend of French and American oak chips: CFr wine aged with French oak chips; CAm wine aged with American oak chips.

We can observe how the slow release of phenolic compounds in CFr is reflected also in the blends with the other woods; indeed the blend Fr+Ch showed the highest result among the wine with French oak, but the lowest if we take a look at the wine aged with cherry as we can see in figure 12.

In the French-Cherry group, as for the case of the American-Cherry, the last sampling showed a significant difference among the blend Fr+CH and the CFr but not the CCh.

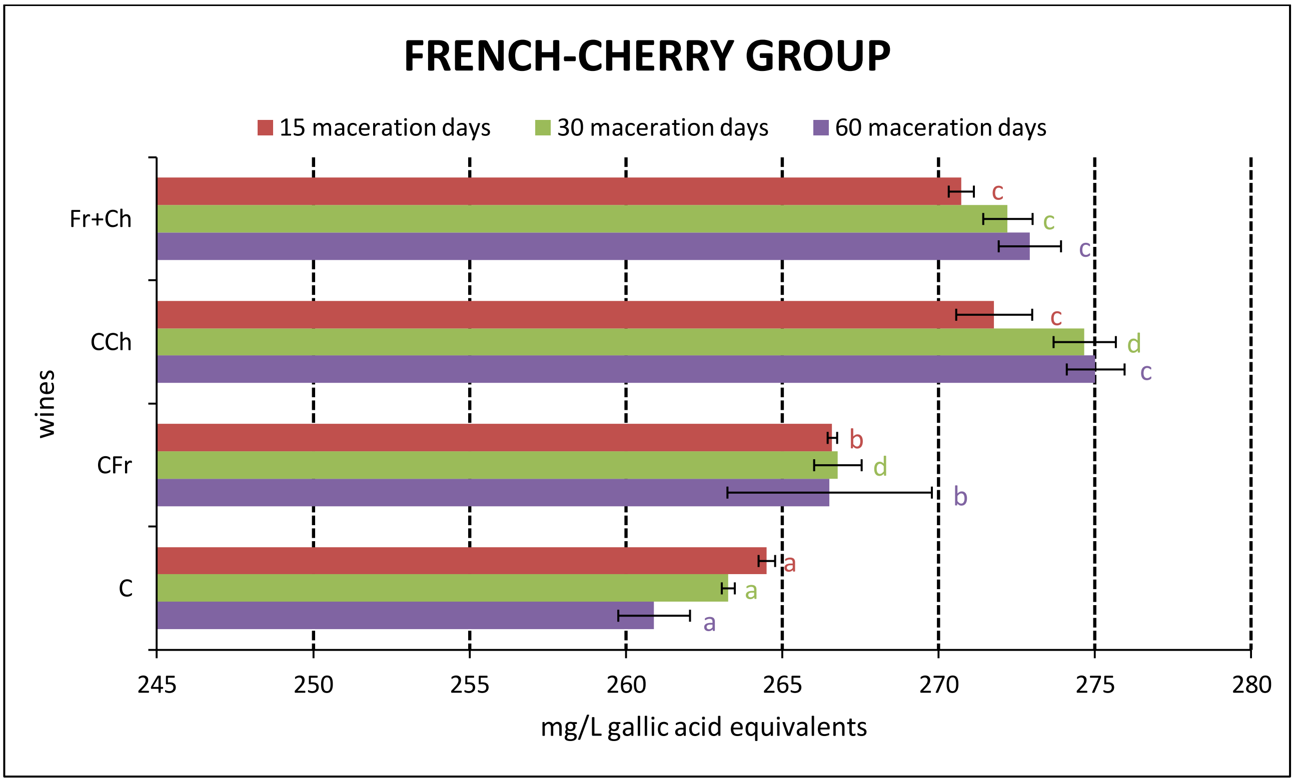


Figure 16- - Evolution of Total phenols in the group with Cherry and French oak wood. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatment. The results with same letters are not significantly different. Legend: C: Control wine; Fr+Ch: wine aged with a blend of French oak and cherry chips, CCh: wine aged with cherry chips, CFr wine aged with French oak chips.

Is interesting to note how the wines with American oak, shows similar behavior in all three treatments, as showed in figure 15, for the extraction evolution. Indeed the sampling after 15 days was the one with highest values, especially for the cherry-american blend, while decreasing in the following samples. In particular the Cherry/American treatment show the highest level of phenols among the wines with American wood, after 60 days, with a value of 273,26 mg/l of gallic acid equivalents, being second only to the control cherry, and underlining not only the high level of extraction from cherry, but also its speed of extraction.

A possible explanation for the decrease of the phenols levels in the following samples could be dueto the porosity of the American wood and the nature of the phenols extracted, which could be more prone to degradation respect to the ones extracted from the other woods.

4.2. FLAVONOIDS PHENOLS

The Flavonoids profile is of great interest to observe for its influence on the mouthfeel of the wine, particularly on their astringency.

In the different treatments was shown a trend similar to the total phenols, with the C wine decreasing slightly, while the CC showed the highest final level already after 15 days sample, as showed in the Figure 16. Indeed according to Sanz et al. (2010) the flavonoids are the main phenolic compounds in cherry wood even though their level in wines aged in contact with wood were not unanimous; in the studies of Del Rosso et al.(2009) and Chinnici et al. (2015) their level was lower in wine aged in cherry casks, while a study by de Simon (2014) showed an increase in their level. It has to be taken into account, anyway, that excepted for the study from Jordao et al. (2017) to our knowledge no study on effect of cherry chips on white wine was performed.

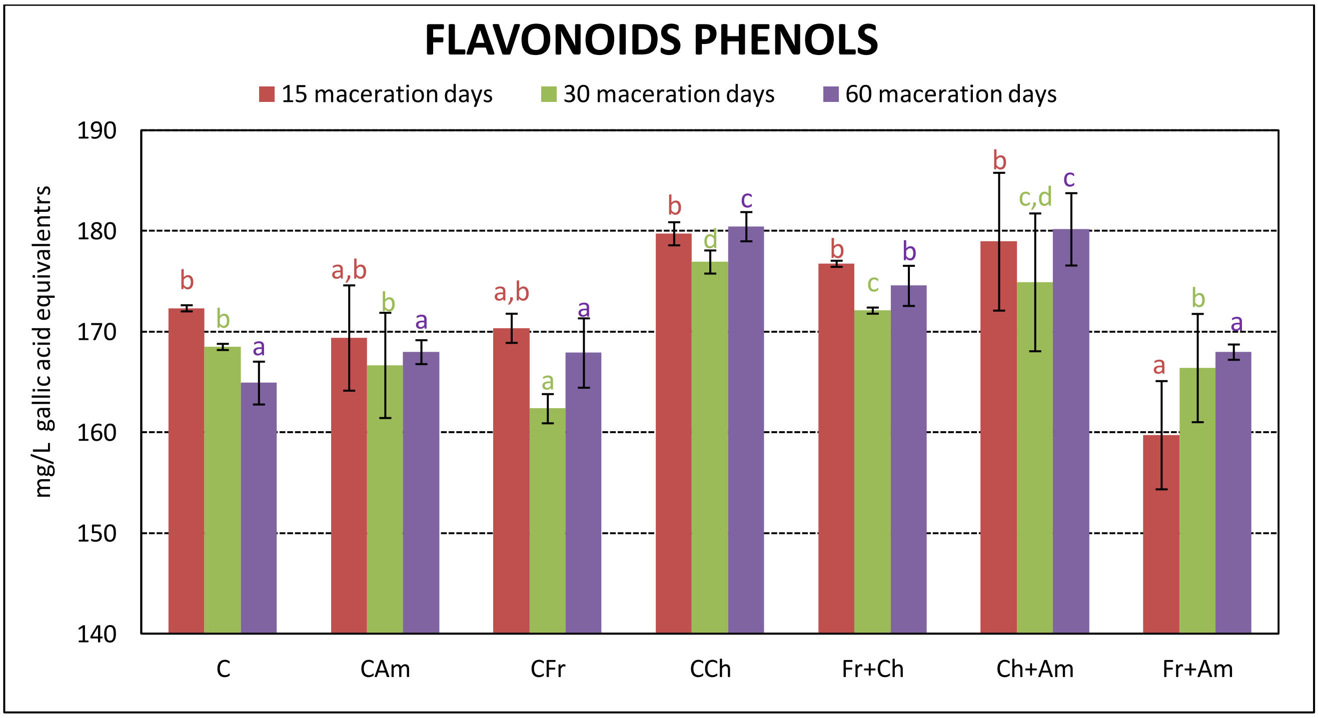


Figure 17- Evolution of flavonoids phenols for the 7 treatments during 60 days. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatments. The results with same letters are not significantly different. Legend: C: Control wine; Ch+Am: wine aged with blend of cherry and American oak chips, Fr+Ch: wine aged with a blend of French oak and cherry chips, CCh: wine aged with cherry chips, Fr+Am wine aged with a blend of French and American oak chips: CFr wine aged with French oak chips; CAm wine aged with American oak chips.

The statistical analysis, differently from the Total Phenols, showed that for some treatments with wood (CAm, CFr and Fr+Am) in the last sampling there was no statistical difference, with the C wine and among them.

In the remaining wines we can observe how the levels of flavonoids do not show differences among the French and American oak which show a similar trend with a steady decrease till the 30 days sampling and a slight increase in the 60 days sampling (167 mg/l for both). As tothe blends, the one which seems to have a higher influence on wine flavonoid level is as for Total phenols, the Ch+Am, as seen in Figure 17, which has the same level of flavonoids as CCh (180 mg/l of gallic acid equivalents), both of these treatments shows level of flavonoids significantly higher from the other treatments, but not between them.The Fr+Ch in the statistical analysis taking in account the all essays is also showing the influence of cherry wood on flavonoid extraction, and even though is significantly higher than the other treatments (including CFr), it issignificantly lower to CCh but not to Ch+Am. in the statistical analysis of the French-Cherry group shown in figure 17 instead the significance disappear for the CCh and Fr+Ch.

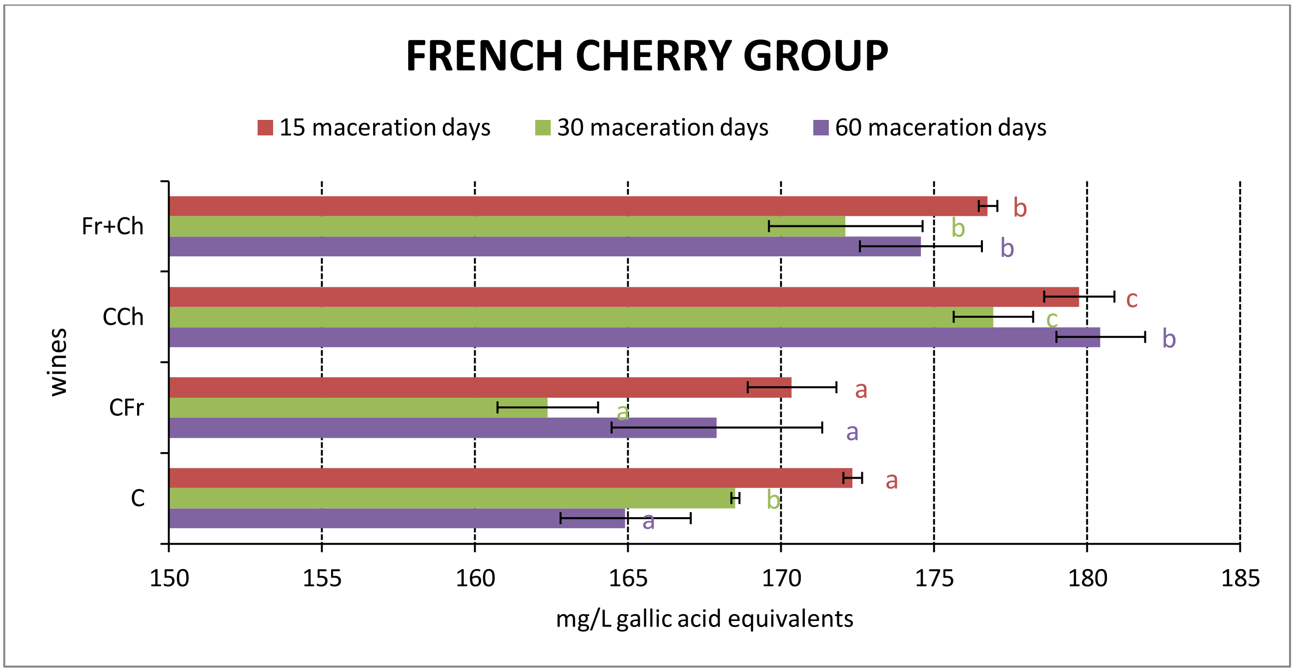


Figure 18 - Evolution of Flavonoids phenols in the group with Cherry and French oak wood. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatment. The results with same letters are not significantly different. Legend: C: Control wine; Fr+Ch: wine aged with a blend of French oak and cherry chips, CCh: wine aged with cherry chips, CFr wine aged with French oak chips.

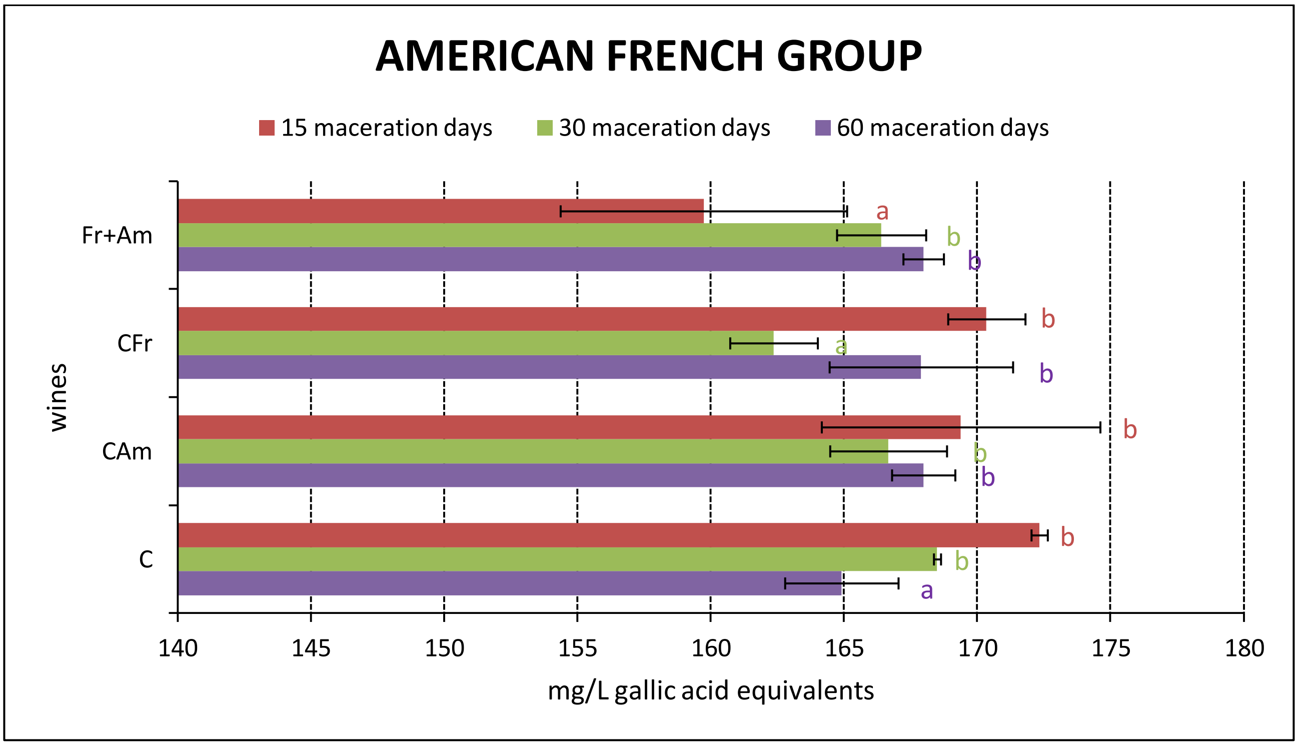


Figure 19 - Evolution of Flavonoids phenols in the group with American and French oak wood. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatment. The results with same letters are not significantly different. Legend: C: Control wine; Fr+Am wine aged with a blend of French and American oak chips: CFr wine aged with French oak chips; CAm wine aged with American oak chips.

In the last sampling the Fr+Am blend showed difference only with C, while statistically no differences were observed among the chips treatments of this group.

The results of statistical analysis among the American cherry group confirm the high level of flavonoids in cherry wood, indeed we can see how the CCh and Ch+Am are the ones being significantly higher than the others, while there is no difference between C and CAm.

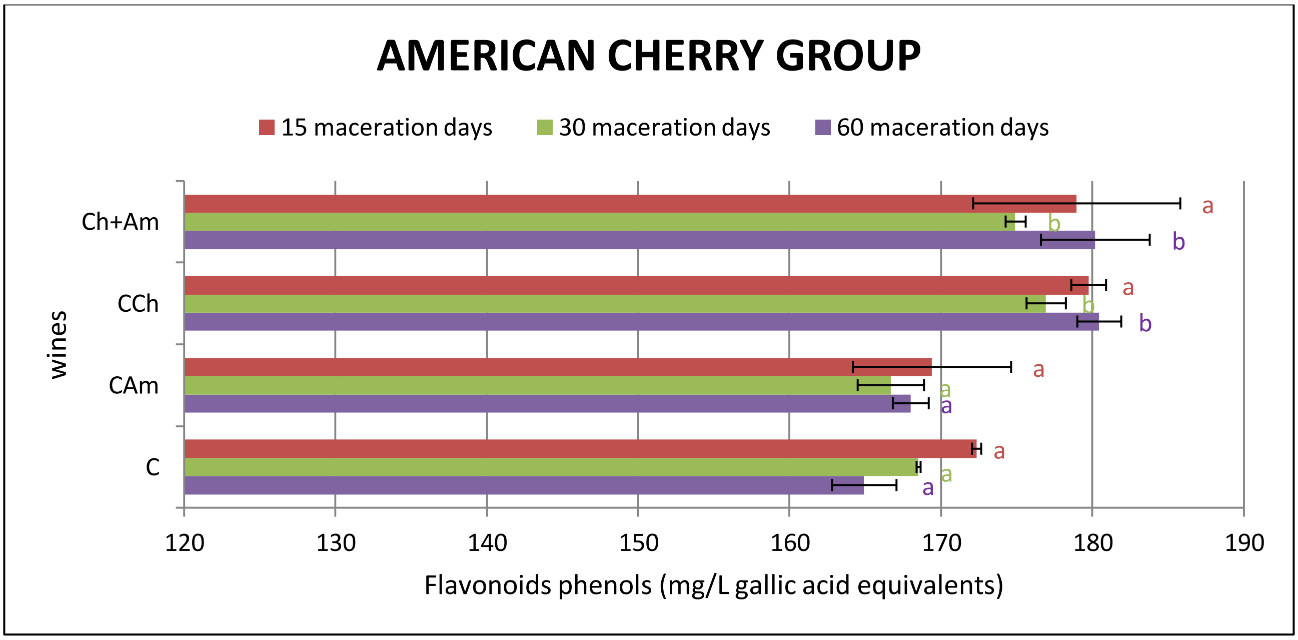


Figure 20- Evolution of Flavonoids phenols in the group with Cherry and American oak wood. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatment. The results with same letters are not significantly different. Legend: C: Control wine; Ch+Am: wine aged with blend of cherry and American oak chips, CCh: wine aged with cherry chips, CAm wine aged with American oak chips.

From the collected results is evident how cherry heartwood is showing more extractable flavonoids (flavan-3-ols (+)-catechin, B-type) among the phenolic constituents in comparison with oaks heartwood as showed by many authors (De Rosso et al., 2009a; Sanz et al., 2011; Delia et al., 2017). Whilst oak heartwood has very low influence on the content of some phenolic compounds like flavonoids in wine (Sanz et al., 2010a; Fernandez de Simon et al., 2014c).

4.3. NON FLAVONOIDS

The non flavonoids class, important for the aroma profile of the wine, also showed interesting results among the different essays.

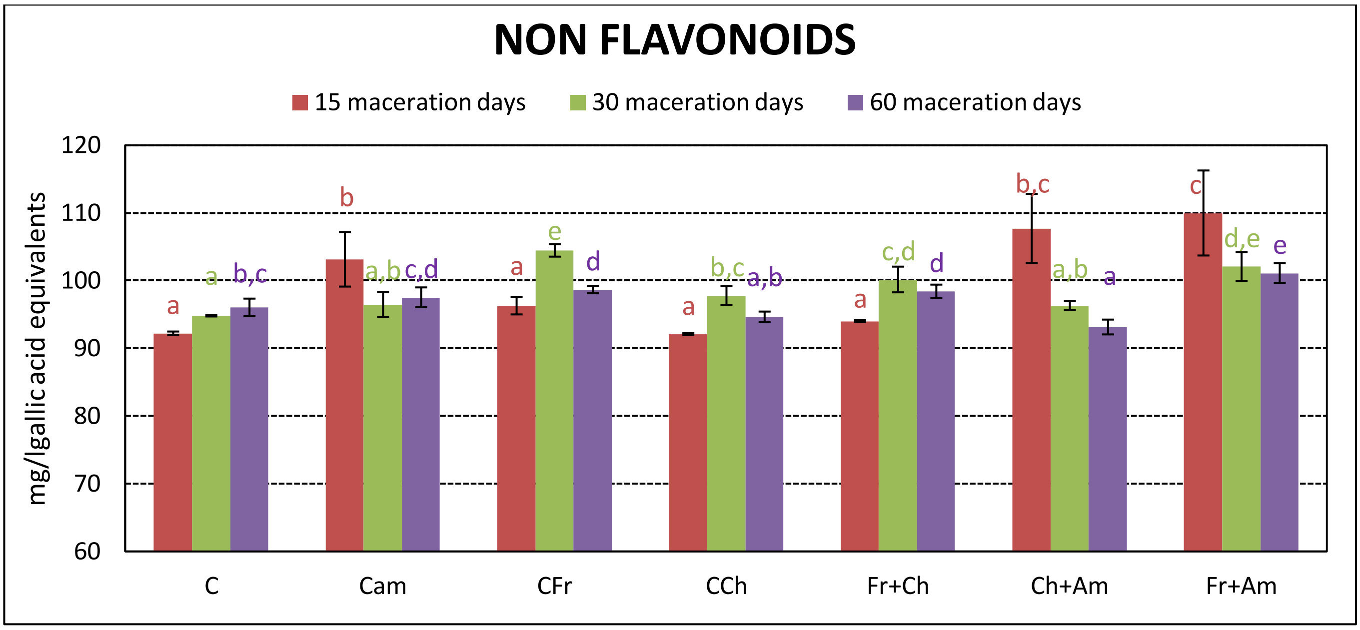


Figure 21- Evolution of flavonoids phenols for the 7 treatments during 60 days. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatments. The results with same letters are not significantly different. Legend: C: Control wine; Ch+Am: wine aged with blend of cherry and American oak chips, Fr+Ch: wine aged with a blend of French oak and cherry chips, CCh: wine aged with cherry chips, Fr+Am wine aged with a blend of French and American oak chips: CFr wine aged with French oak chips; CAm wine aged with American oak chips.

We can observe from figure 20 how, differently from the total phenols and flavonoids profile, the treatment with higher level of Non Flavonoids phenols is a blend , Fr+Am (101 mg/l), and not a wine with cherry wood. It is also visible how the wood seeming to impact more on the non flavonoid level is the French oak, indeed is visible how the treatments Fr+Am, CFr and Fr+Ch are respectively the 1st 2nd and 3rd treatments for final quantity (101, 98 and 98 mg/l), with Fr+Am being significantly higher than the others, while CFr and Fr+Ch are not different among them but significantly higher than all the treatments except Fr+Am, as visible in Figure 21. Differently from the other experiments in this case the essay being significantly the lowest after 60 days of chips maceration is the Ch+Am (93 mg/l).

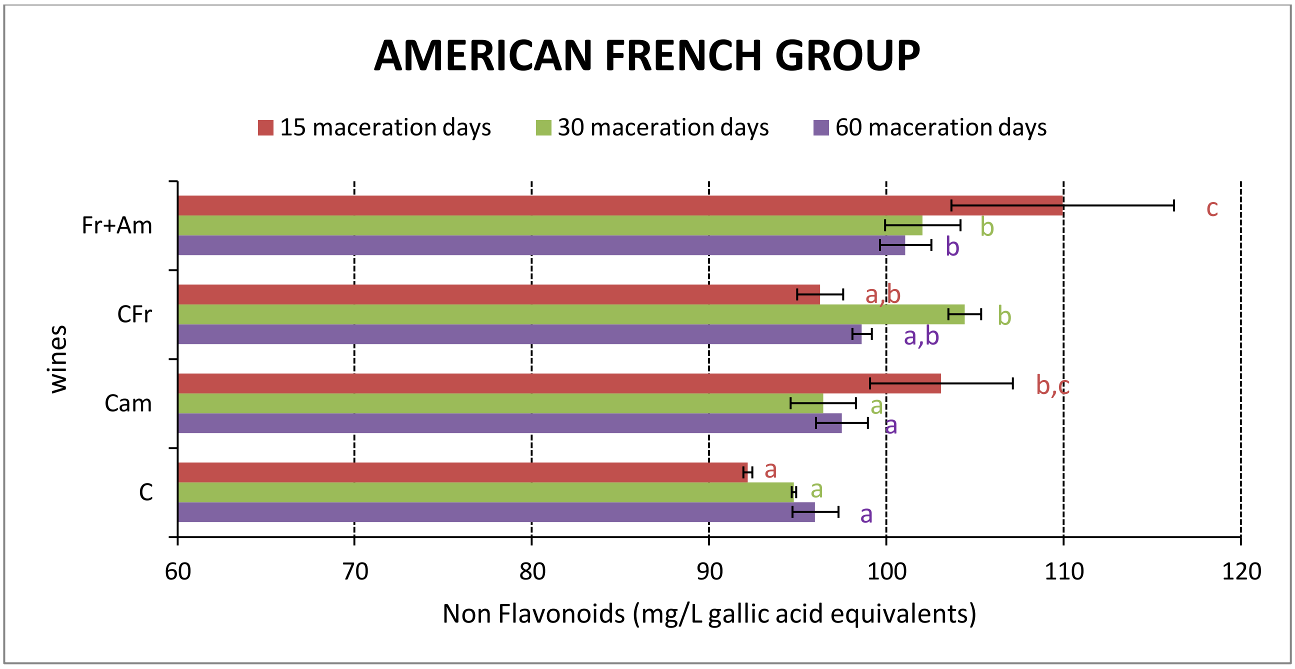


Figure 22-Evolution of Non Flavonoids phenols in the group with French and American oak wood. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatment. The results with same letters are not significantly different. Legend: C: Control wine, Fr+Am wine aged with a blend of French and American oak chips, CFr wine aged with French oak chips, CAm wine aged with American oak chips.

The statistical analysis of the French and American oak group, however shows how there is no significance difference among the treatments (except between Fr+Am and CAm/C).

As was observed for the total phenols results for the non flavonoids the treatments with American wood showed a similar extraction pattern with the highest extraction rate recorded during the first sampling done after 15 days from chips addition and a slight decrease in the following samples, as visible in figure 21.

The analysis of the French oak and cherry group confirmed how the CFr is significantly higher than the rest of the essays (except the blend Fr+Ch), but no other significant difference was detected.

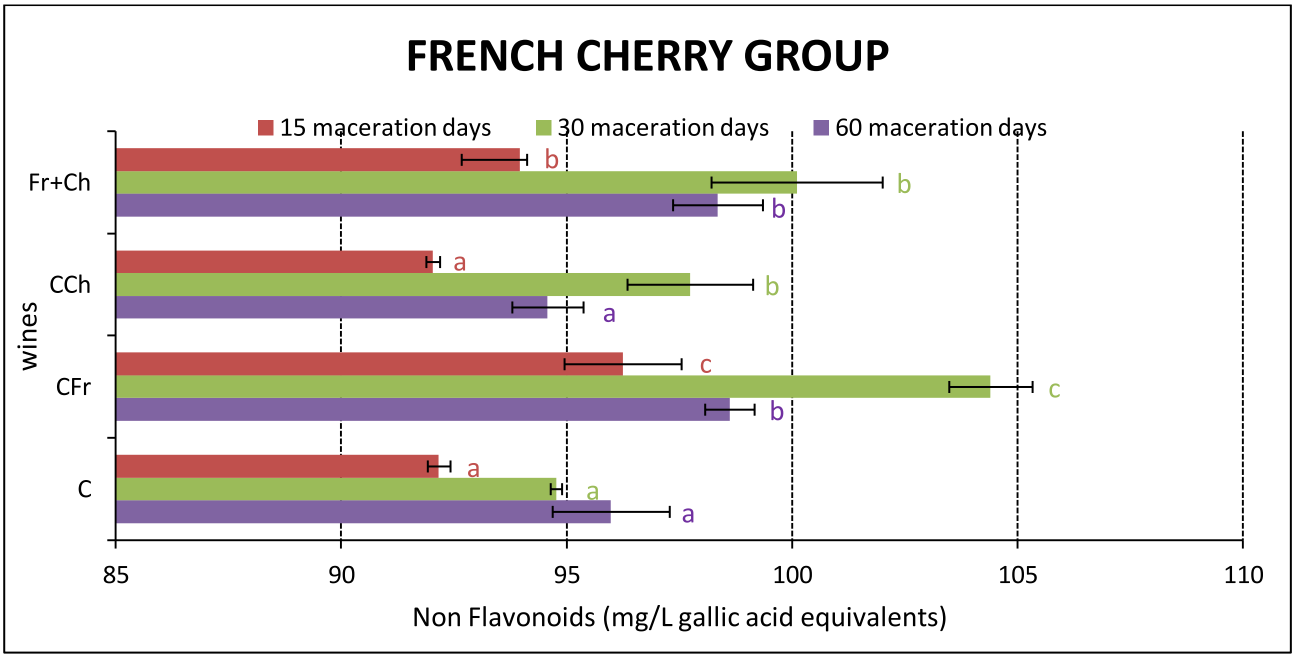


Figure 23- Evolution of Non Flavonoids phenols in the group with French oak and Cherry wood. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatment. The results with same letters are not significantly different. Legend: C: Control wine, Fr+Ch: wine aged with a blend of French oak and cherry chips, CCh: wine aged with cherry chips, CFr wine aged with French oak chips.

Also the comparison among the American oak and cherry group didn't show significant differences among the groups as visible in figure 23.

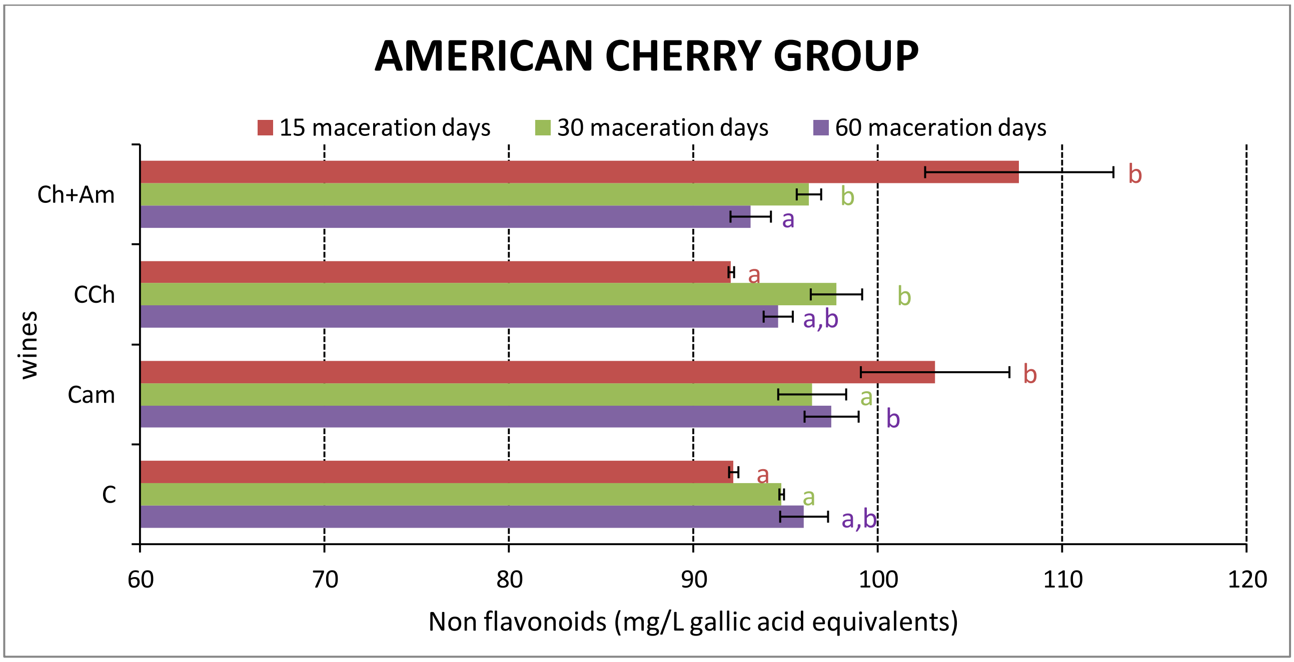


Figure 24- Evolution of Non Flavonoids phenols in the group with Cherry and American oak wood. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatment. The results with same letters are not significantly differentLegend: C: Control wine; Ch+Am: wine aged with blend of cherry and American oak chips, CCh: wine aged with cherry chips, CAm wine aged with American oak chips.

The non flavonoids profile showed a pattern of extraction different from the total and flavonoids phenols where the wines with cherry wood showed the higher extraction rate, while as showed by many studies the non flavonoids profile shows the wines in contact with oak to be richer (Sanz et al., 2010a; Fernandez de Simon et al., 2014c).

4.3.COLOR

The chromatic characteristic of a wine is relevant because of the value that this parameter has on the quality perception of the consumers (Chinnici et al., 2011). Therefore is fundamental to see the changes observed in the color evolution of the wine with different chips.

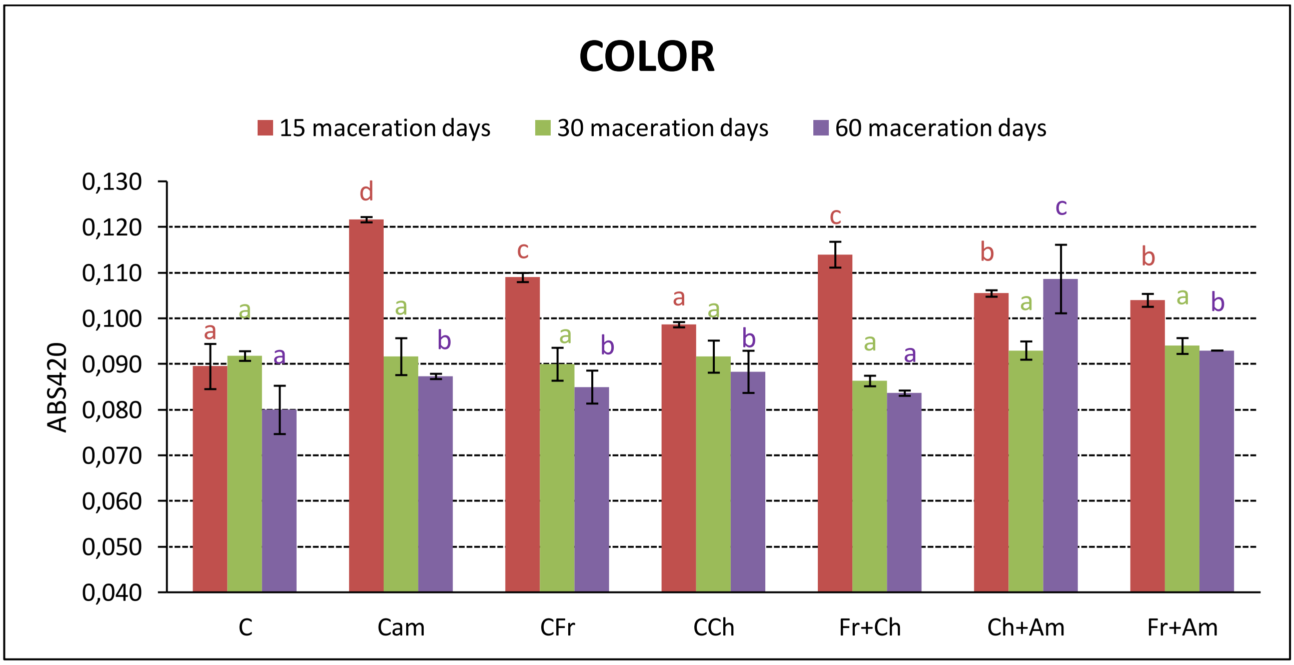


Figure 25- Evolution of Color for the 7 treatments during 60 days. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatments. The results with same letters are not significantly different. Legend: C: Control wine; Ch+Am: wine aged with blend of cherry and American oak chips, Fr+Ch: wine aged with a blend of French oak and cherry chips, CCh: wine aged with cherry chips, Fr+Am wine aged with a blend of French and American oak chips: CFr wine aged with French oak chips; CAm wine aged with American oak chips.

In almost all the wines there was an increase in the Color hue (among the first and the last sampling), except for the C and Fr+Ch where there was a slight decrease, respectively (0,85 to 0,8 and 0,85 to 0,83) and the CFr where there was no difference between the first and the last sampling. In the previous study from Delia et al. (2017) similar observation were detected for wine with French chips even though the statistical analysis didn’t show difference with the other treatments in the study (except for the Acacia, not present in this study).

As expected the C wine showed the lowest value with an Absorbance at 420 nm of 0,80, while the wine with the highest final value for color intensity was the CAm with 0,109 followed by Fr+Am (0,93) and both CAm with CCh (0,88). The statistical analysis showed just Ch+Am being significantly higher from all the other essays, while Fr+Ch showed no differences with the C wine (unique among the wines with wood).

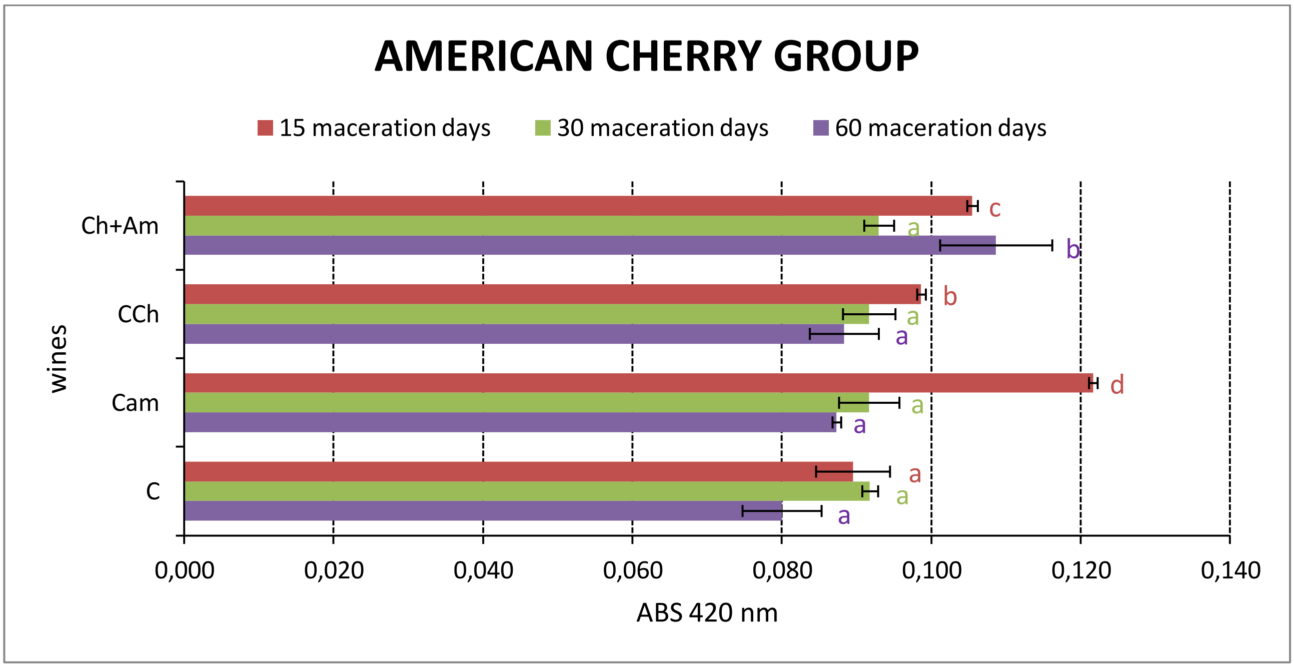


Figure 26- Evolution of Color for the American-Cherry group. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatments. The results with same letters are not significantly different. Legend: C: Control wine; Ch+Am: wine aged with blend of cherry and American oak chips, CCh: wine aged with cherry chips,; CAm wine aged with American oak chips.

In the Analysis run for the American Cherry group the results confirm the significance of the Ch+Am respect to the other treatments.

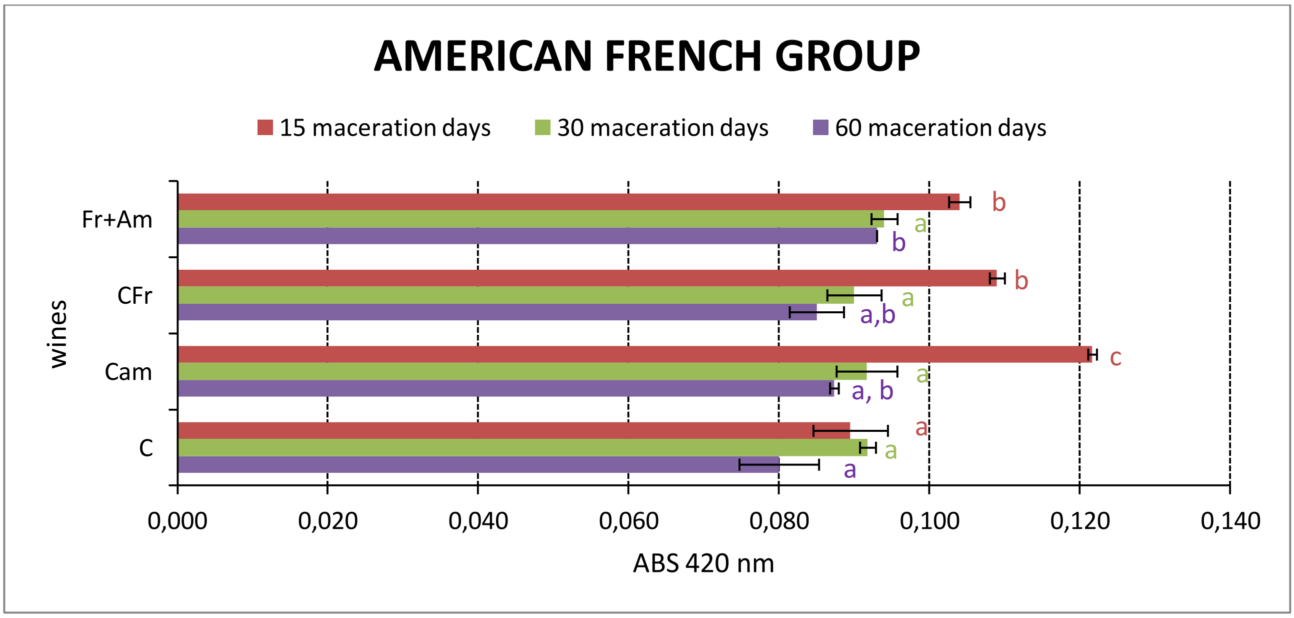


Figure 27- Evolution of Color for the American-French group. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatments. The results with same letters are not significantly different.

Legend: C: Control wine, Fr+Am wine aged with a blend of French and American oak chips: CFr wine aged with French oak chips; CAm wine aged with American oak chips.

The comparison among the American-French group shows, instead a significance difference among the F+A and the C treatment but not between the others, as seen in Figure 25.

The last group considered, the French-Cherry group, didn't show any significant differences among the last sampling, as visible in Figure 27.

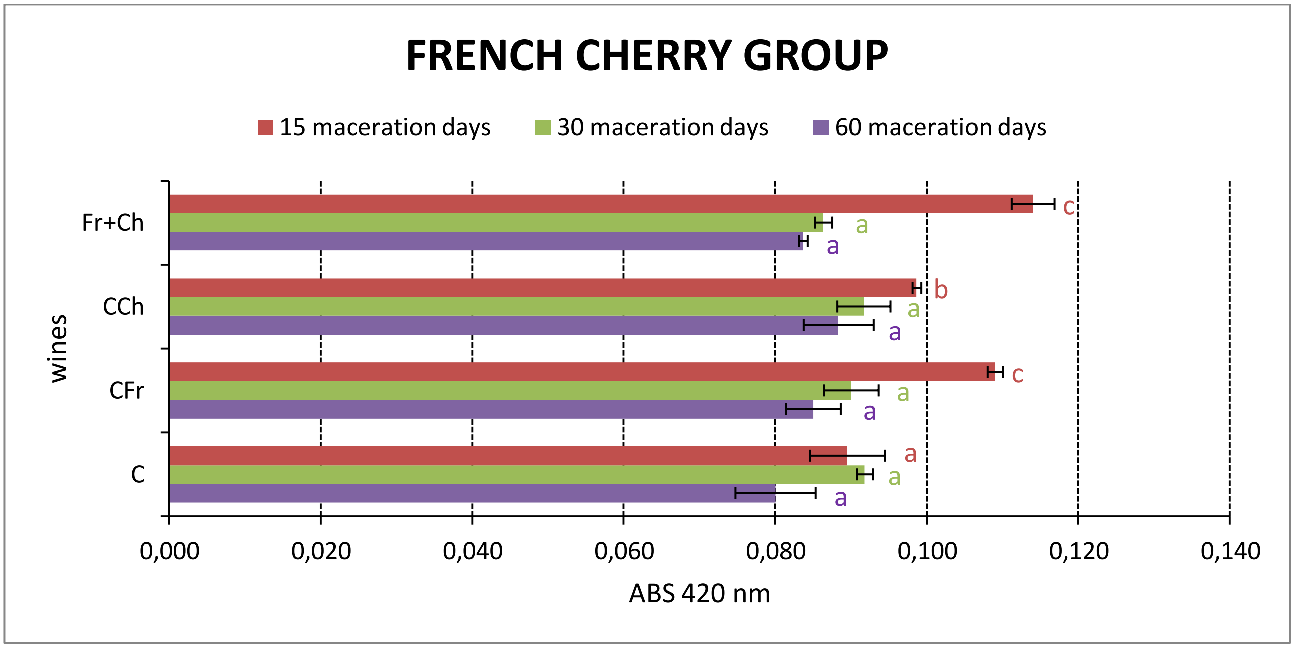


Figure 28- Evolution of Color for the Cherry-French group. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatments. The results with same letters are not significantly different. Legend: C: Control wine, Fr+Ch: wine aged with a blend of French oak and cherry chips, CCh: wine aged with cherry chips, CFr wine aged with French oak chips.

These results confirm the contribution that phenolics substances, and hence wood contact, show to have on wine Color (Lee &Jaworsky, 1987), which, according to Vivas et al. (2008) is linked not only to the release of such compounds, but also to the interaction and condensation of wood originated molecules, namely phenolic aldehydes and catechins.

4.4.DETERMINATION OF SUSCEPTIBILITY TO BROWNING (MADERIZATION TEST)

Among the various phenomena which are detrimental for wine quality the browning of the white ones is among the most serious. It is strictly related to the their phenols level, which being antioxidant are highly reactive with oxygen. Infact, while individual phenols can react very differently, the total content of phenols in a wine is a rough measure of its capacity to take up oxygen, its ability to withstand oxidation, and its capacity to change when exposed to oxygen. (Singleton, 1987).

In this optic the assessment of the influence of the different wine chips on browning is fundamental, in order to determine the advantages and disadvantages of each wood.

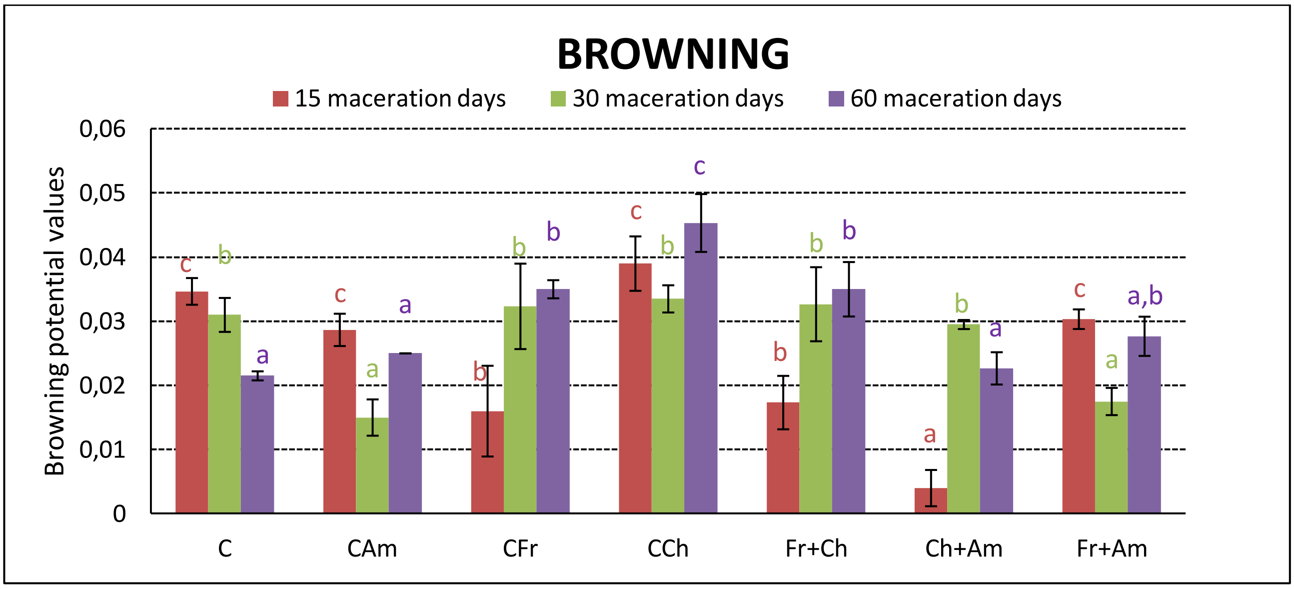


Figure 29- Evolution of browning potential for the 7 treatments during 60 days. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatments. The results with same letters are not significantly different. Legend: C: Control wine; Ch+Am: wine aged with blend of cherry and American oak chips, Fr+Ch: wine aged with a blend of French oak and cherry chips, CCh: wine aged with cherry chips, Fr+Am wine aged with a blend of French and American oak chips: CFr wine aged with French oak chips; CAm wine aged with American oak chips.

It is worth to remember that in the case of this test the higher is the value obtained the higher is the susceptibility to browning.

All the wines showed a decrease in the browning potential except the CCh, confirming the important role that phenols have on the browning, indeed the CCh was the essay with higher final result regarding the total phenols. The other results go together with this statement, since, even though show a decrease from the initial browning potential, they all exhibit a final value which is higher than the C, hence a higher susceptibility to browning, with CFr and Fr+Ch following the CCh. This last result is in accordance with Delia et al. (2017) where the wines with French oak and cherry showed the highest browning potential. It has to be taken into account however that this relationship is not always linear, as in the case of CAm, being the second treatment for total phenols values, but the one with lowest browning potential after the C treatment. Hence more studies need to be done in order to understand the relations existing between the phenolic profile and browning susceptibility.

The statistical analysis showed that CCh is significantly higher than the rest of the treatments, followed by CFr and Fr+Ch significantly higher from the others (except CCh), but not among them. The rest of the treatments didn't show significant differences from the C.

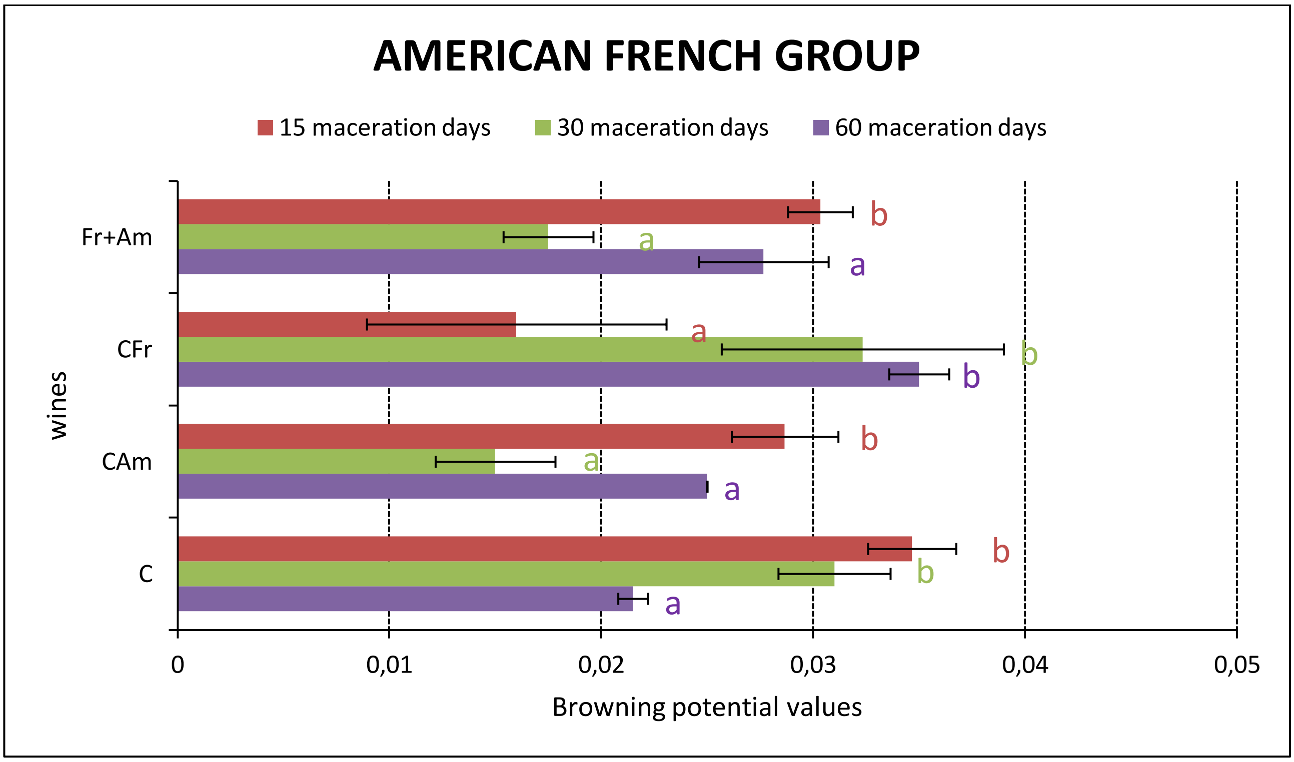


Figure 30- Evolution of browning potential for the American-French group. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatments. The results with same letters are not significantly different. Legend: C: Control wine; F+A wine aged with a blend of French and American oak chips: CF wine aged with French oak chips; CA wine aged with American oak chips.

The statistical analysis of the French-American group, in accordance with overall analysis showed just the CF being statistically higher than the other treatments.

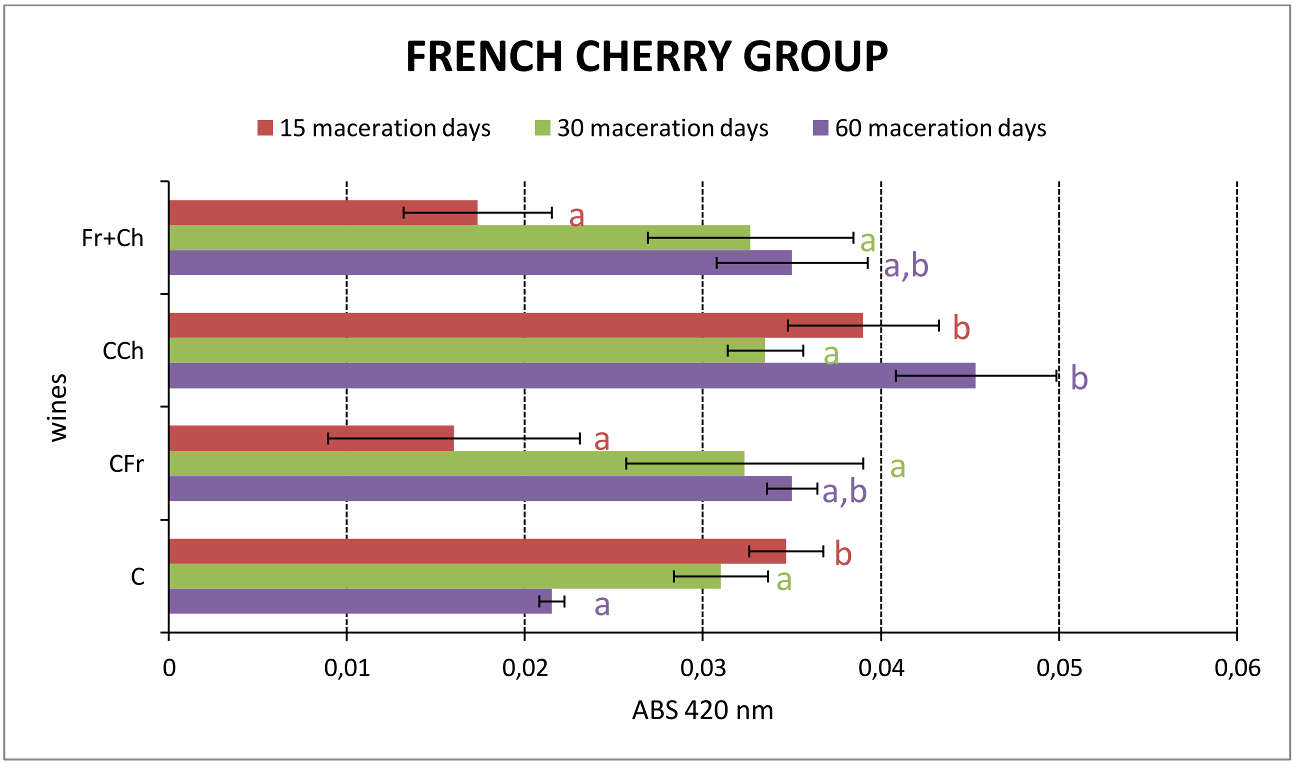


Figure 31- Evolution of browning potential for the Cherry-French group. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatments. The results with same letters are not significantly

different. Legend: C: Control wine, Fr+Ch: wine aged with a blend of French oak and cherry chips, CCh: wine aged with cherry chips, CFr wine aged with French oak chips.

As to the French-Cherry group the differences detected were only the ones between every treatment with wood and the C treatment, without differences between them (figure 30). While in the American Cherry group reported in figure 31 the CCh is the only treatment significantly different from the C at the end of the samplings.

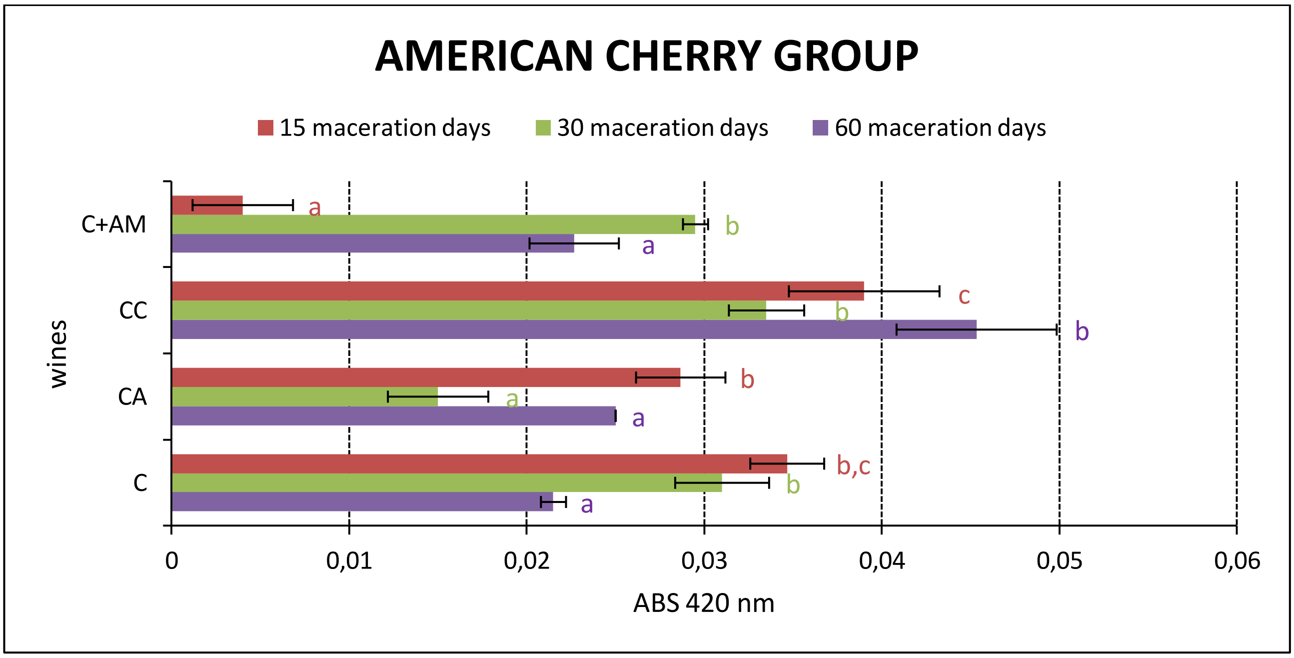


Figure 32- Evolution of browning potential for the Cherry-American group. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatments. The results with same letters are not significantlydifferent. Legend: C: Control wine; Ch+Am: wine aged with blend of cherry and American oak chips, CCh: wine aged with cherry chips, CAm wine aged with American oak chips.

4.5. TANNING POWER

Astringency is not a characteristic typical of white wines, because of the lower phenolic content (ranging from 0 to 200 mg/l against 800 to 1600 mg/l in red wines), but also because of the vinification technique, which, in most of the world wine regions, does not include contact with the seed and the skin, avoiding extraction of the phenols responsible for astringency (Ough, 1969; Singleton et al., 1975; Arnold et al., 1980).

In the case of contact with wood however there is an increase in phenolic substances as we have previously seen, including those responsible for astringency (tannins), for this reason a study about the effect of this extraction on wine astringency becomes important in order to assess the differences existing between the different wood blends.

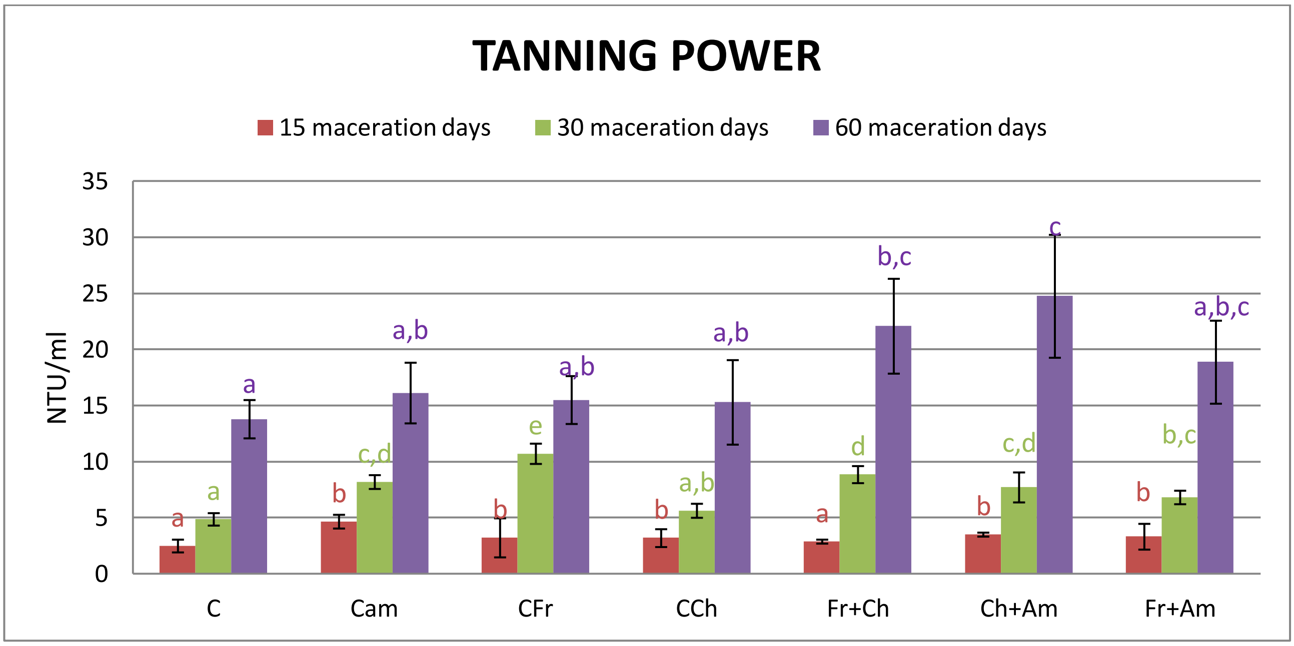


Figure 33- Evolution of Tanning power for the 7 treatments during 60 days. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatments. The results with same letters are not significantly different. Legend: C: Control wine; Ch+Am: wine aged with blend of cherry and American oak chips, Fr+Ch: wine aged with a blend of French oak and cherry chips, CCh: wine aged with cherry chips, Fr+Am wine aged with a blend of French and American oak chips: CFr wine aged with French oak chips; CAm wine aged with American oak chips.

The essay which reached the highest value was the Ch+Am one with 24,75 NTU/ml followed by Fr+Ch (22,08 NTU/ml) and Fr+Am (18,88 NTU/ml). It is interesting to note how the treatments with highest values were by far the one with blend of different wood species, even though the Ch+Am is the only one being significantly different from all the control treatments (CFr, CAm, CCh), with the other blends showing significance just with the C (the case of Fr+Ch) or with none (Fr+Am). Finally no differences among the control treatments were found.

In the study from Delia et al. (2017) after one month the wine with higher values of tannicity was the one with French oak, followed by the one with American oak with cherry having the lower value. Even though the sampling in this study were done at different time (15-30-60 in our case, 20-28 in Jordaostudy) we can see a similar behavior with the control wines, indeed if we see the difference among the first 2 samplings, after 15 and 30 days, we can see how the CFr shows the highest increase, while the CCh the lowest, with CAm being intermediate.

Hence we can observe how the pattern of tannicity in all the essays undergoes a huge increase between the 30 and 60 days of chips maceration, in many cases more than doubling its value. As for other analysis observed the blend between Cherry and American oak (Ch+Am) shows the highest value, suggesting a highly efficient extraction with combination of this blends.

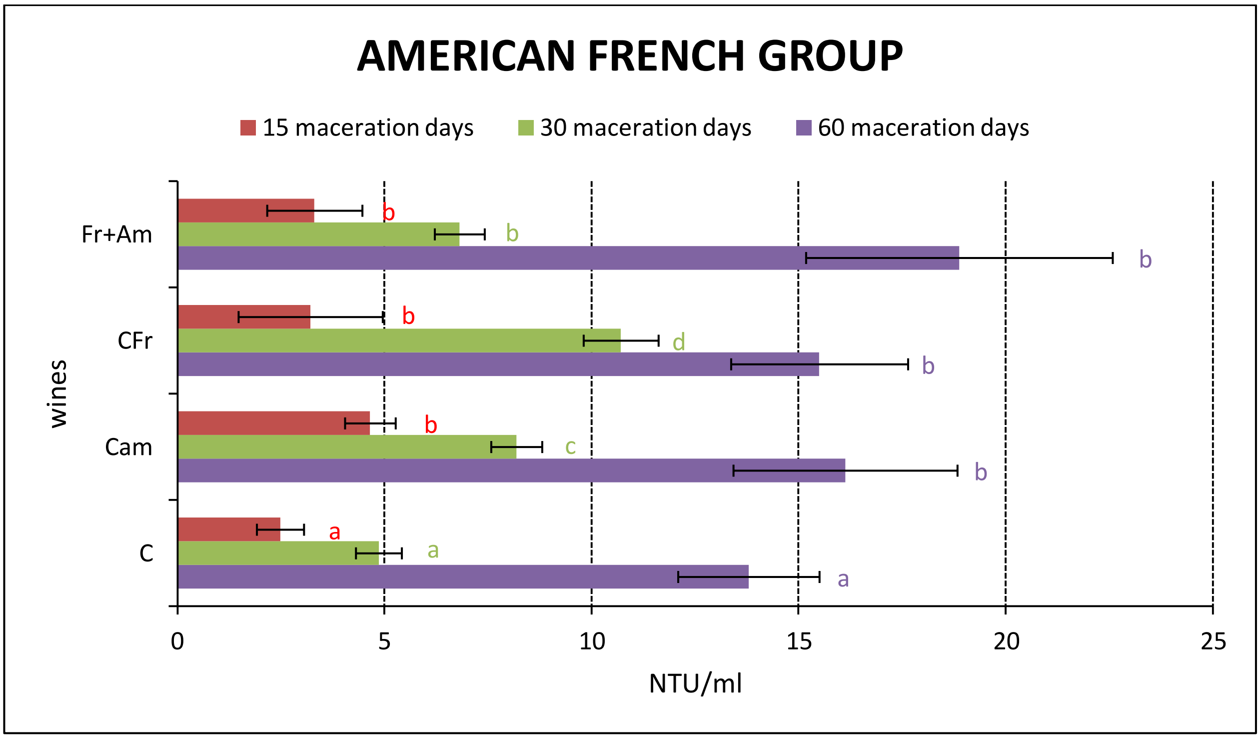


Figure 34 - Evolution of Tanning power for the American-French group. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatments. The results with same letters are not significantly different. Legend: C: Control wine, Fr+Am wine aged with a blend of French and American oak chips: CFr wine aged with French oak chips; CAm wine aged with American oak chips.

The statistical analysis considering the species of wood showed a similar pattern but with some differences; indeed in all three cases the blends (F+A, F+CH and C+AM) showed to be significantly higher than the C treatment as visible in figures 33, 34 and 35. However in the case of the American- French group all the wood treatments (CA, CF and F+A) showed significant difference, while for French-Cherry group the blend (F+CH) is the only one different from all the others, eventually the American-Cherry group showed a difference only among the C+AM and the C but not among the other treatments.

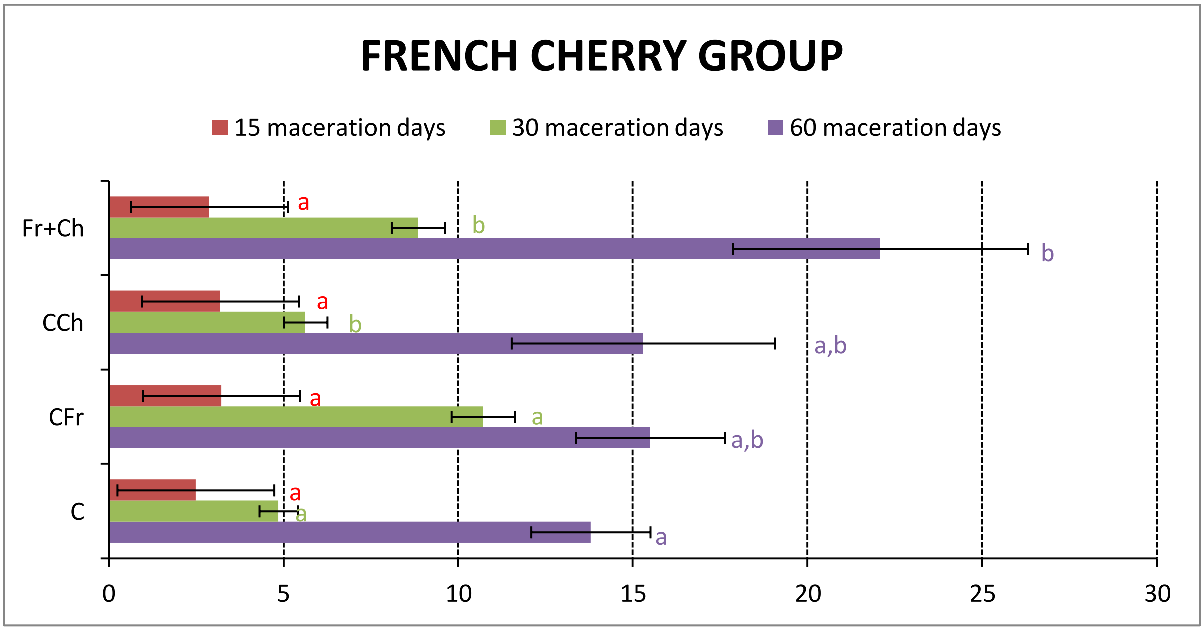


Figure 35 - Evolution of Tanning power for the French-Cherry group. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatments. The results with same letters are not significantly

different. Legend: C: Control wine; Fr+Ch: wine aged with a blend of French oak and cherry chips, CCh: wine aged with cherry chips, CFr wine aged with French oak chips;

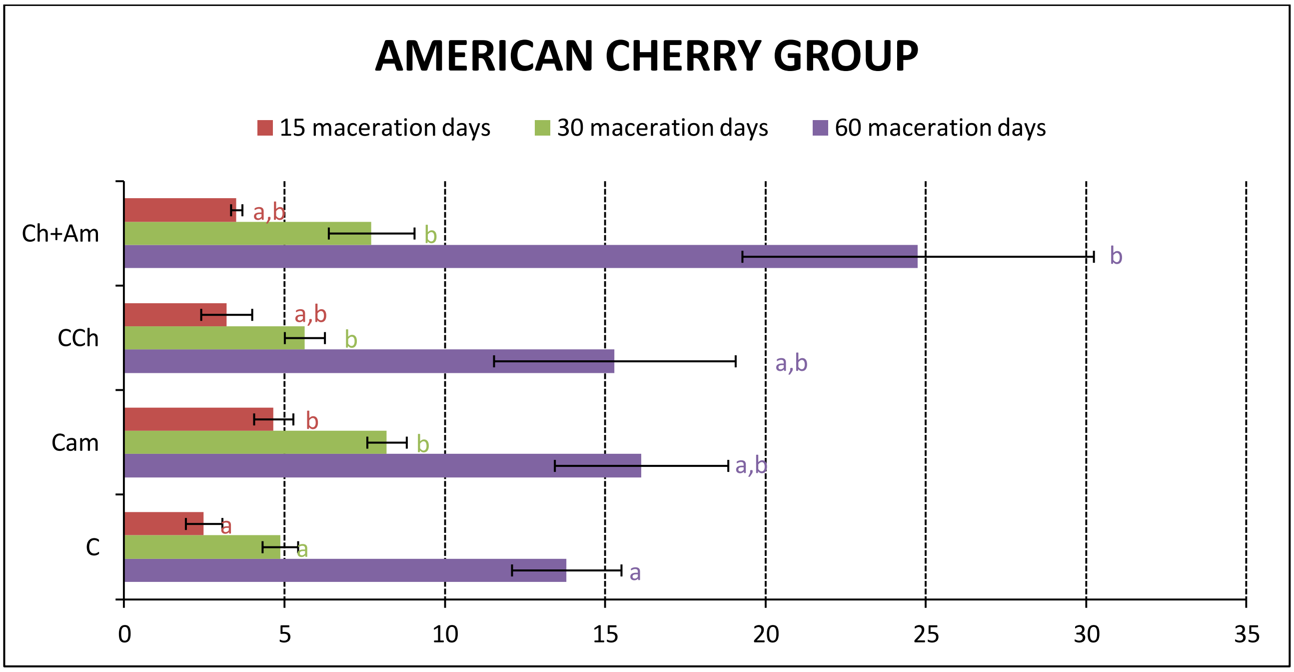


Figure 36 - Evolution of Tanning power for the American-Cherry group. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatments. The results with same letters are not significantly different. Legend: C: Control wine; Ch+Am: wine aged with blend of cherry and American oak chips, CCh: wine aged with cherry chips, CAm wine aged with American oak chips.

Hence looking at the data and the statistical analysis run, we can say that after a maceration of 60 days of wood chips in our wine the tannicity underwent a huge increase. The treatments with blends of different woods showed higher values, but not every was significantly higher than the C or than the other treatments.

In the next chapter the sensory analysis results will be discussed, will be interesting to see if the tannicity obtained through the chemical analysis will show similarity with the perception of astringency observed by the tasters.

4.6. SENSORY ANALYSIS

A sensorial analysis was run for the different treatments at each sampling (15-30-60 days of maceration) evaluating the influence of the wood species and blends on some aroma and taste characteristics of the wines. In order to simplify the observation of the data, the parameters were divided among aroma and taste attributes, the last chapter will be dedicated to the global appreciation of the wines tasted in order to assess those which, on a general scale, were more appreciated.

4.6.1. AROMA

The pictures below show spider diagrams of the mean scores about aroma attributes of the wines involved in this experiment. In these diagrams the centre of the figure represents low intensity of each attribute increasing to an intensity of 5 at the perimeter. When (*) is present it means that a statistical difference was detected among the different essays.

15 days of maceration

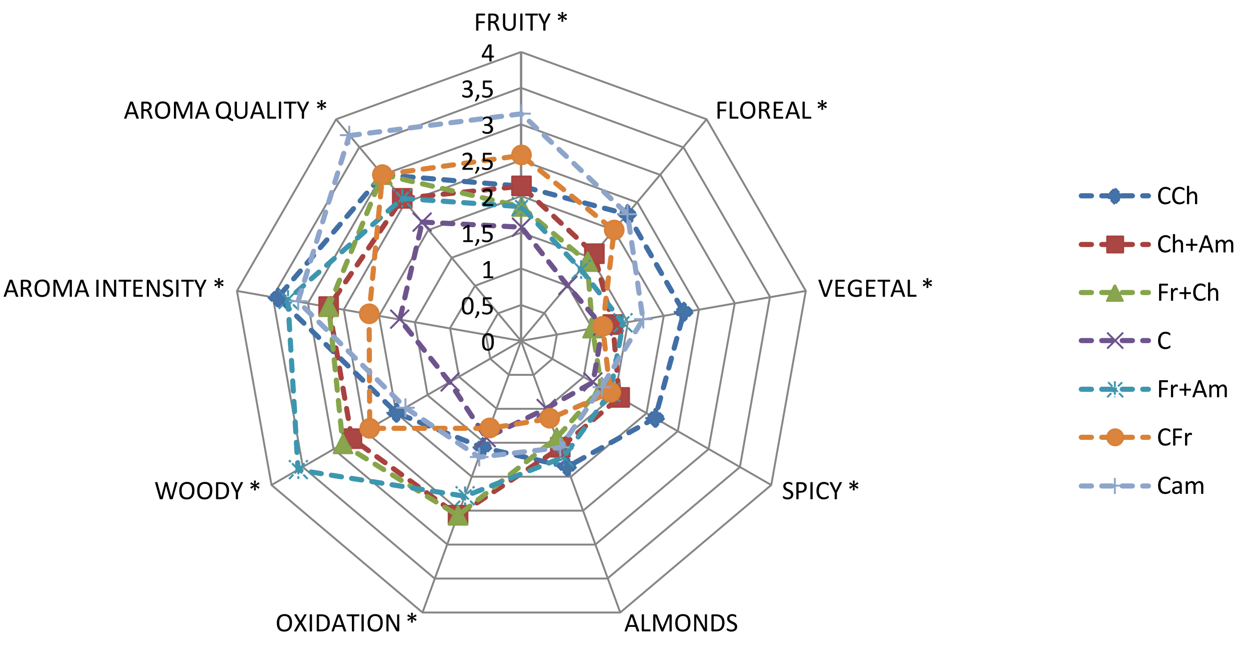


Figure 37- Sensory analysis results for aroma parameters of the studied wines, at 15 maceration days. Legend: C: Control wine; Ch+Am: wine aged with blend of cherry and American oak chips, Fr+Ch: wine aged with a blend of French oak and cherry chips, CCh: wine aged with cherry chips, Fr+Am wine aged with a blend of French and American oak chips: CFr wine aged with French oak chips; CAm wine aged with American oak chips.

We can observe how the aroma parameters of the wines present high variability with significance in almost every parameter, except for the almond character. In particular CAm presents higher ranks for Aroma quality, and Fruity character, being significantly different from the other essays, while for Vegetal and Spicy CCh is significantly higher. It is interesting to observe how the oxidation note after 15 days shows CFr as the lowest one, while for the woody character the Fr+Am treatment was the one with significantly higher than the rest. As a general pattern we can see higher variability in this graph if compared to the ones of 30 and 60 days, where the wines become more and more homogeneous without huge differences among them.

30 days of maceration

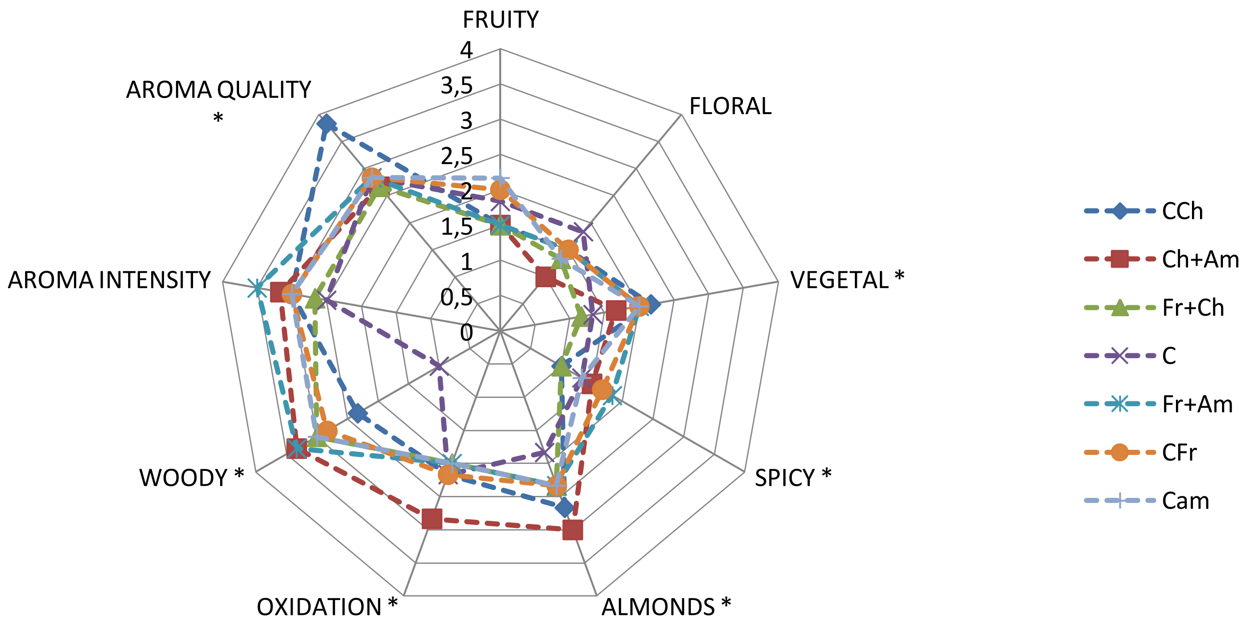


Figure 38-Sensory analysis results for aroma parameters of the studied wines, at 30maceration days. Legend: C: Control wine; Ch+Am: wine aged with blend of cherry and American oak chips, Fr+Ch: wine aged with a blend of French oak and cherry chips, CCh: wine aged with cherry chips, Fr+Am wine aged with a blend of French and American oak chips: CFr wine aged with French oak chips; CAm wine aged with American oak chips.

After 30 days of maceration the situation changed completely with much less parameters showing significant differences. The most evident change is the increased aroma quality of the CCh treatment which is significantly higher than the rest of the treatments; it shows also marked almonds and vegetal traits (even though significantly higher just than the C in both cases). The Ch+Am essay showed the oxidation and Almonds note as increased, being significantly higher than the rest in the case of oxidation, while just of the C in the case of Almonds.

60 days of maceration

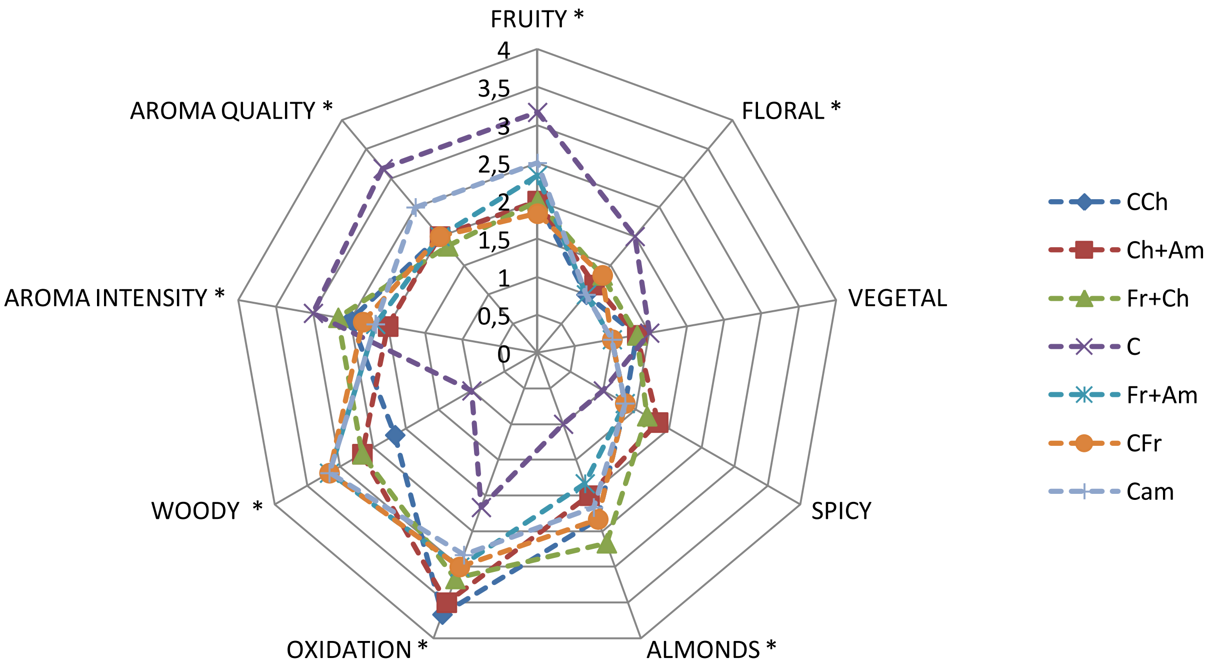


Figure 39- Sensory analysis results for aroma parameters of the studied wines, at 60 maceration days. Legend: C: Control wine; Ch+Am: wine aged with blend of cherry and American oak chips, Fr+Ch: wine aged with a blend of French oak and cherry chips, CCh: wine aged with cherry chips, Fr+Am wine aged with a blend of French and American oak chips: CFr wine aged with French oak chips; CAm wine aged with American oak chips.

In the 60 days tasting the presence of woody characteristics began to be invasive and the characteristics of the C wine started to differ significantly from the rest of the treatments, as evident in Figure 38. The C essay shows to be significant higher than the rest of the treatments in Fruity and floral notes parameter, being also the significantly most appreciated among all the wines as for aroma quality, followed by CAm while the other wines didn't show significance among them. As expected the woody, oxidation and almonds note of the C were the lowest (significance difference in all the three parameters).

Ch+Am confirm is tendency for oxidation notes, as already observed in the 30 days sampling showing, together with CCh the highest notes, with the statistical analysis showing that CCh is significantly higher than all the wines, while Ch+Am shows difference just with CAm and C.

4.6.2 TASTE

For the taste parameters the difference among the treatments, on the contrary of what seen for the aroma, increased with the days of maceration. Nevertheless significance was found in many parameters already from the 15 days of maceration, conversely to what observed in the study of Delia et al. (2017), where statistical differences in the sensory analysis were not found in the aroma nor in the taste parameters.

Starting from tasting of the samples after 15 days, we can see how a statistically significant difference was found only on the evaluation of the flavor quality, where C essay showed the highest result (difference found with all the other treatments except CCh and CAm), and acidity, where there were statistical differences with the C being the one with higher perception of acidity, as visible in figure 39. The previously cited study from Delia et al. (2017), didn't show any significance in acidity perception between the wood and control wines. This seems to contrast with the increasing of Total Acidity detected in wine in contact with wood due to the release of phenolic acids and other substances (Ribereau-Gayon et al., 2010), eventhough the provision in our case is negligible because of the short maceration period, in anycase there shouldn't be drop in acidity in the wines in contact with wood.

A possible explanation to these results could be the increasing roundness and complexity of wines in contact with wood (Julien, et al. 2011), producing a softening effect on the perception of acidity.

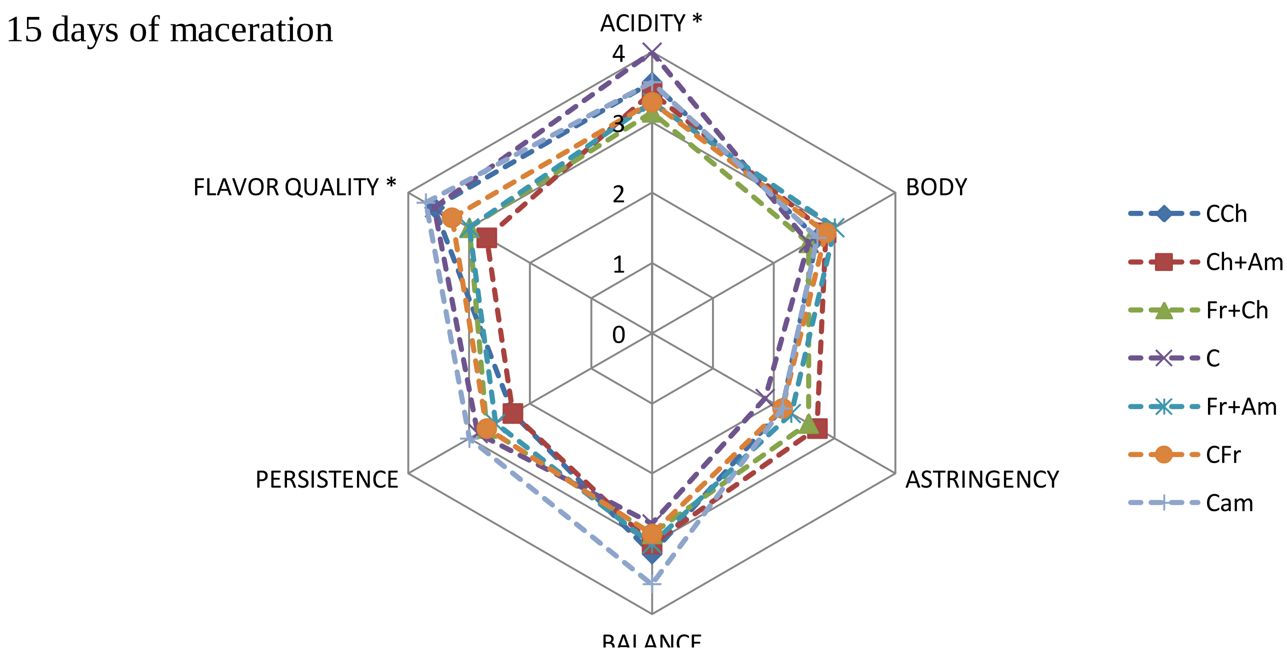


Figure 40- Sensory analysis results for taste parameters of the studied wines, at 15 maceration days. Legend: C: Control wine; Ch+Am: wine aged with blend of cherry and American oak chips, Fr+Ch: wine aged with a blend of French oak and cherry chips, CCh: wine aged with cherry chips, Fr+Am wine aged with a blend of French and American oak chips: CFr wine aged with French oak chips; CAm wine aged with American oak chips.

In the tasting of 30 days we can see how there is still a significant difference among the acidity of C, higher, and the rest of the treatments, while for the body and astringency parameters the opposite tendency is observed, C is significantly lower than the rest of the treatments, as would be expected for a wine in contact with wood. The observation for astringency are similar, being all higher in wines in contact with wood, with the exception of Fr+Am and Fr+Ch which are not statistically different if compared to any of the other treatments. As for the balance of the flavor CCh resulted significantly higher than the rest.

30 days of maceration

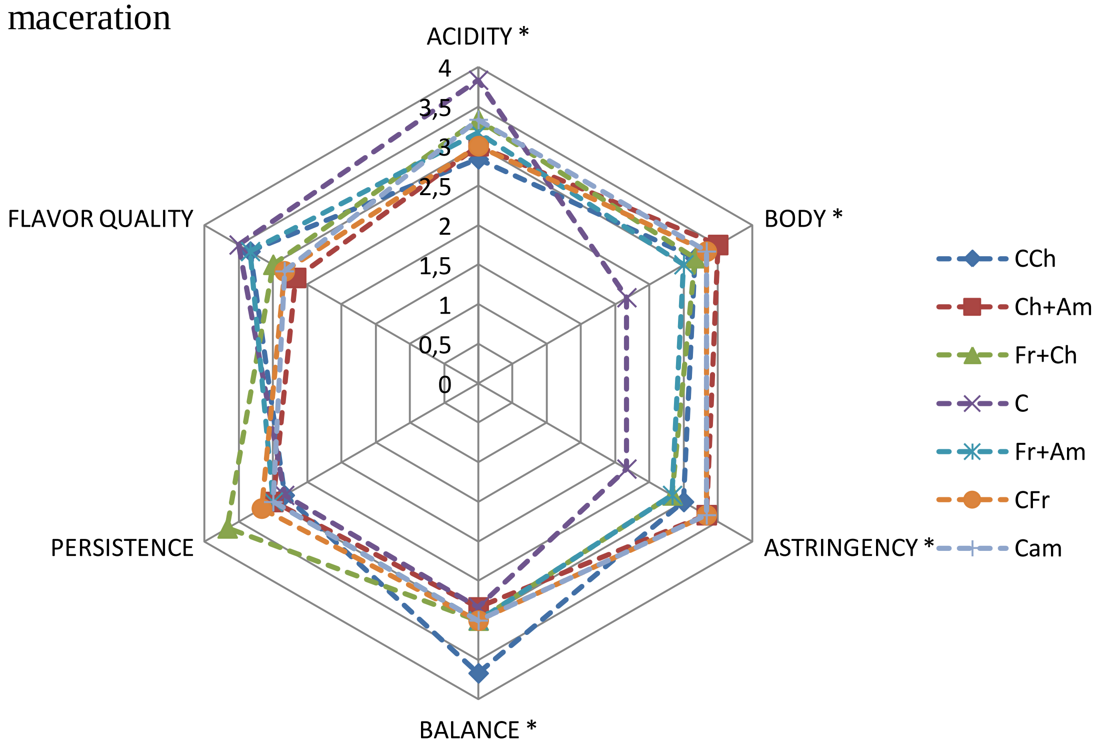


Figure 41-Sensory analysis results for taste parameters of the studied wines, at 30 maceration days. Legend: C: Control wine; Ch+Am: wine aged with blend of cherry and American oak chips, Fr+Ch: wine aged with a blend of French oak and cherry chips, CCh: wine aged with cherry chips, Fr+Am wine aged with a blend of French and American oak chips: CFr wine aged with French oak chips; CAm wine aged with American oak chips.

In the graph of the wines after 60 days of maceration we can see how the differences become more and more wide, with the statistical analysis founding significant differences among the wines in all the observed parameters, as visible in figure 41. The higher variability of the results of the statistical analysis doesn't let us give an exact path to the characters; the C is still showing the highest acidity.

60 days of maceration

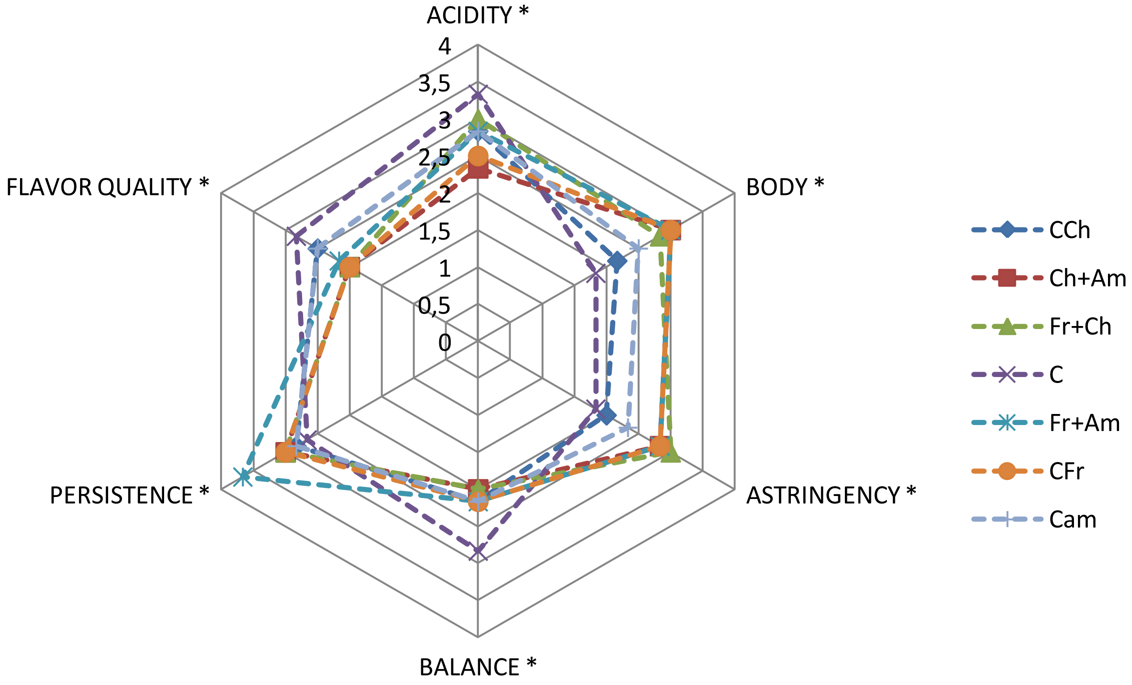


Figure 42- Sensory analysis results for taste parameters of the studied wines, at 60 maceration days. Legend: C: Control wine; Ch+Am: wine aged with blend of cherry and American oak chips, Fr+Ch: wine aged with a blend of French oak and cherry chips, CCh: wine aged with cherry chips, Fr+Am wine aged with a blend of French and American oak chips: CFr wine aged with French oak chips; CAm wine aged with American oak chips.

4.6.3. GLOBAL APPRECIATION

Another parameter taken into account during the sensory evaluation of the wines was the global appreciation of the wine, in order to give an idea of the overall quality of the wine.

From the results shown in Figure 42 we can see how the appreciation of the wine decrease with the increasing of the contact time between the wine and the wood, with the exception of the C and in less measure Fr+Ch. In the tasting of 15 days the wines with higher scores were CAm, significantly higher than all the rest of the treatments except CCh, and the latter which showed to be significantly more appreciated than the rest of the wines except for the already mentioned CAm and CFr. the wine with French oak (CFr) finally is significantly different than all the wines except the previously named.

The tasting after 30 maceration days see the decrease of the appreciation of all the wines if compared to the previous tasting, except for the C and Fr+Ch. The statistical analysis in this case even though showed differences, there was not a result significantly higher than the others, anyway C was the most appreciated together with CCh, while no differences were observed between the other treatments.

The last tasting showed a decrease for all the essays, showing how the C wine is the one getting the highest score also in this case, being significant higher than all the treatments except CCh and Fr+Am.

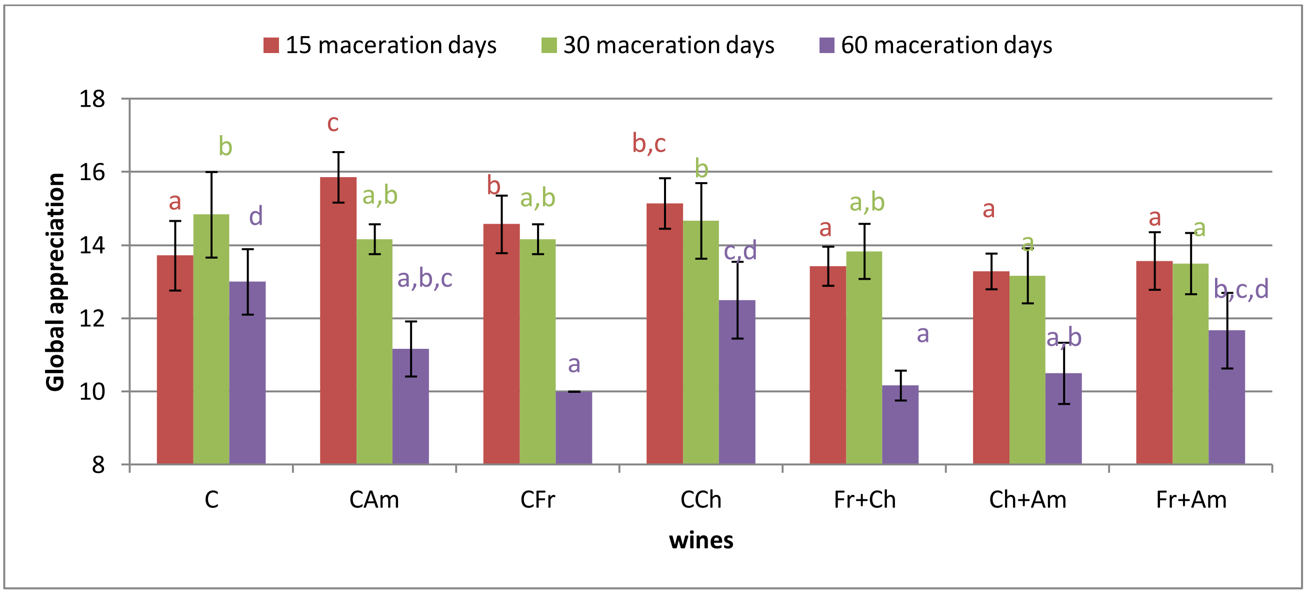


Figure 43- Evolution of Global appreciation for the 7 treatments during 60 days. The statistical analysis was run for data from the same maceration days and not among the different sampling of the same treatments. The results with same letters are not significantly different. Legend: C: Control wine; Ch+Am: wine aged with blend of cherry and American oak chips, Fr+Ch: wine aged with a blend of French oak and cherry chips, CCh: wine aged with cherry chips, Fr+Am wine aged with a blend of French and American oak chips: CFr wine aged with French oak chips; CAm wine aged with American oak chips.

The sensory analysis of the wines at different maceration days showed how a prolonged maceration with wood can be detrimental for a wine not prepared for the contact with wood (especially in relation with the role of lees already mentioned in the introduction). This seems to be the case for all the wood independently from the species or blend used, even though the wine with cherry (CCh) and the blend of French and American oak (Fr+Am) seemed to resist better to the lost of global quality.

5. CONCLUSIONS

In this study were evaluated the effects of chips from different wood species and their blends added after the alcoholic fermentation on the quality of a wine obtained from Encruzado grape variety.

The chemical analysis showed interesting results highlighting how the compounds extracted from the wood changed according to the species of wood used and its characteristic. The cherry wood chips showed the highest extractability for the total phenols profile, with the wines having these chips presenting an extreme phenolic concentration. The analysis for the determination of Flavonoids phenols confirmed what observed for the total phenols, being the Flavonoids one of the main class of phenolic compounds (Ribéreau-Gayon, et al. 2006) with the wines with cherry wood having the highest level of such kind of compounds. On the contrary the essays with the highest amount of non flavonoids phenols, were the one with French oak, even though in this case the French/American oak blend was the one with the final highest level (after 60 days of maceration with wood).

The color parameters showed as expected an increase in color hue in almost all the wines, except the Control and French/cherry blend, with the blend of Cherry and American showing the highest increase in color, highlighting once again the link between the phenols and the color in white wine.

The maderization test showed generally a decrease in the browning potential of the wines, with exception of CCh which confirming the strict-link existing between the total phenols and the browning potential of a white wine. Is also to be mentioned that all the wines in contact with wood presented, eventually, a higher browning potential than the C wine, as expected and suggested by many studies.

The tannin power results showed a huge increase in the tannicity of the wines, especially between the 30 days and the 60 days measurement, and saw the blends of wood collecting the highest measurement, with the Ch+Am being significantly higher than all the single wood wines and the Control.

Considering the amount of chips used (0,5 g/l), very low if considered the average use in winemaking for white wines (0.5-2 g/l), the more marked results were to be expected on the sensory analysis. The wines with the wood, indeed, showed interesting results on the aroma and taste parameters, with the American wood giving the best results already after 15 days, showing a higher aroma quality and higher fruity and floral notes; together with the Cherry wood it was more appreciated than the others. While the taste parameters didn’t show any significance on the 15 days.

After 30 days the most appreciated wines was still the one with cherry, but also the control, because of the increased woody notes of the other essays, which decreased the aroma quality. The taste also showed an increase of the balance for the cherry wood, while the acidity perception remained the highest for the control.

In the last sampling after 60 days of maceration the differences of the aroma parameters among the wood wines became less and less marked, while the control wine showed significant differences with the rest of the wines, having the highest aroma quality, the highest fruity and floral notes.

Also the global appreciation showed the Control being the first among the wines, with the Cherry wood being the second.

Hence the use of blend woods showed interesting results on the chemical level, with the Cherry and its blend giving more on the flavanoid profile, while French and its blends on the non flavanoids profile. On the sensory analysis the wines showed the best results on global appreciation after 15 days of contact, while started to decrease with increasing days of contact between the wine and the wood. The essays with best feedback at this stage were the one with Cherry and the one with American oak, while at 30 days the Control wine showed the best results, followed by the cherry.

Concluding, these results suggest that the contact between the wine and the wood for all the species and blends taken in consideration should be between 15 and 30 days in wine obtained by Encruzado. This is the case for a white wine obtained with traditional winemaking techniques, separated by the lees where the impact of wood cannot be mitigated and altered by their presence, as already mentioned in the introduction, hence the short maceration period is suggested in order to increase the wine quality without compromising its varietal aroma, neither to modify negatively the taste perception or even the yellow color.

Moreover we can state that species of wood alternative to oak such as cherry, and blends of wood from different species, are of huge interest for producing wines with peculiar attributes on the taste and aroma profile able to create curiosity and eventually value for customers. Nevertheless more research is needed in order to determine the optimal time of contact and the best amount of wood from the different botanical species to be used in white wine from different varieties.

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