Evaluation of Carcavelos Fortified Wine Aged in Portuguese (*Quercus pyrenaica*) and French (*Quercus robur*) Oak at Medium and High Toast

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Dedicated to my family and friends for their support throughout my studies.

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Abstract

Adega do Casal Manteiga is a publicly owned winery by the Municipality of Oeiras that produces Carcavelos fortified wine. The effects of botanical species (*Quercus pyrenaica*, and *Quercus robur*) and toasting method (medium and high) on a single vintage wine that has been aged for 8 years is examined. A total of twenty barrels were used with 5 replicates for each factor. The barrels were fabricated and toasted using the same cooperage, J.M. Gonçalves in Portugal. Significant differences were seen between the species *Q. robur* and *Q. pyrenaica* were shown impact on total phenolic content including both flavonoids and non-flavonoids. The total phenols of the wine aged in *Q. pyrenaica* barrels were significantly higher than in the *Q. robur* counterparts with differences of 61.3 mg/L eq. gallic acid and 75.1 mg/L eq. gallic acid for medium and high toast, respectively. *Q. pyrenaica* contained more flavonoids than *Q. robur* with a difference of 35.9 mg/L eq. gallic acid at high toast and 34.2 mg/L eq. gallic acid at medium toast. Regarding non-flavonoid compounds *Q. pyrenaica* showed 39.2 mg/L gallic acid equivalents more than *Q. robur* at high toast and 27.1 mg/L gallic acid equivalents more at medium toast. This difference in non-flavonoids was only statistically significant with the high toast barrels. The degree of toasting had significant effects on the Flavonoid content of the wine, as well as the tanning power. Flavonoid content increased for both *Q. pyrenaica* (Δ37.2 mg/L eq. gallic acid) and *Q. robur* (Δ35.5 mg/L eq. gallic acid) in the wines that were aged in barrels that underwent higher toasting compared to medium toasting. The tannin power decreased for both *Q. pyrenaica* (Δ13.66 NTU/mL) and *Q. robur* (Δ22.78 NTU/mL) when the toasting increased.

Keywords: Aging, Carcavelos D.O.P., Fortified Wine, Oak wood, Toasting

Resumo

A Adega do Casal Manteiga é propriedade pública a cargo do município de Oeiras, dedicada à produção de Vinho de Carcavelos. Os efeitos das espécies botânicas (*Quercus pyrenaica* e *Quercus robur*) e do grau de tosta (médio e forte) foram estudados para um vintage de Vinho de Carcavelos envelhecido durante 8 anos.Para este efeito foram utilizadas 20 barricas, com 5 repetições para cada fator. As barricas foram produzidas na tanoaria J.M Gonçalves (Portugal) onde foi feito também o tratamento térmico. Verificaram-se diferenças significativas entre as espécies *Q. robur* e *Q. pyrenaica* que mostraram impacto na composição fenólica total, incluindo flavonóides e não-flavonóides. O valor de fenóis totais no vinho envelhecido em *Q. pyrenaica* foi significativamente superior relativamente às suas contrapartes em contato com *Q. robur*, com diferenças de 61.3 mg/L eq. de ácido gálhico e 75.1 mg/L eq. de ácido gálhico para tosta média e forte, respetivamente. O carvalho *Q. pyrenaica* mostrou valores superiores de flavonóides relativamente ao *Q. robur*, com diferenças de 35.9 mg/L eq. de ácido gálhico em tosta forte e 34.2 mg/L eq. de ácido gálhico em tosta média. Quanto aos compostos não-flavonóides, *Q. pyrenaica* mostrou mais 39.2 mg/L eq. de ácido gálhico que o *Q. robur* no caso de tosta forte, e mais 27.1 mg/L eq. de ácido gálhico no caso da tosta média. A diferença constatada neste tipo de compostos apenas foi estatisticamente significativa nas barricas de tosta forte. O grau de tosta teve efeitos significativos no teor de flavonóides do vinho, assim como no poder tanante. Os compostos flavonóides aumentaram tanto para *Q. pyrenaica* (∆37.2 mg/L eq. de ácido gálhico) como para *Q. robur* (∆35.5 mg/L eq. de ácido gálhico) nos vinhos envelhecidos em barricas sujeitas a tosta forte, em comparação com a tosta média. O poder tanante diminuiu tanto em *Q. pyrenaica* (∆13.66 NTU/mL) como em *Q. robur* (∆22.78 NTU/mL) quando o grau de tosta foi superior.

Palavras-chave: Carvalho, D.O.P Carcavelos, envelhecimento, tosta, vinho generoso

Resumo Alargado

O objetivo deste trabalho foi de avaliar os efeitos de barricas novas de carvalho *Quercus pyrenaica* e *Quercus robur*, com tosta média e forte, num vinho de Carcavelos. A espécie *Quercus pyrenaica* não tem sido extensivamente estudada, e poucos trabalhos a comparam diretamente com *Quercus robur*. Os estudos feitos com esta espécie referem-se a vinhos secos, aparas ou aduelas, e não existe pesquisa presentemente publicada em vinho generoso Carcavelos.

A Adega do Casal Manteiga é propriedade pública a cargo do município de Oeiras, dedicada à produção de Vinho de Carcavelos. Os efeitos das espécies botânicas (*Quercus pyrenaica* e *Quercus robur*) e do grau de tosta (médio e forte) foram estudados para um vintage de Vinho de Carcavelos envelhecido durante 8 anos. Para este efeito foram utilizadas 20 barricas, com 5 repetições para cada fator. As barricas foram produzidas na tanoaria J.M Gonçalves (Portugal) onde foi feito também o tratamento térmico.

Verificaram-se diferenças significativas nos envelhecidos em *Q. pyrenaica* e *Q. robur*, relativamente aos fenóis totais, flavonóides e não-flavonóides. Ao comparar ambas as espécies, *Q. pyrenaica* mostrou valores superiores de fenóis totais, flavonóides e não-flavonóides relativamente ao *Q. robur*, tanto no tratamento de tosta média como de tosta forte. Os fenóis totais do vinho envelhecido em barricas de *Q. pyrenaica* foram significativamente superiores à contraparte de *Q. robur*, com diferenças de 61.3 mg/L eq. de ácido gálhico e 75.1 mg/L eq. de ácido gálhico, para tosta média e forte, respetivamente.

Q. pyrenaica mostrou também mais compostos flavonóides que *Q. robur*, com diferenças de 35.9 mg/L eq. de ácido gálhico para tosta forte e 34.2 mg/L eq. de ácido gálhico para tosta média. Quanto aos não-flavonóides, *Q. pyrenaica* teve valores superiores ao *Q. robur*, com mais 39.2 mg/L eq. de ácido gálhico para tosta alta e mais 27.1 mg/L eq. de ácido gálhico em tosta média. Nas barricas sujeitas a tosta forte, a madeira tem um impacto significativo. Com tosta média, o efeito da madeira não é significativo. A espécie da madeira não aparentou afetar o poder tanante ou a intensidade da cor nos vinhos.

O grau de tosta mostrou mudanças significativas no poder tanante e no teor em flavonóides no vinho, para ambas as espécies de madeira. O método de tosta não mostrou efeitos

significativos no teor de fenóis totais no vinho. Os compostos flavonóides aumentaram tanto para *Q. pyrenaica* (∆37.2 mg/L eq. de ácido gálhico) e para *Q. robur* (∆35.5 mg/L eq. de ácido gálhico), nos vinhos envelhecidos em barricas sujeitas a tosta forte, em comparação com tosta média. O poder tanante diminuiu com o aumento da tosta, para ambos *Q. pyrenaica* (∆13.66 NTU/mL) e *Q. robur* (∆22.78 NTU/mL).

As análises não evidenciaram efeitos significativos da espécie de madeira ou grau de tosta no caso da densidade dos vinhos, acidez total, acidez volátil, grau alcoólico, extrato seco total e intensidade da cor. O vinho generoso “Carcavelos” produzido pela Adega do Casal Manteiga é tipicamente envelhecido durante 10 anos antes do engarrafamento. Quando este vinho terminar o seu envelhecimento, poderá fazer-se uma nova análise com o vinho nestas barricas, possivelmente incluindo análise de HPLC para aprofundar a composição fenólica e obter uma melhor comparação. Para além disso, deveria incluir-se ainda uma análise sensorial referente aos vinhos depois de terminados.

Palavras-chave: Carvalho, D.O.P Carcavelos, envelhecimento, tosta, vinho generoso

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List of Abbreviations

Δ	Difference / Change
µL	Microliters
Abs	Absorbency
ABV	Alcoholic strength by Volume
ANOVA	Analysis of Variance
AOC / AOP	Appellation d'Origine Contrôlée / Protégée
AVA	American Viticulture Association
B	Baume
C	Celsius
Ca.	Circa
cm	Centimeters
CO^2	Carbon Dioxide
DO	Denominación de Origen / Denominação de Origem
DOC	Denominazione di Origine Controllata / Denominação de Origem Controlada
DOCG	Denominazione di Origine Controllata e Garantita
DOP	Denominación de Origen Protegida / Denominação de Origem Protegida
g	Grams
HCL	Hydrochloric Acid
HPLC	High-performance liquid chromatography
H_2SO_4	Sulfuric Acid
HSO_3^-	Bisulfate ion
$K_4Fe(CN)_6.3H_2O$	potassium ferrocyanide (II) Trihydrate
LBV	Late Bottle Vintage

Max	Maximum
meq	Milliequivalent
Min	Minimum
mL	Mililters
Mol	Molar
NaOH	Sodium Hydroxide
nm	Nanometers
OIV	International Organization of Vine and Wine
PCA	Principal Component Analysis
R^2	Correlation coefficient
SD	Standard Deviation
SO^2	Sulfur Dioxide
T	Temperature
TA	Total Acidity
VA	Volatile Acidity
$ZnSO_4.7H_2O$	Zinc Sulfate Heptahydrate

Bibliographic Report

1 General Introduction

The purpose of this study was to examine the effects of two species of new oak barrels and their toast on Carcavelos fortified wine. Typically fortified wines are aged in old barrels for the purpose of having a neutral effect on the wine. New barrels are sometimes used for white fortified wines for the extraction of some flavors and aromas but are not widespread.

In this study, a single experimental plan was carried out. The objective was to examine the evolution of a single vintage fortified wine aged in French and Portuguese Oak, at medium and high toast. The effects of the species of wood as well as toast, on Carcavelos wine could then be examined.

High quality fortified wines spend long periods of time aging in wood barrels. During this time, the wine undergoes important physical and chemical changes. Many constituents are extracted from the wood during the aging process. Oxygenation is considered indispensable for sweet fortified wines as it develops aromas. This process develops the wines' color, aroma and flavor. Furthermore, the species and geographical origin of the wood, along the the toasting performed by the cooper, have proven to impact the final wine (Chira & Teissedre, 2014; Doussot *et al.,* 2002; Jackson, 2014; Navarro *et al.,* 2016; Velikova & Dodd, 2016). Samples of the wine were taken after 8 years of maturation in barrel.

This Master Thesis was carried out as part of an experimentation being performed at Quinta do Marquês, made in collaboration with Adega do Casal Manteiga, Municipality of Oeiras and the Instituto Superior de Agronomia (ISA), Universidade de Lisboa. The work was completed under the supervision of Professor Jorge M. Ricardo da Silva and coordination with the winemaker Tiago Lopes Correia. The main focus of the internship was to perform a chemical analysis of the fortified wine.

2 Fortified Wines

2.1 History

Fortified wines were originally developed as a response to fermentation problems in warm climates. Warm regions can provide a naturally optimal temperature for yeast activity. This velocious yeast metabolization of the sugar rich grapes further increases the temperature of the must and subsequently, the yeast dies. The dead yeast results in stuck fermentations and unstable wines allowing for microbial spoilage from lactic acid bacteria and spoilage yeasts. Stopping the alcoholic fermentation with the addition of brandy produced wines that were sweet with a pleasant taste (Ribereau-Gayon *et al.,* 2006a). Furthermore, adding brandy provided protection against other spoilage problems of the time, mainly preservation of the wine for long term storage and during transportation. The addition of brandy also allowed for conservation of the wine during long journeys at sea.

2.2 Characteristics

The main characteristics of a fortified wine are a high alcohol and sugar contents. These goals are achieved through the addition of brandy with an Alcoholic strength by volume (ABV) of 77% at a critical point during fermentation. The addition point is based upon the initial baume of the must and usually follows a protocol chart developed by the winery's engineers.

The Office International de la Vigne et du Vin (OIV) defines fortified wines as having an alcoholic content from 15% to 22% alcohol by volume (ABV) and with a sugar level above 12 grams per litre (g/L). Ribereau-Gayon *et al.,* (2006a) elaborates that two categories of fortified wine exist:

1. Spirituous wines receive only brandy or rectified food-quality alcohol during fermentation;
2. Syrupy sweet wines can receive concentrated must or mistelle in addition to brandy or alcohol.

Each demarcated fortified wine region usually creates its own laws within this generalization (Table 1).

Table 1. Regulations of Alcohol Content by Demarcation for Fortified Wines.

Wine Region	Limit range (% vol.)	
	Min.	Max.
Douro - V. Porto	16.5	22
Carcavelos	17.5	
Moscatel de Setúbal	16.5	
Madeira	17	

Taken from lecture of Fortified Wines by Jorge M. Ricardo-da-Silva

2.3 Demarcations

In 1756 Sebastião José de Carvalho e Melo, 1st Marquis of Pombal created the first association that was designated to create appellations. The organization was called Companhia Geral da Agricultura das Vinhas do Alto Douro. The association certifies that the wine or product from the Douro region has distinguishing qualities compared to other regions. Since then appellations have been adapted all over the world under different names, abbreviations, and rules to organize their respective country's production. The United States uses American Viticultural Area (AVA), France uses Appellation d'Origine Contrôlée/Protégée (AOC/AOP), Italy uses Denominazione di Origine Controllata (DOC) and Denominazione di Origine Controllata e Garantita (DOCG), Spain has Denominación de Origen (DO) or Denominación de Origen Protegida (DOP), and the Portuguese use Denominação de Origem Controlada (DOC) which is now Denominação de Origem Protegida (DOP).

3 Types of Fortified Wine

There are four “Vinhos Generosos” from Portugal which are the classical, or “ancient” fortified (“liquoroso”) wines. These wines are now demarcated as D.O.P. (Denominação de Origem Protegida) and include the regions of Porto, Madeira, Setúbal, and Carcavelos.

3.1 Port Wine

Port wines are made in the Douro region of northern Portugal and are typically aged closer to the coast in Vila de Gaia. Prior to the addition of the brandy typically port wines spend four to five days in a “lagar”. During this time, a protocol for pigeage, or in Portuguese pisagem, is followed. Over time the pigeage of the must increases in intensity to ensure a gradually higher extraction of phenolic compounds. A final pisagem is done to homogenize the must and ensure the fermentation stops. Then, the wine is usually racked the following day. There are two styles of port wine: tawny, and ruby.

The differences between the tawny and ruby style are the aging time and the oxygenation. Ruby style wine is a blend of vintages with the youngest being 1 year old and aged by oxidation for 2-4 years before being aged in a reduced environment. Tawny style is also a blend of vintages but the youngest is 2 years old. Tawny is then oxidatively aged for a minimum of 7 years for reserve. In terms of quality the difference between the tawnys is simply the amount of time aged. The age listed on the bottle is an average of all the years included, unless it is a vintage.

In the case of ruby style, it is both selection and age that makes the determination of quality. The standard is a blend aged and oxidized in the normal way. Reserva is selective blending of some of the best ports and bottled between the second and third years of the youngest vintage in the blend. Late Bottle Vintage (LBV) is from a single vintage of exceptional quality and is bottled between the 4th and 6th year after harvest. Vintage is obtained in the same way as LBV but bottled between the 2^{nd} and 3^{rd} year. There is also a legal limit to the amount of a vintage port. Once the limit is met, the same port can be held and made into a LBV.

3.2 Madeira Wine

Madeira fortified wines come from the Portuguese Madeira islands off the coast of Africa. Originally spirits were added to the wine to prevent spoilage during the transportation across the seas. Inevitably some wines were not sold and were brought back. These wines were observed to have an increased quality compared to when they were originally shipped. The discovered quality increase was attributed to the long periods of heat and movement the wine endured during transportation.

Today this process is simulated using either the serpentine method “estufagem” or the “canteiro” method. In the estufagem method the wine is placed in stainless steel vats which are heated with hot water between 45ºC and 55ºC and moved cyclically for a minimum of three months before being bottled and released to the public no earlier than the 31st of October on the second year after the vintage. Interestingly, research has shown that while this accelerated aging method does decrease the total phenolic content of the wine, the effect is not extreme and at a maximum was decreased by 25% while still being comparable with most white wines. When examined more closely, most individual phenols decreased with the exception of caffeic, ferulic, p-coumaric, gallic and syringic acids. Furthermore when considering the heats effect on color there was no clear trend except for the wine was overheated at 70ºC (Pereira *et al.,* 2013).

In the “Canteiro” method wines are placed in barrels located near the rooftop and are aged for a minimum of two years, but cannot be released until after the third year. Evaluation of wine color under accelerated aging showed that regardless of the aging method of these wines the greatest variation in color was found in the vinification step before both aging procedures. Therefore the quicker method of estufagem may be used by the oenologists without sacrificing color (Carvalho *et al.,* 2015).

Madeira wines can range in style from extra dry to sweet depending on the total amount of sugars contained within them and most commonly appear with a single variety on the label. The single variety label common of Madeira fortified wines stem from the fact that unlike port wines, where many grape varieties are used, Madeira fortified wine contains at least 85% of the variety listed. Madeira wines must also label the age of the youngest year contained within the bottle, similar to scotch whiskey. Under the two listed categories in “The Handbook of Enology Vol 1.”, Madeira wines are under the first with their fermentation being stopped by the addition of brandy. Dissimilar to port wine the brandy is 96% ABV.

3.3 Moscatel de Setúbal

Moscatel de Setúbal wines originate from the Península de Setúbal just south of Lisbon. These wines are primarily white, although Moscatel-Roxo a red variety exists. Wines from here can be classified as either "Setúbal" which must contain a minimum of 67% of their authorized varieties, or "Moscatel de Setúbal" which must contain at least 85% of the Moscatel varieties.

Vinification is similar to Porto wines and alcoholic fermentation is stopped after a few days. The brandy they add can vary between 58-77% alcoholic strength by volume. The maceration period however is extremely long and can last from three to five months. As a result of this long maceration period Moscatel de Setúbal typically adds some SO_2 to the wine. The addition of sulfur dioxide is to protect the limpidity of the wine and inhibit oxidative enzymatic activity. The consumer preference is for clear wines, once oxidation occurs and the wine is no longer clear, it becomes undesirable. After maceration, the wine is then aged in barrels and must mature for a minimum of 18 months before bottling.

3.4 Flor Wines (Sherry)

Flor wines are a kind of fortified wine in that the principal characteristic is biological aging in contact with air by the development of flor yeasts. After alcoholic fermentation, brandy, rectified alcohol or agricultural spirits can be added to the wine as the alcohol content of the finished product must be at minimum 15% by volume. Recently, the minimum alcoholic strength requirement can sometimes be obtained without any addition of brandy. Vinification is based on white winemaking principles without maceration including light juice extraction and clarification of the must. Once the wines are selected for aging, they are racked and filled to $5/6^{ths}$ of its capacity. The high alcoholic content prevents microbiological spoilage, but flor yeasts can still develop on the surface of the wine. During aging, the wine is redistributed and blended using the solera system to create a uniformity when bottling. Depending on the aging method there are different categories of flor wines: biological, mixed, or oxidative. Fino and Manzanilla are biologically aged for at minimum of three to four years and produce a nose with green apple aromas. The mixed wine is Amontillado, where the flor is allowed to die off during aging and is considered the halfway point between fino and oloroso. In oxidatively aged wines such as oloroso or palo cortado, the flor is either thin, underdeveloped or absent.

3.5 Mistelle

Mistelles in Portugal have two categories: Abafados and Jeropigas. These are made from either white grapes with maceration or red grapes without maceration and can appear as white, blonde ("tawny"), or red colors. Abafado is produced at the beginning of maceration when alcoholic fermentation is stopped with the addition of brandy. Fermentation is stopped depending on initial sugar content, alcohol content of the brandy, alcohol and sugar content desired in the finished wine. Jeropiga is from the mixture of a grape must that has not undergone alcoholic fermentation and a brandy.

3.6 Aguardente

Aguardente is a Portuguese wine brandy meaning "fire water". This distillate is produced exclusively from wine or the redistillation of a wine with a finishing ethanol content of usually less than 86%. The goal is for a colorless limpid distillate with no off aromas or flavors. They are achieved through multiple distillations to purify the distillate. Aldehyde composition in aguardente is an important factor when you want maximum extraction of color and tannin compounds. When aguardente is used to stop fermentation in fortified wines the alcoholic strength is most commonly at 77%.

3.7 The Use of Barrels in Fortified Wine

One of the most important practices in obtaining high quality wines and fortified wines is the aging process. The aging process and thus, the role of wood is crucial in the development of the wine as the organoleptic properties developed here make the final product highly valued. During this time, the wines undergo a change in chemical composition and an improvement in sensorial qualities. The complexity and taste of the wine are increased with the extraction of phenolic compounds present in the wood. Typical aging aromas such as dried fruits, spice, curry, and nuts appear from the lactone sotolon, though the precursors are not yet known. The micro-oxygenation through the pores of the wood causes a reduction of astringency and changes the color (Alañón *et al.,* 2011).

Traditionally for fortified wines, and thus in most cases, old wood is used for aging. Recently, depending on the goals of the oenologist, new barrels may sometimes be used for the aging process. Successive use of oak barrels results in a decrease in the extraction of phenols over time as they already have been solubilized into the previous wines. Therefore, the use of new barrels enhances the extraction of these volatile compounds. Research has shown that in new barrels most oak-related aroma compounds are extracted within the first two months of maturation with only small increases after. Furthermore, with regards to sweet fortified wines with high levels of oxidation, among the volatile phenols only vanillin content was shown to increase (Cutzach *et al.,* 2000; Gómez-Plaza*et al.,* 2004).

4 Carcavelos Fortified Wine

4.1 History

Wine production in Carcavelos began in the 18th century. Originally the vineyards were privately owned and grown by Sebastião José de Carvalho e Melo, 1st Marquis of Pombal, on his estate in Oeiras. Carcavelos wine originated from the desire he had to utilize grapes from his residence nearby Oeiras. Before his personal production, the grapes were sold to producers in the Douro, which violated the demarcated regulations from the association he created. Because he was a prime minister and had some power, he created his own regulations permitting Carcavelos wines to be blended with wine from the Douro (Robinson and Harding, 2015). In the 19th century, he established Carcavelos as a winemaking region where he produced wine from his property that became known throughout the world, reaching as far as America. This wine was known as Lisbon wine or “Calcavella” (Terras de Portugal 2017) and soon after, the region was demarcated. Eventually, due to the urbanization of the area, the production declined until a research project was created to rebuild the vineyard and maintain the area's history. Today in order for Carcavelos wines to be an appellation, the wine must age for at minimum two years in wood, followed by at least six months in the bottle. The current winemaker matures the wines for at least ten years in oak (T. Correia, personal communication, 2017).

4.2 Location

Adega do Casal Manteiga, is a publicly owned winery by the Municipality of Oeiras located in Oeiras on the southern tip of the Lisboa wine region. The winery and vineyards are at the top of a hill in the National Agronomy Station that resides in the property that was once owned by Sebastião José de Carvalho e Melo. Currently, Carcavelos has 25 hectares of vineyards shared between four producers (Mendes, 2016). Carcavelos is a micro region surrounded by small hills and as a result of its close proximity to the Atlantic ocean and the Tagus river, the region produces fresh and acidic wines. According to the Villa Oeiras website, the average temperatures range between 11°C in the winter and 23°C in the summer. Some humidity settles in overnight as a result of low temperatures and proximity to the water, but morning winds protect the vines from moisture related attacks (Mendes, 2016). The summer season has an average rainfall of less than 5.2mm which put the vines at risk of water stress. The soil profile, which is composed of sand, clay, and limestone allows for the roots to grow deep and reach the water retaining clay underneath (IVV, 1994).

4.3 Grape Varieties

Adega do Casal Manteiga possesses only 12.5 hectares of vines. Within these hectares are both white and red varieties, but the red varieties are only used for experimentations. Carcavelos produces a fortified white wine composed of the three white varieties Galego Dourado, Boal-Ratinho, and Arinto. Some other varieties are allowed but are not used. The recommended and authorized varieties can be seen in Table 2.

Table 2. Carcavelos Grape Varieties.

Recommended (75% min.)	Authorized (25% max)
White: • Galego Dourado • Boal-Ratinho • Arinto	• Rabo-de-Ovelha • Seara-Nova
Red: • Castelão • Negra-Mole (Preto-Martinho)	Trincadeira-Preta (Espadeiro ou Torneiro)

Table modified from Fortified Wines lecture by Jorge M. Ricardo-da-Silva

Gallego Dourado is the most essential variety of the three whites and originates from Carcavelos and Colares. It is known to produce high alcohol, aromatic, and well rounded wines (Robinson *et al.,* 2012; IVV, 2011). Arinto is a highly adaptive variety and thus has been planted throughout all of the Portugal wine regions. Arinto produces acidic wines with notes of green apple and lemon. When used in a blend, it can add freshness, minerality, and structure. Ratinho also originates from Carcavelos and produces medium alcohol wines with low acidity (Robinson *et al.,* 2012).

Harvest of these varieties finishes between the end of August and mid-September. The grapes are hand picked, destemmed, and pressed pneumatically. The must is then put into stainless steel tanks for alcoholic fermentation. Traditionally alcoholic fermentation either finishes or almost finishes and a mistelle, either Abafado or Jeropiga, is added to obtain a wine with an alcohol content of 18-22% and ± 100g/L of sugar. Therefore the sweetness of the wine is related to the sugars contained within the mistelle added. In some cases a dry wine was used in combination with Aguardente.

Today Carcavelos wines are made using the same methodology as port wines. An addition of aguardente with an ABV of 77% is used to stop the fermentation process. Adega do Casal Manteiga sources their aguardente from Torrejana, S. A. Distillery. Once fermentation has stopped, the wine is placed into either Portuguese or French oak barrels for maturation.

5 The Use of Oak in Oenology

5.1 History

Wooden barrels have been used for centuries as containers and were probably developed as an evolution of skills by the crafters of buckets, tanks, or from the watertight construction of boats. Depictions of barrels can be dated back to as early as circa 2630 B.C. as seen in the tomb of Hesi-Re, and 1400 B.C in the tomb of Rekhmire in Thebes (Quibell, 1913). These containers were constructed from palm-wood and according to Herodotus (ca. 485 - 425 B.C.), some were used to transport wine, among other goods, down the Euphrates from Armenia (Jackson, 2014).

Throughout Europe and England, oak barrels for fermentation, storage, and transportation of wine have been uncovered. These barrels are the first indisputable archaeological evidence for the use of oak wood cooperage with hoops and they have been dated back to the Imperial Roman times. According to Wine Science (2014), “Roman barrels were typically longer and thinner than barrels today. They possessed an average ratio of about 1:3, in contrast to the more typical, current standard of 1:1:4.”. In addition to oak, other types of wood such as Chestnut (*Castanea sativa*) and Acacia (*Robinia pseudoacacia*) have historically been used for the construction of large fermenters and storage vessels in oenology. The rise of modern oenological technology such as stainless steel fermenters, bag-in-box and bottles for transportation, has further specialized the use of oak in oenology (Jackson, 2014).

Modern technologies and advancement in the field of oenology have also given us a greater appreciation for all aspects of the oak including cooperage demands, clarification, stabilization, and micro-oxygenation. The discovery of this knowledge brought with it the rise of oak in oenology compared to alternative woods. Zhang explains, “With the passage of time, winemakers discovered that wine aging in oak barrels was not only convenient, but also improved wine quality by improving their appearances, flavors and mouthfeel, therefore, aging wines in oak barrels became an indispensable part of making high-quality wines.” (Zhang *et al.,* 2015). Consequently this rise of demand has caused an increase in the cost of wood. Less expensive alternatives such as staves, chips, and oenological tannin additives have gained popularity as a result. These alternatives can provide the woody aromas, flavors, and structure of oak aged wine but will lack some of the physico chemical reactions that help with clarification and stabilization. Furthermore, the wine will still need to be in contact with oxygen since oxidation reactions are indispensable for bringing out the oaky character (Ribéreau-Gayon *et al.,* 2006b).

5.2 The Oak Tree

The oak tree is a northern hemisphere native angiosperm and taxonomically ordered under the family *Fagacea* with the genus *Quercus*. There are approximately 600 species of *Quercus* and the basic composition of Cellulose (40%), hemicellulose (25%) and lignin (20%) does not significantly differ from one species to another (Zhang *et al.,* 2015). According to Wine Science these numbers are around 50%, 20%, and 30% respectively (Jackson, 2014). Despite the vast number of species within the *Quercus* family there only three major woods used in cooperage and intended for wine aging. Specifically American white oak (*Q. alba*) and two European oaks, the sessile oak (*Q. petraea*) and the pedunculate oak (*Q. Robur*) (Navarro *et al.,* 2016). Although these species share the same basic composition and are used for the same purpose in oenology many studies have shown significant differences between their phenolic composition and extractable compounds such as the *cis* and *trans* isomers of β-methyl-γ-octalactone (better known as the whisky-lactone), volatile phenols (e.g., eugenol), phenolic aldehydes (e.g., vanillin), hydrolysable tannins (e.g., ellagitannins) and more (Chira & Teissedre, 2014; Navarro *et al.,* 2016; Zhang *et al.,* 2015). These differences have since been attributed to species, geographic origin, age of the tree, and cooperage practices such as seasoning and toast (Chira & Teissedre, 2014; Doussot *et al.,* 2000; Doussot *et al.,* 2002; Navarro *et al.,* 2016). Doussot elaborates on these differences explaining, pedunculate oak (*Q. robur*) shows higher levels of dry extract, ellagitannins and free ellagic acid but lower aroma compounds such as oak lactones, eugenol, and vanillin compared to sessile oak (*Q. petraea*).

5.3 Origin of the Oak

Research has shown that after aging, wines with different characteristics can be obtained from the same wine. Chira and Teissedre show the forest origin of wood used induced important changes on all the studied variables ($p \leq 0.05$), but especially on whiskey lactone and eugenol concentration. This is attributed to the type of wood used in cooperaging, as well as the toasting process (Chira & Teissedre, 2014). Pedunculate and sessile oak species grow throughout Europe, and within France, *Q. robur* and *Q. petraea* covers 1.86 and 2.32 million hectares respectively (Doussot *et al.,* 2002). French cooperages source their wood from these main areas, all of them dominated by *Quercus petraea*, with the exception of Limousin which is dominated by *Quercus robur* (Jackson, 2014).

- Limousin
- Allier / Tronçais forest
- Central forest
- Nevers / Bertrange
- Vosges
- Argonne
- Bourgogne

Pedunculate oak (*Quercus robur*) can quickly establish itself in sunlit areas and does well on deep, rich, moist soils usually seen in lowlands or river valleys. Once *Q. robur* becomes shaded it is slowly replaced by *Q. Petraea*, its more shade tolerant relative. Winemakers desiring rich tannin and phenolic extraction may choose oak from Limousin. *Q. robur* from the Limousin forest is more dense, less aromatic, wide grained, tannic wood that is generally more suitable for brandy than wine (Jackson, 2014). Furthermore pedunculate oak was shown to have higher levels of dry extract, ellagitannins, and free ellagic acid but lower volatile compounds than sessile oak.

Sessile oak (*Quercus petraea*) prefers drier shallow soils found in Nevers and Allier and is more prevalent in the central regions of France. Winemakers looking for intermediate tannin extraction may prefer using this oak as it generally contains less extractable phenolics than *Q. Robur*. These oaks have high aromatic potential but are less rich in ellagitannin content (Jackson, 2014; Ribéreau-Gayon *et al.,* 2006b).

American oak (*Quercus alba*) is the type of white oak most grown in the United States. This oaks' reputation is usually attributed to the high quantity of low weight phenolic compounds, aroma compounds such as whiskey lactones, and some volatile compounds, but contains low concentrations of phenolic compounds and ellagitannins. Furthermore, after undergoing toasting, *Q. alba* was reported to have a decrease of between 72% and 99.7% of total ellagitannins (Chira & Teissedre, 2014; Jordão *et al.,* 2007). During the fabrication of American oak barrels, staves from similar species of oak such as *Quercus bicolor*, *Quercus lyrata*, and *Quercus stellata* may be included.

Portuguese oak, or Iberian oak (*Quercus pyrenaica and Quercus faginea*) occupies parts of northern Portugal but is also grown throughout the Iberian peninsula. *Q. pyrenaica* oak is not extensively used in winemaking, but is traditionally used for the maturation of Portuguese fortified wines and is highly regarded. Studies have shown this species contains large amounts of extractable compounds and that *Q. pyrenaica* and *Q. faginea* can be comparable to chemical data reported in some French oak species. Furthermore, both *Q. pyrenaica* and *Q. faginea* have been reported to be richer in tannin than *Q. alba* (Cadahia *et al.,* 2001). It has also been reported that *Q. pyrenaica* contains higher extractable ellagitannin content (Jordão *et al.,* 2007) . Furthermore, wine with chips and staves from *Q. pyrenaica* showed higher aromatic intensity, and complexity than American or French oaks (Chira & Teissedre, 2014; Coninck *et al.,* 2006).

5.4 Selection of the Wood

Coopers look for particular aspects of wood for the fabrication of barrels. Selection of wood is usually based on the grain and origin of the wood as a result of different structures between heartwood. Depending on the species, care must be taken to obtain the staves. An example of this is that American heartwood can be sawed, but sawing French oak would make it porous and affect tightness. Therefore, French oak needs to be split to respect the natural graining of the wood. This results in a higher overall loss during stave productions and can be seen in the higher cost of French oak barrels. The wood must be straight grained with fibers running parallel up the trunk, and free of faults that could cause the final product to leak. This results in cooperages looking for slow growing tight grain oaks. The grain of the wood has been shown to correlate shrinkage during heating and thus oxygen permeability. Furthermore, as the wood will eventually be heated and bent into position, the wood needs to have strength and resilience. The wood must also be free of undesirable odors, and depending on the oenologists goals, the phenolic profile of the species needs to be considered. Studies have poorly correlated the width of the grain and extractive content and therefore, generally it is recommended to base the selection on both the geographical origin and the species (Doussot *et al.,* 2000; Doussot *et al.,* 2002; Jackson, 2014; Navarro *et al.,* 2016).

5.5 Seasoning

Once the staves have been selected they undergo the seasoning process. There are two kinds of seasoning: natural and artificial. Depending on the seasoning process the extractable phenolics from the wood has been shown to change the characteristics of the wine aged within it. Seasoning is essentially a drying process where the humidity of the wood accumulates to the ambient humidity, usually between 14% and 18% (Jackson, 2014). During the drying process, the staves are stacked in such a way that allows air to circulate inside the pile.

Traditionally, the natural drying process takes about two to three years and results in the elimination of some undesired substances that can contribute to bitterness and astringency. Additionally, this process also results in a chemical aging due to rain leaching, artificial watering and biological activity such as fungal attacks. Although fungal attacks can reduce the quality of the wood they also have the potential to synthesize aromatic aldehydes and lactones from wood lignins. Some studies have shown ellagitannin concentration to decrease during seasoning. Alternatively studies have also shown volatile compounds such as whiskey lactones, eugenol, and vanillin either increase or remain the same during drying. Furthermore a study showed that artificial drying by a kiln released more tannins in *Q. petraea* than in *Q. robur* (Doussot *et al.,* 2002; Jackson, 2014; Navarro *et al.,* 2016).

5.6 Toasting

Once the staves have been seasoned, they are bent into position through the use of a temperature increase. The temperature increase results in a reduction of rigidity of the lignin and hemicellulose on the inside of the staves. During this process the staves are frequently sprayed with water to prevent cracking and to further soften the wood. Once sufficiently softened, usually around 15 minutes, the staves are pulled together. Some of the tension caused from this bending has been already released through shrinkage from water loss. Additional heating can be applied at this time resulting in changes in the chemical composition of the wood. Three toasting levels are commonly offered by the cooperage: light, medium, and high toast. Depending on the degree of toasting, wood constituents are degraded and broken into other compounds which increase volatile levels. Ellagitannins are easily hydrolyzed and become ellagic and gallic acids. Therefore, as toasting increases, ellagitannins decrease. Medium toasting was shown to drastically reduce ellagitannin content and increase volatiles. After undergoing intense toasting sessions even species with high levels of ellagitannins after seasoning, such as *Q. robur* and *Q. pyrenaica*, were shown to have comparable levels of ellagitannins following the treatment. Finally, the temperature, the length of toasting, and the human element can have an effect on the toasting of each barrel. Overall this will impact the chemical composition of the wood, and therefore, the final wine (Chira & Teissedre, 2014; Doussot *et al.,* 2002; Jackson, 2014; Jordão *et al.,* 2007; Navarro *et al.,* 2016; Pérez-Prieto *et al.,* 2002; Sanz *et al.,* 2012).

6 Chemical Composition of Oak

During wine maturation in oak, a number of compounds are released from the wood. The total amount of extractable compounds are dependent on what is already present in the wood. Research has shown that many extractable compounds reside in the first few millimeters of the wood, and successive use of a barrel reduces the total amount of phenolics extracted into the wine. This is because subsequent wines need to permeate further into the wood to extract the compounds compared to the previous wines (Gómez-Plaza *et al.,* 2004).

Aroma compounds are an important aspect that affects the consumer's perception of the wines' identity and quality. Many of these compounds are extracted from the wood during barrel maturation and are responsible for specific aromas and are therefore, organoleptically important. As a result of toasting, some volatile compounds have been shown to increase such as coconut for the whisky-lactone, clove from eugenol, and vanilla from vanillin (Doussot *et al.,* 2000). Many types of compounds which originate from the wood are oenologically important such as lactones, coumarins, polysaccharides, terpenes, hydrocarbons, or aromatic compounds such as benzoic aldehydes, and phenolic acids. The main polyphenols from wood are hydrolysable tannins e.g., ellagitannins and gallotannins (Jordão *et al.,* 2005).

Overall, the sensorial impact of aroma compounds is based on the threshold data of each individual component. Their interactions with each other, as well as factors such as, other volatiles from oak or microbiological activity, are not taken into account. Furthermore the impact of furanic compounds, such as furfural on aromatics, is still a topic of debate. Furfural and 5-methylfurfural have high sensorial thresholds on their own, and only have a minor impact on aromatics. However, furfural was found to affect the organoleptic properties of whiskey lactones. Levels of 10 ppm furfural and 1 ppm oak lactone were shown to impart a pleasant wood, caramel or vanilla-like odor. As the furfural content increased, caramel and vanilla odors from furual was accentuated, while woody aromas subsequently decreased (Chira & Teissedre, 2014).

6.1 Hydrolysable Tannins

Ellagitannins represent up to 10% of molecular weight of oak heartwood and are usually found in greater concentration in *Q. robur* compared to *Q. petraea*. These tannins contribute to the wood's high durability, and once extracted into wine, protect other constituents against oxidation as they readily absorb dissolved oxygen and facilitate the hydroperoxidation of wine constituents (Doussot *et al.,* 2000; Jordão *et al.,* 2007; Navarro *et al.,* 2016). Some research has shown *Q. robur* to release more ellagitannins than *Q. pyrenaica* and *Q. petraea (*Alañón *et al.,* 2011). Alternatively, analyses from Jordão *et al.,* (2007) show medium grain *Quercus pyrenaica* from the Gerês forest to contain more ellagitannins than medium gain *Q. petraea* from Alleir. Furthermore, the ellagitannin content contained within the barrels is influenced by the geographical origin of the wood, the species, the forest management from the woods' origin, and the number of times the barrel has been used (Alañón *et al.,* 2011; Navarro *et al.,* 2016). Further investigation of ellgaitannin content in oak is required.

6.2 Lactones

The whiskey lactones (*cis*- and *trans*-β-methyl-γ-octalactones) were shown to have a greater presence in *Q. petraea* than in *Q. robur*. Two isomers, *cis* (–) and *trans* (+), are present, and in a hydroalcoholic solution the *cis* isomer is four times more odorant than the *trans* isomer, which increases in concentration after toasting, as a result of lipid oxidation (Doussot *et al.,* 2000; Pérez-Prieto *et al.,* 2002).

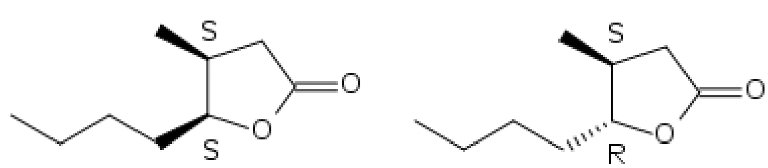

Figure 1. Structural formulas for the *cis* and *trans* isomers of beta-methyl-gamma-octalactone (Left and Right respectively). (Copied from wikipedia under the GNU Free Documentation License)

6.3 Guaiacol and 4-methylguaiacol

The smoky aromas are a result of lignin degradation and are attributed to the compounds guaiacol and 4-methylguaiacol, precursors to eugenol and vanillin. As a result these compounds are indicators for the relative toast of the barrels. Smoking the barrels with wood may also increase these compounds. (Australian Wine Research Institute, 2017; Chira & Teissedre, 2014; Pérez-Prieto *et al.,* 2002)

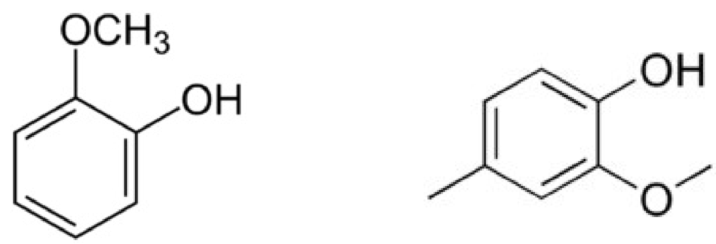

Figure 2. Structural formulas of guaiacol and 4-methylguaiacol (Left and Right respectively). (Copied from wikipedia under the GNU Free Documentation License)

6.4 Eugenol

Eugenol, a volatile phenol, is responsible for the clove aroma. This compound has been shown to be twice as present in French oak compared to American oak (Zhang *et al.,* 2015). Research by Chira & Teissedre (2014) showed *Q. petraea* to contain higher levels of eugenol compared to *Q. robur*. Furthermore, their research explained that toasting the barrel may lead to eugenol degradation.

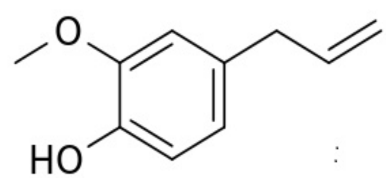

Figure 3. Structural formula of eugenol (Copied from wikipedia under the GNU Free Documentation License)

6.5 Vanillin

Vanillin is a phenolic aldehyde that has a synergistic relationship with the whiskey lactones. This compound can contribute to the perception of vanilla, coffee, dark chocolate, and smoke. Generally, vanillin found in wines, originate during the toasting process and are extracted in larger amounts with increased heating of the wood. (Australian Wine Research Institute, 2017; Chira & Teissedre, 2014; Pérez-Prieto *et al.,* 2002).

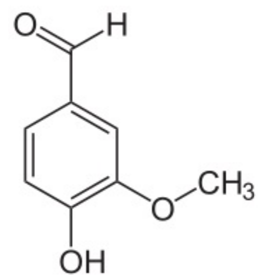

Figure 4. Structural formula of vanillin (Copied from wikipedia under the GNU Free Documentation License)

6.6 Furfural and 5-methylfurfural

Furfural and 5-methylfurfural are generated from the breakdown of cellulose and hemicellulose during toasting. These compounds were shown to be most present at medium toast, before decreasing at higher levels of toast. Their presence in wine are often greater than 1000µg/L, and are perceived as butterscotch and caramel aromas. (Jackson, 2014; Australian Wine Research Institute)

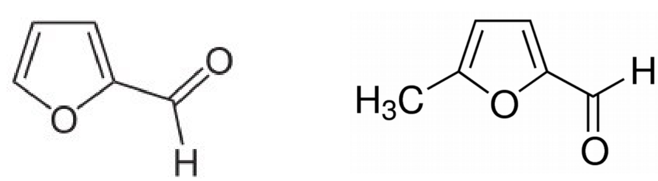

Figure 5. Structural formulas of furfural and 5-methylfurfural (Left and Right respectively). (Copied from wikipedia under the GNU Free Documentation License)

The goal of this work is to examine the effects of new *Quercus pyrenaica* and *Quercus robur* barrels at medium and high toast on Carcavelos fortified wine. *Quercus pyrenaica* has not been extensively researched, and few direct comparisons have been made with *Quercus robur.* In addition, the research available has been on dry wines, oak chips, staves, or seasoned wood. Furthermore, currently there is no published research on Carcevelos fortified wine.

Materials and Methods

7 Description of Materials

7.1 The Wine

The wine used in this study was a 2007 Vintage consisting of three grape varieties (Arinto, Galego Dourado, and Ratinho) which all ripen within one or two days of each other. There is no blend percentage, but Galego Dourado is the main grape variety inside. The wine was produced using classical technology following the D.O.C. rules for Carcavelos wines.The must was vinified in stainless steel vats, and fermentation was stopped with the addition of aguardente sourced from Torrejana, S. A. distillery at 77% ABV. The wine was barreled in 2009, and these analyses were preformed in 2017 at ISA (Instituto Superior de Agronomia, Universidade de Lisboa).

7. 2. Experimental Plan and Conditions

This master thesis originates from an ongoing experiment by the winemaker at Adega do Casal Manteiga. The winemakers plan was to place the same wine into two different species of barrels with two varying degrees of toast that each species shared. After fermentation, the same wine was placed into a total of twenty separate oak barrels. The wine was then aged in the Adega do Casal Manteiga cellar for eight years, where the temperature and humidity stayed within normal levels. The oak barrels were fabricated by the same cooperage, "J.M. Gonçalves" in Palaçoulo, in the northeast of Portugal. Ten barrels were made of Portuguese Nacional oak (*Q. pyrenaica*), and ten barrels from French Limousin oak (*Q. robur*). These barrels were toasted at two separate intensities, either medium toast (~10 to 15 minutes at >150°C) or high toast (~20 to 25 minutes at >200°C). A total of five repetitions were used for each toasting factor accounting for the total of twenty barrels.

8 Chemical Analysis of the Wine

8.1 Determination of pH

pH is a measurement of acids, such as tartaric acids, found in wine. These acids dissociate into hydrogen ions and anions. Due to the small these concentrations of hydrogen ions and anions are so small, a pH scale was created to reflect these figures. Usually for wines it ranges from 3.0 to 4.0. However, the overall scale for an aqueous state ranges from 1-14. The pH can determine grape maturity, affect fermentation rate, microbial activity, tartrate solubility, wine stability for conservation and storage, the interaction of phenolic compounds (impact on the color of wine), and the determination of molecular SO_2 which is the only form effective against microorganisms. This protection is not as strong at a high pH value (lower pH, higher free SO_2). Also, pH has an impact on the flavor and the aroma of wine (Darias *et al.,* 2003).

To measure pH in grape must and wine, a pH meter can be used. The pH meter measures the difference in electrical potential between a pH electrode and a reference electrode. The difference in potential between two electrodes immersed in the liquid under test is measured. The concentration of these free hydrogen ions and anions is measured with an electrode, that is connected to a potentiometer, which calculates the hydrogen ion activity in pH units. The higher the concentration of these free anions the lower the value on the pH scale. One of these two electrodes has a potential that is a function of the pH of the liquid, while the other has a fixed and known potential and constitutes the reference electrode. The selected pH values must encompass the range of values that may be encountered in musts and wines. The pH scale technically is a logarithmic scale that measures the concentration of free hydrogen ions floating in an inspected wine (Mirsky *et al.,* 1929). The pH of each replicate was measured.

8.2 Determination of Density

The density of wine consists of many components. The fermentable sugars account for 95% of the total soluble solids (also °B) which can be determined by the hydrometer. There is an indication of CO_2 presence, which is during fermentation. In this case, the wine has a higher density. It also determines the turbidity of the juice/wine (Zoecklein *et al.,* 1990).

Density (g/mL) is the mass per unit volume of wine or must at 20°C. The specific gravity is the ratio of the density of wine at T °C to the density of water at the same temperature and is denoted by: D^{20oc} (OIV, 2016). Density = (Weight of the substance) / (Volume of the substance) The concentration of dissolved substance in wine is related to specific gravity (Zoecklein *et al.,* 1990). Specific gravity = (Weight of x ml of substance) / (Weight of x ml of water). The wine was poured into a graduated cylinder, and the temperature was taken. The densiometer was then placed in the wine, and the density was recorded.

8.3 Quantification of Total Acidity

The acidity in wine comes from the acids of the grapes and a number of acids produced during and after alcoholic fermentation. The total acidity takes into account all types of inorganic and organic acids. The primary acids found include: acetic, propionic, pyruvic, lactic, succinic, glycolic and galacturonic. The acid and pH are not only related to the perception of sourness. Relative amount of dissociated and undissociated acids, buffer capacity, and the relative amount of the different acids also increase this perception. The potassium bitartrate and calcium tartrate solubility, ionization and formation of hydrolysis esters, polymerization rate and protein instabilities are affected by total acidity (Boulton, 1980). Total acidity has a minor effect on bitterness. The tannin perception decreases with the increase in tartaric acid concentration (Fontoin *et al.,* 2008).

An acid is a substance which dissociates in water to produce hydrogen ions (H^+). Total acidity is a result of the contribution of non-volatile or fixed acids like malic and tartaric along with other acids separated by steam volatilization (Zoechlein *et al.,* 1999). OIV defines total acidity as the sum of titratable acids up to pH 7.0, titrating against an addition of NaOH as a standard alkaline solution, using an indicator such as bromothymol blue. However CO_2 and SO_2 are not to be taken into account while measuring the total acidity (OIV, 2016).

Following the OIV type 1 method 50 mL of wine was placed in a vacuum flask. The vacuum was applied using a water pump while being shaken continuously. In a beaker 25 mL of boiled water, 1 mL of bromothymol blue solution and 5 mL of wine were combined. 0.1 mol/L of sodium hydroxide solution was added until the color changed to blue - green. Two sub-replicates were completed and averaged for each replicate.

8.4 Quantification of Volatile Acidity

Volatile acidity (VA) refers to the acids present in wine that are steam volatile. They are mainly composed of acetic acid with traces of other volatile organics acids. They are present in the wine in the free state and combined as salt. Acetic acid does not occur naturally in grapes, but is formed during the winemaking process as a secondary product. It comes from alcoholic and malolactic fermentation and bacterial diseases. It occurs either by microbial metabolism or chemical oxidation of alcohol. Therefore, VA is viewed as a measure of quality which winemakers should monitor levels throughout the winemaking process. Low VA is desired. High concentrations of acetic acid can cause undesirable sensory effects similar to vinegar. The OIV maximal acceptable limits for dry red wines are below or equal to 20 meq/L.

Volatile acids have a much lower evaporation temperature than water and alcohol. Following the OIV type 1 method the wine was placed in a vacuum flask and shaken to remove carbon dioxide. 20 mL of the wine that was freed of carbon dioxide was placed into a flask containing about 0.5 g of tartaric acid and boiled for an extended period to collect the totality of the volatile acid in a solution to be titrated with sodium hydroxide (NaOH). The distillate was then titrated with sodium hydroxide solution with phenolphthalein as the indicator. The monoprotic base neutralizes the volatile acids collected, and when solution returns to neutral, the indicator phenolphthalein will signal that all of the acids have been neutralized and an endpoint has been reached. The addition of starch and sulfuric acid after will account for any sulfur added to the wine. Four drops of diluted hydrochloric acid, 2 mL of starch solution, and a few crystals of potassium iodide were added. The free sulfur dioxide was then titrated with the iodine solution. Sodium tetraborate solution was then added until the pink coloration reappeared. The combined sulphur dioxide was then titrated with the iodine solution again. Two sub-replicates were completed and averaged for each replicate.

8.5 Quantification of Sulfur Dioxide

The objective of a sulfur dioxide test is to measure the amount of both free and total SO_2 present in a wine. This is important because SO_2 not only protects wine aromas and improves organoleptic taste, but also because there are legal limits (e.g.,150 mg/L for a dry red wine). Free sulfur dioxide is defined as the sulfur dioxide present in the must or wine in the following forms: sulfurous acid (H_2SO_4) and bisulfite ion (HSO_3^-), whose equilibrium is a function of pH and temperature. Total sulfur dioxide is defined as the total of all the various forms of sulfur dioxide present in the wine, either in the free or combined state with their constituents.

Free sulfur dioxide is determined by potentiometric iodometry through direct titration with iodide. 25 mL of wine was combined with 5 mL of sulfuric acid ⅓ (v/v) in a 50 mL beaker. A stirring rod was added and activated magnetically. The electrodes of the semi automatic “*Sulfilyser*” was submerged into the solution, and the iodide was slowly added. The combined sulfur dioxide is subsequently determined by iodometric titration after alkaline hydrolysis. When added to the free sulfur dioxide, it gives the total sulfur dioxide. Sulfur dioxide is important in enology for its antiseptic, antioxidant and antioxidasic characteristics.

8.6 Estimation of Reducing Substances

The Luff Schoorl method considers all the sugars exhibiting ketonic and aldehydic functions, and are determined by their reducing action on an alkaline solution of a copper salt. The remaining amounts of sugar can indicate completion of fermentation, microbial stability (over 2 g/L means there is a risk), the need for SO_2 or other microbial preventative additions, and categorical classification in the market. The gluconic composition is important regarding alcoholic fermentation, stabilization and conservation of wines. Residual sugars, fructose, and glucose can be fermented by lactic bacterias which can cause the increase of acetic acid and volatile acidity.

Following the OIV type IV method, the density was recorded first, and in the case of all the samples the same dilution of 1/25 was applied. The first dilution is 20 mL of wine in a 100 mL flask and then filled with water. 20 mL was then taken from the dilution and added to another 100 mL flask. The wine is clarified and treated with two reagents: Potassium Ferrocyanide (II) Trihydrate ($K_4Fe(CN)_6.3H_2O$) and Zinc Sulfate Heptahydrate ($ZnSO_4.7H_2O$). 5 mL of both reagents were added to the 100 mL flask before being filled with water. The flasks were then agitated and left to sit for 10 minutes. The solution was then filtered and in a 250 mL flask 15 mL of water, 25 mL alkaline solution and 10 mL of the filtrate are combined. The solution was then brought to a boil for 10 minutes. The solution is then cooled and 25 mL of sulfuric acid, 10 mL iodide and 2 mL of starch solution are added. This is then titrated with sodium thiosulfate solution. Copper concentration is determined by reducing the copper with excess iodine and estimating the remaining iodine with a standard thiosulfate solution. Starch is used as an indicator. When there is excess iodine present, the iodine binds to the starch giving it a blue/green/black color. When all the iodine has reacted, the blue/green/black color disappears and the solution takes on a cream appearance. The color change signals the end point of the titration, which can calculate an approximate value for total reducing compounds in the juice / wine. Two sub-replicates were completed and averaged for each replicate.

8.7 Estimation of Alcoholic Strength by Volume

Ethanol is the primary alcohol in wine, and is a result of the alcoholic fermentation. It is the yeast that metabolizes sugar into ethanol and carbon dioxide. Fermentation can be described as a series of redox reactions. Besides ethanol, a number of other monoalcohols and polyalcohols are present in wine. The sensory and physical properties of wine stability depend on the alcoholic strength (Zoecklein *et al.,* 1990). Additional alcohols are also important, like glycerol, methanol, and 3-5 carbon alcohols for sensory and regulatory attributes (Zoecklein *et al.,* 1990).

Knowledge of the percentage of alcohol volume also has a legal importance. Wine labels are required to state the alcohol concentration. The alcohol content is relevant for sensory characteristics (quality factor), antiseptic effect (conservation and preservation factor), and the market value (economic factor).

For measuring the alcohol percentage of the wine, its boiling point is measured and compared with the boiling point of pure water. Due to the alcohol in the wine, this point is decreased. 250 mL of wine was placed in a round bottom distillation flask, and the temperature was recorded. Some glass beads and 8-9 mL of calcium oxide was added. The wine is distilled with alkaline by a suspension of calcium hydroxide. Then the distillate is recovered and returned to the beginning temperature. Water is then added until 250 mL was reached. The solution is then transferred to a large graduated cylinder and measured with a hydrometer. Two sub-replicates were completed and averaged for each replicate.

8.8 Quantification of Total Dry Matter

The total dry matter or total dry extract is a measure of all the present matters (non-volatile) under specified physical condition and may be used as an indicator for fraud.. Total dry extract consists of all the organic substances including anthocyanins, proteins, phenolics, tannins and glycerol and all the mineral compounds, dissolved in wine that are not volatile under normal wine-related condition (Yanniotis *et al.,* 2007). Knowledge about content of dry extract in wine is greatly related to the body of a wine. A high dry extract value is believed to give the wine a fuller body and greater amount of flavor, and should ideally be in balance with the alcohol, acidity and sugar levels. The total dry extract is expressed in g/L. The OIV legal minimum of total dry extract for red wine is 20 g/L. Therefore, besides the important role in wine quality, dry matter has an impact on the viscosity of the wine. Considering this data, viscosity of wine affect pumping, filtration, clarification as well as other processes. Viscosity is also an important quality characteristic, which affects the mouth-feel and modifies other oral sensations, including saltiness, sweetness, bitterness, flavor and astringency (Yanniotis *et al.,* 2007). Total dry matter was calculated following the OIV type IV method. The value is calculated indirectly from the specific gravity of the alcohol free wine and is expressed in g/L (OIV, 2012).

8.9 Estimation of Total Phenols

The phenolic content is important to analyze, especially in red wines. It includes a large group of different chemical compounds which affect the taste, color and mouthfeel. This group can also be divided into two categories: flavonoids and non-flavonoids. Flavonoids characterize red wines more than other features. As such, they constitute more than 80% of their phenolic content (Jackson, 2014).

An estimation of the total phenolics was performed using the Somers and Evans (1977) method. The wine was centrifuged, and 1 mL of wine was diluted with water in a 50 mL vial. A spectrophotometer is used to measure the absorbency of the diluted wine at 280 nm. At 280 nm there is a high absorbency of compounds with a benzene ring, which is common to all phenolic compounds. Three sub-replicates were completed and averaged for each replicate.

8.10 Estimation of Flavonoids

Flavonoids are a group of phenolic compounds that are derived from the flavone structure and include anthocyanins, flavonols, and condensed tannins. Flavonoids are derived from the flavon structure.These compounds are attributed to the major antioxidant activity in wines, and are derived from the skins, seeds, and stems of the grapes. The color, astringency, bitterness, and structure of the wine are impacted by these compounds. Flavonoid content for each barrel was determined by subtracting the non-flavonoid content found from the total phenolic content that was quantified.

8.11 Estimation of Non-Flavonoids

Non-flavonoid compounds, though non colored themselves, are known to enhance and stabilize the color of red wines through intra and intermolecular reactions. These compounds are divided into hydroxybenzoic acids, and hydroxycinnamic acids, volatile phenols, stilbenes and miscellaneous compounds. They can also contribute to wine flavor via volatile phenolic acids and exhibit biological activities as is the case with resveratrol. (Moreno-Arribas & Polo, 2009).

To obtain an estimation of non-flavonoids the Kramling and Singleton (1969) method was used. 10 mL of wine is pipetted into a large test tube followed by 10 mL of 1:4 conc. HCL. 5 mL of standard formaldehyde solution containing 8 mg/ml was then added. The test tube was the sparged with nitrogen and capped. After 24 hours, the sub-replicates were measured. Three sub-replicates were completed and averaged for each replicate.

8.12 Estimation of Tanning Power

Tanning power is the measurement of the potential tannins or tanning capacity of specific tannins, such as proanthocyanidins having specific polymerization degrees, and their interaction with proteins thus influencing the astringent characteristics of the wine. Tanning power is measured using the methodology developed by De Freitas and Mateus (2001). First, a 1:50 dilution with a hydroalcoholic solution (12% v/v, ph 3.2 at 20° C) was made and measured using a turbidimeter (D0). Then, 8 mL of the dilution and 300 µL of albumin were combined in a tube, agitated, and placed into darkness for 30 minutes. A second reading is then taken using the turbidimeter (D1). The final value expressed as NTU/mL is calculated as ((D1-D0)/0.08) (Tavares *et al.,* 2017). Three sub-replicates were completed and averaged for each replicate.

8.13 Determination of Color Intensity

Almost 99.5% of the components in wine are transparent, and only 0.5% are responsible for the coloration of red and white wines. Anthocyanins are the main source of pigmentation in red wines. Copigmentation, the enhancement of visible color due to complexes between anthocyanins and colorless cofactors, also contributes to red wine color. The "chromatic characteristics" of a wine are its luminosity and chromaticity. Luminosity depends on transmittance and varies inversely with the intensity of color of the wine. This test combines three optical densities and shade to compare the relationship between the red and yellow wavelengths. Chromaticity depends on dominant wavelength (distinguishing the shade/hue) and purity. Conventionally, and for the sake of convenience, the chromatic characteristics of red and rosé wines are described by the intensity of color and shade, in keeping with the procedure adopted as the working method. Color is an important factor for consumers and thus winemakers use it as a selling point for their wines (OIV, 2009).

The chromatic characteristics were measured using a spectrophotometric method. Photons in the beam of light are absorbed by the molecules and thus the intensity is reduced. Because this was a fortified white wine, and not a red or rosé, only a measurement of absorbance at 420 nm was taken. Three sub-replicates were completed and averaged for each replicate.

8.14 Statistical Analysis

The software program R and the plugin Rcmdr (R commander) was used to determine the statistical significance of the results. PCA analysis was performed to see which analysis may have significance. Multi-way analysis of variance (multi-way ANOVA's) and the Tukey test ($p < 0.05$) were used to determine significant differences. PCA graphs, ANOVA tables, as well as Tukey test graphs and tables can be seen in annexes 18-26.

Results and Discussion

9 Influence of Botanical Species and Toasting

The wine in this study was analyzed for pH, titratable and volatile acidity, density, alcohol, sugars, SO_2 levels, total phenols, flavonoids, non-flavonoids, tanning power, and color intensity. The annexes contain additional graph comparisons of the results (annexes 1-17), ANOVA tables (annexes 20-26), in addition to Tukey test tables containing the adjusted p values alongside their respective graph visualizations of the differences (annexes 20-26).

9.1 General Physico-Chemical Analysis

The results obtained from the general analyses, including density, pH, total SO_2, total acidity, volatile acidity, alcohol, sugars, and dry extract, of the Carcavelos fortified wine aged for 8 years in new Portuguese oak (*Q. pyrenaica*) and new French oak (*Q. robur*) barrels at medium and high toast can be seen in Table 3.

The total sulfur dioxide content of the wine is low (Table 3). Generally, the total sulfur dioxide content in fortified wines are always low, and there are two main explanations. First the elevated alcoholic content protects the wine from high amounts of microbiological activity and do not have enzymes that need to be inhibited. Secondly in many fortified wines oxygenation is desired as it brings reduced astringency and improves color. This is not always the case though, with the exceptions of namely, the ruby style ports and 'Mostcatel de Setúbal' where the color and clarity are to be kept intact (Alañón *et al.,* 2011).

The volatile acidity, expressed as acetic acid in g/L in Table 3, is high for a white wine but normal for Carcavelos fortified wine. The legal limits for volatile acidity are dependant on the country and the style of wine. According to Fugelsang, in the United States, the legal limit is 1.4 g/L for table reds, 1.2 g/L for table whites, 1.7 g/L for dessert reds, and 1.5 g/L for dessert whites. These limitations change when looking at the state of California where 1.2 g/L is the legal limit for table reds and 1.1 g/L for table whites. Lastly, in the European Union it is 1.2 g/L for table reds and 1.08 g/L for table whites, with the concentrations also subject to variation depending on the country (Fugelsang, 1997; Neeley, 2004).

Table 3. General physico-chemical analysis of the 2007 vintage “Carcavelos” fortified wine barreled in 2009 and aged for 8 years in new French oak and new Portuguese oak barrels

Treatment	Density	pH	Total SO_2 (g/L)	Total Acidity (g/L)	Volatile Acidity (g/L)	Alcohol (%)	Reducing Substances (g/L)	Total Dry Extract (g/L)
NH1	1.0222	3.33	15	6.1 ± 0.1	1.04 ± 0.00	21.4 ± 0.0	72.0 ± 1.4	130.1
NH2	1.0222	3.35	15	6.2 ± 0.0	1.02 ± 0.02	21.1 ± 0.1	70.1 ± 8.0	129.0
NH3	1.0236	3.30	13	6.4 ± 0.1	1.13 ± 0.02	21.7 ± 0.5	71.8 ± 7.6	134.5
NH4	1.0246	3.30	10	6.6 ± 0.2	1.27 ± 0.00	21.1 ± 0.1	68.8 ± 6.1	135.3
NH5	1.0222	3.31	23	6.2 ± 0.3	1.09 ± 0.00	21.2 ± 0.1	65.1 ± 2.8	129.3
NM1	1.0226	3.44	5	6.4 ± 0.1	1.09 ± 0.00	21.1 ± 0.1	65.8 ± 1.8	130.3
NM2	1.0226	3.38	15	6.0 ± 0.0	1.04 ± 0.00	20.7 ± 0.1	66.4 ± 2.8	129.3
NM3	1.0226	3.39	10	6.1 ± 0.3	1.01 ± 0.04	21.2 ± 0.1	66.4 ± 2.8	130.6
NM4	1.0219	3.34	15	6.2 ± 0.2	1.05 ± 0.02	21.2 ± 0.1	59.9 ± 0.9	128.8
NM5	1.0229	3.32	10	6.3 ± 0.0	1.07 ± 0.02	21.3 ± 0.0	54.7 ± 5.5	131.6
LH1	1.0233	3.34	18	7.6 ± 0.1	1.00 ± 0.04	20.9 ± 0.0	68.4 ± 2.8	131.4
LH2	1.0233	3.36	13	6.5 ± 1.2	1.10 ± 0.02	21.5 ± 0.2	67.7 ± 1.8	132.4
LH3	1.0233	3.36	18	5.2 ± 1.0	1.03 ± 0.00	20.8 ± 0.2	67.1 ± 0.9	131.1
LH4	1.0231	3.34	23	6.2 ± 0.3	1.06 ± 0.00	19.7 ± 1.4	72.1 ± 5.2	127.7
LH5	1.0262	3.32	13	6.9 ± 0.0	1.39 ± 0.00	21.1 ± 0.0	62.2 ± 1.4	139.4
LM1	1.0236	3.28	18	6.2 ± 0.1	1.05 ± 0.02	21.7 ± 0.3	64.8 ± 0.5	134.5
LM2	1.0236	3.21	18	6.2 ± 0.1	1.01 ± 0.00	20.5 ± 0.2	64.5 ± 1.8	131.4
LM3	1.0226	3.21	30	6.2 ± 0.0	1.11 ± 0.02	20.7 ± 0.4	62.5 ± 1.8	129.3
LM4	1.0236	3.25	23	6.2 ± 0.1	1.11 ± 0.06	20.8 ± 0.2	62.8 ± 2.3	132.1
LM5	1.0236	3.28	13	6.6 ± 0.0	1.21 ± 0.04	20.8 ± 0.3	60.6 ± 0.9	132.1

Nacional = *Q. pyrenaica*, Limousin = *Q. robur;* NH = Nacional high toast, LH = Limousin high toast, NM = Nacional medium toast, LM = Limousin medium toast; 1-5 = repetition; Results are mean ± SD calculated; Density is calculated in g/cm3; Total acidity is presented in g/L of tartaric acid; Volatile acidity is presented as g/L of acetic acid; Alcohol strength by volume at 20°C.

Normally the analyses in Table 3 are performed as an "identification card" for the wine. As a curiosity, multi-way ANOVA's as well as subsequent Tukey tests were performed on the different analyses. ANOVA's revealed statistically significant differences between the density and pH, when comparing barrels fabricated from *Q. pyrenaica* and *Q. robur.* The Tukey test accepted the null hypothesis for the analysis of density, and only revealed significant differences in pH for *Q. robur* barrels with medium levels of toasting. When comparing pH, there is a maximum difference of 0.23 between *Q. pyrenaica*s' high of 3.44 and *Q. robur*s' low of 3.21. These differences between pH are not that large, and a comparison can be seen in Figure 6. The difference of the averages is even less at only 0.128. It may be possible that more acidic substances are being extracted from the wood increasing the acidity and bringing the pH down. Alternatively, salt substances would have an opposite effect, and would increase the pH.

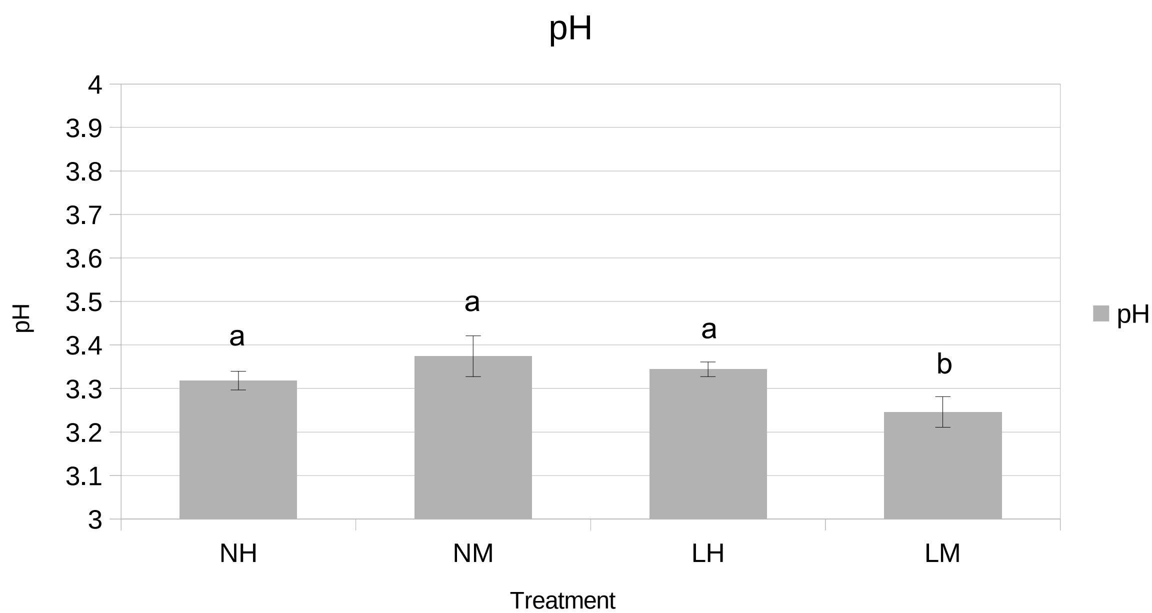

Figure 6. Difference in pH of "Carcavelos" fortified wine aged for 8 years in new medium and high toasted *Q. pyrenaica* and *Q. robur* barrels

Nacional = *Q. pyrenaica*, Limousin = *Q. robur;* NH = Nacional high toast, LH = Limousin high toast, NM = Nacional medium toast, LM = Limousin medium toast; Results shown are mean with SD; Values with the same letters are not statistically different (Tukey test, $p < 0.05$); Tukey test and p values for pH can bee seen in Annex 21

In addition to the difference in pH between the oak species at medium toast, the analysis showed that the toast had a significant effect on the total amount of reducing substances of the wine (Table 3, and Figure 8). Figure 8 shows *Q. pyrenaica* and *Q. robur* to contain more reducing sugars at a high degree of toasting compared to medium toasting. One possible explanation for this is a result of the analytical methodology used, reducing substances. This OIV type IV method is a measurement of the reducing substances within a wine, not a measurement of glucose and fructose. Reducing substances comprise of all the sugars exhibiting ketonic and aldehydic functions and are determined by their reducing action on an alkaline solution of a copper salt (OIV, 2009). Thus ketonic and aldehydic compounds other than sugar, which are present in the wine, can affect this measurement as they could be competing to reduce. Therefore, if an aldehyde or ketone group molecule is more readily available in the wine, there is the possibility that those molecules are donating their electrons and being oxidized resulting in an overall lower measurement of reducing sugars. According to wine science, "prolonged exposure (~25 min, inner surface temperatures > 200°C) chars the innermost layers of the staves, and destroys or limits the synthesis of phenolic and furanilic aldehydes." (Jackson, 2014). Therefore, at higher levels of toasting, fewer furanilic aldehydes will be available to compete with the reducing sugars, and the overall values of measurement can then be expected to be higher. Some amounts of sugar may accumulate from the degradation of hemicellulose hydrolysis, but according to wine science, "it is insufficient to affect taste perception", thus we can conclude that these significant changes cannot be a result of this reaction (Jackson, 2014). Lastly, there is no repeatability given from the OIV using this methodology for sugars.

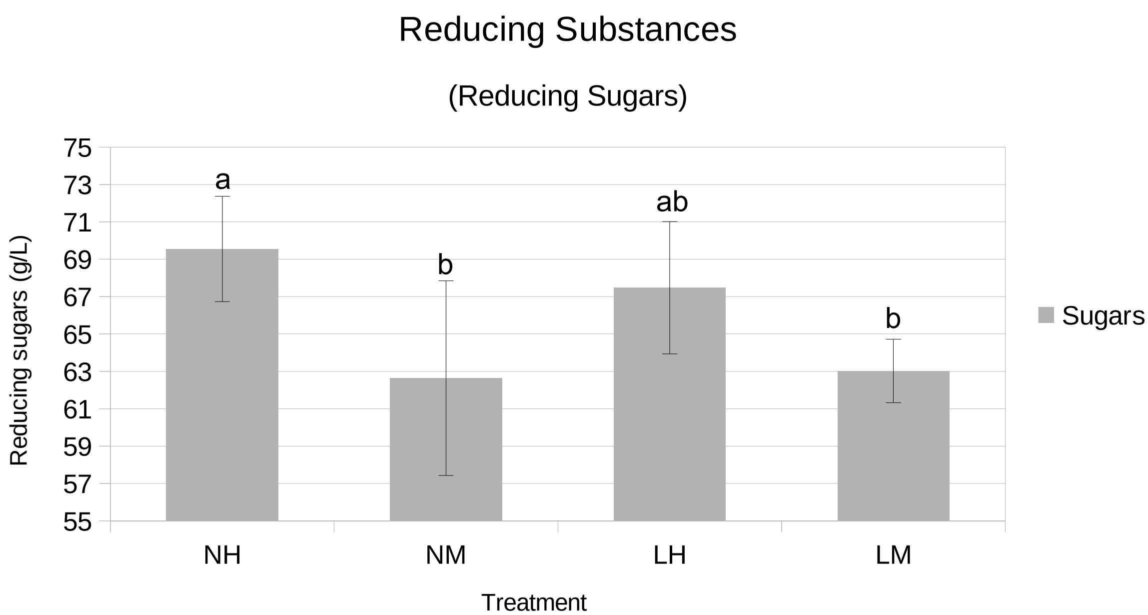

Figure 8. Difference in reducing substances of "Carcavelos" fortified wine aged for 8 years in new medium and high toasted *Q. pyrenaica* and *Q. robur* barrels

Nacional = *Q. pyrenaica*, Limousin = *Q. robur;* NH = Nacional high toast, LH = Limousin high toast, NM = Nacional medium toast, LM = Limousin medium toast; Results shown are mean with SD; Values with the same letters are not statistically different (Tukey test, $p < 0.05$); Tukey test and p values for reducing substances can bee seen in Annex 22

9.2 Extractable Phenolics and Color

The results obtained for total phenols, flavonoid compounds and non-flavonoid compounds of Carcavelos fortified wine aged in new Portuguese oak and new French oak barrels at medium and high toast can be seen in Figure 9. Analysis of variance revealed significant differences in the extraction of phenolics between *Q. pyrenaica* and *Q. robur.* Based on the data in Tables 4 and 5, and shown in Figure 9, *Q. pyrenaica* at both medium and high toast imparted a greater amount of extractable phenolics in the wine than with *Q. robur.*

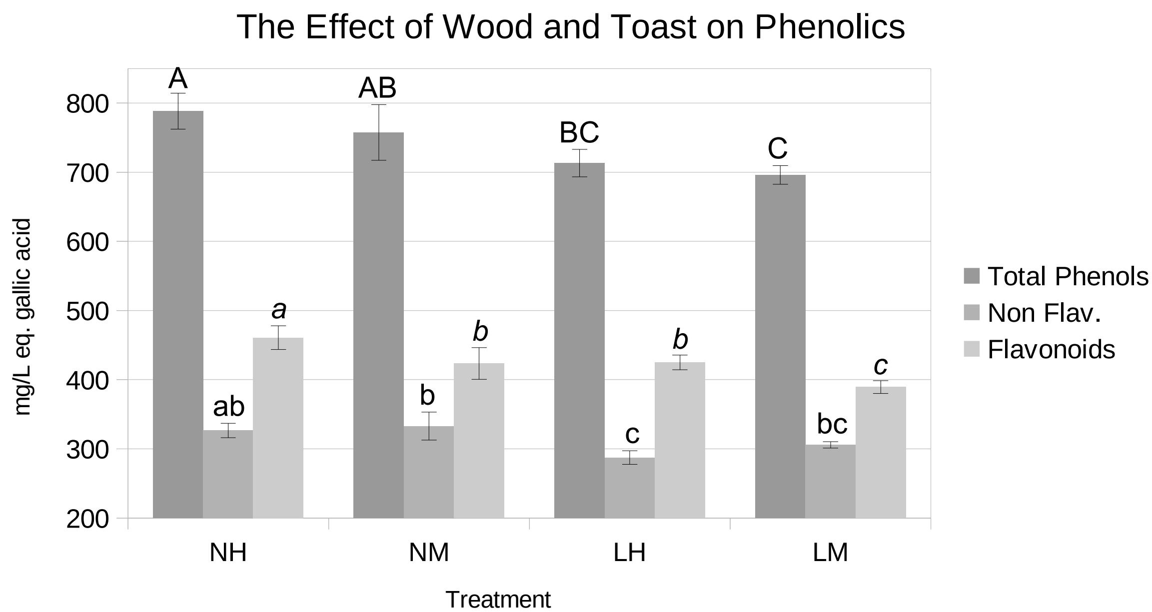

Figure 9. Differences in the phenolic content of "Carcavelos" fortified wine aged for 8 years in new medium and high toasted *Q. pyrenaica* and *Q. robur* barrels

Nacional = *Q. pyrenaica*, Limousin = *Q. robur;* NH = Nacional high toast, LH = Limousin high toast, NM = Nacional medium toast, LM = Limousin medium toast; Results shown are mean with SD expressed as mg/L eq. gallic acid; Values with the same letters are not statistically different (Tukey test, $p < 0.05$); Capital letters for total phenols, lowercase letters for non-flavonoids, italic letters for flavonoids; Tukey test and p values for extractable phenolics can bee seen in Annexes 23-25.

The results of this study are in agreement with established literature where extracted polyphenolics are dependant on the pool present in the wood species and the toast (Cadahía *et al.,* 2001). Numerous studies have shown that the extraction of phenolics from the wood into the wine depends on many factors such as species and geographical origin, as well as cooperaging practices like seasoning and toasting methods (Canas *et al.,* 2008; Chira & Teissedre, 2014; Fernández De Simón *et al.,* 2003; Jordão *et al.,* 2005; Jordão & Laureano, 2005; Zhang *et al.,* 2015).

Previous research has shown significant differences in extractives between *Q. robur* and Q. *petraea*. Moreover, the differences in that research have shown, between the two french oaks used in cooperaging, pedunculate oak (*Q. robur*) to contain higher levels of ellagitannins and free ellagic acid but lower amounts of volatile compounds in comparison to sessile oak (*Q. petraea*) (Doussot *et al.,* 2000; Doussot *et al.,* 2002; Jackson, 2014). Therefore, when comparing Portuguese *Q. pyrenaica* with the French *Q. robur,* it is a comparison to the species of French wood that contains more phenolic compounds and is the less aromatic of the two French oaks (*Q. robur* and *Q. petreaea*).

Quercus pyrenaicas' effects on wine evolution has not been extensively researched in comparison to species such as *Q. petraea*, *Q. robur*, and *Q. alba*. Some research in Spain has shown that, in wood samples after natural seasoning, and also samples that underwent natural seasoning and medium toasting, *Q. robur* contained higher total phenolic and ellagitannin content when compared to *Q. pyrenaica* (Cadahía *et al.,* 2001). A study by Alañón *et al.,* (2001) on the antioxidant capacity and phenolic composition of different woods used in cooperage showed twice as many total phenolics present in wood samples of Spanish *Q. robur* than in *Q. Pyrenaica*. Additionally, research has shown that at medium toasting Spanish *Q. pyrenaica* imparted less volatile compounds and whiskey lactones into wine when in comparison to *Q. robur* (Fernández De Simón *et al.,* 2003).

Research on Portuguese *Q. pyrenaica* has shown that in dry wood French *Q. petraea* contained more *cis* isomers and less *trans* isomers of β-methyl-γ-octalactone. Heating modifies the molecular structure of the wood, and at medium toasting of wood chips, there were two or three times more volatile compounds than in French *Q. petrae*a depending on the type of grain (Jordão *et al.,* 2005). Further research was done on the effect of medium and high toasting on ellagitannin content of *Q. pyrenaica* and *Q. petraea*. Ellagitannins were shown to significantly decrease depending on the degree of toast, with a higher toasting having a stronger effect. Ellagic acid was then shown to increase significantly with this change. When comparing the ellagic acid of *Q. petraea* with *Q. pyrenaica* there was nearly triple the amount extracted from the *Q. pyrenaica* chips than from the *Q. petraea* chips (Doussot *et al.,* 2002; Jordão *et al.,* 2007).

These results from this study are in agreement with the high extraction of phenolics found from *Q. pyrenaica* in the Portuguese studies. However when comparing the total phenolics and volatile compounds of medium toasting between the two species, the results of this study are not in agreement with the Spanish research. This could potentially be a result of the impact of geographical origin, individual tree variation, coarseness of the grain, or seasoning method (Jackson, 2014; Navarro *et al.,* 2016).

Table 4. The influence of wood and toast on phenolic extraction in “Carcavelos” fortified wine aged for 8 years in new French and Portuguese oak barrels at medium and high toast (Phenolic Index)

Treatment	Total Phenols	Non Flav.	Flavonoids
NH1	31.5 ± 0.2	13.1 ± 0.1	18.4
NH2	29.5 ± 0.6	12.1 ± 0.2	17.4
NH3	28.9 ± 0.1	12.2 ± 0.1	16.7
NH4	29.8 ± 0.4	12.5 ± 0.1	17.2
NH5	30.3 ± 0.3	12.4 ± 0.2	17.9
LH1	26.9 ± 0.1	10.7 ± 0.5	16.2
LH2	26.8 ± 0.2	10.9 ± 0.3	15.8
LH3	27.1 ± 0.5	11.0 ± 0.1	16.1
LH4	26.5 ± 0.1	10.6 ± 0.1	15.9
LH5	28.4 ± 0.3	11.6 ± 0.0	16.8
NM1	30.8 ± 0.5	13.4 ± 0.3	17.4
NM2	29.6 ± 0.3	13.5 ± 0.3	16.1
NM3	26.8 ± 0.4	11.9 ± 0.1	14.9
NM4	28.0 ± 0.4	12.0 ± 0.5	16.0
NM5	28.9 ± 0.2	12.6 ± 0.2	16.2
LM1	26.3 ± 0.3	11.6 ± 0.4	14.6
LM2	25.7 ± 0.3	11.4 ± 0.1	14.3
LM3	26.7 ± 0.3	11.7 ± 0.1	15.0
LM4	27.0 ± 0.4	11.9 ± 0.2	15.2
LM5	26.7 ± 0.4	11.6 ± 0.1	15.1

Nacional = *Q. pyrenaica*, Limousin = *Q. robur;* NH = Nacional high toast, LH = Limousin high toast, NM = nacional medium toast, LM = Limousin medium toast, 1-5 = repetition. Results are mean ± SD calculated. Presented as on the phenolic index

Table 5. The influence of wood and toast on phenolic extraction in “Carcavelos” fortified wine aged for 8 years in new French and Portuguese oak barrels at medium and high toast (mg/L gallic acid equivalents)

Treatment	**Total Phenols**	**Non Flav.**	**Flavonoids**
NH1	828.0 ± 3.0	343.3 ± 2.3	483.8
NH2	775.0 ± 15.0	316.5 ± 3.6	457.6
NH3	759.6 ± 1.4	319.4 ± 2.1	439.3
NH4	782.0 ± 8.4	328.4 ± 2.2	452.7
NH5	796.5 ± 7.7	325.2 ± 4.5	470.3
LH1	707.4 ± 12.4	281.5 ± 11.8	425.0
LH2	703.0 ± 5.7	286.1 ± 7.9	416.0
LH3	712.3 ± 10.4	287.5 ± 1.8	423.8
LH4	696.0 ± 8.3	277.9 ± 1.5	417.3
LH5	746.9 ± 5.3	303.6 ± 0.3	442.4
NM1	809.6 ± 2.9	352.4 ± 6.7	456.3
NM2	778.5 ± 4.4	354.4 ± 7.3	423.2
NM3	703.9 ± 12.4	311.4 ± 0.7	391.6
NM4	735.9 ± 0.6	314.5 ± 12.6	420.5
NM5	758.8 ± 7.9	331.5 ± 4.4	426.3
LM1	690.3 ± 7.5	305.4 ± 8.7	384.0
LM2	675.4 ± 5.7	298.9 ± 2.8	375.6
LM3	702.6 ± 7.9	307.9 ± 0.4	393.8
LM4	710.1 ± 8.8	311.4 ± 3.3	397.8
LM5	701.7 ± 9.0	305.2 ± 0.8	395.6

Nacional = *Q. pyrenaica*, Limousin = *Q. robur;* NH = Nacional high toast, LH = Limousin high toast, NM = nacional medium toast, LM = Limousin medium toast, 1-5 = repetition. Results are mean ± SD calculated. Expressed as mg/L eq. gallic acid

9.2.1 Total Phenols

The species of new oak barrels used had a significant effect on the total phenolic content in the wine. *Q. Pyrenaica* at both medium and high toasting showed to impart significantly more total phenolics to the wine compared to *Q. robur. Q. pyrenaica* and *Q. robur* at high toast showed a mean difference of 75.1 mg/L eq. gallic acid having 788.2 and 713.1 mg/L eq. gallic acid, respectively. At medium toasting, there was a difference of 61.3 mg/L eq. gallic acid where *Q. pyrenaica* and *Q. robur* had 757.3 and 696.0 mg/L eq. gallic acid, respectively. This effect is more significant at high toast compared to at medium toast (Figure 10).

The degree of toast had no statistically significant effect on total phenolics within the same species of wood. The mean change between *Q. pyrenaica* at medium and high toasting was 30.9 mg/L eq. gallic acid with high toast having 788.2 mg/L eq. gallic acid and medium toast having 757.3 mg/L eq. gallic acid. The mean change between *Q. robur* was 17.1 mg/L eq. gallic acid with high toast having 713.1 mg/L eq. gallic acid and medium toast having 696.0 mg/L eq. gallic acid.

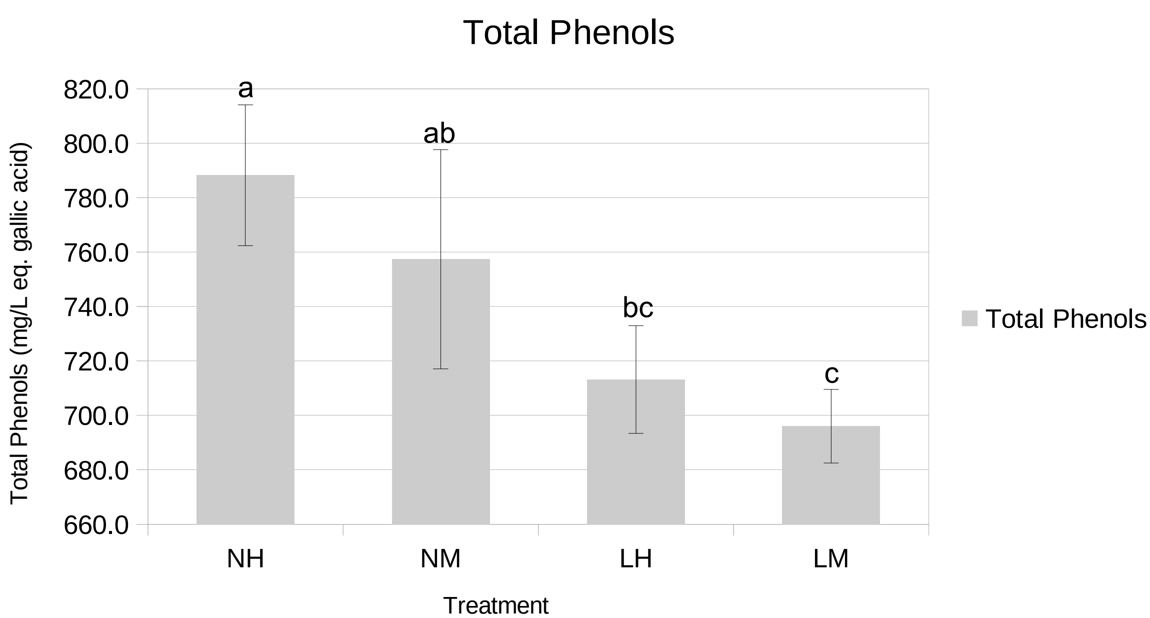

Figure 10. Total phenolic compounds in “Carcavelos” fortified wine aged for 8 years in new oak barrels at medium and high toast

Nacional = *Q. pyrenaica*, Limousin = *Q. robur;* NH = Nacional high toast, LH = Limousin high toast, NM = Nacional medium toast, LM = Limousin medium toast; Results shown are mean with SD expressed as mg/L eq. gallic acid; Values with the same letters are not statistically different (Tukey test, $p < 0.05$); Tukey test and p values for total phenols can be seen in Annex 23

Higher levels of toast did have more total phenolics overall (Figure 10). This can be rationalized by the fact that the total phenolic content imparted on the wine is dependent on the total phenolic content contained within the wood used in cooperage after seasoning the staves. Established research has shown that species of oak used in cooperaging has a significant effect on the total phenolics extracted into the wine (Jackson, 2014; Navarro *et al.,* 2016; Pérez-Prieto *et al.,* 2002).

9.2.2 Non-Flavonoids

The most significant factor for non-flavonoid content in the wine was the species of wood. Figure 11, and Tables 4 and 5 show Portuguese *Q. pyrenaica* to have more non-flavonoid constituents than *Q. robur* at both medium and high toast. The mean difference between Portuguese *Q. pyrenaica* and French *Q. robur* at high toast was 39.2 mg/L eq. gallic acid with values of 326.6 mg/L eq. gallic acid for *Q. pyrenaica* and 287.3 mg/L eq. gallic acid for *Q. robur*. When comparing both species at medium toast there was a difference of 27.1 mg/L eq. gallic acid with a value of 332.9 mg/L eq. gallic acid from *Q. pyrenaica* and 305.8 mg/L eq. gallic acid from *Q. robur*.

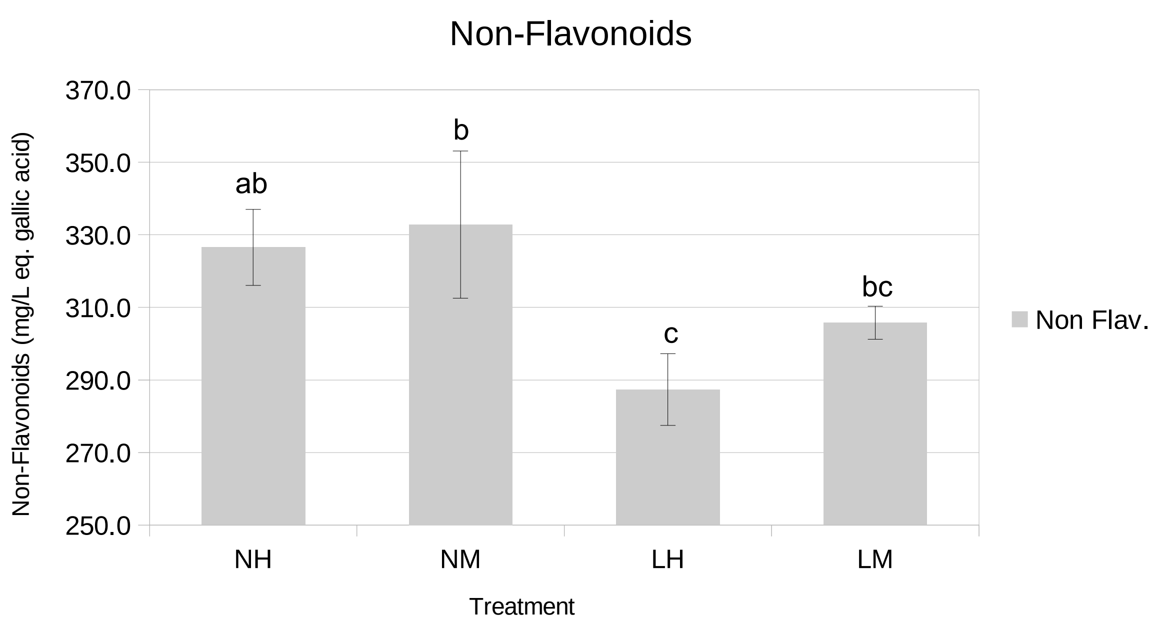

Figure 11. Non-flavonoid content in “Carcavelos” fortified wine aged for 8 years in new Portuguese and French oak barrels at medium and high toast

Nacional = *Q. pyrenaica*, Limousin = *Q. robur;* NH = Nacional high toast, LH = Limousin high toast, NM = Nacional medium toast, LM = Limousin medium toast; Results shown are mean with SD expressed as mg/L eq. gallic acid;Values with the same letters are not statistically different (Tukey test, $p < 0.05$); Tukey test and p values for non-flavonoids can bee seen in Annex 24

Toasting level was only shown to be slightly significant in the ANOVA. When considering the overall effects the values were higher in the medium toast barrels compared to the high toast barrels (Figure 11). A small difference in the wine, from toasting, did appear in the ANOVA (Annex 24). There was a decrease of 18.4 mg/L eq. gallic acid from medium to high toast with mean values of 305.8 and 287.3 mg/L eq. gallic acid respectively, for the wine aged in *Q. robur.* There was also a change in non-flavonoids for the *Q. pyrenaica* barrels which showed a decrease of 6.3 mg/L eq. gallic acid when moving from medium (332.9 mg/L eq. gallic acid) to high (326.6 mg/L eq. gallic acid) toast. The Tukey test later revealed adjusted p values greater than 0.05 for these changes.

Non-flavonoid phenolic compounds include phenolic acids such as vanillin from lignin, caffeic, hydroxycinnamic, benzoic, stillbens, and ellagitannins. Some of the most important non-flavonoids include phenolic acid and ellagitannins which may represent up to 10% of heartwood that is subsequently used in cooperaging (Jordão *et al.,* 2007). Research on ellagitannins have shown these compounds to be extremely hydrolyzable and, at high toasting, decomposition of almost all may occur (Doussot *et al.,* 2002; Jordão *et al.,* 2007). Therefore, the ellagitannin content within the oak is related to the species and to the degree of toasting the staves have been subjected to.

These results are in agreement with previous research. As Tavares *et al.,* (2017) showed that for non-flavonoids, Portuguese oak had the highest values, but was in direct comparison with *Q. petraea* and not *Q. Robur*. The changes showed in this data may be explained as a result of easily hydrolyzed compounds such as ellagitannins within the barrels being decomposed, or other factors such as the role of oxygen, and polymerization reactions in tandem with hydrolysis and precipitation of phenolic compounds over a long extraction (Jordão & Laureano, 2005).

9.2.3 Flavonoids

Tables 4 and 5 show the total flavonoids imparted into the wine. The species of oak and the degree of toasting resulted in significant differences in the wine and can be seen in Figure 12. Overall *Q. pyrenaica* contained more flavonoids than *Q. robur* at both medium and high toast. At high toast there was a mean difference of 35.9 mg/L eq. gallic acid of flavonoids, which showed values of 460.7 and 424.9 mg/L eq. gallic acid for *Q. pyrenaica* and *Q. robur* respectively. A difference of 34.2 mg/L eq. gallic acid with values of 423.6 and 389.4 mg/L eq. gallic acid for *Q. pyrenaica* and *Q. robur,* respectively, was shown at medium toasting levels. *Q. pyrenaica* at medium toast was shown to have comparable levels of flavonoids with *Q. robur* at high toast, with mean values of 423.6 mg/L eq. gallic for *Q. pyrenaica* and 424.9 mg/L eq. gallic acid for *Q. robur.*

Higher toasting treatments resulted in overall more flavonoids within the wine for both *Q. pyrenaica* and *Q. robur*. Portuguese *Q. pyrenaica* oak had a 37.1 mg/L eq. gallic acid increase when changing from medium (423.6 mg/L eq. gallic acid) to high (460.7 mg/L eq. gallic acid) toast. The French *Q. robur* barrels showed a 35.5 mg/L eq. gallic acid increase when changing from medium (389.4 mg/L eq. gallic acid) to high (424.9 mg/L eq. gallic acid) toast. Although these changes are comparable the statistical significance was shown to be greater for the Portuguese *Q. pyrenaica* barrels which had a much lower p value ($p<0.05$) of 0.0096430 compared to the French *Q. robur* barrels with a p value of 0.0134015. This is a result regardless of the lower standard deviation in the French oak compared to the Portuguese oak and can be seen in Figure 12.

These results are in agreement with previous studies that explain changes in non-flavonoids as directly related to the hydrolyzation of ellagitannins and the availability of ellagitannins present after cooperaging. Furthermore that thermal degradation of ellagitannins results in a release of ellagic and gallic acids (Cadahía *et al.,* 2001; Doussot *et al.,* 2002).

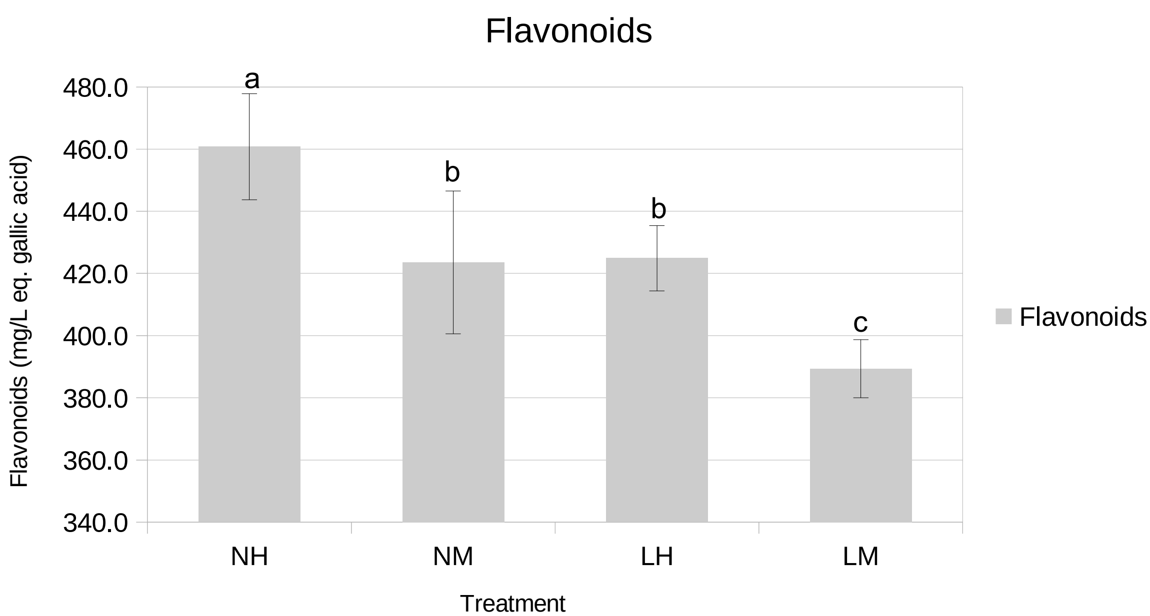

Figure 12. Flavonoid content in “Carcavelos” fortified wine aged for 8 years in new Portuguese and French oak barrels at medium and high toast

Nacional = *Q. pyrenaica*, Limousin = *Q. robur;* NH = Nacional high toast, LH = Limousin high toast, NM = Nacional medium toast, LM = Limousin medium toast; Results shown are mean with SD expressed as mg/L eq. gallic acid; Values with the same letters are not statistically different (Tukey test, $p <0.05$); Tukey test and p values for flavonoids can bee seen in Annex 25

9.2.4 Color Intensity and Tanning Power

The species of oak and the toasting method showed to have no significant effect on the color intensity of the wine. This may be due to not having a geographically large distance between the origins of *Q. pyrenaica* and *Q. robur*. Furthermore, because both species are from the same continent, the difference may not necessarily be as great as when you compare them with a species originating from different continents such as *Q. alba*.

Overall it is clear from Figure 13 and Table 6, the tanning power of the wine is low. This is to be expected as almost all of the tannins can be attributed to the oak. The species of oak showed no significant impact on the tanning capacity of the wine. The wine aged in medium toasted *Q. robur* showed higher tanning power with a difference of 5.12 NTU/mL between *Q. pyrenaica* (25.58 NTU/mL) and *Q. robur* (30.7 NTU/mL). The wine aged in *Q. pyrenaica* high toast barrels was revealed to have a higher tanning power than *Q. robur* with a difference of 4 NTU/mL between *Q. pyrenaica* (11.92 NTU/mL) and *Q. robur* (7.92 NTU/mL).

The toasting method had a significant impact on the tanning power of the wine. Between both *Q. pyrenaica* and *Q. robur* higher toasting levels showed significantly less tanning power when compared to the medium toasted barrels. Moreover *Q. robur* showed a more significant decline of tanning power when in comparison with *Q. pyrenaica. Q. pyrenaica* showed a decrease of 13.66 NTU/mL moving from medium (25.58 NTU/mL) to high (11.92 NTU/mL) toasting. *Q. robur* decreased 22.78 NTU/mL when the toast increased from medium (30.7 NTU/mL) to high (7.92 NTU/mL). The decline as a result of toasting for *Q.pyrenaica*, was shown to be statistically insignificant.

As ellagitannins are easily hydrolyzed, the tanning power should have an inverse relationship with the intensity of the toast. It is clear from Figure 13 and Table 6 that these results are in agreement with the previous assertion. Ellagitannins are also highly reactive with proteins which could have affected the results of this analysis, as this method uses albumin as a reagent in the quantification procedure.

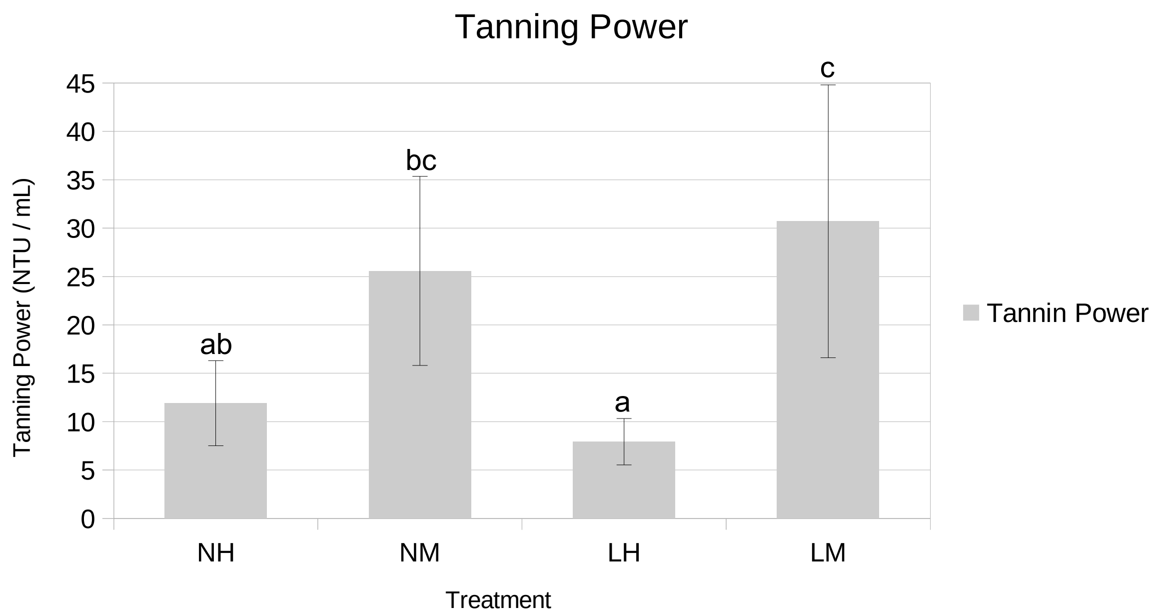

Figure 13. Tanning power of “Carcavelos” fortified wine aged for 8 years in new Portuguese and French oak barrels at medium and high toast

Nacional = *Q. pyrenaica*, Limousin = *Q. robur;* NH = Nacional high toast, LH = Limousin high toast, NM = Nacional medium toast, LM = Limousin medium toast; Results shown are mean with SD expressed at NTU/mL; Values with the same letters are not statistically different (Tukey test, $p < 0.05$); Tukey test and p values for tanning power can be seen in Annex 26

Table 6. The influence of wood and toast on color and tanning power in “Carcavelos” fortified wine aged for 8 years in new French and Portuguese oak barrels at medium and high toast

Treatment	Color Intensity (abs)	Tanning Power (NTU/mL)
NH1	0.988 ± 0.008	14.29 ± 6.45
NH2	0.987 ± 0.008	8.58 ± 04.70
NH3	1.124 ± 0.016	18.33 ± 2.88
NH4	1.211 ± 0.014	10.83 ± 2.11
NH5	1.090 ± 0.001	7.63 ± 2.71
NM1	0.958 ± 0.014	12.04 ± 5.19
NM2	0.939 ± 0.008	20.29 ± 4.18
NM3	1.072 ± 0.002	37.71 ± 18.56
NM4	1.068 ± 0.014	28.38 ± 2.83
NM5	1.071 ± 0.003	29.50 ± 17.75
LH1	1.015 ± 0.025	6.17 ± 2.56
LH2	1.070 ± 0.009	6.58 ± 0.88
LH3	0.991 ± 0.004	7.21 ± 5.67
LH4	1.066 ± 0.008	7.54 ± 3.12
LH5	1.238 ± 0.014	12.08 ± 1.82
LM1	1.102 ± 0.011	22.38 ± 7.40
LM2	1.025 ± 0.003	10.29 ± 4.94
LM3	1.018 ± 0.004	36.50 ± 5.56
LM4	1.130 ± 0.014	39.54 ± 4.88
LM5	1.141 ± 0.006	44.75 ± 7.41

NH = Nacional high toast, LH = Limousin high toast, NM = nacional medium toast, LM = Limousin medium toast, 1-5 = repetition. Results are mean ± SD calculated; Color intensity at 420nm; p - value (<0.05) calculated using the Tukey test.

Conclusion

The goal of this work was to examine the effects of new *Quercus pyrenaica* and *Quercus robur* barrels at medium and high toast on Carcavelos fortified wine. *Quercus pyrenaica* has not been extensively researched, and few direct comparisons have been made with *Quercus robur.* In addition*,* the research available has been on dry wines, oak chips, staves, or seasoned wood. Furthermore, currently there is no published research on Carcevelos fortified wine.

Significant differences between *Q. pyrenaica* and *Q. robur* were found in the wine for total phenols, flavonoids, and non-flavonoid compounds. When comparing both species, *Q. pyrenaica* was shown to have more total phenols, flavonoids, and non-flavonoids than *Q. robur* at both medium and high toast. The total phenols of the wine aged in *Q. pyrenaica* barrels were significantly higher than in the *Q. robur* counterparts with differences of 61.3 mg/L eq. gallic acid and 75.1 mg/L eq. gallic acid for medium and high toast, respectively. *Q. pyrenaica* contained more flavonoids than *Q. robur* with a difference of 35.9 mg/L eq. gallic acid at high toast and 34.2 mg/L eq. gallic acid at medium toast. Regarding non-flavonoid compounds *Q. pyrenaica* showed 39.2 mg/L gallic acid equivalents more than *Q. robur* at high toast and 27.1 mg/L gallic acid equivalents more at medium toast. Furthermore, in the barrels that underwent high toasting, the wood has a significant impact. At medium toast the woods effect is not significiant. The species of wood appeared to have not affected the tannin power or color intensity of the wines.

The degree of toasting showed significant changes in the tanning power and flavonoids content of the wine for both *Q. pyrenaica* and *Q. robur.* The toasting method was shown to have no significant effect on the total phenolic content of the wine. Flavonoid content increased for both *Q. pyrenaica* (Δ37.2 mg/L eq. gallic acid) and *Q. robur* (Δ35.5 mg/L eq. gallic acid) in the wines that were aged in barrels that underwent higher toasting compared to medium toasting. The tannin power decreased for both *Q. pyrenaica* (Δ13.66 NTU/mL) and *Q. robur* (Δ22.78 NTU/mL) when the toasting increased.

Analyses showed no significant effects on the wines density, total acidity, volatile acidity, alcoholic strength, total dry material, and color intensity from the species of wood or the toasting technique. These results are in agreement with other research that shows the total phenolic content as being related to the type of wood species used in cooperaging. This reinforces previous studies that show the total phenolic content extracted into the wine is dependent on the total phenolic content available within the wood after cooperaging. The degree of toasting had significant effects on flavonoid and non-flavonoid content of the wine, as well as the tanning power and reducing sugars content. The changes in phenolic content, seen as a reduction in non-flavonoids with the increase of toasting, and the subsequent increase in flavonoids, which include constituents such as ellagic and gallic acid, may be explained as thermally aided hydrolysis of ellagitannins into ellagic and then gallic acid as it degrades. Moreover, as these tannins are decomposed, the tanning power is reduced.

The “Carcavelos” fortified wine made by the Adega do Casal Manteiga is typically aged for 10 years before bottling. Once this wine has finished aging, another analysis can be made using the wine from these barrels. At that time, an HPLC instrument could be used to examine the individual phenolic constituents more closely to show a more definite comparison. Furthermore a sensorial evaluation should take place as the wine will be completed.

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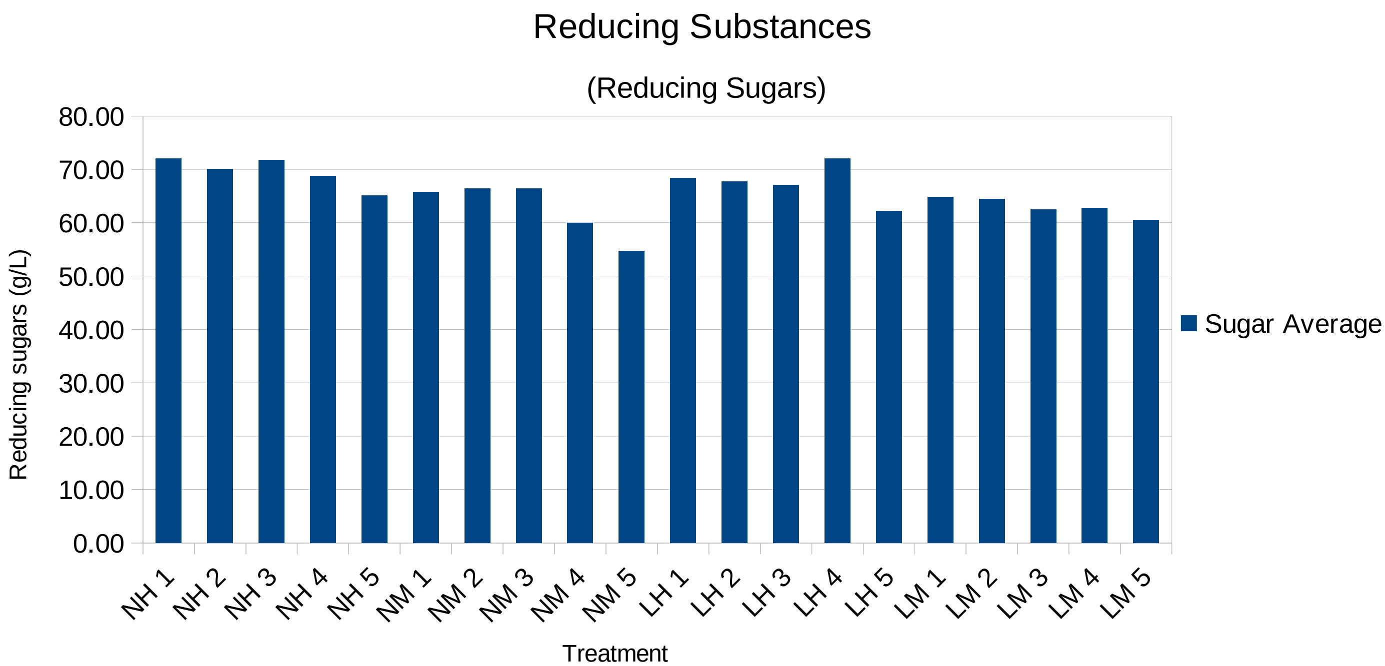
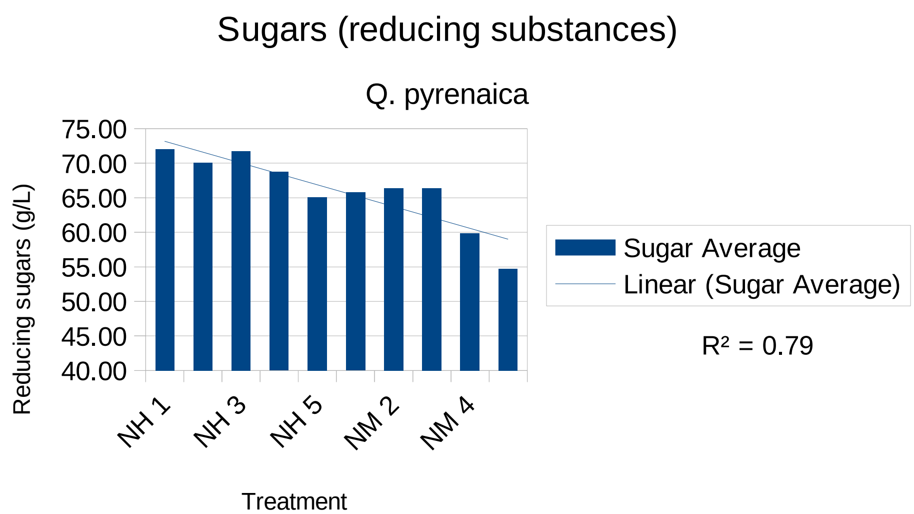
Annexes

Annex 1. Reducing substances of “Carcavelos” fortified wine aged for 8 years



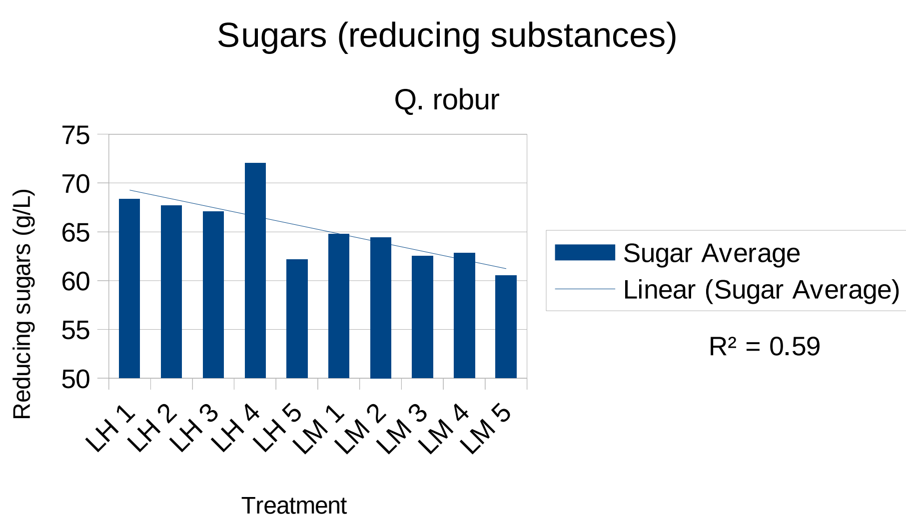
NH = Nacional high toast, LH = Limousin high toast, NM = nacional medium toast, LM = Limousin medium toast, 1-5 = repetition. Results are mean.

Annex 2. Reducing substances of “Carcavelos” fortified wine aged 8 years in *Quercus pyrenaica*



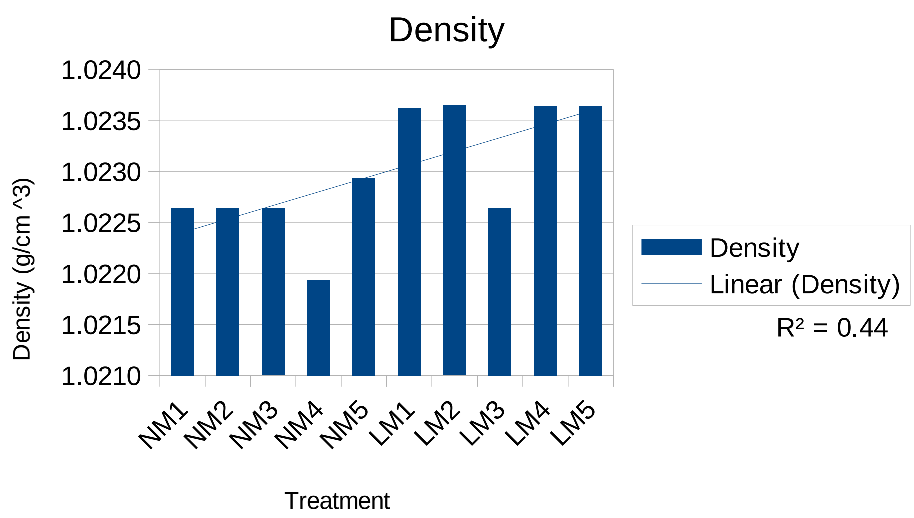
NH = Nacional high toast, NM = nacional medium toast, 1-5 = repetition. Results are mean.

Annex 3. Reducing substances of “Carcavelos” fortified wine aged 8 years in *Quercus robur*



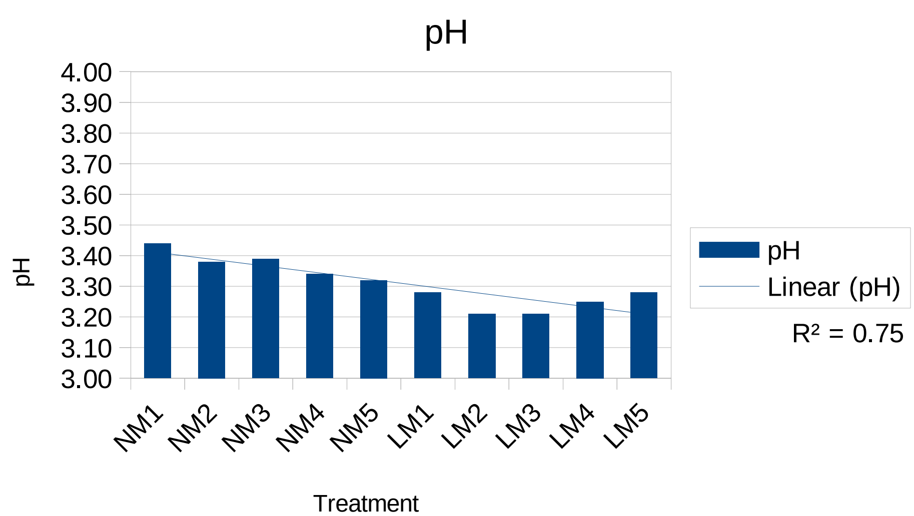
LH = Limousin high toast, LM = Limousin medium toast, 1-5 = repetition. Results are mean.

Annex 4. Density of “Carcavelos” fortified wine aged 8 years in medium toast *Q. pyrenaica* and *Q. robur* barrels



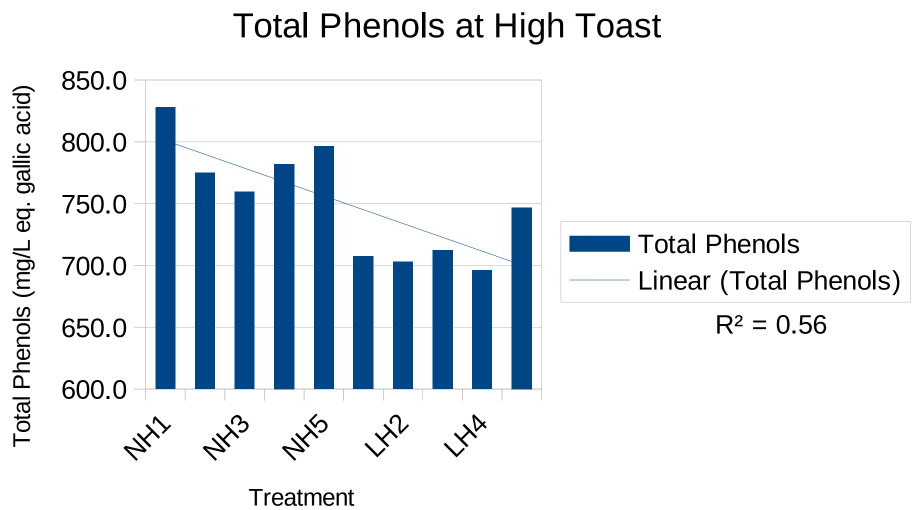
Density of Portuguese Nacional Oak (*Q. pyrenaica*) vs. French Limousin oak (*Q. robur*) at medium toasting.

Annex 5. pH of “Carcavelos” fortified wine aged for 8 years in medium toast *Q. pyrenaica* and *Q. robur* barrels



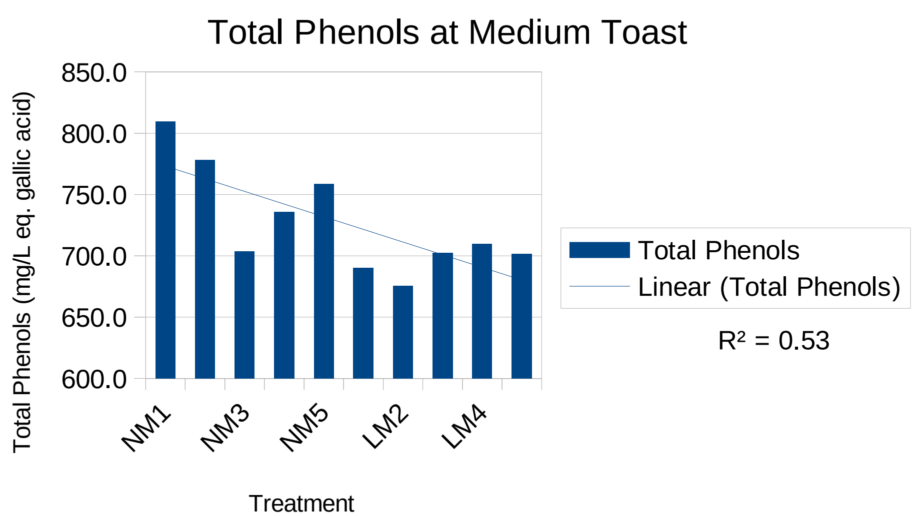
Portuguese Nacional Oak (*Q. pyrenaica*) vs. French Limousin oak (*Q. robur*) on pH at medium toasting.

Annex 6. Total phenols of “Carcavelos” fortified wine aged for 8 years in high toast *Q. pyrenaica* and *Q. robur* barrels



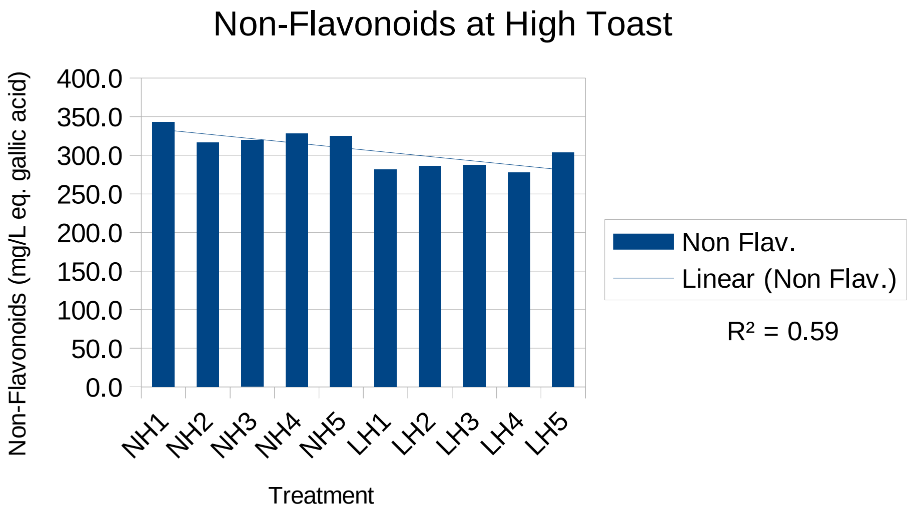
NH = Nacional high toast, LH = Limousin high toast, 1-5 = repetition. Results are mean expressed as mg/L eq. gallic acid.

Annex 7. Total phenols of “Carcavelos” fortified wine aged for 8 years in medium toast *Q. pyrenaica* and *Q. robur* barrels



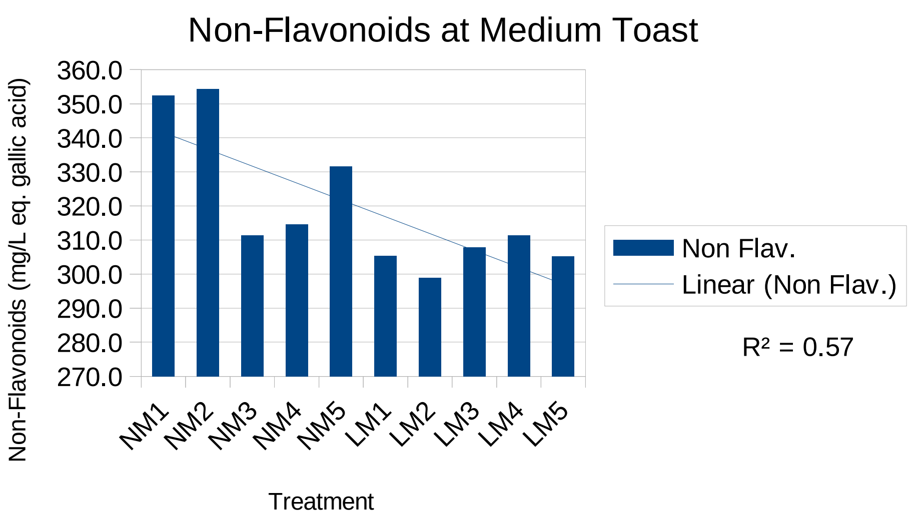
NM = Nacional medium toast, LM = Limousin medium toast, 1-5 = repetition. Results are mean expressed as mg/L eq. gallic acid.

Annex 8. Non-flavonoids of “Carcavelos” fortified wine aged for 8 years in high toast *Q. pyrenaica* and *Q. robur* barrels



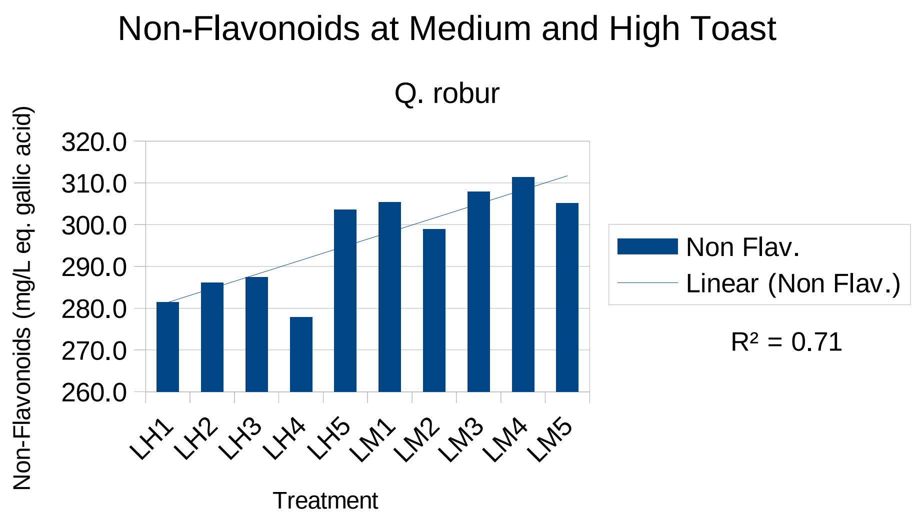
NH = Nacional high toast, LH = Limousin high toast, 1-5 = repetition. Results are mean expressed as mg/L eq. gallic acid.

Annex 9. Non-flavonoids of “Carcavelos” fortified wine aged for 8 years in medium toast *Q. pyrenaica* and *Q. robur* barrels



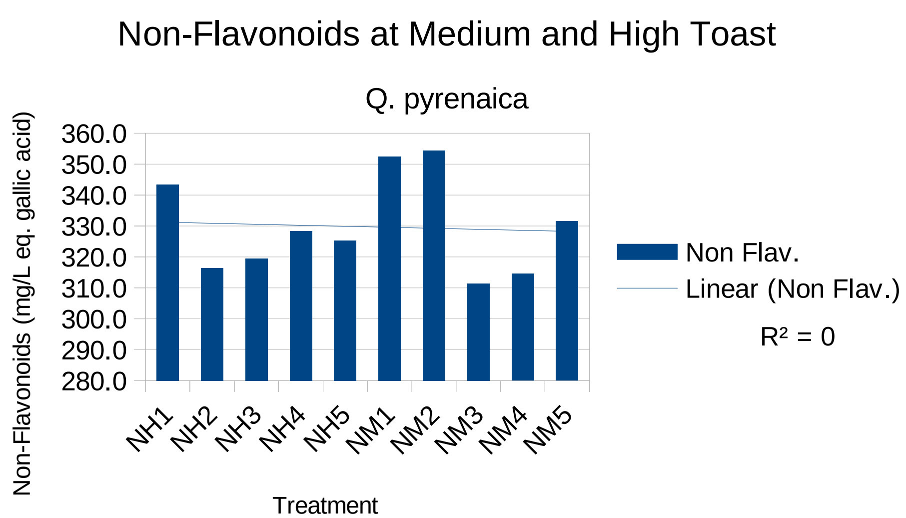
NM = Nacional medium toast, LM = Limousin medium toast, 1-5 = repetition. Results are mean expressed as mg/L eq. gallic acid.

Annex 10. Non-flavonoids of “Carcavelos” fortified wine aged for 8 years in *Q. robur* barrels



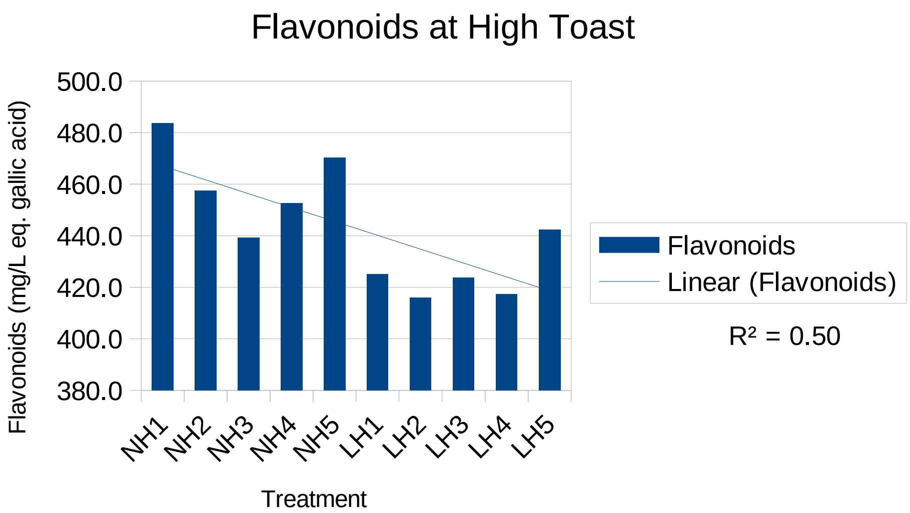
LH = Limousin high toast, LM = Limousin medium toast, 1-5 = repetition. Results are mean expressed as mg/L eq. gallic acid.

Annex 11. Non-flavonoids of “Carcavelos” fortified wine aged for 8 years in *Q. pyrenaica* barrels



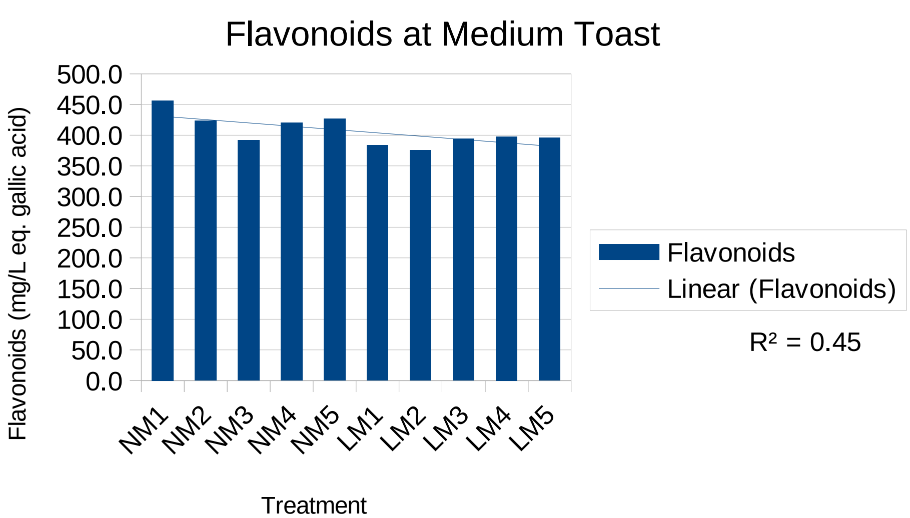
NH = Nacional high toast, NM = Nacional medium toast, 1-5 = repetition. Results are mean expressed as mg/L eq. gallic acid.

Annex 12. Flavonoids of “Carcavelos” fortified wine aged for 8 years in high toast *Q. pyrenaica* and *Q. robur* barrels



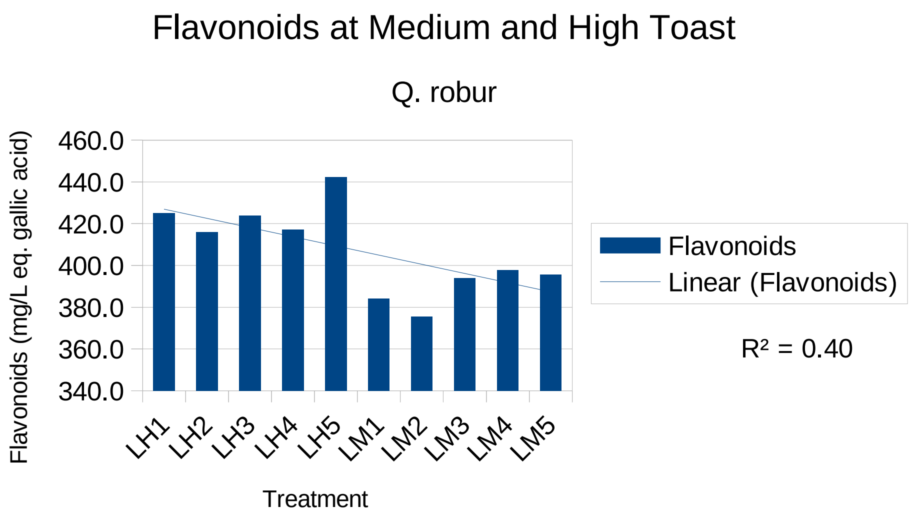
NH = Nacional high toast, LH = Limousin high toast, 1-5 = repetition. Results are mean expressed as mg/L eq. gallic acid.

Annex 13. Flavonoids of “Carcavelos” fortified wine aged for 8 years in medium toast Q. *pyrenaica* and *Q. robur* barrels



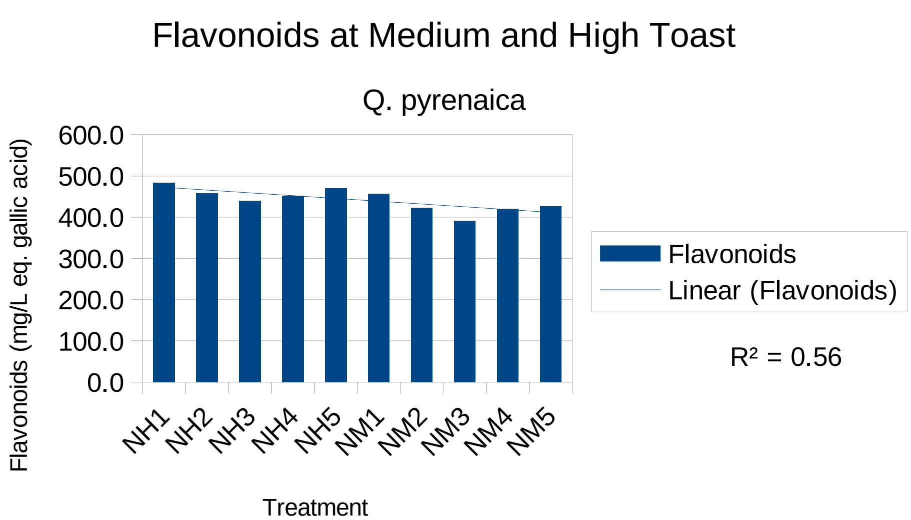
NM = Nacional medium toast, LM = Limousin medium toast, 1-5 = repetition. Results are mean expressed as mg/L eq. gallic acid.

Annex 14. Flavonoids of "Carcavelos" fortified wine aged for 8 years in *Q. robur* barrels



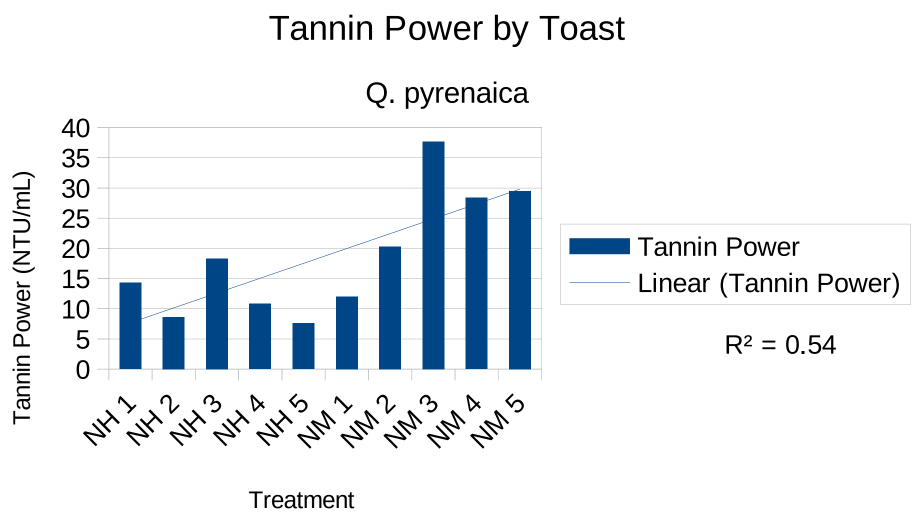
LH = Limousin high toast, LM = Limousin medium toast, 1-5 = repetition. Results are mean expressed as mg/L eq. gallic acid.

Annex 15. Flavonoids of "Carcavelos" fortified wine aged for 8 years in *Q. pyrenaica* barrels



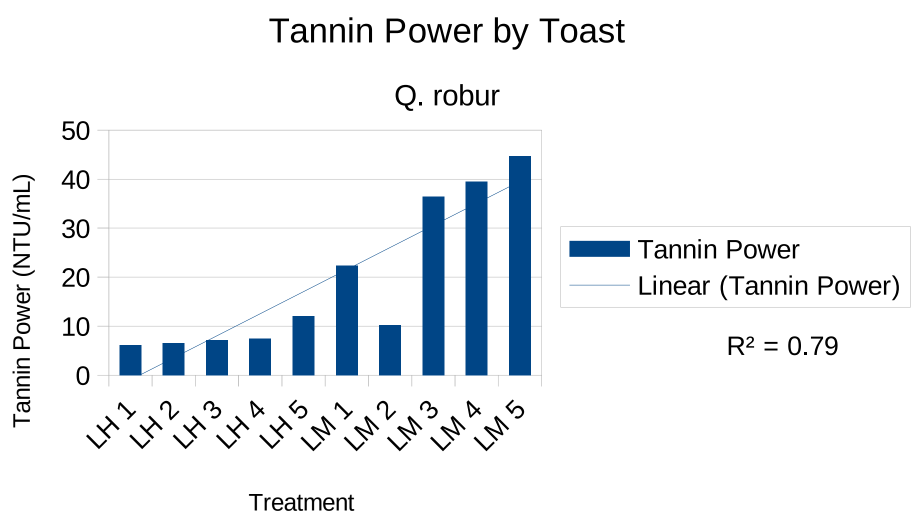
NH = Nacional high toast, NM = Nacional medium toast, 1-5 = repetition. Results are mean expressed as mg/L eq. gallic acid.

Annex 16. Tannin power of “Carcavelos” fortified wine aged 8 years in *Q. pyrenaica* barrels



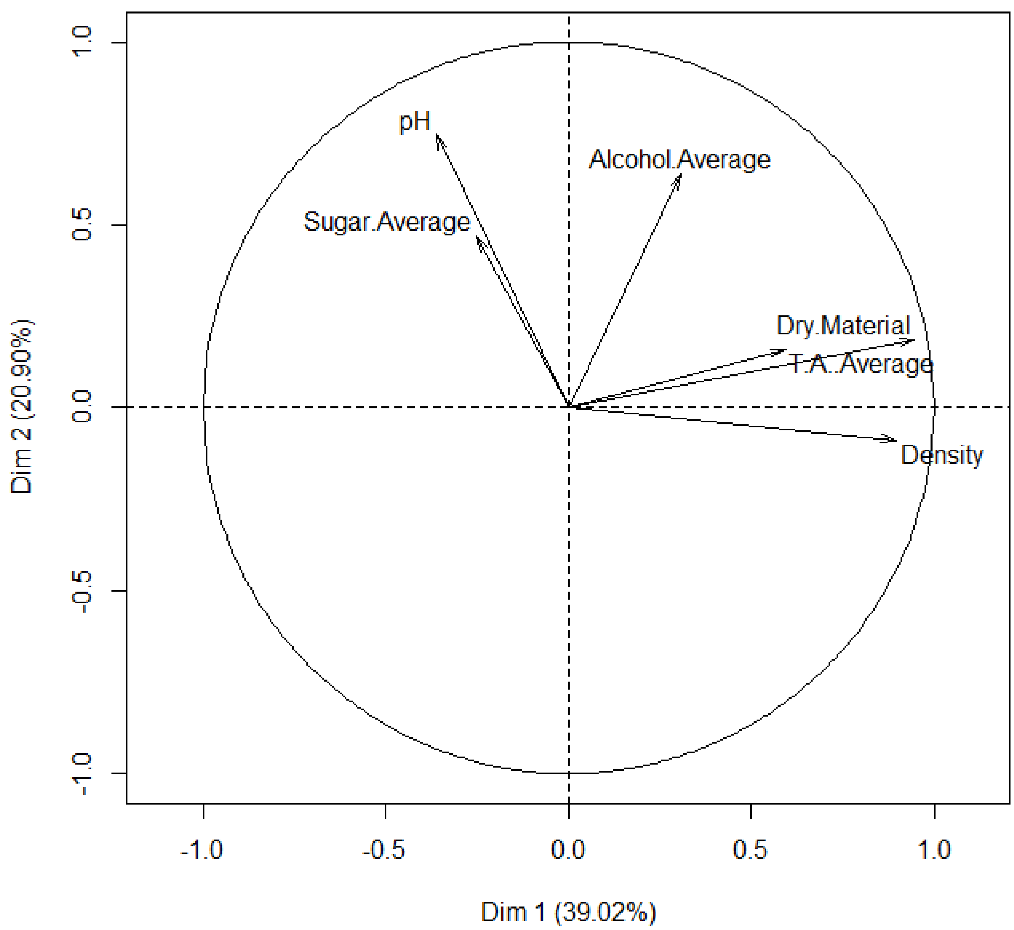
NH = Nacional high toast, NM = nacional medium toast, 1-5 = repetition. Results are mean.

Annex 17. Tannin power of “Carcavelos” fortified wine aged 8 years in *Q. robur* barrels

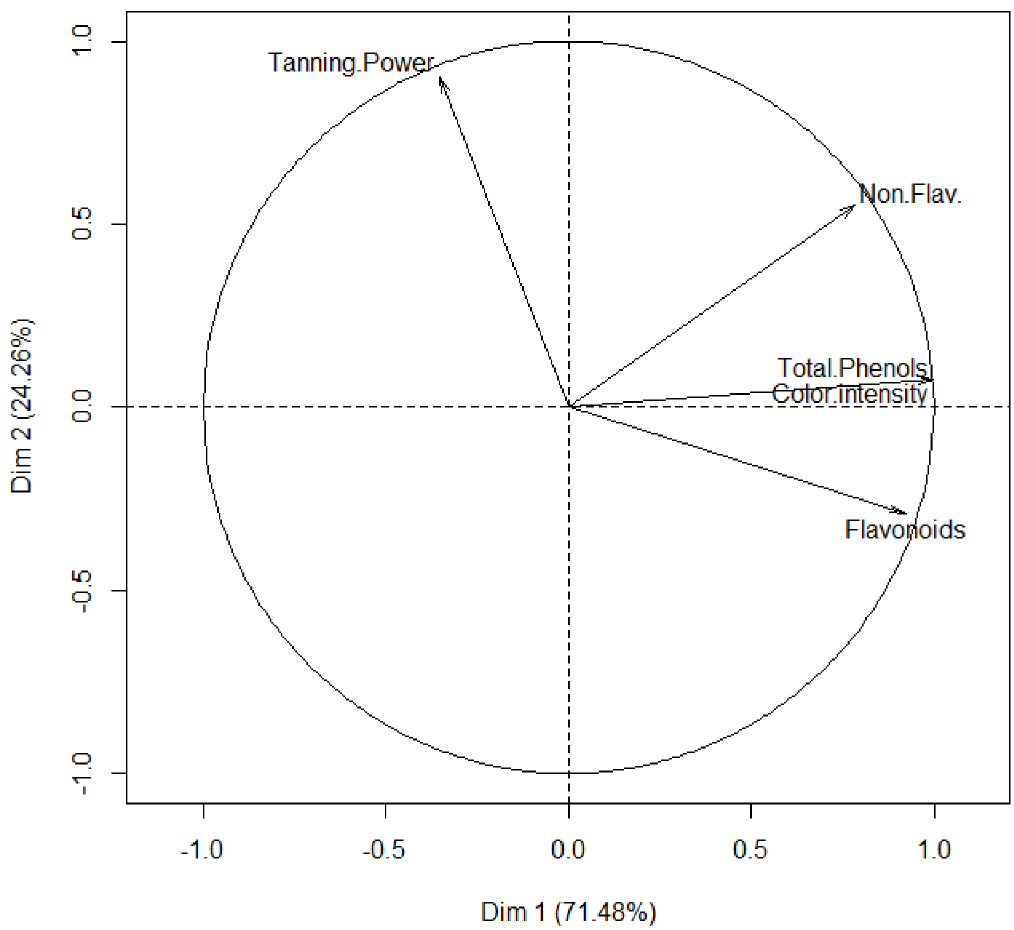


LH = Limousin high toast, LM = Limousin medium toast, 1-5 = repetition. Results are mean.

Annex 18. PCA graph of the basic analyses for “Carcavelos” fortified wine aged for 8 years in new Portuguese and French oak barrels at medium and high toast



Annex 19. PCA graph of the extractable compounds for “Carcavelos” fortified wine aged for 8 years in new Portuguese and French oak barrels at medium and high toast



Annex 20. ANOVA table and Tukey test table and graph for the density of “Carcavelos” fortified wine aged for 8 years in new Portuguese and French oak barrels at medium and high toast

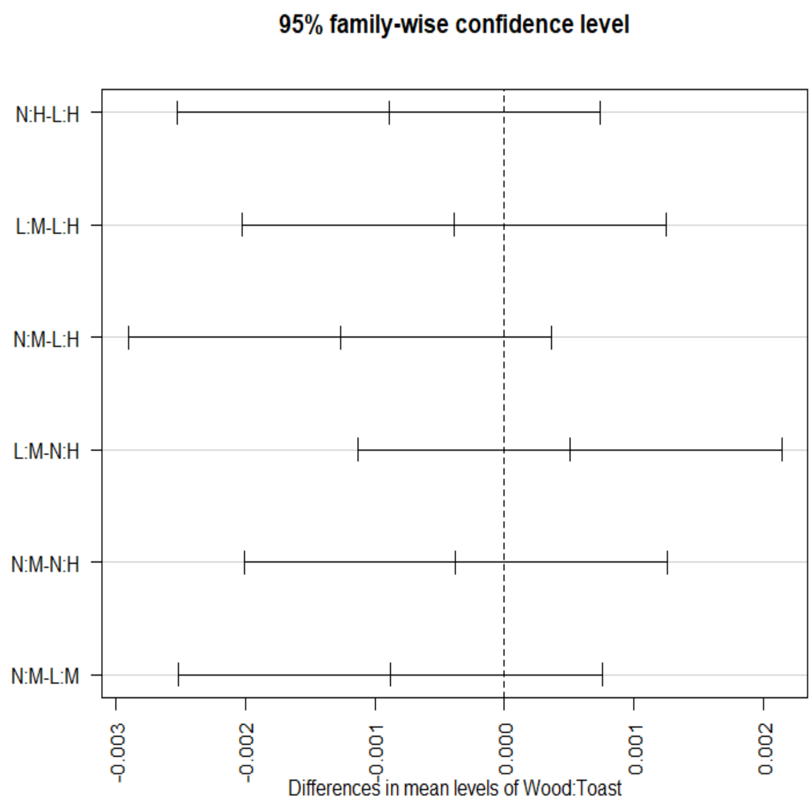
ANOVA table for density

	)f	Sum Sq	lean Sq	: value	'r(>F)
Wood		3.932E-06	3.932E-06	4.814	).0433
Toast		'.32E-07	'.32E-07	).896	).3579
Wood:Toast		)	)	)	).9884
tesiduals	6	.3067E-05	3.17E-07		
Signif. codes:	) '***'	).001 '**'	).01 '*'	).05 '.'	).1 ' '

Tukey table for density

	liff	wr	ıpr	ı adj
I:H-L:H	-0.00089276	-0.002528	).000742479	).4263892
.:M-L:H	-0.00038860	-0.00202384	).001246639	).9032099
I:M-L:H	-0.00126940	-0.00290464	).000365839	).1597146
.:M-N:H	).00050416	-0.00113108	).002139399	).8140680
I:M-N:H	-0.00037664	-0.00201188	).001258599	).9108407
I:M-L:M	-0.00088080	-0.00251604	).000754439	).4376974

Tukey graph for density



Annex 21. ANOVA table and Tukey test table and graph for the pH of “Carcavelos” fortified wine aged for 8 years in new Portuguese and French oak barrels at medium and high toast

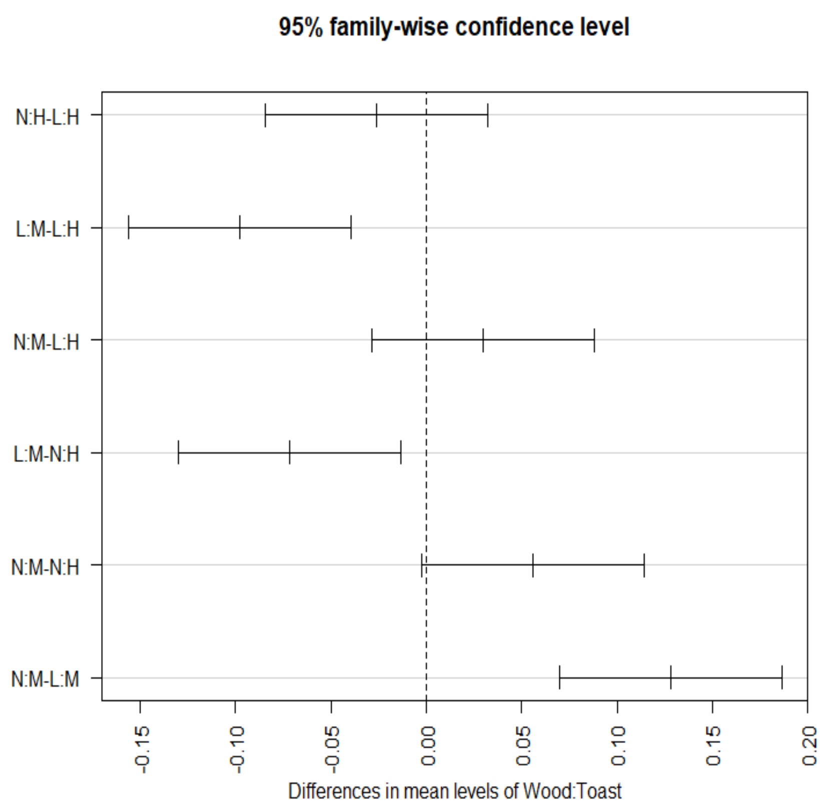
ANOVA table for pH

	)f	Sum Sq	/lean Sq	: value	'r(>F)
Wood		).013005	).013005	2.51	).00275
Toast		).002205	).002205	2.12	).16471
Wood:Toast		).029645	).029645	28.5	5.65E-05
tesiduals	6	).01664	).00104		
Signif. codes:	) '***'	).001 '**'	).01 '*'	).05 '.'	).1 ' '

Tukey table for pH

	diff	wr	upr	adj
I:H-L:H	-0.0260	-0.08435358	).03235358	).5910198
.:M-L:H	-0.0980	-0.15635358	-0.03964642	).0010054
I:M-L:H	).0300	-0.02835358	).08835358	).4765972
.:M-N:H	-0.0720	-0.13035358	-0.01364642	).0133087
I:M-N:H	).0500	-0.00235358	).11435358	).0622726
I:M-L:M	).1280	).069646416	).18635358	).0000595

Tukey graph for pH



Annex 22. ANOVA table and Tukey test table and graph for the reducing substances of "Carcavelos" fortified wine aged for 8 years in new Portuguese and French oak barrels at medium and high toast

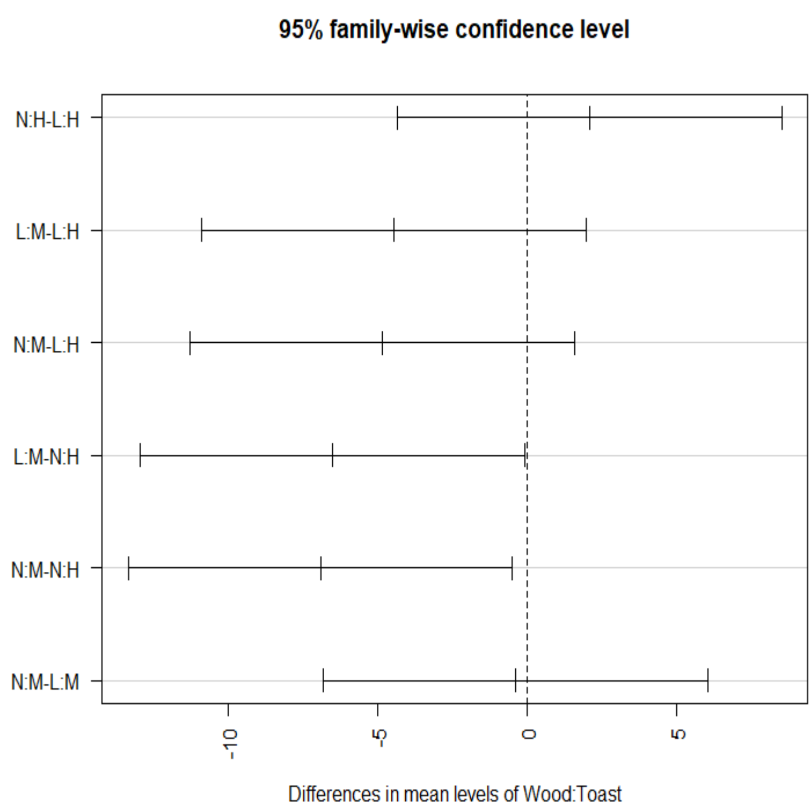
ANOVA table for reducing substances

	)f	Sum Sq	/lean Sq	: value	'r(>F)
Wood		3.56	3.56	).282	).6026
Toast		61.53	61.53	2.803	).002511
Wood:Toast		'.61	'.61	).603	).44868
tesiduals	6	201.87	2.62		
Signif. codes:	) '***'	).001 '**'	).01 '*'	).05 '.'	).1 ' '

Tukey table for reducing substances

	liff	wr	ıpr	› adj
I:H-L:H	2.0775	-4.34972	3.5047195	).7921594
.:M-L:H	-4.4500	-10.87722	.9772195	).2357783
I:M-L:H	-4.8400	-11.26722	.5872195	).1784524
.:M-N:H	-6.5275	-12.95472	-0.1002805	).0458881
I:M-N:H	-6.9175	-13.34472	-0.4902805	).0327300
I:M-L:M	-0.3900	-6.81722	5.0372195	).9980603

Tukey graph for reducing substances



Annex 23. ANOVA table and Tukey test table and graph of the total phenols for “Carcavelos” fortified wine aged for 8 years in new Portuguese and French oak barrels at medium and high toast expressed as gallic acid equivalents

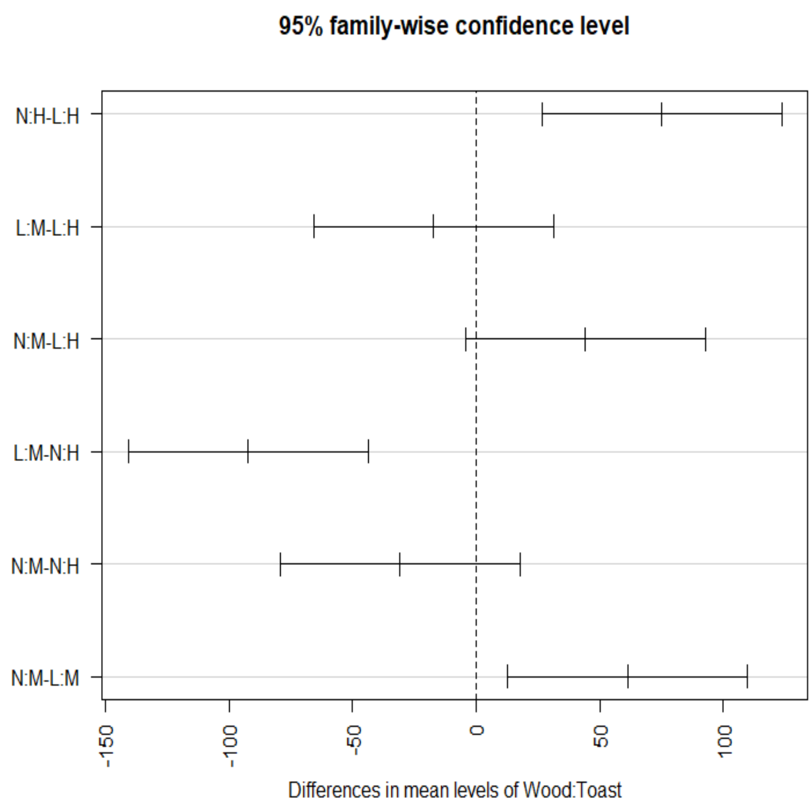
ANOVA table for total phenols

	)f	Sum Sq	lean Sq	value	'r(>F)
Wood		23256	23256	}2.41	}.33E-05
Toast		2887	2877	4.01	).0625
Wood:Toast		237	237	).33	).5735
tesiduals	6	1481	'18		
Signif. codes:	) '***'	).001 '**'	).01 '*'	).05 '.'	).1 ' '

Tukey table for total phenols

	liff	wr	ıpr	adj
l:H-L:H	'5.084210	26.613679	23.55474	).0021297
.:M-L:H	-17.105260	-65.575794	}1.36527	).7461985
l:M-L:H	44.210530	-4.260005	}2.68106	).0802718
.:M-N:H	-92.189470	-140.660005	-43.71894	).0002869
l:M-N:H	-30.873680	-79.344215	7.59685	).2993099
l:M-L:M	}1.315790	2.845258	09.78632	).0111122

Tukey graph for total phenols



Annex 24. ANOVA table and Tukey test table and graph of the non-flavonoids for "Carcavelos" fortified wine aged for 8 years in new Portuguese and French oak barrels at medium and high toast expressed as gallic acid equivalents

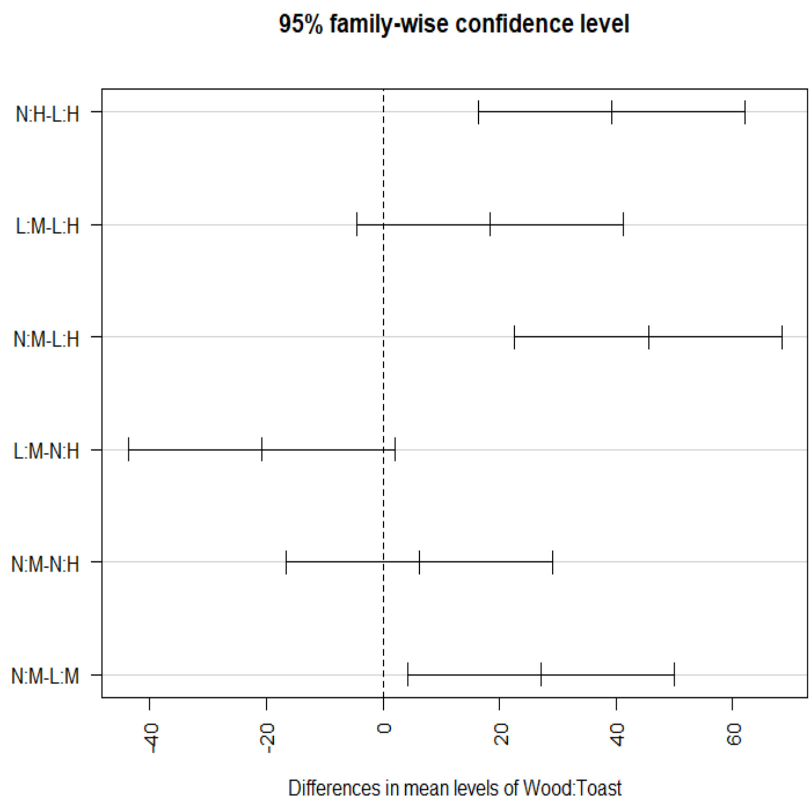
ANOVA table for non-flavonoids

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Wood		5498	5498	34.411	2.39E-05
Toast		763	763	4.777	0.0441
Wood:Toast		84	84	.151	0.2992
Residuals	6	2556	60		
Signif. codes:	0 '***'	0.001 '**'	0.01 '*'	0.05 '.'	0.1 ' '

Tukey table for non-flavonoids

	diff	lwr	upr	p adj
N:H-L:H	39.226316	6.354133	62.098498	0.0008205
L:M-L:H	8.421053	-4.45113	41.293235	0.1385626
N:M-L:H	45.515789	22.643607	68.387972	0.0001768
L:M-N:H	-20.805263	-43.677446	2.066919	0.0813285
N:M-N:H	6.289474	-16.58279	29.161656	0.859382
N:M-L:M	27.094737	4.222554	49.966919	0.0176762

Tukey graph for non-flavonoids



Annex 25. ANOVA table and Tukey test table and graph of the flavonoids for “Carcavelos” fortified wine aged for 8 years in new Portuguese and French oak barrels at medium and high toast expressed as gallic acid equivalents

ANOVA table for flavonoids

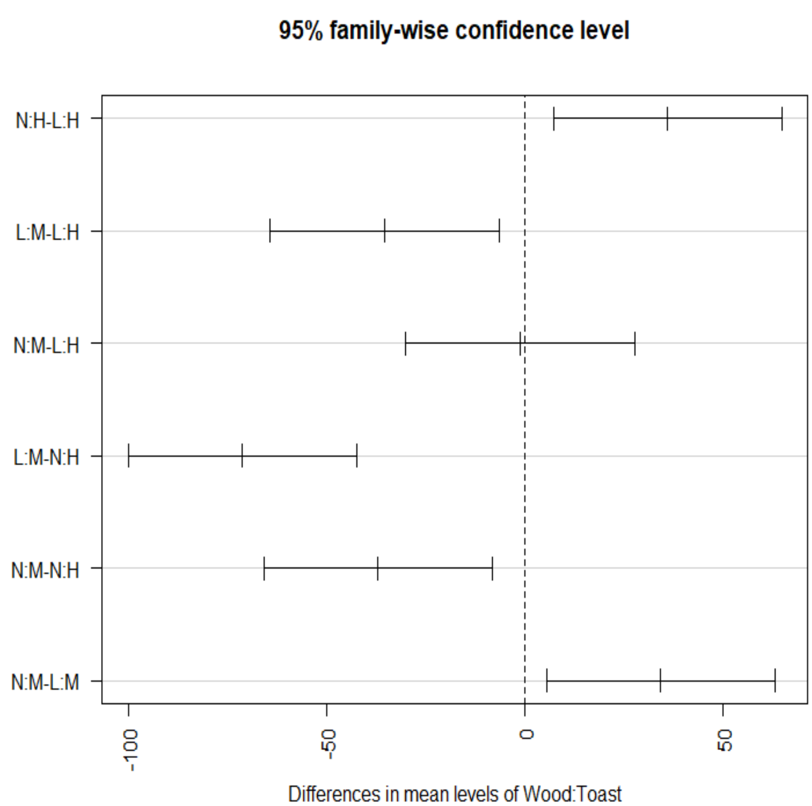
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Wood		6139	6139	24.197	0.000154
Toast		6605	6605	26.034	0.000107
Wood:Toast		3	3	0.013	0.909957
Residuals	6	4059	254		

Signif. codes:	0 '***'	0.001 '**'	0.01 '*'	0.05 '.'	0.1 ' '

Tukey table for flavonoids

	diff	lwr	upr	p adj
N:H-L:H	35.857895	7.036914	64.678875	0.0125388
L:M-L:H	-35.526316	-64.347297	-6.705335	0.0134015
N:M-L:H	-1.305263	-30.126244	27.515718	0.9991888
L:M-N:H	-71.384211	-100.205191	-42.56323	0.0000140
N:M-N:H	-37.163158	-65.984139	-8.342177	0.0096430
N:M-L:M	34.221053	5.400072	63.04203	0.0173990

Tukey graph for flavonoids



Annex 26. ANOVA table and Tukey test table and graph for the tanning power of “Carcavelos” fortified wine aged for 8 years in new Portuguese and French oak barrels at medium and high toast

ANOVA table for tanning power

	)f	Sum Sq	/lean Sq	⁻ value	ᵖr(>F)
Wood		.6	.6	).020	).890306
Toast		659.8	659.8	20.787	).000322
Wood:Toast		04.0	04.0	.302	).270637
tesiduals	6	277.6	79.9		
Signif. codes:	) '***'	).001 '**'	).01 '*'	).05 '.'	).1 ' '

Tukey table for tanning power

	diff	wr	ıpr	ı adj
I:H-L:H	4.00	-12.169344	20.16934	).8925487
.:M-L:H	22.78	5.610656	38.94934	).0048123
I:M-L:H	7.66	.490656	33.82934	).0299261
.:M-N:H	8.78	2.610656	34.94934	).0201863
I:M-N:H	3.66	-2.509344	29.82934	).1137493
I:M-L:M	-5.12	-21.289344	1.04934	).8019385

Tukey graph for tanning power