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Chemical evaluation of Carcavelos fortified wine aged in Portuguese (*Quercus pyrenaica*) and French (*Quercus robur*) oak barrels at medium and high toast

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ABSTRACT

Adega do Casal Manteiga is a winery, publicly owned by the Municipality of Oeiras that produces Carcavelos fortified wine. Carcavelos fortified wine is an appellation of origin and demarcated as D.O.P. (Denominação de Origem Protegida). This study examines the effects of barrels made from 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. Twenty barrels were used, with five 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*, with an impact on total phenolic content, including both flavonoids and non-flavonoids. The total phenols of the wine aged in *Q. pyrenaica* barrels was significantly higher than in the *Q. robur* barrels, and *Q. pyrenaica* contained more flavonoids than *Q. robur* in medium and high toast barrels. *Q. pyrenaica* showed more non-flavonoid compounds than *Q. robur in*high and medium toasted barrels, but this difference in non-flavonoids was only statistically significant in the high toasted 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* and *Q. robur* in the wines that were aged in high tasted barrels compared to those that were medium toasted. The tannin power decreased for both *Q. pyrenaica* and *Q. robur* when the toasting increased.

KEYWORDS

aging, Carcavelos D.O.P., fortified wine, oak wood, toasting

INTRODUCTION

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 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, although 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).

There are four "Vinhos Generosos" from Portugal, which are the classical or "ancient" fortified ("licoroso") wines. These wines are now demarcated as D.O.P. (Denominação de Origem Protegida) and include the regions of Porto, Madeira, Setúbal and Carcavelos. 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 have already been solubilized into the previous wines. Therefore, the use of new barrels enhances the extraction of these volatile compounds, but also non-volatile compounds such as ellagitannins. 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).

The purpose of this study was to examine the effects of two species of new oak barrels and their toasting level on Carcavelos fortified wine. Typically, fortified wines are aged in old barrels because they have 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 barrels at medium and high toast. The effects of the species of wood and the toasting level on Carcavelos wine could then be examined. To the best of our knowledge this is the first time that a scientific study has examined the chemical composition of Carcavelos fortified wine (Appellation of Origin), aged in two different botanical species of Quercus, using new barrels at the beginning of aging, and with two different toasting regimes for each species.

MATERIALS AND METHODS

1. The wine and the Brandy

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. The 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. There is no blend percentage, but Galego Dourado is the main grape variety used. 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 wine brandy, "aguardente", sourced from the only spirit D.O.C. in Portugal, Lourinhã DOC. The Torrejana, S. A. distillery produces this 77% ABV aguardente through the Charante method. This is a high-quality spirit produced in the Lisbon area, allowing for a more sustainable fortified wine with a stronger sense of place. Once fermentation was stopped, the wine was placed into either Portuguese or French oak barrels for maturation. The wine was barreled in 2009, and these analyses were performed in 2017 at the Instituto Superior de Agronomia, Universidade de Lisboa (ISA).

2. Experimental plan and conditions

This work originates from an ongoing large experiment carried out by the winemaker at the Adega do Casal Manteiga winery. Adega do Casal Manteiga is publicly owned by the Municipality of Oeiras and 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, which resides in the property that was once owned by Sebastião José de Carvalho e Melo. Carcavelos is a micro region surrounded by small hills. 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 5.2 mm, which puts 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). The winemaker placed the same wine into two different species of barrels with two varying degrees of toast. After fermentation, the same wine was placed into 20 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 (16–22°C and 60–65% humidity). The oak barrels were fabricated by the same cooperage, "J.M. Gonçalves" in Palaçoulo, in the north-east 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). The barrels were all toasted by direct fire, with temperatures registered using sensors. A total of five repetitions were used for each toasting factor, accounting for the total of 20 barrels.

3. General chemical analysis

The wine in this study was analyzed for pH, titratable and volatile acidity, density, alcohol, sugars, and SO_2 levels. The general physico-chemical characteristics of the wine were measured following analytical methods set forth by the International Organisation of Vine and Wine (OIV).

4. Estimation of phenolics

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 was 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.

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 then sparged with nitrogen and capped. After 24 hours, the sub-replicates were measured. Each replicate represented in the data is an average of three sub-replicates that were measured. The dilution factor was 20, 2.5 by 50 mL. The flavonoid content for each barrel was determined by subtracting the non-flavonoid content found from the total phenolic content that was quantified.

5. Estimation of tanning power

Tanning power was 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 (BSA, bovine serum albumin, ≥ 96 %, Sigma-Aldrich, USA) were combined in a tube, agitated, and placed in 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.

6. Chromatic analysis

The chromatic characteristics were measured using a spectrophotometric method. 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.

7. Statistical analysis

The software program R and the plugin Rcmdr (R commander) was used to determine the statistical significance of the results. Principal component analysis (PCA) was performed to determine which analysis may have significance. Multi-way analysis of variance (multi-way

ANOVA) and the Tukey test ($p < 0.05$) were used to determine significant differences.

RESULTS AND DISCUSSION

1. General physico-chemical analysis and the influences of botanical species and toasting

The Carcavelos fortified wine in this study, aged for 8 years in new Portuguese oak (*Q. pyrenaica*) and new French oak (*Q. robur*) barrels at medium and high toast, was analyzed for pH, titratable and volatile acidity, density, alcohol, sugars, SO_2 levels, total phenols, flavonoids, non-flavonoids, tanning power, and color intensity. Table 1 shows the results obtained from the general analyses, including density, pH, total SO_2, total acidity, volatile acidity, alcohol, sugars, and dry extract.

The total sulfur dioxide content of the wine is low (Table 1). Generally, the total sulfur dioxide content in fortified wines are always low, for two main reasons. First, the elevated alcoholic content protects the wine from high amounts of microbiological activity and does not have enzymes that need to be inhibited. Second, in many fortified wines oxygenation is desired as it brings reduced astringency and improves color. This is not always the case however, with the exception of 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 1, is high for a white wine but normal for Carcavelos fortified wine. The legal limits for volatile acidity are dependent on the country and the style of wine. According to Fugelsang and Edwards (2009), 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 differ in California, where 1.2 g/L is the legal limit for table reds and 1.1 g/L for table whites. Lastly, limits in the European Union are 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 and Edwards, 2009; Neeley, 2004).

Normally the analyses in Table 1 are performed as an "identification card" for the wine. In this study, PCA, multi-way ANOVAs, and subsequent Tukey tests were performed on the different analyses. Figure 1 shows the PCA graph and the variables that may be significant or possibly correlated. ANOVAs revealed 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 statistically 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 the *Q. pyrenaica* high of 3.44 and the *Q. robur* low of 3.21 (Table 1). The difference of the averages is 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.

In addition to the difference in pH between the oak species at medium toast, the analysis showed that the toast level had a significant effect on the total amount of reducing substances of the wine (Table 1): *Q. pyrenaica* and *Q. robur* contain more reducing substances at a high degree of toasting compared to medium toasting. One possible explanation for this is a result of the analytical methodology used: 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 a possibility that those molecules are donating their electrons and being oxidized, resulting in an overall lower measurement of reducing substances. According to Jackson, "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 Jackson states that "it is insufficient to affect taste perception", thus we can conclude that these significant changes cannot be a result of this reaction (Jackson,

TABLE 1. 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 SO2 (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/cm^3. 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.

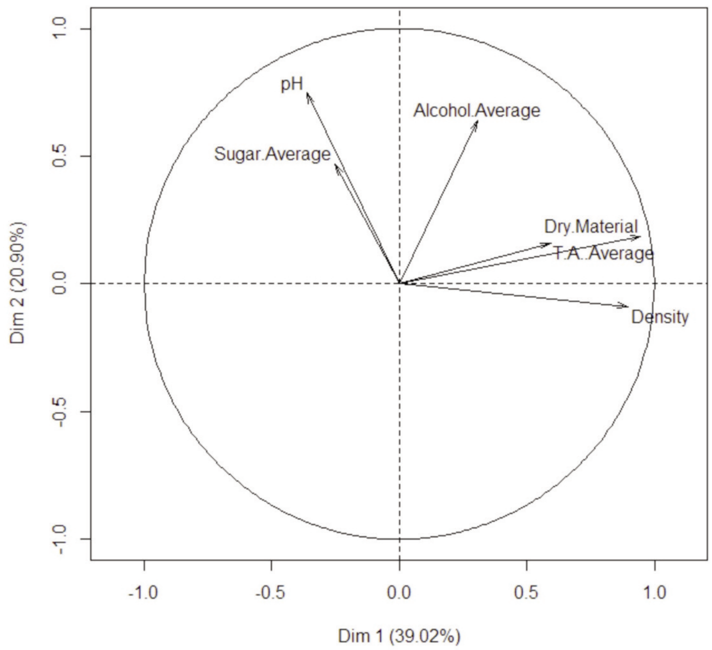

FIGURE 1. PCA graph of the basic analyses for "Carcavelos" fortified wine aged for 8 years in new Portuguese and French oak barrels at medium and high toast levels

2014). Lastly, there is no repeatability given from the OIV using this methodology for reducing substances.

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 Table 2 and Figure 2. PCA was performed to see which analysis may have significance or are possibly correlated (Figure 3). ANOVAs revealed significant differences in the extraction of phenolics between *Q. pyrenaica* and *Q. robur.* Based on the data in Table 2 and Figure 2, both medium and high toast *Q. pyrenaica* imparted a greater amount of extractable phenolics in the wine than *Q. robur* barrels.

The results of this study are in agreement with established literature where extracted polyphenolics are dependent on the pool present in the wood species and the toast level (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 such as seasoning and toasting methods (Canas *et al.,* 2008; Chira and Teissedre, 2014; Fernández De Simón *et al.,* 2003; Jordão *et al.,* 2005a; Jordão *et al.,* 2005b; Zhang *et al.,* 2015).

Previous research has shown significant differences in extractives between two French oaks used in cooperaging, *Q. robur* and *Q. petraea*. Moreover, the differences in that research have shown 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*).

The effects of *Quercus pyrenaicas* on wine evolution have not been extensively researched in comparison to species such as *Q. petraea*, *Q. robur* or *Q. alba*. Research in Spain has shown that, in wood samples after natural seasoning or samples that underwent natural seasoning and medium toasting, *Q. robur* contained higher total phenolic and ellagitannin content than *Q. pyrenaica* (Cadahía *et al.,* 2001).

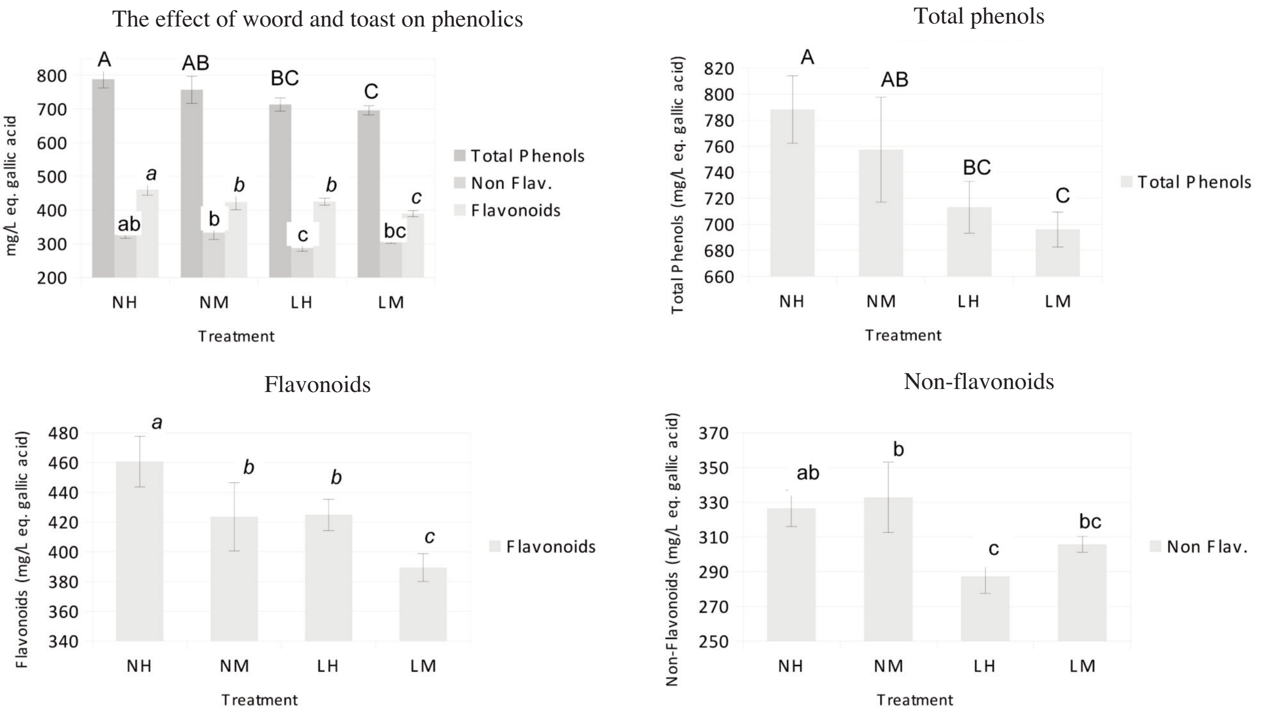

FIGURE 2. Differences in the phenolic content of "Carcavelos" fortified wine aged for 8 years in new medium toasted and high toasted *Q. pyrenaica* and *Q. robur* barrels

TABLE 2. The influence of wood and toast level on phenolic extraction in "Carcavelos" fortified wine aged for 8 years in new medium and high toasted French and Portuguese oak barrels (mg/L gallic acid equivalents)

Treatment	Total phenols	Non-flavonoids	Flavonoids	Color intensity (abs)	Tanning power (NTU/mL)
NH1	828.0 ± 3.0	343.3 ± 2.3	483.8	0.988 ± 0.008	14.29 ± 6.45
NH2	775.0 ± 15.0	316.5 ± 3.6	457.6	0.987 ± 0.008	8.58 ± 04.70
NH3	759.6 ± 1.4	319.4 ± 2.1	439.3	1.124 ± 0.016	18.33 ± 2.88
NH4	782.0 ± 8.4	328.4 ± 2.2	452.7	1.211 ± 0.014	10.83 ± 2.11
NH5	796.5 ± 7.7	325.2 ± 4.5	470.3	1.090 ± 0.001	7.63 ± 2.71
LH1	707.4 ± 12.4	281.5 ± 11.8	425.0	1.015 ± 0.025	6.17 ± 2.56
LH2	703.0 ± 5.7	286.1 ± 7.9	416.0	1.070 ± 0.009	6.58 ± 0.88
LH3	712.3 ± 10.4	287.5 ± 1.8	423.8	0.991 ± 0.004	7.21 ± 5.67
LH4	696.0 ± 8.3	277.9 ± 1.5	417.3	1.066 ± 0.008	7.54 ± 3.12
LH5	746.9 ± 5.3	303.6 ± 0.3	442.4	1.238 ± 0.014	12.08 ± 1.82
NM1	809.6 ± 2.9	352.4 ± 6.7	456.3	0.958 ± 0.014	12.04 ± 5.19
NM2	778.5 ± 4.4	354.4 ± 7.3	423.2	0.939 ± 0.008	20.29 ± 4.18
NM3	703.9 ± 12.4	311.4 ± 0.7	391.6	1.072 ± 0.002	37.71 ± 18.56
NM4	735.9 ± 0.6	314.5 ± 12.6	420.5	1.068 ± 0.014	28.38 ± 2.83
NM5	758.8 ± 7.9	331.5 ± 4.4	426.3	1.071 ± 0.003	29.50 ± 17.75
LM1	690.3 ± 7.5	305.4 ± 8.7	384.0	1.102 ± 0.011	22.38 ± 7.40
LM2	675.4 ± 5.7	298.9 ± 2.8	375.6	1.025 ± 0.003	10.29 ± 4.94
LM3	702.6 ± 7.9	307.9 ± 0.4	393.8	1.018 ± 0.004	36.50 ± 5.56
LM4	710.1 ± 8.8	311.4 ± 3.3	397.8	1.130 ± 0.014	39.54 ± 4.88
LM5	701.7 ± 9.0	305.2 ± 0.8	395.6	1.141 ± 0.006	44.75 ± 7.41

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. Phenolics expressed as mg/L eq. Gallic acid. Color intensity at 420nm. p-value < 0.05 calculated using the Tukey test.

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*.

Further research was done on the effects of medium and high toast on the ellagitannin content of *Q. pyrenaica* and *Q. petraea*. Ellagitannins were shown to significantly decrease depending on the degree of toast, with a higher toast level 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 of medium toast levels between the two species, the results of this study are not in agreement with the Spanish research. This could potentially be an impact of geographical origin, individual tree variation, coarseness of the grain, or seasoning method (Jackson, 2014; Navarro *et al.,* 2016).

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* in both medium and high toasted barrels imparted significantly more total phenolics to the wine compared to *Q. robur.* At high toast, there was a mean difference of 75.1 mg/L eq. gallic acid (*Q. pyrenaica* 788.2

and *Q. robur* 713.1 mg/L eq. gallic acid); at medium toasting, there was a mean difference of 61.3 mg/L eq. gallic acid (*Q. pyrenaica* 757.3 and *Q. robur* 696.0 mg/L eq. gallic acid). This effect is more significant for high toasted barrels than medium toast barrels (Figure 2).

The degree of toast had no statistically significant effect on total phenolics within the same species of wood. The mean change in *Q. pyrenaica* for medium and high toast levels was 30.9 mg/L eq. gallic acid (high toasted barrels 788.2 and medium toasted barrels 757.3 mg/L eq. gallic acid). The mean change in *Q. robur* for medium and high toasting levels was 17.1 mg/L eq. gallic acid (high toasted barrels 713.1 and medium toasted barrels 696.0 mg/L eq. gallic acid).

Higher toast levels did have more total phenolics overall (Figure 2). 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 the 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).

2.2. Non-flavonoids

The most significant factor for non-flavonoid content in the wine was the species of wood. Figure 3 and Table 2 show Portuguese *Q. pyrenaica* to have more non-flavonoid constituents than *Q. robur* at both medium and high toast levels. The mean difference between Portuguese *Q. pyrenaica* and French *Q. robur* in high toasted barrels was 39.2 mg/L eq. gallic acid (*Q. pyrenaica* 326.6 and *Q. robur* 287.3 mg/L eq. gallic acid). There was a difference between both species in medium toasted barrels of 27.1 mg/L eq. gallic acid (*Q. pyrenaica* 332.9 and *Q. robur* 305.8 mg/L eq. gallic acid).

The Toast level was only shown to be slightly significant in the ANOVA. When considering the overall affects, the values were higher in the medium toasted barrels compared to the high toasted barrels (Figure 2). A small difference in the wine from toasting did appear in the ANOVA. There was a decrease of 18.4 mg/L eq. gallic acid from medium to high toast levels, 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 between medium (332.9 mg/L eq. gallic acid) and high (326.6 mg/L eq. gallic acid) toasted barrels. 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 the 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 toast levels, almost all may decompose (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. Tavares *et al.* (2017) showed that for non-flavonoids, Portuguese oak had the highest values, but that 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 *et al.,* 2005a).

2.3 Flavonoids

Table 2 shows the total flavonoids imparted into the wine. The species of oak and the degree of toasting resulted in significant differences in the wine (Figure 2). Overall *Q. pyrenaica* contained more flavonoids than *Q. robur* at both medium and high toast levels. In high toasted barrels there was a mean difference of 35.9 mg/L eq. gallic acid of flavonoids (*Q. pyrenaica* 460.7 and *Q. robur* 424.9 mg/L eq. gallic acid). A difference of 34.2 mg/L eq. gallic acid was shown in medium toast levels (*Q . pyrenaica* 423.6 and *Q. robur* 389.4 mg/L eq. gallic acid). *Q. pyrenaica* at medium toast levels was shown to have comparable levels of flavonoids with

Q. robur at high toast levels (*Q. pyrenaica* 423.6 and *Q. robur* 424.9 mg/L eq. gallic acid).

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 from medium (423.6 mg/L eq. gallic acid) to high (460.7 mg/L eq. gallic acid) toast levels. The French *Q. robur* barrels showed a 35.5 mg/L eq. gallic acid increase from medium (389.4 mg/L eq. gallic acid) to high (424.9 mg/L eq. gallic acid) toast levels. Although these changes are comparable, and the standard deviation was lower in French oak (as seen in Figure 2), 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.

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, and that thermal degradation of ellagitannins results in a release of ellagic and gallic acids (Cadahía *et al.,* 2001; Doussot *et al.,* 2002).

2.4. Color Intensity

The species of oak and the toasting method showed no significant effects on the color intensity of the wine. The same base fortified wine, when kept at the same cellar conditions, shows a tendency for similar wine color intensity and tonality after a considerable aging period, even if the oak species and toasting levels are different.

2.5. Tanning power

Overall it is clear from Figure 4 and Table 2 that 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* barrels 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 high toasted *Q. pyrenaica* 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. In both *Q. pyrenaica* and *Q. robur* higher toasting levels

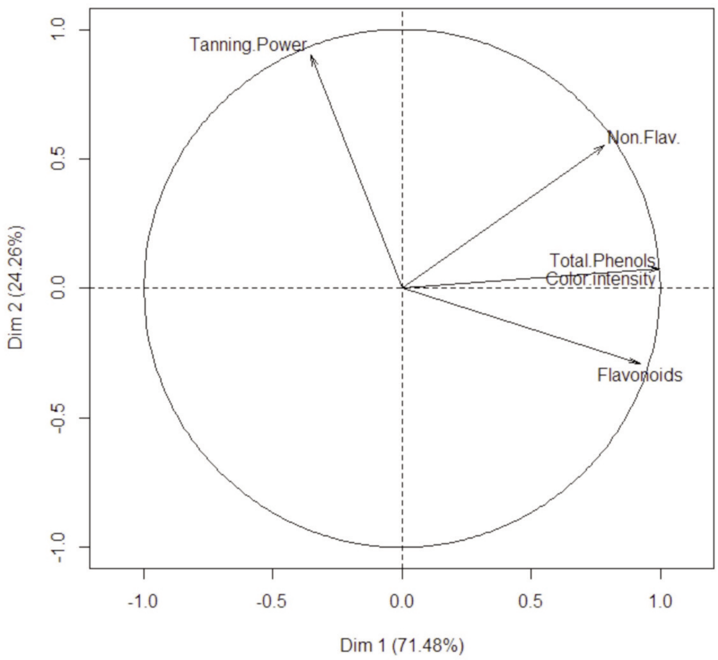

Figure 3. PCA graph of the extractable compounds for "Carcavelos" fortified wine aged for 8 years in new medium or high toasted Portuguese and French oak barrels

showed significantly less tanning power when compared to the medium toasted barrels. Moreover *Q. robur* barrels showed a more significant decline of tanning power than *Q. pyrenaica*: *Q. robur* decreased 22.78 NTU/mL (from 30.7 at medium to 7.92 NTU/mL at high toast levels) and *Q. pyrenaica* decreased 13.66 NTU/mL (from 25.58 at medium to11.92 NTU/mL at high toast levels). For *Q.pyrenaica* the decline caused by toasting 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 4 and Table 2 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.

CONCLUSIONS

The goal of this work was to examine the effects of new medium and high toasted *Q. pyrenaica* and *Q. robur* barrels on Carcavelos fortified wine. *Q. pyrenaica* has not been extensively researched and few direct comparisons have been made with *Q. robur*. In addition, the previous research available was on dry wines, oak chips, staves, or seasoned wood. Furthermore, there currently is no published research on Carcavelos 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 levels. These results are in agreement with other research that shows the total phenolic content is related to the type of wood species used in cooperaging.

Furthermore, in the barrels that underwent high toasting, the wood has a significant impact. At medium toast levels the effect of the wood is not significant.

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 flavonoids content and non-flavonoid content, as well as the tanning power and in reducing the substances 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.

Analyses showed no significant effects of the species of wood or the toasting technique on the wine's density, total acidity, volatile acidity, alcoholic strength, total dry material, or color intensity. A significant difference in pH was seen only in *Q. robur* barrels at medium levels of toast. It may be possible that more acidic substances were extracted from the wood, increasing the acidity and bringing the pH down. Alternatively, salt substances would have an opposite effect and increase the pH.

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 when the wine is completed.

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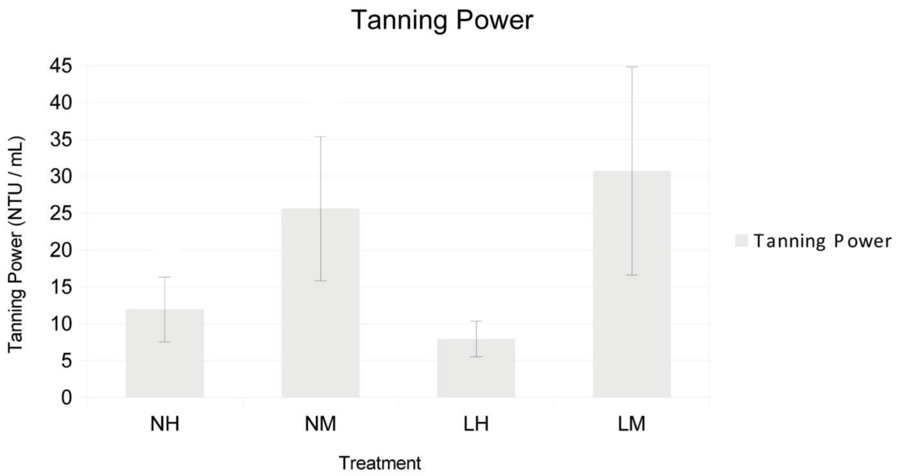

FIGURE 4. Tanning power of "Carcavelos" fortified wine aged for 8 years in medium and high toasted new Portuguese and French oak barrels

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 at NTU/mL. Values with the same letters are not statistically different (Tukey test, $p < 0.05$).

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