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Proanthocyanidin content as an astringency estimation tool and maturation index in red and white winemaking technology

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ABSTRACT

Selecting the appropriate type of barrel for wine maturation but also deciding on the optimum maturation length, is a challenge for winemakers. As different types of barrel woods emerge, it is of great importance for a guideline to be established, which could facilitate winemaking decisions. Since the sensory perception of the finished wine, and particularly the intensity of astringency, is a decisive factor for the quality of a barrel-aged wine, in this experiment, the structural characteristics of wine proanthocyanidins were determined and their correlation with astringency was established. According to the results obtained, the proanthocyanidin content and the type of subunit that is dominant in tannin chains could be used to construct an astringency estimation model. The findings could provide winemakers with a useful tool when deciding how long to mature a specific type of wine in a specific wood container without making it appear coarse and astringent.

1. Introduction

Wine astringency has been an ongoing research topic over the last decade as it is related to wine quality and consequently to consumer preference. It has been described as a "drying, roughing or puckering sensation" elicited by phenols present in wines, more specifically by tannins (Gawel, Iland, & Francis, 2001).

Tannins are a large group of compounds that share certain characteristics such as the ability to precipitate proteins, an attribute to which they owe their name. They can be further separated into two main groups, hydrolysable tannins and condensed tannins. Hydrolysable tannins are oligomeric derivatives of gallic and ellagic acid, and condensed tannins are dimmers or polymers with C–C bonds between flavan-3-ol subunits (Benaiges & Guillén, 2007). In grapes, only condensed tannins can be found, however in wine both groups are present since hydrolysable tannins are extracted into wine from oak during the course of barrel aging. Between condensed tannins, also referred to as proanthocyanidins, the most important ones are flavan-3-ols (catechins) and their condensed forms (+)-catechin (C), (−)-epicatechin (EC), (+)-gallocatechin (GC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG) and (−)-epigallocatechin gallate (EGCG) (Benaiges & Guillén, 2007), all of which can be used as subunits during polymer formation. Although these forms as well as the biosynthesis of flavan-3-ols itself are well studied, the mechanism of polymer formation is still unknown.

Condensed tannin polymers have a terminal subunit and a series of extension subunits linked by interflavanoid bonds (Hanlin, Hrmova, Harbertson, & Downey, 2010). In this skeleton, according to most studies, the main terminal subunit usually is (+)-catechin and the extension subunit (−)-epicatechin. As for the length of the polymer found in grapes, it ranges between five to forty subunits, depending on the source of the tannins, i.e. seed or skin tannins (Hanlin et al., 2010).

Tannin concentration, as well as polymer composition and polymer size are factors of great importance for the winemaker as they have a serious impact on wine mouthfeel. For example, as the polymer chain becomes longer, astringency increases (Vidal et al., 2003). The same happens when the molecular weight of tannins increases, along with their ability to bind with proteins however molecular weight alone is insufficient to characterize proanthocyanidins regarding their influence on astringency (Chira, Jourdes, & Teissedre, 2012).

In order to fully study this group, the degree of polymerization (DP), which corresponds to the average number of flavan-3-ol subunits that

Abbreviations: C, (+)-catechin; EC, (−)-epicatechin; GC, (+)-gallocatechin; EGC, (−)-epigallocatechin; ECG, (−)-epicatechin gallate; EGCG, (−)-epigallocatechin gallate

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compose the tannin chain, was introduced (Guyot, Le Guernevé, Marnet, & Drilleau, 1999). The most successful method for this analysis has been the depolymerization method which consists of treatment of tannins with acid in the presence of a nucleophile such as phloroglucinol and consequent release of terminal and extension subunits (Herderich & Smith, 2005). This way the subunit profile can be revealed with the help of High Performance Liquid Chromatography (HPLC) analysis and structural characteristics of the proanthocyanidins as the mean degree of polymerization (mDP) and the type of individual subunits can be determined.

MDP correlation with astringency sensation, has shown that polymeric procyanidins with a high mDP present higher astringency compared to oligomeric ones that have lower mDP (Sun et al., 2013). Additionally, absolute mDP values have been helpful in characterizing wines not only according to their astringency, but also according to their aging status. Chira et al. (2012), after analyzing 23 vintages of Cabernet Sauvignon, came to the conclusion that wines with an mDP between 2 and 4 are characterized as mellow and slightly astringent, while wines with mDP higher than 4 are perceived as more tannic. Higher mDP also correlated with younger wines (Chira et al., 2012). However, mDP can give no information on the structure of the chain (sequence of the monomers) and it cannot discriminate between tannin mixes, for example a mix consisting of equal parts of tetrameric and octameric tannins can result in the same mDP as a sample of hexameric tannins (Herderich & Smith, 2005). Finally, there has been some debate about whether mDP results should be accompanied by more information on interflavonoid linkages and conversion yield (conversion of proanthocyanidins to known subunits) (Jorgensen, Marin, & Kennedy, 2004).

Tannins extracted from the barrel wood (ellagitannins) have also been found to alter condensed tannin size, and consequently, mDP through complex formation (Watrelot, Badet-Murat, & Waterhouse, 2018). Regarding oak species, European oak wood is the richest in ellagitannins while American oak is the poorest. There are now also a few published results concerning other barrel woods such as Acacia which appear to contain the lowest amounts of ellagitannins (De Simón et al., 2014). As a result, Acacia adds less wood character to the wines while making them suppler and finer textured, an attribute that makes it more appealable for use with white wines (Kozlovic, Jeromel, Maslov, Pollnitz, & Orlić, 2010). On the contrary, Chestnut, is a quite hard wood very rich in hydrolysable tannins and gallic acid (Rooso, Panighel, Vedova, & Stella, 2009). Its high phenolic content coupled with the bigger porosity of the wood, makes it more suitable for shorter aging.

Numerous reports have been made regarding the mDP of grape tannins, and few have dealt with mDP of tannins in bottle aged wines or wines made with different winemaking protocols (Gonzalez-Manzano, Santos-Buelga, Perez-Alonso, Rivas-Gonzalo, & Escribano-Bailon, 2006; Sun, Spranger, Roque-do-Vale, Leandro, & Belchior, 2001). However, there are no reports on the influence of different types of barrel on the evolution of proanthocyanidin composition in wines during maturation. Winemakers have been experimenting with barrel woods other than oak for wine maturation in order to minimize the enhancement of astringency, which is evident especially as far as white wines are concerned. Since white wines are characterized by low phenolic concentrations, changes in their tannin content can dramatically affect their mouthfeel and final quality.

In this study, changes on proanthocyanidin mDP and composition of the wines which stayed in contact with different types of barrel woods for different time periods were monitored. The aim of this project was to improve understanding on the relation between wine proanthocyanidin structure and astringency by exploiting the possible correlations between the sensory and analytical parameters of the wines. In addition, it was of interest to measure the evolution of the astringency of the barrel-aged wines according to the different wood types and contact times and thus to provide information which may be of technological interest.

2. Materials and methods

2.1. Wines and containers

Wines from two red (*Vitis Vinifera* cv Kotsifali and Mandilaria) and two white (*Vitis Vinifera* cv Vilana and Dafni) grape varieties differing greatly in their tannin composition were chosen to mature in barrels made of French and American oak, Acacia and Chestnut, woods that in turn also have been found to present differences in their ellagitannin content (Basalekou, Pappas, Tarantilis, Kotseridis, & Kallithraka, 2017). For comparison reasons, a portion of each wine was kept in inox tanks as a control sample. In order to examine the differences of wood size, inox tanks with French oak sticks (7 g/L oak sticks) immerged in them were also employed. All handlings were performed in duplicate. Chestnut was only used in the case of red wines. Vinifications were carried out during the 2013 vintage and samples were taken from containers every three months starting right after fermentation completion. For the production of red and white wines, the traditional red and white winemaking protocol -respectively- was followed. All analyses were carried out in triplicate. French (Quercus robur) and American (Quercus alba) barrels (225 L) with a medium toasting were purchased from 'Tonnellerie du Monde World Cooperage', Acacia barrels with a light plus (L+) toasting were purchased from Tonnellerie du sud ouest, and Chestnut barrels with a medium toasting were purchased from Tesias Metsovo, Greece. The oak sticks (French oak) were from Seguin Moreau (Oenostick®, V18).

2.2. Sensory analysis

Astringency was assessed by a trained panel consisting of 11 wine experts. Two repetitions of each sample were required, and red and white wines were evaluated in different tasting sessions, with each session furtherly divided into two sets according to the grape variety used in vinification. The judges were winemakers and enologists from the Wines of Crete network. The selection criteria were their experience in wine sensory assessment, however they were all trained in order to familiarize themselves with the term of astringency but also with the intensity scale (Chira et al., 2012). For this reason, aqueous solutions of aluminum sulfate (3 g/L) were prepared. After establishing identification of sensation, scaling training was employed, using model wine solutions containing different concentrations of the training substances (Chira & Teissedre, 2013). The judges were presented with 10 mL samples at room temperature and were asked to rate the intensity of astringency using a 0–10 point scale. Breaks were taken between samples, during which time the panelists could wash their mouths with water in order to minimize the risk of carry over effects.

2.3. Wine classical analyses

Conventional oenological parameters (ethanol content, pH, reducing sugar content, titratable and volatile acidities) were determined in accordance with the official OIV practices (International Organization of Vine and Wine, 2005). Total phenolic content was determined by the Folin-Ciocalteu method (Singleton & Rossi, 1965), total tannins using the method proposed by Ribéreau-Gayon and Stonestreest (1965), and total anthocyanins using the modified Somers assay as described by Mercurio, Dambergs, Herderich, and Smith (2007). All analyses were performed in triplicate.

2.4. Proanthocyanidin composition

Proanthocyanidin composition was determined following acid catalysis with phloroglucinol, as described by Chira et al. (2012). Phloroglucinolysis is based on the acid-catalyzed cleavage -in the presence of phloroglucinol- of flavan-3-ol interflavanoid bonds, after which the terminal unit is released as a flavan-3-ol monomer and C4

phloroglucinol adducts of the extension units are formed (Drinkine, Lopes, Kennedy, Teissedre, & Saucier, 2007). According to the method, 10 mL of wine (50 mL in the case of white wines due to their low phenolic content) were evaporated in vacuum under a low pressure, in a temperature of 40 °C in order to remove excessive alcohol. The wine that remained (approximately 4 mL) in the evaporator flask was re-suspended in water, making up a final volume of 20 mL. For proanthocyanidin isolation this solution was passed from C-18 SPE cartridges (Lichrolut C18, 5 gr octadecyl bonded endcapped silica, 25 mL volume), which were initially activated with 25 mL methanol and 25 mL distilled water. After adding the diluted wine extract, the cartridge was washed with 50 mL of distilled water and left to dry for 15 min. The elution of the compounds of interest was performed by adding 50 mL methanol. In order to obtain dry powder, the elutes were further evaporated under reduced pressure at 30 °C and then lyophilized. Finally, tannin extracts were re-dissolved in methanol to a final concentration of 20 g/L. To perform phloroglucinolysis, 100 μL of the redisolved solution were left to react with 100 μL of phloroglucinol solution (50 g/L phloroglucinol, 10 g/L ascorbic acid, 0.1 N HCl, all in methanol) at 50 °C for 30 min. The reaction was then stopped by adding 1 mL sodium acetate 40 mM. All solvents and reagents used were of high-performance liquid chromatography grade, purchased by Sigma Aldrich (St Louis, MO, USA).

Reaction products were analyzed by a Shimadzu 2010A LC/MS (Shimadzu corporation, Tokyo, Japan) operating in positive ion mode, coupled to a single quadrupole mass spectrometer equipped with an electrospray ion source (Kyraleou, Kallithraka, Chira et al., 2015; Kyraleou, Kallithraka, Koundouras et al., 2015). The source's temperature was 70 °C, the cone voltage at −30 eV, and capillary voltage was at 3.5 kV. Absorbance was recorded at 280 nm and mass spectra in the range of 50–1500 amu. Separation was performed on a reversed-phase XTerra RR C18 (100 × 4.6 mm, 3.5 μm) column (Waters, Massachusetts, USA), with a 20 μL injection volume at a flow rate of 0.5 mL/min, and the elution program was as described by Petropoulos, Kanellopoulou, Paraskevopoulos, Kotseridis, and Kallithraka (2017). All analyses were performed in triplicate. All flavan-3-ol monomer, terminal subunits, and phloroglucinol adducts, extension subunits, were expressed in moles.

Calculations:

mDP = SUM of all subunits (terminal and extension)/SUM of all terminal subunits

Percentage of prodelphinidins (%P) = 100 × (SUM of terminal and extension EGC subunits/SUM of all terminal subunits)

Percentage of galloylation (%G) = 100 × (SUM of terminal and extension ECG subunits/SUM of all terminal subunits)

(%C) = 100 × (SUM of terminal and extension C subunits/SUM of all terminal subunits)

(%EC) = 100 × (SUM of terminal and extension EC subunits/SUM of all terminal subunits)

2.5. Statistical analysis

Statistical analysis was performed using the JMP Statistical Discovery software, version 11. For mDP, %G, %P, %C, %EC and sensory analysis results, mean values and standard error were calculated, and analysis of variance (ANOVA) was performed, using Tukey's comparison tests when samples were significantly different ($p < 0.05$). ANOVA was also used to examine the possible interaction effects between the variables of this experiment. In order to determine whether sensory analysis results could be predicted using the results obtained by chemical analysis, Partial Least Squares (PLS) regression was used. PLS regression fits linear models based on linear combinations (factors) of the explanatory variable, obtained in such a way that maximizes the covariance between the explanatory variables.

3. Results and discussion

3.1. Wine conventional and total phenolic analysis

The alcohol content, titratable and volatile acidity, pH and reducing sugar content of all experimental wine samples are summarized in Table S1. The alcohol content ranged from 12.2 (Kotsifali) to 14.3% (Vilana) (v/v). For titratable acidity the values quantified varied from 4.50 (Dafni) to 6.54 (Vilana) $g\,L^{-1}$ tartaric acid, while pH values ranged from 3.35 (Mandilari) to 3.73 (Dafni). Volatile acidity values ranged from 0.22 (Vilana) to 0.39 (Vilana) $g\,L^{-1}$ acetic acid. These values are relatively low for young wines and they are under the acceptable upper legal limits ($1.2\,g\,L^{-1}$). Reducing sugar content ranged from 1.05 (Dafni) to 2.25 (Dafni) $g\,L^{-1}$ indicating that fermentation was complete in all samples. Total phenols, tannins and anthocyanins of the wines samples are presented in Table S2. As expected, these values differ significantly not only between red and white wines but also between the wines of the same category. In red wines, total anthocyanins ranged from 144.5 to 390.8 mg/L, while total tannins from 1.25 to 3.42 g/L and total phenols from 1024 to 2787 mg/L. In white wines total tannins ranged from 0.27 to 0.35 g/L while total phenols ranged from 289 to 427 mg/L. All values fall within the ranges reported in literature.

3.2. Sensory analysis

A graphical representation of the results of the sensory analysis carried out during this study is given in Fig. 1.

As expected, wines from white grape varieties were perceived as low to medium astringent, while red wines were more astringent (medium to high). In agreement with the findings of Kallithraka, Kim, Tsakiris, Paraskevopoulos, & Soleas (2011), Mandilari wines were the most astringent wines tested. On the other hand, Kotsifali wines, which are characterized by their low anthoycanic and tannin content (Basalekou et al., 2017), have been described as medium astringent with the exception of the wines maturing for 6 months which were perceived as highly astringent. As it can be seen in Fig. 1, the type of the container of the red wines did not result in perceived astringency differences by the panel for all sampling periods. A possible explanation is that astringency of red wines was already high enough that any further increase or decrease could not be easily detected. However, in white wines, the influence of the type of barrel wood on perceived astringency is evident. Interestingly, the only type of container (with the exception of the inert inox tank) that does not seem to influence astringency of white wines, even after 9 months of contact, is Acacia barrel.

Although wine astringency is mainly attributed to phenolic compounds, it could also be influenced or modified by the simultaneous presence of other compounds such as for example organic acids, ethanol, sugars etc. (Kallithraka et al., 2011). According to the results presented in Tables S1 and S2 the chemical composition of the wine samples used in this study was not similar. However, the selection of the samples was based on this high variability since the purpose of this study was to construct a 'model' that could be used for astringency estimation of barrel aged wines irrespectively of the wine color and grape variety.

3.3. Mean degree of polymerization

In order to examine if there is a relation between astringency and mDP, the mDP values of all wines were recorded every three months for a total period of nine months during maturation. In Fig. 2, mean values regardless of the type of container used during maturation can be seen.

It appears that mDP values of white and red wines are quite similar, however there are not any data available concerning white wine mDP values in order to compare with the data obtained in this study. In average, the lowest mDP values recorded were 2.02 and 2.07 (for

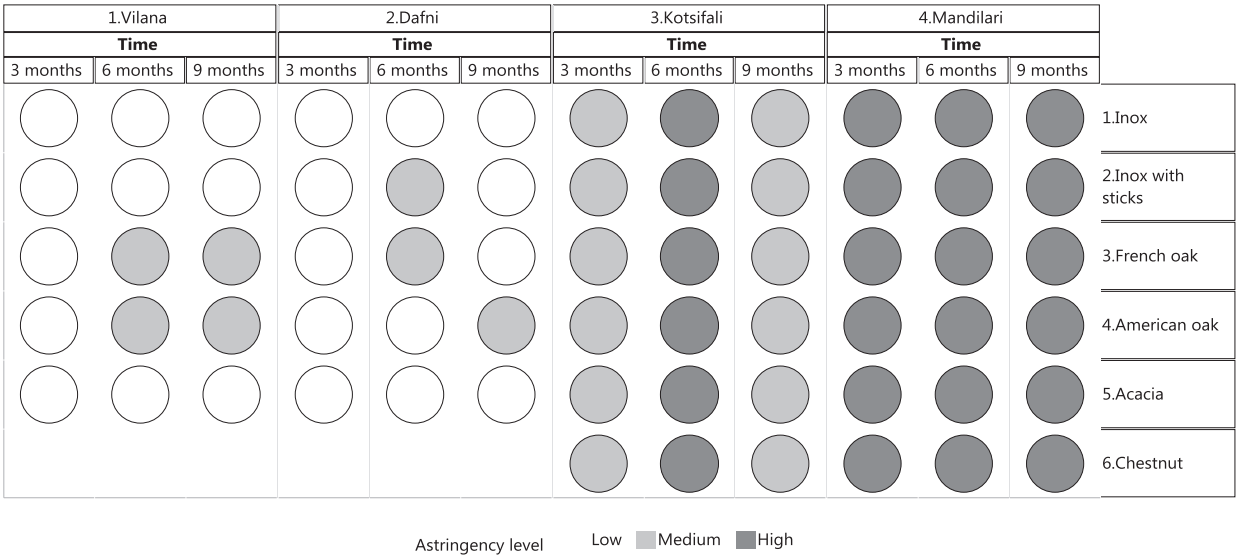

Fig. 1. Astringency levels (low, medium, high) according to variety, type of container and time. For astringency description, the following ranges were used: Low astringency (1–3), Medium Astringency (3.1–5) and High Astringency (5.1–7).

Vilana and Kotsifali respectively), while the highest values obtained were 3.25 and 3.05 (for Dafni during 9 and 3 months of maturation respectively).

To verify if there is a relation between mDP and astringency, statistical analysis was performed. Contrary to the results reported by some authors (Chira et al., 2012; Chira, Pacella, Jourdes, & Teissedre, 2011; Vidal et al., 2003) but also in agreement with other studies (Kyraleou et al., 2016) in this experiment astringency and mDP levels do not seem to correlate as shown in Fig. 3a (R^2 = 0.0085 and Root Mean Square Error = 1.82, p = 0.463).

3.4. Procyanidin composition

Since mDP is a value calculated as a ratio between the sum of all

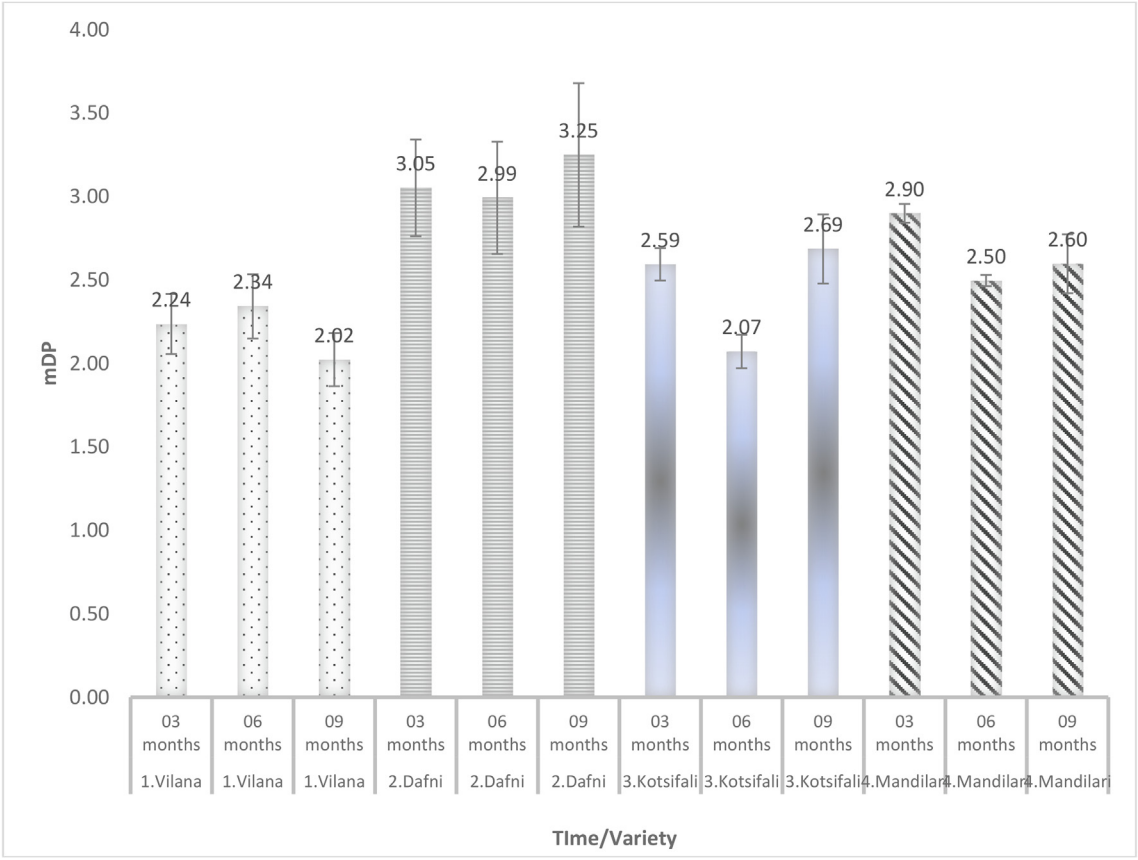

Fig. 2. Mean Degree of Polymerization (mDP) during barrel maturation according to the variety and time of contact (3, 6, or 9 months after fermentation). The mDP values are presented as the average value of all wine samples of each variety independently of the type of container used during maturation.

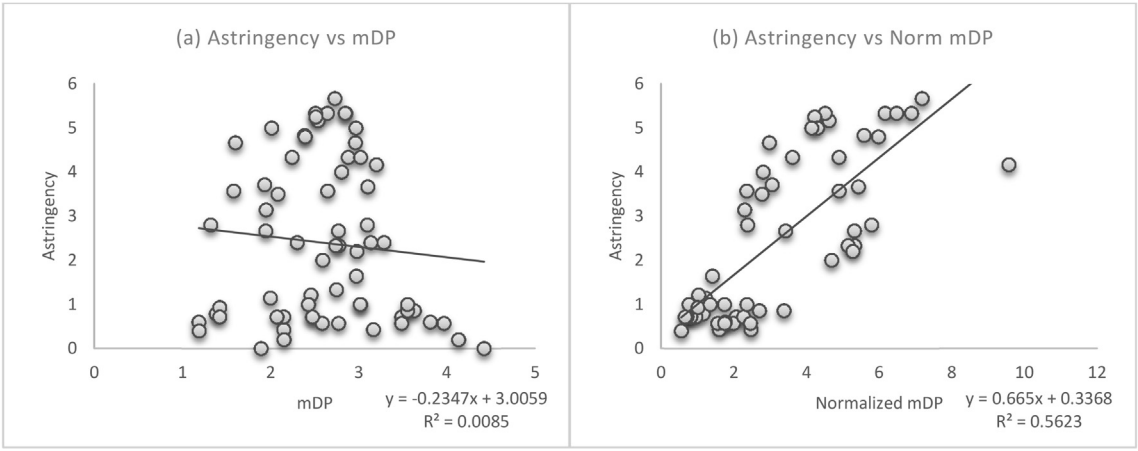

Fig. 3. a and b. Correlation between Astringency and mDP values (a) and Astringency and Normalized mDP values (b).

subunits and the sum of all flavan-3-ol monomers, it does not take into account tannin absolute concentration. Indeed, in this experiment the wine volume used for the analysis of white wine tannin mDP was five times higher than that used for the respective analysis of red wines (50 mL instead of 10 mL used for the analysis of red wines) in order to obtain measurable results.

Since total proanthocyanidin content in wines has also been found to correlate with astringency (Cáceres-Mella et al., 2013), statistical analysis was performed for a second time, after normalizing mDP values according to each wine's total proanthocyanidin content. For this purpose, the pure weight of the tannins collected after the lyophilization step expressed in milligrams per litre was used as a multiplication factor for the actual mDP values (Normalized mDP = proanthocyanidin (mg/L) * mDP/1000). The results obtained revealed a significant correlation with astringency since they also take into account the phenolic content of the wines (Fig. 3b). The resulting equation is the following: Astringency = 0.3368 + 0.665 * Normalized mDP, however with a significant but still low R^2 of 0.56 and RMSE = 1.21 ($p < 0.0001$) suggesting that more information is necessary in order to construct a model based on mDP, which can explain and predict wine astringency.

Moreover, it is important to determine the individual tannin subunit composition of each variety irrespectively of the effect of the container in order to better understand the evolution of tannin structure during maturation and/or aging. For this reason, the percentages of the individual subunits detected in each control wine maturing in inox tanks are shown in Fig. 4.

As mentioned earlier, tannin polymers are mainly composed of C, EC, GC, EGC, ECG and EGCG subunits. The most prominent difference between the four varieties studied is that for the white varieties Vilana and Dafni, and for the red variety Kotsifali the dominant subunit is EGC, whereas in red variety Mandilari the dominant one is EC. (−)-Epicatechin has been found to be significantly more bitter and astringent than C (Kallithraka, Bakker, & Clifford, 1997), while the presence of EGC subunits in proanthocyanidins has been reported to reduce the "coarse" astringent sensation and to be negatively correlated with astringency (Kyraleou et al., 2016; Vidal et al., 2003). (−)-Epicatechin subunits in extension positions, like in the case of Mandilari wines, have also been characterized remarkably important in affecting astringency (Quijada-Morín et al., 2012). These findings are in agreement with the results of the sensory analysis, according to which the most astringent variety, was Mandilari.

The percentages of the subunits detected in white and red wines according to the type of the container and irrespectively of the sampling time are shown in Fig. 5(a and b). During maturation, white wine tannins mainly consist of EGC (terminal or extension) subunits, while the highest percentage was observed in wines maturing in Acacia barrels (Sum of extension and terminal EGC subunits: 63.9%) (Fig. 5a and b). In red wines, EC is the dominant monomer except for the case of Acacia barrels and inox tanks with oak sticks, where the dominant one is EGC.

Acacia barrels and Inox containers with sticks seem to be the only containers among all others of the experiment, where EGC subunits are present in higher percentages between wine tannins in both red and white wines. Previous research reported that the occurrence of terminal EGC subunits could reduce precipitation with salivary proteins (Ricardo-da-Silva et al., 1991), while Quijada-Morín et al. (2012) reported that the amount of EGC in both extension and terminal positions is negatively correlated with the perceived astringency. Recent research on tea tannins reported that EGC is the less astringent than ECG and EGCG (Xu et al., 2018).

3.5. Astringency estimation

In order to examine if the percentage of monomers determined after tannin phloroglucinolysis could provide a more accurate estimation of astringency, Partial Least Squares Regression (PLS) analysis was performed. This type of analysis takes into account all proanthocyanidin subunits and is particularly useful when there are variables that are highly correlated. PLS (NIPALS method) uses the van der Voet T2 test and the leave-one-out validation, after which the optimal number of factors chosen for our experiment was 4. The prediction equation provided by the statistical analysis was the following:

Astringency = [−0.125 * ("%ECG-P")] + [0.158 * ("%ECG")] + [−0.019 * ("%EGC-P")] + [−0.024 * ("%EGC")] + [−0.404 * ("%C-P")] + [−0.035 * ("%C")] + [0.109 * ("%EC-P")] + [−0.065 * ("%EC")] + 4.921.

Using the equation, the values for the "predicted astringency" were calculated for all samples and after, both predicted and actual values were used for correlation purposes (Fig. 6).

According to Fig. 6, the subunit percentages provided a better model for the prediction of astringency, with $R^2 = 0.754$ and Root Mean Square Error = 0.91 ($p < 0.01$). According to our knowledge, this is the first time that a model for the prediction of astringency is provided using both red and white wines in the calibration set, and which could be used as an index during wine maturation.

3.6. General discussion

According to the literature, white wine phenolics are characterized by their ring structure and can be found in different forms based on their patterns of hydroxylation, esterification, glycosylation or conjugation with amino acids (Gawel, Smith, Cicerale, & Keast, 2017). They are mostly monomeric; however, dimmers and trimers can also be found in lower concentrations. As short chained groups, they are

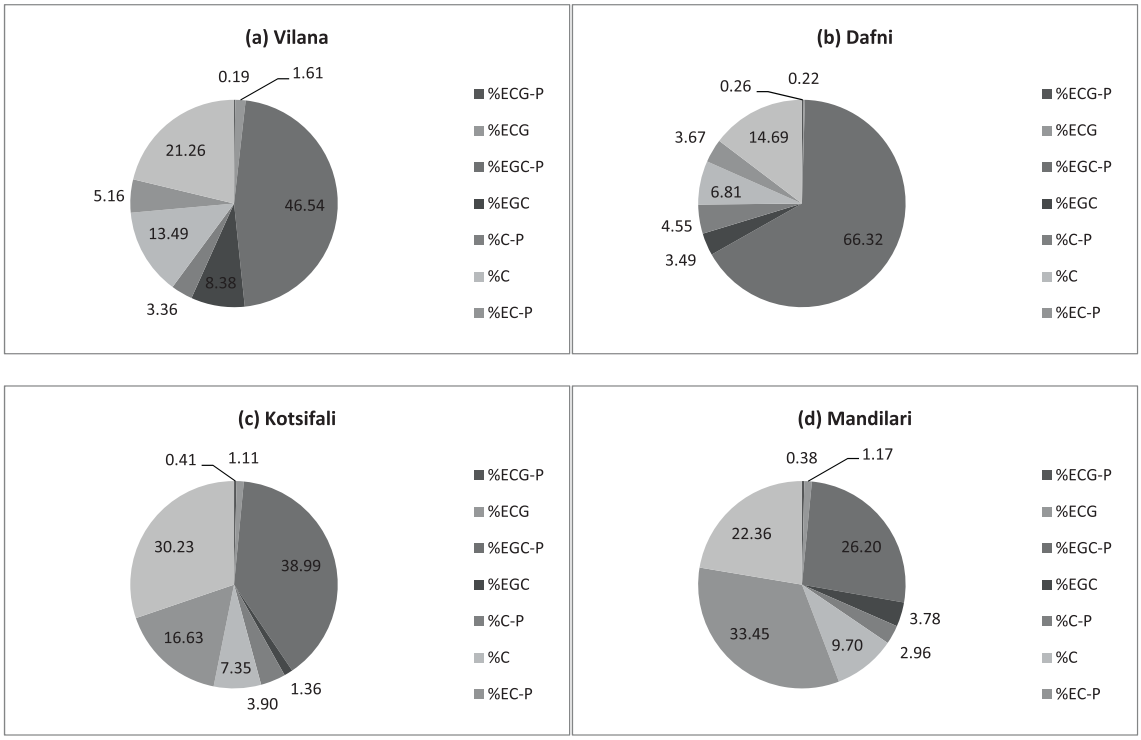

Fig. 4. Percentage of monomer subunits (terminal or extension) per variety for control wines (in inox tanks) 3 months after the end of fermentation (ECG-P, EGC-P, C-P, EC-P: extension subunits, ECG, EGC, C, EC: terminal subunits).

characterized by a low degree of polymerization, with the dominant flavanols being (+)-catechin and its stereoisomer (−)-epicatechin (Gawel et al., 2017).

As richer in tannins, red wines would be expected to contain tannins with higher mDP values than white wines. Rinaldi, Iturmendi, Jourdes, Teissedre, and Moio (2015) observed a significant influence on wine astringency based on the tannin extraction method and grape variety. Red wines are richer in total tannins than white wines, due to the maceration process during fermentation where tannins are extracted from the grape skins and seeds to the must. Maceration for white wines is not so common; however, there are cases where it takes place but usually before alcoholic fermentation begins and for a much shorter period resulting in wines with lower total phenolic concentrations (Gawel et al., 2017). It is possible thus, the astringency of white wines to be similar with that of red wines when tasted at equal tannin concentrations. However, this is not the case since red wines are much richer in tannins compared with white wines. Gonzalez-Manzano et al. (2006) also reported that if the maceration period is not long (as in the traditional red winemaking which was followed for the production of the red wines of this experiment), mostly monomers to trimers can be extracted by the grapes, resulting in an mDP value of 2.3, which agrees with the findings of our research.

MDP values of red wine have been reported to range from 4.9 to 9.8 for Cabernet Franc and Sangiovese wines respectively, while analysis of different vintages of Cabernet Sauvignon revealed a range from 1.8 (vintage 1978) to 7.6 (vintage 2004) (Chira et al., 2012; Gris et al., 2011). Moreover, Carmenere wines had mDP values that ranged from 7.4 to 13.6 during 2004 and 2006 vintages, while, reported mDP for Tinta Miuda red wine was 22.1 (Sun et al., 2001). However, recent research regarding an indigenous Greek red variety (Agiorgitiko) reported lower mDP values, ranging from 1.43 to 2.27 (Petropoulos et al., 2017). Since, according to the literature, this parameter is characterized by high heterogeneity it could hardly be considered as an index to be used for characterization or classification purposes. Indeed, mDP has different values depending on the grape variety, the vintage but also the winemaking technique used (for example stem contact or not, carbonic

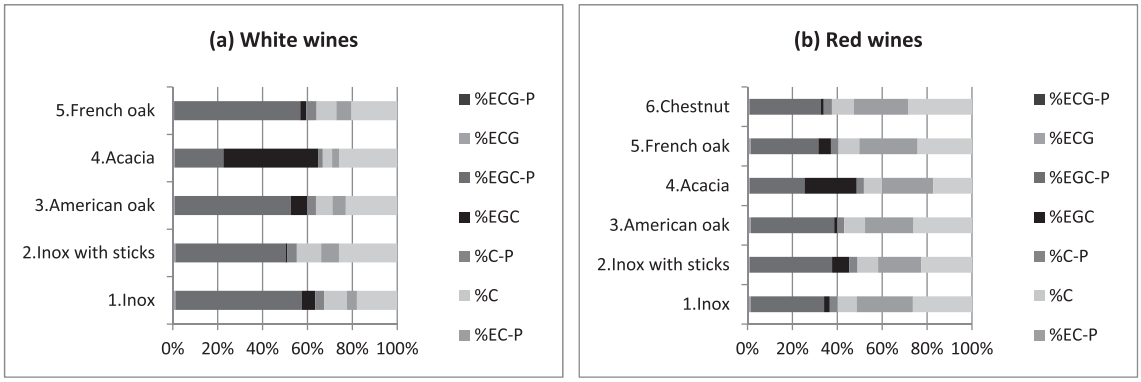

Fig. 5. a and b: percentages of monomer subunits according to the type of container as an average for all samplings (3, 6 and 9 months in barrel) for white (a) and red (b) varieties.

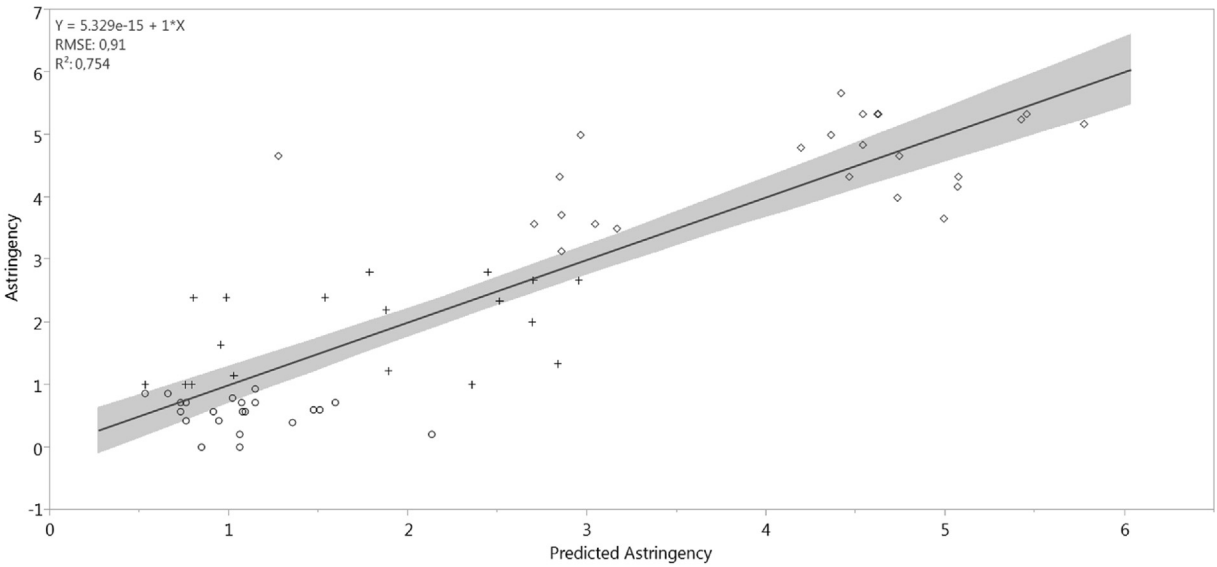

Fig. 6. Predicted versus Actual Astringency results, based on the results of PLS regression analysis.

maceration or classic vinification) (Chira et al., 2012; Sun et al., 2001). Recently, the mDP values of white wine lees (Chardonnay) were determined and they were found to be much higher than the respective values of the red wine lees, even though the initial total phenolic content was lower (Zhijing, Shavandi, Harrison, & Bekhit, 2018) suggesting that a categorization of red and white wines according to their mDP values might not be possible. In accordance with the findings of Zhijing et al. (2018), in this experiment the highest mDP value was recorder for a white wine (Dafni), while this variety also exhibited the highest variation in mDP values depending on the container used during maturation.

According to previous works, the intensity of astringency is related to total concentration of grape phenolic compounds, total proanthocyanidin content, their mean degree of polymerization (mDP) and subunit composition (Chira et al., 2012; Kyraleou et al., 2016; Quijada-Morín et al., 2012). However, comprehensive investigations on the relation between grape proanthocyanidin content and composition with wine or grape sensory properties are rather fragmentary and the results are contradictory. Several published articles have examined either the influence of total tannin or phenolic content (Jeffery, Mercurio, Herderich, Hayasaka, & Smith, 2008; Kallithraka et al., 2011) or the effect of tannin composition (Quijada-Morín, Williams, Rivas-Gonzalo, Doco, & Escribano-Bailón, 2014; Wollmann & Hofmann, 2013) on grape or wine astringency. However, even when both proanthocyanidin concentration and composition have been examined simultaneously (Chira et al., 2011; Ćurko et al., 2014; Kyraleou et al., 2016; Quijada-Morín et al., 2012) the results are not in agreement. Chira et al. (2011) reported that mDP exhibited a higher influence on wine astringency compared with total phenolic compounds or total tannins. In addition, Kyraleou et al. (2016) demonstrated that astringency is more highly correlated with grape total phenolic and proanthocyanidin content than tannin structural composition, whereas the results presented by Quijada-Morín et al. (2012) showed that astringency in wine is more affected by subunit composition than by the total concentration. The presence of galloyl groups (%G), is also a critical factor for astringency. Nevertheless, controversies have also been reported in the literature regarding this issue. %G values correlated positively with perceived astringency in several studies (Chira et al., 2011; Ćurko et al., 2014) while others either report absence of correlation (Kyraleou et al., 2016; Wollmann & Hofmann, 2013) or negative correlation as in the case of grape seed extracts studied by Chira et al. (2015). Interestingly, in the case of EGC, most of the published data are in agreement that it is negatively correlated with astringency perception (Chira et al., 2015; Kyraleou et al., 2016; Quijada-Morín et al., 2012; Vidal et al., 2003). Therefore, the profile of the monomers composing in each polymer is a better approach to estimate astringency as it was also showed in this study (Fig. 6).

According to Fig. 5(a and b), the type and size of wood used during maturation influences the percentage of monomer subunits. In order to examine which of the parameters studied in this experiment exerted the major effect on mDP, Analysis of Variance (Supplementary Table S3) was performed. It was revealed that although mDP was significantly affected by experimental parameters (variety and type of container) the type of container had the highest influence. Moreover, the interaction between the type of container and the variety was also significant indicating that these two experimental parameters are not independent as far as their influence on mDP is concerned.

Indeed, the effect of the variety on proanthocyanidin content is decisive since it determines the concentration and type of tannins found in the wine, and thus their polymerization degree. Regarding time, it is well documented that the extraction of phenolic compounds depends on the time of contact, while their content has been found to also to vary as in relation to oxidation, polymerization but also condensation reactions which are favored by the oxygen diffused into the barrels (Rubio-Bretón, Garde-Cerdán, & Martínez, 2018). The type of container on the other hand, also influences proanthocyanidin content, as different types of wood enrich the wine with different types of tannins, also affecting mDP values (Zhang, Cai, Duan, Reeves, & He, 2015). Total flavonoid content in wines maturing for nine months in barrels of acacia, chestnut and oak, showed different evolution patterns. Wines in contact with oak were characterized by the highest concentrations while those in contact with chestnut by the lowest. Moreover, chestnut has been found to be rich in gallotannins and to contain high amounts of phenolic compounds (Zhang et al., 2015). Changes in proanthocyanidin concentrations of wines aging in oak barrels have also been monitored by Watrelot et al. (2018), who reported that EC extension and terminal units decreased, while EGC (extension) and mDP increased.

4. Conclusions

In this study, the proanthocyanidin mDP values of all wine samples (both red and white) were not significantly correlated with the perceived astringency suggesting that this parameter may not be used as an independent variable for astringency estimation. However, when mDP values are considered in combination with the total proanthocyanidin

content of the wines (normalized values) a better statistical significant correlation can be obtained. Furthermore, the type of subunits comprising the tannin chains possibly exert the strongest influence on the intensity of astringency indicating that their analytical determination might be a valuable tool for astringency estimation during wine maturation in barrels. In addition, as far as the different containers are concerned, Acacia barrels did not result in astringency enhancement of white wines even when used for longer contact periods (at least 9 months) which might be of technological interest to winemakers.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.125135.

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