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Addition of wood chips in red wine during and after alcoholic fermentation: differences in color parameters, phenolic content and volatile composition

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Abstract

Aim: The effect of the time of wood chip addition on phenolic content, color parameters and volatile composition of a red wine made by a native Greek variety (Agiorgitiko) was evaluated.

Methods and results: For this purpose, chips from American, French, and Slavonian oak and Acacia were added in the wine during and after fermentation. Various chemical parameters of wines were studied after one, two and three months of contact with chips. The results showed that the addition of oak chips during alcoholic fermentation did not favor ellagitannin extraction and the reactions involved in tannin condensation and anthocyanin stabilization. Moreover, wines fermented with wood chips contained higher contents of whiskey lactones, eugenol, ethyl vanillate and acetate esters while their ethyl ester content was lower compared with the wines where chip addition took place after fermentation.

Conclusion: When chips are added after fermentation, wines seem to have a greater aging potential compared to the wines fermented with chips due to their higher ellagitannin content and enhanced condensation reactions. On the other hand, color stabilization and tannin polymerization occur faster when chips are added during fermentation, resulting in shorter aging periods suitable for early consumed wines.

Significance and impact of the study: The outcomes of this study would be of practical interest to winemakers since they could improve the control over the wood extraction process.

Keywords: oak chips, Agiorgitiko, Acacia chips, tannins, anthocyanins, ellagitannins, wood derived volatiles

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Introduction

Wine aging in oak wood barrels is an enological practice aiming at improving the overall quality of wine. During this process wine undergoes important modifications mainly due the slow and continuous diffusion of oxygen through the wood pores and the extraction of many wood derived substances which stabilize the color and add organoleptic complexity. Such compounds include polysaccharides, hydrolysable and condensed tannins, and gallic and ellagic acids (Koussissi *et al.*, 2009 ; Chira and Teissedre, 2013a, 2013b). In addition, the aroma complexity is increased due to the extraction of various wood derived volatile compounds which are transferred to the wine and modulate its organoleptic quality.

The type of wood from which a barrel is made contributes to the characteristics of the wine stored in it (Kyraleou *et al.*, 2015). However, the barrel aging process has several disadvantages including longer aging time and higher cost (Gomez Garcia-Carpintero *et al.*, 2012). Considering these, new inexpensive techniques have been developed to simplify and reduce the cost of the aging process. One of these techniques is the addition of small wood chips - commonly known as oak chips - to the wine kept in stainless steel tanks or used barrels (Gomez Garcia-Carpintero *et al.*, 2011). There is no official terminology for oak chips, although the weight/surface area ratio permits some differentiation based on the particle size (Gomez Garcia-Carpintero *et al.*, 2012).

The addition of oak chips has been a widely used technique in USA, Australia and other countries of South America for several years. However, in Europe the use of oak wood pieces in winemaking was not approved until 2006 (Council Regulation EC No. 2165/2005) and since then the addition of oak chips has been generalized.

The oak species most often used in cooperage are *Quercus alba* (American white oak) and two European species, *Quercus robur* L. (pedunculate oak) and *Quercus petraea* Liebl. (sessile oak). Moreover, Slavonian oak wood from *Q. robur* and *Q. petraea* is considered suitable for wine aging (Chira and Teissedre, 2015 ; Kyraleou *et al.*, 2015). Other types of wood as false acacia (*Robinia pseudoacacia*), chestnut (*Castanea sativa*) and cherry (*Prunus avium*) are also being considered as possible sources of wood for aging wines and their derived products.

The wood chips may be added to the grape must or, alternatively, they can be placed into the tanks of finished wine (Gordillo *et al.*, 2013). The addition of oak chips to finished wines and their effect on wine chemical composition and sensory character has been widely studied (Gambuti *et al.*, 2010 ; Chira and Teissedre, 2013a, 2013b, 2015 ; Kyraleou *et al.*, 2015). However, their addition during alcoholic fermentation has been scarcely studied. Gomez Garcia-Carpintero *et al.* (2012), Rodriguez-Bencomo *et al.* (2010) and Soto Vazquez *et al.* (2010) studied the effect of the addition of oak chips during fermentation on volatile content, phenolic composition and chromatic characteristics, respectively. The results concerning the effect of pre-fermentative chip addition on wine color are contradictory. Rodriguez-Bencomo *et al.* (2010) concluded that the addition of oak chips did not favor color stabilization, while Gordillo *et al.* (2013) reported color enhancement and stabilization during the same process. Moreover, the presence of oak chips during alcoholic fermentation enhanced the formation of ethyl esters and fusel alcohol acetates while it reduced the contents of some lactones and volatile phenols in Tempranillo wines (Rodriguez-Bencomo *et al.*, 2010).

The investigation presented herein was undertaken for examining the changes in compositional parameters in Agiorgitiko wine as a result of wood chips addition. Very few studies exist on the evolution of phenolic and volatile compounds of Greek wines that have been in contact with wood pieces after fermentation (Koussissi *et al.*, 2009 ; Kyraleou *et al.*, 2015), while the addition of chips during fermentation has not been studied yet. Moreover, to the best of our knowledge, the present study is the first to compare the changes in phenolic composition and color parameters of wines fermented with chips with those where chip addition took place after fermentation. The outcomes of such study would be of practical interest to winemakers since they could improve the control over the wood extraction process and thus optimize the sensory character of the produced wine.

Materials and methods

1. Samples

The wood pieces used in this study were commercial products supplied by Oak Add-Ins Nadalié (Ludon-Medoc) cooperage. A total of five different types of medium toasted wood chips were considered : American oak (*Q. alba*), French oak (*Q. robur*), Slavonian oak (*Q. robur*), Acacia (*Robinia*

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pseudoacacia) and a mixture (50 : 50) of American and French oak (Kyraleou *et al.*, 2015). Each type of wood fragments was added to either the finished wine or to must in duplicate, at a final concentration of 2.5 g/L.

In this way two sets of samples were prepared : Control (C) (no addition of chips), American oak (Aa, Ad), French oak (Fa, Fd), Slavonian oak (SLa, SLd), Acacia (ACa, ACd) and the mixture of American and French oak (AFa, AFd). Letters a and d indicate whether the wood chip addition took place after (a) or during (d) fermentation. Samples were analyzed after 1, 2 and 3 months in contact with the chips.

Red wine was produced from *Vitis vinifera* cv. Agiorgitiko grapes from the Nemea region in Peloponnese. After crushing and destemming, 60 mg/L SO_2 (as potassium metabisulfite) was added to the grapes (followed by the addition of the different types of wood chips in the case of the second set of samples). The following day, the addition of lyophilized yeasts (commercial strain St Georges, Fermentis) at 20 g/hL (previously hydrated in water 15 min, 38 °C), pectolytic enzymes (Uvazym couleur, Esseco, Italy) at 3 g/hL and nutrients (Superstart) at 20 g/hL took place. The pomace was punched down twice a day for one week. Juice was separated from the pomace when the sugar content reached half of its initial value. For malolactic fermentation, Viniflora *oenos* malolactic culture (6.25 g/hL) was used, and after the completion of fermentation the wines were supplemented with 50 mg/L SO_2 (as potassium metabisulfite) and stored at 18 ± 2 °C in the dark until analyzed. The addition of wood chips took place at the time of pomace separation (in the case of 'd' samples) and just after the end of alcoholic fermentation (in the case of 'a' samples).

Conventional enological parameters of the control wine (ethanol content v/v : 12.9 % ; pH : 3.45 ; titratable and volatile acidity : 4.9 and 0.25 g/L, respectively ; and free and total sulfur dioxide : 25 and 98 mg/L, respectively) were determined according to official OIV methods (OIV, 1990) just after the end of alcoholic fermentation.

2. Spectrophotometric analyses

Several classical analytical parameters (hue, color intensity, total polyphenols - OD280) were determined in wines according to OIV methods (1990). Total phenolic content (TP) (Waterman and Mole, 1994), total anthocyanin content (TA) (Ribéreau-Gayon and Stonestreet, 1966) and proanthocyanidin content (P) (Wallace and Giusti, 2010) were also determined.

For the determination of tannin content two widely used methods were employed. The first method measures tannin concentration (g/L) after heating in acid medium and conversion into cyanidin (TT) (Ribéreau-Gayon *et al.*, 1998), while the second method is a protein based (Bovine Serum Albumin-BSA) tannin precipitation assay (BSA) (Harbertson *et al.*, 2003). All analyses were performed in triplicate.

3. Total ellagitannins

The total ellagitannin (ET) concentration was determined by the quantification of ellagic acid released during acidic hydrolysis (2 h at 100 °C, 2N HCl in methanol) as previously described (Chira and Teissedre, 2013a). Each sample was analyzed in triplicate by HPLC (Kyraleou *et al.*, 2015).

4. Anthocyanin content

The monomeric anthocyanins were determined by HPLC according to the method described by Kyraleou *et al.* (2015).

5. Volatile content

The wood derived volatile compounds were determined according to the method described by Kyraleou *et al.* (2015).

Ethyl esters and acetates were analyzed by GC–MS using the Head-Space Solid Phase Micro Extraction (HS-SPME) methodology. A volume of 25 mL of wine spiked with 25 µg 3-octanol was placed in a 40-mL vial and supplemented with 3 g NaCl. The vial was then placed on a heating stir plate and the solution was equilibrated by magnetic stirring at 750 rpm for 5 min at 30 ºC. The SPME needle was inserted manually through the vial septum and the fiber (DVB/CAR/PDMS, 75 µm) was exposed to the headspace of the sample for 30 min at 30 ºC. The fiber was retracted and the SPME device was inserted into the injector for thermal desorption for 10 min. 3-Octanol was used as an internal standard. Analysis was performed using an Agilent 7890A GC equipped with an Agilent 5873C MS detector. The column used was an HP-5 capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) and the gas carrier was helium with a flow rate of 1 mL/min. The injector and MS-transfer line were maintained at 250 ºC and 260 ºC, respectively. Oven temperature was held at 30 ºC for 5 min and raised to 160 ºC at 4 ºC/min and then to 240 ºC at 20 ºC/min. The selective ion monitoring (SIM) mode was applied, and ethyl

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butanoate (*m/z* 88), ethyl hexanoate (*m/z* 88), ethyl octanoate (*m/z* 88), ethyl 2-methylbutanoate (*m/z* 102), ethyl 3-methylbutanoate (*m/z* 88), isoamyl acetate (*m/z* 43), 3-octanol (*m/z* 59) and 2-phenylethyl acetate (*m/z* 104) were used as quantifier ions.

6. Statistical analysis

All chemical determinations were run in triplicate and values were averaged. The standard deviation (S.D.) was also calculated. The percentage changes were calculated as follows: % change = (measured

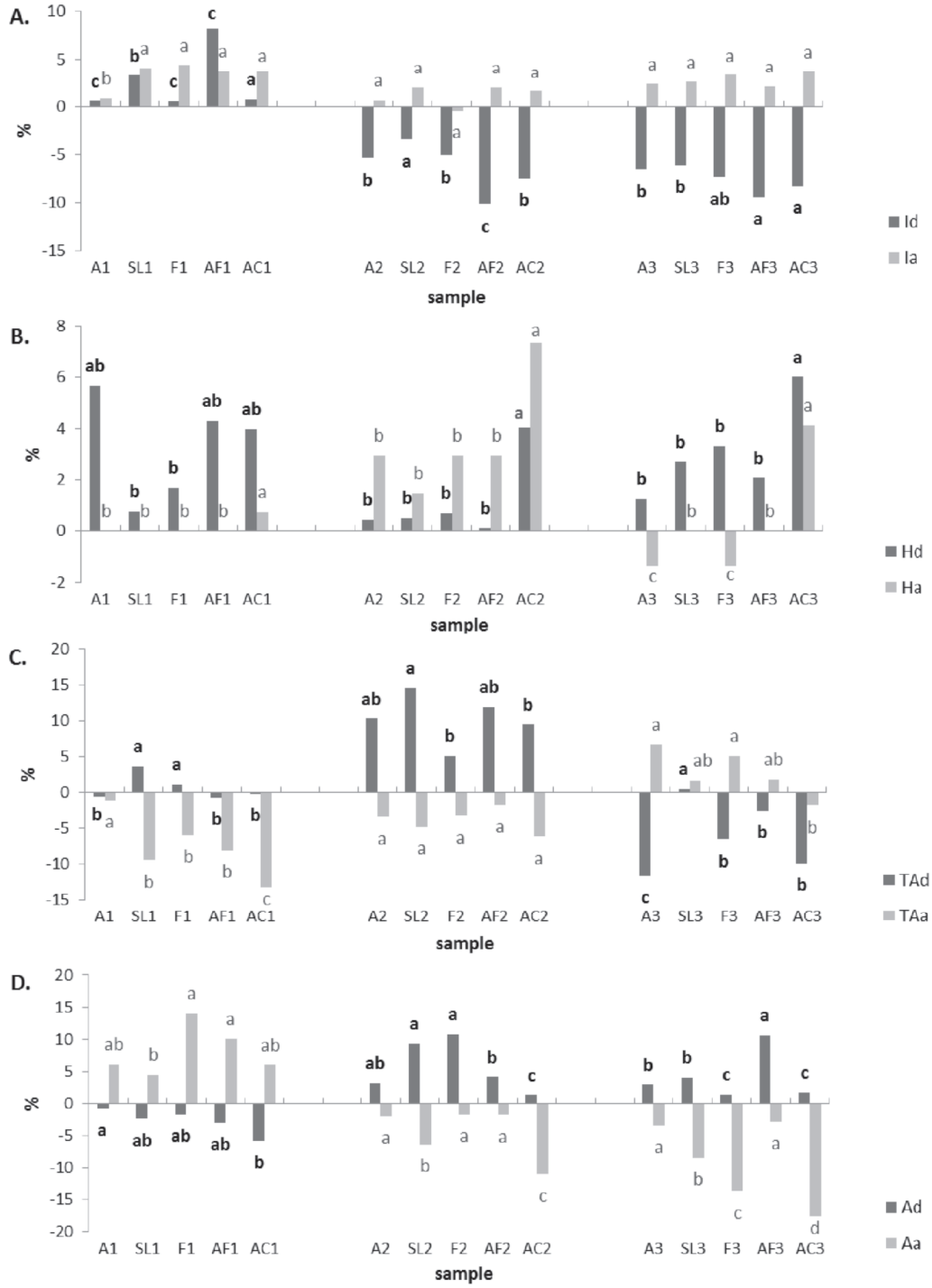


Figure 1 - Percentage changes of the analytical parameters of the wine samples in relation with the control wine: A. color intensity (I), B. hue (H), C. total anthocyanins (TA) and D. sum of individual anthocyanins determined by HPLC (A) after the addition of American (A), French (F), mixture of American-French (AF) and Slavonian (SL) oak and Acacia (AC) chips in wines during (d) and after (a) fermentation.

Numbers 1, 2 and 3 indicate 1, 2 and 3 months of contact time, respectively.

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parameter value of the sample-measured parameter value of the control/measured parameter value of the control) X 100.

Data were subjected to one-way analysis of variance (ANOVA) with Statistica V.7 Software (Statsoft Inc, Tulsa, OK). Comparison of mean values was performed using Tukey's HSD test when samples were significantly different ($p < 0.05$). Principal Component Analysis (PCA) was employed to examine any possible grouping of samples (SPSS 21.0, SPSS Inc).

Results and discussion

1. Color parameters

The percentage changes of the color parameters (color intensity, hue, total anthocyanins and sum of individual anthocyanins determined by HPLC) of Agiorgitiko wine as a result of wood chip addition (during and after fermentation) after 1, 2 and 3 months of contact time are shown in Figure 1. Since it is critical to determine the impact that the two applied techniques have on wine quality, it was thought that calculating the differences between the measured analytical parameter values of the samples and those of the control would be of more practical interest.

Intensity values (Ia) increased in most of the samples as a result of wood chip addition after fermentation. The increase was more profound after the first month followed by the third month. Intermediate changes were observed after the second month of contact with the wood chips. The different wood types did not result in significant differences among the samples (with the exception of A sample during the first month). These results confirm previous studies (Del Alamo Sanza *et al.*, 2004; Gambuti *et al.*, 2010) showing a positive effect of wood aging on red wine color stabilization. However, when chips were added during fermentation, a different pattern was observed. Intensity values (Id) increased after the first month in all the samples but to a lesser extent (with the exception of AF sample) in comparison with the samples where chip addition took place after fermentation. AF sample was characterized by significantly higher I value while F and AC resulted in less increase. However, after two and three months of contact with chips, color intensity values decreased in all the samples compared with the control (Figure 1a). After the second month, AF sample was characterized by significantly lower I value followed by AC while SL was the sample with the lowest I reduction compared with the control. After the third month, AC and AF were also the samples with the highest I reduction while in SL and A samples the reduction was lower. Regarding color hue (tint) (Ha), almost no change was observed after the first month of wood chip addition after fermentation (with the exception of AC sample) (Figure 1b). After two months, Ha of all samples increased in comparison with the control while after three months it increased only in the case of AC sample. In the case of samples where chip addition took place during fermentation, Hd showed a considerable increase after the first and the third months (Figure 1b). After the first month, the highest increase was observed in A sample while the lowest was observed in SL and F samples. The sample that contained the Acacia chips resulted in a significantly higher increase after the second and third months. The remaining wood types did not show any significant differences among them after two and three months.

Moreover, regarding total anthocyanins (TA) (spectrophotometric method), wood chip addition after fermentation resulted in considerably lower amounts compared with the control sample after the first month followed by a lower decrease after the second month (Figure 1c). AC was again the sample that differed significantly from the rest of the samples after the first month, while after the second month no significant differences were observed among samples. Finally, after three months of contact time, TA values were higher in most of the samples when compared with the control, with A and F samples containing the highest amounts. When the addition of wood chips took place during fermentation, TAd values after the first month were decreased (although not statistically significantly) only in the cases of A, AF and AC samples. TAd values of all samples increased after the second month (with SL sample showing the highest increase) while after the third month a decrease was noticed (with the exception of SL sample) (Figure 1c). The highest decrease was observed in the A sample. These results are in agreement with previous studies (Gambuti *et al.*, 2010) where a decrease of total anthocyanins was observed as a result of wood aging. Regarding total anthocyanin concentration (A) determined with HPLC (calculated as the sum of individual peaks), the addition of chips after fermentation increased their content in all the wine samples (with F and AF samples showing significantly higher values) after the first month (in comparison with the control) while after the second and third months a decrease was noticed (AC sample in both cases resulted in a significantly higher decrease of the A value) (Figure 1d). When chip addition took place during fermentation, the opposite pattern was observed. Ad values were decreased after the first month while

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after two and three months control sample contained lower amounts than the rest of the samples. AC was the sample that resulted in the highest decrease (after the first month) and lowest increase (second and third months) of the Ad value. The lower anthocyanin concentration observed in AC sample might be attributed to its particular porous structure which allows higher extraction rates of ellagitannins (Sanz *et al.*, 2012) and consequently faster depletion of monomeric anthocyanins through condensation reactions.

The above results are in agreement with the results obtained by other authors (Soto Vazquez *et al.*, 2010) who observed that the pre-fermentative addition of oak chips did not favor the reactions involved in anthocyanin stabilization and color increase. Wood chip addition during fermentation resulted in wines with lower I values (compared with the control) after

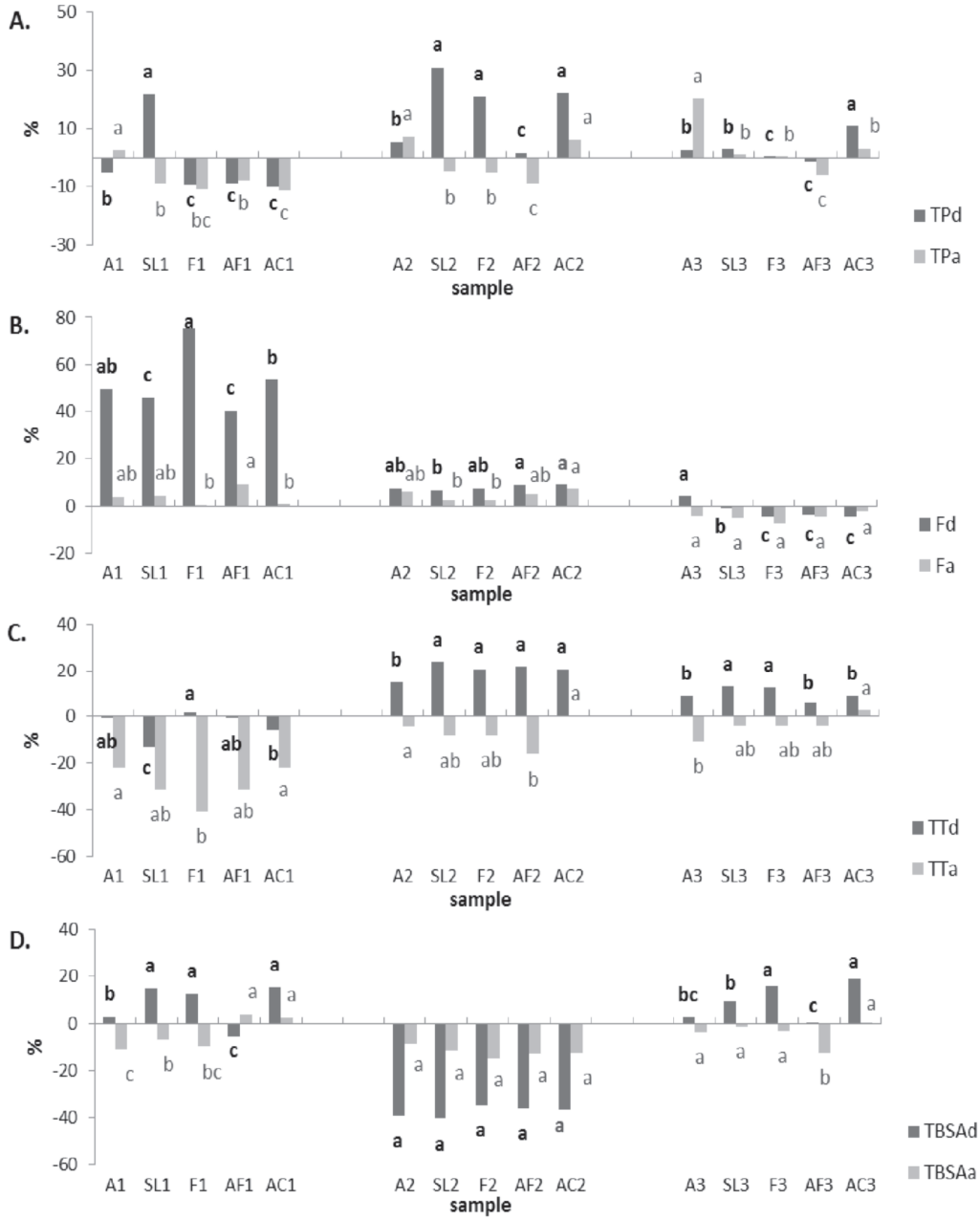


Figure 2 - Percentage changes of the analytical parameters of the wine samples in relation with the control wine: A. total phenols (TP), B. total flavanols (F), C. total tannins (conversion into cyanidin) (TT), D. total tannins (precipitated by BSA) (TBSA) and E. total ellagitannins (ET) after the addition of American (A), French (F), mixture of American-French (AF) and Slavonian (SL) oak and Acacia (AC) chips in wines during (d) and after (a) fermentation.

Numbers 1, 2 and 3 indicate 1, 2 and 3 months of contact time, respectively.

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the second and third months and higher H values after the first and third months when compared with the wines where chip addition took place after fermentation (Figure 1). However, Gordillo *et al.* (2013) observed an increase in wine color intensity when oak chips were added during fermentation, which could be attributed either to the different grape variety studied (Tempranillo) or/and to the shorter aging period used (60 days).

The method used for TA measurement is based mainly on the determination of free anthocyanins and anthocyanin-flavanol adducts while the HPLC method accounts only for the free monomeric anthocyanins. The decrease observed in A is consistent with the participation of monomeric anthocyanins in condensation reactions, implying a positive contribution of wood to color parameters of the wine due to the formation of more stable polymeric complexes (Kyraleou *et al.*, 2015). The main difference among the two techniques studied was that Ad values were lower than the control after the first month of contact with the chips probably due to a faster depletion of monomeric anthocyanins through condensation reactions. In the case of samples where chip addition took place after fermentation, Aa values started to decline after the second month, probably due to a slower polymerization rate between anthocyanins and other phenolic molecules. Moreover, the increase in TA values (compared with the control), attributed probably to the formation of anthocyanin-flavanol adducts, was observed earlier in samples fermented with the chips (second month), compared with the samples where chip addition took place after fermentation (third month). It is thus possible that color stabilization occurs faster (but to a lesser extent) when chips are added during fermentation than after. However, in order to investigate the stability of color over time, further studies are required with longer storage periods.

2. Phenolic content

The percentage changes of the phenolic compounds (total phenols, total flavanols, total tannins measured with the two methods employed and total ellagitannins) of Agiorgitiko wine as a result of wood chip addition (during and after fermentation) after 1, 2 and 3 months of contact time are shown in Figure 2. Total phenolic concentration (TP) decreased after the first month in most of the samples as a result of wood chip addition after fermentation in comparison with the control (with the exception of A sample where an increase was observed) (Figure 2a). SL and AF samples were characterized by a significantly higher decrease. After the second month, SL, F and AF samples contained less while A and AC samples contained more TP than the control. However, the difference was significant only among A and AF samples. Finally, after three months of contact with wood chips, only AF sample contained less TP while all other samples were richer than the control. The highest increase was observed in A and AC samples. When the addition of wood chips took place during fermentation, most of the samples contained less TP after the first month (with the exception of SL which contained significantly more) while after two months all samples contained more TP than the control (the highest increase was observed in SL, AC and F samples). After three months, with the exception of AF sample, all the samples contained higher amounts of TP than the control. The highest increase was observed in AC sample (Figure 2a). These results are in agreement with Del Alamo *et al.* (2004), who reported a progressive decrease of TP in wines during the first months of aging in contact with chips while after the third month an increasing trend was observed. Moreover, Gordillo *et al.* (2013) reported an increase in TP as a result of wood chip addition during fermentation after two months of contact with the wine, which is also in agreement with the results presented above.

Total flavanol concentration (F) of Agiorgitiko wine was higher than that of the control after the first and second months of contact with chips in both cases studied (addition after and during fermentation) (Figure 2b), in agreement with the findings of Gordillo *et al.* (2013) and Soto Vazquez *et al.* (2010). The increase was more profound after the first month in the samples where wood chip addition took place during fermentation (with F containing significantly higher amounts followed by AC), implying less polymerization reactions. After three months of contact, flavanol content showed a decreasing pattern in most of the samples probably due to the transformation of phenols into more condensed forms.

Total tannin concentration of Agiorgitiko wine was determined by two different methods (TT, Tbsa) based on different mechanisms (Figure 2c and d). The first, although it is highly reproducible, tends to overestimate tannin content while the results obtained by the second method are strongly correlated with astringency (Mercurio and Smith, 2008). TT data showed a decrease compared with the control, after the first, second and third months of contact with chips (added after fermentation). The highest decrease was observed for F, AF and SL samples after the first two months, while after the

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third month A sample showed the highest decrease. This decrease is mainly related to polymerization and consequent precipitation reactions of tannins with other compounds such as anthocyanins and flavanols (Gambuti *et al.*, 2010). A different pattern was observed in the case of chip addition during fermentation, in particular after the second and third months where an increase was observed compared with the control. It is thus possible that the condensation reactions between tannins and other phenolic classes (of wine or wood origin) occurred faster when the addition of chips took place during fermentation but to a lesser extent since tannin reduction was not so profound (after the first month) as in the previous case. Regarding protein-precipitable tannin concentration (Tbsa), a different pattern was observed after the first and third months of contact with chips among the two techniques studied. Tbsa content of wines where the addition of chips took place after fermentation decreased in comparison with the control in most of the samples, implying a positive effect of wood addition on wine sensory properties. After the first month, A sample contained significantly less while AF and AC samples contained more Tbsa than the control. After the second month, all the wines studied contained less Tbsa than the control. However, the different wood treatments tested did not result in any significant differences among the samples. Finally, after three months, only AF sample contained significantly less Tbsa than the rest of the samples. When the addition of chips took place during fermentation, an increasing pattern was observed after the first (with the exception of AF sample) and the third months. AC, F and SL (only after the first month) wines contained higher amounts of Tbsa after both samplings. However, after the second month, a decreasing pattern was observed similar (although more profound) to that observed in the case where wood chip addition took place after fermentation. It is possible that increased tannin-anthocyanin interactions might affect the protein-binding capacity of proanthocyanidins, resulting in reduced tannin levels (Kyraleou *et al.*, 2015). This could be further supported by the data of Figure 1 (c and d), where the TAd and Ad contents of wines after two months of contact with wood chips are higher than the control, implying reduced reactivity with tannins. No significant differences were observed among the different treatments, in agreement with the findings of Oberholster *et al.* (2015), who reported no significant differences in Tbsa contents among wines after six months of aging in different oak barrels.

The ellagitannin content (ET) was estimated by the determination of the amount of ellagic acid released after acidic hydrolysis (Figure 2e) (Chira and Teissedre, 2013a). ET increased in all samples as a result of wood chip addition during or after fermentation. In all cases studied AC samples were the richer in ET while A and AF were the poorest, in agreement with Kyraleou *et al.* (2015). The main difference among the two techniques is that ET increase was more profound in the case of chip addition after fermentation and in particular after the second and third months. It has been stated in the literature that the composition of the wine itself may significantly impact the aging process, since pH, alcoholic degree and titratable acidity may have a direct influence on the ethanolysis of wood components (Ortega-Heras *et al.*, 2007). It is thus possible that the lower initial ethanol content of the wines where the addition of chips took place during fermentation might have affected the extraction kinetics of ellagitannins from wood. The above observations suggest that the time at which the chips are added to the wine has an important impact on ET content, which in turn may have consequences on the final wine quality. It is known that the ET content of wines is directly correlated with astringency perception (Chira and Teissedre, 2015), and thus the higher ET levels of the wines when chips are added after fermentation might imply higher astringency. On the other hand, ET might affect polymerization and consequent precipitation reactions with wine phenolic compounds (Gambuti *et al.*, 2010) resulting in color stabilization and improvement of wine sensory properties.

A principal component analysis (PCA) was applied to phenolic related data in order to obtain a possible categorization of the wines. Figure 3 shows the projection of the variables and the wines onto the first two principal components (PC). The first PC explains 37.17 % of the total variance and opposes the percentage changes in total phenols (TP) and total tannins (TT) to those in total protein-precipitable tannins (Tbsa). The second principal component explains 27.54 % of the total variance and opposes the percentage changes in total flavanols (F) to those in total ellagitannins (ET). The PCA allows the discrimination of the wines into three groups (Figure 3). The wines that stayed in contact with the wood chips (added during fermentation) for one and two months are found in the upper left and right parts of the graph, respectively. The rest of the samples are found in the middle part of the graph. Thus wines having spent two months in contact with chips (added during fermentation) are characterized by higher TT and TP and lower Tbsa contents compared with the control. Moreover, wines that stayed in contact for one month with the chips (added during

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fermentation) differ mainly in their Tbsa and F contents in relation to the control. It is possible that most of the tannin condensation and color stabilization reactions, which improve wine quality, occur during the first month, when chip addition takes place during fermentation, due to the limited content of ellagitannins. After that period, anthocyanins and tannins participate less to polymerization reactions probably due to the lack of ellagitannins. Moreover, the polymerized tannin content is lower in this case, leading to less stabilized wines. Finally, all the wines produced by the addition of chips after fermentation and the wines tested after the third month of chip addition during fermentation are not well separated from each other on this graph and are characterized by intermediate changes of their phenolic content. Wine samples taken after three months of contact with chips (added after fermentation) are found on the lower part of this last group and are characterized by their higher ET content. Moreover, the wines that stayed one month in contact with the chips (added after fermentation) are found on the left part of this group as a result of the higher reduction observed in their TP and TT contents. The rest of the wines (after two months and three months of chip addition after and during fermentation, respectively) are found close to each other.

3. Volatile composition

The main contributors to the overall wood aroma acquired by wines during aging in contact with wood belong to very different chemical families, but they all arise from the thermal degradation of wood polysaccharides or from the chemical reactions that take place during aging (Chira and Teissedre, 2013a). The following wood extracted volatile compounds were studied: the two isomers of methyl-γ-octalactone, *cis* (cWL) and *trans* (tWL) (commonly known as whiskey lactones); the volatile phenols guaiacol (Gua), 4-methyl guaiacol (MGua), eugenol (Eg) and isoeugenol (IEg); the aldehyde phenol vanillin (Vn) and its alkyl ester ethyl vanillate

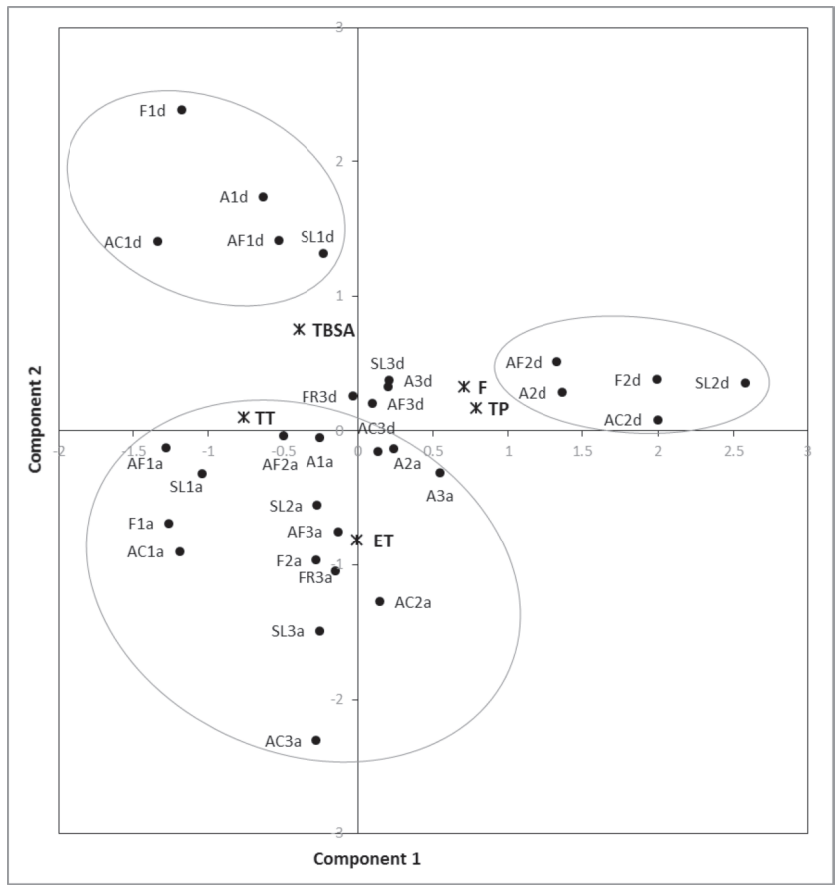


Figure 3 - Biplot of principal components 1 and 2 for mean scores of percentage changes of wine phenolic parameters. Codes are as follows: (TP) total phenolic content, (TT and TBSA) total tannin concentration measured with different methods, (F) total flavanol concentration and (ET) total ellagitannins. Wines: (C) control wine, (A), (F), (AF) and (SL) wines treated with American, French, mixture of American and French, and Slavonian oak wood chips, respectively, and (AC) wines treated with chips from acacia wood during (d) and after (a) fermentation. 1, 2 and 3 indicates 1, 2 and 3 months of aging in contact with wood chips, respectively.

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(EVn); and the phenolic ketone, acetovanillone (AVn).

Figure 4a presents the content of wood extracted compounds for each sample (mean of triplicate measurements). The levels of extracted volatile compounds were quantitatively different depending on the contact time, the type of wood and the time of chip addition. Concerning woody aromas, AC was the sample with the highest total concentration followed by A, whereas SL and AF samples contained less wood extracted volatile compounds (addition of chips after fermentation). When the addition of chips took place during fermentation, A sample was the richest sample followed by AF and AC while SL was the poorer in wood volatile compounds.

In C wine, Vn, Gua and Eg were present in low levels, in accordance with other authors Gomez Garcia-Carpintero *et al.*, 2012; Chira and Teissedre, 2015) who reported low amounts of wood derived compounds in wines not aged in contact with wood.

Esters are mainly synthesized by yeasts during fermentation and are important for the aroma of the wines as they contribute to their 'fruity' and 'floral' character. The following esters were studied during the present study: the ethyl ethers of straight-chain fatty acids: ethyl butanoate (EB), ethyl hexanoate (EH) and ethyl octanoate (EO); the ethyl esters of branched acids: ethyl 3-methylbutanoate (E3mB) and ethyl 2-methylbutanoate (E2mB); and the higher alcohol acetates: isoamyl acetate (IA) and 2-phenylethyl acetate (PA). EB and EH are reported to enrich the wine with strawberry-like aromas, EO with odors of ripe fruit, E2mB with strawberry, apple and anise odors, and E3mB with pineapple, floral and lemon flavors, while IA and PA are described by banana and rose notes, respectively (Sumby *et al.*, 2010).

Figure 4b presents the content of esters determined for each sample (mean of triplicate measurements). The levels of esters were quantitatively different depending mainly on the time of chip addition. Concerning total concentration of ethyl esters, the wines where chip addition took place after fermentation contained higher contents than the wines fermented with oak chips and in some cases than the control. These results are consistent with the results reported by Gomez Garcia-Carpintero *et al.* (2012, 2014) and Rodriguez-Bencomo *et al.* (2010) concerning the control wines but in contrast with the results reported for the wines fermented with chips probably due to the different chemical composition of the wines used or to the different yeast strains employed for fermentation.

In most of the samples, the total concentration of ethyl esters decreased with aging in both control and wood treated samples. It is well known that the fruity and floral notes of wines are less intense as the aging period increases due to chemical modifications of their ester content (Sumby *et al.*, 2010). As esters are produced in excess during fermentation, they gradually hydrolyze during storage until the chemical equilibrium with their corresponding acids and alcohols is achieved. These changes in wine ester content are mainly dependent on pH, alcoholic degree and storage temperature (Garde-Cerdan *et al.*, 2004). The only exception to this trend was the samples treated with Acacia chips (both during and after fermentation) where ester content increased with time. This observation may be attributed to their higher ET content. According to Jordao *et al.* (2012), ellagitannins play an important role in wine oxidation process since they quickly absorb the dissolved oxygen. It is thus possible that the higher ET content of these particular wines preserved the esters from oxidative degradations.

Regarding the higher alcohol acetate content of the samples (Figure 4c), a different trend was observed. After the first and second months, wines treated with chips during fermentation were richer in total acetates than those treated after fermentation. These data are consistent with the results reported by Gomez Garcia-Carpintero *et al.* (2012, 2014) and Rodriguez-Bencomo *et al.* (2010). This effect can be attributed to the action of the wood chips as a carrier for the yeast cells, giving rise to an effect similar to that of the immobilized cells (Gomez Garcia-Carpintero *et al.*, 2012).

However, an interesting observation was that after the third month of aging this trend was reversed, and the samples with chip addition after fermentation were richer than the others. It seems the degradation rate is faster in the case of chip addition during fermentation, maybe due to the lower ET content of these wines and hence the lower protection effect from oxidation.

PCA was applied to the data obtained from GC–MS analysis in order to find out which compounds could be used for sample discrimination. The first two principal components accounted for 64.81 % of the total variance. Figure 5 shows the projection of the variables and the wines onto the first two principal components (PC). The first PC explains 46.84 % of the total variance and opposes tWL, cWL, Eg, EVn,

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EB and PA to Gua, EH and EO. The second principal component explains 17.38 % of the total variance and opposes Vn, MGua and AVn to IEg and IA. The PCA allows the discrimination of the wines into five groups (and one group for the control) based on the different time of chip addition and only in the case of the wines where wood addition took place after fermentation on wood types (Figure 5). Wines where chips were added during fermentation (d) are found in the right part of the graph, well separated from the rest. They are characterized by higher concentrations of some of the wood extracted compounds (cWL, tWL Eg, EVn) and individual esters (E3mB, E2mB, EB, PA) and by lower contents of Gua, EO and EH. Other authors have reported higher concentration of wood derived compounds in wines where chip addition took place after fermentation (Rodriguez-Bencomo *et al.*, 2010; Gomez Garcia-Carpintero *et al.*, 2012), probably due to the different wine matrix or chip toasting level used.

Wines where chip addition took place after fermentation (a) are found in the left part of the graph and are separated into four groups according to the type of wood they were in contact with, in agreement with the results of Kyraleou *et al.* (2015). The first group is formed by the wines treated with AC chips and is found in the upper left part of the graph. These wines are characterized by their high Vn, AVn and MGua and low IA and IEg contents, in agreement with the findings of Chira and Teissedre (2015), who also observed that AC wood resulted in high amounts of Vn and AVn in the corresponding wines. The second group is formed by the wines treated with A chips and is found below the first one. These wines are also characterized by high Vn and AVn contents but lower than those found in AC treated wines. The third group is formed by the wines treated with F chips and is found in the middle left part of the graph. These wines are characterized by their high EO and EH, intermediate Vn, MGua, AVn, IEg and

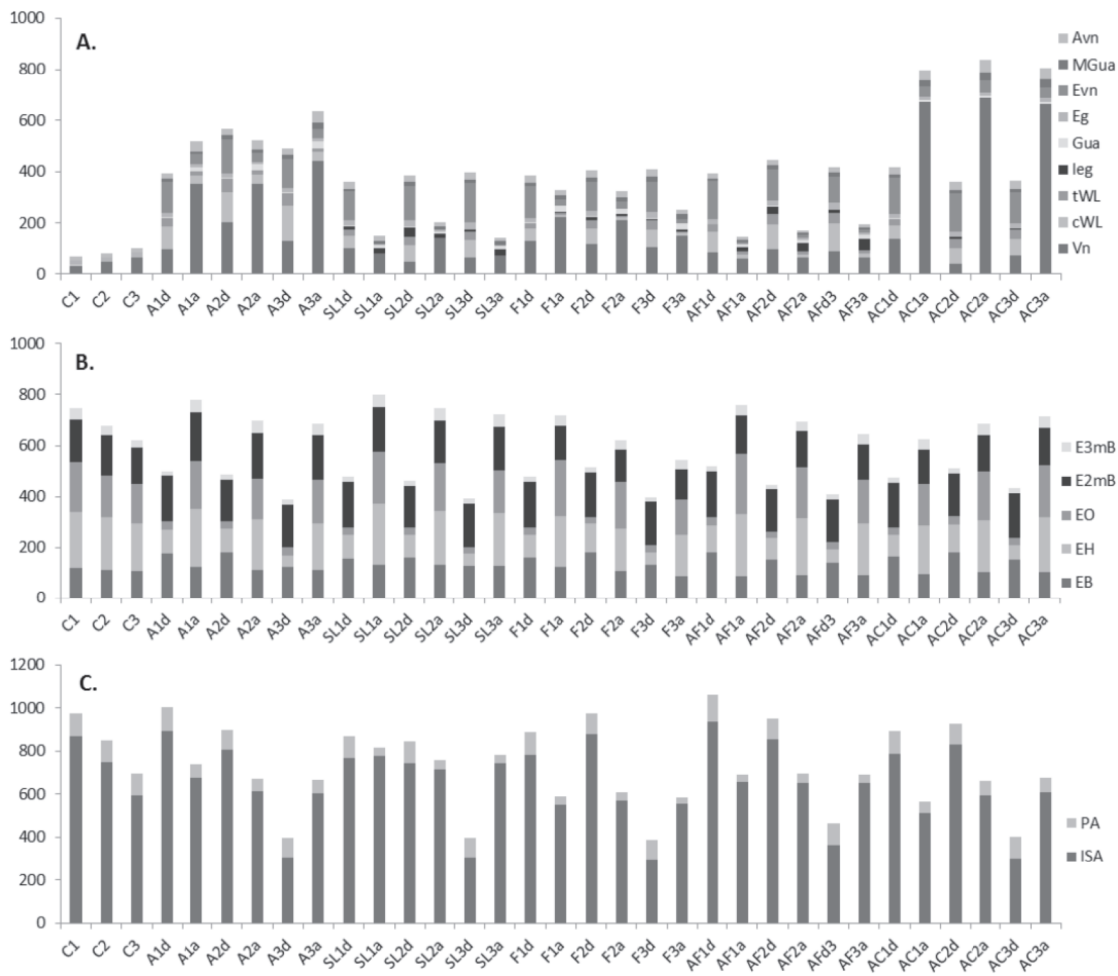


Figure 4 - Volatile content of the wines: wood extracted compounds (A), ethyl esters (B) and acetate esters (C) of the samples after the addition of American (A), French (F), mixture of American-French (AF) and Slavonian (SL) oak and Acacia (AC) chips in wines during (d) and after (a) fermentation.

Numbers 1, 2 and 3 indicate 1, 2 and 3 months of contact time, respectively.

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IA, and low WL, Eg, EVn, EB, E2mB, E3mB and PA contents, in agreement with Pizarro *et al.* (2014). The next group is formed by the wines treated with AF and SL chips after fermentation. They are found in the lower left part of the graph and are characterized by their lower WL and higher IEg contents, in agreement with Chira and Teissedre (2015) and Kyraleou *et al.* (2015). Finally, control wines are grouped together in the lower left part close to the previous group since they contain minor quantities of wood extracted compounds.

In conclusion, the treatment of Agiorgitiko wine with wood chips may affect its quality depending on the time of addition. The addition of wood chips during alcoholic fermentation did not favor ellagitannin extraction and the reactions involved in tannin condensation and anthocyanin stabilization. Wood chip addition during fermentation resulted in wines with lower I and higher H values when compared with the wines where chip addition took place after fermentation. Moreover, color stabilization and tannin polymerization seem to occur faster, but to a lesser extent, when chips are added during fermentation than after. Finally, the wines fermented with wood chips contained higher contents of some of the wood extracted volatiles (whiskey lactones, Eg, EVn), ramified ethyl esters (E3mB, E2mB) and acetates while they were poorer in ethyl esters compared with the wines where chip addition took place after fermentation. Moreover, addition of wood chips after fermentation enhanced the levels of



Figure 5 - Biplot of principal components 1 and 2 for mean scores of wine volatile composition.
Codes are as follows: *cis* whiskey lactone (cWL), *trans* whiskey lactone (tWL), guaiacol (Gua), 4-methyl guaiacol (MGua), eugenol (Eg), isoeugenol (IEg), vanillin (Vn), ethyl vanillate (EVn), acetovanillone (AVn), ethyl butanoate (EB), ethyl hexanoate (EH), ethyl octanoate (EO), ethyl 3-methylbutanoate (E3mB), ethyl 2-methylbutanoate (E2mB), isoamyl acetate (IA) and 2-phenylethyl acetate (PA). Wines: (C) control wine, (A), (F), (AF) and (SL) wines treated with American, French, mixture of American and French, and Slavonian oak wood chips, respectively, and (AC) wines treated with chips from acacia wood during (d) and after (a) fermentation. 1, 2 and 3 indicates 1, 2 and 3 months of aging in contact with wood chips, respectively.

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guaiacol, methyl guaiacol and vanillin. Overall, when chips are added after fermentation wines seem to have a greater aging potential compared with the wines fermented with chips, while stabilization and condensation reactions occur faster (but to a lesser extent) in the latter wines, making them ready for early consumption.

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