Effect of vine-shoot and oak extract foliar grapevine applications on the oenological parameters, phenolic acids and glutathione content of white musts and wines

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Abstract

Aim: An oxidation process frequently occurs during white winemaking, affecting its quality. The aim was to study, for two years (2013 and 2014), the effects that foliar applications of vine-shoot (Airén (AVS) and Moscatel (MVS)) and oak wood (OW) extracts on Airén grapevines have on wine color, and must and wine glutathione, trans-GRP, trans-caftaric acid, and trans-p-coumaric acid content.

Methods and results: These compounds were analyzed by HPLC. The results showed that, in general, foliar application of Airén vine-shoot extracts did not affect glutathione concentration, and the other treatments decreased it. AVS2013 and AVS2014 samples were characterized by a high content of glutathione and trans-GRP, while MVS2014 samples retain high levels of trans-caftaric acid. trans-p-Coumaric acid concentration decreased after AVS2013 treatment, meanwhile in the 2014 season all applications increased its content. The content of these compounds in the wines was similar to those obtained in the musts. OW2013 showed the lowest value of Abs 420, likely because this treatment decreased glutathione concentration in musts.

Conclusions: The foliar applications of vine-shoots and oak extracts on Airén grapevines had a clear effect in reducing the wine alcohol degree and Baumé in case of grapes. This finding is interesting in the context of the current global warming change scenario. Although the two vintages studied were different, an improvement in the color quality was observed in both. The content of glutathione decreased from must to wine: the content in both matrices was lower in treated samples than in control samples, except for Airén extracts in both vintages. This means that glutathione is oxidized, avoiding the oxidation of other must and wine molecules.

Significance and impact of the study: These findings are important in relation to revalorizing waste from the vineyard, and thus being able to improve the quality of the white wine in relation to the oxidation processes that take place in the winemaking process.

Keywords

vine-shoot extracts, foliar application, must, wine, glutathione, colour
INTRODUCTION

It has become mandatory for the wine industry to apply waste products to grapevines, with the aim of reducing chemicals according to sustainable practices (European Parliament, 2008). In this sense, vine-shoots are one of the most important waste products and have been used as organic fertilizers and food additives, to produce paper pulp, or to create solid biofuels, etc. (Jiménez Gómez et al., 1993; Jiménez et al., 2006; Portilla et al., 2008; Mendivil et al., 2013). Sánchez-Gómez et al. (2014) studied the chemical composition of Airén vine-shoot waste aqueous extracts in terms of phenolic, volatile, and mineral compounds, and reported that the extracts showed an interesting composition that may be assimilated by plants. Thereby, Airén or Moscatel vine-shoot extracts applied foliarily to the grapevines improved wine amino acids, phenolic acids, and volatile composition, increased grape yield and decreased the alcohol degree (Sánchez-Gómez et al., 2016a; Sánchez-Gómez et al., 2016b; Sánchez-Gómez et al., 2017).

Several residues are generated in the manufacturing process of oak barrels, which can be added in the form of chips or pellets as an alternative to wine aging. Pardo-García et al. (2014) observed that oak extracts, obtained from oak waste from the cooperages, are interesting applications for the sustainability of the grapevine and oak industry. Thereby, the foliar application of the oak extracts affected grape composition, producing less alcoholic and acidic red wines with higher color intensity, lower shade, a more stable color and a higher content of polyphenols such as gallic acid, hydroxycinnamoyltartaric acids, acylated anthocyanins, flavanols and stilbenes (Pardo-García et al., 2014). In addition, after these applications, several compounds from the oak extracts were assimilated by vines and stored in grapes as glycosidic forms, affecting the wine volatile composition, mainly months after bottle storage (Martínez-Gil et al., 2011; Martinez-Gil et al., 2013).

Color is one of the most important properties of must and wines from white varieties. The oxidation process that occurs frequently in white wines is a well-known problem in the winemaking industry because the color of musts and wines can be modified (Cejudo-Bastante et al., 2010). Sulfur dioxide is usually used to prevent must oxidation and browning development during bottle storage. However, there are strict regulations about sulfur dioxide use in the food industry, because of its toxicity and allergic effects on human health (Lester, 1995). Hydroxycinnamic acids are prone to enzymatic oxidation reactions during the winemaking process, forming o-quinones and leading to color darkening. Thereby, glutathione, is an antioxidant tripeptide formed by three amino acids - cysteine, glutamate and glycine - and present in musts and wines. This tripeptide can regenerate the o-diphenol group of enzymatically oxidized trans-caftaric acid, giving rise to 2-S-glutathionyl-trans-caftaric acid (GRP) and thus inhibiting wine browning (Cheynier et al., 1989; Cejudo-Bastante et al., 2010; Kritzinger et al., 2013). It has been recently reported that bisulfite competes as a nucleophile with glutathione for the o-quinone of trans-caftaric acid (Hayasaka et al., 2017). Thus, the aim of this work was to study the effect that foliar applications of Airén and Moscatel vine-shoot (AVS and MVS) extracts and oak wood (OW) extracts from non-toasted and toasted wood have on Airén grapevines. The effect of these treatments on must and wine glutathione, trans-GRP, trans-caftaric acid, and trans-p-coumaric acid concentration, and on wine color was evaluated during two consecutive vintages.

MATERIALS AND METHODS

1. Vegetal raw material for extract preparation

1.1 Vine-shoot and oak samples

Vine-shoot waste from the Airén Vitis vinifera cultivar in 2013 and from Airén and Moscatel in 2014 were sampled in the Castilla-La Mancha Spanish region, four months after each harvest, by randomized selection. Each year, vine-shoots were dried at room temperature for three months until a final humidity of 6.5 % (g\text{water}/100g\text{sample}). The 2013 oak sample was obtained from a commercial aqueous French oak extract (103 C), hereinafter OW\text{2013} supplied by Protea France S.A.S. (Gensac la Pallue, France). This extract was obtained by macerating French oak chips (Quercus sessiliflora Salisb) for natural seasoning with water at high temperatures, and this was later toasted at medium-intensity levels, as described by Martínez-Gil et al. (2011). In 2014, the French oak wood used was provided by Protea France S.A.S. in the form of non-toasted chips (Quercus sessiliflora Salisb), naturally seasoned for 24 months.

1.2 Extracts preparation

All non-toasted vine-shoot waste samples (2013 and 2014) and the oak chips from 2014, were ground in a hammer miller (RETSCH, SM100 Comfort, Haan, Germany), sieved in a 10-mesh...
to get a homogeneous sawdust, and kept under vacuum at room temperature (25-27°C) until use. To obtain the toasted samples for the 2014 experiment, dried Airén and Moscatel vine-shoots and oak chip materials were heated at 180 °C for 45 min according to the methods in Sánchez-Gómez et al. (2016c) and Sánchez-Gómez et al. (2016d) and the extraction procedure was as described in Sánchez-Gómez et al. (2014). The resulting Airén vine-shoot wastes extracts were as follows: Airén aqueous extract from non-toasted vine-shoots in 2013 (AVS<sub>2013</sub>), Airén aqueous extract from non-toasted vine-shoots in 2014 (AVS<sub>2014</sub>), and Airén aqueous extract from toasted vine-shoots in 2014 (AVS<sub>Toasted</sub>). For Moscatel vine-shoot waste, the resulting extracts were Moscatel aqueous extract from non-toasted vine-shoots in 2014 (MVS<sub>2014</sub>) and Moscatel aqueous extract from toasted vine-shoots in 2014 (MVS<sub>Toasted</sub>). For oak wood, the extracts obtained were French oak wood extract from non-toasted chips in 2014 (OW<sub>2014</sub>) and French oak wood extract from toasted chips in 2014 (OW<sub>Toasted</sub>). For each extract, 1.8 L was prepared.

2. Vines treatments

The white grapevines used in this study were from Airén Vitis vinifera grown at O.D. Jumilla in southwest Spain (38° 35’ 17” N and 1° 16’ 20” W; 511 m above sea level) during the 2013 and 2014 vintages. The grapevines were trained on the traditional bush vines Gobelet system with a row distribution north to south.

The formulations from 2013 (AVS<sub>2013</sub> and OW<sub>2013</sub>) and 2014 (AVS<sub>2014</sub>, AVS<sub>Toasted</sub>, MVS<sub>2014</sub>, MVS<sub>Toasted</sub>, OW<sub>2014</sub> and OW<sub>Toasted</sub>) were prepared using 0.05 % (v/v) of the Agral (Syngenta, Switzerland) adjuvant, which is a superficial wetting agent typically used for treatments with foliar applications, constituted by an inert mixture of polymers. The chemical composition of the extracts from 2013 and 2014, used in these applications, has been discussed in detail in previous works (Sánchez-Gómez et al., 2014; Sánchez-Gómez et al., 2016c; Sánchez-Gómez et al., 2016d).

In 2013, the formulation effect of vine-shoot extracts was tested, and the previous dilutions carried out with oak extracts (Martínez-Gil et al., 2011, 2012) were used as a reference. The AVS<sub>2013</sub> extract was applied once on day 7 post-veraison (AVS<sub>2013</sub>-100) and the second application was carried out with AVS<sub>2013</sub> diluted with water at 50 % and applied twice on days 7 and 14 post-veraison (AVS<sub>2013</sub>-50). The commercial aqueous oak extract (OW<sub>2013</sub>) was applied once on day 7 post-veraison for comparing these results the previous ones from the same oak extract (Martínez-Gil et al., 2011, 2012).

In 2014, the toasted procedure effect and possible vine-shoot varieties effect were tested. Because of the results obtained in 2013, all treatments were applied once. Thus, Airén grapevines were treated with four different vine-shoot extracts (AVS<sub>2014</sub>, AVS<sub>Toasted</sub>, MVS<sub>2014</sub>, MVS<sub>Toasted</sub>) and two oak extracts (OW<sub>2014</sub>, OW<sub>Toasted</sub>), which were applied once on day 7 post-veraison. Figure 1 summarizes the experimental design for the two years.

Each year, the treatments were applied on plants arranged in rows and in an edaphologically homogeneous portion of the plot, avoiding its edges. Each treatment was carried out on six plants randomly selected in the same row, distributed on three replicates with two plants each (n = 3), leaving other rows with untreated plants between the different applications to avoid contamination. Moreover, six additional plants were selected each year as controls, which were treated with water and the adjuvant following the same treatment protocol. The treatments were carried out when the environmental temperature was below 20 ºC, between approximately 7 a.m. and 9 a.m. The grapevines were treated with 300 mL of each formulation per plant by spraying locally over the leaves.

3. Winemaking

Grapes were manually harvested on 26 September 2013 and 9 September 2014 at the optimum technological maturation moment for the grapevines used as the control sample. In both years, the grapes (approximately 20 kg for each treatment) were separately processed. These grapes were destemmed and the whole grape mass was mixed and homogenized (must and solid parts combined). Then, the grape mass from each treatment was placed into 30 L stainless steel tanks and ammonium bisulfite (150 mg/mL) was added to the resulting musts. Maceration was carried out at 11 ºC ± 1 ºC for 17 h.

Skins and seeds from each treatment were removed with a traditional vertical hand-press and the free-run musts were put into stainless steel tanks to finish the alcoholic fermentation. Musts were inoculated with 20 g/L of UCLM S377 active dry yeast strain (Springer Oenologie, Maisons-Alfort Cedex, France) of Saccharomyces bayanus, previously rehydrated and activated according
to the supplier. The alcoholic fermentation took place at 16 °C ± 1 °C. The density was measured each day and alcoholic fermentation was considered complete after 16 days in 2013 and 17 days in 2014, when it was constant at 0.990 g/L and the residual sugars value were below 5 g/L. As no malolactic fermentation was carried out, at this final point three wines from each treatment (33 wines in total: 12 from 2013 and 21 from 2014) were obtained, bottled and stored at 22 °C ± 2 °C.

4. Analytical methods

4.1 Musts and wines oenological parameters

Must parameters such as °Baumé (ºBé), pH, total acidity (g/L of tartaric acid) and malic acid (g/L), along with wines parameters such as alcoholic degree (°A), pH, total acidity (g/L of tartaric acid), volatile acidity (g/L), malic acid (g/L), lactic acid (g/L) and color intensity (Abs 420 nm) were analyzed using equipment based on Fourier Transform-infrared spectroscopy (FT-IR Multispec; TDI, Barcelona, Spain) using the methods of ECC (1990) as reference.

4.2 Determination of glutathione by HPLC

The glutathione analysis was performed using the method described by Garde-Cerdán et al. (2014). This compound was analyzed by reverse-phase HPLC using a liquid chromatograph Agilent 1100 Series (Palo Alto, USA). Each sample (5 mL) was mixed with 100 µL of norvaline (internal standard). The mixture was submitted to an automatic precolumn derivatization with o-phthaldialdehyde (OPA Reagent, Agilent). The injected amount from the derived sample was 10 µL, with a constant temperature of 40 °C. All separations were performed on a Hypersil ODS (250 × 4.0 mm, I.D. 5 µm) column (Agilent).

Two eluents were used as mobile phases: eluent A was 75 mM sodium acetate, 0.018 % triethylamine (pH 6.9) + 0.3 % tetrahydrofuran; eluent B was water, methanol, and acetonitrile (10:45:45, v/v/v). Detection was performed using a fluorescence detector (FLD). The identification of glutathione was performed by comparison of its retention time with that of pure reference standard. The pure reference compound and internal standard were from Sigma-Aldrich (Steinheim, Germany). The results for glutathione correspond to the average of three analyses (n = 3).

4.3 Determination of trans-GRP, trans-caftaric and trans-p-coutaric acids by HPLC-DAD-MS

The analysis was based on methods from Pardo-García et al. (2014) and Sánchez-Gómez et al. (2014). Briefly, the HPLC grade solvents used were water/formic acid/acetonitrile (97.5:1.5:1 v/v/v) as solvent A and acetonitrile/formic acid/solvent A (78.5:1.5:20 v/v/v) as solvent B. The elution gradient was set up for solvent B as follows: 0 min, 5 %; 2 min, 10 %; 4 min, 14 %; 9 min, 14 %; 37 min, 18.5 %; 35 min, 20 %;
50 min, 25 %; 55 min, 50 %; 60 min, 5 %; and 65 min, 5 %. The loop volume was 20 µL.

Identification of trans-GRP (2-S-glutathionyl-trans-caftaric acid or grape reaction product) and trans-caftaric and trans-p-coumaric acids was carried out using MS and DAD detectors in comparison with the corresponding mass fragmentation and UV-Vis spectra. As they were not available, they were quantified at the same wavelength as their analogous hydroxycinnamic acids (trans-cafeic and trans-p-coumaric acids, respectively): trans-GRP and trans-caftaric acid at 324 nm, and trans-p-coumaric acid at 308 nm. Quantification was based on trans-cafeic and trans-p-coumaric acids calibration curves of their respective standards (Sigma-Aldrich) at five different concentrations achieved by UV-Vis signal (0.70-175 mg/L) ($R^2 = 0.92-0.99$).

5. Statistical analysis

Data statistical analysis was performed using the SPSS Version 22.0 statistical package for Windows (SPSS, Chicago, USA). Oenological parameters, glutathione, trans-GRP, trans-caftaric and trans-p-coumaric acids data concentration from musts and wines were processed using variance analysis (ANOVA). Differences between means were compared using the Tukey test at 99.95 % probability level. Canonical discriminant analyses were performed in both years for all treatments with the glutathione, trans-GRP, trans-caftaric and trans-p-coumaric acids concentration in musts and wines.

**RESULTS AND DISCUSSION**

1. Must and wine oenological parameters

The oenological parameters for musts in both years of study are shown in Table 1. In 2013, control must presented significant differences in all parameters studied in relation to the treatments. Control musts showed the highest °Baumé content and the lowest pH, although in this last case pH range values were very close among control and musts from treatments. On the contrary, OW2013 presented the lowest value of °Baumé and the highest total acidity and malic acid content followed in these two last parameters for control must. Musts from grapevines treated with Airén vine-shoot extract (AVS$_{2013-100}$ and AVS$_{2013-50}$) showed intermediate content of alcohol and the lowest values of total acidity and malic acid, and therefore, the highest pH values.

In the 2014 vintage, °Baumé in the control must showed a similar behavior than in the 2013 must, presenting the highest content and significant differences regarding the treatments. Thus, in general, the treatments tested seem to affect the °Baumé content, providing in

| TABLE 1. Oenological parameters of musts at harvest day after the different grapevine treatments applied in 2013 and 2014. | °Baumé | pH | Total acidity (g/L tartaric acid) | Malic acid (g/L) |
|---|---|---|---|---|
| **Must 2013** | | | | |
| Control$_{2013}$ | 10.20 ± 0.00 d | 3.49 ± 0.00 a | 4.81 ± 0.01 c | 4.06 ± 0.05 c |
| AVS$_{2013-50}$ | 9.90 ± 0.00 b | 3.51 ± 0.00 c | 3.96 ± 0.01 a | 2.94 ± 0.05 a |
| AVS$_{2013-100}$ | 10.10 ± 0.00 c | 3.54 ± 0.00 d | 4.65 ± 0.01 b | 3.90 ± 0.05 b |
| OW$_{2013}$ | 9.30 ± 0.00 a | 3.50 ± 0.00 b | 4.99 ± 0.01 d | 4.68 ± 0.05 d |
| **Must 2014** | | | | |
| Control$_{2014}$ | 11.25 ± 0.07 e | 3.57 ± 0.01 b | 2.22 ± 0.01 ab | 0.57 ± 0.04 ab |
| AVS$_{2014}$ | 10.70 ± 0.00 d | 3.60 ± 0.00 d | 2.25 ± 0.04 b | 0.75 ± 0.05 b |
| AVS$_{Toasted}$ | 9.55 ± 0.07 a | 3.56 ± 0.00 a | 2.40 ± 0.01 c | 0.55 ± 0.02 ab |
| MVS$_{2014}$ | 9.80 ± 0.00 b | 3.57 ± 0.01 b | 2.44 ± 0.01 c | 0.56 ± 0.06 ab |
| MVS$_{Toasted}$ | 10.30 ± 0.00 c | 3.55 ± 0.00 a | 2.23 ± 0.03 b | 0.39 ± 0.01 a |
| OW$_{2014}$ | 10.30 ± 0.00 c | 3.58 ± 0.00 c | 2.15 ± 0.01 a | 0.65 ± 0.09 b |
| OW$_{Toasted}$ | 10.40 ± 0.00 c | 3.55 ± 0.01 a | 2.40 ± 0.01 c | 0.70 ± 0.05 b |

The mean values are shown with their standard deviation (n = 3). For each year, different letters in the same column indicate significant differences between treatments (p ≤ 0.05). Control: untreated grapevines, from 2013 (Control$_{2013}$) and 2014 (Control$_{2014}$). AVS, grapevines treated with Airén aqueous extract from non-toasted vine-shoots. From 2013, applied once on day 7 post-veraison (AVS$_{2013-50}$ and AVS$_{2013-100}$) and applied twice, at days 7 and 14 post-veraison, diluted with water at 50 % (AVS$_{2013-50}$); from 2014, applied once on day 7 post-veraison (AVS$_{2014}$). AVS$_{Toasted}$, grapevines treated with Airén aqueous extract from toasted vine-shoots, applied once on day 7 post-veraison in 2014. MVS$_{2014}$, grapevines treated with Moscatel aqueous extract from non-toasted vine-shoots, applied once on day 7 post-veraison in 2014. MVS$_{Toasted}$, grapevines treated with Moscatel aqueous extract from toasted vine-shoots, applied once on day 7 post-veraison in 2014. OW, commercial aqueous oak extract (OW$_{2014}$) applied once on day 7 post-veraison; French oak wood extract from non-toasted chips in 2014 (OW$_{2014}$) and French oak wood extract from toasted chips in 2014 (OW$_{Toasted}$).
TABLE 2. Oenological parameters of wines at the end of alcoholic fermentation after the different grapevine treatments applied in 2013 and 2014.

| Alcoholic degree (%) (v/v) | pH | Total acidity (g/L tartaric acid) | Volatile acidity (g/L) | Malic acid (g/L) | Lactic acid (g/L) | Abs 420 |
|---------------------------|----|---------------------------------|------------------------|------------------|------------------|---------|
| Wines 2013                |    |                                 |                        |                  |                  |         |
| Control 2013              | 9.70 ± 0.13 c | 3.25 ± 0.01 a | 6.81 ± 0.11 a | 0.15 ± 0.02 ab | 3.65 ± 0.03 b | 0.29 ± 0.03 c | 0.52 ± 0.04 b |
| AVS2013-50                | 9.95 ± 0.30 c | 3.28 ± 0.01 b | 6.83 ± 0.08 a | 0.26 ± 0.03 c | 3.40 ± 0.09 a | 0.14 ± 0.02 ab | 0.52 ± 0.03 b |
| AVS2013-100               | 8.96 ± 0.14 b | 3.33 ± 0.01 c | 6.71 ± 0.09 a | 0.11 ± 0.03 a | 3.60 ± 0.09 b | 0.10 ± 0.03 a | 0.58 ± 0.04 b |
| OW2013                    | 8.13 ± 0.36 a | 3.23 ± 0.01 a | 6.83 ± 0.08 a | 0.20 ± 0.04 bc | 3.86 ± 0.07 c | 0.18 ± 0.03 b | 0.45 ± 0.02 a |
| Wines 2014                |    |                                 |                        |                  |                  |         |
| Control 2014              | 12.03 ± 0.05 f | 3.34 ± 0.01 e | 6.08 ± 0.04 b | 0.14 ± 0.02 ab | 1.86 ± 0.02 bc | 0.40 ± 0.02 b | 0.53 ± 0.04 ab |
| AVS2014                   | 10.86 ± 0.03 e | 3.31 ± 0.01 d | 5.67 ± 0.03 a | 0.09 ± 0.01 a | 1.70 ± 0.03 a | 0.39 ± 0.03 ab | 0.47 ± 0.02 a |
| AVS toasted 2014          | 7.90 ± 0.03 a | 3.20 ± 0.01 a | 6.13 ± 0.02 bc | 0.10 ± 0.01 ab | 1.78 ± 0.02 ab | 0.31 ± 0.03 ab | 0.50 ± 0.03 ab |
| MVS2014                   | 9.79 ± 0.02 a | 3.23 ± 0.02 b | 5.95 ± 0.22 b | 0.12 ± 0.08 ab | 1.86 ± 0.13 bc | 0.40 ± 0.12 b | 0.49 ± 0.02 a |
| MVS toasted 2014          | 10.48 ± 0.04 c | 3.28 ± 0.00 c | 6.32 ± 0.11 c | 0.15 ± 0.01 b | 2.25 ± 0.10 d | 0.58 ± 0.02 c | 0.54 ± 0.02 b |
| OW2014                    | 10.67 ± 0.07 d | 3.28 ± 0.01 d | 6.13 ± 0.02 bc | 0.12 ± 0.01 ab | 1.92 ± 0.04 c | 0.36 ± 0.02 ab | 0.55 ± 0.02 b |
| OW toasted 2014           | 10.00 ± 0.17 b | 3.25 ± 0.00 b | 6.08 ± 0.14 b | 0.09 ± 0.01 a | 1.98 ± 0.07 c | 0.29 ± 0.06 a | 0.53 ± 0.02 ab |

The mean values are shown with their standard deviation (n = 3). For each year, different letters in the same column indicate significant differences between treatments (p ≤ 0.05). Control: untreated grapevines, from 2013 (Control2013) and 2014 (Control2014). AVS, grapevines treated with Airén aqueous extract from non-toasted vine-shoots, from 2013, applied once on day 7 post-veraison (AVS502013-100) and applied twice, on days 7 and 14 post-veraison, diluted with water at 50% (AVS502013-50); from 2014, applied once on day 7 post-veraison (AVS502014-100). AVS toasted grapevines treated with Airén aqueous extract from toasted vine-shoots, applied once on day 7 post-veraison in 2014. MVS, grapevines treated with Moscatel aqueous extract from non-toasted vine-shoots, applied once on day 7 post-veraison in 2014. MVS toasted grapevines treated with Moscatel aqueous extract from toasted vine-shoots, applied once on day 7 post-veraison in 2014. OW, commercial aqueous oak extract (OW2013) applied once on day 7 post-veraison; French oak wood extract from non-toasted chips in 2014 (OW toasted 2014) and French oak wood extract from toasted chips in 2014 (OW toasted 2014)
**TABLE 3.** Glutathione, *trans*-GRP and *trans*-caftaric and *trans*-p-coutaric acids content (mg/L) in musts and wines after the different grapevine treatments applied in 2013 and 2014.

| Year | Treatment | Musts | Wine | Musts | Wine | Musts | Wine | Musts | Wine | Musts | Wine |
|------|-----------|-------|------|-------|------|-------|------|-------|------|-------|------|
| 2013 | Control 2013 | 44.69 ± 1.99 c, β | 1.20 ± 0.46 b, α | 10.79 ± 0.06 a, β | 57.46 ± 0.17 d, β | 2013 | AVS 2013 | 45.95 ± 1.97 c, β | 1.26 ± 0.40 b, α | 12.35 ± 0.11 c, β | 47.49 ± 0.47 a, β | 31.41 ± 0.31 b, α | 6.92 ± 0.08 b, α |
|      | AVS 2013 > 50 | 40.86 ± 0.94 b, β | 0.29 ± 0.14 a, α | 12.70 ± 0.07 d, β | 53.41 ± 0.68 b, β | 2013 | AVS 2013 > 100 | 29.31 ± 0.91 a, β | 0.83 ± 0.35 ab, α | 11.31 ± 0.10 b, β | 55.05 ± 0.86 c, β | 30.74 ± 0.31 b, α | 7.02 ± 0.13 b, α |
| 2014 | Control 2014 | 20.18 ± 1.31 c, β | 1.06 ± 0.24 b, α | 6.52 ± 0.02 d, β | 18.87 ± 0.58 a, β | 2014 | AVS 2014 | 21.01 ± 0.18 c, β | 1.15 ± 0.02 b, α | 7.76 ± 0.15 f, β | 26.67 ± 0.87 c, α | 29.16 ± 0.01 ab, β | 9.59 ± 0.30 c, α |
|      | AVS toasted | 17.80 ± 0.23 bc, β | 0.32 ± 0.03 a, α | 4.93 ± 0.08 b, α | 25.55 ± 0.77 c, α | 2014 | MVS 2014 | 15.23 ± 0.02 ab, β | 0.39 ± 0.06 a, α | 7.19 ± 0.02 e, β | 40.60 ± 0.78 e, β | 32.02 ± 0.04 c, β | 10.39 ± 0.30 c, α |
|      | MVS toasted | 15.20 ± 0.41 ab, β | 0.35 ± 0.00 a, α | 7.19 ± 0.06 e, β | 32.21 ± 0.37 d, α | 2014 | OW 2014 | 12.95 ± 0.32 ab, β | 0.47 ± 0.06 a, α | 6.12 ± 0.02 c, β | 22.69 ± 0.63 b, α | 7.91 ± 0.13 b, α | 9.54 ± 0.03 b, α |
|      | OW toasted | 13.50 ± 2.04 b, β | 0.22 ± 0.02 a, α | 4.66 ± 0.10 a, β | 18.13 ± 0.33 d, β | 2014 | MVS toasted | 15.20 ± 0.41 ab, β | 0.35 ± 0.00 a, α | 7.19 ± 0.06 e, β | 32.21 ± 0.37 d, α | 32.96 ± 0.18 c, α | 12.64 ± 0.38 d, β |

For each year, letters in the same column indicate significant differences between treatments and Greek letters indicate, for each compound, differences between musts and wines according to the Tukey test (p ≤ 0.05). The mean values are shown with their standard deviation (n = 3).

Control: untreated grapevines, from 2013 (Control 2013) and 2014 (Control 2014). AVS, grapevines treated with Airén aqueous extract from non-toasted vine-shoots, from 2013, applied once on day 7 post-veraison (AVS 2013 > 100) and applied twice, on days 7 and 14 post-veraison, diluted with water at 50 % (AVS 2013 > 50); from 2014, applied once on day 7 post-veraison (AVS 2014) and applied two times, on days 7 and 14 post-veraison, diluted with water at 50 % (AVS 2014 > 50). AVS toasted: grapevines treated with Airén aqueous extract from toasted vine-shoots, applied once on day 7 post-veraison in 2014 (AVS toasted). MVS, grapevines treated with Moscatel aqueous extract from non-toasted vine-shoots, applied once on day 7 post-veraison in 2014. MVS toasted: grapevines treated with Moscatel aqueous extract from toasted vine-shoots, applied once on day 7 post-veraison in 2014. OW, commercial aqueous oak extract (OW 2013) applied once on day 7 post-veraison; French oak wood extract from non-toasted chips in 2014 (OW 2014) and French oak wood extract from toasted chips in 2014 (OW toasted).
As in 2013 vintage, malolactic fermentation was not carried out, which was confirmed by the high values of malic acid (Table 2): the wines with the MVS\textsubscript{toasted} treatment had a significantly higher value of malic and lactic acid. Although the vintage variability is observed, foliar applications seem to have a clear effect on the alcohol degree, as in both vintages the treated wines were less alcoholic than control ones. Additionally, the formulation effect along with the wood toasting procedure or wood type, does not seem to have an influence on the alcohol degree.

2. Glutathione, trans-GRP and trans-caftaric and trans-\(p\)-coutaric acids content in musts and wines

Glutathione, trans-GRP and trans-caftaric and trans-\(p\)-coutaric acids content in musts and wines for the two years studied after different grapevines treatments are summarized in Table 3. In both studied vintages, the application of Airén vine-shoot extracts (AVS and AVS\textsubscript{toasted}) did not affect glutathione concentration in musts with respect to the control wines. However, in both cases, the treatment carried out with the oak wood extracts (OW and OW\textsubscript{toasted}) significantly decreased the glutathione content in the musts compared to control samples. In relation to the Moscatel vine-shoot extracts (MVS and MVS\textsubscript{toasted}), their effect was similar to those obtained by the OW extracts, as glutathione concentration in musts decreased compared to control samples. The concentration of all these extracts decreased considerably after alcoholic fermentation, even below 2 mg/L (Table 3). In the 2013 vintage, wines from grapevines treated with AVS\textsubscript{2013-100} showed the lowest glutathione content with respect to the control wines. However, in the 2014 vintage, wines elaborated from the grapevines treated with different treatments presented lower glutathione concentration than control samples, except for AVS\textsubscript{2014} wines. The different behavior in the glutathione concentration could be due to the climatic conditions, among other reasons, as those affect the vines development and by the pathways that modify or activate vine secondary mechanisms due to the different extract applications.

All treatments applied during the 2013 vintage increased significantly the trans-GRP content in musts, with respect to controls. In this regard, the most effective treatments were both vine-shoot extracts (AVS\textsubscript{2013-50} and AVS\textsubscript{2013-100}) compared to the oak wood extract application (OW\textsubscript{2013}) (Table 3). In the 2014 vintage, the content of trans-GRP in musts was increased by the application of non-toasted Airén vine-shoot extract (AVS\textsubscript{2014}) and from both Moscatel vine-shoot extracts, non-toasted and toasted. The rest of the treatments, such as application of oak wood extracts (OW\textsubscript{2014} and OW\textsubscript{toasted}) and AVS\textsubscript{toasted} produced a decrease in trans-GRP content respect to control musts. trans-GRP concentration also decreased after alcoholic fermentation, but to a lesser extent than glutathione (Table 3). In the 2013 vintage, wines from grapevines treated with AVS\textsubscript{2013-50} and AVS\textsubscript{2013-100} had higher trans-GRP content than the control and OW\textsubscript{2013} wines. In 2014, the highest trans-GRP concentration was found in the AVS\textsubscript{2014} wines, while the lowest content was found in OW\textsubscript{toasted} wines.

In the first year of study, the concentration of trans-caftaric acid decreased in the musts from grapevines treated with vine-shoot and OW extracts (Table 3). However, in the 2014 vintage, all treatments except OW\textsubscript{toasted} increased trans-caftaric acid content in the musts compared to control samples. In this way, the most effective treatments were the vine-shoot extracts from Moscatel (MVS\textsubscript{2014} and MVS\textsubscript{toasted}). trans-caftaric acid concentration during the 2013 vintage decreased after alcoholic fermentation, but it increased or remained similar in the 2014 season. It is possible that during the 2013 winemaking this compound was involved in enzymatic oxidation reactions. trans-Caftaric acid forms o-quinone leading to color darkening (Ferreira-Lima \textit{et al.}, 2016). So, wines from 2013 vintage elaborated from grapevines treated with AVS\textsubscript{2013-50} and OW\textsubscript{2013} had higher trans-caftaric acid content than the control and AVS\textsubscript{2013-100} wines. In the 2014 vintage, wines from untreated grapevines AVS\textsubscript{2014} and OW\textsubscript{2014} showed lower trans-caftaric acid content than AVS\textsubscript{toasted}, MVS\textsubscript{2014} and MVS\textsubscript{toasted} wines.

The treatments did not affect trans-\(p\)-coutaric acid concentration in the 2013 vintage musts, except AVS\textsubscript{2013-50} (Table 3), which showed lower content compared to the control and the rest of the treatments. However, in the 2014 vintage all treatments increased trans-\(p\)-coutaric acid content compared to untreated grapevines: MVS\textsubscript{2014} and MVS\textsubscript{toasted} were the most effective treatments. This was similar for trans-caftaric acid, as after alcoholic fermentation, its concentration increased in all samples and in both vintages except for MVS\textsubscript{2014} and MVS\textsubscript{toasted} (Table 3). The increase in the trans-\(p\)-coutaric acid content from must to wine was also reported by Portu \textit{et al.} (2015) when jasmonate foliar application was carried out on
whether trans-p-coumaric acid, whose content was similar during both vintages, the concentration of the rest of the compounds studied in the Airén musts was higher in 2013 than in 2014 vintage. trans-p-Coumaric acid is an ester formed from trans-p-coumaric acid and tartaric acid. Acid esters are formed in simple hydrogen-ion-catalyzed esterification (Margalit, 2004).
and it has been shown that their formation and the hydroxycinnamic acid production depends on pH (Edwards et al., 1985; Hall and De Luca, 2007). As for the glutathione concentration, the differences found in the phenolic acid composition could be attributed to the physiological impact of the use of the extracts on biosynthesis of the compounds analyzed in must and wines, related to the secondary mechanism.

In an attempt to differentiate the treatments of both vintages in musts and wines according to glutathione, trans-GRP and trans-caftaric and trans-p-coumaric acids compounds, complementary discriminants analyses were also carried out (Figure 2). Figure 2a shows the graphical representation of the must samples in the 2013 vintage for each treatment. The two canonical discriminant functions explained 97.60 % of the total variance, reaching 84.40 % with the first. In case of function 1, trans-GRP was the compound that contributed more to the differentiation with a negative correlation. For function 2, glutathione had the highest weight, also with a negative correlation. Figure 2b shows the discriminant analysis for wines from 2013 vintage, which presented a wide distance between control, OW\textsubscript{2013} and AVS\textsubscript{2013}-100, with respect to AVS\textsubscript{2013}-50 treatment. Function 1 and 2 explained 90.50 % and 9.40 % of the total variance, respectively. trans-GRP and trans-caftaric acid were the compounds that contributed more to the differentiation with a positive correlation in function 1, whereas for function 2 trans-p-coumaric acid had a higher weight and a positive correlation. According to the information provided by the variables related to functions 1 and 2, it could be concluded that musts from AVS\textsubscript{2014} were described by trans-GRP, while musts from MVS\textsubscript{2014} retain higher levels of trans-caftaric acid. With respect to the wines, AVS\textsubscript{2014} and control samples were described by trans-caftaric acid and trans-GRP, while MVS\textsubscript{2014} samples were characterized by trans-GRP, AVS\textsubscript{Toasted}\textsuperscript{*} MVS\textsubscript{Toasted}\textsuperscript{*} OW\textsubscript{2014} and OW\textsubscript{Toasted} wine samples presented similar behavior.

**Acknowledgments:** Many thanks for the financial support given by the Ministerio de Economía y Competitividad-FEDER of the Spanish Government to the Project AGL2012-33132. T.G.-C. wishes to thank the MINECO for her Ramón y Cajal contract. Also, R.S.-G. is grateful for her predoctoral contract (PREDUCML17) from Universidad de Castilla-La Mancha. A.G.-D. and E.P.P.-Á. thank the Gobierno de La Rioja for her research personal formation grant. We wish to thank the Bodega San Isidro (Winery in Murcia, southwest Spain) for allowing us to use its vineyards and Francisco Pardo, Diego Cutillas and Juan Miguel Baños for their technical assistance during winemaking and Manuel Márquez (Viticultural Service Company) for selecting and supplying the vine-shoots.

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