Applying rosemary extract and caffeic acid to modify the composition of Monastrell wines

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Abstract
This work studies the effect of applying rosemary extract and caffeic acid on the polyphenolic and aromatic composition of Monastrell wines, as well as the influence of traditional winemaking or incorporating prefermentative maceration. For this purpose, three treatments were carried out in triplicate. In one of them, rosemary extract was applied on the clusters 10 days before harvest, caffeic acid was applied in the same way in another, and, finally, this acid was applied to grape before crushing. Each treatment was run by both traditional vinification and vinification with prefermentative maceration. After making wines, they were monitored for 12 months after fermentation. The application of rosemary extract, and that of caffeic acid but to a lesser extent, increased the color, the concentration of anthocyanins, and the percentage of polymerized anthocyanins, while prefermentation maceration gave rise to wines with a higher concentration of condensed tannins and polyphenols. Applying rosemary extract and caffeic acid in the vineyard also increased the concentration of esters and other compounds that favor wine aromatic quality, which was also enhanced by prefermentative maceration.

Keywords Rosemary extract · Caffeic acid · Wine · Polyphenols · Aromas

Introduction
The polyphenolic and aromatic composition is a determining factor for organoleptic wine properties. It is often accepted that both color and structure define the quality of red wines, with anthocyanins, flavonols, and their polymeric pigments being the phenolic compounds with the strongest sensory impact. In young wine, color is due mainly to grape anthocyanin composition, and also to extraction intensity during maceration. In contrast, the color of aged red wine is a consequence of the formation of stabler pigments [1, 2].

Copigmentation effect plays an important role in color stability [3, 4]. During copigmentation, associations are formed between red compounds of the grapes (anthocyanins) and other mostly colorless compounds. The reactions lead to hyperchromic, hypsochromic, or bathochromic changes, and to the formation of vertical stacking structures, which prevent water molecules entering these complexes and, thus, protect anthocyanins from hydration by shifting the equilibrium to colored forms [5–10].

Copigmentation also influences the oxidation, condensation or polymerization reactions of phenolic compounds as it decreases the kinetics of the reactions that occur during wine aging [11, 12]. These anthocyanin copigmentation reactions can also act as a first phase in the formation of stable polymeric pigments [13, 14].

From a structural point of view, the best copigments are those containing aromatic nuclei with a flat conformation, because it allows them to approach and associate with anthocyanins [15]. The molecules that act as copigments exist naturally in grapes and musts, and are mainly phenols in both non-flavonoid and flavonoid compounds, but also alkaloids, amino acids, organic acids, polysaccharides, metals, etc. [4]. Phenolic compounds possess π-conjugated systems that facilitate their combination with anthocyanin via π–π-stacking interactions [16]. Of all the non-flavonoid copigments, hydroxycinnamic acids have the highest copigmentation potential, because they constitute the main acyl groups in the structure of acylated anthocyanins. Of them all, caffeic acid stands out, because it esterifies the molecule of both malvidol 3-glucoside and peonidol 3-glucoside at position 6. Caffeic acid derives from caftaric
acid hydrolysis, which can be induced by exposing grapes to sunlight. Its action as a copigment has been studied in many research works in model systems [17–23] and as an additive in winemaking [24–30]. These studies show a strong copigmenting effect of caffeic acid when interacting with anthocyanins in both synthetic media and wine. Although caffeic acid has been shown to be a good copigment, its supplementation in vine and winemaking is not authorized by OIV.

To enhance the effect of copigmentation, it is necessary to have a higher concentration of copigments and pigments in wines, as well as a higher copigment/pigment ratio. To fulfill these objectives, strategies can be adopted in viticulture and winemaking fields [27, 29]. To do so, many authors have studied the cofermentation of different grape cultivars and the addition of copigments in the prefermentation stage [31–33].

To increase the contact between phenolic compounds in must and to improve copigmentation reactions, carrying out a prefermentation maceration phase could be interesting [34, 35]. Prefermentative maceration increases the extraction and stabilization of polyphenolic compounds in the liquid phase (anthocyanins and low-molecular-weight condensed tannins) and reduces the extraction intensity during the fermentation process to avoid extracting bitter condensed tannins from seeds [36, 37]. If cooling takes place with carbonic snow, the freezing of skin results in the lysis and disorganization of grape cells, and provides an easy outlet for phenolic and aromatic compounds [38, 39]. Several authors have found that prefermentative maceration leads to an improvement in wine aromatic composition and have described an increase in not only the concentration of esters, but also in fruit and floral aromas [40, 41]. All this can be attributed to an easier extraction of these compounds, but also to the fact that fermentation by non-<em>Saccharomyces</em> autochthonous yeasts may begin at a low temperature, which generates more varietal aromas [40, 42].

The prefermentative supplementation of copigments, combined with prefermentative cold maceration techniques, has shown a synergistic effect on copigmentation processes and color stability by demonstrating that their joint effect on the color of wines is superior to that obtained when applied alone prefermentative maceration [43, 44]. The effect of the supplementation of copigments could be more effective if they interacted with grape components during the ripening process [18, 43]. Recent studies have shown that the foliar application of biotic and abiotic elicitors to the vines can modify grape composition. Several plant extracts can contribute to increase the concentration of polyphenols [45–48]. Extracts from oak, algae, grapevine shoots, methyl jasmonate, chitosan, and yeast have demonstrated their effectiveness in increasing the concentration of amino acids in must [49, 50], enhancing polyphenolic stability [51–54], and rising the concentration of volatile compounds in wine [55–60].

According to Del Pozo-Isfran [61], the application of rosemary extract to vineyards prolongs the half-life of anthocyanins during wine conservation. Talcott et al. [62] and Brenes et al. [63] also verified that adding rosemary extract to grapes juice increases the formation of the complexes of copigments with anthocyanins and results in not only hyperchromic changes in wine, but also improves its antioxidant capacity. Bimpilas et al. [48] applied rosemary extract and other natural extracts rich in hydroxycinnamic acids, and observed an increase in anthocyanins and color intensity, which were greater than those observed with caffeic acid addition. On the other hand, Darici et al. [64] found a significant increase in the concentration of esters in Cabernet Sauvignon wines treated with rosemary extract. Different studies have determined the composition of the extracts of rosemary (<em>Rosmarinus officinalis</em> L.) [65–67] to establish its complex composition, it being rich in flavonoids, phenolic acids and terpenoids, with carnosic acid as the predominant compound, and also found caffeic acid and its ester, rosminaric acid. Perhaps, the action of rosemary extract was due to a more complex composition, rich in flavonoids and other polyphenols.

Given its long ripening cycle of Monastrell variety, their grapes must be harvested with a high sugar concentration to achieve good polyphenolic maturity, which allows to obtain a stable color and a balanced tannic concentration. This results in wines with high alcohol contents, which are not well accepted by consumers, and is contrary to the decreasing alcohol consumption policy. These wines have very low acidity, which imposes touch-ups that tend to do away with wine’s organoleptic balance. This situation, together with the high concentration of this variety’s polyphenoloxidase enzymes, poses serious winemaking problems by hindering color stability and wine’s polyphenolic balance.

The purpose of this work is to compare the effect of adding rosemary extracts rich in flavonoids and caffeic acid and the direct application of caffeic acid to vineyards and on prefermentative addition. To increase the contact between the copigments and the grapes, prefermentative maceration has been tested as an alternative to traditional red wine vinification. The aim is to achieve not only a stabler color, but also a better polyphenolic and aromatic balance, in Monastrell wines. The application of these techniques can be a very useful tool for designing winemaking systems that guarantee crop sustainability by always taking quality improvement as a fundamental objective. Spraying with natural plant extracts can also be very interesting for organic viticulture [68].
Materials and methods

Grape and wine samples

The trial was carried out in the “Valencian Denomination of Origin” (Fontanars, Spain), for 2 consecutive years (2016 and 2017). The plant material was cv. Monastrell variety (syn. Mourvedre VICV-7915) vines grafted onto Richter-110R rootstocks, planted in rainfed in 2005 and spaced \( 1.5 \times 3 \) m (2.200 vines/ha). Vines were trained to a vertical trellis on a bilateral cordon system with East-South orientation. The soil has a sandy loam texture, highly calcareous, and of low fertility.

The trials’ experimental design was factorial and performed in randomized complete blocks with three replicates. Each block had all experiments (three). The elementary plots contained 30 vines each for those receiving treatment, and 60 vines for those not receiving treatment.

The assay involved three experiments in the vineyard: (1) grapes without treatment; (2) treated grapes with rosemary extract; (3) treated grapes with caffeic acid.

The ripening of the fruits was monitored to apply both rosemary extract and caffeic acid at the optimum time, when the harvest’s anthocyanin potential allowed copigmentation to be effective in grapes. Based on previous experience, 10 days before the estimated harvest was considered the optimum time for copigmentation reactions to occur. Rosemary extract and caffeic acid were applied with a non-ionic surfactant to promote adherence of the products to grape skin (Montana wax at 20%, 2.5 mL/L). The rosemary extract and caffeic acid were previously dissolved in water up to a concentration appropriate to apply about 90 mg of caffeic acid per kg of grapes. Applications were made by spraying in the grape clusters area.

Caffeic acid was purchased from Sigma-Aldrich and rosemary extract was supplied by Acofarma (Spain). The caffeic acid concentration in the rosemary extract was determined by HPLC to calculate the amount to be applied, using a similar concentration to that of the treatment with pure caffeic acid.

The cluster were collected in 20-kilogram boxes 10 days after applying copigments treatments. Caffeic acid at 90 mg/kg dose was applied to half of the untreated grapes on the selection table before destemming and crushing. The remaining of the untreated grapes were used for the control wines. The grapes treated with rosemary extract, caffeic acid in the vineyards, and caffeic acid before fermentation and control were vinified following two vinification protocols: one with prefermentative maceration at 5–6 °C for 5 days, followed by traditional fermentation; the other with traditional fermentation without prefermentative maceration. All the vinifications were carried out in triplicate (Fig. 1).

Grapes were processed in a pallet destemmer-roller crusher. Pulp was placed in 50-liter tanks. A commercial Saccharomyces cerevisiae yeast (Enartis Ferm Red Fruit) was inoculated for fermentation (30 g/hL). The fermentation temperature was 27–28 °C and two pumping overs were made.

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**Fig. 1** Experimental design
performed daily. After a 10-day maceration-fermentation, low-pressure pressing was carried out, and wine was blended with the first-pressing. Malolactic fermentation was favored by the prior addition of 1 g/L of Oenococcus oeni bacteria (Lalvin 31, Lallemand). Having completed malolactic fermentation, and after sulfiting at 30 mg/L of free sulfur, wines were racked and homogenized, and the aromatic and polyphenolic wine composition was monitored for 12 months.

**Determination of common parameters**

The common parameters (density, ethanol, pH, total acidity, volatile acidity, and sulfurous) were determined according to Official Methods (Commission Regulation (EEC) 1990) [69]. Total soluble solids (TSS) ('Brix) was determined by refractometry and reducing sugars by the Fehling method (Blouin [70]).

**Determination of phenolic compounds**

The wines' phenolic composition was determined by a Jasco V-530 UV–visible spectrophotometer and a Jasco MD2010 Plus high-performance liquid chromatography (HPLC) instrument coupled with a diode array detector (DAD) (Jasco LC-Net II/ADC, Tokyo, Japan). All the measurements were taken in triplicate. Color intensity, hue, and the gelatin index (which is directly linked to astringency) were estimated by the methods described by Glories [71]. Total polyphenols were calculated according to Folin’s method [72]. Condensed tannins were determined following the method developed by Ribéreau-Gayon [73]. The method reported by Boulton was used to analyze the contribution of the copigmented, free and polymeric anthocyanins to total wine color [74]. The DMACH index, which indicates the degree of condensed tannins polymerization, was calculated according to Vivas and Glories [75].

Individual phenolic compounds were quantified by HPLC by the method of Jensen et al. [76]. HPLC was used to quantify phenolic acids, flavan-3-ols, major anthocyanidins and acylated anthocyanins [76]. Total anthocyanins were calculated as the sum of anthocyanidins and acylated anthocyanins. After centrifugation and filtration, wine samples were injected directly into the HPLC (20 μL). Separation was performed at 40 °C in a Gemini NX column (Phenomenex, Torrance, CA, USA): 5 μm, 250 mm × 4.6 mm. Solvents were trifluoroacetic acid at 0.1% (A) and acetonitrile (B). The elution gradient was as follows: 100% A (min 0); 90% A + 10% B (min 5); 85% A + 15% B (min 20); 82% A + 18% B (min 25); 65% A + 35% B (min 30). Individual chromatograms were extracted at 280 nm (3- flavanols and phenolic acids), 320 nm (phenolic acids), 360 nm (flavonols), and 520 nm (anthocyanins). For quantification purposes, calibration curves were obtained with commercially available standards: flavan-3-ols (Fluka, Milwaukee, WI, USA), caffeic acid (Fluka, Milwaukee, WI, USA), and malvidin-3-O-mono glucoside (Sigma-Aldrich, St Louis, MO, USA). The same method was followed for the quantification of caffeic acid in extracts.

**Determination of aromatic compounds**

The aromatic wine composition was determined in an HP-6890 gas chromatograph (GC) (Hewlett Packard, Palo Alto, CA, USA) equipped with a split/splitless capillary injection port and a flame ionization detector (FID). Separations were performed inside a Phenomenex ZB-Wax plus column (60 m × 0.25 mm × 0.25 μm). The column temperature was initially set at 40 °C. This temperature was left for 5 min before being raised to 102 °C at a rate of 4 °C/min; to 112 °C at a rate of 2 °C/min; to 125 °C at a rate of 3 °C/min; and this temperature was left for 5 min and then raised to 160 °C at a rate of 3 °C/min; to 200 °C at a rate of 6 °C/min; and this temperature was left for 30 min. The carrier gas was helium that flowed at a rate of 3 mL/min. Injections were performed in the 1:20 split mode (2 μL injection volume) with an FID. Injections were performed in triplicate. Volatile compounds were identified by comparing retention times to standard compounds. In addition, Kovats retention indices (KI) for the GC peaks corresponding to substance identification were calculated by interpolating the retention time of the standard alkane (C8–C20) (Fluka Buchs, Schwiez, Switzerland), and analyzed under the same chromatographic conditions. The calculated KI were compared to those reported in the literature for the same stationary phase. Sample preparation was carried out following the method proposed by Ortega et al. [77] with the modifications specified by Hernández-Orte et al. [78].

**Statistical analysis**

The statistical analysis was carried out using Centurion XVI.II for Windows (Statgraphics Technologies, Inc., The Plains, VA, USA). Interactions between different treatments were performed by a multifactorial analysis of variance (ANOVA). The data corresponding to the control wines and the wines from the copigmentation treatments with rosemary extract and caffeic acid were processed by a simple ANOVA to evaluate whether the application of copigments influenced wine composition. The data corresponding to the wines made by traditional vinification, and those by prefermentation maceration followed by traditional vinification, were processed with a simple ANOVA to establish whether prefermentation maceration modified wine composition. The Duncan's test was used to separate means (p value < 0.01) when the ANOVA test was significant.
Results and discussion

This article shows the polyphenolic and aromatic concentrations of wines at the end of the 12-month storage period. No differences have been observed in the oenological parameters in the must and in the wine at the end of fermentation. The statistical analysis included a multifactorial ANOVA to assess the overall effect of the factors co-pigments and vinification techniques and the interaction among them. One way ANOVA also was performed to assess the individual effect of the different co-pigments and vinification techniques applied.

Common parameters in Monastrell musts and wines

In 2016, the must analyses did not show significant differences in Brix degree (23.8–24.34), pH (3.43–3.54) or total acidity (5.78–5.91 g/L as tartaric acid). A similar situation was observed in 2017 musts (Brix degree: 24.41–24.86; pH: 3.55–3.64; and total acidity: 5.21–5.39 g/L in tartaric acid). This indicates that the co-pigmentation treatments did not affect the technological maturity of the grapes. The small differences observed can be attributable to the intrinsic variability of the vineyard. However, differences have been observed between vintages, since 2017 was a warmer year and riper grapes were obtained.

The alcohol degree of the wines produced in 2016 was between 13.8 and 14.15%. The pH values differed only by 0.11 units (3.58–3.69), and the total acidity variation was 0.42 g/L (5.25–5.67 g/L in tartaric acid). The wines were fermented to dryness with residual sugars ranging between 1.45 and 2.32 g/L, in line with those levels usually reported [79]. The volatile acidity was between 0.38 and 0.60 g/L, and the total sulfur was between 70 and 78 mg/L, values that are common in industrial wines [80]. In 2017, the wines showed a higher alcohol content (14.19–14.41%), higher pH (3.62–3.75) and lower total acidity (4.79–5.08 g/L as tartaric acid). The volatile acidity (between 0.45 and 0.65 g/L) and sulfuric (85–93 mg/L) were as also within the expected values, as well as the residual sugars, which did not exceed 2.41 g/L. The small differences observed for the oenological parameters of the wines indicate that the copigmentation treatments and winemaking practices applied do not have significant influence in these parameters.

Polyphenolic composition of Monastrell wines

Table 1 shows the multifactorial ANOVA data for the experimental factors addition of copigments considered. In each column, an F-ratio values can be compared to one another in each column, because the number of comparisons was the same in all cases. A high F-ratio value means the factor has a stronger effect on the variable.

A slight interaction between factors was observed for some polyphenolic parameters. For some of these compounds, this denotes that the effect of the applied copigments was slightly different depending on the vinification

Table 1 Multifactorial variance analysis for the applied copigments (Copig), the winemaking technique (Tech) and their interaction, for the polyphenolic compounds of Monastrell wines in 2016 and 2017

| Compounds                       | Interaction Copig × Tech | Copigments 2016 | Copigments 2017 | Winemaking Techniques 2016 | Winemaking Techniques 2017 |
|--------------------------------|--------------------------|----------------|----------------|---------------------------|---------------------------|
| Color intensity                | ns                       | 5.22*          | 3.23           | ns                        | ns                        |
| Hue (%)                        | 4.21*                    | ns             | ns             | ns                        | ns                        |
| Copigmented anthocyanins (%)   | ns                       | ns             | ns             | ns                        | ns                        |
| Polymerized anthocyanins (%)   | ns                       | 9.48**         | 11.54**        | ns                        | ns                        |
| Free anthocyanins (%)          | 3.78*                    | 14.11**        | 7.84**         | ns                        | ns                        |
| Malvidin (mg/L)                | ns                       | ns             | 7.89**         | ns                        | ns                        |
| Peonidin (mg/L)                | ns                       | ns             | 4.56*          | ns                        | ns                        |
| Petunidine (mg/L)              | ns                       | ns             | 3.57*          | 3.71*                     | ns                        |
| Cyanidin (mg/L)                | ns                       | ns             | 3.38*          | ns                        | ns                        |
| Delphinidin (mg/L)             | ns                       | ns             | 5.52**         | ns                        | 11.77**                   |
| Total anthocyanins (mg/L)      | 12.41***                 | 14.27***       | 4.80*          | ns                        | ns                        |
| Condensed tannins (g/L)        | 18.12***                 | ns             | ns             | 11.94**                   | 4.18*                     |
| Total polyphenols (g/L)        | ns                       | 4.79*          | ns             | 23.86**                   | 6.63**                    |
| Folin Index                    | ns                       | ns             | ns             | 4.26*                     | ns                        |
| DMACH Index (%)                | 14.81***                 | ns             | ns             | ns                        | ns                        |
| Gelatin Index (%)              | ns                       | 7.91**         | ns             | ns                        | ns                        |

In each row, the numbers denote significant differences according to Duncan’s test (*p < 0.05; **p < 0.01; ***p < 0.001)
Table 2  Means, standard deviations, and variance analyses of the polyphenolic parameters of Monastrell wines depending on the applied copigments during each season, and the average for 2016 and 2017

| Parameters                     | Copigment              | 2016        | 2017        | Average 2016–2017 | Year (p value) | Interaction Copig × Year (p) |
|-------------------------------|------------------------|-------------|-------------|-------------------|----------------|-------------------------------|
| Color intensity               | Control                | 9.13 ± 1.01a| 11.63 ± 0.90a| 10.73 ± 1.31a     | ns             | ns                            |
|                               | Rosemary extract       | 11.12 ± 0.20b| 12.62 ± 0.85a| 11.87 ± 0.52b     |                |                               |
|                               | Caffeic vineyard       | 10.77 ± 1.42b| 12.29 ± 1.26a| 11.53 ± 1.38ab    |                |                               |
|                               | Caffeic winery         | 10.15 ± 0.66ab| 11.95 ± 0.48a| 11.05 ± 0.79ab    |                |                               |
| Hue (%)                       | Control                | 75.68 ± 3.95a| 68.70 ± 2.57a| 72.19 ± 4.83a     | ns             | ns                            |
|                               | Rosemary extract       | 76.15 ± 0.94a| 68.99 ± 2.29a| 71.37 ± 4.00a     |                |                               |
|                               | Caffeic vineyard       | 73.55 ± 2.19a| 69.43 ± 2.38a| 71.49 ± 3.07a     |                |                               |
|                               | Caffeic winery         | 74.21 ± 2.37a| 67.88 ± 2.15a| 71.05 ± 3.93a     |                |                               |
| Copigmented anthocyanins (%)  | Control                | 9.87 ± 1.25a | 16.55 ± 1.63a| 13.21 ± 3.72a     | 21.38***       | ns                            |
|                               | Rosemary extract       | 10.82 ± 1.40a| 15.92 ± 2.00a| 14.22 ± 3.06a     |                |                               |
|                               | Caffeic vineyard       | 10.50 ± 1.88a| 15.60 ± 2.62a| 13.05 ± 3.43a     |                |                               |
|                               | Caffeic winery         | 9.66 ± 2.23a | 15.36 ± 1.81a| 12.51 ± 3.54a     |                |                               |
| Polymerized anthocyanins (%)  | Control                | 50.47 ± 3.27a| 47.64 ± 4.85a| 49.05 ± 4.26a     | ns             | ns                            |
|                               | Rosemary extract       | 56.49 ± 0.15b| 51.89 ± 2.86b| 54.19 ± 1.15b     |                |                               |
|                               | Caffeic vineyard       | 57.15 ± 4.27bc| 49.89 ± 6.62ab| 53.52 ± 6.93b     |                |                               |
|                               | Caffeic winery         | 60.20 ± 3.30c | 54.70 ± 5.20b | 57.45 ± 5.38c     |                |                               |
| Free anthocyanins (%)         | Control                | 39.66 ± 3.41b| 35.82 ± 6.04a| 37.74 ± 5.14a     | ns             | ns                            |
|                               | Rosemary extract       | 32.68 ± 1.53a| 32.19 ± 4.16a| 31.59 ± 3.43a     |                |                               |
|                               | Caffeic vineyard       | 32.35 ± 3.99a| 34.51 ± 9.91a| 33.93 ± 7.31a     |                |                               |
|                               | Caffeic winery         | 30.15 ± 2.30a| 29.94 ± 6.17a| 30.05 ± 4.51a     |                |                               |
| Malvidin-3-O-monoglucoside (mg/L) | Control              | 34.99 ± 7.35a| 49.13 ± 2.24a| 42.06 ± 15.45a    | ns             | 6.29**                        |
|                               | Rosemary extract       | 56.64 ± 5.48b| 51.89 ± 2.86b| 54.19 ± 1.15b     |                |                               |
|                               | Caffeic vineyard       | 39.36 ± 8.63ab| 54.28 ± 6.95b| 46.82 ± 7.15ab    |                |                               |
|                               | Caffeic winery         | 41.67 ± 8.72ab| 55.66 ± 4.21b| 48.67 ± 5.96ab    |                |                               |
| Peonidin-3-O-monoglucoside (mg/L) | Control              | 2.53 ± 0.56a | 3.18 ± 0.68a | 2.85 ± 0.62a      | ns             | 6.07*                         |
|                               | Rosemary extract       | 4.08 ± 1.12b | 3.14 ± 1.07a | 3.45 ± 0.56b      |                |                               |
|                               | Caffeic vineyard       | 2.77 ± 0.86a | 3.18 ± 0.86a | 2.98 ± 0.89a      |                |                               |
|                               | Caffeic winery         | 2.51 ± 0.96a | 3.13 ± 1.12a | 2.82 ± 1.06a      |                |                               |
| Petunidine-3-O-monoglucoside (mg/L) | Control              | 4.54 ± 1.24a | 4.38 ± 0.56a | 4.46 ± 1.33a      | ns             | ns                            |
|                               | Rosemary extract       | 7.14 ± 2.35b | 5.52 ± 2.17b | 6.06 ± 2.26b      |                |                               |
|                               | Caffeic vineyard       | 4.51 ± 2.35a | 4.72 ± 1.58a | 4.61 ± 1.97a      |                |                               |
|                               | Caffeic winery         | 3.77 ± 1.04a | 5.29 ± 1.78b | 4.53 ± 1.61a      |                |                               |
| Cyanidin-3-O-monoglucoside (mg/L) | Control              | 2.04 ± 0.33a | 2.07 ± 0.20a | 2.05 ± 0.38a      | ns             | ns                            |
|                               | Rosemary extract       | 3.09 ± 0.92b | 2.31 ± 0.72a | 2.57 ± 0.84b      |                |                               |
|                               | Caffeic vineyard       | 1.84 ± 0.57a | 2.45 ± 0.54a | 2.14 ± 0.58a      |                |                               |
|                               | Caffeic winery         | 2.03 ± 0.81a | 2.06 ± 0.67a | 2.05 ± 0.72a      |                |                               |
| Delphinidin-3-O-monoglucoside (mg/L) | Control              | 3.74 ± 0.84a | 4.87 ± 0.78a | 4.30 ± 0.97a      | ns             | 4.32*                         |
|                               | Rosemary extract       | 6.20 ± 2.72b | 4.59 ± 2.18a | 5.13 ± 1.38b      |                |                               |
|                               | Caffeic vineyard       | 4.15 ± 1.50ab| 4.84 ± 1.23a | 4.49 ± 1.49a      |                |                               |
|                               | Caffeic winery         | 3.75 ± 0.78a | 4.27 ± 1.58a | 4.01 ± 1.43a      |                |                               |
| Total anthocyanins (mg/L)     | Control                | 223.59 ± 17.39a| 303.21 ± 53.02a| 263.40 ± 56.07a    | 72.83***       | ns                            |
|                               | Rosemary extract       | 245.50 ± 7.54b| 349.30 ± 17.53b| 297.40 ± 75.43a    |                |                               |
|                               | Caffeic vineyard       | 242.05 ± 24.52b| 338.10 ± 31.26b| 290.08 ± 88.35a    |                |                               |
|                               | Caffeic winery         | 237.83 ± 21.82ab| 332.24 ± 43.91ab| 285.04 ± 77.44a    |                |                               |
| Condensed tannins (g/L)       | Control                | 2.01 ± 0.08a | 1.98 ± 0.07a | 2.00 ± 0.11a      | ns             | ns                            |
technique that was followed. Nevertheless, the low F-ratio values in most parameters allowed us to jointly process the data according to the applied copigment or winemaking technique.

Polyphenolic compounds were affected by the application of different copigments, and also by the followed vinification techniques, albeit to a lesser extent, for the 2 study years. For the effect of applying copigments, the polyphenolic parameters related to the concentration of anthocyanins and their different fractions were those with the highest F-ratio values and, therefore, the more markedly effected, as observed by other researchers [5, 16]. The parameters related to the concentration of condensed tannins were less affected by the treatments with different copigments. However, we found that the vinification techniques influenced these compounds, especially in the 2016 vintage in which grapes were less ripe.

Table 2 shows the behavior of wines 12 months after they were made according to the applied copigments. This behavior slightly differed to that observed after malolactic fermentation and at the beginning of the conservation process (data not shown). However, the differences increased in the percentage of polymerized anthocyanins of the wines treated with copigments, especially when caffeic acid was added shortly before L. The copigmenting effect of caffeic acid on alcoholic fermentation has been highlighted by other authors, as well as its implication for the increase in the percentage of polymerized anthocyanins throughout conservation [29, 81, 82].

On the anthocyanins concentration, the minority anthocyanins determined decreased at 12 months compared to the values obtained after malolactic fermentation because of the condensation and polymerization reactions with proanthocyanidins and other compounds. Such polymerization is facilitated by the presence of copigments [48]. The wines of 2016 treated with copigments, especially with rosemary extract, were those with the highest color and the highest concentration of total anthocyanins, malvidin-3-O-monoglucoside, and the rest of the minority anthocyanins determined. These results agree with those reported by other studies when rosemary extract was applied. The initial formation of a higher percentage of copigmented anthocyanins was enhanced [65, 66] and the half-life of anthocyanins during wine conservation was prolonged [64]. When applying rosemary extract to the clusters, Bimpilas et al. [48] reported an increase in both anthocyanins and color intensity. This increase was greater than that observed with caffeic acid, which they attributed to the more complex composition of extracts rich in hydroxycinnamic acids and flavonoids. The higher anthocyanins concentration brought about by rosemary extract is not accompanied by significant changes in the

Table 2 (continued)

| Parameters          | Copigment       | 2016         | 2017         | Average 2016–2017 | Year (p value) | Interaction Copig × Year (p) |
|---------------------|-----------------|--------------|--------------|-------------------|----------------|-----------------------------|
| Total polyphenols (g/L) | Control        | 2.06 ± 0.92a | 2.21 ± 0.68a | 2.14 ± 0.86a      | ns             | ns                          |
|                     | Rosemary extract| 2.14 ± 0.07a | 2.55 ± 0.37a | 2.35 ± 0.31a      | ns             | ns                          |
|                     | Caffeic vineyard| 1.96 ± 0.50a | 2.66 ± 0.63a | 2.31 ± 0.66a      | ns             | ns                          |
|                     | Caffeic winery  | 1.99 ± 0.25a | 2.37 ± 0.86a | 2.18 ± 0.68a      | ns             | ns                          |
| Folin Index         | Control         | 54.38 ± 7.92a| 48.43 ± 1.95a| 51.40 ± 6.36a     | ns             | ns                          |
|                     | Rosemary extract| 54.44 ± 7.16a| 53.60 ± 8.72a| 53.88 ± 7.91a     | ns             | ns                          |
|                     | Caffeic vineyard| 49.92 ± 1.80a| 54.33 ± 2.02a| 52.13 ± 1.86a     | ns             | ns                          |
|                     | Caffeic winery  | 50.08 ± 2.94a| 48.76 ± 3.20a| 49.42 ± 3.43a     | ns             | ns                          |
| DMACH Index (%)     | Control         | 47.71 ± 17.06a| 47.33 ± 8.93a| 47.52 ± 14.21a    | ns             | 3.96*                       |
|                     | Rosemary extract| 42.27 ± 4.50a| 56.77 ± 17.66a| 49.52 ± 17.21a   | ns             | ns                          |
|                     | Caffeic vineyard| 47.74 ± 6.22a| 50.43 ± 8.56a| 49.08 ± 7.36a     | ns             | ns                          |
|                     | Caffeic winery  | 49.34 ± 6.72a| 53.67 ± 7.62a| 51.50 ± 7.29a     | ns             | ns                          |
| Gelatin Index (%)   | Control         | 24.16 ± 3.74a| 37.30 ± 10.47a| 30.73 ± 10.18a    | ns             | 13.76***                    |
|                     | Rosemary extract| 23.54 ± 2.11a| 43.70 ± 8.78a| 37.98 ± 11.03a    | ns             | ns                          |
|                     | Caffeic vineyard| 20.97 ± 3.07a| 38.82 ± 9.55a| 29.89 ± 11.38a    | ns             | ns                          |
|                     | Caffeic winery  | 22.62 ± 2.04a| 39.49 ± 8.73a| 31.06 ± 10.65a    | ns             | ns                          |

In each column, different letters denote significant differences based on Duncan’s test, and the numbers with asterisk denote significant differences between treatments (*p < 0.05; **p < 0.01; ***p < 0.001)

For the data analysis across years, the statistical significance of the effects of year, and copigments (Copig) by year interaction, are also indicated.
concentrations of polyphenols and condensed tannins, or in the quality parameters of condensed tannins.

In the 2017 vintage, grape maturity was better than in 2016, as manifested by a higher concentration of total polyphenols and anthocyanins, and by more astringent of tannins. Notwithstanding, the behavior of the wines of the 2016 and 2017 vintages was similar (Table 2). The interaction between the addition of copigments and vintage was minimal.

The vinification techniques barely influenced the parameters related to the color and anthocyanin concentration of wines after 12-month storage time (Table 3). However, they affected the composition of condensed tannins and total polyphenols, so that the wines made with

### Table 3

| Compounds                         | Winemaking techniques | 2016       | 2017       | Average 2016–2017 | Year (p value) | Interaction Tech × Year (p) |
|-----------------------------------|-----------------------|------------|------------|-------------------|----------------|-----------------------------|
| Color intensity                   | T                     | 10.14 ± 1.12a | 11.33 ± 1.10a | 10.73 ± 1.25a    | 22.33***      | ns                          |
|                                   | PM                    | 9.72 ± 0.80a  | 10.97 ± 0.76a | 10.44 ± 0.97a    |                |                             |
| Hue (%)                           | T                     | 74.82 ± 3.30a | 68.85 ± 2.74a | 71.83 ± 2.45a    | 77.54***      | ns                          |
|                                   | MT                    | 74.59 ± 2.12a | 68.65 ± 1.87a | 71.09 ± 3.55a    |                |                             |
| Copigmented anthocyanins (%)      | T                     | 9.84 ± 1.64a  | 16.04 ± 2.15a | 12.94 ± 3.67a    | 32.49***      | ns                          |
|                                   | PM                    | 10.52 ± 1.86a | 15.67 ± 1.89a | 13.55 ± 3.16a    |                |                             |
| Polymerized anthocyanins (%)      | T                     | 53.32 ± 5.58a | 50.80 ± 6.90a | 52.06 ± 6.31a    | 13.21***      | ns                          |
|                                   | PM                    | 52.28 ± 2.64a | 44.86 ± 2.86a | 48.37 ± 4.88a    |                |                             |
| Free anthocyanins (%)             | T                     | 36.84 ± 5.27a | 34.16 ± 8.23a | 35.00 ± 7.05a    | ns             | 4.46*                       |
|                                   | PM                    | 37.20 ± 2.17a | 39.47 ± 3.23a | 38.08 ± 3.70a    |                |                             |
| Malvidin-3-O-monoglucoside (mg/L) | T                     | 38.78 ± 16.96a| 56.16 ± 14.02a| 47.47 ± 17.67a   | 10.69**       | ns                          |
|                                   | PM                    | 37.19 ± 7.25a | 49.57 ± 14.35a| 44.69 ± 12.73a   |                |                             |
| Peonidin-3-O-monoglucoside (mg/L) | T                     | 2.67 ± 0.17a  | 3.59 ± 0.82a  | 3.13 ± 1.09a     | ns             | 5.17*                       |
|                                   | PM                    | 3.02 ± 0.63a  | 2.72 ± 1.29a  | 2.91 ± 1.10a     |                |                             |
| Petunidine-3-O-monoglucoside (mg/L)| T                    | 4.88 ± 2.26a  | 6.15 ± 1.56b  | 5.51 ± 2.02a     | ns             |                             |
|                                   | PM                    | 4.43 ± 1.56a  | 4.31 ± 1.64a  | 4.47 ± 1.67a     |                |                             |
| Cyanidin-3-O-monoglucoside (mg/L) | T                     | 2.08 ± 0.81a  | 2.33 ± 0.61a  | 2.21 ± 0.72a     | ns             |                             |
|                                   | PM                    | 2.20 ± 0.66a  | 1.81 ± 0.66a  | 2.02 ± 0.69a     |                |                             |
| Delphinidin-3-O-monoglucoside (mg/L)| T                   | 3.98 ± 2.16a  | 5.01 ± 1.63b  | 4.50 ± 1.95b     | ns             |                             |
|                                   | PM                    | 3.85 ± 0.94a  | 3.28 ± 1.19a  | 3.63 ± 1.24a     |                |                             |
| Total anthocyanins (mg/L)         | T                     | 209.80 ± 29.30a| 313.71 ± 54.77a| 261.75 ± 70.60a  | 80.82***      | ns                          |
|                                   | PM                    | 219.08 ± 14.34a| 334.12 ± 56.68a| 271.60 ± 77.87a  |                |                             |
| Condensed tannins (g/L)           | T                     | 1.80 ± 0.43a  | 2.38 ± 0.24a  | 2.09 ± 0.32a     | ns             | 8.52**                      |
|                                   | PM                    | 2.47 ± 0.59b  | 2.53 ± 0.45b  | 2.54 ± 0.53b     |                |                             |
| Total polyphenols (g/L)           | T                     | 1.96 ± 0.06a  | 1.87 ± 0.11a  | 1.91 ± 0.10a     | ns             |                             |
|                                   | PM                    | 2.10 ± 0.10b  | 1.96 ± 0.10b  | 2.02 ± 0.12b     |                |                             |
| Folin Index                       | T                     | 50.14 ± 4.57a | 51.28 ± 5.97a | 50.71 ± 5.26a    | ns             | 6.93*                       |
|                                   | PM                    | 54.21 ± 5.89b | 48.88 ± 4.17a | 51.51 ± 5.62a    |                |                             |
| DMACH Index (%)                   | T                     | 50.50 ± 10.46a| 44.08 ± 11.08a| 47.29 ± 11.09a   | ns             | 12.32**                     |
|                                   | PM                    | 43.29 ± 8.65a | 57.52 ± 13.79b| 51.17 ± 13.52a   |                |                             |
| Gelatin Index (%)                 | T                     | 23.24 ± 2.84a | 38.89 ± 10.20a| 31.06 ± 10.84a   | 79.62***      | ns                          |
|                                   | PM                    | 23.03 ± 3.62a | 40.77 ± 8.44a | 33.44 ± 11.07a   |                |                             |

*T*, traditional winification; PM, prefermentative maceration

In each column, different letters denote significant differences based on Duncan’s test, and the numbers with asterisk denote significant differences between treatments (*p < 0.05; **p < 0.01, ***p < 0.001)

For the data analysis across years, the statistical significance of the effects of year, and techniques (Tech) by year interaction, are also indicated

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prefermentative maceration obtained the highest concentration of these compounds in both studied vintages. This cannot be attributed to greater extraction, since after post-malolactic fermentation was not been observed (data not shown). Therefore, it can be attributed only to the greater polyphenolic stability caused by prefermentative maceration, as interpreted by Favre et al. [83].

The prefermentation maceration was designed to improve the extraction of pigments, condensed tannins, and aromas from skin to must by assuming that aqueous extraction increases the extraction of color and its subsequent stability. However, some major controversies appear as to the effect of prefermentative maceration on wine color. While some studies show a positive effect of prefermentation maceration on both color and stability, and also on sensory quality of the wine [36–38, 84], others indicate that cold prefermentation maceration scarcely affects color [85, 86], or may even have a negative effect by diminishing color intensity and phenolic composition [87, 88]. There are a divergent behavior displayed in relation to different polyphenolic compounds as prefermentative maceration increases proanthocyanidins, but lowers anthocyanin content compared to wines made by the traditional maceration [83, 89]. Grape variety, terroir, its degree of maturity, and vinification techniques may bring about the variable effects of prefermentative maceration techniques.

The application of rosemary extract or caffeic acid to clusters, together with prefermentative maceration followed by traditional vinification, seemed to affect the polyphenolic composition of Monastrell wines as it gave rise to wines with a higher concentration of anthocyanins, condensed tannins and polyphenols. This fact not only improved the color of the wines at 12 months, but also contributed to maintain color longer, as indicated by the increased percentage of polymerized anthocyanins.

### Aromatic composition of Monastrell wines

A multifactorial ANOVA is shown in Table 4. The interaction observed between factors on wine aromatic composition was very low, which allowed the data to be processed together in accordance with the applied copigment or vinification technique. Applying copigments affected the vast majority of the concentrations of analyzed aromatic compounds. Winemaking techniques also had a marked effect on the concentration of aromatic compounds in wines for

| Compounds            | Interaction Copig × Techniques | Copigments | Winemaking techniques |
|----------------------|--------------------------------|------------|-----------------------|
|                      | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 |
| Alpha-pinen         | ns   | 5.32* | 3.28* | 3.26* | 8.94** | 7.08** |
| Beta-pinen          | ns   | ns   | 4.56* | 4.38* | ns   | ns   |
| Ethyl isovaleriato  | ns   | ns   | ns   | ns   | ns   | ns   |
| Isoamyl acetate     | ns   | ns   | ns   | ns   | ns   | ns   |
| Ethyl hexanoate     | 4.96* | 9.97** | 5.07* | 4.28* | ns   | ns   |
| n-Amyloalcohol      | 3.57* | 4.31* | ns   | ns   | 45.11*** | 12.11*** |
| Hexil acetate       | ns   | ns   | 4.4.71*** | 46.36*** | 49.91*** | ns   |
| Ethyl lactate       | ns   | ns   | 4.18* | 3.38* | 10.84*** | 8.05** |
| Cis 3-hexenol       | ns   | ns   | 7.13** | 8.70** | 6.40* | ns   |
| Ethyl octanoate     | ns   | ns   | ns   | ns   | 14.16** | 15.49** |
| 1.2 Propylene glycol| 6.51* | ns   | 15.12*** | 6.82** | ns   | ns   |
| Ethyl 3-hydroxybutyrate| 3.12* | 7.83** | 4.48* | 3.89* | ns   | ns   |
| Linalol              | ns   | ns   | 12.65*** | 13.09*** | ns   | ns   |
| Ethyl decanoate     | ns   | ns   | ns   | ns   | 50.09*** | 39.33*** |
| Diethyl succinate   | 11.13*** | ns   | 9.02** | 4.12* | ns   | ns   |
| 2-Phenyl acetate    | 9.23** | ns   | 99.09*** | 4.05* | 6.81** | 31.53*** |
| 2 Methoxyphenol     | ns   | ns   | 3.55* | ns   | ns   | ns   |
| γ- Octalactone      | 5.32** | 9.28** | 11.56** | 9.78** | 11.74*** | 9.15** |
| 2 Phenylethanol     | ns   | ns   | ns   | ns   | 15.14*** | 13.64*** |
| Eugenol             | ns   | ns   | 5.93* | 6.10** | ns   | ns   |
| Decanoic acid       | 19.55*** | 21.15*** | ns   | ns   | 87.97*** | 79.92*** |
| Vanillin            | ns   | ns   | 4.12* | 8.75** | ns   | ns   |

In each row, the numbers denote significant differences according to Duncan’s test (*p < 0.05; **p < 0.01; ***p < 0.001)
| Compounds (µg/L)         | Copigments          | 2016       | 2017       | Average 2016–2017 | Year p value | Interaction Copig × Year (p) |
|-------------------------|---------------------|------------|------------|--------------------|--------------|-----------------------------|
| Alpha-pinen             | Control             | 41.37 ± 6.27a | 46.48 ± 7.05b | 43.93 ± 6.37b | ns           | ns                          |
|                         | Rosemary extract    | 31.81 ± 9.20a | 32.74 ± 11.97a | 32.27 ± 10.32a |              |                             |
|                         | Caffeic vineyard    | 36.06 ± 3.49a | 39.63 ± 3.83ab | 37.84 ± 3.99ab |              |                             |
|                         | Caffeic winery      | 34.30 ± 9.55a | 37.69 ± 10.50ab | 35.99 ± 9.85a |              |                             |
| Beta-pinen              | Control             | 14.37 ± 1.24ab | 16.15 ± 1.40ab | 15.26 ± 1.57ab | ns           | ns                          |
|                         | Rosemary extract    | 17.55 ± 7.46b | 19.50 ± 8.20b | 18.62 ± 7.63b |              |                             |
|                         | Caffeic vineyard    | 14.01 ± 2.81ab | 15.39 ± 3.08ab | 14.70 ± 2.94ab |              |                             |
|                         | Caffeic winery      | 10.41 ± 4.27a | 11.44 ± 4.69a | 10.92 ± 4.36a |              |                             |
| Ethyl isovaleriato      | Control             | 18.60 ± 5.97a | 20.90 ± 6.71a | 19.75 ± 6.25b | ns           |                             |
|                         | Rosemary extract    | 13.83 ± 3.58a | 15.20 ± 3.94a | 14.52 ± 3.70a |              |                             |
|                         | Caffeic vineyard    | 18.96 ± 6.48a | 20.84 ± 7.13a | 19.90 ± 6.65b |              |                             |
|                         | Caffeic winery      | 18.20 ± 3.62a | 18.62 ± 5.07a | 18.41 ± 4.26  |              |                             |
| Isoamyl acetate         | Control             | 442.84 ± 71.14a | 437.57 ± 79.93b | 440.21 ± 78.37b | 3.78*        | ns                          |
|                         | Rosemary extract    | 351.97 ± 134.60a | 386.78 ± 147.92ab | 369.37 ± 137.80a |              |                             |
|                         | Caffeic vineyard    | 359.25 ± 44.99a | 394.78 ± 49.44a | 377.01 ± 49.21a |              |                             |
|                         | Caffeic winery      | 365.16 ± 90.46a | 401.27 ± 99.41ab | 383.22 ± 93.69a |              |                             |
| Ethyl hexanoate         | Control             | 171.84 ± 37.88a | 215.55 ± 20.09b | 193.70 ± 32.08a |              |                             |
|                         | Rosemary extract    | 145.37 ± 45.54a | 179.75 ± 50.05a | 162.56 ± 46.82a |              |                             |
|                         | Caffeic vineyard    | 152.15 ± 22.18a | 167.20 ± 24.38a | 159.67 ± 23.82a |              |                             |
|                         | Caffeic winery      | 144.37 ± 40.67a | 158.65 ± 44.69a | 151.51 ± 41.93a |              |                             |
| n-Amyl alcohol          | Control             | 43.52 ± 7.33a | 48.90 ± 8.24b | 46.21 ± 8.03a | ns           | ns                          |
|                         | Rosemary extract    | 359.75 ± 134.60a | 386.78 ± 147.92ab | 369.37 ± 137.80a |              |                             |
|                         | Caffeic vineyard    | 359.25 ± 44.99a | 394.78 ± 49.44a | 377.01 ± 49.21a |              |                             |
|                         | Caffeic winery      | 365.16 ± 90.46a | 401.27 ± 99.41ab | 383.22 ± 93.69a |              |                             |
| Hexil acetate           | Control             | 4.48 ± 1.60a | 6.85 ± 1.86a | 5.67 ± 2.08a | ns           | ns                          |
|                         | Rosemary extract    | 7.01 ± 2.71b | 8.46 ± 2.18b | 7.73 ± 2.50ab |              |                             |
|                         | Caffeic vineyard    | 26.35 ± 4.66c | 28.46 ± 4.90c | 27.41 ± 4.75c |              |                             |
|                         | Caffeic winery      | 11.70 ± 5.43b | 12.91 ± 5.91b | 12.31 ± 5.52b |              |                             |
| Ethyl lactate           | Control             | 9710.64 ± 1630b | 10,910.83 ± 1832b | 10,310.74 ± 1786b |              |                             |
|                         | Rosemary extract    | 7359.68 ± 2259a | 8087.56 ± 2483a | 7723.62 ± 2324a |              |                             |
|                         | Caffeic vineyard    | 10,009.14 ± 1819b | 10,999.05 ± 1999b | 10,504.10 ± 1916b |              |                             |
|                         | Caffeic winery      | 7829.04 ± 2637ab | 8603.34 ± 2898ab | 8216.19 ± 2707a |              |                             |
| Cis 3-hexenol           | Control             | 9.90 ± 3.06a | 12.74 ± 2.56b | 11.32 ± 3.10b | ns           | ns                          |
|                         | Rosemary extract    | 8.63 ± 2.49a | 9.62 ± 2.74a | 9.12 ± 2.58a |              |                             |
|                         | Caffeic vineyard    | 13.54 ± 3.55b | 14.88 ± 3.90b | 14.21 ± 3.67c |              |                             |
|                         | Caffeic winery      | 7.98 ± 1.80a | 8.02 ± 2.37a | 8.00 ± 2.04a |              |                             |
| Ethyl octanoate         | Control             | 20.81 ± 7.66a | 22.96 ± 9.00a | 21.89 ± 8.15a | ns           | ns                          |
|                         | Rosemary extract    | 26.29 ± 17.08a | 29.19 ± 18.51a | 27.74 ± 17.27a |              |                             |
|                         | Caffeic vineyard    | 25.52 ± 17.55a | 28.00 ± 19.29a | 26.76 ± 17.86a |              |                             |
|                         | Caffeic winery      | 19.86 ± 3.46a | 21.02 ± 4.55a | 20.44 ± 3.95a |              |                             |
| 1,2 Propylene glycol    | Control             | 170.82 ± 64.27c | 188.84 ± 73.91c | 179.83 ± 67.55c | ns           | ns                          |
|                         | Rosemary extract    | 53.09 ± 20.05a | 58.34 ± 22.03a | 55.71 ± 20.53a |              |                             |
|                         | Caffeic vineyard    | 84.70 ± 35.79b | 84.39 ± 46.41ab | 84.54 ± 40.04b |              |                             |
|                         | Caffeic winery      | 135.15 ± 66.17c | 134.51 ± 86.62bc | 134.83 ± 74.46bc |              |                             |
| Ethyl 3-hydroxybutyrate | Control             | 69.52 ± 16.21b | 78.11 ± 18.21b | 73.82 ± 17.24b | 3.41*        | ns                          |
|                         | Rosemary extract    | 50.51 ± 9.66a | 55.51 ± 10.61a | 53.01 ± 10.14a |              |                             |
|                         | Caffeic vineyard    | 55.78 ± 10.15a | 61.29 ± 11.15a | 58.53 ± 22.37a |              |                             |
|                         | Caffeic winery      | 55.92 ± 13.31a | 61.45 ± 14.63a | 58.68 ± 13.81a |              |                             |
both the studied vintages, as other researchers have shown [40, 41].

Table 5 includes the concentrations of the volatile compounds in wines 12 months after they were made in accordance with the applied copigments. The application of rosemary extract and caffeic acid to clusters produced higher hexyl acetate, diethylsuccinate, 2-phenylacetate, γ-octolactone, and vanillin concentrations. There are

| Compounds       | Copigments       | 2016          | 2017          | Average 2016–2017 | Year p value | Interaction Copig × Year (p) |
|-----------------|------------------|---------------|---------------|-------------------|--------------|-------------------------------|
| Linalol         | Control          | 48.94 ± 7.87c | 54.99 ± 8.84b | 51.97 ± 8.67b     | ns           | ns                            |
|                 | Rosemary extract | 48.91 ± 5.48c | 49.76 ± 7.80b | 49.36 ± 6.59b     |              |                               |
|                 | Caffeic vineyard | 39.26 ± 3.06b | 42.14 ± 3.54ab| 40.80 ± 3.53b     |              |                               |
|                 | Caffeic winery   | 30.86 ± 9.57a | 34.79 ± 12.86a| 32.83 ± 11.00a    |              |                               |
| Ethyl decanoate | Control          | 291.37 ± 68.23a | 327.38 ± 76.67a | 309.38 ± 72.53a | ns           | ns                            |
|                 | Rosemary extract | 241.41 ± 95.13a | 265.28 ± 104.53a | 253.34 ± 97.34a |              |                               |
|                 | Caffeic vineyard | 288.23 ± 63.96a | 316.73 ± 70.28a | 302.48 ± 66.56a  |              |                               |
|                 | Caffeic winery   | 255.43 ± 52.23a | 280.69 ± 57.39a | 268.06 ± 54.59a  |              |                               |
| Diethyl succinate | Control          | 1074.88 ± 182.62a | 1207.73 ± 205.19a | 1141.31 ± 199.79a | ns           | ns                            |
|                 | Rosemary extract | 1607.26 ± 473.77b | 1766.22 ± 520.63b | 1686.74 ± 487.83c |              |                               |
|                 | Caffeic vineyard | 1375.41 ± 362.21ab | 1511.44 ± 398.04ab | 1443.43 ± 374.29b |              |                               |
|                 | Caffeic winery   | 1129.71 ± 219.92a | 1241.44 ± 241.67a | 1185.57 ± 230.55a |              |                               |
| 2-Phenyl acetate | Control          | 11.33 ± 2.36a | 15.93 ± 2.89a  | 13.63 ± 3.48a     | 5.92*        | ns                            |
|                 | Rosemary extract | 20.03 ± 7.40b | 24.80 ± 5.30b  | 22.41 ± 6.69b     |              |                               |
|                 | Caffeic vineyard | 14.51 ± 3.14ab | 21.99 ± 7.51b  | 18.25 ± 6.77b     |              |                               |
|                 | Caffeic winery   | 19.62 ± 7.53b | 18.87 ± 11.85b | 19.24 ± 9.60b     |              |                               |
| 2-Methoxyphenol | Control          | 525.48 ± 201.87a | 590.42 ± 226.83a | 577.95 ± 210.13b | ns           | ns                            |
|                 | Rosemary extract | 450.13 ± 140.90a | 557.86 ± 126.37a | 504.00 ± 140.75a |              |                               |
|                 | Caffeic vineyard | 555.21 ± 112.10ab | 610.12 ± 123.18a | 582.66 ± 117.26b |              |                               |
|                 | Caffeic winery   | 631.08 ± 152.08a | 693.50 ± 167.12a | 662.29 ± 157.68b |              |                               |
| γ-Octolactone   | Control          | 409.24 ± 106.78a | 491.28 ± 111.22a | 450.26 ± 113.52a | ns           | ns                            |
|                 | Rosemary extract | 544.26 ± 177.37b | 598.09 ± 194.91ab | 571.18 ± 182.16b |              |                               |
|                 | Caffeic vineyard | 592.43 ± 132.92b | 651.02 ± 146.06b | 621.72 ± 138.26b |              |                               |
|                 | Caffeic winery   | 450.80 ± 168.39ab | 495.39 ± 185.04a | 473.09 ± 172.46a |              |                               |
| 2-Phenylethanol | Control          | 28,581.14 ± 5246.47a | 32,113.64 ± 5894.91a | 30,347.39 ± 5691.18ab | ns           | ns                            |
|                 | Rosemary extract | 31,519.23 ± 7745.04a | 34,636.51 ± 8511.03a | 33,077.87 ± 8024.27a |              |                               |
|                 | Caffeic vineyard | 26,938.34 ± 2179.57a | 29,602.57 ± 2395.13a | 28,270.45 ± 2605.16a |              |                               |
|                 | Caffeic winery   | 26,452.40 ± 7589.87a | 29,068.57 ± 8340.52a | 27,760.49 ± 7821.21a |              |                               |
| Eugenol         | Control          | 95.14 ± 13.78a | 106.90 ± 15.49ab | 101.02 ± 15.41a   | ns           | ns                            |
|                 | Rosemary extract | 81.58 ± 10.49a | 88.66 ± 11.53a  | 85.12 ± 11.26a    |              |                               |
|                 | Caffeic vineyard | 89.63 ± 28.13b | 100.47 ± 30.92a | 95.05 ± 29.10a    |              |                               |
|                 | Caffeic winery   | 100.83 ± 17.39b | 110.80 ± 19.11b | 105.82 ± 18.39a   |              |                               |
| Decanoic acid   | Control          | 48.28 ± 26.65a | 54.24 ± 29.95a  | 51.26 ± 27.56a    | 11.16**      | 4.36*                         |
|                 | Rosemary extract | 52.15 ± 33.73a | 59.55 ± 39.72a  | 55.85 ± 35.80a    |              |                               |
|                 | Caffeic vineyard | 48.62 ± 6.79a | 53.43 ± 7.46a   | 51.03 ± 7.32a     |              |                               |
|                 | Caffeic winery   | 46.34 ± 20.57a | 49.55 ± 24.90a  | 47.95 ± 22.13a    |              |                               |
| Vanillin        | Control          | 38.53 ± 16.63a | 43.29 ± 18.69a  | 40.91 ± 17.27a    | ns           | ns                            |
|                 | Rosemary extract | 62.13 ± 14.22b | 68.27 ± 15.63c  | 65.20 ± 14.78c    |              |                               |
|                 | Caffeic vineyard | 52.55 ± 16.75b | 59.10 ± 17.93b  | 55.82 ± 17.10b    |              |                               |
|                 | Caffeic winery   | 48.09 ± 23.42ab | 52.85 ± 25.74ab | 50.47 ± 23.90ab   |              |                               |

In each column, different letters denote significant differences based on Duncan’s test, and the numbers with asterisk denote significant differences between treatments (* p < 0.05; ** p < 0.01; *** p < 0.001)

For the data analysis across years, the statistical significance of the effects of year, and copigments (Copig) by year interaction, are also indicated.

Table 5 (continued)
Table 6  Means, standard deviations and variance analyses of the aromatic compounds of Monastrell wines depending on winemaking technology applied during each season, and the average for 2016 and 2017

| Compounds (µg/L) | Winemaking techniques | 2016   | 2017   | Average 2016–2017 | Year p value | Interaction Tech × Year (p) |
|-----------------|------------------------|--------|--------|-------------------|--------------|-----------------------------|
| Alpha-pinen     | T                      | 39.23 ± 7.21a | 43.37 ± 8.06b | 41.30 ± 7.81b | ns            | ns                          |
|                 | PM                     | 32.54 ± 7.51a | 34.90 ± 9.85a | 33.72 ± 8.70a |              |                             |
| Beta-pinen      | T                      | 12.59 ± 1.36a | 13.92 ± 1.56a | 13.26 ± 1.59a | ns            | ns                          |
|                 | MT                     | 15.67 ± 6.81a | 17.32 ± 7.48a | 16.50 ± 7.08a |              |                             |
| Ethyl isovaleriato | T              | 16.81 ± 3.04a | 18.59 ± 3.44a | 17.70 ± 3.32a | ns            | ns                          |
|                 | PM                     | 17.98 ± 6.90a | 19.19 ± 7.96a | 18.58 ± 7.35a |              |                             |
| Isoamyl acetate | T                      | 329.66 ± 75.43a | 364.68 ± 85.16a | 347.17 ± 81.11a | 3.49*        | ns                          |
|                 | PM                     | 429.95 ± 32.91b | 475.53 ± 97.27b | 452.74 ± 93.24b |              |                             |
| Ethyl hexanoate | T                      | 147.33 ± 39.81a | 163.17 ± 45.78a | 155.25 ± 42.96a | ns            | ns                          |
|                 | PM                     | 169.53 ± 32.91a | 187.40 ± 36.52a | 178.47 ± 35.38a |              |                             |
| n-Amyloalcohol  | T                      | 47.94 ± 7.77b  | 52.97 ± 8.53b  | 50.45 ± 8.43b  | 4.08*        | ns                          |
|                 | PM                     | 31.78 ± 7.93a  | 35.17 ± 9.05a  | 33.48 ± 8.55a  |              |                             |
| Hexil acetate   | T                      | 9.51 ± 8.64a   | 11.31 ± 8.90a  | 10.41 ± 8.68a  | ns            | ns                          |
|                 | PM                     | 15.26 ± 9.45b  | 17.03 ± 9.51a  | 16.14 ± 9.37a  |              |                             |
| Ethyl lactate   | T                      | 9796.13 ± 1702a| 10,826.04 ± 1891b | 10,311.08 ± 1845a |              |                             |
|                 | PM                     | 7658.12 ± 2429a| 8474.36 ± 2722a | 8066.24 ± 2571a |              |                             |
| Cis 3-hexenol   | T                      | 8.83 ± 2.49a   | 10.26 ± 3.20a  | 9.55 ± 2.91a   | ns            | ns                          |
|                 | PM                     | 11.19 ± 3.92a  | 12.37 ± 4.34a  | 11.78 ± 4.11a  |              |                             |
| Ethyl octanoate | T                      | 16.06 ± 2.53a  | 17.24 ± 2.50a  | 16.65 ± 2.54a  | ns            | ns                          |
|                 | PM                     | 30.18 ± 14.73b | 33.34 ± 16.17b | 31.76 ± 15.30b |              |                             |
| 1,2 Propylene glycol | T     | 126.87 ± 84.97a | 136.37 ± 98.89a | 131.62 ± 90.83a | ns            | ns                          |
|                 | PM                     | 95.01 ± 37.03a | 96.67 ± 44.50a | 95.84 ± 40.28a |              |                             |
| Ethyl 3-hydroxybutyrate | T | 53.91 ± 9.69a | 59.60 ± 10.89a | 56.76 ± 10.54a | ns            | ns                          |
|                 | MT                     | 61.95 ± 16.56a | 68.58 ± 18.87a | 65.26 ± 17.78* |              |                             |
| Linalol         | T                      | 40.84 ± 9.93a  | 39.95 ± 14.38a | 40.37 ± 12.30a | ns            | ns                          |
|                 | PM                     | 42.75 ± 10.85a | 47.89 ± 10.72a | 45.32 ± 10.93a |              |                             |
| Ethyl decanoate | T                      | 215.60 ± 42.88a| 238.49 ± 48.82a | 227.05 ± 46.67a | 5.13*        | ns                          |
|                 | PM                     | 322.62 ± 50.98b| 356.55 ± 56.97b | 339.59 ± 55.90b |              |                             |
| Diethyl succinate | T               | 1275.97 ± 467.10a | 1408.10 ± 509.41a | 1342.04 ± 485.43a |              |                             |
|                 | PM                     | 1317.66 ± 284.36a| 1455.31 ± 309.47a | 1386.49 ± 300.60a |              |                             |
| 2-Phenyl acetate | T                      | 14.39 ± 5.65a  | 16.78 ± 3.11a  | 15.58 ± 4.64a  | 5.32*        | ns                          |
|                 | PM                     | 23.36 ± 9.33b  | 29.02 ± 8.15b  | 26.19 ± 9.09b  |              |                             |
| 2 Methoxyphenol | T                      | 538.85 ± 147.23a | 628.06 ± 135.73a | 583.46 ± 146.48a |              |                             |
|                 | PM                     | 542.10 ± 179.51a | 597.88 ± 195.00a | 569.99 ± 186.53a |              |                             |
| γ-Octolactone   | T                      | 432.73 ± 119.05a | 478.30 ± 132.24a | 455.51 ± 125.92a |              |                             |
|                 | PM                     | 565.63 ± 170.79b | 639.59 ± 167.36b | 602.61 ± 170.53b |              |                             |
| 2 Phenylethanol | T                      | 31,758.42 ± 4420b | 35,098.37 ± 4915b | 33,428 ± 4901b | ns            | ns                          |
|                 | PM                     | 24,987.13 ± 5857a | 27,612.28 ± 6448a | 26,299.71 ± 6205a |              |                             |
| Eugenol         | T                      | 98.12 ± 27.74a  | 108.45 ± 30.63a | 103.28 ± 29.22a | ns            | ns                          |
|                 | PM                     | 90.48 ± 16.55a  | 99.97 ± 18.18a  | 95.22 ± 17.77a  |              |                             |
| Decanoic acid   | T                      | 68.45 ± 12.75b  | 76.78 ± 15.95b  | 72.61 ± 14.82b  | ns            | ns                          |
|                 | PM                     | 29.25 ± 10.44a  | 31.61 ± 12.41a  | 30.43 ± 11.35a  |              |                             |
| Vanillin        | T                      | 49.51 ± 15.56a  | 54.70 ± 17.09a  | 52.11 ± 16.29a  | ns            | ns                          |
|                 | PM                     | 51.14 ± 22.82a  | 57.06 ± 24.86a  | 54.10 ± 23.66a  |              |                             |

T, traditional winemaking; PM, prefermentative maceration

In each column, different letters denote significant differences based on Duncan's test, and the numbers with asterisk denote significant differences between treatments (*p < 0.05; **p < 0.01; ***p < 0.001)

For the data analysis across years, the statistical significance of the effects of year, and techniques (Tech) by year interaction, are also indicated.
organoleptically important effect, because esters are related to fruit and floral aromas, $\gamma$-octalactone to coconut aroma, and vanillin to vanilla, which were all positive for aromatic wine quality.

The application of rosemary extract and caffeic acid during grape ripening could have stimulated the biosynthesis of aromatic precursors and led to the formation of these compounds. The effect of caffeic acid was much less evident when it was applied in the vineyard before fermentation. Darici et al. [64] also found a significant increase in the concentration of esters, higher alcohols, and terpenic compounds in Cabernet Sauvignon wines treated with plant extracts (rosemary extract and blueberry extract). The flavonoids, phenolic compounds, and their derivatives, which are naturally found in the structure of these extracts, have been shown to be effective in preventing the auto-oxidation of aromatic compounds [90, 91]. Also, a biostimulating effect of the formation of aromatic compounds on grapes was also observed when eucalyptus extract, almond skins extract, benzothiadiazole, methyl jasmonate, and chitosan were applied to vineyards, obtaining wines with a higher concentration of diazole, methyl jasmonate, and chitosan were applied to vineyards for the formation of these compounds may be affected mainly from the fermentation process, the substrates in the grapes for the formation of these compounds may be affected by the treatment received by the grapes, thus affecting their final concentrations in wines.

Regarding the effect of vinification techniques (Table 6), the concentration of only some compounds, such as $n$-amylalcohol, 2-phenylethanol and decanoic acid, was higher in the wines made by traditional vinification. Instead other compounds like isoamyl acetate, hexyl acetate, cis-3-hexenol, ethyl octanoate and decanoate, 2-phenylacetate, and $\gamma$-octolactone, which are very important for organoleptic wine quality, appeared at higher concentrations in the wines subjected to prefermentation maceration. These results agree with those obtained by Alvarez et al. [38], Selli et al. [95], and De Santis and Frangipane [96]. These researchers attributed the higher aromatic concentration of pre-fermentatively macerated wines to the extractive effect of this technique on the skin components, and perhaps also to the multiplication of non-\textit{Saccharomyces} native cryophilic yeasts and their influence on the release of certain aromas, especially volatile esters [97, 98].

## Conclusions

The application of rosemary extract and, to a lesser extent, caffeic acid, to cv. Monastrell grapes increased the concentrations of anthocyanins and the percentage of polymerized anthocyanins of its respective wines, which not only contributes to more intense color, but also to stabler color. The stronger effect of rosemary extract may be due to its complex composition, because, apart from containing caffeic acid, it also contains a significant amount of other flavonoids. The application of these products did not modify the concentration of total polyphenols and condensed tannins, which increased when wines were made by pre-fermentation maceration.

Applying rosemary extract or caffeic acid to clusters before harvest increased the concentrations of a remarkable number of esters related to wine quality, an effect that was not observed when applying caffeic acid in winery, before the fermentation. The pre-fermentation maceration of the grapes treated with the studied copigments also increased the concentrations of other esters and acetates, which are considered positive for wine quality.

Taking into account the results obtained in the two studied vintages, the combination of applying rosemary extract or caffeic acid in the vineyards, together with pre-fermentation maceration, positively affects wine polyphenolic concentration and increases the concentration of positive aromatic compounds.

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## Declarations

### Conflict of interest

The authors declare that they have no conflict of interest.

### Compliance with ethics requirements

This study does not contain any experiment with human participants or animals.

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