European Food Research and Technology
https://doi.org/10.1007/s00217-022-04005-y

ORIGINAL PAPER

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

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Received: 21 December 2021 / Revised: 11 March 2022 / Accepted: 12 March 2022

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

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Published online: 17 May 2022

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 prefermentation 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-*Saccharomyces* 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 (*Rosmarinus officinalis* 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, rosmarinic 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 × 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

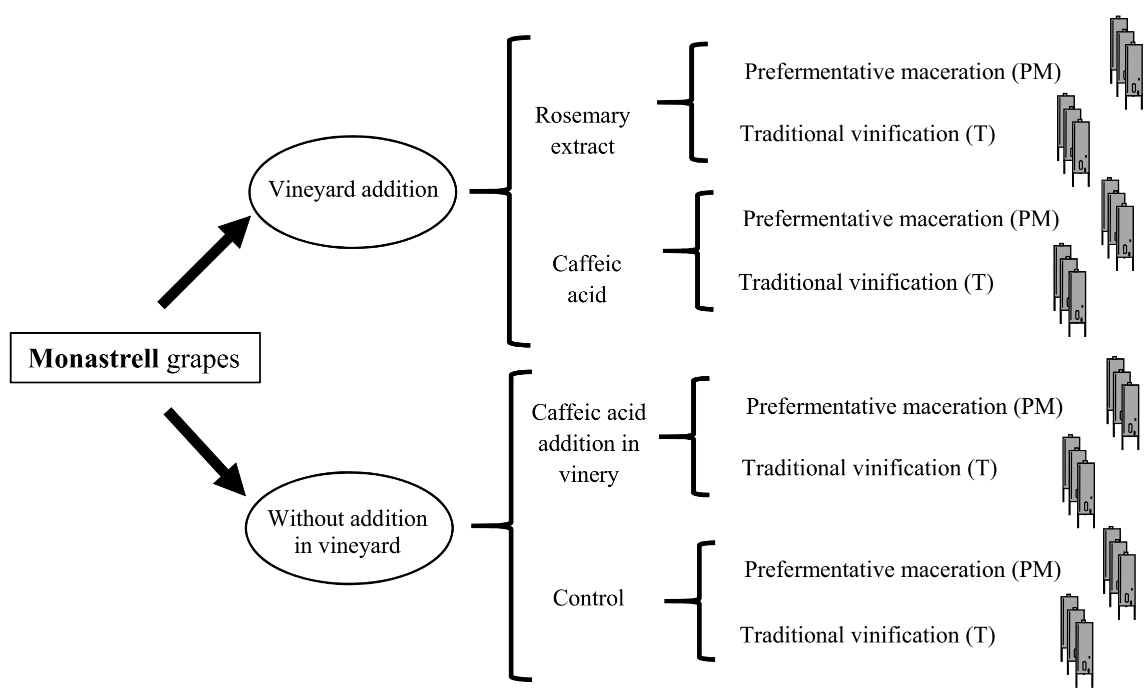

Fig. 1 Experimental design

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 1g/hL 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) (ºBrix) 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*-monoglucoside (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 oenogical 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 sulfurous 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 sulfurous (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 vinification technique (Tech) and their interaction, for the polyphenolic compounds of Monastrell wines in 2016 and 2017

Compounds	Interaction Copig × Tech		Copigments		Winemaking Techniques	
	2016	2017	2016	2017	2016	2017
Color intensity	ns	5.22*	3.23*	ns	ns	ns
Hue (%)	4.21*	ns	ns	ns	ns	ns
Copigmented anthocyanins (%)	ns	ns	ns	ns	ns	ns
Polymerized anthocyanins (%)	ns	9.48**	11.54**	9.22**	ns	ns
Free anthocyanins (%)	3.78*	14.11**	7.84**	ns	ns	ns
Malvidin (mg/L)	ns	ns	7.89**	7.47**	ns	ns
Peonidin (mg/L)	ns	ns	4.56*	ns	ns	ns
Petunidine (mg/L)	ns	ns	3.57*	3.71*	ns	10.57**
Cyanidin (mg/L)	ns	ns	3,38*	ns	ns	ns
Delphinidin (mg/L)	ns	ns	5.52**	ns	ns	11.77**
Total anthocyanins (mg/L)	12.41***	14.27***	4.80*	ns	ns	ns
Condensed tannins (g/L)	18.12***	ns	ns	ns	11.94**	4.18*
Total polyphenols (g/L)	ns	4.79*	ns	ns	23.86**	6.63**
Folín Index	ns	ns	ns	ns	4.26*	ns
DMACH Index (%)	14.81***	ns	ns	ns	ns	ns
Gelatin Index (%)	ns	7.91**	ns	ns	ns	ns

In each row, the nunbers 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 ± 8.62ab	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	58.40 ± 3.50b	57.52 ± 4.21b		
	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 ± 0.30a	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
	Rosemary extract	1.96 ± 0.11a	1.99 ± 0.12a	1.97 ± 0.11a		
	Caffeic vineyard	1.99 ± 0.03a	1.93 ± 0.10a	1.96 ± 0.08a		
	Caffeic winery	2.09 ± 0.14a	1.92 ± 0.15a	2.00 ± 0.16a		

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		
	Caffeic vineyard	1.96 ± 0.50a	2.66 ± 0.63a	2.31 ± 0.66a		
	Caffeic winery	1.99 ± 0.25a	2.37 ± 0.86a	2.18 ± 0.68a		
Folín 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		
	Caffeic vineyard	49.92 ± 1.80a	54.33 ± 2.02a	52.13 ± 1.86a		
	Caffeic winery	50.08 ± 2.94a	48.76 ± 3.20a	49.42 ± 3.43a		
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		
	Caffeic vineyard	47.74 ± 6.22a	50.43 ± 8.56a	49.08 ± 7.36a		
	Caffeic winery	49.34 ± 6.72a	53.67 ± 7.62a	51.50 ± 7.29a		
Gelatin Index (%)	Control	24.16 ± 3.74a	37.30 ± 10.47a	30.73 ± 10.18a	13.76***	ns
	Rosemary extract	23.54 ± 2.11a	43.70 ± 8.78a	37.98 ± 11.03a		
	Caffeic vineyard	20.97 ± 3.07a	38.82 ± 9.55a	29.89 ± 11.38a		
	Caffic winery	22.62 ± 2.04a	39.49 ± 8.73a	31.06 ± 10.65a		

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

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 were minimized in the percentage of the copigmented anthocyanins. After fermentation, this percentage was significantly lower in the control wines, but 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 3 Means, standard deviations, and variance analyses of the polyphenolic parameters of Monastrell wines depending on winemaking technology applied during each season, and the average for 2016 and 2017

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 ± 4.25a	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 A		
Free anthocyanins (%)	T	36.84 ± 5.27a	34.16 ± 8.23a	35.00 ± 7.05a a a	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 ± 1.16a	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	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	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	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	ns
	PM	2.10 ± 0.10b	1.96 ± 0.10b	2.02 ± 0.12b		
Folín 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

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 4 Multifactorial variance analysis for the applied copigments (Copig), the vinification technique and their interaction, for the aromatic compounds of Monastrell wines in 2016 and 2017

Compounds	Interaction Copig × Techniques		Copigments		Winemaking techniques	
	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*	3.48*	ns	ns
Ethyl isovaleriato	ns	ns	ns	ns	ns	ns
Isoamyl acetate	ns	ns	ns	3.19*	20.44***	11.76**
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***	46.35***	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- Octolactone	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 (*$p<0.05$; **$p<0.01$; ***$p<0.001$)

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 prefermentation 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

Table 5 Means, standard deviations, and variance analyses of the aromatic compounds of Monastrell wines depending on the applied copigments during each season, and the average for 2016 and 2017

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	17.75 ± 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	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	ns	ns
	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	37.65 ± 13.15a	41.37 ± 14.45ab	39.51 ± 13.49a		
	Caffeic vineyard	43.54 ± 11.53a	47.85 ± 12.67b	45.69 ± 11.91a		
	Caffeic winery	34.73 ± 11.69a	38.17 ± 12.85a	36.45 ± 12.00a		
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	ns	ns
	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		

Table 5 (continued)

Compounds (µg/L)	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 ± 398.04b	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 ± 201.87ab	590.42 ± 226.83a	557.95 ± 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 ± 152.08b	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

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

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	ns	ns
	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[a]		
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	339.58 ± 55.90b		
Diethyl succinate	T	1275.97 ± 467.10a	1408.10 ± 509.41a	1342.04 ± 485.43a	ns	ns
	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	ns	ns
	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	ns	ns
	PM	565.63 ± 170.79b	639.59 ± 167.36b	602.61 ± 170.53b		
2 Phenylethanol	T	31,758.42 ± 4420b	35,098.37 ± 4915b	33,428.40 ± 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 signifficant 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, γ-octolactone 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 vinery 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 terpenes, acetals, and esters [90, 93, 94]. These studies have shown that the application of plant extracts and elicitors in the vineyard caused an increase in higher alcohols and esters in the wines, and although these compounds originate 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*-amyloalcohol, 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 γ-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-S*accharomyces* 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 prefermentation 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 prefermentation 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 prefermentation maceration, positively affects wine polyphenolic concentration and increases the concentration of positive aromatic compounds.

Acknowledgements The authors are grateful to Ministry of Education and Science (Spain) and the Conselleria de Innovación, Universidades, Ciencia y Sociedad Digital of Valencian Community (Spain).

Funding Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature.

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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