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Original article

Application of green tea extract and catechin on the polyphenolic and volatile composition of Monastrell red wines

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Summary The present work studies the effect of adding catechin and plant extract rich in catechin, *Camellia sinensis* (green tea) extract, on the volatile and polyphenolic composition of red wines made from Monastrell grapes. The aim of this research is to study the way to increase the red wine quality made with Monastrell variety of grapes. In one of them, the green tea extract was applied to grapes in vineyards 10 days before harvest. In another, catechin was applied to vineyards in the same way, and finally, catechin was applied during grape processing. Each trial was fermented by traditional vinification and prefermentative maceration for 5 days prior to alcoholic fermentation. A simple ANOVA analysis was used to evaluate if the application of copigments and winemaking techniques influenced wine composition. In addition, a multifactorial ANOVA was used to study the copigment treatment effect x winemaking technique interaction. Wine polyphenolic and volatile composition were analysed 12 months after fermentation. The application of green tea extract and catechin to vineyards increased (13%–16%) the concentration of anthocyanins in relation to the control to a greater extent when the initial polyphenolic concentration of grapes was lower. The volatile concentration rose when copigments (green tea extract and pure catechin) were applied to vineyards, in particular, hexyl acetate, ethyl octanoate and vanillin. The application of techniques like prefermentative maceration enhances the effects of copigments added to vineyards. The combination of the application of green extract or pure catechin with prefermentative cold maceration, had positive effects increasing polyphenols and volatile compounds concentration.

Keywords aromas, catechin, copigmentation, green tea extract, polyphenols, prefermentative maceration, wine.

Introduction

Of the major polyphenolic compounds found in red grapes, anthocyanins provide mainly wine colour, while tannins confer astringency and structure. Considering that colour is a factor that is normally related to red wine quality, ensuring that this colour remains stable over time is very important to increase its acceptance. During the maceration process, anthocyanins, tannins and other minor polyphenolic compounds (flavonols and cinnamic acids) are extracted. All the phenolic compounds present in wine will interact through condensation and polymerisation reactions to reduce astringency and to stabilise colour (Vivas *et al*., 1994). In addition to these reactions, the copigmentation effect also plays an important role, which contributes to colour stability (Berké & Freitas, 2007). During copigmentation, associations are formed between red compounds of the grapes (anthocyanins) and other colourless compounds. Copigmentation processes also occur later during winemaking and storage. They prevent the oxidation of anthocyanins (Heras-Roger *et al*., 2016) and influence the condensation and polymerisation reactions of phenolic compounds, by increasing their stability (Boulton, 2001). Molecules that favour copigmentation must have an aromatic ring with a planar structure (Zhao *et al*., 2020). Catechin monomers and procyanidins (condensed tannins) have been shown to interact differently with malvidin 3-O-glucoside (oenin) depending on their conformation. The dimers with a C4–C6 interflavonoid linkage are associated more closely with oenin than with their C4–C8 analogues (Berké & Freitas, 2007).

To promote copigmentation and to make its effects more noticeable and long-lasting, it is necessary to have a higher concentration of copigments and pigments in wine so that the copigment/pigment ratio can

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increase. To achieve these objectives, strategies in both viticulture and oenology can be modified. Foliar treatments performed by applying elicitors increase anthocyanin content in wine (Gil-Muñoz *et al.*, 2018). In the oenological field, the strategy to enhance copigmentation consists of increasing the copigment/pigment molar ratios, for which cofermentation of different varieties has been studied by Gómez Gallego *et al.* (2012). Prefermentative application of cofactors (gallic acid, caffeic acid, rutin, catechin, grape skin, grape seed tannin) increases copigmentation reactions and produces wines with marked colour intensity and a higher anthocyanin concentration over time (Aleixandre-Tudó *et al.*, 2013; Teixeira *et al.*, 2013; Zhang *et al.*, 2015). The efficacy of adding copigments may increase if they are applied during the grapes ripening period, which may also favour anthocyanins synthesis. Recently, Bimpilas *et al.* (2016) compared the application of pure copigments and plant extracts as a source of some copigments in the prefermentative stage. These authors observed that the application of rosemary extract (rich in caffeic acid) led to a greater increase in wine colour intensity compared with the other wines treated with pure rosmarinic acid because of this plant extract has a more complex composition (flavonoids and hydroxycinnamic acids) than rosmarinic acid, which favours and increases the copigmentation phenomenon.

Catechin action has been tested by many authors as a copigment to obtain higher wine colour stabilisation over time (González-Manzano *et al.*, 2008; Liu *et al.*, 2021). Despite the good results, applying catechin in the way of an extract of some plant rich in catechin has not yet been tested. Catechin is present in green tea, furthermore, is rich in (−)-epigallocatechin gallate, (−)-epigallocatechin, (−)-epicatechin gallate and (−)-epicatechin, and also contains other favonols (myricetin, quercetin, kaempferol) and corresponding glycosides, phenolic acids (gallic, p-coumaric, chlorogenic) and tannins (González-Manzano *et al.*, 2008).

Another strategy to increase polyphenolic wine content is to apply prefermentative maceration techniques. The technique consists of macerating red grapes at low temperature by preventing alcoholic fermentation for a time period lasting between 4 and 7 days, and then fermenting at a temperature between 25–28 °C. The most recent studies show that prefermentative maceration increases the anthocyanins concentration at the beginning of the maceration stage, which allows the contact time with the solid parts during alcoholic fermentation to shorten and avoids extracting astringent and bitter tannins from seeds. The most recent results show a general increase in polyphenolic compounds, which gives wines with higher colour density, anthocyanin concentration (Heredia *et al.*, 2010; Aleixandre-Tudó *et al.*, 2013; Casassa & Sari, 2015). However, the obtained tannin concentration results are disparate, with increases in some assays (González-Neves *et al.*, 2013) or decrease that result in lowering tannin mDP and bitterness (Álvarez *et al.*, 2006; Aleixandre-Tudó *et al.*, 2016; Lukić *et al.*, 2017). Cold prefermentative maceration can also be an inefficient technique for extracting polyphenols, with no differences from the traditional vinification process (Casassa & Sari, 2015; Panprivech *et al.*, 2015). Recently, some authors have studied the effect of prefermentative maceration combined with prefermentative copigment treatment, showing a synergistic impact in colour stability, having demonstrated that the concentration of anthocyanins and their copigments influence as much on wine colour as the cold soak (Zhang *et al.*, 2018) prefermentative maceration. In addition, prefermentative maceration also acts on volatile red wine content by modifying content because it allows the initial development of non-*Saccharomyces* yeasts (Casassa & Sari, 2015) prior to alcoholic fermentation. According to recent essays carried out with the Merlot variety, the increase in esters exceeds 20% (ethyl hexanoate, ethyl octanoate, ethyl decanoate, diethyl succinate, ethyl laurate), 43% in octanoic acid and 67% in decanoic acid. Similar results have been obtained by Gambacorta *et al.* (2019) after applying prefermentative maceration to the red Aglicano, Primitivo and Nero di Troia varieties.

It is a fact that Monastrell is a grape variety, which needs a very good ripening of the grape, to obtain a good concentration of phenolic compounds and, therefore, get a good colour, with the disadvantage that more alcoholic wines are elaborated (Moreno-Olivares *et al.*, 2022). In addition, in this variety polyphenol oxidase (tyrosinase) is present (Sánchez-Ferrer *et al.*, 1988; Valero *et al.*, 1989; Núñez-Delicado *et al.*, 2005). Monastrell wines have a low acidity and a high pH, which complicate its winemaking and conservation. The study herein presented with the catechin copigment treatment to grapes (in way to green tea extract or pure) and in the winery after processing, would allow an early harvesting and, therefore, to reduce alcohol content, but a polyphenolic and volatile balance for Monastrell wines. If the application of catechin can be done by spraying a natural extract (green tea), it could be most interesting for organic viticulture (Bulgari *et al.*, 2015). In the present work, a comparative study of the effect of catechin as a pure compound in the way of green tea extract applied to grapes while ripening was carried out combined with cold maceration prefermentative technique. The application of these techniques will be a very useful methodology to improve winemaking systems that guarantee crop sustainability, rising the concentration levels of polyphenol and aroma in wines as a fundamental objective.

Material and methods

Site description and experimental design

The plant material chosen was cv Monastrell variety (syn Mourvedre VICV-7915) vines. The plot is located in the 'Valencian Denomination of Origin' (Fontanares, Spain). They were grafted onto Richter-110 (110R) rootstocks. The vines were driven on a single trellis, a double cordon royal pruning system and cultivated in rainfed in 2005 and spaced 1.5 × 3 m (2.200 plants/ha). The soil has a sandy loam texture, high calcareous and low fertility. In two consecutive vintages, grapes were treated in 2016 and 2017. The experimental design of assays was of a factorial type in randomised complete blocks with three replications. The elementary plots included 30 treated vines in each assay. Each block had every experiments (three). The assay involved three experiments in the vineyard (grapes without treatment, grapes treated with green tea extract, grapes treated with pure catechin). The green tea extract and catechin copigments were applied by monitoring polyphenolic grape maturity at the optimal time of anthocyanins synthesis to achieve the greatest effectiveness of copigments. Based on a previous experience (Lizama *et al.*, 2007; Álvarez *et al.*, 2009), 10 days prior to harvest was considered the optimum time for copigmentation reactions to occur. The concentration of catechin in green extract was determined. Both the green tea extract and the (+) catechin (Fluka, Milwaukee, WI, USA) were previously dissolved in water until a concentration of about 90 mg/kg grapes and was applied by foliar spraying to bunches. To promote adherence of copigments to skin, the applied solution contained a non-ionic surfactant (Montana was 20% at 20%, 2.5 mL/L). These products were applied 30 days after version.

Green tea extract analyses

Prior to treatment, the amount of catechin in the commercial green tea extract (ACOFARMA, Terrasa, Barcelona, Spain) was quantified by the method of (Choung *et al.*, 2014), with some modifications. A chromatographic analysis was carried out using reversed-phase HPLC-DAD MD-2010 Plus (JASCO, Tokyo, Japan), equipped with a Gemini-NX C18, 5 µm (250 X 4.6 mm) Phenomenex column (Torrance, CA, USA). The column temperature was 30 °C, the mobile phase was 0.1% (v/v) phosphoric acid/water (solvent A) and methanol 100% (solvent B). The gradient program was as follows: 0–25 min: 20% B; 26–50 min: 30% B; 51 min: 20% B; 55 min: 20% B. Contents of catechin in green tea extract were calculated on the basis of the calibration curves of authentic catechin (Fluka, Milwaukee, WI, USA) ($y = 1x10^7x + 624\,439$, $R^2 = 0.9942$); The HPLC elutes were monitored by absorbance at 280 nm. Results were expressed as ppm in extract green extract ethanol solution. The injection volume was 20 µL. Catechin was prepared in aqueous solution at the 0.5 g/L concentration so that after having been sprinkled on grapes, its concentration would be 90 mg/kg grapes. The catechin concentration in the green tea extract (952–1002 mg catechin/L) was determined to adjust the dilution of extracts to the 0.5 g/L concentration of pure catechin so that when extracts were applied, the previously mentioned results would be obtained. With the obtained results, it was adjusted to a dilution to apply 90 mg/kg to grapes in the field.

Winemaking process

Monastrell grapes were harvested 10 days after applying treatments (soluble solids 25–25 °Brix). After manually harvesting, grapes were put in 20-kg boxes. Every year grapes were processed in a pallet destemmer-roller crusher, and the paste was placed in 50 L tanks. Every winemaking assays were performed in triplicate (Fig. 1). In addition to adding copigment, two maceration types were applied during vinification (traditional at Temp = 27–28 °C and cold soak at Temp = 6 °C for 5 days prior to maceration at Temp = 27–28 °C). Six microvinifications were carried out on the control grapes without the field treatments, three through cold soak and three with traditional vinification. Likewise, six microvinifications were performed for each field assays to apply the catechin copigment directly or in the way of green tea extract. With the untreated grapes in the field, six microvinifications were carried out with prefermentative application in winery of the catechin copigment at a dose of 90 mg/kg of grapes. Ten days after alcoholic fermentation began, wine was pressed at low pressure and blended with the wine from the first pressing. *Oenococcus oeni* bacteria (Lalvin 31 by Lalleman) were added at 1 g/hL to promote malolactic fermentation.

In all the assays, the selected *Saccharomyces cerevisiae*, var. *bayanus*, Enartis Ferm Red Fruit (Sepsa-Enartis, La Rioja, Spain) yeasts were used (20 g/hL). Maceration-fermentation took place at 27–28 °C for all the treatments by applying two daily pump-overs with the same guidelines for them all. Wines were devatted 10 days after fermentation began. In the wines subjected to cold soak, prefermentation maceration was carried out at 6 °C for 5 days, followed by traditional fermentation.

Ten 10 days after alcoholic fermentation began, wine was pressed at low pressure and mixed with the drained wine. At that time, 1 g/hL of *Oenococcus oeni* bacteria (Lalvin 31 from Lallemand S.A. Montreal, Canada) was added for malolactic fermentation. Having completed malolactic fermentation, and after sulphiting at 30 mg/L of free sulphur dioxide, wines were racked and homogeniesd. After 12 months, the

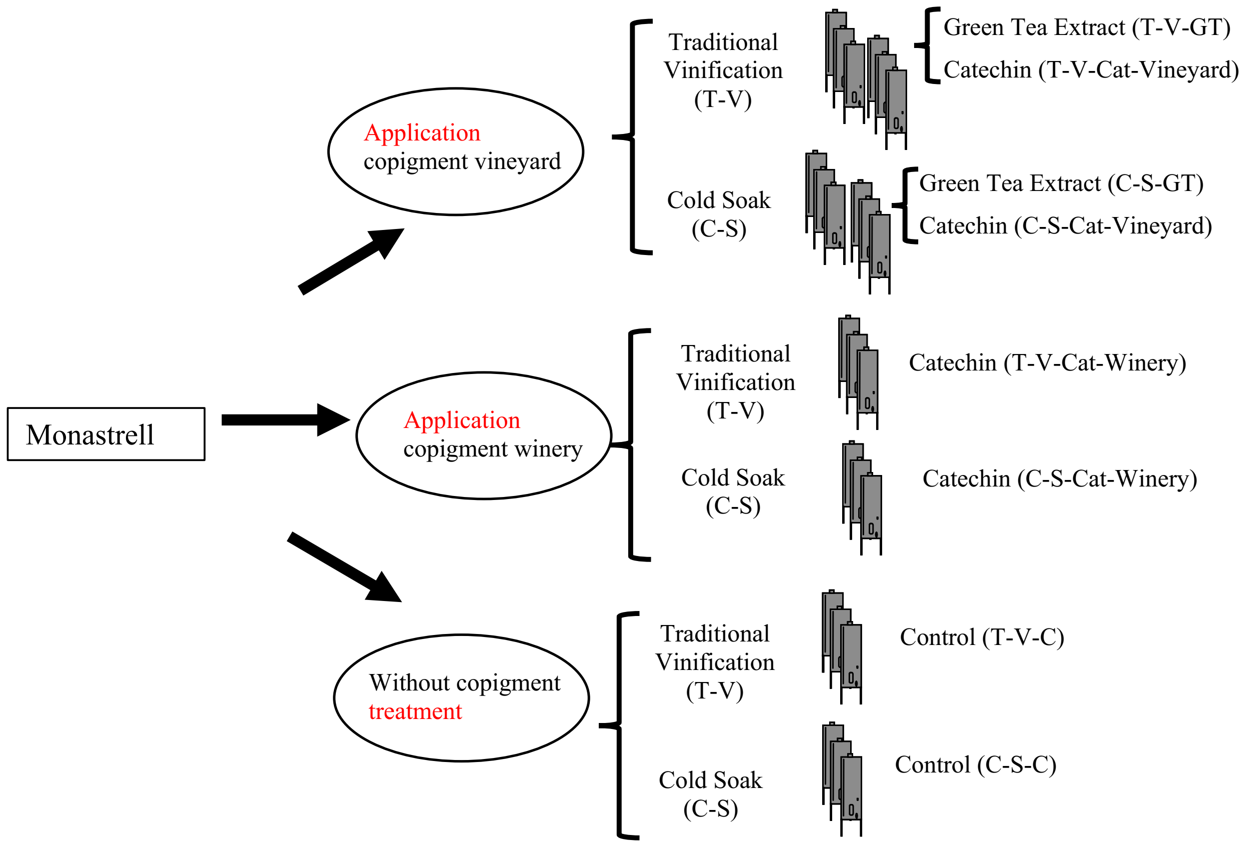

Figure 1 Copigment treatment and vinification experimental design.

polyphenolic and volatile composition of wines were determined.

Twenty-four vinifications per year were carried out by following eight protocols in triplicate (the two experimental treatments in the vineyard, one treatment with the prefermentative application of copigments and one untreated control). All the protocols were followed with traditional maceration and cold prefermentative maceration.

Physico-chemical parameters

The physico-chemical parameters in must and wine (density, ethanol, pH, total and volatile acidity, SO_2 content) were performed according to the Official Methods established by OIV (2003). Total soluble solids determination was performed by refractometry and reducing sugars were established by the Fehling method (Lane & Eynon, 1923).

Phenolic parameters

The wine phenolic composition was determined by a JASCO V-630 UV–Visible spectrophotometer and a JASCO MD2010 Plus HPLC, coupled with a diode array detector (DAD) (JASCO LC-Net II/ADC, Tokyo, Japan). All the measurements were taken in triplicate. Colour density, hue, TPI (Total Phenol Index) and the gelatine index (astringency) were estimated by the methods described by Glories (1984). Condensed tannins were determined by the method of Ribéreau-Gayon & Stonestreet (1966). The Folin–Ciocalteu assay was determined according to Singleton & Rossi (1965). The method reported by Boulton (1996) was followed to analyse the contribution of the copigmented, free and polymeric anthocyanins to the total wine colour. The DMACH Index (degree of tannin polymerisation) was calculated according to Vivas *et al.* (1994).

HPLC was used to quantify individual phenolic compounds (phenolic acids, flavan-3-ols, flavonols, major anthocyanidins, acylated anthocyanins) according to Boido *et al.* (2006).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 carried out on a Gemini NX (Phenomenex, Torrance, CA, USA): 5 µm, 250 mm x 4.6 mm i.d. column at 40 °C. Solvents were 0.1% trifluoroacetic acid (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 520 nm. For quantification purpose, calibration curves were obtained with malvidin-3-glucoside (S-0911, Extrasynthèse, Genay, France). The calibration curves of malvidin-3-glucoside was: (y = 236 316x - 499 676, R^2 = 0.9994).

Volatile compounds extraction and identification

The quantification of volatile compounds was performed on all the wines in this study after a 12-month storage time. Volatile compounds were analysed by the procedure proposed by Ortega *et al.* (2001) with slight modifications. A volume of 2.7 mL of samples was transferred to a 10 mL screw-capped centrifuge tube containing 4.05 g of ammonium sulphate (Panreac, Barcelona). Then 6.3 mL of milliQ water (Panreac) were added. In addition, 20 μL of internal standard solution: 2-butanol, 4-methyl-2-pentanol and 2-octanol 140 μg/mL each from Sigma-Aldrich (Steinheim, Germany), in absolute ethanol and 0.25 mL of dichloromethane, both of LiChrosolv quality from Merck (Darmstadt, Germany). The tube was shaken mechanically for 120 min and was then centrifuged at 2900 g for 15 min. The dichloromethane phase was recovered and transferred to the autosampler vial and analysed. The chromatographic analysis was carried out in an HP-6890, equipped with a ZB-Wax plus column (Phenomenex, Torrance, CA, USA) (60 m × 0.25 mm × 0.25 μm). The column temperature, initially set at 40 °C, was left at this temperature for 5 min to then be 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. Then this temperature was maintained 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 was then kept at this temperature for 30 min. The carrier gas was helium, which was fluxed at a rate of 3 mL/min. Injection was done in the split mode 1:20 (injection volume 2 μL) with a flame-ionisation-detector (FID detector). In addition, Kovats retention indices (KI) were calculated for the GC peaks corresponding to identify substances by the interpolation of the retention time of normal alkane (C8 e C20) by Fluka Buchs, Schwiez, (Switzerland), analysed under the same chromatographic condition. The calculated KI were compared to those reported in the literature for the same stationary phase. Volatiles were identified by comparing them to the linear retention index of pure standard compounds and by comparing the experimental mass spectra to those reported in the library. Compound identification was accepted with a probability higher than 80%. Volatiles were quantified using relative areas related to 2-octanol as internal standards.

Statistical analysis

Statistical analysis was performed with CENTURION XVI.II for Windows (Statpoint Technologies, Inc., Virginia, USA). The data corresponding to the control and the wines from the vineyard treatments with green tea extract and pure catechin were processed by a simple ANOVA to evaluate if the application of copigments influenced wine composition and to also select the variables that most influenced the differences between them. The data corresponding to the wines elaborated by traditional vinification, and those by cold soak followed by traditional vinification, were processed by a simple ANOVA to establish it cold soak process had modified wine maceration. The Duncan test was run to separate means (P value < 0.01) when the ANOVA test was significant. In addition, a multifactorial ANOVA was used to study the copigment treatment effect x winemaking technique interaction.

Results and discussion

The wines made in 2016 and 2017 were analysed at after the 12 months storage. To study the influence of factors (application of copigment and maceration technique), simple ANOVA was performed with each factor vs. the control wines.

In order to jointly process data according to the applied copigment (green tea extract and catechin in the field and when entering the winery), the interaction between copigments and winemaking techniques after 12 months of ageing was previously tested for the analysed compounds. Table 1 shows the multifactorial ANOVA data for the experimental factors application of copigments considered and vinification techniques, and also for their interaction, in 2016 and 2017, and for the polyphenolic and volatile compounds of wines after 12 months of ageing. In each column, a F-ratio values can be compared to one another in each column because the number of comparisons was the same in every cases. A high F-ratio value means the factor has a stronger effect on the variable.

According to the multifactorial ANOVAs, the polyphenolic and volatile compounds were affected by the green tea extract and catechin copigment treatments, while the winemaking techniques (T-V and C-S) did not practically affect polyphenolic compounds, but affected volatile compounds. Very few interactions were observed in wine polyphenolic and volatile compounds due to the application of copigments and winemaking techniques. The interactions were year-

Table 1 Multifactorial variance analysis for the applied copigments, the vinification technique and their interaction, for the polyphenolic compounds and volatile compounds of Monastrell wines after de 12 month of ageing in 2016 and 2017 vintages

	Interaction Copig × Techniq		Copigments		Winemaking Techniques	
Compounds	2016	2017	2016	2017	2016	2017
Colour density	ns	ns	ns	ns	ns	ns
Hue (%)	4.16*	5.05*	3.76*	ns	85.28***	3.74*
Copigmented anthocyanins (%)	9.97**	4.23*	ns	ns	ns	ns
Polymerised anthocyanins (%)	ns	ns	ns	ns	ns	ns
Free anthocyanins (%)	ns	ns	ns	ns	30.78***	ns
Malvidin (mg/L)	4.56*	7.28*	ns	ns	ns	ns
Peonidin (mg/L)	3.56*	5.07*	ns	ns	ns	ns
Petunidin (mg/L)	3.38*	ns	ns	ns	ns	ns
Cyanidin (mg/L)	3.49*	6.71*	ns	ns	ns	3.40*
Delphinidin (mg/L)	5.52*	ns	ns	ns	ns	ns
Total anthocyanins (mg/L)	4.80*	ns	ns	ns	12.41***	ns
Condensed tannins (g/L)	ns	ns	11.94**	ns	18.66***	ns
Total polyphenols (g/L)	ns	14.80***	23.86***	ns	ns	ns
Folin Index	ns	ns	4.26	ns	ns	ns
DMACH Index (%)	ns	ns	ns	ns	14.89***	ns
Gelatine Index (%)	ns	ns	ns	ns	ns	ns
Alpha-pinen (μg/L)	6.93*	3.26*	ns	ns	ns	5.32*
Beta-pinen (μg/L)	ns	ns	ns	ns	5.17*	6.96*
Ethyl isovalerate (μg/L)	ns	ns	ns	ns	ns	ns
Isoamyl acetate (μg/L)	7.67*	ns	4.78*	11.76**	ns	6.57*
Ethyl hexanoate (μg/L)	ns	4.28	6.65*	9.68**	ns	9.97**
n-amyl alcohol (μg/L)	6.12*	ns	6.93*	ns	ns	ns
Hexyl acetate (μg/L)	9.60**	46.35***	13.61**	ns	15.68***	ns
Ethyl lactate (μg/L)	ns	3.38*	4.23*	ns	ns	ns
Cis 3-hexenol (μg/L)	6.57*	8.70**	ns	ns	ns	ns
Ethyl octanoate (μg/L)	5.42*	4.45*	14.65***	ns	ns	ns
1.2 propylene glycol (μg/L)	8.93**	6.83*	ns	ns	ns	17.3***
Ethyl 3-hydroxybutyrate (μg/L)	ns	3.89*	13.76***	7.45**	ns	ns
Linalool (μg/L)	9.61**	13.09**	ns	ns	ns	ns
Ethyl decanoate (μg/L)	ns	ns	10.16**	39.11***	ns	ns
Diethyl succinate (μg/L)	ns	4.12*	27.41***	11.56**	ns	ns
2 phenyl acetate (μg/L)	ns	4.05*	ns	ns	ns	ns
2 metoxyphenol (μg/L)	6.11*	ns	ns	ns	21.38***	ns
γ-octalactone (μg/L)	ns	ns	9.57**	9.15**	ns	9.28**
2 phenylethanol (μg/L)	ns	ns	34.01***	13.64**	ns	ns
Eugenol (μg/L)	4.87*	6.10*	ns	ns	ns	ns
Decanoic acid (μg/L)	ns	ns	16.10**	79.92***	11.08***	21.15***
Vanillin (μg/L)	18.11***	ns	ns	ns	ns	ns

In each row, different letters denote significant differences according to Duncan's test $*P < 0.05$, $**P < 0.01$; $***P < 0.001$; as determined by ANOVA. ns means do not significant exist difference between treatments; DMACH, means degree of proanthocyanidins polymerisation; PVPP, polyvinylpolypyrrolidone.

dependent and few compounds had shown aforementioned interaction for the 2 years 2016 and 2017. Hence data can be interpreted jointly according to the applied copigment without having to take into account the winemaking technique and *vice versa*.

Common must and wine parameters in Monastrell

Composition must and wine were determined, different tanks did not present significant differences in their maturity. Specifically, 2016 Brix degree (23.9–24.2); pH (3.45–3.55), total acidity (5.6–5.9 g/L tartaric acid). A similar situation was observed in 2017 must: Brix degree (24.3–24.7); pH (3.55–3.65), total acidity (5.3–5.4 g/L tartaric acid). The values obtained show that the copigmentation treatments did not affect the grape technological maturity; the small differences observed can be attributable to the intrinsic variability of the vineyard. Nevertheless, significant differences have been observed between vintages, whereas 2017 was a warmer year than 2016 and ripest grapes were obtained.

Likewise, the wines produced were analysed. The 2016 wine values obtained were alcohol degree was between 13.9%–14.2%, pH (3.55–3.70) and total acidity (5.3–5.6 g/L tartaric acid). In the end of alcoholic fermentation, the residual sugars ranged between 1.5–2.3 g/L. (Ribéreau-Gayon *et al.*, 2006a). Wines volatile acidity were between 0.40 and 0.59 g/L acetic acid, and the sulphurous values ranged from 75–80 mg/L, which are common in industrial wines (Ribéreau-Gayon *et al.*, 2006b). On the other hand, in 2017, as a result of the high temperatures, the wines showed higher alcohol content (14.0%–14.2%), higher pH values (3.64–3.75) and lower total acidity (5.2–5.4 g/L tartaric acid). The volatile acidity (0.48–0.69 g/L acetic acid) and sulphurous (80–89 mg/L) and residual sugars (1.5–2.1 g/L). The small differences in the oenological parameters of wines indicate that neither copigmentation treatments nor winemaking practices did not cause significant differences in common parameter wines.

Effect of copigment treatment (green tea extract in the vineyard, pure catechin in the vineyard, pure catechin when entering the winery) on Monastrell wine polyphenolic composition after 12 months of ageing

Table 2 shows the means and standard deviations together with the ANOVA for the polyphenolic compounds studied after 12 months of ageing depending on the way to apply the copigments. This performance is different at that moment that observed after malolactic fermentation and at the start of conservation process (data not shown). After 12 months of ageing, the concentration of malvidin and other anthocyanins significantly lowered. This same result has been reported by Zhang *et al.* (2021), but their concentration remained higher in the wines treated with copigments in the 2016 vintage than in the 2017 one thanks to the presence of catechin, which helps to stabilise malvidin, (González-Manzano *et al.*, 2008; Zhao *et al.*, 2020; Liu *et al.*, 2021), especially when grapes were treated with the green tea extract and catechin when entering the winery, such polymerisation is facilitated by presence of copigments (Bimpilas *et al.*, 2016). The total anthocyanins concentration remained stable during wine storage and was higher in the wines treated with the green tea extract and catechin (Teixeira *et al.*, 2013). The wines treated with copigments, were with the highest concentration of total anthocyanins, malvidin-3-*O*-monoglucoside and the rest of minority anthocyanins determined.

The condensed tannins concentration was significantly lower in the wines treated with the green tea extract, while the polyphenols concentration (TPI and Folin Index) after 12 months storage was similar for all the wines. No differences were obtained for the degree of tannin polymerisation, or for tannin astringency in the two vintages studied (Table 2). However, the application of catechin as a copigment should have increased the degree of tannin polymerisation according to González-Manzano *et al.* (2008) by reducing astringency. Instead a reduction in astringency and an increase in the degree of tannin polymerisation occurred during the ageing time. In the 2017 vintage, grape maturity was better than 2016, manifested by higher concentration of total anthocyanins and by more astringent of tannins; however, the wines behaviour was similar. The interaction between the application of copigments and vintage was minimal.

Effect of application of copigment (green tea extract in vineyard, pure catechin in the vineyard, pure catechin when entering the winery) on Monastrell wine volatile composition after 12 months of ageing

Wine volatile composition was also determined at the end of the conservation process (Table 3) 12 months after bottling. In order to carry out a comparative analysis of the results and to establish whether applications of the tested copigments resulted in significant differences in volatile compounds, Table 3 shows the different behaviours of the analysed volatile compounds depending on the way and time of copigment treatment. No significant differences were found for grape supplementation with the green tea extract and catechin in eight of the studied volatile compounds: β pinen, ethyl isovalerate, ethyl hexanoate, ethyl lactate, ethyl 3-hydroxybutyrate, ethyl decanoate, diethyl succinate, ɣ́-octalactone, 2-phenylethanol and decanoic acid. These compounds had the mildest effect on wine volatile composition (Saerens *et al.*, 2010). Only the concentration of ethyl octanoate was higher in all the wines from the grapes treated with the green tea extract and catechin, while linalool and eugenol concentrations were higher in the wines from the grapes treated with green tea and hexyl acetate, 2-phenyl acetate and vanillin in the wines treated with catechin in the field. These results are important because the esters present in wine vastly vary and contribute significantly to aroma, even though they are not present at high concentrations. Esters confer wine a fresh fruity aroma (Ivit *et al.*, 2018). Hexyl acetate and 2-phenylethyl acetate contribute floral aromas and ethyl octanoate contribute fruity aromas (Saerens *et al.*, 2010; Holt *et al.*, 2012). At the organoleptic level, linalool (floral aroma), vanillin (vanilla aroma), eugenol (spicy aroma) are also important. Other volatile compounds appear at lower concentrations when grapes are treated with the green tea extract, such as α-pinene and isoamyl acetate. The flavonoids, phenolic compounds

Table 2 Means, standard deviations and variance analyses of the polyphenolic parameters of Monastrell fermentation depending on the applied copigments during each season after de 12 month of ageing and the average for 2016 and 2017 vintages

Compounds	Copigment	2016	2017	Average 2016–2017	Year (*P*-value)	Interaction copig × year (*P*)
Colour density	Control	8.53 ± 1.01a	11.63 ± 0.90a	10.08 ± 1.31a	26.12***	ns
	Green tea extract	8.69 ± 0.01a	10.53 ± 0.94a	9.58 ± 1.58a		
	Catechin vineyard	8.53 ± 0.40a	11.35 ± 0.78a	9.94 ± 1.57a		
	Catechin winery	8.23 ± 0.25a	11.08 ± 0.79a	9.65 ± 1.57a		
Hue (%)	Control	75.68 ± 3.95a	68.70 ± 2.57ab	72.19 ± 4.83a	27.42***	10.22**
	Green tea extract	82.92 ± 0.21b	65.19 ± 4.46a	71.10 ± 9.43a		
	Catechin vineyard	80.34 ± 1.89b	70.82 ± 1.98b	75.58 ± 5.26a		
	Catechin winery	81.36 ± 0.92b	66.93 ± 2.56a	74.14 ± 7.68a		
Copigmented anthocyanins (%)	Control	9.87 ± 1.25a	16.55 ± 5.59a	13.21 ± 3.72a	39.35***	ns
	Green tea extract	13.60 ± 1.64b	24.50 ± 3.02b	20.87 ± 5.94b		
	Catechin vineyard campo	12.21 ± 2.07b	27.38 ± 6.95b	19.79 ± 4.35b		
	Catechin winery	12.46 ± 2.56b	34.75 ± 11.77b	23.60 ± 10.15b		
Polymerised anthocyanins (%)	Control	50.47 ± 3.27a	47.64 ± 4.85a	49.05 ± 4.26a	ns	ns
	Green tea extract verde	48.51 ± 0.54a	44.07 ± 3.06a	46.55 ± 2.57a		
	Catechin vineyard	44.76 ± 1.74a	45.47 ± 2.14a	45.12 ± 2.00a		
	Catechin winery	44.03 ± 1.20a	45.86 ± 4.62a	44.95 ± 3.39a		
Free anthocyanins (%)	Control	39.66 ± 4.41a	35.82 ± 6.04a	37.74 ± 5.14a	ns	ns
	Green tea extract	43.88 ± 1.36a	41.49 ± 3.49a	42.29 ± 3.11a		
	Catechin vineyard	43.03 ± 1.52a	40.35 ± 3.12a	41.69 ± 2.75a		
	Catechin winery	43.51 ± 2.53a	37.66 ± 5.23a	40.58 ± 4.99a		
Malvidin (mg/L)	Control	44.99 ± 7.35a	63.13 ± 2.24b	49.06 ± 15.45a	3.74*	ns
	Green tea extract	67.84 ± 4.24c	62.05 ± 24.02b	63.98 ± 19.50b		
	Catechin vineyard	54.14 ± 6.49b	56.56 ± 13.96ab	55.35 ± 10.59ab		
	Catechin winery	49.76 ± 10.59abb	42.17 ± 18.41a	45.97 ± 15.03a		
Peonidin (mg/L)	Control	2.53 ± 0.56a	3.18 ± 0.68a	2.85 ± 1.04a	4.14*	ns
	Green tea extract	4.80 ± 0.41c	3.53 ± 1.39b	3.95 ± 1.29a		
	Catechin vineyard	3.71 ± 0.37b	3.08 ± 0.68a	3.39 ± 0.62a		
	Catechin winery	3.99 ± 1.19b	2.35 ± 0.95a	3.17 ± 1.34a		
Petunidine (mg/L)	Control	4.54 ± 1.24a	4.38 ± 0.56a	5.46 ± 1.33a	5.83*	ns
	Green tea extract	7.97 ± 0.85c	6.43 ± 2.14a	6.95 ± 1.92b		
	Catechin vineyard	6.65 ± 0.94bc	5.45 ± 1.77a	6.05 ± 1.50ab		
	Catechin winery	5.84 ± 1.63b	4.71 ± 1.90a	5.27 ± 1.81a		
Cyanidin (mg/L)	Control	2.04 ± 0.33a	2.07 ± 0.30b	2.05 ± 0.38a	12.42**	6.76*
	Green tea extract	2.93 ± 0.17b	2.15 ± 0.57b	2.41 ± 0.60a		
	Catechin vineyard	2.49 ± 0.27b	1.84 ± 0.25ab	2.17 ± 0.42a		
	Catechin winery	2.69 ± 0.53b	1.53 ± 0.55a	2.11 ± 0.79a		
Delphinidin (mg/L)	Control	3.74 ± 0.84a	4.87 ± 0.78a	4.30 ± 0.97a	ns	ns
	Green tea extract	5.83 ± 1.40c	4.70 ± 1.89a	5.07 ± 1.77a		
	Catechin vineyard	5.44 ± 0.77b	4.28 ± 1.11a	4.86 ± 1.10a		
	Catechin winery	4.59 ± 1.02ab	3.59 ± 1.65a	4.09 ± 1.42a		
Total anthocyanins (mg/L)	Control	223.59 ± 17.39a 59 ± 17.39a	303.21 ± 53.02a	263.21 ± 53.02a	2.49*	ns
	Green tea extract	251.68 ± 18.37b	298.27 ± 57.41a	282.74 ± 52.11ab		
	Catechin vineyard	253.27 ± 16.05b	354.01 ± 46.10b	303.64 ± 41.79b		
	Catechin winery	251.03 ± 15.37bb	316.24 ± 35.92a	283.63 ± 42.97ab		
Condensed tannins (g/L)	Control	2.01 ± 0.08b	1.98 ± 0.07b	1.95 ± 0.11b	ns	ns
	Green tea extract	1.74 ± 0.05a	1.59 ± 0.16a	1.64 ± 0.15a		
	Catechin vineyard	2.05 ± 0.08b	1.92 ± 0.10b	1.99 ± 0.11b		
	Catechin winery	2.13 ± 0.13c	1.84 ± 0.09b	1.98 ± 0.18b		
Total polyphenols (g/L)	Control	2.06 ± 0.92a	2.21 ± 0.68a	2.14 ± 0.86b	ns	ns
	Green tea extract verde	2.62 ± 0.80a	2.45 ± 0.30a	2.51 ± 0.49a		
	Catechin vineyard	2.21 ± 0.34a	2.78 ± 0.70a	2.49 ± 0.61a		
	Catechin winery	2.49 ± 0.78a	2.73 ± 0.64a	2.61 ± 0.70a		

Table 2 (*Continued*)

Compounds	Copigment	2016	2017	Average 2016–2017	Year (*P*-value)	Interaction copig × year (*P*)
Folin Index	Control	54.38 ± 7.92a	48.43 ± 1.95a	51.40 ± 6.36a	6.71*	ns
	Green tea extract	52.66 ± 5.97a	46.30 ± 5.88a	48.42 ± 6.44a		
	Catechin vineyard	59.87 ± 5.98a	50.71 ± 7.50a	55.29 ± 8.08a		
	Catechin winery	54.89 ± 3.18a	53.20 ± 8.47a	54.05 ± 6.25a		
DMACH Index (%)	Control	47.71 ± 17.06a	47.33 ± 8.93a	47.52 ± 14.21a	ns	3.39*
	Green tea extract	40.04 ± 9.75a	60.89 ± 16.79a	53.94 ± 17.62a		
	Catechin vineyard	50.70 ± 6.03a	48.00 ± 11.62a	49.35 ± 9.05a		
	Catechin winery	50.99 ± 13.26a	48.91 ± 12.46a	49.95 ± 12.48a		
Gelatin Index (%)	Control	24.16 ± 3.74a	52.10 ± 15.77a	38.00 ± 17.90a	34.13***	ns
	Green tea extract	22.54 ± 2.14a	52.44 ± 15.40a	42.47 ± 19.20a		
	Catechin vineyard	23.63 ± 5.66a	47.78 ± 16.99a	35.70 ± 17.46a		
	Catechin winery	22.06 ± 1.49a	49.96 ± 15.36a	36.01 ± 17.86a		

For the data analysis across years, the statistical significance of the effects of year and copigments by year interaction, are also indicated. In each column, different letters denote significant differences based on Duncan's test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). ns means do not significant difference exist between treatments; DMACH, means degree of proanthocyanins polymerisation; PVPP, polyvinylpolypyrrolidone.

and their derivatives, which are naturally found in the structure of these extracts, have been shown to be effective in preventing auto-oxidation of volatile compounds (García-Ruiz *et al.*, 2013). The application of plant extracts and elicitors in the vineyard caused an increase in higher alcohols and esters in the wines, and although this compounds originate mainly from the fermentation process, the formation of these substrates may have been affected by the grapes treatment, thus affecting their final concentrations in wines. A biostimulating effect of the formation of volatile compounds on grapes was also observed when eucalyptus extract, almond skins extract, benzothiadiazole, methyl jasmonate, chitosan, were applied to vineyards, obtaining wines with a higher concentration of terpenes, acetals and ester (Yildirim *et al.*, 2005; Vitalini *et al.*, 2014; D'Onofrio *et al.*, 2018).

Effect of winemaking techniques (traditional and cold soak) on the Monastrell polyphenolic composition of wines after 12 months of ageing

Table 4 shows the means and standard deviations together with the ANOVA for the polyphenolic compounds studied at the end of storage depending on the applied winemaking technique (T-V and C-S).

In the wines from the 2016 vintage, wine conservation modified the effect of the winemaking techniques because once malolactic fermentation had ended, the tested maceration techniques did not affect the colour-related parameters (Table 4). At 12 months, the colour of the prefermentative macerated wines was more intense, and their hue was lesser. These results coincide with those found by González-Neves *et al.* (2016). In contrast, the observed behaviour of condensed tannins and total polyphenols remained as their values were higher in the prefermentally macerated wines (C-S) at both the end of malolactic fermentation and after storage (Panprivech *et al.*, 2015; Favre & Gonzalez-Neves, 2017). Conversely in the 2017 vintage wines, there were no significant differences according to winemaking techniques, except for the concentration of condensed tannins. The greater grape maturity of this vintage minimised the effect of prefermentative maceration, which other authors have also observed (Álvarez *et al.*, 2006).

Effect of winemaking techniques (traditional and cold soak) on Monastrell wine volatile composition after 12 months of ageing

Table 5 shows the volatile composition of the wines treated with the green tea extract when the preservation process ended 12 months after bottling. The behaviour noted in both the 2016 and 2017 vintages was exactly the same as statistically significant differences were found. Twenty-two volatile compounds were studied. In some of them (10), no significant effect was noted for the applied maceration technique (T-V and C-S) on wine volatile composition; for example α and β pinen, ethyl isovalerate, cis 3 hexenol, 1,2 propylene glycol, linalool, 2-phenyl ethyl acetate, 2-meoxyphenol (guaiacol) eugenol and vanillin. However, a significant effect of prefermentative maceration on the concentration of some of the studied compounds, such as isoamyl acetate, ethyl hexanoate, n-amylalcohol, hexyl acetate, ethyl lactate, ethyl octanoate, 3-ethyl hydroxybutyrate, ethyl decanoate, diethyl succinate, 2-

Table 3 Means, standard deviations and variance analyses of the volatile compounds of Monastrell wines depending on the applied copigments during each season after de 12 month of ageing and the average for 2016 and 2017 vintages

Compounds (µg/L)	Copigments	2016	2017	Average 2016–2017	Year *P*-value	Interaction copig × year (*P*)
Alpha-pinen	Control	41.37 ± 6.27b	46.48 ± 7.05b	43.93 ± 6.97b	4.52*	ns
	Green tea extract	29.47 ± 5.29a	33.11 ± 5.94a	31.29 ± 5.75a		
	Catechin vineyard	32.22 ± 7.58a	36.20 ± 8.51ab	34.21 ± 8.05a		
	Catechin winery	30.62 ± 3.32a	34.40 ± 3.73ab	32.51 ± 3.93a		
Beta-pinen	Control	14.37 ± 1.24a	16.15 ± 1.40a	15.26 ± 1.57a	ns	ns
	Green tea extract	16.78 ± 8.79a	18.68 ± 10.00a	17.73 ± 9.15a		
	Catechin vineyard	18.47 ± 6.79a	20.76 ± 7.63a	19.61 ± 7.08a		
	Catechin winery	16.92 ± 7.14a	19.01 ± 8.02a	17.97 ± 7.41a		
Ethyl isovaleriate	Control	18.60 ± 5.97a	20.90 ± 6.71a	19.75 ± 6.25a	ns	ns
	Green tea extract	17.84 ± 3.59a	20.42 ± 3.90a	19.13 ± 3.86a		
	Catechin vineyard	22.50 ± 10.02a	16.86 ± 4.57a	19.68 ± 14.33a		
	Catechin winery	15.09 ± 2.82a	16.95 ± 3.17a	16.02 ± 3.05a		
Isoamyl acetate	Control	442.84 ± 71.14b	497.57 ± 79.93b	470.21 ± 78.37b	5.22*	ns
	Green tea extract	307.31 ± 66.42a	345.29 ± 74.63a	326.30 ± 71.01a		
	Catechin vineyard campo	352.43 ± 30.39a	395.99 ± 34.15a	374.21 ± 38.49a		
	Catechin winery	356.38 ± 54.68a	400.43 ± 61.44a	378.41 ± 60.61a		
Ethyl hexanoate	Control	191.84 ± 17.88a	215.55 ± 20.09b	203.70 ± 22.08a	4.61*	ns
	Green tea extract	182.32 ± 65.55a	204.86 ± 73.66a	193.59 ± 68.36a		
	Catechin vineyard	158.93 ± 24.06a	178.57 ± 27.03a	168.75 ± 26.72a		
	Catechin winery	161.55 ± 13.63a	181.52 ± 15.31a	171.53 ± 17.39a		
n-amyl alcohol	Control	43.52 ± 7.33b	45.74 ± 14.04a	44.63 ± 10.88b	ns	ns
	Green tea extract	27.30 ± 4.96a	29.48 ± 6.73a	28.39 ± 5.82a		
	Catechin vineyard	40.08 ± 10.17b	45.04 ± 11.43a	42.56 ± 10.76b		
	Catechin winery	39.51 ± 9.00b	44.39 ± 10.11a	41.95 ± 9.59b		
Hexil acetate	Control	8.56 ± 0.80a	10.30 ± 5.43a	9.43 ± 3.86a	ns	ns
	Green tea extract	13.68 ± 5.32b	9.53 ± 2.57a	11.60 ± 4.57a		
	Catechin vineyard	15.44 ± 2.88b	17.19 ± 3.39b	16.32 ± 3.17b		
	Catechin winery	8.98 ± 1.35a	9.68 ± 1.92a	9.33 ± 1.64a		
Ethyl lactate	Control	9710.64 ± 1630a	10910.83 ± 1832a	10310.74 ± 1786a	4.32	ns
	Green tea extract	11517.11 ± 3642a	12940.62 ± 4093a	12228.88 ± 3814a		
	Catechin vineyard	9609.38 ± 1559a	10797.06 ± 1751a	10203.22 ± 1715a		
	Catechin winery	10115.65 ± 1485a	11365.90 ± 1668a	10740.78 ± 1657a		
Cis 3-hexenol	Control	11.81 ± 2.24b	17.48 ± 5.14b	14.65 ± 4.82b	6.05*	ns
	Green tea extract	9.14 ± 4.31ab	11.01 ± 3.29ab	10.07 ± 3.83ab		
	Catechin vineyard	7.90 ± 1.52a	6.96 ± 1.40a	7.43 ± 1.49a		
	Catechin winery	14.13 ± 3.45c	15.88 ± 3.87b	15.00 ± 3.66b		
Ethyl octanoate	Control	20.62 ± 7.83a	25.32 ± 6.73a	22.97 ± 7.46a	ns	ns
	Green tea extract	25.69 ± 6.81ab	27.51 ± 8.69a	26.60 ± 7.60a		
	Catechin vineyard	38.99 ± 12.59c	44.59 ± 13.43c	41.79 ± 12.30b		
	Catechin winery	32.23 ± 10.44bc	33.19 ± 17.34b	32.71 ± 13.84ab		
1.2 propylene glicol	Control	170.82 ± 64.27c	188.84 ± 73.91c	179.83 ± 67.55c	ns	ns
	Green tea extract	82.94 ± 12.58a	79.75 ± 14.99a	81.34 ± 13.47a		
	Catechin vineyard	131.10 ± 35.71b	147.30 ± 40.1bc	139.20 ± 37.64b		
	Catechin winery	90.34 ± 18.79a	101.51 ± 21.1b	95.92 ± 20.15a		
Ethyl 3-hidroxibutirate	Control	69.52 ± 16.21a	78.11 ± 18.21b	73.82 ± 17.24a	4.29*	ns
	Green tea extract	56.68 ± 20.44a	67.48 ± 19.89a	62.08 ± 20.26a		
	Catechin vineyard	54.98 ± 9.83a	59.97 ± 13.57a	57.48 ± 11.74a		
	Catechin winery	60.66 ± 5.74a	68.15 ± 6.45a	64.40 ± 7.05a		
Linalol	Control	48.94 ± 7.87b	54.99 ± 8.84ab	51.97 ± 8.67ab	3.56*	ns
	Green tea extract	58.43 ± 11.49c	65.65 ± 12.91b	62.04 ± 12.38b		
	Catechin vineyard	39.59 ± 7.11a	41.92 ± 9.38a	40.76 ± 8.13a		
	Catechin winery	39.55 ± 5.14a	43.73 ± 6.70a	41.64 ± 6.16a		
Ethyl decanoate	Control	291.37 ± 68.23a	327.38 ± 76.67a	309.38 ± 72.53a	ns	ns
	Green tea extract	273.52 ± 122.76a	307.32 ± 137.93a	290.42 ± 127.34a		

Table 3 (*Continued*)

Compounds (μg/L)	Copigments	2016	2017	Average 2016–2017	Year *P*-value	Interaction copig × year (*P*)
	Catechin vineyard	318.75 ± 55.51a	358.15 ± 62.37a	338.45 ± 60.56a		
	Catechin winery	271.21 ± 39.42a	304.73 ± 44.29a	287.97 ± 44.05a		
Diethyl succinate	Control	1074.88 ± 182a	1207.73 ± 205a	1141.31 ± 199a	6.76*	5.29*
	Green tea extract	1284.17 ± 411a	1682.07 ± 689ab	1483.12 ± 585a		
	Catechin vineyard	1144.56 ± 197a	1286.03 ± 221ab	1215.30 ± 215a		
	Catechin winery	1499.20 ± 373a	1684.50 ± 419b	1591.85 ± 395a		
2- phenylacetate	Control	23.58 ± 5.17a	0.60 ± 0.05a	11.20 ± 12.64a	18.95***	23.17***
	Green tea extract	23.68 ± 5.91a	16.32 ± 7.46c	20.00 ± 13.15a		
	Catechin vineyard	43.48 ± 2.04b	6.13 ± 2.32b	24.80 ± 22.67a		
	Catechin winery	31.51 ± 5.77a	0.06 ± 0.01a	15.75 ± 16.74a		
2- metoxyphenol	Control	525.48 ± 201.87b	590.42 ± 226.83b	557.95 ± 210.13b	ns	ns
	Green tea extract	518.39 ± 105.40b	582.46 ± 118.43b	550.42 ± 113.24b		
	Catechin vineyard	306.29 ± 78.86a	281.78 ± 141.40a	294.04 ± 111.33a		
	Catechin winery	532.11 ± 68.35b	597.88 ± 76.80b	564.99 ± 78.02b		
γ-octolactone	Control	409.24 ± 106.78a	541.28 ± 117.50a	475.26 ± 128.11a	ns	ns
	Green tea extract	390.95 ± 250.81a	439.27 ± 281.81a	415.11 ± 258.92a		
	Catechin vineyard	560.23 ± 87.13b	596.96 ± 70.19a	578.60 ± 78.75b		
	Catechin winery	408.26 ± 61.22a	520.20 ± 94.33a	464.23 ± 36.15a		
2- phenylethanol	Control	28581.14 ± 52467a	32113.64 ± 5894a	30347.39 ± 5691a	3.17*	ns
	Green tea extract	29509.15 ± 10500a	33156.31 ± 11797a	31332.75 ± 10952a		
	Catechin vineyard	25514.60 ± 3431a	28668.09 ± 3855a	27091.34 ± 3883a		
	Catechin winery	29181.39 ± 5705a	32788.08 ± 6410a	30984.74 ± 6151a		
Eugenol	Control	95.14 ± 13.78a	106.90 ± 15.49a	101.02 ± 15.41a	ns	ns
	Green tea extract	146.60 ± 61.01b	164.72 ± 68.56b	155.66 ± 63.39b		
	Catechin vineyard	90.31 ± 10.45a	101.47 ± 11.74a	95.89 ± 12.19a		
	Catechin winery	108.18 ± 16.01a	121.55 ± 17.99b	114.86 ± 17.84a		
Decanoic acid	Control	48.28 ± 26.65a	54.24 ± 29.95a	51.26 ± 27.56a	ns	ns
	Green tea extract	39.46 ± 15.03a	42.05 ± 13.32a	40.75 ± 13.78a		
	Catechin vineyard	51.75 ± 20.27a	58.14 ± 22.78a	54.95 ± 21.09a		
	Catechin winery	45.73 ± 20.51a	68.86 ± 13.58a	57.29 ± 20.61a		
Vanillin	Control	38.53 ± 16.63a	43.29 ± 18.69a	40.91 ± 17.27a	ns	ns
	Green tea extract	53.08 ± 16.55b	59.64 ± 18.60a	56.36 ± 17.34a		
	Catechin vineyard	131.72 ± 47.44d	148.00 ± 65.66c	139.86 ± 51.73b		
	Catechin winery	77.60 ± 37.11c	87.19 ± 41.70b	82.40 ± 38.45ab		

For the data analysis across years, the statistical significance of the effects of year, and copigments by year interaction, are also indicated. In each column, different letters denote significant differences based on Duncan's test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). ns means do not significant difference exist between treatments.

phenylethanol, ɣ-octalactone and vanillin, was observed.

The results of this study showed that cold prefermentative maceration resulted in a significant increase in the concentration of seven esters (isoamyl acetate, ethyl hexanoate, hexyl acetate, ethyl octanoate, ethyl 3-hydroxybutyrate, ethyl decanoate, diethyl succinate) and ɣ-octalactone.

Studies by other authors (Álvarez *et al*., 2006; Selli *et al*., 2006) have shown that the wines made by the cold prefermentative maceration technique had higher concentrations of total acetates and esters. This effect could be explained by the fact that applying cold soak allows cryophilic *Saccharomyces* yeasts that to develop, which can influence the release of certain aromas, especially volatile esters. (Cai *et al*., 2014; Casassa & Sari, 2015) This could be one of the advantages of this technique. In addition, other studies have indicated that by the cold soak technique, non-*Saccharomyces* indigenous yeasts (*Hanseniapora*) can initiate fermentation by generating varietal aromas, while reducing herbaceous notes.

In the present study, compounds n-amylalcohol, 2-phenylethanol, hexyl acetate and decanoic acid were lower concentration in the wines in which cold prefermentative maceration was performed (Table 5) than in those processed by traditional winemaking. These results agree with those obtained by Mihnea *et al*. (2015), who observed a higher concentration of some higher alcohols in the wines obtained by the

Table 4 Means, standard deviations and variance analyses of the polyphenolic parameters of Monastrell wines depending on winemaking technology applied during each season after de 12 month of ageing and the average for 2016 and 2017 vintages.

Compounds	Winemaking techniques	2016	2017	Average 2016–2017	Year (*P*-value)	Interaction TechxYear (*P*)
Colour density	T-V	8.36 ± 0.59a	11.14 ± 0.84a	9.75 ± 1.58a	10.28**	ns
	C-S	9.15 ± 1.18b	11.15 ± 1.00a	10.29 ± 1.47a		
Hue (%)	T-V	81.30 ± 1.40b	69.08 ± 3.11a	75.19 ± 6.64a	18.43***	ns
	C-S	77.49 ± 4.35a	66.74 ± 3.74a	71.35 ± 6.70a		
Copigmented anthocyanins (%)	T-V	12.51 ± 2.57a	30.50 ± 6.89a	21.51 ± 10.47a	44.19***	ns
	C-S	10.88 ± 2.11a	23.88 ± 9.12a	18.30 ± 9.54a		
Polymerised anthocyanins (%)	T-V	44.98 ± 2.38a	46.16 ± 5.18a	45.57 ± 4.01a	ns	ns
	C-S	47.04 ± 4.71a	44.36 ± 2.12a	45.51 ± 3.66a		
Free anthocyanins (%)	T-V	42.51 ± 1.46a	38.49 ± 6.62a	40.50 ± 5.14a	ns	ns
	C-S	42.08 ± 4.23a	39.17 ± 2.56a	40.42 ± 3.61a		
Malvidin (mg/L)	T-V	50.42 ± 14.58a	60.64 ± 16.42a	55.53 ± 16.14a	ns	ns
	C-S	47.98 ± 11.61a	51.31 ± 18.80a	49.88 ± 15.94a		
Peonidin (mg/L)	T-V	3.45 ± 1.02a	3.50 ± 0.92a	3.48 ± 0.96a	ns	ns
	C-S	3.81 ± 1.11a	3.07 ± 1.32a	3.39 ± 1.27a		
Petunidine (mg/L)	T-V	5.94 ± 1.92a	6.21 ± 1.53a	6.07 ± 1.71a	ns	ns
	C-S	6.09 ± 1.29a	5.28 ± 1.93a	5.63 ± 1.71a		
Cyanidin (mg/L)	T-V	2.36 ± 0.42a	2.07 ± 0.41a	2.22 ± 0.43a	ns	ns
	C-S	2.64 ± 0.52a	1.92 ± 0.66a	2.23 ± 0.70a		
Delphinidin (mg/L)	T-V	4.67 ± 1.33a	4.76 ± 1.19a	4.71 ± 1.24a	ns	ns
	C-S	4.89 ± 1.06a	3.96 ± 1.60a	4.36 ± 1.45a		
Total anthocyanins (mg/L)	T-V	248.28 ± 16.37a	315.86 ± 48.46a	282.07 ± 49.44a	6.17*	ns
	C-S	238.10 ± 24.32a	320.00 ± 55.71a	284.90 ± 60.57a		
Condensed tannins (g/L)	T-V	2.05 ± 0.61a	2.81 ± 0.52a	2.43 ± 0.68a	3.70*	8.73*
	C-S	2.85 ± 0.56b	2.69 ± 0.70a	2.76 ± 0.64b		
Total polyphenols (g/L)	T-V	1.94 ± 0.14a	1.74 ± 0.20a	1.84 ± 0.20a	8.19**	ns
	C-S	2.12 ± 0.11b	1.87 ± 0.11a	1.98 ± 0.16a		
Folin index	T-V	53.00 ± 5.41a	50.91 ± 7.58a	51.95 ± 6.56a	4.71*	8.28*
	C-S	59.65 ± 5.40b	48.41 ± 5.56a	53.23 ± 7.82a		
DMACH index (%)	T-V	53.56 ± 12.48a	45.57 ± 14.87a	49.56 ± 14.10a	ns	ns
	C-S	41.54 ± 8.85a	52.00 ± 14.48a	47.52 ± 13.27a		
Gelatine index (%)	T-V	31.75 ± 17.41a	53.63 ± 14.95a	42.69 ± 19.45a	28.12***	ns
	C-S	26.91 ± 12.92a	47.51 ± 15.35a	38.68 ± 17.51a		

For the data analysis across years, the statistical significance of the effects of year, and techniques by year interaction, are also indicated. In each column, different letters denote significant differences based on Duncan's test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). ns means do not significant difference exist between treatments; DMACH, means degree of proanthocyanins polymerisation; PVPP, polyvinylpolypyrrolidone; T-V, traditional winemaking; C-S, cold soak prefermentative maceration.

prefermentative maceration technique González-Neves *et al.* (2015) made a similar observation and indicated the possibility of this effect resulting from the action of non-*Saccharomyces* yeasts during the cooling period.

Conclusions

The application of green tea extract to vineyards, and of catechin to a lesser extent, led to a higher concentration of anthocyanins (especially malvidin-3-*O*-glucoside) in the Monastrell grapes of the two studied vintages. Although the application of copigments did not affect wines' colour intensity, colour stability was greater 12 months after alcoholic fermentation. The concentration of copigmented anthocyanins and total anthocyanins increased. The stronger effect of green tea extract may be due to its complex composition because, apart from containing catechin, it also contains a significant amount of other flavonoids. However, the application of green tea extract resulted in a lower concentration of tannins in wines and, thus, reduced astringency, although this effect did not occur when the added copigment was catechin in vineyards and during grape processing, which can be a tool to reduce the astringency of red varieties that are too tannic or have a late polyphenolic ripening. Harvesting these grapes, with high sugar concentration, will give rise to wines with high alcoholic content (not accepted by today's consumers).

Table 5 Means, standard deviations and variance analyses of the volatile compounds of Monastrell wines depending on winemaking technology applied during each season after de 12 month of ageing and the average for 2016 and 2017 vintages

Compounds (µg/L)	Winemaking techniques	2016	2017	Average 2016–2017	Year *P*-value	Interaction Techn x Year (*P*)
Alpha-pinen	T-V	32.47 ± 8.16a	36.48 ± 9.17a	34.47 ± 8.70a	ns	ns
	C-S	34.37 ± 6.46a	38.62 ± 7.26a	36.50 ± 7.10a		
Beta-pinen	T-V	17.32 ± 7.00a	19.38 ± 7.95a	18.35 ± 7.35a	ns	ns
	C-S	15.95 ± 6.05a	17.92 ± 6.80a	16.94 ± 6.41a		
Ethyl isovalerate	T-V	16.08 ± 3.01a	18.25 ± 3.43a	17.17 ± 3.38a	ns	ns
	C-S	20.94 ± 14.38a	19.32 ± 8.90a	20.13 ± 10.91a		
Isoamyl acetate	T-V	328.53 ± 60.72a	369.13 ± 68.22a	348.83 ± 66.79a	3.18*	ns
	C-S	400.95 ± 69.95b	450.51 ± 78.60b	425.73 ± 77.40b		
Ethyl hexanoate	T-V	157.90 ± 31.27a	177.41 ± 35.13a	167.65 ± 34.18a	6.34*	ns
	C-S	189.43 ± 37.60b	212.84 ± 42.25b	201.13 ± 41.10b		
n-amyl alcohol	T-V	41.83 ± 11.08b	46.40 ± 13.52a	44.12 ± 12.11a	ns	ns
	C-S	33.38 ± 6.50a	35.92 ± 8.83a	34.65 ± 7.74a		
Hexyl acetate	T-V	9.34 ± 2.20a	9.60 ± 2.87a	9.47 ± 2.48a	ns	ns
	C-S	13.99 ± 4.54b	13.75 ± 5.32a	13.87 ± 4.87b		
Ethyl lactate	T-V	11030.80 ± 2738b	12394.16 ± 3076a	11712.48 ± 2947a	5.11*	ns
	C-S	9445.61 ± 1413a	10613.05 ± 1588a1	10029.33 ± 1593a		
Cis 3-hexenol	T-V	11.52 ± 3.71a	15.01 ± 6.26a	13.27 ± 5.28a	ns	ns
	C-S	9.97 ± 3.87a	10.66 ± 3.56a	10.31 ± 3.67a		
Ethyl octanoate	T-V	22.86 ± 6.28a	25.66 ± 6.04a	24.26 ± 6.13a	ns	ns
	C-S	35.91 ± 12.11b	39.64 ± 16.09a	37.78 ± 14.14a		
1.2 propylene glycol	T-V	123.04 ± 64.48a	131.52 ± 77.16a	127.28 ± 69.06a	ns	ns
	C-S	114.56 ± 34.75a	127.17 ± 37.87a	120.86 ± 36.32a		
Ethyl 3-hydroxybutyrate	T-V	52.36 ± 9.43a	59.82 ± 10.10a	56.09 ± 12.48a	5.97*	ns
	C-S	68.56 ± 14.71b	77.04 ± 16.53b	72.80 ± 15.99b		
Linalol	T-V	44.84 ± 11.42a	48.74 ± 14.24a	46.79 ± 12.85a	ns	ns
	C-S	48.42 ± 10.93a	54.40 ± 12.28a	51.41 ± 11.84a		
Ethyl decanoate	T-V	250.76 ± 46.42a	281.76 ± 52.15a	266.26 ± 51.05a	ns	ns
	C-S	326.66 ± 83.15b	367.04 ± 93.42b	346.85 ± 89.38b		
Diethyl succinate	T-V	1021.79 ± 178a	1148.08 ± 200a	1084.93 ± 197a	4.87*	ns
	C-S	1479.62 ± 300b	1782.09 ± 441b	1630.85 ± 402b		
2 phenyl acetate	T-V	27.00 ± 10.52a	13.06 ± 12.25a	19.65 ± 11.40a	11.87***	ns
	C-S	34.33 ± 7.12a	18.16 ± 14.60a	26.25 ± 17.45a		
2 metoxyphenol	T-V	429.50 ± 250.37a	507.58 ± 242.67a	493.54 ± 208.81a	6.82*	ns
	C-S	461.63 ± 131.43a	518.69 ± 147.67a	490.16 ± 140.53a		
γ- octalactone	T-V	366.88 ± 154.63a	433.29 ± 185.49a	400.08 ± 168.90a	9.72**	ns
	C-S	517.46 ± 118.28b	615.57 ± 68.27b	566.51 ± 107.28a		
2 phenylethanol	T-V	332939.80 ± 5790b	37011.01 ± 6505b	34975.40 ± 6401b	3.11*	ns
	C-S	223453.34 ± 2968a	26352.07 ± 3335a	24902.71 ± 3437a		
Eugenol	T-V	121.76 ± 50.23a	136.81 ± 56.44a	129.29 ± 53.11a	ns	ns
	C-S	98.35 ± 14.90a	110.50 ± 16.74a	104.43 ± 16.77a		
Decanoic acid	T-V	58.22 ± 20.53b	73.52 ± 37.25b	65.62 ± 20.30b	5.98*	ns
	C-S	34.38 ± 11.98a	38.63 ± 13.46a	36.51 ± 12.72a		
Vanillin	T-V	42.95 ± 14.67a	48.26 ± 16.48a	45.60 ± 15.58a	ns	ns
	C-S	107.52 ± 96.26a	120.81 ± 99.39a	114.17 ± 91.38a		

For the data analysis across years, the statistical significance of the effects of year, and techniques by year interaction, are also indicated. In each column, different letters denote significant differences based on Duncan's test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). ns means do not significant difference exist between treatments.

T-V, traditional winemaking; C-S, cold soak prefermentative maceration.

The application of copigments together with prefermentative maceration performance resulted in wines with a higher concentration of polyphenols. Although it did not enhance wine colour, it contributed to maintain colour longer, as indicated by the higher percentage of polymerised anthocyanins. In addition, the combination of applying green tea extract or catechin to vineyards, together with prefermentative maceration, increased the concentration of an important number of volatile compounds responsible for positive wine aromas.

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Author contributions

Juan Alberto Anaya: Investigation, Methodology, Formal analysis, Writing-Original Draft. **Inmaculada Álvarez:** Conceptualization, Visualization, Supervision. **María José García:** Methodology, Formal analysis. **Victoria Lizama**: Writing-Original Draft, Supervision, Validation.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical approval

This study does not contain any experiment with human participants or animals. Ethics approval was not required for this research.

Peer review

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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