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Impact of rutin and buckwheat (*Fagopyrum esculentum*) extract applications on the volatile and phenolic composition of wine

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Abstract

The aim of this research is to study the possibility of increasing the quality of red wins made with the Monastrell grape variety. A methodology was established to improve the concentration levels of polyphenol and aroma in these wines. Among flavonoids, the copigmenting effect of rutin stands out, and was tested in both winery and field applications. Buckwheat extract (*Fagopyrum esculentum*), in which rutin is the main flavonoid, may be of interest for viticulture given its biological activity.

This paper focuses on researching the effect of applying the prefermentative vegetable extract of buckwheat (*Fagopyrum esculentum*) on the concentration of polyphenols and aroma compounds in vineyards. Simultaneously, a study was carried out to compare the effect of pure copigment (rutin) when applied in vineyards and cellars. Traditional vinification was done, plus prefermentative cold maceration.

The application of buckwheat extract, and rutin extract to a lesser extent, to Monastrell grapes increased the concentration of malvidin and other anthocyanins, and total anthocyanins. After 12-month storage, no differences were observed in the percentage of copigmented, polymerised and free anthocyanin, the total polyphenol concentration, and the tannin quality parameters like DMACH (aldehyde p-dimethylaminoacimaldehyde) and the Gelatin Index. The concentrations of diethyl succinate, 2 phenylethyl acetate, vanillin and ethyl octanoate increased, while other compounds decreased when the copigment was added. The maceration technique followed during the vinification process had very little effect on polyphenolic compounds. The prefermentative maceration slightly increasing the concentration of total polyphenols, but had no effect on the parameters related to colour, anthocyanin concentration, nature of anthocyanins, their binding state or tannin quality parameters. The results showed that cold prefermentation maceration increased the concentration of some volatile compounds, including alcohols and esters, which should be considered important contributors to Monastrell wine aroma.

The combination of the applying buckwheat extract and pure rutin together, and prefermentative cold maceration, positively affects the polyphenolic concentration and increases the concentration of quality volatile compounds.

Keywords: buckwheat, rutin, copigmentation, prefermentation maceration, wine, polyphenols, volatile compounds.

1. Introduction

Phenolic composition is a determining factor for red wine organoleptic properties. The anthocyanins extracted from grape skin during maceration are the compounds that most strongly influence red wine color, and are also responsible for blue and purple tones (Mazza and Brouillard, 1990). It is generally accepted that an increase in the colour and phenolic structure of wines also implies their higher quality. The cultivation techniques applied in vineyards, grape variety, its degree of ripeness, and the followed vinification techniques all determine both concentration and composition in the polyphenols of wines and, thus, wine colour.

Red wine colour depends on the concentration of anthocyanins and their state. This state depends on several factors, one of which is the copigmentation phenomenon. Copigmentation is defined as the association between anthocyanins and other less coloured phenolic compounds, which results in a complex structure that increases wine's red color intensity. This effect is very important in young wines because it is responsible for 30-50% of their colour (Markovic et al., 2005 b; Heras-Roger et al., 2016)

Copigmentation reactions act on the colouration of anthocyanins via a hyperchromic effect and a bathochromic effect (Baranowski and Nagel, 1983; Brouillard et al., 1989; Bloor and Falshaw, 2000). A rise in the concentration of copigments intensifies colour, which is due to the less coloured forms of free anthocyanins that displace towards the coloured forms. In addition, the formed copigmented anthocyanins contribute to greater colour intensity than the flavilium cation.

Among the non-flavonoid copigments, hydroxycinnamic acids have the highest copigmentation potential. In this group, caffeic acid stands out as a copigmentation factor that plays an important role in red wine colour because it is naturally present in grapes (Darias-Martín et al., 2001, 2002; Schwarz et al., 2005; Álvarez et al., 2006, 2009). Flavonoid compounds constitute the most important group of polyphenols in grapes and wines. Of flavonoids, the copigmenting effect of rutin, tested in both model solutions and wines (Baranac et al., 1996; Hermosín et al., 2005 a; Álvarez et al., 2006, 2009), has marked impact on wine colour. Finally, the copigmenting effect of 3-flavanols should be highlighted (Boulton, 2000), which are very significant in (-) epicatechin (Liao et al., 1992).

To enhance the copigmentation effect, both the concentration of copigments and pigments in wines, and the copigment/pigment ratio, need to be high. To increase the effect, strategies can be implemented in viticulture (Álvarez et al., 2009; Aleixandre-Tudó et al., 2013) and in oenology. Many authors have studied the cofermentation of different grape types and the prefermentative addition of copigments (Mirabel et al., 1999; Rustioni et al., 2012; Gombau et al., 2016; Vallazo-Valleumbrocio et al., 2017; Zhang et al., 2018). Prefermentative copigment supplementation, combined with cold prefermentative maceration, has a synergistic effect on copigmentation processes and color stability, and it has been demonstrated that the concentration of anthocyanin pigments and their copigments can have as much influence on wine color as the applied winemaking techniques. These results have been found by Schwarz et al., (2005), Lizama et al., (2007) and Álvarez et al., (2009), who demonstrated that their joined effect was superior than when only prefermentative maceration was applied (Lizama et al., 2007; Parrado et al., 2007).

Winemaking techniques strongly influence the extraction of grape components by affecting the concentration and composition of red wines. Temperature, maceration duration, and the presence or absence of ethanol, are factors that affect these characteristics and copigmentation phenomena (Gómez-Míguez and Heredia, 2004). Prefermentative cold maceration allows greater and better polyphenolic extraction by influencing the increase in the concentration of anthocyanins, the ionisation index and their copigmentation. It affects, among others, colour stability by slowing down the fermentation process and disorganising skin cell membranes by facilitating the release of aromatic and phenolic compounds (Reynols et al, 2001; Gómez-Míguez and Heredia, 2004; Parenti et al., 2004; Alvarez et al., 2004, 2005).

Prefermentative cold maceration has been used to increase the concentration of phenolic compounds in must (Okubo et al., 2003; Zamora, 2004). This directly affects colour stability by also facilitating the formation of polymeric structures and the condensation of tannins which, in turn, confer wine structure and roundness (Reynols et al., 2001; Gómez-Miguez et al., 2006a; Álvarez et al., 2004 and 2005; González-Neves et al., 2015). Prefermentative maceration allows rapid extraction of anthocyanins and low-molecular tannins in the aqueous phase by allowing a reduction in extraction intensity during the fermentation process to, thus, minimise the risk of tannin extraction from seeds, especially with grapes with a lower degree of maturity (Gil-Muñoz *et al.*, 2009; González-Neves *et al.*, 2010).

The copigment supplementation effect could be more effective if they interact with grape components during the ripening process (Dimitric-Markovic et al (2003a); Schwarz el al, 2005). The application of copigments to vineyards by using plant extracts rich in certain copigments such us Rosemary extract rich in flavonoids and caffeic acid (Talcott el al., 2003; Brenes et al., 2005; Del Pozo-Insfran, 2006; Bimpilas et al., 2016); green tea extract rich in catequines (Alvarez et al., 2015), along with incorporating prefermentative maceration techniques that enhance copigmentation induced in the field (Lizama et al., 2007), could advance the anthocyanin maturity of grapes and lead to greater subsequent polyphenolic polymerisation, which would allow harvesting without having to wait for the usual overripening stages.

Buckwheat (*Fagopyrum esculentun)* can be used as a good source of dietary rutin (Ohsawa and Tsutumi, 1995; Kitabayashi et al., 1995a, b; Watanabe et al., 1997; Watanabe, 1998).

Buckwheat plants exhibit marked biological activity for being rich in flavonoids, phenolic acids, tannins, phytosterols and phagopyrins. Rutin is one of the many known flavonoids with substantial biological activity (ÇelíK et al. 2018).

The purpose of this work is to compare the effect of adding buckwheat extracts rich in flavonoids and rutine and the direct application of pure rutine to vineyards (in the grape clusters area) on prefermentation addition. The aim is to achieve a better polyphenolic and aromatic balance in Monastrell wines. The application of this technique can be a very useful tool for designing winemaking systems that guarantee crop sustainability by always taking quality improvement as a fundamental objective.

The application of this technique can act as a very useful tool for designing wine production systems that guarantee crop sustainability by always considering quality improvement as a fundamental objective. Spraying with natural plant extracts can also be most interesting for organic viticulture (Bulgari et al., 2015).

2. Material and Methods

2.1 Site description and experimental design

The study was carried out for two consecutive years (2016 and 2017) with the Monastrell variety that belongs to the "Valencian Denomination of Origin" (Fontanars, Spain).

The plant material was cv. Monastrell variety (syn. Mourvedre VICV-7915) vines grafted onto Richter-110 rootstocks, planted and rainfed in 2005 and spaced 1.5x3 m (2,200 vines/ha). Vines were trained on vertical trellises in a bilateral cordon system with an east-southern orientation. Soil has a sandy loam texture, and is highly calcareous and of low fertility.

In order to apply the buckwheat extract (rich in rutin) and pure rutin at the optimum time, polyphenolic grape ripening was monitored to determine grape harvests’ anthocyanin potential to allow effective copigmentation in grapes. Based on previous experience (Lizama et al., 2007; Alvarez et al., 2009), 10 days before the estimated harvest was taken as the optimum time for copigmentation reactions to occur. The buckwheat extract and rutin were applied to different plots, together with a non-ionic surfactant to promote adherence (Montana wax 20% at 20%, 2.5 mL/L) that favoured adherence to grape skins. The rutin concentration in the extract was determined. Rutin 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. This concentration follows the recommendations established by other authors (González et al., 2009; Bimpilas et al., 2016). The rutin concentration in the buckwheat extract (932 mg rutin/L) was determined to adjust the dilution of extracts to the 0.5g/L concentration of pure copigment so that when extracts were applied, the aforementioned results would be obtained. These products were previously dissolved in water until a concentration of about 90 mg of rutin per kg of grapes was reached. Applications were carried out by spraying in the grape clusters area. Products were applied using a hand-sprayer 30 days after veraison.

Rutin was purchased from SIGMA-ALDRICH Rutinhydrate, Minimum (R-5143). The buckwheat extract (Tr) was prepared in the laboratory of the Food Technology Department at the UPV (Polytechnic University of Valencia) by alcoholic extraction (ethanol) of buckwheat flour, supplied commercially by Laboratorios GUINAMA. Buckwheat was ground in a grinder. The obtained powder (250 g) was placed inside a 1 L Erlenmeyer flask with 500 mL of 9/1 ethanol/water mixture. It was left at room temperature for 24 h. Then it was filtered and the liquid fraction was placed inside a rotary evaporator at 50 ºC and 200 revolutions per minute to concentrate the extract until an approximate volume of 50 mL was obtained. The extract was left in a refrigerator until used.

The rutin concentration in the buckwheat extract was determined by HPLC to calculate the amount to be added to applications, which came close to the pure copigment concentration. The experimental design of the trials was the factorial type in randomised complete blocks with three replicates. The experiment utilised a randomised block design with two treatments (bukwheat extract and pure rutin) and three replications per treatment. Each replicate had 30 grapevines spread over five consecutive rows of seven plants each. Only the three inner rows were utilised for sampling, with the two outer rows used as borders and 120 each for those not receiving treatment.

The assay involved three experiments in the vineyard: 1) grapes without treatment; 2) treated grapes with buckwheat extract; 3) treated grapes with pure rutin.

2.2 Winemaking process

Ten days after applying copigments, grapes were harvested in 20-kilogram boxes. Grapes were processed in a paddle destemmer-roller crusher and paste was placed in 50-litre tanks. Prefermentative maceration was carried out at 5-6 ºC for 5 days, followed by traditional fermentation. A commercial *Saccharomyces cerevisiae yeast* (Enartis Ferm Red Fruit) was inoculated for fermentation (30 g/hL). The fermentation temperature was 27-28 ºC and two pump-overs were performed daily.

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.

Having completed malolactic fermentation, and wines were racked and homogenised after sulphite treatment at 30 mg/L of free sulphur. Twelve months later, the polyphenolic and aromatic wine composition was determined.

Twenty-four vinifications per year were carried out following eight protocols in triplicate: two experimental treatments in vineyards with buckwheat extract (90 mg/kg of grapes); one treatment with the prefermentative addition of copigments (rutine pure 90 mg/kg of grapes); one control without treatment. All the protocols were carried out with traditional maceration and cold prefermentative maceration.

2.3 Determination of technology and grape phenolic maturity

The following determinations were made: total acidity and pH following official methods (Commission Regulation (EEC), 1990); total soluble solids (TSS) (ºBrix) by refractometry; phenolic maturity of grapes according to Saint-Cricq de Gaulejac et al. (1998).

2.4 Phenolic parameters by spectophotometry

Wine phenolic composition was determined in a JASCO V-530 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 intensity, hue, IPT (Total poliphenols Index) and the Gelatin Index (astringency) were estimated by the methods described by Glories (1984). Condensed tannins were determined by the method developed by Ribéreau-Gayon (1979). The Folin-Ciocalteu assay was run according to Singleton and Rossi (1965). The method reported by Boulton (2001) was followed to analyse the contribution of the copigmented, free and polymeric anthocyanins to total wine colour. The DMACH Index (degree of tannin polymerisation) was calculated according to Kanha and Glories (1994).

2.5 Anthocyanins analysis by HPLC

The individual anthocyanins compounds were quantified by HPLC via the method of Boido et al. (2006). Total anthocyanins were calculated as the sum of glucoside anthocyanins and acylated anthocianins. After centrifugation and filtration, wine samples were injected directly into the HPLC (20 µL). Separation was carried out in a Gemini NX (Phenomenex, Torrance, CA, USA) 5 µm, 250 mm x 4.6 mm i.d. column at 40 ºC. 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, calibration curves were obtained with a commercially available standard: malvidin-3-glucoside (Sigma-Aldrich, St Louis, MO, USA). The content of anthocyanins was calculated on the basis of the calibration curves of authentic malvidin-3-glucoside ($y = 236316x - 166569$, $R^2 = 0.9994$)

2.6 Rutin buckwheat flour extract analyses by HPLC

Rutin (Sigma-Aldrich, St Louis, MO, USA) was determined by the modified method (Qin et al, 2010). The chromatographic analysis was carried out by a reversed phase HPLC-DAD MD-2010 Plus (JASCO, Tokyo, Japan), equipped with a Gemini-NX C18, 5 µm (250 X 4.6 mm) Phenomenex (Torrance, CA, USA). The mobile phase consisted of 0.1% TFA in deionised water (v⁄v) (solvent A) and acetonitrile (solvent B). The gradient programme was as follows: 0–28 min: 20-26% B; 28-44 min: 26-100% B; 44-52 min: 100% B; 52-56 min: 100-20% B; and 56-80 min: 20% B. Rutin content as calculated on the basis of the calibration curves of authentic rutin ($y = 45036x + 127808$, $R^2 = 0.99873$). The HPLC elutes were monitored by absorbance at 316 nm. The results were expressed as ppm in ethanol solution.

2.7 Volatile compounds extraction and identification

Volatile compounds were analysed by the procedure proposed by Ortega et al. (2001) with the slight modifications specified by Hernandez-Orte et a. (2014). A volume of 2.7 mL of the samples was transferred to a 10-mL screw-capped centrifuge tube that contained 4.05 g of ammonium sulphate (Panreac, Barcelona) to which the following compounds were added: 6.3 mL of milliQ (Panreac), 20 µL of a standard internal solution (2-octanol from Aldrich at 140 µg/mL in absolute ethanol from LiChrosolv-Merck) and 0.25 mL of dichloromethane (Li-Chrosolv-Merck). The tube was shaken mechanically for 120 min and to then be centrifuged at 2,900 g for 15 min. The dichloromethane phase was recovered with a 0.5-mL syringe, transferred to the autosampler phial and analysed. The chromatographic analysis was carried out in a HP-6890, equipped with a ZB-Wax plus column (60m×0.25mm x0.25 μm) from Phenomenex. The column temperature, initially set at 40°C and maintained at this temperature for 5 min, was then 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 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 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 (gas chromatography) peaks corresponding to identify substance by the interpolation of the retention time of normal alkane (C8 - 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. Semiquantitative data were obtained by calculating the relative peak área in relation to that of the internal standard (2 octanol).

2.8 Statistical analysis

A statistical analysis was performed with CENTURION XVI.II for Windows (Statgraphics Technologies, Inc., The Plains, VA, USA). A multifactorial ANOVA was carried out to determine interactions between treatments. The data corresponding to the control wine and the wines from the field treatments with the buckwheat extract and rutin were processed by a simple ANOVA to evaluate whether the copigment application influenced the phenolic and aromatic wine composition. The data corresponding to the wines made by traditional maceration, and those by prefermentative maceration followed by traditional vinification, were processed by a simple ANOVA to establish whether prefermentative cold maceration would modify phenolic and aromatic wine composition. The Duncan test was used to separate means (p-value <0.01) when the ANOVA test was significant.

3. Results and Discussion

To jointly process the data of the wines supplemented with the buckwheat extract, pure rutin in the field and pure rutin in the winery, the existence of interactions between the results obtained from treatments with copigments and the applied vinification techniques (traditional or prefermentative maceration) was initially tested. Table 1 shows the multifactorial analysis of variance (MANOVA) results for the factors copigment addition and winemaking technique, and the results for their interaction, for 2016 and 2017, in the polyphenolic and volatile compounds of the wines analysed 12 months after bottling.

The results showed that the polyphenolic and volatile compounds in wines were generally affected applyings in the vineyards and also by the followed vinification technique. However, there was only a slight interaction between these variables (interaction was year-dependent and a few compounds showed this interaction for the two study years), which allowed data to be jointly processed according to the applied copigment or the followed vinification technique.

3.1. Technology and grape phenolic maturity

In 2016, the technological and polyphenolic maturity of grapes that were allocated to the different tanks did not present significant differences in their technological maturity (Brix degree between 23.8-24.34; pH between 3.43-3.54; total acidity between 5.78-5.91 g/L expressed as tartaric acid), nor in grape phenolic maturity (color intensity between 10.45-11.37; anthocyanin concentration between 287-304 mg/L; polyphenol concentration between 2.21-2.35 g/L). A similar situation was observed in the 2017 grapes (Brix degree between 24.41-24.86; pH between 3.55-3.64; total acidity between 5.21-5.39 g/L expressed as tartaric acid; color intensity between 11.23-11.41; anthocyanin concentration between 311-325 mg/L; polyphenol concentration between 2.23-2.36 g/L). This showed that the co-pigmentation treatments did not affect technological grape maturity because the small differences were random and attributable to the minor variability between vineyard plots. It is true that differences were observed between the two study years because 2017 was warmer and, therefore, grape maturity was greater.

3.2. Effect of copigments on the polyphenolic and aromatic composition of Monastrell wines

Table 1 shows that the polyphenolic compounds were affected by the application of copigments, and also by the followed vinification techniques to a lesser extent, in the two study years. The copigments applitations significantly affected the polyphenolic parameters related to the concentration of anthocyanins and their different fractions and were, therefore, those that had a more marked effect on grapes after the spraying of copigments, which falls in line with other researchers (Boulton, 2001; Karna et al., 2005). The parameters related to tannin concentration and tannin quality were less affected by the treatments with the different copigments and winemaking techniques. Applying copigments affected the concentration of some volatile compounds, while the vinification technique affected mainly the 2017 vintage, when grapes matured more.

The addition of copigments affected half the volatile compounds in the two study years: α-pinen, ethyl isovalerate, isoamyl acetate, ethyl hexanoate, hexyl acetate ethyl octanoate, ethyl decanoate, diethyl succinate, 2 phenylethyl acetate, 2 methoxyphenol, decanoic acid, vanillin. Most of these compounds are esters that strongly influence the wine organoleptic profile because they are the main markers of the fermentative aroma of young wines.

There were only a few interactions between copigments and the maceration techniques, which enabled the data to be processed according to copigment or winemaking technique.

Table 2 shows the means and standard deviations, together with the ANOVA, for the polyphenolic compounds studied at 12-month storage depending on the copigments treatments, and copigments by the year interaction.

The 2017 vintage wines supplemented with buckwheat extract and rutin contained a higher concentration of polyphenolic compounds (malvidin and total anthocyanins), which could be attributed to better ripening caused by the vintage effect. No significant colour differences were observed in the 2016 or 2017 wines, although hue in 2016 was slightly higher in the wines from the copigment treatments. In the 2017 vintage, only the wines treated with rutin in the field had a higher hue than the controls. The studies by Gonzalez et al. (2010) have revealed that the field applitation of rutine confers finished wines a higher hue.

After 12-month storage, the wines from the grapes treated with buckwheat extract and rutin in the 2016 vintage contained higher concentrations of malvidin, peonidin, petunidin, delphinidin and total anthocyanins compared to the control wines. The fractions of the detailed anthocyanins were clearly lower in the control wine from the 2016 vintage *versus* the treated wines. Of all these, malvidin had the most abundant, especially in the wines pretreated with buckwheat extract and rutin in both the field and the cellar. According to a study by Baranac et al. in 1996, rutin has a high copigmentation affinity with malvidin, which would explain why the concentration of the copigmented anthocyanins was lower in the control wine than in the other treatments with rutin added in either the vineyard or before processing.

In the 2017 wines, compared to the control, a significant increase was observed for the total content of anthocyanins when the buckwheat extract was used, but to a lesser extent in relation to the addition of pure rutin in either the vineyard or cellar. None of the individual anthocyanin forms significantly increased when the extract was employed. Addition of pure rutin in the winery significantly increased the malvidin concentration in wines.

In 2016 vinthage the concentration of the condensed tannins was lower in the wines from grapes treated with the buckwheat extract than in controls. The 2017 vintage wines reated with the buckwheat extract had a lower proportion of condensed tannins, but the difference was not significant.

The fact that adding buckwheat increased the concentration of anthocyanins, and lowered that of tannins, could be partly due to the presence of small amounts of ethanol in the extract, which would stimulate ethylene formation in plants and could contribute to increased anthocyanin synthesis (Chervin et al., 2001; Gallegos et al., 2006, González et al., 2009). Ethylene is responsible for the accumulation of anthocyanins in grapes during ripening (Chervin et al., 2006; Muñoz-Robredo et al., 2013), but the observed increase in anthocyanin concentration can also be attributed to anthocyanin stability caused by the rapid polymerisation and copigmentation of its anthocyanins in the presence of rutin. This is due to the rapid polymerisation of the anthocyanins after malolactic fermentation (data not shown) which contributed to the stability of the anthocyanins during storage. Over time the anthocyanins of all treatments have polymerised so that there is no difference after 12 months, but the rapid polymerisation has contributed to the stability of the anthocyanins over time. This drop in condensed tannins can also be attributed to the presence of small amounts of etanol.

After 12-month storage, no differences among experimental treatments were observed in the percentage of the copigmented, polymerised and free anthocyanins, the total concentration of polyphenols or the tannin quality parameters. These results do not agree with the studies conducted by Gonzalez et al. (2010), who have shown that rutine spraying on bunches at the end of ripening can increase polyphenol and anthocyanin contents in grapes and wines, which improves colour intensity and stability. According to these authors vineyard treatments could at least be as interesting as the prefermentative treatment on must.

The means of the concentration of 22 studied volatile compounds in wine are shown in Table 3. The values was quite homogeneousin the two studied vintages as the ANOVA indicated.

The volatile compounds of the wines from treatmensts with buckwheat and rutin were differentiated according to their behaviour.

Table 3 indicates that the concentrations of β-pinen, n-amyl alcohol, ethyl lactate, 1,2-propylene glycol, 2-phenylethanol, ƴ-octolactone and eugenol, with no significant differences among experimental treatments in of the two studied vintages. The concentration of cis 3-hexenol, ethyl octanoate, linalol and decanoic acid only showed significant differences in one of the two vintages.

Some compounds came at higher concentrations in the wines from the grapes treated with the buckwheat extract and rutin, such as diethyl succinate, 2 phenyl ethyl acetate, vanillin and ethyl octanoate. These compounds are related to wine quality. This effect is important in organoleptic terms because esters are related to fruity and floral aromas, vanillin to vanilla aroma, and they are all positive for wine aromatic quality (Belda, 2017). Several studies (Garcia-Ruiz et al. 2013; D'Onofrio et al., 2018; Vitalini et al., 2014) have shown that the application of plant extracts and elicitors in vineyards increases higher alcohols and esters in wines. Darici et al. (2020) found a significant increase in the concentration of esters in Cabernet sauvignon wines that they treated with rosemary extract. Moreover, the application of an aminopolysaccharide like chitosanto vineyards increases the levels of acetals and total alcohols in wines, while the application of benzothiadizole confers more acetals and total esters (Vitalini, 2014).

This study indicates a clearly significant effect of applying the buckwheat extract, lowering the concentration of α-pinene, ethyl isovalerate, isoamyl acetate, ethyl hexanoate and ethyl decanoate in wines. Likewise, the addition of rutin in the field or winery allowed wines to be obtained with lower concentrations of hexyl acetate, ethyl 3-hydroxybutyrate and 2 methoxyphenol. These compounds are extremely important in the aromatic wine profile because they confer floral and fruity aromas (Englezos et al., 2016).

Flavonoids, phenolic compounds and their derivatives, which are naturally found in the structure of these extracts, have been demonstrated as being effective in preventing the auto-oxidation of volatile compounds (Garcia-Ruiz et al. 2013; Yıldırım et al., 2005). A biostimulating effect of the formation of volatile compounds on grapes has also been observed when eupcalyptus extract, almond skin extract, benzothiadiazole, methyl jasmonate and chitosan, were applied to vineyards, and wines were obtained with a higher concentration of terpenes, acetals and esters (Garcia-Ruiz et al. 2013; D'Onofrio et al., 2018; Vitalini et al., 2014). These studies have shown that the application of plant extracts and elicitors in vineyards leads to increased higher alcohols and esters in wines and, although these compounds originate mainly from the fermentation process, the substrates in grapes for the formation of these compounds can be affected by the treatment applied to grapes and, thus, affect their final concentrations in wines.

Studies by Chervin et al (2001, 2002) demonstrate that spraying ethanol solution on bunches of grapes stimulates ethylene production in plants. The buckwheat extracts employed in that study could have contained small quantities of ethanol, which could have caused this effect and affected the concentration of some volatile compounds.

Studies by Gonzalez et al., 2009, report that the application of hydroalcoholic ethanol solutions to bunches increases the skin/pulp ratio by more than 15% compared to the controls. Different studies (Segade et al., 2016; Giacosa et al., 2019) , demonstrate that increased grape skin thickness is related to a higher concentration of the compounds found in grape skin, such as tannins, anthocyanins and precursors of aromas, as well as greater extractability of volatile compounds.

3.3 Effect of winemaking techniques on polyphenolic and aromatic wine composition

Table 1 shows that phenolic compounds were not significantly affected by the winemaking technique followed in the two study years, although the total polyphenol index was affected. A significant effect appeared in both vintages for wine aromatic composition.

In the two studied vintages, prefermentative cold maceration significantly affected the concentrations of n-amylalcohol, cis-3 hexenol, ethyl octanoate, ethyl 3- hydroxybutyrate, diethyl succinate, 2-phenyl ethyl acetate, and decanoic acid, compounds that affect the aromatic profile of wines.

Only a few interactions take place between copigments and the maceration techniques, which enabled data to be processed according to copigment or the winemaking technique.

Table 4 shows the means and standard deviations, together with the ANOVA, of the polyphenolic compounds studied in the wines treated with buckwheat extract and rutin once the storage period had finished, and in accordance with the applied vinification technique.

No significant differences were observed for most of the concentrations of polyphenolic compounds in the wines from the grapes treated with buckwheat extract and rutin, except for the concentration of total polyphenols and Folin Index in the wines made with prefermentative maceration. In these wines, condensed tannins also had a higher value, but the difference with traditionally made wines was not significant. This difference would not be attributable a greater extraction, but only to a greater polyphenolic stability caused by prefermentative maceration.

In view of the results obtained in the wines from the buckwheat extract and rutin treatments, we can state that the maceration technique used in winemaking barely affected polyphenolic compounds. The prefermentative maceration slightly increased the concentration of condensed tannins and total polyphenols, but did not affect the parameters related to colour, anthocyanin concentration, nature of anthocyanins, their pigment polymerization, or the quality parameters of tannins. The main advantage of prefermentative maceration over the traditional winemaking technique is its greater capacity to extract anthocyanins and to facilitate copigmentation reactions (Vazquez et al. (2010).

Many studies have been carried out about the application of the prefermentative maceration technique to winemaking and its effect on phenolic compounds. Several authors have reported increases of intensity and colour stability in wines made with prefermentative maceration, such as Álvarez et al. (2006) who used Monastrell grapes and Gómez-Míguez et al. (2007) who worked with Syrah grapes. Other studies indicate negative or diverse effects when applying this technique, such as Budic-Leto et al. (2003) in the winemaking of Babic grapes and González-Neves et al. (2009) in Tannat. The colour intensity and quality of Tannat wines obtained with prefermentative maceration had less than those made by traditional maceration, but have higher concentration of tannins and total phenols

(Favre et al., 2013). Variety grapes, ripeness and winemaking techniques may be responsible for the different effects of prefermentative maceration on phenolic wine composition.
The maceration technique followed in the vinification of the wines treated with the buckwheat extract and pure rutin significantly affected the concentrations of 12 studied volatile compounds (Table 5). The results showed that traditional vinification increased the concentrations of β-pinen, n-amylalcohol, 2 phenylethanol and decanoic acid.

Esters are a very important group of compounds for wine aroma,. They are generated by yeasts during alcoholic fermentation and are related to fruity notes (Etievant 1989). The results showed that prefermentation cold maceration increased the concentration of some esters, such as hexyl acetate (apple, pear), ethyl octanoate (pineapple, pear, floral), ethyl 3-hydroxybutyrate and diethyl succinate (caramel), which should be considered important contributors to Monastrell wine aroma (Alvarez et al. 2006; Cai et al., 2014; Aleixandre Tudó et al., 2016).

Alvarez et al. (2006), Moreno et al., (2013) and Aleixandre-Tudó et al., (2016) studied the effect of cold prefermentative maceration on volatile wine composition under different conditions. They all generally describe improvements in the aromatic composition, a higher ester concentration, and enhanced fruity, floral and caramel aromas.

Mihnea et al. (2015) observed a higher concentration of some alcohols in the wines obtained by cold prefermentative maceration. González-Neves et al. (2015) reported a similar observation and posed the possibility of this effect resulting from the action of non-Saccharomyces yeasts during the cooling period.
These results agree with those obtained by Alvarez et al. 2005, Selli et al. 2006 and De Santis and Frangipane, 2010. These researchers attributed the higher aromatic concentration of pre-fermentatively macerated wines to the extractive effect of this technique on skin components.
These results could also be explained by not only the cold maceration technique allowing the development of cryophilic yeasts, but also their influence on the release of certain aromas, especially volatile esters, which would be one of the advantages of this technique, as cited by other authors (Charpentier and Feuillat, 1998, Casassa and Sari 2015; Cai et al. 2014). Other studies have also stated that. At this low temperature, fermentation by non-Saccharomyces autochthonous yeasts, possibly of the genus Hanseniapora, can start the fermentation and generate varietal aromas, while herbaceous notes diminish (Cai et al., 2014; Gonzalez Neves et al. 2015). However, the present study noted a higher cis-3-hexenol concentration.

4. Conclusions

The application of buckwheat extract and pure rutin to a lesser extent, to Monastrell grapes increases the concentrations of malvidin and other studied anthocyanins, as well as that of total anthocyanins. However, the concentration of condensated tannins is lower in the wines from grapes treated with the buckwheat extrac than in the control winest. The application of these products does not modify the concentration of the percentage of the copigmented, polymerised and free anthocyanins, the total concentration of polyphenols, or the quality parameters of tannins.

There is no clear effect of adding copigments on the volatiles composition of wine becaus, as far as the quality-related compounds are concerned, their concentration increases in some cases, but lowers in others.

The maceration technique used in winemaking barely influenced the polyphenolic compounds, with prefermentative maceration, slightly increasing the concentration of condensed tannins and total polyphenols, but does not affect the parameters related to colour, anthocyanin concentration, nature of anthocyanins, their pigments polymerisation or tannin quality parameters. The results reveal that prefermentation cold maceration increases the concentrations of some esters, and other compounds, which should be considered important contributors to wine aroma.

Considering the results obtained in the two studied vintages, the combination of applying buckwheat extract or pure rutin, together with the prefermentative cold maceration, positively affects the polyphenolic concentration and increases the concentration of quality volatile compounds.

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Table 1. Multifactorial variance analysis for the applied copigments, the vinification technique and their interaction for the polyphenolic and volatile compounds of Monastrell wines in 2016 and 2017.

Compound	Copigments		Winemaking Techniques		Interaction Copig x Techniques	
	2016	2017	2016	2017	2016	2017
Colour Intensity	3.59*	ns	ns	ns	10.71*	ns
Hue (%)	ns	4.20*	13.01***	ns	ns	3.55*
Copigmented anthocyanins (%)	ns	9.62**	ns	ns	ns	ns
Polymerised anthocyanins (%)	ns	11.14**	ns	ns	5.30*	ns
Free anthocyanins (%)	ns	ns	ns	ns	ns	ns
Malvidin (mg/L)	9.88**	3.78*	ns	ns	4.90*	26.09**
Peonidin (mg/L)	5.10*	ns	ns	ns	ns	ns
Petunidine (mg/L)	6.29*	ns	ns	10.36**	ns	4.92*
Cyanidin (mg/L)	ns	5.64*	ns	ns	ns	9.08**
Delphinidin (mg/L)	3.97*	4.22*	ns	6.23*	ns	ns
Total anthocyanins (mg/L)	5.95*	7.77**	ns	ns	5.87*	ns
Condensed tannins (g/L)	11.53***	ns	ns	ns	ns	ns
Total polyphenols (g/L)	25.07***	ns	11.16**	4.21*	7.36*	6.22*
Folín Index	ns	ns	7.63**	ns	ns	ns
DMACH Index (%)	ns	5.62*	ns	ns	ns	ns
Gelatin Index (%)	ns	ns	ns	ns	ns	ns
α-pinen	8.69**	11.06**	ns	13.00**	3.15*	ns
β-pinen	ns	ns	8.97**	ns	9.70*	12.56**
Ethyl isovalerate	4.88*	4.48*	ns	ns	ns	ns
Isoamyl acetate	6.41**	8.07**	ns	ns	ns	ns
Ethyl hexanoate	4.17*	5.17*	ns	ns	ns	ns
n-Amylalcohol	ns	ns	6.41**	10.69**	14.2**	4.69*
Hexyl acetate	14.21***	22.40**	7.03**	ns	6.32*	ns
Ethyl lactate	ns	ns	ns	ns	ns	ns
Cis 3-hexenol	7.49**	ns	14.93***	16.45***	ns	ns
Ethyl octanoate	8.72**	5.46*	4.33*	19.31***	ns	ns
1,2 Propylene glycol	ns	ns	7.87**	ns	5.69*	ns
Ethyl 3-hydroxybutyrate	ns	3.18*	16.56***	15.87***	7.66**	ns
Linalol	ns	3.47*	ns	4.53*	ns	11.26**
Ethyl decanoate	3.75*	4.29*	ns	ns	ns	ns
Diethyl succinate	3.19*	4.21*	16.00***	15.00***	ns	ns
2 Phenyl ethyl acetate	24.35***	24.35***	4.92*	4.92*	ns	6.54*
2 Methoxyphenol	8.85**	8.88*	ns	6.00*	ns	ns
γ- Octolactone	ns	ns	ns	ns	ns	ns
2 Phenylethanol	ns	ns	6.21*	ns	ns	2.64***
Eugenol	ns	ns	ns	ns	5.62*	ns
Decanoic acid	3.21*	ns	29.76***	20.99***	ns	ns
Vanillin	7.52**	5.18*	ns	ns	12.87*	3.45*

In each row. different letters 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 with the average for 2016 and 2017.

Parameters	**Copigment**	**2016**	**2017**	**Average 2016-2017**	**Year (p-value)**	**Copig. x Year (p) interaction**
Colour Intensity	Control	9.83±1.01a	11.63±0.90a	10.73±1.29a	26.85***	3.28*
	Buckwheat extract	10.79±0.16a	11.94±0.87a	10.82±1.03a		
	Rutin vineyard	10.42±1.19a	11.84±0.97a	10.42±1.48a		
	Rutin winery	11.10±1.82a	11.75±0.61a	11.12±1.12a		
Hue (%)	Control	75.68±3.95a	68.70±2.57a	72.19±4.85a	10.37**	ns
	Buckwheat extrac	78.47±1.09a	68.80±1.57a	72.02±4.95a		
	Rutin vineyard	76.50±5.35a	69.95±0.77a	73.23±5.01a		
	Rutin winery	74.91±1.08a	65.79±5.79a	70.35±5.42a		
Copigmented anthocyanins (%)	Control	9.87±1.25a	16.55±1.63a	13.21±3.72a	26.54***	ns
	Buckwheat extrac	10.30±0.36a	18.72±3.77a	14.51±6.38a		
	Rutin vineyard	11.28±2.60a	14.42±9.87a	12.85±6.74a		
	Rutin winery	11.68±1.99a	15.15±10.04a	13.41±9.16a		
Polymerised anthocyanins (%)	Control	50.47±3.27a	47.64±1.95a	49.05±4.12a	6.89*	ns
	Buckwheat extrac	49.12±0.49a	51.92±6.92a	49.66±3.86a		
	Rutin vineyard	48.72±4.12a	49.69±7.34a	49.21±4.06a		
	Rutin winery	49.46±4.14a	45.14±6.90a	47.30±3.68a		
Free anthocyanins (%)	Control	39.66±3.41a	35.82±6.04a	37.74±5.02a	ns	ns
	Buckwheat extrac	40.58±0.54a	38.70±3.66a	39.32±3.08a		
	Rutin vineyard	40.00±3.98a	39.93±3.11a	39.96±3.45a		
	Rutin winery	38.86±4.29a	39.68±1.91a	39.27±3.24a		
Malvidin (mg/L)	Control	34.99±7.35a	49.13±2.24a	42.06±15.45a	4.25*	8.02*
	Buckwheat extrac	55.78±5.24c	49.00±20.00a	49.26±16.89a		
	Rutin vineyard	47.77±12.88bc	54.67±14.14ab	51.22±13.55a		
	Rutin winery	42.33±10.34b	56.04±6.72b	49.18±8.47a		
Peonidin (mg/L)	Control	2.53±0.56a	3.18±0.68a	2.85±1.01a	ns	ns
	Buckwheat extrac	3.83±0.48b	2.65±1.08a	3.05±1.07a		
	Rutin vineyard	3.53±0.69b	3.30±0.60a	3.41±0.64a		
	Rutin winery	3.58±1.09b	2.88±0.76a	3.23±0.98a		
Petunidine (mg/L)	Control	4.54±1.24a	4.38±0.56a	4.46±1.31a	ns	ns
	Buckwheat extrac	6.98±0.82b	4.79±2.11a	5.52±2.04a		
	Rutin vineyard	6.02±1.53b	5.01±2.39a	5.52±2.01a		
	Rutin winery	6.10±1.66b	4.58±1.20a	5.34±1.60a		
Cyanidin (mg/L)	Control	2.04±0.33a	2.07±0.30b	2.05±0.38a	6.47*	ns
	Buckwheat extrac	2.67±0.30a	1.86±0.47a	2.13±0.57a		
	Rutin vineyard	2.55±0.40a	1.91±0.63a	2.23±0.61a		
	Rutin winery	2.57±0.89a	2.14±0.55ab	2.36±0.75a		
Delphinidin (mg/L)	Control	3.74±0.84a	4.87±0.78b	4.30±0.95a	ns	ns
	Buckwheat extrac	5.15±1.10b	3.52±1.48a	4.06±1.54a		
	Rutin vineyard	5.09±1.21b	4.69±0.99b	4.89±1.09a		
	Rutin winery	5.19±1.69b	3.72±1.10ab	4.45±1.57a		
Total anthocyanins (mg/L)	Control	223.59±17.39a	303.21±53.02a	263.40±54.34a	85.21***	3.02*
	Buckwheat extrac	245.15±15.37b	341.01±30.34b	307.39±49.37b		
	Rutin vineyard	234.48±17.05ab	359.23±61.43bc	296.86±77.76ab		
	Rutin winery	247.20±25.90b	368.73±27.00c	307.96±77.43b		
	Control	2,01±0,08b	1,98±0,07a	2,00±0,11a		

Condensed tannins (g/L)	Buckwheat extrac	1.86±0.03a	1.86±0.05a	1.86±0.04a	28.52***	ns
	Rutin vineyard	1.99±0.09b	1.97±0.08a	1.98±0.08a		
	Rutin winery	2.16±0.05c	1.91±0.13a	2.03±0.16a		
Folin Index	Control	2.06±0.92a	2.21±0.68a	2.14±0.86a	14.33**	ns
	Buckwheat extrac	2.09±0.35a	2.71±0.24a	2.51±0.40a		
	Rutin vineyard	2.00±0.44a	2.68±0.50a	2.34±0.75a		
	Rutin winery	2.04±0.18a	2.54±0.62a	2.29±0.52a		
DMACH Index (%)	Control	54.38±7.92a	48.43±1.95a	51.40±6.18a	15.65***	ns
	Buckwheat extrac	55.44±7.00a	52.09±6.92a	53.21±6.82a		
	Rutin vineyard	57.54±5.09a	51.91±7.34a	54.72±6.76a		
	Rutin winery	60.22±4.46a	50.85±6.90a	55.53±7.41a		
Gelatin Index (%)	Control	47.71±17.06a	47.33±8.93a	47.52±13.77a	ns	ns
	Buckwheat extrac	47.53±4.69a	56.73±12.86a	53.67±11.48a		
	Rutin vineyard	54.19±11.13a	49.75±6.87a	51.97±11.00a		
	Rutin winery	54.81±8.89a	52.28±13.03a	53.54±10.85a		

For the data analysis across years the statistical significance of the effects of year, and the copigments x year interaction, are also indicated. In each column. different letters denote significant differences based on Duncan’s test (* $p < 0.05$; ** $p < 0.01$. *** $p < .001$)

Table 3. Means, standard deviations and variance analyses of the volatile compounds of Monastrell wines depending on the applied copigments during each season with the average for 2016 and 2017.

Compounds (µg/L)	Copigments	2016	2017	Average 2016-2017	Year p-value	Copig. x Year (p) interaction
α-pinen	Control	41.37±6.27b	46.48±7.05c	43.93±6.97c	ns	ns
	Buckwheat extrac	23.37±5.57a	24.54±5.61a	23.95±5.43a		
	Rutin vineyard	34.54±8.16b	36.74±8.68b	35.64±8.22b		
	Rutin winery	34.79±8.28b	37.01±8.81b	35.90±8.34b		
β-pinen	Control	14.37±1.24a	16.15±1.40a	15.26±1.57a	ns	ns
	Buckwheat extrac	13.32±0.61a	14.17±0.65a	13.74±0.75a		
	Rutin vineyard	17.09±7.97a	18.18±8.48a	17.63±7.97a		
	Rutin winery	15.12±7.93a	16.08±8.44a	15.60±7.92a		
Ethyl isovalerate	Control	17.35±3.41ab	20.90±6.71ab	19.12±5.46b	ns	ns
	Buckwheat extrac	11.16±0.80a	11.68±1.22a	11.42±1.03a		
	Rutin vineyard	29.02±17.47b	30.87±18.59b	29.94±17.45c		
	Rutin winery	18.95±6.48ab	20.18±6.93ab	19.56±6.51b		
Isoamyl acetate	Control	442.84±71.14b	497.57±79.93c	470.21±78.37c	ns	ns
	Buckwheat extrac	269.24±67.16a	286.43±71.44a	277.83±67.57a		
	Rutin vineyard	376.42±63.56b	400.45±67.62b	388.43±64.00b		
	Rutin winery	365.17±109.21b	388.48±116.26b	376.82±109.66		
Ethyl hexanoate	Control	191.84±17.88b	215.55±20.09b	203.70±22.08b	ns	ns
	Buckwheat extrac	131.73±10.03a	140.14±10.67a	135.93±10.90a		
	Rutin vineyard	181.52±44.02b	193.11±46.83b	187.32±44.31b		
	Rutin winery	179.34±56.20b	190.79±59.78b	185.07±56.36b		
n-Amylalcohol	Control	43.52±7.33a	48.90±8.24a	46.21±8.03b	ns	ns
	Buckwheat extrac	39.14±6.18a	37.32±3.04a	38.23±5.69a		
	Rutin vineyard	40.17±14.26a	42.74±15.17a	41.45±14.29a		
	Rutin winery	35.42±9.66a	39.01±15.44a	37.21±12.58a		
Hexyl acetate	Control	9.36±3.08ab	11.15±2.50b	10.26±2.86b	6.34*	ns
	Buckwheat extrac	12.40±4.50b	15.40±2.74c	13.90±3.92c		
	Rutin vineyard	7.48±0.88a	7.83±1.21a	7.65±1.04a		
	Rutin winery	17.08±3.00c	19.79±4.82d	18.43±4.12d		
Ethyl lactate	Control	9710.64±1630a	10910.83±1832a	10310.74±1786b	ns	ns
	Buckwheat extrac	8457.77±1951a	8997.63±2075a	8727.70±1965a		
	Rutin vineyard	8931.41±1859a	9501.50±1978a	9216.45±1878a		
	Rutin winery	7824.32±2173a	8323.75±2311a	8074.04±2182a		
Cis 3-hexenol	Control	10.84±2.34a	12.24±2.77a	11.54±2.58a	ns	ns
	Buckwheat extrac	10.09±2.47a	9.79±3.18a	9.94±2.75a		
	Rutin vineyard	16.52±4.57b	16.16±6.32a	16.34±5.33b		
	Rutin winery	16.04±4.02b	12.25±6.00a	14.14±5.31b		
Ethyl octanoate	Control	23.75±4.95a	23.12±8.85a	23.43±6.93a	ns	ns
	Buckwheat extrac	31.67±10.07b	31.60±12.84b	31.63±11.15a		
	Rutin vineyard	33.08±20.52b	33.29±22.96b	33.19±21.04a		
	Rutin winery	27.18±19.27b	30.85±19.35b	29.01±18.75a		
1,2 Propylene glycol	Control	161.32±70.89a	163.84±99.34a	162.58±83.38a	ns	ns
	Buckwheat extrac	129.39±34.69a	158.22±82.06a	143.81±71.10a		
	Rutin vineyard	138.35±37.18a	147.18±39.55a	142.76±37.36a		
	Rutin winery	129.79±62.70a	138.07±66.70a	133.93±55.59a		
	Control	69.52±16.21b	78.11±18.21b	73.82±17.24b		

Ethyl 3-hydroxybutyrate	Buckwheat extrac	69.05±26.45b	73.46±28.14b	71.26±26.48b	ns	ns
	Rutin vineyard	50.29±8.80a	53.50±9.36a	51.90±8.94a		
	Rutin winery	55.96±11.50^{a}a	59.53±12.24ab	57.74±11.62a		
Linalol	Control	48.94±7.87a	54.99±8.84b	51.97±8.67b	ns	ns
	Buckwheat extrac	53.13±27.78a	53.90±25.68b	53.51±25.84ab		
	Rutin vineyard	39.01±6.71a	36.75±11.02a	37.88±8.89a		
	Rutin winery	41.77±6.21a	44.43±6.61ab	43.10±6.35b		
Ethyl decanoate	Control	291.37±68.23b	327.38±76.67b	309.38±72.53b	ns	ns
	Buckwheat extrac	190.03±59.42a	202.16±63.21a	196.09±59.60a		
	Rutin vineyard	297.60±78.29b	316.60±83.29b	307.10±78.70b		
	Rutin winery	254.11±80.68ab	270.94±85.83ab	262.82±80.31b		
Diethyl succinate	Control	1074.88±182^{a}	1207.73±205a	1141.31±199.7	ns	ns
	Buckwheat extrac	1449.86±438b	1755.17±702b	1602.52±587.4		
	Rutin vineyard	1136.09±284ab	1208.61±302a	1172.35±286.1		
	Rutin winery	1362.94±324ab	1449.94±345ab	1406.44±327.23a		
2 Phenyl ethyl acetate	Control	18.03±9.02a	19.18±9.59a	18.60±9.01a	ns	ns
	Buckwheat extrac	25.99±11.26ab	27.65±11.98b	26.82±11.27b		
	Rutin vineyard	27.55±5.47b	29.31±5.81b	28.43±5.53b		
	Rutin winery	49.89±2.73bc	53.07±2.90c	51.48±3.18c		
2 Metoxyphenol	Control	525.48±201.87c	590.42±226c	557.95±210.13	ns	ns
	Buckwheat extrac	563.08±313.79c	599.02±333c	581.05±313.52		
	Rutin vineyard	102.43±11.16a	118.58±30.58a	111.04±11.37a		
	Rutin winery	311.33±159.21b	331.20±169b	321.27±159.13		
γ-Octolactone	Control	234.24±80.24a	291.28±252a	275.26±231.52	4.97*	ns
	Buckwheat extrac	402.46±252.06a	374.95±320a	394.96±187.16		
	Rutin vineyard	343.85±105.72a	365.80±112a	354.83±106.05		
	Rutin winery	318.02±153.30a	243.77±262a	417.96±150.46		
2 Phenylethanol	Control	28581.14±5246a	32113.64±5894a	30347.39±569	ns	ns
	Buckwheat extrac	26271.36±4237a	27948.25±4507a	27109.81±431		
	Rutin vineyard	26477.97±4464a	28168.06±4749a	27323.02±453		
	Rutin winery	27951.50±7656a	29735.64±8144a	28843.57±769		
Eugenol	Control	95.14±13.78a	106.90±15.49a	101.02±15.41a	ns	ns
	Buckwheat extrac	103.25±19.23a	109.85±20.45a	106.55±19.48a		
	Rutin vineyard	88.31±15.63a	87.57±16.63a	87.94±15.83a		
	Rutin winery	93.09±12.88a	99.04±13.70a	96.07±13.20a		
Decanoic acid	Control	48.28±26.65a	54.24±29.95a	51.26±27.56a	6.33*	ns
	Buckwheat extrac	72.31±58.17ab	76.93±61.88a	74.62±58.06a		
	Rutin vineyard	86.89±53.95ab	92.43±57.39a	89.66±53.88a		
	Rutin winery	100.09±51.35b	106.48±54.63a	103.28±51.33a		
Vanillin	Control	34.78±15.86a	38.29±17.42a	36.54±16.20a	ns	ns
	Buckwheat extrac	68.44±14.48c	70.30±13.10bc	69.37±13.37c		
	Rutin vineyard	47.28±14.71ab	53.20±33.41b	50.24±25.12b		
	Rutin winery	62.91±17.80bc	74.67±11.38c	68.79±15.65c		

For the data analysis across years, the statistical significance of the effects of year, and the copigments x year interaction, are also indicated. In each column. different letters denote significant differences based on Duncan's test (* $p < 0.05$; ** $p < 0.01$. ***

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

Compounds	Winemaking techniques	2016	2017	Average 2016-2017	Year (p-value)	Technique x Year (p) interaction
Colour Intensity	T	9.89±1.67a	11.55±0.91a	10.72±1.57a	26.60***	ns
	MP	10.30±0.50a	11.22±0.72a	10.82±0.77a		
Hue (%)	T	78.28±2.95a	68.94±1.96a	73.61±5.35a	12.63***	ns
	MT	73.17±5.07a	67.68±3.40a	70.03±3.98a		
Copigmented anthocyanins (%)	T	10.97±1.45a	22.44±5.14a	16.70±6.91a	73.56***	ns
	MP	10.70±2.52a	22.77±9.40a	17.60±9.42a		
Polymerised anthocyanins (%)	T	48.77±3.32a	45.83±4.25a	47.30±4.04a	26.83***	ns
	MP	50.45±3.60a	44.36±2.36a	46.97±4.22a		
Free anthocyanins (%)	T	40.26±3.51a	38.19±5.44a	39.23±4.62a	ns	ns
	MP	38.85±3.47a	38.87±2.33a	38.86±2.82a		
Malvidin (mg/L)	T	46.51±11.66a	58.59±11.29a	52.55±12.85a	4.76*	ns
	MP	39.97±11.18a	47.33±14.33a	44.03±13.12a		
Peonidin (mg/L)	T	3.27±0.84a	3.39±0.68a	3.33±0.76a	ns	ns
	MP	3.35±0.99a	3.11±1.19a	3.21±1.09a		
Petunidine (mg/L)	T	5.82±1.63a	6.08±1.27b	5.95±1.45b	ns	6.13*
	MP	5.68±1.59a	4.30±1.82a	4.89±1.83a		
Cyanidin (mg/L)	T	2.38±0.60a	2.17±0.36a	2.28±0.50a	6.38*	ns
	MP	2.49±0.60a	2.01±0.67a	2.21±0.67a		
Delphinidin (mg/L)	T	4.73±1.41a	4.87±0.96a	4.80±1.19a	ns	ns
	MP	4.76±1.36a	3.93±1.09a	4.25±1.34a		
Total anthocyanins (mg/L)	T	237.36±17.76a	349.06±52.41a	293.21±68.57a	107.84***	ns
	MP	235.41±25.91a	342.03±56.85a	296.34±70.40a		
Condensed tannins (g/L)	T	1.96±0.41a	2.80±0.59a	2.38±0.69a	28.90***	ns
	MP	2.34±0.67a	2.82±0.57a	2.62±0.65a		
Total polyphenols (g/L)	T	1.97±0.12a	1.85±0.06a	1.91±0.11a	17.59***	ns
	MP	2.11±0.06b	1.96±0.09b	2.04±0.11b		
Folin Index	T	54.14±5.12a	52.23±6.95a	53.18±6.09a	17.44***	8.21**
	MP	61.05±5.51b	53.41±4.83a	57.25±7.73a		
DMACH Index	T	52.26±9.49a	44.70±14.22a	48.48±12.49a	ns	ns
	MP	50.64±15.01a	49.34±11.48a	49.90±12.86a		
Gelatin Index (%)	T	41.06±15.20a	40.09±19.16a	40.57±17.02a	ns	ns
	MP	47.47±22.57a	45.90±17.44a	46.57±19.42a		

For the data analysis across years, the statistical significance of the effects of year, and the techniques x year interaction, are also indicated. In each column. different letters denote significant differences based on Duncan's test (* $p< 0.05$; ** $p < 0.01$. *** $p < 0.001$).T: traditional. MP: prefermentation maceration.

Table 5. Means, standard deviations and variance analyses of the volatile compounds of Monastrell wines depending on winemaking technology applied during each season with the average for 2016 and 2017.

Compounds (µg/L)	Winemaking techniques	2016	2017	Average 2016-2017	Year p-value	Technique x Year (p)) interaction
α-pinen	T	33.91±10.67a	36.70±11.91a	35.30±11.21a	ns	ns
	MP	33.12±8.43a	35.69±9.81a	34.41±9.09a		
β-pinen	T	17.99±5.90b	19.34±6.12b	18.67±5.96b	ns	ns
	MT	11.95±3.11a	12.95±3.58a	12.45±3.34a		
Ethyl isovalerate	T	16.77±5.67a	18.05±6.33a	17.41±5.95a	ns	ns
	MP	21.46±14.55a	23.77±15.70a	22.93±15.01a		
Isoamyl acetate	T	329.55±76.50a	356.42±87.08a	342.98±81.78a	ns	ns
	MP	397.29±108.68a	430.04±123.5a4	413.66±115.66a		
Ethyl hexanoate	T	170.99±46.74a	184.98±52.44a	177.98±49.38a	ns	ns
	MP	171.23±39.24a	184.82±42.51a	178.03±40.83a		
n-Amyl alcohol	T	41.90±11.42b	48.04±11.86b	44.97±11.87b	ns	ns
	MP	33.22±7.60a	35.95±8.84a	34.58±8.23a		
Hexyl acetate	T	9.54±4.27a	12.51±6.09a	11.03±5.39a	ns	ns
	MP	13.61±4.41b	14.58±4.66a	14.09±4.49a		
Ethyl lactate	T	8781.28±1832a	9489.57±2088a	9135.43±1965	ns	ns
	MP	8680.79±2117a	9377.28±2335a	9029.04±2221		
Cis 3-hexenol	T	10.85±1.75a	9.59±1.45a	10.22±1.71a	ns	ns
	MP	15.90±4.92b	15.63±5.77b	15.76±5.28b		
Ethyl octanoate	T	20.70±2.76a	19.51±2.62a	20.10±2.71a	ns	ns
	MP	37.14±17.49b	39.92±18.39b	38.53±17.71b		
1,2 Propylene glycol	T	161.57±54.94a	148.60±104.9a8	155.09±82.68a	ns	ns
	MP	117.85±29.47a	105.06±45.29a	111.46±38.14a		
Ethyl 3-hydroxybutyrate	T	50.44±9.36a	54.53±10.93a	52.48±10.23a	ns	ns
	MT	71.97±18.98b	77.77±21.00b	74.87±19.91b		
Linalol	T	39.63±9.31a	40.64±13.47a	40.14±11.40a	ns	ns
	MP	50.29±19.31a	52.89±17.79a	51.59±18.31a		
Ethyl decanoate	T	242.76±41.71a	262.05±46.32a	252.40±44.45a	ns	ns
	MP	274.09±106.56a	296.49±117.01a	285.29±110.68a		
Diethyl succinate	T	1056.54±240a	1138.35±252a	1097.45±245.73a	ns	ns
	MP	1455.35±318b	1672.37±490b	1563.86±421.46b		
2 Phenyl ethyl acetate	T	25.14±14.98a	26.74±15.93a	25.94±15.23a	ns	ns
	MP	35.59±11.44a	37.86±12.17a	36.73±11.67a		
2 Metoxyphenol	T	318.27±239.46a	349.04±272.9a6	333.65±253.07a	ns	ns
	MP	454.92±288.08a	470.57±303.3a9	463.00±291.23a		
γ- Octolactone	T	345.79±120.71a	387.86±194.10a	373.08±183.48a	4.11*	ns
	MP	303.49±202.78a	250.04±272.7a4	348.42±173.93a		
2 Phenylethanol	T	29515.09±548b	31880.68±6184a	30697.88±5872a	ns	ns
	MP	25125.90±4425a	27102.12±4760a	26114.01±4631a		
Eugenol	T	91.66±12.44a	100.10±14.82a	96.38±13.98a	ns	ns
	MP	94.24±20.38a	101.58±21.48a	97.91±20.93a		
Decanoic acid	T	118.16±38.18b	126.79±39.27b	122.47±38.35b	20.46***	14.88***
	MP	35.62±14.03a	38.25±14.65a	36.94±14.17a		
Vanillin	T	58.36±19.57a	58.54±26.01a	58.45±22.64a	ns	ns
	MP	48.34±20.03a	59.69±23.91a	54.02±22.45a		

For the data analysis across years, the statistical significance of the effects of year, and the techniques x year interaction, are also indicated. In each column, different letters denote significant differences based on Duncan's test (* $p < 0.05$; ** $p < 0.01$. *** $p < 0.001$). T: traditional. MP: pre-fermentation maceration.