iVES OENO One vine & wine

DOI: https://doi.org/10.20870/oeno-one.2025.59.1.8348

OPEN ACCESS

ORIGINAL RESEARCH ARTICLES

Balancing phenolic maturity and alcohol content: The impact of seed removal on red wines

A.L. Pérez Mendoza[†,1], A. Martínez-Moreno[*,†,1], E. Gómez-Plaza[1], A.B. Bautista-Ortín[1]

[1] Department of Food Science and Technology, Faculty of Veterinary Sciences, University of Murcia, Campus de Espinardo, 30100 Murcia, Spain.

[†] These authors contributed equally to this work and share first authorship

Article number: 8348

*correspondence:
martinezamoreno@gmail.com

Associate editor:
Stamatina Kallithraka

Received:
8 October 2024

Accepted:
7 February 2025

Published:
25 February 2025

This article is published under the **Creative Commons licence** (CC BY 4.0).

Use of all or part of the content of this article must mention the authors, the year of publication, the title, the name of the journal, the volume, the pages and the DOI in compliance with the information given above.

ABSTRACT

Climate change is significantly impacting in red grape ripening in warm climate regions, producing an imbalance between technological and phenolic maturation, due to the delay in the accumulation of phenolic compounds. That forces the winery to harvest the grape with a high sugar concentration, obtaining wines with a high phenolic compound content, but also with a high alcohol content. However, nowadays, consumers prefer to have wines with low alcohol content. A solution to reduce the alcohol content could be the obtention of wines from less ripe grapes, although in this case, the presence of immature seeds could increase wine astringency and bitterness. Seed removal of these unripe grapes could be a good solution, although it should not be forgotten that tannins are very important to assure wine colour stability through polymerisation with anthocyanins and the reduction of the concentration of these compounds may affect the potential of these wines for ageing. For that, the objective of this work is to evaluate how the total removal of seeds from unripe grapes from the Monastrell variety affects the phenolic and aroma compound concentration, colour and sensorial quality of the wines after 6 months of ageing in the bottle. Results showed that the removal of seeds from immature grapes significantly reduced astringency and enhanced the wine's sensory quality without negatively affecting the colour intensity of the wine. These findings suggest that total seed removal in unripe grapes can be a valuable oenological strategy to mitigate the challenges posed by climate change and produce Monastrell wines with a lower alcohol content without compromising their quality and offering the market the wine demanded by the consumer.

KEYWORDS: tannin, astringency, anthocyanin, oenological technique, alcohol content reduction

INTRODUCTION

Climate change is modifying environmental conditions in all wine-growing areas of the world. Its effects can be positive for vineyards located in cold areas, but for those located in hot and dry areas, such as in the Region of Murcia, the consequences can be negative, both for the volume of production and for the quality of grapes and wines.

High temperatures accelerate sugar accumulation and acid degradation in grapes compared to the rates observed under typical Mediterranean climate conditions (Mira de Orduña, 2010; Martínez-Moreno *et al.,* 2023) and there is a growing imbalance between technological maturity and phenolic maturity as a consequence of this climate change. Therefore, the wine obtained from these grapes reaches a higher alcoholic degree, presents fewer organic acids (mainly malic), a higher pH and a lower anthocyanin content and, therefore, a lower colour (Resco *et al.,* 2016; van Leeuwen & Destrac-Irvine, 2017), since the skins, and especially seeds remain phenolically immature.

In fact, the alcoholic content of wines has increased steadily since the eighties, and today it is normal to find red wines with 15 % ethanol. The alcohol concentration in wine is important for several reasons as ethanol is essential for the ageing, stability and organoleptic properties of wine (Pickering *et al.,* 1998). However, a high ethanol content also presents a series of technical drawbacks such as I) problems in alcoholic fermentation, with slow development of microbiological activity due to inhibition or death of yeasts and stops in the process, II) problems in malolactic fermentation, due to the lower activity of *Oenococcus oeni*, which delays the stabilisation of the wines and increases some unfavourable sensory characteristics, III) lower volatility of some aromatic compounds that can lead to a lower aromatic complexity of these wines. To this, the inconveniences for the consumer must be added, such as excessive warming sensation during tasting, lack of fruit flavour and freshness (possibly due to the greater solubility of the volatile components of the wine that affects the threshold detection and the distribution in the headspace (Robinson *et al.,* 2009)), excessive caloric content, negative effects of alcohol on human health, severe controls for driving vehicles and price increases in those countries whose taxes are linked to the ethanol content of the drink.

For all these reasons, there is clear evidence that wine consumers demand wines with a lower alcoholic content. Therefore, the studies of Mora *et al*. (2021) concluded that, when exploring young consumer attitudes to different red wines, they showed a preference for soft red wines (described as lower ratio total polyphenol index/polysaccharides), and with floral and fruity aromas and low alcohol content. The studies of Deroover *et al*. (2021) as well as the sector report published by Alimarket Gran Consumo (2020) highlight some trends that are beginning to consolidate in the wine sector, among them the demand for calmer wines (with less alcohol content), that will increase in response to a growing trend for healthier beverages.

One potential approach winemakers can use to reduce alcohol levels in wine is to harvest grapes with lower sugar content but to produce a high-quality wine with these grapes requires solving the problem of their low phenolic maturity at this stage, and especially the seeds, which will give wines with a high astringency and bitterness (Keller, 2020). However, it should not be forgotten that tannins are very important to assure wine colour stability through polymerisation with anthocyanins (Singleton & Trousdale, 1992) and this reduction in their concentration may affect the potential of these wines for ageing. We conducted a previous study (Bautista-Ortín *et al.,* 2014) elaborating a Monastrell wine from well-ripen grapes where all the seeds were removed from the grapes, and the results were quite promising. The absence of seed tannins did not affect anthocyanin concentration, and the tannin concentration was 40 % lower than in a control wine. The copigmentation (CA) study showed that both wines had a similar extent of CA phenomena, although the colour intensity was higher when seed tannins were present. Moreover, the panellist evaluated the wines elaborated without seeds as being fruitier, less astringent and with an overall higher quality. However, these wines showed a high alcohol content, which does not align with current consumer preferences for lower-alcohol wines (Sekhon & Sun, 2024). For that, in this work Monastrell wines were elaborated from unripe grapes with seed total removal and their phenolic content, chromatic and sensorial characteristics were evaluated and compared with respect to those wines obtained of unripe and optimal ripe grapes in the presence of seeds after 6 months of ageing in the bottle to verify if this oenological practical may be an alternative to mitigate challenges posed by climate change and produce Monastrell wines with a lower alcohol content without compromising their quality.

MATERIALS AND METHODS

1. Vineyard Site and Grape Samples

Monastrell red grapes were harvested from a vineyard in the Aceniche Valley, located within the D.O. Bullas, region of Murcia, Spain. The Monastrell vines, planted in 1990, are spaced 2.5 by 3.0 meters apart (1333 vines per hectare). The vineyard sits at an altitude of 850 meters above sea level and experiences hot summers and very cold winters, with occasional snowfall. The average annual temperature was 15.9 °C, and the annual precipitation was 336 mm. The vines are cultivated using a bush training system and are grown under rainfed conditions (a cultivation method in which vines depend entirely on natural rainfall, without any irrigation).

2. Wine Production (Microvinification)

Monastrell grapes with varying degrees of ripeness (12 and 14° Baumé) from the same plot were used to carry out the different vinifications. Once the grapes were harvested, they were transported to the experimental winery at the University of Murcia and placed in a refrigeration chamber for a few hours. Subsequently, the

grapes were crushed and destemmed, distributed into 10-litre stainless steel tanks. Seed removal treatment was manually performed only in unripe grapes. The other two vinification processes, involving the presence of seeds, were also conducted with both unripe and ripe grapes. All vinifications were conducted in triplicate. Fermentation for all wines took place at 22 °C using Viniferm CT007 (Agrovin SA, Alcázar de San Juan, Ciudad Real, Spain) as the fermentation yeast at a dosage of 25 g/hL. Actimax Plus (25 g/hL, Agrovin SA, Alcázar de San Juan, Ciudad Real, Spain) and Actimax Natura (25 g/hL, Agrovin SA, Alcázar de San Juan, Ciudad Real, Spain) were also added to prevent fermentation stops.

During the 7 days of maceration, the cap was submerged twice daily. After the corresponding time, the must wine was racked and pressed. The free-run and press must wines were mixed and reintroduced into different tanks. After the completion of alcoholic fermentation (AF), the wines were racked, sulphited and cold stabilised for a month. After this period, the wines were racked again, sulphited and bottled in 375 mL bottles. Analysis of chromatic parameters, phenolic and aroma compounds and sensorial was carried out after 6 months of wine ageing in the bottle.

3. Analytical Determinations

3.1. Physicochemical parameters

The wines were characterised by measuring the alcohol content, pH and acidity according to the European Community methods (Regulation Commission, 1990).

3.2. Spectrophotometric analysis

The chromatic parameters were evaluated using a UV/visible spectrophotometer (Helios Alpha model from Thermo Spectronic, Cambridge, United Kingdom). The colour intensity (CI) of the undiluted wine was determined as the sum of absorbances at 620 nm, 520 nm and 420 nm according to Glories (1984).

3.3. Determination of tannins by HPLC

Determination of tannin total concentration and composition (mean apparent degree of polymerisation, percentage of galloylation and the percentage of epigallocatechin) was carried out by the phloroglucinolysis method according to the conditions proposed by Busse-Valverde *et al*. (2010). The following equipment was used: a Waters 2695 HPLC system (Waters, Milford, MA) coupled to a Waters 2996 photodiode array detector and an Atlantis dC18 column (250 × 4.6 mm, 5 µm packing) protected with a guard column of the same material (20 mm × 4.6 mm, 5 µm packing). The injection volume was 10 µL. A water/formic acid mixture (98:2, v/v) was used as solvent A and acetonitrile/solvent A (80:20 v/v) as solvent B, at a constant flow rate of 0.8 mL/min and an oven temperature of 30 °C. Elution conditions were as follows: 100 % A for 5 min, linear gradient from 100 to 90 % A in 30 min and gradient from 90 to 80 % in 30 min, followed by washing and re-equilibration of the column.

3.4. Determination of phenolic compounds by HPLC

The separation of anthocyanins and vitisins in wine was carried out in a Waters Acquity Arc liquid chromatograph (Waters, Milford, MA, USA) equipped with a Waters 2998 diode array detector (Waters, Milford, MA, USA). The column used was a Poroshell120 EC-C18 core-shell column (150 mm × 2.1 mm, 2.7 µm, Agilent Technologies, Santa Clara CA, USA). The composition of the mobile phases was: 1 % formic acid in water (A) and 1 % formic acid in 1:1 (v/v) methanol:acetonitrile (B). The elution gradient started with 100 % A for 2 min, linear increase from 0 to 15 % B in 33 min, from 15 to 21 % B in 15 min and from 21 % to 30 % B in 20 min followed by washing and re-equilibration of the column. The column oven was maintained at 55 °C, the flow rate was 0.3 mL/min and the injection volume was 5 µL. Compound identification and quantification were performed at 520 nm using malvidin-3-glucoside chloride as an external standard.

3.5. Determination of volatile compounds

For the analysis of Monastrell wine volatile compounds, 10 mL of wine was added to a 30 mL headspace glass vial. A total of 4 grams of sodium chloride (NaCl) and 25 µL of internal standard (2-octanol, Sigma-Aldrich) were added to the same vial. The vial was tightly sealed with a PTFE-lined cap. The solution was homogenised with a vortex shaker (IKA, Konigswinter, Germany). Gas chromatographic determination of volatile analyses was performed using a Gas Chromatograph HP 5890 GC system coupled to an HP 5972 quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA). In total, 2 µL of extract was injected in split mode. An HP InnoWax (50 m × 0.32 mm, 0.25 µm) capillary column (Agilent) was used for the analysis. The injector temperature was programmed at 40 °C for 5 min, raised to 225 °C at 3 °C/min, and then held at that temperature for 5 min. The detector runs at electronic impact mode (70 eV), with an acquisition range (m/z) from 50 to 200 amu. Peak tentative identification was carried out by comparing mass spectra with those of the mass library (Wiley 6.0, Chichester, UK) and comparing the calculated retention indices with those published in the literature. Semiquantitative data were obtained by calculating the relative peak area (total ion count signal or that of selected fragments in the case of some coeluted compounds) in relation to that of the internal standard.

3.6. Sensory analysis

Wines were subjected to sensory evaluation using a descriptive analysis. Prior to the sensory analysis, the wine from the three different replications for each experience was pooled to have a representative sample and to avoid differences among the replications. A total of 12 staff members with high experience in wine sensory analysis participated. They had several training sessions to establish and define suitable descriptors for the formal evaluation of wines. Wine samples were presented in 40 mL aliquots and coded and served in standard wine glasses. The evaluations took place in a controlled room maintained at 20 °C, free from any odour, noise or distraction, with the booth illuminated with a white light.

4. Statistical analysis

The statistical analysis of the data was performed using the package Statgraphics Centurion XVI.3 (Statpoint Technologies, Inc., The Plains, VA, USA). Physicochemical parameters, phenolic and volatile compounds and sensory analysis data were processed using the variance analysis (ANOVA) ($p \leq 0.05$). The differences between means were compared using Tukey's test.

RESULTS AND DISCUSSION

1. Physicochemical parameters

The physicochemical characteristics of the wines (alcohol, pH and total acidity) are shown in Table 1. The 12° Baumé grape wines produced with seeds (CW12) and without seeds (WW12) did not exhibit significant differences in their physicochemical parameters. Both wines had an average alcohol content of 12.4 % vol. In contrast, the 14° Baumé grape wines (CW14) demonstrated a higher alcohol content of 14.6 %. The pH values were 3.43 for the CW12 and WW12 wines and 3.58 for the CW14 wines, while the total acidity was close to 5.9 g/L for the CW12 and WW12 wines and 5.59 g/L for the CW14 wines.

2. Tannin concentration and composition

The concentration and composition of tannins were measured by the phloroglucinolysis method (Table 2). The table shows the content of condensed tannins (TT), their degree of polymerisation (mDP) and the percentage of subunits of epigallocatechin (%EGC) and galloylation degree (%Gal). CW14 wines had the highest concentration of TT and mDP with a value of 539.90 mg/L and 7.57, respectively. As expected, the lowest tannin concentration was found in WWS12 wine. This wine showed the highest content of EGC with a percentage of 18.9 % while CW12 wine obtained the lowest EGC values at 15.2 %. Moreover, the CW12 wine had a higher galloylation percentage (proportion of galloylated flavanol subunits to total flavanol subunits) than CW14 and WWS12 with values of 3.43 %, 2.61 % and 1.87 %, respectively. This indicates that higher concentrations of seed tannin diffused into the CW12 wine more effectively than into CW14, likely due to the reduced lignification of the unripe seeds, which allows better tannin diffusion from the seeds to the must/wine, while the intensive lignification in ripe seeds makes them more waterproof (Cadot *et al.,* 2006).

TABLE 1. Physicochemical characteristics of Monastrell red wines.

Treatment	Alcohol (%vol)	pH	Total acidity (g/L)
CW 12	12.30±0.72a	3.43±0.08a	5.87±0.12b
WWS 12	12.50±0.41a	3.42±0.05a	5.94±0.17b
CW 14	14.64±0.13b	3.58±0.03b	5.59±0.06a

Different letters within the same column indicate significant differences ($p < 0.05$).

TABLE 2. Concentration and composition of tannins determined by the phloroglucinolysis method on Monastrell red wines.

	TT (mg/L)	mDP	%EGC	%Gal
CW 12	334.89± 2.52b	3.69±0.02a	15.25±0.29a	3.43±0.22c
WWS 12	203.18± 25.5a	4.31±0.30b	18.93±2.99b	1.87±0.46a
CW 14	539.90± 36.47c	7.57±0.26c	16.33±1.37ab	2.61±0.07b

TT: total tannins, mDP: mean degree of polymerization, % EGC: percentage of epigallocatechin, %Gal: percentage of galloylation. Different letters within the same column indicate significant differences ($p < 0.05$).

These results align with the different tannin compositions of the skins and seeds previously reported in the literature (Busse-Valverde *et al.,* 2010; Rodríguez Montealegre *et al.,* 2006). The elevated concentration of tannins in CW14 wine is likely attributable to the higher ethanol content, which may enhance the solubilisation of cell walls from the skin and seed cuticle facilitating tannin diffusion into the wine. Canals *et al*. (2005) also observed an increase in phenolic compounds and tannins in wines made from riper grapes. Moreover, tannins derived from seeds possess a lower mean degree of polymerisation (mDP) compared to those from skins (Guaita *et al.,* 2017). Therefore, the removal of seeds in WSS12 wine resulted in a reduction in tannin content and a rise in mDP relative to CW12. This significant increase in mDP has also been previously reported in wines made with early seed removal from red varieties such as Merlot (Lee *et al.,* 2008) or Gaglioppo (Guaita *et al.,* 2017). Additionally, seed tannins generally contain higher levels of galloylated forms than those from skin (Souquet *et al.,* 1996), which resulted in a significant decrease in the galloylation percentage of these compounds in WWS12 wines.

It is important to not forget that the concentration and composition of tannins play a crucial role in the sensory characteristics of red wines (Gawel, 1998), significantly influencing their quality (Gómez-Plaza *et al.,* 2016). However, a high concentration of tannins, along with elevated levels of galloylation and low mDP values, can enhance the perception of astringency and bitterness (Vidal *et al.,* 2003). These effects, however, may be moderated by the presence of (-)-epigallocatechin within the tannin structure (Chira *et al.,* 2009; Fernández *et al.,* 2007).

3. Concentration of anthocyanins and vitisins

Table 3 shows free and polymeric anthocyanin, vitisin and total anthocyanin concentrations in wines made from grapes with different ripening stages (CW12 and CW14) and wine made with total seed removal (WWS12) determined by HPLC. Regarding total anthocyanin content, no significant differences were observed between CW12 and WW12, while CW14 presented the highest concentration of these compounds. Other studies also found no differences in anthocyanin concentration when seeds were removed in Gaglioppo (Guaita *et al.,* 2017) or Monastrell (Bautista-

TABLE 3. Phenolic Compounds (mg/L) measured by HPLC on Monastrell red wines.

Compounds	CW 12	WWS 12	CW14
Free anthocyanins			
Del-3-glucoside	6.16±0.02b	3.75±0.02a	5.66±0.67b
Cyan-3-glucoside	2.58±0.00c	0.98±0.02a	2.05±0.21b
Pet-3-glucoside	9.22±0.09b	5.71±0.01a	9.48±1.05b
Peond-3-glucoside	7.35±0.06b	3.63±0.03a	8.02±0.85d
Malv-3-glucoside	46.10±0.01b	26.77±0.05a	58.31±6.73c
Malv-(6-acetyl)-3-glucoside	2.43±0.04b	0±0a	2.95±0.42c
Peond-(6-coumaryl)-3-glucoside	0.39±0.01b	0±0a	0.64±0.14c
Malv-(6-coumaryl)-3-glucoside	3.72±0.02b	0±0a	5.02±0.74c
Σ free anthocyanins	77.98±0.14b	40.87±0.09a	92.16±5.8c
Vitisins			
Del-3-glucoside-pyruvic acid	0.19±0.00a	0.50±0.00b	0.14±0.05a
Cyan-3-glucoside-pyruvic acid	0.15±0.02a	0.18±0.02a	0.12±0.02a
Pet-3-glucoside-pyruvic acid	0.84±0.03a	1.48±0.02b	0.92±0.12b
Peond-3-glucoside-pyruvic acid	0.55±0.01a	1.08±0.01b	0.63±0.07a
Malv-3-O-glucoside-pyruvic acid (Vitisin A)	5.55±0.02a	10.11±0.0c	7.29±0.87b
Malvidin-3-O-glucoside-acetaldehyde (Vitisin B)	0.89±0.03b	1.08±0.01c	0.57±0.08a
Malv-6-acetyl-3-glucoside-pyruvic acid	0.10±0.00a	0.33±0.01c	0.23±0.06b
Malv-6-coumaryl-3-glucoside-pyruvic acid	0.97±0.02a	1.75±0.00c	1.30±0.21b
Σvitisins	9.28±0.13a	16.55±0.08c	11.25±1.32b
Polymeric anthocyanins	25.52±0.21b	41.84±0.13c	24.10±1.19a
Σ Total anthocyanins	112.79±0.49a	99.27±0.27a	127.51±12.3b

Different letters within the same row indicate significant differences ($p < 0.05$).

Ortín *et al.,* 2014) wines, or even an increase in anthocyanin content in Merlot wines (Lee *et al.,* 2008), although in this last study, the seeds were removed when the sugar content during fermentation was close to 10° Brix. The greater accumulation of these compounds in the skin together with a higher degradation of the cell walls when the grape ripening increases would explain the higher presence of anthocyanins in CW14 wines, as also reported in previous studies conducted by Bautista-Ortín *et al*. (2006) with the Monastrell variety.

CW14 wine exhibited the highest content of free anthocyanins (92.16 mg/L) followed by CW12, while the WWS12 presented the lowest concentration. However, in all wines malvidin-3-glucoside was the predominant anthocyanin, while other anthocyanins, such as malvidin-(6-acetyl)-3-glucoside, peonidin-(6-coumaroyl)-3-glucoside and malvidin-(6-coumaroyl)-3-glucoside were not detected in WWS12 wine, although they were present in CW12 and CW14 wines. Regarding vitisins, seed removal significantly increased the content of these compounds in the wine, almost doubling the values shown by CW12 and exceeding CW14's concentration by 32 %. In all wines the malvidin-3-O-glucoside-pyruvic acid (Vitisin A) showed the highest concentration, reaching values of 10.11 mg/L for WWS12, 7.29 mg/L for CW14 and 5.55 mg/L for CW12. Additionally, a greater formation of vitisin B in CW12 was also observed compared to CW14, although WWS12 again exhibited the highest concentration of this compound. Notably, WWS12 wine had a significantly higher content of polymeric anthocyanins than the other two wines, while no differences were found between CW14 and CW12.

The absence of seed tannins in the WW12 wine compared to CW12 and CW14 wines may have promoted that the anthocyanins were more readily available for the ongoing formation of low molecular mass pigments (vitisins) and polymeric pigments resulting in condensation products with tannins or even among them alone as it was reported by Bindon *et al*. (2014a). These same authors indicated although these pigments formed only by anthocyanins are more bleachable by bisulfite than those formed with tannins, they contribute a strong 520 nm absorbance at wine pH and moreover could be important intermediates in the pathway for pigmented polymer formation in wine, which includes downstream condensation reactions with tannins and/or other polyphenols. Bindon *et al*. (2014b) reported that the formation kinetics of wine polymeric pigments during ageing are not necessarily dependent upon tannin concentration, as

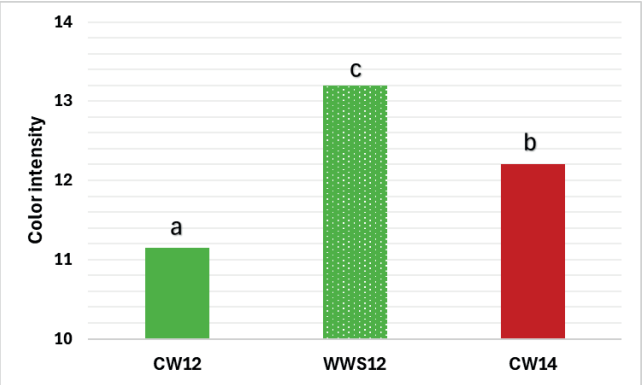


FIGURE 1. Color Intensity (CI) spectrophotometrically measured on the Monastrell red wines.

Different letters indicate significant differences between treatments ($p < 0.05$).

TABLE 4. Major volatile compounds on the Monastrell red wines.

	CW 12	WWS 12	CW14
Alcohol	11306±1421ab	9399±1148a	13211±1761b
Esters	9248±407a	10482±657b	10977±633b
Aldehydes and ketones	158±31b	136±30a	154±29ab
Terpenes	132±12a	131±12a	167±14b
Acids	625±625c	576±115b	469±82a
Σ volatile compounds	21471±5513a	20635±5307a	24980±6529b

Different letters within the same row indicate significant differences ($p < 0.05$). The concentration of volatile compounds is expressed in µg of 2-octanol equivalents/L.

the evolution of tannin pigmentation was similar in wines with both low and high anthocyanin and tannin concentrations. However, some studies suggest that enhanced grape skin tannin extraction during vinification can lead to higher overall wine tannin concentration and increased polymeric pigment formation (Cortell *et al.,* 2005; Cortell *et al.,* 2007; Ristic *et al.,* 2010). Therefore, further studies are needed to understand the role of absent seed tannins in the incorporation of skin tannins into pigmented polymers.

4. Colour intensity

The colour intensity of the studied wines is depicted in Figure 1. The wine produced with seeds removed prior to maceration (WWS12) showed the highest colour intensity (13.2), followed by the wine made from riper grapes (CW14). In contrast, the lowest colour intensity was observed in the wine produced with seeds from less ripe grapes (CW12). The increased colour intensity in WWS12 wine may be attributed to its higher content of both low and high polymerisation pigments, which positively impact the wine´s colour, enhancing its colour intensity (Cheng *et al.,* 2023). While Lee *et al*. (2008) reported no visually noticeable differences in colour between control wines and those with seed removal, their findings showed control wines to be slightly lighter (larger L* value) and more orange. However, the results of this study demonstrate clear differences in colour intensity, underscoring the influence of seed removal and grape ripeness on wine colouration.

5. Aroma compounds

To date, few works have been published regarding the aroma profile of seed-removed red wines. In this research, the aroma composition of Monastrell wines elaborated with grapes at different maturity levels, with and without seeds, was analysed after 6 months of bottle ageing. More than 80 volatile compounds were identified (Table S1). The volatile compounds were classified into 5 chemical groups: alcohols, esters, aldehydes and ketones, terpenes and acids (Table 4). Alcohols and esters, followed by acids represented the aroma compounds at higher concentrations. The CW14 wine, made from riper grapes, exhibited higher concentrations of alcohols, esters and terpenes, while CW12 wine, made from less ripe grapes, had higher concentrations of aldehydes, ketones and acids. As expected, the wine from the ripest grapes (CW14) had higher levels of aroma compounds, as grape ripening naturally leads to an increase in alcohols, esters and terpenes in the corresponding wines (Torchio *et al.,* 2016). These results are very similar to those reported by Gu *et al*. (2022), which observed similar increases in alcohols, esters and terpenes across different red wine varieties as grapes ripened. Interestingly, although CW14 wine showed a significant increase in esters compared to CW12, the seedless WWS12 wine had an even higher ester concentration than its control (CW12) and closely resembled the riper CW14 wine. Esters, known for their fruity aromas, are produced during fermentation through the esterification of alcohols and fatty acids by yeast metabolism (Yilmaztekin *et al.,* 2015). The observed increase in esters in the WWS12 wine may be attributed to the higher concentration of fatty acids present in this wine. Fatty acids are well-established precursors to aromatic esters, with higher concentrations of fatty acids leading to increased ester formation (Bertrand, 1981). Additionally, the manual removal of seeds during the WWS12 treatment likely facilitated the release of fatty acids into the wine, further promoting ester synthesis. Previous research has demonstrated that different pre-fermentation techniques significantly influence fatty acid concentrations in the must (Chen *et al.,* 2023). Specifically, these studies reported a notable increase in fatty acid levels when techniques such as destemming and crushing were employed. Hence, the results obtained in this study suggest that seed removal during vinification of less ripe grapes (which typically have a lower alcohol content) can enhance fruity aromas, thereby improving wine quality. The higher concentration of acids in CW 12 wines compared to CW 14 wines may be due to variations in the grape must composition between the two, reflecting differences in grape maturity and chemical profile (Schreirer, 1979)

5. Sensory analysis

The sensory evaluation of seedless Monastrell wines is shown in Figure 2. Significant differences were noted for all descriptors except colour intensity. The WWS12 wines

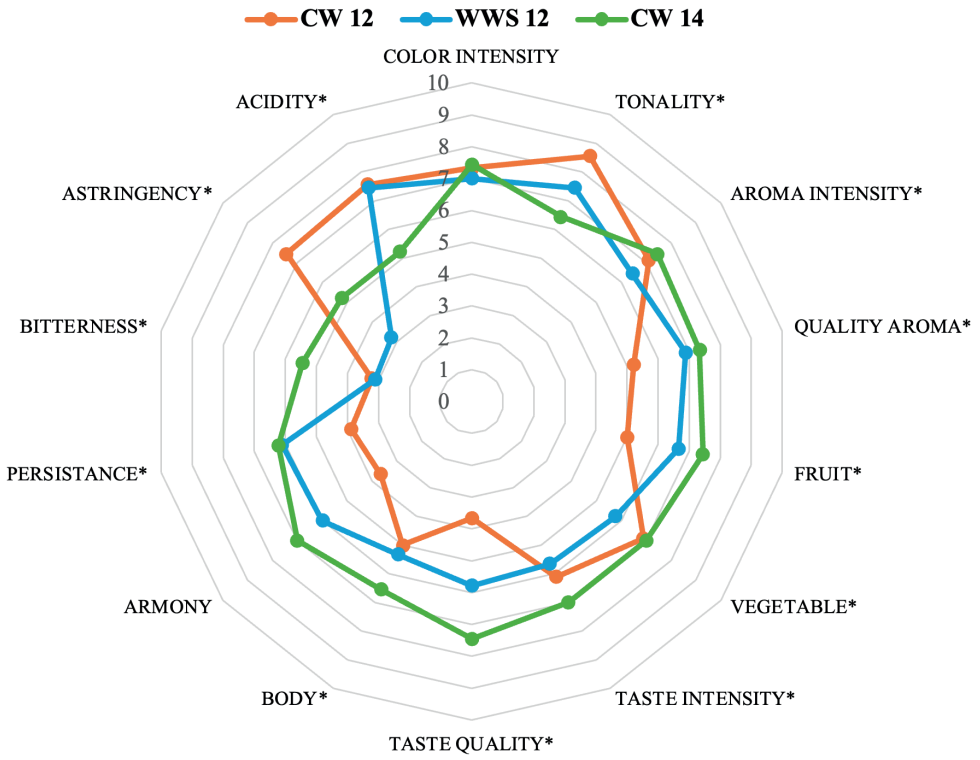


FIGURE 2. Descriptive sensory analysis of the Monastrell red wines studied.

* denotes significant differences (*p*-value < 0.05).

displayed higher tonality scores (tonality evolved from violet-purple to ruby-garnet) compared to CW14, with tasters perceiving a more violet/purple colour in the seedless wine. Additionally, both CW14 and WWS12 wines received similar, and significantly higher, aroma quality scores compared to CW12 wines. This trend extended to mouthfeel attributes such as taste quality and persistence. In terms of aroma descriptors, WWS12 wine scored the highest in fruitiness and, the lowest in vegetal aromas, which are often associated with lower-quality red wines. Similar observations were made by Cliff *et al*. (2012), who found that grape seed extract suppressed fruity aromas. Furthermore, this increase in fruitiness score is consistent with the significant increase in esters in the WWS12 wine, reaching values similar to those obtained in CW14 (Table 4). Regarding taste description, the WWS12 wine showed the lowest astringency scores, followed by CW14. The WWS12 wine also exhibited less bitterness than CW14, aligning with previous findings in red wines with seed removal (Bautista-Ortín *et al.,* 2014; Canals *et al.,* 2008). The reduced bitterness in wines from unripe grapes likely stems from lower levels of galloylated phenolics, which interact more strongly with bitter taste receptors due to their lipophilic nature (VanderWeide *et al.,* 2022), as well as increased levels of (-)-epigallocatechin in the tannin structure (Chira *et al.,* 2009; Fernández *et al.,* 2007). Moreover, the WWS12 wine significantly improved the persistence and harmony scores, achieving values comparable to the CW14 wine. Overall, seed removal during vinification had a marked impact on the sensory attributes of the resulting wines, particularly enhancing aroma and taste perceptions, leading to a more balanced and pleasant sensory experience.

CONCLUSION

The study demonstrates that removing seeds during vinification significantly impacts the wine's colour, phenolic content and sensory profile. The seedless wine (WWS12) showed reduced tannin levels, increased epigallocatechin and decreased astringency and bitterness compared to the wines made with seeds (CW12 and CW14). This absence of seeds facilitated the development of polymeric pigments and vitisins, enhancing colour intensity in WWS12, likely due to the increased availability of anthocyanins and their interactions, which contribute to the formation of stable colour compounds during winemaking. Additionally, seed removal promoted fruity aromas, supported by the higher ester content, creating a sensory experience comparable to that of wines made from fully ripe grapes (CW14). This technique also improved flavour persistence and harmony, suggesting seed removal could enhance the balance and quality of wines made from underripe grapes. These findings emphasise the importance of adapting winemaking techniques to grape ripeness and conditions, with seed removal offering a potential strategy to elevate wine quality when using grapes not fully ripen. Further research is needed to explore the role of skin tannins and anthocyanins or anthocyanins alone in polymeric pigment formation and how these wines' aromatic profiles evolve over time. Moreover, as the complete removal of seeds is a technique that poses significant challenges in commercial wineries, future research should explore innovative solutions, including the development of specialised equipment, to make this process more feasible and efficient.

REFERENCES

Bautista-Ortín, A. B., Busse-Valverde, N., López-Roca, J. M., Gil-Muñoz, R., & Gómez-Plaza, E. (2014). Grape seed removal: Effect on phenolics, chromatic and organoleptic characteristics of red wine. *International Journal of Food Science and Technology, 49*(1), 34–41. https://doi.org/10.1111/ijfs.12270

Bautista-Ortín, A. B., Fernández-Fernández, J. I., López-Roca, J. M., & Gómez-Plaza, E. (2006). The effect of grape ripening stage on red wine color. *Oeno One, 40*(1), 15-24.

Bertrand, A. (1981). Formation des substances volatiles au cours de la fermentation alcoolique. Incidence sur la qualité du vin. In Colloque Soc. Fr. Microbiol (pp. 251-267).

Bindon, K. A., McCarthy, M. G., & Smith, P. A. (2014b). Development of wine colour and non-bleachable pigments during the fermentation and ageing of (*Vitis vinifera* L. cv.) Cabernet Sauvignon wines differing in anthocyanin and tannin concentration. LWT−Food Science and Technology, 59, 923−932.

Bindon, K., Kassara, S., Hayasaka, Y., Schulkin, A., & Smith, P. (2014a). Properties of wine polymeric pigments formed from anthocyanin and tannins differing in size distribution and subunit composition. Journal of Agricultural and Food Chemistry, 62(47), 11582-11593

Busse-Valverde, N., Gómez-Plaza, E., López-Roca, J. M., Gil-Muñoz, R., Fernández-Fernández, J. I., & Bautista-Ortín, A. B. (2010). Effect of different enological practices on skin and seed proanthocyanidins in three varietal wines. Journal of Agricultural and Food Chemistry, 58(21), 11333–11339. https://doi.org/10.1021/jf102265c

Cadot, Y., Miñana-Castelló, M. T., & Chevalier, M. (2006). Anatomical, histological, and histochemical changes in grape seeds from *Vitis vinifera* L. cv Cabernet franc during fruit development. Journal of Agricultural and Food Chemistry, 54(24), 9206–9215. https://doi.org/10.1021/jf061326f

Canals, R., Llaudy, M. C., Valls, J., Canals, J. M., & Zamora, F. (2005). Influence of ethanol concentration on the extraction of color and phenolic compounds from the skin and seeds of tempranillo grapes at different stages of ripening. Journal of Agricultural and Food Chemistry, 53(10), 4019–4025. https://doi.org/10.1021/jf047872v

Canals, R., del Carmen Llaudy, M., Canals, J. M., & Zamora, F. (2008). Influence of the elimination and addition of seeds on the colour, phenolic composition and astringency of red wine. European Food Research and Technology, 226, 1183-1190.

Chen, X., Liu, S., Yuan, J., Zhu, Y., Yuan, C., & Ren, Y. (2023). Application of different pre-fermentation techniques in the winemaking using Guankou table grape (*Vitis vinifera*× *Vitis labrusca*). Food Bioscience, 51, 102272.

Chira, K., Schmauch, G., Saucier, C., Fabre, S., & Teissedre, P. L. (2009). Grape variety effect on proanthocyanidin composition and sensory perception of skin and seed tannin extracts from Bordeaux wine grapes (cabernet Sauvignon and merlot) for two consecutive vintages (2006 and 2007). Journal of Agricultural and Food Chemistry, 57(2), 545–553. https://doi.org/10.1021/jf802301g

Cheng, S., Wu, T., Gao, J., Han, X., Huang, W., You, Y., & Zhan, J. (2023). Color myth: anthocyanins reactions and enological approaches achieving their stabilization in the aging process of red wine. Food Innovation and Advances, 2(4), 255-271.

Cliff, M. A., Stanich, K., Edwards, J. E., & Saucier, C. T. (2012). Adding grape seed extract to wine affects astringency and other sensory attributes. Journal of Food Quality, 35(4), 263–271. https://doi.org/10.1111/j.1745-4557.2012.00448.x

Cortell, J. M., Halbleib, M., Gallagher, A. V., Righetti, T. L., & Kennedy, J. A. (2005). Influence of vine vigor on grape (*Vitis vinifera* L. cv. Pinot noir) and wine proanthocyanidins. Journal of Agricultural and Food Chemistry, 53(14), 5798-5808.

Cortell, J. M., Halbleib, M., Gallagher, A. V., Righetti, T. L., & Kennedy, J. A. (2007). Influence of vine vigor on grape (*Vitis vinifera* L. Cv. Pinot Noir) anthocyanins. 2. Anthocyanins and pigmented polymers in wine. Journal of Agricultural and Food Chemistry, 55(16), 6585-6595.

Deroover, K., Siegrist, M., Brain, K., McIntyre, J., & Bucher, T. (2021). A scoping review on consumer behaviour related to wine and health. Trends in Food Science and Technology, 112 (March), 559–580. https://doi.org/10.1016/j.tifs.2021.03.057 eBooks. https://doi.org/10.5772/intechopen.1004462

Souquet, J., Cheyner, V.I.R., Brossaud, F., & Moutounet, M. (1996). Polymeric proanthocyanidins from grape skins. Phytochemistry 43(2), 509–512.

Fernández, K., Kennedy, J. A., & Agosin, E. (2007). Characterization of *Vitis vinifera* L. Cv. Carménère grape and wine proanthocyanidins. Journal of Agricultural and Food Chemistry, 55(9), 3675–3680. https://doi.org/10.1021/jf063232b

Gawel, R. (1998). Red wine astringency: A review. Australian Journal of Grape and Wine Research, 4(2), 74–95. https://doi.org/10.1111/j.1755-0238.1998.tb00137.x

Glories, Y. (1984). La couleur des vins rouges. 2e partie : mesure, origine et interprétation. OENO One, 18(4), 253. https://doi.org/10.20870/oeno-one.1984.18.4.1744

Gómez-Plaza, E., Olmos, O., & Bautista-Ortín, A. B. (2016). Tannin profile of different Monastrell wines and its relation to projected market prices. Food Chemistry, 204, 506–512. https://doi.org/10.1016/j.foodchem.2016.02.124

Gu, X., Zhang, X., Wang, K., Lv, X., Li, R., & Ma, W. (2022). GC–MS Untargeted Analysis of Volatile Compounds in Four Red Grape Varieties (Vitis vinifera L. cv) at Different Maturity Stages near Harvest. Foods, 11(18), 2804.

Guaita, M., Petrozziello, M., Panero, L., Tsolakis, C., Motta, S., & Bosso, A. (2017). Influence of early seeds removal on the physicochemical, polyphenolic, aromatic and sensory characteristics of red wines from Gaglioppo cv. European Food Research and Technology, 243(8), 1311–1322. https://doi.org/10.1007/s00217-017-2842-1

Keller, M. (2020). The science of grapevines. Academic press. 1st ed.; Keller, M., Ed.; Elsevier Academic Press: London, UK, 2010; p. 400

Lee, J., Kennedy, J. A., Devlin, C., Redhead, M., & Rennaker, C. (2008). Effect of early seed removal during fermentation on proanthocyanidin extraction in red wine: A commercial production example. Food Chemistry, 107(3), 1270–1273. https://doi.org/10.1016/j.foodchem.2007.08.041

Martínez-Moreno, A., Martínez-Pérez, P., Bautista-Ortín, A. B., & Gómez-Plaza, E. (2023). Use of unripe grape wine as a tool for reducing alcohol content and improving the quality and oenological characteristics of red wines. OENO One, 57(1), 109–119.

Mira de Orduña, R. (2010). Climate change associated effects on grape and wine quality and production. Food Research International, 43(7), 1844–1855. https://doi.org/10.1016/j.foodres.2010.05.001

Mora, M., Dupas de Matos, A., Vázquez-Araújo, L., Puente, V., Hernando, J., & Chaya, C. (2021). Exploring young consumer´s attitudes and emotions to sensory and physicochemical properties of different red wines. Food Research International, 143(March). https://doi.org/10.1016/j.foodres.2021.110303

Pickering, G. J., Heatherbell, D. A., & Barnes, M. F. (1998). Optimising glucose conversion in the production of reduced alcohol wine using glucose oxidase. Food Research International, 31(10), 685–692. https://doi.org/10.1016/S0963-9969(99)00046-0

Regulation Comission, E. (1990). Commission Regulation ECC. Commission Regulation (EEC) No 2676/90 of 17 September 1990 Determining Community Methods for the Analysis of Wines.

Resco, P., Iglesias, A., Bardají, I., & Sotés, V. (2016). Exploring adaptation choices for grapevine regions in Spain. Regional Environmental Change, 16(4), 979–993. https://doi.org/10.1007/s10113-015-0811-4

Ristic, R., Bindon, K., Francis, L. I., Herderich, M. J., & Iland, P. G. (2010). Flavonoids and C-13-norisoprenoids in *Vitis vinifera* L. cv. Shiraz: relationships between grape and wine composition, wine colour and wine sensory properties. Australian Journal of Grape and Wine Research, 16(3), 369-388.

Robinson, A. L., Ebeler, S. E., Heymann, H., Boss, P. K., Solomon, P. S., & Trengove, R. D. (2009). Interactions between wine volatile compounds and grape and wine matrix components influence aroma compound headspace partitioning. Journal of Agricultural and Food Chemistry, 57(21), 10313–10322. https://doi.org/10.1021/jf902586n

Rodríguez Montealegre, R., Romero Peces, R., Chacón Vozmediano, J. L., Martínez Gascueña, J., & García Romero, E. (2006). Phenolic compounds in skins and seeds of ten grape *Vitis vinifera* varieties grown in a warm climate. Journal of Food Composition and Analysis, 19(6–7), 687–693. https://doi.org/10.1016/j.jfca.2005.05.003

Schreirer, P. (1979). Flavor composition of wines: A review. Critical Reviews in Food Science andNutrition,12, 59–111. doi: 10.1080/10408397909527273

Sekhon, K., & Sun, Q. (2024). Opportunities and challenges for low-alcohol wine. In IntechOpen.

Singleton, V. L., & Trousdale, E. K. (1992). Anthocyanin-tannin interaction explaining differences in polymeric phenols between white and red wines. American Journal Enology and Viticulture, 43, 63−70.

Torchio, F., Giacosa, S., Vilanova, M., Río Segade, S., Gerbi, V., Giordano, M., & Rolle, L. (2016). Use of response surface methodology for the assessment of changes in the volatile composition of Moscato bianco (*Vitis vinifera* L.) grape berries during ripening. Food Chemistry, 212, 576–584. https://doi.org/10.1016/j.foodchem.2016.05.191

Van Leeuwen, C., & Destrac-Irvine, A. (2017). Modified grape composition under climate change conditions requires adaptations in the vineyard. Oeno One, 51(2), 147–154. https://doi.org/10.20870/oeno-one.2016.0.0.1647

VanderWeide, J., Del Zozzo, F., Nasrollahiazar, E., Kennedy, J. A., Peterlunger, E., Rustioni, L., & Sabbatini, P. (2022). Influence of freezing and heating conditions on grape seed flavan-3-ol extractability, oxidation, and galloylation pattern. Scientific Reports, 12(1), 1–9. https://doi.org/10.1038/s41598-022-07925-7

Vidal, S., Francis, L., Guyot, S., Marnet, N., Kwiatkowski, M., Gawel, R., Cheynier, V., & Waters, E. J. (2003). The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. Journal of the Science of Food and Agriculture, 83(6), 564–573. https://doi.org/10.1002/jsfa.1394

Yang C.X., Wang Y.J., Wu B.H., Fang J.B., Li S.H. (2011). Volatile compounds evolution of three table grapes with different flavour during and after maturation. Food Chemistry.128:823–830. doi: 10.1016/j.foodchem.2010.11.029

Yilmaztekin, M., Kocabey, N., & Hayaloglu, A. A. (2015). Effect of maceration time on free and bound volatiles of red wines from cv. karaoğlan (*Vitis vinifera* L.) grapes grown in Arapgir, Turkey. Journal of Food Science, 80(3). https://doi.org/10.1111/1750-3841.12767