THE EXTRACTION KINETICS OF ANTHOCYANINS AND PROANTHOCYANIDINS FROM GRAPE TO WINE IN THREE DIFFERENT VARIETIES

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Abstract

Aims: To study the influence of the grape variety (Syrah, Monastrell and Cabernet Sauvignon) on the extraction kinetics of phenolic compounds (proanthocyanidins (PAs) and anthocyanins) during a skin maceration period of 20 days.

Methods and results: Anthocyanins and PAs were analyzed by HPLC in three wine varieties during maceration time (20 days). The results showed that anthocyanin extraction followed the same kinetics for the three varieties. In the case of PAs, Syrah must-wine showed the highest concentration of these compounds, which were seen to be mainly skin-derived. Monastrell must-wine presented the lowest concentration of total PAs and the lowest percentage of skin-derived PAs. Cabernet Sauvignon must-wine obtained intermediate values for total PAs, although these compounds were more polymerized.

Conclusion: Grape variety and extractability play a very important role in the wine PA profile. Thus, the highest values of total PAs were observed in the wines of Cabernet Sauvignon and especially Syrah.

Significant and impact of the study: Knowledge of the kinetics of each variety during the maceration step may help manage the composition of wines in light of consumer preference.

Key words: anthocyanin, proanthocyanidin, grape, wine, kinetic

Résumé

Objectifs : Etudier l'influence de la variété de raisin (Syrah, Monastrell et Cabernet Sauvignon) sur la cinétique d'extraction des composés phénoliques (anthocyanes et proanthocyanidines (PAs)) pendant une période de macération de la peau de 20 jours.

Méthodes et résultats : Les anthocyanes et les PAs ont été analysées par HPLC dans trois variétés de vins pendant le temps de macération (20 jours). Les résultats ont montré que l'extraction des anthocyanes a suivi la même cinétique pour les trois variétés. Pour les PAs, le moût-vin de Syrah a montré la plus forte concentration de ces composés, qui provenaient principalement de la peau. Le moût-vin de Monastrell a présenté la plus faible concentration de PAs totales et le plus faible pourcentage de PAs provenant de la peau. Le moût-vin de Cabernet Sauvignon a obtenu des valeurs intermédiaires pour les PAs totales, mais ces composés étaient plus polymérisés.

Conclusion : La variété de raisin et l'extractibilité jouent un rôle très important dans le profil proanthocyanidinic des vins. Les valeurs les plus élevées de PAs totales ont été observées dans les vins de Cabernet Sauvignon et, en particulier, dans les vins de Syrah.

Importance et impact de l'étude : La connaissance de la cinétique de chaque variété lors de l'étape de macération peut aider à gérer la composition des vins à la lumière de la préférence des consommateurs.

Mots clés: anthocyane, proanthocyanidine, raisin, vin, cinétique

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INTRODUCTION

Phenolics, mainly anthocyanins and proanthocyanidins (PAs), are important compounds in red wine quality because they influence colour, mouthfeel and aging ability. Studies have shown that overall intensity and persistence are positively correlated with astringency, and therefore to PA content (Mercurio *et al.*, 2010). An optimal extraction of phenolic compounds from the skin and seeds of grape berries is crucial to ensure colour stabilization in the resulting wines and to impart desirable mouthfeel properties. The diffusion of the different polyphenols from grape to must-wine and, consequently, their extractability and final concentration at the end of the maceration process largely depends on their location in the berry and the characteristics of the different grape varieties, especially those related to the concentration in phenolic compounds and the extractability of these polyphenols.

Anthocyanins are located in the skins, in the upper cellular layers of the hypodermis, while PAs are found in both skins and seeds. Skin PAs are mainly located in the skin cell vacuoles (Amrani-Joutei *et al.*, 1994), while seed PAs are found in the epidermis, the outer integument and the inner integument (Cadot *et al.*, 2006). PAs with upper dihydroxylated unit are called procyanidins and are present in grape skin and seeds, and PAs with upper trihydroxylated unit are called prodelphinidins and are present only in grape skin (Prieur *et al.*, 1994; Souquet *et al.*, 1996). Grape skin PAs have a higher mean degree of polymerization (mDP) and a lower proportion of galloylated subunits than seed PAs.

Both the quantity and the extractability of anthocyanins and tannins increase throughout the grape ripening. The tannins and anthocyanins form different complexes with the cell wall components during berry development (Geny *et al.*, 2003). As the berry ripens these complexes are broken up more easily than in unripe berries. During the maceration step of the winemaking process, phenolic compounds are extracted from grapes into wine. Anthocyanins are mainly extracted in the first days of fermentative maceration (González-Neves *et al.*, 2008), and most skin PAs, due to their localization, are also solubilized, together with anthocyanins. However, the extraction of seed PAs takes longer. Seed PAs are diffused more slowly and require longer maceration times, while their extraction is favoured by the presence of ethanol (Canals *et al.*, 2005; González-Manzano *et al.*, 2004; Del Llaudy *et al.*, 2008). This different extraction dynamics of PAs from grape into wine is therefore an important aspect to be considered when managing the maceration time (Singleton and Draper, 1964; Boulton, 1995), although it may also be influenced by the grape variety and the technological process applied at the winery, e.g., the extent of berry crushing, pectinolytic enzyme addition, the use of dry ice or the application of low prefermentative temperature (Canals *et al.*, 2008; Cerpa-Calderón and Kennedy, 2008; Busse-Valverde *et al.*, 2011; Hernández-Jiménez *et al.*, 2012; Bautista-Ortín *et al.*, 2013).

Grape variety is one of the factors that have an important influence on the phenolic (PAs and anthocyanins) concentration and composition of the wine. Gonzalez-Neves *et al.* (2008) studied the influence of grape variety on the extraction of anthocyanins during fermentation on skins in three varieties (Cabernet Sauvignon, Merlot and Tannat) and concluded that the anthocyanin fingerprint of the young wines obtained in classical fermentation is characteristic of each variety, although their initial evolution follows general tendencies. In the case of PAs, Busse-Valverde *et al.* (2010) reported differences in the PA composition of Monastrell, Syrah and Cabernet Sauvignon wines that were more related to the variety than the winemaking technique. A more recent study of these authors (Busse-Valverde *et al.*, 2012) with wines of the same three varieties and elaborated using different maceration times again reported that the proportion of skin- or seed-derived PAs in wine and the extraction percentage clearly depend on the variety, even more than on skin contact time. The authors suggested that this could be due to the existence of a different initial concentration of grape PAs, a difference in composition or their different degrees of extractability, which would be associated with the composition of the berry cell walls of the different varieties.

It has been shown that the extraction of skin and seed PAs is incomplete (Bindon *et al.*, 2010a; Busse-Valverde *et al.*, 2010, 2012). The type of PAs, interactions among themselves and with other molecules, their adsorption to cell walls and their oxidation, among other factors, could influence their extraction (Bindon *et al.*, 2010b; Hanlin *et al.*, 2010), and all of these may be influenced by variety.

The objective of this study was to determine how the grape variety affects the extent of skin and seed phenolic diffusion from grapes of Monastrell, Cabernet Sauvignon and Syrah to wine during 20 days of fermentative maceration and the final qualitative and quantitative composition of these wines at the moment of pressing.

MATERIALS AND METHODS

Grapes from *Vitis vinifera* L. cvs. Monastrell (also known as Mourvèdre), Cabernet Sauvignon and Syrah were harvested in 2010 from a commercial vineyard in Jumilla (SE Spain). Grapes were carefully harvested into 20-kg boxes and transported to the winery.

1. Physicochemical determinations in grapes

Grape analysis involved the traditional flesh measurements. Total soluble solids (ºBrix) were measured using a digital refractometer (Atago RX-5000). Titratable acidity and pH were measured using an automatic titrator (Metrohm, Herisau, Switzerland) with 0.1 N NaOH. The methodology for carrying out these analyses is described in EEC regulation no. 2676/90.

2. Vinifications

All the vinifications were made in triplicate in 100-L stainless steel tanks using 90 kg of grapes. Before alcoholic fermentation started, total acidity was corrected to 5.5 g/L and selected yeasts were added (Levuline GALA, Oenofrance, France, 10 g of dry yeast/100 kg of grapes). The fermentative pomace contact period was 20 days.

All the vinifications were conducted at 25±1 °C. Throughout the fermentation pomace contact period, the cap was punched down twice a day and the temperature and must density were recorded. At the end of this period, the wines were pressed at 1.5 bars in a 75-L tank membrane press.

Free-run and press wines were combined and stored at room temperature, the cap was punched down twice a day and the temperature and must density were recorded. The analyses were carried out every 2 days during the maceration period.

3. Determination of anthocyanins in grapes and wines

Grapes were peeled with a scalpel and the skins and seeds were stored at -20 °C until analysis. Samples (2 g) were immersed in methanol (40 mL) in hermetically closed tubes and placed on a stirring plate at 150 rpm and 25 °C. After two hours, the methanolic extracts were filtered through a 0.45-µm membrane and analyzed by high-performance liquid chromatography (HPLC). Samples of wine were similarly filtered and directly analyzed by HPLC according to Bautista-Ortín *et al.* (2005).

4. Determination of proanthocyanidins in grapes and wines

The seeds and skins of 10 berries were separated from the mesocarp and rinsed with distilled-deionized water. Whole seeds and skins were extracted separately in covered Erlenmeyer flasks with 10 mL of 2:1 acetone/water at room temperature for 24 h on an orbital shaker at 200 rpm. To minimize PA oxidation, solutions were spurged with nitrogen and the extraction was carried out in the dark. Following extraction, the extract was concentrated under reduced pressure at 35 ºC to remove acetone, and then lyophilized to a dry powder. This powder was redissolved in 1 mL methanol in a volumetric flask.

Skin and seed PAs were determined according to the method described by Kennedy and Jones (2001) with some modifications, as follows. A solution of 0.2 N HCl in methanol, containing 100 g/L phloroglucinol and 20 g/L ascorbic acid was prepared (phloroglucinolysis reagent). The methanolic extract was reacted with the phloroglucinolysis reagent (1:1) in a water bath for 20 minutes at 50 ºC and then combined with 2 volumes of 200 mM aqueous sodium acetate to stop the reaction. Blanks from grape seeds and skins were analyzed using the methanolic extracts without the phloroglucinolysis reagent in order to not infra-estimate the mDP and the total PA content.

For wines, the samples were prepared by an optimization of the method described by Pastor del Río and Kennedy (2006). For this, 5 mL of wine was evaporated in a CentriVap concentrator (Labconco, USA), redissolved in 3 mL water and then passed through a C18-SPE column (1 g, Waters, Milford, USA), previously activated with 10 mL methanol followed by 20 mL water. The cartridge was washed with 20 mL water, and the compounds of interest were eluted with 10 mL methanol, evaporated, and then dissolved in 1 mL methanol. Phloroglucinolysis was then carried out as described above.

HPLC analysis followed the conditions described by Busse-Valverde *et al.* (2010).

PA cleavage products were estimated using their response factors relative to (+)-catechin, which was used as the quantitative standard. These analyses allowed the total PA content, the apparent mDP and the percentage of each constitutive unit to be determined. The mDP was calculated as the sum of all subunits (flavan-3-ol monomer and phloroglucinol adducts, in moles) divided by the sum of all flavan-3-ol monomers (in moles).

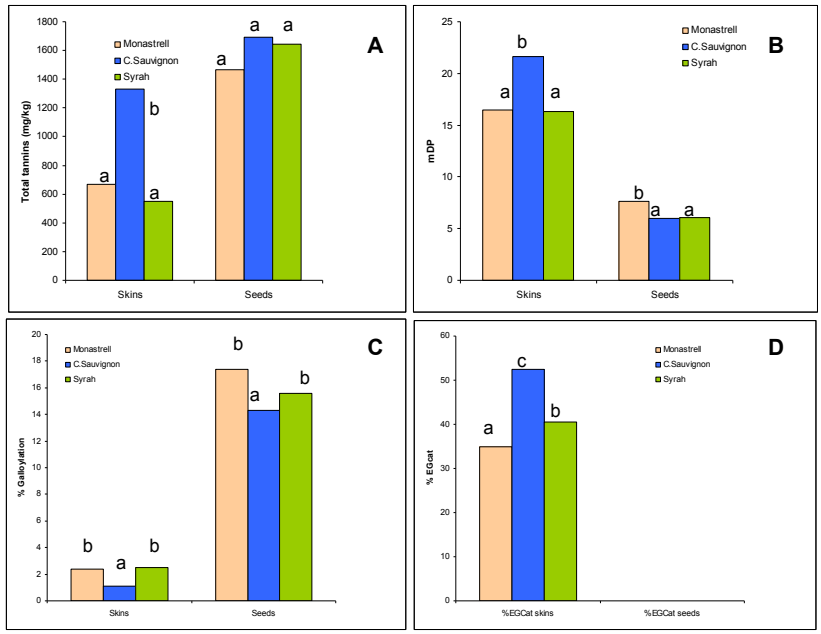

Figure 1. Mean concentration and composition values of skin and seed proanthocyanidins in berries of the three studied varieties.

The PAs extracted from skin and seeds during winemaking were determined following the method proposed by Peyrot des Gachons and Kennedy (2003).

5. Statistical data treatment

Significant differences among samples and for each variable were assessed by analysis of variance (ANOVA). A Duncan test was used to separate the means ($p<0.05$) when the ANOVA test was significant, using the statistical package Statgraphics 5.0 Plus.

RESULTS AND DISCUSSION

1. Physicochemical determinations in grapes

The physicochemical data of grapes at the moment of harvest are shown in Table 1. Similar results were found in pH and acidity parameters (mg/L). But in contrast, statistical differences were found in ºBrix, indicating that the Monastrell grapes were the least mature and Syrah grapes the most mature. Finally, the size of the berries was different among the three varieties studied; Cabernet Sauvignon grapes showed the smallest berries and Monastrell grapes the biggest berries. These differences can influence in the maceration process, besides grape variety.

2. Phenolic profile of grape for the three varieties

Table 2 shows the anthocyanin composition of the grapes of the three varieties studied at the time of harvest and the relative percentage of the different kinds of compounds. When the results were expressed as µg/g of skin, the highest values were observed in Syrah grapes, intermediate values in Monastrell grapes and lowest values in Cabernet Sauvignon grapes. If the results are expressed as mg/kg of berry, total anthocyanins were also higher in Syrah grapes (1555 mg/kg berry), followed by Cabernet Sauvignon (946 mg/kg berry) and then Monastrell (938 mg/kg berry). Romero-Cascales *et al.* (2005) found the highest anthocyanin values expressed as µg/g of skin or mg/kg of berry in Monastrell and Syrah grapes, while Cabernet Sauvignon grapes had the lowest concentration.

Table 1. Physicochemical characteristics in grapes at harvest

	Weight 100 g	pH	Acidity	ºBrix
Monastrell	122.02 b ± 1.37	3.57 a ± 0.07	5.26 a ± 0.01	24.2 a ± 0.05
C. Sauvignon	80.39 a ± 3.12	3.66 a ± 0.05	5.03 a ± 0.04	24.7 ab ± 0.06
Syrah	105.24 ab ± 2.45	3.57 a ± 0.09	5.41 a ± 0.17	25.1 b ± 0.10

Table 2. Concentration of anthocyanins in berries of the three varieties at harvest

	Total anthocyanins (µg/g skin)	Total anthocyanins (mg/kg berry)	% Non acylated	% Acylated	% Dihydroxylated	% Trihydroxylated
Monastrell	10611.8 b[1]	937.6 a	64.8 c	35.1 a	30.7 c	69.2 a
C. Sauvignon	8882.0 a	945.9 a	43.7 b	56.3 b	10.7 b	89.3 b
Syrah	18796.9 c	1554.6 b	31.4 a	68.6 c	7.0 a	93.0 c

1 Different letters in the same row indicate different levels of significance according to Duncan test ($p<0.05$).

The highest non-acylated anthocyanin percentage and the lowest proportion of acylated anthocyanins were found in Monastrell grapes. A similar result was also observed by Romero-Cascales *et al.* (2005) and García-Beneytez *et al.* (2002). Syrah and Cabernet Sauvignon grapes had higher percentages of acylated anthocyanins (69 and 56%, respectively). For the three varieties, trihydroxylated monoglucosides were present in higher proportions than dihydroxylated monoglucosides although the highest percentage of the latter was observed in Monastrell grapes, in which the values were statistically different from those obtained for Syrah and Cabernet Sauvignon. It has been stated that grapes in which the trihydroxylated anthocyanins (delphinidin, petunidin and malvidin) are predominant are much more pigmented and provide wines with a more stable colour (Fernández-López *et al.*, 1998; Romero-Cascales *et al.*, 2005).

Figure 1 shows the total PA content (mg/kg berry), mDP of PAs, galloylation percentage and the percentage of extension (-)-epigallocatechin for the three varieties studied at the time of harvest. As regards skins, the highest PA content corresponded to Cabernet Sauvignon grapes, the same being true in the case of seeds, although no statistical differences were found among varieties. The mDP was always higher in skin than seed PAs, the most polymerized PAs being those of Cabernet Sauvignon grape skins and Monastrell grape seeds. The galloylation percentage was higher in seed PAs than in those from skin. Cabernet Sauvignon grapes showed the lowest levels of tannins to a statistically significant extent. Cabernet Sauvignon grapes presented the highest content of prodelphinidins, while Monastrell grapes showed the lowest content of these compounds. It seems that the biosynthesis of flavanols and PAs in the grape is highly regulated at the variety level, which leads to differences that could play a significant role in the technological and sensory properties of the resulting wines (Mattivi *et al.*, 2009).

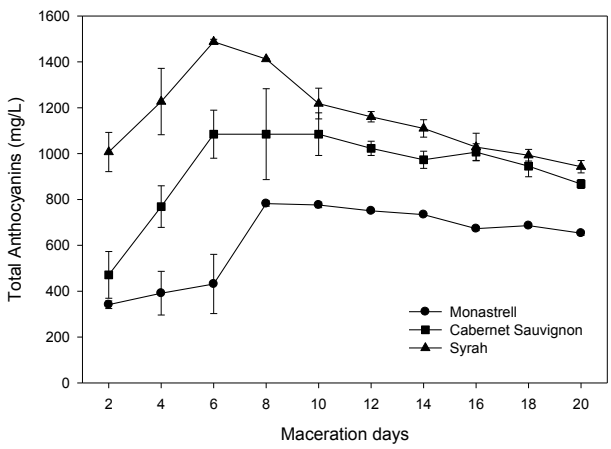

Figure 2. Evolution of total anthocyanins during the maceration period of the three studied varieties.

3. Total anthocyanins extracted during the maceration period

The total anthocyanin concentration was analyzed every two days during the maceration period, which lasted 20 days (Figure 2). During this time, anthocyanin extraction followed a similar kinetics in all three varieties, the concentration increasing from the beginning of maceration until reaching a maximum and then decreasing slowly until the end of the process. The main differences for the three varieties were found during the first days of maceration. The anthocyanin concentration increased rapidly in Syrah and Cabernet Sauvignon must-wines, reaching the highest concentration at day 6 (1488 mg/L and 1085 mg/L, respectively). In Monastrell grapes, anthocyanin extraction into the wine was slower, reaching a maximum value at day 8, although lower than in the other varieties (783 mg/L). The extraction of anthocyanins from grape skins was clearly incomplete. So, considering our winemaking conditions, the real percentage of extraction of skin anthocyanins at the moment of maximum extraction in the wines was 80% for Cabernet Sauvignon grapes, 67% for Syrah grapes and 58% for Monastrell grapes. It has been suggested that an equilibrium based on adsorption-desorption is established between the anthocyanin concentration of the grape and the wine and, when this equilibrium

has been reached, no more anthocyanins can be extracted from grape skins into the wine (Boulton, 2001). The lower extraction of anthocyanins from Monastrell grapes compared with Syrah and Cabernet Sauvignon grapes can be attributed to the cell wall composition and the more rigid structure of this variety (Romero-Cascales *et al.*, 2005; Ortega-Regules *et al.*, 2008).

After, anthocyanin levels decreased until the end of maceration, at which time the Syrah and Cabernet Sauvignon wines showed close values, while Monastrell wines continued to show the lowest anthocyanin content. A similar result to that obtained in this study with Monastrell variety was also found by Bautista-Ortín *et al.* (2004), although these authors pointed to a more pronounced decrease in the anthocyanin content after the maximum value had been reached.

The anthocyanin composition of the musts may be modified significantly by the loss of an important fraction of these molecules through adsorption of the yeasts. These anthocyanins precipitate along with the dead yeasts once fermentation has finished, becoming part of the lees (Vasserot *et al.*, 1997; Morata *et al.*, 2003). Other causes can be the re-fixation on the skins, the fixation on the seeds, and the reactions of the oxidation, condensation and cycloaddition in which anthocyanins are involved. In our case, the three varieties obtained similar alcohol content and total acidity at the end of alcoholic fermentation (data not shown), so these parameters did not affect to extraction kinetics.

4. Proanthocyanidin extraction during maceration

Figures 3 and 4 show the concentration of total PAs, mDP, the concentration of skin-seed derived PAs, galloylation percentage and percentage of extension epigallocatechin during the 20 days of fermentative maceration for the three varieties. Samples were analyzed every two days.

Tannin extraction can be described as a diffusion process (Boulton, 1995), although skin and seed tannins have different extraction kinetics. Most of the tannins in the skins, due to their location, begin to be solubilized together with anthocyanins, although removal is a slow process. In contrast, the seeds have a slow diffusion rate, which is accelerated to half of fermentation, when alcohol facilitates dissolution of the cuticle. In our study, the extraction kinetics of total PAs was rapid during the first few days of maceration; it then stabilized with a slow extraction kinetics (as in the case of Monastrell variety), before decreasing until the end of the process. After two days of maceration, no differences were observed in the PA content among the must-wines of the three varieties, although differences became more evident as maceration time progressed, perhaps due to the different diffusion rates of the PAs in the different varieties. This diffusion rate was highest for the PAs of Syrah, followed by those of Cabernet Sauvignon and Monastrell.

The highest total PA extraction rate was reached on day 10 for Cabernet Sauvignon wine (950 mg/L), day 12 for Syrah wine (1053 mg/L) and day 18 for Monastrell wine (649 mg/L). The values then remained stable in Cabernet Sauvignon and Syrah wines, although near the end of the maceration period, the total PA concentration diminished slightly.

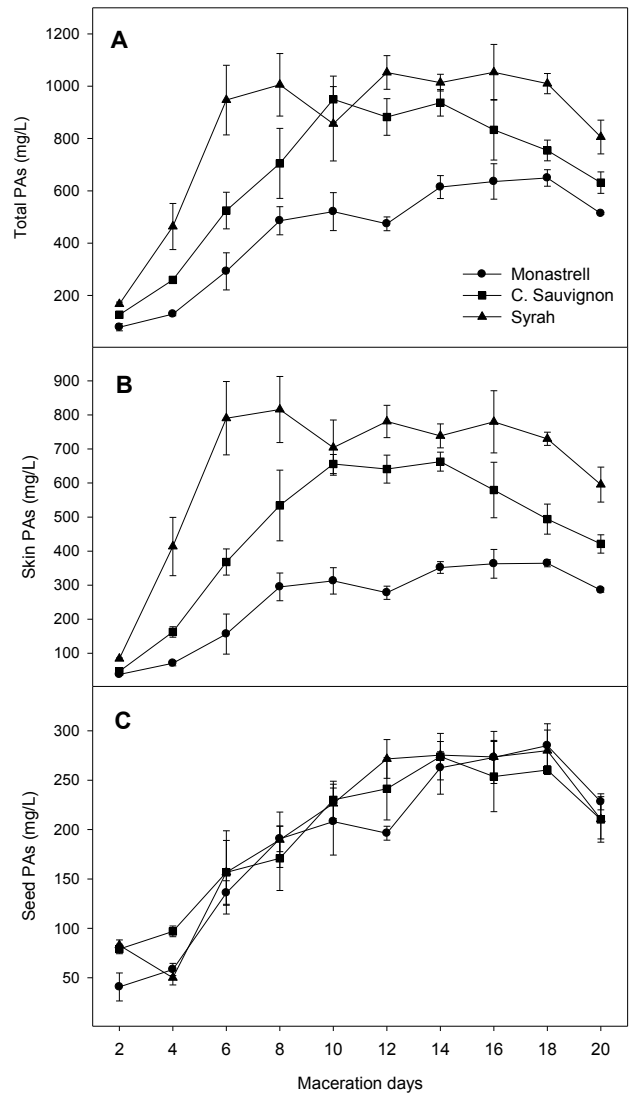

Figure 3. Proanthocyanidin concentration during maceration period.

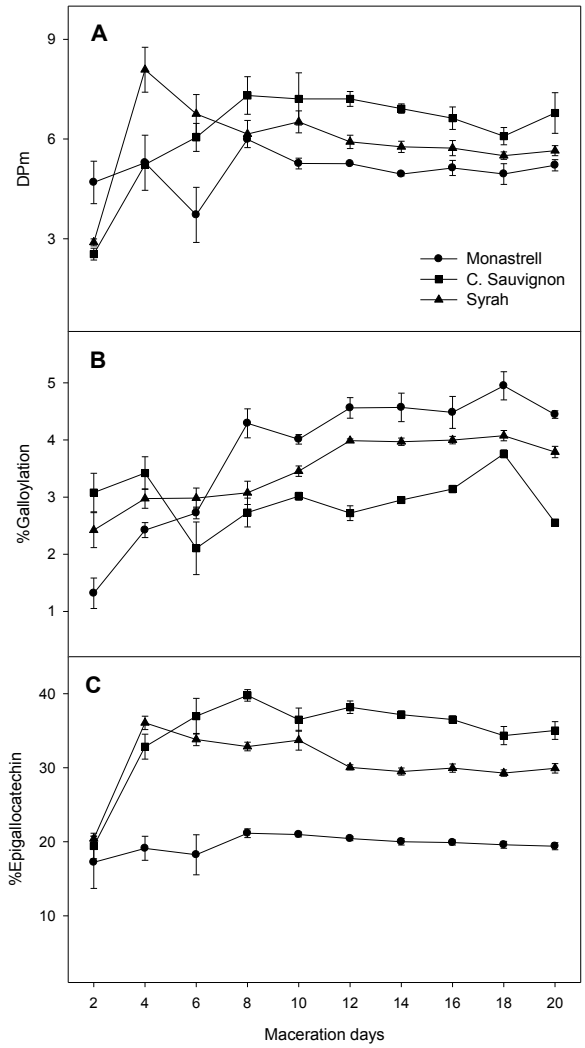

Figure 4. Proanthocyanin composition concentration during maceration period.

Throughout the maceration period, the Syrah wine presented the highest total PA content, although the Syrah grapes had shown the lowest skin PA content and similar seed PA content to the other varieties. The Monastrell grapes produced wines with the lowest PA content, while Cabernet Sauvignon grapes gave wines with intermediate values. The PA content found in wines represents only a part of the PAs present in grapes and, although this content increases in wine with maceration time, it also depends on the variety, since the Cabernet Sauvignon grape, which presented the highest PA content, did not produce the wine with the highest PA concentration. It appears that the extraction of tannins is highly variable, depending on the variety, so that measurement of the total tannin content in grape may not always be useful for predicting the tannin content of the resulting wine, as also observed by Adams and Scholz (2009) and Bautista-Ortín *et al.* (2013). Romero-Cascales *et al.* (2005) also demonstrated that Syrah grapes, which showed a lower skin PA value than Monastrell grapes and a similar seed PA value, produced a wine with a similar PA content to that of Monastrell. These authors concluded that the content of PAs in wine did not correlate with the content of these compounds in grapes but did so with phenolic extractability. This parameter, total PA content, appears to be closely related to the different composition of the cell walls in the different varieties (Ortega-Regules *et al.*, 2006).

Taking into account the PA composition measured in grapes and following the method described by Peyrot des Gachons and Kennedy (2003), it was possible to determine the skin and seed contribution to the PA composition of must-wine (Figure 3, B and C). The extraction of skin PAs during maceration also presented a very different extraction kinetics for the three varieties, but quite similar to that observed in the case of total PAs. The highest skin extraction values were obtained for Syrah wines, followed by Cabernet Sauvignon wines and, lastly, Monastrell wines. Monagas *et al.* (2003) also indicated that wine PAs had a more similar profile to skin PAs than those of seeds. In Syrah wines, skin PA extraction reached a maximum at day 8 and in Cabernet Sauvignon must-wines at day 10, and, in both wines, after a plateau, the concentration decreased slightly until the end of the maceration period. In Merlot grapes, Cerpa-Calderón and Kennedy (2008) found the maximum proportion of PAs extracted from skin on the 9th day of maceration, followed by a stabilization of the concentration until day 18, after which no further extraction occurred. These authors suggested that this may reflect the continued adsorption of extracted PAs to suspended cell wall material and, possibly, yeast cells. In the case of our Monastrell wines, the extraction of skin PAs continued until day 18, just two days before maceration finished, in a process that was slower and in which the quantity extracted was lower than for the other varieties. The lower extraction in this variety may be related to the greater amount of cell wall material and thicker walls, as found by Ortega-Regules *et al.* (2008), in Monastrell compared with Syrah and Cabernet Sauvignon, which may act as a stronger barrier to the extraction of PAs located inside skin cells.

As regards seed-derived PAs, the extraction kinetics was similar for the three varieties; their extraction was increasing from the beginning to the end of the maceration period. The amounts obtained for the three varieties were much lower compared with the skin-derived PAs, especially for Cabernet Sauvignon

J. Int. Sci. Vigne Vin, 2016, 50, n°2, 91-100

and Syrah. In the case of Monastrell, the values of both skin and seed PAs were closer. These results agree with those obtained by González-Manzano *et al*. (2004), Del Llaudy *et al*. (2008) and Cerpa-Calderón and Kennedy (2008).

The mDP of the PAs (Figures 4, A) increased more quickly for Syrah wine, reaching a maximum at day 4, while Cabernet Sauvignon and Monastrell reached the maximum value two days later (day 6). The values of this parameter then decreased slightly for Monastrell, but decreased by close to two units for Syrah, before remaining stable until day 18 of maceration. In Cabernet Sauvignon there was also a slight decrease in mDP values, which occurred several days later than in Monastrell and Syrah. At the end of the maceration process, the mDP values slightly increased in all three varieties.

The highest mDP of PAs during the first days of maceration was reached in Syrah wine followed by Cabernet Sauvignon wine, while from half way through maceration the highest mDP value was seen in Cabernet Sauvignon, the PAs of Syrah wine showing intermediate values. This result may be due to the skin PAs of Cabernet Sauvignon being more polymerized.

Therefore, mDP of the PAs present in wine was seen to be most affected during the first few days of maceration, which does not coincide with the findings of Del Llaudy *et al.* (2008), who found no major changes in mDP values after application of 25 days of maceration.

The increase in mDP values coincided with an increase in the extraction of skin-derived PAs in the wines. However, during the maceration period, an increase in the skin-derived PAs was not always reflected in the state of PA polymerization. This may be due to several causes: i) the simultaneous extraction of seed-derived PAs, which are less polymerized and may compensate the values of mDP, ii) the adsorption of these compounds mainly by suspended flesh cellular wall material, for which the very high molecular weight skin PAs have a high affinity (Bindon *et al.*, 2010b), iii) a process of oxidation (Poncet-Legrand *et al.*, 2010) and interaction with other phenolic compounds of wine and iv) the low efficiency of the hydroalcoholic medium (as is the must-wine) in the extraction of high molecular weight PAs, as previously shown by Bautista-Ortín *et al.* (2013).

The galloylation percentage of the PAs (Figure 4, B) affects both the bitterness and astringency of wines (Lesschaeve and Noble, 2005). This parameter increased for all varieties until day 18, probably due to the increase in the contribution of seed PAs, which are more galloylated than those of skin. It then fell slightly until the end of maceration, probably due to the release of gallic acid units from their corresponding galloylated precursors (Singleton and Trousdale, 1983).

Although the PAs of Monastrell wine during the first days of maceration showed lower galloylation percentage values than the wines of the other varieties, this parameter then increased very quickly, reaching the highest value at the moment of pressing. The PAs of Cabernet Sauvignon wine presented the lowest galloylation percentage values. These results are consistent with those measured in the grapes.

The evolution of the extension (-)-epigallocatechin percentage during maceration reflects the extraction of prodelphinidins into the wine (Figure 4, C). This parameter increased during the first days of maceration and then diminished slightly until the end of the process. This decrease could be related to the oxidation of PAs, in which this subunit is present, since it has been suggested that the flavan-3-ols tri-hydroxyls are more susceptible to oxidation during the fermentation process than di-hydroxyls, such as (+)-catechin and (-)-epicatechin, due to differences in the redox potential (Nanjo *et al.*, 1996). This would explain the lower proportion of skin PAs in must-wine during maceration (Aron and Kennedy, 2007), although the contribution of seed PAs, which do not contain (-)-epigallocatechin, may also contribute to the decrease.

The percentage of prodelphinidins differed in the must-wines of the three varieties. Syrah wine reached the maximum value at day 4 and Cabernet Sauvignon and Monastrell wines four days later, Cabernet Sauvignon must-wines showing the highest value of this parameter and Monastrell the lowest, which, again, indicates the difficulty of extracting the phenolic compounds from the skins of Monastrell grapes. Francis *et al.* (2002) reported that a high prodelphinidin content in a wine can decrease the astringency sensation and soften the mouthfeel of the wine.

CONCLUSION

It is clear that grape variety and extractability play a very important role in the wine PA profile. Thus, the highest values of total PAs were observed in the wines of Cabernet Sauvignon and, especially, Syrah due to a greater contribution of PAs from grape skins. This was reflected in higher mDP values and higher percentage of (-)-epigallocatechin, together with a

low galloylation percentage. In contrast, Monastrell wine presented the lowest PA values; PAs were also less polymerized, but showed a high percentage of galloylation and a lower percentage of (-)-epigallocatechin due to the greater contribution of seeds compared to skins.

Therefore, as regards the phenolic content and composition of the resulting wines, it is important to know how each variety behaves during the maceration step. This will enable winemakers to choose the most suitable maceration time according to the type of wine desired, since these two parameters will have a great influence on the final organoleptic characteristics of the wine.

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