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INFLUENCE OF FERMENTOR TYPE ON POLYPHENOL EXTRACTION IN RED WINES PRODUCED FROM CABERNET SAUVIGNON

George Adrian COJOCARU, Arina Oana ANTOCE

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
Faculty of Horticulture, Department of Bioengineering of Horti-Viticultural Systems,
59 Marasti Blvd., District 1, Bucharest, Romania

Corresponding author emails: arina.antoce@horticultura-bucuresti.ro; aantoce@yahoo.com

Abstract

Phenolic composition and colour of red wines produced from Cabernet Sauvignon grape variety were determined by means of specific spectrophotometric measurements in order to establish the influence of the maceration technique on the final wine quality. The results showed that the type of fermentor used during maceration-fermentation in red winemaking influence significantly the levels of anthocyanins, copigmentation colourless anthocyanins, polymeric pigments, flavones or total phenols. The age of vines had also specific influences which are thoroughly discussed in the paper. The highest values regarding total pigments (coloured, copigmented and colourless) were observed in wines from 3 years old vines, but they are less representative from the technological viewpoint. However, an increase in total phenols and total coloured pigments was observed when the 4 years old vines are compared with 5 years old vines. The type of fermentor used for the maceration-fermentation process is the most important for the total coloured pigments and total phenols extraction, these parameters ensuring structure and stable colour for wines. The highest levels of total coloured pigments were found in wines produced with horizontal fermentors with inner agitator, while the lowest values were obtained by using roto-fermentors, showing that the first are more suitable for producing well-coloured young wines. The results regarding colour intensity and hue revealed an increased colour intensity and lower hue when horizontal fermentors with inner agitator are used and lower colour intensity and higher hue when roto-fermentors are used. Although a higher hue values obtained when roto-fermentors are used means a higher oxidation, these fermentors also lead to a higher tannin extraction, being recommended when the resulted wines are intended for ageing.

Key words*: spectrophotometer, anthocyanin, pigments, maceration, red wine.*

INTRODUCTION

Colour, given by the anthocyanins free and combined with other polyphenols, is one of the most important intrinsic characteristic of red wines, being the first attribute evaluated during wine-tastings. Structure too, given by other specific polyphenols, is also important in red wine sensory appreciation and for the wine capacity to age. Certain technologies and treatments applied during vinification can influence the colour and the overall concentration of certain phenolic compounds in red wines. The oenologists are able to decide the appropriate technologies and treatments, in accordance with the desired final product characteristics (Cojocaru and Antoce, 2011; Antoce and Cojocaru, 2015; González-Neves et al., 2015; Gómez-Plaza et al., 2000; Busse-Valverde et al., 2011). Among the possible interventions, the effect of maceration-fermentation technique on wine colour and phenolic compounds is widely studied (Busse-Valverde et al., 2011; Casassa and Harbertson, 2014; González-Neves et al., 2013; Gambuti et al., 2009; Koyama et al., 2007; Gil-Munoz et al., 1999). Many important parameters of wines are influenced by maceration-fermentation process, including the polyphenol compounds extraction (Casassa and Harbertson, 2014; González-Neves et al., 2013; Gambuti et al., 2009; Koyama et al., 2007; Gil-Munoz et al., 1999; Jackson, 2008; Rakonczás et al., 2015). During maceration, along with anthocyanin and tannin extraction from grape skins potassium content is also increased, which leads to an increase of pH and changes in the total titratable acidity due to the potassium hydrogen tartrate precipitation (Drăghici and Râpeanu, 2011; Rakonczás et al., 2015; Peng et al., 1996), changes that can also affect the colour. Temperature, duration, homogenisation, aeration and enzyme addition during maceration are also important factors that have direct effect on extraction rate and final concentration in polyphenols or some other cellular constituents (Casassa et al., Harbertson, 2014; González-Neves et al., 2013; Gambuti et al., 2009; Koyama et al., 2007; Gil-Munoz et al., 1999; Jackson, 2008; Ribéreau-Gayon et al., 2006). Thus, for each style of wine, these parameters should be controlled when a certain maceration industrial technology is applied.

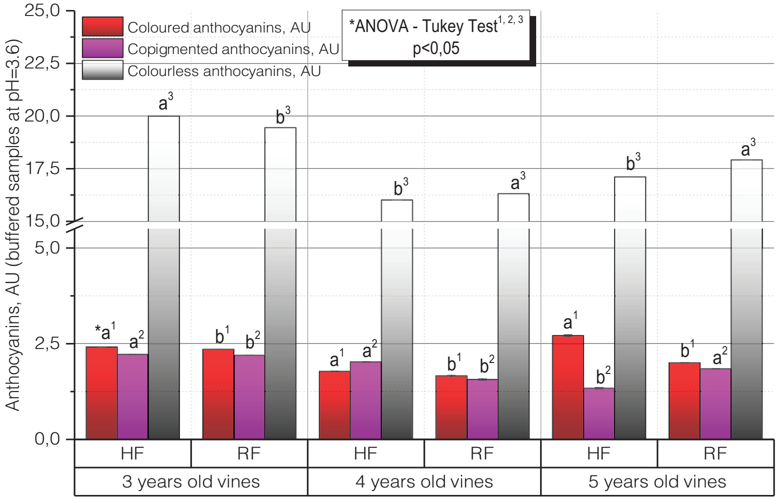
MATERIALS AND METHODS

The grapes for this study were harvested on October 2013 in Vrancea wine region from parcels containing Cabernet Sauvignon of 3, 4 and 5 years of age. Vinification of grapes involved minimal oenological intervention to reduce the influences induced during vinification in order to assess the effect of maceration techniques on phenolic composition and colour of wine. Grape batches were treated with 30 mg/kg with sulphur dioxide for antioxidant protection and then crushed and destemmed. The resulted grape mash batches from each parcel (with grapes from vines of 3 different ages) were transferred with progressive cavity pumps and each divided for maceration-fermentation in two type of fermentors: roto-fermentors (RF) and horizontal tank with inner agitator (HF). During the first day of maceration, all analysed tanks were treated with a preparation of pectolytic enzyme in dose of 1 g/q (Zymorouge G, AEB Spindal), for an enhanced extraction of tannins and pigments and better colour stabilisation. Cap management during maceration-fermentation was programmed in both types of tanks and achieved by 5 minutes of rotation for 3 times/day. The maceration and alcoholic fermentations were conducted at 24-28°C without inoculation (using the wild grape microflora) for 15 days, after which the marc batches were pressed with a horizontal press at 0.4 Bars. The resulted wines were then gravitationally clarified, racked and analysed to determine the parameters related to colour and phenolic composition. Six types of wines were obtained and used to compare the influence of maceration technique on phenolic composition. Wine samples were analysed in triplicate by assessing the main spectrophotometric parameters usually used to describe the colour and phenolic composition. The spectrophotometric methodology requires standardization of wine pH to 3.6 and filtration with PES membrane with 0.45 µm pore size. Each spectrophotometric measurement was performed with a UV - VIS double beam spectrophotometer Specord 250 from Analytik Jena AG using the software WinAspect version 2.2.7. In accordance to the parameter determined quartz or glass cuvettes were used. All the results were calculated to account for dilution and conventionally referred to the optical path of 10 mm and expressed in absorbance units (Antoce and Cojocaru, 2015). ***Monomeric anthocyanin*** concentration was determined as the difference in optical densities at 520 nm of the wine diluted to 5% and buffered to pH=3.6 in order to exclude copigmented anthocyanins (Boulton, 2001; Levengood and Boulton, 2004) and the same wine treated with sulphur dioxide to bleaching in order to exclude polymeric pigments and other resistant pigments (Jacobson, 2006; Eldridge and Liles, 1997). ***Copigmented anthocyanin*** concentration was determined as the difference in optical densities at 520 nm of the wine sample treated with an excess of acetaldehyde and of the same wine diluted down to 5% and buffered to pH=3.6. The excess of acetaldehyde reacts with the free SO_2 in wine, preventing any bleaching effect on the existent pigments, thus giving an estimation of total colour at 520 nm (Boulton, 2016, 2010 and 1996; Levengood and Boulton, 2004; Jacobson, 2006; Eldridge and Liles, 1997). ***Polymeric pigments and bleaching resistant pigments*** were determined as optical densitiy at 520 nm of the wine buffered at pH=3.6 and treated with an excess of SO_2. The effect of SO_2 is to bleach all monomeric and copigmented anthocyanins, but leave unaffected (coloured) the polymeric pigments and other non-bleachable derivatives of anthocyanins, which are formed through the addition of compounds such as pyruvate, acetaldehyde, hydroxycinnamates or vinylflavanols to the C4 and 5 hydroxyl positions of anthocyanins, generally known as pyranoanthocyanins (Somers, 1971; Boulton, 1996; Levengood and Boulton, 2004;

Harbertson and Spayd, 2006; Jacobson, 2006; Remy et al., 2000; Eldridge and Liles, 1997; Bakker et al., 1997; Bakker and Timberlake, 1997; Romero and Bakker, 1999; Mateus et al., 2001; Mateus and De Freitas, 2001; Cameira-dos-Santos et al., 1996; Fulcrand et al., 1996, 1997, 1998; Es-Safi et al., 1999; Benabdeljalil et al., 2000; Lu and Foo, 2001; Hayasaka and Asenstorfer, 2002; Schwarz et al., 2003). ***Colourless anthocyanins*** were determined as the difference in optical densities at 520 nm of the wine sample treated with excess of HCl and the same wine treated with excess of acetaldehyde. The addition of excess of HCl allows quantifying of total anthocyanins (coloured and colourless) by destroying the effect of concentration and the equilibrium formed at certain pH, while the acetaldehyde allows quantifying of total coloured anthocyanins (Jacobson, 2006; Eldridge and Liles, 1997). ***Flavone cofactors*** were determined at 365 nm, being the maximum of absorption wavelength for flavonoids involved in copigmentation phenomenon, especially quercetin and kaempferol (Eldridge and Liles, 1997; Merken and Beecher, 2000; Harbertson and Spayd, 2006). ***Total phenols*** were determined on a diluted wine sample (1/100) at 280 nm and corrected with a factor of 3.9 representing the non-polyphenol substances absorbing at the same 280 nm wavelength (Somers, 1998). ***Colour intensity*** and ***hue*** was calculated as the sum of absorbance determined at 420, 520, and 620 nm and, respectively, the ratio between absorbance at 420 nm and 520 nm (Ribéreau-Gayon et al., 2006; OIV, 2016) with mention that samples were buffered at pH=3.6 for standardization. The detailed spectrophotometric methodology is described in previous works (Boulton, 2016 and 1996; Levengood and Boulton, 2004; Jacobson, 2006; Eldridge and Liles, 1997). The Origin 10.0 software program was used for data processing. Analysis of variance (ANOVA) was applied to the results with posthoc Tukey Test for the comparison of means, at 0.05 significance level.

RESULTS AND DISCUSSIONS

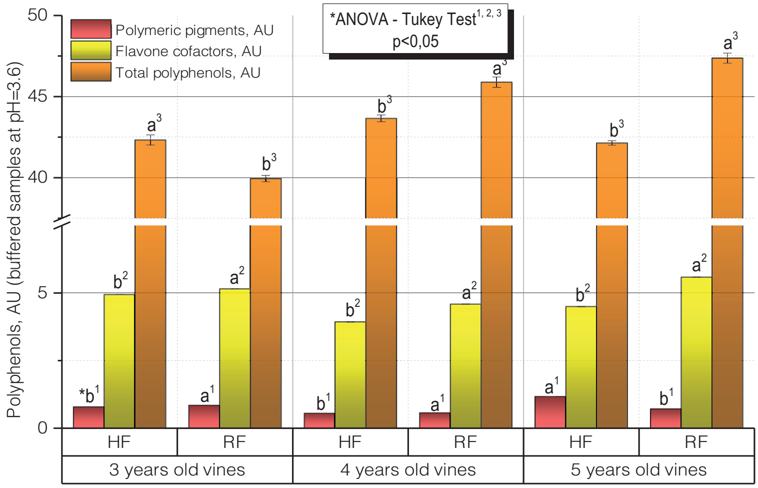
The results presented in figure 1 show the variation of coloured, copigmented and colourless anthocyanins in Cabernet Sauvignon wines produced with grapes coming from vine plantations of 3, 4 and 5 years of age, each macerated in two types of tanks. As expected, we can observe slight differences in coloured anthocyanin between vines with different ages, the highest value being observed in wines produced from 5 year old vines using the horizontal fermentor with inner agitator and the lowest being observed in wines produced from 4 year old vines using the roto-fermentor. Generally, when the horizontal fermentor with inner agitator was used, slightly higher content in coloured monomeric anthocyanin was observed in wine samples, irrespective to vine age, compared to wines produced with roto-fermentors. However, regarding the copigmented anthocyanin, the highest content was observed in wines produced from 3 year old vines, while the lowest content was observed in wines produced from 5 year old vines. This case is similar for monomeric coloured anthocyanin regarding the type of fermentor used with the exception of the copigmented anthocyanins observed in the wines produced from 5 years old vines. The lower values of copigmented anthocyanins obtained in the horizontal fermentor with inner agitator are well explained by the larger values of polymeric pigments shown in figure 2. Generally, aside of the enhancement in absorbance it is believed that the copigmented anthocyanins are do not affect the rate of polymerization reactions during wine ageing (Bimpilas et al., 2016; Boulton, 2001). A possible pathway of polymeric pigments formation was suggested by previous works, considering that the hydrophobic stacking interaction between anthocyanin chromophores and the so-called copigments, could be the first step in the formation of a covalent bond between the anthocyanin and its copigment (Brouillard and Dangles, 1994). However, the polymerization reactions during ageing are involved in formation of a more stable coloured pigments (Jackson, 2008; Somers, 1971). Between colourless hydrated form of anthocyanins and coloured flavylium cation an equilibrium forms based mainly on the wine pH, determined not only by the thermodynamic constants of anthocyanin molecules, but also by the concentration, solvent and temperature. The colourless hydrated form of anthocyanin can act as nucleophile, being involved in condensation reactions with flavan-3-ols as electrophiles in wines, leading to larger red pigments of flavanol-anthocyanin ($F - A^+$) (Cheynier et al., 2000; Brouillard and Delaporte, 1977).



***ANOVA results:** the population means for **age of vines** are significantly different; the population means for **type of fermentor** are significantly different; the interaction between **age of vines** and **type of fermentor** is significant.

**HF - Horizontal fermentor with inner agitator; RF - Roto-fermentor;

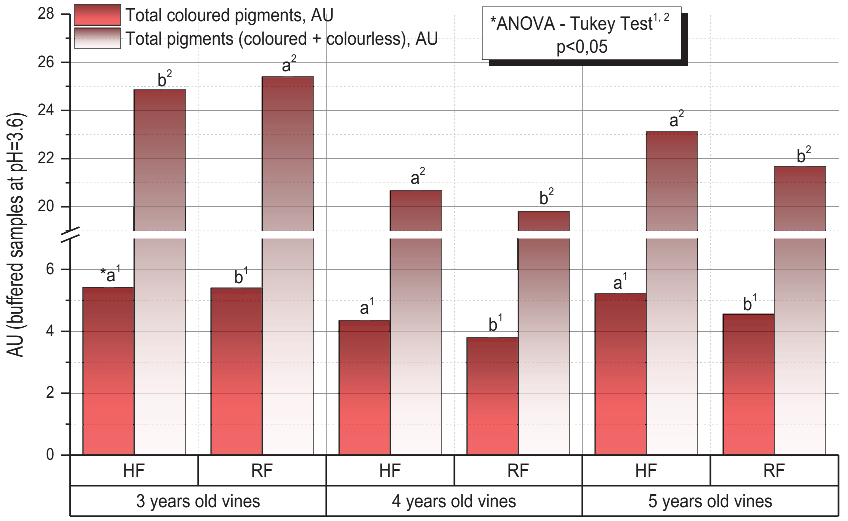
Figure 1. Variation of anthocyanin content in Cabernet Sauvignon wines produced with two types of maceration techniques from grapes with different age of vine plantations



***ANOVA results:** the population means for **age of vines** are significantly different; the population means for **type of fermentor** are significantly different; the interaction between **age of vines** and **type of fermentor** is significant.

**HF - Horizontal fermentor with inner agitator; RF - Roto-fermentor;

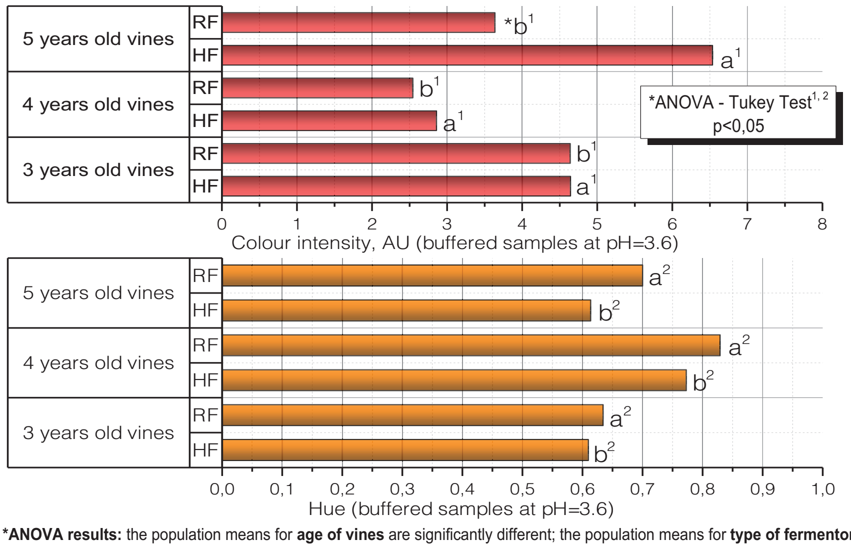
Figure 2. Variation of certain polyphenols content in Cabernet Sauvignon wines produced with two types of maceration techniques from grapes with different age of vine plantations



***ANOVA results:** the population means for **age of vines** are significantly different; the population means for **type of fermentor** are significantly different; the interaction between **age of vines** and **type of fermentor** is significant.

**HF - Horizontal fermentor with inner agitator; RF - Roto-fermentor;

Figure 3. Variation of total coloured pigments and total pigments (coloured + colourless) in Cabernet Sauvignon wines produced with two types of maceration techniques from grapes with different age of vine plantations



***ANOVA results:** the population means for **age of vines** are significantly different; the population means for **type of fermentor** are significantly different; the interaction between **age of vines** and **type of fermentor** is significant.

**HF - Horizontal fermentor with inner agitator; RF - Roto-fermentor;

Figure 4. Variation of colour intensity and hue in Cabernet Sauvignon wines produced with two types of maceration techniques from grapes with different age vine of plantations

On the other hand, the red flavylium cation (coloured anthocyanins) can act as electrophile, being involved in condensation reactions with flavan-3-ols as nucleophile in wines, leading to larger red pigments of anthocyanin-flavanol (A^+ - F) (Cheynier et al., 2000; Remy et al., 2000).

Our results regarding colourless (hydrated) form of anthocyanins presented in figure 1 show greater values for 3 years old vines, which explains why the total content of anthocyanins (coloured, copigmented and colourless) presented in figure 3 is greater in wines produced from 3 years old vines. This phenomenon is probably due to the fact that being in the first year of production the 3 years old vines have low yields, thus accumulating more anthocyanin per grape. However, the effect of concentration, standardization of pH to 3.6 and alcoholic concentration affects this specific equilibrium of anthocyanin forms, leading to the results presented in figure 1. The polymeric pigments, flavone cofactor and total phenolic compounds are presented in figure 2.

The level of total phenols in the grapes and wines is influenced significantly by the age of vines, being greater in wines produced from 4 and 5 years old vines. Due to the high concentration of tannins (figure 2) in wines produced from 5 years old vines, in the case of a higher concentration of coloured anthocyanins (as flavylium cations) – the wines produced in horizontal fermentor (figure 1), the formation of polymeric pigments of anthocyanin-flavanol (A^+ - F) type seem to be favoured (figure 2). However, the other mechanism for the production of flavanol-anthocyanin type of polymeric pigments (F - A^+) seems to be slower in the wines from 5 years old vines. This was not the case in the wine samples produced from 3 years old vines, where we have found higher concentrations of colourless hydrated anthocyanin (figure 1), but similar values for polymeric pigments with wines produced for 5 years old vines (figure 2). Thus, based on a single study, the observed results on polymeric pigments formation are not very conclusive. Another study have already shown that certain winemaking parameters (increased temperature during storage or pH = 3.8) can lead to formation of anthocyanin-flavanol (A^+ - F) polymeric pigments (Fulcrand et al., 2006).

Generally, observations on wine samples produced from 4 and 5 years old vines, showed that the roto-fermentors tend to extract more phenols (tannins) than the horizontal fermentors with inner agitator (figure 2).

Also, the results regarding total coloured pigments presented in figure 3, are not well correlated with colour intensity of wine samples (figure 4). However, when horizontal fermentors were used, greater values were observed in both types of analyses irrespective of the age of vines and, correspondingly, lower values when roto-fermentors were used. Total pigments (coloured and colourless) are generally higher when horizontal fermentors with inner agitator were used (figure 3). Only in wines produced from 3 years old vines we have observed higher concentrations of total pigments (coloured and colourless) with the use of roto-fermentor. The results regarding hue of colour in figure 4, revealed that wines produced with roto-fermentors are more oxidized, with more yellow tones than wines produced with horizontal fermentors with inner agitator.

CONCLUSIONS

Polyphenol extraction in wines is influenced significantly by the type of fermentor used, but the age of vines, especially when they are very young, can also have a major effect on wine quality. Different equilibrium between anthocyanin forms (coloured, copigmented and colourless) could be observed in relation with age of vines and also the type of fermentor used during the process of maceration. Generally, the content of total pigment (coloured, copigmented and colourless) was higher in wine samples produced from 3 years old vines, possibly due to a low yield and higher anthocyanin accumulation/grape, but this case is less representative from the technological point of view. An increasing trend was observed in the case of total coloured pigments in the case of the 4 and 5 years old vines, with similar behaviours regarding the colour intensity, even though not correlated to the vine age. The type of fermentor used during maceration-fermentation has a significant effect on extraction of anthocyanins and tannins and implicitly big impact the structure and colour in red wines. An increased extraction of total phenols and a decreased total coloured pigments was observed in wines produced with roto-fermentors, and an opposite effect was observed in wines produced with horizontal fermentors with inner agitator. Colour intensity has a similar behaviour with total coloured pigments, more intense colour being observed when wines are produced with horizontal fermentors. The analyses regarding hue of colour showed that roto-fermentors produced wines with increased values, which suggest an advanced oxidation compared to the wines from horizontal fermentors with inner agitator. However, although the level of total coloured pigments and colour intensity are founded in greatest concentrations when wines are produced with horizontal fermentors, and also, the hue values are lower than in wines from roto-fermentors, we recommend this later type of fermentor to be used when wines with better polypehnolic structure meant for aging are desired. The horizontal fermentors, with a higher extraction of anthocyanins are more suitable for the production of intensely coloured wines designed to be commercialised young.

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