

# ANTIOXIDANT POTENTIAL AND PHENOLIC COMPOSITION OF WHITE AND RED WINES

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## Summary

Many *in vitro* and *in vivo* researches show, that wine, especially red one, contains natural phenolic antioxidants that have beneficial effects on our health. Polyphenols are not only important for the antioxidant properties but also influence the sensorial properties of wine. Antioxidant potential (AOP), contents of total and individual phenolic compounds (flavonoids, tannins and anthocyanins), colour parameters and content of sulphur dioxide were determined in a number of Slovenian white and red wines of different vintages from 2001 to 2004. In the presented work AOP of wine, expressed as mol/l, was determined spectrophotometrically at wavelength 517 nm using stable free radical DPPH<sup>•</sup> (1,1-diphenyl-2-picrylhydrazyl).

Correlations between AOP and phenolic compounds were established. Content of total and individual phenols were found to be characteristic of the grape variety. In accordance with the literature data we confirmed that wines with greater content of total phenols had also significantly greater AOP. Slovenian red wines had significantly higher content of total and individual phenols, and consequently a higher AOP in comparison to white wines. Certain older wines (2001-2003) of different varieties investigated had higher AOP than younger (2004) what was more obvious in white wines than in red ones.

In the red wines tested the correlations between AOP and phenolic compounds were in the following order: total phenols > flavonoids > tannins > anthocyanins. Cabernet Sauvignon contained the highest content of total phenols and had greater AOP than Refošk and Merlot. The best correlations for all investigated red wines of vintage 2004 were between AOP and flavonoids ( $R^2=0.97$ ) and total phenol content ( $R^2=0.95$ ). Correlations between AOP and tannins ( $R^2=0.80$ ) and anthocyanins content ( $R^2=0.40$ ) were worse. Mentioned correlations for white wines were as followed: total phenols > tannins > flavonoids. During the ageing of the bottled red wines the content of anthocyanins decreased and consecutively the intensity of colour as well as the hue of colour.

**Key words:** wines, antioxidant potential, DPPH, phenolic compounds, colour parameters, maturation

## Introduction

In the recent years polyphenols in wine have raised a lot of attention due to their health-promoting effects and the antioxidant role they play in biological and food systems. Results of certain studies have indicated that these effects could be ascribed to the antioxidant properties of polyphenols, an important group of secondary plant compounds. Phenolic compounds that are important in terms of the wine quality (in particular, colour and astringency) could be classified into two groups: flavonoids and non-flavonoids. Wine is a complex mixture of polyphenols and detailed analysis of polyphenols, preferentially by LC-MS, demands expensive equipment and many different standards for quantitative and qualitative analysis of wine polyphenols.

Despite mentioned technical feasibility for detailed analysis, wine polyphenols are still frequently analysed by classical spectrophotometric tests like Folin-Ciocalteu method, where redox potential of reducing substances is measured. Absorbance read at around 750 nm corresponds to the concentration of reduced heteropolyphosphotungstate-molybdate. In wine, where polyphenols are the main reducing agents present, absorbance is directly proportional to the concentration of total polyphenols. Absorbance of the wine sample is compared to the absorbance of the standard polyphenol, for example gallic acid, and expressed as mg of gallic

acid per litre of wine (mg GAE/l). Method itself is relatively fast, as it takes around two hours to obtain the result.

Mode of action of polyphenols “*in vivo*” could be attributed to their ability to quench free radicals and prevent excessive damage of cellular components. As classical Folin-Ciocalteu method does not provide an insight into the ability of substance to stabilize free radical, methods that would test such ability of polyphenols have been introduced. One of the methods that have gain a lot of attention is based on ability of substances to quench purple coloured DPPH<sup>•</sup> free radical, by donating hydride ion to the radical, into the colourless DPPH<sub>2</sub>. Decrease in absorbance at around 515 nm is directly proportional to the antioxidant potential of the substance tested. Method is currently used for determination of antioxidant potential of many pure antioxidants and especially complex mixtures of antioxidants from various food samples. In the last few years DPPH method has been increasingly used for assessing the antioxidant potential of wine. In the proposition of fast method for AOP determination of wine by O.I.V. it is suggested that antioxidant potential of wine is expressed as ED<sub>50</sub> index, a dilution of wine required to decrease the initial concentration (absorbance) of DPPH<sup>•</sup> in a test by 50%. Method is generally considered to be faster than Folin-Ciocalteu assay, and absorbances are usually measured between 15 minutes and two hours after the addition of the antioxidant, when equilibrium is supposed to be reached.

### Materials and methods

In the first part of the experiment the 40 samples of Slovenian wines (20 red and 20 white, respectively of different varieties and vintages from 2001 to 2004) from market place were examined. In the second part of the experiment we have focused only on the red wines of vintage 2004 (total 45 samples; 15 samples of each variety Refošk, Merlot and Cabernet Sauvignon).

**DPPH as an assay of AOP.** It is known that DPPH<sup>•</sup> reacts with different antioxidants at different molar ratios (Brand-Williams *et al.*, 1995). Substances with higher numbers of hydroxyl groups and those having hydroxyl groups in *ortho* positions in the aromatic rings usually quench more DPPH<sup>•</sup> molecules on molar basis. Molar ratios of quenched DPPH<sup>•</sup>/antioxidant can simply be determined by measuring the absorbance of remaining DPPH<sup>•</sup> in the solutions where known concentrations of antioxidant were added and subtracting those absorbances from the control where only DPPH<sup>•</sup> is present. In order to avoid experimental artefacts, antioxidants should not exceed the concentration needed to quench more than 70% of DPPH<sup>•</sup>. Molar extinction coefficient of the reduced form (DPPH<sub>2</sub>) at 517 nm is less than 5% of that of the oxidised form (DPPH<sup>•</sup>). If the contribution of the oxidised form is neglected, decrease of absorbance is proportional to the concentration of the quenched DPPH<sup>•</sup>. In the literature different molar extinction coefficients for DPPH<sup>•</sup> in methanol or ethanol can be found, ranging from 10900 to 12500 l x mol<sup>-1</sup> x cm<sup>-1</sup> (Molyneux, 2004). We have used a value of 12000 l x mol<sup>-1</sup> x cm<sup>-1</sup> in our calculations. Concentration of quenched DPPH<sup>•</sup> (mol/l) was determined as follows:

$$c_{\text{DPPH quenched}} = \frac{A_{\text{DPPH}} - A_{\text{DPPH + antioxidant}}}{12000 \text{ l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1} \cdot 1 \text{ cm}} \quad (1)$$

**Total phenols** content were determined by Folin-Ciocalteu colourimetric method (Singleton and Rossi, 1965). The absorbance was measured at 765 nm after incubation of the solution (diluted sample of red wine or undiluted sample of white wine, sodium carbonate and Folin-Ciocalteu reagent) for 2 hours. Concentration is expressed as mg of gallic acid equivalent per litre of wine determined from a standard calibration curve.

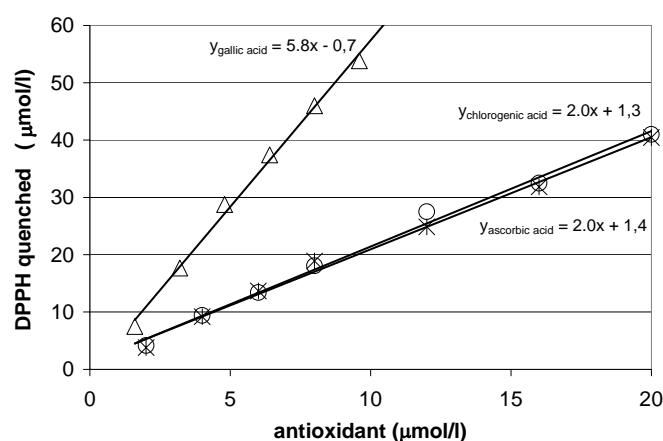
**Flavonoids** content were determined by proposed method (Ough and Amerine, 1988) using formaldehyde for their precipitation. The flavonoids content, expressed in mg GAE/l, was calculated as the differences between total phenols and non-flavonoids. Total **tannins** determination is based on the precipitation of tannin with methylcellulose and ammonium sulphate. The tannins content, expressed in mg GAE/l, was calculated as the differences between total phenols and non-tannins.

Total **anthocyanins** (free and bleached by SO<sub>2</sub> form) are determined using pH variation procedures. Concentration was calculated as the difference in absorbance measured at 520 nm in 1 cm optical path, multiplying by factor 388 (Ribéreau-Gayon *et al.*, 2000).

For **colour-related parameters** a direct measurement of absorbance (A) of the filtered wines (0.45 µm) at 420, 520, and 620 nm was carried out using spectrophotometer. Colour intensity was calculated as the sum of 420, 520, and 620 nm absorbances (referred to as 1 cm optical path lengths); colour hue was quantified as the ration between the absorbances at 420 and 520 nm (Glories, 1984).

## Results and discussion

From the plots where the concentration of quenched DPPH<sup>•</sup> is depicted as a function of the antioxidant added, molar ratio of quenched DPPH<sup>•</sup>/antioxidant can be graphically determined, as the slope of the line fitted to experimental data (figure 1).



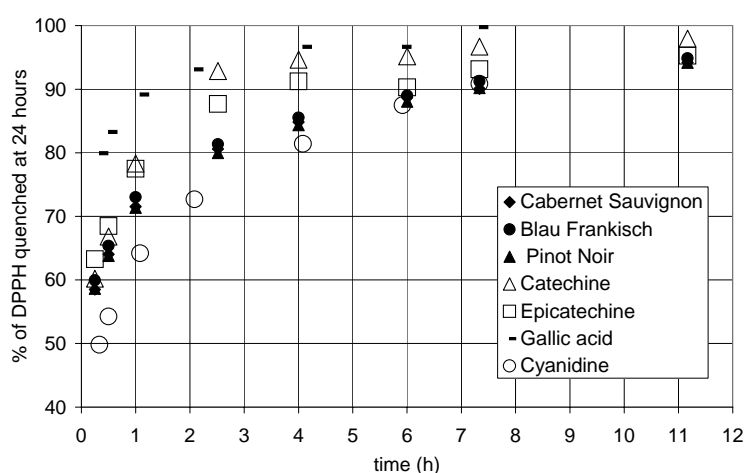
**Figure 1: Relationship between molar concentration of DPPH<sup>•</sup> and different antioxidants**

Determined molar ratios of DPPH<sup>•</sup>/gallic acid = 5.8; DPPH<sup>•</sup>/chlorogenic acid = 2.0 and DPPH<sup>•</sup>/ascorbic acid = 2.0 are in accordance with the literature data (Brand-Williams *et al.*, 1995; Villaño *et al.*, 2007).

Wine is a complex mixture of polyphenols and discrete values for individual polyphenols cannot be determined by such a plot. Nevertheless the linear relationship between quenched DPPH<sup>•</sup> and antioxidants is still present, indicating that AOP of complex mixture as wine can simply be expressed as the amount of wine needed to quench certain amount of moles of DPPH<sup>•</sup>. Multiplying the volume of the solution in the assay with  $c_{\text{DPPH quenched}}$ , moles are obtained. From the dilution factor of the original wine and known amount of that solution added to the assay, volume of the original wine needed to quench calculated amount of DPPH<sup>•</sup> can be obtained. AOP of wine can than be expressed as a ratio of those two values as moles of DPPH<sup>•</sup> quenched per litre of wine (mol/l). Wines with higher concentration of antioxidants will have to be more diluted before the assay, to quench the same amount of DPPH<sup>•</sup>. Dilution factor will be higher and amount of the original wine in the test therefore lower. Smaller volumes will result in higher determined AOP of that wine.

**Kinetics of DPPH reaction with antioxidants.** Determination of AOP with DPPH<sup>•</sup> is considered to be relatively fast method. Some antioxidants, as ascorbic acid, react very fast and equilibrium (absorbance remains constant) is obtained in a few seconds. Some other antioxidants react relatively slow and equilibrium is reached only within hours (Brand-Williams *et al.*, 1995).

Kinetics of DPPH<sup>•</sup> quenching with certain major wine polyphenols, gallic acid, which is often used as polyphenol standard in the analysis of wine antioxidants, and three red wines (vintage 2004) was determined in the time span of 24 hours. As DPPH<sup>•</sup> is gradually degraded also in the absence of antioxidants, absorbance of both, control samples with DPPH<sup>•</sup> and samples where antioxidants or wines were added, was measured within the course of experiments and values subtracted. Results shown in figure (2) are expressed as a % of maximal difference in certain assay ( $A_{\text{DPPH}} - A_{\text{DPPH}+\text{antioxidant}}$ ), which was determined after the 24 hours of incubation.

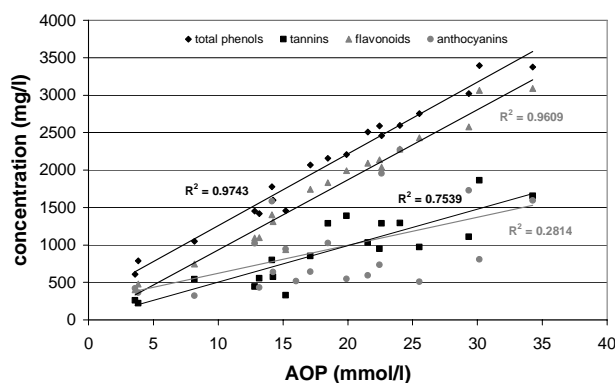


**Figure 2: Time dependence of DPPH quenched (%) in selected wines and standards**

Polyphenols and wines tested show relatively slow kinetics. The slowest kinetics is observed with cyanidine, a representative of anthocyanins, where less than 75% of DPPH<sup>•</sup> is quenched in two hours, which is considered to be relatively long incubation time for the assay. The flavanols, (–)-epicatechine and (+)-catechine, show faster kinetics, as within two hours slightly less than 90% of DPPH<sup>•</sup> is quenched. All three wines show very similar kinetic behaviour, which is still relatively slow. Only around 80% of DPPH<sup>•</sup> is quenched after 2 hours, meaning that AOP of such wines is underestimated for at least 20%, if measured in two hours or less after addition of wine. This is a significant drawback of the DPPH method, as time needed for incubation is relatively long compared to the classical Folin-Ciocalteu assay, where total signal is obtained within this period.

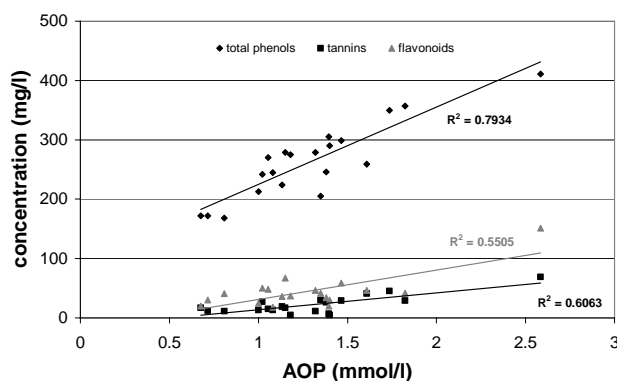
### **Correlations of AOP and phenolic compounds in the investigated wines**

Content of total phenols, tannins, flavonoids and antocyanins determined in 20 red wines of different vintages varied from 607 to 3920 mg/l, 222 to 2649 mg/l, 400 to 3457 mg/l and from 323 to 2270 mg/l, respectively. Colour intensity and hue was determined from 2.7 to 13.9 and from 0.48 to 0.90, respectively. The AOP was in wide range from 3.6 to 34.3 mmol/l. The highest correlation between AOP and phenolic compounds was determined for the content of total phenols (0.974), followed by flavonoids (0.961), tannins (0.754) and anthocyanins (0.281) as shown in figure 3.



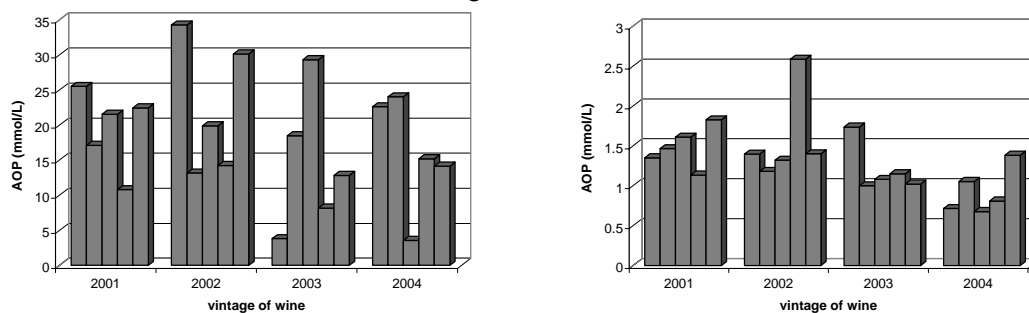
**Figure 3: Correlation of phenolic compounds and antioxidant potential for different varieties and vintages of red wines**

In white wines the content of phenolic compounds was significantly lower and varied for total phenols, tannins and flavonoids from 168 to 311 mg/l, 5-69 mg/l and from 16 to 170 mg/L, respectively. Colour intensity (absorbance at 420 nm) varied from 0.04 to 0.19, while the AOP was in the range from 0.68 to 2.58 mmol/l. The correlation between AOP and phenolic compounds was in the changed order of precedence and also not as tight as in the red wines: total phenols (0.793), tannins (0.606) and flavonoids (0.551) as shown in figure 4.



**Figure 4: Correlation of phenolic compounds and antioxidant potential for different varieties and vintages of white wines**

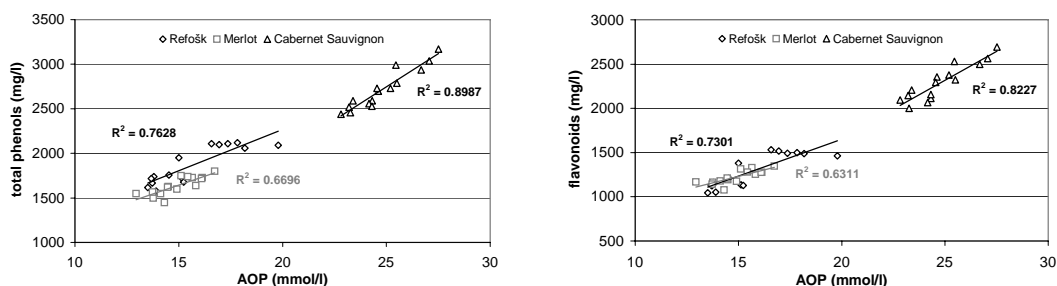
Slovenian red wines had significantly higher content of total and individual phenols, and consequently a higher AOP in comparison to white wines irrespective of the vintage. Certain older wines (2001-2003) of different varieties investigated had higher AOP than younger (2004) what was more obvious in white wines than in red ones as shown on figure 5.



**Figure 5: Antioxidant potential of red (left) and white (right) wines with regard to vintage**

We determined the similar composition of phenolics in red wines of vintage 2004 than in the wines of the previous three years. Total phenols were in the range 1448-3169 mg/l, tannins

429-1455 mg/l, flavonoids 1044-2692 mg/l and anthocyanins 226-715 mg/l, while the colour intensity and hue were much closer and varied from 12.3 to 21.9, and 0.43 to 0.52, respectively. The AOP was in the relatively narrow range from 13.0 to 27.5 mmol/l. The best overall correlations between AOP and phenolics were established for the flavonoids (0.969) and total phenols (0.954) with the comparable  $R^2$ . Correlations for tannins (0.802) and anthocyanins (0.396) were worse, yet still better than for red wines of different vintages. Overall correlations mentioned above were referring to all 45 wines, however when we looked on the particular variety (figure 6), we established that higher correlations were observed total phenols than for flavonoids, as expected. Phenolic composition of red wines and AOP is shown in table 1.



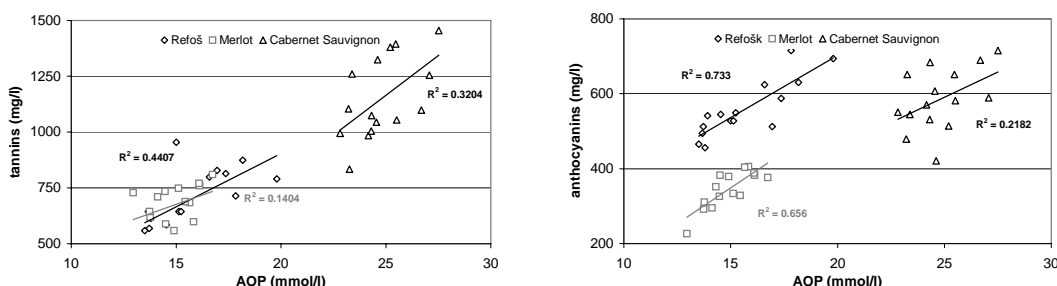
**Figure 6: Correlation of phenolic compounds (left) and flavonoids (right) with antioxidant potential in red wines of different varieties of vintage 2004**

Phenolic composition of individual variety had the influence on determined correlations of anthocyanins and tannins with AOP. As shown in figure 7, the anthocyanins were better correlated in wine of variety Refošk ( $R^2=0.733$ ), which could be ascribed to the higher ratio of anthocyanins/total phenols (30.0%) in comparison to Merlot (21.1%) and Cabernet Sauvignon (21.6%), where the value of  $R^2$  was lower (0.656 and 0.218, respectively). Despite high overall correlation of tannins with AOP (0.802), for merlot only  $R^2 = 0.140$ , was determined.

**Table 1: The average value and standard deviation of antioxidant potential and content of phenolics in investigated red wines**

| Parameter (unit)     | Refošk      | Merlot      | Cabernet Sauvignon |
|----------------------|-------------|-------------|--------------------|
| AOP (mmol/l)         | 15.7 ± 1.98 | 14.9 ± 1.06 | 24.8 ± 1.44        |
| Total phenols (mg/l) | 1868 ± 210  | 1637 ± 103  | 2717 ± 226         |
| Flavonoids (mg/l)    | 1291 ± 193  | 1225 ± 78   | 2294 ± 207         |
| Tannins (mg/l)       | 698 ± 142   | 674 ± 78    | 1151 ± 182         |
| Anthocyanins (mg/l)  | 559 ± 77    | 346 ± 50    | 586 ± 83           |

Determined molar ratios of DPPH<sup>•</sup>/total phenols were  $1.43 \pm 0.06$  for Cabernet sauvignon,  $1.55 \pm 0.07$  for Merlot and  $1.56 \pm 0.05$  for Refošk.



**Figure 7: Correlation of tannins (left) and anthocyanins (right) with antioxidant potential in red wines of different varieties of vintage 2004**

Relatively tight correlations between the Folin-Ciocalteu assay of wine phenolics and determined AOP of wines with DPPH method indicate that not much additional information is gained when both methods are applied. Relatively slow kinetics observed for the red wines with DPPH method, where only 80% of the signal is gained after two hours, which is time needed for the Folin-Ciocalteu assay, does not fully prove the use of DPPH<sup>•</sup> as a substance that can be applied for the fast determination of AOP of wine.

## Literature

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