

Changes of Selected Antioxidant Parameters of Red Wines during Maturation

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Abstract

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The effects of red wine maturation on the contents of selected parameters of bioactive compounds in wine were determined. Samples of Alibernet, Cabernet Sauvignon, and Torysa wines were studied by spectrophotometric analysis after 3, 7, 11, 19, and 28 months of aging and the selected parameter content was statistically evaluated. Statistically highly significant changes in total polyphenol content, total anthocyanin content, antioxidant activity, and wine colour density were found depending on the aging according to the used statistical analyses. The obtained results can be used for the optimisation of the wine aging process and they allow producers to time the optimal date of wine release onto the market, depending on the desired content parameters.

Keywords: aging; anthocyanins; Cabernet Sauvignon; polyphenols; *Vitis vinifera* L.

Epidemiological and experimental studies have revealed that a mild up to moderate drinking of wine, particularly red wine, attenuates the cardiovascular, cerebrovascular, and peripheral vascular risk. Both wines and grapes can attenuate cardiac diseases such as atherosclerosis and ischemic heart disease. Wine polyphenols could reinforce the endogenous antioxidant system and thereby diminish oxidative damage (BERTELLI & DIPAK 2009).

Antioxidants neutralise free radicals and thus protect the organism from the oxidative damage of lipids, proteins, and nucleic acids (GUERERRO *et al.* 2010). Anthocyanins also inhibit the malignant cell survival (COOKE 2005). Antioxidant activity is currently considered to be one of the most significant characteristics of red wines and it is associated with the content of polyphenols such as flavonoids, phenolic acids, stilbenes, coumarins, and lignoids (CIMINO *et al.* 2007; RADOVANOVIC *et al.* 2009). Antioxidant activity is dependent mainly on total phenolics. The total phenolic contents of red wine samples exhibited a good correlation ($P < 0.01$) with antioxidant properties (BÜYÜKTUNCEL *et*

al. 2014). Any variation in the vinification process that would introduce a difference in the phenolic composition of wine should influence its antioxidant activity (AGATONOVIC-KUSTRIN *et al.* 2015). Grape variety largely determines such components as phenolic content, antioxidant activity, and mineral content with the exception of vitamins (KONDRASHOV *et al.* 2009).

Vine breeders and wine producers can try to produce grape products and wines with the highest content of phenolic antioxidants and antioxidant activity, as well as with the optimal organoleptic properties (LACHMAN *et al.* 2009). Phenolic compounds are among the most important quality parameters of wines, and they contribute to organoleptic characteristics such as colour, astringency, and bitterness (PAIXAO *et al.* 2007); they are significantly influenced by the environment, and therefore they are a good reflection of terroir (LAMPÍŘ & PAVLOUŠEK 2013). Consequently, numerous papers have focused on the determination of antioxidant capacities of wines and correlation with their polyphenol content (BÜYÜKTUNCEL *et al.* 2014). Anthocyanins, synthesised via

the flavonoid pathway, are a class of crucial phenolic compounds which are fundamentally responsible for the red colour of grapes and wines (HE *et al.* 2010). The proportion and amount of each anthocyanin is greatly influenced by cultivar type and viticultural conditions. This obviously influences both the hue and the colour stability, which are directly affected by the hydroxylation and methylation pattern of the B ring of the anthocyanins (JACKSON 2014). The individual anthocyanins are not very stable, and they are susceptible to degradation. This is affected by numerous factors such as pH, temperature, structure, light, oxygen, solvents, enzymes, and metallic ions (TSENG *et al.* 2006). The process of maceration that occurs during fermentation has an influence on the extraction of grape polyphenolics into the wine, increasing their content and diminishing the colour (IVANOVA *et al.* 2012). The finished wines showed concentrations of tannins and anthocyanins that generally mirrored observed differences in phenolic concentrations of the skin and seeds (CASASSA *et al.* 2015).

During aging, the wine colour changes from bright red to deep red and then a reddish-brown hue. This is mainly attributed to various reactions, such as anthocyanin and other phenolic compounds, acetaldehyde-mediated condensation, co-pigmentation, and self-association (PUŠKÁŠ & UROŠ 2012).

Wine aging is a well-known winemaking practice for the improvement of red wine quality and results in changes in phenolic, colour, and carbonyl components (GARCÍA-CARPINTERO *et al.* 2012). Aging is the main factor influencing the antioxidant activity and total polyphenol (TP) content of wines (LACHMAN *et al.* 2009). The wine with lower phenolic levels is more susceptible to oxidation (JACKSON 2014). During wine storage the phenolic composition is continuously modified because of several interactions involving these phenolic compounds (SUN *et al.* 2011). Phenolic compounds are also the major targets of hydroperoxyl radicals. However, sulphur dioxide in wine can scavenge hydrogen peroxide quickly and irreversibly under winemaking conditions, thereby leaving the organic fraction of wine protected (ELIAS & WATERHOUSE 2010). During aging, carbonyls are well known to take part in wine reactions, with potential benefits to the colour of red wines (ELIAS *et al.* 2008).

The aim of the research was to find the influence of red wine maturation on the content of selected parameters of bioactive substances in wine – total polyphenol content, total anthocyanin content, antioxidant activity, and wine colour density.

MATERIAL AND METHODS

Wine samples. All evaluated wines come from the south-Slovakian wine-growing region from the village of Strekov. Harvest date for Torysa and Cabernet Sauvignon was on October 6, 2012 and for Alibernet on October 20, 2012 with sugar content of 25°Bx. Grapes were healthy, without rot damage. Following harvest, the grapes were destemmed, crushed, and slightly sulphured. The mash was inoculated with selected yeasts of *Saccharomyces cerevisiae* enriched with special yeast nutrition. The mash was macerated for 10 days, followed by closed membrane pneumatic pressing. Young wine was treated after malolactic fermentation with the bacteria *Oenococcus oeni*, slightly sulphured, and let for maturation in stainless steel tanks of 1500 l in volume, from which also samples of take-offs were made. Analyses were done in January 2013, May 2013, September 2013, May 2014, and February 2015. The content of each sample was 500 ml and it was taken from the middle part of the tank. In all tested wines the alcohol content ranged from 14.3% to 14.7%, total acidity was adjusted to 6.5 g/l and reducing sugar content ranged from 0 g/l to 0.3 g/l. Content of free SO₂ was measured every two months and adjusted to 30 mg/l. The pH of wines ranged from 3.2 to 3.4.

Chemicals and instruments. All analysed parameters – total polyphenol content, total anthocyanin content, antioxidant activity, and wine colour density in wines were determined by UV/VIS spectrophotometry (Shimadzu UV/VIS-1240; Shimadzu, Tokyo, Japan). The chemicals used for all analyses were: Folin-Ciocalteu reagent, monohydrate of gallic acid p.a., anhydrous sodium carbonate p.a., citric acid p.a., dodecahydrate of disodium hydrogen phosphate, 35% hydrochloric acid p.a., ethanol p.a., methanol p.a., 1,1-diphenyl-1-picrylhydrazyl (DPPH) radical p.a., and Trolox (pure).

Sample analysis. Total polyphenol content (TPC) was determined by a modified method of SINGLETON and ROSSI (1965). Absorbance of sample solutions was measured against a blank at 765 nm. TPC in wines was calculated as the amount of gallic acid equivalent (GAE) in mg per 1 l of wine.

Total anthocyanin content (TAC) was assessed by a modified pH differential method of LAPORNIK *et al.* (2005). TAC was calculated from the difference in absorbance values between both solutions and expressed as the amount of anthocyanins in mg per 1 l of wine.

Antioxidant activity (AA) was assessed by the method of BRAND-WILLIAMS *et al.* (1995) using

the DPPH (1,1-diphenyl-1-picrylhydrazyl) radical. Absorbance was read at 515.6 nm and antioxidant effectiveness was expressed as % inhibition of DPPH and also as Trolox equivalent calculated from the calibration curve.

Wine colour density (WCD) was assessed by the method of SUDRAUD (1958) as the sum of the absorbances at 420 and 520 nm. The absorbance of wine samples was measured for the 0.2 cm path length of glass cells.

All chemical analyses were performed as four parallels. The samples of wines were filtered through paper filter (Filtrak, Grade 390) before analysis.

Statistical analysis. The analysis of variance (ANOVA), multifactor analysis of variance (MANOVA), and the multiple range test were done using the Statgraphic Centurion XVII software (StatPoint Inc., Warrenton, USA).

RESULTS AND DISCUSSION

Very highly significant differences were found between the evaluated varieties by the *LSD* test (at $P < 0.001$) (Figure 1), when average values of all observed parameters were taken in account.

Statistically highly significant changes in the total polyphenol content, total anthocyanin content, antioxidant activity, and wine colour density depending on aging were also revealed. The analysis has shown that in almost all observed parameters the measured values were significantly higher in the varieties with dark-coloured skin and also flesh, in contrast to AA of the Cabernet Sauvignon variety, which has the dark-coloured skin, but the flesh is translucent. This phenomenon can be explained by the substances responsible for AA, which are largely presented mainly

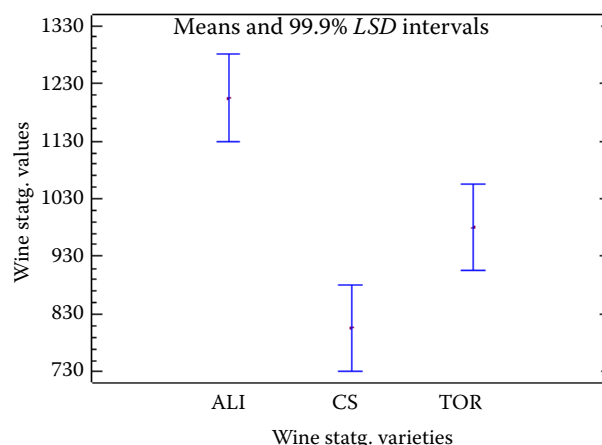


Figure 1. Differences in the studied varieties in dependence on the evaluated characteristics – total polyphenol content, total anthocyanin content, antioxidant activity, and wine colour density* after 28 months of aging ALI – cv. Alibernet, CS – cv. Cabernet Sauvignon, TOR – cv. Torysa; *average values of chosen parameters

in the skin, which was also confirmed by LACHMAN *et al.* (2009), where the decrease was caused mainly by condensation reactions of flavanols.

Total polyphenol content (TPC). The highest average TPC was found in Alibernet variety, with an average value of 3766.4 mg/l, followed by Torysa variety with 3151.8 mg/l. The lowest TPC was measured in Cabernet Sauvignon, 2581.4 mg/l, which was by about 31% lower content than in Alibernet variety. Overall, the highest value was measured in cv. Alibernet with 3859.1 mg/l in the third month of aging. The absolute lowest value, 2332.4 mg/l, was measured in Cabernet Sauvignon after 28 months of aging. The values of TPC ranged in our case in the interval from 2332.4 mg/l to 3859.1 mg/l.

A significant statistical difference between aging and TPC in all three varieties in each measurement

Table 1. Statistical analyses of total polyphenol content (mg/l) and antioxidant activity (% DDPH) in observed wines (2012)

Wine age	Total polyphenol			Antioxidant activity		
	Alibernet	Cabernet Sauvignon	Torysa	Alibernet	Cabernet Sauvignon	Torysa
3 months	3859 ± 23 ^e	2635 ± 23 ^c	3606 ± 47 ^d	72.5 ± 2.1 ^c	81.5 ± 1.3 ^c	70.4 ± 2.0 ^c
7 months	3662 ± 73 ^a	2545 ± 37 ^b	3333 ± 73 ^c	71.2 ± 0.7 ^c	81.1 ± 0.5 ^c	69.2 ± 0.8 ^c
11 months	3723 ± 52 ^b	2646 ± 52 ^d	3331 ± 38 ^c	70.0 ± 1.2 ^c	81.7 ± 1.5 ^c	55.9 ± 1.1 ^a
19 months	3825 ± 23 ^d	2749 ± 69 ^e	2749 ± 23 ^b	66.4 ± 0.4 ^b	76.5 ± 0.5 ^b	59.8 ± 0.6 ^b
28 months	3763 ± 38 ^c	2332 ± 38 ^a	2740 ± 25 ^a	44.4 ± 1.0 ^a	76.4 ± 0.5 ^b	54.3 ± 0.9 ^a

Means ± standard deviation; column values with different lowercase letters in superscript are significantly different at $P < 0.001$ by *LSD* in ANOVA (Statgraphic); % DDPH – antioxidant effectiveness expressed as % inhibition of 1.1-diphenyl-1-picrylhydrazyl radical

period was found after 7 and 11 months of aging, except for cv. Torysa, where no change was observed (Table 1). Therefore it can be concluded that the TPC continuously varied during wine aging.

No statistically significant relationship between TPC and AA was proved in any variety during the evaluation period. Therefore it can be concluded, if the TPC decreases during aging, the TAC need not decrease regularly. According to LACHMAN *et al.* (2009), a less significant relationship between antioxidant properties and total phenolics and only a weak relationship between antioxidant activity and total anthocyanin content were also reported. On the other hand, following the results of KONDRASHOV *et al.* (2009), a significant positive relationship between TAC and TPC values was indicated in 6 Cabernet Sauvignon and 4 Merlot wines. The relatively constant total polyphenol content in our results is in accordance with results of LACHMAN *et al.* (2009) suggesting the relevance of qualitative changes of phenolics. By evaluation of TPC and TAC relationships only a weak dependence was confirmed in Torysa variety ($R^2 = 0.7427$), while for the varieties Alibernet and Cabernet Sauvignon no conclusive correlation between the tested TPC and TAC values was found. When assessing a dependence between TPC and WCD, only a weak dependence was also found in Torysa variety ($R^2 = 0.7781$). For the varieties Alibernet and Cabernet Sauvignon no conclusive relationship was indicated. As suggested, the aging is the main factor influencing the AA and TPC content of wines, which was also confirmed by BALGA *et al.* (2014) and GALANAKIS *et al.* (2015).

Antioxidant activity (AA). According to statistical analyses a very highly significant difference in the aging impact on AA changes was observed after 11 months of aging between cvs Alibernet and Cabernet Sauvignon and after 7 months in cv. Torysa (Table 1). Until that time no significant differences in AA were noted (homogeneous groups were created in the framework of each evaluated variety).

From the practical point of view no AA change was revealed in cvs Alibernet and Cabernet Sauvignon during 11 months of aging and in cv. Torysa for 7 months of aging. After that time large differences in changes in AA content were determined. According to KALLITHRAKA *et al.* (2006) a low and statistically insignificant correlation between anti-radical activity determined by DPPH assay and TAC was also revealed. It is assumed that the antioxidant capacity of wine is strongly correlated with the type

of phenolic compounds present in wines. That points out the predominant role of phenols in antioxidant activity (BÜYÜKTUNCEL *et al.* 2014).

Total anthocyanin content (TAC). The highest average value of TAC was reached in cv. Alibernet (983.96 mg/l), the lowest in Cabernet Sauvignon (562.82 mg/l). The highest TAC value was measured in cv. Alibernet (1520 mg/l) after 3 months of aging, the lowest TAC value was measured in cv. Torysa (208.5 mg/l) after 19 months of aging. BALGA *et al.* (2014) reported that the TAC in their study ranged from 376.97 mg/l to 998.47 mg/l. The impact of aging on TAC was significantly demonstrated for all three varieties. The TAC values were relatively different at the beginning of the experiment (Figure 2), but the TAC values were relatively equal (about 300 mg/l) in all chosen varieties at the end of the experiment, after 28 months of aging.

According to the correlation analysis of TAC and WCD a statistically significant dependence for variety Cabernet Sauvignon ($R^2 = 0.846$) was confirmed, whereby for varieties Alibernet and Torysa no conclusive relationship between TAC and WCD was found. Therefore it can also be stated that in the variety with dark-coloured skin and translucent flesh a decrease in TAC was also followed by a decrease of WCD, while for varieties with the dark skin and flesh this relationship was not confirmed.

Based on statistical evaluation, significant differences between aging and TAC content in cv. Alibernet were found in each measured period. It can be said that the content of TAC during wine storage was continuously decreasing. Similar results were also obtained in Cabernet Sauvignon and a linear decrease of TAC was confirmed. In cv. Torysa a significant TAC change was also observed with a linear decrease of TAC after 3, 7, and 11 months of aging. After 28 months of aging a significant increase of TAC was found.

During wine maturation and aging, the concentration of monomeric anthocyanins in red wines declines constantly, especially of the acylated anthocyanins (GÓMEZ-MÍGUEZ *et al.* 2007). In spite of the steady decline observed in the anthocyanin pigments during winemaking and maturation of wine in this period of time the loss of anthocyanins is due rather to their transformation into derived pigments than to degradation (GARCÍA-PUENTE RIVAS *et al.* 2005). The TAC reduction was attributed to condensation reactions, which was also confirmed by MONAGAS *et al.* (2006). They stated that the occurrence of

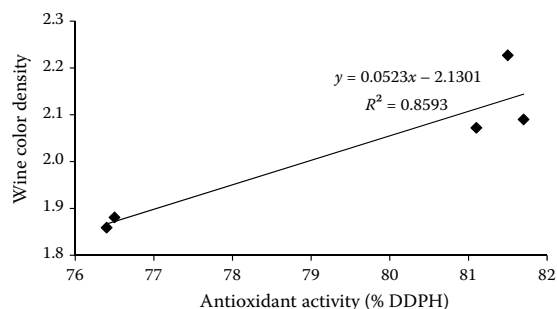


Figure 2. Changes in total anthocyanin content (TAC) during 28 months of aging

condensation reactions during aging in bottles was connected with disappearance of monomeric anthocyanins, increase of catechins and procyanidins and decrease of low-polymerised polyphenols.

Wine colour density (WCD). Wine colour density was calculated as the sum of $A_{420\text{nm}}$ and $A_{520\text{nm}}$. The highest average values of WCD were measured in cv. Alibernet (4.5368 a.u.) and the lowest in cv. Cabernet Sauvignon (2.0258 a.u.). The absolute maximum value of WCD was measured in cv. Alibernet (5.584 a.u.) after 3 months of aging. The absolute lowest value was measured in cv. Cabernet Sauvignon (1.859 a.u.) after 28 months of aging.

The impact of aging on WCD in all three varieties was not confirmed, no statistically significant difference was found out in any variety. The influence of aging did not change the WCD, all evaluated groups were homogeneous.

CONCLUSIONS

Very highly significant differences between the evaluated varieties were found out by the LSD test (at $P < 0.001$) was, when average values of all observed parameters were taken in account. Statistically highly significant changes in the content of TPC, TCA, TAC, and WCD depending on the aging time were also observed.

The analysis has shown that almost all measured parameters were significantly higher in varieties with the dark-coloured skin and flesh, besides the antioxidant activity in Cabernet Sauvignon variety, which has the dark-coloured skin but the flesh is translucent. This phenomenon can be explained by the substances responsible for antioxidant activity, which are not present in the flesh, but mainly in the skin.

The obtained results can be used in the optimisation process of wine aging and allow producers

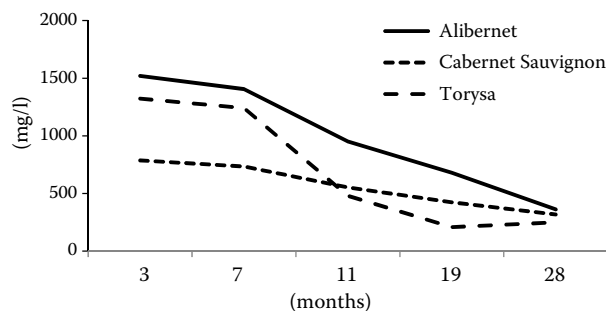


Figure 3. Changes in wine colour density during 28 months of aging in the varieties Alibernet 2012, Cabernet Sauvignon 2012, and Torysa 2012

to time the optimal date of wine release onto the market, depending on the desired content parameters. For example, the best consumption wine age for the varieties Alibernet and Cabernet Sauvignon in relation to maximum antioxidant activity is until 11 months and in variety Torysa until 7 months, after this time the antioxidant activity is significantly lower. In relation to the total anthocyanin content the best way is to consume the wines as soon as possible. In relation to the total polyphenol content of variety Torysa is the wine that keeps its polyphenol content significantly unchanged until 11 months.

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