ESTABLISHING OF CHROMATIC AND ANTIOXIDANT CHARACTERISTICS OF SOME RED WINES FROM MINIS VINEYARD

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Abstract: Were characterized in relation to the antioxidant and chromatic properties the red wines processing in Minis vineyard from tree grapes varieties: Cabernet Sauvignon, Merlot and Pinot Noir harvested in 2004. It was determined the total antioxidant capacity, total polyphenols content and monomeric anthocyanins amount, as well as the main chromatic characteristics for young and aged in bottle for 12 months red wines. Total antioxidant capacity it was determined by FRAP method (expressed as mM Fe^{2+}/L). The polyphenols content it was determined by Folin-Ciocalteu method (expressed such as mM gallic acid/L). The monomeric anthocyanins it was evaluated by differential pH method and the chromatic properties by standardized method and by Glories method. It was obtained that the chromatic and antioxidant characteristics of red wines present the distinctive values in rapport with wine's evolution stage and grapes variety. By ageing, the antioxidant capacity decreases due to diminishing of total polyphenols content, especially monomeric forms of anthocyanins. The highest values for antioxidant capacity were founding in young red wine (particularly from Cabernet Sauvignon grape's variety). The antioxidant capacity was highly correlated with the polyphenols amount (R= 0.9731).

Keywords: *red wines, chromatic parameters, polyphenols, monomeric anthocyanins, total antioxidant capacity*

INTRODUCTION

It was established that a medium wine consumption, especially red wine, brings in diet some substances that have protective role against cardiovascular diseases [1, 2]. Epidemiological studies consistently show a decrease in mortality risk associated with wine intake and red wine in particular. Red wine is a rich source of phenolic compounds, many of them having antioxidant properties. Some of the most noteworthy are catechin, epicatechin, quercetin, and some of the proanthocyanidin dimers and trimers [3, 4]. Red wine consumption significantly reduces the risk of coronary heart disease, except at high levels of intake. Phenols are a general class of aromatic compounds that contain one or more hydroxyl groups. Their presence in grapes is well known but still a subject of study, whereas much of their physiological function is yet unknown. In winemaking, an understanding of the phenolic make up of grapes is crucial in that phenolic compounds dictate the color, astringency, bitterness, and taste of the wine produced. It may be tell, that the polyphenols contribute to the definition of organoleptic quality, to the food-hygiene and to the wines particularization [5 - 7].
Quality and quantity of polyphenols are related to the grape variety, vineyard, weather, soil, viticultural treatments, and wine making technologies. The level and quality of red wine's coloring can be determined based on spectrophotometric and chromatographic analyses. Through wines ageing in the bottle, due to oxidation and condensation processes, it was diminished the monomer polyphenols content (which has the antioxidant properties); during the time of wine storage in bottle take place the structural changes, and one of the most studied of those changes concern red wine color evolution, called "*wine ageing*". For an aged wine, it has been demonstrated that initially present grape pigments slowly turn into new more stable red pigments. That phenomenon goes on for weeks, months and years [8 - 11].
At international level it was monitoring the evolution of chromatic characteristics during wine's evolution, it was studied the influence of different factors (biological, biochemical, technological) regarding wine quality [8 - 10], but there are only few researches about the antioxidant characteristics and correlation that can established between antioxidant and chromatic characteristics of red wines. It was established that there is a strong correlation between polyphenols content and wine antioxidant capacity [12 - 14]; also, it was established that origin place (vineyard), grape's variety and evolution stage have a remarkable influence about these parameters [12].
In this paper are presented and discussed the results obtained in the case of chromatic and antioxidant characteristics determination for some red wines from Minis vineyard. On the basis of obtained results it was established some correlations between chromatic

and antioxidant characteristics, which helps to estimate of red wines quality and authenticity.

MATERIAL AND METHOD

The sample for analysis

In this study were analyzed red wines obtained in Minis wine making centers in 2004 harvest year. The selected wines were obtained from Cabernet Sauvignon (CS), Pinot Noir (PN) and Merlot (M) grapes varieties.
Two red wines categories were analysed: young and aged in bottle for one year.
For these wines it was determined the chromatic parameters, total antioxidant capacity, total polyphenols content and monomeric anthocyanins or free anthocyanins content, as well as the chromatic characteristics.

Reagent and equipment

All chemicals and reagents were analytical grade or pure quality purchased from Merck, Fluka, Sigma and Chimopar. For reagents preparation and for dilution was used bidistilled water. Absorbance determination for FRAP and total polyphenol content was made using Spectrophotometer Specord 205 by Analitik Jena.

Determination of Total Antioxidant Capacity (Adaptation of FRAP method) [15]

It was used: acetate buffer, 300 mM/L, pH = 3.6 (3.1 g $CH_3COONa\cdot3H_2O$ and 16 mL conc. acetic acid per 1L off buffer solution); TPTZ (2,4,6-tripyridyl-s-triazine) solution 10 mM/L (0.31 g TPTZ in 100 mL HCl 40 mM) - prepared freshly before of utilization; $FeCl_3$ solution 20 mM/L (0.54 g $FeCl_3\cdot6H_2O$ in 100 mL bidistilled water) - prepared freshly before of utilization; FRAP working solution (25 mL acetate buffer, 2.5 mL TPTZ solution, 2.5 mL $FeCl_3$ solution) - prepared freshly always utilization; Standard solution - Mohr salt 1 mM/L: 0.393 g $(NH_4)_2\cdot Fe(SO_4)_2\cdot6H_2O$ in 1000 mM bidistilled water.
Aqueous solution of known Fe concentration was used for calibration, in a range of 0.05 – 0.4 mM/L. For the preparation of calibration curve 0.5 mL aliquot of 0.05; 0.1; 0.15; 0.20; 0.25; 0.30; 0.35; 0.40 μM Fe^{2+}/mL aqueous as Mohr salt solution were mixed with 2.5 mL FRAP working solution. FRAP reagent was used as blank.
1 mL from diluted wines in bidistilled water 1:100 (v/v) was mixed with the same reagents as described above, and after 10 min absorbance was read at λ = 593 nm.
The total antioxidant capacity (TAC) in wine samples in Fe (II) equivalents was calculated.
Calibration curve ecuation was: $Y = -0.02404 + 3.41362\cdot X$. Correlation coefficient (R) for calibration curve was 0.9991.

Determination of phenolic compounds [16]

The content of total polyphenolic compounds (P) was determined by Folin-Ciocalteu method [4]. It was used: Folin-Ciocalteu′s phenol reagent solution 1:10 (v/v) in bidistilled water, Na_2CO_3 solution 7.5%; Standard solution - gallic acid 10 mM/L: 1.8755 g acid galic in 1000 mL ethanol 96% (v/v).

For the preparation of calibration curve 0.5 mL aliquot of 0.05; 0.1; 0.2; 0.3; 0.4; 0.5; 0.6 μM/mL aqueous gallic acid solution were mixed with 2.5 mL Folin-Ciocalteu reagent and 2.0 mL Na_2CO_3 solution. 1 mL from diluted 1:50 (v/v) wines was mixed with the same reagents as described above, and absorption was read after 2 h at $\lambda = 750$ nm versus a blank solution (0.5 ml bidistilled water, 2.5 mL Folin-Ciocalteu′s phenol reagent and 2.0 mL Na_2CO_3 solution). Total content of polyphenols in wines in gallic acid equivalents (GAE) was calculated.

Calibration curve ecuation was: $Y = -0.10164 + 1.92242 \cdot X$. Correlation coefficient (R) for calibration curve was 0.9980.

Determination of total monomeric anthocyanins [17]

The total monomeric anthocyanins (AM), or free anthocyanins was spectrometric determined by differential pH method. Anthocyanins pigments undergo reversible structural transformations with a change in pH manifested by strikingly different absorbance spectra. The colored oxonium form predominates at pH 1.0 and the colorless hemiketal form at pH 4.5. The pH-differential method is based on this reaction, and permits accurate and rapid measurement of the total monomeric anthocyanins, even in the presence of polymerized degraded pigments and other interfering compounds. The pigments content was calculated as cyanidin-3-glucoside [5].

Chromatic properties evaluation

Were established by standardized method A and B [18] and by Glories method [19].

By using standard method A were obtained the transmittances at the following length wave: 445, 495, 550, 625 in ratio with blank sample (distillated water). With determined values will be determinate chromatic tristimulus X, Y, Z for point coordination (x, y) and from in colors spectral representation was obtained length wave dominant (λd). In the basis of this λd can be established the hue of wine color. In conformity with this method brown wines have λd between 585 – 598 nm. For red wines λd is between 599 – 650 nm, for red–scarlet λd is 540 – 585 nm [18].

Standard methods B permits color expression through intensity and hue. Color intensity, IC, is based on the relation $IC = A_{420} + A_{520}$, where: A_{420} – absorption at 420 nm and A_{520} – absorption at 520 nm. The hue is expressed on angle α value ($tg\ \alpha = A_{520} - A_{420}$) as follows: the wines with α between 0˚ – 51˚ are red wines, the wines with α between 52 – 80˚ are red-scarlet wines; the wines with negative angles are brown wines [18].

Glories method [19] is much more exact, has in calculation the contribution of blue-mauve pigments to the total color of wines. This method establishes other parameters for red wine color evaluation: color intensity (IC*), color tonality (T), and the contribution of each color (red, yellow and blue) to wine color. These parameters are calculated using the formulas:

$$IC^{*}=A_{420}+A_{520}+A_{620}; \quad T=\frac{A_{420}}{A_{520}};$$

$$420\% = \frac{A_{420}}{IC^{*}}\times 100; \quad 520\% = \frac{A_{520}}{IC^{*}}\times 100; \quad 620\% = \frac{A_{620}}{IC^{*}}\times 100$$

where 420%, 520% and 620% represent respectively the contribution of yellow, red and black color to the total wine color.

RESULTS AND DISCUSSIONS

In table 1 are presented the chromatic parameters obtained by application of A and B standardized methods. The results obtained by using A and B methods are in perfect accordance: the wine shade identified by these methods is the same.

For all young red wines the red shades were obtained. Through ageing the color of two wines (Cabernet Sauvignon and Merlot) became brown.

The data from table 2 show the chromatic structure obtained by Glories method. By this method application, it was determined the percent with that each pigment category (yellow, red and blue) contribute to the total wine color. On the base of each pigments category contribution to the wine color it can be appreciated the wine shade.

From obtained data it was observed that the pigments structure reflects exactly the chromatic features of analyzed red wines.

In general case, for wine with red shade, the red pigment class takes part in higher percent (over 40%) to underline the wine color; for wine with brown shade, the red pigments percent decreases (under 40%) being accompanied by the increasing of yellow-orange pigments percent. For aged red wine the yellow pigments percent increase and the red pigments percent decrease, the both pigments classes are more equilibrate in aged wine. The class of blue pigments takes part at total color of wine in a percentage of 10 – 16%.

Although all analyzed samples were taken from the same harvest year, 2004, the wines Cabernet Sauvignon and Merlot from Minis vineyard get the brown color after ageing for 1 year. It was observed that the chromatic structure considerably varies during wine's ageing: the percent of yellow pigments which take part to the red wine total color formation is higher than red pigments percent.

From the results showed in tables 1 and 2 it was observed that by aging of wines appears a tendency of decreasing for absorbance at λ = 520 nm and of increasing for absorbance at λ = 420 nm and 620 nm.

This tendency of young red wines to have a highly absorption at 520 nm it was verified for all analyzed wine types.

The highest values for absorbance at 520 nm were registered for Cabernet Sauvignon wine.

As a rule, for red aged wines, the absorbance at 520 nm decreases while the absorbance at 420 nm and 620 nm increases, due to the shift from monomeric to polymeric anthocyanins [10].
IC and IC* have the same direction of evolution (the values decreased for aged wines). Through ageing, the tonality increases concomitantly with the decreasing of color intensity for all grapes varieties.

Table 1. *Chromatic properties of red wine determined by standardized A and B methods*

Grape variety		Method A		Method B					
		λd	Wine color	A_{420}	A_{520}	IC	tg α	α	Wine color
Minis[1]	CS	597	brown	4.2213	3.7246	7.9459	-0.4967	-26.41	brown
	M	594	brown	3.9941	3.4219	7.416	-0.5722	-29.78	brown
	PN	604	red	3.1517	3.16	6.3117	0.0083	0.48	red
Minis[2]	CS	619	red	3.6681	4.6139	8.282	0.9458	43.40	red
	M	612	red	3.4905	4.258	7.7485	0.7675	37.51	red
	PN	614	red	3.1073	3.8243	6.9316	0.717	35.64	red

[1] - aged red wines
[2] - young red wines

Table 2. *Chromatic properties of red wine determined by Glories method*

Grape variety		A_{420}	A_{520}	A_{620}	IC*	T	Chromatic structure		
							% yellow pigments	% red pigments	% blue pigments
Minis[1]	CS	4.2213	3.7246	1.5787	9.5246	1.13	44.32	39.11	16.57
	M	3.9941	3.4219	1.4003	8.8163	1.17	45.30	38.81	15.88
	PN	3.1517	3.16	0.9006	7.2123	1.00	43.70	43.81	12.49
Minis[2]	CS	3.6681	4.6139	1.5209	9.8029	0.80	37.42	47.07	15.51
	M	3.4905	4.258	1.3061	9.0546	0.82	38.55	47.03	14.42
	PN	3.1073	3.8243	0.8304	7.762	0.81	40.03	49.27	10.70

[1] - aged red wines
[2] - young red wines

For all cases, the highest values of color intensity, and implicit, the smallest values for tonality, were registered for the young red wine from Cabernet Sauvignon grapes. The smallest values for IC* were observed for aged red wine. These results are in accordance with data obtained in other studies [8, 10].
The tonality present values in the range 0.8 – 1.0 for wines with red shade and the values at least 1.0 for wines with brown shade. In the case of Minis vineyard, for values of tonality at least 1.0, the wines are aged and for values small than 1.0 the wines are young.

In table 3 are presented the values of total antioxidant capacity, total polyphenols content and total monomeric anthocyanins. From these results it was observed that, by ageing for one year, the total antioxidant capacity decreases with 13 – 29%. In all cases, the polyphenols content has the same direction of evolution with total antioxidant capacity. Particularly, it was obtained a different evolution depending on the grapes variety used for wine making. The highest values for antioxidant capacity were found in young red wine (particularly from Cabernet Sauvignon grape's variety).

Table 3. *The values of polyphenols, total antioxidant capacity and monomeric anthocyanins for analyzed wines*

Grape variety		Polyphenols (P) [mM acid gallic/L]	Total antioxidant capacity (TAC) [mM Fe^{2+}/L]	Monomeric anthocyanins (AM) [mg/L]
Minis [1]	CS	22.51	27.11	101.89
	M	19.14	25.28	80.12
	PN	15.91	19.17	71.98
Minis [2]	CS	29.49	35.09	190.15
	M	26.03	29.14	175.23
	PN	22.41	26.93	136.16

[1] - aged red wines
[2] - young red wines

Note: Each value from table 3 represents the average of three determinations.

Through ageing the polyphenols content, the total antioxidant capacity and the total monomeric anthocyanins content decreased. The highest values of monomeric anthocyanins were found in red wine from Cabernet Sauvignon grape variety, followed by red wines from Merlot and Pinot Noir grape varieties. For young wines, the monomeric anthocyanins amounts were in the range of 136 – 190 mg/L. Through ageing for one year, the content of monomeric anthocyanins decreases until 71 – 101 mg/L. The values of anthocyanins content are very different related to grape's variety and wine evolution stage.

After processing the obtained data with Origin Program there were established the linear correlations of the followed type: TAC = f(P) with R=0.9731 and AM = f(P) with R = 0.9491, different from the data obtained for dependence TAC = f(IC*) with R = 0.8754 and TAC = f(AM) with R = 0.8114. The linear dependence established between TAC and P is graphically represented in figure 1.

From these correlations, results that the decreasing of total antioxidant capacity by ageing (evaluated by FPAP method) is highly correlated to diminishing of polyphenols contents, particularly on decreasing of monomeric or free anthocyanins. These results have the role to confirm the resulted obtained in international researches [13, 14]. The obtained results must be analyzed in relation with evolution stage of wine and grapes variety, these factors being crucially for antioxidant and chromatic features of red wine color underlining.

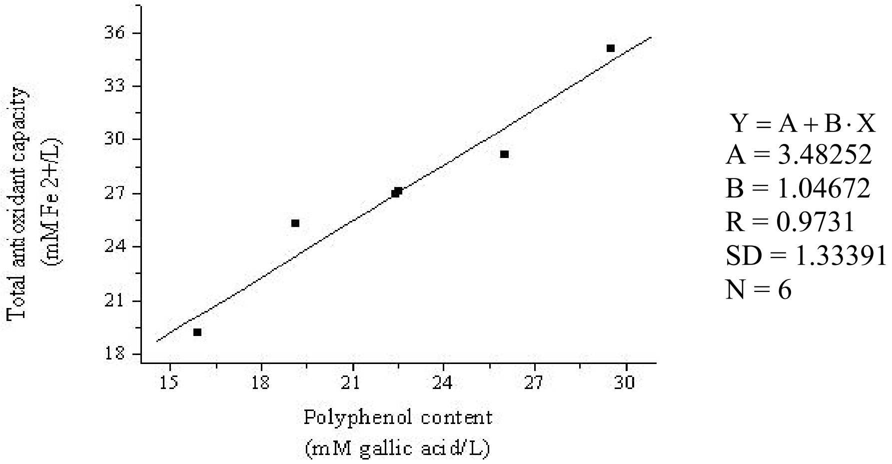

Figure 1. *The linear dependence established between total antioxidant capacity and polyphenols content*

CONCLUSIONS

For all young red wines were obtained the red shades. Through ageing the color of two wines (Cabernet Sauvignon and Merlot) became brown. The pigments structure, evaluated by Glories method reflects exactly the chromatic features of analyzed red wines obtained by A and B standardized methods. Through ageing, the tonality increases concomitantly with the decreasing of color intensity for all grapes varieties. The highest values of color intensity and implicit, the smallest values for tonality were registered for red wine from Cabernet Sauvignon grape variety and the smallest values for Pinot Noir. For red aged wines, the absorbance at 520 nm decreases while the absorbance at 420 nm and 620 nm increases. Total antioxidant capacity, polyphenols and anthocyanins content showed different values in relation to with grape's variety and evolution stage of red wine. Through ageing the total antioxidant capacity, total polyphenols content and total monomeric anthocyanins decrease. Decreasing of total antioxidant capacity is due to diminishing of total polyphenols content, especially monomeric forms of anthocyanins. The polyphenols content has the same evolution direction of with total antioxidant capacity. Between these parameters it was observed a linear correlation (R=0.9731).

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