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THE ANALYSIS OF CHROMATIC AND ANTIOXIDANT CHARACTERISTICS OF SOME RED WINES FROM RECAS VINEYARD

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Abstracts

Were characterized in relation to the antioxidant and chromatic properties the red wine processing in Recas vineyard from black grapes harvested in 2004 and 2005. 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 acid gallic/L). The monomeric anthocyanins it was evaluated by differential pH method and the chromatic properties by standardized method and by Glories method. 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 decrease due to diminishing of total polyphenols content, especially monomeric anthocyanins forms. 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= 0.9774).

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 (Ricardo, 1991; Burns, 2000; Tedesco, 2001; Berke, 2003). Epidemiological studies consistently show a decrease in mortality risk associated with wine intake and red wine in particular. Red wines are a rich source of phenolic compounds, many of which have antioxidant properties. Some of the most noteworthy are catechin, epicatechin, quercetin, and some of the proanthocyanidin dimers and trimers (Kaner, 1994; Campos, 1996; Teissedre, 2000).

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 (Mazza, 1999; Landrault, 2001).

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 (Pérez-Magariño, 2004; Tsai, 2004).

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 (Ollala, 1996; Pascu, 2005), but there are only few researches about the antioxidant characteristics and correlation that can established between antioxidant and chromatic characteristics of red wines (Monagas, 2006). It was established that there is a strong correlation between polyphenols content and wine antioxidant capacity (Pelegrini, 2000; Landrault, 2001; Tsai, 2004); also, it was established that origin place (vineyard), grape's variety and evolution stage have a remarkable influence about these parameters (Ollala, 1996, Landrault, 2001).

In this paper it was presented and discussed the results obtained in the case of chromatic and antioxidant characteristics determination for some red wines from Recas 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.

Experimental

In this study were analyzed red wines obtained in Recas vineyard from black grapes harvested in 2004 and 2005. The selected wines were obtained from Cabernet Sauvignon, Pinot Noir and Merlot 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. Absorption 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)* (Benzie and Strain, 1996). It was used: acetate buffer, 300 mM/L, pH = 3.6 (3.1 g $CH_3COONa \cdot 3H_2O$ 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 20mM/L (0.54 g $FeCl_3 \cdot 6H_2O$ 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 1mM/L: 0,393 g $(NH_4)_2 \cdot Fe(SO_4)_2 \cdot 6H_2O$ 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. absorption was read at λ= 593 nm. The total antioxidant capacity (TAC) in wine samples in Fe (II) equivalents was calculated. Calibration curve equation was: $Y = -0.02404 + 3.41362 \cdot X$. Correlation coefficient (R) for calibration curve was 0.9991.

Determination of phenolic compounds (Singleton and Rossi, 1965). 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 10mM/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 λ= 750 nm in rapport with 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 equation was: $Y = -0.10164 + 1.92242 \cdot X$. Correlation coefficient (R) for calibration curve was 0.9980.

Determination of total monomeric anthocyanins (Giusti, 2000). 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.

Chromatic properties were established by standardized method A and B (Culegere de standarde române comentate, 1987) and by Glories method (Glories, 1984).

Results and Discussions

In the table 1 it was presented the chromatic parameters obtained by application of A and B standardized methods. It can be observed that 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 it was obtained the red shades. Through ageing for one year the color of all analyzed samples was unchanged (red), indifferently of harvested year. The data from the 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 aged red wines the yellow pigments percent and the blue pigments percent increases and the red pigments percent decreases. The chromatic structure is more equilibrated in aged wines. This thing is due to the different stabilization of color by ageing. The color stabilization can be attributed the diminishing of anthocyanins content and formation of combinations between tannin and anthocyanins, polymer compounds, and intermolecular associations which have the red color. (Pascu, 2005). 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 the table 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 520nm decreases while the absorbance at 420nm and 620nm increases, due to the shift from monomeric to polymeric anthocyanins (Pascu, 2005). 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. 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 study (Pelegrini, 2000; Pascu, 2005). The tonality present values in the range 0.7-0.8 for young red wines and more 0.9 for aged wines. In the case of Recas vineyard, for values of tonality at least 0.9 the wines are aged and for value small than 0.9 the wines are young.

In the 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). Through ageing the polyphenols content, the total antioxidant capacity and the total monomeric anthocyanins content decreased. The highest values of monomeric anthocyanins were founding 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, harvest year 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=0.9774, TAC=f(IC*) with R=0.9428 and IC*=f(P) with R=0.91853. 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 with diminishing of polyphenols contents. Also, between TAC and IC* was established of linear dependence and P is highly correlated with IC*. The decreasing of color intensity has that result the diminishing of TAC, a small polyphenols content is accompanied of the low color intensity. These results have the role to confirm the resulted obtained in international researches (Tsai, 2004). These results must be analyzed in relation with evolution stage and harvest year of wine and grapes variety, these factors being crucially for antioxidant and chromatic features of red wine color underlining.

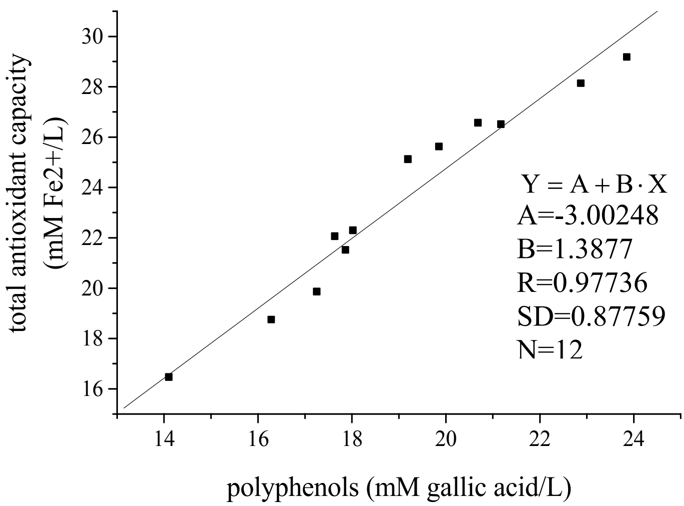

Figure 1. The linear dependence of total antioxidant capacity against polyphenols contents

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}	I.C	tgα	α	Wine color
2004									
Recas[1]	Cabernet Sauvignon	617	red	3.1881	3.3040	6.4921	0.1159	6.61	red
	Merlot	614	red	3.0071	3.0241	6.0312	0.017	0.97	red
	Pinot Noir	613	red	2.6147	2.9141	5.5288	0.2994	16.67	red
Recas[2]	Cabernet Sauvignon	631	red	3.1106	3.9651	7.0757	0.8545	40.51	red
	Merlot	625	red	2.7510	3.5448	6.2958	0.7938	38.44	red
	Pinot Noir	622	red	2.4622	3.3872	5.8494	0.925	42.77	Red
2005									
Recas[1]	Cabernet Sauvignon	614	red	3.3147	3.4013	6.7160	0.0866	4,95	red
	Merlot	620	red	3.1473	3.2071	6.3544	0.0598	3.421	red
	Pinot Noir	624	red	2.7795	3.0147	5.7942	0.2352	13.24	red
Recas[2]	Cabernet Sauvignon	635	red	3.2147	3.8492	7.0639	0.6345	32.40	red
	Merlot	631	red	3.0079	3.6122	6.6201	0.6043	31.14	red
	Pinot Noir	626	red	2.6789	3.3971	6.076	0.7182	35.69	red

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

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

Grape variety		A_{420}	A_{520}	A_{620}	I.C*	T	Chromatic structure		
							% yellow pigments	% Red pigments	% Blue pigments
2004									
Recas[1]	Cabernet Sauvignon	3.1881	3.3040	0.7210	7.3131	0.96	44.19	45.81	10.00
	Merlot	3.0071	3.0241	0.7716	6.8028	0.99	44.20	44.45	11.35
	Pinot Noir	2.6147	2.9141	0.6413	6.1701	0.90	42.38	47.23	10.39
Recas[2]	Cabernet Sauvignon	3.1106	3.9651	0.6547	7.7304	0.78	40.24	51.29	8.47
	Merlot	2.7510	3.5448	0.7244	7.0202	0.78	39.19	50.49	10.32
	Pinot Noir	2.4622	3.3872	0.6123	6.4617	0.73	38.10	52.42	9.48
2005									
Recas[1]	Cabernet Sauvignon	3.3147	3.4013	0.8146	7.6306	0.97	43.44	44.57	10.68
	Merlot	3.1473	3.2071	0.7103	7.0647	0.98	44.55	45.40	10.05
	Pinot Noir	2.7795	3.0147	0.5894	6.3836	0.92	43.54	47.23	9.23
Recas[2]	Cabernet Sauvignon	3.2147	3.8492	0.7208	7.7847	0.84	41.30	49.45	9.26
	Merlot	3.0079	3.6122	0.5873	7.2074	0.83	41.73	50.12	8.15
	Pinot Noir	2.6789	3.3971	0.4283	6.5043	0.79	41.19	52.23	6.58

[1] – aged red wines; [2] - young red wine

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

Grape variety		Total polyphenols (mM acid gallic/L)	Antioxidant capacity (mM Fe^{2+}/L)	Monomeric anthocyanins (mg/L)
2004				
Recas[1]	Cabernet Sauvignon	21.19	26.48	99.05
	Merlot	18.04	22.27	83.13
	Pinot Noir	14.12	16.44	78.21
Recas[2]	Cabernet-Sauvignon	23.87	29.15	175.23
	Merlot	19.21	25.09	140.22
	Pinot Noir	17.27	19.83	115.10
2005				
Recas[1]	Cabernet Sauvignon	19.87	25.59	81.42
	Merlot	17.65	22.03	76.43
	Pinot Noir	16.30	18.72	71.29
Recas[2]	Cabernet Sauvignon	22.89	28.11	152.23
	Merlot	20.70	26.54	135.29
	Pinot Noir	17.88	21.49	120.47

[1] *– aged red wines*
[2] *- young red wine*

Conclusions

For all young red wines it was obtained the red shades. Through ageing the color of wines remains unchanged. 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 520nm decreases while the absorbance at 420nm and 620nm increases. Total antioxidant capacity, polyphenols and anthocyanins content showed different values in relation to with grape's variety, harvest year and evolution stage of red wine. Through ageing the total antioxidant capacity, polyphenols content and total monomeric anthocyanins decrease. The polyphenols content has the same evolution direction of with total antioxidant capacity (between these parameters it was observed a linear correlation R=0.9774). Between TAC and IC* was established of linear dependence and P is highly correlated with IC*. The decreasing of color intensity has that result the diminishing of TAC, a small polyphenols content is accompanied of the low color intensity.

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