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ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT OF SOME ROMANIAN RED WINES

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ABSTRACT. The positive health effects of the consumption of wines are due to their phenolic constituents with significant antioxidant activity. The antioxidant activity evaluation of is of major interest to compare the potential antioxidant value of different food, to evaluate their antioxidant intake. The aims of this study were to evaluate the influence of wine variety, vintage year and winery on the atioxidant characteristics of some Romanian red wines. "Fetească Neagră", "Cabernet Sauvignon" and "Merlot" varieties of red wines, produced in three different vintage year by different Romanian wineries were investigated in terms of antioxidant activity and total phenolic content, by UV-VIS spectrophotometry. The results of this study showed that the wine variety, the vintage year and the winerie affected the antioxidant activity and the phenolic content of wines. Also, linear correlations were found between the antioxidant activity of wines determined by DPPH and ABTS assays, and the antioxidant activity and the total phenolic content of wines.

Keywords: *red wine, antioxidant activity, phenolic content, spectrophotometry*

INTRODUCTION

The positive health effects of the consumption of wines are due to their phenolic constituents with significant antioxidant activity [1]. Moreover, phenolic compounds are one of the most important quality parameters of wine, since they contribute to their organoleptic characteristics, particulary color, astringency and bitterness [2,3]. Phenolic compounds present different antioxidant activities, so their presence or absence and their concentration variations will influence the antioxidant activity of wines. Studies have shown that the polyphenol content and the antioxidant activity of wines depend on many factors, such as: grape varieties, soil, climatic conditions, [4], oenological techniques, ageing process etc. [5].

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There are many analytical methods for assessing the antioxidant activity of foodstuffs but unfortunately, none of these is a standardized one. The most frequently used methods are spectrophotometric that employ chromogenic compounds of radical nature [6-8]. They are mostly based on oxidizing-reducing reactions, phenolic compounds functioning as reduction agents, being able to donate hydrogen radical or electron [9]. In these assays, free radicals react with antioxidant compounds leading to the decrease of radical concentration, and then absorbance measurements are performed. Folin-Ciocalteu method is commonly used only for assessment of the total phenolic content (the sum of phenolic compounds) of wines [10].

The aims of this study were to evaluate the influence of wine variety, vintage year and winery on the antioxidant characteristics and total phenolic content of some Romanian red wines, produced in three different vintage years by different Romanian wineries. Moreover, the correlation between these characteristic was done.

RESULTS AND DISCUSSION

The antioxidant activities of the analyzed wines, expressed as mg vit C/mL of wine (in case of DPPH assay) and μmol Trolox/mL of wine (when ABTS assay was used) and the total phenolic content (mg of gallic acid/mL of wine) were calculated according the calibration curves equations (Table 1) and were presented in Table 2.

Table 1. Calibration curves equations

Assay	Equation	r^2
DPPH	$A_0 - A_s = 8.121 \times$vit C (mg/mL) – 0.123	0.9920
ABTS	$A_0 - A_s = 4.290 \times$Trolox (μmol/mL) – 2.890	0.9702
TPC	$A_s = 0.009 \times$gallic acid (μg/mL) + 0.125	0.9841

A_0 - absorbance of the bank sample
A_s - absorbance of the analyzed wine

Table 2. The antioxidant activities and the total phenolic content of the analyzed red wines

Wine	Antioxidant activity		TPC (mg gallic acid/mL wine)
	mg vit C/mL wine	μmol Trolox/mL wine	
MPM2006	2.839±0.002	1.302±0.001	3.533±0.022
MPM2007	2.779±0.001	1.277±0.001	3.048±0.003
MPM2008	2.741±0.002	1.338±0.001	3.789±0.078
MR2006	2.039±0.001	1.080±0.001	2.850±0.003
MR2007	2.453±0.001	1.179±0.003	2.050±0.012

Wine	Antioxidant activity		TPC (mg gallic acid/mL wine)
	mg vit C/mL wine	µmol Trolox/mL wine	
MR2008	2.338±0.001	1.188±0.001	2.250±0.078
MSC2006	1.850±0.009	1.024±0.001	1.939±0.032
MSC2007	2.135±0.020	1.225±0.001	3.091±0.087
MSC2008	1.886±0.003	1.120±0.001	1.933±0.012
CSO2006	2.102±0.001	1.155±0.001	2.315±0.045
CSO2007	1.894±0.001	1.123±0.002	2.105±0.012
CSO2008	2.033±0.004	1.159±0.001	2.176±0.034
CSURL2006	2.407±0.009	1.238±0.001	2.478±0.101
CSURL2007	2.051±0.006	1.082±0.001	1.904±0.008
CSURL2008	2.213±0.007	1.245±0.001	2.309±0.036
CSVM2006	2.713±0.003	1.330±0.001	4.220±0.038
CSVM2007	2.724±0.002	1.344±0.001	4.029±0.093
CSVM2008	2.522±0.004	1.301±0.001	2.909±0.103
FNDM2006	1.905±0.004	1.169±0.003	1.796±0.006
FNDM2007	1.816±0.001	1.114±0.002	1.781±0.059
FNDM2008	2.455±0.002	1.276±0.001	2.676±0.056
FNSC2006	1.626±0.007	1.057±0.001	1.220±0.014
FNSC2007	2.104±0.015	1.207±0.003	1.989±0.033
FNSC2008	1.985±0.004	1.162±0.001	1.948±0.008
FNURL2006	1.705±0.001	1.070±0.001	1.454±0.008
FNURL2007	2.094±0.002	1.194±0.001	2.018±0.006
FNURL2008	2.016±0.001	1.202±0.002	2.504±0.064

Data expressed as mean ± standard deviation
M-Merlot, CS-Cabernet Sauvignon, FN-Fetească Neagră
PM-Prince Mircea, R-Recaş, SC-SERVE Ceptura, O-Oprişor, URL-Urlaţi, VM-Vânju-Mare, DM-Dealu Mare

The antioxidant activity of wines, determined using DPPH free radical varies between 1.626 mg vit C/mL wine for FNSC2006 and 2.839 mg vit C/mL wine for MPM 2006. Comparing the antioxidant activity of wines from different grape varieties, determined by DPPH method, the results show that, regardless of vintage year and wineries, wines FN have the lowest antioxidant activity ranging from 1.626 mg vit C/mL wine (FNSC2006) to 2.455 mg vit C/mL wine (FNDM2008). Results also show that the antioxidant activities of the same variety of wines are usually influenced by vineyard and the vintage year:

- for M variety, regardless vintage year, wines from PM vineyard have the highest antioxidant activity, followed by wines from R and SC vineyards. Also, MPM wines possess the highest antioxidant activities among all analyzed wines;

- for CS variety, the antioxidant activities of VM wines > URL wines > O wines;
- among the wines of the FN variety, the antioxidant activities of FN wines variety do not differ significantly from one vineyard to another and from one year to another. FNSC2006 wine possesses the lowest antioxidant activity (1.626 mg vit C/mL wine), and FNDM2008 wine has the highest antioxidant activity (2.455 mg vit C/mL wine).

When $ABTS^{\bullet+}$ free radical was used, the antioxidant activity of wines ranged between 1.024 µmol Trolox/mL wine (MSC2006) and 1.344 µmol Trolox/mL wine (CSVM2007).

The antioxidant activities evaluated by DPPH and ABTS assays were correlated. Good correlation coefficient were found between the antioxidant activities of M wines (r^2 = 0.8089, y=0.6216 + 0.224 x), CS wines (r^2 = 0.8853, y=0.5557 + 0.289 x) and FN wines (r^2 = 0.9399, y=0.6185 + 0.2276 x), determined by DPPH and ABTS assays. Also, a good correlation between the antioxidant content determined with DPPH and $ABTS^{\bullet+}$ was obtained when all wines were considered (Figure 1).

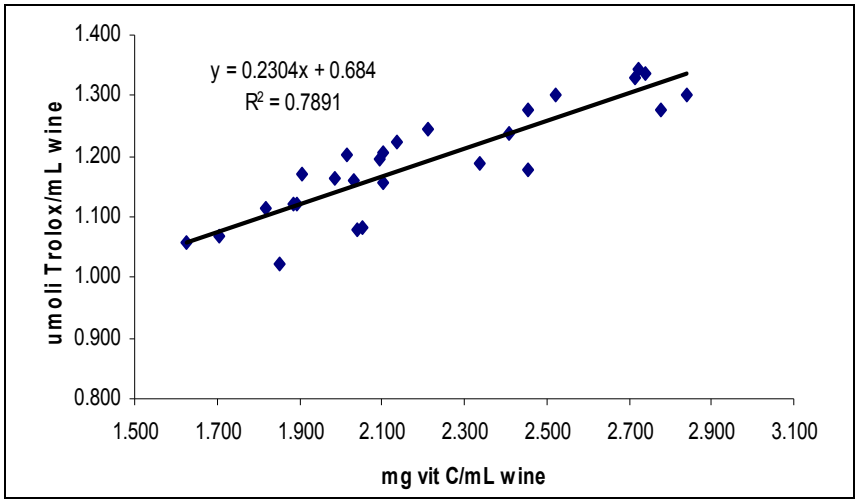

Figure 1. The correlation between the antioxidant activities of wines evaluated by DPPH and ABTS assays

As literature shows [7, 11, 12], differences between the antioxidant activities of wines evaluated with DPPH and ABTS assays may be attributable to the fact that every individual phenolic compound contained in wine causes a different response to each specific radical used in the assay.

DPPH and $ABTS^{\bullet+}$ radicals have different stereochemical structures and different paths of genesis and thus they give, after the reaction with the antioxidants, qualitatively different responses to the inactivation of each radical [12]. The obtained results support the finding of Prior and Cao [13], that different methods should be used in parallel for the estimation of the antioxidant activity of samples.

Polyphenols are important constituents of wines, significantly contributing to their antioxidant activity. The results presented in Table 1 show that the phenolic content of the analyzed wines widely vary, ranging between 1.220-4.220 mg gallic acid/mL wine. The total phenolic content was correlated with the antioxidant activity determined both by DPPH and ABTS assays, considering each sort of wine as well as all wines (Table 3).

Table 3. Correlation coefficients between the TPC and the antioxidant activity determined by DPPH and ABTS assays

	r^2	
	DPPH assay	ABTS assay
M wines (n=9)	0.4935	0.7653
CS wines (n=9)	0.8370	0.6327
FN wines (n=9)	0.8061	0.8609
Total of wines (n=27)	0.7245	0.6773

The results show that when the sorts of wines are considered, the TPC of M wines are better correlated with the antioxidant activities determined by the ABTS assay. In case of FN wines, the TPC are almost similarly correlated with their antioxidant content investigated by both ABTS and DPPH assays. The TPC of CS wines are better correlated with the antioxidant activity evaluated with DPPH; phenolic compounds contribute with 83.70% to the antioxidant activity of CS wines. Results also show that, when all wines are considered, a moderate correlation between the TPC values and the antioxidant activities determined by DPPH and ABTS assays is obtained. However, the lack of strong correlation between these assays support the idea that different sorts of wines contain different classes of phenolic compounds with different behavior against DPPH and $ABTS^{\bullet+}$ radicals.

CONCLUSIONS

DPPH and ABTS assays can be applied for the determination of the antioxidant activity of red wines. Differences between the antioxidant activities of wines evaluated with DPPH and ABTS assays may be due to the different behavior of every individual phenolic compound from wine against

different radicals used in the assays. MPM wines and CSVM wines present high antioxidant activities and total phenolic contents. The antioxidant activity and the phenolic content of the analyzed wines depend on the wine variety, the vintage year.

EXPERIMENTAL PART

Material and methods

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2-azinobis (3-ethylbenzthiazolin-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ascorbic acid (vit C), gallic acid Folin-Ciocalteu's reagent and $K_2S_2O_8$ were purchased from Merck (Darmstadt, Germany). Na_2CO_3 and methanol were obtained from Reactivul Bucureşti (Bucharest, Romania) and Chimopar (Bucharest, Romania), respectively. All chemicals and solvent were analytical grade.

Sample collection

Twenty-seven Romanian red wines of "Fetească Neagră" (FN), "Cabernet Sauvignon" (CS) and "Merlot" (M) varieties, produced in 2006, 2007 and 2008 vintage years were chosen from commercial wineries: Oprişor – O, Urlaţi –URL, Vânju Mare – VM, Prince Mircea – PM, Recaş – R, SERVE Ceptura – SC, Dealu Mare – DM. They were used directly, without any treatment.

Instrumental

The absorbance measurements were performed with T80+ UV/VIS Spectrophotometer (PG-Instruments).

Free radical scavenging assays

DPPH assay

0.25 mL of wine appropriately diluted with distilled water were added to 3.0 mL of 0.09 mg/mL methanolic solution of DPPH. After 20 min, the absorbance of the reaction mixture was measured at 517 nm. Each wine was analyzed triplicate. Calibration was performed in the 0.150 - 0.275 mg vit C/mL concentration range, following the same procedure. The obtained calibration curve was used for antioxidant activity calculation.

ABTS assay

The first step in ABTS assay was the generation of the $ABTS^{\bullet+}$ cationic radical. The $ABTS^{\bullet+}$ was obtained from the reaction of 7 mmol/L ABTS diammonium salt solution with 2.45 mmol/L $K_2S_2O_8$ solution added 1:1 (v/v). The reaction mixture was incubated for 24 h at room temperature, in dark place. Then, 0.5 mL of wine were added to 3 mL $ABTS^{\bullet+}$ solution. Absorbance measurements

were done after 20 min, at 734 nm. Calibration was performed in the 1.10 - 1.35 µmol of Trolox/ mL concentration range, following the same procedure. The obtained calibration curve was used for antioxidant activity calculation.

Total phenolic content

Total phenolic content (TPC) of wine samples were determined according to Folin-Ciocalteu method, using gallic acid as standard. 1.5mL of Folin-Ciocalteu reagent (0.2 mol/L) was added at 0.3 mL of wine appropriately diluted with distilled water. The reaction mixture was allowed to react for 5 min and then, 1.2 mL of 0.7mol/L Na_2CO_3 solution were added. Sample was incubated at room temperature, in dark place for 120 min, and its absorbance was measured at 760 nm. The experiment was performed three times for each wine. Calibration was performed in the 0 - 100 µg gallic acid/ mL concentration range, following the same procedure. The obtained calibration curve was used for antioxidant activity calculation.

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