STUDY ON FREE RADICAL SCAVENGING AND TOTAL POLYPHENOLS OF SOME ROMANIAN WINES

Maria CIOROI[1], Lucian Tudor MIRON[1], Gabriela RAPEANU[1], Nicoleta STANCIUC[1], Elena POSTOLACHE[1], Constanta VICOL[1]

[1]Dunarea de Jos University, 111 Domneasca Street, 800201, Galati
maria.cioroi@ugal.ro

Abstract: *Phenolic compounds contribute to organoleptic characteristics such as colour, astringency and bitterness of grapes and wines. Some relevant analytical parameters of the wines produced by R & D Station for Viticulture and Wine Bujoru and Regional Laboratory for quality control and hygiene wine Odobesti, are given in this paper.*
Free radical scavenging activity was determined for several red and white wines from different grape varieties cultivated in the south-east of Romania vineyards region. The free radical scavenging activity of the wine samples was analysed by using the 2,2,-diphenyl-1-picrylhydrazyl (DPPH) assay. The absorbance was read at 515 nm.
The amount of polyphenols in selected wines was investigated by means of UV–VIS methods according to the Folin–Ciocalteu colorimetric method.
The mean concentration of total polyphenols (TPs) content of the analyzed red wines was 639.5205 mg/L gallic acid equivalent and for the white wines was 180.5233 mg/L gallic acid equivalent. The investigated red wines showed antioxidant behaviour in the range of 89.85% to 76.60%. The free radical scavenging activity of the wines was correlated to total polyphenol compounds content. The polyphenolic compounds content in the investigated wines contributes to their antioxidant activity.
The hypothesis of a protective effect of red wine is supported by obtained results which show that the red wines with higher amounts of polyphenols possess higher antioxidant properties.

Keyword: *antioxidant capacity, DPPH, gallic acid, red wines, white wines, Tg Bujor, Odobesti*

Introduction

Polyphenols are an important group of secondary plant compounds. They play an important role in the plant itself as protective agents against fungus attack and UV irradiation. According to several epidemiological studies they have positive effects on human health: they decrease the incidence of coronary heart disease, reduce platelet aggregation, and provide antioxidative and anti-carcinogenic protection [1, 2]. From a winemaking perspective, polyphenols serve as antioxidants, colouring components (especially anthocyanins in red wine), and contribute to the mouthfeel and bitterness of wine [3, 4].

Phenolic compounds represent one of the most important quality parameters of grapes and wines since they contribute to organoleptic characteristics such as colour, astringency and bitterness.

Recent studies indicate that consumption of a small amount of red wine on a regular basis reduces the risk of coronary heart disease and atherosclerosis, and this benefit is attributed to the antioxidant properties of the polyphenol compounds [5].

Several studies suggest that some phenolic compounds, when ingested at high concentrations, may exhibit certain roles in

carcinogenicity, genotoxicity, thyroid toxicity and can be connected with oestrogenic activity [6, 7].
The aim of this study was to determine the free radical scavenging activity of several wines produced in south-east of Romania vineyards and to correlate this with the total polyphenolic content.

Materials and methods

2.1. Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH) in free radical form was obtained from Sigma Chemical Co. (St. Louis, MO). Folin–Ciocalteu reagent, gallic acid, quercetin and ethanol were purchased from Merck Co. (Germany). All reagents were analytical grade.

2.2. Wine samples

The wines were produced from different grape varieties grown in the south-east of Romania. Thirty-three wines were supplied by R&D Station for Viticulture and Wine Bujoru and Regional Laboratory for quality control and hygiene wine Odobesti.

2.3. Determination of total phenolic content

The total phenol content in selected wine samples was determined spectrophotometrically according to the Folin–Ciocalteu colorimetric method [8] (Singleton & Rossi, 1965) using gallic acid as a standard polyphenol: 0.1 mL of wine was mixed with 7.9 mL distilled water and 0.5 mL of Folin–Ciocalteu reagent. After 1 min, 1.5 mL of 20% Na_2CO_3 was added. The absorbance was measured after 120 min at 760 nm. The concentration of the total phenolic compounds in the wines was expressed as gallic acid equivalents (mg/L). The results in every assay were obtained from three parallel determinations.

2.4. Free radical scavenging activity

The free radical scavenging activity of the wine samples was analyzed by using the 2,2,-diphenyl-1-picrylhydrazyl (DPPH) assay [9,10,11] The antioxidant assay is based on the measurement of the loss of DPPH colour by the change of absorbance at 515 nm caused by the reaction of DPPH with the tested sample. The reaction was monitored by a UV/VIS spectrophotometer. A volume of 0.1 mL of wine appropriate diluted with distilled water and 3.9 mL of freshly prepared DPPH (0.025 g/L) in ethanol was introduced and put into a small vat at room temperature. After 60 min incubation period at room temperature, the absorbance was read as against the blank at 515 nm. The determinations were performed in triplicate.

2.5. Statistical analysis

All the measurements were carried out in triplicate, and presented as mean values. The direction and magnitude of the correlation between the variables was quantified by the correlation factor r^2. The correlations were considered statistically significant, if the p-value was less than 0.05.

Results and Discussion

1. General characterizations of wines

Some relevant analytical parameters of the wines produced by R & D Station for Viticulture and Wine Bujoru and Regional Laboratory for quality control and hygiene wine Odobesti, are given in Table 1. The wines differ in many analytical parameters such as the content of alcohol, total extract, total and free SO_2, reducing sugar, total acidity (as tartaric acid), volatile acidity (as acetic acid) and specific weight.

Table 1.

Some relevant analytical parameters in some Romanian wines

Wine	Specific weight (g/cm^3)	Alcohol content (vol%)	Total acidity (tartaric acid) (g/l)	Volatile acidity (acetic acid) (g/l)	Total extract (g/l)	Reducing sugar (g/l)	Total SO2 (mg/l)	Free SO2 (mg/l
Babeasca Neagra (Veritas Panciu, 2009)	0.9925	14.73	8.23	2.22	29.6	4.22	103.4	6.63
Cabernet Sauvignon (Vincon Vrancea, 2007)	0.9919	14.04	5.27	0.66	26.15	1.73	25.25	4.59
Merlot (Vincon Vrancea, 2007)	0.9916	14.15	6.09	0.66	25.66	1.73	8.04	9.95
Babeasca Neagra (Veritas Panciu, 2007)	0.9905	14.92	6.37	0.64	25.0	0.23	28.02	5.34
Cabernet Sauvignon (Murfatlar, 2007)	0.9932	12.18	4.57	0.59	23.9	0.72	60.59	6.63
Pinot Gris (Veritas Panciu, 2007)	0.9885	15.43	5.42	0.54	21.4	0.14	69.66	5.36
Cabernet Sauvignon (Vincon Vrancea, 2009)	0.9908	15.48	5.79	0.80	27.5	0.76	160.3	19.38
Cabernet Sauvignon (Vincon Vrancea, 2008)	0.9955	12.53	10.02	0.56	31.0	1.11	20.41	5.10
Pinot Gris (Veritas Panciu, 2009)	0.9892	16.60	5.5	1.20	37.7	11.48	191.75	7.14
Merlot (Vincon Vrancea, 2009)	0.9900	14.88	6.09	4.47	23.7	1.21	321.7	23.46
Babeasca Neagra (Veritas Panciu, 2008)	0.9891	15.01	6.14	0.58	21.6	0.16	19.13	4.08
Merlot (Vincon Vrancea, 2008)	0.9929	12.45	10.82	4.47	24.1	0.36	135.99	10.71
Pinot Gris (Veritas Panciu, 2008)	0.9892	14.23	5.42	1.13	19.8	0.16	33.1	5.61
Cabernet Sauvignon (Murfatlar, 2008)	0.9936	11.28	7.56	1.8	22.3	0.1	37.42	3.83
Aligote (Vin Club, 2008)	0.9889	13.01	4.41	0.54	15.2	0.15	66.74	3.83
Galbena de Odobesti (SCDVV, 2008)	0.9909	12.10	7.56	1.06	17.8	0.21	80.27	4.59
Cabernet Sauvignon (Vincon Vrancea, 2009)	0.9953	12.23	6.52	3.64	29.6	0.76	160.3	23.97
Muscat Ottonel – 2009 Dealurile Bujorului	0.9892	13.0	5.5	0.39	23.8	14.0	84	20
Băbească gri -2009 Dealurile Bujorului	0.9898	10.3	5.9	0.60	23.3	34.0	248	55
Băbească neagră – 2009 Dealurile Bujorului	0.9892	14.2	6.1	0.54	23.4	3.8	128	35
Merlot 2009 Dealurile Bujorului	0.9968	14.1	6.3	0.60	28.9	2.9	76	29
Fetească neagră- 2008 Dealurile Bujorului	0.9890	11.9	6.4	0.58	28.4	2.6	96	22
Cabernet Sauvignon – 2008, Dealurile Bujorului	0.9889	12.2	5.8	0.45	25.2	1.6	85	30

2. Total polyphenols

The concentration of the total phenolic compounds in the wines was expressed as gallic acid equivalents (mg/L). The equation of standard curve was y = 0.4317x – 0.024 and R^2 = 0.9934.

The total polyphenols content of selected wines, obtained using the Folin–Ciocalteu reagent, is presented in Table 2. The mean concentration of the total polyphenols (TPs) content of the red wines was 639.5205 mg/L gallic acid equivalent. The total polyphenols content on the white wines was 180.5233 mg/L gallic acid equivalent.

Table 2.

The total polyphenols content of selected wines, obtained using the Folin–Ciocalteu reagent

No	Wines	TP (mg gallic acid equivalent/L)
1.	Babeasca Neagra (Veritas Panciu, 2009)	448.5692
2.	Cabernet Sauvignon (Vincon Vrancea, 2007)	616.396
3.	Merlot (Vincon Vrancea, 2007)	845.321
4.	Aligote (Vin Club, 2007)	160.8662
5.	Babeasca Neagra (Veritas Panciu, 2007)	256.7672
6.	Cabernet Sauvignon (Murfatlar, 2007)	517.4014
7.	Pinot Gris (Veritas Panciu, 2007)	409.8995
8.	Cabernet Sauvignon (Vincon Vrancea, 2009)	696.0557
9.	Cabernet Sauvignon (Vincon Vrancea, 2008)	544.4702
10.	Pinot Gris (Veritas Panciu, 2009)	372.0031
11.	Merlot (Vincon Vrancea, 2009)	620.263
12.	Babeasca Neagra (Veritas Panciu, 2008)	353.4416
13.	Feteasca Regala (Vin Club, 2009)	252.9002
14.	Merlot (Vincon Vrancea, 2008)	501.9335
15.	Pinot Gris (Veritas Panciu, 2008)	516.628
16.	Cabernet Sauvignon (Murfatlar, 2008)	623.3565
17.	Feteasca Regala (Vin Club, 2008)	366.5893
18.	Aligote (Vin Club, 2008)	146.9451
19.	Galbena de Odobesti (SCDVV, 2008)	208.0433
20.	Feteasca Regala (Vin Club,2007)	160.8662
21.	Galbena de Odobesti (SCDVV, 2007)	343.3875
22.	Cabernet Sauvignon (Vincon Vrancea, 2009)	583.9134
23.	Aligote (Vin Club, 2009)	137.6643
24.	Galbena de Odobesti (SCDVV,2009)	126.0634
25.	Sarba (Dealurile Bujorului, 2009)	600.1547
26.	Muscat Ottonel (Dealurile Bujorului, 2009)	209.5901
27.	Babeasca gri (Dealurile Bujorului, 2009)	344.9343
28.	Babeasca neagra (Dealurile Bujorului, 2009)	478.7316
29.	Feteasca Regala (Dealurile Bujorului, 2009)	397.5251
30.	Feteasca alba (Dealurile Bujorului, 2009)	161.6396
31.	Merlot (Dealurile Bujorului, 2009)	1474.091
32.	Feteasca neagra (Dealurile Bujorului, 2009)	1139.985
33.	Cabernet Sauvignon (Dealurile Bujorului,2009)	1089.714

The maximum values of PT was 1474.091 mg/L of gallic acid equivalent on the red wine Merlot (SCDVV Bujoru, 2009) and minimum value on Galbena de Odobesti (SCDVV, 2009) was 126.0634 mg/L GAE.

The total polyphenols content of the red wines samples analyzed is in range of the levels quoted in the literature for wines grown in different countries such as in the Czech Republic 874–2262 mg/L GAE [12], Spain 1010–2446 mg/L GAE [13].

The grape phenolic content and also the total polyphenol compounds on wines could be affected by the numerous viticulture and wine-making factors. The phenol composition has an important role in stabilizing lipid oxidation and is associated with antioxidant activity.

3. Antioxidant capacity

The free radical scavenging activity of the wine samples was analyzed by using the 2.2,-diphenyl-1-picrylhydrazyl (DPPH) assay.

Wine is a complex mixture of polyphenols and the antioxidative capacity can be expressed as the amount of wine needed to quench a certain amount of DPPH radical. The decrease in the absorbance at 515 nm is taken as a measure of the extent of radical-scavenging.

The inhibition of DPPH in percent (I %) of each wine sample was calculated from the decrease of absorbance according to the relationship:

$$I(\%) = \frac{Ablank - Asample}{Ablank} \times 100$$

where A_{blank} is the absorbance of control reaction (ethanol–water with DPPH radical) and A_{sample} is the absorbance of the tested wine sample. Quercetine was used as standard polyphenol in antioxidant capacity assay. The equation of standard curve was $y = 7.0028x - 0.1399$ with value $R^2 = 0.9935$.

All the wine samples showed a different radical scavenging activity after incubation with a free DPPH radical solution. The values of I% for red wines are in the range between 89.85 (Pinot Gris - Veritas Panciu, 2008) .and 76.6 (Merlot - Bujoru, 2009). Also for white wines inhibition (I%) was in the range from 12.96 (Galbena de Odobesti - SCDVV, 2009) to 35.29 (Galbena of Odobesti - SCDVV, 2008)

The percentage of DPPH radical scavenging activity was plotted as against the total phenolic content of the wine samples in Fig. 1. For the investigated wines, the correlation between antioxidant activity and the total phenolic content was $r^2 = 0.4781$, (SD = 0.025, n = 33).

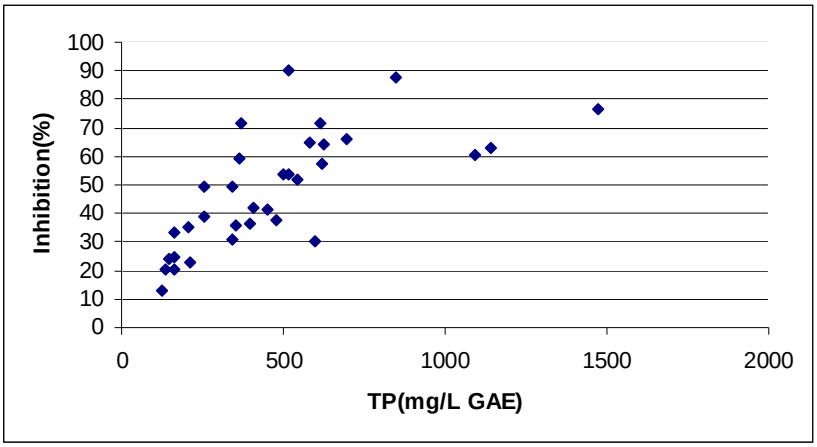

Figure 1. The percentage of DPPH radical scavenging activity plotted against total phenolic content of the tested wine samples

The polyphenol compounds content in the investigated wines contributes to their antioxidant activity. Since structural features of phenolic compounds are responsible for the antioxidant activity, the antioxidant activities of the tested wines may be related to their total phenols content. The different antioxidant activity of tested wines is dependent especially on the grape varieties.

Conclusion

The present work regarding the free radical scavenging and total polyphenols of some Romanian wines some general has drawn the following conclusions:

- According to the obtained results, the free radical scavenging ability of Romanian wines is correlated to total polyphenol content.
- High amounts of polyphenols and significant antioxidant activity were observed in the red wines produced from vine varieties from the South-East Romanian regions.
- The significant differences in the antioxidant activity between the analyzed wines were confirmed.
- It is important to determine which group of phenolic compounds is most significant in antioxidant activities of wines. This aspect will be a subject of our next research activity.

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