CORRELATION BETWEEN ANTIOXIDANT ACTIVITY AND TOTAL POLYPHENOLS CONTENT OF AUTOCHTHONOUS WINE VARIETY - FETEASCĂ NEAGRĂ

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Abstract

Autochthonous wine variety Fetească Neagră is a red wine known for high quality rich flavor, pleasant and complex associated with black currant flavor and balanced taste, consistent and vigorous. Red wines produced in four different wine growing regions of Romania were analysed for total polyphenols content and antioxidant activity. The total polyphenols content was determined by Folin - Ciocalteu method and antioxidant activity was measured DPPH method. Determined values of antioxidant activity in observed wines were within the interval 56.74 – 94.08% inhibition of DPPH (average value was 70.92% inhibition of DPPH) and total polyphenol content ranged from 1776 to 2935 mg gallic acid/liter (average content was 2218 mg gallic acid/L). Our results showed that the antioxidant activity of tested wines were directly influenced by the total phenolic contents. As a result, we mention that wines with a higher total phenolic contents showed the highest values of antioxidant activity. Particularly, autochthonous wine variety Fetească Neagră had the highest total polyphenols content, the highest concentration of important antioxidants and significantly higher antioxidant activity. There was a very high correlation between antioxidant activity and total polyphenols content in all of the tested wines.

Key words: red wine, Fetească Neagră, antioxidant activity, polyphenols, spectrophotometry

The polyphenols, such as flavanols ((+)-catechin, (-)-epicatechin etc.), flavonols (quercetin, rutin, myricetin, etc.), anthocyanins (malvidin-3-O-glucoside), oligomeric and polymeric proanthocyanidins, phenolic acids (gallic acid, *p*-coumaric acid, caffeic acid, ferulic acid, syringic acid, vannilic acid, *trans*-cinnamic acid, etc.), stilbenes (*trans*-resveratrol) found in large quantities, especially in red wine.

From the point of view of the quality of the wine, the phenolic compounds that form in the grapes define the color, flavor and taste. Their evolution should be seen as a result of the relationship between speed biosynthesis and the transformation that takes place during grape maturation under the influence internal factors (genetic) and external stakeholders biotope (climate, soil, terrain).

During the winemaking process from grape skins only a small fraction of phenolic compounds (30 - 50%) is extracted.

The color, smell and taste of the future wine are due to enzymatic transformations of polyphenols from crushed grapes - compounds that suffer from contact of must with oxygen - but also of transformations polymerization during storage

and aging of wine - polyphenols in wine are not identical to those extracted from grapes that are originally contained in the must (Pomohaci N. *et al*, 2000). The total content of phenolic compounds in red wines is very variable ranging (Zou H.L. *et al*, 2002).

In biological of properties, terms polyphenols have multiple health benefits and disease prevention (so called French paradox), such as cardioprotective, vasodilatory actions, antiinflammatory, anti-mutagenic, anti-carcinogenic, antiviral, antibacterial properties, neuroprotective, antiatherogenic and hepatoprotective activities. (Estruch R., 2000, Santos - Buelga C., Scalbert A., 2000, Minussi, R.C. et al, 2003, King R. E. et al, 2006, Geana E.I. et al, 2011, Georgiev V. et al, 2014). These biological properties are attributed mainly to their powerful antioxidant antiradical activity.

The autochthonous grape variety Fetească Neagră is famous for their rich flavour profile that contributes in resulting superior quality red wines (Banc R. et al, 2014, Hosu A. et al, 2014). Feteasca Neagră is one of the oldest grapes varieties found nowadays in Romania. The aroma of this wine variety is pleasant and complex.

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associated to the aroma of black currants. The taste is generally balanced, consistent and vigorous. Acidity is an appropriate characteristic of the variety, leaving a pleasant and persistent flavour. The high alcoholic potential, dry extract, dyes, as well as acidity, are in perfect harmony, which gives the Feteasca Neagra red wine finesse and smoothness unique, standing out as a quality wine (Coldea T.E. *et al*, 2015).

The purpose of this study was to determine, evaluate and correlate the content of total polyphenols and antioxidant activity of red wine

samples - Fetească Neagră, originating from different Romanian vineyard areas.

MATERIAL AND METHOD

Wine samples. We analyzed five samples of Fetească Neagră wine from different regions of Romania, purchased on the local market, harvest year 2012 (table 1). All samples were analyzed in 2015 shortly after opening glass packaging containers.

The wine samples of Fetească Neagră from different Romania wine regions

Table 1

Sample	Wine region	Vineyard	Quality	Alcohol content [%]				
P1	Dobrogea	Murfatlar	DOC - CMD	13.5				
P2	Muntenia	Tohani (Dealu Mare)	DOC - CMD	11.5				
P3	Moldova	Cotești	DOC - CMD	12.5				
P4	Banat	Recaș	DOC - CMD	14.5				
P5	Muntenia	Valea Călugărească (Dealu Mare)	DOC - CMD	11.5				
DOC – CMD - quality designation for grapes harvested at full maturity.								

Chemicals and equipment. 2,2-diphenyl-1-picrylhydrazil radical (DPPH), methanol, carbonat de sodiu, gallic acid, Folin - Ciocalteu's reagent were prepared in a mixture of phosphotungstic acid $(H_3PW_{12}O_{40})$ and phosphomolybdic acid $(H_3PMo_{12}O_{40})$,. All other reagents used were of analytical grade purity from Sigma-Aldrich (Germany).

Absorbance measurements were recorded on a UV-VIS-NIR spectrophotometer (UV-3600, Shimadzu, Japan). All samples were analysed in 10 mm quartz cells at room temperature.

DPPH radical-scavenging assay. The method DPPH•, based on the reaction with electron donating or hydrogen radicals (H•) producing compounds/antioxidants according to the following reaction were used:

DPPH• + Aox-H \rightarrow DPPH-H + Aox• purple colourless where: Aox-H = Ph-OH,

Ph – polyphenolic compound.

The DPPH radical-scavenging effect was analysed using the procedure described by Menković N. et al, 2014. Fractions diluted in appropriate solvents (10–100 μ L) were dispensed into a set of test tubes and the final volume was adjusted to 5 mL. Finally, 0.5 mL of a 0.004% methanolic DPPH solution was transferred into each test tube. The absorbances were recorded at 518 nm after 5 min incubation at room temperature in the dark, against methanol as the blank. The percent inhibition (antioxidant effectiveness) was calculated against the control solution, containing methanol instead of a test solution. The IC50 (concentration providing 50% inhibition) values were calculated use the dose inhibition curve in

linear range by plotting the volume (μ L) of wine needed to reduce the initial concentration of DPPH radical by 50 %.

Total phenolic content. Total polyphenolic content of the samples was determined by Folin-Ciocalteu method proposed in 1965 by Singleton V.L. and Rossi J. A. This method is based on the reduction of a phosphotungsten- phophomolybdate complex by phenolics to blue reaction products. Absorbance was measured at 765 nm. The content of total polyphenols in wines was calculated as amount of gallic acid equivalent (GAE) in mg per 1 litre of wine.

Statistical analysis. The samples were analyzed in three replicated and the results were expressed as mean ± standard deviation using Microsoft Excel 2013 software.

RESULTS AND DISCUSSIONS

Antioxidant activity in analysed wine samples was in range 56.74 – 94.08% inhibition of DPPH, Figure 1. Average value of antioxidant activity was 70.92% inhibition of DPPH. The strongest antiradical activity was found in red wine Fetească Neagră (Valea Călugărească - Dealu Mare) P5 sample, which had the highest content of phenolic compounds and the lowest activity was obtained for red wine Fetească Neagră (Recaș) sample P4 who had the lowest phenolic content. The high content of phenolic compounds in red wines contribute to their increased antioxidant activity.

Antioxidant activity expressed as IC_{50} ranged from 1.885 to 7.51 μL of wine. These IC_{50}

values follow the same order of antioxidant activity as the IC_{50} values similar to those have been reported for some Hungarian red wines (average value of 7.58 μL of wine, Lugasi A. and Hovari J., 2003) and for some Italian red wines (average value of 8.39 μL of wine, Giovanelli G., 2005)

Total polyphenol content in analysed wine samples was in the range from 1776 to 2935 mg galic acid/L, *Figure 2*. Average content of total polyphenol content was 2218 mg galic acid/L. The results obtained in determining the total polyphenol content in Romanian wines analyzed by Folin - Ciocalteu recorded variations between samples of red wine. The total polyphenol content varied between different samples of wine, depending on environmental factors in the

vineyard, wine processing techniques, soil and atmospheric conditions during the ripening and aging processes grains. Total polyphenol content of red wine is high due to better extraction of phenolic compounds through a longer contact time with the skins and seeds of grapes, fermentation conditions and temperature processing. The literature has shown that total phenolic content of red wine Fetească Neagră was 1.09 mg galic acid/mL (Coldea T.E. et al, 2015). Also among red wines, Banc R., 2013 has obtained significant differences between the values of total polyphenol content, for example phenolic content of Fetească Neagră wine - Tohani 2010 (2359 mg galic acid/L) was three times higher than Băbească Neagră wine - Panciu 2011 (801 mg galic acid/L).

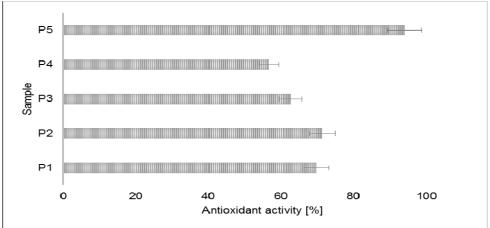


Figure 1 Antioxidant activity of red wines - Fetească Neagră

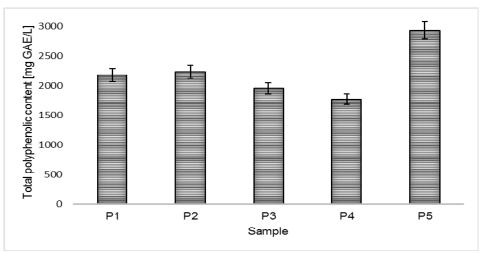


Figure 2 Contents of polyphenolic compounds of red wines - Fetească Neagră

The total content of phenolic compounds and antioxidant activity, measured as radical scavenging capacity were comparable to wines produced from international varieties of grapes. The total phenolic contents determined by the Folin-Ciocalteu method and the total antioxidant capacity determined by the DPPH for red wines

from other countries are as follow in table 2. Such large differences in total phenolic content between the investigated red wines from different countries are likely to result from grape varieties, vineyard location, climate, soil type, different wine processing techniques and ageing.

Table 2
The total phenolic contents and the total antioxidant capacity for red wines in literature

Total phenolic [mg GAE/L]	Countries	References	Total antioxidant capacity by DPPH assay [mmol Trolox equivalents per litre of wine]	Countries	References
1019–2446 1313 - 2389	Spanish	Sánchez-Moreno C. et al, 1999 Fernández-Pachón M.S. et al, 2004	4.65 - 17.41	Spanish	Fernández-Pachón M.S., et al, 2004
621.7 - 3200.3	Greek	Kallithraka S. et al, 2006	9.51 - 12.39	South African	De Beer D. <i>et al</i> , 2003
2340 – 3730 1794 - 4614	Sicilian	Di Majo D. <i>et al</i> , 2008 Dugo G. <i>et al</i> , 2006	-	-	-
2220 - 2390	California	Jewell W.T. and Ebeler S.E., 2001	-	1	-
1390–1600	Finland	Heinonen M. et al, 1998	-	=	-
1365–3326 3314 - 4177 1921–3659	Italian	Simonetti P. et al, 1997 Minussi R.C. et al, 2003 Stevanato R. et al, 2004	-	-	-
938–1820 1724 - 1936 1788 - 3070	Portuguese	Schoonen J.W. and Sales M.G.F., 2002 Paixao N. et al, 2007 Jordao A.M. et al, 2010	-	-	-
1460 – 3380 1218 - 3444	Slovakian	Stasko A. <i>et al</i> ,2008 Bajčan D. <i>et al</i> , 2016	13.22 - 17.74 69.0 – 84.2 % inhibition of DPPH	Slovakian	Stasko A. <i>et al</i> ,2008 Bajčan D. <i>et al</i> , 2016
1012 - 3264 554 - 2669	Croatian	Seruga M. <i>et al</i> , 2011 Vrcek I.V. <i>et al</i> , 2011	9.2 - 37.8 4.94 - 20.64	Croatian	Seruga M. <i>et al</i> , 2011 Vrcek I.V. <i>et al</i> , 2011
3.2 - 5.9	Brazilian	Lucena A.P.S. et al, 2010	2.6 - 6.3	Brazilian	Nixdorf S.L. and Hermosin-Gutierrez I., 2010
1402 - 3130 860 - 2710	China	Li H. <i>et al</i> , 2009 Jiang B. and Zhang Z.W. 2012	4.19 - 17.17 3.86 - 6.18 3.64 - 8.06 4.73 - 31.05	China	Li H. <i>et al</i> , 2009 Xi Z.M., <i>et al</i> , 2013
963 - 2262	Czech Republic	Stratil P., <i>et al</i> , 2008	2.91 - 8.62	Czech Republic	Stratil P., et al, 2008
1460 – 3380 1181 – 3589	Australian	Stasko A. <i>et al</i> ,2008 Yoo Y.J., <i>et al</i> , 2011	13.22 - 17.74 8.51 - 18.85	Australian	Stasko A. <i>et al</i> ,2008 Yoo Y.J., <i>et al</i> , 2011
1602 - 1968	Serbian	Radovanovic A.N. <i>et al</i> , 2012	3.64 - 8.06	Serbian	Radovanovic A.N. et al, 2012
1837 – 3467 2491.28 – 3845.19	Turkish	Porgali E. and Buyuktuncel E., 2012 Cavuldak Ö. A., et al, 2013	60,68 – 87,58 % inhibition of DPPH	Turkish	Cavuldak Ö. A., et al, 2013

The correlation between total polyphenolic content and antioxidant activity of red wine samples we tested is shown in Figure 3. Our results show that the antioxidant activity of wines tested were directly influenced by the total phenolic contents, the wines with a higher total phenolic contents presented the highest values of antioxidant activity. The same high correlation between total polyphenolic content and antioxidant activity was shown by many other researchers in their studies (Büyüktuncel E. *et al*, 2014, Leahu A. *et al*, 2014, Paixao N. *et al*, 2007, Vrcek I.V. *et al*, 2011).

Some studies have shown that the antioxidant activity of wine seems to be related more to the type of individual phenolic compounds than the total content of polyphenols (Geana E.I. *et*

al, 2011, Ma T.-T. et al, 2014, Tudorache A. et al, 2014).

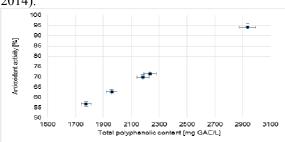


Figure 3 Correlation between antioxidant activity and total polyphenols content of red wines - Fetească Neagră

Our study confirms that tested autochthonous wine variety Fetească Neagră is a good source of antioxidants and hence a moderate consumption may have a beneficial effects on human health.

In spite of such differences between the results obtained by different authors, practically all published papers on this subject suggests that the predominant source of the antioxidant activity of red wines derives from different polyphenolic compounds presented in the wines and depends strongly on their total polyphenol content.

CONCLUSIONS

Red wines Fetească Neagră produced in four different wine growing regions of Romania were analysed for total polyphenols content and antioxidant activity. The total polyphenols content was determined by Folin-Ciocalteau method and antioxidant activity was measured DPPH method. Determined values of antioxidant activity in observed wines were within the interval 56.74 -94.08% inhibition of DPPH (average value was 70.92% inhibition of DPPH) and total polyphenol content ranged from 1776 to 2935 mg gallic acid/liter (average content was 2218 mg gallic acid/L). The results presented in this study showed that the antioxidant activity of wines tested were directly influenced by the total phenolic contents, becuse as wines with a total phenolic contents greater had the highest values of antioxidant activity.

In conclusion, autochthonous wine variety Fetească Neagră had the highest total polyphenols content, the highest concentration of important antioxidants and significantly higher antioxidant activity. There was a very high correlation between antioxidant activity and total polyphenols content in all of the wines tested. The determinations of total phenolic compounds and the total antioxidant activity of wine tell more about the health effect of a particular wine and can be used as criteria of quality and beneficial health effect.

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