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The Content of Total Polyphenols of the Selected Red Wines from the Territory of Tuzla Canton in Correlation with Antioxidant Activity



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ABSTRACT

Wine is an agricultural foodstuff product and its antioxidant activity is based on the action of natural antioxidants. The most important antioxidants in grapes and wine are phenolic compounds which not only make wine medicinal but also have an important role in organoleptic characteristics. Numerous studies have shown that moderate consumption of wine, especially of red wine, is beneficial for the cardiovascular system and for human health. These effects are mainly attributed to polyphenols in wine. In this research, the total content of polyphenols in the red wine samples has been determined *via* Folin-Ciocalteu (FC) method. The total antioxidant activity in red wines is established via indirect FRAP method (Ferric Reducing Antioxidant Power) with $\text{FeSO}_4 \times 7\text{H}_2\text{O}$ as the standard. The highest concentration of total content of polyphenols was in the "Postup" wine sample, Pelješac Vineyard: 2450.62 mg/L GAE; while the lowest total content of polyphenols was in the sample of the wine from an individual producer from the area of Kalesija: 600.63 mg/L GAE. Large differences in the content of polyphenols in the analyzed red wines from different areas are probably due to the differences in grape brands, vineyard location, climate, soil type, as well as the different methods of wine making and aging..

INTRODUCTION

Phenolic and polyphenol compounds are essential ingredients of wine whose presence and concentrations in wine influence on organoleptic properties and are often associated with beneficial effects on health. Polyphenolic compounds present in wine have a great influence on the sensory characteristics and quality of the wine¹. They are important components of the red wine that contribute to its color, taste and structural characteristics. Anthocyanin polymeric (tannins) are responsible for bitterness and astringency².

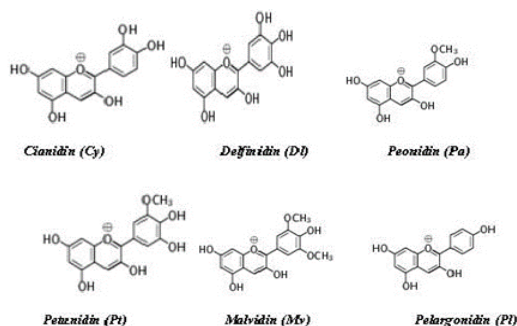


Figure 1. Basic classes of anthocyanins present in black wine

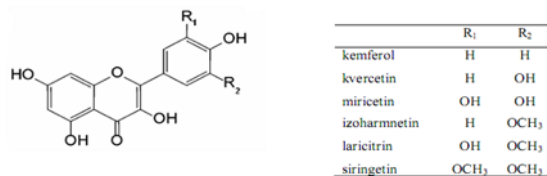


Figure 2. Flavanola grape plant³

Maximum quantities of total phenolic substances are generally limited to 500 mg / L for white, 2.000 mg / L for pink and 5.500 mg / L for black wines (NN 34/95). Polyphenolic compounds are present in all parts of the cluster, and their extraction into wine is primarily dependent on the variety, conditions of cultivation of grape and processing of fermentation. Furthermore, the procedures applied during care (stabilization and refinement) and the conditions of matured and preserving of wines (selection of barrels, bottles .) affect the changes taking place on polyphenols and their share in wine, and hence on the organoleptic properties of the wine.

It has been found that the phenolic components of the wine have a very high antioxidant potential. Their oxidative power is twenty times higher than vitamin E and fifty times higher than vitamin C⁴. Wine conception significantly reduces the possibility of cancer formation,

favorably affects the increase in so-called positive cholesterol i.e High Density Cholesterol (HDL) and at the same time contributes to the reduction of hazardous Low Density Cholesterol (LDL) cholesterol, the cause of heart disease and blood vessels, lowers blood pressure, reduces the ability to create stones in small quantities, while in large quantities impairs calcium absorption⁵.

MATERIAL AND METHODS

The study covered 8 samples of red wine (Table 1) of different geographical origin. Seven wine samples from the Tuzla Canton area and one sample from the area of Croatia were analyzed. Analyzed samples are taken from individual manufacturers and can not be obtained in free sale. For comparison, the total polyphenol content of seven commercially available samples of red wine (Table 2) was measured. Six samples from the territory of Bosnia and Herzegovina and one sample from the territory of Croatia. For all samples, the geographical origin of the vine is indicated as the initial raw material for the production of wine, and if the wine from the free sale is indicated by the manufacturer. During the research, all wines were kept in a refrigerator in dark bottles, and the analyses were carried out between January and March 2014. The concentration of total phenols was determined by the spectrophotometric Folin-Ciocalteu method with malonic acid as standard. The antioxidant capacity of red wines was determined by indirect FRAP (Ferric Reducing Antioxidant Power) method with $\text{FeSO}_4 \times 7\text{H}_2\text{O}$ as standard. The method is based on the reduction of the Fe^{3+} ferri ferrum into the ferro Fe^{2+} form in the presence of antioxidants, where at low pH the intensely blue colored ferro tripyridyl triazine complex has an absorption peak at 595 nm.

Table 1. Analyzed individual producers red wine samples

Sample no.	Name	Producers	Years of harvest	Volume of bottles	Origin
1.	Domestic vine I	Family A	2011	0,75	Brač
2.	Frankovka	Family B	2012	0,75	Tuzla
3.	Cabernett Sovignon	Family B	2012	0,75	Tuzla
4.	Frankovka	Family B	2011	0,75	Tuzla
5.	Domestic vine II (Slovenian)	Family C	2012	0,75	Požarnica
6.	Domestic vine III (Moldava)	Family C	2012	0,75	Požarnica
7.	Domestic vine IV	Family D	2012	0,75	Caparde
8.	Domestic vine V	Family E	2012	0,75	Kalesija

Table 2. Analyzed commercial red wine samples

No Sample	Name	Producer	Years of harvest	Volume of bottles (L)	Origin
1.	Postup	Istravino	2008	0,75	Vinogorje Pelješac
2.	Blatina	Vinarija Čitluk	2011	0,75	Čitluk
3.	Vranac Hepok	Vinarija Hepok	2011	0,75	Mostarsko vineyards
4.	Romanca	Vinarija Čitluk	2009	0,75	Čitluk
5.	Hercegovački Vranac	Vinarija Čitluk	2011	0,75	Čitluk
6.	Blatina Stojić	Vinarija Stojić	2011	0,75	Mostarsko vineyards
7.	Hercegovačka Blatina	Vinarija Čitluk	2010	0,75	Mostarsko vineyards

The content of total polyphenols in red wine samples was examined by the Folin-Ciocalteu (FC) method

In order to determine the content of total phenols in the prepared samples, the Folin-Ciocalteu method⁶ was used. The method is based on the oxidation of phenolic compounds by the addition of Folin-Ciocalteu reagent (Kemika) and the formation of a colored product. The Folin-Ciocalteu reagent is a mixture of phospho wolphramic acid and phosphomolybdic acid⁷. This reagent oxidizes the phenolic compounds, and it itself is reduced to the mixture wolfram oxide and molybdenum oxide, which are blue colored. The measurement was performed on the UV / VIS spectrometer "UVmini-1240V SHIMADZU".

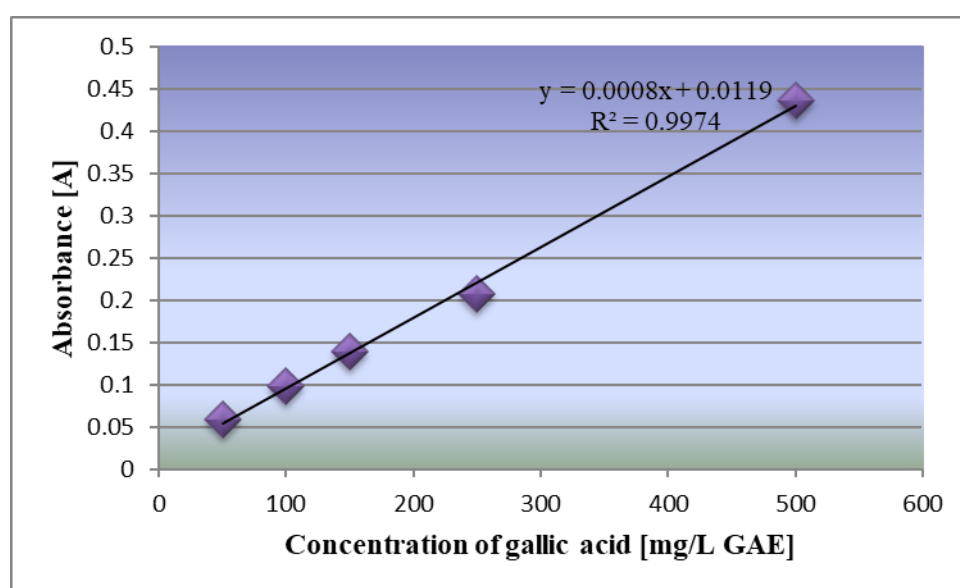


Figure 3. Curve for determining the content of total polyphenols

RESULTS AND DISCUSSION

The results obtained show that there is a big difference in the concentration of phenolic compounds in the investigated wines. The highest concentration of total phenols was measured in the wine sample of Postup, Vinogorje Pelješac and is 2450,62 mg / L GAE. The lowest concentration was measured in the wine of individual producers from the region of Kalesija and amounted to 600.63 mg / L GAE. The lower content of the phenol has been demonstrated in wine samples from the area of Požarnica (869,36 mg / L GAE and 725,63 mg / L GAE) and in the wine from the Caparde area (650,63 mg / L GAE) (Figure 4). According to the literature data, the concentration of total phenols in black wines, determined by the same method, as a standard range from 180 mg / L GAE to 3000 mg / L GAE^{6,8}. Their

average value is about 2160 mg / L GAE⁹. By comparing the average phenol content in commercially available wines and individual producer wines, it can be concluded that the phenol content in commercially available wines is slightly higher (Figure 5).

The content of phenol according to our research in commercially available wines (average 1766,69 mg / L GAE) and individual producer wines (1088,81 mg / L GAE) does not deviate significantly from other investigated wines (584,57 mg / L GAE - 2169, 74 mg / L GAE)¹⁰ (2402 mg / L GAE - 3183 mg / L GAE)¹¹. By comparing the average phenol content in wine samples from the Tuzla Canton area (six samples) and other examined samples (eight samples), it can be concluded that their content is significantly smaller, but does not differ from the literature data (Figure 6).

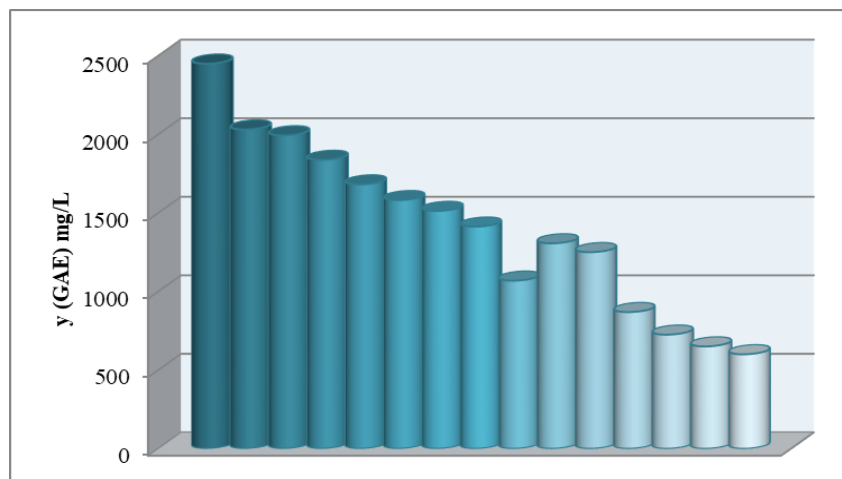


Figure 4. Content of total polyphenols in analyzed samples of red wine

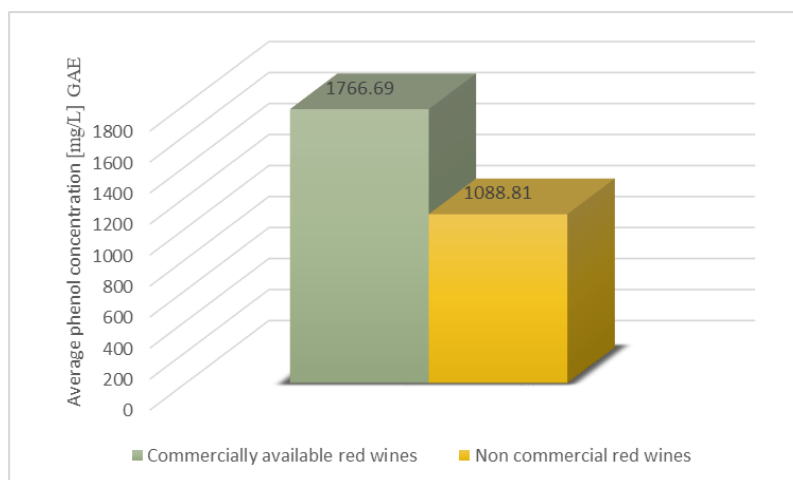


Figure 5. The average content of phenols in the wine of individual producers and in other examined samples

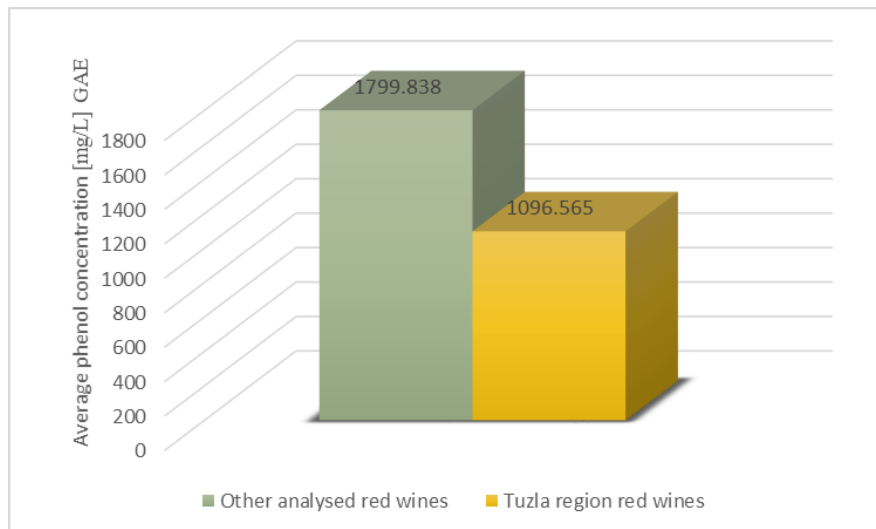


Figure 6. Average content of phenols in wine from the TK region and in other tested samples

The share of polyphenolic compounds in grapes and wine depends on a large number of factors such as cultivar, ecological conditions of farming, applied agro-technical and ampelos technical procedures in the vineyard, and vinification techniques^{12,13}.

We assume that a different technological process for the production of commercially available wines and wine samples taken from the individual producer has also caused a lower content of phenols in wines from the TK area. The obtained values of the total polyphenol content in black wines are comparable with the literature data. For comparison, Table 3 and Figure 7 show the phenol content in wine samples from different areas.

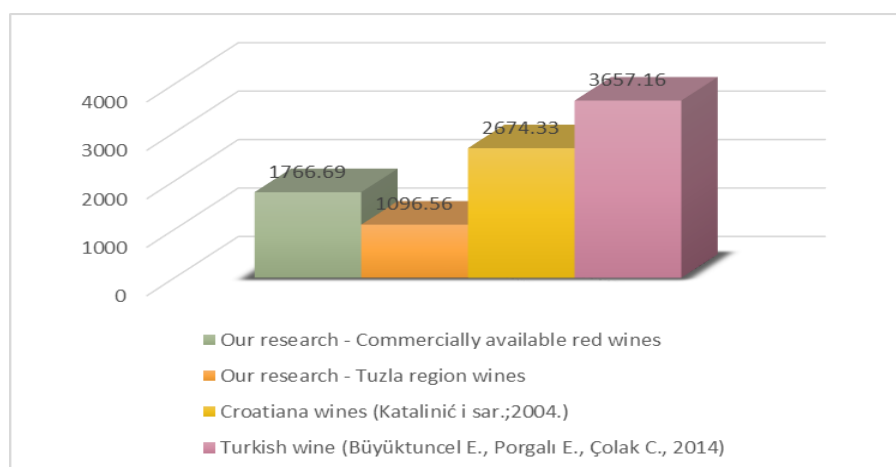


Figure 7. The average content of phenol in wine from the TK area and different wine samples

Table 3. Content of phenol in wine samples from different areas¹⁴

	Red wine
Minussi <i>et al.</i> (2003) (Italian wines)	3314 - 4177
M. S. Fernandez-Pachon <i>et al.</i> (Spanish wines)	1313 - 2389
Kallithraka <i>et al.</i> (2006) (Greek wines)	622 - 3200
Dugo <i>et al.</i> (2006) (Sicilian wines)	1794 - 4614
Paixao <i>et al.</i> (2007) (Portuguese wines)	1724 - 1936
Di Majo <i>et al.</i> (2008) (Italian wines)	2340 - 3730
Stratil <i>et al.</i> (2008) (Czech Republic wines)	963 - 2262
H. Li <i>et al.</i> (2009) (China wines)	1402 - 3130
Lucena <i>et al.</i> (2010) (Brazilian wines)	3200 - 5900
Jordao <i>et al.</i> (2010) (Portuguese wines)	1788 - 3070
Seruga <i>et al.</i> (2011) (Croatian wines)	1012 - 3264
Yoo <i>et al.</i> (2011) (Australian wines)	1181 - 3589
Jiang <i>et al.</i> (2012) (China wines)	860 - 2710
Radovanovic <i>et al.</i> (2012) (Serbian wines)	1602 – 1968
Porgalı <i>et al.</i> (2012) (Turkish wines)	1837 - 3467

In order to confirm the connection between the total antioxidative activity of the wine and the content of total phenols, a correlation analysis was made. The influence of phenolic compounds on the antioxidant activity of black wines is shown by high correlation $r = 0.890244$ (Figure 8). The highest value of antioxidant activity was shown by the wine sample Postup Vinogorje Pelješac ($a = 62.77 \text{ mmolFeII/L}$), which also contains the highest concentration of total phenols ($(\gamma \text{ GAE}) = 2450.62 \text{ mg/L}$) among the analyzed wines. The lowest antioxidant activity (as well as the lowest concentration of total phenols ($\gamma \text{ (GAE)} = 600.63 \text{ mg/L}$) showed a sample of individual producer's wines from the Kalesija region ($a=10.54 \text{ mmolFeII/L}$). In many studies^{15,16}, a high degree of positive correlation of the content of phenolic soluble and antioxidative activity of wine has been demonstrated³. Data that are in accordance with the results of other researchers¹¹ confirm the fact that high levels of phenol contribute to greater antioxidant activity. The deviations in the ratio of antioxidant activity and the concentration of total phenols (Figure 8) also influence their composition is different in each wine¹⁷.

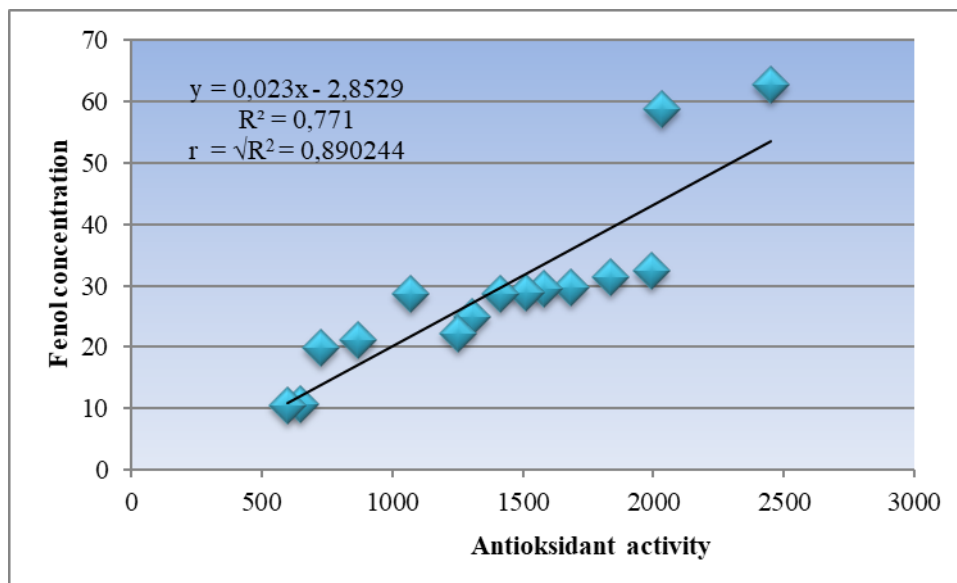


Figure 8. Correlation between total antioxidant activity of red wine and polyphenol content

CONCLUSION

Black wine is a rich source of different groups of phenolic compounds, among which the anthocyanins are responsible for the sensory properties of the wine. The phenol content according to our research in commercially available wines (average 1766,69 mg / L GAE) and individual producer's wines (1088,81 mg / L GAE) does not deviate significantly from other investigated wines. Our research has shown that commercially available wines from the Herzegovina region contain higher concentrations of polyphenols from wines from the TK region taken from individual producers. This can be explained by the fact that the concentration of polyphenol in grapes depends on light and temperature and their interaction^{18,19}. Namely, the higher exposure of the berry to the light causes more polyphenols, while too high temperatures can reduce the total polyphenols¹⁸. The phenol concentration depends on the technological process of wine production. The amount of polyphenolic compounds that will be extracted into the wine depends on the variety, temperature and duration of contact with the skin, and most of all about the conditions of maceration²⁰. The increase in temperature significantly accelerates the extraction of phenolic compounds²¹, which can be explained by the degradation of cell walls and accelerates the expiration of cellular content. Based on the collected data from wine producers from the TK region, we know that sulfur dioxide is not added to sample 5, sample 6, sample 7, and sample 8, although sulfur dioxide intensifies the extraction of phenolic compounds, primarily the

anthocyanin from the lining, as it disrupts cell membranes and facilitates exposure soluble components. These samples also showed the smallest content of total phenols. The technological process of wine production affects the average antioxidant activity of the wine, the content of total phenols, flavonoids and nonflavonoids. Samples of commercially available wines are richer sources of antioxidants than wines from individual producers, which most likely depend on the variety, temperature and duration of contact with the subcutaneous tissue, and most of all on the conditions of maceration. Based on all of the above, it is clear that the wines of individual producers, which during the production were not exposed to high temperatures, showed a lower content of free phenols, and thus lower antioxidant activity. Statistical analysis of the obtained results calculated Pearson's correlation coefficient of 0.89024. The positive correlation between the concentration of total phenols and the antioxidant activity of the wine confirms the fact that wines with a higher concentration of total phenols have a stronger antioxidant activity.

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