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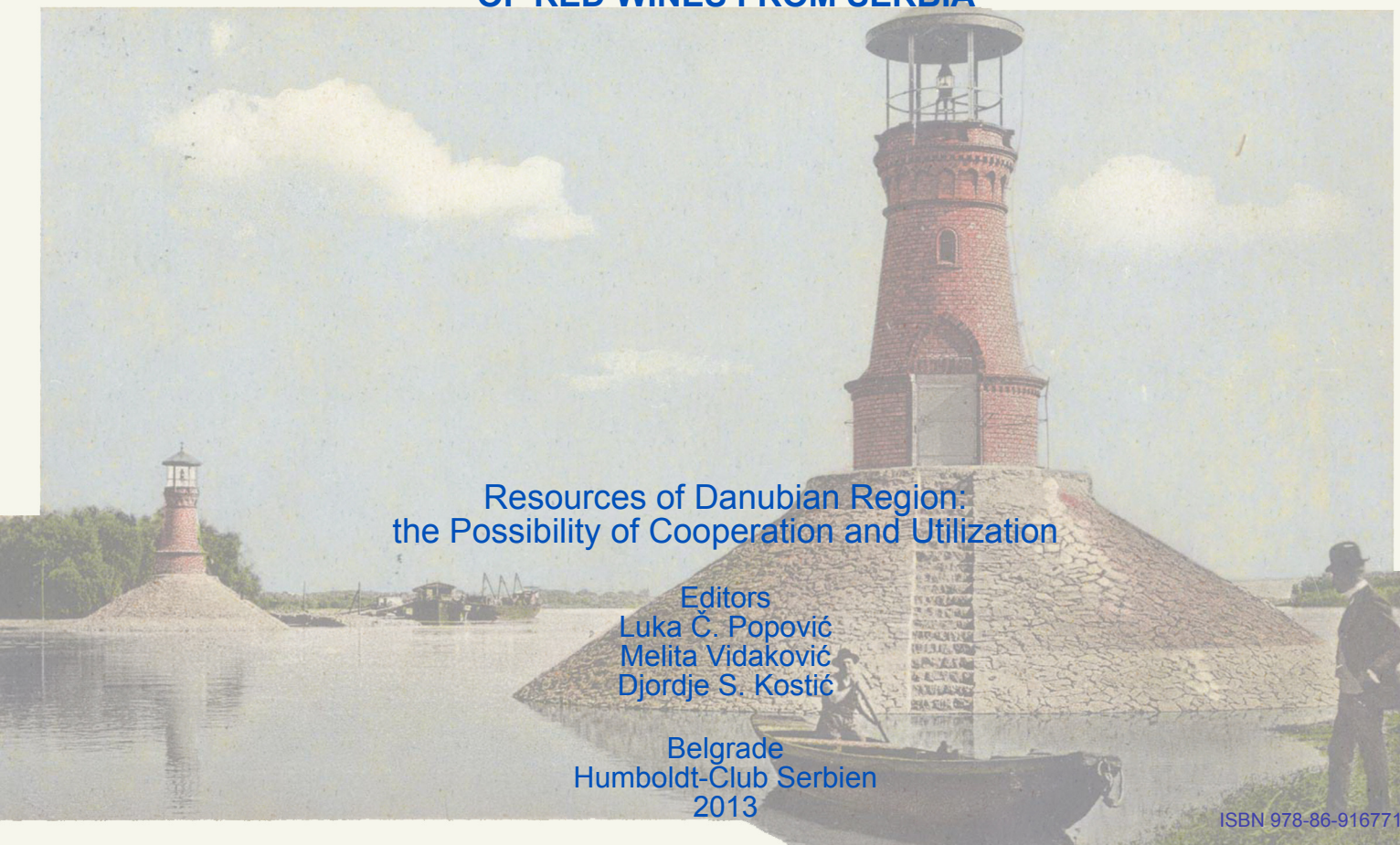
ANALYSIS OF PHENOLIC CONTENT AND ANTIOXIDATIVE CAPACITY OF RED WINES FROM SERBIA



Resources of Danubian Region:
the Possibility of Cooperation and Utilization

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Abstract. Red wine is a rich source of phenolic compounds and it is well known that regular wine consummation in reasonable amounts can improve health. The aim of this study was to conduct preliminary determination of total phenolic and total flavonoid content as well as antioxidative capacity of wines produced in winery «Radovanović» from Krnjevo, Serbia.
Total phenolic content varied from 2046.67 to 2156.06 mg/l expressed as gallic acid equivalents. The amounts of flavonoids ranged from 67.3 to 73.5 % of total phenolic content. Also, all samples showed evident antioxidant effect.
Keywords: red wine, phenols, flavonoids, antioxidants

Introduction

Wine is one of the most popular alcoholic beverages, which has very important role in many European and Mediterranean cultures. It is well known that wine, with addition of different herbs, was used in traditional medicine for treatment of many diseases. In modern times, interest for wine has increased when epidemiological studies confirmed that regular moderate consumption of red wine could decrease mortality from coronary diseases ("the French paradox"). It is suggested that phenolic compounds present in wine are mainly responsible for this effect (Renauld and Lorgeril, 1992, Bertelli, 1995).

Phenolic components present in wine affect the sensorial characteristics such as appearance, taste, mouth-feel and fragrance. They may come from the fruit (skins and seeds) and wine stems, production by yeast metabolism, or extraction from wood cooperage. Also, many researches suggested that these compounds, which possess antioxidant activity, could have positive role in prevention of diseases such as coronary heart disease and carcinogenesis. Final composition of phenols in wine depends on their content in grapes, cultivar, vintage, climate, the extraction parameters, winemaking technology, etc. Two main groups of phenols present in wines include the flavonoids and the non-flavonoids (Soleas *et al*., 1997).

Flavonoids are primarily from the skins, seeds, and stems of the fruit (Stockley and Hoj, 2005). In red wines, they commonly constitute > 85% of the phenol content (≥ 1,000 mg/L) (Soleas *et al*., 1997). Flavonoids can express many different physiological effects. Some of the most important include antioxidant, anti-inflammatory, antitumor, anti-bacterial and anti-atherosclerotic properties. Also, they

can inhibit a number of enzymes present in human body (i.e. cycloxygenase, elastase) (Agrawal, 2011).

The aim of this work is preliminary investigation of total phenolic and total flavonoid content as well as antioxidative capacity of red wine produced in one of the best Serbian winery.

Materials and Methods

All samples were produced in winery "Radovanović" from Krnjevo, Serbia. Wines samples were made from Cabernet Sauvignon grape variety using oak barrels from woods of different geographical origin. Barrels were made from American, Serbian, Croatian and French oak. Analysis was performed on twenty wine samples (five from each barrel type).

Analysis was performed using Agilent 8453 UV-Visible Spectroscopy System (Germany). Folin Ciocalteu's phenol reagent was purchased from (Fluka Biochemica, Switzerland). Substances and reagents used in experiment were potassium chloride (Zorka Pharma, Serbia), cc hydrochloric acid (Lachner, Czech Republic), sodium acetate (Poch, Poland), ethanol 96% (Zorka Pharma, Serbia), formaldehyde 37% (Carbo-Erba, Italy), phloroglucinol-2-hydrate (Centrohem, Serbia), sodium carbonate (Sinex laboratory, Serbia), gallic acid (Alfa Aesar, England) and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) (Sigma-Aldrich, Germany).

Total phenolic content

The content of total phenolic compounds in wine samples was determined by the Folin-Ciocalteu's (FC) method using gallic acid as standard. This method is based on the reduction of a phosphowolframate–phophomolybdate complex by phenolics to blue reaction products. Prior to analysis, all wine samples were filtered through membrane filter (0.45 µm) and adequately diluted. Volume of 100 µl of sample or gallic acid standard was mixed with 500 µl of Folin reagent (Folin-Ciocalteu:water, 1:10, v/v) and left for 6 minutes. After that, 400 µl of 7.5% Na_2CO_3 was added, vortexed, and left in dark for 2h. The absorbance of the standards and samples was measured at 740 nm. The TPC content of the sample was expressed as mg of gallic acid equivalents per litre of sample (mg GAE/l).

Total flavonoid content

To 5 ml of sample, 2.5 ml of HCl:H_2O (1:4, v/v) solution and 2.5 ml of 37% formaldehyde were added and left for 2 h. Then, 2 ml of phloroglucinol (10 mg/ml) is mixed into samples and left for 24h at room temperature. Supernatant was filtered through 0,45 µm filter and total phenolic content was determined by the Folin-Ciocalteu's (FC) method previously described. The amount of flavonoids was calculated as the difference between total phenols and non-flavonoids in sample. The flavonoid content was expressed in mg/l gallic acid equivalents.

2,2-diphenyl-1-picrylhydrazyl radical (DPPH) test

The radical scavenging activities of wines were evaluated after the reaction with DPPH. For this purpose, all samples were filtered through membrane filter (0.45 μm) and diluted with ethanol. Free radical scavenging activity of wines was determined using the modified method proposed by Soler-Rivas *et al*. (2000).

Radical scavenging capacity (RSC) was calculated as follows:

$$\%RSC = 100 - \frac{Asample \cdot 100}{Ablank}$$

The free radical scavenging activity was expressed as the mean scavenging concentration IC_{50}.

Results and Disscusion

Total phenolic and flavonoid contents as well as antioxidative capacities of analyzed samples are presented in Table 1.

Total phenolic content (TPC) was in range from 2046.67 (Croatian oak) to 2156.07 (Serbian oak) mg/l GAE. Previously published results showed that total phenolic content in average red wine can vary from 900-2500 mg/l GAE, but higher values (over 5000 mg/l GAE) were also observed (Cimino *et al*., 2007, Ivanova *et al*. 2010). Generally, it is recognized that wines with higher phenolic content have higher potential for expression of beneficial effects on human health. Some of the levels quoted in the literature for total phenolic content of red wines from different European countries are: Czech Republic (874-2262), Greece (1710-2825), Italy (3314-4177), Spain (1637-2717), Slovenia (1010-2446) (Radovanović *et al*., 2009).

Obtained values in this study are up to two times higer then those observed for red wines from the region. Namely, study conducted by Ivanova *et al*. (2010) showed that red wine made from the same variety from Macedonia had 1185 mg/l GAE. Similarly, published results for Cabernet Sauvignon wine from Croatia showed lower concentration of total phenols (1400 mg/l GAE) (Rastija *et al*. 2009). On the other hand, in another paper published by Katalinić et al. (2004), red wine from the same variety produced in Croatia had over 3000 mg/l GAE. These differences could be consequence of many different factors including climate conditions, vintage year as well as winemaking technology.

Total flavonoid content of analyzed samples was in range from 1378.54 to 1563.92 mg/l GAE. These values are higher than those published for Cabernet Sauvignon wine from Macedonia (910 mg/l GAE), but lower than the ones for Croatian Cabernet Sauvignon (2814 mg/l GAE) (Ivanova *et al*., 2010, Katalinić *et al*., 2004). The amounts of flavonoids ranged between 67,36 and 73,47 % of total phenolic content, which confirms that flavonoids are main phenolic components in red wine. Also, all red wines tested in this study had evident antioxidant effect (0,59-0,69 μl/ml).

Statistical analysis (ANOVA test) of obtained data showed that there is no significant difference ($p > 0.05$) between samples based on the origin of the wood barrel in which wines were produced. So, there

is no clear influence of this parameter on total phenolic and flavonoid content as well as antioxidant potential. These results corroborate with previous study by De Simon *et al*. **Table 1.** Total phenolic content, total flavonoid content and antioxidative capacity of analyzed samples

Wine	TPC (mg/l GAE)	Flavonoids (mg/l GAE)	IC_{50} (µl/ml)
S1	2062.47	1506.80	0.66
S2	2096.2	1512.66	0.60
S3	2141.53	1544.19	0.65
S4	2156.07	1563.92	0.59
S5	2134.33	1537.07	0.60
average	2118.12	1532.93	0.62
F1	2074.87	1477.86	0.62
F2	2063.40	1422.12	0.65
F3	2058.40	1469.15	0.65
F4	2094.73	1514.27	0.66
F5	2096.13	1520.77	0.64
average	2077.51	1480.83	0.64
A1	2106.66	1473.23	0.69
A2	2136.06	1516.83	0.66
A3	2127.20	1555.39	0.66
A4	2127.53	1547.31	0.64
A5	2086.2	1532.792	0.64
average	2116.88	1525.11	0.66
H1	2105.40	1541.54	0.64
H2	2112.27	1541.85	0.68
H3	2053.07	1481.40	0.62
H4	2046.67	1378.54	0.63
H5	2096.20	1482.66	0.59
average	2082.72	1485.20	0.63

S-Serbian oak
F-French oak
A-American oak
H-Croatian oak
mg/l GAE- mg/l gallic acid equivalents
IC_{50}-concentration necessary for inhibition of 50% of DPPH radical

(2003) in which no significant differences were observed in total phenolic content between wines produced in French, American and Spanish oak barrels. Still, in some studies it has been shown that origin of wood barrels for wine production can influence organoleptic characteristics of wine (Frangipane *et al*., 2007). Therefore, further investigation could be focused on analysis of potential influence of barrel origin on these characteristics of wines (sensory analysis, gas chromatography for volatile compounds etc.).

Conclusion

Phenolic and flavonoid content and antioxidative capacity are important characteristics, which could be in part, responsible for positive effects of moderate wine consumption on human health. In this paper, red wines made from Cabernet Sauvignon grape variety in wood barrels of different geographical origin were analyzed (winery "Radovanović", Serbia). Results confirmed presence of significant amounts of phenolic and flavonoid components in all samples. Also, wine samples showed evident *in vitro* antioxidative effect. On the other hand, influence of wood origin on measured parameters was not obvious. These results provide useful information about biologically active components in red wines produced in Serbia and their potential for beneficial effects on human health.

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