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Impact of Yield on Total Polyphenols, Anthocyanins, Reducing Sugars and Antioxidant Potential in White and Red Wines Produced from Montenegrin Autochthonous Grape Varieties

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Abstract The aim of the present research is to determinate the impact of grape yield (6 t ha^{-1}, 8 t ha^{-1}, 10 t h^{-1}, 12 t ha^{-1} and 15 t ha^{-1}) on the quality of wine produced from red and white grape varieties Vranac, Kratošija, Krstač, and Žižak. For that purpose the quality parameters i.e. the total polyphenols, anthocyanins, reducing sugars and antioxidant potential of wine were determined. The antioxidant activity was assessed using 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) test and reducing power assay. The results of correlation analysis showed that there is a poor response of quality parameters to variation in yield. For most quality parameters the maximum value was determined at yield of 8 t ha^{-1}. The highest total polyphenols content, concentration of anthocyanins, reducing sugars and antioxidant activity expressed red wine Vranac that practically in all studied quality parameters was followed by variety Kratošija, Žižak and Krstač.

Keywords Crop Yield, Wine, Phenolic Compounds, DPPH-Test, Reducing Power Assay, Correlation

1. Introduction

Viticulture has a long tradition and important role in agriculture sector of Montenegro. Autochthonous grapevine varieties in Montenegro are very important because the viticulture and winemaking sector of Montenegro is based on the breeding of these varieties and on the production of grape and wines of these varieties. Vranac and Kratošija are the most widespread black grapevine varieties, which have been bred in Montenegro for a long time ago. Wines of these varieties are of premium quality, Vranac has dark red ruby color, full body, fruity taste and pleasant astringency and it has potential of lying down and maturing. Kratošija is characterized by an intense ruby-red color and aroma of red berry fruits and an extremely pleasant taste; it has a light and harmonious structure and smooth finish. Krstač is white wine with light gold color with greenish reflection and nicely expressed fruity aroma of peach and pear, while Žižak has green-yellow color, good body, fullness and aroma of southern fruit and it is recommended to blend it with Krstač.

The efforts to achieve the optimum crop load in order to reach expected wine quality is still the most discussed viticulture matter. It is difficult to propose proper number of buds per vine for a defined soil type, climatic conditions, scion/rootstock combination and trellis. There is permanent necessity for such investigation, especially in regions where these connections are poorly understood. It is also widely believed by the vine growers that high-yielding vines produce lower-quality wines. Tomić *et al*. [1] did similar investigations on Kratošija variety and concluded that the yield of 12 t ha^{-1} achieved satisfying quality of grape and wine, while 8 distinguished with wine quality, but because of economic reasons this load could be recommended only for production of grape on small surfaces and production of high quality wine. This thought should be considered carefully as it depends primarily on fertility and quality of the cultivar and growing conditions of the vineyard [2,3]. In some cases an increase in crop load coupled with improvements in canopy microclimate led to improvements in berry composition. It is clear that the relationship between yield and quality is not a straightforward one and wine grape growers should investigate their own optimum yields that will still produce quality wine grapes [4]. Also the wines from low-yield vineyards were considered, by the tasters, to have better sensory quality than the wines from high-yield vineyards [5].

One of the indicators of the wine quality is its antioxidant

activity. Antioxidants are substances important for the stability of food, as well as in defence mechanisms of biological systems. Cells of aerobic organisms are constantly exposed to the effects reactive oxygen species (free radicals). These species are highly reactive and cause aging of cells, mutagenesis, carcinogenesis and coronary heart disease by destabilizing membranes, DNA damage, and destruction of proteins and oxidation of low density lipoproteins. Most polyphenols have in their structure, two continuous hydroxyl groups on the aromatic ring, called a catechol group, which has a great affinity for free radicals on nearby molecules. The polyphenolic contents of wine consist of flavonoids and non flavonoids and depend on the grape variety, vineyard location, cultivation system, climate, and soil type, vine cultivation practices, harvesting time, production process, and aging [6]. The polyphenolic molecules have a functional role in that they behave as antioxidants against the free radicals and show a physiological role as well; in fact, they increase the antioxidant capacity in the human body after red wine consumption [7]. Phenolic compounds in grapes come mainly from the seeds and from the skins [8].

Several studies have shown that low crop level can increase anthocyanins and total phenolic compound of fruit and wine [9,10,11]. Anthocyanins contribute little to the taste of wine. However, because anthocyanins readily polymerize with tannins, they play an important role in tannin retention during aging.

Reducing sugars cause minor interferences and must be corrected. Sulfites also cause interference but the magnitude is variable. It is usually not an important factor except for white wines with medium to high sulfite levels (>50 mg L^{-1}) and low phenol levels (<250 mg L^{-1}). Folin-Ciocalteu method for total phenols determination in wine is responsive to any reducing substance, so if applied to other types of samples, large errors could be encountered [12].

2. Objectives

Reduction of yield at 6, 8, 10, 12 and 15 t ha^{-1}, of two white (Krstač and Žižak) and two red (Vranac and Kratošija) Montenegrin autochthonous grape varieties was done in experimental vineyards in order of examining impact on the enological potential and quality of their wines. To investigate wine quality after six months of maturation the total polyphenols content, concentration of anthocyanins, reducing sugars, reducing power and $DPPH^{\bullet}$ scavenging ability were determined, as well as basic chemical composition of grape must (sugar level, total acidity, pH value) and produced young wine (alcohol, total acidity, tartaric acid, pH, volatile acidity, total dry and sugar-free extract, total and free SO_2, and in red wines also color intensity and color hue). To our knowledge, no work has been reported before on these quality parameters of wine from above mentioned Montenegrin autochthonous grape varieties in relation to yield.

3. Materials and Methods

3.1. Agro-biological Conditions and Winemaking Technology

Wines produced from two white (Krstač and Žižak) and two red (Vranac and Kratošija) Montenegrin autochthonous grape varieties were investigated. The grape varieties were planted in the vineyards on Ćemovsko field (2400 ha) in the growing season 2010. The distance of planting in the vineyard where Vranac and Žižak are located is 2.6 m × 1.2 m and vines were formed in the shape of double horizontal cordon. Paulsen 1103P rootstock was used for Vranac and Kratošija varieties; Kober 5BB was used for Žižak, while for Krstač variety Rihter 110 rootstock was used. The mixed pruning is applied depending of the planned yield. The distance of planting of Krstač and Kratošija is 2.6 m × 0.7 m. Also the mixed pruning is used depending of the planned yield and vines were formed in the shape of single Guyot. All standard agro-technical operations were applied and vineyards were in good and healthy condition.

In order to achieve planned yield, vines were loaded with different number of buds by winter pruning. For all examined yields we followed necessary indicators of real and potential fertility. These indicators were followed on 15 vines (three repetitions of 5 vines) for every combination of planned yield. Potential fertility as one of the most important characteristics of variety was determined in May, in the time when it is easy to notice flowers, and based on reached data; fertility coefficients (potential buds fertility coefficient, shoot fertility coefficient and absolute shoot coefficient) were calculated. In order to realize tasks, we have followed the most important agro-biological, economics and technological characteristics of five examined planned yields (6, 8, 10, 12 and 15 t ha^{-1}). Yield was determined by measuring of picked grapes weight in phase of technological maturity.

Processing of grape was performed in the microvinification cellar, according to the standard procedure for making white and red wines. The grapes were harvested manually. For the vinification we used an average grape sample of each yield as follows: 100 kg of Vranac, 350 kg of Kratošija, 200 kg of Krstač and 200 kg of Žižak, respectively. Potassium metabisulfite was added (10 g 100 kg^{-1} of grapes) and was purchased from Agroterm KFT, Hungary. All enzymes, wine yeasts and yeast nutrients were obtained from Lallemand, Australia. Lallzyme Cuvee Blanc for maceration (2 g 100 kg^{-1} of grapes) and Lallzyme HC for clarification (1 g hL^{-1} of must) were added during vinification of white wines, while Lallzyme EX V (1 g 100 kg^{-1} of grapes) for maceration was added during red wine vinification. Yeast Lalvin ICV-D47 (30 g hL^{-1} of must) was used for alcoholic fermentation of Krstač and Žižak varieties, Lalvin BM 4x4 (30 g hL^{-1} of must) was used for Kratošija, while Lalvin BDX (30 g hL^{-1} of must) was used for Vranac variety. Yeast nutrient, Fermaid E (25 g hL^{-1}) was added during fermentation and Go-ferm protect (30 g hL^{-1}) protect was

used for yeast preparation.

3.2. Reagents and Solvents

Ethanol (96%), trichloroacetic acid, sodium carbonate, potassium hexacyanoferrate(III) and gallic acid (98%), hydrochloric acid, acetic acid, sodium acetate, citric acid monohydrate, crystalline sodium carbonate, sulphuric acid, starch, sodium thiosulphate pentahydrate were obtained from Merck (Darmstadt, Germany). DPPH$^{\bullet}$ reagent and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich GmbH (Steinheim, Germany). Potassium dihydrogen phosphate, potassium chloride and disodium hydrogen phosphate were obtained from Kemika (Zagreb, Croatia). Iron(III) chloride, copper(II) sulphate and potassium iodide were purchased from Carlo Erba (Milano, Italy). For preparation of solutions ultrapure water (MiliQ, Millipore) was used.

3.3. Basic Chemical Composition of Grape Must and Young Wine

For determination of basic chemical parameters, sugar level, alcohol, total and volatile acidity, tartaric acid, pH value, total dry and sugar-free extract, total and free SO_2, color intensity and color hue, the reference methods of European Union [13] were used.

3.4. Folin-Ciocalteu Assay

The Folin-Ciocalteu assay was performed according to a slightly modified method by [14]. Briefly, 200 μL of a sample solution at different concentrations were mixed with 125 μL of freshly prepared Folin-Ciocalteu reagent diluted with water (1:2, v v^{-1}) and 125 μL of sodium carbonate solution (20%, w v^{-1}). The final mixture was supplemented to 1 mL with Milli-Q water. After 40 min the absorbance at 765 nm was measured against blank. The mass concentration of phenolic compounds in wine (γ_P) was expressed in gallic acid equivalents as mg of gallic acid per L of wine. The determination was conducted in triplicate and results were averaged.

3.5. Determination of Total Content of Anthocyanins

The total monomeric anthocyanins were determined using the pH differential method [15]. Determining of the appropriate dilution factor for the sample was done by diluting with potassium chloride buffer, pH 1.0, until the absorbance of the sample at the $\lambda_{vis\text{-}max}$ was within the linear range of the spectrophotometer. The final volume of the sample was divided by the initial volume to obtain the dilution factor (DF). Two dilutions of the sample were prepared, one with potassium chloride buffer, pH 1.0, and the other with sodium acetate buffer, pH 4.5, diluting each by the previously determined DF. These dilutions were equilibrated for 15 min. The absorbance of each dilution were measured on a model AGILENT 8453 Dioda Array Spectrophotometer (Alpha Omega Technologies, Inc.) at the $\lambda_{vis\text{-}max}$ and at 700 nm (A_{700}) (to correct for haze), against a blank cell filled with distilled water. The absorbance of the diluted wine sample (A) was calculated as follows:

$$A = \left(A\lambda_{vis-\max} - A_{700}\right)_{pH\,1.0} - \left(A\lambda_{vis-\max} - A_{700}\right)_{pH\,4.5} \quad (1)$$

The mass concentration of anthocyanins (γ_A) was expressed in cyanidin-3-glucoside equivalents as mg of per litre of wine sample and calculated according to the following formula:

$$\gamma_A = \frac{(A \cdot MW \cdot DF \cdot 1000)}{(\varepsilon \cdot 1)} \quad (2)$$

Where MW is the molecular weight, DF is the dilution factor and ε is the molar absorptivity.

3.6. Determination of Reducing Sugars

Reducing sugars were determined according to the method of OIV-MAE-AS311-01-SUCRED [16]. 25 mL of the alkaline copper salt solution was mixed with 15 mL of water and 10 mL of the clarified solution of wine sample in a 300 mL conical flask. A few small pieces of pumice stone were added. The mixture was kept boiling for exactly 10 minutes. After cooling, 10 mL of potassium iodide solution, 30% (m v^{-1}), 25 mL of sulfuric acid, 25% (m v^{-1}), and 2 mL of starch solution were added. Titration was done with sodium thiosulphate solution, 0.1 mol L^{-1}. A blank titration in which the 25 mL of sugar solution is replaced by 25 mL of distilled water was carried. The mass concentration of reducing sugars was expressed in grams of invert sugar per litre of wine sample (γ_{RS}).

3.7. Determination of Antioxidant Activity

3.7.1. Reducing Power Assay

The reducing power was determined according to the method of Juntachote *et al*. [17]. An aliquot of a sample solution (0.5 mL) at different concentrations was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.8), 2.5 mL of potassium hexacyanoferrate(III) solution (1%, w v^{-1}) and 2.5 mL trichloroacetic acid solution (20%, w v^{-1}). After centrifugation at 13000 min^{-1} for 15 min on a model 5415C Centrifuge (Eppendorf, Hamburg, Germany), the supernatant (2.5 mL) was mixed with 2.5 mL of Milli-Q water and 1 ml of iron(III) chloride solution (1%, w v^{-1}). The absorbance at 740 nm (A_{740}) was measured on a model 8453 Hewlett Packard UV-Visible spectrophotometer (Hewlett Packard, Waldbronn, Germany) with a 1-cm cell against blank. The reducing power of the investigated samples was quantitatively expressed as the slope of the lines (not shown) representing the dependence of A_{740} on the concentration of the phenolic compound in the reaction mixture and denoted

as the coefficient of reducing power (C_R). The slope was obtained by linear regression analysis. Each determination was performed in triplicates and results of C_R were expressed in $(mg\ L)^{-1}$.

3.7.2. The DPPH$^{\bullet}$ Radical Scavenging Method

The DPPH$^{\bullet}$ radical-scavenging effectiveness was determined according to Brand-Williams *et al*. [18]. DPPH$^{\bullet}$ solution (0.1 mM, 2.9 mL) in 96% ethanol was added to 0.1 mL solution of sample at different concentrations. After 30 min, the absorbance was measured at 517 nm (A_{s517}). Ethanol (96%) was used as a blank. The absorbance of the control solution (A_{c517}) consisting of 0.1 mL of 96% ethanol and 2.9 mL of DPPH$^{\bullet}$ solution was also measured. A decrease in absorbance from A_{c517} to A_{s517} occurs when antioxidant reacts with the DPPH$^{\bullet}$ radical and radical becomes a stable molecule. DPPH$^{\bullet}$ scavenging ability is expressed as concentration of DPPH$^{\bullet}$ in mmol L^{-1} being scavenged by antioxidants in wine sample ($c_{DPPH\ \bullet}$). Triplicate analyses were run for each wine sample.

3.8. Statistical Analysis

Experimental data were arranged with software EXCEL XP and statistically analyzed by SAS program (SAS Software, Version 8.01, 1999) using GLM (General Linear Models). Average values for experimental groups were calculated using Duncan test (Duncan procedure; SAS Software 1999). The significance level for comparison was 5%. The statistical model included effects of varieties and yields on all investigated parameters.

4. Results and Discussion

The results of basic chemical composition of grape must (sugar level, total acidity, pH value) and young wine produced (alcohol, TA, tartaric acid, volatile acidity, pH value, total dry and sugar-free extract, total and free SO_2, color intensity and hue) are shown in Table 1. The results of analysis for determination of total polyphenols content, concentration of anthocyanins, reducing sugars, reducing power and DPPH$^{\bullet}$ scavenging ability in relation to yield of variety (6 t ha^{-1}, 8 t ha^{-1}, 10 t h^{-1}, 12 t ha^{-1} and 15 t ha^{-1}) are shown in Table 2.

In all parameters listed in Table 2 the p-value was ≤ 0.001, which means statistically very high significant differences; different letters indicate significant differences (95% confidence) between wines. Collecting the data for all varieties, depending on the yield, we determined the maximum value of most parameters at 8 t ha^{-1} (Table 2), except for C_R.

Therefore, with the aim to elucidate the impact of variety on investigated wine quality parameters Figure 1 presents the comparison for the content of reducing sugars, total polyphenols, mass concentration of anthocyanins, reducing power and DPPH$^{\bullet}$ scavenging ability in investigated wines for the yield of variety of 8 t ha^{-1}.

Different letters in index indicate significant differences (95% confidence) between wines; n.d. – not determined.

As observed from Figure 1A in reducing sugars content red wines with Vranac having the highest amount of γ_{RS} overestimated white wines. Except for variety Vranac, where we can observe the highest amount of reducing sugars at the lowest yield with coefficient of determination for the linear relationship between mentioned parameters (R^2) being 0.62, we could not confirm any dependence between reducing sugars content and the yield ($R^2 < 0.1$). Concerning polyphenols content (Figure 1B) the best results are shown for wine of red variety Vranac, after that is Kratošija, what is in agreement with previous results by [19,20]. Expectedly, white wines have appreciably lower content of polyphenols. The levels of the different classes of phenolic compounds in grapes are subject to a large variability. The phenolic composition depends on grape variety (as we can see Fig. 1), but also on additional factors, such as climate and even on vine location in the same vineyard, enological practices, the storage conditions, etc [21,22,6,23]. Red wines have, typically, eight times more phenolic compounds than white wines [8]. Phenolic compounds in white wines are consisting of hydroxycinnamates and flavanols (catechin, epicatechin). The reason why red grapes have so many more phenolic compounds than white grapes are because red grapes have anthocyanins, which are absent in white grapes [8]. Also, red wine production includes the procedure of maceration, which is not applied for white wine production and therefore white wines contained lower amounts of polyphenols [19]

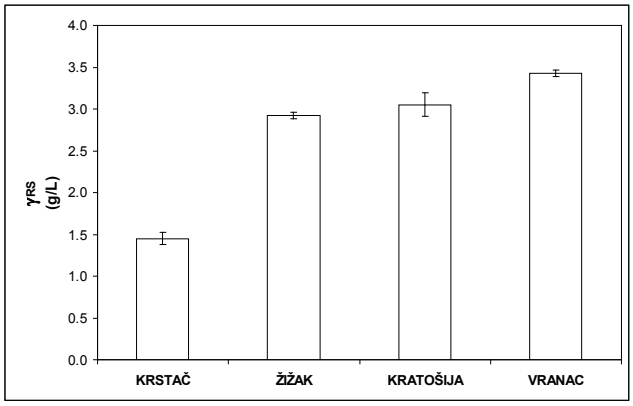
Table 1. Basic chemical composition of grape must and young wine produced in relation to yield and variety

Yield of variety (t ha^{-1})	Grape must			Produced young wine after alcoholic fermentation completion										
	sugar (%)	total acidity (g L^{-1})	pH	alcohol (%vol)	total acidity (g L^{-1})	tartaric acid (g L^{-1})	volatile acidity (g L^{-1})	pH	total dry extract (g L^{-1})	sugar-free extract (g L^{-1})	total SO_2 (mg L^{-1})	free SO_2 (mg L^{-1})	color inten-sity	color hue
KRATOŠIJA														
6	23.6	8.19	3.58	15.00	5.77	1.87	0.55	3.73	34.4	31.2	64	19	2.8	0.26
8	22.8	7.59	3.69	14.45	5.62	1.40	0.50	3.73	29.4	25.3	33	13	2.33	0.28
10	22.8	7.44	3.55	14.19	5.73	1.95	0.40	3.77	30.0	28.9	58	20	2.69	0.29
12	22.0	7.63	3.5	13.44	5.90	2.36	0.40	3.61	28.4	27.4	46	18	2.45	0.34
15	23.0	7.62	3.45	13.40	5.77	2.28	0.35	3.63	28.7	27.9	54	19	2.28	0.27
VRANAC														
8	23.4	5.51	3.64	14.15	8.10	3.23	0.20	3.39	37.8	32.5	47	13	5.33	0.23
10	22.3	5.64	3.65	13.90	7.38	2.84	0.20	3.31	32.0	27.7	19	15	5.59	0.26
12	23.1	5.80	3.65	13.40	8.13	4.63	0.20	3.29	34.4	29.9	31	9	6.01	0.24
15	21.2	5.96	3.65	13.38	8.10	4.78	0.20	3.27	34.4	30.0	67	14	5.53	0.21
KRSTAČ														
6	18.8	4.14	3.43	12.78	6.15	3.76	0.2	3.33	20.1	19.3	20	9	n.d.	n.d.
8	18.6	3.79	3.44	12.78	6.00	3.85	0.15	3.34	20.1	19.4	31	10	n.d.	n.d.
10	18.6	4.13	3.44	12.69	6.07	4.57	0.20	3.33	20.6	20.1	28	12	n.d.	n.d.
12	18.8	4.20	3.39	12.90	6.15	4.27	0.15	3.34	20.3	19.7	23	10	n.d.	n.d.
15	18.6	4.37	3.35	12.90	6.15	4.00	0.15	3.29	20.3	19.7	20	10	n.d.	n.d.
ŽIŽAK														
6	21.2	6.49	3.41	13.87	7.70	4.29	0.10	3.21	23.7	22.2	17	6	n.d.	n.d.
8	21.5	7.53	3.37	13.78	7.50	3.76	0.10	3.29	25.8	22.4	23	6	n.d.	n.d.
10	21.5	6.76	3.39	13.86	7.80	4.22	0.15	3.26	23.2	21.8	17	6	n.d.	n.d.
12	18.6	6.72	3.57	13.34	8.13	4.04	0.25	3.25	25.5	21.4	13	3	n.d.	n.d.
15	20.2	7.55	3.47	13.26	8.23	4.36	0.15	3.23	23.5	22.6	10	4	n.d.	n.d.

Total acidity is expressed as tartaric acid, volatile acidity is expressed as acetic acid; n.d. – not determined.

Table 2. Content of total polyphenols (γ_P), mass concentration of anthocyanins (γ_A), reducing sugars (γ_{RS}), reducing power (C_R) and DPPH$^{\bullet}$ scavenging ability ($c_{DPPH^{\bullet}}$) in relation to yield and variety (Duncan test, $\alpha = 5\%$)

Yield of variety (t ha^{-1})	γ_P (mg gallic acid L^{-1})	γ_A (mg cyanidin-3-glucoside L^{-1})	γ_{RS} (g L^{-1})	C_R (mg L)$^{-1}$	$c_{DPPH^{\bullet}}$ (mmol L^{-1})
KRATOŠIJA					
6	1851±17^{a}	164±2^{a}	2.58±0.04^{b}	0.971±0.027^{c}	154±1^{a}
8	1601±44^{b}	158±2^{b}	3.05±0.14^{a}	0.927±0.031^{d}	139±0^{b}
10	1380±8^{c}	147±1^{c}	2.95±0.07^{a}	0.977±0.017^{c}	118±0^{c}
12	1265±13^{d}	145±2^{c}	2.53±0.04^{b}	1.033±0.017^{b}	108±1^{e}
15	1316±40^{d}	146±1^{c}	2.58±0.04^{b}	1.082±0.014^{a}	111±1^{d}
Average	1493.0±16.4^{b}	152.1±0.7^{b}	2.75±0.04^{a}	1.008±0.008^{b}	126.6±0.6^{b}
VRANAC					
8	2032±82^{a}	223±5^{b}	3.43±0.04^{a}	1.099±0.028^{b}	166±1^{a}
10	1829±18^{b}	237±3^{a}	1.70±0.07^{b}	1.135±0.009^{a}	159±1^{b}
12	1693±50^{c}	201±1^{c}	1.45±0.07^{c}	1.089±0.019^{c,b}	146±2^{c}
15	2072±19^{a}	223±6^{b}	1.45±0^{c}	1.061±0.009^{c}	157±2^{b}
Average	1906.5±30.1^{a}	221.0±2.2^{a}	2.01±0.03^{c}	1.096±0.009^{a}	157.2±0.7^{a}
KRSTAČ					
6	228±2^{c}	n.d.	1.50±0.21^{a}	0.086±0.002^{c}	8.66±0.28^{b}
8	259±3^{a}	n.d.	1.45±0.07^{a}	0.093±0.001^{b}	9.02±0.06^{a}
10	255±7^{b,a}	n.d.	1.30±0^{a}	0.097±0.002^{a}	8.99±0.03^{a}
12	247±6^{b}	n.d.	1.30±0.07^{a}	0.100±0.001^{a}	9.17±0.01^{a}
15	248±6^{b}	n.d.	1.50±0^{a}	0.098±0.002^{a}	9.01±0.03^{a}
Average	253.9±2.1^{d}	n.d.	1.33±0.27^{d}	0.096±0.000^{c}	9.04±0.02^{d}
ŽIŽAK					
6	269±7^{c}	n.d.	1.70±0.07^{d,c}	0.088±0.005^{c}	12.3±0.2^{c}
8	260±2^{d}	n.d.	2.93±0.04^{b}	0.098±0.002^{b}	12.4±0.2^{c}
10	280±6^{b}	n.d.	1.78±0.04^{c}	0.101±0.001^{b}	13.1±0^{b}
12	290±1^{a}	n.d.	3.75±0.07^{a}	0.097±0.002^{b}	13.9±0.3^{a}
15	277±3^{b}	n.d.	1.63±0.04^{d}	0.109±0.002^{a}	13.3±0.1^{b}
Average	275.1±2.6^{c}	n.d.	2.36±0.02^{b}	0.098±0.001^{c}	13.0±0.1^{c}
Average on varieties					
6	793.0±9.0^{c}	164.0±2.4^{d}	1.91±0.06^{c}	0.384±0.011^{c}	58.6±0.4^{e}
8	1038.2±32.7^{a}	190.2±3.5^{a}	2.71±0.07^{a}	0.554±0.016^{b}	81.8±0.3^{a}
10	936.2±9.8^{c}	192.1±2.0^{a}	1.93±0.05^{c}	0.578±0.007^{a}	74.9±0.3^{b}
12	886.5±24.4^{d}	173.4±1.0^{c}	2.19±0.02^{b}	0.592±0.009^{a}	69.9±0.8^{d}
15	978.2±16.9^{b}	184.5±3.3^{b}	1.79±0.02^{c}	0.587±0.007^{a}	72.4±1.0^{c}



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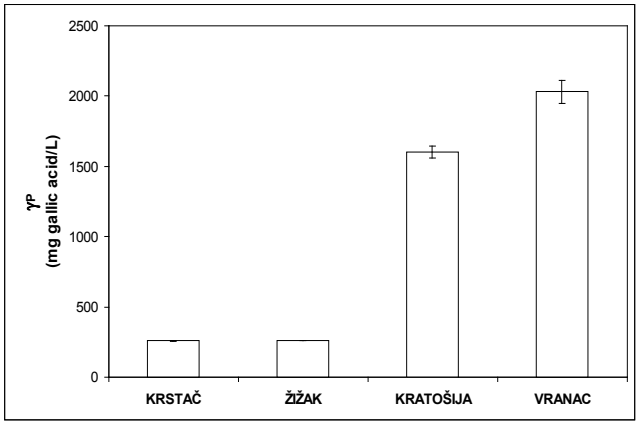

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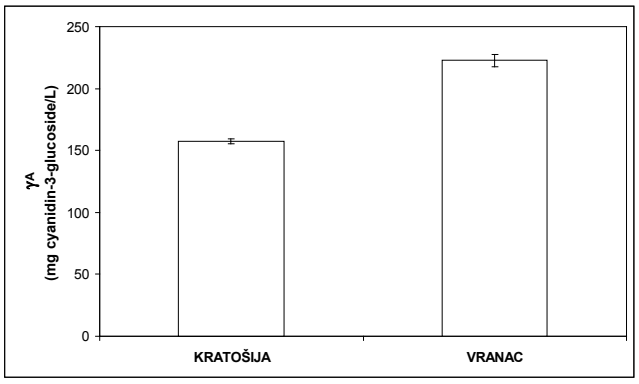
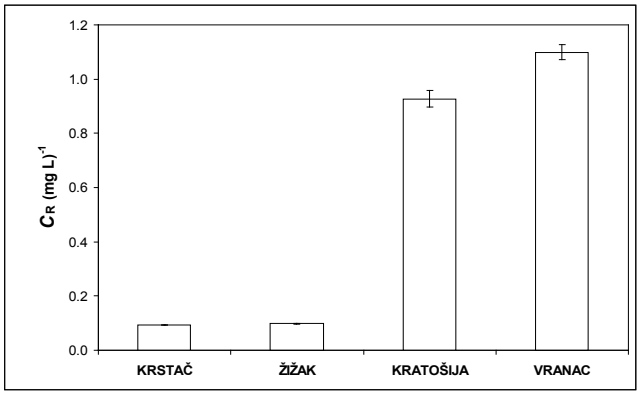
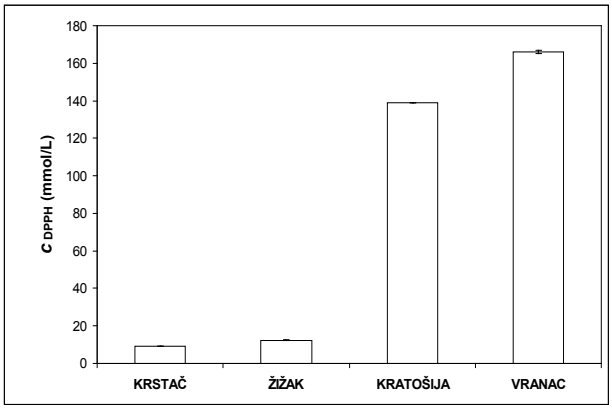


(C)



(D)



(E)

Figure 1. The content of reducing sugars (ɣ RS) (A), polyphenols, expressed as mass concentration of gallic acid (ɣ P) (B), concentration of anthocyanins expressed as cyanidin-3-glucoside (ɣ A) (C), reducing power (CR) (D) and DPPH scavenging ability (cDPPH) (E) in wines Krstač, Žiž ak, Kratoš ija and Vranac with yield of 8 t ha-1

In red wines investigated in our research, the highest amount of anthocyanins has Vranac (Fig. 1C) that in average (by collecting the data for all yields) contained statistically significant higher mass concentration of these compounds (γ_A = (221 ± 3.6) mg L^{-1}; N = 12) in comparison to Kratošija (γ_A = (152 ± 1.6) mg L^{-1}; N = 15). The presence and concentration of each group of anthocyanins is varietal specific, changes with environmental conditions and viticultural practices and has a huge impact on the color and color stability of a wine.

It is generally believed that heavy crop loads have a negative effect on grape composition and subsequent wine quality. Prajitna *et al*. [24] and Kamiloglu [25] in their investigations found out that the total phenols and total anthocyanins significantly increased in wines from low yield crops. It was also determined that the highest increase occurred in the treatment with 60% of clusters removed while yield reduction affected not only the total anthocyanin concentration but also the anthocyanin profile in wines [26]. The yield reduction from 8 t ha^{-1} to 4 t ha^{-1} resulted in a higher concentration of the flavan-3-ol (+)-catechin, and the flavanols isorhamnetin-3-O-galactoside and isorhamnetin-3-O-glucoside in berry skins [27]. On the other side, some authors have found higher content of phenolic compounds in wines from vineyards with a high yield then in those with low yield [5]. As it could be observed from Table 1 in investigated wines, with except for variety Kratošija, there is a poor response of total phenolic content to variation in yield with R^2 being less than 0.33. However, in wine of variety Kratošija a decrease in a crop load resulted in higher phenolic content (R^2 = 0.76) but also in higher amount of anthocyanins (R^2 = 0.74).

The ability of phenols to act as antioxidants depends on the degree of conjugation, number and distribution of functional group and molecular weight. Monohydroxybenzoic acids are very weak antioxidants; while trihydroxybenzoic acid, gallic acid is the best antioxidant of all hydroxybenzoic acids. Most polyphenols

have in their structure, two continuous hydroxyl groups on the aromatic ring, called a catechol group, which has a great affinity for free radicals on nearby molecules. Wine is rich in substances with the catechol group. The catechol reacts with the oxygen-free radical to form a semiquinone, then a quinone. As a result, the free radicals are scavenged and no longer available to oxidize other compounds. On Figure 1 we can see that red wines express notably stronger ability to scavenge DPPH$^{\bullet}$ radical than white wines. DPPH$^{\bullet}$ concentrations being scavenged by the same amount of wine are for red wines more than 10-times higher than those for white wines. A strong positive linear dependence of DPPH$^{\bullet}$ scavenging ability on the total polyphenols content was observed for Kratošija ($R^2 = 0.98$) and Žižak ($R^2 = 0.68$). For Kratošija it was confirmed that the DPPH$^{\bullet}$ scavenging ability strongly depends also on the amount of anthocyanins ($R^2 = 0.96$). Further, the correlation studies show that the yield of variety Žižak positively affected the DPPH$^{\bullet}$ scavenging ability of wine ($R^2 = 0.60$), while for Kratošija a negative relationship has been found ($R^2 = 0.82$). DPPH$^{\bullet}$ scavenging abilities of Vranac (negative correlation; $R^2 = 0.31$) and Krstač (positive correlation; $R^2 = 0.32$) correlated poorly to the yield of variety.

The ability of a compound to transfer an electron to reduce a substance is a good indicator of its antioxidant activity. Further, it may be also indicative in defining the reaction mechanism of an antioxidant in a given environment. According to reducing power, wines Krstač, Žižak and Kratošija at the yield of 8 t ha^{-1} show comparable values that are slightly lower than that of variety Vranac (Fig. 1). For variety Žižak we have observed a slight positive correlation among C_R and the yield ($R^2 = 0.64$) on the other side C_R of variety Vranac correlated negatively with the yield ($R^2 = 0.39$). The yield of variety Krstač and Kratošija only poorly influenced the reducing power of wines ($R^2 < 0.64$ and 0.70, respectively). The correlation studies between C_R and the content of total polyphenols revealed a good relationship with $R^2 = 0.93$ (for all investigated varieties and yields). We have also found good correlation between DPPH$^{\bullet}$ scavenging ability of wines and their reducing power ($R^2 < 0.95$). This observation indicates that the substances responsible to reduce Fe^{3+} to Fe^{2+} are capable to scavenge DPPH$^{\bullet}$ radical satisfactorily what suggests that these substances might react with DPPH$^{\bullet}$ radical through electron transfer reaction mechanism. We have tried to define also any impact of the content of reducing sugars on C_R and find out, surprisingly, that for Krstač, Žižak and Kratošija a poor negative correlation exists ($R^2 = 0.08$, 0.04 and 0.36, respectively), while for Vranac very weak positive effect of reducing sugars on C_R was observed ($R^2 = 0.03$).

According to Duncan's multiple range test for the yields we could confirmed the highest mean values of $c_{DPPH\,\bullet}$, γ_P and γ_{RS} at yield of 8 t ha^{-1} and all of mentioned parameters differed significantly among all investigated yields. The highest mean value of C_R was determined at 15 t ha^{-1} and it was comparable value to the yields of 10 and 12 t ha^{-1}. The γ_A in red wines was the highest at 10 t ha^{-1} and comparable to the yield of 8 t ha^{-1}, while at lower and higher yields the mean value were significantly lower and different (95% confidence). According to Duncan's multiple range tests for the varieties the significant difference existed between wines practically in all investigated parameters, except for C_R of white wines.

The statistically very high significant influence ($p \leq 0.001$) among varieties, yields and cumulative impact of variety and yield was determined in total polyphenols, reducing sugars, reducing power and DPPH$^{\bullet}$ scavenging ability. According to Pearson correlation coefficients we could confirmed the strongest correlations between γ_P and $c_{DPPH\,\bullet}$ (0.9962, $p < 0.0001$, $N = 57$), followed by correlations between $c_{DPPH\,\bullet}$ and C_R (0.9764, $p < 0.0001$, $N = 57$ data), γ_P and C_R (0.9660, $p < 0.0001$, $N = 57$ data) for all wine samples, even between $c_{DPPH\,\bullet}$ and γ_A for only red wine (0.8423, $p < 0.0001$, $N = 27$).

Psarra *et al*. [28] in their investigation on 26 wines assessed slightly poorer correlation between antioxidant parameters (DPPH$^{\bullet}$ scavenging ability and ferric reducing power) and total polyphenols content with correlation coefficient values amounting 0.8115 and 0.5956, respectively. According to authors provided the evidence that antioxidant activity of wine depended on relative amounts of individual polyphenols and further on synergistic action between them.

5. Conclusion

In general the highest content of total polyphenols, anthocyanins and reducing sugars as well as the strongest reducing power and DPPH$^{\bullet}$ scavenging ability was determined for wine of Vranac variety, lower values were obtained for Kratošija that was followed by Žižak and Krstač. Despite the maximum value of most parameters was determined at the yield of 8 t ha^{-1} as compared to values at yield of 6 t ha^{-1}, 10 t h^{-1}, 12 t ha^{-1} and 15 t ha^{-1} a poor correlation among yield and quality parameters has been found. That means that any further reduction in crop load did not result in higher contents of total polyphenols, anthocyanins as well as reducing sugars in wine and also did not improve its antioxidant potential. What is an appropriate yield depends on a number of factors, but the most important are the style of wine that is being aimed at, and the grape variety itself.

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