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Original research paper

THE EFFECTS OF *PROKUPAC* VARIETY CLONES AND VINIFICATION METHOD ON THE QUANTITY OF RESVERATROL IN WINE

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ABSTRACT: The focus of this study was to investigate the effects of clones (subvarieties) of autochthonous Serbian grape variety *Prokupac*, along with the influence of vinification method on the content of *trans*- and *cis*-resveratrol as well as on total phenolic content (TPC) in wines. Wines were made from four clones of *Prokupac* variety (PR1, 40/1, PR6 and PR7) by application of different periods of maceration duration (1, 5 and 10 days). The effects of different species of selected wine yeasts and glucosidase enzymes on the quantity of resveratrol and TPC in wine made from PR6 variety were also investigated. The content of *trans*-resveratrol varied from 0.27 mg/L to 1.46 mg/L. The highest content of resveratrol was determined in *Prokupac* clone PR6, and the lowest in PR7 clone. An increase in resveratrol and TPC content was observed in all clones when the duration of maceration was prolonged. Wine produced by application of β enzyme preparation and 299 yeast had the highest concentration of total resveratrol (4.23 mg/L). The TPC was the highest in the wine made by combined application of yeast 299 and OE enzyme. The obtained results showed that by adequate selection of varieties, prolonged duration of maceration, application of appropriate species of yeast and enzyme preparations, it is possible to increase the content of resveratrol and other phenolic compounds in wine.

Key words: *antioxidants, polyphenols, yeast, enzymes, maceration, HPLC*

INTRODUCTION

Resveratrol (3,5,4′-trihydroxystilbene) is a polyphenol which appears as a *cis*- and a *trans*- isomer, free or in a glucosidase bound form as a 3-mono-D-glucoside called piceid or polydatin (3,5,4'-trihydroxystilbene-3-β-mono-D-glucoside).

Resveratrol is a phenolic phytoalexin with antifungal properties. It belongs to the class of antibiotics synthetized by certain parts of plants as a response to an attack of illness, in cases of abiotic stress (in the presence of heavy metal ions) (Vitrac et

al., 2002) and tissue damage or UV radiation effects (Pervaiz, 2003). *Trans*-resveratrol occurs naturally in grapes however, *cis*-resveratrol and its glucoside are present in wines of diverse origin, analysed by different technology. It was detected, that vinification process causes, that some amount of *trans*-resveratrol transforms into its *cis*- form. Nevertheless, *trans*- form protected from light can be stable for months, except in high pH, while *cis*-resveratrol protected from light was stable only in neutral pH (Cvejic et al., 2010). *Trans*-resveratrol is mostly synthesised in grape berry epidermis while its content in the pulp is quite low (Rodríguez-Delgado et al., 2002). In the past few years, a symmetrical resveratrol dimer was discovered and named palidol (Vitrac et al., 2002). It is also considered to be a metabolic product which appears as a response to a microbiological attack. Another stilbene, piceatannol glucoside (3,5,3',4'-tetrahydroxystilbene-3-D-glycoside) called astringin, was also detected in *Vitis vinifera* cultivar cells and in wine (Moreno-Labanda et al., 2004). Both forms of resveratrol have similar properties however, *cis*-isomer with a lesser extent and both forms are present in red wines examined in many researches published in different journals (Tan et al., 2009).

In the past decade or so, numerous beneficial effects of resveratrol and other phenolic compounds on human health were pointed out in literature. Indeed it is possible to emphasize anticarcinogen, cardioprotective, vasodilatory and antioxidant activity. There is also evidence of positive effect on the patient with diseases such as diabetes mellitus, Alzheimer's, Parkinson's, multiple sclerosis, while beside it exhibits ability of thrombocytes aggregation inhibition, as well as anti-inflammatory activity (Čakar et al., 2018a; Gojkovic-Bukarica et al., 2008).

It is stated in literature that the total content of resveratrol in wine may exceed 30 mg/L (Flamini, 2003) and that white wines almost regularly contain less than 1 mg/L while its content in red wines varies from 0.9 to 8.7 mg/L (La Torre et al., 2004). The highest concentration of resveratrol was found in grape epidermis (skin) with the content ranging from 50 to 100 μg/g (Jang et al., 1997). More resveratrol in red wines than in white ones is also due to the technological process of manufacturing. White wine implies a very short contact of grape juice with epidermis (Jeandet et al., 1995). Grape juice as a non- fermented product is not a significant source of resveratrol due to the lack of alcohol which improves extraction of resveratrol and other phenolic compounds from grape skin (Careri et al., 2004; Čakar et al., 2018b).

The literature data indicate that, almost all countries study quantities of resveratrol in their autochthonous varieties. Today modern oenology applies selected wine yeasts which have different glucosidase activity, to achieve a higher content of free resveratrol in wine by application of yeasts with higher glucosidase activity. In addition, the usage of glucosidase enzyme preparations which are added to must in the process of alcoholic fermentation showed a significant contribution, too. Accordingly, the objective of this study was to determine the quantities of resveratrol (*cis-, trans*- and total) and total phenolic content (TPC) in wines obtained from four different clones of Serbian autochthonous variety *Prokupac.* Wines were produced by using different pure yeast cultures and glucosidase enzyme preparations during the vinification procedure. This is for the first time that data on the quantities of resveratrol in *Prokupac* are made public. Such data would be a contribution to the planned expansion of plantations of some clones of this wine which has proved to be resistant to viral diseases.

MATERIALS AND METHODS

Grapes used in vinification

Prokupac is a Serbian autochthonous wine variety. It is characterized with less strong vigour and good yielding capacity. *Prokupac* shoots are developed and strong, with a vertical rise, can be grown at training systems with small trunk. The fertility is good; the lowest buds on shoot are very fertile, therefore, short pruning provides an excellent yield in practice. Test analysis on the most significant economical viruses from a reference laboratory

in Bari, confirmed the "natural virus free" status for all four clones. Grapes of four different clones (PR 1, 40/1, PR 6 and PR 7) of the autochthonous variety *Prokupac* were processed at the Radmilovac Experimental Station of the Faculty of Agriculture, University of Belgrade. These clones differ in some economic and technological properties such as: the time of ripening in relation to plants from the population; the content of sugar and acids in must, alcohol in wine; mechanical analysis of grape clusters and berries and quality of wine. Grapes of all *Prokupac* clones used in this study were picked at the same time due to the nature of experiment. The average content of sugar in must varied from 18.5% (in clone PR1) to 23.0% (in clone PR6), and the content of total acids varied between 5.70 g/L (in clone PR6) and 10.45 g/L (in clone PR1) marked as wine acid. The content of alcohol in wine goes along with the quantity of sugar in grapes and varied between 10.16 vol% and 13.42 vol%. The specific gravity of must was determined by hydrometer expressed in Oechsle degree. The obtained results were used to calculate sugar % by using Saleron formula. Titratable acids were determined by volumetric titration using NaOH (Tanner and Brunner, 1979). Alcohol content in wines expressed in vol% was determined by digital mechanical analyser (DMA), Anton Paar DMA 35, Graz, Austria (Stabinger et al., 1967).

The vinification

Grapes were mashed, separated from leaves and stems and such prepared must of each variety was inoculated with 20 g/hL of UVAFERM BDX yeast (BDX) (Lallemand, Canada). Then potassium metabisulfite ($K_2S_2O_5$) was added in the amount of 5 g per every 100 kg of must and the must was set for fermentation by microvinification (10kg of grapes per microvinification without replicates) using pigéage system twice a day. Fermentation with maceration (solid – liquid contact) lasted for one, five and ten days at average temperature of 25 °C. After that the liquid part was separated and fermentation continued without contact with the solids during the next 14 days. The effect of yeast strain on the quantity of resveratrol and total phenolic content was studied on PR6 clone of *Prokupac* variety. The musts of selected clone were inoculated in separate vinifications with BDX, UVAFERM 299 (299) (Lallemand, Canada) and Lalvin Rhône 2056 (2056) (Lallemand, Canada) yeasts and exposed to enzyme preparations of different glucosidase activity - Lallzyme Beta (β) (Lallemand, Canada) and Lallzyme OE (OE). After alcoholic fermentation was completed (14 days at 25 °C) wines were racked and samples were stored until the further analysis.

Standards and reagents

The standards used for the quantification were *trans*-resveratrol purified to 99% purity from Sigma Aldrich (Steinheim, Germany) and *cis*-resveratrol obtained by isomerisation of the *trans*- form under the influence of UV radiation. The isomerisation conducted by exposing *trans*-resveratrol (C= 100 mg/L in methanol) to daylight for ten hours, and about 70% of *trans*-resveratrol was transformed to the *cis*-isomer (Kolouchová-Hanzlíková et al., 2004, Cvejic et al., 2010). All other chemicals and reagents of analytical grade were purchased from Sigma Aldrich (Steinheim, Germany). The Premium Syringe Filters (Captiva) Regenerated Cellulose (0.45 µm, 15 mm) were obtained from Agilent Technologies (Santa Clara, CA, USA). Water HPLC grade was provided by Ultrapure Water System Arium pro UV Sartorius (Göttingen, Germany).

Wine sample preparation

The wine is a complex matrix and to decrease its influence during the quantification of resveratrol, solid-phase extraction (SPE) was applied. Wine samples were filtered through syringe filter and SPE was conducted by using the Oasis HLB 6CC 200 mg cartridges (Waters, Milford, MA, USA) with some modification of method (Ferreiro-Gonzales et al., 2014). The obtained solutions of wine samples after SPE were used for the further analysis.

HPLC analysis

The content of free *trans*-resveratrol and its *cis*- isomer in wine were determined by

applying reverse-phase high performance liquid chromatography (HPLC) with UV detection. In quantification of *cis*- and *trans*-resveratrol in wine the external standard method was applied. The HPLC system consisted of the following components: Hitachi system of two constant flow pumps (Model 655A-11); Hitachi gradient controller (Model L-5000); Hitachi auto sampler (Model 655A-40); Gilson UV detector; Bishoff Hyper chrome analytical column, (250 x 4.6 mm, 5 µm ODS Hypersil) and CSW32 data processing software package. The binary gradient consisted of solvent A: acetonitrile–acetic acid–water (20:2:78 v/v); and solvent B: acetonitrile–acetic acid–water (90:2:8 v/v) as follows: linear gradient from 0% to 10% B in 8 min, 10–15% B in 12 min, 15–30% B in 10 min, 30–50% B in 5 min, 50–100% B in 5 min, and 2 min 100% B isocratic. The flow rate was 1 mL/min, the column temperature was maintained at 25°C, and the injection volume was 50 µL. Detection wavelengths for *trans*-resveratrol was 306 nm and for its *cis*-isomer it was 286 nm (Cvejic et al., 2010). The analysis was performed in triplicate.

Total phenolic content (TPC)

The total phenolic content (TPC) was determined by Folin-Ciocalteu method. The results are expressed in mg/L of equivalents of gallic acid (mg/L GAE) (Burns et al., 2001). The analysis was performed in triplicate.

Statistical analysis

The statistical analysis were performed using the software SPSS Statistics V20.0 (IBM, Chicago, IL, USA; 2014); two-way ANOVA, with Tukey *post hoc* test to compare the influence of extraction after different time intervals and vinification procedures on the content of total resveratrol, its isomers and TPC.

RESULTS AND DISCUSSION

Resveratrol and isomers in wine obtained from different clones of Prokupac variety

The influence of maceration duration and clone on the content of *trans*-resveratrol in produced wines was estimated by application of two-way ANOVA analysis. Indeed duration of maceration affected the content of *trans*-resveratrol ($p < 0.05$); the highest content was found in those wine samples in which maceration lasted 10 days whereas lower content was in the samples obtained after 5 days of maceration (Table 1). The samples prepared after 1 day of maceration had the lowest content of *trans*-resveratrol. Similarly, it was found that different clones affected the content of *trans*-resveratrol in all cases ($p < 0.05$). Wines produced from clone PR6 had the highest content of *trans*-resveratrol after 1, 5 and 10 ($p < 0.05$) days of maceration (Table 1).

Table 1.
Trans-resveratrol and total phenolic content extraction rate for *Prokupac* clones

***Prokupac* clones**	**Maceration duration** (days)	***Trans*-resveratrol** (mg/L)	**Total phenolic compounds as gallic acid** (mg/L)
PR1	1	0.35±0.02	426.80±24.0
41/1	1	0.41±0.01	407.30±27.1
PR6	1	0.48±0.03[a]	447.40±23.0[a]
PR7	1	0.27±0.01	421.70±25.5
PR1	5	0.50±0.02	586.20±27.8[a]
41/1	5	0.60±0.02	534.80±26.2
PR6	5	0.63±0.03[a]	504.00±25.4
PR7	5	0.49±0.02	529.70±26.0
PR1	10	0.94±0.04[A]	1100.50±51.2[A]
41/1	10	1.17±0.05[A]	1172.50±57.0[A]
PR6	10	1.46±0.02[Aa]	1265.00±63.2[Aa]
PR7	10	0.82±0.04[A]	894.80±39.0[A]

[A] *Means significantly different from the means of the samples macerated after 1 and 5 days ($p < 0.05$)*
[a] *Means significantly different within the same maceration period ($p < 0.05$)*
Values are Mean±Standard deviation (n = 3)

The clone PR6 after 10 days of maceration had the highest content of *trans*-resveratrol (1.46 mg/L ($p < 0.05$)) which was the maximum content throughout the all analysed samples in study. On the other hand, the lowest content of resveratrol was detected in clone PR7 (0.82 mg/L) ($p < 0.05$) after 10 days of maceration, while the similar observation was noticed in samples after 1 and 5 days of maceration ($p < 0.05$). *Cis*-resveratrol was not detected in any of the wines obtained from the four different clones of *Prokupac* variety during the different maceration duration.

The obtained results can be compared with literature data which suggest that grape varieties Pinot noir and Merlot contain high concentrations of stilbene (Goldberg et al., 1995), followed by Cabernet sauvignon, Muscat, Concord, Grenache and Tempranillo (Moreno-Labanda et al., 2004). Resveratrol concentration in wine produced from Cabernet sauvignon depends on the climate region. If grapes have been grown in a dry and warm climate where fungal infection attacks are rare, it will contain less resveratrol in comparison with those grown in colder and more humid climate regions such as Bordeaux, France and Canada (Soleas et al., 1997). It is believed that higher resveratrol content is a genetic property of red grape va-rieties (Rodríguez-Delgado et al., 2002). Generally, in all grape varieties it was observed a tendency of increased resveratrol concentration with extending maceration period, which is in accordance with previous investigations. It is known that white wines contain lower concentrations of resveratrol in comparison to red wines. In order to examine the effect of the technological process of wine production on the quantity of resveratrol, experiments were carried out. The white wine was produced following the procedure for red wine. Resveratrol quantity in must was 0.18 mg/L, and after maceration and fermentation during 24 hours, its level increased to 0.89 mg/L. After five days of maceration and fermentation of Chardonnay grapes, the quantity of resveratrol in white wine increased up to 2.41 mg/L. In rosé wines the quantity of *trans*-isomers of resveratrol varied from 0.005 to 1.19 mg/L (Romero-Pérez et al., 1996). The findings from a previous study on Pinot noir and Chardonnay varieties suggested that there was three times more resveratrol in red wine -than in white wine even when processed by the same procedure as red wine. It was concluded that red varieties of grapes genetically contain more resveratrol than those of white. According to the reported data, very low concentration of *trans*-resveratrol (about 0.1 mg/L) was found by analysis of 100 white wine variants. (Romero-Pérez et al., 1996).

It is very important to localize resveratrol in grapes. In view of the fact that resveratrol is mostly located in the epidermis of the berry, prolonged „skin contact“ and intensive pomace maceration are preconditions for its good extraction (Darias-Martín et al., 2000). Studies on kinetics of extraction of *trans*-resveratrol point out that the transfer of resveratrol from the epidermis to the liquid part (juice) reaches its maximum value after the fourth day of must fermentation (Atanacković et al., 2012). On the contrary, the maximum extraction of *trans*-resveratrol was reached after the tenth day of fermentation and maceration of must for Aglianico and Piedirosso. With Nerello Mascalese maximum extraction of resveratrol occurred only after the twentieth day from the beginning of fermentation. This is explained by varying rate of ethanol content which assists in the extraction of resveratrol (Gambuti et al., 2004). The must of Aglianico and Piedirosso varieties developed the maximum concentration of alcohol on the tenth day from the beginning of fermentation, unlike the must of Nerello Mascalese. During the maceration of Syrah grape variety, *trans*-resveratrol ranged from 0.4 to 2.2 mg/L (must to must after 10 days of maceration) reaching up to 0.6 mg/L in the wine. The maximum level observed for *trans*-resveratrol was reached with 10 days of maceration (2.2 mg/L) (Alencar et al., 2018). The investigation conducted on grape varieties Pinot noir and Chardonnay from the province of Bourgogne consisted of maceration in fermentation during 7 days at 25 °C, and fermentation of must. Major portion of resveratrol passed into wine du-

ring maceration. Wine obtained from must maceration of Chardonnay variety had higher content of total resveratrol (1.11 mg/L) than wine obtained by classical fermentation of grape juice (0.12 mg/L). Similar results for resveratrol content were obtained for Pinot noir variety wines produced after fermentation of grape juice (less 0.23 mg/L) and must maceration (3.04 mg/L). More free resveratrol was obtained from Pinot noir after fermentation with yeasts which possess higher glycolsidase activity (Eder et al., 2000). The study on Cabernet sauvignon and Merlot from Chile indicated that thermal vinification by heating to 60 °C for an hour contributed to the extraction of phenolic compounds much more than classical vinification procedures. The rate of extraction of *trans*-resveratrol into wine by thermal vinification process exceeded the procedures of bedewing «remontage» and submerging «pigéage» (Burns et al., 2001). Contrary to these findings, the research by Kocabey et al. (Kocabey et al., 2016), in which Karaoglan red wine samples were macerated for 5, 10 and 15 days. Only one stilbene (*trans*-resveratrol) was determined in wine samples and its concentration (2.19–2.68 mg/L) was not influenced by maceration duration ($p > 0.05$). Prolonged maceration time, usage of enzymatic preparation are useful to increase the concentration of phenolic compounds and consequently, the antioxidant activity of wine improves (Čakar et al., 2018b; Ivanova-Petropulos et al., 2016).

The influence of vinification procedure on the content of total resveratrol and its isomers

To evaluate the influence of vinification procedure on the content of resveratrol, different pure wine yeasts culture and enzyme preparations were applied. Clone PR6 was used in different vinification procedures due to the fact that it had the highest content of *trans*-resveratrol in preliminary quantification among all other clones. The influence of different yeasts and enzyme preparations for vinification on the content of *cis*- and *trans*- resveratrol was estimated by application of two-way ANOVA analysis. The results infer that different yeasts affected the content of two resveratrol isomers ($p < 0.05$). The highest quantity of *cis*- and *trans*- resveratrol was found in the samples produced with yeast 299 ($p < 0.05$) (Table 2). The application of enzymatic preparation β in vinification with different yeast showed the highest values for *cis*-resveratrol content ($p < 0.05$), while the highest values for *trans*-isomer were shown for enzymatic preparation OE (Table 2). The highest content of *cis*-resveratrol was observed in the sample produced with yeast 299 and β preparation (3.59 mg/L), while the lowest content was found in the sample produced with the same yeast but without any enzymatic preparation (0.73 mg/L). On the other hand, the highest content of *trans*-resveratrol was in the sample produced with yeast 299 and OE preparation (1.56 mg/L), while the lowest was found in sample produced with yeast 2056 in combination with enzymatic preparation *β* (0.48 mg/L).

The two-way ANOVA analysis indicated the influence of vinification on the content of total resveratrol. Different yeasts affected the total resveratrol content; the highest quantity was determined in the samples produced with yeast 299 ($p < 0.05$). Usage of enzymatic preparation β gave the highest content of total resveratrol in the samples produced with different yeasts ($p < 0.05$) (Table 2).

The highest resveratrol content was observed in the sample produced with 299 yeast and β enzymatic preparation (4.23 mg/L) ($p < 0.05$), whereas the lowest was produced with the same yeast but without any enzyme preparations (1.51 mg/L) ($p < 0.05$).

The obtained results are supported by the literature data that reveal that the activity of glucosidase enzymes increases the content of free isomers of resveratrol and other phenolic compounds influencing the biological activity of natural products (Čakar et al., 2018b). Previous investigations reported that the concentration of free *trans*- and *cis*- forms of resveratrol in grape juice and wine is affected by *β*-glucosidase enzymes. These enzymes originnate from grapes, although they may also be subsequently added to must. Slowed down hydrolysis also brings about chan-

ges of free and glycoside linked resveratrol during aging in oak wood (Dourtoglou et al., 1999). In this study, the ratio between *trans*- and *cis*-isomers (Table 3) was in all cases lower than 1, except for the wine obtained with BDX, yeast 299 and yeast 299 combined with OE enzyme preparation. In all analysed wines, *cis*-resveratrol was dominant, except in three wine samples mentioned before. For the sake of comparison, in Austrian wines obtained from Blaufränkisch variety, vintages between 1993 and 1997, *trans*- vs. *cis*-resveratrol ratio ranged from 0.70 to 1.70 (Eder et al., 2000). The effect of UV radiation on plant tissues played a significant role in the metabolism of phenolic compounds where UV light decreases biosynthesis of enzymes. Those enzymes are involved in biosynthesis of flavonoids which protect plants against an overdose of ultraviolet light which can turn the metabolism of phenolic compounds in different direction. The study of Napoleon table grape evaluated the influence of UVB and UVC radiation on the composition and metabolism of phenolic compounds. It was determined that the quantity of stilbene of berry epidermis, *trans*-resveratrol, *cis*-resveratrol and *trans*-resveratrol-D-glucoside (piceid), 10 mg of which was found in each kilogram of fresh sample, increased with prolonged grape storage under the influence of UV radiation. UVB radiation induced quick biosynthesis of these components during the first five days of grape storage in cooled state (Cantos et al., 2000).

Table 2.
Effect of yeast species (BDX, 299, and 2056) and enzymes (β and OE) on the content of total resveratrol, resveratrol isomers and TPC in vinification of clone PR6 (*Prokupac* variety)

Combination of yeasts and enzymes	***Cis*-resveratrol** (mg/L)	***Trans*-resveratrol** (mg/L)	**Total resveratrol** (mg/L)	**Total phenolic compounds (TPC) as gallic acid** (mg/L)
BDX	0.81±0.02	1.02±0.07	1.83±0.01	1645.60±85.50^{Aa}
BDX + *β*	2.13±0.06	0.75±0.04	2.88±0.03^{a}	1016.20±60.50^{A}
BDX + OE	0.98±0.07	0.70±0.03	1.68±0.04	1445.00±70.00
299	0.73±0.04	0.78±0.02	1.51±0.07	1151.92±65.50
299 + *β*	3.59±0.05	0.64±0.01	4.23±0.04^{Aa}	961.60±55.00
299 + OE	1.46±0.04	1.56±0.01	3.02±0.03^{A}	1774.20±89.00^{Aa}
2056	1.17±0.06	0.98±0.06	2.15±0.02^{Aa}	1258.90±68.00
2056 + *β*	1.30±0.05	0.48±0.04	1.78±0.01	903.00±60.00
2056 + OE	1.05±0.02	0.98±0.03	2.03±0.02	1609.60±70.00^{a}

^{A} *Means significantly different within the vinification with different yeast and same method (p < 0.05)*
^{a} *Means significantly different within the vinification with same yeast and different method (p < 0.05)*
Values are Mean±Standard deviation (n = 3)

Table 3.
Trans/cis ratio and % of cis-resveratrol in vinification of clone PR6 (*Prokupac* variety)

Combination of yeasts and enzymes	**trans/cis**	**% cis-resveratrol**
BDX	1.26^{Aa}	44.26
BDX + *β*	0.35	73.96
BDX + OE	0.71	58.33
299	1.07	48.34
299 + *β*	0.18	84.87
299 + OE	1.07^{A}	48.34
2056	0.84	54.42
2056 + *β*	0.37	73.03
2056 + OE	0.93^{a}	51.72

^{A} *Means significantly different within the vinification with different yeast and same method (p < 0.05)*
^{a} *Means significantly different within the vinification with same yeast and different method (p < 0.05)*
BDX, 299, and 2056 are vinification yeast species
β, and OE are enzyme preparations with different glucosidase activity

The influence of clone selection and vinification procedure on TPC content

The influence of maceration duration and clone selection on TPC in the produced wines was estimated by application of two-way ANOVA analysis. As expected, duration of maceration influenced the TPC content ($p < 0.05$): the highest content was found in the wine samples produced with 10-day maceration, while lower TPC was measured in the samples obtained after shorter maceration period (Table 1). Similarly, it was found that different clones affected the content of TPC in all cases ($p < 0.05$). Wines produced from clone PR6 had the highest TPC after 1 and 10 ($p < 0.05$) days of maceration, while PR1 was the highest in TPC after 5 days (Table 1). Similarly to the quantity of *trans*-resveratrol, clone PR6 after 10 days of maceration reached the highest TPC (1265.00 mg/L GAE) ($p < 0.05$) which was the maximum content throughout the all analysed samples in study. On the other hand, the lowest TPC was detected in clone PR7 (894.80 mg/L GAE) ($p < 0.05$) after 10 days of maceration, in PR6 after 5 days ($p < 0.05$), and in clone 41/1 after 1 day of maceration ($p < 0.05$).

As in the case of resveratrol, clone PR6 was used to study the effect of vinification procedures on TPC content because PR6 was the highest in TPC. It can be observed that that different yeast strains affected the content of TPC ($p < 0.05$) (Table 2). The application of enzymatic preparation OE in vinification with different yeasts contributed to the highest values for TPC ($p < 0.05$) in comparison with the enzymatic preparation *β* and vinification without enzymatic preparation (Table 2). The yeasts 299 and 2056 in combination with OE enzymatic preparation produced the highest TPC ($p < 0.05$). It is interesting to highlight that wine produced only with yeast BDX had the highest TPC in comparison with the samples produced with same yeast but with two other enzymatic preparations (*β* and OE) (Table 2). The highest TPC content in the study was observed in the sample produced with yeast 299 and OE enzymatic preparation (1774.20 mg/L GAE), while the lowest in the sample produced with yeast 2056 and *β* enzymatic preparation (903.00 mg/L). The enzymatic preparation *β* possesses higher glycosidolytic activity which resulted in higher content of resveratrol aglycone. Beside glycosidolytic activity, OE possesses pectolytic activity, too. It affects degradation of pectin from grape skin which intensifies liberation of phenolic compounds in wine. Enzymatic preparations in combination with yeasts which possess higher glycosidolytic activity increased the content of phenolic compounds in wine. From practical point of view, it can be concluded that both enzymatic preparations (OE and β) can be recommended to wine producers.

Similarly to resveratrol, the TPC also increased along increased duration of maceration. A study that compared maceration of Cabernet Sauvignon for 7, 13 and 21 days found that TPC increased with prolonged time of skin contact (Sener and Yildirium, 2012). The length of maceration also influenced TPC in Teran wines which varied from 1455 to 2718 mg/L of GAE (Plavsa et al., 2012). In both cases (*trans*-resveratrol and TPC content), maceration of must in fermentation lasted 10 days. This is further supported by the study indicating that the content of selected phenolic compounds and TPC depends on vinification procedure (Čakar et al., 2019).

CONCLUSIONS

In this study, the wine samples produced from PR6 clone of *Prokupac* variety were found to be the best sources of *trans*-resveratrol in comparison to other tested clones. The study leads to the conclusion that by application of different methods of primary grape processing and vinification it is possible to influence and increase the quantity of resveratrol (*cis*-, *trans*- and total) in wine. The obtained results pointed out that by adequate selection of variety, prolonged duration of maceration, application of suitable yeast species and enzyme preparations, it is possible to increase the content of resveratrol and other phenolic components in wine.

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УТИЦАЈ ВРСТЕ КЛОНА ПРОКУПЦА И ПОСТУПКА ВИНИФИКАЦИЈЕ НА САДРЖАЈ РЕЗВЕРАТРОЛА У ВИНУ

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Сажетак: Циљ овог истраживања био је да се испита утицај различитих клонова аутохтоне српске сорте Прокупац као и поступак винификације на садржај *trans*- и *cis*-резвератрола и садржај укупних полифенола (СУП) у винима. Вина су произведена од четири клона сорте Прокупац (PR1, 40/1, PR6 и PR7) применом различитих дужина трајања мацерације (1, 5 и 10 дана) за сваки клон. Такође је испитиван утицај различитих изабраних квасаца и ензимских препарата гликозидаза на садржај резвератрола и СУП у винима произведеним од клона PR6. Садржај *trans*- и *cis*- резвератрола је одређен HPLC методом са UV детектором уз претходну течно чврсту екстракцију (SPE). Одређивање СУП је урађено методом по Фолин-Чокалтеу. Садржај *trans*- резвератрола је био од 0,27 mg/L до 1,46 mg/L. Највиши садржај резвератрола је био у клону PR6 док је најнижи PR7. Повећање садржаја резвератрола и СУП је примећено код свих клонова када је мацерација дуже трајала. Вино произведено применом ензимског препарата гликозидаза β и квасца 299 је имало највиши садржај укупног резвертрола (4,23 mg/L). Највиши СУП је био у винима произведеним применом ензимског препарата гликозидаза ОЕ и квасца 299. Добијени резултати указују да је избором одговарајућег клона, дужине мацерације, примене одговарајућег квасца и ензимског препарата могуће повећати садржај резвератрола и других фенолних једињења у вину.

Кључне речи*: антиоксиданси, полифеноли, квасац, ензими, мацерација, HPLC*

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