Vitis **54** (Special Issue), 169–171 (2015)

Resveratrol concentration in 'Vranac' wines

B. Radović[1)], V. Tešević[1)], V. Kodžulović[2)] and V. Maraš[2)]

[1)] Chemical Faculty, University of Belgrade, Belgrade, Serbia
[2)] Biotechnical Faculty, University of Montenegro, Podgorica, Montenegro

Summary

Red wine is the main source of resveratrol. It occurs in both, isomeric (*trans*- and *cis*-) and in its glucosides forms. The grapevine variety, climate, conditions and duration of wine storage, and phytosanitary conditions of grape are some of the factors that influence content of resveratrol. This study analyzed *trans*- and *cis*-resveratrol isomers concentrations and *trans*-resveratrol-3-O-glucoside and *cis*-resveratrol-3-O-glucoside concentrations among three clone candidates and population of autochthonous Montenegrin grapevine variety 'Vranac'. Data from period of three years were collected and analyzed with UPLC-MS/MS. Results showed no statistically significant difference ($p = 0.169$) in concentration of resveratrol among three clone candidates and population of grapevine variety 'Vranac'.

Key words: clones; UPLC-MS/MS; stilbenes; grapevine populations.

Introduction

'Vranac' is an autochthonous grapevine variety of Montenegro, with a significant importance for the viticulture and winemaking industries, and for the Montenegrin economy in general. 'Vranac' grapes have been used for the production of premium quality red wines. However, since 2004, researchers worked on developing clones with improved agro-biological, economical and technological characteristics. As part of this project three year (2010-2012) research was conducted in order to determine the concentration of resveratrol in wine of three putative 'Vranac' clones.

Resveratrol is a member of the stilbene family and it can exist in *cis*- or *trans*- configurations (*trans*-3, 5, 4'-trihydroxystilbene), or as glucosylated forms called piceid (resveratrol-3-O-beta-mono-D-glucoside) (Figure). Both *trans*- and *cis*-resveratrol are known as phenolic bioactive wine constituents. Phenolic compounds are an important group of substances in wine that contribute to several sensory characteristics, such as color, flavor and astringency (Frémont 2000). Furthermore, it has been reported that phenols have multiple biological effects, such as antioxidant activity, anti-inflammatory action, inhibition of platelet aggregation and antimicrobial activity (Đekić *et al.* 2008).

Concentration of resveratrol in wine depends on multiple factors. It is known that resveratrol is synthesized in the plant in response to infection caused by fungal diseases such as *Botrytis cinerea* or due to abiotic stress such as UV radiation excess. It is assumed that the UV-B radiation stimulates the production of enzymes responsible for the synthesis of stilbene, in order to protect berry cells from UV-induced damages (Langcake *et al.* 1976). Red wines and grapes present higher levels of resveratrol than white grape products (Moreno *et al*. 2008). *Cis*-isomer is produced by UV irradiation of the *trans*-isomer (Moreno *et al*. 2008). It is generally absent or only slightly detectable in grapes, but both isomers are present in variable amounts in commercial wines (Cvejić *at al.* 2010).

Cis-resveratrol was not reported at first as a natural constituent of grape berries (Jeandet *et al*. 1991, Jeandet *et al*. 1995). However, later, Moreno *et al*. 2008, detected *cis*-isomer in red grape skins. Moreover, *cis*-resveratrol and its glucoside have been detected in wines. More likely, *cis*-resveratrol is derived from its *trans*-isomer during vinification (Cvejić *et al.* 2010) even if the mechanisms of its formation during winemaking are still unclear (Dourtoglou *et al*. 1999).

Piceid is a derivative of resveratrol. The average content of piceid was found ten times higher than that of resveratrol in the root of *Polygonum cuspidatum* and in red wine (Dan *et al. 2013*). Additionally, piceid was the most abundant form of resveratrol in nature (Regev-Shoshani *et al.* 2003). A number of studies have suggested that piceid may also have anti-carcinogenic effects (Soleas *et al*. 2001), inhibition of platelet aggregation (Chung

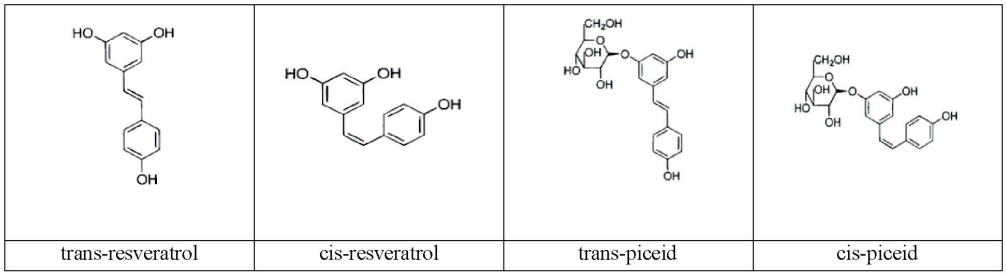

Figure: Structures of free and glycosylated *trans*- and *cis*- resveratrol forms.

Correspondence to: Dr. B. Radovic, Jelene Balšić 18, 81000 Podgorica, Montenegro. E-mail: radovicbojan081@gmail.com

et al. 1992, Orsini *et al.* 1997), and anti-oxidation activity (Brohan *et al*. 2011). This work aimed at developing new 'Vranac' clones with high concentrations in resveratrol and its glucoside. The goal of the study was to determine concentrations of stilbenes (*trans*- and *cis*-resveratrol and its piceid) in three developed clones and to statistically test the difference in resveratrol concentrations between clones and population of 'Vranac' variety.

Material and Methods

Wines from the 'Vranac' population and its three putative clones were produced in the experimental winery of Plantaze Co. Standard vinifications were performed in Ganimede® tanks. Potassium meta-bisulfite (Agroterm KFT, Hungary) was added (8 g·100 kg^{-1} of grapes). All enzymes, wine yeast, lactic acid bacteria and yeast nutrients were purchased from Lallemand, Australia. In more details, Enoferm BDX (30 g·hL^{-1}) ; Go-ferm protect (30 g·hL^{-1}); Lalvin EX-V (2 g·100 kg^{-1}); Fermaid E (25 g·hL^{-1}) were added during fermentation. For malolactic fermentation commercial lactic acid bacteria Lalvin VP41 was used for inoculation.

The analyses were performed on four wine samples produced in three years (2010, 2011, 2012).*Trans*-resveratrol standard was purchased from Sigma Chemical Co. (St. Louis, MO). *Cis*-resveratrol is not commercially available and was therefore obtained through UV-photoisomerization (280-310 nm, for 10 h) of a standard solution (100 mg·L^{-1}) of *trans*-resveratrol. Under these conditions, 80 % of the *trans*-resveratrol was converted into the *cis*-isomer.

UPLC MS/MS analyses: Analyses of resveratrol was carried out on Waters Acquity UPLC H-Class (WAT-176015007) equipped with a quaternary pump Waters Quaternary Solvent Menager; an injector Waters Sample Menager-FTN (Flow Through Needle); a column compartment with a ZORBAX Eclipse XDB C18 column (150 × 4,6 mm; 5 µm); a Photodiode Arraydetector Waters 2998 PDA and a mass detector Waters TQ (Tandem Quadrupole, WAT-176001263). For data acquisition and processing, the software MassLynx® V4.1 was used.

The mobile phase consists of 0.2 % fumaric acid in H_2O (A) and acetonitrile (B). The chromatographic separation was performed using a six stage linear gradient: from 5 % to 16 % of B in 20 min, from 16 to 40 % of B in 8 min, from 40 % to 70 % of B in 4 min, from 70 to 100 % of B in 4 min, and at 100 % of B during 9 min, and back to 5 % of B in 9 min, with a total flow rate of 0.7 mL·min^{-1}. The total gradient time was 55 min. The separation temperature was set up at 25 °C. UV-visible spectra were recorded in the wavelength range from 190 nm to 600 nm. Injection volume was 10 µL. MS Scan standard resveratrol was recorded in ESI^- and ESI^+ mode. The transition m/z 229→ 107 was monitored in ESI^+ MRM mode (Multiple Reaction Monitoring Mode) under the following conditions: capillary voltage at 3.5 kV, the cone voltage 34 V, collision energy of 24 eV, the source temperature of 150 °C, desolvation temperature of 450 °C, flow of desolvation gas 800 L·h^{-1}. The samples were analyzed without prior preparation: they were only filtered through a filter Econofilter 25/0.45 µm.

Calibration graphs were obtained by plotting the peak area against the concentration. Five standards of *trans*- and *cis*-resveratrol were used covering the range 0.05-5 mg·L^{-1}. Concentration for *trans*- and *cis*-piceid were obtained from calibration graphs for *trans*- and *cis*-resveratrol respectively. The resveratrol concentration among the 4 'Vranac'-types (Clone 1, Clone 2, Clone 3 and Population) over three years were compared and statistically tested using repeated measures ANOVA ($p = 0.05$). SPSS® 15.0 software was used for statistical analysis.

Results and Discussion

The results of resveratrol concentration variability among the 4 ,Vranac'-types, obtained from the wine analysis, are summarized in the Table. Results showed that the highest average concentration of *trans*-piceid (1.123 mg·L^{-1}) was found in C lone III, while the highest average concentration of *cis*-piceid (1.193 mg·L^{-1}) was found in the Population sample. The highest average concentration of *trans*-resveratrol (0.600 mg·L^{-1}) among all the samples was found in the Population sample. The highest average concentration of total resveratrol (3.119 mg·L^{-1}) was found in Clone I.

Resveratrol concentrations among the 4 'Vranac'-types were statistically tested using repeated measure analysis of variance (rANOVA) at $p = 0.05$. Results showed that there was no statistically significant difference in total concentration of resveratrol among the tested 'Vranac'-types ($F(3,6) = 2.373$, $p < 0.169$). Similarly, results showed that

Table

Concentrations (mean and st. dev.) of *trans*-resveratrol (mg·L^{-1}), *cis*-resveratrol (mg·L^{-1}), *c*-resveratrol-3-O-glucoside (mg·L^{-1}) and *cis*-resveratrol-3-O-glucoside (mg·L^{-1}) in wine of population of 'Vranac' variety and its putative clones in period 2010- 12 in Plantaze A.D. winery, Podgorica, Montenegro

Resveratrol (mg·L^{-1})	Population	putative Clone I	putative Clone II	putative Clone III
trans-resveratrol	0.600 (0.129)	0.485 (0.084)	0.414 (0.062)	0.457 (0.107)
cis-resveratrol	0.380 (0.058)^{a}	0.318 (0.049)	0.295 (0.084)	0.190 (0.174)
trans-resveratrol-3-O-glucoside	0.834 (0.344)	1.123(0.196)	0.837 (0.237)	1.179 (0.067)
cis-resveratrol-3-O-glucoside	0.506 (0.162)^{a}	1.193 (0.323)^{b}	0.870 (0.090)^{ab}	0.988 (0.030)^{b}
Total	2.320 (0.317)	3.119 (0.596)	2.416 (0.180)	2.914 (0.317)

In the same line, when reported, means followed by the same letter are not statistically different.

there was no statistically significant difference in concentration of trans-resveratrol among the tested ‘Vranac’-types ($F(3,6) = 2.730$, $p < 0.169$) either.

The only statistically significant difference found concerned in the variability in *cis*-resveratrol concentration among the tested ‘Vranac’-types ($F(3,6) = 29.876$, $p < 0.032$). However, Tuckey post-hoc tests (with Bonferroni adjustment for multiple comparisons) showed that neither *cis*-resveratrol concentration among ‘Vranac’-types was statistically significant at $p = 0.05$. Finally, results showed that there was a statistically significant difference in concentration of *cis*-resveratrol-3-O-glucoside among the tested ‘Vranac’-types ($F(3,6) = 70.087$, $p < 0.033$). Tuckey post-hoc tests (with Bonferroni adjustment for multiple comparisons) showed that a statistically significant difference in *cis*-resveratrol-3-O-glucoside concentration among the tested ‘Vranac’-types was found between the Population wines and Clone III pair (at $p = 0.024$), and between Clone II and Clone III pair (at $p = 0.039$). Similarly, the results showed that there was a statistically significant effect of concentration of *cis*-resveratrol among the tested ‘Vranac’-types ($F(3,6) = 29.876$, $p < 0.032$). However, Tuckey post-hoc tests (with Bonferroni adjustment for multiple comparisons) showed that *cis*-resveratrol concentration among groups was not statistically significant at $p = 0.05$.

The study was conducted with the primary focus being directed on the determination of *trans*-resveratrol and *cis*-resveratrol levels and their piceid in ‘Vranac’ wines. While there are numerous factors that could have impacted the total content of resveratrol (such as environmental and climate conditions), results indicate that ‘Vranac’ clones have the same capacity to produce resveratrol concentrations in wine as the ‘Vranac’ population.

References

Brohan, M.; Jerkovic, V.; Collin, S.; 2011: Potentiality of red sorghum for producing stilbenoid-enriched beers with high antioxidant activity. J. Agric. Food Chem. **59**, 4088-4094.

Chung, M. I.; Teng, C. M.; Cheng, K. L.; Ko, F. N.; Lin, C. N.; 1992: An antiplatelet principle of *Veratrum formosanum*. Planta Med. **58**, 274-276.

Cvejić, J.; Đekić, S.; Petrović, A.; Atanackovic, A.; Jović, S.; Brceski, I.; Gojković-Bukarica, L.; 2010: Determination of *trans*- and *cis*-Resveratrol in Serbian commercial wines. J. Chromatogr. Sci. Vol. **48**, 229-234.

Dan, S.; Ying, C.; Miao, L.; Daozhou, L.; Han, C.; Bangle, Z.; Siyuan, Z.; Tiehong, Y.; Qibing, M.; 2013: Comparision of piceid and resveratrol in antioxidation and antiproliferation activities *in vitro*. PLoS ONE **8**, e54505.

Đekić, S.; Milosavljević, S.; Vajs, V.; Jović, S., Petrović, A.; Nikićević, N.; Manojlović, V.; Nedović, V.; Tešević, V.; 2008: *Trans*- and *cis*-resveratrol concentration in wines produced in Serbia, J. Serb. Chem. Soc. **73**, 1027-1037.

Dourtoglou, V. G.; Makris, D. P.; Bois-Dounas, D.; Zonas C.; 1999: Trans-resveratrol concentration in wines produced in Greece. J. Food Comp. Anal. **12**, 227-233.

Fremont, L.; 2000: Biological effects of resveratrol. Life Sci. **66**, 663-673.

Jeandet, P.; Bessis R.; Gautheron, B.; 1991: The production of resveratrol (3.5.4’-trihydroxystilbene) by grape berries in different developmental stages. Am. J. Enol. Vitic. **42**,41-46.

Jeandet, P.; Bessis, R.; Maume, B. F.; Meunier, P.; Peyron, D.; Trollat, P.; 1995: Effect of enological practices on the resveratrol isomer content of wine. J. Agric.Food Chem. **43**, 316-319.

Jeandet, P.; Bessis, R.; Sbaghi, M.; Meunier, P.; Trollat, P.; 1995: Resveratrol content of wines of different ages: Relationship with fungal disease pressure in the vineyard. Am. J. Enol. Vitic. **46**, 1-4.

Langcake, P.; Pryce, R. J; 1976: The production of resveratrol by Vitis-vinifera and other members of the Vitacee as a response to infection or injury. Phys. Plant Pathol. **9**,77-86.

Moreno, M.; Castro, E.; Falqué, E.; 2008: Evolution of trans- and cis- resveratrol content in red grapes (*Vitis vinifera* L. cv Menciá, Albarello and Merenzao) during ripening. Eur. food Res. Technol. **227**,667-674.

Orsini, F.; Pelizzoni, F.; Verotta, L.; Aburjai, T.; Rogers C.B.; 1997: Isolation, synthesis, and antiplatelet aggregation activity of resveratrol 3-O-beta-Dglucopyranoside and related compounds. J. Nat. Prod. **60,** 1082-1087.

Regev-Shoshani, G.; Shoseyov, O.; Bilkis, I.; Kerem, Z.; 2003: Glycosylation of resveratrol protects it from enzymic oxidation. Biochem. J. **374**, 157-163.

Soleas, G. J.; Goldberg, D. M.; Grass, L.; Levesque, M.; Diamandis, E. P.; 2001: Do wine polyphenols modulate p53 gene expression in human cancer cell lines? ClinBiochem **34**, 415-42.