Phenolic natural products of the wines obtained from three new Merlot clone candidates

Dragan Vujovic\(^a\), Boris Pejin\(^b\), Jelena Popovic Djordjevic\(^c\), Milovan Velickovic\(^a\) and Vele Tesevic\(^c\)

\(^a\)Faculty of Agriculture, University of Belgrade, Belgrade, Serbia; \(^b\)Institute for Multidisciplinary Research – IMSI, University of Belgrade, Belgrade, Serbia; \(^c\)Faculty of Chemistry, University of Belgrade, Belgrade, Serbia

ABSTRACT
This work aimed to evaluate the total contents of polyphenolics (the Ribereau-Gayon–Maurié procedure), anthocyanins (using pH differential method) and tannins (the Nègre procedure) as well as the content of phenolic acids (using UPLC/MS chromatography), respectively of the wines obtained from three new Merlot clone candidates in the perennial clonal selection. The aforementioned chemical parameters were determined in the samples covering the period 2009–2012. In comparison both with the standard Merlot wine (mother vine) and the wines obtained from other two clone candidates, the Merlot wine of the clone candidate No. 022 was found to have the highest total content of all three examined components 1.89 ± 0.05 g/L (polyphenolics), 185.59 ± 5.00 mg/L (anthocyanins) and 1.11 ± 0.03 g/L (tannins), as well as six phenolic acids including gallic acid (25.49 ± 0.27 mg/L). These findings are in good agreement with the observed trend for the viticultural parameters indicating the clone candidate No. 022 as more promising than mother.

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1. Introduction
The clonal selection of grapevine, aiming to improve the existing varieties, is one of the most important factors in viniculture development. Till date, the key results with clones

CONTACT
Dragan Vujovic \(\text{draganv@agrif.bg.ac.rs}\); Boris Pejin \(\text{borispejin@imsi.rs}\)

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have been achieved in terms of the yield and quality of grapes and wine. Merlot, a dark blue-coloured wine grape, is among the varieties that have been grown for hundreds of years all over the world. However, it is still of great importance, enjoying a high reputation both among producers and consumers. During such a long period, some individual vines within varieties were exposed to different influences (such as different types of soil, pathogenic organisms, high or low temperatures as well as chemical compounds) causing the heterogeneity within the population which pointed out the need for the clonal selection (Ruhl et al. 2004; Keller 2010). This work aimed to evaluate the total contents of polyphenolic compounds (the Ribereau-Gayon–Maurié procedure), anthocyanins (by spectrophotometric procedure, using pH differential method) and tannins (the Nègre procedure) (Danicic 1988; Lee et al. 2005) as well as selected phenolic acids (using UPLC/MS chromatography with TQ analyser), respectively of the wines obtained from three new Merlot clone candidates in the perennial clonal selection. The wine samples analysed herein covered the period 2009–2012.

2. Results and discussion

In comparison both with the standard Merlot wine (originating from mother vine) and the wines obtained from other two clone candidates, the Merlot wine of the clone candidate No. 022 was found to have the highest total content of all three examined components: 1.89 ± 0.05 g/L (polyphenolics), 185.59 ± 5.00 mg/L (anthocyanins) and 1.11 ± 0.03 g/L (tannins). Additionally, the same wine sample was found to contain the highest content of the analysed phenolic acids: 25.49 ± 0.27 mg/L (gallic acid), 4.59 ± 0.14 mg/L (caffeic acid), 4.43 ± 0.09 mg/L (vanillic acid), 3.31 ± 0.05 mg/L (protocatechuic acid), 2.89 ± 0.03 mg/L (ellagic acid) and 0.18 ± 0.00 mg/L (ferulic acid). This is especially true for gallic acid and caffeic acid (Table 1).

Wines from the region of Dalmatia, known for their high content of polyphenolics (reaching more than 3 g/L) are analysed using the Folin-Ciocalteu method. However, the clone candidate No. 022 has offered wine with the enhanced content of these compounds, compared with the Merlot one originating from Croatia (1260 ± 23 mg GAE/L) (Šeruga et al. 2011). Additionally, the aforementioned wine also contained higher content of anthocyanins, compared with another Merlot wine sample (146.2 mg/L) from the same country (Katalinić et al. 2004). On the other hand, a sample of Argentinian Merlot wine was found to have a smaller content of total tannins (1051.9 ± 37 mg/L) than the wine sample discussed herein (Casassa et al. 2015).

Table 1. Chemical composition of selected samples of Merlot wines.

| Parameters              | Standard Merlot winea | Merlot 022 wineb | Merlot 025 wineb | Merlot 029 wineb |
|-------------------------|-----------------------|-----------------|-----------------|-----------------|
| Total polyphenolics (g/L) | 1.88 ± 0.08           | 1.89 ± 0.05     | 1.84 ± 0.06     | 1.78 ± 0.04     |
| Total anthocyanins (mg/L) | 169.89 ± 4.07         | 185.59 ± 5.00   | 181.07 ± 4.68   | 176.20 ± 4.33   |
| Total tannins (g/L)     | 1.06 ± 0.04           | 1.11 ± 0.03     | 1.10 ± 0.02     | 1.06 ± 0.05     |
| Gallic acid (mg/L)      | 22.64 ± 0.23          | 25.49 ± 0.27    | 20.71 ± 0.21    | 21.44 ± 0.22    |
| Caffeic acid (mg/L)     | 2.08 ± 0.07           | 4.59 ± 0.14     | 2.18 ± 0.07     | 1.80 ± 0.05     |
| Vanillic acid (mg/L)    | 4.17 ± 0.06           | 4.43 ± 0.09     | 4.32 ± 0.08     | 4.24 ± 0.05     |
| Protocatechuic acid (mg/L) | 2.31 ± 0.02       | 3.31 ± 0.05     | 2.95 ± 0.05     | 2.76 ± 0.03     |
| Ellagic acid (mg/L)     | 2.32 ± 0.02           | 2.89 ± 0.03     | 2.12 ± 0.01     | 1.97 ± 0.01     |
| Ferulic acid (mg/L)     | 0.14 ± 0.00           | 0.18 ± 0.00     | 0.16 ± 0.00     | 0.09 ± 0.00     |

aMother wine. bClone candidate wine.
Cardiovascular diseases are known to be the leading group ‘killer diseases’. Among them, hypertension is the most common heart chronic illness which the world has been facing in the last years. The importance of hypertension lies in the fact that it forms one of the main risk factors for coronary heart disease, atherosclerosis and peripheral vascular disease (Das 2004). The improved content of polyphenolic compounds including phenolic acids, the compounds that are tightly linked with cardiovascular physiology, may contribute to the medicinal properties (more precisely, to antihypertensive and cardioprotective activities) of the Merlot wine originating from the clone candidate No. 022 (Tenore & Ciampaglia 2013; Correia & Jordão 2015). Finally, these findings are in good agreement with the observed trend for the viticultural parameters indicating the aforementioned clone candidate as more promising than mother.

3. Experimental
See Supplementary material.

4. Conclusion
The chemical findings presented herein are in good agreement with the observed trend for the viticultural parameters, indicating that Merlot clone candidate No. 022 may be considered as more promising than mother. An accurate analysis of chromatograms could characterise Merlot wines on the basis of geographic origin (Barbera et al. 2013); the used strain of the yeast Saccharomyces cerevisiae may also affect the aromatic profile of the fermented must (Zhu et al. 2014). Further research work will be directed towards determination of the content of bioelements (macro, micro and ultra-micro metals) in all three wine samples.

Disclosure statement
No potential conflict of interest was reported by the authors.

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