SUPPLEMENTARY MATERIAL

Multielement analysis and antioxidant capacity of Merlot wine clones developed in Montenegro

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Abstract

The overall aim of this paper was to compare the multielement composition and antioxidant capacity of two Montenegrin Merlot wines obtained from specific vine clones (VCR1 and VCR 101) along with commercial Merlot wine throughout the consecutive vintages in 2010 and 2011. Elemental composition was analysed using inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS)]. Additionally, antioxidant capacity was assessed by cyclic voltammetry. VCR 1 wine from 2011 stood out for its elemental composition. On the other hand, antioxidant capacity of VCR 101 wines was the highest one for the both vintages. According to the experimental data obtained, all three wines are good source of essential elements. Finally, these wines are products with significant antioxidative activity and specific geographical origin.

Keywords: Antioxidant capacity; clonal selection; cyclic voltammetry; elemental composition; Merlot

3. Experimental

3.1. Standards and solvents

0.1 M KCl (Merck, Darmstadt, Germany) was used as supporting electrolyte in cyclic voltammetry analysis. Multielement standards were prepared in-house by mixing of certified traceable ICP grade single element standards (Merck-CertiPUR). For sample dilution and preparation of standards, ultrapure water (MilliQ, Millipore) and ultrapure acids (nitric acid, hydrochloric acid, Merck-Suprapure) were applied. The other chemicals and solvents used of analytical grade were purchased from Merck (Darmstadt, Germany).

3.2. Wine samples

The samples of commercial wines (2010 and 2011) along with two red wine clones of Merlot variety were obtained from developing sector of "Plantaže 13. juli" A.D. winery (Podgorica, Montenegro). The samples were selected according to a vintage (2010 or 2011), and labeled as Merlot bottled, Merlot VCR1 and Merlot VCR 101. All the analysed samples were produced

under the same conditions and using standard vinifications (Radović et al. 2015). They were stored at 10 ºC in the dark (prior to analysis) and analysed immediately after opening.

3.3. Multielement analysis

ICP-OES (Thermo Scientific, United Kingdom), model 6500 Duo, equipped with a CID86 chip detector was applied for determination of major elements (calcium, sodium, potassium, magnesium) and iron. This instrument operated sequentially with both radial and axial torch configurations. Analyses were done according to previously described method (Šelih et al, 2014).

Operation conditions and selected wavelengths were as follow:

- nebulizer: concentric
- spray chamber: cyclonic
- radio frequency power: 1150 W
- principal argon flow rate: 12 L/min
- auxiliary argon flow rate: 0.5 L/min
- nebulizer flow rate: 0.5 L/min
- sample flow rate: 1 mL/min
- Selected wavelenght: Ca (373.6 nm); K (766.4 nm); Mg (279.5 nm); Na (589.5 nm); Fe (259.9 nm)

Iteva software controlled the entire system.

ICP-MS (iCAP Q, Thermo Scientific X series 2) was used for the analysis of trace and ultratrace elements. These analyses were also performed according to previously described method (Hopfer et al, 2015).

Operation conditions and measured isotopes were as follow:

- Rf power: 1548 W
- gas flows: 13.90, 1.09 and 0.80 L/min
- acquisition time: 3 × 50 sec
- points per peak: 3
- dwell time: 10 msec
- detector mode: pulse

- replicates: 3
- measured isotope: Zn (66); Al (27); Mn (55); Cu (65); Ba (138); Cr (53); Ni (60); Pb (208); Sb (121); V (51); Se (82); Co (59); Tl (205); As (75); Cd (112)

Qtegra instrument control software was used for controlling of the entire system. Internal standards were ^{45}Sc, ^{115}In and ^{159}Tb.

Multielement stock solution containing 1000 mg/l of major elements was used to prepare intermediate multi-element standard solutions for ICP-OES measurements, while multielement stock solution containing 10 mg/l of each element was used to prepare intermediate multielement standard solutions for ICP-MS measurements. The samples were diluted (1:10) in water containing 2% (v/v) of nitric acid. The standards were prepared with ethanol at a concentration of 1% (v/v) to be equal with diluted samples and nitric acid at a concentration of 2%.

3.4. Cyclic voltammetric determination of antioxidant capacity

Cyclic voltammograms were recorded on a CHI760B instrument (CHInstruments, Austin, Texas, USA). The cell was equipped with GC electrode, an accessory platinum electrode of larger area (Model CHI221, cell top including Pt wire counter electrode) and an Ag/AgCl reference electrode (Model CHI111). All measurements were taken at ambient temperature. Prior to each run, the surface of the glassy carbon electrode was freshly abraded with 1.0, 0.3 and 0.05 μm alumina powder rinsed with redistilled water and degreased in ethanol in ultrasonic bath. The scan was taken in the potential range between 0 mV and 1.3 V with a scan rate 100 mV/s.

Q_{600} factor was calculated as the area below voltammetric anodic peak. Antioxidant composite index (ACI) was calculated as percentage of the obtained Q_{600} factor compared with the best Q_{600} factor according to the following equation:

$$\text{ACI} = \text{Sample } (Q_{600}) / \text{Best } (Q_{600}) \times 100$$

An ACI value of 100 was assigned to the best score for Q_{600} factor obtained for the voltammetric measurements.

References

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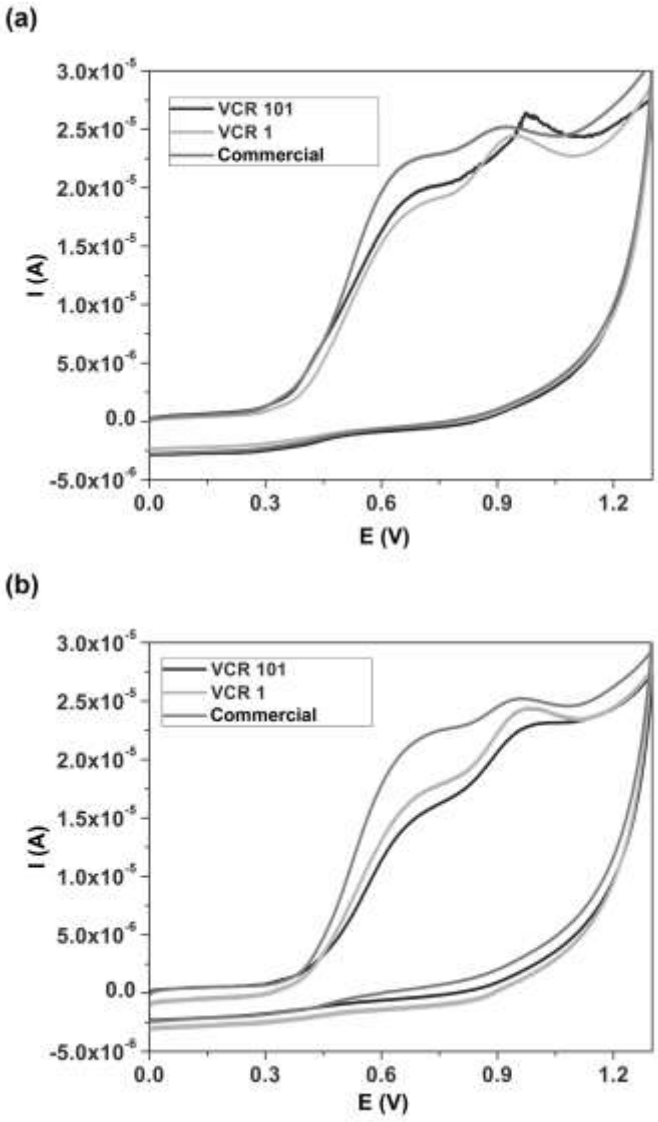

Supplementary Figure: Cyclic voltammograms obtained for the analysed wine samples during 2010 (a) and 2011 (b) vintages. *VCR1/VCR101– the wines obtained from specific vine clones.

Table S1. The contents of major and trace elements in analysed wine samples determined by ICP-OES or ICP-MS.

	Sample	MAJOR (mg/L)				TRACE (µg/L)				
		K	**Ca**	**Mg**	**Na**	**Fe**	**Zn**	**Al**	**Mn**	**Cu**
2010	**Comm**	480 ± 3	51.5 ± 0.6	45.3 ± 0.2	11.7 ± 0.4	1001 ± 2	390.3 ± 0.4	443.5 ± 0.8	477.3 ± 0.4	24.4 ± 0.7
	VCR1	439 ± 6	52.3 ± 0.7	42.8 ± 0.2	9.3 ± 0.4	868 ± 1	735.8 ± 0.6	337.8 ± 0.4	321.2 ± 0.4	126.4 ± 0.5
	VCR101	473 ± 5	52.1 ± 0.5	42.1 ± 0.2	9.6 ± 0.5	870 ± 1	542.1 ± 0.6	250.9 ± 0.3	292.1 ± 0.3	113.4 ± 0.7
2011	**Comm**	515 ± 5	45.8 ± 0.4	49.2 ± 0.2	14.7 ± 0.5	1088 ± 4	531.4 ± 0.4	495.5 ± 0.5	545.7 ± 0.5	22.9 ± 0.4
	VCR1	757 ± 8	78.3 ± 0.5	78.9 ± 0.5	14.3 ± 0.7	1609 ± 2	982.9 ± 0.8	983.8 ± 0.8	362.9 ± 0.4	520.1 ± 0.5
	VCR101	422 ± 6	55.6 ± 0.6	39.5 ± 0.2	10.4 ± 0.6	1203 ± 1	678.2 ± 0.5	484.0 ± 0.7	329.5 ± 0.4	245.0 ± 0.4

*Comm – commercial wine, VCR1/VCR101– the wines obtained from novel vine clones. All values are represented as mean ± SD (triplicate).

Table S2. The content of ultratrace elements in analysed wine samples determined by ICP-MS.

	Sample	ULTRATRACE (µg/L)										
		Ba	**Cr**	**Ni**	**Pb**	**Sb**	**V**	**Se**	**Co**	**Tl**	**As**	**Cd**
2010	**Comm**	79.8 ± 0.2	34.6 ± 0.2	16.9 ± 0.2	12.2 ± 0.1	5.73 ± 0.03	7.53 ± 0.02	2.61 ± 0.20	1.04 ± 0.01	1.33 ± 0.01	1.06 ± 0.04	0.35 ± 0.00
	VCR1	48.2 ± 0.2	50.0 ± 0.3	21.6 ± 0.2	27.1 ± 0.3	4.60 ± 0.02	1.55 ± 0.02	3.52 ± 0.05	0.79 ± 0.00	0.37 ± 0.01	0.17 ± 0.00	0.34 ± 0.01
	VCR101	47.4 ± 0.4	56.4 ± 0.5	19.8 ± 0.1	14.0 ± 0.2	6.76 ± 0.03	1.74 ± 0.03	4.39 ± 0.08	0.75 ± 0.02	0.42 ± 0.03	0.37 ± 0.03	0.22 ± 0.01
2011	**Comm**	61.9 ± 0.2	45.5 ± 0.2	19.2 ± 0.2	7.2 ± 0.1	5.38 ± 0.02	0.94 ± 0.03	5.16 ± 0.30	1.85 ± 0.01	1.49 ± 0.02	0.30 ± 0.01	0.22 ± 0.01
	VCR1	63.7 ± 0.3	22.6 ± 0.3	38.7 ± 0.3	72.6 ± 0.5	4.78 ± 0.02	0.83 ± 0.02	ND	1.70 ± 0.02	0.62 ± 0.00	1.36 ± 0.05	0.64 ± 0.03
	VCR101	39.1 ± 0.2	32.0 ± 0.6	21.0 ± 0.2	18.6 ± 0.1	4.56 ± 0.02	0.69 ± 0.01	0.63 ± 0.01	1.16 ± 0.01	0.43 ± 0.03	ND	0.14 ± 0.00

*ND – not detected. Comm – commercial wine, VCR1/VCR101– the wines obtained from novel vine clones. All values are represented as mean ± SD (triplicate).

Table S3. Antioxidant potency composite index (ACI) of the selected wine samples determined by cyclic voltammetry and scaled to relative percentages.

Sample			Q_{600}	ACI
Merlot	2010	Commercial	1.428	79.08
		VCR1	1.206	66.78
		VCR101	1.501	83.11
	2011	Commercial	1.506	83.39
		VCR1	1.141	63.17
		VCR101	1.806	100.00

*VCR1/VCR101– the wines obtained from novel vine clones.