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SUPPLEMENTARY MATERIAL

Comparative analytical study of the selected wine varieties grown in Montenegro

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ABSTRACT

Samples of the selected red wine varieties grown in Montenegro (Merlot, Cabernet Sauvignon and Vranac; vintages 2010-2012) were compared according to total phenolic content, anti-DPPH radical activity, phenolic profile and elemental composition. All the samples showed profound anti-DPPH radical activity, due to high content of total phenolic compounds (R = 0.92). The most abundant phenolics were catechin and gallic acid with the highest values recorded for Merlot 2012 (43.22 and 28.65 mg/L, respectively). In addition to this, the content of essential elements including the potentially toxic ones was within healthy (safe) level for all the samples analysed. This study has actually pointed out Merlot wine variety as the best quality one, though all three varieties may be used as safe and health-promoting nutritional products.

KEYWORDS: Red wines; phenolic compounds; DPPH; LC–MS/MS; ICP; metals

3. Experimental

3.1. Standards and solvents

Methanol and formic acid of HPLC grade were purchased from Merck (Darmstadt, Germany). DPPH and acetonitrile (HPLC grade) were delivered from Sigma-Aldrich (Steinheim, Germany). Folin-Ciocalteu's reagent was purchased from Merck (Darmstadt, Germany). Standards of catechin, epicatechin, quercetin, myricetin, protocatechuic and 4-hydroxy benzoic acid were purchased from Fluka AG (Buch, Switzerland). Gallic and caffeic acid as well as kaempherol and resveratrol were supplied from Sigma-Aldrich (Steinheim, Germany). Ultrapure water (TKA Germany MicroPure water purification system, 0.055 μS/cm) was used in liquid chromatography (LC) analisys. The 0.22 μm Econofilters were purchased from Agilent Technologies (Santa Clara, CA, USA).

Multi-element standards were prepared in-house by mixing of certified, traceable, ICP grade single element standards (Merck-CertiPUR). Other chemicals and solvents were of analytical grade and purchased from Merck (Darmstadt, Germany).

3.2. Wine samples

All the samples of commercial red wines were provided by "Plantaže 13. juli" A.D. vinery (Podgorica, Montenegro), and selected according to variety (Merlot, Cabernet Sauvignon and Vranac) and vintage (2010-2012). Standard vinifications were performed according to Radović et al. 2015. Samples were stored at 10 ºC in the dark were analysed immediately after their opening.

3.3. Spectrophotometric analysis of total phenolic content

The total phenolic content was determined by UV-VIS spectrophotometry using modified Folin-Ciocalteu procedure (Singleton et al. 1998). Absorbance was measured at 740 nm with a UV-VIS spectrophotometer (GBC Cintra 40). This analysis was performed in triplicate. The aforementioned content (calculated using gallic acid as standard) is expressed as gallic acid equivalents (mg GAE/L).

3.4. Determination of anti-DPPH radical activity

The anti-DPPH radical activity was determined by UV-VIS spectrophotometry (Gorjanović et al. 2010). Absorbance was measured at 517 nm on UV-VIS spectrophotometer (GBC Cintra 40). Four different dilutions of each wine sample were made with each one evaluated in triplicate. The obtained results are expressed as EC_{50}^{-1} values (reciprocal dissolution of the wine sample able to scavenge 50% of $DPPH^{\bullet}$).

3.5. LC-MS/MS profiling of phenolic compounds

The wine samples filtered through 0.22 μm Econofilters (Agilent Technologies, Santa Clara, CA, USA) were directly injected into analysing system including liquid chromatograph (Waters Acquity UPLC H-Class; WAT-176015007; Milford, MA USA) with ultraviolet detector [Waters 2998 PDA (Photodiode Array)] interfaced to a mass detector [Waters TQ (Tandem Quadropole), WAT-176001263)]. For acquisition and processing data, MassLynx V4.1 software was performed.

Separation of phenolic compounds was done on ZORBAX Eclipse XDB C18 column (150 × 4.6 mm; 5 μm) using 0.2% (v/v) formic acid in deionised water (solvent A) and acetonitrile (solvent B). Elution program was: 5% - 16% linear gradient of solvent B (20 min); 16% - 40% linear gradient of solvent B (28 min); 40% - 70% linear gradient of solvent B (32 min); 70% - 98% linear gradient of solvent B (36 min); constant at 98% solvent B (45 min); 98% - 5% linear gradient of solvent B (46 min); constant at 5% solvent B (55 min), due to the column reconditioning (Radović et al. 2015). Column temperature was maintained at 25 ºC, while mobile phase flow rate was 0.7 ml/min. After separation, phenolics were analysed and quantified using PDA and mass detectors. ESI source of mass detector operated at 150 °C under capillary voltage of 3.5 kV, cone voltage in the range of 20 - 60 V and collision energy from 10 eV to 56 eV, depending on the compound tested (Table S5). Tuning of the mass spectrometer for all analysed phenolics was conducted with the IntelliStart feature of MassLynx V4.1.

Identification and quantification of phenolic compounds was conducted according to retention times, UV maxima and multiple reaction monitoring transitions in ESI- or ESI+ mode (Table S5). Concentration of determined compounds is expressed in mg/L.

3.6. Multi-element analysis

Major elements (calcium, sodium, potassium, magnesium) and iron were measured by ICP-OES (Thermo Scientific, United Kingdom, model 6500 Duo, equipped with a CID86 chip detector). The analysis was done as previously described (Šelih et al. 2014; Vadalá et al. 2016). The conditions were as follow: radio frequency power 1150 W; principal argon flow rate 12 L/min; auxiliary argon and nebuliser flow rates 0.5 L/min; and sample flow rate 1 L/min. Nebuliser with concentric flow and cyclonic spray chamber were used. For each metal, specific wavelength was selected (373.6 nm for Ca; 766.4 nm for K; 279.5 nm for Mg; 589.5 nm for Na; 259.9 nm for Fe). The entire system was controlled by Iteva software.

The fifteen trace and ultratrace elements were analysed by ICP-MS (iCAP Q, Thermo Scientific X series 2). The analysis was performed as previously described (Hopfer et al. 2015). The conditions were as follow: Rf power 1548 W, gas flows 13.90, 1.09 and 0.80 L/min, acquisition time 3 × 50 sec. Detector was operating in pulse mode with dwell time of 10 msec (3 points per peak were recorded). The entire system was controlled with Qtegra instrument control software. ^{45}Sc, ^{115}In and ^{159}Tb were used as internal standards. The standards were prepared with ethanol at concentration 1% (v/v).

3.7. Statistical analysis

The experimental data obtained were analysed using two-way ANOVA (Stat Soft Statistica 10.0) [the factors were wine variety (levels: Merlot, Cabernet Sauvignon, Vranac) and vintage (levels: 2010, 2011 and 2012)], followed by Tukey HSD post-hoc test. The results carried out in triplicate are presented as mean ± STD. Values of $p \leq 0.05$ were considered statistically significant. The direction and magnitude of the correlation between the total phenolic content and anti-DPPH radical activity were quantified by the correlation factor R.

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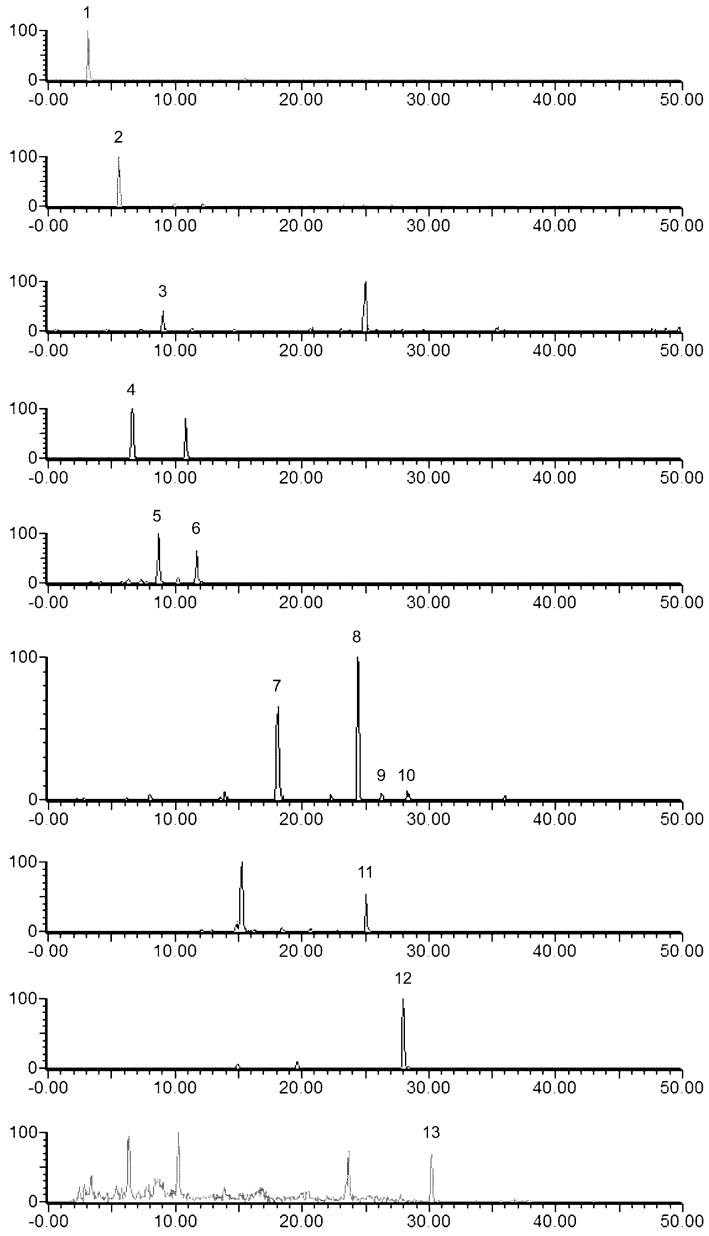

Figure S1. Representative LC-MS/MS chromatograms for the phenolic compounds analysed. Given numbers refer to the chromatographic peak obtained by monitoring of mass transition characteristic for specific phenolic compound (1 – Gallic acid; 2 – Protocatechuic acid; 3 – 4-Hydroxy benzoic acid; 4 – Caffeic acid; 5 – Catechin; 6 – Epicatechin; 7 – *trans*-Resveratrol; 8 – *cis*-Resveratrol; 9 – *trans*-Piceid; 10 – *cis*-Piceid; 11 – Myricetin; 12 – Quercetin; 13 – Kaempherol); y-axis refers to the relative abundance of the specific phenolic compound and x-axes represents time axes.

Table S1. Total phenolic content and anti-DPPH radical activity of the red wines varieties selected.

	Sample	Total phenolic content (mg/L)	EC_{50}^{-1} ($\%^{-1}$)
2010	Vranac	1513.0 ± 54.6 $^{a, A}$	22.6 ± 1.2 $^{a, A}$
	Merlot	2184.5 ± 93.7 $^{a, B}$	42.7 ± 1.4 $^{a, B}$
	Cabernet Sauvignon	2529.4 ± 80.6 $^{a, C}$	58.8 ± 2.5 $^{a, C}$
2011	Vranac	2362.1 ± 77.6 $^{b, A}$	59.2 ± 1.5 $^{b, A}$
	Merlot	2988.6 ± 142.2 $^{b, B}$	66.7 ± 3.1 $^{b, B}$
	Cabernet Sauvignon	2873.4 ± 93.2 $^{b, C}$	61.7 ± 2.7 $^{a, B}$
2012	Vranac	2204.4 ± 82.3 $^{b, A}$	41.3 ± 1.6 $^{c, A}$
	Merlot	3730.2 ± 164.7 $^{c, B}$	73.5 ± 3.7 $^{c, B}$
	Cabernet Sauvignon	2593.8 ± 95.0 $^{b, C}$	59.2 ± 2.4 $^{a, C}$

*Value for total phenolic content is expressed as gallic acid equivalent (mg GAE/L).

Anti-DPPH radical activity is expressed as EC_{50}^{-1}, the value that represents the reciprocal dissolution of wine sample able to scavenge 50% of $DPPH^{\bullet}$.

All values are represented as mean ± SD (triplicate).

Different letters within each column shows statistically significant differences (Tukey HSD test, $p < 0.05$).

The small letters represent statistically significant differences within the same variety through different vintages, while the big letters show statistically significant differences between different varieties in the same vintage.

Table S2. The content of the phenolic compounds in the wine samples examined, determined by LC-MS/MS.

Phenolic compound	Vr	Me	CS	Vr	Me	CS	Vr	Me	CS
		2010			2011			2012	
					(mg/L)				
Gallic acid	16.41 ± 0.41^{a,A}	10.23 ± 0.22^{a,B}	15.39 ± 0.36^{a,C}	15.10 ± 0.28^{b,A}	13.39 ± 0.34^{b,B}	21.59 ± 0.35^{c,C}	12.58 ± 0.32^{c,A}	28.65 ± 0.42^{c,B}	13.94 ± 0.26^{c,C}
Protocatechuic acid	2.09 ± 0.09^{a,A}	1.52 ± 0.05^{a,B}	1.46 ± 0.12^{a,C}	1.66 ± 0.10^{b,A}	1.05 ± 0.07^{b,B}	1.09 ± 0.03^{b,C}	1.80 ± 0.13^{b,A}	0.55 ± 0.03^{c,B}	1.25 ± 0.07^{b,C}
4-Hydroxy benzoic acid	0.40 ± 0.02^{a,A}	0.16 ± 0.01^{a,B,C}	0.22 ± 0.02^{a,A,C}	0.05 ± 0.01^{b,A}	0.12 ± 0.02^{a,A}	0.22 ± 0.01^{a,A}	ND	0.04 ± 0.01^{a,A}	0.09 ± 0.00^{a,A}
Caffeic acid	2.34 ± 0.10^{a,A}	2.85 ± 0.11^{a,B}	4.91 ± 0.25^{a,C}	1.25 ± 0.16^{b,A}	10.63 ± 0.12^{b,B}	1.51 ± 0.04^{b,A}	1.59 ± 0.07^{b,A}	2.70 ± 0.11^{a,B}	1.50 ± 0.06^{b,C}
Catechin	7.41 ± 0.22^{a,A}	16.98 ± 0.36^{a,B}	20.88 ± 0.33^{a,C}	9.17 ± 0.22^{b,A}	23.10 ± 0.21^{b,B}	23.14 ± 0.28^{b,C}	9.85 ± 0.13^{b,A}	43.22 ± 0.33^{c,B}	19.00 ± 0.26^{c,C}
Epicatechin	2.96 ± 0.04^{a,A}	9.04 ± 0.15^{a,B}	11.96 ± 0.10^{a,C}	4.97 ± 0.19^{b,A}	9.46 ± 0.23^{a,B}	11.33 ± 0.11^{b,C}	4.29 ± 0.10^{c,A}	21.74 ± 0.28^{b,B}	9.41 ± 0.15^{c,C}
trans-Resveratrol	0.46 ± 0.02^{a,A}	0.24 ± 0.01^{a,B}	0.21 ± 0.01^{a,C}	0.33 ± 0.02^{b,A}	0.26 ± 0.01^{a,B}	0.20 ± 0.01^{a,C}	0.55 ± 0.02^{c,A}	0.28 ± 0.01^{b,B}	ND
cis-Resveratrol	0.11 ± 0.01^{a,A}	0.26 ± 0.01^{a,B}	0.38 ± 0.02^{a,C}	0.05 ± 0.01^{b,A}	0.54 ± 0.02^{b,B}	ND	0.22 ± 0.03^{c,A}	ND	0.23 ± 0.01^{c,A}
trans-Piceid	2.08 ± 0.07^{a,A}	1.51 ± 0.03^{a,B}	0.64 ± 0.01^{a,C}	2.22 ± 0.02^{b,A}	1.48 ± 0.01^{a,B}	0.32 ± 0.03^{b,C}	1.27 ± 0.05^{c,A}	0.77 ± 0.03^{b,B}	0.69 ± 0.02^{a,C}
cis-Piceid	1.84 ± 0.04^{a,A}	1.60 ± 0.05^{a,B}	1.25 ± 0.05^{a,C}	1.27 ± 0.01^{b,A}	3.00 ± 0.04^{b,B}	1.04 ± 0.02^{b,C}	1.50 ± 0.03^{c,A}	1.29 ± 0.05^{c,B}	0.63 ± 0.02^{c,C}
Myricetin	1.32 ± 0.05^{a,A}	1.59 ± 0.01^{a,B}	1.16 ± 0.01^{a,C}	1.81 ± 0.03^{b,A}	1.02 ± 0.05^{b,B}	0.75 ±0.01^{b,C}	4.15 ± 0.10^{c,A}	0.76 ± 0.02^{c,B}	0.86 ± 0.01^{b,C}
Quercetin	0.82 ± 0.03^{a,A}	1.89 ± 0.01^{a,B}	2.19 ± 0.04^{a,C}	3.37 ± 0.05^{b,A}	3.92 ± 0.07^{b,B}	2.27 ± 0.02^{a,C}	2.98 ± 0.07^{c,A}	6.03 ± 0.12^{c,B}	2.22 ± 0.03^{a,C}
Kaempherol	ND	ND	0.11 ± 0.02^{a,B}	0.20 ± 0.01^{b,A}	0.30 ± 0.02^{b,B}	0.14 ± 0.01^{a,A}	0.07 ± 0.00^{c,A}	0.15 ± 0.01^{c,B}	0.20 ± 0.02^{b,C}

*ND – not detected; Vr – Vranac variety; Me – Merlot variety; CS – Cabernet Sauvignon variety. All values are presented as mean ± SD (triplicate).
Different letters within each column shows statistically significant differences (Tukey HSD test, $p < 0.05$). The small letters represent statistically significant differences within the same variety through different vintages, while the big letters show statistically significant differences between different varieties in the same vintage.

Table S3. The contents of major and trace elements in the wine samples selected, determined by ICP-OES or ICP-MS.

Sample		MAJOR (mg/L)				TRACE (µg/L)				
		Ca	K	Mg	Na	Al	Fe	Mn	Zn	Cu
2010	Vr	$63.1 \pm 0.7^{a,B}$	$479 \pm 5^{a,A}$	$46.4 \pm 0.3^{a,A}$	$9.8 \pm 0.3^{a,A}$	$939.8 \pm 1.2^{a,A}$	$1113 \pm 3^{a,A}$	$483.8 \pm 0.4^{a,A}$	$988.3 \pm 0.7^{a,A}$	$375.1 \pm 0.5^{a,A}$
	Me	$51.5 \pm 0.6^{a,B}$	$480 \pm 3^{a,A}$	$45.3 \pm 0.2^{a,A}$	$11.7 \pm 0.4^{a,BC}$	$443.5 \pm 0.8^{a,B}$	$1001 \pm 2^{a,B}$	$477.3 \pm 0.4^{a,B}$	$390.3 \pm 0.4^{a,B}$	$24.4 \pm 0.7^{a,B}$
	CS	$62.6 \pm 0.7^{a,A}$	$803 \pm 8^{a,B}$	$80.6 \pm 0.5^{a,B}$	$10.6 \pm 0.4^{a,AD}$	$452.1 \pm 0.7^{a,C}$	$971 \pm 2^{a,C}$	$529.1 \pm 0.5^{a,C}$	$496.2 \pm 0.4^{a,C}$	$57.7 \pm 0.5^{a,C}$
2011	Vr	$57.5 \pm 0.4^{b,A}$	$525 \pm 6^{b,A}$	$38.5 \pm 0.3^{b,A}$	$10.3 \pm 0.4^{ab,A}$	$715.8 \pm 0.8^{b,A}$	$1027 \pm 3^{b,A}$	$491.2 \pm 0.4^{b,A}$	$1095.6 \pm 0.7^{b,A}$	$38.6 \pm 0.4^{b,A}$
	Me	$45.8 \pm 0.4^{b,B}$	$515 \pm 5^{b,A}$	$49.2 \pm 0.2^{b,B}$	$14.7 \pm 0.5^{b,B}$	$495.5 \pm 0.5^{b,B}$	$1088 \pm 4^{b,B}$	$545.7 \pm 0.5^{b,B}$	$531.4 \pm 0.4^{b,B}$	$22.9 \pm 0.4^{a,B}$
	CS	$42.4 \pm 0.3^{b,C}$	$496 \pm 5^{b,B}$	$47.7 \pm 0.3^{b,C}$	$12.3 \pm 0.3^{b,C}$	$629.4 \pm 0.5^{b,C}$	$1223 \pm 3^{b,C}$	$655.4 \pm 0.5^{b,C}$	$449.8 \pm 0.5^{b,C}$	$16.6 \pm 0.7^{b,C}$
2012	Vr	$57.1 \pm 0.7^{b,A}$	493 ± 6	$41.4 \pm 0.5^{c,A}$	$9.1 \pm 0.3^{ac,A}$	$530.6 \pm 0.6^{c,A}$	$1041 \pm 2^{c,A}$	$440.3 \pm 0.3^{c,A}$	$551.7 \pm 0.5^{c,A}$	$124.9 \pm 1.5^{c,A}$
	Me	$62.2 \pm 0.5^{c,B}$	614 ± 5	$74.4 \pm 0.5^{c,B}$	$14.6 \pm 0.5^{b,B}$	$1172.3 \pm 2.5^{c,B}$	$2186 \pm 4^{c,B}$	$752.0 \pm 0.4^{c,B}$	$1114.7 \pm 0.8^{c,B}$	$267.3 \pm 1.7^{b,B}$
	CS	$43.7 \pm 0.5^{b,C}$	464 ± 5	$49.3 \pm 0.5^{c,C}$	$13.4 \pm 0.4^{b,C}$	$658.4 \pm 0.7^{c,C}$	$1134 \pm 3^{c,C}$	$700.5 \pm 0.5^{c,C}$	$419.3 \pm 0.5^{c,C}$	$17.7 \pm 0.5^{b,C}$

*Vr – Vranac variety; Me – Merlot variety; CS – Cabernet Sauvignon variety. All values are presented as mean ± SD (triplicate).
Different letters within each column shows statistically significant differences (Tukey HSD test, $p < 0.05$). The small letters represent statistically significant differences within the same variety through different vintages, while the big letters show statistically significant differences between different varieties in the same vintage.

Table S4a. The content of ultratrace elements in the wine samples selected, determined by ICP-MS.

Sample		ULTRATRACE (µg/L)					
		V	Cr	Ni	Co	As	Se
2010	Vr	$1.44 \pm 0.02^{a,A}$	$34.6 \pm 0.2^{a,A}$	$37.0 \pm 0.3^{a,A}$	$0.99 \pm 0.00^{a,A}$	$0.37 \pm 0.02^{a,A}$	$3.77 \pm 0.20^{a,A}$
	Me	$7.53 \pm 0.02^{a,B}$	$34.6 \pm 0.2^{a,A}$	$16.9 \pm 0.2^{a,B}$	$1.04 \pm 0.01^{a,B}$	$1.06 \pm 0.04^{a,B}$	$2.61 \pm 0.20^{a,A}$
	CS	$17.49 \pm 0.05^{a,C}$	$40.8 \pm 0.3^{a,B}$	$18.8 \pm 0.2^{a,c}$	$1.12 \pm 0.01^{a,C}$	$2.07 \pm 0.04^{a,C}$	ND
2011	Vr	$0.70 \pm 0.02^{b,A}$	$21.5 \pm 0.2^{b,A}$	$27.1 \pm 0.3^{b,A}$	$0.93 \pm 0.00^{b,A}$	ND	ND
	Me	$0.94 \pm 0.03^{b,B}$	$45.5 \pm 0.2^{b,B}$	$19.2 \pm 0.2^{b,B}$	$1.85 \pm 0.01^{b,B}$	$0.30 \pm 0.01^{b,B}$	$5.16 \pm 0.30^{b,B}$
	CS	$0.53 \pm 0.02^{b,C}$	$37.5 \pm 0.5^{b,C}$	$25.7 \pm 0.3^{b,C}$	$1.24 \pm 0.01^{b,C}$	ND	$0.33 \pm 0.02^{a,B}$
2012	Vr	$0.86 \pm 0.03^{c,A}$	$29.1 \pm 0.2^{c,A}$	$17.6 \pm 0.2^{c,A}$	$0.86 \pm 0.00^{c,A}$	$0.03 \pm 0.01^{b,A}$	$2.55 \pm 0.20^{a,A}$
	Me	$1.10 \pm 0.06^{c,B}$	$54.6 \pm 0.3^{c,B}$	$48.1 \pm 0.5^{c,B}$	$3.01 \pm 0.02^{c,B}$	$4.90 \pm 0.02^{c,B}$	$3.22 \pm 0.30^{a,A}$
	CS	$0.58 \pm 0.02^{b,C}$	$37.6 \pm 0.3^{b,C}$	$20.0 \pm 0.2^{c,C}$	$1.01 \pm 0.01^{c,C}$	ND	ND

*ND – not detected; Vr – Vranac variety; Me – Merlot variety; CS – Cabernet Sauvignon variety. All values are presented as mean ± SD (triplicate).

Different letters within each column shows statistically significant differences (Tukey HSD test, $p < 0.05$). The small letters represent statistically significant differences within the same variety through different vintages, while the big letters show statistically significant differences between different varieties in the same vintage.

Table S4b. The content of ultratrace elements in the wine samples selected, determined by ICP-MS.

Sample		ULTRATRACE (µg/L)				
		Cd	Sb	Ba	Tl	Pb
2010	Vr	$0.43 \pm 0.01^{a,A}$	$6.23 \pm 0.03^{a,A}$	$56.2 \pm 0.1^{a,A}$	$1.37 \pm 0.01^{a,A}$	$102.9 \pm 0.2^{a,A}$
	Me	$0.35 \pm 0.00^{a,B}$	$5.73 \pm 0.03^{a,B}$	$79.8 \pm 0.2^{a,B}$	$1.33 \pm 0.01^{a,B}$	$12.2 \pm 0.1^{a,B}$
	CS	$0.27 \pm 0.02^{a,C}$	$5.59 \pm 0.02^{a,C}$	$75.1 \pm 0.1^{a,C}$	$1.53 \pm 0.00^{a,C}$	$9.9 \pm 0.1^{a,C}$
2011	Vr	$0.35 \pm 0.02^{b,A}$	$7.06 \pm 0.03^{b,A}$	$31.0 \pm 0.3^{b,A}$	$1.29 \pm 0.01^{b,A}$	$43.4 \pm 0.2^{b,A}$
	Me	$0.22 \pm 0.01^{b,B}$	$5.38 \pm 0.02^{b,B}$	$61.9 \pm 0.2^{b,B}$	$1.49 \pm 0.02^{b,B}$	$7.2 \pm 0.1^{b,B}$
	CS	$0.25 \pm 0.01^{b,C}$	$4.93 \pm 0.01^{b,C}$	$79.2 \pm 0.2^{b,C}$	$1.19 \pm 0.01^{b,C}$	$7.6 \pm 0.1^{b,C}$
2012	Vr	$0.14 \pm 0.01^{c,A}$	$4.86 \pm 0.01^{c,A}$	$27.6 \pm 0.1^{c,A}$	$1.29 \pm 0.00^{b,A}$	$17.7 \pm 0.3^{c,A}$
	Me	$0.52 \pm 0.00^{c,B}$	$6.58 \pm 0.03^{c,B}$	$118.8 \pm 0.3^{c,B}$	$1.68 \pm 0.01^{c,B}$	$53.7 \pm 0.3^{c,B}$
	CS	$0.18 \pm 0.01^{b,C}$	$7.49 \pm 0.03^{c,C}$	$72.8 \pm 0.3^{c,C}$	$1.06 \pm 0.02^{c,C}$	$7.2 \pm 0.2^{b,C}$

*ND – not detected; Vr – Vranac variety; Me – Merlot variety; CS – Cabernet Sauvignon variety. All values are presented as mean ± SD (triplicate).

Different letters within each column shows statistically significant differences (Tukey HSD test, $p < 0.05$). The small letters represent statistically significant differences within the same variety through different vintages, while the big letters show statistically significant differences between different varieties in the same vintage.

Table S5. Basic parameters of the analytes applied for the analysis of the compounds selected.

Phenolic compound	Retention time (min)	Molecular formula	ESI mode	Quantification transition	Cone voltage (V)	Collision energy (eV)
Gallic acid	3.2	$C_7H_6O_5$	-	169→125	30	20
Protocatechuic acid	5.9	$C_7H_6O_4$	-	153→109	30	20
4-Hydroxy benzoic acid	9.4	$C_7H_6O_3$	-	137→93	30	20
Caffeic acid	7.1	$C_9H_8O_4$	-	179→135	30	20
Catechin	9.1	$C_{15}H_{14}O_6$	+	291→139	26	20
Epicatechin	12.1	$C_{15}H_{14}O_6$	+	291→139	26	16
trans-Resveratrol	26.5	$C_{14}H_{12}O_3$	+	229→107	34	24
cis-Resveratrol	28.5	$C_{14}H_{12}O_3$	+	229→107	34	24
trans-Piceid	18.7	$C_{20}H_{22}O_8$	+	229→107	34	24
cis-Piceid	24.7	$C_{20}H_{22}O_8$	+	229→107	34	24
Myricetin	25.3	$C_{15}H_{10}O_8$	+	319→153	52	38
Quercetin	28.1	$C_{15}H_{10}O_7$	-	301→151	30	20
Kaempherol	30.3	$C_{15}H_{10}O_6$	+	287→153	56	36