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Polyphenolic compounds in seeds from some grape cultivars grown in Serbia

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Abstract: Seed extracts from eight grape cultivars (*Vitis vinifera*) growing in Serbia were screened for their polyphenolic composition by means of HPLC/ /PDA/ESI/MS analysis. The study revealed 34 phenolic compounds belonging to the following groups: flavan-3-ol monomers, proanthocyanidins, flavonols, hydroxycinnamic acid and hydroxybenzoic acid derivatives. The quantities of the main constituents were determined using PDA/HPLC. Qualitative and quantitative differences among the cultivars were observed.

Keywords: *Vitis vinifera*; grape seeds; HPLC/PDA/ESI/MS; flavanol monomers; proanthocyanidins; flavonols; hydroxycinnamic acid; hydroxybenzoic acid derivatives.

INTRODUCTION

Many agricultural by-products are composed of plant tissues rich in phytochemicals, with valuable chemical and biological properties. Examples are by--products from wine processing,[1–3] such as marcs, stems, dregs (a sludgy residue deposited on the bottom of fermentation vats) and grape-seeds, which represent rich sources of polyphenolics.

Phenols represent the third most abundant constituent in grapes after carbohydrates and fruit acids.[4] The composition of phenolics depends on whether the extraction is performed on whole grape, pulp, skin or seeds. The total extractable phenolics in grapes are present at only about 10 % or less in pulp, 60–70 % in the seeds and 28–35 % in the skin. The phenol content of seeds may range from 5 to 8 % by weight.[5]

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The phenolic compounds in grapes can be divided into two main groups: phenolic acids (localized mainly in the skin and pulp) and flavonoids. The most common phenolic acids in grape include cinnamic and benzoic acid derivatives. Flavonoids include colorless flavan-3-ols, flavonols and red and blue anthocyanins.[5] The most abundant phenolics isolated from grape seeds and skins are flavan-3-ols (catechin and epicatechin) and their oligomers and polymers (proanthocyanidins). The outer seed coat contains the majority of both the monomeric and polymeric flavan-3-ols (2 to 5 times more than the endosperm).[6] Grape skins also contain anthocyanins which contribute to their red or blue color.[7,8]

Various conditions (time, solvent, and the manner) for the extraction of polyphenols from grape seeds are described in the literature. Due to the acidic lability of interflavan linkages within proanthocyanidins and the susceptibility of polyphenols to oxidation, a valid extraction method should provide for the complete as possible extraction of the polyphenolics while limiting their degradation.[9] Methanol/water[10,11] or acetone/water systems[12] are the common solvents used for extracting polyphenols from grape seeds. In particular, lower molecular weight polyphenols, such as phenolic acids, anthocyanins, and flavanol monomers and oligomers, are well extracted with methanol, while the higher molecular weight flavanols are better extracted with aqueous acetone than with methanol.[13–16]

Several methods for the analysis of polyphenols have been proposed in the literature. Most of them are based on high performance liquid chromatography (HPLC) coupled with either a photodiode array (PDA) detector or a mass spectrometer (MS). Reverse phase columns are favorable, using acetonitrile and acidic water solutions as eluents.[17] Since UV detection depends upon the chemical structure of a molecule, several wavelengths could be selected for monitoring. Red-colored anthocyanins show an absorbance maximum at around 520 nm; yellow-colored flavonols display an absorbance maximum at around 360 nm; hydroxycinnamic acids can be specifically detected by their high absorbance around 320 nm. Flavan-3-ols show no specific absorbance and have a maximum around 280 nm, as do all the above-mentioned phenolics.[18]

Many studies proved that procyanidins and other polyphenolics from grape seed could be the key compounds responsible for various beneficial effects for human health.[19,20] These effects are mainly associated with the antioxidant activity of the phenolic compounds, which act as reducing agents by trapping free radicals, by acting as chelators, by donating hydrogen, and by quenching singlet oxygen. These highly reactive species are present in biological systems and may oxidize lipids, proteins, nucleic acids, which may initiate degenerative heart disease. In addition, grape seed polyphenolics possess various potent biological effects, such as antitumor, antibacterial, antiviral, anti-inflammatory, enzyme-inhibiting effects.[21–24] Waste products of the winery and grape juice industry derived from grape seeds represent a rich source of polyphenols.[5,25] It is well

known that the concentration of polyphenolic compounds in grapes depends on the grape cultivar,[26,27] and other factors, such as ripening time, climate, soil and location of growth.[28]

The aim of this study was to determine the polyphenolic composition of grape seed extracts from *Vitis vinifera* L. cv., Smederevka, Prokupac, Serbian original varieties, and Italian Riesling, Traminer, Black Burgundy, Gamay Noir, Muscat Hamburg and Gamay Bojadiser, all grown in the same geographical area and vintage. Two grape cultivars, Italian Riesling and Traminer, have a yellow--green colored grape berry used for production of high-quality white wines. The cultivar Smederevka is an autochthonous grape cultivar of Serbia, with lightly yellow and green colored berries. This cultivar is used for production of quality white wines as well as for all kinds of blending, because the grapes accumulate a high level of acids. Since the grape is well transportable and the pulp is crispy, it is also used fresh. Black Burgundy and Gamay Noir are purple-colored grape cultivars, used in production of high-quality red wines. Muscat Hamburg is the most widespread table grape in Serbia. This grape can be used fresh as well as for wine and grape brandy production. Prokupac is also an autochthonous grape cultivar of Serbia. Its berries are navy-blue colored with plenty of dots, and it is used for the production of quality rosé wines. Gamay Bojadiser grapes are full of colored materials, and it is mostly used for blending. An HPLC/PDA/MS method was used for the polyphenols analysis. The similarities and differences between the polyphenolic compositions of grape seed extracts from different cultivars are discussed.

EXPERIMENTAL

Plant material

Seeds from eight grape cultivars, including Italian Riesling, Traminer, Smederevka, Black Burgundy, Gamay Noir, Muscat Hamburg, Prokupac and Gamay Bojadiser were examined. All studied cultivars were grown in the vicinity of Belgrade (experimental orchard of Radmilovac, property of Faculty of Agriculture, University of Belgrade). The experimental vineyard was raised in 1995 (cultivars Smederevka and Gamay Noir), and 1996 (cultivars Italian Riesling, Traminer, Black Burgundy, Muscat Hamburg, Prokupac and Gamay Bojadiser). The distance of sowing was 3×1 m, with two rows support, and the training system was a "double-branched asymmetrical cordone",[29] the tree being 90 cm high. Approximately 20 clusters (about 5 kg of grape) were collected in late summer 2008, from 10 different plants. All the samples were collected when the Brix values were in range 22.5–24.5°.

Chemicals

Gallic acid, catechin, epicatechin, caffeic acid, ellagic acid, and rutin were purchased from Sigma Aldrich (St. Louis, MO, USA). All chemicals and solvents were of analytical grade. The HPLC water was purified by a Milli-Q System.

Sample preparation

The seeds from the berries were manually separated from pulp and dried on filter paper. The samples of whole, dried seeds (20 g) were macerated in 120 mL of 50 % MeOH, and 1

mL of rutin solution (2.78 mg mL^{-1} in MeOH, internal standard) was added. The mixtures were sonicated in an ultrasonic bath for 8 h. The extracts were filtered through filter paper, evaporated (to 1 mL) at 45 °C under reduced pressure and filtered through a 0.45 mm cellulose filter (Millipore). The filtrate was then transferred into a vial and filled up with 50 % MeOH to a volume of 1.5 mL.

HPLC/PDA Analysis

HPLC analysis of extracts was performed using an Agilent 1200 chromatograph equipped with a PDA model G1315B, a Bin pump model G1312A, an autosampler model G1313A and a RR Zorbax Eclipse Plus C18 column (1.8 μm, 150 mm×4.6 mm). The mobile phase A was 0.2 % formic acid in water and the mobile phase B was acetonitrile. Elution was performed at 0.95 mL min^{-1} with the following gradient program of solvent B: 0–20 min, 5–16 %; 20–28 min, 16–40 %; 28–32 min, 40–70 %; 32–36 min, 70–99 %; 36–45 min, 99 % and 45–46, min. 99–5 %.[30] The injection volume was 10 μL. Wavelengths of 280 nm (for flavan-3-ols and benzoic acid derivatives) and 360 nm (for flavonols and cinnamic acid derivatives) were selected for detection.

Quantification of the compounds was realized using calibration curves obtained by HPLC of pure standards: gallic acid, caffeic acid, (+)-catechin, (-)-epicatechin, and ellagic acid. Rutin was used as an internal standard. Some compounds were quantified as equivalents of the most similar chemical structures: gallic acid for gallic acid glucoside, gentisic acid glucoside, protocatechuic acid, *p*-hydroxybenzoic acid and methyl gallate; caftaric acid as caffeic acid; (+)--catechin for proanthocyanidin dimers and trimers and their monogallates; (–)-epicatechin for epicatechin gallate; ellagic acid for ellagic acid pentoside.

LC/MS analysis

LC/MS analysis was performed on an Agilent MSD TOF coupled to an Agilent 1200 series HPLC, using the same column and gradient program as were employed for the HPLC/ /PDA analysis. Mass spectra were acquired using an Agilent ESI-MSD TOF. The drying gas (N2) flow was 12 L min^{-1}; the nebulizer pressure was 310.264 kPa and the drying gas temperature was 350 °C. For ESI analysis, the parameters were: capillary voltage, 4000 V; fragmentor, 140 V; skimmer, 60 V; Oct RF V 250 V, for negative modes. The mass range was from 100 to 2000 *m*/*z*. Data processing was realized with the software Molecular Feature Extractor and Mass Profiler.

Statistical analysis

All the experiments were performed in triplicate. Significant differences between the means were separated by analysis of variance (ANOVA) followed by Tukey's test. Computations were realized using Origin software package version 7.0.

RESULTS AND DISCUSSION

The rapid resolution HPLC column and the appropriate gradient program afforded the separation of some 34 phenolic compounds in less than 30 min. Identification of the compounds was based on the UV spectra and molecular formula obtained from accurate mass measurements, both measured on the HPLC/ /PDA/ESI/MS equipment, which also involved comparison of these data with those of the metabolites previously reported for grape seed extracts.[23,31] The identified phenolic compounds could be classified into the following groups: flavanol monomers (catechin and epicatechin), proanthocyanidins, flavonols,

hydroxycinnamic acids, and hydroxybenzoic acid derivatives (Table I, Fig. 1.). However, owing to the unavailability of authentic compounds, with exception of gallic acid, ellagic acid, catechin and epicatechin, the peaks could be tentatively assigned but without determination of stereochemistry.

TABLE I. LC/MS Data of grape seed extracts (GSEs)

Peak	t_R min	Compound	Class of compound[a]	λ_{max} nm	Species	Mass	Molecular formula
1	3.2	Gallic acid	HB	220, 272	M–H, 2M–H	170.0215	$C_7H_6O_5$
2	4.0	Proanthocyanidin trimer	PC	200, 218, 228*sh*, 236*sh*, 280	M–2H, M–H	866.2058	$C_{45}H_{38}O_{18}$
3	4.3	Gallic acid glucoside	HB	218, 256	M–H, 2M–H	332.0744	$C_{13}H_{16}O_{10}$
4	5.0	Gentisic acid glucoside	HB	216, 252	M–H, 2M–H	316.0794	$C_{13}H_{16}O_9$
5	5.8	Protocatechuic acid	HB	218, 260, 292*sh*	M–H, 2M–H	154.0266	$C_7H_6O_4$
6	7.3	Caftaric acid	HC	300*sh*, 324	M–H, 2M–H	312.0481	$C_{13}H_{12}O_9$
7	8.2	*p*-Hydroxybenzoic acid	HB	278, 312	M–H, 2M–H	138.0317	$C_7H_6O_3$
8	8.3	Proanthocyanidin dimer	PC	200, 216, 228*sh*, 280	M–H, 2M–H	578.1424	$C_{30}H_{26}O_{12}$
9	9.5	Proanthocyanidin dimer	PC	200, 216, 228*sh*, 280	M–H, 2M–H	578.1424	$C_{30}H_{26}O_{12}$
10	9.8	Methyl gallate	HB	220, 272	M–H, 2M–H	184.0372	$C_8H_8O_5$
11	10.1	(+)-Catechin	FM	200, 218, 226*sh*, 278	M–H, 2M–H	290.0790	$C_{15}H_{14}O_6$
12	10.9	Proanthocyanidin trimer	PC	200, 218, 228*sh*, 236*sh*, 280	M–2H, M–H	866.2058	$C_{45}H_{38}O_{18}$
13	11.3	Proanthocyanidin trimer	PC	200, 218, 228*sh*, 236*sh*, 280	M–2H, M–H	866.2058	$C_{45}H_{38}O_{18}$
14	11.7	Proanthocyanidin dimer	PC	200, 216, 228*sh*, 280	M–H, 2M–H	578.1424	$C_{30}H_{26}O_{12}$
15	11.8	Caffeic acid	HC	246, 298*sh*, 326	M–H	180.0423	$C_9H_8O_4$
16	12.7	Proanthocyanidin dimer	PC	200, 216, 228*sh*, 280	M–H, 2M–H	578.1424	$C_{30}H_{26}O_{12}$
17	13.9	Proanthocyanidin trimer monogallate	PC/HB	200, 218, 278	M–2H, M–H	1018.2168	$C_{52}H_{42}O_{22}$
18	14.3	(–)-Epicatechin	FM	200, 218, 226*sh*, 278	M–H, 2M–H	290.0790	$C_{15}H_{14}O_6$
19	15.4	Proanthocyanidin dimer monogallate	PC/HB	200, 218, 278	M–H, 2M–H	730.1534	$C_{37}H_{30}O_{16}$
20	15.8	Proanthocyanidin trimer	PC	200, 218, 228*sh*, 236*sh*, 280	M–2H, M–H	866.2058	$C_{45}H_{38}O_{18}$

TABLE I. Continued

Peak	t_R min	Compound	Class of compound[a]	λ_{max} nm	Species	Mass	Molecular formula
21	16.5	Proanthocyanidin trimer	PC	200, 218, 228*sh*, 236*sh*, 280	M–2H, M–H	866.2058	$C_{45}H_{38}O_{18}$
22	17.4	Proanthocyanidin dimer monogallate	PC/HB	200, 218, 278	M–H, 2M–H	730.1534	$C_{37}H_{30}O_{16}$
23	17.5	Syringic acid	HB	276	M–H, 2M–H	198.0528	$C_9H_{10}O_5$
24	20.6	Ellagic acid pentoside	HB	254, 300*sh*, 360	M–H, 2M–H	434.0485	$C_{19}H_{14}O_{12}$
25	21.9	Ellagic acid	HB	254, 298*sh*, 368	M–H	302.0063	$C_{14}H6O_8$
26	21.9	(–)-Epicatechin gallate	FM/HB	200, 218, 278	M–H, 2M–H	442.0900	$C_{22}H_{18}O_{10}$
27	22.0	Taxifolin	FL	232, 254, 290, 330*sh*	M–H, 2M–H	304.0583	$C_{15}H_{12}O_7$
28	22.7	Quercetin-3-*O*-glucuronide	FL	256, 264*sh*, 356	M–H, 2M–H	478.0747	$C_{21}H_{18}O_{13}$
29	23.1	Astilbin	FL	292, 326*sh*	M–H, 2M–H	450.1162	$C_{21}H_{22}O_{11}$
30	22.9	Quercetin-3-*O*-glucoside	FL	256, 268*sh*, 300*sh*, 360	M–H, 2M–H	464.0955	$C_{21}H_{20}O_{12}$
31	23.8	Kaempferol rutinoside	FL	266, 320*sh*, 350	M–H	594.1585	$C_{27}H_{30}O_{15}$
32	24.7	Isorhamnetin-3-*O*-glucoside	FL	256, 266*sh*, 302*sh*, 350	M–H, 2M–H	478.1111	$C_{22}H_{22}O_{12}$
33	24.3	Quercetin 3-*O*-rhamnoside	FL	256, 266*sh*, 302*sh*, 350	M–H, 2M–H	448.1006	$C_{21}H_{20}O_{11}$
34	27.4	Quercetin	FL	256, 268*sh*, 300*sh*, 370	M–H, 2M–H	302.0427	$C_{15}H_{10}O_7$

[a]HB – hydroxybenzoic acid derivative, FM – flavanol monomers, PC – proanthocyanidins, FL – flavonols, HC – hydroxycinnamic acids

All the identified compounds exhibited quasi-molecular ion $[M–H]^–$, as the dominant ion species in the mass spectrum. The exception were procyanidin trimers, where doubly charged $[M–2H]^{2–}$ species were dominant. Cluster ions, such as $[2M–H]^–$, were also observed for most of the compounds.

The amounts of phenolic compounds are presented in Tables II and III. The range of free gallic acid varied from 4 to 23 mg per 100 g of grape seeds. While white grape cultivars (Italian Riesling, Traminer and Smederevka) showed high gallic acid contents (over 17 mg per 100 g), the colored cultivars possessed significantly lower contents (below 10 mg per 100 g). This is in accordance with data published for some white and red grape varieties from Spain.[32] Except in Italian Riesling, glucosides of gallic acid were found in all the studied cultivars. In addition, protocatechuic acid was detected in the white grape cultivars and Gamay Noir. The presence of ellagic acid or ellagic acid glycoside was con-

firmed in Muscat Hamburg and Prokupac seeds. This finding is surprising because it was hitherto believed that the presence of ellagic acid is unique for muscadine grapes (*Vitis rotundifolia*) among the *Vitis* varieties.[33,34]

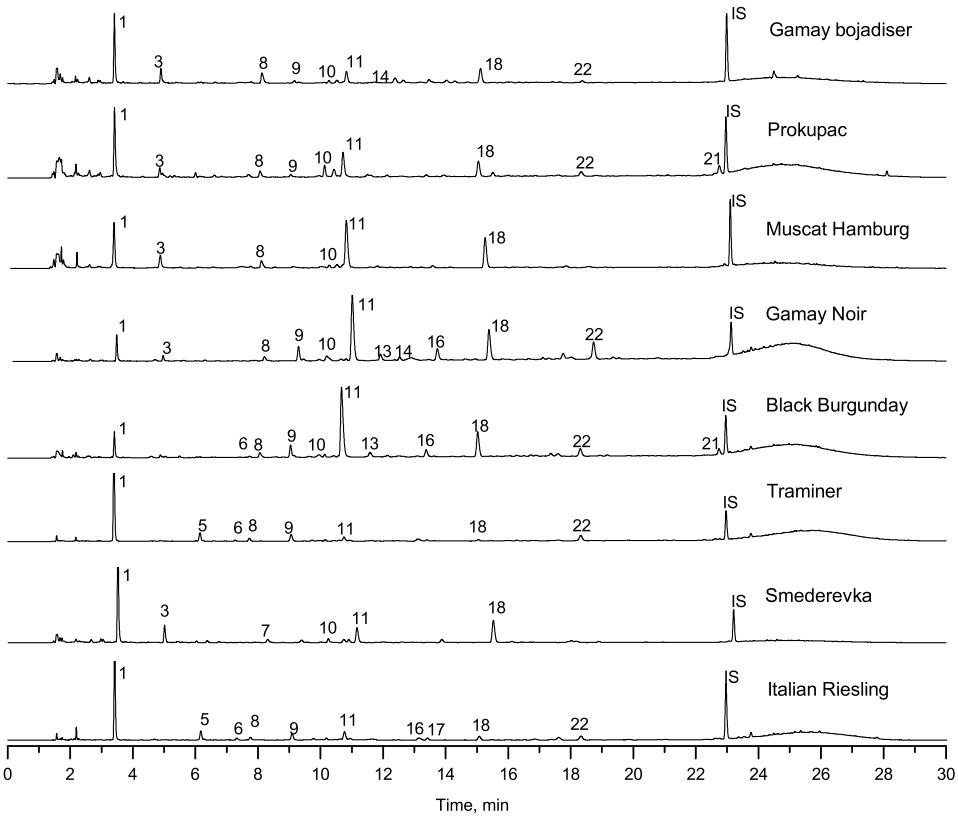

Fig. 1. LC/UV Chromatograms of grape seed extracts (λ = 280 nm).

In comparison with other classes of polyphenolic compounds, hydroxycinnamic acid derivatives were present in lower amounts in the seeds. This is in accordance with data from the literature claiming that hydroxycinnamic acids are localized mainly in the skin and pulp.[5] No hydroxycinnamic acids derivatives were detected in the cultivar Smederevka, while caftaric acid (ester of caffeic acid with tartaric acid) was found in the remaining cultivars. Underivatized caffeic acid was only found in the seeds of Traminer.

The presence of taxifolin (dihydroquercetin) and its glycoside astilbin was confirmed in Italian Riesling and Traminer cultivars, while in Black Burgundy only astilbin was detected. It should be noted that the presence of such types of flavonols is very rare in grape extracts.[35] Kaempferol rutinoside, quercetin or their glycosides were found in all the studied cultivars. Isorhamnetin-3-*O*-gluco-

TABLE II. Hydroxybenzoic acid, hydroxycinnamic acids derivatives and flavonols content in GSEs (IR - Italian Riesling, SM – Smederevka, TR – Traminer, BB - Black Burgundy, GN - Gamay Noir, MH - Muscat Hamburg, PR – Prokupac, GB - Gamay Bojadiser)

Peak	t_R min	Compound	IR		SM		TR		BB		GN		MH		PR		GB	
			Value[a]	*SD*	Value	*SD*	Value	*SD*	Value	*SD*	Value	*SD*	Value	*SD*	Value	*SD*	Value	*SD*
1	3.2	Gallic acid	17.67	0.23	18.62	0.35	22.48	0.41	4.30	0.10	5.15	0.05	5.84	0.05	8.12	0.10	9.45	0.05
3	4.3	Gallic acid glucoside	nd	–	3.51	0.10	0.59	0.04	1.04A*	0.06	1.54B*	0.05	4.50	0.10	1.29AB*	0.10	5.10	0.10
4	5.0	Gentisic acid glucoside	nd	–	0.76A	0.03	nd	–	nd	–	0.64AB	0.06	nd	–	0.54B	0.05	1.25	0.05
5	5.8	Protocatechuic acid	1.96A	0.05	0.78	0.10	2.44	0.03	nd	–	0.84A	0.05	nd	–	nd	–	nd	–
6	7.3	Caftaric acid	1.04A	0.04	nd	–	0.81A	0.01	2.67B	0.15	2.90B	0.10	7.04	0.05	1.87	0.08	6.62	0.11
7	8.2	*p*-Hydroxybenzoic acid	nd	–	1.43	0.15	nd	–	0.24	0.05	nd	–	nd	–	nd	–	nd	–
10	9.8	Methyl gallate	nd	–	1.49	0.10	Traces	–	1.44	0.05	1.42	0.08	3.06	0.06	2.10	0.10	1.35	0.05
15	11.8	Caffeic acid	nd	–	nd	–	2.04	0.03	nd	–	nd	–	nd	–	nd	–	nd	–
23	17.5	Syringic acid	Traces	–	nd	–	Traces	–	nd	–	nd	–	nd	–	nd	–	nd	–
24	20.6	Ellagic acid pentoside	nd	–	nd	–	nd	–	nd	–	nd	–	nd	–	1.24	0.05	nd	–
25	21.9	Ellagic acid	nd	–	nd	–	nd	–	nd	–	nd	–	2.36	0.05	1.26	0.05	nd	–
27	22.0	Taxifolin	Traces	–	nd	–	Traces	–	nd	–	nd	–	nd	–	nd	–	nd	–
28	22.7	Quercetin-3-*O*-glucuronide	Traces	–	nd	–	Traces	–	Traces	–	Traces	–	nd	–	Traces	–	nd	–
29	23.1	Astilbin	Traces	–	nd	–	Traces	–	Traces	–	nd	–	nd	–	nd	–	nd	–
30	22.9	Quercetin-3-*O*-glucoside	Traces	–	nd	–	nd	–	nd	–	nd	–	Traces	–	Traces	–	Traces	–
31	23.8	Kaempferol rutinoside	Traces	–	Traces	–	Traces	–	nd	–	Traces	–	Traces	–	Traces	–	Traces	–
32	24.7	Isorhamnetin-3-O--glucoside	nd	–	nd	–	nd	–	nd	–	nd	–	nd	–	nd	–	Traces	–
33	24.3	Quercetin 3-*O*--rhamnoside	Traces	–	nd	–	nd	–	nd	–	nd	–	nd	–	nd	–	nd	–
34	27.4	Quercetin	nd	–	nd	–	nd	–	nd	–	Traces	–	Traces	–	Traces	–	nd	–

[a]Expressed in mg/100g of dry grape seeds. Values with the same letters within the row are not significantly different ($p < 0.05$, without asterisk or $p < 0.01$, with asterisk)

TABLE III. Flavanol monomers and Proanthocyanidins content in GSEs (IR – Italian Riesling, SM – Smederevka, TR – Traminer, BB – Black Burgundy, GN – Gamay Noir, MH – Muscat Hamburg, PR – Prokupac, GB – Gamay Bojadiser)

Peak	t_R min	Compound	IR		SM		TR		BB		GN		MH		PR		GB	
			Value[a]	*SD*	Value	*SD*	Value	*SD*	Value	*SD*	Value	*SD*	Value	*SD*	Value	*SD*	Value	*SD*
2	4	Proanthocyanidin trimer	4.48	0.08	Traces	–	Traces	–	2.45	0.05	3.34	0.05	nd	–	1.40	0.10	nd	–
8	8.3	Proanthocyanidin dimer	29.50	0.59	0.75	0.05	6.85	0.09	11.27A	0.25	10.42A	0.33	17.38	0.13	5.25	0.05	15.29	0.10
9	9.5	Proanthocyanidin dimer	5.22	0.06	Traces	–	2.18	0.07	7.76	0.08	17.34	0.15	1.56	0.06	4.24	0.05	8.65	0.05
11	10.1	(+)-Catechin	42.41	0.52	6.85	0.10	17.21	0.01	134.82	1.11	145.04	1.00	107.81	1.41	26.14	0.15	37.26	0.64
12	10.9	Proanthocyanidin trimer	nd	–	nd	–	5.14	0.12	nd	–	nd	–	nd	–	4.35	0.05	17.28	0.20
13	11.3	Proanthocyanidin trimer	14.08	0.37	0.96	0.05	Traces	–	6.37	0.08	13.06	0.08	nd	–	nd	–	nd	–
14	11.7	Proanthocyanidin dimer	10.37	0.55	1.52	0.08	Traces	–	8.82	0.08	22.04	0.05	23.25	0.15	5.45	0.05	15.46	0.15
16	12.7	Proanthocyanidin dimer	21.35	0.56	2.90	0.10	8.36	0.05	10.40	0.20	16.69	0.10	29.30	0.19	6.11	0.10	32.74	0.25
17	13.9	Proanthocyanidin trimer monogallate	14.38	0.54	0.78	0.11	nd	–	nd	–	8.00	0.11	nd	–	Traces	–	25.98	0.43
18	14.3	(–)-Epicatechin	29.47	0.50	39.18	0.76	9.83	0.06	60.39	0.54	91.67	0.59	86.31	0.16	23.49	0.50	57.65	0.48
19	15.4	Proanthocyanidin dimer monogallate	14.49	0.50	0.88	0.08	nd	–	4.36	0.05	5.75	0.05	16.25	0.13	traces	–	nd	–
20	15.8	Proanthocyanidin trimer	nd	–	nd	–	nd	–	4.05	0.05	5.65	0.05	nd	–	traces	–	nd	–
21	16.5	Proanthocyanidin trimer	50.59	1.28	Traces	–	Traces	–	8.27	0.11	13.24	0.25	Traces	–	4.27	0.08	27.68	0.16
22	17.4	Proanthocyanidin dimer monogallate	50.77	1.15	1.35	0.05	20.24	0.20	16.70	0.10	40.62	0.78	nd	–	12.15	0.15	32.08	0.11
26	21.9	(–)-Epicatechin gallate	21.56	0.51	1.07	0.08	Traces	–	15.54	0.25	9.80	0.19	7.90	0.10	12.85	0.31	nd	–
		C+E	71.88	1.02	46.03	0.86	27.04	0.06	195.21	1.63	236.72	1.58	194.13	1.56	49.63	0.65	94.91	1.05
		C/E	1.44	0.01	0.17	0.00	1.75	0.01	2.23	0.00	1.58	0.00	1.25	0.01	1.11	0.02	0.65	0.01
		Proanthocyanidin dimer	66.45	1.73	5.17	0.23	17.39	0.21	38.25	0.60	66.49	0.63	71.49	0.52	21.05	0.25	72.13	0.55
		Galloylated proantocyanidins	101.20	2.71	4.07	0.31	20.24	0.20	36.59	0.40	64.18	1.13	24.15	0.23	24.99	0.46	58.06	0.54

[a]Expressed in mg/100g of dry grape seeds. Values with the same letters within the row are not significantly different ($p < 0.05$, without asterisk or $p < 0.01$, with asterisk)

side was detected only in Gamay Bojadiser (Table II). Such flavonols have been already reported in grapes extracts.[32,36]

The most abundant phenolic compounds in the grape seed extracts were monomeric flavan-3-ols and proanthocyanidins, as found by other authors.[26,32,37] Generally, the content of flavan-3-ol monomers (catechin and epicatechin) was higher in the colored than in the white grape cultivars (Table III). Only Smederevka and Gamay Bojadiser possessed greater amounts of epicatechin than catechin, while the catechin/epicatechin ratio for most cultivars was between 1 and 2. The exception was Black Burgundy which contained more than two times more catechin than epicatechin. Muscat Hamburg, Gamay Bojadiser, Italian Riesling and Gamay Noir were the richest in proanthocyanidin dimers, while Italian Riesling possessed a high amount of galloylated proantocyanidins. On the other hand, Smederevka possessed a very low amount of proanthocyanidin dimers and galloylated proanthocyanidins.

CONCLUSIONS

Statistically significant difference in the contents of some polyphenolic compounds between the studied cultivars was noticed. From these findings, it may be concluded that the amounts and distribution of various phenolic compounds in grape seeds depend directly on the cultivar, as the other factors, such as ripening time, climate, soil and location of growth, were the same for all the studied cultivars. This is the first time the presence of ellagic acid or ellagic acid glycoside in some *Vitis vinifera* cultivars was evidenced. The variation of the composition of the phenolic compounds from certain cultivar could be used in industry to make specific food additives or dietary supplements.

И З В О Д

ПОЛИФЕНОЛНА ЈЕДИЊЕЊА ИЗ СЕМЕНКИ ОСАМ СОРТИ ГРОЖЂА ГАЈЕНИХ У СРБИЈИ

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Помоћу HPLC/PDA/ESI/MS анализе је испитан полифенолни састав екстраката семенки осам сорти грожђа (*Vitis vinifera*) гајених у Србији. Утврђено је присуство 34 фенолна једињења која припадају следећим групама: флаванолски мономери, проантоцијанидини, флавоноли, деривати хидроксициметне и деривати хидроксибензоеве киселине. Квантитативни садржај главних састојака је одређен уз помоћ PDA/HPLC. Примећене су квалитативне и квантитативне разлике између појединих сорти.

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REFERENCES

1. F. Bonilla, M. Mayen, J. Merida, M. Medina, *Food Chem.* **66** (1999) 209
2. J. L. Torres, R. Bobet, *J. Agric. Food Chem.* **49** (2001) 4627
3. J. L. Torres, B. Varela, M. T. García, J. Carilla, C. Matito, J. J. Centelles, M. Cascante, X. Sort, R. Bobet, *J. Agric. Food Chem.* **50** (2002) 7548
4. V. L. Singleton, in *Proceedings of the University of California, Davis, Grape and Wine Centenary Symposium*, University of California, Davis, CA, 1980, p. 215
5. J. Shi, J. Yu, J. E. Pohorly, Y. Kakuda, *J. Med. Food* **6** (2003) 291
6. J. H. Thorngate, V. L. Singleton, *Am. J. Enol. Viticult.* **45** (1994) 259
7. R. C. Silva, J. Rigaud, V. Cheynier, A. Chemina, *Phytochemistry* **30** (1991) 1259
8. C. Prieur, J. Rigaud, V. Cheynier, M. Moutounet, *Phytochemistry* **36** (1994) 781
9. H. Zou, P. Kilmartin, M. Inglis, A. Frost, *Aust. J. Grape Wine Res.* **8** (2002) 163
10. C. F.-A. E. M. Santos-Buelga, M. T. Escribano-Bailon, *Food Chem.* **53** (1995) 197
11. D. Rusjan, Z. Korosec-Korusa, *Acta Chim. Slov.* **54** (2007) 114
12. G. Spagna, R. N. Barbagallo, P. G. Pifferi, *J. Agric. Food Chem.* **48** (2000) 4619
13. L. Foo, L. Porter, *J. Sci. Food Agric.* **32** (1981) 711
14. R. Hemingway, G. McGraw, *J. Wood Chem. Technol.* **3** (1983) 421
15. I. McMurrough, D. Madigan, M. R. Smyth, *J. Agric. Food Chem.* **44** (1996) 1731
16. S. Guyot, N. Marnet, D. Laraba, P. Sanoner, J. F. Drilleau, *J. Agric. Food Chem.* **46** (1998) 1698
17. S. Muñoz, M. Mestres, O. Busto, J. Guasch, *Anal. Chim. Acta* **628** (2008) 104
18. S. Gómez-Alonsoa, E. García-Romeroa, I. Hermosín-Gutiérrez, *J. Food Comp. Anal.* **20** (2007) 618
19. P. M. Aron, J. A. Kennedy, *Mol. Nutr. Food Res.* **52** (2008) 79
20. S. E. Rasmussen, H. Frederiksen, K. S. Krogholm, L. Poulsen, *Mol. Nutr. Food Res.* **49** (2005) 159
21. X. Terra, J. Valls, X. Vitrac, J. M. Merrillon, L. Arola, A. Ardevol, C. Blade, J. Fernandez-Larrea, G. Pujadas, J. Salvado, M. Blay, *J. Agric. Food Chem.* **55** (2007) 4357
22. C. Agarwal, R. Veluri, M. Kaur, S. C. Chou, J. A. Thompson, R. Agarwal, *Carcinogenesis* **28** (2007) 1478
23. M. Stanković, V. Tešević, V. Vajs, N. Todorović, S. Milosavljević, D. Gođevac, *Planta Med.* **74** (2008) 730
24. N. Nair, S. Mahajan, R. Chawda, C. Kandaswami, T. C. Shanahan, S. A. Schwartz, *Clin. Diagn. Lab. Immunol.* **9** (2002) 470
25. I. S. Arvanitoyannis, D. Ladas, A. Mavromatis, *Int. J. Food Sci. Techol.* **41** (2006) 475
26. E. Bakkalbaşı, O. Yemiş, D. Aslanova, N. Artık, *Eur. Food Res. Technol.* **221** (2005) 792
27. T. Fuleki, J. M. R. da Silva, *J. Agric. Food Chem.* **45** (1997) 1156
28. J. A. Kennedy, M. A. Matthews, A. L. Waterhouse, *Phytochemistry* **55** (2000) 77
29. A. Nakalamić, *Jugoslovensko vinogradarstvo i vinarstvo* **4** (1991) 7 (in Serbian)
30. D. Gođevac, V. Tešević, V. Vajs, S. Milosavljević, M. Stanković, *Food Chem. Toxicol.* **47** (2009) 2853
31. E. Cantos, J. C. Espin, F. A. Tomas-Barberan, *J. Agric. Food Chem.* **50** (2002) 5691
32. R. Rodriguez Montealegre, R. Romero Peces, J. L. Chacon Vozmediano, J. Martinez Gascueña, E. Garcia Romero, *J. Food Compos. Anal.* **19** (2006) 687
33. S. U. Mertens-Talcott, J. H. Lee, S. S. Percival, S. T. Talcott, *J. Agric. Food Chem.* **54** (2006) 5336

34. J. H. Lee, J. V. Johnson, S. T. Talcott, *J. Agric. Food Chem.* **53** (2005) 6003
35. C. Cavaliere, P. Foglia, R. Gubbiotti, P. Sacchetti, R. Samperi A. Lagana, *Rapid Commun. Mass Spectrom*. **22** (2008) 3089
36. E. Obreque-Slier, A. Pena-Neira, R. Lopez-Solis, F. Zamora-Marin, J. M. Ricardo-da Silva, O. Laureano, *J. Agric. Food Chem.* **58** (2010) 3591
37. A. I. Mandić, S. M. Đilas, G. S. Ćetković, J. M. Čanadanović-Brunet, V. T. Tumbas, *Int. J. Food Prop.* **11** (2008) 713.