ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2013, *58*(6), 533-541

THE PHENOL CONTENT, ANTIOXIDANT ACTIVITY AND METAL COMPOSITION OF THE SERBIAN VINEYARD PEACH

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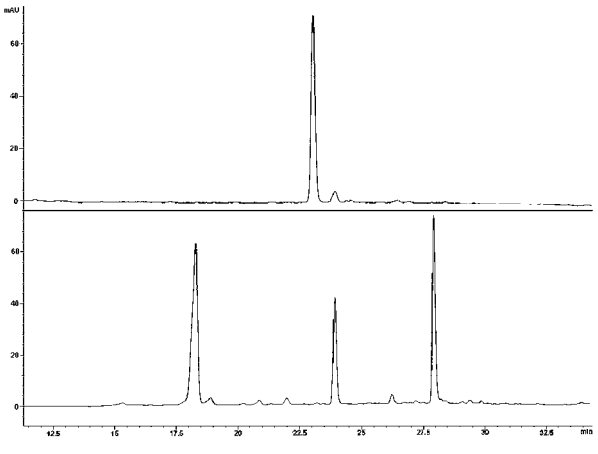
Received February 20, 2013

The present study was performed to evaluate the antioxidant capacity, the polyphenol and metal content of the vineyard peach which was cultivated in Southern Serbia. The antioxidant capacity of the vineyard peach extracts was evaluated using 2,2-diphenil-1-picrylhydrazil radical-scavenging assay. The polyphenol contents of vineyard peach samples were 89.1 to 355.0 mg GAE/100 g f.w. The high polyphenol content was significantly correlated with the high antioxidant capacity. Vineyard peaches contained only cyanidin based pignents. The predominant hydroxicinnamic acid is caffeic acid. Flavonols identified in vineyard peach samples were quercetin derivates. The metal levels were analysed by inductively coupled plasma atomic emission spectrometry method (ICP-OES). The trace levels of metals such as Na, K, Mg, Ca, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn, and Cd were determined in all analyzed vineyard peach samples. Generally, the vineyard peaches cultivated in Southern Serbia are a rich source of phenolics, which show evident antioxidant activity. Also, they are a rich source of minerals required for a human organism.



INTRODUCTION

Epidemiological studies have consistently shown that a high uptake of fruits/vegetables is associated with the reduced risk of developing chronic diseases. Among phytonuntrients responsible for this health benefit are phenolic compounds.[1] Their preventative effects against the development of degenerative diseases, such as cancer,[2] cardiovascular diseases,[3] neural degeneration,[4] diabetes and obesity[5] have been reported. Phenolic compounds are generally strong antioxidants and the primary explanation of their action is the protection of cell constituents against oxidative damage through the scavenging of free radicals, thereby averting their deleterious effects on nucleic acids, proteins and lipids in cells.[6]

There are many factors that influence the phenolic content and the antioxidants capacity of peaches. Results obtained from several studies suggest that the composition and content of phenolic compounds in peaches are influenced by the cultivar, the growing season and the growing location. The variation in total phenolics, anthocyanins, flavonoids and fruit mass among genotypes was much greater than that observed

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between growing seasons, indicating that genetics plays a more important role than the growing season in influencing phenolic content in peaches.[7]

Likewise, peaches can be a source of phenolics with important physiological functions in the human organism. The daily consumption of fruit contributes significantly in the requirements in the human organism for essential elements such as K, Ca, Mg, Cr, Co, Fe, F, I, Cu, Mn, Ni, Se and Zn.[8] However, the concentration levels of elements in plants and fruits are directly related to their interaction with all environmental, geological and biological systems.[9] The ingestion of heavy metals through food can cause accumulation in organisms, producing serious health hazards such as injury to the kidney, symptoms of chronic toxicity, renal failure and liver damage.[10]

There are many investigations regarding the physical and chemical properties of peaches, mostly their antioxidant capacity, phenol, acids, as well as anthocyanin and mineral contents.[11-14] There are no studies on the composition and physical properties of the vineyard peach growing in Serbian climatic conditions. The objective of the present study is to evaluate the content of phenolics and minerals as quality markers as well as antioxidant capacity in the mentioned vineyard peaches.

MATERIALS AND METHODS

Chemicals

Standards of gallic, ferulic, p-coumaric, and caffeic acids, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cyaniding 3-glucoside and cyaniding 3-rutinoside were purchased from Extrasynthese (Ganay, France). DPPH were purchased from Sigma-Aldrich (Steinheim, Germany). Trolox and Folin-Ciocalteu's phenol reagent were obtained from Merck (Darmstadt, Germany). Other chemicals and solvent were of analytical grade. Ultra-scientific (USA) ICP multi-element standard solutions of about 20.00 ± 0.10mg/L were used as a stock solution for calibration. De-ionized water was used for the preparation of all solutions throughout the period experiment. De-ionized water was produced with a MicroMed high purity water systems (TKA Wasseraufberei tungssystem GmbH).

Fruit samples

Five different vineyard peach varieties[15] (Table 1) from 8-year-old tree were selected. After fruit set, three trees were selected from three different vineyards, for each variety and then 50 fruits were snipped. The samples of each varieties with skins and pulps in a blender were homogenized. The samples were packed in polyethylene bags and stored at -20°C. Before analysis the fruit was thawed at room temperature.

Preparation of the methanol extracts

The polyphenolic compounds from vineyard peach samples were extracted using conventional solvent extraction procedure. Ten grams of homogenized samples were extracted in an ultrasound bath with 30 ml of methanol solution containing 0.1% HCl. The contact time was 60 min. After the extraction, the samples were filtered with Whatman No.1 filter paper and the residual tissue was washed with 2x20 ml of solvent. The filtrates were combined in the total extract. Finally, the obtained vineyard peach extracts were collected in graduated vessels of the same volume at 100 ml. The obtained extracts were used for spectrophotometric and HPLC measurements. The extraction was done in triplicate for each vineyard peach varieties.

Determination of total phenolic compounds

Folin-Ciocalteu reagent was used to determine the total phenolic compounds (TP).[16] A volume of 1 mL of vineyard peach extract, diluted 5-6 times with some solvent (to obtain absorbance within the range of the prepared calibration curve), was mixed with 0.5 mL of Folin-Ciocalteu reagent previous diluted with distilled water (1:2). A volume of 2 mL of 20% sodium carbonate solution was added to the mixture, shaken thoroughly and diluted to 10 mL by adding the distilled water. The mixture was to stand for 120 min and the blue color formed was measured at 760 nm with a spectrophotometer (UV/Vis spectrometer agilent 8454; agilent, Santa Clara, CA, USA). Gallic acid (GA) was used as a standard for the calibration curve. The concentrations of gallic acid in the solution from which the curve was prepared were 0, 50, 100, 150, 250, and 500 mg/L (R^2=0,996). The content of TP was expressed as mg of gallic acid equivalent GAE/100g of fresh weight (f.w.). All measurements were carried out in 3 repetitions.

Table 1

Morphological fruit characteristics of vineyard peach types

Sample	Types	Location	Fruit shape[1]	Skin pubescence[2]	Skin ground colour[3]	Skin blush[4]
1	I / 8	Sićevo	4	7	5	4
2	I / 1	Trgovište	3	5	4	0
3	I / 5	Brestovac	3	7	4	0
4	I / 14	Kamenica	5	7	5	4
5	II / 8	Rastovnica	3	7	5	5

[1]Fruit shape: 3-rounded, 4-ovate, 5-oval; [2] Skin pubescence: 5-intermediate, 7-high; [3]Skin ground colour: 4-cream-yellow, 5-yellow; [4]Skin blush: 0-absent, 4-red-mottled, 5-partly red.

Determination of total flavonoids

The total flavonoids (TF) assay was performed as previously described by Yang[17] with minor modifications. A volume of 1 mL of diluted extracts or standard solution of gallic acid (50-500 mg/L) was placed in a 10-mL volumetric flask, then 4 mL of dd H_2O, and after 5 min 0.3 ml of $NaNO_2$ (5%) and 1.5 mL of $AlCl_3$ (2%) were added. The mixture was shaken and 5 min later 2 ml of 1M solution of NaOH were added, again well shaken. The absorbance was measured at 510 nm against the blank. The results were calculated according to the calibration curve for gallic acid (R^2=0.998). The content of TF was expressed as mg gallic acid equivalent GAE/100g f.w. All samples were analyzed in triplicate.

Measurements of DPPH$^{\bullet}$ scavenging activity

The free radical scavenging capacity of vineyard peach extracts was determined according to the previously reported procedure using the stable (DPPH) radicals.[18] The method was based on the reduction of stable DPPH nitrogen radicals in the presence of antioxidants. An aliquot of vineyard peach extracts or methanol solution of Trolox (10-30 mM) was mixed with 2.5 mL of 0.1 mM DPPH methanolic solution. The mixture was thoroughly vortexed, kept in the dark for 30 min, and after that the absorbance was measured at 515 nm against a blank of methanol without DPPH. The results were calculated according to the calibration curve for Trolox (R^2=0.994). DPPH values, derived from triplicate analysis, were expressed as mmol of TE/100g f.w.

HPLC-DAD determination of phenolics composition

The individual phenolics were analyzed by the direct injection of the extracts (previously filtered through a 0.45 μm pore size membrane filter) into a Agilent 1200 chromatographic system equipped with a quaternary pump, and Agilent 1200 DAD with radiofrequency identification tracking technology for flow cells, a UV lamp, an 8 μL flow cell, and automatic injector and ChemStation software. The column temperature was 30^{o}C. After injecting 5 μL of sample extract, the separation was performed in the Agilent/eclipse XDBC-18 4.6x150 mm column. Two solvents were used for the gradient elution: A-(H_2O+5% HCOOH) and B-(80% ACN+5%HCOOH+H_2O). The elution program used was as follows: from 0 to 10 min 0% B, from 10 to 28 min gradually increases 0-25% B, from 28 to 30 min 25% B, from 30 to 35 min gradually increases 25-50% B, from 35 to 40 min gradually increases 50-80% B, and finally for the last 5 min gradually decreases 80-0% B. The detection wavelengths were 320 and 520 nm. The identification and quantization of the various phenolic compounds were performed by means of calibration curves obtained with standard solutions of cyanidin 3-glucoside, cyaniding 3-rutinoside, caffeic acid, p-coumaric acid, and ferulic acid. The results are expressed as mg/1000 g of f.w.

Determination of metal contents

Metal analysis were carried out on iCAP 6000 inductively coupled plasma optical emission spectrometer (Thermo Scientific, Cambridge, UK) which use an Echelle optical design and a charge injection device solid-state detector. The operating conditions are shown in Table 2. Also, Table 3 lists the emission line selected for each of the element, based upon tables of known interferences and baseline shifts and empirical observations made during the samples preparation.

Dry ashing method was carried out in a VIMS (Serbia) electric furnace equipped with microprocessor program of temperature IVIGOS3123 (±1^{o}).

Table 2

Operational parameters for ICP-OES measurements

Parameters	
Flush pump rate	100 rpm
Analysis pump rate	50 rpm
RF power	1150 W
Nebulizer gas	0.7 $Lmin^{-1}$
Coolant gas flow	12 $Lmin^{-1}$
Auxiliary gas flow	0.5 $Lmin^{-1}$
Plasma view	Dual mode

Table 3

Emission wavelength, linear working range, LOD, LOQ and correlation coefficient of the calibration for each mineral determination

Mineral	Wavelength (nm)	LOD^1/mgL^{-1}	LOQ^2/mgL^{-1}	Correlation coefficient
K	766.490	0.607	2.023	0.99990
Na	818.326	0.4047	1.3489	1.00000
Ca	393.366	0.480	1.600	0.99040
Mg	279.553	0.360	1.201	0.99894
Fe	259.940	0.0539	0.1799	0.99992
Cu	324.754	0.1326	0.4421	0.99955
Zn	202.548	0.1138	0.3794	0.99967
Mn	257.610	0.1985	0.6619	0.99900
Cr	284.325	0.0723	0.2412	0.99987
Cd	226.502	0.0826	0.2755	0.99983
Co	230.786	0.1021	0.3405	0.99973
Pb	220.353	0.2325	0.7751	0.99864
Ni	231.604	0.1138	0.3794	0.99969

[1]LOD – limits of detection, [2]LOQ – limits of quantification

Prior to ICP-OES analysis, 15 g of homogenized fruit samples was placed in porcelain vessels and heated for 12 h. The vessels with the residues obtained after the vaporization of the water and of the most organic compounds were then ashed in a furnace for 24 h. The furnace was programmed to raise temperature starting from 50°C to 450°C in the first 16 h, after which it was kept at constant 500°C until the end of the process. The residues were treated with 1 mL conc. HNO_3 and heated in a furnace again until a complete mineralization of the sample was attained (~ 10 h). The white obtained ashes were dissolved in 5% (*v/v*) HNO_3 to a total volume of 50 mL. The emission wavelengths used, the correlation coefficient for the calibration straight line, and the detection and quantification limits found for each metal in the ICP-OES determination are presented in Table 3. Results are expressed as milligrams of metal per liter of working solution.

The detection and quantification limits, given by LOD=3xSD/*m* and LOQ=10xSD/*m*, respectively, where SD is the standard deviation of reagent blank and *m* is the slope of the calibration graph.

Statistical analysis

The data were reported as mean±standard deviation (SD) with triplicate determinations. The significance of inter-group differences was determined by the analysis of variance (ANOVA). The *p* value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

It is well known that phenolic compounds exist in many fruits, which have attracted a great deal of public and scientific interest because of the health promoting effects as antioxidant. Recent studies have shown that peaches are good sources of antioxidant phenolic compounds.[7,11,13] Table 4 shows the total phenolic (TP) contents of the vineyard peach samples, which were significantly ($p<0.05$) different. The total phenolic content ranged from 89.10 to 355.9 mg GAE/100g f.w. The phenolic content of sample 1 was significantly different from the others ($p<0.05$). significant differences were found in total phenolic content

when comparing between sample 2 and sample 3, sample 4 and sample 5 ($p<0.05$); however, no significant differences in total phenolic content were found between sample 3, sample 4 and sample 5 in this study, there was a 3.98-fold difference in total phenolic content between the highest and lowest ranked samples, sample 4 and sample 1 ($p<0.05$). It is well known that the number of factors influence the concentration of the active constituents particularly phenolic compounds present in the fruits. Some of the notable factors are the time and period of collection, geographical origin and climatic conditions.

The total flavonoids (TF) of the five types vineyard peach were measured (Table 4). the sample 4 presented highest flavonoid content 9281.70 ± 3,67 mg gallic acid equivalents / 100g f.w, $p<0.05$), followed by sample 3, sample 5, sample 2 and sample 1. Significant differences were found in the total flavonoid content between all samples. An approximately 5.70-fold difference in the total flavonoid content was found between the highest and lowest ranked varieties, sample 4 and sample 1 ($p<0.05$).

The mean concentration of the phenolic content of Serbian vineyard peaches was 262.4 mg GAE/100g f.w. This is higher or similar than the levels quoted in the literature from peaches cultivars grown in different countries. The interval range for the polyphenolic content of similar extracts reported by others is 300-449 mg GAE/100 g f.w. in USA,[19] 25.62-41.03 mg GAE/100 g f.w. in Chile,[20] 48.33-80.3 mg GAE/100 g f.w. in California[21], 40.7 mg GAE/100 g f.w. in Croatia[22] and 39.3-43.8 mg GAE/100 g f.w. in Turkey.[23]

Total antioxidant activity (TAA) of the five types of the vineyard peach, expressed as mill moles (mmol) of Trolox equivalents per 100 g f.w., is shown in Table 4. The total antioxidant activity values of the five vineyard peach types varied from 1.04 to 1.15 mmol TE/100 g f.w. The data were consisted with the literature.[22,24] A significant difference was found among samples 3, 4, 5 and sample 1. The present study reveals a very good correlation between the total antioxidant activity and total phenolics (R^2=0.9729). Also, the significant correlation between the total flavonoid content, and antioxidant activity (R^2=0.9683) of the tested vineyard peach samples was confirmed.

Identification of major phenolic compounds

Five types of the vineyard peach were selected for further studies, including the identification of the major phenolic compounds. The compound were separated and tentatively identified by using reversed-phase HPLC-DAD (Table 5). The major groups of phenolic compounds detected were: hidroxycinnamic acids and anthocyanins.

The HPLC chromatogram of 3 acids, i.e., caffeic acid, p-coumaric acid, and ferulic acid, in the vineyard peach extract, recorded at 320 nm with a diode array detector, is shown in Fig. 1. The quantification of hidroxycinnamic acids in the analyzed samples is shown in Table 5 the caffeic acid contents ranged from 1.10 to 14.75 mg/kg f.w (mean 9.23 mg/kg f.w.), p-coumaric acid contents from 0.17 to 3.12 mg/kg f.w. (mean 1.78 mg/kg f.w.), and ferulic acid contents from 0.8 to 8.96 mg/kg f.w. (means 5.85 mg/kg f.w.), thus caffeic acid was predominant in all the vineyard peach types investigated, representing 54.69 % of the total hidroxycinnamic acid content, as demonstrated for a number peaches[25] while p-coumaric acid (9.70 %) was much less abundant.

Table 4

Polyphenols and antioxidant activity in selected types of vineyard peach

Sample	Types	Total phenols mg GAE/100g f.w.	Total flavonoids mg GAE/100g f.w.	Antioxidant activity mmol TE/100g f.w.
1	I / 8	89.10 ± 3.84^{c}	45.07 ± 3.82^{e}	1.04 ± 0.03^{c}
2	I / 1	197.10 ± 11.62^{b}	105.07 ± 2.58^{d}	1.09 ± 0.02^{dc}
3	I / 5	339.40 ± 19.18^{a}	260.80 ± 10.03^{b}	1.15 ± 0.01^{a}
4	I / 14	355.00 ± 21.12^{a}	281.70 ± 3.67^{a}	1.14 ± 0.01^{ad}
5	II / 8	331.20 ± 18.53^{d}	205.97 ± 4.81^{c}	1.12 ± 0.01^{abd}

Table 5

Hydroxycinnamic acids and anthocyanins (mg/kg f.w.) of vineyard peach samples

Compound	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
		Hydroxycinnamic acids			
caffeic acid	1.10 ± 0.05	5.77 ± 0.07	12.28 ± 0.09	14.75 ± 0.12	12.23 ± 0.11
p-coumaric acid	0.17 ± 0.02	0.73 ± 0.05	2.08 ± 0.04	3.12 ± 0.07	2.80 ± 0.05
ferulic acid	0.83 ± 0.02	3.29 ± 0.07	7.67 ± 0.08	8.96 ± 0.01	8.52 ± 0.15
Total	2.10	9.79	22.02	26.83	23.55
			Anthocyanins		
cyanidin-3-glucoside	1.42 ± 0.04	ND	ND	3.89 ± 0.05	0.79 ± 0.04
cyanidin-3-rutinoside	0.12 ± 0.01	ND	ND	0.32 ± 0.01	0.10 ± 0.01
Total	1.54	/	/	4.21	0.89
			Flavonols		
quercetin derivative 1	3.88 ± 0.11	8.29 ± 0.23	4.88 ± 0.16	2.01 ± 0.09	2.80 ± 0.10
quercetin derivative 2	3.06 ± 0.15	6.38 ± 0.20	3.65 ± 0.12	1.92 ± 0.08	2.01 ± 0.06
Total	6.94	14.67	8.53	3.93	4.81

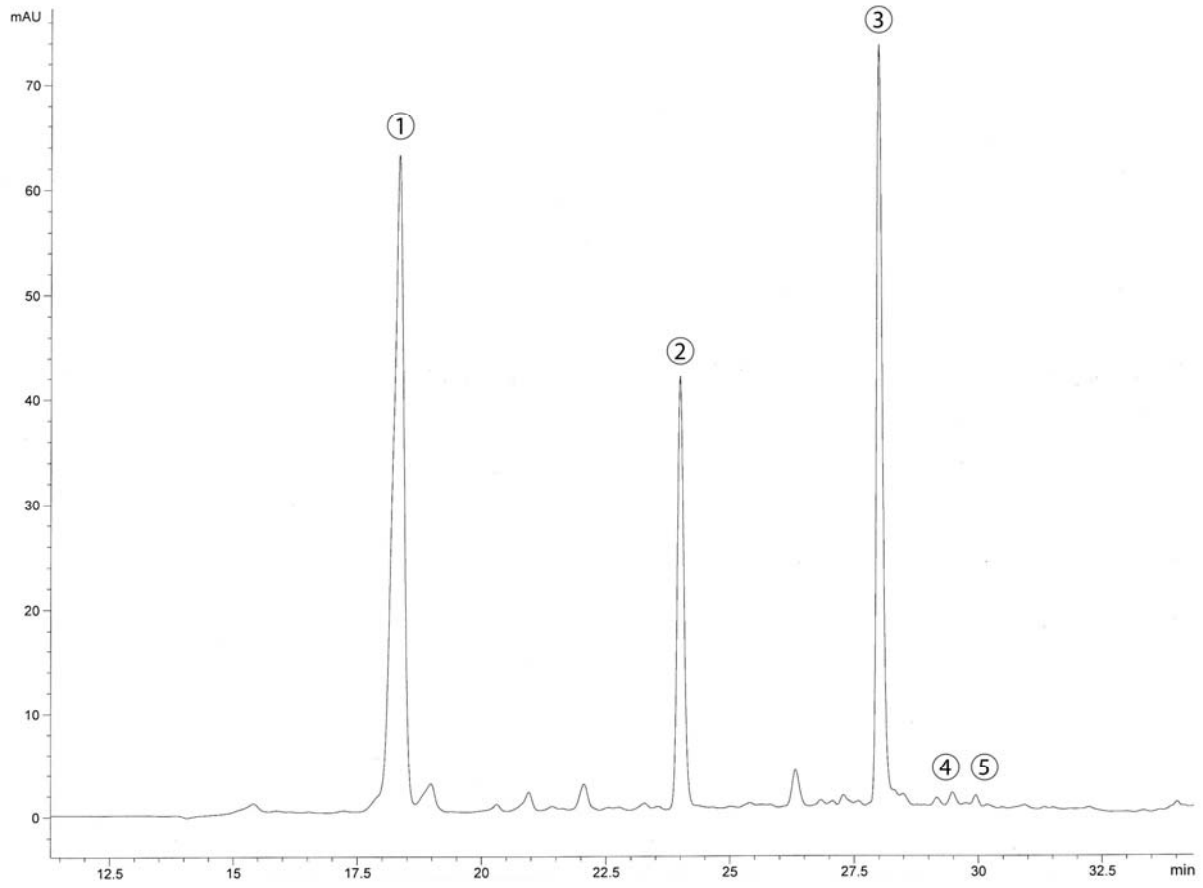

Fig. 1 – HPLC chromatogram of vineyard peach extract detected at 320 nm. Compounds: (1) caffeic acid, (2) p-coumaric acid, (3) ferrulic acid, (4) quercetin derivative 1, (5) quercetin derivative 2.

The HPLC chromatogram of two anthocyans in the vineyard peach methanol extract, reported at 520 nm with a diode array detector, is shown in Fig 2. The vineyard peaches contained only cyanidin based pigments: cyanidin-3-glucoside (2.03 mg/kg f.w, 91,12%) and cyanidin-3-rutinoside (0.18 mg/kg f.w, 8.88 %), which is consistent with our results.[12] Previous studies have shown the occurrence of quercetin 3-glucoside, 3-galactoside and 3-rutinoside in peaches, as well as

some kaempferol derivates.[26] The study of these vineyard peach samples has confirmed the occurrence of the quercetin derivates, although the kaempferol analogues were not detected. These results are in accordance with those reported by Tomas-Barberan.[12]

Metal content in vineyard peach

The IPC-DES was employed to determine 13 elements (Na, K, Mg, Ca, Cr, Cu, Fe, Mn, Ni, Zn and Cd) in the vineyard peaches. The best are chosen, and at the same time, the working wavelengths on the bases of the next criteria: relative intensity of signal as a function of sensitivity of methods; relative error and relative standard deviation of standard solution signal; and interfering effect of other elements also present in the real samples.[27] The results of the five vineyard peach types are summarized in Tables 6 and 7. Two groups of elements were established for all the data obtained. The major mineral concentrations in the different vineyard peach types are given in Table 6. The major mineral concentrations of the vineyard peaches were established as sodium, potassium, calcium and magnesium, and potassium concentrations were found to be of higher levels. The vineyard peach types I/14 and I/8 are rich in calcium. The potassium and magnesium concentrations of vineyard peach types I/14 were found to be the highest compared with others. Sodium concentration ranged from 25.960 mg/kg (sample 4) to 38.440 mg/kg (sample 1).

The minor and heavy metal concentrations of the vineyard peach as listen in Table 8 are expressed in micrograms per 100 grams f.w. the mineral concentrations of samples were found to be different depending on the several types of the copper and manganese vineyard peach. Among the minor metals determined, iron concentrations were found to be highest. The highest minor and heavy metal levels of the vineyard peach samples were measured with 517.0-997.0 μg/100g f.w. Fe, 82.65-119.15 μg/100 g f.w Cu and 48.90-105.17 μg/100 g f.w. Vineyard peach types I/1 and the highest lead concentration with 10.40 μg/100 g f.w. and zinc with 97.00 μg/100 g f.w. Vineyard peach types I/8 had the highest nickel concentration with 23.85 μg/100 g f.w. These data are in accordance with those found in literature.[27-29] Cadmium was not detected in vineyard peach type I/5.

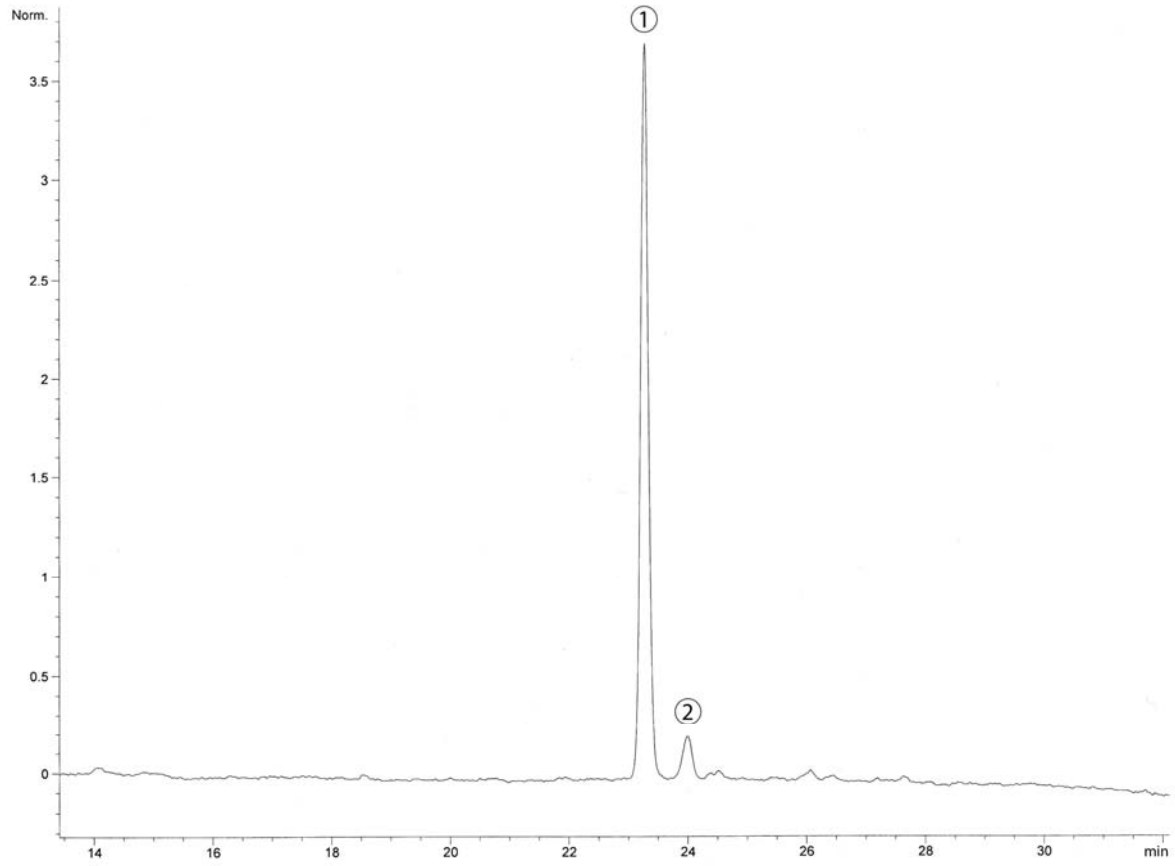

Fig. 2 – HPLC chromatogram of vineyard peach extract detected at 520 nm.
Compounds: (1) cyanidin-3-glucoside, (2) cyanidin-3-rutinoside.

Table 6

Mayor mineral concentrations (mg/100g f.w.)

Sample	K	Na	Ca	Mg
1	87.5 ± 1.35	3.84 ± 0.23	17.84 ± 0.42	10.86 ± 0.30
2	155.1 ± 1.89	3.09 ± 0.48	13.92 ± 0.38	14.29 ± 0.15
3	189.0 ± 2.05	2.75 ± 0.22	12.99 ± 0.52	13.92 ± 0.19
4	257.2 ± 3.22	2.59 ± 0.17	20.08 ± 0.48	15.70 ± 0.28
5	158.4 ± 1.78	2.85 ± 0.15	16.43 ± 0.28	12.97 ± 0.22

Table 7

The minor and heavy metal concentrations (μg/100 g f.w.)

Sample	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Cd
1	0.45±0.01	3.55±0.18	82.65±2.13	517.0±4.32	48.90±1.13	23.85±0.92	2.00±0.10	52.05±1.02	0.10±0.02
2	0.70±0.03	6.45±0.17	82.85±2.08	997.0±5.28	89.90±2.05	9.75±0.78	10.40±0.22	97.00±1.17	0.10±0.01
3	0.50±0.03	3.30±0.22	88.20±1.67	687.0±3.05	63.65±1.24	8.85±0.32	4.75±0.13	67.60±0.98	/
4	0.60±0.02	2.75±0.13	75.20±1.35	636.5±2.98	105.15±2.68	16.35±0.65	8.09±0.58	44.45±0.73	0.15±0.03
5	0.30±0.01	6.10±0.28	119.15±0.92	566.0±3.52	58.25±1.27	5.25±0.19	2.45±0.17	47.85±0.68	0.05±0.02

The differences in minor and heavy metals and major minerals of vineyard peach samples may be clue to growth conditions, genetic factors, soil properties, geographical variations and analytical procedures. Metals are present in foods either naturally or as a result of human activities such as agricultural practices, industrial emissions car exhausts or contamination during manufacture. The results of heavy metal concentrations were compared with the values recommended by the World Health Organization (WHO; World Health Organization: Evaluation of certain food additives and contaminants, Technical report series et al. 1993) and EU Directives (Commission of the European Communities; Commission Regulation (EC) No.221/2002 for the 6 February 2002) for fruits. Based on the WHO health criteria and EU Directive, there are no health risks with respect to concentrations of lead, nickel, copper, cadmium, and chromium in fruits analyzed in this study.

CONCLUSIONS

This study shows that although all the analyzed vineyard peaches contained phenolic compounds and flavonoids, their contents are markedly different among the vineyard peach types. All vineyard peach types represent a potential source of natural antioxidants, but type II/14 showed a better performance. Also, vineyard peaches have high content of K, Ca and Mg, but low in Na, as well as other essential minerals Mg, but low in Na, as well as other essential minerals (Fe, Mn, Ca) in low concentrations and the levels of the toxic elements are negligible. The data obtained indicate that vineyard peaches are rich in various essential elements and might be considered as an important dietary mineral supplementation for individuals deficient in nutritional elements.

Acknowledgements: The research was financed by the Ministry of Education and Sciences, the Republic of Serbia, projects No. 31060 and 172047.

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