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Phenolic and mineral profile of Balkan indigenous apple and pear cultivars

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Abstract: The aim of this study was the determination of phenolic compounds, and mineral nutrients in the pulp and peel obtained from Balkan indigenous apple and pear cultivars. The phenolic composition, assessed by HPLC-DAD and spectrophotometric methods varied significantly between the cultivars for both peel and pulp. Among the apples, the Mekica cultivar had the highest total phenolic content while Šećerlija and Zlatna Parmenka had the lowest. In the case of the pear samples, the maximum total phenolic content was found in Crna Takiša while the minimum content was recorded for Bela Arapka. In most of the investigated samples, chlorogenic acid in apples and arbutin in pears were the major detected polyphenolic compounds. With regard to the mineral analysis, K was the most abundant followed by P, Mg, Ca and S. Iron was the dominant microelement in apple peel and pulp samples, while in pear samples the principal microelement was B. The obtained results provide detailed information on the chemical composition of the tested apple and pear cultivars and thereby, could encourage their wider cultivation and consumption.

Keywords: cultivar, autochthonous, apple, pear, peel.

INTRODUCTION

Due to excessive and widespread agricultural modernization, many autochthonous cultivars have been neglected and substituted with more productive and new international cultivars.[1] Accordingly, the global diet currently relies on a reduced number of species and varieties. It is becoming homogenized and separated from Serbian cultural food traditions. This not only endangers the preservation of biodiversity, but could also "open the door" to overall epidemics of certain pests and pathogens.[2]

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On the contrary, indigenous cultivars represent the local germplasm cultivated mainly in the marginal areas. Generally, they are resilient to the local environment and represent a favorable source for crop genetic variability, resistance to biotic and abiotic stresses, as well as for phenological and quality characteristics. Since traditional cultivars mostly do not meet transport and storage demands, they are not cultivated for large-scale production.[3] Therefore, although indigenous cultivars could be more nutritious than newer ones, they are economically less important.[4]

Owing to its geographical location, the Republic of Serbia has advantageous natural conditions and areas for planting a large number of fruit species and cultivars.[5] Indigenous *Malus* and *Pyrus* varieties are found in Serbia mainly on individual farms in hilly–mountainous regions. Due to the ageing of orchards and the abandonment of agricultural practices, there is a great possibility that certain genotypes will disappear in the future.[6]

Increased consumption of fruits has been recommended as one of the essential components of a healthy diet for the prevention of chronic diseases.[7] There are numerous reports on health-promoting benefits of phenolic compounds present in apples and pears,[8,9] and as a result of a recent study,[10] there is a new claim "an apple or pear a day helps keep the stroke at bay".

Studies dealing with the phenolic composition of apples and pears are often restricted to a few cultivars[3,11] that are very popular with customers. Little attention has been given to autochthonous apple and pear cultivars,[12,13] while there are only few reports,[14] on the chemical profile of those grown in Serbia. Besides phytochemicals, an important part of the nutritional information of fruits is their mineral content. The significant impact of metal ions on human health is demonstrated by the fact that the function of more than one-third of all human proteins depend on them.[15] As with polyphenols, the cultivar significantly influences the mineral content of the fruits.[16]

The aim of this study was the chemical analysis of phenolic compounds mineral nutrients in the pulp and peel obtained from Serbian indigenous apple and pear cultivars. This work is important for an understanding of their nutritional potential and for a consequential expansion of their cultivation and use.

EXPERIMENTAL

Chemicals

Chlorogenic acid (95 %, titration), rutin hydrate (94 %, HPLC), hyperoside (97 %, HPLC), isoquercitrin (90 %, HPLC), phloridzin (99 %, HPLC), gallic acid (97.5 %, titration), arbutin (96 %, HPLC) and 4-(dimethylamino)cinnamaldehyde (*p*-DMACA, ≥98.0%, HPLC) were purchased from Sigma Aldrich (St. Louis, MO, USA), and quercitrin (98.5 %, HPLC) from Extrasynthese (Genay, France). Acetonitrile (99.8 %) and formic acid (98–100 %) were supplied by Sigma–Aldrich (Steinheim, Germany). Water was deionized by using a Milli-Q system (Millipore, Bedford, MA, USA). All other reagents were of analytical grade.

Plant material

The apple and pear cultivars investigated in this study were harvested in two regions in Serbia: Ljig in central Serbia (apples Kolačara, Streknja, Šećerlija, Zlatna Parmenka and Senabija) and on Zlatibor Mountain in the western part of Serbia (apples Masnjača, Mekica, Kožara, Kraljica and Šarenka, as well as pear cultivars Bela Arapka, Bronzara, Bela Takiša, Crna Takiša). Samples were collected at the full maturity stage during 2014. Ten apples from each cultivar were picked randomly, placed in polyethylene bags and transported to the Institute for Medicinal Plant Research. In order to obtain uniform sample, damaged fruits were removed. After rinsing with water, the seeds and stems were removed, and the peel was mechanically separated from the pulp. Peel and pulp were blended in a Philips microblender HR2860 to obtain a paste that was further lyophilized. The obtained lyophilized samples (one per each cultivar) were stored at 4 °C until further analysis.

Extraction procedure

Ultrasound-assisted extraction was carried out at room temperature. Two grams of lyophilized pulp and peel samples were extracted with 25 mL of 70 % methanol for 30 min in a Bandelon Sonorex RK52 ultrasonic bath (Bandelon Electronic, Munich, Germany) operating at room temperature. After filtration, the obtained extracts were used for the determination of the total phenolic content, the total proanthocyanidins content and HPLC analysis.

Total phenolic content

Determination of total phenolic content (*TPC*) was conducted according to a previously described method.[17] An aliquot of sample (20 μL) was mixed with 1580 μL of distilled water, 100 μL of Folin–Ciocalteu reagent and 300 μL of 20 % Na_2CO_3, followed by incubation for 2 h at room temperature. After incubation, the absorbance was recorded at 765 nm. The contents of total phenolics were calculated using a standard curve for gallic acid and are expressed as milligrams of gallic acid equivalents per 1 g of dry sample (mg GAE g^{-1} dry weight (dw)).

Total proanthocyanidin content

The content of total proanthocyanidin compounds (*TPR*) in the samples was determined spectrophotometrically using *p*-DMACA method with slight modifications.[18] The investigated extracts were mixed with 80 μL of *p*-DMACA reagent (2 mL), methanol (25 mL) and a drop of glycerol. After 7 min, the absorbance at 640 nm was measured. The contents of proanthocyanidins in the samples are expressed as milligrams of catechin equivalents per 1 g of dry sample (mg CE g^{-1} dw). The p-DMACA reagent was prepared immediately before use, and contained 1 % (*w*/*V*) *p*-DMACA in a cold mixture of methanol and HCl (4:1).

HPLC analysis

Phenolic compounds in the tested extracts were determined by comparing the retention times and absorption spectra (200–400 nm) of unknown peaks with those of pure standards injected under the same conditions. The standards used were chlorogenic acid, phloretin 2′-*O*--glucoside (phloridzin), quercetin 3-*O*-galactoside (hyperoside), quercetin 3-*O*-rutinoside (rutin), quercetin 3-*O*-rhamnoside (quercitrin) and quercetin 3-*O*-glucoside (isoquercetine). The HPLC-DAD analysis was performed on an Agilent 1200 Series HPLC (Agilent Technologies, Palo Alto, CA, USA) equipped with Lichrospher® 100 RP 18e column (5 μm, 250 mm×4 mm). Mobile phase A was formic acid in water (1 %) and mobile phase B was acetonitrile. The injection volume was 30 μL, and the flow rate 1 mL min^{-1} with a gradient program as follows: 5–15 % B 0–5 min, 15–20 % B 5–8 min, 20 % B 8–12 min, 20–30 % B 12–15 min, 30 % B 15–17 min, 30–35 % B 17–20 min, 35 % B 20–22 min, 35–100 % B 22–

–25 min. The stop time of the analysis was 25 min and column temperature was 25 °C. For the quantitative analysis of phenolic compounds, a calibration curve was obtained by injection of known concentrations (5–400 μg mL^{-1}) of different standard compounds: arbutin (y = = 11560.8x – 0.5, R^2 = 1), chlorogenic acid (y = 83506.1x–58.1, R^2 = 0.998), quercetin 3-*O*- -rutinoside (y = 26573.7x + 70.4, R^2 = 0.999), quercetin 3-*O*-galactoside (y = 64124.3x + + 172.7, R^2 = 0.997), quercetin 3-*O*-glucoside (y = 69710.5x+15.7, R^2 = 0.999), quercetin 3-*O*-rhamnoside (y = 57551.6x + 46.7, R^2 = 0.999) and phloretin 2′-*O*-glucoside (y = 58862.7x + 51.9, R^2 = 0.999). Quantification was performed based on DAD results, using 280 nm for chlorogenic acid and phloretin 2′-*O*-glucoside and 350 nm for the flavonoid compounds. The investigated samples were analyzed in triplicate. The acceptable level of method precision was shown by the percentage relative standard deviation (*RSD*%) lower than 5 %.

Determination of the mineral elements

The contents of mineral elements were determined as described previously by Pavlović *et al.*[19] Lyophilized peel and pulp samples were subjected to microwave digestion using an Ethos 1 microwave system (Advanced microwave digestion system, Milestone, Italy). One gram of apple or pear sample, 1.0 mL of 30 % H_2O_2, and 7.0 mL of concentrated ultrapure HNO_3 (69.0–70.0 %)were mixed and transferred into the microwave digestion vessel. After the effervescence had subsided, the sample was cooled for 5 min, transferred into a clean volumetric flask, and diluted to 25 mL with ultrapure H_2O. A blank was prepared in the same way. All analyses were performed in triplicate on a Thermo Scientific iCAP 6500 Duo ICP (Thermo Fisher Scientific, Cambridge, UK).

Statistical analysis

The phenolic composition analyses were performed in triplicate and the data are presented as mean ± standard deviation. Differences between the group means and their significance were verified using one-way ANOVA. Statistical significance was set at $p < 0.05$. On the other hand, the data for the elemental analysis were obtained from one measurement.

RESULTS AND DISCUSSION

Phenolic composition

The phenolic concentration is commonly linked with nutritional and sensory attributes of fruits.[20] Apples and pears are one of the rare food types with precise data about their phenolic composition. The polyphenolic profiles of all apple cultivars are practically the same but concentrations may range from 0.1 to 5 g of total polyphenols per kg fresh weight and may be as high as 10 g kg^{-1} in certain varieties of cider apples.[21] In the case of pears, the situation is similar, there are reports for *TPC* ranging from 3.02 to 4.58 g per kg fresh weight.[22]

In the present investigation, the content of total phenolics (*TPC*) and total proanthocyanidins (*TPR*) showed significant variation among the tested cultivars (Tables I and II). The *TPC* in apple and pear peel samples determined by the Folin–Ciocalteu assay ranged from 2.82 to 12.24 mg GAE g^{-1} dw and from 3.81 to 8.11 mg GAE g^{-1} dw, respectively. In the case of apple and pear pulp, TPC varied between 1.55 to 5.95 mg GAE g^{-1} dw and 1.38 to 3.50 mg GAE g^{-1} dw, respectively. Among the apples, the Mekica cultivar had the highest *TPC* while Šećerlija and Zlatna Parmenka had the lowest. In the case of pear samples the max-

TABLE I. Total phenolic content, total proanthocyanidin content and content of individual phenolic compounds in investigated apple samples ($n = 3$); Data are presented as means±SD, * – sample from Ljig, ** – sample from Zlatibor, nd – not detected, tr – traces

Sample	TPC, mg GAE/g dw	TPR, mg catechin/g dw	Chlorogenic acid µg/g dw	Phloretin 2'-*O*-glucoside µg/g dw	Quercetin 3-*O*-galactoside µg/g dw	Quercetin 3-*O*-rutinoside µg/g dw	Quercetin 3-*O*-rhamnoside µg/g dw	Quercetin 3-*O*-glucoside µg/g dw
Kolačara* peel	4.47±0.06	1.67±0.01	336.13±3.28	191.72±3.82	173.11±2.87	24.15±1.14	84.15±1.78	205.36±1.57
Kolačara* pulp	2.75±0.21	1.34±0.01	441.97±5.16	55.18±1.69	nd	nd	tr	nd
Streknja* peel	7.36±0.05	4.52±0.04	601.62±6.45	374.53±5.14	292.88±3.15	tr	321.35±3.58	316.27±2.96
Streknja* pulp	3.00±0.04	1.44±0.07	339.18±4.43	26.05±0.38	nd	nd	tr	tr
Šećerlija* peel	2.82±0.05	2.01±0.09	96.79±1.87	18.40±0.72	tr	tr	103.90±2.01	tr
Šećerlija* pulp	3.49±0.11	1.58±0.04	652.14±5.16	25.61±0.77	nd	nd	tr	tr
Zlatna Parmenka* peel	4.98±0.07	0.71±0.02	470.23±4.81	345.23±3.78	435.23±5.17	20.38±0.45	377.10±3.98	23.26±0.87
Zlatna Parmenka* pulp	1.55±0.03	0.77±0.01	336.50±4.57	21.79±0.98	tr	nd	tr	0.58±0.01
Senabija* peel	4.07±0.05	2.33±0.03	93.68±1.72	60.13±1.54	tr	tr	tr	5.04±0.04
Senabija* pulp	3.21±0.01	1.31±0.07	419.78±3.85	36.23±1.24	tr	nd	tr	tr
Masnjača** peel	6.24±0.02	3.13±0.10	147.68±2.12	365.68±2.99	230.11±2.15	11.58±0.34	858.15±8.74	146.65±1.12
Masnjača** pulp	1.81±0.01	1.00±0.03	148.42±2.87	26.24±1.11	nd	nd	94.08±0.95	nd
Mekica** peel	12.24±0.07	4.84±0.08	371.56±3.15	1310.35±14.87	1016.23±9.57	504.20±4.32	2129.47±18.65	323.98±2.87
Mekica** pulp	5.95±0.03	2.08±0.06	1530.99±14.78	310.65±3.52	tr	nd	99.56±1.13	tr
Kožara** peel	7.17±0.09	2.79±0.06	688.69±5.84	1008.38±11.34	251.00±2.78	tr	544.77±6.23	13.57±0.01
Kožara** pulp	3.26±0.01	1.76±0.01	666.73±6.14	150.69±1.24	tr	nd	92.62±0.95	tr
Kraljica** peel	9.59±0.05	3.67±0.16	565.72±5.13	461.07±4.11	966.87±8.74	148.14±1.21	1376.33±12.97	180.39±1.25

TABLE I. Continued

Sample	TPC, mg GAE/g dw	TPR, mg catechin/g dw	Chlorogenic acid µg/g dw	Phloretin 2'-*O*-glucoside µg/g dw	Quercetin 3-*O*-galactoside µg/g dw	Quercetin 3-*O*-rutinoside µg/g dw	Quercetin 3-*O*-rhamnoside µg/g dw	Quercetin 3-*O*-glucoside µg/g dw
Kraljica** pulp	3.96±0.03	2.24±0.09	1034.43±11.56	98.31±1.24	nd	nd	tr	tr
Šarenka** peel	11.42±0.07	7.62±0.13	198.49±2.22	1147.22±10.28	589.78±4.73	207.03±2.03	1541.15±16.14	344.51±3.21
Šarenka** pulp	5.03±0.02	3.84±0.04	693.06±5.78	247.62±2.16	nd	nd	109.58±0.99	3.25±0.02
ANOVA								
Cultivar	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Fruit part	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

TABLE II. Total phenolic content, total proanthocyanidin content and content of individual phenolic compounds in investigated pear samples ($n = 3$); data are presented as means±SD , ** – sample from Zlatibor, nd – not detected, tr – traces

Sample	TPC, mg GAE/g dw	TPR, mg catechin/g dw	Chlorogenic acid µg/g dw	Arbutin µg/g dw	Quercetin 3-*O*-galactoside µg/g dw	Quercetin 3-*O*-rutinoside µg/g dw	Quercetin 3-*O*-rhamnoside µg/g dw	Quercetin 3-*O*-glucoside µg/g dw
Bela Takiša** peel	5.95±0.08	3.82±0.08	505.82±5.21	657.63±6.14	186.03±1.52	36.71±0.20	187.65±1.64	172.71±1.23
Bela Takiša** pulp	2.89±0.01	1.08±0.02	395.65±3.14	455.21±3.84	nd	nd	nd	nd
Bela Arapka** peel	8.11±0.04	5.17±0.07	1716.77±18.21	716.9±6.89	81.80±0.56	108.58±1.05	847.29±8.25	225.61±2.25
Bela Arapka** pulp	3.50±0.26	1.44±0.03	405.49±3.98	638.06±6.15	tr	nd	tr	nd
Bronzara** peel	5.77±0.06	2.95±0.03	1078.75±10.12	554.66±5.24	tr	69.56±0.52	146.06±1.21	48.77±0.45
Bronzara** pulp	1.94±0.01	0.88±0.03	286.30±2.14	308.93±3.11	nd	nd	nd	nd
Crna Takiša** peel	3.81±0.06	0.40±0.02	416.27±4.28	591.73±4.97	31.69±0.21	77.66±0.78	109.72±1.02	89.84±0.78
Crna Takiša** pulp	1.38±0.15	0.11±0.00	248.40±2.15	303.48±3.02	nd	nd	nd	nd
ANOVA								
Cultivar	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Fruit part	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

imum *TPC* was found for Bela Arapka while the minimum *TPC* was recorded for Crna Takiša. The present findings are consistent with those of other researchers. For example Huber and Rupasinghe,[23] reported that *TPC* for Red Delicious, Empire and Royal Gala peel extracts were 3.80, 4.82 and 4.84 mg g^{-1} dw, respectively. Regarding commercial apple cultivars, Alarcon-Flores *et al*.[24] showed that the Pink Lady variety had the highest concentration of phenolic compounds (4.11 mg g^{-1} dw) followed by Ambrosia (4.01 mg g^{-1} dw). For peel samples of ten Chinese pear cultivars, Li *et al*.[25] showed variations in the *TPC* from 2.64 to 11.21 mg GAE g^{-1} dw. The results from the present study were also comparable with the results obtained for three Pakistan pear varieties, Nakh, Nashpati and Bartlett, for which the TPC ranged from 3.81 to 8.11 mg GAE g^{-1} dw in peel and from 3.34 to 3.56 mg GAE g^{-1} dw in pulp.[26] In the same investigation, the TPC was approximately 6–20 times lower in pulp samples compared to peel. In the present study, the TPC was generally 2–3 fold higher in peel compared to pulp samples, in both apples and pears.

As important bioactive constituents, the *TPR* content was also determined, and found to range from 0.71 to 7.62 mg catechins g^{-1} dw and from 0.77 to 3.84 mg catechins g^{-1} dw in apples peel and pulp, respectively, and from 0.40 to 5.17 mg catechins g^{-1} dw and from 0.11 to 1.44 mg catechins g^{-1} dw in pear peel and pulp, respectively, (Tables I and II). It could be observed that the *TPR* content was generally lower in pear peel and pulp samples than in the investigated apple samples. In addition, in comparison with the pulp samples, the *TPR* content was higher in the peel samples. Although the obtained values for the *TPR* content were high, it was difficult to compare the results with existing literature data since different methodologies for quantification were used. For example, the TPR content in the cultivar Champion determined by HPLC analysis was 1.65 mg g^{-1} dw.[13]

Chemical analysis of the individual phenolic compounds in the investigated pear and apple samples was conducted using the HPLC-DAD technique and the results are presented in Table I. Based on retention time and spectrum information, seven phenolic compounds were identified, *i.e*., one phenolic acid (chlorogenic acid), one phenolic glucoside (arbutin), four flavonols (quercetin 3-*O*-rutinoside, quercetin 3-*O*-galactoside, quercetin 3-*O*-rhamnoside and quercetin 3-*O*-glucoside) and one dihydrochalcone (phloretin 2′-*O*-glucoside). For each individual phenolic compound, ANOVA evaluated significant differences in dependence on the variety considered. Among the identified phenolic compounds, in peel and pulp samples obtained from apples collected in Ljig, chlorogenic acid was the dominant compound (ranging from 93.68 to 601.62 μg g^{-1} dw in peel and from 336.50 to 652.14 μg g^{-1} dw in pulp). The situation was similar with pulp samples of apples collected from Zlatibor mountain (148.42 to 1530.99 μg g^{-1} dw) while for the peel samples (with the exception of the cultivar Kraljica) the situation was slightly different. Namely, phloretin 2′-*O*-glucoside (in

Kožara peel) or quercetin 3-*O*-rhamnoside (Masnjača, Mekica, Kraljica and Šarenka peel) were prevalent compounds followed by chlorogenic acid (from 147.68 to 688.69 μg g^{-1} dw). In general, the content of chlorogenic acid was significantly higher in apple pulp compared to apple peel. The exceptions were cultivars Zlatna Parmenka and Streknja, in which the content was approximately 1.5 fold higher in peel, and Masnjača, in which, chlorogenic acid was equally present in peel and pulp. For some cultivars (Streknja, Zlatna Parmenka, Kožara and Kraljica), the content of chlorogenic acid was quite high compared with the results obtained by other authors for some commercial cultivars. Awad and de Jager[27] reported values between 160 and 330 μg g^{-1} dw in peel of Jonagold cultivar and Lata *et al.*[11] values 260 and 280 μg g^{-1} dw for peel samples of the cultivars Elstar and Granny Smith, respectively. In addition, the obtained values for Streknja, Kožara, Šećerlija and Kraljica peel are much higher than those reported in previous studies for peel samples of Fuji, Royal and Pink Lady when the content of chlorogenic acid ranged from 280 to 430 μg g^{-1} dw.[24] Among apple phenolics, chlorogenic acid is one of the most important substrates for polyphenol oxidase (PPO); its oxidation generates pigments that can co-oxidize other substances.[28] Therefore, apple cultivars with a low concentration of chlorogenic acid would be more appropriate for producing apple juice, to minimize enzymatic browning, and to control the stability of the final product. For this reason, it could be stated that the cultivars Mekica and Kraljica are less suitable for producing apple juice.

Arbutin and chlorogenic acid were detected as the major phenolic compounds in case of pear peel and pulp samples. Such results were consistent with previous data.[29] In peel and pulp samples of Bela and Crna Takiša, arbutin was the main phenolic compound, as well as in pulp samples obtained from the cultivars Bela arapka and Bronzara, while chlorogenic acid prevailed in their peel samples. In a study conducted by Li *et al.*,[25] arbutin was the dominant compound in 11 Chinese cultivars in peel (ranging from 323.3 to 6982.0 μg g^{-1} dw) and in pulp (ranging from 92.8 to 2077.0 μg g^{-1} dw).

Quercetin glycosides were the only flavonols found in analyzed apple and pear samples. In some cultivars, they were the most abundant phenolic group mainly identified in the peel of the investigated apple and pear cultivars, while they were present only in traces in the pulp. Significant differences in the contents of these compounds were found among the tested cultivars. The exception was quercetin 3-*O*-rhamnoside with a content of around 100 μg g^{-1} dw in cultivars Masnjača, Mekica, Kožara and Šarenka. Among the apple peel samples, the highest contents of individual flavonols were detected in Mekica, while in the case of the pear samples, Bela arapka was the cultivar with the highest content. Senabija among apples and Bronzara and Crna Takiša among pears had the lowest levels of these compounds. In most of the pear and apple samples, quercetin

3-*O*-rhamnoside was the dominant flavonol, the exception being the apple cultivar Kolačara, in which quercetin 3-*O*-glucoside was dominant. The quercetin 3-*O*-rhamnoside contents in apple peel samples of the studied cultivars (from traces to 2129 μg g^{-1} dw) were comparable with previously obtained results for cultivars Jonagold (2660 μg g^{-1} dw) and Elstar (580 μg g^{-1} dw).[8] On the other hand, the levels of quercetin 3-*O*-glucoside (5 to 344 μg g^{-1} dw) were lower compared with those of the commercial cultivars Jonagold and Elstar for which values of 530 and 700 μg g^{-1} dw were found.[8] In the case of the apple pulp samples, the contents of quercetin 3-*O*-rhamnoside in Masnjača, Mekica, Kožara and Šarenka pulp (≈100 μg g^{-1} dw) were significantly higher compared to the commercial cultivars Royal, Fuji, Golden and Pink Lady that contained from 2.7 to 28.6 μg g^{-1} dw.[24] In terms of the content of flavonols in pears, the peel of Bela Arapka was the richest one, followed by the peel sample of Bela Takiša.

Macro and microelement analysis

Besides phytochemicals, an important part of nutritional information is the concentration of essential elements. Minerals play a key role in different physiological functions of the body, especially in regulation processes. Numerous factors, such as soil type and conditions as well as the variety, may cause variations in mineral contents of fruits. In this study, the content of most of the investigated elements was significantly different depending on the species, cultivar, and part of the fruit examined (Tables III and IV). Generally, peel samples showed a higher mineral content compared to pulp. These results are in accordance with previous results.[30,31] The most common mineral in the tested apple and pear samples was potassium with contents ranging from 4.12 to 8.86 and from 6.27 to 8.36 mg g^{-1} dw in apple and pear peel, respectively. In pulp, the potassium content was lower varying from 3.86 to 7.49 and from 4.59 to 6.59 in apple and pear samples, respectively. The concentration of K was the highest in the cultivar Šećerlija among the apples and regarding the pears, the cultivar Bela Takiša was the richest in this element. Remarkable contents were also observed for P, Mg, Ca and S. These data are consistent with the results obtained by other authors.[31,32] The highest content of P and S in peel and pulp was obtained for the cultivar Šećerlija, while the concentration of Ca was highest in Zlatna Parmenka. The present results for macroelements were in agreement with those reported by Manzoor *et al.*[31] for cultivars Golden Delicious and Red Delicious. On the other hand, the contents of Mg and Ca determined in the present investigation were lower than those reported by Ekholm *et al.*[16] for apple peel (0.7 and 0.5 mg g^{-1} dw, respectively) and pulp (0.5 and 0.4 mg g^{-1} dw, respectively). Considering the pear samples, the highest contents of Ca, Mg and P were recorded in Crna Takiša cultivar, while Bela Arapka was the one richest in P. The contents of Ca in the

pear peel samples were remarkably higher compared to those found in the apple peel samples.

TABLE III. The concentration of macroelements (expressed as mg g^{-1} dw) in the apple and pear samples (n = 1); * samples from Ljig, ** samples from Zlatibor

Sample	Mineral				
	Ca	K	Mg	P	S
Apple cultivar					
Kolačara* peel	0.28	7.42	0.39	0.68	0.29
Kolačara* pulp	0.17	6.09	0.24	0.44	0.17
Streknja* peel	0.29	7.32	0.46	0.82	0.33
Streknja* pulp	0.17	6.05	0.21	0.39	0.16
Šećerlija* peel	0.36	8.86	0.50	1.09	0.43
Šećerlija* pulp	0.28	7.49	0.31	0.58	0.22
Zlatna Parmenka* peel	0.69	4.87	0.48	0.56	0.24
Zlatna Parmenka* pulp	0.36	5.69	0.25	0.54	0.17
Senabija* peel	0.33	7.43	0.45	0.69	0.30
Senabija* pulp	0.27	6.71	0.30	0.41	0.16
Masnjača** peel	0.37	6.68	0.65	0.99	0.36
Masnjača** pulp	0.13	5.03	0.23	0.50	0.24
Mekica** peel	0.59	6.24	0.60	0.79	0.38
Mekica** pulp	0.28	3.86	0.18	0.38	0.13
Kožara** peel	0.28	4.12	0.90	0.31	0.24
Kožara** pulp	0.07	5.46	0.30	0.22	0.11
Kraljica** peel	0.28	7.19	0.62	0.78	0.36
Kraljica** pulp	0.09	5.40	0.22	0.63	0.24
Šarenka** peel	0.49	7.28	0.42	0.74	0.29
Šarenka** pulp	0.22	5.45	0.17	0.33	0.10
Pear cultivar					
Bela Takiša** peel	0.52	8.36	0.42	0.72	0.23
Bela Takiša** pulp	0.21	6.59	0.30	0.75	0.18
Bela Arapka** peel	1.29	8.11	0.58	0.85	0.42
Bela Arapka** pulp	0.24	6.34	0.31	0.72	0.26
Bronzara** peel	0.50	6.27	0.44	0.59	0.35
Bronzara** pulp	0.17	4.59	0.25	0.56	0.19
Crna Takiša** peel	1.43	7.39	0.56	0.95	0.27
Crna Takiša** pulp	0.36	5.66	0.33	0.84	0.23

Trace elements with contents higher than 1 μg g^{-1} dw were Al, B, Ba, Cu, Fe, Mn, Na and Zn, while the other microelements were lower in the investigated samples. According to the obtained data, Fe was the dominant microelement in the apple peel and pulp samples, while B was the principal microelement in the pear samples. Appreciable amounts of Fe were recorded in the peel samples of Kolačara and Šećerlija. The iron values in the investigated apple samples were lower compared to some commercial ones.[31] On the other hand, they were comparable with the values obtained for Romanian autochthonous cultivars when the

TABLE IV. The concentration of microelements (expressed as μg/g dw) in apple and pear samples (n = 1); LLD – lower than limit of detection, * – sample from Ljig, ** – sample from Zlatibor

Sample	Mineral																			
	Al	As	B	Ba	Cd	Co	Cr	Cu	Fe	Li	Mn	Mo	Na	Ni	Pb	Sb	Se	Sr	Zn	V
Apple cultivar																				
Kolačara* peel	2.88	0.01	7.36	1.17	LLD	0.01	0.18	1.18	11.21	0.02	3.18	0.02	3.66	0.21	LLD	LLD	LLD	1.11	1.03	0.003
Kolačara* pulp	0.73	LLD	5.58	0.72	LLD	0.004	0.42	1.07	7.59	0.01	2.00	0.01	0.44	0.29	LLD	LLD	LLD	0.89	0.87	LLD
Streknja* peel	1.51	0.04	3.89	1.29	LLD	LLD	0.13	1.19	7.54	0.01	3.06	LLD	0.02	0.18	0.09	LLD	LLD	0.67	0.85	LLD
Streknja* pulp	0.73	0.02	2.34	1.04	LLD	0.001	0.57	1.87	6.47	0.01	1.60	LLD	LLD	0.54	0.02	0.05	LLD	0.70	1.15	LLD
Šećerlija* peel	4.95	0.04	14.86	0.74	LLD	0.02	0.27	2.36	16.55	0.01	3.22	0.04	4.37	0.47	LLD	LLD	LLD	0.48	1.93	0.03
Šećerlija* pulp	0.96	LLD	12.69	0.76	LLD	0.01	LLD	2.22	7.79	0.004	1.95	0.02	4.54	0.18	LLD	LLD	LLD	0.68	1.67	LLD
Zlatna Parmenka* peel	17.64	0.02	8.11	3.50	LLD	LLD	0.19	1.48	11.41	0.01	2.47	0.03	2.35	0.21	LLD	LLD	LLD	2.38	1.20	0.01
Zlatna Parmenka* pulp	1.60	LLD	5.80	1.72	LLD	0.002	0.52	1.49	5.52	0.003	1.22	0.02	3.06	0.27	LLD	LLD	LLD	1.50	0.92	LLD
Senabija* peel	2.48	0.01	6.44	3.64	LLD	LLD	0.34	2.73	9.56	0.01	1.80	0.01	0.65	0.30	0.05	LLD	LLD	1.39	1.52	0.01
Senabija* pulp	0.54	0.01	4.93	3.51	LLD	LLD	0.09	1.90	5.08	0.01	1.10	LLD	LLD	0.33	LLD	LLD	LLD	1.72	0.87	LLD
Masnjača** peel	0.89	LLD	14.40	0.36	LLD	0.01	0.01	1.58	9.62	LLD	2.82	0.04	1.60	0.53	LLD	LLD	LLD	0.28	1.26	LLD
Masnjača** pulp	0.13	LLD	9.61	0.16	LLD	LLD	LLD	1.35	5.74	LLD	1.06	LLD	LLD	0.08	LLD	0.02	LLD	0.20	0.57	0.004
Mekica** peel	0.89	0.02	7.70	0.84	LLD	LLD	LLD	1.91	6.95	0.002	2.84	0.06	0.66	0.23	LLD	LLD	LLD	0.27	2.24	0.01
Mekica** pulp	0.15	LLD	3.36	0.37	LLD	LLD	LLD	1.39	3.51	LLD	0.99	0.02	LLD	0.09	LLD	LLD	LLD	0.19	0.92	LLD
Kožara** peel	1.72	0.01	8.40	0.22	LLD	LLD	0.01	1.32	9.21	0.003	2.68	LLD	1.09	0.89	LLD	0.01	LLD	0.21	0.73	0.003
Kožara** pulp	0.08	LLD	6.53	0.04	LLD	LLD	LLD	0.76	2.66	LLD	0.81	LLD	LLD	0.20	LLD	LLD	LLD	0.08	0.39	LLD
Kraljica** peel	0.92	0.05	16.96	0.24	LLD	LLD	0.01	1.69	8.95	0.002	2.47	LLD	1.78	0.48	0.29	LLD	LLD	0.32	0.92	LLD
Kraljica** pulp	0.10	0.02	10.01	0.08	LLD	LLD	LLD	1.16	4.81	LLD	0.84	LLD	1.02	0.19	LLD	LLD	LLD	0.16	0.52	LLD
Šarenka** peel	0.53	LLD	13.56	0.53	LLD	LLD	LLD	1.63	5.25	LLD	2.66	0.05	LLD	0.10	LLD	LLD	LLD	0.21	1.59	0.01
Šarenka** pulp	0.20	LLD	7.66	0.22	LLD	LLD	LLD	0.98	1.90	LLD	1.18	0.01	LLD	0.02	LLD	LLD	LLD	0.13	0.54	LLD

TABLE IV. Continued

Sample	Mineral																					
	Al	As	B	Ba	Cd	Co	Cr	Cu	Fe	Li	Mn	Mo	Na	Ni	Pb	Sb	Se	Sr	Zn	V
Pear cultivar																						
Bela Takiša** peel	1.15	0.02	10.05	0.52	0.01	0.01	LLD	3.90	7.72	0.002	2.10	0.01	0.89	0.64	0.09	LLD	LLD	0.18	5.34	LLD
Bela Takiša** pulp	0.17	LLD	6.03	0.26	0.01	0.01	LLD	2.55	2.49	LLD	1.10	0.003	0.10	0.52	LLD	LLD	LLD	0.11	2.82	LLD
Bela Arapka** peel	0.86	0.02	10.39	1.34	0.01	0.01	LLD	5.20	9.60	0.004	3.79	0.02	LLD	0.43	LLD	LLD	LLD	0.44	6.43	LLD
Bela Arapka** pulp	0.23	LLD	7.34	0.45	0.01	0.03	LLD	4.27	4.30	LLD	1.65	0.01	LLD	0.44	LLD	LLD	LLD	0.15	4.55	LLD
Bronzara** peel	1.67	0.04	5.13	0.39	0.03	0.03	LLD	3.23	6.05	0.002	2.12	0.04	1.73	0.61	LLD	LLD	LLD	0.13	3.49	LLD
Bronzara** pulp	0.69	LLD	2.70	0.20	0.02	0.06	LLD	1.68	2.56	LLD	0.87	0.01	0.86	0.50	LLD	LLD	LLD	0.09	2.71	LLD
Crna takiša** peel	0.83	0.01	11.50	1.29	0.02	0.02	LLD	4.51	5.17	0.005	3.36	0.01	3.15	0.21	LLD	LLD	LLD	0.44	3.50	0.003
Crna takiša** pulp	0.49	LLD	8.15	0.59	0.01	0.01	LLD	2.44	2.29	LLD	1.40	0.01	1.40	0.11	LLD	LLD	LLD	0.20	1.78	0.004

Fe content ranged from 2.45 to 3.64 μg g^{-1} dw.[33] The content of B was highest in apple cultivars Šećerlija and Kraljica, while the cultivar Crna Takiša exhibited the highest B content (11.50 and 8.15 μg g^{-1} dw in peel and pulp samples, respectively) among the pears.

According to the obtained data, the Al content was higher in the fruit samples from Ljig compared to that in the samples collected on Zlatibor Mountain. While the lowest Al content in apple peel was observed for the cultivar Šarenka, the cultivar Zlatna Parmenka was statistically superior as a source of Al. For Zn and Cu, the data showed that pear samples were a better source compared to apples.

The investigated non-nutritive toxic elements that are known to have deleterious effects even in small quantities (below 100 ppm) were As, Sb, Cd, Pb and Se.[34] Se was below the limit of detection in all the investigated samples, as were Sb in the pear samples and Cd in the apple samples. The content of the remaining elements was generally higher in the peel of the fruits than in the pulp for both apples and pears.

The shown variability in the mineral contents could be ascribed to differences in cultivation conditions, such as soil fertility, pH, climate and seasonal variations. However, the differential capacity of the cultivars to absorb metal ions from soils and/or the specific capacity of redistribution within the overall plant could be other reasons for the exhibited variability.

CONCLUSIONS

The results of the present study provide valuable data regarding the phenolic and mineral contents of Balkan apple and pear cultivars. The concentrations of the identified polyphenols and minerals were significantly different depending on the apple or pear cultivar for both peel and pulp. Although it is difficult to directly compare the content of apple phenolics between different studies due to variations caused by different growth periods, geographic locations, genetic diversity, methodology and many other factors,[11] the cultivars Mekica, Šarenka (among the apples) and Bela Arapka (among the pears) could be considered as rich sources of phenolics according to the results of this study. Based on these data, mentioned cultivars show high potential for juice production while the cultivars Senabija and Šećerlija, due to their lower phenolic content, are recommended for direct consumption. Balkan autochthonous apple and pear varieties could be considered as a good source of dietary minerals.

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ИЗВОД

ФЕНОЛНИ И МИНЕРАЛНИ ПРОФИЛ АУТОХТОНИХ ВАРИЈЕТЕТА ЈАБУКА И КРУШАКА СА БАЛКАНА

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Циљ ове студије била је хемијска анализа фенолних једињења, као и минералних састојака присутних у узорцима коре и меса балканских аутохтоних сорти јабука и крушака. Фенолне компоненте одређене применом HPLC-DAD и спектрофотометријских техника значајно су варирале у зависности од варијетета у узорцима коре и меса. Међу јабукама варијетет Мекица имао је највећи садржај укупних фенола, док је у варијететима Шећерлија и Златна парменка он био најнижи. У случају крушака Црна такиша је била богат извор ове групе једињења, док је у варијетету Бела арапка забележена најнижа концентрација. У већини испитиваних варијетета хлорогенска киселина (код јабука) и арбутин (код крушака) била су доминантна фенолна једињења. Анализом елемената показано је да је K најзаступљенији, следе P, Mg, Ca и S. Гвожђе је доминантан микроелемент у кори и месу јабука, док је у узорцима крушака то B. Добијени резултати дају детљну информацију о хемијском саставу испитиваних варијетета и на тај начин стимулишу њихово шире гајење и примену у исхрани.

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