Research Article

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Distribution of elements in seeds of some wild and cultivated fruits. Nutrition and authenticity aspects

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Abstract

BACKGROUND: The compositional, functional, and nutritional properties of fruits are important for defining their quality. Fruit seeds should be better exploited as they are also considered to be a good source of bioactive components. Twenty macro, micro, and trace elements were identified and quantified in the seeds of 70 genuine wild and cultivated fruit species/cultivars by inductively coupled plasma atomic emission spectrometry and inductively coupled plasma mass spectrometry. Sophisticated chemometric techniques were also used to establish criteria for the classification of the analyzed samples.

RESULTS: Calcium and P were the most abundant elements, followed by K and Na. The content of microelements and trace elements differed among the different cultivars/genotypes. The content of Ba, Pb, and Sr was significantly higher in wild fruits, whereas Fe, Mg, Mn, Ni and Zn content was higher in cultivated fruits.

CONCLUSION: All of the statistical procedures that were used – Kruskal–Wallis, Mann–Whitney U-test, and principal component analysis (PCA) – confirm a unique set of parameters that could be used as phytochemical biomarkers to differentiate fruit-seed samples belonging to different cultivars/genotypes according to their botanical origin. This kind of investigation may contribute to intercultivar/genetic discrimination and may enhance the possibilities of acquiring a valuable authenticity factor.

Supporting information may be found in the online version of this article.

Keywords: elements; berries seeds; ICP-OES; ICP-MS; nutritional properties; authenticity

INTRODUCTION

A long tradition of fruit cultivation and a favorable climate in Serbia enable very successful and diverse fruit production. Optimal natural conditions in certain regions of the country allow good results in terms of quality, yield, and revenue for many berry cultivars. Consequently, fruit growing is a very important branch of agriculture, which accounts for about 11% of the country's total agricultural production (Statistical Office of the Republic of Serbia).[1]

Furthermore, Serbia represents a rich gene pool of wild (indigenous) species, with over 100 wild fruit species classified in 15 families and 26 genera. Despite the fact that they are progenitors of cultivated varieties, indigenous fruits are used as rootstocks for fruit production, small- or large-scale organic or conventional production, afforestation and erosion prevention, timber production, landscape architecture, bee pastures, and for other purposes.[2]

Berry fruits and related species have a very important place in the daily diet of humans due to their high vitamin, mineral, polysaccharide, essential oil, and phytochemical content, and especially due to their antioxidants.[3] Not only the fruit, but even the seeds are considered to be a good source of different bioactive components.[4] Owing to their nutritional composition and potential health benefits, fruit seeds have been gaining attention in recent years.[5,6]

The seed is the primary reproductive organ developed from an ovule after fertilization, whose function is to maintain the species.[7,8] Fruit seeds are typically rich in vitamin and mineral content; hence an important function of a seed is to act as an energy and nutrient source for the new plant. Seed germination as the early stage of plant development presents a period when there is increased sensitivity to metals.[9] It was pointed out that *Viola*, *Populus,* and *Salix* species accumulate considerable amounts of metals in the seeds, while the higher concentration of heavy metals in seeds of *Helianthus annus* or *Populus alba* do not affect germination and seedling vigor.[9] The presence of the elements in all the parts of a plant, including the seed, depends on the way in which plants acquire, distribute, metabolize, and use mineral matter. The mineral composition of fruits depends not only on the species or varieties but also on the growing conditions such as

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soil type and geographical origin. For example, differences among mulberry species from different areas were observed depending on mineral composition.[10]

Minerals are micronutrients that are essential for the growth, maintenance, and proper functioning of the human body. An adequate intake of micronutrients is essential for health and to prevent or treat various diseases, such as bone demineralization and arterial hypertension, and to maintain overall cardiovascular health.[11] As the body cannot synthesize essential and trace elements, they must be ingested in regular amounts as a part of the human diet.[12,13] A significant part of the total intake of elements in the human body occurs by digesting fruits, especially berry fruits, as one of the best dietary sources of bioactive compounds.[14] Berry seeds are consumed together with the fruit and hence it is important to know the content of micronutrients or toxic metals and their contribution to daily intake.

In addition to its nutritional values, the authenticity of food products has recently become a topical issue for consumers and producers.[15] Awareness of product authentication is increasing due to the importance of food quality assurances, which are essential to protect consumers and to avoid competition that can destabilize markets.[16] One aspect of the authenticity of fruits is description, such as descriptions of geographical and botanical origin. Possible markers that could be used for fruit discrimination are macro and micro elements.

Given the importance of the consumption of berry fruits with proven benefits for health, high sensory qualities, and a rich presence of bioactive substances, there is a need for studies of the variability of nutrients among cultivars. In addition, as fruit seeds can be obtained as byproducts from processing companies, more information about cultivars' seeds and their composition is required. The aim of this work is to characterize fruit seeds from 70 different genuine Serbian wild and cultivated fruit species/cultivars by evaluating their composition in terms of the presence of elements. Several cultivars of raspberries, gooseberries, blackberries, strawberries, blueberries, currants, aronia, as cultivated fruits, and 26 species of wild fruit were analyzed. Knowledge of the qualitative and quantitative presence of elements in fruit seeds can be a powerful tool in the authentication of botanical and geographical origin. A second objective of this work was to establish criteria for element-based classification and differentiation of cultivated and wild fruits. This kind of investigation may contribute to intercultivar discrimination and enhance the possibility of acquiring an important means of establishing authenticity. To the best of the authors' knowledge there been have no published articles containing information about the composition of fruit seeds in terms of elements for such a high number of different wild and cultivated cultivars.

EXPERIMENTAL

Materials and methods

Reagents and chemicals

All glassware was soaked in 10% HNO_3 for a minimum of 12 h and was rinsed with distilled water. All chemicals were of analytical grade and were supplied by Merck (Darmstadt, Germany). Ultrapure water was prepared by passing doubly deionized water through a water purification system (MicroPure water purification system, 0.055 μS cm^{-1}, TKA, Thermo Fisher Scientific, Niederelbert, Germany) to remove carbon contamination.

A multielement stock solution containing 0.5000 g L^{-1} of major elements was used to prepare intermediate multielement standard solutions for inductively coupled plasma atomic emission spectrometry (ICP-OES) measurements. Multielement stock solution containing 1.000 g L^{-1} of minor and trace elements was used to prepare intermediate multielement standard solutions.

Standard reference materials were used to check the accuracy and precision of the instrument conditions: ERM-CD281 (rye grass) Institute for Reference Materials and Measurements (IRMM). Differences between certified values and quantified concentrations were below 10%. The results are presented in Table S1 (supplementary material).

Plant material

The cultivated berry cultivars (Table 1) were produced and picked from commercial orchards in regions that were suitable for fruit growing. The indigenous fruits, belonging to the spontaneous Serbian flora, were collected from diverse locations (Table 2). They were identified according to the taxonomical criteria of Mratinić and Kojić.[17] All selected trees/bushes were well formed, without any pest symptoms.

Thirty mature fruits per species/cultivars were harvested randomly according to shape and color uniformity, from all cardinally oriented branches (or bushes) with different directions around the plant. Selected fruits were harvested at the fully ripe stage of maturity for each species. After short transportation in cold containers, seeds were removed from the fleshy mesocarp. After a few days of air drying, seeds were stored in paper bags in a dark place until the analysis.

Sample collection and preparation

A total of 70 wild and cultivated fruit seed samples were collected in summer/autumn 2014 in Serbia.

Fruit seeds samples were ground to powder using a mill with liquid nitrogen. The samples were prepared for analysis by wet digestion procedure. Samples of about 0.25 g of fruit seeds were transferred into precleaned cuvettes and then 7 mL of 65% HNO_3 and 1 mL of 30% H_2O_2 were added. The samples were heated for 6 h at 85 °C. After cooling, the samples were quantitatively transferred into a 25 mL volumetric flask and diluted with ultrapure water.

Element assessment

All fruit seed samples were analyzed in duplicate and the concentration of elements were determined in triplicates. The concentration of major elements (Al, Ca, Fe, K, Na, Mg, S, and P) was determined by ICP-OES (Thermo Fisher Scientific, Waltham, USA), model 6500 Duo, equipped with a CID86 chip detector. This instrument operates sequentially with both a radial and an axial view of plasma. The operating RF power was 1150 W, argon plasma gas flow rate was 12 L min^{-1}; auxiliary 0.5 L min^{-1}; nebulizer 0.5 L min^{-1}. The entire system was controlled with Iteva software. The selected wavelengths for analyzed elements were 308.2 nm (Al), 422.6 nm (Ca), 238.2 nm (Fe), 285.2 nm (Mg), 769.8 nm (K), 818.3 nm (Na), 718.2 nm (P) and 180.7 nm (S).

Analytical measurements of other elements were made using an ICP-MS, iCAP Q, (Thermo Scientific Xseries2, UK). The entire system was controlled with Qtegra Instrument Control Software. Instrumental conditions and measured isotopes are given in Table S2 (supplementary material).

Table 1. The cultivated berry cultivars

Species	Cultivar	Origin	Pedigree
Strawberry			
Fragaria ananassa Duch.	Capri	Italy	'CIVRI-30' × R6R1 – 26
	Albion	USA	Diamante × Cal 94.16 – 1
	Irma	Italy	Don × sel. ISF 89.33.1.
	Alba	Italy	Honeoye × Tudla
	Premy	Italy	Unknown
	Asia	Italy	Maya × NF101
	Joly	Italy	Clery × Darselect
	Laetitia	Italy	Unknown
	Garda	Italy	Complex crossings that are including cultivars Addie, Alba, Belrubi, Cardinal and Holiday
	Clery	Italy	Sweet Charlie × Marmolada
	Roxana	Italy	Surprise des Halles × Senga Sengana
	Brilla		Complex crossings that are including cultivars Alba, Brighton, Darselect, Cesena and Tribute
	Jeny	Italy	Unknown
	Arosa	Italy	Chandler × Marmolada
	VR 4	Italy	Unknown
Raspberry			
Rubus idaeus L.	Glen Ample	Scotland	Complex crossings that are including cultivars Glen Prosen and Meeker
	Meeker	USA	Willamette × Cuthbert
	Tulameen	Canada	Nootka × Glen Prosen
	Willamette	USA	Newburgh × Lloyd George
Blackberry			
Rubus *fruticosus* L.	Cacak's thornless	Sebia	Dirksen Thornless × Black Saten
	Loch Ness	Scotland	Unknown
Rubus *allegheniensis* L.	Triple crown	USA	Carbondale 47 × Arkansas 545
Black currant			
Ribes nigrum	Ben Sarek	Scotland	Goliath × Ojebyn
	Malling Juel	England	Unknown
	Ojebin	Sweden	Unknown
	Ometa	Switzerland	Westra × *R. nigrum*
	Čačanska crna	Serbia	Seedling of Malling Jet
	Silmu	The Netherlands	Unknown
	Tenah	The Netherlands	(Goliath × R.n.) × R.n.) × Brödtorp
	Titania	Sweden	Altajskaja Desertnaja × (Consort × Kajaanin Musta)
	Triton	Sweden	Altajskaja Desertnaja × (Consort × Kajaanin Musta)
	Tsema	The Netherlands	Goliath × R.n.) × R.n.) × Brödtorp
Interspecies hybrid	Ben Nevis	Scotland	(Brödtrop × Janslunda) × (Consort × Magnus)
	Bona	Poland	Öjebyn × S/12 (Ribes dikuscha × Climax)
Blueberry			
Vaccinium corymbosum L.	Bluecrop	USA	GM-37 (Jersey × Pioneer) × CU-5 (Stanley × June)
	Brigita blue	Australia	Unknown
	Duke	USA	(Ivanhoe × Earliblue) × (E 30 × E 11)
	Patriot	USA	LS3 × Earliblue
	Spartan	USA	Earliblue × US 11 – 93
Gooseberry			
Ribes uva-crispa L.	Hinnonmaki Red	Finland	Unknown
	Hinnonmaki Yellow	Finland	From wild gooseberry
Chokeberry (Aronia)			
Aronia arbutifolia	Nero	Check Republic	Unknown
Cape Gooseberry (Goldenberry)			
Physalis peruviana		Peru	Unknown
Goji Berry			
Lycium barbarum L.		China	Unknown

Table 2. The indigenous wild fruits

Species	Latin name	Location	Geographic coordinates		Altitudes
			Longitude	Latitude	
White mulberry	*Morus alba*	New Belgrade	20°43′E	44°81′N	78
Black mulberry	*Morus nigra*	New Belgrade	20°43′E	44°81′N	78
Single-seeded hawthorn	*Crategus monogina*	New Belgrade	20°43′E	44°81′N	78
Hungarian hawthorn	*Crataegus nigra*	Stara Mt, Zlot	21°99′E	43°58′N	513
Wild cherry	*Prunus avium*	Kušici	20°10′E	43°50′N	1000
Dogwood	*Cornus sanguinea*	Rudnik Mt.	20°32′E	44°07′N	635
Common yew	*Taxus baccata*	Zemun	20°41′E	44°45′N	79
Blackthorn	*Prunus spinosa*	Jakovo	20°15′E	44°45′N	64
Blackthorn	*Prunus spinosa*	Saranovo	20°51′E	44°13′N	250
Danewort	*Sambucus ebulus*	Rudnik Mt.	20°32′E	44°07′N	635
Elderberry	*Sambucus nigra*	Deliblato Sands	21°05′E	44°53′N	250
Filed rose	*Rosa arvensis*	Suva Mt.	22°10′E	43°10′N	1100
Dog rose	*Rosa canina*	Zlatar Mt.	19°47′E	43°24′N	1000
Barberry	*Berberis vulgaris*	Zemun	20°41′E	44°84′N	79
Bilberry	*Vaccinium myrtilus*	Golija Mt.	20°16′E	43°20′N	1100
Bilberry	*Vaccinium myrtilus*	Tara Mt.	19°18′E	43°54′N	1300
Alpine currant	*Ribes alpinum*	Stara Mt., Radičevac	22°43′E	44°01′N	680
European dewberry	*Rubus caesius*	Suva Mt.	22°10′E	43°10′N	900
Blackberry	*Rubus hirtus*	Golubac	21°37′E	44°39′N	179
Red raspberry	*Rubus idaeus*	Kopaonik Mt.	20°49′E	43°16′N	1200
Gooseberry	*Ribes grossularia*	Stara Mt., Radičevac	22°43′E	44°01′N	680
Common juniper	*Juniperus communis*	Zlatar Mt.	19°47′E	43°24′N	850
Wild strawberry	*Fragaria vesca*	Zlatibor Mt.	19°40′E	43°38′N	700
Wayfaring tree	*Viburnum lantana*	Djerdap George	22°31′E	44°40′ N	320
Guelder-rose	*Viburnum opulus*	Tara Mt.	19°18′E	43°54′N	700
Wild cape gooseberry	*Physalis sp.*	Ljig	20°14′E	44°13′ N	210

Statistical analysis

Descriptive statistics, the Mann–Whitney U-test, and the Kruskal–Wallis one-way analysis of variance by ranks test were performed using a demo version of NCSS statistical software.[18] Principal component analysis (PCA) was carried out by means of PLS ToolBox, v.6.2.1, for MATLAB 7.12.0 (R2011a). Principal component analysis was conducted as an exploratory data analysis using a singular value decomposition algorithm (SVD) with a 0.95 confidence level for Q and T^2 Hotelling limits for outliers. By using only a limited number of principal components (PCs), the dimensionality of the retention data space was reduced, further analysis was simplified, and the parameters were grouped according to similarities.

RESULTS AND DISCUSSION

Elements profiles of fruit seeds – nutrient aspect

Elements that have an important role in vital biochemical and physiological functions in living organisms are recognized as essential for life. At least 17 elements are known to be essential nutrients for plants. The soil supplies elements N, P, K, Ca, Mg, and S in relatively large amounts, and Fe, Mn, B, Mo, Cu, Zn, Cl, and Co, in smaller amounts.[19] Nutrients must be available not only in sufficient amounts but also in appropriate ratios. The human body has a certain requirement for these essential elements, but their excessive intake can produce toxic effects.[20] Unlike cultivated fruits, wild varieties of berries survive and reproduce in natural conditions, without direct human influence. The fact that they were not treated with any kind of fertilizers or chemicals make them natural products with great nutritional value and excellent quality.[21]

Cultivated fruits

The contents of macro- and microcomponents in cultivated fruit seeds (raspberry, blackberry, blueberry, blackcurrant, strawberry), expressed as g kg^{-1} dw, are presented in Fig. 1. Vertical bars denote 0.95 confidence intervals. The results for the mineral content in gooseberry ($n = 2$), chokeberry ($n = 1$), cape gooseberry ($n = 1$) and goji berry ($n = 1$) seeds are not shown on the figure due to the small number of samples; however, they are discussed in the text.

Among the cultivars investigated, blackcurrant contained the highest total amount of macronutrients, with K and P content dominating – this was at least twice as high as that of other berry fruits (4.98 and 6.61 g kg^{-1} dw, respectively, Fig. 1). A similar trend was also observed in the seeds of gooseberry (3.87 and 5.40 g kg^{-1} dw for K and P, respectively), chokeberry (3.12 and 4.55 g kg^{-1} dw for K and P, respectively) and goji berry (3.34 and 5.35 g kg^{-1} dw for K and P, respectively). Unlike these cultivars, all of the other samples that were investigated contained Ca as the most abundant element. Cultivated berry fruits (strawberry, blackcurrant, blackberry and bilberry) from Finland[22] had a much higher level of K compared to berry fruits grown in Serbia, which can be explained in terms of different agroclimatic conditions, and different organ analysis in both studies. Strawberry seeds contained similar amounts of the macroelements Ca, Mg, and Na as reported in a study by Grzelak-Blaszczyk *et al.*;[23] however, there was a lower content of K. Aronia, cultivar 'Nero', contained

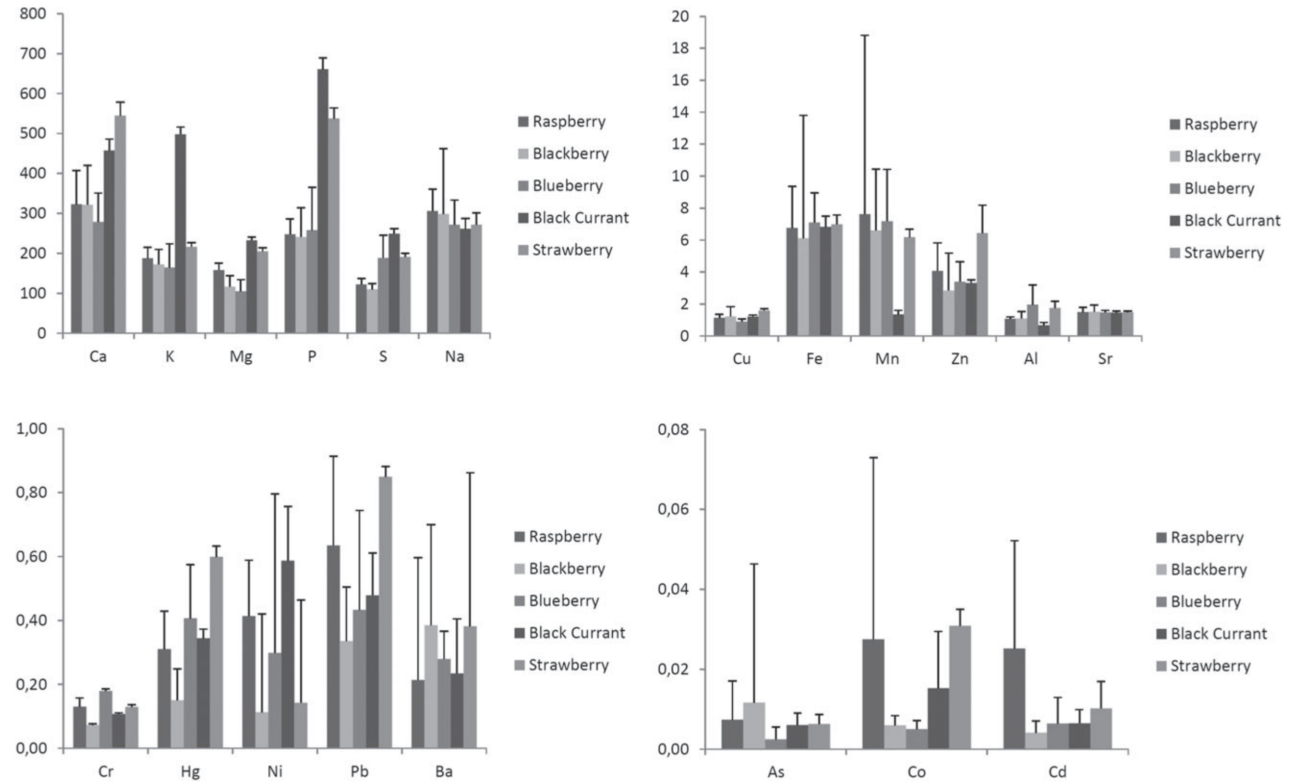

Figure 1. Contents of macro- and microcomponents in cultivated fruit seeds (raspberry, blackberry, blueberry, black currant, strawberry).

the highest level of Mg. Blackcurrant and gooseberry cultivars collected highest levels of S.

In addition to higher total amounts of macroelements, blackcurrant also contains a four to six times lower amount of Mn and 1.5–4 times higher amount of Ni (Fig. 1). Iron content in the fruit seeds that were examined was the highest in the 'Triplecrown' blackberry (0.097 g kg^{-1} dw) followed by the 'Tulameen' raspberry (0.091 mg/100 g dw), which makes such seeds suitable for consumption by people with anemia. Strawberry seeds contained more Zn than other berry fruits, and more compared to strawberry seeds from the study by Grzelak-Blaszczyk *et al.*[23] (0.064 *vs*. 0.043 g kg^{-1} dw). Among the cultivated species, blueberry contains the highest amount of Al (0.0197 g kg^{-1} dw). Distribution of some toxic metals, such as Hg, Pb and Ni was unique for particular berry cultivars with a wide range of concentrations (Hg – from 0.0015 g kg^{-1} dw for blackberry to 0.0060 g kg^{-1} dw for strawberry; Ni – from 0.0011 g kg^{-1} dw for blackberry to 0.0059 g kg^{-1} dw for blackcurrant; Pb – from 0.0034 g kg^{-1} dw for blackberry to 0.0085 g kg^{-1} for strawberry). The 'Clery' strawberry accumulated the highest level of Pb in seeds, probably due to mineral fertilizers, such as superphosphate.[24] Blackberry seeds generally contained lower amounts of heavy metals, Cr, Hg, Ni, and Pb (Fig. 1).

In addition to the specific uptake of macroelements by certain cultivars, each berry fruit shows uniform distribution of predominant minerals, i.e. the variability in the data is small (Fig. 1). On the other hand, the majority of micro- and trace elements are widely spread around the central value indicating high variability in the content.

The Kruskal–Wallis test was used to compare the medians and variance of 20 elements, as variables, for five botanical species of cultivated berries as a single factor. The Kruskal–Wallis one-way analysis of variance tests if samples originate from the same distribution. When the Kruskal–Wallis test led to significant results, the multiple-comparison Z-value test was performed to identify where the differences occurred or how many differences actually occurred. The results are presented in Table S1 (supplementary material). Based on the Kruskal–Wallis test several elements, such as Al, Ba, Ca, Co, Cr, Cu, K, Mg, Mn, Ni, P, S, and Zn were identified as parameters discriminating fruit-seed samples according to the botanical species. Samples of strawberry and currant seeds were separated from the other botanical species, based on the content of these elements.

Wild fruits

Statistical observations of wild fruit seeds (listed in Table 2) were not possible owing to their low numbers but, to point out on some characteristics of these rare and previously not investigated species, the profile of the elements that they contain is discussed.

The most abundant element was Ca, with an average content of 5.49 g kg^{-1} in wild species and 4.41 g kg^{-1} in cultivated fruits (Table 3). Although both breeding methods indicate high variability among data, the content of Ca in wild fruits was far more spread around the central value – the ranges were between 1.02 (wild *Physalis peruviana*) and 13.96 g kg^{-1} dw (*Fragaria vesca*) – compared to ranges of cultivated fruits – 1.55 (cultivated goldenberry) to 7.06 g kg^{-1} dw (Aronia cv. 'Nero'). Very high Ca content was found in samples of wild red currant, juniper, and dogwood. Together with green vegetables, nuts, and dried fruits, seeds of cultivated and wild fruit proved to be a good source of Ca.

Phosphorus was present in similar amounts to Ca, with higher average content in cultivated species (4.91 g kg^{-1} dw) compared to wild fruits (3.34 g kg^{-1} dw) (Table 3). These differences were more pronounced taking into account the appropriate medians (5.33 g kg^{-1} dw and 2.94 g kg^{-1} dw for cultivated and wild fruits,

Table 3. Parameters of descriptive statistics obtained from metal content analysis (g kg^{-1} dw) of fruit seed samples

		Al	As	Ba	Ca	Cd	Co	Cr	Cu	Fe	Hg	K
Cultivated fruit	Mean	0.0135	0.000060	0.0038	4.41	0.00012	0.00020	0.00126	0.0132	0.069	0.0041	2.96
	Median	0.0112	0.000052	0.0029	4.56	0.00005	0.00012	0.00104	0.0130	0.066	0.0026	2.26
	Stdev	0.0086	0.000055	0.0056	1.30	0.00020	0.00017	0.00070	0.0028	0.012	0.0041	1.37
	Min	0.0033	0.000004	0.0003	1.55	0.00001	0.00001	0.00016	0.0069	0.042	0.0001	0.84
	Max	0.0405	0.000273	0.0394	7.06	0.00115	0.00086	0.00412	0.0192	0.097	0.0210	5.47
Wild fruit	Mean	0.0180	0.000050	0.0109	5.49	0.00012	0.00012	0.00130	0.0128	0.054	0.0036	2.67
	Median	0.0087	0.000029	0.0070	4.97	0.00006	0.00010	0.00103	0.0130	0.051	0.0033	2.49
	Stdev	0.0249	0.000054	0.0217	2.84	0.00012	0.00007	0.00087	0.0030	0.017	0.0015	1.17
	Min	0.0041	0.000004	0.0009	1.02	0.00002	0.00005	0.00065	0.0076	0.026	0.0018	1.13
	Max	0.1141	0.000176	0.1151	13.96	0.00050	0.00031	0.00491	0.0170	0.090	0.0067	5.01
Man-Whitney U-test[a]	*P*	0.4085	0.2355	0.0004	0.2288	0.4695	0.1446	0.7428	0.6617	<0.0001	0.2242	0.5274
	H_0	Accept	Accept	Reject	Accept	Accept	Accept	Accept	Accept	Reject	Accept	Accept

		Mg	Mn	Na	Ni	P	Pb	S	Sr	Zn
Cultivated fruit	Mean	1.94	0.049	2.73	0.0031	4.91	0.0060	1.98	0.0151	0.047
	Median	2.06	0.053	2.74	0.0020	5.33	0.0044	2.02	0.0148	0.038
	Stdev	0.49	0.032	0.46	0.0024	1.66	0.0056	0.49	0.0022	0.024
	Min	0.70	0.002	1.42	0.0006	1.16	0.0007	1.04	0.0111	0.018
	Max	2.68	0.172	3.57	0.0082	7.55	0.0369	2.73	0.0249	0.125
Wild fruit	Mean	1.52	0.035	2.69	0.0017	3.34	0.0065	1.67	0.0178	0.038
	Median	1.43	0.020	2.70	0.0013	2.94	0.0066	1.53	0.0169	0.034
	Stdev	0.58	0.039	0.60	0.0018	1.50	0.0029	0.61	0.0069	0.024
	Min	0.54	0.007	1.18	0.0001	1.27	0.0023	0.81	0.0095	0.013
	Max	2.99	0.189	3.97	0.0054	6.52	0.0142	2.66	0.0445	0.132
Man-Whitney U-test[a]	*P*	0.0027	0.0800	0.7382	0.0261	0.0003	0.0366	0.0329	0.0324	0.0449
	H_0	Reject	Accept	Accept	Reject	Reject	Reject	Reject	Reject	Reject

[a] Differences between two sets of data is significant when *P* value is less or equal to 0.05.

respectively). However, its presence in cultivated species could be the result of the application of fertilizers containing phosphorus. Regarding wild fruits, bird cherry seeds stored the highest level of P (6.52 g kg^{-1} dw), followed by wild goldenberry (5.64 g kg^{-1} dw) and wild gooseberry (5.20 g kg^{-1} dw).

Compared to Ca and P, the content of K and Na was lower (approximately 2.70–2.90 g kg^{-1} dw), very similar, and present in equal amount in fruits of both growing conditions. This supports the position of Marzouk and Kassem[25] who proved that type of fertilizing makes no difference to K storage in fruits. Bird cherry (*Prunus avium*) stored the highest level of K (5.02 g kg^{-1} dw) compared with other wild fruits. The level of K was in third place compared with the other elements accumulated in seeds. This contrasts with the results of Chaves *et al.*[26] who studied vegetable seeds, and Pereira *et al.*[27] who studied berry fruits. These authors claimed that K is a prevalent mineral element in seeds as it is more mobile in xylem and phloem than other elements and the effect of plant transpiration on K distribution in the plant is negligible. The sodium level ranged from 0.118 g kg^{-1} dw in wild strawberry seeds to 0.397 g kg^{-1} dw in white mulberry seeds.

A specific mineral profile concerning macroelements was obtained for wild strawberry seeds, which contain a very high amount of Ca (13.96 g kg^{-1} dw), and lower amounts of K (2.47 g kg^{-1} dw), Mg (1.76 g kg^{-1} dw), and Na (1.18 g kg^{-1} dw).

The macronutrients Mg and S were present in similar amounts, in a range of 1.50–2.00 g kg^{-1} dw, with higher content in cultivated fruits. These levels were comparable to Mg levels in berries consumed in Australia.[28] and in Pakistan, with similar amounts of Mg in fruit flesh and seeds.[29] According to Hicsonmez *et al.*[30] the Mg content in plant seeds is not related to the other elements, revealing that Mg concentration is independent from the other element concentrations. A similar finding to that of Marakoglu *et al.*[31] was that blackthorn seeds were the richest in S compared to other wild fruit species. Again, the content of S was identical in fruit flesh and seeds. The fact that our study encompassed just seeds whereas the previous one included both seed and fruit flesh can make it hard to compare the two studies. However, the amounts of some elements, such as Mg and S, are comparable.

Plants could accumulate sufficient quantities of most of the trace elements that are crucial for their normal growth. The content of micronutrients decreased in the following order: Fe, Zn ∼ Mn, Sr, and Cu. With the exception of Sr, all other elements were present in higher amounts in cultivated species.

According to Boudraa *et al.*[32] *Crataegus monogyna* fruits are especially rich in Fe, but in our study bilberry (*Vaccinium myrtilus*) was the best source of this mineral among the wild fruits. The manganese level was also the highest in bilberry seeds (0.189 g kg^{-1} dw), which agrees with Ekholm *et al.*[22] but is much higher than the results for Spada *et al.*[33] Wild fruits may also be sources of dietary copper, providing values of over 0.016 g kg^{-1} dw – for example *Prunus avium* and *Prunus spinosa*. The zinc level was the highest in *Crategus monogina* seeds (0.131 g kg^{-1} dw) followed by 'Albion' strawberries (0.125 g kg^{-1} dw). This is contrary to the findings of Ekholm *et al.*[22] who claimed that raspberry is the main source of this element. Zinc is an antagonist of some other metals, such as Cd, Pb, and Ni, and can reduce the negative effects of these toxic elements.[34]

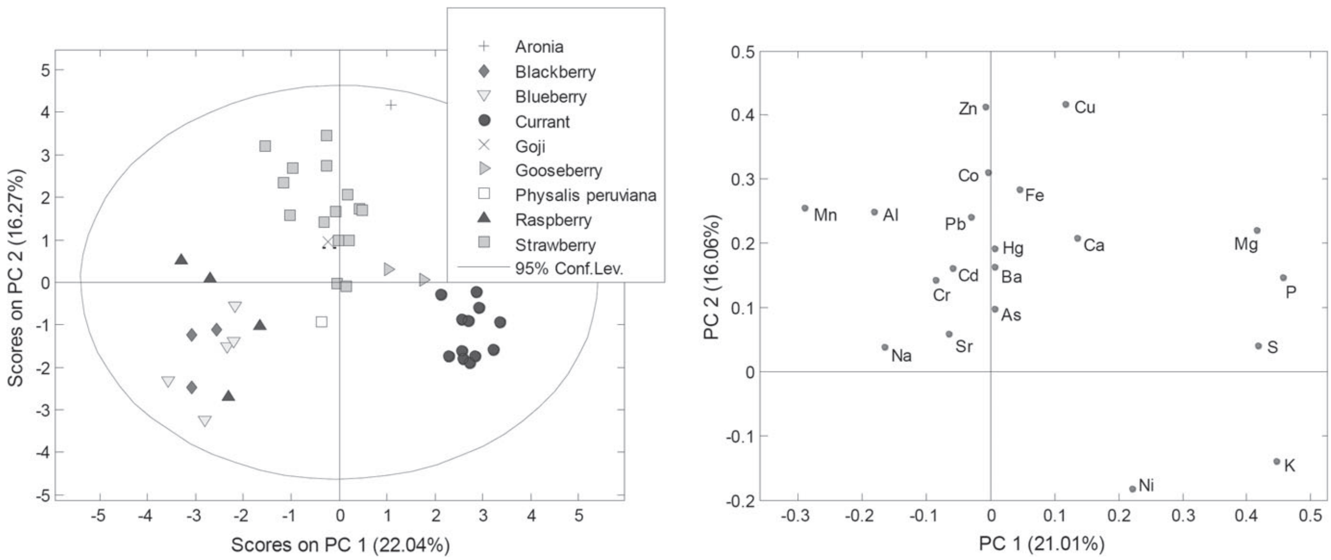

Figure 2. Principal component analysis (PCA) – element-based classification and differentiation of berries cultivars, (a) score plot, (b) loading plot.

Some wild fruits show a specific uptake of certain elements. Extremely high Al content was found in white mulberry (0.114 g kg^{-1} dw) and wild blueberry (0.059 g kg^{-1} dw). The range of Al content in all the other samples was between 0.0046 and 0.0175 g kg^{-1} dw. Wild blueberry also contained a higher amount of Mn (0.153 g kg^{-1} dw), and danewort contained more Zn (0.132 g kg^{-1} dw) than other wild and cultivated fruits.

The Mann–Whitney U test was used to discover if the two populations (cultivated and wild fruit) had the same distribution of metal content (Table 3). A nonparametric test was used due to the significant deviation from the normal distribution of each of the studied variables. Statistically significant differences between cultivated and wild fruit seeds were observed in the content of following elements: Ba, Fe, Mg, Ni, P, Pb, S, Sr, and Zn. The content of Ba, Pb, and Sr was significantly higher in wild fruits, while higher Fe, Mg, Mn, Ni, and Zn content was found in cultivated fruits. The connection of Ba with Sr, Ca, and Pb has already been explained by Lamb *et al.*[35] by the fact that it belongs to the second element group and shares several chemical characteristics with Ca (its content is also higher in seeds of wild fruits in this study but the difference is not significant) and Sr and has some similarity with Pb. Further, Hicsonmez *et al.*[30] showed significant correlation between content of Sr and Ba. From the viewpoint of plant physiology and plant nutrition, it is well known that both the uptake and the effect of stable Sr^{2+} on plants growth depends on the concentration of Ca^{2+}.[36]

The fact that the cultivated fruits stored a higher level of Fe, Mg, Mn, Ni, and Zn in their seeds compared to wild fruits can be explained by the fact that animal manure results in higher iron content, while applying organic manures, enriched with NPK, resulted in higher zinc and manganese content.[37] This is to be expected as organic manures enhance soil properties and soil fertility[38] and might lead to an increase in available nutrients and their uptake. Thus, micronutrients were the elements that distinguished the growing method.

Authenticity assessment

Principal component analysis is the most frequently used pattern-recognition technique, used to obtain basic insight into the data structure and to determine parameters with the highest influence on classification and differentiation of objects. In the current study, PCA was performed in two directions – first to classify cultivated fruits according to the element profile of the seeds, and second to differentiate cultivated and wild fruit seeds from certain botanical species.

Principal component analysis applied to data about the elements in 44 samples of cultivated fruit seeds resulted in a seven-component model, which explains 77.70% of the total data variance. In the case of natural samples, when the variability among the samples is relatively high and a diverse set of parameters (variables) is considered, it is common to find the low overall data variance captured by a few PCs. Mutual projections of factor scores and their loadings for the first two PCs have been presented in Fig. 2. Score plots of models (Fig. 2(a)) suggested the existence of three distinctive groups of objects. Samples of first and second clusters were separated along the PC1 direction. Cultivars of raspberry and blackberry (both species are part of the genus *Rubus*) and blueberry seeds formed the first cluster on the lower left part of the plot; the second compact group is made of gooseberry and currant seeds (both belonging to the *Ribes* genus) in the right part of plot. The third group of samples was separated from the first two groups along the PC2 axis and contained samples of strawberry seeds, aronia, *Physalis peruviana,* and goji on the upper part of the plot.

Loading plot (Fig. 2(b)), a plot of the direction vectors that define the model, revealed that the highest positive influences on PC1 have variables Mg, P, S, and K. The high content of these elements in gooseberry and currant seeds mainly affects their separation from the other samples. The highest negative influences on PC1 have variables Mn, Al, and Na. Variables that potentially have the highest influence on separation along PC2 were Zn, Cu, Cd, Co, Pb, Hg, and Cr, indicating their higher content in seeds of strawberry, aronia, *Physalis peruviana,* and goji.

Principal component analysis was also performed in order to establish the differences between cultivated and wild fruit seeds for botanical species such as strawberry, currant, blueberry, blackberry, and raspberry. Mineral uptake is guided by the growing method and botanical origin. In case of strawberry, currant, and blueberry seeds, samples of wild fruit are clearly separated from the cultivated ones, while due to the smaller number of blackberry and raspberry samples these clusterings were not so conspicuous (Fig. S1). Regarding strawberries, this was expected due to the fact that cultivars belong to the octaploid (2n = 8x = 56) species *Fragaria ananassa,* while the wild accession belongs to the diploid (2n = 2x = 14) species *Fragaria vesca*. A similar situation occurs with blueberries where cultivars belong to *Vaccinium corymbosum* L. whereas wild genotypes belong to *Vaccinium myrtillus* L. In currants, cultivars belong to *Ribes nigrum* and the wild ones are *Ribes alpinum*. Similar results regarding discrimination of cultivated and wild strawberry and blueberry species was obtained, but based on the polyphenolic profile.[39] However, the accumulation of elements in particular wild species is specific (Fig. S2b). Wild strawberries showed higher content of Ca, Sr, and Ba compared to cultivated ones. Similar findings were observed in samples of wild currant seeds with the addition of Mn and wild raspberry with the addition of Al. On the other hand, the samples of wild blueberry were rich in the microelements Ba, Zn, Al, Mn, Cu, As, and Pb, whereas wild blackberry contained higher amount of Zn, P, Mg, Cd, Hg, K, Pb, S, and Cr. This can be explained by the fact that the mineral content of forest (wild) fruits depends on many factors such as genotype, region, microclimatic conditions, and soil characteristics,[22] which together suggest that Serbian wild fruit seeds have a specific trace element composition.

In conclusion, large amounts of fruit seeds that are discarded annually in the juice or conserve industries present a potential waste of a valuable resource. The aim of studying the chemical profile of fruit seeds is to point out on the benefits of this byproduct and to raise consumers' awareness about their health benefits. Such investigations may also contribute to intercultivar/genetic discrimination and increase the possibility of defining the factors that could be used as markers for authenticity assessment.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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