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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: Compositional, functional and nutritional properties of fruits are important for defining its quality. Regarding the fact that fruit seeds are also considered to be a good source of bioactive components, their exploitation should be greater. Twenty macro, micro and trace elements were identified and quantified in seeds of 70 genuine wild and cultivated fruits species/cultivars by inductively coupled plasma atomic emission spectrometry and inductively coupled plasma mass spectrometry. Additionally, sophisticated chemometric techniques were applied to establish criteria for classification of analysed samples.

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

CONCLUSION: All employed statistical procedures (Kruskal-Wallis, Mann-Whitney U-test, and PCA) confirm unique set of parameters that could be used as phytochemical biomarkers to differentiate fruit seeds samples belonging to different cultivars/genotypes according to their

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botanical origin. This kind of investigation may contribute for the inter-cultivar/genetic discrimination and enhancing the possibilities of acquiring a valuable authenticity factor.

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

## INTRODUCTION

A long tradition of fruit cultivation and favourable climatic conditions in Serbia enables a very successful and diverse fruit production. Optimal natural conditions in certain regions of 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 the 11% of the total country 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/conventional) production, afforestation and erosion prevention, timber production, landscape architecture, bee pasture and others.[2]

Berry fruits and related species have very important place in human daily diet due to the high content of vitamins, minerals, polysaccharides, essential oils and phytochemicals, especially 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 are 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 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 vigour.[9] The content of the elements in all parts of the plant, including the seed, depends on the way in which plants acquire, distribute, metabolize and use mineral matter. Additionally, the mineral composition of fruits depended, not only on the species or varieties, but also on the growing conditions such as soil type and geographical origin. Namely, differences among mulberry species from different area were observed based 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 the health and for the prevention or treatment of various diseases, such as bone demineralization, arterial hypertension, and overall cardiovascular risk.[11] As the body cannot synthesise essential and trace elements, they must be ingested in regular amounts as a part of the human diet.[12,13] The significant part of the total intake of elements in the human body is carried out by fruits, especially by berry fruits as one of the best dietary sources of bioactive compounds.[14] Berries seeds are consumed together with a fruit and hence it is important to know the content of micronutrients or toxic metals and their contribution on daily intake.

In addition to its nutritional values, authenticity aspect of food products became topic issue in recent years, for both - consumers and producers. [15] Awareness of product authentication is increasing due to the importance of food quality assurances which is essential in order to protect the consumers and to avoid competition that can create a destabilized market.[16] One of the aspects of authenticity of fruits is originality with respect to description, such as geographical and botanical origin. Possible markers that could be used for fruit discrimination are macro and micro elements.

Regarding the importance of consumption of berry fruits with proven health-beneficial properties, high sensory values and rich composition in bioactive substances, there is a need for studies relating the variability of nutrient among cultivars. In addition, regarding the fact fruit seeds can be obtained as a byproduct from processing companies more information of cultivar`s seeds and its composition is required. The aim of this work is to characterize fruit seeds from 70 different genuine Serbian wild and cultivated fruits species/cultivars by evaluation of their elements composition. 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 elements content of fruit seeds can be powerful tool in authentication of botanical and geographical origin. Second objective of this work is to establish criteria for element-based classification and differentiation of cultivated and wild fruits. This kind of investigation may contribute for the inter-cultivar discrimination and enhancing the possibilities of acquiring an important authenticity factor. According to our knowledge, there have no published article that comprises information about the elements composition of fruit seeds of 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 minimum 12 h and rinsed with distilled water. All chemicals were of analytical grade and were supplied by Merck (Darmstadt, Germany). Ultra-pure water was prepared by passing doubly de-ionized water through water purification system ((MicroPure water purification system, 0.055 μS/cm, TKA, Thermo Fisher Scientific, Niederelbert, Germany) to remove carbon contamination.

Multi-element stock solution containing 0.5000 g/L of major elements was used to prepare intermediate multielement standard solutions for inductively coupled plasma atomic emission spectrometry (ICP-OES) measurements. Multi-element stock solution containing 1.000 g/L of minor and trace elements was used to prepare intermediate multi-element standard solutions.

Standard reference materials were used in order 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) are produced and picked from the commercial orchards in regions which are suitable for fruit growing. The indigenous fruits, belonging to the spontaneous Serbian flora, were collected from diverse locations (Table 2). The identification was done according to the taxonomical criteria of Mratinić and Kojić.[16] All selected trees/bushes were well formed, without any pest symptoms.

Thirty mature fruits per species/cultivars were harvested randomly according to shape and colour uniformity, from all cardinally-oriented branches (or bush) with different directions around the plant. Selected fruits were harvested at the fully ripe maturity stage for each species. After short transportation in cold containers, seeds were removed from the fleshy mesocarp. After few days of air drying, seeds were stored in paper bags on 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 mill with liquid nitrogen. The samples were prepared for analysis by wet digestion procedure. About 0.25 g fruit seeds sample was transferred into pre-cleaned cuvette and then 7 mL of 65% $HNO_3$ and 1 mL of 30% $H_2O_2$ were added. The sample was heated for 6 hours at 85 °C. After cooling, the samples were quantitatively transferred into a 25 mL volumetric flask and diluted with ultra-pure water.

**Element assessment**

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

Analytical measurements of other elements were performed 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).

**Statistical Analysis**

Descriptive statistics, Mann Whitney U-test and 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) has been carried out by means of PLS ToolBox, v.6.2.1, for MATLAB 7.12.0 (R2011a). PCA was carried out as an exploratory data analysis by using a singular value decomposition algorithm (SVD) and a 0.95 confidence level for $Q$ and $T^2$ Hotelling limits for outliers. 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 which have 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. Human body has a certain requirement for these essential elements, but their excessive intake can produce toxic effects.[20] Contrary to cultivated fruits, wild varieties of berries are survived and reproduce in real nature, 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*

Contents of macro- and microcomponents in cultivated fruit seeds (raspberry, blackberry, blueberry, black currant, strawberry), expressed as g/kg dw, are presented in Figure 1. Vertical bars denote 0.95 confidence intervals. Results of 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 were discussed in the text.

Among investigated cultivars, black currant contained the highest total amount of macronutrients, with domination of K and P content which are at least 2-fold higher than those of other berry fruits (4.98 and 6.61 g/kg dw, respectively, Figure 1). Similar trend was also observed in seeds of gooseberry (3.87 and 5.40 g/kg dw for K and P, respectively), chokeberry (3.12 and 4.55 g/kg dw for K and P, respectively) and goji berry (3.34 and 5.35 g/kg dw for K and P, respectively). Contrary to mentioned cultivars, all others investigated samples contained Ca as the most abundant element. Cultivated berry fruits (strawberry, black currant, blackberry and bilberry) from Finland [22], have much higher level of K compared to berry fruits grown in Serbia, which can be explained with different agroclimatic conditions, and different organ analysis in both studies. Strawberry seeds contained similar amount of macroelements Ca, Mg and Na as reported in study of Grzelak-Blaszczyk et al.[23], but however lower content of K. Aronia, cultivar 'Nero', gained the highest level of Mg. Regarding S, black currant's and gooseberry's cultivars collected top levels of this element.

In addition to higher total amount of macroelements, black currant shows specific uptake of Mn and Ni compared to other cultivars, it contains 4 to 6 times lower amount of Mn and 1.5 to 4 times higher amount of Ni (Figure 1). Iron content in the examined fruit seeds was the highest in blackberry 'Triplecrown' (0.097 g/kg dw) followed by raspberry 'Tulameen' (0.091 mg/100 g dw), which makes referred seeds suitable for consumption by people with anaemia. Strawberry seeds contained more Zn than other berry fruits, and more compared to strawberry seeds from study of Grzelak-Blaszczyk et al.[23] (0.064 *vs*. 0.043 g/kg dw). Among cultivated species blueberry contains the highest amount of Al (0.0197 g/kg dw). Distribution of some toxic metals, such as Hg, Pb and Ni, were unique for particular berry cultivar with wide range of concentrations (Hg – from 0.0015 g/kg dw for blackberry to 0.0060 g/kg dw for strawberry; Ni - from 0.0011 g/kg dw for blackberry to 0.0059 g/kg dw for black currant; Pb - from 0.0034 g/kg dw for blackberry to 0.0085 g/kg for strawberry). Strawberry 'Clery' accumulated the highest level of Pb in seeds, probably due to the mineral fertilizers, such as superphosphate.[24] Blackberry seeds generally contained lower amount of heavy metals, Cr, Hg, Ni and Pb (Figure 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 of data are small (Figure 1). Contrary, 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 variances of 20 elements, as variables, for five botanical species of cultivated berries as a single factor. Kruskal–Wallis one-way analysis of variance tests if samples originate from the same distribution. When the Kruskal–Wallis test led to significant results, multiple-comparison Z-value test was performed to identify where the differences occurred or how many differences actually occurred. Results are presented in Table S1 (Supplementary material). Based on 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 governing a separation of fruit seed samples according to the botanical species. Samples of strawberry and currant seeds were separated from the other botanical species, based on these elements content.

*Wild fruits*

Low sample numbers of wild fruit seeds (listed in Table 2) disable any statistical observations, but in order to point out on some characteristics of these rare and previously not investigated species, their elements content profile are discussed.

The most abundant element was Ca, with the average content of 5.49 g/kg in wild species and 4.41 g/kg 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 range were between 1.02 (wild *Physalis peruviana*) and 13.96 g/kg dw (*Fragaria* *vesca*), compared to range of cultivated fruits 1.55 (cultivated goldenberry) to 7.06 g/kg 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 amount as Ca, with higher average content in cultivated species (4.91 g/kg dw) compared to wild fruits (3.34 g/kg dw) (Table 3). These differences were more pronounced taking into account the appropriate medians (5.33 g/kg dw and 2.94 g/kg dw for cultivated and wild fruits, respectively). However, its presence in cultivated species could be result of the application of phosphorus content fertilizers. Regarding wild fruits, bird cherry seeds stored the highest level of P (6.52 g/kg dw) followed by wild goldenberry (5.64 g/kg dw) and wild gooseberry (5.20 g/kg dw).

Compared to Ca and P, the content of K and Na were lower (approximately 2.70 – 2.90 g/kg dw), mutually very similar and present in equal amount in fruits of both growing conditions. This goes in a favour with the statement of Marzouk and Kassem[25] who proved that type of fertilizing makes no difference among K storage in fruits. Bird cherry (*Prunus avium*) stored the highest level of K (5.02 g/kg dw) compared to other wild fruits. Level of K was on the third place comparing to the other elements accumulated in seeds, which is different from the results of Chaves et al.[26] who studied vegetable seeds, and Pereira et al.[27] who studied berry fruits. Namely, these authors claimed that K is 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. Sodium level ranged from 0.118 g/kg dw in wild strawberry seeds to 0.397 g/kg dw in white mulberry seeds.

Specific mineral profile concerning macroelements was obtained for wild strawberry seeds which contain very high amount of Ca (13.96 g/kg dw), and lower content of K (2.47 g/kg dw), Mg (1.76 g/kg dw) and Na (1.18 g/kg dw).

Macronutrients Mg and S were present in similar amount, in range 1.50 – 2.00 g/kg dw, with higher content in cultivated fruits. These levels were comparable to Mg level in berries consumed in Australia.[28] and in Pakistan indicated similar amounts of this element in fruit flesh and seeds.[29] According to the Hicsonmez et al. [30] the content of Mg in plant seeds is not related with the other elements, revealing that Mg concentration is independent from the other element concentrations. Similarly as the results of Marakoglu et al.[31], blackthorn seeds was 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 while the previous one included both seed and fruit flesh can contribute to the result's differences and make it hard for comparison. However, the amount of some elements, as it is pointed out with Mg and S, are comparable.

Plants could sufficiently accumulate most trace elements which 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 amount 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 within wild fruits. Manganese level was also the highest in bilberry seeds (0.189 g/kg dw), which is in coincidence with Ekholm et al.[22], but much higher compare to the results of Spada et al.[33] Wild fruits may also be copper sources for the diet, providing values over 0.016 g/kg dw, as for example *Prunus avium* and *Prunus spinosa*. Zinc level was the highest in *Crategus monogina* seeds (0.131 g/kg dw) followed by strawberry 'Albion' (0.125 g/kg dw). This is opposite to the Ekholm et al.[22] who claimed that raspberry is the main source of this element. Zn is an antagonist of some other metals, such as Cd, Pb, Ni and can reduce negative effects of these toxic elements. [34]

Some wild fruits show specific uptake of certain elements. Extremely high content of Al was marked in white mulberry (0.114 g/kg dw) and wild blueberry (0.059 g/kg dw). The range of content of Al in all other samples were between 0.0046 and 0.0175 g/kg dw. Wild blueberry also contains higher amount of Mn (0.153 g/kg dw), while danewort contains more Zn (0.132 g/kg dw) compared to other wild and cultivated fruits.

The Mann-Whitney U test was used to compare if the two populations (cultivated and wild fruit) have the same distribution of metal content (Table 3). Non-parametric test was used due to the significant deviation from normal distribution of each of the studied variables. Statistically significant difference between cultivated and wild fruit seeds was observed in the content of following elements: Ba, Fe, Mg, Ni, P, Pb, S, Sr, and Zn. The content of Ba, Pb and Sr were significantly higher in wild fruits, while higher content of Fe, Mg, Mn, Ni and Zn were found in cultivated fruits. Connection of Ba with Sr, Ca and Pb is already explained by Lamb et al. [35] by the fact that it belongs to second element group and shares several chemical characteristics with Ca (also higher in seeds of wild fruits in this study but the difference is not significant) and Sr and additionally shows similarity with Pb. Further, Hicsonmez et al.[30] showed significant correlation between content of Sr and Ba. Also, from the viewpoint of plant physiology and plant nutrition, it is well known that both the uptake and the effect of stable $Sr^{2+}$ is in the relationship to the chemically related $Ca^{2+}$.[36]

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

**Authenticity assessment**

Principal component analysis is the most often used pattern recognition technique, applied to get the basic insight in the data structure and to determine criteria for classification and differentiation of objects. In the current study, PCA was performed in two directions, first to classify cultivated fruits according to seeds' elements profile, and second to differentiate cultivated and wild fruit seeds from certain botanical specie.

PCA applied on data of elements content in 44 samples of cultivated fruit seeds, resulted in seven-component model which explains 77.70% of total data variance. In the case of natural samples, when the variability among the samples is relatively high and diverse set of parameters (variables) is considered, it is not unusual to get the low overall data variance captured by few PCs. Mutual projections of factor scores and their loadings for the first two PCs have been presented in Figure 2. Score plots of models (Figure 2a) suggested the existence of three distinctive groups of objects. Samples of first and second cluster were separated along the PC1 direction. Cultivars of raspberry and blackberry (both species are part of the genus *Rubus*) and blueberry seeds formed first cluster at the lower left part of plot, the second compact group is made of gooseberry and currant seeds (both belonging to the *Ribes* genus), in the right part of plot. Third group of samples was separated from the first two groups along the PC2 axis and contained samples of strawberry seeds, aronia, *Physialis peruviana* and goji at the upper part of plot.

Loading plot (Figure 2b) revealed that the highest positive influences on PC1 have variables Mg, P, S and K. 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, which potentially have the highest influence on separation along the PC2 were Zn, Cu, Cd, Co, Pb, Hg, Cr indicating its higher content in seeds of strawberry, aronia, *Physialis peruviana* and goji.

PCA 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 clustering were not so conspicuous (Figure S1). Regarding strawberry this was expected due to the fact that cultivars are belonging to octaploid (2n = 8x = 56) species *Fragaria ananassa*, while the wild accession belongs to the diploid (2n = 2x = 14) species *Fragaria vesca*. Similar is with blueberry where cultivars are belonging to the *Vaccinium corymbosum* L. while wild genotypes to the *Vaccinium myrtillus* L. In currants, cultivars are belonging to the *Ribes nigrum* and the wild one is *Ribes alpinum*. Previously, according to Milivojević et al. [39], similar results regarding discrimination of strawberry and blueberry species was also done, but based on the polyphenolic profile. However, the accumulation of elements in particular wild species is specific (Figure S2b). Wild strawberries showed higher content of Ca, Sr and Ba compared to cultivated ones. Similar observation was noticed in samples of wild currant seeds with addition of Mn and wild raspberry with addition of Al. Contrary, the samples of wild blueberry were reached in the content of microelements Ba, Zn, Al, Mn, Cu, As and Pb, while wild blackberry contained higher amount of Zn, P, Mg, Cd, Hg, K, Pb, S and Cr. This evidence is explainable with the fact mineral content of forest (wild) fruits depend on many factors such as genotype, region, microclimatic conditions and soil characteristics[22], which all together suggest that the Serbian wild fruit seeds have specific trace element composition.

In conclusion, large amount of fruit seeds that are discarded annually in juice or conserve industries present a potential waste of a valuable resource. The aim of the studies that concern chemical profile of fruit seeds is to pointed out on the benefits of this byproduct and to arise consumers awareness about their health-beneficial effects. Additionally, such kind of investigation may contribute for the inter-cultivar/genetic discrimination and enhancing the possibilities of acquiring a valuable authenticity factor.


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## *Appendix* A. Supplementary material

Supplementary data associated with this article can be found, in the online version.

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## Figure caption

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

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

**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 |

| Species | Cultivar | Origin | Pedigree |
|---|---|---|---|

**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 |

**Table 3**. Parameters of descriptive statistics obtained from metal content analysis (g/kg 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 |

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

| | | **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.

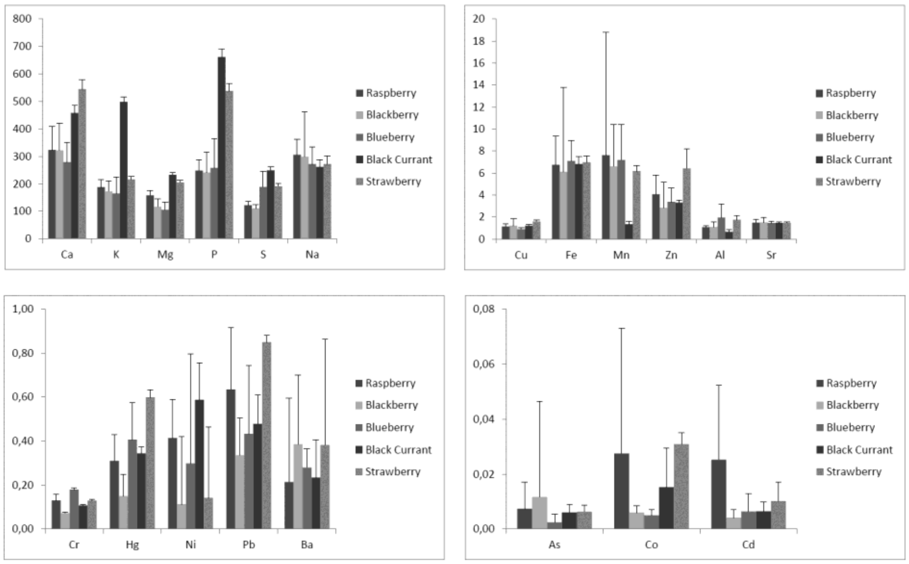


**Figure 1**.

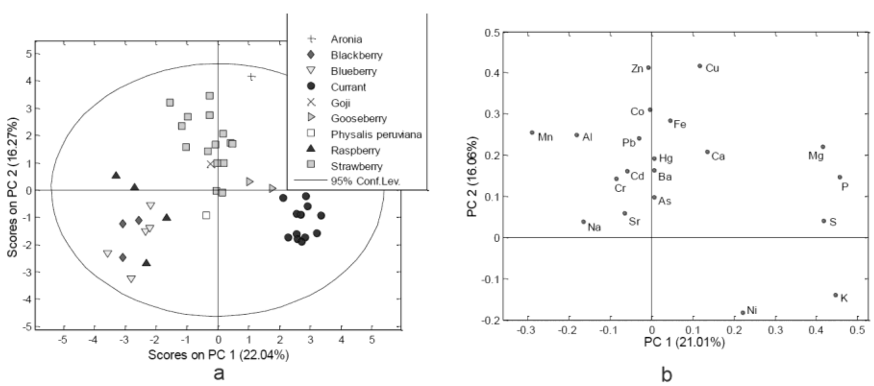


**Figure 2.**