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Estimation of differences in trace element composition of Bulgarian summer fruits using ICP-MS

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Abstract. *The content of potentially essential and toxic elements: chromium, manganese, iron, copper, nickel, cadmium and arsenic in Bulgarian fruits such as aronia, morello, cherry, raspberry, nectarine peach, apple type „akane" and pear type „early gold" were investigated. By using the ICP-MS we found that raspberry has the highest content of iron (4635.9 ± 53.2 µg kg^{-1}), manganese (5690.9 ± 31.7 µg kg^{-1}) and chromium (150.2 ± 2.5 µg kg^{-1}), while the richest in copper is the nectarine (887.5 ± 31.19 µg kg^{-1}). The content of toxic elements (nickel, cadmium and arsenic) is in amount significantly below the permissible standards. Single ANOVA and subsequent Dunkan's test were used to define the fruit and to estimate the significance of chemical elements. The test for multidirectional comparisons indicated that for five of the investigated seven elements: iron, copper, nickel, cadmium, and arsenic the fruits are statistically distinguishable. According to hierarchical cluster analysis the fruits are into one cluster.*

Keywords: trace element content, fruits, Dunkan multidirectional analysis, cluster analysis

Introduction

Fruits are an essential part of dietary intake of people. Their regular consumption is generally associated with reduced risks of numerous functional disorders, free radical scavenging, detoxification, and stimulation of the immune system. They have low energy content, while very high is their nutrient value. Increased consumption of fruits and vegetables can help replace foods high in saturated fats, sugar and salt and thus improve the intake of most micronutrients and dietary fiber. Daily consumption of fresh vegetable food (4400 g/day) is recommended to help prevent major non-communicable diseases such as cardiovascular diseases and certain cancers (WHO, 2003; Joanna, 1999).

Knowledge of the mineral composition of fruits is required for different purposes. They present the complete nutritional value content and demonstrate trends in agriculture. This element composition is used as a bio-indicator to monitor pollution trends (Fraga, 2005). The mineral and trace element contents of fruits are known to be affected by the culture of plant, soil conditions, weather conditions during the growing period, use of fertilizers and the state of the fruit maturity at harvest (Szefer at al., 2007; Ekholm et al., 2014; Giannenas et al., 2009; Järup, 2003). According to those factors Bulgarian fruits have specific mineral composition.

Contaminated food is for most people the main source of exposure to toxic elements. Heavy metals are dangerous because they tend to bio-accumulate. The study of toxic elements in Bulgarian fruit completes their elemental composition and shows differences in the ability of individual products to accumulate in cells. Furthermore, it is important to compare these levels with European health standards (EC 1881/2006). Our previous studies demonstrated that ICP-MS analysis provides a rapid analysis and possibility for simultaneous multi-element determination (Toncheva, 2014). Researches by other authors have shown that this technology dominates as the most suitable methodology for quantification in fruits (Zhang et al., 2014; Ekholm et al., 2014, Balabanova et al., 2016). The data on the mineral composition of Bulgarian fruits are scanty and their enrichment with ISP-MS analysis represents research interest.

The purpose of this investigation was to determine the contents of essential and potentially health risk trace elements in traditional Bulgarian summer fruits. The main objective of this study is also to evaluate by using the cluster analysis the similarity and distances of the investigated fruits based on the mineral content and some chemical characteristics.

Materials and methods

Plant material

The most popular fruits grown in Bulgaria were selected. Fruits such as cherry, morello, raspberry, nectarine peach, aronia, apple type "akane" and pear type "early gold" were analyzed. All samples were purchased from retail stores, supermarkets and market squares in Plovdiv area. Fruits were purchased at the peak of their season. A total of three subsamples weighing 0.2 – 0.5 kg were obtained of each food item. One pooled sample was prepared representing each item and the samples were prepared as for consumption, i.e. only the edible parts were analyzed. The apples

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and pears were peeled and core removed. All samples was dried, homogenized and stored at -18°C until analysis.

Analysis of moisture content and carbohydrate content

The moisture content of the fruit was determined by weighing the samples before and after drying at 97°C for 16 h (ISO 712:2009). The content of total soluble carbohydrates and monosaccharides was determined by the method of Schoorl (Luzkanov et al., 1994). A 10 g sample of research fruit was dissolved in water (t = 80°C, 30 min). Proteins there were precipitated with $Pb(CH_3COO)_2.3H_2O$ from Sigma Aldrich Chemie (Schnelldorf, Germany). The quantity of monosaccharides and total soluble carbohydrates (after hydrolysis) were determined with copper-alkaline solution.

Analysis of trace element contents in summer fruits

Sample preparation to determine the content of trace elements in the fruits was carried out by mineralization of organic matter of all samples with concentrated nitric acid HNO_3 and 30% H_2O_2 (Toncheva et al., 2014). Fruit samples (0.5 g) were mineralized with 5ml of cold concentrated nitric acid HNO_3 (Merck, Darmstadt, Germany). Then the sample was heated in plate for 60 min. To each sample after 20 min was added additionally 1ml concentrated nitric acid HNO_3. After cooling was added 3ml 30% H_2O_2 (Chimtex Ltd, Dimitrovgrad, Bulgaria) and the sample was thermally treated further for 50 min. Furthermore, it was cooled and quantitatively transferred into a flask of 50 ml and diluted with ultrapure water.

An ICP-MS system was used for simultaneous multi-element detection of chromium (Cr), manganese (Mn), iron (Fe), cadmium (Cd), nickel (Ni), copper (Cu) and arsenic (As). Quadrupole mass spectrometer was used with inductively coupled plasma ICP-MS Agilent 7700 (Tokyo, Japan), Oktopole Reaction System (ORS) and helium as a collision gas. Operating conditions for ICP mass spectrometer are shown in Table 1.

For the purpose of the analysis external calibration was made against aqueous multi-element standard solutions in the concentration range 10-1000 µg L^{-1}, prepared after appropriate dilution of the ICP multi-element standard solution VI (110580 Merck, Darmstadt, Germany). To control any non-spectroscopic multiplicative obstacles, monitoring signal Rh (CPAchem Bulgaria) was included, introduced as an internal standard for all test and standard solutions.

Table 1. Operating conditions for ICP-MS research

Plasma conditions		
1.	RF power	1.5 kW
2.	Argon plasma gas flow	15 $L.min^{-1}$
3.	Auxiliary gas flow	0.9 L. min^{-1}
4.	Nebulizer gas flow	0.95 L. min^{-1}
5.	Sample flow rate	0.34 mL. min^{-1}
6.	Nebulizer type	Micromist (Glass Expansion)
7.	Spray chamber	Scott double pass, Peltier cooled 2°C
8.	Interface cones	Ni
9.	Collision gas	He
	Collision gas flow	3.5 $mL.min^{-1}$
10.	Mass spectrometer settings	
11.	Resolution	Normal
12.	Acquisition mode	Peak hoping
13.	Channels per mass	1
14.	Dwell time	100 ms
15.	Replicate	5

Statistical analysis

The differences in metal content of the investigated fruits were proven by application of mathematical-statistical analysis. The data base for investigated types of fruits was composed of the average of three independent repetitions for each sample. The programme "SPSS" was used for their processing. The experimental data were estimated by using the average values for each investigated chemical element, standard deviation (s) and variation coefficients (CV,%). One way (ANOVA) dispersion analysis was made. The difference between all average values for the variants was estimated by test for the smallest differences (LSD) at significance levels α = 0.5, 0.1, 0.0, 0.01. The grouping of the variants was made by Dunkan test for multidirectional comparisons.

Hierarchical cluster analysis with a measure of similarity of Euclidean distance was used to compare the types of products on the basis of all investigated parameters (Ward, 1963; Duran and Odelle, 1977). In order to avoid the difference of the dimensions of the studied traits, the data were standardized. The results of the clustering are presented graphically via dendrogram.

Results and discussion

Raspberry, morello, cherry, nectarine peach, summer apple type "akane", pear type "early gold" and aronia, are the most famous Bulgarian fruit from the beginning of the summer season. Basic chemical parameters of these foods (Table 2) were investigated and variation analysis was made for the obtained data. The results for average value, standard deviation and the other statistical parameters are presented. We found that Bulgarian cherries contain 3% more total carbohydrates, apples and pears contain 25% more protein than the same fruits analyzed by american authors (Atkins, 1996). This difference can be explained by sortable and soil characteristics. Aronia contains the highest dry matter content, while the richest in protein is raspberry followed by cherry. The lowest content of total carbohydrates belongs to pear type "Early gold" (5.2% ± 0.1) and the highest belongs to cherry (14.2 ± 0.2%). Correlation between moisture content and trace element composition of the fruit was not established.

It is known that plant foods have rich element composition. Our research focuses on seven trace elements and a database of the trace element content of Bulgarian summer fruits was created (Table 3). Raspberry has the highest content of Fe, Mn and Cr. Daily consumption in 100 g of this Bulgarian foodstuff can provide the body 1% of provisional maximum tolerable daily intake (PTDI) of Fe as well as 4.5% of provisional maximum tolerable daily intake of Cr and 25 % of the Recommended Daily Allowance (RDAs) of Mn (WHO, 1996, Commission Directive 2008/100/EC).

Iron is a mineral that is naturally present in many foods. The biological role of Fe is determined by its ability to pass in different oxidation states. The iron in foods of plant origin is mostly present in the form of insoluble complexes of Fe^{3+} with phytic acid, phosphates, oxalates and carbonates. Therefore, it is involved in a number of vital processes, such as transport of oxygen, electron transfer, DNA synthesis, etc. (Shenkin, 2008). The content of this element in fruits

Table 2. Dry matter, protein and carbohydrate content in Bulgarian summer fruits

Type			Dry matter, %	Protein, %	Total carbohydrate, %	Mono-saccharides,%
1	Morello	$\bar{x}$	14.97[c]	1.43[c]	8.03[d]	8.03[e]
		$S_{\bar{x}}$	0.15	0.15	0.07	0.03
		S	0.15	0.25	0.12	0.06
		CV, %	1.00	17.48	1.49	0.75
2	Cherry	$\bar{x}$	18.53[d]	13.37[e]	14.23[f]	13.30[g]
		$S_{\bar{x}}$	0.26	0.12	0.15	0.17
		S	0.26	0.21	0.25	0.30
		CV, %	1.4	1.57	1.76	2.26
3	Aronia	$\bar{x}$	23.07[e]	1.03b	8.83[e]	8.80[f]
		$S_{\bar{x}}$	0.15	0.03	0.03	0.12
		S	0.15	0.06	0.06	0.20
		CV, %	0.65	5.83	0.68	2.27
4	Nectarine	$\bar{x}$	14.57[c]	0.70[a]	7.27[c]	4.40[a]
		$S_{\bar{x}}$	0.20	0.06	0.03	0.12
		S	0.20	0.10	0.06	0.20
		CV, %	1.37	14.29	0.83	4.55
5	Apple "akane"	$\bar{x}$	13.03[b]	0.50[a]	8.80[e]	6.93[d]
		$S_{\bar{x}}$	0.03	0.06	0.12	0.09
		S	0.03	0.10	0.20	0.15
		CV, %	0.23	20	2.27	2.16
6	Pear "early gold"	$\bar{x}$	12.07[a]	0.50[a]	5.20[a]	4.90[b]
		$S_{\bar{x}}$	0.09	0.06	0.06	0.06
		S	0.09	0.10	0.10	0.10
		CV, %	0.75	20	1.92	2.04
7	Raspberry	$\bar{x}$	18.67[d]	2.10[d]	6.50[b]	6.47[c]
		$S_{\bar{x}}$	0.15	0.06	0.12	0.09
		S	0.14	0.10	0.20	0.15
		CV, %	0.75	4.76	3.08	2.32

Means with the same letter are not significantly different

varies from 1300 μg kg^{-1} to 50000 μg kg^{-1}. The highest iron content has raspberry (4635.9 ± 53.2 μg kg^{-1}) followed by the morello (2825.30 ± 29.3 μg kg^{-1}) and the lowest - pear type "early gold" (227.3 ± 2.0 μg kg^{-1}). The Bulgarian raspberries are a richer source of iron than Polish fruits (Szefer et al., 2007). The investigated summer apple, nectarine and raspberries accumulate from the soil more iron than similar English products (Mayer, 1997). With the exception of raspberry all Bulgarian fruits have approximately the same content of Fe as the analogical products from Finland and China (Ekholm et al., 2007; Zhang et al., 2014).

Chromium enhances insulin action, it is essential for normal blood glucose and lipid metabolism (Ochiai, 2011). The highest level of chromium was found in raspberry (150.2 ± 2.5 μg kg^{-1}) and the smallest content – in the cherry (35.6 ± 0.2 μg kg^{-1}). According to Szefer and Grembecka the chromium content is in the range between 50 μg kg^{-1} and 180 μg kg^{-1}. All investigated fruits belong to this range.

Copper is an important essential element for human health. It is a component of many enzymes, participates in the iron metabolism and promotes the absorption of proteins and hydrocarbons (Ochiai, 2011). It is known that the fruits produced in Europe have copper content in the range of 240 to 650 μg kg^{-1}. The most of Bulgarian fruits are in the pointed interval. Highest content of copper was found in nectarine (887.5 ± 31.2 μg kg^{-1}), followed by raspberry (723.9 ± 9.4 μg kg^{-1}). The Bulgarian early apples, early pears and nectarines accumulate higher levels of copper, but lower iron content than that of analogues from Poland (Szefer et al., 2007). Morello is a poor source of this potentially essential element. It's significant to be mentioned that Cu above certain concentration established by regulations have negative effects on the human body (WHO, 1996). In our case, the foodstuffs we investigated do not contain excessive amounts of Cu.

In most Bulgarian fruits the content of essential trace element Mn is less than 1000 μg kg^{-1}. Aronia is a very good source of manganese (4117.4 ± 42.9 μg kg^{-1}). Daily consumption in 100 g of aronia can provide about 20% of provisional maximum tolerable daily intake of Mn. The same amount of morello can give the human body approximately 3% of the Recommended Daily Allowance (RDAs) of this element (WHO, 1996; Commission Directive, 2008). The Bulgarian pear "early gold", nectarine and raspberries are richer sources of manganese than the similar Polish fruits (Szefer et al., 2007).

The levels of the potentially health risk metals (Cd, Ni and As) are significantly low, being much less than or just about 0.5 mg/kg for Ni in all samples. Nickel content in our samples of necktarine and pear is lower than the Polish peach (1 mg kg^{-1}) and pear (1 mg kg^{-1}), but Polish aronia (120 μg kg^{-1}) is poorer in this element. Finnish raspberry samples (30 μg kg^{-1}) and peach (10 μg kg^{-1}) have slightly higher values of cadmium content than the Bulgarian fruit (Szefer et al., 2007; Ekholm et al., 2007). According to the European legislation

Table 3. Trace element content in traditional Bulgarian summer fresh fruits in µg/kg

Type			Cr	Fe	Ni	Mn	Cu	Cu	As
1	Morello	$\bar{x}$	93.63^{d}	2825.3^{d}	58.03^{a}	375.77^{a}	166.63^{a}	4.30^{d}	12.40^{f}
		$S_{\bar{x}}$	4.10	29.30	4.27	10.35	3.02	0.49	0.52
		S	7.10	140.18	7.40	17.93	5.24	0.85	0.9
		CV, %	18.26	4.97	12.75	4.77	3.14	19.63	7.26
2	Cherry	$\bar{x}$	35.6^{a}	458.2^{b}	12.80^{a}	218.60^{a}	631.33^{cd}	0.27^{a}	8.17^{e}
		$S_{\bar{x}}$	0.20	7.65	0.17	6.63	4.01	0.03	0.38
		S	0.70	13.25	0.30	11.49	6.95	0.06	0.65
		CV, %	1.97	2.92	2.34	5.26	1.1	22.22	7.96
3	Aronia	$\bar{x}$	83.3^{c}	924.4^{c}	326.40^{c}	4117.40^{c}	556.00^{c}	27.53^{e}	2.50^{c}
		$S_{\bar{x}}$	4.50	10.68	13.71	42.90	68.56	1.39	0.52
		S	7.80	18.50	23.75	108.18	118.74	2.40	0.9
		CV, %	9.36	2.00	7.28	2.64	21.36	8.72	36
4	Nectarine	$\bar{x}$	59.8^{b}	236.7^{a}	228.47^{b}	366.67^{a}	887.50^{f}	2.87^{cd}	0.23^{a}
		$S_{\bar{x}}$	2.66	12.79	49.10	8.42	31.19	0.38	0.03
		S	4.61	22.15	85.05	14.58	54.02	0.65	0.06
		CV, %	7.71	9.36	37.23	3.98	6.11	22.65	26
5	Apple "akane"	$\bar{x}$	54.5^{b}	573.9^{b}	66.37^{a}	344.05^{a}	464.00^{b}	0.10^{a}	2.10^{c}
		$S_{\bar{x}}$	0.69	14.38	4.40	2.97	2.75	0.00	0.21
		S	1.20	24.90	7.63	5.15	4.76	0.00	0.36
		CV, %	2.20	4.34	11.50	1.50	1.03	0.0	17.14
6	Pear "early gold"	$\bar{x}$	41.8^{a}	227.3^{a}	38.83^{a}	852.00^{b}	715.40^{de}	0.40^{ab}	1.03^{b}
		$S_{\bar{x}}$	1.30	2.10	3.67	28.12	8.89	0.06	0.09
		S	2.25	8.55	6.36	48.70	15.40	0.10	0.15
		CV, %	5.38	3.74	16.38	5.72	2.15	25.0	14.56
7	Raspberry	$\bar{x}$	150.2e	4635.9^{e}	344.7^{c}	5690.9^{d}	723.90^{e}	2.13^{bc}	5.93^{d}
		$S_{\bar{x}}$	2.50	53.2	1.36	31.70	9.47	0.20	0.47
		S	4.35	197.45	2.36	69.70	16.40	0.35	0.81
		CV, %	2.90	4.26	0.68	6.58	2.27	16.43	13.66

Means with the same letter are not significantly different

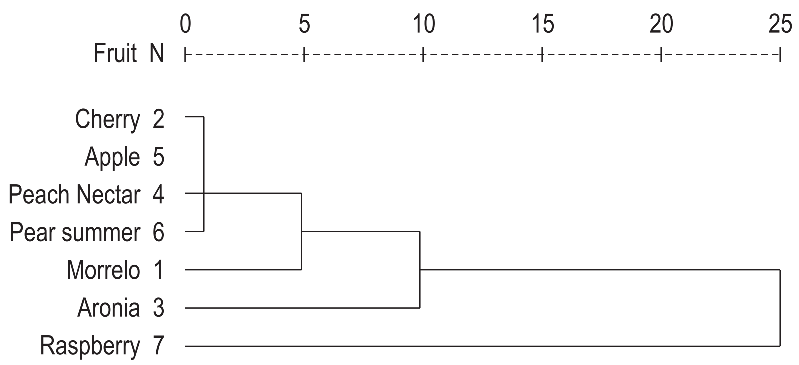

Figure 1. Hierarhical cluster analysis. Dendrogram using average distance between groups.

the permissible levels of toxic elements such as Cd, Ni and As in fruits is 0.05 mg kg^{-1}, 0.5 mg kg^{-1} and 0.5 mg kg^{-1} respectively (EC, 1881/2006, BG 31/ 29 July 2004). The content of the above elements in Bulgarian fruits from the Plovdiv region is significantly below these standards. The existence of statistically important differences in the content of the considered metals in summer fruits was examined through one-way analysis of variance. Statistically significant differences between the different types of fruits were found. They were measured by Duncan analysis (Figure 1).

Each chemical element was evaluated separately. The investigated samples are divided into 7 groups: the first is made of fruits which are with the smallest content of the respective metal (group a), followed by the group with higher content (group b). Fruit which has the highest statistically significant differences of the corresponding element falls within group g. Some fruits belong to two successive groups which is statistically possible.

Duncan's test arranges summer fruits according to iron content in 5 groups (Table 3). The pear „early gold" and nectarine are poor in Fe (group a), followed by cherry and apple type „akane" (group b). The raspberry (group *e*) is the most distant from all investigated samples, followed by morello (group d). According to its Cd content, Ni-content and As-content, the fruits are divided into 5 groups, 3 groups, 6 groups, respectively. Regarding the content of manganese and chromium fruits are statistically divided into four and five groups. Duncan's test shows that the fruits are statistically different according to content of Fe, Cu, Ni, Cd, Cr, Mn and As. According to Ni and Mn, cherry, morello and apple type akane belong to one group (a). According to the other chemical parameters, all studied variants are statistically different and nectarine, apple type „akane“, pear „early gold" belong to the same group (a).

Conclusion

Bulgarian early summer fruits are a reliable source of essential micronutrients – iron, chromium and manganese. From our foodstuffs, raspberry has the highest content of these elements. The content of potentially health risk elements in the products is considerably below European norms. The lowest content of Ni, As and Cd has the cherry. Duncan test for multidirectional comparisons demonstrates that of five of the investigated seven elements: Fe, Cu, Ni, Cd and As fruits have distinguishable particles. About content of Cr and Mn, all fruit are statistically indistinguishable. According to the determined contents of the elements a dose of consuming amounts should be implemented in accordance with the Recommended Daily Allowance (RDAs) regulated with Directive 2008/100 of the Commission of the European Communities.

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Abstract should be not more than 350 words. It should be clearly stated what new findings have been made in the course of research. Abbreviations and references to authors are inadmissible in the summary. It should be understandable without having read the paper and should be in one paragraph.

Keywords: Up to maximum of 5 keywords should be selected not repeating the title but giving the essence of study.

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Discussion: The objective of this section is to indicate the scientific significance of the study. By comparing the results and conclusions of other scientists the contribution of the study for expanding or modifying existing knowledge is pointed out clearly and convincingly to the reader.

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Todorov N and Mitev J, 1995. Effect of level of feeding during dry period, and body condition score on reproductive performance in dairy cows,IXth International Conference on Production Diseases in Farm Animals, September 11–14, Berlin, Germany.

Thesis:

Hristova D, 2013. Investigation on genetic diversity in local sheep breeds using DNA markers. Thesis for PhD, Trakia University, Stara Zagora, Bulgaria, (Bg).

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