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DIFFERENTIATION OF BULGARIAN WILD FRUITS ACCORDING TO THEIR MINERAL CONTENT

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Abstract

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The content of 12 essential and toxic elements (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Pb) in Bulgarian forest fruits as bilberry (*Vaccinium myrtillus*), lingonberry (*Vaccinium vitis-idaea*), cornel (*Cornus mas*) and howthorn (*Crataegus laevigata*) was investigated. The highest content of Zn was found in cornels (10.67±0.04 mg kg^{-1}), while the richest in Fe is lingonberry (37.1±0.3 mg kg^{-1}), followed by the hawthorn (10.42±0.05 mg kg^{-1}). Content of toxic metals (Pb, Ni) are in amount significantly below the permissible standards. The lowest content of total carbohydrates belongs to hawthorn (5.9±0.1%), while the highest in cornels (11.2±0.1%). The differences in metal content of the investigated fruits were proven by application of mathematical-statistical analysis. Discriminant analysis was used to define the fruit and to estimate the significance of individual indexes in modelling. Canonical variables, included by degree of importance in discriminant functions for examined group of wild edible fruits are Fe, Mn, Cu, and Zn.

Key words: trace element content, wild fruits, ICP-MS, discriminant analysis

Introduction

Large number of wild and cultured fruits from different countries (Australia, Finland, Canada etc.) have been investigated because of their rich content of vitamins, proteins and minerals (Konczak and Roulle, 2011; Miller et al., 1993; Brand et al., 1983). Wild fruits are investigated mostly for the content of phenolic compounds, organic acids, vitamin C, flavonoids, antioxidant stability etc. (Hertog et al., 1994; Ishige et al., 2001; Kiselova et al., 2006; Alexieva et al., 2012). It is well known that the fruits are important for assuring of microelements in different diets, for preparation of confectionary and drinks, and also for activation of proteins in the human body (Bertini et al., 2001). The addition of forest fruits to the composition of cakes and drinks enriches their mineral content. The last action is possible due to the presence of substantial quantities of microelements in them. Some of microelements in fruits are essential as Fe, Cu, Co, Mn, Se, Mo, Ni and Zn, others as Pb and As are bad for the human health (Ochiai, 2011; WHO, 1996; Kozlowski et al., 2009).

Mineral content of forest fruits depends on many factors such as product type, climate, region of flourishing, climatic conditions, soil and more. Geological origin of the soil also affects their mineral composition and it varies according to different countries (Sanches-Castillo et al., 1998; Ekholm et al., 2007). These facts suggest that the Bulgarian forest fruits also have specific trace element composition. Quantitative

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assessment of this mineral composition is of interest to food chemistry both in terms of establishing the levels of content and comparing these levels with European health standards ((EC) 1881/2006, BG 31/ 29 July 2004).

The aim of this work is to create a database for the content of essential and toxic elements in typical Bulgaria forest fruits of lingonberry, bilberry, cornel and hawthorn as well as to examine the possibility for application of mathematical - statistical modelling of different groups of wild berries.

Materials and Methods

Plant material

Forest fruits as bilberry *(Vaccinium myrtillus),* lingonberry *(Vaccinium vitis-idaea)*, cornel (*Cornus mas*) and hawthorn (*Crataegus laevigata)* were purchased from Bulgarian markets at the peak of their season. Randomly four different samples from each type of berries were taken. The samples were frozen and were stored at –20°C until analysis. Before the analysis the samples were thawed and in hawthorn and cornels the pits were removed with plastic knife.

Analysis of moisture content and carbohydrate content

The moisture content of the fruit was determined by drying at 105°C to constant weight (ISO 712:2009). The content of total soluble carbohydrates and monosaccharides was determined by the method of Schoorl (Luzkanov et al., 1994). 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.

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

Analysis of trace element contents in wild forest fruits

Sample preparation to determine the content of trace elements in the fruits was carried out by mineralization with concentrated nitric acid HNO3 (Merck, Darmstadt, Germany) and with 30% H2O2 (Chimtex Ltd, Dimitrovgrad, Bulgaria) (Toncheva et al., 2014).

An ICP-MS system was used for simultaneous multi-element detection of vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), molybdenum (Mo) and lead (Pb). Quadrupole mass spectrometer was used with an 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 nonspectroscopic multiplicative obstacles, monitoring signal Rh (CPAchem Bulgaria) was included, introduced as an internal standard for all test and standard solutions. The data of measurements are presented as the mean value of eightfold replicates ± expanded uncertainty.

Statistical analysis

The data base for considered four types of berries was derived from four independent samples with a repetition of each of them twice. In the experimental data averaged results of 8 repetitions of the experiment are presented. The programme “Statistica” was used for their processing. The distribution of considered indicators was established with the criterion of Kolmogorov-Smirnov. The distribution is normal and for the establishment of statistically significant differences between the indicators of different types of fruit the criterion of Tukey for multiple comparisons was applied.

Stepwise linear discriminant analysis with a priori equal probabilities for goal in the groups was used for modeling groups of fruits (Bondar and Statyukha, 1976; Lakin, 1990; McLachlan, 1992; Vandeginste, 1998).

Results and Discussion

Table 2
Moisture and carbohydrate content in Bulgarian wild forest fruits

Fruits	Bilberry	Cornels	Lingonberry	Hawthorn
Moisture content,%	83.3±0.8	81.4±0.7	85.4±0.7	68.1±0.7
Total carbohydrate content,%	7.9±0.2	11.2±0.1	9.0±0.1	5.9±0.1
Monosaccharides content,%	7.1±0.2	11.0±0.3	8.8±0.2	5.2±0.2
Total sugar content,%	0.8±0.2	0.2±0.1	0.2±0.1	0.7±0.1

The wild berries studied – bilberry, cornels, lingonberry, hawthorn are typical for Bulgaria. For their complete characterization basic chemical parameters (Table 2) were investigated. We found that Bulgarian red berries and lingonberries contain quantity of total carbohydrates similar to that found in them by another authors [Atkins, 1996]. The lowest content of total carbohydrates belongs to *Crataegus laevigata* (5.9%±0.1) and the highest – *Cornus mas* (11.2±0.1%). Furthermore the investigated fruits *Crataegus laevigata* has also the lowest moisture. Correlation between moisture content and trace element composition of the fruit was not established.

Wild fruits can be used to control the pollution trends in the environment. Database the trace elemental content in Bulgarian wild forest fruits was created (Table 3). The toxic trace elements content are discussed together with the benefits of the essential elements for human health.

The elements arsenic (As), selenium (Se), vanadium (V) and cobalt (Co) are not included in Table 3, since in all the fruits traces were found with content less than 300 μg kg^{-1} for arsenic (As) and selenium (Se) and less than 50 μg kg^{-1} for vanadium(V) and cobalt (Co) respectively.

The highest content of zinc (Zn) in investigated forest fruits was found in *Cornus mas* (10.67±0.04 mg kg^{-1}). Zinc content in cornel is about twice less than in raspberry (20 mg kg^{-1}), identified as the richest source of this trace element between all forest fruits in Finland (Ekholm et al., 2007). However from the same study can be seen that Zn content in Bulgarian lingonberry is 2.5 times poorer than the one in Finland. The samples from Finland contain Zn about 8 mg kg^{-1}.

Iron (Fe), a physiologically essential element, is important to haemoglobin in red blood cells which transports oxygen from the lungs in body tissues. Iron (Fe) content in the examined forest fruits from Bulgaria ranges from 3.95±0.08 mg kg^{-1} to 37.1 ±0.3 mg kg^{-1}. Similar iron content is observed for wild fruits in Mexico (from 4 mg kg^{-1} to 44 mg kg^{-1}) (Sanchez-Castillo, 1998). The highest iron content from investigated fruits is found in *Vaccinium vitis-idaea* (37.1±0.3 mg kg^{-1}), followed by *Cornus mas* (10.42±0.05 mg kg^{-1}). This makes referred berries suitable for consumption by people with anaemia.

Cobalt as a part of vitamin B_{12} is necessary for the production and function of cells. Cobalt and nickel in minimal doses are beneficial to human health, but at high levels they are toxic. Australian wild fruits contain cobalt (Co) from 16 μg kg^{-1} to 84 μg kg^{-1} (Konczak, 2011), while in Bulgarian berries it is less than 50 μg kg^{-1}.

The permissible level of lead in forest fruits according the European legislation is 0.2 mg kg^{-1} [(EC) 1881/2006, BG 31/ 29 July 2004]. The Australian fruits contents between 40 μg kg^{-1} and 2080 μg kg^{-1} lead (Konczak, 2011), while those from Finland contain between 50 μg kg^{-1} and 160 μg kg^{-1} (Ekholm et al., 2007). The highest content of lead in Bulgarian forest fruits was found in bilberry – 194±8 μg kg^{-1}, and the lowest – the hawthorn – about 114±13 μg kg^{-1}. The content of lead (Pb) in Bulgarian forest fruits is according permissible level in the European legislation. It is slightly higher than that of the Finland

Table 3
Trace element content in Bulgarian wild forest fruits

Element	Bilberry	Cornels	Lingonberry	Hawthorn
	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}	μg kg^{-1}
Cr	75±2	78±3	121±13	72 ±8
Fe[a]	5.83±0.02	3.95±0.08	37.1±0.3	10.42 ±0.05
Mn	20.1±0.5[a]	418±27	35.9±0.3[a]	3.45 ±0.03[a]
Ni	189±8	566±11	100±21	110±28
Cu[a]	2.13±0.03	1.22±0.03	1.49±0.06	1.25±0.04
Zn[a]	9.83±0.08	10.67±0.04	2.59±0.05	1.37±0.04
Mo[a]	2.2±0.1	1.69±0.02	1.5±0.2	1.49±0.2
Pb	194±8	150±11	125±8	114±13

[a] – concentration in mg kg^{-1}

Table 4
Content of Fe, Cu, Zn and Mn at 100 g Bulgarian forest fruits as a percentage of the RDA

Element	RDA,	Bilberry		Cornels		Lingonberry		Hawthorn	
	mg	mg 100g^{-1}	%	mg 100g^{-1}	%	mg 100g^{-1}	%	mg 100g^{-1}	%
Fe	14.0	0.583	3.5	0.395	2.8	3.700	24.2	1.040	7.4
Zn	10.0	0.983	9.8	1.017	10.7	0.259	2.6	0.137	1.4
Cu	1.0	0.213	21.3	0.149	14.9	0.125	12.5	0.125	12.5
Mn	2.0	2.010	100.0	0.042	2.0	3.590	179.5	0.345	17.3

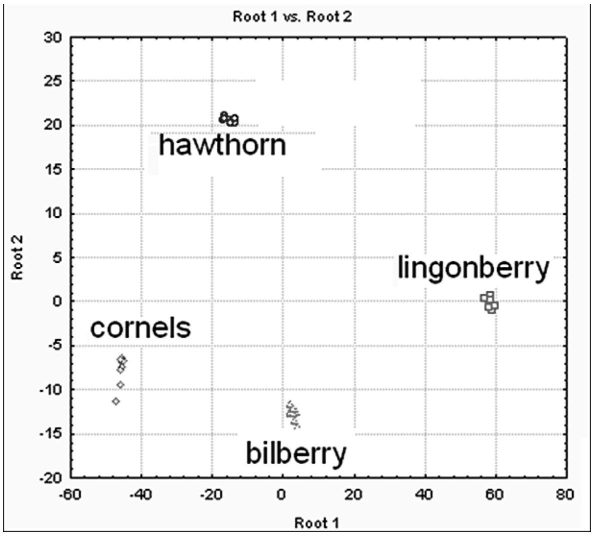

Figure 1. Placement of wild berries by the first two canonical variables

fruits, but it is lower than the lead (Pb) content in the fruits from Australia and Mexico (Sanchez-Castillo, 1998).

The facts before mean that the Bulgarian forest fruits from the region of Velingrad contain toxic metals (Pb, Ni) in amount significantly below the permissible standards. That results proof that they are ecologically clean.

Bulgarian *Vaccinium myrtillus* is a very good supplier of Mn (Table 4). The organism needs manganese for number of metabolic processes. The consumption of 100 g product provides about 100% of the RDA. And consumption of 100 g of *Vaccinium vitis-idaea* provides 20% of the RDA of iron and more than 100% of the RDA of manganese [CD 2008/100/EC].

The existence of statistically important differences in the content of considered metals in forest fruits is examined through the criterion of Tukey. Statistically significant differences between the different types of wild berries were found, which give rise to modelling them. The incremental linear discriminant analysis was applied with grouping variable „type forest fruit“. Linear discriminant functions were obtained by "Backward stepwise method", assuring 100% readability of each fruit. In order of importance for modeling metals involved in are: Fe, Mn, Cu and Zn. The coefficients of discriminant functions (Table 4) allow to determining the affiliation of an unknown sample of berries to some of indicated groups.

Canonical analysis was applied for better representation of the data. The results are presented on Figure 1.

To further clarify the differences between the various groups berries Mahalanobis distances were determined between centroids of individual groups represented in Table 5.

Matrix of distances given in Table 5 is asymmetric because above the main diagonal the distances between groups are presented, provided that the discriminant function includes all parameters and data below the main diagonal provided that the four most important parameters selected by the algorithm are included. This explains the larger values above the main diagonal than below it.

Selecting four parameters as the main of the algorithm is motive to distinguish berries only by these four minerals and reduce the number of necessary measurements.

Mahalanobis distances are more indicative for the different groups than presenting then canonical variables (which are mostly used for visualization) because they give an idea of the dynamics of change of the distance between the centroids of the individual groups. In examining the contents of all studied

Table 5
Discriminant functions describing wild berries

	Hawthorn	Lingonberry	Cornels	Bilberry
Fe	0.0020	0.0155	–0.0010	0.0070
Mn	0.0239	0.1964	–0.0298	0.0797
Cu	0.0405	0.2351	–0.1224	0.0368
Zn	–0.0113	–0.1234	0.1563	0.0530
Constant	–70.6690	–3833.7318	–52.3414	–1121.0963

Table 6
Mahalanobis distances for samples of hawthorn, cornels, lingonberry and bilberry

		Hawthorn	Lingonberry	Cornels	Bilberry
1	Hawthorn	–	7777	2740	2303
2	Lingonberry	6714	–	15197	4491
3	Cornels	1996	12502	–	3550
4	Bilberry	1651	3734	2741	–

minerals in selected berries closest-located groups are the hawthorn and bilberry, followed by hawthorn and cornels and most remote, are lingonberry and cornels (Table 6)

Conclusions

The analysis of mineral composition of wild edible fruits (hawthorn, cornel, bilberry and lingonberry) by discriminant analysis revealed the possibility of their arrangement in separate groups according to trace element content. The lowest content of total carbohydrates in the studied Bulgarian fruits belongs to hawthorn (5.9±0.1%), while the rich in carbohydrates is cornels (11.2±0.1%). Determining parameters to distinguish different groups of wild fruits according to their trace element content are Fe, Mn, Cu and Zn. According to four specified parameters in modelling berries closest-located groups are the hawthorn and bilberry, followed by hawthorn and cornels, and most remote is lingonberry and cornels.

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