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Mathematical analysis of the trace element content of Bulgarian fruits

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The content of 8 essential and toxic elements (Cr, Mn, Fe, Ni, Cu, As, Cd, Pb) in most famous Bulgarian fruits as strawberry, white cherry, peach, apricot, green apple, pear, blackberry, fig, prune, green and blue-black grapes, melon, watermelon, quince and pumpkin were investigated. The highest content of Fe was found in quince (35.7±0.2 mg kg^{-1}), followed by green grapes (16±0.2 mg kg^{-1}). Strawberry was rich in Mn (10.5±0.1 mg kg^{-1}), while green grapes had the highest content of Cu (921±2 µg kg^{-1}). The contents of toxic metals Ni, As and Cd were significantly below the maximum levels for contaminants in food. Excessive lead content was observed in strawberry (369±1 µg kg^{-1}) and fig (181±2 µg kg^{-1}). K-Cluster analysis with three groups (K = 3) was carried out. The first cluster was occupied with white grapes, the third cluster included white cherry, melon and watermelon, and all the other fruits were in the second cluster. The differences in metal content of the investigated fruits were proven by application of statistical analysis. Discriminant analysis was used to obtain a model of Bulgarian fruits which contained the following canonical variables ordered by level of significance - Cr, Fe, Mn, Cu, Cd and Pb.

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

INTRODUCTION

Fruits are very vital components of human nutrition. They are good sources of fiber, minerals, carbohydrates, organic acids, enzymes and vitamins, contain more than 20 different minor and trace elements. Most of them like Mn, Cu, Fe and Cr, are mostly cofactors of many enzymes and thus have very important role in several physiological functions of humans, they facilitate normal metabolism and growth. Any deficiency of these essential elements causes disturbances in the whole physiological system [1, 2].

The amount of metals in fruits is normally very small, but their contents can be significantly altered according to soil, air pollution and resulting from manufacturing and packaging processes [3, 4]. Furthermore metals such as Cd, Pb, Cr and Ni may contaminate the environment and thus into fruits at various levels causing health problems [5]. For some metals effects on the human body are cumulative and it is necessary to control their level in the food [6]. Thus for monitoring of fruits quality in manufacture and trade good measurements are always required.

Different techniques have been employed for carrying out the determination of trace elements in fruits across the world namely: stripping potentiometry [7], flame furnace atomic absorption spectrometry [8, 9], flow injection spectrometric methods [10], atomic fluorescence spectrometry [11], capillary zone electrophoresis [12], inductively coupled plasma mass spectrometry (ICP-MS) [13, 14].

ICP-MS is a methodology which provides a rapid analysis and possibility for simultaneous multi-element analysis. This technology dominates as the most suitable methodology for quantification in fruits according researches by other authors [3, 15]. Our experience to study the wild fruits [16] also shows that it gives satisfactory results.

Because the data on the element composition of traditional Bulgarian fruits are limited, in this work using ICP-MS technique we determined 8 elements in 15 most common Bulgarian fruits. Mathematical model of the composition was developed.

MATERIALS AND METHODS

Plant material

The most popular fruits available in Bulgaria were selected. Foodstuffs as strawberry, white cherry, peach, apricot, green apple, pear, black berry, fig, prune, green and blue-black grapes, melon, watermelon, quince and pumpkin were analyzed. All products are purchased from retail stores, supermarkets and market squares in Plovdiv areas. Fruits were purchased at the peak of their season. A total of three subsamples weighing 0.2-0.3 kg were obtained of each food item. Randomly from them one sample representing each fruit was arranged. Before analysis all the products were washed with ultrapure water to remove the dust and soil particles and the fertilizers residues.

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The samples for analysis were prepared ready for consumption, i.e. only the edible parts were analyzed.

For element content determination all samples were dried (18 h at 80 ^oC) and after homogenization were stored at -18 ^oC. Prior instrumental analysis, the dried fruit (~0.5 g) were treated on hot plate with concentrated HNO_3 (Merck, Darmstadt, Germany) and 30% H_2O_2 (Chimtex Ltd, Dimitrovgrad, Bulgaria) according the procedure described in ref. [16].

Analysis of moisture content and carbohydrate content in fruits

The moisture content of the fruit was determined by weighing the samples before and after drying at 97 ^oC for 16 h (ISO 712:2009). The content of total soluble carbohydrates and monosaccharides was determined by the method of Schoorl [17].

Determination of element contents in fruits

An inductively coupled plasma quadrupole mass spectrometer (Agilent 7700 Tokyo, Japan), with an octopole reaction system (He collision gas) was used for simultaneous multi-element detection of Cr, Mn, Fe, Cd, Ni, Cu, As and Pb in sample solutions. Operating conditions for ICP mass spectrometer are the same as in our previous publication [16]. The calibrants in concentration range 10-1000 μg L^{-1} were prepared from ICP multi-element standard solution VI (110580 Merck, Darmstadt, Germany) after appropriate dilution in 0.1 % (v v^{-1}) HNO_3. Rhodium (CPAchem Bulgaria) was used as an internal standard for correction of both matrix effect and instrumental drift.

Statistical analysis

Program “Statistica” was used for the data processing. The data distribution was normal according to the criterion of Kolmogorov - Smirnov [18, 19]. Discriminant analysis with a priori equal probabilities was used for modeling groups of fruits [20, 21]. K-means clustering was applied for comparing the Bulgarian fruits. [22, 23]. This method classifies data through fixed apriori number of clusters [24].

RESULTS AND DISCUSSION

Dry matter, protein and carbohydrate content of fruits

Table 1 shows basic chemical parameters of Bulgarian fruits. Their profiles in the fruits are typical for these types of foods [25, 26]. Slightly higher levels of the protein content in grape, cherry and apple were observed. This difference can be explained by sortable and soil characteristics. A green grape contains the highest carbohydrate content (16.7%±0.1) and proteins (1.1%±0.1). Correlation between moisture content and trace element composition of the fruit was not established.

Element contents in fruit samples

The element contents with corresponding combined uncertainties (U) - in fresh fruits are summarized in Table 2. The studied fruits green and red grapes have the richest composition in terms of essential elements. These foodstuffs have a high content of Fe, Mn, Cr and Cu. The main trends in Bulgarian fruits are similar to those observed by other authors [3, 4, 27]. The highest content of any elements of all fruits was shown by Fe.

Iron is essential trace element. Iron participates in two important processes in body such as transport of oxygen and electron transfer, due to iron-binding proteins [1]. Iron content in the examined Bulgarian fruits ranges from 1.03±0.02 mg kg^{-1} to 35.7±0.2 mg kg^{-1}. Similar iron content (from 2 mg kg^{-1} to 48 mg kg^{-1}) is observed for fruits by Finland and Poland [3, 4]. The investigated grapes, prunes and strawberries accumulate from the soil more Fe than similar English products [27]. The highest Fe content was found in quinces (35.7±0.2 mg kg^{-1}), followed by green grapes (16±0.2 mg kg^{-1}). Daily consumption of 100 g Bulgaria quinces provides approximately 5% of the Recommended Daily Allowance (RDAs) for Fe. This value for consumption of 100g Bulgarian grapes is approximately 2.5% of RDAs [28].

Chromium is a mineral that humans require in trace amounts to enhance the action of insulin. The highest Cr content was found in blue-black grapes (220±3 μg kg^{-1}) and the smallest - in melons (15±0.3 μg kg^{-1}). Bulgarian and Chinese strawberries have similar content of this metal. Bulgarian strawberries have a low content of Cr (54±0.8 μg kg^{-1}) compared to these by China (88.3±4.5 μg kg^{-1}) [15]. Daily consumption of 100 g Bulgaria blue-black grape provides approximately 10% of the Recommended Daily Allowance (RDAs) for Cr.

The amount of the essential elements Mn and Cu is in the range from 0.8 mg kg^{-1} to 10.5 mg kg^{-1} for Mn and in the range from 140.0 μg kg^{-1} to 921 μg kg^{-1} for Cu.

Bulgarian fruits have approximately the same content of Mn as the analogical products from Finland and Poland, while the content of copper is lower than those analogs [3, 4]. The content of toxic metals Ni, As and Cd were in amount significantly below the maximum levels for contaminants in food. Excessive lead content was observed in strawberry (368.6±10.5 μg kg-1) and fig (180.7 ±20.5 μg kg-1) [29, 30].

Table 1. Dry matter, protein and carbohydrate content in Bulgarian fruits

№	**Fruits**	**Dry matter**, %	**Protein**, %	**Total carbo-hydrate**, %	**Mono-saccharides**, %	**Total sugar**, %
1	Strawberries	8.35±0.19	0.7±0.1	8.2±0.1	6.3±0.1	1.9±0.1
2	Cherries white	17.00±0.12	0.9±0.1	14.5±0.2	13.4±0.2	0.9±0.1
3	Blackberries	15.50±0.10	0.9±0.1	6.3±0.1	6.1±0.1	0.2±0.1
4	Peaches	17.09±0.21	0.7±0.1	8.7±0.1	3.2±0.1	5.5±0.1
5	Apricots	14.24±0.25	0.6±0.1	7.3±0.1	4.4±0.1	2.9±0.1
6	Green apples	13.17±0.13	0.5±0.1	8.8±0.2	6.9±0.1	1.9±0.1
7	Pears	12.14±0.15	0.5±0.1	5.1±0.1	4.9±0.1	0.2±0.1
8	Figs	21.05±0.10	0.9±0.1	13.5±0.1	13.0±0.1	0.5±0.1
9	Prunes	21.56±0.15	0.4±0.1	10.3±0.1	9.1±0.1	1.2±0.1
10	Green grapes	19.30±0.12	1.1±0.1	16.7±0.1	16.6±0.1	0.1±0.1
11	Blue-Black grapes	19.00±0.15	1.1±0.1	14.0±0.1	13.7±0.1	0.3±0.1
12	Watermelons	9.24±0.10	0.6±0.1	6.6±0.1	3.1±0.1	3.5±0.1
13	Melons	7.98±0.10	0.3±0.1	4.9±0.1	2.1±0.1	2.8±0.1
14	Quinces	16.65±0.15	0.4±0.1	9.1±0.1	8.4±0.1	0.7±0.1
15	Pumpkin	23.91±0.14	1.1±0.1	4.4±0.1	1.4±0.1	3.0±0.1

Table 2. Trace element content in Bulgarian fruits

		Cr		**Fe**		**Mn**		**Ni**		**Cu**		**As**		**Cd**		**Pb**	
№	Fruits	**Conc. µg/kg**	±U	**Conc. mg/kg**	±U	**Conc mg/kg**	±U	**Conc µg/kg**	±U	**Conc µg/kg**	±U	**Conc. µg/kg**	±U	**Conc. µg/kg**	±U	**Conc µg/kg**	±U
1	Straw-berries	**54**	0.8	**5.08**	0.08	**10.5**	0.1	**26.3**	0.3	**140**	1	**1.9**	0.04	**8.2**	0.1	**369**	1
2	Cherries white	**57**	0.2	**1.30**	0.01	**0.91**	0.02	**46.9**	0.7	**631**	2	**8.2**	0.2	**0.3**	0.02	**103**	1
3	Black-berries	**70**	0.5	**8.57**	0.10	**3.19**	0.07	**99.0**	0.2	**663**	4	**5.9**	0.3	**4.8**	0.3	**61**	0.5
4	Peaches	**120**	0.2	**7.20**	0.10	**3.90**	0.03	**163**	4	**128**	1	**2.0**	0.1	**4.4**	0.1	**64**	1
5	Apricots	**87**	0.6	**1.58**	0.07	**2.48**	0.02	**152**	3	**851**	9	**0.2**	0.03	**2.7**	0.1	**68**	0.1
6	Green apples	**61**	0.5	**6.87**	0.02	**2.24**	0.03	**35.6**	0.1	**297**	2	**0.7**	0.01	**0.4**	0.02	**28**	0.6
7	Pears	**87**	0.9	**13.2**	0.2	**2.27**	0.02	**91**	2	**838**	8	**3.0**	0.2	**2.1**	0.01	**95**	1
8	Figs	**41**	0.3	**11.2**	0.2	**2.85**	0.02	**169**	2	**255**	2	**3.3**	0.2	**5.9**	0.3	**181**	2
9	Prunes	**55**	0.5	**13.9**	0.1	**2.32**	0.02	**90**	1	**451**	3	**14.6**	0.5	**1.9**	0.1	**110**	1
10	Green grapes	**126**	1	**16**	0.2	**8.2**	0.1	**146**	1	**921**	2	**2.5**	0.1	**3.2**	0.04	**58**	0.3
11	Blue-Black grapes	**220**	3	**10.6**	0.1	**5.8**	0.1	**134**	2	**751**	9	**6.0**	0.2	**4.6**	0.05	**88**	0.5
12	Water-melons	**33**	1	**1.03**	0.02	**1.01**	0.02	**42.2**	0.4	**202**	1	**2.1**	0.1	**5.2**	0.1	**102**	1
13	Melons	**15**	0.3	**1.04**	0.08	**0.99**	0.01	**40.3**	0.4	**487**	2	**0.9**	0.07	**1.3**	0.1	**34**	2
14	Quinces	**128**	1	**35.7**	0.2	**2.07**	0.02	**172**	1	**287**	2	**2.3**	0.09	**6.1**	0.1	**42**	0.2
15	Pumpkin	**74**	1	**9.3**	0.2	**0.81**	0.01	**177**	1	**567**	3	**6.8**	0.2	**12.2**	0.1	**133**	2

The Scheffe criterion shows significant statistical differences in the studied fruits. The availability of these differences in mineral content in the fruits provides the reason for a subsequent modeling of its origin. A linear discriminant analysis was applied with grouping parameter "type of Bulgarian fruit". The obtained model includes the following parameters arranged as Cr, Fe, Mn, Cu, Cd, and Pb. Cluster analysis was applied with the method of K –means clusters. Most suitable was the grouping in 3 clusters. The fruits included in them had similar values of trace element content, which differentiate them from the products in the other clusters. In Table 3 is shown product participation in each of the 3 clusters and also the percentage distribution of the fruits into the three clusters.

The first cluster contains only green grapes, because this product is the richest of essential elements. The second contains the predominant part of the products. The third cluster contains four fruits, which have almost equal content of the Ni, Fe and Mn - watermelon, melon, apricot and white cherry.

Canonical analysis was performed for a better visualization of the results. The groups of the products are presented in Figure 1. The first two canonical variables are crucial for model.The figure confirms our hypothesis for the presence of significant differences between the separate groups of fruits. The analysis of the Mahalonobis distances (Table 4) between the three groups shows that the second and the third group are close to each other and are relatively far from the group of the white grape. If we trace the projections of the clouds of the various groups upon the first canonical variable (which plays important role in discrimination of the groups) – the white cherry, apricot, melon, water melon are projected on the positive, while the fruits of the second group are projected close to zero in the negative direction. The white grape is projected in the negative direction.

Table 3. Distribution of type summer fruits in clusters

Fruits in clusters	Number of fruits	Distribution of the fruits, %
1	2	3
Green grapes	1	7.7
Blackberry, peach, apple, pear, black grapes, pumpkin, fig, prune	8	61.5
White cherry, apricot, melon, water melon	4	30.8

Table 4. The mahalonobis distances for different fruits groups

Mahalanobis distances			
Groups	G_1	G_2	G_3
1	2	3	4
G_1	0.00	83.54	218.69
G_2	83.54	0.00	58.44
G_3	263.81	58.44	0.00

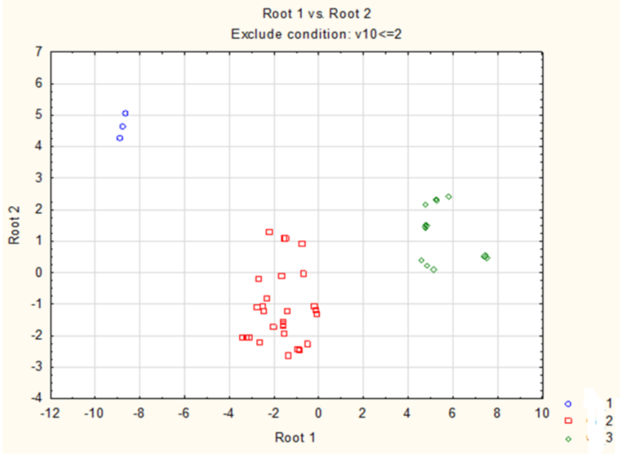

Figure 1. Disposition of the groups of fruits in the plane of the first two canonical variables

CONCLUSION

The highest Fe content in Bulgarian fruits was found in quinces (35.7±0.2 mg kg^{-1}), followed by green grapes (16±0.15 mg kg^{-1}). Differences in the values of trace element content for different groups of fruits were statistically proven. This makes efficient the use of the discriminant analysis for qualitatively differentiate of various fruits. The content of the elements Cr, Fe, Mn, Cu, Cd and Pb determines the specificity of Bulgarian fuits.

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МАТЕМАТИЧЕСКИ АНАЛИЗ НА СЪДЪРЖАНИЕТО НА МИКРОЕЛЕМЕНТИ В БЪЛГАРСКИ ПЛОДОВЕ

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(Резюме)

В състава на най-известните български плодове - ягода, бяла череша, праскова, кайсия, зелена ябълка, круша, къпина, смокиня, синя слива, бяло и черно грозде, диня, пъпеш, дюля и тиква е определено съдържанието на микроелементи (Cr, Mn, Fe, Ni, Cu, As, Cd, Pb). Най-високо съдържание на Fe е установено за дюля (35.7±0.2 mg kg^{-1}), следвана от бяло грозде (16±0.2 mg kg^{-1}). Ягодата е богата на Mn (10.5±0.1 mg kg^{-1}), докато бялото грозде показа най-високо съдържание на Cu (921 ±2 μg kg^{-1}). Съдържанието на токсични метали Ni, As и Cd е в количества значително по-ниски от максимално допустимите нива на замърсители в храни. Наблюдава се завишено съдържание на олово в ягода (369±1 μg kg^{-1}) и смокиня (181±2 μg kg [1]). Направен е К-клъстерен анализ с три групи (K = 3). Първият клъстер съдържа бяло грозде, третият включва бяла череша, пъпеш и диня, а всички останали плодове са във втория клъстер. Разликите в съдържанието на микроелементи са проверени с прилагане на дискриминантен анализ. Математическият модел на елементния състав на български плодове включва следните канонични променливи подредени по ниво на значимост: Cr, Fe, Mn, Cu, Cd и Pb.

Ключови думи: *съдържание на микроелементи, плодове, масспектрометрия с индуктивно свързана плазма, дискриминантен анализ.*