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Comparative analysis for macro and trace elements content in goji berries between varieties from China and R. Macedonia

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Abstract. The goji fruits (*Lycium barbarum* L.) are known for their high mineral content: Ca, Mg, P, Fe, Zn, Cu, P, Se, etc., with a positive incidence of high importance on the human body. Considering these, the present paper summarizes data for 16 macro and trace elements contents (Ca, Mg, K, Na, P, Fe, Zn, Cu, Se, Ge, Pb, Ni, Cd, As, Bi and Hg) in *Lycium barbarum* L. planted in China and in R. Macedonia. The collected samples were totally digested with application of microwave digestion system. Total concentration of the selected elements was determined using inductively coupled plasma with mass spectrometry (ICP-MS). The major elements content (Ca, K, P and Mg) ranges from 507 mg/kg to 3877 mg/kg for samples from R. Macedonia and from 269 mg/kg to 5047 mg/kg for Chinese samples. Statistically, significant differences for the analyzed elements between Macedonian and Chinese variety were obtained for Na, Mg, K, Ca, Fe, Ni, Cu, Se, Cd and Pb. Multivariate statistic was applied for revealing the dominant element association: F1 (Na-Mg-Fe-Ni-Se-Bi), F2 (Zn-Hg-Pb), F3 (P-K), F4 (Ca-Ge-Cu) and F5 (As-Cd). The total elements content for the analyzed elements dominates in Chinese samples (7965 mg/kg) vs. Macedonian samples (7661 mg/kg). The content of the potentially toxic elements (As, Bi, Cd and Pb) doesn't exhibit the maximum allowed limit for these kind of foodstuff. Multivariate assessment for elements content reveals a significant correlation within the geographical origin of samples. Two dominant components (Ca-Mg-P-K-Na-Fe-Zn-Cu-Se and Na-Ni) were extracted for Chinese samples vs. Cd-Pb-As-Bi-Hg dominant for the Macedonian goji berries samples.

Keywords: Goji berry, *Lycium barbarum*, major elements, trace elements, ICP-MS

Introduction

Gouqizi ("goo-chee-zee") or gouqi, the Mandarin name for wolfberry (*Lycium barbarum* L.), is a redorange berry of the Solanaceae nightshade family that includes tomato, eggplant, chili pepper and potato. In vernacular English, gouqi has become "goji". For at least 2000 years, goji berry has grown wild in China and used in common recipes and traditional Chinese medicine (Seeram, 2008, 2010; Zhang et al., 2014). The Chinese revere goji berry as a national treasure among the most nutrient dense of the nation's plants. This reputation has stimulated scientific investigation about its potential health benefits and systematic cultivation, commercialization and now increasing export to first-world countries mainly in Australasia, Europe and the USA. The benefits on human health, the therapeutical features of the goji berries are associated to a large number of hypoglycaemia, immune-modular, antihypertensive, hepatic, anti-ageing, anti-fatigue, anti-oxidant, etc. treatments (Wang et al., 2010; Mirecki et al., 2015). Previously investigation reports data for goji berries contain significant percentages of a day's macronutrient needs – carbohydrates, protein, fat and dietary fiber. Almost 70% of the mass of a goji berry exists as carbohydrate, 12% as protein, and 10% each as fiber and fat, giving a total caloric value of 370 for a 100 gram serving (Potterat, 2010; Amagase and Farnsworth, 2011; Zhang et al., 2014). Only few papers have described the mineral content of goji berries planted in China (Llorent-Martínez et al., 2013; Chen et al., 2014; Endes et al., 2015; Nascimento et al., 2015), but exhaustive research in this area is still necessary. The remarkable nutritive features of the goji berries are also due to the important content of minerals, some of which are essential for the normal functioning of the human body: Ca, Mg, K, P, Fe, Zn, Cu, Cr, I, Se, etc. (Seeram,

2010; Mirecki et al., 2015). We need to mention the fact that accidentally – because of geogenous or anthropic causes (soil and climate conditions, the existence of some pollutants, some improper processing, etc.) these fruit can also contain some toxic elements (Hg, Pb, As, Cd, etc.) that have negative impact on the human body (Gogoasa et al., 2014). The bioavailability of metals in soil is a dynamic process that depends on specific combinations of chemical, biological, and environmental parameters (Mirecki et al., 2015; Balabanova et al., 2015). Metals distribution in plants is quite heterogenous and is controlled by genetic, environmental and toxic factors.

The main purpose of this investigation was to determine the major and trace elements content in goji berries variety (*Lycium barbarum* L.) planted in different geographical places (China and Republic of Macedonia). The investigation was used to provide the possible elements enrichments/deficiencies in dependence on the different geographical origin of the samples. The obtained data can give an overview for the nutritive value of this foodstuff taking into account the Recommended Daily Allowance (RDAs) for minerals established by the Commission of the European Communities and recommended daily intake from given in the report of the World Health Organization (WHO, 1996; Commission of the European Communities, Directive 2008/100/EC, 2008).

Materials and methods

Within this study multi-element quantification of 16 elements (Ca, Mg, K, Na, P, Fe, Zn, Cu, Se, Ge, Pb, Ni, Cd, As, Bi and Hg) was conducted. The investigation was carried out using goji berries planted in China but commercially available in R. Macedonia

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Table 1. Operating conditions for the applied microwave digestion system

Step Unit	Initial temperature, °C	Final temperature, °C	Ranging Time, minute	Time hold, minute	Power, W
1	25	100	15	5	800 (75%)
2	100	150	10	5	800 (100%)
3	150	180	10	10	1600 (85%)

(supplied from the local markets) and goji berry planted in R. Macedonia. In order of determination of elements content, method development was introduced too.

Analytical procedures

Five commercially available samples of dry goji berry from China and the same number of samples grown in R. Macedonia were brought to the laboratory, and the samples were totally digested using closed microwave digestion system (MARS 5, CEM corporation, Matthews, NC, USA). For sample preparation, 0.5 g of goji berry dry sample was digested with 5 mL nitric acid (69.0%, w/w) and 2 mL hydrogen peroxide (30.0%, w/w). Additionally, blank samples and duplicates were prepared too. Samples were made up to a final volume of 25 mL with ultrapure deionised water. Three steps program was created for total dissolving of goji berry samples (Table 1).

The quadrupole inductively coupled plasma with mass spectrometer (Q-ICP-MS) was used for all isotopic measurements (model 7500cx, Agilent technologies, Santa Clara, CA, USA). The instrument was tuned for standard robust plasma conditions, equipment with Micromist nebulizer. Tuning was performed by optimizing the signal measured counts per ratio (CPS) for ^7Li , ^{89}Y , and ^{205}Tl , from aqueous standard solution (Tune solution) that contains 10 ng/mL Li, Y, Co, Ce and Tl, per each element. For the ICP-MS analysis the following isotopes were selected: ^{23}Na , ^{24}Mg , ^{31}P , ^{39}K , ^{42}Ca , $^{56}\text{Fe}/^{57}\text{Fe}$, ^{60}Ni , ^{63}Cu , ^{66}Zn , ^{72}Ge , ^{75}As , ^{77}Se , ^{114}Cd , ^{202}Hg , $^{206}\text{Pb}/^{207}\text{Pb}/^{208}\text{Pb}$ and ^{209}Bi . Quality assurance was assumed using certified reference material SRM-3287, blueberry dry fruit (National Institute of Standards & Technology, NIST, Charleston, CS, USA) for: Ag, Ca, Cu, Fe, Mg, Ni, P, Pb, Zn. The obtained data showed satisfactory sensibility for the applied method with recovery in range of 87.5–109%. The limits of quantification were estimated as follows: 0.1 mg/kg for Na, Mg, P, K and Ca, 0.05 mg/kg for Fe, 0.0008 mg/kg for Fe, Ni, Cu and Zn, 0.00015 mg/kg for Ge, As, Se and Cd and 0.00005 mg/kg for Hg, Pb and Bi.

Data processing

The obtained values for the contents of the investigated elements were statistically processed using basic descriptive statistics. Multivariate statistic method (cluster and R-mode factor analyses) was used to reveal the associations of the chemical elements. The factor analysis was performed on variables standardized to zero mean and unit standard deviation. To understand the complex connection between the elements contents in analyzed samples chemometric technique of PCA was applied (Yu, 2005). It is based on eigenanalysis of the covariance or correlation matrix. Each sample has a score along each model component which shows the location of the sample in this model and can be used to detect sample patterns, groupings, similarities or differences (Gergen and Harmanescu, 2012). In PAST software, the PCA routine finds the eigenvalues and eigenvectors of the variance-covariance (var-covar) matrix or the correlation matrix. Var-covar is used if all variables are measured in the same units (content in mg/kg). Correlation (normalized var-covar) is used if the variables are measured in different unit. In this way it implies normalizing all

variables using division by their standard deviations (Balabanova et al., 2015).

Results and discussion

Optimization of the analytical method

The ICP-MS system was optimized using the typical tuning condition for high and variable sample matrices (plasma conditions optimized for 0.65% CeO/Ce). No attempt was made to optimize any parameter for the targeted removal of any specific interference. A flow of 5.5 mL/min He gas (only) was added to the cell for the collision mode measurements. Normal background components of the argon plasma gas and aqueous sample solution (Ar, O, H), together with the additional components of the blank sample matrix (HNO_3 and H_2O_2), led to formation of several high intensity background peaks in the no-gas mode spectrum, notably $^{40}\text{Ar}^{16}\text{O}$, $^{40}\text{Ar}^{38}\text{Ar}$, $^{40}\text{Ar}^{18}\text{O}$ and $^{40}\text{Ar}^2$, from the plasma, but also $^{40}\text{Ar}^{18}\text{OH}$, $^{40}\text{Ar}^{12}\text{C}$, $^{38}\text{Ar}^{16}\text{OH}$, $^{40}\text{Ar}^{12}\text{CH}$, $^{40}\text{Ar}^{12}\text{C}$, $^{40}\text{Ar}^{13}\text{C}$, $^{38}\text{Ar}^{12}\text{C}^{14}\text{N}$, $^{40}\text{Ar}^{14}\text{N}$ were qualitatively determined as polyatomic interferences from the matrix. Their higher intensity background peaks show why several interfered elements (^{56}Fe , ^{58}Ni , ^{60}Ni , ^{64}Zn , ^{77}Se , ^{78}Se and ^{80}Se) were traditionally measured in helium mode. Satisfied accuracy for the applied measurements was obtained. The obtained data showed satisfactory sensibility with recoveries in range of 87.5–109%. The estimated limits of quantification are as follow: 0.1 mg/kg for Na, Mg, P, K and Ca, 0.05 mg/kg for Fe, 0.0008 mg/kg for Fe, Ni, Cu and Zn, 0.00015 mg/kg for Ge, As, Se and Cd and 0.00005 mg/kg for Hg, Pb and Bi.

Characterization for the selected elements

Basic descriptive stats for all 16 elements are summarized in Table 2. For major elements content, such as Ca, Mg, Na and K significant differences occur between the varieties from Macedonia and China. Goji berries samples from China are enriched with Na, Mg and Ca, while for K content is the opposite. The potassium content in goji berry can reach up to 1.5% as shown by Endes et al. (2015). Spanish analysis showed that goji berries are very much enriched (1.3 – 1.7%) with this macronutrient (Llorent-Martínez et al. 2013). The present investigation showed much lower potassium content in the range of 0.2 – 0.5% (Table 2). Llorent-Martínez et al. (2013) reported that goji berries in Spain contain 330 – 710 mg/kg, as median between the Chinese and Macedonian goji berries (Table 2). The sodium and magnesium content ranges from 15 to 816 mg/kg and from 632 to 1452 mg/kg, respectively. For the phosphorus content in goji fruits from Macedonia was obtained mean value of 2234 mg/kg, as well as for the goji fruits from China (2258 mg/kg).

An oxygen carrier of hemoglobin, iron is also a cofactor for enzymes involved in numerous metabolic reactions (Seeram, 2008). When intake is deficient, low iron levels cause iron deficiency anemia affecting millions of children worldwide (WHO, 1996; Potterat, 2010). Goji berry iron is 50% higher in content than in flax seeds (Seeram, 2008). The present study shows that the iron

Table 2. Basic statistics for elements content (values given in mg/kg)

Elements	Varieties from China				Varieties from R. Macedonia				t	P*
	min	max	Mean	SD	min	max	Mean	SD		
Na	406	816	638	180	15.2	27.2	21.4	4.22	7.84	0.001*
Mg	1009	1452	1209	120	632	825	707	66.7	8.61	0.001*
P	1846	2541	2258	261	2059	2781	2234	160	-0.70	0.52
K	2261	3877	3004	573	3823	5047	4310	493	-4.83	0.008*
Ca	507	1034	751	265	269	478	331	51	3.96	0.01*
Fe	63.3	82.6	75.6	10	20.9	60	38.9	16.3	4.97	0.008*
Ni	1.67	4.87	3.67	1.63	0.21	0.6	0.4	0.16	5.52	0.005*
Cu	8.91	14.3	11.5	1.9	7.45	10.8	9.31	1.06	3.43	0.02*
Zn	10.4	17.6	14.3	3.69	7.5	13.3	9.44	1.59	2.65	0.05
Ge	0.12	0.34	0.2	0.12	0.12	0.32	0.25	0.09	-0.92	0.41
Se	0.025	0.061	0.042	0.011	0.005	0.018	0.01	0.005	4.49	0.01*
As	0.007	0.036	0.018	0.015	0.006	0.18	0.06	0.08	-1.04	0.35
Cd	0.003	0.033	0.012	0.017	0.055	0.093	0.079	0.016	-5.92	0.004*
Hg	<LOQ	0.00024	0.00008	0.000014	<LOQ	0.00025	0.00006	0.00001	0.99	0.37
Pb	0.015	0.096	0.054	0.028	0.008	0.028	0.022	0.01	3.08	0.03*
Bi	0.001	0.046	0.012	0.025	0.015	0.06	0.039	0.014	-1.95	0.12

t-T-test for dependent samples; * – marked differences are significant at $p < 0.05$

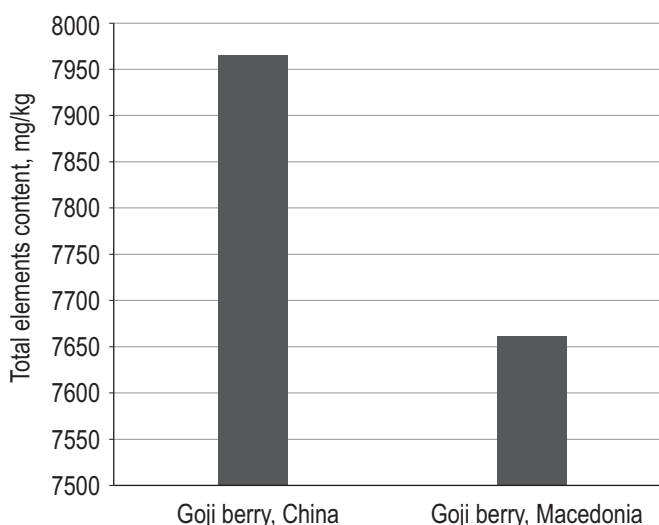
content in goji berry ranges from 20 – 60 mg/kg in Macedonian samples to 63 – 83 mg/kg in goji berry from China. The comparative analysis indicates significant difference between the iron content in goji berry varieties from Macedonia vs. China ($p = 0.008$).

Zinc content in all analyzed samples ranges from 7.5 to 17.5 mg/kg (Table 2). The metabolic role of this element is correlated with some proteins synthesis. DNA and functions of over 100 enzymes, zinc is also involved in critical cell activities such as membrane transport, repair and growth, especially in infants (Potterat, 2010; Amagase and Farnsworth, 2011). Some previous investigations conducted with Chinese goji berry showed similar results (mean value of 8.27 mg/kg) given from Endes et al. (2015). Some other cases showed that zinc in goji berries from China, Tibet, and other parts of Asia are characterized with contents lower than 15 mg/kg (Nascimento et al., 2015). The present study showed that there is no significant variation in zinc content between Macedonian and Chinese goji berry (Table 2). Very similar to zinc, copper content in the analyzed samples ranges from 7.5 – 14.3 mg/kg. It's significant to be mentioned that Zn and Cu above certain concentrations established by regulations have negative effects on the human body (WHO, 1996). In our case, the samples we analysed do not contain excessive amounts of Zn and Cu (WHO, 1996).

Selenium content in goji berry ranges from 5 µg/kg (minimum content obtained from Macedonian goji berry) to 62 µg/kg (maximum content obtained from Chinese goji berry). Sometimes called the „antioxidant mineral“, selenium is often included in supplements. Several reported data for selenium content in goji berry suggest significant variations in samples with different geographical origin, ranging from 5 to 500 µg/kg (Chen et al., 2014; Endes et al., 2015). Llorent-Martínez et al. (2013) assumed not detectable selenium contents (<3 µg/kg) in analyzed goji samples from Spanish supermarkets. Comparative analysis showed significant variation between Se content in samples from R. Macedonia vs. China. Macedonian samples were more enriched with Se (mean value 42 µg/kg) compared to Chinese samples

(mean value 5 µg/kg), where the enrichment factor is higher than 8.

The germanium content in the analyzed samples ranges from 120 to 340 µg/kg. For comparison, some conducted scientific investigations with some Chinese food stuffs and supplements reported that the aloe vera tablet, ginseng tablet and ginger tablet contained 20.8, 5.48 and 9.96 mg/kg. Foods found to contain germanium were potato, garlic and carrot, having 1.85, 2.79 and 0.60 mg/kg of germanium, respectively. McMahon et al. (2006) reports, that soya mince contains significant enrichments of germanium compared to other cereals (9.39 mg/kg). In this way, goji berries are almost 10 times enriched with germanium compared to the abovementioned foodstuff. The geographical origin of the goji berry fruit has no significance impact on germanium content. The potentially health risk elements (As, Cd, Hg, Pb and Bi) analyzed in goji berry fruits occur in trace content and does not exceed the

**Figure 1.** Total elements content, Macedonian goji berry vs. Chinese goji berry

maximum recommended value from WHO (1996).

Considering total element content of the analyzed minerals (Na, Mg, P, K, Ca, Fe, Ni, Cu, Zn, Ge, Se, As, Cd, Hg, Pb and Bi) between the goji berries planted in Macedonia (7661 mg/kg) and China (7965 mg/kg), enrichment occurs in favor of Chinese varieties (Figure 1). The t-test dependent samples identified that there is no significant difference for the total element content of the analyzed elements for $p < 0.05$ ($t = 0.18$, $p = 0.85$).

Determination of elements associations

The applied Factor analysis (FA) singled out five dominant geochemical elements associations due to their lithogenic distribution in the planting environs: F1 (Na-Mg-Fe-Ni-Se-Bi), F2 (Zn-Pb-Hg), F3 (K-P), F4 (Ca-Ge-Cu), F5 (As-Cd). The total variance for the dominant factor loading was 87.7%. Bioaccumulation of elements primarily depends on their bioavailability in the soil (Intawongse and Dean, 2006; Alloway, 2012; Balabanova et al., 2015). This in turn is conditioned by the lithogenic distribution of elements in soil for the given area of cultivation (Seeram, 2008; Balabanova et al., 2015). Most regions are characterized by specific geochemistry of the elements that helps in determining the geographical origin of the species. The most dominant association was Na-Mg-Fe-Ni-Se-Bi with the strongest loading value for Ni (Table 3). The bioavailability of the mentioned elements is significantly correlated with the geochemistry of the elements in soil (Marschner, 2002). Heavier metals such as Pb and Zn including mercury associate in the second factor (F2). Usually, accumulation of these elements is strongly correlated with heavy metal pollution of soil. Association of Pb and Zn is a known lithological phenomenon, especially for agricultural areas that relays on very old volcanic rocks (Balabanova et al., 2013). Phosphorus and potassium are dominant in the characterization of the plant tissue, as demonstrated in the analysis of goji berry samples. This is

Table 3. Matrix of dominant rotated factor loadings

Element	F1	F2	F3	F4	F5
Na	0.71	0.32	0.07	0.58	0.11
Mg	0.78	-0.11	0.15	0.49	0.20
Fe	0.74	-0.15	-0.10	0.56	0.18
Ni	0.94	-0.15	-0.13	0.04	0.12
Se	0.76	0.41	0.02	0.44	0.11
Bi	-0.63	0.49	-0.02	-0.45	-0.06
Zn	-0.32	0.79	0.18	-0.20	-0.34
Hg	0.03	0.92	-0.05	0.01	0.12
Pb	0.44	0.68	-0.18	0.40	-0.05
P	0.04	-0.01	0.99	-0.05	0.09
K	-0.31	0.01	0.61	-0.50	0.04
Ca	0.20	-0.08	0.06	0.93	0.15
Ge	0.22	0.25	-0.28	0.87	0.03
Cu	0.32	-0.39	0.15	0.70	0.42
As	-0.17	0.02	-0.09	-0.13	-0.97
Cd	-0.54	0.05	0.15	-0.77	-0.89
Prp.Totl	27.3	21.5	17.6	11.2	10.1

F1- ... F5 – Factor loadings; Prp.Totl – Principal total variance in %

also confirmed by literature, which mentions that the distribution of P and K in different vegetal products (fruits or vegetables) is determined by the nature of the products and elements analysed as well as by the cultivation conditions (Seeram, 2008). Extraction of As-Cd as single association is probably correlated with several

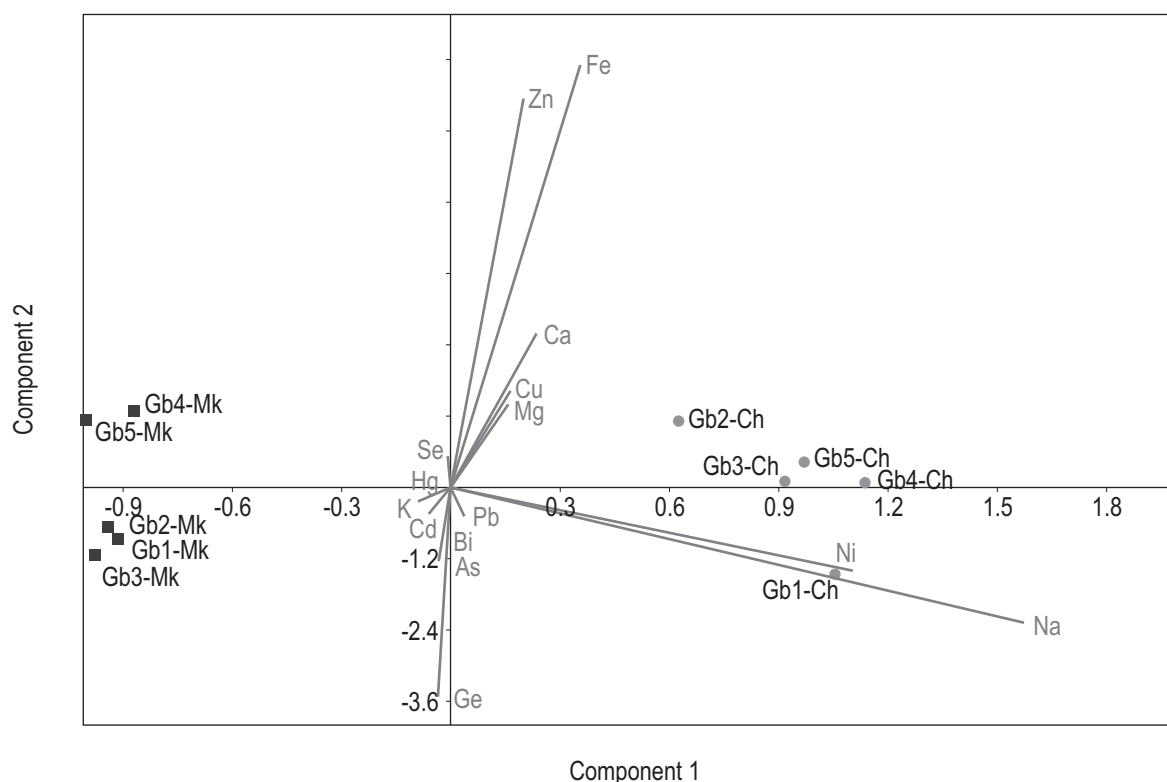


Figure 2. Biplot for PCA: elements content vs. goji berry country origin

anthropogenic activities. Increased concentrations of As and Cd in agricultural soils are known to come from human activities (Taylor, 1997), such as the application of phosphate fertilizer, sewage sludge, wastewater, and pesticides.

Inter-correlation of elements content vs. geographical origin of goji berry samples

Principal component analysis gave very precise classification and dependence of goji berry samples on the geographical origin. Three dominant factors were identified with total variance of 81.9%. Locality has a significant influence on element contents and was expressed with the applied PCA (Figure 2). As dominant principal components were identified the following principal components: Ca-Mg-P-K-Na-Fe-Zn-Cu-Se and Na-Ni strongly correlated with Chinese goji berry samples. On the other hand, the potential health risk elements (Cd, Pb, As, Bi and Hg) were dominant for the Macedonian goji berries samples. The distribution of the last mentioned component was extended with germanium distribution, which is probably correlated with the lithological impact of the planting region.

Conclusion

Experimental results from the determination of some mineral microelements in goji berries point out important amounts of essential elements (Zn>Fe>Cu>Se>Ge) and very small, negligible amounts of toxic elements (As, Bi, Hg, Pb and Cd). In terms of mineral imply between types of goji berry grown in Macedonia and China significant difference between the elements content of Na, Mg, K, Ca, Fe, Ni, Cu, Se, Cd and Pb has been established. Thus the mineral uptake from soil vs. bioavailability should be furthermore examined. Multivariate analysis allowed determination of the complex connection of test specimens and country of origin. In this way definite lithological connection was determined for the elements Ca, Mg, Na, Fe, Zn, Cu and Ni with significant correlation for the Chinese goji berry samples, while the elements Cd, Pb, As, Bi and Hg where much dominant in the Macedonian goji berry. Macedonian species were enriched with germanium content. However, we recommend caution when systematically using this fruit as a mineral supplement because improper, excessive consumption can overload the body with certain minerals or nutrients that could have unwanted side effect. According to the determined contents of the analyzed elements a dose of consuming amounts should be implemented in accordance with the Recommended Daily Allowances (RDAs) regulated with the Directive 2008/100/EC from Commission of the European Communities.

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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:

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