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MINERALS, TRACE ELEMENTS AND FLAVONOIDS CONTENT IN WHITE AND COLOURED KIDNEY BEAN

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ABSTRACT

The aim of the study was to determine the contents of mineral elements (Ca, K, Mg, N, P), trace elements (Cu, Fe, Mn, Cd, Pb, Ni, Co, Cr, and Zn), and certain phenolic compounds (flavonoids, isoflavones) in the fourteen varieties of cultivated raw kidney beans (*Phaseolus vulgaris* L.). Heavy metals and mineral elements were analyzed by AAS methods, phenolics were detected by HPLC. Compared with vegetables, legumes proved to be a good source of many minerals and elements, e.g., the contents of K, Mg, P, Fe, and Zn varied in the ranges 13.02 - 23.08 g.kg^{-1}, 1.41 - 2.52 g.kg^{-1}, 3.81 - 5.26 g.kg^{-1}, 0.064 - 0.121 g.kg^{-1}, and 0.026 - 0.037 g.kg^{-1} dw, respectively. Kidney beans, both species, contained no or few amounts of phenolics (only four cultivars) and the levels of kaempferol and apigenin were comparable to isoflavone genistein and quite higher than isoflavon daidzein content.

Keywords: kidney bean, AAS, HPLC

INTRODUCTION

The kidney bean (*Phaseolus vulgaris* L.) is one of the most important food legumes, consumed worldwide as pods of green beans or culinary processed seeds of dry beans. Nowadays, common kidney beans are regarded as functional foods that contribute health benefits and have therapeutic properties. The bean consumption is associated with a reduction risk of cardiovascular disease, diabetes mellitus, obesity, and diseases of digestive tract. The common bean is rich and inexpensive source of proteins, carbohydrates, dietary fibers to millions of peoples. In additon to being an important source of protein, legumes are also reported to be a good source of minerals (K, P, Ca, Mg) and trace elements. Metals, such as iron, zinc and manganese are essential metals, since they play an important role in biological systems. Cu and Zn are essential micronutients, they can be toxic when taken in excess. Lead and cadmium are non essential metals as they are toxic, even in trace **(Gençcelep et al., 2009)**.

The potential health benefits of bean have also been attributed to presence of secondary metabolites such as phenolic compounds that possess antioxidant properties **(Luthria, Pastor-Corrales, 2006)**. Nowadays, polyphenolics are being studied for their potential role in the prevention and treatment of a number of chronic diseases including certain forms of cancer, osteoporosis, and heart disease, and also for their ability to relieve menopausal symptoms. Phenolics are known as natural antioxidants and due to qualitative diversity, they are divided to some groups: phenolic acids, flavonoids, isoflavons, tannins etc. The most important of these phenolic compounds in beans are flavonols quercetin and kaempferol, flavon apigenin and some phenolic acids (e.g. *p*-coumaric acid or ferulic acid). Composition and content of phenolic acids in beans published several authors **(Troszyńska et al., 2006; Amarowicz, Pegg, 2008; Kalogeropoulos et al., 2010)**, but there are few reports about flavonoids content of kidney bean. The individual flavonoid content of green and yellow bean was reported **Hempel, Bohm (1996)** and **Price et al. (1998)**, but this report did not include flavonoid composition of dry been seeds. Our attention was also focused on isoflavons, which are typical for phenolic composition of soyabean, and their presence in foods and their consumption is associated with estrogenic and potencial anticancerogenic properties.

In this study, the levels of minerals, essential micronutrients and non-essential heavy metals in kidney been samples were determined by AAS. Chosen isoflavons – daidzein, genistein, and flavonoids - kaempferol, apigenin – were determined by HPLC·

MATERIAL AND METHODS

Materials

Eleven varieties of white bean and three coloured cultivars (Ultima, Viola, Kreola) were purchased from fy Legusem Horná Streda (Slovak Republic).

Methods

Minerals, trace elements. Major mineral elements (K, Ca, Mg) and trace elements (Fe, Mn, Zn, Cu, Co, Ni, Cr, Pb, Cd) were determined using a Varian AA240FS atomic absorption spectrometer equipped with a D2 lamp background correction system, using an air–acetylene flame. Between 0.9 and 1.1 g of dried sample was weighed into digestion tubes and HNO_3 were added. The samples were incinerated in a Nabertherm muffle furnace. Ashes were dissolved in nitric acid and passed through an ash-free, acidwashed filter paper and diluted to a certain volume with water. Determinations were carried out in duplicate. Minerals concentrations were determined on a dry weight basis as g.kg^{-1}, microelements values as mg.kg^{-1}.

Phosphorus. Phosphorus was measured using tartare antimonylo–potassium and molybdate ammmonium reagent on the UV-1800 UV-VIS spectrophotometer (Shimadzu). In short, 1 mL of the concentrated acid solution obtained for the determination of minerals was transferred into volumetric flask, and 8 mL of tartate–molybdate reagent was added. The volume was adjusted to 50 mL by adding of distilled water. The absorbance at 666 nm was measured after 60 min.

Nitrogen. Nitrogen was determined by the Kjeldahl method. The method consisted of mineralizing the sample with concentrated sulphuric acid and alkalizing with sodium hydroxide solution. The ammonium liberated was collected by distillation and recovered in boric acid solution. Subsequent titration with hydrochloric acid made it possible to calculate the initial amount of ammonium present in sample.

Flavonoids. The flavonoids – genistein, daizein, kaempferol and apigenin – were determined as aglycons according to **Wang, Murphy (1990)** with some modifications. Acid hydrolysis was chosen to convert the selected flavonoids conjugates into their aglycons. Ten grams of dried, milled sample was placed in a stoppered Erlenmayer flask containing 1M HCl. After 2 hours hydrolysis at 100 ^{o}C methanol was added. The sample was filtered under vacuum, the eluate was evaporated to dryness, redissolved in the mobile phase and determined by HPLC (L 6200 A, Merck-Hitachi) using the RP C18 column (LiChroCART 125-4 Purospher RP18e, 5 µm, Merck) with methanol : 1mM acetate ammonium (6:4), as a mobile phase. Identification of flavonoids was based on retention time UV spectra by comparision with commercial standards.

RESULTS AND DISCUSSION

In this study, the existence of five minerals was determined in two species of bean, K was predominant, followed by P, Mg, Ca (Tables 1, 2). The values in kidney bean seeds show imbalance between the potassium content and other minerals. Mineral supplementation can be used as an alternative approach to correct this imbalance **(Tůma et al., 2004)**. On the other hand, high potassium content in the diet contributes to regulation of water and salt balance in organism. The mean Ca:P ratio in beans, being ≈ 0.2, reveals a high concentration of phosphorus compared to calcium. This ratio should not be less than 1.0 **(Iqbal et al., 2006)**. Calcium values are lower than those in the literature **(Augustin et al., 1981; Campos-Vega et al., 2010)**. The Mg, K, P levels are adequate. The very high values of nitrogen are associated with bacteria *Rhizobium* activity and nitrogen fixation in roots. Kjeldahl-N was determined and crude protein content in seeds was calculated by the factor 6,25. On the basis results, protein content in bean seeds is between 18,2 - 24,9 % which corresponds with a data **Vojtaššáková et al. (1999)**.

The determination of macro- and trace elements in foodstuffs is an important part of nutritional and toxicological analyses. Cadmium and lead are best known for their toxicological properties. Pb and Cd can be accumulated in biological systems becoming potential contaminants along the alimentary chain. Copper, chromium, iron, and zinc in adequate amounts are essential micronutrients for human health. These elements play an important role in human metabolism, and interest in these elements is increasing together with reports of relationships between trace element status and oxidative diseases. On the other hand, e.g. Cu and Zn are essential micronutrients, they can be risk elements when taken in excess.

Table 1 Minerals content (g.kg^{-1}) in white and coloured beans

Cultivar	Type	Ca	Mg	K
Lucka	w	1.24	2.03	18.12
Sara	w	0.33	2.04	23.08
Sina	w	0.86	2.31	14.76
Diana	w	0.91	1.84	13.02
Petra	w	0.82	1.80	14.76
Anka	w	1.76	2.12	15.25
Gesta	w	0.90	2.52	15.70
Salva	w	0.78	2.08	14.89
Gama Z	w	0.86	2.21	15.88
Masai	w	0.99	2.21	20.63
Luna	w	0.67	1.69	15.34
mean±SD	w	0.92±0.37	2.07±0.24	16.49±2.96
minimum	w	0.33	1.69	13.02
maximum	w	1.76	2.52	23.08
Ultima	c	0.91	1.41	13.92
Kreola	c	0.44	1.62	14.86
Viola	c	0.59	1.54	15.1
mean±SD	c	0.65±0.23	1.52±0.11	14.63±0.62
minimum	c	0.466	1.409	13.92
maximum	c	0.906	1.619	15.1

w-white, c-coloured, SD-standard deviation, minimum and maximum content

Table 2 Minerals content (g.kg^{-1}) and dry mater (DM) in % in white and coloured beans

Cultivar	Type	P	N	DM
Lucka	w	4.20	34.69	92.80
Sara	w	3.81	30.71	92.30
Sina	w	4.42	29.13	91.30
Diana	w	3.85	39.9	92.10
Petra	w	4.86	28.5	92.10
Anka	w	4.03	32.9	92.55
Gesta	w	4.52	35.63	91.35
Salva	w	4.13	38.82	91.95
Gama Z	w	4.32	36.76	93.30
Masai	w	4.95	33.75	92.30
Luna	w	5.26	38.78	92.95
mean±SD	w	4.39±0.46	34.51±3.93	92.20±0.70
minimum	w	3.81	28.5	91.30
maximum	w	5.26	39.9	93.30
Ultima	c	4.33	35.89	91.65
Kreola	c	4.71	30.85	90.75
Viola	c	4.94	36.36	92.40
mean±SD	c	4.66±0.30	34.37±3.05	91.20±0.64
minimum	c	4.33	30.85	90.75
maximum	c	4.94	36.36	92.4

w-white, c-coloured, SD-standard deviation, minimum and maximum content

Table 3 Trace elements content ($mg.kg^{-1}$) in white and coloured bean

Cultivar	Fe	Mn	Zn	Cu	Co	Ni	Cr	Pb	Cd	Na
Lucka	121.17	20.74	31.95	8.41	0.75	3.12	0.32	0.7	0.14	51.45
Sara	76.7	16.03	36.83	9.75	0.38	1.78	0.38	0.76	0.15	33.53
Sina	107.66	16.32	36.09	7.72	0.44	6.68	0.49	0.93	0.13	37.34
Diana	64.87	14.44	31.48	7.76	0.49	4.34	0.16	0.87	0.08	40.12
Petra	110.96	14.82	26.65	8.14	0.49	2.01	0.16	0.81	0.12	36.8
Anka	114.58	19.71	28.2	8.21	0.54	2.64	0.32	0.92	0.14	36.52
Gesta	106.51	15.65	33.01	11.38	0.55	6.56	0.22	0.76	0.11	56.75
Salva	85.37	15.6	37.35	8.21	0.38	4.13	0.11	0.76	0.13	44.15
Gama	108.73	15.81	32.74	8.89	0.69	2.89	0.32	0.85	0.16	52.3
Masai	103.14	16.52	28.43	8.5	0.76	4.55	0.32	0.7	0.17	58.28
Luna	110.7	17.64	29.26	8.6	0.81	3.17	0.27	0.86	0.13	25.28
mean±SD	100.94±17.50	16.66±1.96	31.99±3.65	8.41±1.05	0.57±0.15	3.80±1.64	0.28±0.11	0.81±0.08	0.13±0.02	42.96±10.52
minimum	64.87	14.44	26.65	7.72	0.38	1.78	0.11	0.70	0.08	25.28
maximum	121.17	20.74	37.35	11.38	0.81	6.68	0.49	0.93	0.17	58.28
Ultima	91.27	13.2	28.42	8.18	0.55	3.49	0.22	0.71	0.12	53.68
Kreola	96.47	13.66	29.75	8.1	0.33	4.02	0.16	0.66	0.12	52.26
Viola	77.11	11.9	32.41	8.71	0.38	1.35	0.38	0.65	0.12	41.77
mean±SD	88.28±	12.92±0.91	30.19±2.03	8.33±0.33	0.42±0.11	2.95±1.41	0.25±0.11	0.67±0.03	0.12±0.00	42.96±6.51
minimum	77.11	11.90	28.42	8.1	0.33	1.35	0.16	0.65	0.12	41.77
maximum	96.47	13.66	32.41	8.71	0.55	4.02	0.38	0.71	0.12	53.68

The Codex Alimentarius has set a limit for the maximum levels of chosen risk elements in legumes; for cadmium, lead, chromium, cuprum and nickel are maximum values 0.1; 1.0; 4.0; 15.0; 6.0 $mg.kg^{-1}$, respectively.
Limits for contaminants in the slovak food commodities are harmonized with EU limits **(Cimboláková, Nováková, 2009)**.

The concentration levels of the elements (Fe, Mn, Zn, Cu, Co, Ni, Pb, Cr, Cd, Na) measured in our two beans species are given in Table 3. The order of the levels of the elements in the samples was determined to be: Fe > Na > Zn > Mn > Cu > Ni > Pb > Co > Cr > Cd. The levels of essential elements in tested beans were higher than those of toxic elements. With respect to Fe, the concentrations obtained by us were similar to the concentration published by some researches **(Augustin et al., 1981; Campos-Vega et al., 2010)**.

The iron values of the samples varied from 64.87 to 121.17 $mg.kg^{-1}$ – higher values than data found in the literature, because the composition of legume depends, not only on the species or varieties, but also on the growing conditions, such as soil and other conditions. Higher average values of iron were found in white species of bean. Sodium was found in relatively higher amounts. Nevertheless, Na : K ratios were significantly below 1.5 in all the samples studied (mean 0.0026 and 0.0033 in white and coloured beans, respectively), which is very interesting and recommended from the point of view of nutrition, since the intake of sodium chloride and diets with a high Na : K ratio have been related to the incidence of hypertension **(Rupérez, 2002)**. **Kalafová et al. (2009)** examined effect of zinc and nickel supplementation on mineral blood parameters in animal organism. Authors found the insignificant highest concentrations of potassium and slightly lower value of sodium in blood of tested animal after supplementation Ni^{2+} + Zn^{2+} in comparison with groups without zinc application.

Legumes are known as zinc accumulators **(Gençcelep et al., 2009)** and average zinc concentrations of our tested beans ranged from 26.65 to 37.35 $mg.kg^{-1}$. The maximum and minimum zinc levels were found in white beans. Zinc has been recognized e.g. as a co-factor of the superoxide dismutase enzyme, which is involved in protection against oxidative processes too. The copper concentration (7.72–11.38 $mg.kg^{-1}$) was under the maximum levels of chosen risk elements in Codex Alimentarius valid in Slovak Republic. Copper can be found in many enzymes, some of which are essential for Fe metabolism and there are probably direct correlation between the dietary Zn and Cu ratio and the incidence of cardiovascular disease **(Campos-Vega et al., 2010)**. The manganese contents of samples were between 11.90 and 20.74 $mg.kg^{-1}$. The lowest and highest manganese concentrations were found in cv. Viola (coloured) and cv. Lucka (white), respectively. Chromium content ranged from 0.11 $mg.kg^{-1}$ to 0.49 $mg.kg^{-1}$. Manganese and chromium are recognised as essentials trace elements for humans and their metabolic roles are studied (e.g. Mn-containing enzym system, Cr in relationship to insulin function and carbohydrate metabolism). The nickel and cobalt levels in bean samples were found to be in the range of 1.35-6.68 $mg.kg^{-1}$ and 0.33-0.81 $mg.kg^{-1}$, respectively. Nickel is an essential mineral element that may accumulate to toxic levels in soils due to anthropogenic activities. The toxic risk elements contents were between 0.65-0.93 $mg.kg^{-1}$ (Pb) and 0.08-0.17 $mg.kg^{-1}$ (Cd). Most of the trace elements present in bean, their content is generally below the limit values or few higher (Cd) as the maximum level allowed in **Codex Alimentarius** valid in Slovak Republic.

Tables 4 and 5 show the amount of isoflavons and flavonoids in the hydrolysed samples of bean seeds. The results show that in 10 cultivars from a total of 14 values were not measured at detectable levels of flavonoids. Comparing data in Tables 4, 5 suggest that the beans have not large differences in content of phenolic substances, regardless of the type variety. Isoflavones genistein and daidzein are largely undetectable.The presence of isoflavones in raw bean seeds was not confirmed by **Diaz-Batala et al. (2006)**. **Espinosa-Alonso et al. (2006)** were found isoflavones in only low levels. The major flavonoids in tested beans are kaempferol and apigenin. These flavonoids were determined in 4 genotypes and their content ranged from 7.657 to 16.556 mg. kg^{-1} DW (kaempferol), 7.529 to 13.710 mg. kg^{-1} DW (apigenin). The most favorable results have been found in cultivars Lucka and Sara, which have all identified observed flavonoids. Sara bean cultivar contains higher levels of flavonoids than the cultivar Lucka and the differences are statistically significant. The content of the flavonoids of both varieties in order of decreasing apigenin > kaempferol> genistein > daidzein. In view of the content of macro-and microcomponents for the consumer, white cultivars Lucka and Sara seems to be the best among the bean cultivars.

Table 4 Isoflavons content (mg.kg^{-1}) in white and coloured beans

Cultivar	Type	Daidzein	Genistein
Lucka	w	3.190 ± 0.01 a	10.090 ± 0.42 a
Sara	w	3.860 ± 0.08 b	12.120 ± 0.40 b
Sina	w	nd	nd.
Petra	w	nd	nd.
Diana	w	nd	nd.
Anka	w	nd	nd.
Gesta	w	nd	nd.
Salva	w	nd	nd.
Gama Z	w	nd	nd.
Masai	w	nd	nd.
Luna	w	nd	nd.
Ultima	c	nd	9.985 ± 0.36 c
Viola	c	nd	nd.
Kreola	c	nd	nd.

w-white, c-coloured, nd.-under detection
Values in the same column with different letters present significant differences at $p < 0.05$ using t-test for independent samples. Each column contains mean of four replication ± standard deviation.

Table 5 Flavonoids content (mg.kg^{-1}) in white and coloured beans

Cultivar	Type	Kaempferol	Apigenin
Lucka	w	11,050 ± 0,33 a	12,630 ± 0,22 a
Sara	w	13,320 ± 0,30 b	13,710 ± 0,20 b
Sina	w	16,556 ± 0,35 c	8,268 ± 0,18 c
Petra	w	nd.	nd.
Diana	w	nd.	nd.
Anka	w	nd.	nd.
Gesta	w	nd.	nd.
Salva	w	nd.	nd.
Gama Z	w	nd.	nd.
Masai	w	nd.	nd.
Luna	w	nd.	nd.
Ultima	c	7,657 ± 0,15 d	7,529 ± 0,10 d
Viola 311	c	nd.	nd.
Kreola	c	nd.	nd.

w-white, c-coloured, nd.-under detection
Values in the same column with different letters present significant differences at $p < 0.05$ using t-test for independent samples. Each column contains mean of four replication ± standard deviation.

CONCLUSION

Beans, as one of the most frequent consumed crops for us, are long appreciated for its composition and nutrient content, but generally its nutritional properties are little discussed and presented. As a conclusion of this study, it can be said that legume are a valuable agricultural product, based on their rich and beneficial composition. These results may be useful for the evaluation of dietary information and concluded that legumes are the good source of minerals.

Our results also show that although cultivated kidney bean is not good source of flavonoids, but probably contain another types of phenolics (phenolic acids, anthocyans in coloured bean). For this reason is a need to identify and quantify these important antioxidant compounds in beans for use in breeding programs focused on nutrition and health.

Dry bean is an integral part of diets in a significant portion of the world population, but the potential benefits of consuming beans from a nutraceutical standpoint have largely been overlooked. Since dry beans contain compounds that may have significant antioxidant potential, it will be useful to investigate their identification, potential and maximizing their use in food industry.

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