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DIFFERENCES IN FYTOCHEMICALS CONTENT IN COLOURED CULTIVARS OF COMMON BEAN (*PHASEOLUS VULGARIS* L.)

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ABSTRACT

Nowadays, legume production is increasing as the world population but its consumption is declining due to dietary habit changes. Industry is finding new uses of legumes and creating new foods with a positive effect on health and well-being of humans. Key components in legume (like protein, soluble and insoluble fibre, resistant starch, and bioactive polyphenols) make beans suitable for a wide range of food product applications. The main phenolic compounds present in legume flours are flavonoids. The study is focused on determination of bioactive compounds content in seeds of four varieties of common bean. The samples of plant material were collected from the area of Piešťany (Slovakia). The samples of common bean seeds were homogenized and a methanolic extract was prepared. These extracts were used for analyses. The total content of polyphenols was determined spectrophotometrically and the content of individual phenolic by using the RP-HPLC/DAD method. The content of risky metals in samples was determined using the AAS method. TPC in studied varieties of common bean were determined in the range from 2156.87 mg.kg^{-1} (NEGRA) to 1362.70 mg. kg^{-1} (ZENIT). Highest concentration of phenolic acids was determined in ellagic acid, where the average value was 0.21845 mg.kg^{-1} DM (dry matter) and the lowest in *trans*- caffeic acid, with the average value 0.02380 mg.kg^{-1} DM. Highest concentration of flavonoids was determined in vitexin, where the average value was 0.02480 mg.kg^{-1} DM and the lowest in kaempferol, with the average value 0.00545 mg.kg^{-1} DM. Highest macro elements amount was determined in white beans. For potassium it was 12277 mg.kg^{-1} (ZENIT) and the lowest was in manganese, and it was 10.70 mg.kg^{-1} (RUBIN) in red beans. From the analyzed varieties we have found that they are important source of polyphenol substances with a demonstrable bioactive effect.

Keywords: common bean, polyphenols, variety, macroelements, microelements

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most widely produced legume specie in the world, for its edible beans, used both as dry seed and as unripe fruits. The leaf is occasionally used as a vegetable, and the straw is used for fodder **(Li *et al*., 2016)**. Common bean (*P. vulgaris*) is one of the most important legume crops, providing as much as 15% of total daily calories and 36% of total daily protein in parts of Africa and the America **(Nemli *et al*., 2015)**. In Canada, both white and coloured beans exist. The different market classes of coloured beans include pinto, brown, cranberry, azuki, dark and light red kidney, small red, pink, and black **(Pitura *et al*., 2019)**. Common bean is the most important grain legume for human direct consumption **(Herrera *et al.* 2019)** and is considered as a key source of minerals (4–6% ash) **(Pitura *et al*., 2019)** like iron and zinc, proteins (20–27%), and complex of carbohydrates (up to 60%), dietary fiber (up to 28%), vitamins, as well as bioactive compounds (such as polyphenols, phytosterols and saponins) **(Aguilera *et al*., 2011; Herrera *et al.* 2019**).
Research suggests that the beans with darker coloured seed coats have higher total flavonoid contents, when compared to those with lighter coloured seed coats. More than 200 flavonol aglycones have been identified in plants. Among these, quercetin (3,5,7,3′,4′-pentahydroxyflavone), kaempferol (3,5,7,4′-tetrahydroxyflavone), myricetin (3,5,7,3′,4′,5′-hexahydroxyflavone), and isorhamnetin (3,5,7,4′-tetrahydroxy-3′-methoxy flavone) are the most abundant in food. It is generally believed that bean seed coat colour is due to the presence of flavonols, as well as the other phenolic compounds, including anthocyanins and tannins. Flavonols in beans are mainly concentrated in the seed coat. Beans with white seed coats do not contain flavonol compounds **(Pitura *et al*., 2019).**

Phenolic compounds, commonly found in edible and non-edible plants, have been reported to possess multiple biological effects, including antioxidant activity. In the human diet, polyphenols make the most important contribution to antioxidant activity **(Alshikh *et al*.,2015)**.
As antioxidants, phenolic compounds are able to decrease oxygen concentration, intercept singlet oxygen **(Alshikh *et al*.,2015)**, prevents or remove oxidative damage to a target molecule. Antioxidants are an inhibitor of the oxidation process, even at relatively small concentration and thus have diverse physiological role in the body. A variety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables and tea **(Yadav *et al*., 2016)**. Numerous of publications have reported the health benefits of phenolic compounds in disease risk reduction, for example as the protection against cancer **(Mirali *et al*., 2014)**, including those of breast, colon, prostate, and stomach as well as cardiovascular disease and various inflammatory disorders **(Mirali *et al*., 2014)**.
Common bean also contain components such as organic acids and tocopherols, which are considered as bioactive components. Their effects have health benefits related to the antihypertensive, antibacterial and antioxidant potential, and the protective effects in oxidative stress-induction, such as the carcinogenesis inhibitory effect **(Arribas *et al*., 2019)**. This plant has been used as carminative, diuretic, emollient and also in the treatment of diabetes, diarrhea, dysentery, and kidney problems **(Kuete, 2014)**.

MATERIAL AND METHODS

Studied plant material was obtained from the Plant Research Canter in Piešťany (Slovakia). We analysed four varieties (NEGRA- black beans, RUBIN- red beans, INKA- colour beans, ZENIT- white beans) of common beans (*Phaseolus vulgaris* L.) The total content of polyphenol substances, individual phenols, as well as the content of macro and microelements were determined in common beans seeds.

Plant material

Seeds of four common beans cultivars (NEGRA, RUBIN, INKA, ZENIT) were manually separated, then dried at 105ºC to a constant weight and finally pulverized (Grindomix 200 GD, Retsch, Germany).

Determination of risk elements content

Plant samples (1 g) were mineralized in a closed microwave digestion system (Mars X-Press 5, CEM Corp., USA) in a mixture of 5 cm^3 HNO_3 (Suprapur, Merck, Germany) and 5 cm^3 deionized water (0.054 µS/cm, Simplicity 185, Millipore, UK). Digestive conditions for the applied microwave system comprised heating to 160 °C for 15 minutes and kept it constant for 10 minutes. A blank sample was treated in the same way. The digested substances were subsequently filtered through a quantitative filter paper Filtrak 390 (Munktell, Germany) and filled up with deionized water to a volume of 50 cm^3. Contents of risk elements (Zn, Cu, Ni, Cr, Pb and Cd) were determined by F-AAS and GF-AAS method (Varian AA Spectr DUO 240FS/240Z/UltrAA, Varian, Australia) and expressed as mg/100 DM
(dry matter). The graphite furnace technique was used for the determination of Pb and Cd, whereas the flame AAS method was used for the determination of Zn, Cu, Ni and Cr. Gained results were evaluated according to FC SR (Food Codex of the Slovak Republic valid in Slovakia) as well as according Commission Regulation (EC) 1881/2006.

Determination of phenolic acids and flavonoids
Chemicals

Standard chemicals (*trans*- caffeic acid, syringic acid, ellagic acid, *trans* p-coumaric acid, *trans*-sinapic acid, ferulic acid, vitexin, resveratrol, daidzein, kaempferol), methanol (HPLC grade), acetonitrile (gradient HPLC grade) and phosphoric acid (ACS grade) were purchased from Sigma-Aldrich (Sigma Aldrich Chemie GmbH, Steiheim, Germany). Deionized water (0.054 $µS.cm^{-1}$) was treated in a Simplicity 185 purification system (Millipore SAS, Molsheim, France).

Preparation of calibration solutions and samples

Standard solutions were prepared by dissolving 0.5 mg of each standard in 10 mL of methanol. The lyophilized samples (2 g) were after homogenization extracted with 20 mL of 80% methanol at laboratory temperature for 8h, by horizontal shaker (Unimax 2010; Heidolph Instrument GmbH, Germany). Extract was filtered through Munktell no. 390 paper (Munktell & Filtrac, Germany) and stored in closed 20 mL vial tubes until the analysis. Prior the injection the standard solutions and sample extracts were filtered through syringe filter Q-Max (0.45 µm, 25 mm; Frisenette ApS, Knebel, Denmark).

HPLC analyses

Phenolic acids and flavonoids were determined by using an Agilent 1260 Infinity high performance liquid chromatograph (Agilent Technologies, Waldbronn, Germany) and DAD detector (G1315C). All HPLC analyses were performed on a Purosphere reverse phase C18 column (4 mm × 250 mm × 5 µm) (Merck, KGaA, Darmstadt, Germany). The detection wavelength was 320 nm for *trans*-caffeic acid, syringic acid, vitexin, ellagic acid, *trans* p-coumaric acid, *trans*-sinapic acid, ferulic acid, resveratrol, and 372 nm for daidzein and kaempferol.

Spectrophotometric determination of total polyphenol content (TPC)

TPC was determined by the method of **Lachman *et al*. (2003)** and expressed as milligrams of gallic acid equivalent per kilogram (mg $GAE.kg^{-1}$) dry weight. Gallic acid is usually used as a standard unit for phenolics content determination because of its wide spectrum of phenolic compounds. The total polyphenol content was estimated using the Folin-Ciocalteau assay and the absorbance was measured at 765 nm wavelength against blank (Spectrophotometer Shimadzu UV-1800; Shimadzu, Kyoto, Japan). The concentration of polyphenols was calculated from a standard curve plotted with known concentration of gallic acid.

Statistical analysis

The statistical differences between individual species were evaluated using a non-parametric statistical DUNN'S Multiple pairwise comparisons Kruskal-Wallis test at the level of significance $p < 0.05$. All calculations were performed by using MS Excel 2016 and XLSTAT (Addinsoft, 2014).

RESULTS AND DISCUSSION

Common bean (*Phaseolus vulgaris* L.) is an important source of macro- and microelements (Table 1 and 2), which are extremely important for correct process of metabolism. Their lack of inhibition of physiological processes in living organisms can cause various diseases **(Bonafaccia *et al*., 2003)**.

Table 1 The content of macroelements in common bean seeds ($mg.kg^{-1}$ DM)

Spiecies	Variety	K	Na	Ca	Mg	P	Fe	Mn
black beans	NEGRA	10837	42.10	298.5	321.4	4744	77.60	15.40
red beans	RUBIN	10985	48.20	144.6	255.7	3957	58.10	10.70
colour beans	INKA	10892	157.4	406.3	304.8	3326	85.90	24.30
white beans	ZENIT	12277	19.20	205.3	309.9	3262	51.00	18.10
Average		**11248**	**66.73**	**263.7**	**279.9**	**3822**	**68.15**	**17.13**

Table 2 The content of microelements in common bean seeds (mg kg^1 DM)

Species	Variety	Zn	Cu	Co	Ni	Cr
black beans	NEGRA	15.3	6.40	0.10	5.40	2.70
red beans	RUBIN	17.8	8.40	0.10	5.90	0.60
colour beans	INKA	17.0	7.10	0.40	3.30	1.00
white beans	ZENIT	16.2	3.80	1.10	1.70	0.70
Average		**16.6**	**6.43**	**0.43**	**4.08**	**1.25**

In Table 1 and 2 there are presented results of selected micro- and macroelements content in common bean seeds. **Pedrosa *et al*. (2015)** were also dealing same topic as we, determination of the mineral contents in common dry beans (*Phaseolus vulgaris* L.). They find out that the average content of Fe in common beans was 60.25 $mg.kg^{-1}$, Zn 21.60 $mg.kg^{-1}$, and according to this, our results are in correspondence with their results that they have obtained. **Pedrosa *et* al.** (2015) determinated the average value for Ca 1.60 $mg.kg^{-1}$, Mg 1.46 $mg.kg^{-1}$, Na 0.02 $mg.kg^{-1}$. Comparing to this findings, the results that we have obtained for this three elements (Ca, Mg and Na) were significantly higher (Table 1 and 2). This can be caused by cultivation locality, variety, conditions of land and agricultural interventions **(Bonafaccia *et al*., 2003)**.
In Table 3 are presented the results of the content of selected heavy metals in common beans seeds. The highest content of cadmium in the seeds was measured in the variety of the NEGRA-black beans (0.50 $mg.kg^{-1}$). The lowest amount of cadmium was measured in the seeds of variety RUBIN-red beans (0.08 $mg.kg^{-1}$). The limit amount of cadmium, stated by The Food Code of the SR, is 0.1 $mg.kg^{-1}$.

Table 3 The content of risk metals in common bean seeds ($mg.kg^{-1}$ DM)

Species	Variety	Pb	Cd	Hg
black beans	NEGRA	1.40	0.50	0.003
red beans	RUBIN	1.60	0.08	0.002
colour beans	INKA	1.80	0.10	0.003
white beans	ZENIT	1.50	0.10	0.002
Limit (FC SR)		**0.2**	**0.1**	**0.05**

FC SR- Food Code of the Slovak republic,

The Pb content in the seeds ranged from 1.40 $mg.kg^{-1}$ (NEGRA- black beans) to 1.80 $mg.kg^{-1}$ (INKA-colour beans). In all seeds of every variety, according to the legislation (FC SR: Pb 0.2 mg kg^1), all the measured values were above this limit. Regarding to Hg content, all of the sample values were under the limit (0.05 $mg.kg^{-1}$). Our results confirmed that all of our selected species (black, red, colour and white beans) have the ability to accumulate the heavy metals in seeds. Heavy metals are one of the biggest problems of environment in the world. They have a tendency to accumulate in living organisms. The huge amount of heavy metals is toxic or carcinogenic for human, animals, plants and microorganisms **(Vardhan *et al*., 2019)**.

Table 4 The average contents of phenolic acids in common bean seeds (mg.kg^{-1} DM)
• Multiple pairwise comparisons Kruskal-Wallis Tests,

Spieces	Variety	tCA	SYA	ELA	tpCA	tSI	FA
black beans	NEGRA	0.0242^b	0.1080^b	0.1602^b	0.0241^b	0.0388^b	0.1436^b
red beans	RUBIN	0.0108^b	0.1168^b	0.2405^b	0.0107^b	0.0215^b	0.0657^b
colour beans	INKA	0.0130^b	0.0991^b	0.1867^b	0.0368^b	0.0354^b	0.1059^b
white beans	ZENIT	0.0472^b	0.1865^b	0.2864^b	0.0251^b	0.0387^b	0.0751^b

Different letters (a, b and c) between the variables show statistically significant differences (p <0,05).
• a 0,05 >p > 0,01
• b 0,01 >p > 0,001
• c p < 0,001
• tCA- *trans*- caffeic acid, SYA- syringic acid, ELA- ellagic acid, tpCA- *trans* p-coumaric acid, tSI- *trans*- sinapic acid, FA- ferulic acid

Table 5 The average contents of flavonoids in common bean seeds (mg.kg-1 DM)

Spieces	Variety	VIT	RES	DAI	KAE
black beans	NEGRA	0.0209^b	0.0046^b	0.0064^b	0.0046^b
red beans	RUBIN	0.0234^b	0.0024^b	0.0032^b	0.0054^b
colour beans	INKA	0.0259^b	0.0059^b	0.0039^b	0.0082^b
white beans	ZENIT	0.0290^b	0.0106^b	0.0089^b	0.0036^b

• Multiple pairwise comparisons Kruskal-Wallis Tests,
Different letters (a, b and c) between the variables show statistically significant differences (p <0,05).
• a 0,05 >p > 0,01
• b 0,01 >p > 0,001
• c p < 0,001
• VIT- vitexin, RES- resveratrol, DAI- daidzein, KAE- kaempferol

In Table 4 and 5 are presented the results of polyphenols content in common bean seeds (mg.kg^{-1} DM). *Trans*- caffeic acid amount in seeds of selected varieties varied from 0.0108 mg.kg^{-1} (RUBIN) to 0.0474 mg.kg^{-1} (ZENIT). **Giusti *et al*., (2017)** find out that the values of *trans*- caffeic acid in seeds of selected varieties was under the detection limit, which is not corresponding with our results. The highest content of syringic acid was measured in the seeds of variety (ZENIT) 0.1865 mg.kg^{-1} and the lowest content was measured in the variety (INKA) 0.0991 mg.kg^{-1}. Based on this information, it is possible to create the following order of varieties: ZENIT>RUBIN>NEGRA>INKA (Table 4). **Giusti *et al*., (2017)** published that the content of syringic acid was recorded in selected cultivars of beans in the interval from 1.8 mg.kg^{-1} to 12.6 mg.kg^{-1} DM. The content of ferulic acid was varied from 1.7 mg. kg^{-1} to 14.6 mg.kg^{-1} DM.
The content of *trans* p-coumaric acid was under the limit of detection. The highest content of *trans* sinapic acid was measured in the seeds of variety (NEGRA) 0.0388 mg.kg^{-1} and the lowest content was in the variety (RUBIN) 0.0215 mg.kg^{-1} (Table 4). Our results are lower, when compared to **Lopez *et al.* (2013)**, who has published the content of *trans* sinapic acid 0.1 mg.kg^{-1} DM in dark beans (*Phaseolus vulgaris* L.). The highest content of kaempferol was measured in seeds of variety (INKA) 0.0082 mg.kg^{-1} and the lowest content was in variety (NEGRA) 0.0046 mg.kg^{-1} (Table 5). **Lopez *et al*. (2013)** find out in their research that the content of kaempferol in dark bean seeds was under the detection limit, which is not corresponding with our results. Polyfenols content in common bean seeds was significantly lower, comparing to **Lopez et al. (2013)**. It may be caused by cultivation locality, variety, conditions of land and agricultural interventions **(Bonafaccia *et al*., 2003)**.
The statistical differences between individual species were evaluated using a non-parametric statistical DUNN'S Multiple pairwise comparisons Kruskal-Wallis test at the level of significance p <0.05. All of our statistical significant differences were at the significant level from 0.01 to 0.001 (letter b). There were found statistically significant differences in *trans*- caffeic acid between varieties RUBIN-red beans and ZENIT-white beans; syringic acid was between varieties INKA-colour beans and ZENIT-white beans; ellagic acid between varieties NEGRA-black beans and ZENIT- white beans; *trans* p-coumaric acid between varieties INKA-colour beans and RUBIN-red beans; ferulic acid between varieties RUBIN-red beans and NEGRA- black beans. In case of measured *trans*-sinapic acid values, there were found statistically significant differences between varieties RUBIN-red beans/ZENIT-white beans/NEGRA-black beans (Table 4). In case of measured flavonoids resveratrol and daidzein between varieties RUBIN-red beans and ZENIT-white beans; kaempferol between varieties INKA-colour beans and ZENIT-white beans and vitexin between varieties NEGRA-black beans and ZENIT- white beans (Table 5).
Pitura *et al*. (2019) reported that in general, black bean seed coats had the greatest phenolic acid content followed from light to dark red bean that we can confirm with our findings. **Padhi *et al.* (2017)** published that the content of total polyphenols was recorded in selected cultivars of beans in the intervals from 1590 mg.kg^{-1} to 4330 mg.kg^{-1} DM. Comparing with our determined values of total polyphenols, their results were in similar interval. Our results are in correspondence with the results of **Giusti *et al*., (2017)** who indicated the content of total polyphenols in beans from 1120 mg.kg^{-1} to 3880 mg.kg^{-1} DM. There were found statistically significant differences in contents of total polyphenols between varieties ZENIT- white beans and NEGRA-black beans. Statistically significant highest TPC (p <0.05) was recorded in seeds in variety NEGRA-red beans (2156.87 mg GAE.kg^{-1} DM). Statistically significant lowest content of total polyphenols
(p <0.05) was recorded in seeds in variety ZENIT-white beans (1362.70 mg GAE.kg^{-1} DM).

Table 6 The average contents of total polyphenols (mg GAE.kg^{-1} DM) in selected species and varieties common bean seeds.

Species	Variety	TPC (mg GAE.kg^{-1} ± SD) DM
black beans	NEGRA	2156.87±105.2^b
red beans	RUBIN	1587.00±59.68^b
colour beans	INKA	1892.60±155.9^b
white beans	ZENIT	1362.70±59.13^b

TPC- the contents of total polyphenols, SD- the standard deviation
Multiple pairwise comparisons Kruskal-Wallis Tests,
Different letters (a, b and c) between the variables show statistically significant differences (p <0,05)
• a 0,05 >p > 0,01
• b 0,01 >p > 0,001
• c p < 0,001

CONCLUSION

Common bean (*Phaseolus vulgaris* L.) is one of the most widely wide-spread legume specie in the world and that´s why the aim of this work was to determinate the content of selected bioactive compounds. Common bean is very important nowadays. We have confirmed that the beans are characterized by an increased ability to cumulate some of the risk elements in the seeds. From the obtained results we found out that the limit values for Pb and Cd content, provided by the Slovak Food Code, exceeded in all seeds of the common bean varieties that were analysed. Seeds of the common beans are also characterized by a different content of bioactive substances (*trans*- caffeic acid 0.0108-0.0472 mg.kg^{-1}, syringic acid 0.0991-0.1865 mg.kg^{-1}, vitexin 0.0209-0.0290 mg.kg^{-1}, ellagic acid 0,1602-0,2864 mg.kg^{-1}, *trans* p-coumaric acid 0.0107-0.0368 mg.kg^{-1}, *trans*-sinapic acid 0.0215-0.0388 mg.kg^{-1}, ferulic acid 0.0657-0.1436 mg.kg^{-1}, resveratrol 0.0024-0.0106 mg.kg^{-1}, daidzein 0.0032-0.0089 mg.kg^{-1} and kaempferol 0.0036- 0.0082 mg.kg^{-1}). The highest macro element amount in common bean seeds was measured in K (12277 mg.kg^{-1}, ZENIT white beans) and the lowest amount was detected in Mn (10.70 mg.kg^{-1}, RUBIN red beans). The highest microelements amount in common bean seeds was Zn (17.8 mg.kg^{-1}, RUBIN red beans) and the lowest Co (0.40 mg.kg^{-1}, INKA colour beans).
Our chemical analyses revealed that the coloured seeds could be an excellent source of natural antioxidants, with demonstrating significantly higher content of total polyphenols. From the analysed varieties, NEGRA-black beans can be considered as the most important source of polyphenol substances with a demonstrable bioactive effect. Our results confirm that the type of crop, colour of the seeds also variety have an influence on the content of polyphenol compounds.

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