Selected legumes as a source of valuable substances in human nutrition

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Summary

The aim of the study was to determine the contents of nutrients, risk metals, total polyphenol content (*TPC*), total antioxidant activity (*TAA*) as well as selected phenolics content in 7 varieties of *Cicer arietinum* L., 11 varieties of *Lupinus albus* L. and 3 varieties of *Lathyrus sativus* L. The contents of nutrients and risk elements was determined by atomic absorption spectrometry, *TPC* values were estimated using the Folin-Ciocalteu reagent and *TAA* using the 2,2-diphenyl-1-picrylhydrazyl radical were determined spectrophotometrically. Content of caffeic acid, *trans*-ferulic acid, myricetin and genistein was determined by high-performance liquid chromatography with diode array detector. The determined *TPC* values (expressed as gallic acid equivalents) were in the range from 436 $mg \cdot kg^{-1}$ dry weight (dw) in chickpea to 7 863 $mg \cdot kg^{-1}$ dw in white lupin. The *TAA* values (expressed as Trolox equivalents) were in the range from 3.78 $mmol \cdot kg^{-1}$ dw (chickpea) to 17.5 $mmol \cdot kg^{-1}$ dw (grass pea). Content of genistein was in the range from 0.30 $mg \cdot kg^{-1}$ dw (grass pea) to 2.78 $mg \cdot kg^{-1}$ dw (chickpea). Caffeic acid (242–502 $mg \cdot kg^{-1}$ dw), *trans*-ferulic acid (1.39–5.90 $mg \cdot kg^{-1}$ dw) and myricetin (7.38–12.5 $mg \cdot kg^{-1}$ dw) were determined only in white lupin. The results confirmed that legume species that are not extensively used in our population can be a valuable source of bioactive compounds in human nutrition.

Keywords

chickpea; white lupin; grass pea; phenolic compound; macroelement; microelement; antioxidant activity

Legumes are plants taxonomically classified under the family Fabaceae, previously known as Leguminosae [1]. They yield one to 12 seeds or grains of various sizes, colours and forms, which are protected inside a pod [2]. The Fabaceae family constitutes the third largest family of flowering plants, comprising more than 650 genera and about 18 000 species [1].

Many legume species, such as chickpea (*Cicer arietinum* L.), white lupin (*Lupinus albus* L.), grass pea (*Lathyrus sativus* L.) or lentil (*Lens culinaris* L.) are important cultivated plants and their seeds can be used in human nutrition, animal feed or for production of plant-based oils [3]. From a nutritional point of view, grain legumes are considered important sources of plant proteins, essential minerals, carbohydrates, vitamins, bioactive compounds [1] and phytochemicals such as phenolic acids, anthocyanins, proanthocyanidins and flavonols [4]. The latter were identified in various species of legumes [2]. Their biological activity means that they have a positive effect on living organisms, tissues and cells. Bioactive compounds are foodstuff components that are involved in growth, biochemical reactions, mechanisms and development and provide significant health benefits [5]. Bioactive compounds also include antioxidants such as polyphenols. Antioxidants protect human body against oxidation induced by free radicals, superoxides, oxygen radicals and other substances [5]. These substances help prevent chronic diseases such as diabetes, coronary heart diseases and many types of cancers.

Grain legume seeds are important for human

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nutrition as they contain slow-release carbohydrates, where starch is the major carbohydrate in legumes [6]. The content of starch is 40–50 % of their total weight. The digestion rate for starch in cereals is higher than that in legumes. It has been found that grain legume seeds in human consumption provide several health benefits for various metabolic syndromes [7]. These crops could be valuable functional and/or medicinal food [8].

Legumes have unique nutritional and health-promoting properties related to the content of phytochemicals including polyphenols [3]. Polyphenols are secondary metabolites diverse in structure and with a wide phylogenetic distribution [9] that are valuable functional components in foods [10]. They are classified into different groups according to the number of phenol rings and to structural elements that bind these rings to one another [9]. Plant polyphenols are antioxidants against free radicals or reactive oxygen species because of their high redox potentials, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators [11]. Chemically, most of antioxidants belong to phenolics, sulphated polysaccharides, carotenoids, fatty acids or terpenoids [9]. Bioactive compounds from plants are signalling compounds [12], as protectants or attractants. The active substances such as flavonoids are examples of bioactive compounds that protect the plant from free radicals generated during biochemical process of capturing solar energy [13]. More than 8 000 polyphenols have been identified till present. These bioactive substances commonly found in leafy vegetables, fruits, tea, coffee, wine are present also in legumes [12].

Legumes contain also a wide range of antinutritive compounds with a negative effect on human health, such as lectins, phytates, protease inhibitors or allergens, which reduce assimilation of nutrients, could in some cases cause negative physiological effects or could be even toxic [14, 15]. Phytates in legumes affect biological availability of nutrients, reducing intestinal absorption and biological availability of some essential minerals, such as calcium, magnesium, iron and zinc [16]. These compounds can be eliminated by cooking but partly can be eliminated also by soaking or germination of legume seeds. On the other hand, several antinutritive substances, such as phytates, are responsible for some health benefits as they participate in lowering the levels of saccharides, cholesterol and triacylglycerides in blood [16].

The objective of this study was to determine the contents of valuable substances, such as phenolic compounds, macroelements and microelements, and to determine the content of risk metals in selected legumes. The obtained results were evaluated according to the valid legislation in Slovakia [17] as well as according to Commission Regulation (EC) 1881/2006 [18].

MATERIALS AND METHODS

Plant material

Samples of the plant material (seeds) were collected at a full maturity stage from Research Institute of Plant Production, National Agricultural and Food Centre in Piešťany (Slovakia). These comprised 11 varieties (Alban, Astra, R-933, Satmarean, Nelly, Pop I, Los Palacios, Primorskij, Solnečnyj, Weibit, Wtd) of white lupin (*Lupinus albus* L.), 7 varieties (Krajova z Kralovej, Maskovsky Bagovec, Businsky, Slovak, Beta, Alfa, Irenka) of chickpea (*Cicer arietinum* L.) and 3 varieties (Arida, Krajova z Kralovej, Cachticky cicer) of grass pea (*Lathyrus sativus* L.). The plants were conventionally cultivated in the same locality. Seeds of the plant material were manually separated, then dried at 105 °C to a constant weight and finally pulverized by a knife mill (Grindomix 200 GD; Retsch, Haan, Germany). Legumes were individually packed in paper packages and stored separately in a dry place with good ventilation, without sun radiance at room temperature.

Chemicals and reagents

Standard chemicals (caffeic acid, *trans*-ferulic acid, myricetin and genistein), methanol (gradient high-performance liquid chromatography (HPLC) grade), acetonitrile (gradient HPLC grade), phosphoric acid (American Chemical Society reagent grade) were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). HNO_3 (Suprapur) was obtained from Merck (Darmstadt, Germany). Folin-Ciocalteu reagent, gallic acid, 6-hydroxy-2,5,7,8-tetra-methylchromane-2-carboxylic acid (Trolox) and 2,2-diphenyl-1-picrylhydrazyl radical (•DPPH) were obtained from Merck. Deionized water (0.054 $\mu S \cdot cm^{-1}$) was obtained from Simplicity 185 purification system (Millipore SAS, Molsheim, France).

Determination of heavy metals content

Samples (1 g) were mineralized in a closed microwave digestion system Mars X-Press 5 (CEM, Cologno Al Serio, Italy) in a mixture of 5 ml HNO_3 and 5 ml deionized water. Digestion conditions comprised heating to 160 °C for 15 min, keeping the temperature constant for 20 min and then 20 min cooling. A blank sample was treated in the same way. The digested substances were subsequently filtered through a quantitative filter paper No. 390 (Munktell Filtrak, Bärenstein, Germany) and filled up with deionized water to a volume of 50 ml. Contents of selected elements (Zn, Cu, Ni, Cr, Pb, Fe, Mn, Co and Cd) were determined by flame atomic absorption spectroscopy (F-AAS) and graphite furnace atomic absorption spectrometry (GF-AAS) using Varian DUO 240FS/240Z/UltrAA (Varian, Palo Alto, California, USA) and expressed as milligrams per kilogram of dry weight (dw). The graphite furnace technique was used for the determination of Pb and Cd, whereas the F-AAS method was used for the determination of Zn, Cu, Ni, Fe, Mn, Co and Cr. Multielement standard for GF-AAS (16 elements, Merck) was used.

Preparation of extracts

The samples (5 g) were homogenized in a laboratory mixer (Kinematica, Luzern, Switzerland) and then extracted with 50 ml of 80% (v/v) methanol for 8 h in a Twisselman extractor (Behr Labor-Technik, Düsseldorf, Germany) operating at a temperature near the boiling point of the solvent. The extract was filtered through No. 390 paper into 50 ml vials and stored for 24 h until the analyses. Prior to injection, the standard solutions and sample extracts were filtered through a syringe microfilter Q-Max (pore size 0.45 μm, diameter 25 mm; Frisenette, Knebel, Denmark).

Determination of total polyphenol content

Total polyphenol content (*TPC*) was determined by the method of Lachman et al. [19] and expressed as milligram of gallic acid equivalents (GAE) per kilogram dw. *TPC* was estimated using the Folin-Ciocalteu reagent. Briefly, an aliquot of the extract, blank or standard was pipetted in a 50 ml flask, the Folin-Ciocalteu reagent (2.5 ml) was added and the mixture was allowed to react for 3 min under continuous stirring. Finally, a solution of sodium carbonate (7.5 ml) was added and mixed thoroughly. The volume was then made up to 50 ml with distilled water and left to stay at room temperature for 2 h when a coloured blue complex was formed. The absorbance was measured at 765 nm wavelength against blank (spectrophotometer UV-1800, Shimadzu, Kyoto, Japan). *TPC* was calculated from a standard curve constructed with gallic acid solutions of known concentrations.

Determination of individual phenolics content

Phenolic compounds were determined by a modified method of Gabriele et al. [20] using Agilent 1260 Infinity HPLC system (G1315C; Agilent Technologies., Santa Clara, California, USA). All HPLC analyses were performed on a Purosphere reverse phase C18 column (250 mm × 4 mm × 5 μm; Merck). The detection wavelength was 320 nm for caffeic acid and *trans*-ferulic acid, and 372 nm for myricetin and genistein.

Determination of total antioxidant activity

Total antioxidant activity (*TAA*) was determined by the method of Brand-Williams et al. [21] using of DPPH radical (•DPPH, Merck), which is a stable radical in solution and appears purple coloured, absorbing at 515 nm in methanol. The initial concentration of •DPPH solution (A_0) was recorded and absorbance was read at 515 nm using Shimadzu UV-VIS 1800 spectrophotometer. Antioxidants present in the methanol extract of the sample reduced •DPPH and changed the colour of the solution proportionally to the antioxidant concentration. *TAA* was expressed as millimoles of Trolox equivalents (TE) per kilogram dw.

Statistical analysis

Each analysis was done in four repetitions. The results were statistically evaluated by one-way and multifactor ANOVA (analysis of variance – multiple range tests, method: 95.0 percent least significant difference using the statistical software Statgraphics Centurion XVI.I (Statpoint Technologies, Warrenton, Virginia, USA), the data being considered significantly different when $p < 0.05$. Calculations were performed using MS Excel 2016 (Microsoft, Redmond, Washington, USA) and XLSTAT (Addinsoft, New York, New York, USA).

RESULTS AND DISCUSSION

Defficiency in certain minerals in human nutrition, either macroelements or microelements, may cause diseases or functional disorders [22]. Legumes may be in particular an important source of microelements [23]. In Tab. 1 and Tab. 2, our analytical results on the contents of selected micro- and macroelements in chickpea, white lupin and grass pea seeds are presented.

The highest average content of Cu and Fe was determined in grass pea (8.23 mg·kg^{-1} and 47.6 mg·kg^{-1}, respectively), while the highest Mn, Cr, Ni and Co content was found in white lupin (572, 0.93, 3.99 and 0.30 mg·kg^{-1}, respectively). Chickpea contained the highest (22.4 mg·kg^{-1}) average amount of Zn (Tab. 1). According to our results (Tab. 2), chickpea contained the highest

Tab. 1. Contents of microelements in chickpea, white lupin and grass pea seeds.

Variety	Cu	Zn	Mn	Fe	Cr	Ni	Co
	[mg·kg-1]						
Chickpea							
Krajova	7.10 b	23.0 e	38.0 f	45.2 b	0.50 b	2.90 d	1.00 b
Maskovsky	7.60 d	22.9 d	29.0 c	45.3 c	0.50 b	2.20 a	0.90 a
Businsky	9.90 f	23.7 g	30.9 d	47.9 e	0.60 c	2.80 c	0.90 a
Slovak	7.90 e	23.6 f	25.8 a	47.9 e	0.50 b	2.90 d	0.90 a
Beta	7.20 c	20.3 a	27.8 b	45.5 d	0.40 a	2.60 b	1.00 b
Alfa	6.70 a	21.7 c	38.5 g	50.4 f	0.70 d	3.30 f	1.00 b
Irenka	7.10 b	21.5 b	33.5 e	34.5 a	0.60 c	3.10 e	0.90 a
Mean	7.64 B	22.4 C	31.9 A	45.2 B	0.54 A	2.83 B	0.94 B
White lupin							
Alban	6.20 c	19.0 c	484 a	35.6 e	1.70 g	3.20 b	0.20 b
Astra	7.40 h	22.6 g	609 j	39.4 j	1.30 g	4.30 g	0.40 d
R-933	6.80 f	19.9 d	607 k	36.8 f	1.00 d	4.80 j	0.40 d
Satmarean	6.90 g	24.1 i	590 f	38.0 h	1.00 d	4.50 h	0.10 a
Nelly	6.80 f	22.6 g	552 d	40.0 k	0.80 c	3.70 e	0.30 c
Pop I	6.20 c	20.3 e	536 b	35.5 d	0.60 b	3.50 d	0.40 d
Los Palacios	6.60 e	19.9 d	568 e	34.9 b	1.10 e	4.60 i	0.20 b
Primorskij	6.60 e	23.7 h	605 i	38.2 i	0.60 b	5.20 k	0.30 c
Solnečnyj	6.30 d	18.1 a	591 g	35.0 c	0.50 a	3.30 c	0.50 e
Weibit	6.10 b	21.2 f	601 h	36.9 g	0.80 c	3.80 f	0.30 c
Wtd	5.40 a	18.5 b	548 c	32.4 a	0.80 c	3.00 a	0.20 b
Mean	6.48 A	20.9 B	572 B	36.6 A	0.93 B	3.99 C	0.30 A
Grass pea							
Arida	7.40 a	16.8 b	12.4 b	36.2 a	0.20 a	1.70 b	0.40 b
Kralova	8.80 c	16.6 a	11.9 a	48.7 b	0.30 b	1.50 a	0.20 a
Cachticky	8.50 b	17.3 c	14.2 c	57.9 c	0.40 c	2.00 c	0.20 a
Mean	8.23 B	16.9 A	12.8 A	47.6 B	0.30 A	1.73 A	0.26 A

Values are given per kilogram of dry weight. Different small letters (a–k) show statistically significant differences ($p < 0.05$) between varieties in each of the investigated legumes as examined by least significant difference test. Different capital letters (A–C) show statistically significant differences ($p < 0.05$) between the investigated legume species as examined by least significant difference test.

average amounts of K and P (7368 mg·kg^{-1} and 2172 mg·kg^{-1}), while in white lupin, the highest contents of Na and Ca were determined (164 mg·kg^{-1} and 2823 mg·kg^{-1}). In chickpea, the highest average Mg content (1110 mg·kg^{-1}) was determined.

Our results for Cu, Zn and Fe are in correspondence with results presented by Akinyele and Shokunbi [24] and Sanchez-Castillo et al. [25], on the other hand our values for Ni, Cr and Mn are higher. Prusinki [26] reported that white lupin contained Cr at 11.3–176 mg·kg^{-1}, Mn at 1675–4095 mg·kg^{-1}, Fe at 78–93 mg·kg^{-1}, Co at 16.2–16.6 mg·kg^{-1}, Ni at 12–15.6 mg·kg^{-1}, Cu at 4.8–9.9 mg·kg^{-1} and Zn at 40.3–53.6 mg·kg^{-1}. These values were lower when compared to our results, but contents for K, Na, Mg and Ca were higher in comparison with our results. Venkidasamy et al. [27] found out that the contents of mineral elements in white lupin and grass pea were lower in comparison with our assessed contents, except for P content in white lupin and the values for Zn, Cu, P, Na in chickpea seeds that were consistent with our results. These differences could be caused by cultivation locality, variety or conditions of land and agricultural treatments [28]. Our results confirmed the statistical significant differences between varieties in each of the investigated legume species.

In Tab. 3, the results of selected risk metal content in chickpea, white lupin and grass pea seeds are presented. The maximum permitted amounts of metals in foods are Zn 50.0 mg·kg^{-1}, Cu 15.0 mg·kg^{-1}, Ni 3.0 mg·kg^{-1}, Cr 4.0 mg·kg^{-1}, Pb 0.2 mg·kg^{-1} and Cd 0,1 mg·kg^{-1} (limit values for legumes according to the legislation of the Slovakia [17], for Pb and Cd maximum level according to Commission Regulation 1881/2006 [18] are given). Excessive accumulation of risk metals in plant foods grown in contaminated regions can

Tab. 2. Contents of macroelements in chickpea, white lupin and grass pea seeds.

Variety	K	Na	Ca	Mg	P
	[mg·kg-1]				
Chickpea					
Krajova	7161 c	56.0 c	1300 d	1048 c	2151 e
Maskovsky	7086 b	47.1 b	984 a	1009 b	2675 g
Businsky	7496 d	10.1 a	1153 b	1299 g	2542 f
Slovak	6988 a	81.0 e	1433 e	969 a	2065 c
Beta	7728 g	95.0 g	1293 c	1116 e	1770 a
Alfa	7503 e	69.3 d	1620 g	1066 d	1917 b
Irenka	7616 f	90.5 f	1611 f	1262 f	2083 d
Mean	7368 A	64.1 A	1342 A	1110 B	2172 A
White lupin					
Alban	7586 d	223 j	3128 j	1190 j	1853 c
Astra	8450 h	163 f	2568 b	1073 c	2868 k
R-933	6890 a	128 b	2646 d	1008 b	1283 a
Satmarean	7150 b	132 c	2416 a	1217 k	1928 e
Nelly	8153 f	115 a	2749 e	1123 h	1806 b
Pop I	8488 j	188 i	2890 f	1161 i	2561 j
Los Palacios	8457 i	188 i	2919 h	1107 g	1945 g
Primorskij	8305 g	173 g	3030 i	1107 f	1860 d
Solnečnyj	7948 e	182 h	2641 c	1101 e	1979 i
Weibit	7494 c	149 d	2902 g	996 a	1938 f
Wtd	8495 k	160 e	3162 k	1088 d	1969 h
Mean	7947 B	164 C	2823 B	1107 B	1999 A
Grass pea					
Arida	8915 b	103 a	1738 c	1017 b	2729 b
Kralova	9270 c	127 c	1366 b	1026 c	2734 c
Cachticky	8809 a	126 b	1340 a	930 a	2327 a
Mean	8998 C	119 B	1481 A	991 A	2597 B

Values are given per kilogram of dry weight. Different small letters (a–k) show statistically significant differences ($p < 0.05$) between varieties in each of the investigated legumes as examined by least significant difference test. Different capital letters (A–C) show statistically significant differences ($p < 0.05$) between the investigated legume species as examined by least significant difference test.

seriously affect global food security and quality of foods [29].

In all samples of chickpea, the content of Pb was under the limit of detection. The Pb content in white lupin seeds measured in varieties Satmarean, Nelly, Weibit and Wtd exceeded the limit value given in the legislation (0.20 mg·kg^{-1} dw) [17, 18]. The hygienic limit for Pb in the seeds was exceeded in each of the investigated varieties of grass pea. The determined contents of Cd in chickpea seeds in the varieties Businsky, Slovak and Beta were under the detected limit. The Cd contents in the other chickpea varieties were lower than the maximum permitted Cd amount of 0.10 mg·kg^{-1}. The hygienic limit for Cd was exceeded in two white lupin varieties (Nelly and Pop I), while in varieties Alban, Astra and R-933, the Cd content was under the detected limit. Only in Kralova variety of grass pea, the determined Cd content exceeded the hygienic limit. The content of Hg in all varieties of chickpea, white lupin as well as grass pea was lower compared to the maximum permitted Hg content of 0.05 mg·kg^{-1}. In Businsky variety of chickpea, the Hg content was under the limit of detection.

Legumes are a good source of bioactive phenolic compounds, which play significant roles in many physiological as well as metabolic processes in the human organism [30]. The average contents of selected polyphenols determined by HPLC are shown in Tab. 4. The content of monitored substances was in a wide range depending on the type of legume. Only in varieties of white lupin, caffeic acid, *trans*-ferulic acid and myricetin were determined. The highest content of caffeic acid was determined in Los Palacios variety (502 mg·kg^{-1} dw) and the lowest content was determined in Alban variety (242 mg·kg^{-1} dw). The content of *trans*-ferulic acid in white lupin varieties was in the range from 1.39 mg·kg^{-1} (Solnečnyj variety) to

Tab. 3. Contents of risk metals in chickpea, white lupin and grass pea seeds.

Variety	Pb	Cd	Hg
	[mg·kg-1]		
Chickpea			
Krajova	< *LOD*	0.03 ± 0.01 bc	0.002 ± 0.001 b
Maskovsky	< *LOD*	0.05 ± 0.01 c	0.003 ± 0.001 c
Businsky	< *LOD*	< *LOD*	< *LOD*
Slovak	< *LOD*	< *LOD*	0.002 ± 0.000 b
Beta	< *LOD*	< *LOD*	0.001 ± 0.000 a
Alfa	< *LOD*	0.01 ± 0.00 a	0.002 ± 0.000 b
Irenka	< *LOD*	0.03 ± 0.01 bc	0.001 ± 0.001 a
Mean	< *LOD*	0.02 A	0.002 A
White lupin			
Alban	< *LOD*	< *LOD*	0.009 ± 0.001 f
Astra	0.10 ± 0.01 a	< *LOD*	0.005 ± 0.000 c
R-933	0.10 ± 0.01 a	< *LOD*	0.006 ± 0.000 h
Satmarean	0.30 ± 0.05 b	0.09 ± 0.01 d	0.005 ± 0.000 c
Nelly	0.30 ± 0.05 b	0.10 ± 0.01 e	0.004 ± 0.000 b
Pop I	< *LOD*	0.10 ± 0.01 e	0.007 ± 0.001 d
Los Palacios	< *LOD*	0.08 ± 0.01 c	0.008 ± 0.001 e
Primorskij	0.10 ± 0.01 a	< *LOD*	0.005 ± 0.001 c
Solnečnyj	0.10 ± 0.01 a	< *LOD*	0.013 ± 0.001 g
Weibit	0.30 ± 0.05 b	0.03 ± 0.01 a	0.003 ± 0.001 a
Wtd	0.30 ± 0.05 b	0.06 ± 0.01 b	0.003 ± 0.000 a
Mean	0.15 A	0.04 B	0.005 B
Grass pea			
Arida	0.30 ± 0.04 a	0.08 ± 0.01 b	0.004 ± 0.001 b
Kralova	0.40 ± 0.04 b	0.10 ± 0.01 c	0.002 ± 0.000 a
Cachticky	0.50 ± 0.06 c	0.06 ± 0.01 a	0.002 ± 0.000 a
Mean	0.40 B	0.08 C	0.003 AB
Limit [17]	0.20	0.10	0.05
LOD	0.02	0.001	0.000020
LOQ	0.040	0.010	0.000040

Values are given per kilogram of dry weight. Values represent mean ± standard deviation. Different small letters (a–h) show statistically significant differences ($p < 0.05$) between varieties in each of the investigated legumes as examined by least significant difference test. Different capital letters (A–C) show statistically significant differences ($p < 0.05$) between the investigated legume species as examined by least significant difference test.
LOD – limit of detection, *LOQ* – limit of quantitation.

Tab. 4. Average contents of selected polyphenols in chickpea, white lupin and grass pea seeds.

Variety	CA	*t*FA	MYR	GEN
	[mg·kg-1]			
Chickpea				
Krajova	< *LOD*	< *LOD*	< *LOD*	2.35 bc
Maskovsky	< *LOD*	< *LOD*	< *LOD*	2.10 bc
Businsky	< *LOD*	< *LOD*	< *LOD*	2.23 bc
Slovak	< *LOD*	< *LOD*	< *LOD*	1.98 ab
Beta	< *LOD*	< *LOD*	< *LOD*	2.44 cd
Alfa	< *LOD*	< *LOD*	< *LOD*	2.78 d
Irenka	< *LOD*	1.34 a	< *LOD*	1.69 a
Mean	< *LOD*	0.19 A	< *LOD*	2.22 C
White lupin				
Alban	242 a	4.38 f	7.72 ab	0.70 a
Astra	473 h	7.42 h	9.11 c	0.90 a
R-933	457 g	4.79 f	7.38 a	0.65 a
Satmarean	477 i	5.90 g	12.50 e	0.69 a
Nelly	331 c	3.54 e	8.16 b	0.84 a
Pop I	452 f	5.70 g	7.35 a	0.70 a
Los Palacios	502 k	2.88 d	7.91 ab	0.80 a
Primorskij	420 e	1.79 ab	9.96 d	0.80 a
Solnečnyj	312 b	1.39 ab	9.66 cd	0.79 a
Weibit	419 d	2.16 bc	< *LOD*	0.69 a
Wtd	489 j	2.63 cd	7.35 a	0.89 a
Mean	416 A	3.87 B	7.92 A	0.77 B
Grass pea				
Arida	< *LOD*	< *LOD*	< *LOD*	0.30 a
Kralova	< *LOD*	< *LOD*	< *LOD*	0.30 a
Cachticky	< *LOD*	< *LOD*	< *LOD*	0.39 a
Mean	< *LOD*	< *LOD*	< *LOD*	0.33 A

Values are given per kilogram of dry weight. Different small letters (a–k) show statistically significant differences ($p < 0.05$) between varieties in each of the investigated legumes as examined by least significant difference test. Different capital letters (A–C) show statistically significant differences ($p < 0.05$) between the investigated legume species as examined by least significant difference test.
CA – caffeic acid, *t*FA – *trans*-ferulic acid, MYR – myricetin, GEN – genistein, *LOD* – limit of detection.

7.42 mg·kg^{-1} (Astra variety), while the myricetin content was in the range from 7.35 mg·kg^{-1} (Wtd and Pop I varieties) to 12.5 mg·kg^{-1} (Satmarean variety). The myricetin content in Weibit variety was under the limit of detection. In all varieties of the other investigated legumes, the contents of caffeic acid, *trans*-ferulic acid and myricetin were under the limits of detection with exception of Irenka variety of chickpea, where the determined content of *trans*-ferulic acid was 1.34 mg·kg^{-1}. Genistein was determined in all of our analysed samples. The highest content of genistein in chickpea was 2.78 mg·kg^{-1} dw in variety Alfa and the lowest (1.69 mg·kg^{-1} dw) in variety Irenka. The highest content of genistein in white lupin was determined in variety Astra (0.90 mg·kg^{-1} dw) and the lowest one in variety R-933 (0.65 mg·kg^{-1} dw). In grass pea, the genistein content was in the range from 0.30 mg·kg^{-1} (Arida and Kralova varieties) to 0.39 mg·kg^{-1} (Cachticky variety). Statistically lowest content of genistein was determined in samples of grass pea and the statistically highest one in samples of chickpea.

TPC and antioxidant activity values are given in Tab. 5. The highest *TPC* (expressed as GAE) was recorded in seeds of white lupin of variety Satmarean (7863 mg·kg^{-1} dw) and the lowest one was recorded in seeds of chickpea of variety Alfa 436 (mg·kg^{-1} dw). The highest *TAA* (expressed as TE) was recorded in seeds of grass pea of variety Kralova (17.5 mmol·kg^{-1} dw) and the lowest value was recorded in seeds of white lupin of variety Beta (3.78 mmol·kg^{-1} dw). The value ranges for *TPC* (expressed as GAE) in chickpea, white lupin and grass pea were 436–972 mg·kg^{-1}, 5629–7863 mg·kg^{-1} and 654–1186 mg·kg^{-1}, respectively. The determined values of *TAA* (expressed as TE) were in the range of 3.78–9.20 mmol·kg^{-1} for chickpea, 10.4–15.5 mmol·kg^{-1} for white lupin and 13.4–17.5 mmol·kg^{-1} for grass pea. For comparison, *TPC* was previously determined to be in the range of 650–9600 mg·kg^{-1} dw in selected cultivars of legumes [28]. Gusti et al. [31] determined *TPC* in selected varieties of chickpea in the range 720–1200 mg·kg^{-1} dw. Those previously published values are comparable to our results. Our values are higher compared to Wang et al. [32], who determined *TPC* in the range 362–1540 mg·kg^{-1} dw and *TAA* in the range 3.221–7.107 mmol·kg^{-1} dw (expressed as TE) in selected cultivars of legumes. Our results are in correspondence with the results of Xu et al. [33], who determined *TPC* of 980 mg·kg^{-1} dw for chickpea. Padhi et al. [34] reported that *TPC* of chickpea ranged from 1470 mg·kg^{-1} dw to 2870 mg·kg^{-1} dw and *TAA* was in the range from 32.36 mmol·kg^{-1} dw to 120.16 mmol·kg^{-1} dw. Our results were higher when compared to those values. It is well known that the content of health-beneficial compounds in plants is affected by many factors such as agrochemical treatments, climatic conditions, and also storage conditions and cultivars [35].

Tab. 5. Total polyphenols content and antioxidant activity in selected species and varieties of chickpea, white lupin and grass pea seeds.

Variety	*TPC* [mg·kg^{-1}]	*TAA* [mmol·kg^{-1}]
Chickpea		
Krajova	803^{cd}	9.20^{b}
Maskovsky	746^{bc}	4.51^{a}
Businsky	717^{b}	4.22^{a}
Slovak	839^{d}	5.39^{a}
Beta	473^{a}	3.78^{a}
Alfa	436^{a}	5.97^{a}
Irenka	972^{e}	9.05^{b}
Mean	712^{A}	6.02^{A}
White lupin		
Alban	7289^{d}	11.9^{bcd}
Astra	7768^{ef}	15.5^{f}
R-933	6412^{bc}	15.5^{f}
Satmarean	7863^{f}	12.3^{d}
Nelly	5629^{a}	11.0^{abc}
Pop I	7544de	14.2^{e}
Los Palacios	6476^{bc}	10.8^{ab}
Primorskij	6369^{bc}	11.2^{a}
Solnečnyj	6221^{bc}	12.4^{d}
Weibit	6629^{c}	12.1^{cd}
Wtd	6577^{c}	10.4^{a}
Mean	6798^{B}	12.5^{B}
Grass pea		
Arida	1186^{c}	13.9^{a}
Kralova	795^{b}	17.5^{b}
Cachticky	654^{a}	13.4^{a}
Mean	878^{A}	14.9^{C}

Values represent mean. Different small letters (a–f) show statistically significant differences ($p < 0.05$) between varieties in each of the investigated legumes as examined by least significant difference test. Different capital letters (A–C) show statistically significant differences ($p < 0.05$) between the investigated legume species as examined by least significant difference test.
TPC – total polyphenols content expressed as milligrams of gallic acid equivalents per kilogram of dry weight, *TAA* – total antioxidant activity expressed as millimoles of Trolox equivalents per kilogram of dry weight.

CONCLUSIONS

Risk metals should be eliminated from all foodstuffs of plant origin. In this study, none of the determined Cd and Hg contents in seeds of chickpea, white lupin and grass pea present risk to the consumer with regard to maximum levels given by the Slovak legislation [17]. On contrary, the limit values for Pb content given in the legislation [17, 18] were exceeded in seeds of white lupin in varieties Satmarean, Nelly, Weibit, Wtd and in all investigated varieties of grass pea. This represents an increased risk at regular consumption of white lupin and grass pea as foodstuffs. It is known that Pb can entry into water, soil as well as plants in form of dry or wet deposition mainly from polluted atmosphere. Via long distance transport, Pb can get from remote industrial emission sources to clean places, where it can contaminate the plant production. Our results confirmed that legumes are able to accumulate some risky metals in seeds. On the other hand, legumes are a food raw material that provides a wide range of nutritive components, trace elements and bioactive substances. The determined average values of *TPC* (expressed as GAE) varied from 436 mg·kg^{-1} dw in chickpea (variety Alfa) to 7863 mg·kg^{-1} dw in white lupin (variety Satmarean). The highest *TAA* (expressed as TE) was recorded in grass pea seeds variety Kralova (17.5 mmol·kg^{-1} dw) and the lowest one in chickpea variety Beta (3.78 mmol·kg^{-1} dw). It can be concluded that legumes are a rich source of polyphenols with chemoprotective properties and can be a valuable source of bioactive compounds in human nutrition.

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