Varietal influence on the content of biologically valuable compounds in selected legumes

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Summary

The present study surveyed antioxidant properties of selected legumes less utilized by our population. These were determined spectrophotometrically by various methods, namely, using radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), the ferric reducing antioxidant power (FRAP) and total polyphenols contents (*TPC*) using Folin–Ciocalteu reagent. The contents of individual phenolics were determined by high performance liquid chromatography with diode array detector in methanolic extracts from 11 varieties of white lupin, 3 varieties of grass pea and 7 varieties chickpea. The varieties of legumes were a major source of variation in content of phenolic substances and total antioxidant activity (*TAA*). The highest content of phenolic acids had white lupin var. Solnečnyj with caffeic acid at 568 $mg \cdot kg^{-1}$ dry weight (dw) and the lowest content of quercetin had grass pea var. Krajova at 0.163 $mg \cdot kg^{-1}$ dw. *TPC* (expressed as gallic acid equivalent) values were in the range from 1 273 $mg \cdot kg^{-1}$ dw (grass pea) to 8 004 $mg \cdot kg^{-1}$ dw (white lupin). The results confirmed that the variety, but also the crop species, significantly differ in the content of biologically valuable components in selected legumes. The present study provides data on the phytochemical composition of legumes and the antioxidant properties of these compounds.

Keywords

legume; white lupin; chickpea; grass pea; antioxidant activity; flavonoid; polyphenol

Legumes are annual crops from family Fabaceae that represent a significant source of proteins of plant origin for human nutrition and livestock [1]. This large plant family includes approximately 18 000 species with seeds of various shapes, sizes and colours, characterized by the content of various biologically active compounds that are protected inside the pod [1, 2]. Seeds of plants such as chickpea (*Cicer arietinum* L.), white lupin (*Lupinus albus* L.) or grass pea (*Lathyrus sativus* L.) can be used for human nutrition, animal feeding and for plant-based oil production [3]. Legumes have been the staple food implemented into the nutrition of various cultures worldwide. Their high nutrition value and low cost have made them an interesting source of biologically active compounds, proteins, starch and phytochemicals [4].

Plant foodstuffs and legume seeds contain a large number of phytochemicals, such as phenolic acids or flavonoids [5]. Flavonoids and phenolic acids form the most important group of secondary metabolites and extra-nutritional constituents in plants [6]. The consumption of plant foods provides a diverse range of flavonoids, which can act synergistically as antioxidants, anticancer and anti-inflammatory agents [7]. Phenolic acids and flavonoids are effective as reducing agents, scavengers of free radicals and quenchers of singlet oxygen. Besides this, flavonoids and

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phenolic acids play a relevant role by the reduction of risk of cancer development and also other diseases of civilization incidence. Thus, integrating of legumes into dietary patterns is important for promoting human health [6].

It is known that the content of phenolic compounds and antioxidant activity of the plants vary among species and cultivars. Significant differences among wild grown and cultivated varieties of some plants have been reported. LAMPART-SZCZAPA et al. [8] investigated the total polyphenols contents of bitter and sweet lupin seeds, while the bitter varieties exerted higher values when compared to sweet varieties.

Legumes contain a wide spectrum of antinutritive compounds with a negative effect on human organism, which is rather limiting for using legumes in diet [9]. Selection of cultivars with a low content of antinutritive compounds or utilization of proper processing methods and technological treatments deactivate or eliminate most of them in seeds, which may help to increase the use of legumes in human diet [10]. Content and composition of biologically active and antinutritive compounds in plant seeds is known to be affected mainly by variety, conditions of processing, conditions of storage and by climatic conditions [11].

The present study aimed to gain knowledge that can help the consumer to make the right choice by selecting of plant raw material in the way to maximalize the intake of phytochemicals beneficial to human organism. Due to the possible benefit of biologically active compounds in legumes for the health of consumer, the chosen variety and also legume species are very important factors. Therefore, it is relevant to describe the composition and content of biologically active compounds in various varieties and legume species to increase their consumption. The aim of this study was to determine the content of biologically valuable compounds, polyphenols and flavonoids, and to evaluate the influence of variety on the content of these compounds in selected types of legumes.

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 included 3 old regional varieties (Arida, Krajova from Kralová, Cachticky) of grass pea (*Lathyrus sativus* L.), 7 old regional varieties (Krajova, Maskovsky Bagovec, Businsky, Slovak, Beta, Alfa, Irenka) of chickpea (*Cicer arietinum* L.) and 11 old regional varieties (Alban, Astra, R-933, Satmarean, Nelly, Pop I, Los Palacios, Primorskij, Solnečnyj, Weibit, Wtd) of white lupin (*Lupinus albus* L). The plants were conventionally cultivated in Piešťany, Slovakia. 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). Ground samples were individually packed in paper packages and stored separately in a dry place with good ventilation, without sun light at laboratory temperature 22 °C for a maximum 78 h.

Chemicals and reagents

Standard chemicals (4-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, *trans*-ferulic acid, kaempferol, myricetin, quercetin, genistein and apigenin), 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). Deionized water (0.054 $\mu S\cdot cm^{-1}$) was obtained from Simplicity 185 purification system (Millipore SAS, Molsheim, France). Potassium persulphate, acetic acid, sodium acetate, 40 $mmol\cdot l^{-1}$ hydrochloric acid, ferric chloride hexahydrate, Folin-Ciocalteu reagent, gallic acid, 6-hydroxy-2,5,7,8-tetra-methylchromane-2-carboxylic acid (Trolox) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate)) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) were obtained from Merck (Darmstadt, Germany).

Preparation of extracts

Seeds for analysis were homogenized by a laboratory mixer (Kinematica, Luzern, Switzerland) for 30 s to a fine powder. An amount of 5 g of it was weighed on an analytical balance into a cellulose thimble and extracted in 50 ml 80% methanol for 8 h in Twisselman extractor (BehrLabor-Technik, Düsseldorf, Germany). The extracts were filtered using filter paper (130 $g\cdot m^{-2}$; Filtrak, Thermalbad Wiesenbad, Germany) into 50 ml centrifuge tubes and stored for 24 h until the analyses. Before injection, the standard solutions and sample extracts were filtered through a syringe microfilter Q-Max (cellulose acetate, pore size 0.45 μm, diameter 25 mm; Frisenette, Knebel, Denmark).

Total polyphenols content

The total polyphenols content (*TPC*) was determined in methanol extracts following the method of Lachman et al. [12] by spectrophotometry using the Folin-Ciocalteu assay. An aliquot portion of the prepared extract (2 ml) was pipetted into a volumetric flask (50 ml). The sample was diluted with distilled water, 2.5 ml Folin-Ciocalteu reagent was added and thoroughly mixed. After 3 min of reacting, 7.5 ml of 20% sodium carbonate was added and the flask was mixed again. Then, distilled water was added till the mark, the sample was mixed and left to react for 2 h (formation of a blue complex took place). Calibration curve was prepared from the solution of gallic acid (5 $\mu g \cdot ml^{-1}$). Absorbance of blue solutions against control at a wavelength of 765 nm was determined using UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The results were calculated as milligram per kilogram of gallic acid equivalents (GAE) on dry weight (dw) basis.

Individual phenolics content

Phenolic compounds were determined by a modified method of Gabriele et al. [13] 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 4-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, *trans*-ferulic acid and 372 nm for kaempferol, myricetin, quercetin, genistein and apigenin.

Total antioxidant activity

Total antioxidant activity (*TAA*) was determined by three different methods using radicals DPPH, ABTS and the ferric reducing antioxidant power (FRAP), respectively.

DPPH radical-scavenging capacity was determined by the method of Brand-Williams et al. [14]. Into a cuvette, 3.9 cm^3 of DPPH solution was pipetted and the absorbance was determined (A_0 means initial concentration of DPPH solution). Then, 0.1 cm^3 of the extract was added (according to individual plant species). The absorbance of the mixed solution was assessed by spectrophotometer UV-1800 in relation to value A_t (in time intervals; after 1 min till 10 min) at 515.6 nm. Radical-scavenging capacity of at defined time was calculated from the formula that presents *TAA*. *TAA* was expressed as millimoles of Trolox equivalents (TE) per kilogram dry weight.

Determination of antioxidant activity using ABTS radical was achieved by the method of Paulova et al. [15]. The 0.1 $mol \cdot l^{-1}$ acetate buffer (pH 4.3) was prepared and then 3 ml of ABTS solution was pipetted into cuvettes. Then, 50 μl of the extract from tested crops was added. After 20 min (reduction of radical cation ABTS), absorbance was measured at 734 nm. Trolox was used as the standard. The results were calculated as millimoles of Trolox per kilogram of the sample based on the calibration curve.

FRAP was determined by the method of Paulova et al. [15] using of TPTZ radical. Acetate buffer (0.1 $mol \cdot l^{-1}$, pH 3.5) was prepared and aliquots of the solutions were mixed with a FRAP reagent followed by spectrophotometric measurement of the absorbance of the reaction mixture after incubation at 37 °C for 10 min at 593 nm against the blank. FRAP assay has so far been widely used to directly test the total antioxidant potential of several foods and plant extracts based on the reduction of complexes of TPTZ with ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), which are almost colourless. The solution will eventually turn slightly brownish forming blue ferrous complexes after complete reduction. *TAA* was expressed as millimoles of Trolox equivalents (TE) per kilogram dw.

Statistical analysis

Each analysis was done in four replicates and the results were as mean. For statistical evaluation of experimental data of assessed *TPC* as well as phenolic compounds and antioxidant activity, both statistical methods of one-way analysis of variance and two-way analysis of variance were used and we observed the relation of more than two of the middle values of basic files using the statistical software Statgraphics Centurion XVI.I (Statpoint Technologies, Warrenton, Virginia, USA). Statistical differences were evaluated using least significant difference (LSD) test at a level of significance of 0.05. These calculations were performed with MS Excel 2016 (Microsoft, Redmond, Washington, USA) and XLSTAT 2014 (Addinsoft, New York, New York, USA).

RESULTS AND DISCUSSION

In Tab. 1A, average values of *TPC* (expressed as GAE) and *TAA* (expressed as TE) in seeds of lupin varieties are presented. The *TPC* ranged from 6 522 $mg \cdot kg^{-1}$ dw (Nelly) to 8 004 $mg \cdot kg^{-1}$ dw (Alban). Values of varieties were similar to each other. Statistically significant differences were identified for *TPC* values among varieties of lupin in 5 content values, i. e. R-933/Satmarean; Satmarean/Nelly; Satmarean/Primorskij; Satmarean/Solnečnyj; Satmarean/Wtd. The varieties could be ranked in the following order on the basis of *TPC*:

Alban > Weibit > Satmarean > Pop I > Astra > Los Palacios > Primorskij > Solnečnyj > Wtd > R-933 > Nelly.

The highest average *TAA* value determined by the DPPH method in tested varieties of lupin was determined for Solnečnyj variety (17.8 mmol·kg-1 dw). On the contrary, the lowest value was determined for Wtd variety, which was by 41.8 % lower in comparison with Solnečnyj variety. Our assessments were in accordance with the results of Boudjou et al. [16] who reported the *TAA* value (DPPH method) for white lupin of 16.7 mmol·kg-1 dw. The *TAA* values determined by the FRAP method were relatively similar to each other, in a range from 1.82 mmol·kg-1 dw (Astra) to 2.44 mmol·kg-1 dw (Solnečnyj). Antioxidant activity assessed by the ABTS method was the highest (9.27 mmol·kg-1 dw) in variety Weibit and the lowest in variety Alban (7.14 mmol·kg-1 dw).

Statistically significant differences among *TAA* values of varieties were found only for the DPPH method, specifically, for pairs of varieties Alban/Wtd, Astra/Primorskij, Astra/Wtd, R-933/Primorskij, R-933/Wtd, Satmarean/Wtd, POP I/Wtd, Los Palacio/Wtd, Primorskij/Solnečnyj, Weibit/Wtd and Solnečnyj/Wtd.

Tab. 1B presents average values of *TPC* and *TAA* for chickpea. *TPC* values ranged from 1 273 mg·kg-1 dw (variety Krajova) to 1 763 mg·kg-1 dw (variety Irenka). No statistically significant differences in the contents of polyphenols among varieties of chickpea were determined. The varieties could be ranked on the basis of *TPC* values in the order Krajova > Maskovsky Bagovec > Alfa > Beta > Businsky > Slovak > Irenka. *TPC* values in chickpea determined in this study were considerably higher than those reported previously by Mahbub et al. [17].

The highest *TAA* value determined by the DPPH method was in variety Irenka (16.9 mmol·kg-1 dw) and the lowest value in variety Beta (8.6 mmol·kg-1 dw). No statistically significant differences in *TAA* values determined by this method among chickpea varieties were found. Similarly, no statistically significant differences were found among chickpea varieties in *TAA* values determined by the FRAP method, while their interval was in a range 1.57–2.03 mmol·kg-1 dw. Relatively similar conclusions were observed also by evaluation of *TAA* values determined by the ABTS method, where the differences were very small. The highest *TAA* value was determined in varieties Businsky and Slovak (9.62 mmol·kg-1 dw) and the lowest in variety Beta (8.78 mmol·kg-1 dw). Also here, no statistically significant differences in the values were found. Harlen and Jati [18] referred an average *TAA* value determined by the ABTS method for chickpea 6.48 mmol·kg-1 dw, which was a lower value than our average *TAA* value (9.35 mmol·kg-1 dw). In addition, lower values (6.0 mmol·kg-1 dw) were reported also by Marathe et al. [19] for chickpea (ABTS method).

TPC values determined in this study in seeds of grass pea (Tab. 1C) were in range 1 475–1 578 mg·kg-1 dw, while the highest value

Tab. 1. Total polyphenols content and antioxidant activity in seeds of selected legumes varieties.

A. White lupin

Variety	*TPC* [mg·kg-1]	*TAA* [mmol·kg-1]		
		DPPH	ABTS	FRAP
Alban	8 004 ab	17.0 bc	7.14 a	2.00 a
Astra	7 495 ab	16.7 c	8.08 a	1.82 a
R-933	6 653 a	15.8 c	8.31 a	1.93 a
Satmarean	7 816 b	14.5 bc	8.10 a	2.32 a
Nelly	6 522 a	15.2 abc	7.28 a	1.95 a
Pop I	7 502 ab	14.4 bc	8.26 a	1.97 a
Los Palacios	7 030 ab	14.5 bc	7.39 a	2.29 a
Primorskij	6 778 a	11.7 ab	7.95 a	2.28 a
Solnečnyj	6 706 a	17.8 c	8.36 a	2.44 a
Weibit	7 838 ab	16.4 bc	9.27 a	1.84 a
Wtd	6 692 a	9.9 a	8.81 a	1.89 a

B. Chickpea

Variety	*TPC* [mg·kg-1]	*TAA* [mmol·kg-1]		
		DPPH	ABTS	FRAP
Krajova	1 273 a	14.8 a	9.11 a	1.68 a
Maskovsky Bagovec	1 411 a	10.1 a	9.38 a	1.65 a
Businsky	1 603 a	13.5 a	9.62 a	1.77 a
Slovak	1 686 a	14.5 a	9.62 a	1.99 a
Beta	1 534 a	8.6 a	8.78 a	1.80 a
Alfa	1 447 a	13.5 a	9.32 a	1.57 a
Irenka	1 763 a	16.9 a	9.59 a	2.03 a

C. Grass pea

Variety	*TPC* [mg·kg-1]	*TAA* [mmol·kg-1]		
		DPPH	FRAP	ABTS
Arida	1 480 a	14.6 a	2.21 a	10.7 a
Krajova from Kralová	1 475 a	11.6 a	2.11 a	10.8 a
Cachticky	1 578 a	9.2 a	2.50 a	10.7 a

Values represent mean. Different letters in superscript show statistically significant differences ($p < 0.05$) between varieties.

TPC – total polyphenols content expressed as milligrams of gallic acid equivalents per kilogram of dry weight, *TAA* – total antioxidant activity expressed as micromoles of Trolox equivalents per gram of dry weight determined by methods using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and as ferric reducing antioxidant power (FRAP).

was determined for variety Cachticky and the lowest value for variety Krajova from Kralová. The results showed that there were no significant differences in *TPC* values among grass pea varieties. The order of tested varieties based on *TPC* was Cachticky > Arida > Krajova from Kralová.

Variety Arida exerted the highest *TAA* value determined by the DPPH method (14.6 $mmol\cdot kg^{-1}$ dw) and, on contrary, the lowest value of *TAA* was determined for variety Cachticky (9.1 $mmol\cdot kg^{-1}$ dw). The range of *TAA* values determined by the FRAP method was from 2.1 $mmol\cdot kg^{-1}$ dw (Krajova form Kralová) to 2.5 $mmol\cdot kg^{-1}$ dw (Cachticky). Varieties of grass pea Arida and Cachticky exerted the same *TAA* values determined by the ABTS method (10.7 $mmol\cdot kg^{-1}$ dw), while *TAA* value was slightly higher in variety Krajova from Kralová (10.8 $mmol\cdot kg^{-1}$ dw). No statistically significant differences were found among varieties in *TAA* values determined by any of the used methods.

Tab. 2A presents the contents of phenolic acids in tested varieties of white lupin. The highest content of 4-hydroxybenzoic acid was determined in variety Solnečnyj with a value of 12.32 $mg\cdot kg^{-1}$ dw. On the contrary, the lowest value was determined for R-933 variety (3.679 $mg\cdot kg^{-1}$ dw). Similar results were obtained also for caffeic acid, where the highest value was determined again in seeds of variety Solnečnyj (568.3 $mg\cdot kg^{-1}$ dw), but the lowest value of caffeic acid was determined for variety Pop I (370.2 $mg\cdot kg^{-1}$ dw). Alban variety had the highest content of *trans-p*-coumaric acid with a value of 2.147 $mg\cdot kg^{-1}$ dw. This acid was found in small amounts in variety Primorskij (0.891 $mg\cdot kg^{-1}$ dw). Variety Alban had the highest content of *trans*-ferulic acid (11.84 $mg\cdot kg^{-1}$ dw) and the lowest content of this compound was determined for variety Primorskij (3.819 $mg\cdot kg^{-1}$ dw).

Summarizing the contents of phenolic acids in selected varieties of white lupin, the orders could be assembled: 4-hydroxybenzoic acid – Solnečnyj > Los Palacios > Nelly > Alban > Primorskij > Weibit > Pop I > Wtd > Satmarean > Astra > R-933; caffeic acid – Solnečnyj > Los Palacios > Alban > Satmarean > Weibit > R-933 > Primorskij > Wtd > Astra > Nelly > Pop I; t*rans-p*-coumaric acid – Alban > Weibit > Nelly > Satmarean > Wtd > Astra > Solnečnyj > Los Palacios > Pop I > Primorskij; *trans*-ferulic acid – Alban > Solnečnyj > Astra > Los Palacios > Satmarean > Nelly > Wtd > R-933 > Pop I > Weibit > Primorskij.

Statistically significant differences in contents of 4-hydroxybenzoic acid were found between the following varieties: Solnečnyj/R-933, Solnečnyj/Astra, Solnečnyj/Satmarean, Solnečnyj/Wtd, Solnečnyj/Pop I, Solnečnyj/Weibit, Solnečnyj/Primorskij, Solnečnyj/Alban, Solnečnyj/Nelly, Los Palacios/R-933 and Los Palacios/Astra. Statistically significant differences in contents of caffeic acid were found between the following varieties: Solnečnyj/Pop I, Solnečnyj/Nelly, Solnečnyj/Astra, Los Palacios/Pop I, Los Palacios/Nelly, Los Palacios/Astra, Alban/Pop I, Alban/Nelly and Satmarean/Pop I. Statistically significant differences in contents of *trans*-ferulic acid were found between the following varieties: Alban/Primorskij, Alban/Weibit, Alban/Pop I, Alban/R-933, Alban/Wtd, Alban/Nelly, Alban/Satmarean, Alban/Los Palacios, Solnečnyj/Primorskij, Solnečnyj/Weibit, Solnečnyj/Pop I, Solnečnyj/R-933, Solnečnyj/Wtd, Astra/Primorskij, Astra/Weibit, Astra/Pop I, Astra/R-933, Astra/Wtd, Los Palacios/Primorskij, Los Palacios/Weibit, Satmarean/Primorskij and Nelly/Primorskij. No significant differences in the content of *trans-p*-coumaric acid among varieties were found.

Ruiz-López et al. [20] found out higher contents of 4-hydroxybenzoic acid in seeds of white lupin, while our results were lower (22.770 $mg\cdot kg^{-1}$ dw). Also, the contents of caffeic acid and *trans-p*-coumaric acid were also considerably lower (0.580 $mg\cdot kg^{-1}$ dw and 0.110 $mg\cdot kg^{-1}$ dw, respectively).

In Tab. 2B, the content values of phenolic acids in varieties of chickpea are reported. The highest content of 4-hydroxybenzoic acid was determined in seeds of variety Krajova (40.90 $mg\cdot kg^{-1}$ dw) and the lowest value was determined in variety Businsky (14.32 $mg\cdot kg^{-1}$ dw). Variety Irenka had the highest content of caffeic acid (3.336 $mg\cdot kg^{-1}$ dw and the lowest content of caffeic acid was determined for variety Maskovsky Bagovec (0.873 $mg\cdot kg^{-1}$ dw). Only in two varieties, Krajova and Irenka, *trans-p*-coumaric acid was detected (at 1.800 $mg\cdot kg^{-1}$ dw and 4.179 $mg\cdot kg^{-1}$ dw, respectively). Only in variety Irenka, *trans*-ferulic acid was detected (at 0.672 $mg\cdot kg^{-1}$ dw).

The tested chickpea varieties could be ranked on the basis of the content of phenolic acids in the following order: 4-hydroxybenzoic acid – Krajova > Beta > Alfa > Slovak > Irenka > Maskovsky Bagovec > Businsky; caffeic acid – Irenka > Slovak > Krajova > Alfa > Beta > Businsky > Maskovsky Bagovec; *trans-p*-coumaric acid – Irenka > Krajova.

Statistically significant differences in contents of 4-hydroxybenzoic acid were found between the following varieties: Krajova/Businsky, Beta/Businsky, Alfa/Businsky and Slovak/Businsky. Statistically significant differences in contents of caffeic

Tab. 2. Average contents of selected polyphenols in seeds of selected legumes varieties.

A. White lupin

Variety	Content [mg·kg^{-1}]			
	4- Hydroxybenzoic acid	Caffeic acid	*trans-p*-Coumaric acid	*trans*-Ferulic acid
Alban	6.180 ^{bc}	543.8 ^{ab}	2.147 ^{a}	11.840 ^{a}
Astra	3.713 ^{c}	432.8 ^{bcd}	1.146 ^{a}	9.034 ^{ab}
R-933	3.679 ^{c}	452.6 ^{abcd}	1.095 ^{a}	4.812 ^{cde}
Satmarean	4.464 ^{bc}	524.9 ^{abc}	1.583 ^{a}	6.866 ^{bcd}
Nelly	6.782 ^{bc}	417.9 ^{cd}	1.874 ^{a}	6.834 ^{bcd}
Pop I	5.192 ^{bc}	370.2 ^{d}	0.979 ^{a}	4.775 ^{cde}
Los Palacios	9.415 ^{ab}	566.6 ^{a}	1.109 ^{a}	7.730 ^{bc}
Primorskij	5.596 ^{bc}	451.5 ^{abcd}	0.891 ^{a}	3.819 ^{e}
Solnečnyj	12.320 ^{a}	568.3 ^{a}	1.123 ^{a}	9.350 ^{ab}
Weibit	5.404 ^{bc}	456.5 ^{abcd}	2.118 ^{a}	4.267 ^{de}
Wtd	5.136 ^{bc}	446.2 ^{abcd}	1.298 ^{a}	5.273 ^{cde}

B. Chickpea

Variety	Content [mg·kg^{-1}]			
	4- Hydroxybenzoic acid	Caffeic acid	*trans-p*-Coumaric acid	*trans*-Ferulic acid
Krajova	40.90 ^{a}	1.954 ^{ab}	1.800 ^{b}	< *LOD*
Maskovsky Bagovec	27.51 ^{ab}	0.873 ^{b}	< *LOD*	< *LOD*
Businsky	14.32 ^{b}	1.026 ^{b}	< *LOD*	< *LOD*
Slovak	40.02 ^{a}	2.181 ^{ab}	< *LOD*	< *LOD*
Beta	40.04 ^{a}	1.530 ^{ab}	< *LOD*	< *LOD*
Alfa	40.03 ^{a}	1.681 ^{ab}	< *LOD*	< *LOD*
Irenka	36.56 ^{ab}	3.336 ^{a}	4.179 ^{a}	0.672 ^{a}

C. Grass pea

Variety	Content [mg·kg^{-1}]			
	4- Hydroxybenzoic acid	Caffeic acid	*trans-p*-Coumaric acid	*trans*-Ferulic acid
Arida	7.139 ^{a}	14.58 ^{a}	4.184 ^{b}	1.791 ^{a}
Krajova from Kralová	5.432 ^{a}	28.75 ^{a}	4.023 ^{b}	1.543 ^{a}
Cachticky	1.653 ^{a}	52.25 ^{a}	8.231 ^{a}	2.630 ^{a}

Values are given per kilogram of dry weight. Different letters in superscript show statistically significant differences ($p < 0.05$) between varieties.
LOD – limit of detection.

acid were found between Irenka/Maskovsky Bagovec and Irenka/Businsky. A statistically significant difference in contents of *trans-p*-coumaric acid was also found between varieties Irenka/Krajova, which were the only varieties with content of this acid greater than the detection limit of the analysis.

Authors Domínguez-Arispuro et al. [21] reported the content of *trans*-ferulic acid (1.16 mg·kg^{-1} dw) and *trans-p*-coumaric acid (1.18 mg·kg^{-1} dw) in seeds of chickpea. Those values were higher at *trans*-ferulic and lower at *trans-p*-coumaric than the values obtained in this study.

In Tab. 2C, the results of determination of phenolic acids contents in selected varieties of grass pea are presented. The highest content of 4-hydroxybenzoic acid was assessed in variety Arida (7.139 mg·kg^{-1} dw) and the lowest content had the variety Cachticky (1.653 mg·kg^{-1} dw). The dominating phenolic acid in variety Arida was caffeic acid (52.25 mg·kg^{-1} dw). Contents of *trans-p*-coumaric acid ranged from 4.023 mg·kg^{-1} dw (Krajova from Kralová) to 8.231 mg·kg^{-1} dw (Cachticky). The highest content of *trans*-ferulic acid was determined in variety Cachticky (2.630 mg·kg^{-1} dw) and similar values were determined in varieties Arida (1.791 mg·kg^{-1} dw) and Krajova from Krajová (1.543 mg·kg^{-1} dw), respectively. On the basis of contents of phenolic acids, the tested grass pea varieties could be ranked in the order: 4-hydroxybenzoic acid – Arida > Krajova from Kralová > Cachticky; caffeic acid – Cachticky > Krajova from Kralová > Arida;

trans-p-coumaric acid – Cachticky > Arida > Krajova from Kralová; *trans*-ferulic acid – Cachticky > Arida > Krajova from Kralová. Statistically significant differences among varieties were confirmed only for content of *trans-p*-coumaric acid in varieties Cachticky/Krajova from Kralová and Cachticky/Arida.

In Tab. 3A, contents of flavonoids in white lupin varieties are given. The highest content of myricetin was determined for variety Solnečnyj (16.45 mg·kg^{-1} dw) and the lowest content was determined for variety Weibit (8.923 mg·kg^{-1} dw). The highest content of quercetin was determined for variety Solnečnyj (1.202 mg·kg^{-1} dw) and the lowest content was determined for variety Weibit (0.215 mg·kg^{-1} dw). The highest content of apigenin was determined for variety Alban (3.786 mg·kg^{-1} dw) and the lowest content was determined for variety R-933 (0.900 mg·kg^{-1} dw). Genistein content ranged from 0.711 mg·kg^{-1} dw (Primorskij) to 0.870 mg·kg^{-1} dw (Nelly). The highest content of kaempferol was determined for variety Solnečnyj (0.915 mg·kg^{-1} dw) and the lowest content for variety Pop I (0.340 mg·kg^{-1} dw).

On the basis of results on flavonoids content in varieties of white lupin, these could be ranked in the following order: myricetin – Solnečnyj > Alban > Los Palacios > Satmarean > Primorskij > Astra > Nelly > Wtd > R-933 > Pop I > Weibit; kvercetín – Solnečnyj > Los Palacios > Wtd > Pop I > Alban > Astra > Primorskij > Satmarean > Nelly > R-933 > Weibit; apigenin – Alban > Los Palacios > Solnečnyj > Weibit > Wtd > Primorskij > Satmarean >

Tab. 3. Average contents of selected flavonoids in seeds of selected legumes varieties.

A. White lupin

Variety	Content [mg·kg^{-1}]				
	Myricetin	Quercetin	Apigenin	Genistein	Kaempferol
Alban	16.43 ^{a}	0.360 ^{bc}	3.786 ^{a}	0.822 ^{a}	0.582 ^{a}
Astra	11.87 ^{bc}	0.360 ^{bc}	1.128 ^{bcd}	0.855 ^{a}	0.617 ^{a}
R-933	10.24 ^{bc}	0.216 ^{c}	0.900 ^{d}	0.747 ^{a}	0.418 ^{a}
Satmarean	12.92 ^{abc}	0.312 ^{c}	1.350 ^{bcd}	0.755 ^{a}	0.573 ^{a}
Nelly	10.98 ^{bc}	0.263 ^{c}	1.225 ^{bcd}	0.870 ^{a}	0.384 ^{a}
Pop I	8.96 ^{c}	0.398 ^{bc}	1.036 ^{cd}	0.764 ^{a}	0.340 ^{a}
Los Palacios	14.27 ^{ab}	0.997 ^{a}	2.717 ^{ab}	0.754 ^{a}	0.589 ^{a}
Primorskij	12.19 ^{abc}	0.330 ^{bc}	1.624 ^{bcd}	0.711 ^{a}	0.844 ^{a}
Solnečnyj	16.45 ^{a}	1.202 ^{a}	2.563 ^{abc}	0.809 ^{a}	0.915 ^{a}
Weibit	8.92 ^{c}	0.215 ^{c}	1.951 ^{bcd}	0.726 ^{a}	0.757 ^{a}
Wtd	10.78 ^{bc}	0.871 ^{ab}	1.885 ^{bcd}	0.797 ^{a}	0.854 ^{a}

B. Chickpea

Variety	Content [mg·kg^{-1}]				
	Myricetin	Quercetin	Apigenin	Genistein	Kaempferol
Krajova	*< LOD*	1.989 ^{ab}	3.604 ^{a}	1.175 ^{a}	*< LOD*
Maskovsky Bagovec	*< LOD*	1.972 ^{ab}	3.315 ^{a}	1.050 ^{a}	*< LOD*
Businsky	*< LOD*	3.091 ^{a}	4.627 ^{a}	1.113 ^{a}	*< LOD*
Slovak	*< LOD*	2.676 ^{ab}	4.365 ^{a}	0.992 ^{a}	*< LOD*
Beta	*< LOD*	1.246 ^{b}	2.021 ^{a}	1.218 ^{a}	*< LOD*
Alfa	*< LOD*	1.399 ^{b}	2.198 ^{a}	1.389 ^{a}	*< LOD*
Irenka	*< LOD*	1.276 ^{b}	3.095 ^{a}	0.846 ^{a}	*< LOD*

C. Grass pea

Variety	Content [mg·kg^{-1}]				
	Myricetin	Quercetin	Apigenin	Genistein	Kaempferol
Arida	1.175 ^{a}	*< LOD*	1.971 ^{a}	0.164 ^{a}	*< LOD*
Krajova from Kralová	1.386 ^{a}	*< LOD*	2.031 ^{a}	0.163 ^{a}	*< LOD*
Cachticky	1.563 ^{a}	*< LOD*	2.020 ^{a}	0.196 ^{a}	*< LOD*

Values are given per kilogram of dry weight. Different letters in superscript show statistically significant differences ($p < 0.05$) between varieties.
LOD – limit of detection.

Nelly > Astra > Pop I > R-933; genistein – Nelly > Astra > Alban > Solnečnyj > Wtd > Pop I > Satmarean > Los Palacios > R-933 > Weibit >Primorskij and kaempferol – Solnečnyj > Wtd > Primorskij > Weibit > Astra > Los Palacios > Alban > Satmarean > R-933 > Nelly > Pop I.

Statistically significant differences in content of myricetin were found between varieties Solnečnyj/Weibit, Solnečnyj/Pop I, Solnečnyj/R-933, Solnečnyj/Wtd, Solnečnyj/Nelly, Solnečnyj/Astra, Alban/Weibit, Alban/Pop I, Alban/R-933, Alban/Wtd, Alban/Nelly, Alban/Astra, Los Palacios/Weibit and Los Palacios/Pop I. Statistically significant differences in content of quercetin were found between varieties Solnečnyj/Weibit, Solnečnyj/R-933, Solnečnyj/Nelly, Solnečnyj/Satmarean, Solnečnyj/Primorskij, Solnečnyj/Astra, Solnečnyj/Alban, Solnečnyj/Pop I; Los Palacios/Weibit, Los Palacios/R-933, Los Palacios/Nelly, Los Palacios/Satmarean, Los Palacios/Primorskij, Los Palacios/Astra, Los Palacios/Alban, Los Palacios/Pop I, Wtd/Weibit, Wtd/R-933, Wtd/Nelly and Wtd/Satmarean. Statistically significant differences in content of apigenin were found between varieties Alban/R-933, Alban/Pop I, Alban/Astra, Alban/Nelly, Alban/Satmarean, Alban/Primorskij, Alban/Wtd, Alban/Weibit, Los Palacios/R-933, Los Palacios/Pop I and Solnečnyj/R-933. There were no significant differences in the contents of genistein and kaempferol among the tested varieties.

Our obtained values of genistein content were higher than those reported by Valentinuzzi et al. [22], who reported the genistein content of 0.170 $mg\cdot kg^{-1}$ dw. Magalhaes et al. [23] reported the apigenin content in seeds of white lupin in the range from 0.63 $mg\cdot kg^{-1}$ dw to 1.96 $mg\cdot kg^{-1}$ dw, which is consistent with our determined values. Ruiz-López et al. [20] determined the apigenin content of 1.190 $mg\cdot kg^{-1}$ dw in seeds of white lupin, which is consistent with our determined values. D'Agostina et al. [24] investigated also the content of genistein in seeds of lupin, but their values (0.2113 $mg\cdot kg^{-1}$ dw) are lower than those obtained in the present study.

Tab. 3B presents data on the contents of flavonoids in chickpea varieties. The highest value was determined for variety Businsky (3.091 $mg\cdot kg^{-1}$ dw), and the lowest value for variety Beta (1.246 $mg\cdot kg^{-1}$ dw). Content of apigenin in individual varieties was in the range from 2.021 $mg\cdot kg^{-1}$ dw (Beta) to 4.627 $mg\cdot kg^{-1}$ dw (Businsky). The highest value of genistein was determined for variety Alfa (1.389 $mg\cdot kg^{-1}$ dw) and the lowest value for variety Irenka (0.846 $mg\cdot kg^{-1}$ dw). Myricetin and kaempferol were not detected in seeds of chickpea. On the basis of gained data on the content of flavonoids in chickpea varieties, these could be ranked in the following order: quercetin – Businsky > Slovak > Krajova > Maskovsky Bagovec > Alfa > Irenka > Beta; apigenin – Businsky > Slovak > Krajova > Maskovsky Bagovec > Irenka > Alfa > Beta; genistein – Alfa > Beta > Krajova > Businsky > Maskovsky Bagovec > Slovak > Irenka. Statistically significant differences between varieties were found only for quercetin content in the case of Businsky/Beta, Businsky/Irenka and Businsky/Alfa. Domínguez-Arispuro et al. [21] carried out quercetin determination in chickpea and reported contents in the range from 1.849 $mg\cdot kg^{-1}$ dw to 3.005 $mg\cdot kg^{-1}$ dw, which is consistent with our determined values.

Flavonoids contents in tested varieties of grass pea are presented in Tab. 3C. The highest content of myricetin was determined for variety Cachticky (1.563 $mg\cdot kg^{-1}$ dw) and the lowest content for variety Krajova from Kralová (1.386 $mg\cdot kg^{-1}$ dw). Content values of apigenin were in the range from 1.971 $mg\cdot kg^{-1}$ dw (Arida) to 2.031 $mg\cdot kg^{-1}$ dw (Krajova from Kralová). The highest content of genistein was determined for variety Cachticky (0.196 $mg\cdot kg^{-1}$ dw) and the lowest content was determined for variety Krajova from Kralová (0.163 $mg\cdot kg^{-1}$ dw). Quercetin and kaempferol were not detected in seeds of grass pea. No significant differences in contents of flavonoids were found among varieties. Based on the obtained values, varieties could be ranked in the following order: myricetin – Cachticky > Krajova from Kralová > Arida; apigenin – Krajova from Kralová > Cachticky > Arida.

CONCLUSIONS

The highest values of *TPC* (expressed as GAE) were in Alban variety – 8 004 $mg\cdot kg^{-1}$ dw (white lupin), Irenka variety – 1 763 $mg\cdot kg^{-1}$ dw (chickpea) and Cachticky variety – 1 578 $mg\cdot kg^{-1}$ dw (grass pea). The highest *TAA* value (based on DPPH and FRAP) was determined for Solnečnyj variety (white lupin) and Irenka (chickpea). By ABTS, the highest values were determined for varieties Weibit (white lupin), Businsky (chickpea), Slovak (chickpea) and Krajova from Kralová (grass pea). The highest *TAA* values were determined for three grass pea varieties by the three methods. Regarding phenolic acids contents, the varieties of white lupin Solnečnyj (the highest contents of 4-hydroxybenzoic and caffeic acids) and Alban (with the highest contents of *trans-p*-coumaric and *trans*-ferulic acids) might be considered as their most valuable sources. Chickpea variety Krajova had the highest content of 4-hydroxybenzoic acid, while Irenka variety had the highest contents of caffeic, *trans-p*-coumaric and *trans*-ferulic acids. Grass pea variety Arida had the highest content of 4-hydroxybenzoic acid and variety Cachticky had the highest contents of caffeic, *trans-p*-coumaric and *trans*-ferulic acids. Regarding flavonoids, it could be stated that the most valuable varieties were those of white lupin Solnečnyj (the highest contents of myricetin, quercetin and kaempferol), Alban (the highest content of apigenin) and Nelly (the highest content of genistein). Chickpea variety Businsky had high contents of quercetin and apigenin, while the variety Alfa had the highest content of genistein. Grass pea variety Cachticky had the highest contents of myricetin and genistein and variety Krajova from Kralová had the highest content of apigenin. Based on these facts, we could suggest varieties of white lupin Solnečnyj and Alban, variety of chickpea Irenka and variety of grass pea Cachticky as perspective crops regarding their high contents of antioxidants. The obtained results confirmed that contents of biologically valuable compounds differ between crop types and varieties. They also demonstrate that the less popular legumes can be a valuable source of biologically active substances in human nutrition. Utilization of these legumes has good prospects particularly in the production of so-called functional foodstuffs with high content of chemoprotective components that could have a positive inmpact on consumers' health.

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REFERENCES

1. Moreno-Valdespino, C. A. – Luna-Vital, D. – Camacho-Ruiz, R. M. – Mojica, L.: Bioactive proteins and phytochemicals from legumes: mechanisms of action preventing obesity and type-2 diabetes. Food Research International, *130*, 2020, article 108905. DOI: 10.1016/j.foodres.2019.108905.
2. Rajhi, I. – Baccouri, B. – Rajhi, F. – Mhadhbi, H. – Flamini, G.: Monitoring the volatile compounds status of whole seeds and flours of legume cultivars. Food Bioscience, *41*, 2021, article 101105. DOI: 10.1016/j.fbio.2021.101105.
3. Šibul, F. – Orčić, D. – Vasić, M. – Anačkov, G. – Nađpal, J. – Savić, A. – Mimica-Dukić, N.: Phenolic profile, antioxidant and anti-inflammatory potential of herb and root extracts of seven selected legumes. Industrial Crops and Products, *83*, 2016, pp. 641–653. DOI: 10.1016/j.indcrop.2015.12.057.
4. Maphosa, Y. - Jideani, V. A..: The role of legumes in human nutrition. In: Chávarri Hueda, M. (Eds.): Functional food – Improve health through adequate food. Zagreb : InTech, 2017, pp. 103–l21. ISBN: 978-953-51-3440-4. DOI: 10.5772/66263.
5. Kan, L. – Nie, S. – Hu, J. – Wang, S. – Bai, Z. – Wang, J. – Zhouc, Y. – Jianga, J. – Zenga, O. – Song, K.: Comparative study on the chemical composition, anthocyanins, tocopherols and carotenoids of selected legumes. Food Chemistry, *260*, 2018, pp. 317–326. DOI: 10.1016/j.foodchem.2018.03.148.
6. Ghasemzadeh, A. – Ghasemzadeh, N.: Flavonoids and phenolic acids: Role and biochemical activity in plants and human. Journal of Medicinal Plants Research, *5*, 2011, pp. 6697–6703. DOI: 10.5897/JMPR11.1404.
7. Dennis, K. K. – Liu, K. H. – Uppal, K. – Go, Y. M. – Jones, D. P.: Distribution of phytochelatins, metal-binding compounds, in plant foods: A survey of commonly consumed fruits, vegetables, grains and legumes. Food Chemistry, *339*, 2021, article 128051. DOI: 10.1016/j.foodchem.2020.128051.
8. Lampart-Szczapa, E. – Korczak, J. – Nogala-Kalucka, M. – Zawirska-Wojtasiak, R.: Antioxidant properties of lupin seed products. Food Chemistry, *83*, 2003, pp. 279–285. DOI: 10.1016/S0308-8146(03)00091-8.
9. Olkowski, B. I. – Classen, H. L. – Wojnarowicz, C. – Olkowski, A. A.: Feeding high levels of lupine seeds to broiler chickens: plasma micronutrient status in the context of digesta viscosity and morphometric and ultrastructural changes in the gastrointestinal tract. Poultry Science, *84*, 2005, pp. 1707–1715. DOI: 10.1093/ps/84.11.1707.
10. Diaz, D. – Morlacchini, M. – Masoero, F. – Moschini, M. – Fusconi, G. – Piva, G.: Pea seeds (*Pisum sativum*), faba beans (*Vicia faba* var. minor) and lupin seeds (*Lupinus albus* var. multitalia) as protein sources in broiler diets: effect of extrusion on growth performance. Italian Journal of Animal Science, *5*, 2006, pp. 43–53. DOI: 10.4081/ijas.2006.43.
11. Wu, H. – Gu, J. – Bk, A. – Nawaz, M. A. – Barrow, C. J. – Dunshea, F. R. – Suleria, H. A.: Effect of processing on bioavailability and bioaccessibility of bioactive compounds in coffee beans. Food Bioscience, *45*, 2021, article 101373. DOI: 10.1016/j.fbio.2021.101373.
12. Lachman, J. – Proňek, D. – Hejtmánková, A. – Pivec, V. – Faitová, K.: Total polyphenol and main flavonoid antioxidanrs in different onion (*Allium cepa* L.) varietes. Scientia Horticulturae, *30*, 2003, pp. 142–147. DOI: 10.17221/3876-HORTSCI.
13. Gabriele, M. – Pucci, L. – Árvay, J. – Longo, V.: Anti-inflammatory and antioxidant effect of fermented whole wheat on TNFα-stimulated HT-29 and NF-κB signaling pathway activation. Journal

of Functional Foods, *45*, 2018, pp. 392–400. DOI: 10.1016/j.jff.2018.04.029.
14. Brand-Williams, W. – Cuverlier, M. E. – Berset, C.: Use of free radical method to evaluate antioxidant activity. LWT – Food Science and Technology, *28*, 1995, pp. 25–30. DOI: 10.1016/S0023-6438(95)80008-5.
15. Paulova, H. – Bochoráková, H. – Táborská, E.: Metody stanovení antioxidační aktivity přírodních látek in vitro. (In vitro methods for estimation of the antioxidant activity of natural compounds.) Chemické Listy, *98*, 2004, pp. 174–179. ISSN: 0009-2770. <http://www.chemicke-listy.cz/ojs3/index.php/chemicke-listy/article/view/2144/2144> In Czech.
16. Boudjou, S. – Oomah, B. D. – Zaidi, F. – Hosseinian, F.: Phenolics content and antioxidant and anti-inflammatory activities of legume fractions. Food Chemistry, *138*, 2013, pp. 1543–1550. DOI: 10.1016/j.foodchem.2012.11.108.
17. Mahbub, R. – Francis, N. – Blanchard, C. – Santhakumar, A.: The anti-inflammatory and antioxidant properties of chickpea hull phenolic extracts. Food Bioscience, *40*, 2021, article 100850. DOI: 10.1016/j.fbio.2020.100850.
18. Harlen, W. C. – Jati, I. R. A. (Ed.): Antioxidant activity of anthocyanins in common legume grains. In: Watson, R. R. – Preedy, V. R. – Zibadi, S. (Eds.): Polyphenols: Mechanisms of action in human health and disease. 2nd ed. Amsterdam : Elsevier, 2018, pp. 81-92. ISBN: 978-0-12-813006-3. DOI: 10.1016/B978-0-12-813006-3.00008-8.
19. Marathe, S. A. – Rajalakshmi, V. – Jamdar, S. N. – Sharma, A.: Comparative study on antioxidant activity of different varieties of commonly consumed legumes in India. Food and Chemical Toxicology, *49*, 2011, pp. 2005–2012. DOI: 10.1016/j.fct.2011.04.039.
20. Ruiz-López, M. A. – Barrientos-Ramírez, L. – Garcia-López, P. M. – Valdés-Miramontes, E. H. – Zamora-Natera, J. F. – Rodríguez-Macias, R. – Vargas-Radillo, J. J.: Nutritional and bioactive compounds in Mexican lupin beans species: A mini-review. Nutrients, *11*, 2019, article 1785. DOI: 10.3390/nu11081785.
21. Domínguez-Arispuro, D. M. – Cuevas-Rodríguez, E. O. – Milán-Carrillo, J. – León-López, L. – Guttiérrez-Dorado, R. – Reyes-Moreno, C.: Optimal germination condition impacts on the antioxidant activity and phenolic acids profile in pigmented desi chickpea (*Cicer arietinum* L.) seeds. Journal of Food Science and Technology, *55*, 2018, pp. 638–647. DOI: 10.1007/s13197-017-2973-1.
22. Valentinuzzi, F. – Cesco, S. – Tomasi, N. – Mimmo, T.: Effect of aluminium exposure on the release of organic acids and genistein from the roots of *Lupinus albus* L. plants. Rhizosphere, *1*, 2016, pp. 29–32. DOI: 10.1016/j.rhisph.2016.07.002.
23. Magalhaes, S. C. – Taveira, M. – Cabrita, A. R. – Fonseca, A. J. – Valentao, P. – Andrade, P. B.: European marketable grain legume seeds: Further insight into phenolic compounds profiles. Food Chemistry, *215*, 2017, pp. 177–184. DOI: 10.1016/j.foodchem.2016.07.152.
24. D'agostina, A. – Boschin, G. – Resta, D. – Annicchiarico, P. – Arnoldi, A.: Changes of isoflavones during the growth cycle of *Lupinus albus*. Journal of Agricultural and Food Chemistry, *56*, 2008, pp. 4450–4456. DOI: 10.1021/jf8003724.

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