Changes in the content of bioactive compounds induced by the addition of biologically effective preparations in selected legumes

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Summary

The influence of biologically effective preparations on the content of phenolic compounds and the total polyphenolics content in legumes was evaluated. The control variant (A), variant B with inoculant Rizobin LF (Legume Technology, Nottingham, United Kingdom), variant C with growth regulator Lexin (Lexagro, Piešťany, Slovakia) and variant D with a combination of Rhizobin LF and Lexin were analysed. White lupine contained the most phenolic compounds in variant D. In variant B in white lupine, we determined the lowest content of caffeic acid 62.94 mg·kg^{-1} of dry weight (DW), *trans*-ferulic acid 3.69 mg·kg^{-1} DW and myricetin 9.11 mg·kg^{-1} DW. The content of 4-hydroxybenzoic acid in chickpea ranged from 4.52 mg·kg^{-1} DW in variant D to 17.25 mg·kg^{-1} DW in variant C. The highest content of 4-hydroxybenzoic acid in grass pea was in variant B. Variant C contained 1.89 mg·kg^{-1} DW and variant D 1.79 mg·kg^{-1} DW of 4-hydroxybenzoic acid. The highest total polyphenolics content in grass pea was in variant A, 171.19 mg·kg^{-1} DW. It can be concluded that the variety and use of biologically effective preparations can have a positive effect on the content of phenolic compounds and polyphenols.

Keywords

white lupine; chickpea; grass pea; bioactive compound; biologically effective preparation

Legumes are classified as dicotyledonous plants that belong to Leguminosae family with approximately 19 000 species. Throughout the world, they are widely cultivated since ancient times. They are a rich source of micronutrients, dietary fibre and protein with positive effects on the health of consumers.

Legumes establish nitrogen-fixing symbiosis with a variety of soil bacteria collectively called rhizobia, which are members of several families and genera [1]. All these bacteria are Gram-negative aerobic rods with the ability to induce nodules in stems and roots of legumes where, after their transformation into bacteroid, they can fix atmospheric nitrogen. Nodes can be formed on roots or on stems. They are indeterminate with apical meristematic growth or determined with growth by expansion of infected cells from the central zone of the nodes. Rhizobia form root nodules on the host legume and, thereby, provide plants with nitrogen in exchange for a portion of the carbohydrates formed by the plant. However, plants for their proper growth need to absorb most of the nitrogen in the form of nitrates or ammonium. Approximately 80 % of biologically fixed nitrogen comes from symbiosis of legumes with Rhizobiaceae [2].

Of the total amount of nitrogen found in legume plants, 50–60 % comes from symbiotic fixation (bean 60 %, soya 50 %, pea 50 %, lentil 50 %). To be able to grow legumes, we need to have suitable conditions for the development

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and activity of nitrogen-fixing bacteria. In conditions with insufficient occurrence of free rhizobia in the soil, inoculation (bacterization) of seeds is performed. This is a process of applying bacteria to seeds leading to adequate amounts of effective strains. Inoculants are vaccines for legume seeds and field crops, which are also a comparatively cheap and environmentally friendly source of nitrogen and phosphorus. Seed treatment is a biological, chemical and physical (mechanical) process used to mitigate the negative effects of various external or internal influences. It improves their germination and vigour, which promotes formation of a healthy plant with an increased production potential [3]. The process of seed treatment can be combined with the inoculation of legumes. It can be therefore said that such process is a comparatively cheap and highly effective method of plant protection and stimulation of growth [4]. The effective preparations of this type belong various growth regulators, enzymes, compounds associated with plant bioenergetics or photosynthetic pigments producing protein complexes that participate in conversion of light to energetically rich chemical compounds [5].

Legumes are nutritionally valuable crops, containing proteins with essential amino acids, complex carbohydrates, dietary fibre, unsaturated lipids, vitamins and essential minerals for the human diet [6]. Another property that makes legumes important in the human diet is the content of bioactive compounds of which phenolic compounds, saponins, peptides and small proteins are the most relevant [7]. Phenolic compounds in legumes such as pea, chickpea, bean or lentil are phenolic acids such as hydroxybenzoic acids, hydroxycinnamic acids or flavonoids such as flavonols or isoflavones, primarily daidzein and genistein [8]. In a recent study by Patto et al. [9] total polyphenolics content (*TPC*) in grass pea was determined to range from 0.46 g·kg^{-1} to 1.02 g·kg^{-1}, expressed as milligrams of gallic acid equivalents (GAE) per kilogram dry weight (DW). In general, dark varieties of legume seeds tend to contain more phenolic compounds than light ones [10]. These compounds exert a wide range of beneficial properties regarding metabolism, homeostasis and cell proliferation modulation. Moreover, they have antioxidant activities, which are essential for the prevention of oxidative stress conditions and diseases [11]. Among the most common bioactive compounds of legumes are flavonoids, especially in legume species that have coloured seed coats. They form the main class of polyphenols with the highest antioxidant potential. This group encompasses anthocyanins and anthoxanthins. In addition, they exhibit high free radical-scavenging capacity, anti-inflammatory and anticancer activities and a positive impact on immune response [12].

Chickpea (*Cicer arietinum* L.) is one of the oldest known pulse crops. Chickpea cultivation is known worldwide, with most of the production occurring in the Indian subcontinent. Globally, chickpea is ranked as the second-most produced legume crop, with over 14 million tonnes harvested in 2019 according to the FAOSTAT database (Food Agricultural Organization, Rome, Italy). Chickpea plays an important role in maintaining soil fertility by fixing nitrogen in the amount of up to 140 kg per hectare and year. Chickpea requires a relatively low amount of nitrogen because 70 % nitrogen is from symbiotic nitrogen fixation [13]. Several health benefits of chickpea are attributed to the presence of bioactive components in it. The main phytochemicals in chickpea include flavonoids, specifically isoflavonoids and 5-deoxyisoflavonoids, carotenoids, phenolic acids, stilbenes and lignans [14, 15]. The flavonoids and phenolics are mostly present in the chickpea seed coat.

Grass pea (*Lathyrus sativus* L.) is a legume that has been cultivated since ancient times in the eastern Mediterranean region and has also spread to the southern parts of Europe, North Africa and Asia. Grass pea is characterized by high yield, high protein content (290 mg·kg^{-1}) and plays an important role in low-input farming systems. It is not demanding and is resistant to extreme environments, from drought to flooding [16]. Grass pea seeds are characterized by good nutritional properties. According to Mullan et al. [17], grass pea is characterized by the content of starch, proteins, lipids and minerals at levels similar to fava bean and pea. However, grass pea seeds contain a neurotoxin, β-N-oxalyl-1-α,β-diamino-propionic acid (β-ODAP). This non-protein amino acid causes neurolathyrism, a neurological disease in both humans and domestic animals [18]. Grass pea seeds after removal of anti-nutritional compounds can be a material for obtaining protein preparations [19].

Lupin is a representative of the legume family which includes over 400 species, from which only four are of agronomic interest, namely, white lupin (*Lupinus albus* L.), blue (or narrow-leafed) lupin (*L. angustifolius* L.), yellow lupin (*L. luteus* L.) and pearl (or Tarwi) lupin (*L. mutabilis* L.). [20]. Studies on the use of lupin seeds in animal and human diets showed that it can compete with soya seeds. Lupin seeds contain approximately 440 mg·kg^{-1} of proteins, 130 mg·kg^{-1} of lipids and a large group of bioactive compounds [21]. White lupin seeds are generally classified as

sweet or bitter depending on the alkaloids level, which ranges from 0.1 mg·kg^{-1} to 40 mg·kg^{-1} [22]. Lupin seeds are characterized by low levels of antinutrients such as trypsin inhibitors, phytic acid, saponins or lectins [23].

Although the number of studies is still low, in the last decade, those analysing the changes in the bioactive compounds content of legumes after rhizobial inoculation are gaining interest. Considering that legumes are currently considered as functional foods, it is important to carry out more studies about the differences in the bioactive compound profiles and/or contents after the inoculation of various legumes with distinct rhizobia [24]. Therefore, this study was performed to assess the effect of inoculants on TPC, phenolic acids and flavonoids in seeds of selected varieties of white lupin, chickpea and grass pea.

MATERIALS AND METHODS

Plant material

Seeds were provided by the Gene Bank of Slovakia (Research Institute of Plant Production, National Agricultural and Food Centre, Piešťany, Slovakia). The plant material was sown on the field experimental plots of the Research Institute of Plant Production in Piešťany, Slovakia. The evaluated material consisted of 11 foreign varieties of white lupin Alban (France), Astra (Chile), R-933 (Poland), Satmarean (Romania), Nelly (Hungary), Pop I. (Poland), Los Palacios (Spain), Primorskij (Russian Federation), Solnecnyj (Ukraine), Weibit (Germany) and WTD (Poland), 3 Slovakian varieties of chickpea Krajova z Kralovej, Maskovsky Bagovec and Businsky, along with 3 Slovakian varieties of grass pea Arida, Krajova z Kralovej and Cachticky cicer. The location of Piešťany belongs to the maize production area. The average annual temperature is 9.2 °C and the long-term average precipitation is 625 mm. The climate is typically lowland, slightly dry and slightly windy. The plants were conventionally grown in the same location. The latitude and longitude for the experimental field were 48°35'08" N and 17°48'56" E. Four variants were sown from each genotype, specifically, variant A was control, variant B with inoculant Rizobin LF (Legume Technology, Nottingham, United Kingdom), variant C with growth regulator Lexin (Lexagro, Piešťany, Slovakia) and variant D with a combination of Rizobin LF and Lexin. Seed samples were collected at full maturity, then cleaned, dried at 105 °C to constant weight and finally crushed with a knife mill Grindomix 200 GD (Retsch, Haan, Germany). Each analysis was performed using 1 g of the average sample in three replicates.

Biologically effective preparations

For inoculation, inoculant Rizobin LF was used. It is intended for *Faboideae* with *Bradyrhizobium japonicum* as the active ingredient. Seed inoculation was applied by manually mixing the seeds at a rate of 350 g per hectare just before sowing in variant B.

Variant C contained stimulator Lexin at a dose of 0.25 l per hectare. Lexin is a universal stimulator and a regulator of growth and fertility of agricultural crops. It contains humic and fulvic acids and the growth hormone auxin.

Variant D contained both the inoculant and the growth stimulator.

Chemicals and reagents

Standard chemicals (ferulic acid, caffeic acid, genistein and myricetin), acetonitrile (gradient HPLC grade), phosphoric acid (American Chemical Society reagent grade) and methanol (gradient HPLC grade) were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Nitric acid (Suprapur), Folin-Ciocalteu reagent, gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,2-diphenyl-1-picrylhydrazyl radical (•DPPH) were obtained from Merck (Darmstadt, Germany). Deionized water (0.054 μS·cm^{-1}) was prepared by Simplicity 185 cleaning system (Millipore SAS, Molsheim, France).

Extract preparation

Samples of 5 g were homogenized using a laboratory mixer (Kinematica, Luzern, Switzerland) and extracted with 50 ml of 80% (v/v) methanol for 8 h in a Twisselman extractor (Behr Labor-Technik, Düsseldorf, Germany), which works at a temperature close to the boiling point of the solvent. The obtained extract was then filtered through No. 390 paper (Filtrak, Thermalbad Wiesenbad, Germany) into 50 ml vials and stored for 24 h until the analysis. Standard solutions before injection and sample extracts were filtered through a Q-Max cellulose acetate membrane microfilter (pore size 0.45 μm, diameter 25 mm; Frisenette, Knebel, Denmark).

Total polyphenolics content determination

Total polyphenolics content (*TPC*) was determined by the spectrophotometric method of Lachman et al. [25] and expressed as milligrams of GAE per kilogram DW. *TPC* was estimated using the Folin-Ciocalteu reagent. An aliquot of the extract, blank or standard, was pipetted to a 50-ml flask, added 2.5 ml of Folin-Ciocalteu reagent and

mixed. After 3 min, sodium carbonate solution (7.5 ml) was added. The volume was added up to 50 ml with distilled water. After 2 h, the samples were centrifuged for 10 min at 1 968 ×*g*. Absorbance was measured by a Shimadzu UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan) at 765 nm. *TPC* was calculated from a standard curve constructed using gallic acid.

Individual phenolics content

To determine the content of selected phenolic compounds, the method of GABRIELE et al. [26] was used. Infinity HPLC system G1315C (Agilent Technologies., Santa Clara, California, USA) was used with a Purosphere C18 reverse phase column (250 mm × 4 mm × 5 μm, Merck). Detection wavelengths were 320 nm for 4-hydroxybenzoic acid, caffeic acid and trans-ferullic acid, and 372 nm for myricetin, rutin and genistein. Data were collected and processed using Agilent Open Lab Chem Station software for LC 3D systems (Agilent Technologies).

Statistical analysis

Each parameter was tested in three repetitions. The statistical program Statgraphics Centurion XVI (Statpoint Technologies, Warrenton, Virginia, USA) was used. The results were statistically evaluated by one-way and multifactor ANOVA. Differences between mean values were assessed by the least significant difference (LSD) interval test at a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

The presented study was focused on the evaluation of the content of selected phenolic acids, flavonoids and *TPC* in varieties of selected legumes under various treatments (variants A–D). The determined average values of phenolic acids and flavonoids in white lupin seeds are shown in Tab. 1. Myricetin was detected in all 11 varieties of white lupin, *trans*-ferulic acid was detected in only two cultivars (Alban and Astra) and apigenin in two cultivars (Weibit and WTD). 4-Hydroxybenzoic acid was not detected in any of the monitored varieties. RUIZ-LOPEZ et al. [27] determined the average value of apigenin in the dry matter of white lupin seeds at 1.19 mg·kg^{-1} DW, which was less than the values determined by us in individual variants. The content of caffeic acid statistically significantly differed between variant B and variant D. Within variants A, B, C, D, there was no statistically significant difference in the content of apigenin in the monitored varieties of white lupin. The myricetin content in the evaluated variants had the following order: variant B < variant A < variant C < variant D. Analyses of variance showed statistically significant differences between variant A / variant C; variant B / variant C, D; variant C / variant D in myricetin content in individual white lupin cultivars.

Differences between white lupin cultivars in the content of phenolic acids in individual variants were statistically evaluated in Tab. 2. The evident statistical differences were found in Alban, Astra, Los Palacios, Weibit and WTD varieties in the content of caffeic acid in all variants A, B, C, D. SIGER et al. [28] determined a lower content of caffeic acid (0.58 mg·kg^{-1} and 0.09 mg·kg^{-1} DW) in two lupin seed cultivars compared to our results. Caffeic acid content 0.580 mg·kg^{-1} DW according to RUIZ-LÓPEZ et al. [27] was again significantly lower compared to our results. Those authors reported the content of 4-hydroxybenzoic acid of 22.77 mg·kg^{-1} DW in the seeds of lupin, while it was not detectable in any cultivar in our study. *Trans*-ferulic acid was detectable only in the varieties Alban and Astra, where it differed statis-

Tab. 1. Content of phenolic acids and flavonoids in white lupin.

	Variant A	Variant B	Variant C	Variant D	Average	$LSD_{0,05}$
4-Hydroxybenzoic acid [mg·kg^{-1}]	< *LOD*	< *LOD*	< *LOD*	< *LOD*	< *LOD*	< *LOD*
Caffeic acid [mg·kg^{-1}]	65.58^{ab}	62.94^{a}	71.51^{ab}	74.80^{b}	68.71	11.59
trans-Ferulic acid [mg·kg^{-1}]	4.06^{b}	3.69^{a}	4.73^{c}	5.03^{c}	4.38	0.32
Apigenin [mg·kg^{-1}]	2.43^{a}	2.49^{a}	2.61^{a}	2.76^{a}	2.57	0.52
Myricetin [mg·kg^{-1}]	9.13^{a}	9.11^{a}	9.47^{b}	10.66^{c}	9.59	0.18

The presented results are cumulative for all selected varieties of white lupine. The values are expressed per kilogram of dry weight and as arithmetic mean ($n = 3$) by least significant difference interval test. Same letters in superscript in row mean that there are no statistically significant differences between the values at the significance level of $\alpha = 0.05$.
Variant A – control, variant B – with addition of inoculant Rizobin LF, variant C – with addition of growth regulator Lexin, variant D – with addition of combination of Rizobin LF and Lexin.
LOD – limit of detection. $LSD_{0.05}$ – least significant difference at the level $\alpha = 0.05$.

Tab. 2. Content of phenolic acids in selected varieties of white lupin.

White lupin variety	Caffeic acid [mg·kg^{-1}]				*trans*-Ferulic acid [mg·kg^{-1}]			
	Variant A	Variant B	Variant C	Variant D	Variant A	Variant B	Variant C	Variant D
Alban	95.85^{a}	104.78^{b}	110.37^{c}	114.01^{d}	3.68^{a}	3.94^{b}	4.25^{c}	4.27^{c}
Astra	64.50^{a}	78.74^{b}	102.55^{c}	111.72^{d}	4.43^{a}	3.44^{b}	5.20^{c}	5.80^{d}
R-933	33.70^{a}	110.22^{c}	75.04^{b}	83.98^{bc}	< *LOD*	< *LOD*	< *LOD*	< *LOD*
Satmarean	33.70^{a}	110.22^{b}	139.22^{b}	131.95^{b}	< *LOD*	< *LOD*	< *LOD*	< *LOD*
Nelly	85.70^{b}	31.09^{a}	35.30^{a}	100.94^{b}	< *LOD*	< *LOD*	< *LOD*	< *LOD*
Pop I.	33.86^{b}	2.54^{a}	2.17^{a}	1.97^{a}	< *LOD*	< *LOD*	< *LOD*	< *LOD*
Los Palacios	2.48^{c}	2.30^{b}	2.03^{a}	2.86^{d}	< *LOD*	< *LOD*	< *LOD*	< *LOD*
Primorskij	59.23^{ab}	57.12^{ab}	109.62^{b}	44.73^{a}	< *LOD*	< *LOD*	< *LOD*	< *LOD*
Solnecnyj	14.95^{ab}	11.24^{a}	62.92^{c}	57.62^{bc}	< *LOD*	< *LOD*	< *LOD*	< *LOD*
Weibit	38.03^{a}	66.49^{c}	56.40^{b}	89.86^{d}	< *LOD*	< *LOD*	< *LOD*	< *LOD*
WTD	31.10^{b}	20.07^{a}	81.81^{c}	92.28^{d}	< *LOD*	< *LOD*	< *LOD*	< *LOD*

Values are expressed per kilogram of dry weight and represent average values of 3 samples. For each phenolic acid, same letter in superscript in row means that there are no statistically significant differences between the values at the significance level of $\alpha = 0.05$.
Variant A – control, variant B – with addition of inoculant Rizobin LF, variant C – with addition of growth regulator Lexin, variant D – with addition of combination of Rizobin LF and Lexin.
LOD – limit of detection.

Tab. 3. Content of flavonoids in selected varieties of white lupin.

White lupin variety	Apigenin [mg·kg^{-1}]				Myricetin [mg·kg^{-1}]			
	Variant A	Variant B	Variant C	Variant D	Variant A	Variant B	Variant C	Variant D
Alban	< *LOD*	< *LOD*	< *LOD*	< *LOD*	8.60^{a}	9.12^{ab}	8.29^{a}	10.17^{b}
Astra	< *LOD*	< *LOD*	< *LOD*	< *LOD*	8.23^{b}	7.80^{a}	11.21^{d}	9.97^{c}
R-933	< *LOD*	< *LOD*	< *LOD*	< *LOD*	8.45^{b}	8.15^{b}	7.31^{a}	7.83^{ab}
Satmarean	< *LOD*	< *LOD*	< *LOD*	< *LOD*	8.45^{a}	8.15^{a}	13.29^{c}	11.26^{b}
Nelly	< *LOD*	< *LOD*	< *LOD*	< *LOD*	8.87^{b}	9.41^{d}	8.24^{a}	9.14^{c}
Pop I.	2.22^{c}	1.68^{b}	1.48^{a}	2.13^{c}	9.91^{a}	13.59^{d}	10.71^{b}	12.46^{c}
Los Palacios	< *LOD*	< *LOD*	< *LOD*	< *LOD*	10.32^{c}	9.62^{b}	9.02^{a}	10.66^{d}
Primorskij	< *LOD*	< *LOD*	< *LOD*	< *LOD*	6.33^{a}	8.60^{b}	10.84^{c}	16.65^{d}
Solnecnyj	< *LOD*	< *LOD*	< *LOD*	< *LOD*	14.95^{c}	11.24^{a}	11.30^{a}	11.62^{b}
Weibit	3.21^{a}	5.02^{b}	3.69^{a}	3.22^{a}	5.67^{b}	7.36^{c}	5.31^{a}	8.61^{d}
WTD	1.86^{ab}	1.57^{a}	2.65^{b}	2.12^{ab}	4.19^{b}	3.69^{a}	8.62^{c}	8.85^{d}

Values are expressed per kilogram of dry weight and represent average values of 3 samples. For each flavonoid, same letter in superscript in row means that there are no statistically significant differences between the values at the significance level of $\alpha = 0.05$.
Variant A – control, variant B – with addition of inoculant Rizobin LF, variant C – with addition of growth regulator Lexin, variant D – with addition of combination of Rizobin LF and Lexin.
LOD – limit of detection.

tically significantly in variants A, B, C, D. In other varieties of white lupin, *trans*-ferulic acid was undetectable.

From flavonoids, apigenin and myricetin were detected in white lupine seeds in this study (Tab. 3). Apigenin was detected only in three white lupin cultivars (Pop I., Weibit and WTD). Myricetin was detected in the Astra, Nelly, Pop I., Los Palacios, Primorskij, Weibit and WTD varieties in all monitored variants A, B, C, D. In the R-933 and Satmarean varieties, the same values of myricetin were determined in variant A (8.45 mg·kg^{-1} DW) and variant B (8.15 mg·kg^{-1} DW). According to Vollmannova et al. [29], the content of flavonoids in lupin cultivars was myricetin 11.15–21.19 mg·kg^{-1} DW and apigenin 1.10–2.61 mg·kg^{-1} DW. In the case of myricetin, we determined lower values in culti-

Tab. 4. Content of 4-hydroxybenzoic acid in selected varieties of chickpea.

Chickpea variety	4-Hydroxybenzoic acid [mg·kg^{-1}]			
	Variant A	Variant B	Variant C	Variant D
Krajova z Kralovej	14.244^{a}	13.942^{a}	17.251^{b}	12.733^{a}
Maskovsky Bagovec	9.753^{b}	12.306^{c}	7.416^{a}	9.794^{b}
Businsky	5.798^{b}	12.559^{d}	10.195^{c}	4.525^{a}

Values are expressed per kilogram of dry weight and represent average values of 3 samples. Same letter in superscript in row means that there are no statistically significant differences between the values at the significance level of $\alpha = 0.05$.
Variant A – control, variant B – with addition of inoculant Rizobin LF, variant C – with addition of growth regulator Lexin, variant D – with addition of combination of Rizobin LF and Lexin.

vars Alban, R-933, Nelly, Weibit and WTD in all variants A, B, C, D.

Of the evaluated phenolic acids and flavonoids, only 4-hydroxybenzoic acid was detected in chickpea varieties and variants A, B, C, D (Tab. 4). Caffeic acid, *trans*-ferulic acid, apigenin and myricetin were below the detection limit. Thus, our results do not match the claim of Singh et al. [30] that inoculation of chickpea with *Mesorhizobium* strain did not cause a significant increase in the antioxidant potential but significantly increased the flavonoids content in the seeds. The average content of 4-hydroxybenzoic acid in individual variants A, B, C, D ranged from 4.52 mg·kg^{-1} DW in variant D to 17.25 mg·kg^{-1} DW in variant C. Statistically significant differences at the confidence level of 95.0 % were found based on multiple comparisons using multifactor ANOVA between all variants.

Another evaluated crop was grass pea, where we analysed 4-hydroxybenzoic acid in variants A, B, C, D (Tab. 5). A statistically significant difference in the content of 4-hydroxybenzoic acid was proven by using multifactor ANOVA in all variants A, B, C, D. Studies show that the content of phenolic compounds and the antioxidant activity of plants depend on the species and cultivar. At the same time, significant differences were observed between wild plants and cultivated varieties. In our study, *TPC* was determined in selected legumes (Tab. 6). Based on the results of the analyses, we can compile the following order of *TPC* in individual variants: A < B < C < D. According to Siger et al. [28], *TPC* values of the seeds of two lupin cultivars (4 915 mg·kg^{-1} and 6 276 mg·kg^{-1} DW, respectively), were significantly higher than those reported in this study. A statistically significant difference in *TPC* was observed in all evaluated variants of white lupin. Lampart-Szczapa et al. [31] determined *TPC* in hot and sweet lupin seeds. In our cultivars, *TPC* of white lupin ranged from 2 287.12 mg·kg^{-1} (in WTD) to 4 647.10 mg·kg^{-1} (in Satmarean). Tirdilová et al. [32] determined *TPC* values in white lupin from 5 629 mg·kg^{-1} to 7 765 mg·kg^{-1}, which does not correspond to our results. *TPC* values of hot cultivars were shown to be higher compared to sweet cultivars of white lupin. In the selected chickpea varieties, phenolic acids caffeic acid and *trans*-ferulic acid were undetectable. We detected only 4-hydroxybenzoic acid. Apigenin and myricetin in chickpea were undetectable, too. *TPC* values in individual chickpea variants decreased in the order B < D < A < C. Wang et al. [33] deter-

Tab. 5. Content of phenolic acids and flavonoids in grass pea.

	Variant A	Variant B	Variant C	Variant D	Average	$LSD_{0,05}$
4-Hydroxybenzoic acid [mg·kg^{-1}]	< *LOD*	2.50^{c}	1.89^{b}	1.79^{a}	2.06	0.07
Caffeic acid [mg·kg^{-1}]	< *LOD*	< *LOD*	< *LOD*	< *LOD*	< *LOD*	< *LOD*
trans-Ferulic acid [mg·kg^{-1}]	< *LOD*	< *LOD*	< *LOD*	< *LOD*	< *LOD*	< *LOD*
Apigenin [mg·kg^{-1}]	< *LOD*	< *LOD*	< *LOD*	< *LOD*	< *LOD*	< *LOD*
Myricetin [mg·kg^{-1}]	< *LOD*	< *LOD*	< *LOD*	< *LOD*	< *LOD*	< *LOD*

The presented results are cumulative for all selected varieties of grass pea. The values are expressed per kilogram of dry weight and as arithmetic mean ($n = 3$) by least significant difference interval test. Same letters in superscript in row mean that there are no statistically significant differences between the values at the significance level of $\alpha = 0.05$.
Variant A – control, variant B – with addition of inoculant Rizobin LF, variant C – with addition of growth regulator Lexin, variant D – with addition of combination of Rizobin LF and Lexin.
LOD – limit of detection. $LSD_{0.05}$ – least significant difference at the level $\alpha = 0.05$.

Tab. 6. Total polyphenols content in the monitored varieties of selected legumes.

Crop	Total polyphenols content [mg·kg^{-1}]					
	Variant A	Variant B	Variant C	Variant D	Average	$LSD_{0,05}$
White lupin	2 891.92 ^{a}	2 987.52 ^{a}	3 234.62 ^{ab}	3 577.10 ^{b}	3 172.79	423.36
Chickpea	408.80 ^{ab}	669.24 ^{b}	327.88 ^{a}	416.28 ^{ab}	427.26	266.11
Grass pea	171.19 ^{b}	129.93 ^{ab}	136.24 ^{ab}	115.44 ^{a}	138.20	55.71

The presented results are cumulative for all selected varieties of legumes. The values are expressed as milligrams of gallic acid equivalents per kilogram of dry weight and as arithmetic mean (n = 3) by least significant difference interval test. Same letters in superscript in row mean that there are no statistically significant differences between the values at the significance level of α = 0.05.
Variant A – control, variant B – with addition of inoculant Rizobin LF, variant C – with addition of growth regulator Lexin, variant D – with addition of combination of Rizobin LF and Lexin.
$LSD_{0.05}$ – least significant difference at the level α = 0.05.

mined *TPC* in the range of 362–1 540 mg·kg^{-1}, which are values comparable to our values in individual variants. *TPC* values in the analysed chickpea varieties were significantly lower in the cultivar of Kralova z Kralovej, Maskovsky Bagovec and Businsky in comparison with the results of Xu et al. (1 440 mg·kg^{-1}) [34].

Grass pea was characterized by the lowest *TPC* in individual variants compared to lupin and chickpea. The highest *TPC* was in variant A and with the addition of biologically effective preparations it decreased. Within the varieties, *TPC* decreased in the order Arida > Cahchticky cicer > Krajova z Kralovej. It is well established that the content of beneficial compounds depends on many factors influencing the plants, such as agrochemicals, climatic conditions or storage conditions, and may vary between cultivars [35].

CONCLUSIONS

The presented study was aimed at determining the influence of biologically effective preparations on the content of bioactive substances in selected legume varieties. The presented study was aimed at determining the influence of biologically effective preparations on the content of bioactive substances in selected legume varieties. It can be concluded that the use of biologically effective preparations had no clear effect on the content of phenolic compounds and polyphenols in legumes. The effect was different both in varieties and in individual types of legumes.

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