Comparison of the impact of the years on the content of bioactive substances of selected legumes

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Abstract
Legumes are an interesting source of biologically valuable components. The aim of this study was to assess influence of the year on the content of phenolic compound, total polyphenol content and antioxidant activity of white lupine, chickpea and grasspea. The highest values of caffeic acid content were found in white lupine variety Los Palacios (734.64 $mg.kg^{-1}$ DW), Solnecnyj (896.02 $mg.kg^{-1}$ DW) in 2019. The content of trans-ferulic acid were the highest in cultivars Alban (19.34 $mg.kg^{-1}$ DW), Sonecnyj (19.03 $mg.kg^{-1}$ DW) in 2019. The highest content of myricetin were in selected genotypes Solnecnyj (25.86 $mg.kg^{-1}$ DW) and Los Palacios (23.76 $mg.kg^{-1}$ DW) in 2019. At the same time, statistical differences in the content of phenolic compounds in varieties of white lupine were evident. In the selected cultivars of chickpea and grass, the values of the content of phenolic compounds were below the detection limit. In 2019, the highest antioxidant activity and total content of polyphenols was found in all monitored types of legumes.

Keywords: white lupine; chickpea; grass pea; bioactive substances; years

INTRODUCTION

Pulses are annually grown crops that yield one to twelve grains/seeds within a pod and are often promoted in diet owing to their low cost and many beneficial nutritional effects (Craig, 2009). The year 2016 has been declared as the International Year of Pulses (IYP) by the UNO to promote the use of legumes in human nutrition (FAO).

Pulses are rich in macronutrients such as proteins (usually 21–26 %), carbohydrates and are low in calories and fat (Marinangeli & Jones, 2011).

Bioactive compounds are usually the non-nutrient food constituents that typically occur in small quantities (when compared with macronutrients) in cereals, legumes, and fruits as well as vegetables. Pulses contain plenty of bioactive substances which have metabolic effects on human body upon consumption (Rochfort & Panozzo, 2007). Some of these substances play important role in defence mechanisms of the plants against environmental conditions or predators, while others act as reserve compounds that are accumulated (mainly for germination) in seeds as energy stores. Besides that, bioactive components include dietary fibres, resistant starches, and bioactive phytochemicals (mainly polyphenols and phytosterols) which make pulses suitable for application in wide range of food products. Other bioactive components include phytates, lectins and enzyme inhibitors that are often considered as anti-nutritional factors (Kabagambe et al., 2005).

The influence of change in climate and associated factors (biotic and abiotic) affects the crop production and yield, by declining average yields of major crop plants around the world (Yadav et al., 2015).

MATERIAL AND METHODS

Material for analysis

For this research we used 11 varieties of white lupine of foreign origin, 6 samples of chickpea and 3 samples of grass pea of Slovak origin were used. The plant material was grown on field experimental plots of the National Agricultural and Food Center - Research Institute of Planned Production in Piešťany (GPS coordinates 48.5917973 and 7.827155). The size of individual plots was 5.2x1.5 m. Field trials of lupine, grass pea and chickpea were grown in 2018-2021. But chickpeas were not grown in 2018.

Analysis methods

Preparation of extracts

Extracts for further analysis were prepared using the Twisselman extractor.

Determination of total phenol content

The total polyphenol content (TPC) of selected legume samples was determined by a standard spectrophotometric method according to Lachman et al. (2003) using Folin-Ciocalteu reagent. Results were expressed as milligram equivalents of gallic acid (GAE) per kilogram dry weight (dw).

Determination of the content of individual phenolic substances

We used the HPLC method according to Germ et al. (2019). The detection wavelengths were 320 nm (caffeic acid), 320 nm (transferulic acid), 320 nm (myricetin), 372 nm (genistein) and 265 nm (apigenin). The data obtained were processed using Agilent Open Lab ChemStation software for LC3D systems.

Determination of total antioxidant activity

To determine the antioxidant activity, we used a generally accepted method based on the use of DPPH (2.2-diphenyl-1-picrylhydrazyl) according to Brand-Williams et al. (1995).

Meteorological data and characteristics of the meteorological station

The supplier of the automatic weather station is the company Microstep MIS https://www.microstep-mis.com/. The MicroStep MIS automatic meteorological station is in a fenced area, the sensors are located according to Slovak Hydrometeorological Institute standards (SHMÚ) standards valid at the time of installation. The distance between the meteorological station and the nearest building is approx. 80 meters. Meteorological data is played once every 24 hours. Wind speed, wind direction, air temperature at a height of 2 meters above the ground, ground temperature, soil temperature, air humidity, soil humidity, sunshine, solar radiation, usable photosynthetic radiation, precipitation is measured. The data logger can record data for about 2 years. Once a month, the data is transformed into a file in xls format to simplify further processing.

Statistical methods

The obtained results were evaluated by the statistical program STATGRAPHICS Centurion XVI, 2009 from Stat Point Technologies, Incwww.STATGRAPHICS.com. We used the one-factor ANOVA method, Kruskal Walis test.

RESULTS AND DISCUSSION

Our work was focused on monitoring the influence of individual years 2018-2021 on the content of selected phenolic compounds, total phenol content and total antioxidant activity in white lupine, grass pea and chickpea seeds. We detected the content of caffeic acid and transferulic acid only in selected varieties of white lupine. In the evaluated varieties of chickpea and grass pea were content of fenolic acids undetectable. Table 1 shows the content of caffeic acid in individual varieties of white lupine. Content of caffeic acid ranged from 242.25 $mg.kg^{-1}$ DW Alban (FRA) to 510.92 $mg.kg^{-1}$ DW Los Palacios (ESP) in 2018. In 2019, the values of the caffeic acid content were higher in comparison with 2020 and 2021. The Solnecnyj variety (UKR) showed the highest caffeic acid content in 2019 (896.02 $mg.kg^{-1}$ DW) and in 2020 (131.52 $mg.kg^{-1}$ DW). The lowest content of caffeic acid was detected in the Los Palacios variety 2.48 mg.kg^{-1} DW in 2020. The content of caffeic acid was decreasing in individual varieties of white lupine in the following order: Pop I. (POL)>Los Palacios (ESP)> Solnecnyj (UKR)> Satmarean (ROM)> Wtd (POL)> Astra (CHL)> Alban (FRA)> Primorskij (RUS)>Nelly (HUN)>R-933 (POL)> Weibit (DEU) in 2021. Statistical evaluation confirmed significant differences in content of caffeic acid for the varieties Alban, Primorskij, Satmarean, Solnecnyj, Nelly, Weibit between the years 2018/2019, 2018/2020, 2018/2021 and 2019/2020, 2019/2021, 2020/2021. Astra and R-933 varieties showed statistically significant differences caffeic acid content in 2018/2019, 2018/2020, 2018/2021, 2019/2020, 2019/20. According to Vollmannova et al., 2021 the content of caffeic acid in selected varieties of white lupine ranged from 443 mg.kg^{-1} DW to 766 mg.kg^{-1} DW which is comparable to our results in 2018 and 2019.

Lupine seeds are characterized by a high content of biologically valuable substances with a high antioxidant potential, such as tannins and flavonoids (Zielinska, et al., 2008). From a nutritional point of view, white lupine has the highest oil content and lower alkaloid content compared to blue or yellow lupine Sujak et al., 2006. Phenols present in legume seeds are represented by phenolic acids, flavonoids, and condensed tannins (Singh et al., 2017). The content of trans-ferulic acid in 11 varieties of white lupine is shown in Table 2. Of the evaluated varieties, it had the highest content of trans-ferulic acid variety Astra (CHL) 7.42 mg.kg^{-1} DM. In 2019, the values ranged from 1.66 mg.kg^{-1} DM Pop I. (POL) to 19.34 mg.kg^{-1} DM Alban (FRA). In 2020, the content of trans-ferulic acid was detectable only in varieties Alban, Astra, R-933 and Weibit. In the other genotypes, the values were below the detection limit. It was the same in 2021, when the content of trans-ferulic acid was measured only in the varieties Alban, Astra, Satmarean. The statistical evaluation confirmed the evident differences in the content of trans-ferulic acid between the years 2018 and 2019 in all varieties except the R-933 variety. Statistical differences of the content of trans-ferulic acid were found for the Alban and Astra varieties between years 2018/2019, 2018/2020, 2018/2021, 2019/2020, 2019/2021, 2020/2021.

In the seeds of selected cultivars of white lupin, we evaluated from flavonoids the content of myricetin, Table 3. The content of myricetin ranged in 2018 from 6.43 mg.kg^{-1} DM Los Palacios (ESP) to 12.48 mg.kg^{-1} DM Satmarean (ROM). The year 2019 was again manifested by an increase in myricetin content in all varieties except Nelly and PopI varieties. In 2019, the highest value of myricetin content was detected in the variety Solnecnyj (UKR) 25.86 mg.kg^{-1} DM. Cultivar Weibit showed the lowest myricetin content in 2020, 2021, while in 2018 the value was below the detection limit. Through statistical evaluation, we detected significant differences in myricetin content in the genotypes Alban, Nel-

Table 1. Influence of years on the content of caffeic acid in selected variety of white lupine (mg. kg $^{-1}$ DM)

Crop	Influence of the years on the content of caffeic acid in white lupine seeds			
White lupine	2018	2019	2020	2021
Alban	242.25^{c}	645.79^{d}	95.85^{b}	74.92^{a}
Astra	473.32^{c}	302.95^{b}	64.5^{a}	86.14^{a}
R-993	457.32^{c}	355.94^{b}	33.70^{a}	24.44^{a}
Satmarean	477.20^{c}	573.80^{d}	85.70^{a}	97.09^{b}
Nelly	330.49^{d}	156.98^{c}	85.69^{b}	49.03^{a}
Pop I	452.30^{c}	151.143^{b}	33.86^{a}	138.87^{b}
Los Palacios	510.92^{c}	734.64^{d}	2.48^{a}	111.87^{b}
Primorskij	419.62^{c}	449.21^{d}	59.23^{b}	53.46^{a}
Solnečnyj	311.52^{c}	89.,02^{d}	131.52^{b}	101.34^{a}
Weibit	418.84^{d}	370.46^{c}	38.03^{b}	15.59^{a}
Wtd	489.87^{d}	405.41^{c}	31.10^{a}	88.82^{b}

The values in the table are expressed as arithmetic mean (n = 4). The different letters (a, b, c, d) between the variables show statistically significant differences (p <0.05) between individual years using Analysis of Variance., Kruskal Walis test.

Table 2. Influence of years on the content of trans-ferulic acid in selected variety of white lupine (mg. kg $^{-1}$ DM)

Crop	Influence of the years on the content of trans-ferulic acid in white lupine seeds			
White lupine	2018	2019	2020	2021
Alban	4.38^{c}	19.34^{d}	3.69^{b}	2.66^{a}
Astra	7.42^{c}	10.94^{d}	4.43^{b}	2.96^{a}
R-993	4.79^{a}	4.47^{a}	4.61^{a}	*<LOD*
Satmarean	5.89^{b}	7.90^{c}	*<LOD*	3.86^{a}
Nelly	3.54^{a}	7.25^{b}	*<LOD*	*<LOD*
Pop I	5.70^{b}	1.66^{a}	*<LOD*	*<LOD*
Los Palacios	2.75^{a}	11.59^{b}	*<LOD*	*<LOD*
Primorskij	1.79^{a}	3.18^{b}	*<LOD*	*<LOD*
Solnečnyj	1.39^{a}	19.03^{b}	*<LOD*	*<LOD*
Weibit	2.16^{a}	2.89^{b}	5.67^{c}	*<LOD*
Wtd	2.63^{a}	7.43^{b}	*<LOD*	*<LOD*

The values in the table are expressed as arithmetic mean (n = 4). The different letters (a, b, c, d) between the variables show statistically significant differences (p <0.05) between individual years using Analysis of Variance., Kruskal Walis test. *LOD* – limit of detection.

Table 3. Influence of years on the content of myricetin in selected variety of white lupine (mg. kg $^{-1}$ DM)

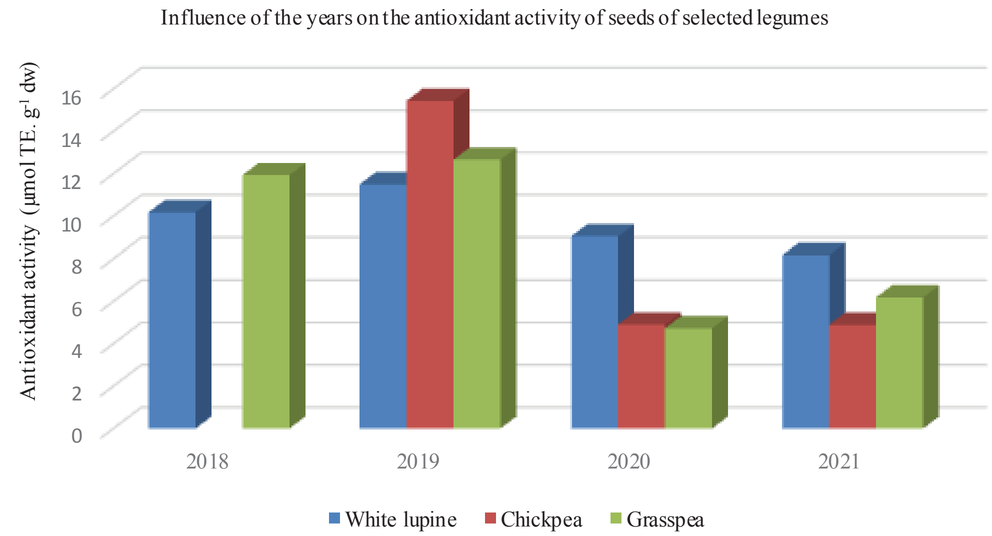
Crop	**Influence of the years on the content of myricetin in white lupine seeds**			
White lupine	**2018**	**2019**	**2020**	**2021**
Alban	7.72^{b}	20.38^{d}	8.61^{c}	7.01^{a}
Astra	9.11^{b}	10.34^{c}	8.24^{a}	8.74^{b}
R-993	7.38^{a}	9.41^{c}	8.44^{b}	*<LOD*
Satmarean	12.48^{c}	12.56^{c}	8.88^{b}	6.75^{a}
Nelly	8.16^{c}	4.17^{b}	8.87^{d}	2.95^{a}
Pop I	7.35^{b}	4.55^{a}	9.91^{c}	11.76^{d}
Los Palacios	6.43^{a}	23.76^{d}	10.32^{b}	14.60^{b}
Primorskij	9.96^{c}	13.25^{d}	6.33^{b}	4.68^{a}
Solnečnyj	9.66^{a}	25.86^{c}	14.95^{b}	10.13^{b}
Weibit	*<LOD*	8.94^{c}	3.21^{b}	2.05^{a}
Wtd	7.35^{c}	11.29^{d}	4.19^{a}	6.79^{b}

The values in the table are expressed as arithmetic mean (n = 4). The different letters (a, b, c, d) between the variables show statistically significant differences (p <0.05) between individual years using Analysis of Variance., Kruskal Walis test. *LOD* – limit of detection.

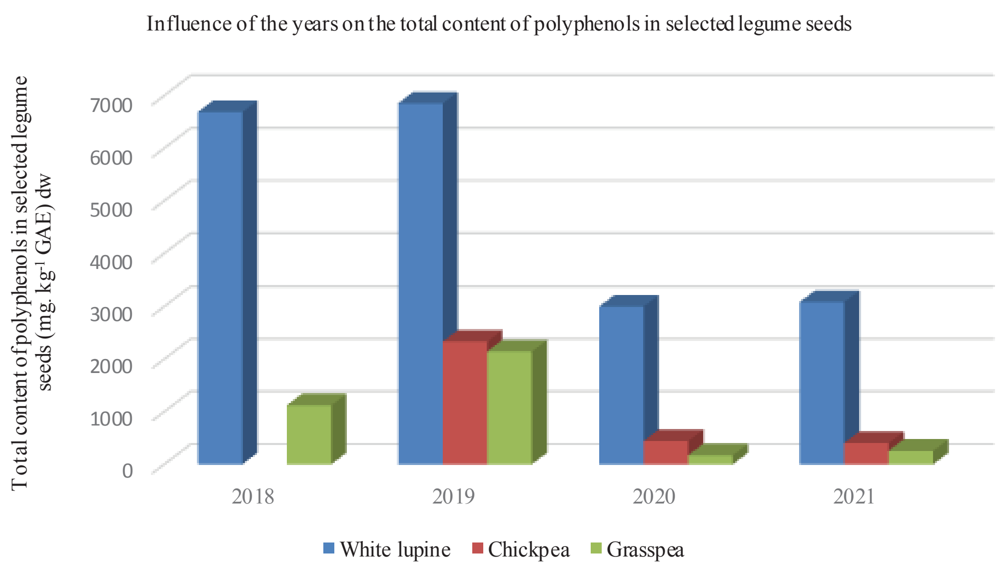
ly, Pop I, Primorskij, Weibit and Wtd between the years 2018/2019, 2018/2020, 2018/2021, 2019/2020, 2019/2021, 2020/2021.

Using the DPPH method, we determined the antioxidant activity of the seeds in selected cultivars of white lupin, chickpea, and grass pea Graph. 1, which was the highest in 2019 among all evaluated types of legumes. We did not evaluate chickpea, as the seeds were not grown in 2018. Antioxidant activity values decreased in the following order: chickpea (15.44 µmol TE. g^{-1} DW)> grass pea (12.66 µmol TE. g^{-1} DM) >white lupine (11.5 µmol TE. g^{-1} DW) in 2019. By comparing the individual years, we can conclude that the antioxidant activity of selected cultivars of white lupine was the highest in 2018, 2020, 2021 compared to chickpea and grass pea.

By comparing individual years, we found that the total content of polyphenols was the highest in the monitored cultivars of white lupine compared to the content of total polyphenols in chickpea and grass pea Graph 2. The values of the content of to-



Graph 1. Average antioxidant activity seeds of selected legumes (µmol TE. g^{-1} DW) in the evaluated years 2018-2021



Graph 2. Average total content of polyphenols in the seeds of selected legumes (mg. kg^{-1} GAE) dw in the evaluated years 2018-2021

Table 4. Meteorological data in RIPP Piešťany

Month	Temperature °C				Rainfall /mm				Sunshine /(min.)			
	2018	2019	2020	2021	2018	2019	2020	2021	2018	2019	2020	2021
April	13.7	12.5	11.1	8.3	17.5	0.4	0.4	0.7	746.8	562.0	198.3	306.9
May	17.5	12.6	13.3	13.4	27.2	3.3	1.6	4.1	824.3	1163.0	621.5	651.8
June	20.4	23.1	18.9	21.7	106.0	9	2.4	0.4	930.9	697.0	848.6	410.7
July	21.6	21.7	20.7	22.9	39.0	1.4	1.6	1.4	767.8	523.0	681.6	712.4
August	23.4	22.8	21.9	19.1	33.2	2.6	1.9	3.4	572.9	905.0	793.2	-
September	17.0	16.4	17.0	17.4	104.0	1.2	3.9	0	945.2	802.0	929.2	-

RIPP - (Research Institute of Plant Production) tal polyphenols decreased for lupine in individual years in the following order: 2019 (6852.35 mg. kg^{-1} GAE)>2018 (6683.84 mg. kg^{-1} GAE)> 2021 (3081.1 mg. kg^{-1} GAE)> 2020 (2998.62 mg. kg^{-1} GAE). According to Siger et al., 2012, were determined the values of TPC in the seeds of 2 lupine cultivars (4915 and 6276 mg. kg^{1} GAE dry matter), which corresponds to our measured TPC values for lupine. At the same time, we can state that content of total polyphenols was the highest in all monitored types of legumes in 2019.

The different values of the observed parameters in the individual years of the duration of the experiment indicate a significant influence of the year on the content of polyphenolic compounds, the values of antioxidant activity in the seeds of the studied legumes. Of the evaluated years, the year 2019 was interesting for our work, when the highest values of the monitored parameters were recorded. This year was characterized by the lowest average amount of precipitation in the months of april - september 1.63 mm (Table 4). This year, the second highest average temperature in the months of april - september was 18.18 °C, while the highest average temperature in 2018 was 18.93 °C. The year 2019 was also characterized by the second highest average value of the length of sunshine of 775.33 min.

CONCLUSION

Our work was focused on comparing the effect of year on the content of phenolic compounds, antioxidant activity and the total content of polyphenols in selected types of legumes. We found that the values of the content of phenolic compounds change depending on the vintage. The content caffeic acid was interesting in white lupine in variety Los Palacios, Sonecnyj. The highest content of trans-ferulic acid and myricetin was recorded in the varieties Alban, Astra, Sonecnyj and Los Palacios. In the selected cultivars of chickpea and grass pea, the values of the phenolic compound content were below the detection limit. The total content of polyphenols and antioxidant activity varied depending on the type of legume and on the year. The highest antioxidant activity and total content of polyphenols was detected in 2019 in all monitored types of legumes. Due to their unique and very rich chemical composition and high nutritional value, leguminous seeds can be an important component of the daily diet. In this aspect, special attention should be paid to lupine seeds, which until now have been mainly used as animal feed and are currently gaining importance as a nutraceutical component.

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