

## THE VARIABILITY OF THE TOTAL POLYPHENOLS CONTENT AND THE ANTIOXIDANT ACTIVITY IN THE VARIETIES OF SELECTED LEGUMES

Mária Timoracká\*<sup>1</sup>, Marek Šnirc<sup>1</sup>, Janette Musilová<sup>1</sup>, Iveta Čičová<sup>2</sup>

### Address(es):

<sup>1</sup> Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Food Sciences, Tr. A. Hlinku 2, 94901 Nitra, Slovakia, +421376414862.

<sup>2</sup> Gene Bank of the Slovak Republic, Research Institute of Plant Production in Piešťany, Bratislavská cesta 122, 921 68 Piešťany, Slovakia.

\*Corresponding author: [maria.timoracka@uniag.sk](mailto:maria.timoracka@uniag.sk)

<https://doi.org/10.55251/jmbfs.9399>

### ARTICLE INFO

Received 31. 8. 2022  
Revised 11. 11. 2022  
Accepted 16. 11. 2022  
Published 21. 12. 2022

Regular article



### ABSTRACT

The goal was to determine the variability of the total polyphenols content and the antioxidant activity in varieties of selected dry legume seeds, which were obtained from the soil of Piešťany. The soil in the Piešťany locality had a neutral soil reaction and was moderately humic. Of the risk elements, the total cadmium content exceeded the limit value by the Law No. 220/2004, but did not exceed the threshold value by the European Commission (2006). In the case of mobile forms of lead, the limit value by Law No. 220/2004 exceeded by 180%. The determination of the heavy metal content in legumes was performed using a flame AAS (instrument Varian AA Spectr DUO 240FS/240 Z/UltrAA). Based on the obtained results, we found the achievement of limit value of lead in 3 varieties of chickpea (Maškovský Bagovec, Kráľová from Krajová and Bušínský) and in 3 varieties of beans (Albena, Luna and Sina). For all 3 types of legumes, the difference between the individual varieties was statistically demonstrated when determining the total polyphenols content and the antioxidant activity values. Between species has been confirmed a statistically significant difference. The order of legumes based on the average decreasing content of total polyphenols is lentil>chickpea>bean and on antioxidant activity lentils>beans>chickpeas. The dependence of antioxidant activity on the total polyphenols has been confirmed in lentil varieties, in the case of chickpeas this relationship is statistically insignificant and a lower mean dependence was determined in beans.

**Keywords:** legumes, polyphenols, antioxidant activity, variability, heavy metals, soil

### INTRODUCTION

From point of food safety and therapeutic effects against pathological conditions that subsequently lead to oxidative damage, interest of people in natural antioxidants has recently increased (Sánchez-Bonet *et al.*, 2021). Natural antioxidants in food are increasingly in demand due to the toxicity of synthetic antioxidants (Asati *et al.*, 2022) as exemplified by research on various plants, including legumes. Polyphenols are common constituents of foods of plant origin and are major antioxidants in the human diet (Shem-Tov *et al.*, 2011). Dry legumes are a source of bioactive polyphenols and also contribute to polyphenol intake from other food; the health benefits of consuming legumes such as antioxidant activity could be effective for the expansion of their food uses (Heiras-Palazuelos *et al.*, 2013). Taking into consideration the present health condition of population and prevalence of morbidity and mortality in the Slovak Republic, it is desirable to develop foodstuffs which are natural source of bioactive substances and have positive impact on health of consumers (functional food) (Bojňanská *et al.*, 2012, Umar *et al.*, 2012). According to Xu *et al.* (2007), phenolic compounds contribute to the overall antioxidant activity of plant foods. Significant positive correlation was observed between phenolics and antioxidant activity by DPPH scavenging activity in the various legume (Han and Bike, 2008). Polyphenols exhibit antioxidant activity through various mechanisms, such as chelating transition metal ions (as copper and iron) (Tian *et al.*, 2022), scavenging free radicals such as inhibiting oxidative enzymes. Under certain circumstances, polyphenols can exhibit oxidation reactions by chelating transition metals by increasing their catalytic activity or by reducing metal ions, leading to an increased ability to generate free radicals. The disposition of polyphenols to act as prooxidants can cause a reduction in food quality (Tian *et al.*, 2022) under given conditions. Musilová (2009) published that growth location, variety type and soil contamination can affect the amount of polyphenols. Also according to Tian *et al.* (2022) reported the effect of the concentration of polyphenols on the pro-oxidative or antioxidative activity depends on the environmental conditions. The presence and the action of heavy metals can started up an oxidative stress in plants. Heavy metals can present a threat to the environment, but also to humans. According Kisa *et al.* (2016) there are few studies on the relationship between heavy metal and phenolic compounds, no general conclusion about the heavy metal stress on phenolic compound levels.

Therefore, at first we also focused on heavy metals determination in the soil and in selected legume due to the elimination of the influence of heavy metals as stress factors for the formation of polyphenols and their antioxidant activity (Segev *et al.*, 2010). The main objective of this study was to determine the variability of the total polyphenols content and the antioxidant activity values in the varieties of chosen legumes, the effect of varieties on the total polyphenols and the antioxidant activity, the dependence of antioxidant activity on total polyphenols, an inter-variety and inter-species differences.

### MATERIAL AND METHODS

#### Samples

Samples of 9 different white bean (*Phaseolus vulgaris* L.) varieties: Albena, Ema, Fabia, Gesta, Gracia, Luna, Petra, Salva, Sina; samples of 6 different chickpea (*Cicer arietinum* L.) varieties: Kráľová z Krajovej, Maškovský Bagovec, Bušínský, Slovák, Beta, Alfa and 6 lentils (*Lens culinaris* L.) varieties: Slovenská zemplinská, Trebišovská, Slovenská modrá, Hrotovická veľkozrná, Nelka, Renka were investigated. All the varieties originated from the experimental station of the Research Institute of Crop Production in Piešťany – an important cultivation area for crop conservation in Slovakia. Legume samples were collected at full ripeness. The seeds were manually cleaned and stored at the room temperature (normal conditions) for analysis. The sample of soil were collected from the same sampling point as legums samples.

#### Heavy metals content determination

In soil samples from observed localities the exchangeable reaction (pH/KCl), the available nutrients (potassium, magnesium, phosphorus) and mobile forms of Ca according Mehlich II., and content of humus were determined. Results of soil analysis were evaluated by Decree of the Ministry of Agriculture and Rural Development of the Slovak Republic No. 151/2016. Pseudototal content of risk metals (including all residual metal fraction) was assessed in soil extract by *aqua regia* and content of mobile forms of chosen heavy metals in soil extract by  $\text{NH}_4\text{NO}_3$  ( $c = 1 \text{ mol} \cdot \text{dm}^{-3}$ ).

*Plant material* - The samples of legumes after their drying, regulation and decomposition by  $\text{HNO}_3$  on the microwave digestion were used. Determinations

of metal content were analysed in a Varian AA240Z atomic absorption spectrometer. The graphite furnace technique was used for the determination of Cd and Pb.

The results of soil were evaluated according to **Law No. 220/2004** - the legislative valid in the Slovak republic as well as threshold values proposed by **European Commission (EC) (2006)** and the content of heavy metals in legume seeds were evaluated according to the Food Codex of the Slovak Republic as well as according to **EU Commission No. 2021/1317** (for Pb) and **EU Commission No. 2021/1323** (for Cd).

**Total polyphenols content determination**

Total polyphenol content was determined according to **Lachman et al. (2003)**. Folin-Ciocalteu solution was used as a reagent for the colorimetric assay of polyphenolic antioxidants. The sample solutions were measured at 765 nm (instrument Shimadzu spectrophotometer 710, Kyoto, Japan). The total polyphenols contents were expressed as gallic acid equivalents in mg.kg<sup>-1</sup> of dry matter.

**Antioxidant activity determination**

The antioxidant activity was determined using free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) according to **Brand-Williams et al. (1995)**. The solutions of samples were measured at 515 nm spectrophotometrically using a Shimadzu spectrophotometer (Japan). Results were expressed as % DPPH inhibition.

**Statistical analysis**

All analysis were performed in six replicates. Descriptive analysis includes mean±standard deviation. All statistical analyzes were performed by XLSTAT 2014.5.03 statistical software using Dunn's test. Mean comparisons between the total polyphenol content and the antioxidant activity were performed using the LSD or Kruskal-Wallis test (P<0.05).

**RESULTS AND DISCUSSION**

**The heavy metals content determination**

Locality Piešťany lies in the north-south valley of the Váh river in the northern extremity of the Danube lowland. The city lies 162 m above sea level. This area is characteristically lowland, dry, relatively warm and slightly windy. The soil is predominantly sandy-clay. The soil had 232 mg K.kg<sup>-1</sup>(a good content), 295 mg Mg.kg<sup>-1</sup> (a high content of magnesium) and 18 mg P.kg<sup>-1</sup>(a low content of phosphorus ), by medium level of humus and neutral soil reaction. From point of view positive nutrient content and satisfactory soil reaction a soil in Piešťany is suitable for growing legumes.

The measured contents of heavy metals in the soil (Table 1) from the Piešťany locality were compared with the limit values set by **Law No. 220/2004** about the soil protection and the use of agricultural land and the threshold values recommended by the **European Commission (2006)**.The total content of heavy metals in the *Aqua regia* extract was exceeded only in the case of cadmium. The determined value of the cadmium content was 0.98 mg.kg<sup>-1</sup> and exceeded the limit value established by **Law No. 220/2004** by 40%. The contents of the other analyzed elements were below the limit values determined by **Law No. 220/2004**. From the point of view of heavy element intake by plants, is important content of available and potentially available forms of heavy metals. The content of mobile forms of heavy metals in the ammonium nitrate solution in the soil from Piešťany was satisfactory, with the exception of the lead content of 0.28 mg.kg<sup>-1</sup>, which exceeded the limit value by 180%.

**Table 1** The contents of heavy metals in *Aqua regia* and nitrate ammonium reagent (c=1 mol.dm<sup>-3</sup>) in the soil of Piešťany locality (mg.kg<sup>-1</sup>)

	Zn	Cu	Cr	Cd	Pb	Co	Ni
<i>Aqua regia</i>	82.8	25.3	35	0.98	26.8	13.8	46.6
*Limit value	150	60	70	0.7	70	15	50
*Critical value	200	100	100	1.5	100	-	70
<i>Nitrate ammonium</i>	0.08	0.11	0.04	0.04	0.28	0.16	0.17
*Threshold value	2	1	-	0.1	0.1	-	1.5

**Legend:** \*Limit value and \*Critical value by the **Law No. 220/2004** and \*Threshold value recommended by the **European Commission (2006)**

Any of the determination of heavy metals content in the soil not guarantee that the plants growing on this soil will always contain their tolerable amounts. It is therefore crucial in terms of hygiene, whether the heavy metals accumulate in parts of plant used for consumption (**Rattan et al., 2005**). The subject of research was 6 chickpea varieties, 6 lentil varieties and 9 white bean varieties. We compared the content of only toxic metals - Pb and Cd - determined in selected types of legumes

from the Piešťany location with the limit values evaluated by the Codex Alimentarius of the Slovak Republic as well as by the **EU Commission No. 2021/1317** (for Pb) and **No. 2021/1323** (for Cd). Results are shown in the Tables 2-3. The contents of toxic elements Cd and Pb in lentil varieties were below the detection limit. The content of toxic elements Cd in chickpea varieties were below the detection limit, however, a low concentration of 0.02 mg.kg<sup>-1</sup> was measured in the Slovak variety. The content of toxic elements Cd in white bean varieties were below the detection limit with exception Petra variety; the value of Cd was exceeded by 150% and by 1150% according to the **Codex Alimentarius (2022)** valid in the Slovakia and to the Regulation of the **EU Commission No. 2021/1323**, respectively. **Hodálová (2014)** reported that the limit values of Cd (0.1 mg.kg<sup>-1</sup>) were exceeded in all observed bean varieties by an average of 31.25%. In our case of the toxic element Pb, the results reached the limit value (0.2 mg.kg<sup>-1</sup>) according to the law valid in Slovakia in the 3 chickpea varieties - Bušínský, Maškovský Bagovec and Kráľová z Krajová as well as in the 3 white bean varieties - Albena, Luna, Sina. On the basis of **EU Commission Regulation No. 2021/1317**, the limit values were exceeded by 100% in the same varieties. Heavy metals such as lead and cadmium enter to the food chain by uptake by edible plants, thus our legumes samples are relatively safe, therefore we can continue to evaluate the variability of the content of bioactive substances.

**Table 2** The content of toxic elements – lead and cadmium (mg.kg<sup>-1</sup>) in lentil and chickpea varieties

Lentil varieties	Pb	Cd	Chickpea varieties	Pb	Cd
<i>Slovenská zemplínska</i>	und.	und.	<i>Kráľová z Krajovej</i>	0.2	und.
<i>Trebišovská</i>	und.	und.	<i>Maškovský Bagovec</i>	0.2	und.
<i>Slovenská modrá</i>	und.	und.	<i>Bušínský</i>	0.2	und.
<i>Hrotovická veľkozrná</i>	und.	und.	<i>Slovák</i>	und.	0.02
<i>Nelka</i>	und.	und.	<i>Beta</i>	0.1	und.
<i>Renka</i>	und.	und.	<i>Alfa</i>	und.	und.
*Limit value	0.2	0.1		0.2	0.1
**Limit value	0.1	0.02		0.1	0.02

**Legend:** \*Limit value by the **Codex Alimentarius valid in the Slovak Republic (2022)** and \*\* Limit value by the **EU Commission No. 2021/1317** (for Pb) and **No. 2021/1323** (for Cd), und. - undetected

**Table 3** The content of toxic elements - lead and cadmium (mg.kg<sup>-1</sup>) in white bean varieties

White bean varieties	Pb	Cd
<i>Albena</i>	0.2	und.
<i>Ema</i>	0.1	und.
<i>Fabia</i>	0.1	und.
<i>Gesta</i>	0.1	und.
<i>Gracia</i>	0.1	und.
<i>Luna</i>	0.2	und.
<i>Petra</i>	und.	0.25
<i>Salva</i>	und.	und.
<i>Sina</i>	0.2	und.
*Limit value	0.2	0.1
**Limit value	0.1	0.02

**Legend:** \*Limit value by the **Codex Alimentarius valid in the Slovak Republic (2022)** and \*\* Limit value by the **EU Commission No. 2021/1317** (for Pb) a **No. 2021/1323** (for Cd), und. - undetected

**The total polyphenols content and the antioxidant activity determination**

The values of the total polyphenols content (mg.kg<sup>-1</sup>) and the antioxidant activity (in %) in the observed varieties of lentil seeds were expressed as the average value±standard deviation of 6 measured values (Table 4). Table 4 also shows statistical significance between individual lentil varieties. There was a statistically difference between the individual varieties of lentils in the total polyphenols content and the percentage antioxidant activity. Lentils are an excellent food with antioxidant properties. We determined the values of total polyphenols in lentils in range of 1333.7-1972.9 mg.kg<sup>-1</sup>. The Slovenská modrá variety had the highest average total polyphenols content (1972.9 mg.kg<sup>-1</sup>) and the highest average antioxidant activity (33.57%).

**Fратиanni et al. (2014)** verify the total polyphenols contents in lentils, while the results correspond with our values. In dry lentil seeds, **Alshikh et al. (2015)** reported the content of total polyphenols in the range of 1370–5530 mg.kg<sup>-1</sup> depending on the cultivar. We determined the values of antioxidant activity in lentils in the range of 17.48-33.57%. The values are similar to those established by **Fратиanni et al. (2014)** - an average of 23%. **Xu et al. (2007)** report that the total content of polyphenols in lentils harvested in the cold season is lower (486-960 mg.kg<sup>-1</sup>). **Kalogeropoulos et al. (2010)** report amounts of polyphenols of 258-259 mg.kg<sup>-1</sup> in cooked lentils. These values are lower than the values reported for

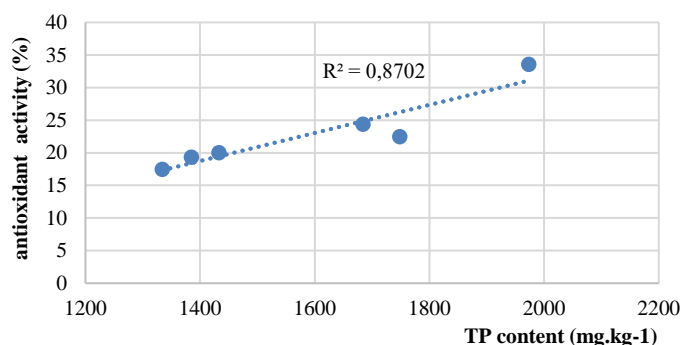
unheated legumes **Kalogeropoulos et al. (2010)**. **Xu and Changa (2008)** report in their study an observed decrease in total polyphenols after soaking and cooking peas, lentils and chickpeas. More pigmented varieties tend to contain higher levels of phenolic compounds (such as anthocyanins) than less pigmented varieties (**Alshikh et al., 2015; Balwinder et al., 2017**).

**Table 4** The total polyphenols content (mg.kg<sup>-1</sup>) and the antioxidant activity (%) in selected lentil varieties (n=6)

Variety	Total polyphenols	Antioxidant activity
Slovenská zemplinská	1748.1± 8.0 <sup>c</sup>	22.50±0.95 <sup>a,b,c</sup>
Trebišovská	1684.1±4.7 <sup>b,c</sup>	24.41±0.73 <sup>b,c</sup>
Slovenská modrá	1972.9±22.6 <sup>c</sup>	33.57±1.31 <sup>c</sup>
Hrotovická veľkozrná	1432.6±10.8 <sup>a,b,c</sup>	20.01±0.85 <sup>a,b,c</sup>
Nelka	1333.7±16.1 <sup>a</sup>	17.48±0.77 <sup>a</sup>
Renka	1384.7±14.9 <sup>a,b</sup>	19.36±0.49 <sup>a,b</sup>

**Legend:** mean±standard deviation, different small letters (a,b,c) in a column show statistically significant differences (P< 0.05) between varieties, the same small letters in a column show statistically non-significant differences (P< 0.05) between varieties

Between the content of total polyphenols and the antioxidant activity, a higher medium dependence is confirmed in the case of lentils (R<sup>2</sup>=0.8702). As the content of polyphenols increases, the antioxidant activity in lentils increases. Lentils are a legume with good antioxidant properties. This explains 87% of the variability of the model. This relationship is shown graphically in **Figure 1**.



**Figure 1** A relationship between the total polyphenols (TP content) and the antioxidant activity in selected lentil varieties

The values of the content of total polyphenols (mg.kg<sup>-1</sup>) and antioxidant activity (%) in the selected varieties of chickpea seeds were expressed as the average value±standard deviation of 6 measured values (Table 5). Table 5 also shows statistical significance between individual lentil varieties. There was a statistically difference between the individual varieties of lentils in the content of total polyphenols and the antioxidant activity. The highest content of total polyphenols was recorded in the Alfa variety (964.7 mg.kg<sup>-1</sup>), the lowest in the Slovák variety (796.6 mg.kg<sup>-1</sup>). In the Alfa variety, the amount of total polyphenols was 17.4% higher than in the Slovák variety. A significantly lower content of total polyphenols (183 mg.kg<sup>-1</sup>) was measured by **Fратиanni et al. (2014)**. In chickpea varieties, **Fernandez-Orozco et al. (2009)** found a content of total polyphenols of 540 mg.kg<sup>-1</sup>. On the contrary, **Han and Baik (2008)** reported a significantly higher content of total polyphenols in chickpea varieties (2200 mg.kg<sup>-1</sup>). This inconsistency can be caused by the diverse climate and geographical area, or different chickpea cultivars. **Xu et al. (2007)** reported a total polyphenol content of 980 mg.kg<sup>-1</sup> in chickpea harvested in the cooler season. The content of phenolics in natural matter is variable in dependence on the type of horticultural crop, also individual varieties of crops (**Drewnowski and Gomez-Carneros, 2000**). The highest antioxidant activity value was found in the Bušínský variety (5.01%) and the lowest in the Mašíkovský Bagovec variety (0.61%). The value of antioxidant activity in the Bušínský variety was 87.8% higher than in the Mašíkovský Bagovec variety. **Umar et al. (2022)** indicates a slightly higher value of the antioxidant activity (4.17-4.95%).

The values of the total polyphenols (mg.kg<sup>-1</sup>) and the antioxidant activity (%) in the observed varieties of bean seeds were expressed as the average value±standard deviation of 6 measured values (Table 6). Table 6 also shows statistical significance between individual white bean varieties. A statistically significant difference was showed between the individual varieties of white beans in the content of total polyphenols and the antioxidant activity. The Fabia variety had the highest average content of total polyphenols (836.7 mg.kg<sup>-1</sup>) and the highest average antioxidant activity (11.65%). As one variety, the Albena variety had the lowest average content of total polyphenols (501.7 mg.kg<sup>-1</sup>) and the lowest average antioxidant activity (2.05%). The content of total polyphenols is 40% higher in the variety Fabia than in the variety Albena.

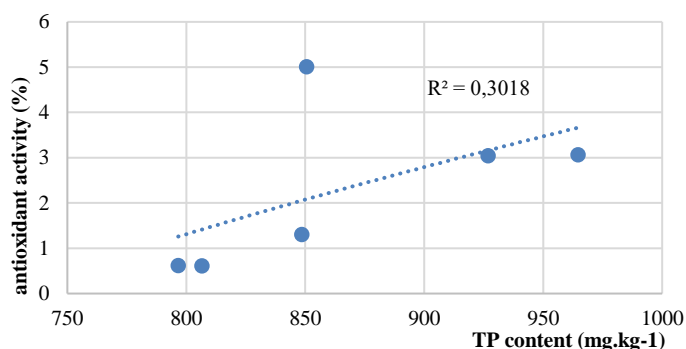
**Luthria and Pastor-Corrales (2006)** reported a total polyphenol content of 191–483 mg.kg<sup>-1</sup> in bean varieties. **Xu et al. (2007)** reported total polyphenol content 570–699 mg.kg<sup>-1</sup> for common beans harvested in a cooler season.

**Table 5** The total polyphenols content (mg.kg<sup>-1</sup>) and the antioxidant activity (%) in selected chickpea varieties (n=6)

Variety	Total polyphenols	Antioxidant activity
Králová z Krajovej	926.9±42.3 <sup>b</sup>	3.04±1.65 <sup>a,b</sup>
Mašíkovský Bagovec	806.6±15.4 <sup>a</sup>	0.61±0.47 <sup>a</sup>
Bušínský	850.6±7.3 <sup>a,b</sup>	5.01±0.64 <sup>b</sup>
Slovák	796.6±14.6 <sup>a</sup>	0.62±0.60 <sup>a</sup>
Beta	848.6±31.5 <sup>a,b</sup>	1.30±0.77 <sup>a,b</sup>
Alfa	964.7±25.6 <sup>b</sup>	3.06±0.90 <sup>a,b</sup>

**Legend:** mean±standard deviation, different small letters (a or b) in a column show statistically significant differences (P< 0.05) between varieties, the same small letters in a column show statistically non-significant differences (P< 0.05) between varieties

**Figure 2** graphically shows the relationship between the content of total polyphenols and antioxidant activity in selected chickpea varieties. This relationship is statistically insignificant (R<sup>2</sup>=0.3018). The content of total polyphenols in chickpeas does not affect its antioxidant activity. Model variability is explained at 30%. **Xu et al. (2007)** reported that the variable content of phenols may have a different degree of influence on the antioxidant activity in each type of legume. **Quintero-Soto et al. (2018)** identified twenty phenolic compounds and their levels showed a great variability among the chickpea genotypes.



**Figure 2** A relationship between the total polyphenols (TP content) and the antioxidant activity in selected chickpea varieties

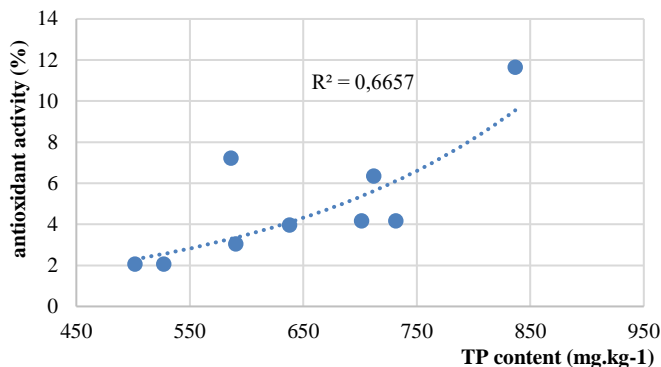
**Table 6** The total polyphenols content (mg.kg<sup>-1</sup>) and the antioxidant activity (%) in selected white bean varieties (n=6)

Variety	Total polyphenols	Antioxidant activity
Albena	501.7±6.5 <sup>a</sup>	2.06±0.81 <sup>a</sup>
Ema	637.8±16.0 <sup>a,b,c</sup>	3.95±0.22 <sup>a,b,c</sup>
Fabia	836.7±15.1 <sup>d</sup>	11.65±1.27 <sup>c</sup>
Gesta	701.2±11.7 <sup>b,c,d</sup>	4.16±1.04 <sup>a,b,c</sup>
Gracia	526.9±9.7 <sup>a,b</sup>	2.05±1.02 <sup>a</sup>
Luna	590.5±7.2 <sup>a,b,c</sup>	3.04±0.42 <sup>a,b</sup>
Petra	586.3±3.8 <sup>a,b,c</sup>	7.21±1.44 <sup>b,c</sup>
Salva	712.1±5.8 <sup>b,c,d</sup>	6.34±0.43 <sup>b,c</sup>
Sina	731.5±3.3 <sup>c,d</sup>	4.16±0.65 <sup>a,b,c</sup>

**Legend:** mean±standard deviation, different small letters (a,b,c or d) in a column show statistically significant differences (P< 0.05) between varieties, the same small letters show statistically non-significant differences (P< 0.05) between varieties

There is a lower medium dependence (R<sup>2</sup>=0.638) between the total polyphenols and the antioxidant activity in selected bean varieties. The content of total polyphenols in beans has a partial effect on the antioxidant activity of beans and **Duodu (2011)** reached the same conclusion. The variability of the model is thus explained to 63.8%. Results are shown in the **Figure 3**.





**Figure 3** A relationship between the total polyphenols (TP content) and the antioxidant activity in selected white bean varieties

From point of view of the total polyphenol content and the antioxidant activity legumes between species were compared. The cross-species comparison showed a highly statistically significant influence of the type of legume on the content of total polyphenols and the antioxidant activity. The high significant correlations between the antioxidant activity and the phenolic composition were confirmed also by **Canas et al. (2008)**. The highest average content of total polyphenols in the selected legume varieties was found in lentils (1592.7 mg.kg<sup>-1</sup>), followed by chickpeas with a content of 865.7 mg.kg<sup>-1</sup> and the lowest amount of total polyphenols was found in beans (647.2 mg.kg<sup>-1</sup>). In lentils, the content of polyphenols was higher by 59.36% than in beans. The antioxidant activity was again the highest in lentils at 22.9%, the lowest was in chickpeas at 2.3%, and the antioxidant activity of beans at 5%. Results are shown in the Table 7. **Xu et al. (2007)**, **Fратиanni et al. (2014)**, **Alshikh et al. (2015)** report that the legumes they tested, lentils had the highest concentration of phenolic components and the antioxidant activity values.

**Table 7** The average of total polyphenols content (mg.kg<sup>-1</sup>) and the antioxidant activity (%) in investigated legumes

crop	total polyphenols (mg.kg-1)	antioxidant activity (%)
lentil	1592.7 <sup>c</sup>	22.9 <sup>c</sup>
chickpea	865.7 <sup>b</sup>	2.3 <sup>a</sup>
white bean	647.2 <sup>a</sup>	5.0 <sup>b</sup>

**Legend:** mean±standard deviation, different letters (a,b,c) in a column show statistically significant differences (P< 0.05) between legume species, the same letters in a column show statistically non-significant differences (P< 0.05)

## CONCLUSION

Soil hygiene and risky toxic metals contents in observed legumes in the current study were evaluated, then were investigated internal variability of total polyphenols content and antioxidant activity. The total content of cadmium and mobile forms of lead in the soil of the monitored location exceeded the limit value set by Act no. 220/2004 by 40% and 180%, respectively, but met the European Commission (EC) limit (2006). The toxic metals lead and cadmium were below the detection limit in all legume varieties. Limit values of lead were analyzed in the varieties Buinsk, Maškovský Bagovec, Kráľová z Krajová (chickpeas) and Luna, Sina, Albena (beans) (white beans). The cadmium content in the chickpea varieties was under the detection limit, except for the variety Slovák. Only the Petra variety of white beans had exceeded the limit value for cadmium. Pollutants such as Pb and Cd can enter the food chain through the uptake of plant foods, so in out legume samples are relatively suitable for human consumption, but heavy metal determination and control in plant foods is essential.

Lentil varieties had the highest total polyphenol content and the highest percentage of antioxidant activity, suggesting that lentils are foods with excellent antioxidant properties. We observed that bean varieties had the lowest levels of total polyphenols. The antioxidant activity in bean cultivars, on the other hand, was more than the percentage of antioxidant activity in chickpea varieties. Individual varieties of the selected legumes have statistically significant differences in total polyphenol content and antioxidant activity. Internal variability was confirmed in the selected legume cultivars; the variety influences total polyphenol content and antioxidant activity. In lentils, the relationship between antioxidant activity and total polyphenols was stated. The total polyphenol content of chickpeas has little or no effect on the antioxidant activity value. In beans, a partial effect of total polyphenol content on antioxidant activity was confirmed. Variability in total polyphenol content affects antioxidant activity differently in each variety and type of legume. Based on the findings, we can conclude that the type and variety of legumes affect antioxidant activity and total polyphenol content. According to our results and dietary and bioactive compound assessment, we can advise lentils as an excellent material for developing modern food products.

**Acknowledgments:** The project was supported by VEGA 1/0722/19.

## REFERENCES

- Alshikh, A., Costa de Camargo, A., Shahidi, F. (2015). Phenolics of selected lentil cultivars: Antioxidant activities and inhibition of low-density lipoprotein and DNA damage. *Journal of Functional Foods*, 18 (1), 1022-1038. [10.1016/j.jff.2015.05.018](https://doi.org/10.1016/j.jff.2015.05.018)
- Asati, V., Deepa, P. R., Sharma, P. K. (2022). Desert legume *Prosopis cineraria* as a novel source of antioxidant flavonoids / isoflavonoids: Biochemical characterization of edible pods for potential functional food development. *Biochemistry and Biophysics Reports*, 29 (1), 101210. <https://doi.org/10.1016/j.bbrep.2022.101210>
- Balwinder, S., Jatinder, P. S., Amritpal, K., Narpinder, S. (2017). Phenolic composition and antioxidant potential of grain legume seeds: A review. *Food Research International*, 101,1-16. <https://doi.org/10.1016/j.foodres.2017.09.026>
- Bojňanská, T., Francáková, H., Lišková, M., Tokár, M. (2012). Legumes – the alternative raw materials for bread production. *Journal of Microbiology, Biotechnology Food Sciences*, 1 (special issue), 876-886. <https://doi.org/10.15414/jmbfs.2015.4.special3.18-22>
- Brand-Williams, W., Cuvelier, M. E., Berste, C. (1995). Use free radical method to evaluate antioxidant activity. *LWT - Food Sci. Technol.*, 28 (1), 25-30.
- Canas, S., Casanova, V., Belchior, P. (2008). Antioxidant activity and phenolic content of Portuguese wine aged brandies. *Journal of Food Composition and Analysis*, 21 (8), 626-633. <https://doi.org/10.1016/j.jfca.2008.07.001>
- Drewnowski, A. and Gomez-Carneros, C. (2000). Bitter taste, phytonutrients, and the consumer: a review. *American Journal of Clinical Nutrition*, 79, 727-747. <https://doi.org/10.1093/ajcn/72.6.1424>
- Decree of the Ministry of Agriculture and Rural Development of the Slovak Republic No. 151/2016 laying down details of agrochemical soil testing and storage and use of fertilizers. 2016.
- European Commission (EC) (2006). Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.
- EU Commission No. 2021/1317. Commission Regulation (EU) 2021/1317 of 9 August 2021 amending Regulation (EC) No 1881/2006 as regards maximum levels of lead in certain foodstuffs.
- EU Commission No. 2021/1323. Commission Regulation (EU) 2021/1323 of 10 august 2021 amending Regulation (EC) No 1881/2006 as regards maximum levels of cadmium in certain foodstuffs.
- Duodu, K. G. (2011). Effects of Processing on Antioxidant Phenolics of Cereal and Legume Grains. American Chemical Society (ACS) , vol. 1089. eISBN: 9780841226418. [DOI: 10.1021/bk-2011-1089.ch003](https://doi.org/10.1021/bk-2011-1089.ch003)
- Fernandez-Orozco, R., Frias, J., Zielinski, H., ... & Vidal-Valverde, C. (2009). Evaluation of bioprocesses to improve the antioxidant properties of chickpeas. *LWT- Food Science and Technology*, 42 (4), 885-892. <https://doi.org/10.1016/j.lwt.2008.10.013>
- Fратиanni, F., Cardinale, F., Cozzolino, A., ... & Nazzaro, F. (2014). Polyphenol composition and antioxidant activity of different grass pea (*Lathyrus sativus*), lentils (*Lens culinaris*), and chickpea (*Cicer arietinum*) ecotypes of the Campania region (Southern Italy). *Journal of Functional Foods*, 7 (1), 551-557. <https://doi.org/10.1016/j.jff.2013.12.030>
- Han, H. and Baik, B. H. 2008. Antioxidant activity and phenolic content of lentils (*Lens culinaris*), chickpeas (*Cicer arietinum* L.), peas (*Pisum sativum* L.) and soybeans (*Glycine max*), and their quantitative changes during processing. *International Journal of Food Science and Technology*, 43 (11), 1971-1978. <https://doi.org/10.1111/j.1365-2621.2008.01800.x>
- Heiras-Palazuelos, M. J., Ochoa-Lugo, M. I., Gutiérrez-Dorado, R.,... & Reyes-Moreno, C. (2013). Technological properties, antioxidant activity and total phenolic and flavonoid content of pigmented chickpea (*Cicer arietinum* L.) cultivars. *Int J Food Sci Nutr.*, 64(1):69-76. <https://doi.org/10.3109/09637486.2012.694854>
- Hodálová, T. 2014. Analýza obsahu ťažkých kovov v pôdach a ich vstup do konzumných častí strukovín: diplomová práca. Nitra: SPU v Nitre. 70 p.
- Kalogeropoulos, N., Chiou, A., Ioannou, M., ... & Andrikopoulos, K. (2010). Nutritional evaluation and bioactive microconstituents (phytosterols, tocopherols, polyphenols, triterpenic acids) in cooked dry legumes usually consumed in the Mediterranean countries. *Food Chemistry*, 121 (3), 682-690. <https://doi.org/10.1016/j.foodchem.2010.01.005>
- Kisa, D., Elmastas, M., Lokman, O., Kayir, O. (2016). Responses of the phenolic compounds of Zea mays under heavy metal stress. *Appl Biol Chem*, 59 (6), 813–820. <https://doi.org/10.1007/s13765-016-0229-9>
- Lachman, J., Hejtánková, A., Dudjak, E. (2003). Content polyphenolic antioxidants and phenolcarboxylic acids in selected parts of yacon. In *Vitamins 2003*. Pardubice : University Pardubice, pp. 89-97. ISBN 80-7194-549-8.
- Luthria, D. L., Pastor-Corales, M.A. (2006). Phenolic acids content of fifteen dry edible bean (*Phaseolus vulgaris* L.) varieties. *Journal of Food Composition and Analysis*, 19 (2), 205-211. <https://doi.org/10.1016/j.jfca.2005.09.003>

- Musilová, J. (2009). Vzťah vybraných rizikových kovov k nutričným komponentom ľuľka zemiakového (*Solanum tuberosum* L.) : habilitačná práca. Nitra : SPU v Nitre, 120 p.
- Quintero-Soto, F., Saracho-Peña, A. G., Chavez-Ontiveros, J., ... & Lopez-Valenzuela, J. A. (2018). Phenolic profiles and their contribution to the antioxidant activity of selected chickpea genotypes from Mexico and ICRISAT collections. *Plant Foods Hum. Nutr.*, 73(2), 122-129. <https://doi.org/10.1007/s11130-018-0661-6>
- Rattan, R. K., Datta, S. P., Chhonkar, P. K., Suribabu, K., Singh, A. K. (2005). Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater – a case study. *Agriculture, Ecosystem and Environment*, 109, 310-322. <http://dx.doi.org/10.1016/j.agee.2005.02.025>.
- Sánchez-Bonet, D., García-Oms, S., Belda-Antolí, M., ... & Cejalvo-Lapeña D. (2021). RP-HPLC-DAD determination of the differences in the polyphenol content of *Fucus vesiculosus* extracts with similar antioxidant activity. *Journal of Chromatography B*, 1184 (1), 1184:122978. <https://doi.org/10.1016/j.jchromb.2021.122978>
- Segev, A., Badani, H., Kapulnik, Y., Shomer, I., Oren-Shamir, M., Galili, S. (2010). Determination of polyphenols, flavonoids, and antioxidant capacity in colored chickpea (*Cicer arietinum* L.). *J Food Sci*, 75(2), 115-9. <https://doi.org/10.1111/j.1750-3841.2009.01477.x>
- Shem-Tov, Y., Badani, H., Segev, A., Hedvat, I., Galili, S., Hovav, R. (2011). Determination of total polyphenol, flavonoid and anthocyanin contents and antioxidant capacities of skins from peanut (*Arachis Hypogaea*) lines with different skin colors. *Journal of Food Biochemistry*, 36 (3), 301-308. <https://doi.org/10.1111/j.1745-4514.2011.00539.x>
- Tian, L., Zhang, S., Yi, J., ... & McClements, D. J. (2022). Factors impacting the antioxidant/prooxidant activity of tea polyphenols on lipids and proteins in oil-in-water emulsions. *Lebensmittel-Wissenschaft & Technologie*, 156 (1), 113024. <https://doi.org/10.1016/j.lwt.2021.113024>
- Umar, A., Khan, M. T., Bukhari, S., Kiani, R. K. (2022). Antioxidant activity and total phenolic contents of dried and germinated legumes. *researchSquare*, 1, 1-13. <https://doi.org/10.21203/rs.3.rs-1239490/v1>
- Xu, B., Chang, S. (2008). Effect of soaking, boiling, and steaming on total phenolic content and antioxidant activities of cool season food legumes. *Food Chemistry*, 110 (7), 1-13. <https://doi.org/10.1016/j.foodchem.2008.01.045>
- Xu, B., Yuan, H. S., Chang, K. C. S. (2007). Comparative analyses of phenolic composition, antioxidant capacity, and color of cool season legumes and other selected food legumes. *Journal of Food Science*, 72 (2), 167-177. <https://doi.org/10.1111/j.1750-3841.2006.00261.x>