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TOTAL POLYPHENOL CONTENT AND ANTIOXIDANT CAPACITY CHANGES IN DEPENDENCE ON CHOSEN GARDEN PEA VARIETIES

Alžbeta Hegedűsová, Ivana Mezeyová, Mária Timoracká, Miroslav Šlosár, Janette Musilová, Tünde Juríková

ABSTRACT

The green pea is ranged between the crops with high antioxidant potential. This potential is connected with phytochemical components mainly with polyphenols. All these bioactive chemicals have disease-fighting properties. In real human diet there is no usually possibility of fresh garden pea consumption during the whole year. The total polyphenol content is significantly changed among other things by processing methods. Focus on variety, bio-fortification and other specific agricultural inputs, could be the right method of total polyphenol contents and total antioxidant capacity increasing. The main objective of the present work was to consider the changes of total polyphenols content in dependence on variety and to evaluate an antioxidant potential six garden pea varieties arranged by the ripening point of view. Variety ´Exzeleus´ belongs to very early type, ´Premium´ is early maturing, ´Flavora´ is middle ripening variety and the last three varieties ´Utrio´, ´Jumbo´ and ´Ambassador´ are middle late types of varieties. Every variety was grown in four replications, i.e. on 24 m^2 total plot in Botanical garden of Slovak University of Agriculture in Nitra during 2013. Total polyphenols were determined by the Lachman´s method and expressed as mg of gallic acid equivalent per kg fresh mater. Total antioxidant capacity was measured by the Brand-Williams method using a compound DPPH (2.2-diphenyl-1-pikrylhydrazyl)). The highest value was reached in case of variety ´Jumbo´ 1179.995 ±28.081 mg/kg, the lowest value in case of ´Premium´ 674.505 ±26.541 mg/kg. When evaluating an antioxidant capacity in chosen varieties of garden pea, the interval estimated by our trail ranged from 0.523 ±0.206% (´Exzeleus´) to 6.844 ±0.591% (´Flavora´). Following the both observed parameters, TPC and TAC, variety ´Flavora´ (as a member of middle ripening varieties) seems to be the most optimal from the human nutrition point of view. The various varieties had significant influence on TPC and TAC according to used statistical analyzes. Within the all observed varieties, when they were arranged by ripening, there was estimated significant difference only in case of garden pea varieties ´early – middle late´. Other couples didn´t show any statistical important differences in total polyphenol content.

Keywords: garden pea; total polyphenols; antioxidant capacity

INTRODUCTION

Green peas (*Pisum sativum* L.) as a good source of vegetable protein, vitamins, fiber and micronutrients belong to Pea family – Fabaceae (or Leguminosae). The legumes are rich source for lysine and tryptophan but low in sulphur-containing amino acids, methionine and cysteine. Plant proteins are cheaper than the animal proteins; therefore, the people consume legume seeds worldwide as major source of protein **Petchiammal et al., (2014)**. According to increasing scientific studies the green pea is ranged between the crops with high antioxidant potential. This potential is connected with phytochemical components mainly with polyphenols. Molecular structure of phenolic contributes to the antioxidant activity. Polyphenols are known to exhibit stronger antioxidant activity than monophenols **Troszynska et al., (2002)**. The main factor responsible for the delayed research on polyphenols is the variety and the complexity of their chemical structure **D'Archivio et al., (2007)**. Furthermore, prooxidant effects of polyphenols have been described **Elbling et al., (2005**), having opposite effects on basic cell physiological processes: if as antioxidants they improve cell survival, as pro-oxidant they may induce apoptosis and block cell proliferation **Lambert et al., (2005)**. Among micronutrients, peas have high contents of ascorbic acid, β-carotene, thiamine and riboflavin and, compared to other vegetables, peas are rich in iron **Nilson et al., (2004)**. Field peas are a potential dietary source of Fe, however true animal/human Fe bioavailability studies are required **Amarakoon et al., (2014)**. One of the the great aptitude of peas is ability to accumulate Se in the grain, which shows a great potential to be used as a "functional food" in Se biofortification programs **Poblaciones et al., (2013)** An increase of Se levels by fertilization translated into a much greater Se accumulation in the pea grain than in other cereals such as breadmaking wheat **Broadley et al., (2010)**.

All these bioactive chemicals have disease-fighting properties. Consumption of foods rich in antioxidant polyphenols is significantly associated with reduced risk of various non-communicable human diseases, including diabetes **(Talukdar, 2013)**. Epidemiological studies and

associated meta-analyses strongly suggest that long term consumption of diets rich in plant polyphenols offer protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases **(Pandey and Rizvi, 2009)**. Important biological activities have now been suggested for these bioactive compounds like enhancement of the antioxidant, antimutagenic, anticarcinogenic and anti-hyperglycemic effects, which makes pulses an important crop for human health **(Singh and Basu, 2012)**. Legumes in generally can be considered as a therapeutic functional foods due to their significant content of functional proteins and carbohydrates and their extraordinary reserve of secondary metabolites and bioactive constituents that are beneficial for managing and preventing several chronic illnesses in humans **Fratianni et al. (2014)**. The benefit of these plants is related to the secondary metabolites that are produced by the plants even though plants produced these secondary metabolites for the benefits of the plant itself as defense against infection and injury, but it was found that the secondary metabolites have benefits to the human health and curing human diseases **(Mushtaq and Wani, 2013)**. The antioxidant activity of plant polyphenols can retard the development of most major age-related degenerative diseases such as cancers, diabetes, cardiovascular disease, and neurodegenerative diseases **(Lee, 2013; Seo et al., 2012)**. On the other hand, because of their sweet taste and starchy texture full of health-protective phytonutrients the pea belongs between the first vegetables, which are used for baby nourishment.

The content of mentioned bioactive compounds is significantly changed among other things by processing methods. The raw seeds of pea are the most potent antioxidant suppliers and free radical scavengers **Nithiyanantham et al., (2012)**. In real human diet there is no usually possibility of fresh garden pea consumption during the whole year. Most of time the pea is cooked, canned, dried or microwaved before it is consumed. These processes lead to changes in physical characteristics and chemical composition of pea. According to **Turkmen et al., (2005)** there was noticed small decrease of total antioxidant activity and total polyphenols content after boiling, steaming and microwaving of pea in comparison with fresh mass. As well as in case of **(Xu and Chang, 2008)** various steps of processing resulted in significant decrease in total phenolic content (TPC) and DPPH free radical scavenging content increasing. Focus on variety, bio-fortification and other specific agricultural inputs, could be the right method of total polyphenol contents increasing.

The main objective of the present work was to consider the changes of total polyphenols content in dependence on variety and to evaluate an antioxidant potential in chosen varieties of garden pea.

MATERIAL AND METHODOLOGY

For small area field trials there were chosen six garden pea varieties from the ripening point of view. Variety ´Exzeleus´ belongs to very early type, ´Premium´ is early maturing, ´Flavora´ is middle ripening variety and the last three varieties ´Utrio´, ´Jumbo´ and ´Ambassador´ are middle late types of varieties. Place of one trial plot was 1 m^2. Every variety was grown in four replications, i.e. on 24 m^2 total plot in Botanical garden of Slovak University of Agriculture in Nitra during 2013. Agrochemical characteristic of soil substrate are figured in table 1. Harvest of pea garden yield was made in phenological stage ´technological ripeness´ and it was dried by lyophilization.

Total polyphenol content estimation (TPC)

Total polyphenols were determined by the method of **Lachman et al., (2003)** and expressed as mg of gallic acid equivalent per kg fresh mater. Gallic acid is usually used as a standard unit for phenolic content determination because a wide spectrum of phenolic compounds. The total polyphenol content was estimated using Folin-Ciocalteau assay. The Folin-Ciocalteau phenol reagent was added to a volumetric flask containing 100 μL of extract. The content was mixed and 5 mL of a sodium carbonate solution (20%) was added after 3 min. The volume was adjusted to 50 mL by adding of distilled water. After 2 hours, the samples were centrifuged for 10 min and the absorbance was measured at 765 nm of wavelength against blank. The concentration of polyphenols was calculated from a standard curve plotted with known concentration of gallic acid.

Total antioxidant capacity

Total antioxidant capacity was measured by the **Brand-Williams et al., (1995)** method-using a compound DPPH (2.2-diphenyl-1-pikrylhydrazyl)).

The 2.2-diphenyl-1- pikrylhydrazyl ($DPPH^{\cdot}$) was pipetted to cuvette (3.9 m^3), then the value of absorbance, which corresponded to the initial concentration of $DPPH^{\cdot}$ solution in time Ao was written. Then 0.1 cm^3 of the followed solution was added and then 2. was immediately started to measure 1. the dependence A = f(t). The solution in the cuvette 2. was mixed and measured 1. the absorbance of 1, 5 and 10 minutes at 515.6 nm in the spectrophotometer Shimadzu UV/VIS - 1240. The percentage of inhibition reflects how antioxidant

Table 1 Agrochemical characteristic of soil substrate in mg/kg.

Agrochemical characteristic	pH (H_2O)	pH (KCl)	Cox (%)	Hum. (%)	
	7.55	6.36	1.39	4.01	
Nutrients	Nan	K	Ca	Mg	P
	13.3	285.0	5630.0	364.0	252.5

compound are able to remove DPPH˙ radical at the given time.
Inhibition (%) = (Ao - At / Ao) x 100

Statistical analyzes

The analysis of variance (ANOVA), the multifactor analysis of variance (MANOVA) and the multiple Range test were done using the Statgraphic Centurion XV (StatPoint Inc. USA).

RESULTS AND DISCUSSION

On the base of reached results there were estimated changes in the total polyphenols content and also changes in total antioxidant capacity values in dependence on chosen pea varieties from the consumer point of view. The results of submitted task were compared and evaluated with scientific knowledge of foreign and home authors.

1 Evaluation of total polyphenol content and values of antioxidant capacity in chosen pea varieties in field conditions

Following the total polyphenol content parameter (as it is figured in table 2), the highest value was reached in case of ´Jumbo´ variety with 1179.995 ±28.081 mg/kg. When comparing this variety from total antioxidant capacity point of view, there was estimated value 1.707 ±0.291%. This is on the lower level of observed antioxidant capacity interval. The maximum value in total antioxidant capacity was measured in case of ´Flavora´ variety, with 6.844 ±0.591%. On the other hand, ´Flavora´ reached 849.717 ±16.310 mg/kg in total polyphenol content, which is on the third place behind the variety ´Premium´ (674.505 ±26.541 mg/kg) with the lowest polyphenol content and ´Ambassador´ with the value 791.572 ±3.493 mg/kg. The results are similar as in case **Han et al., (2007**) where total phenolic content in case of green pea was estimated to 1200 mg/kg. According to mentioned authors, the total phenolic content (PC) was 12 mg/g in lentils, 2.2 mg/g in chickpeas, 2.3 mg/g) in soybeans, 2.5 mg/g in yellow peas and 1.2 mg/g) in green peas. The total phenolic content according to various sources was ranged. The differences in their TPC could be due to the ecotype, the geographical region where they grow and different extraction conditions. When the concentrations of the phenolic compounds was expressed as mg equivalents of gallic acid/g of sample (dry matter basis), as it was in our case, the values of the garden pea occurred between the lentils (variety ´San Gerardo´ with 1098 ±0.87 µg/g ±SD and ´Colliano´ with 1594 ±1.97 µg/g ±SD) and chickpea (´Sassano 147 ±0.11µg/g ±SD, ´Castelcivita´ 183 ±0.02 µg/g ±SD) **Fratianni et al. (2014)**.

When comparing legumes from the TAC point of view, according to **Petchiammal et al., (2014)** horse gram (brown and black), cowpea (brown), common bean and masur (black) showed high protein content and also exhibited good DPPH scavenging activity, ferric reducing and reducing power activity. Comparatively, pea (white and green) and chick pea (white, green, brown) showed less antioxidant capacity.

The total polyphenols content as concentrated on the ripening according to figure 1, the highest value was estimated in case of ´Jumbo´ variety, which is middle late type of variety. There is also ´Utrio´ and ´Ambassador´, which are middle late ripening, but variety ´Utrio´ reached the total polyphenol content value 876.337 mg/kg and Ambassador 791.572 mg/kg.

From the antioxidant capacity point of view, the highest value was reached in case of middle type variety ´Flavora´ with the value 6,844 %. The lowest value was found in case of very early type variety – ´Exzeleus´ with the 0,523%. In comparison with results of **Kavalcova et al., (2014)**, where the interval of statistically significant highest value of antioxidant activity was recorded in onion (from 20.22 ±0.53 to 25.76 ±0.53) and statistically significant the lowest value of antioxidant activity was recorded in garlic (from 4.05 ±0.20 to 5.07 ±0.47), our results in peas are lower, except of the variety ´Flavora´, which is comparable with the values of garlic **Halvorsen et al., (2002)** also observed that TAC in peas was relatively low among legumes and vegetables.

2 Analyses of interactions between observed features in chosen pea varieties

2.1 Statistical evaluation of total polyphenol content differences significance within the frame of chosen varieties

When comparing all pea varieties, following the Table 3, there were significant differences according to used statistical methods on the all three types observed confidence levels between almost the all observed varieties. Only in case of couples ´Exzeleus´ vs. ´Utrio´, ´Exzeleus´ vs. ´Flavora´ and ´Utrio´ vs. ´Flavora´ there were not found any.

Table 2 Total phenolic content and antioxidant capacity (average and average deviation values) in chosen pea varieties after harvest in dray mass (mg/kg DM*) .

Varieties (ripening)	TPC (mg/kg)*	TAC (%)*
´Exzeleus´ (very early)	861.454 ±11.653	0.523 ±0.206
´Premium´ (early)	674.505 ±26.541	1.797 ±0.329
´Flavora´ (middle)	849.717 ±16.310	6.844 ±0.591
´Ambassador´ (middle late)	791.572 ±3.493	1.908 ±0.412
´Utriuo´ (middle late)	876.337 ±16.164	2.130 ±0.435
´Jumbo´ (middle late)	1179.995 ±28.081	1.707 ±0.291

(**TPC –** total polyphenols content (mg/kg), **TAC** – total antioxidant capacity (%), **DM** – dry mass)

Within the all observed varieties, when they were arranged by ripening, there was estimated significant difference only in case of garden pea varieties ´early – middle late´. Other couples didn´t show any statistical important differences in total polyphenol content.

Following to **Amarakoon et al., (2014**) significant genotypic and environmental variation was not observed (p >0.05) with respect to concentrations of phenolic in field pea. Similar, according the results of **Timoracka et al., (2010)** the differences of flavonoid contents in individual pea varieties were not significant.

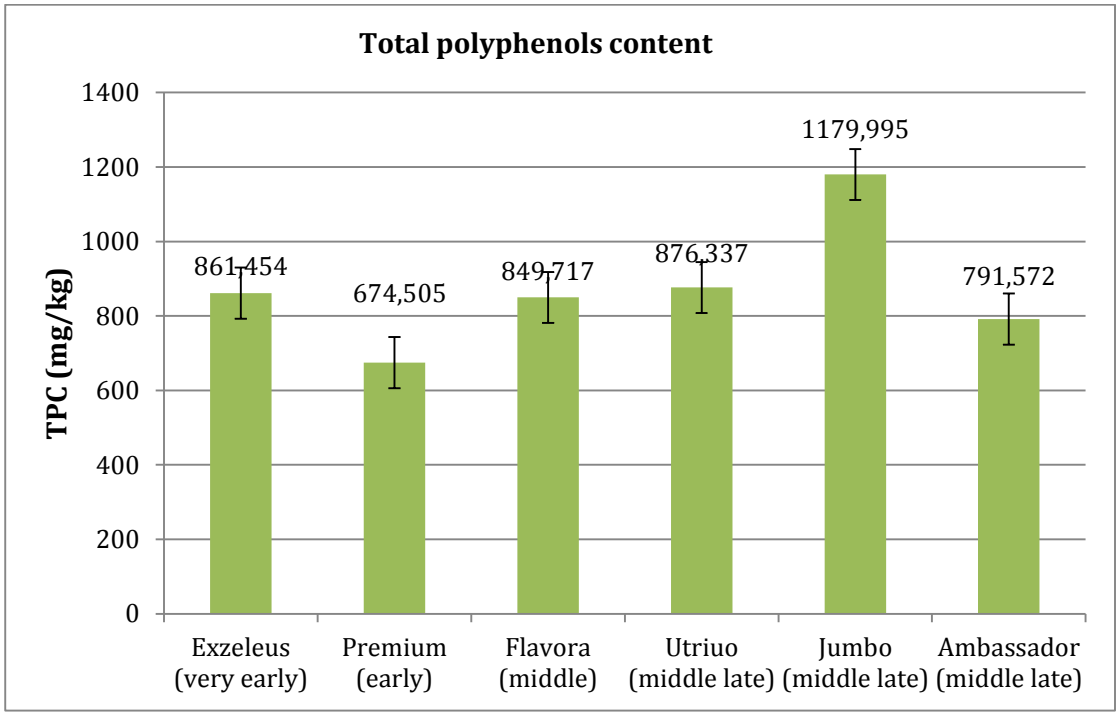

Figure 1 Average content of total polyphenols TPC (mg/kg) in chosen pea varieties arranged by ripening.

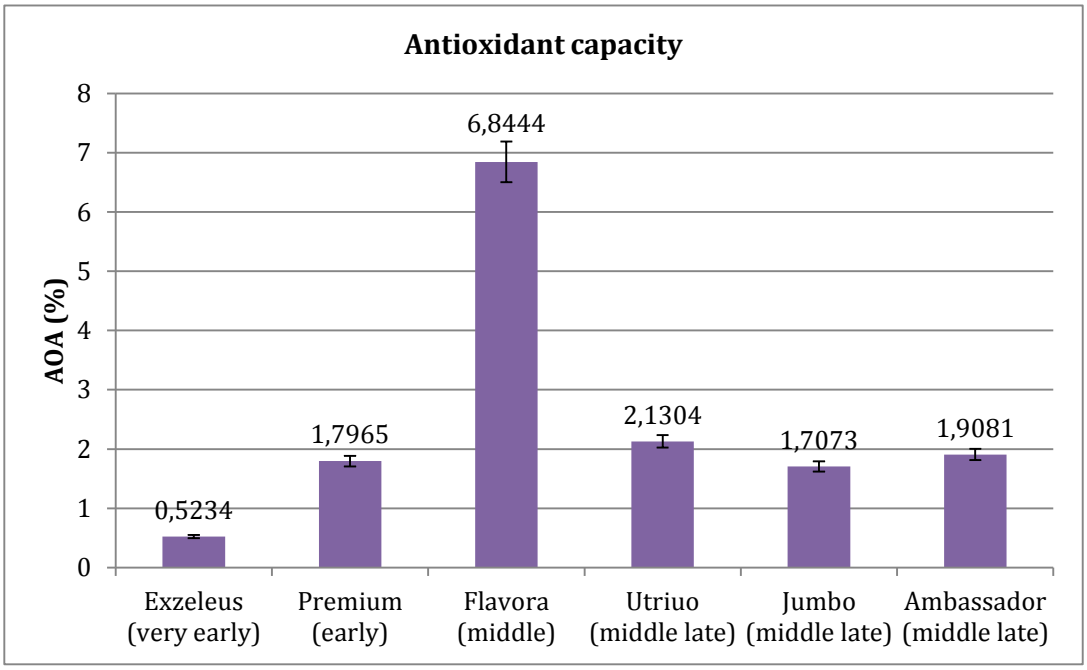

Figure 2 Average content of total antioxidant capacity TAC (%) in chosen pea varieties arranged by ripening.

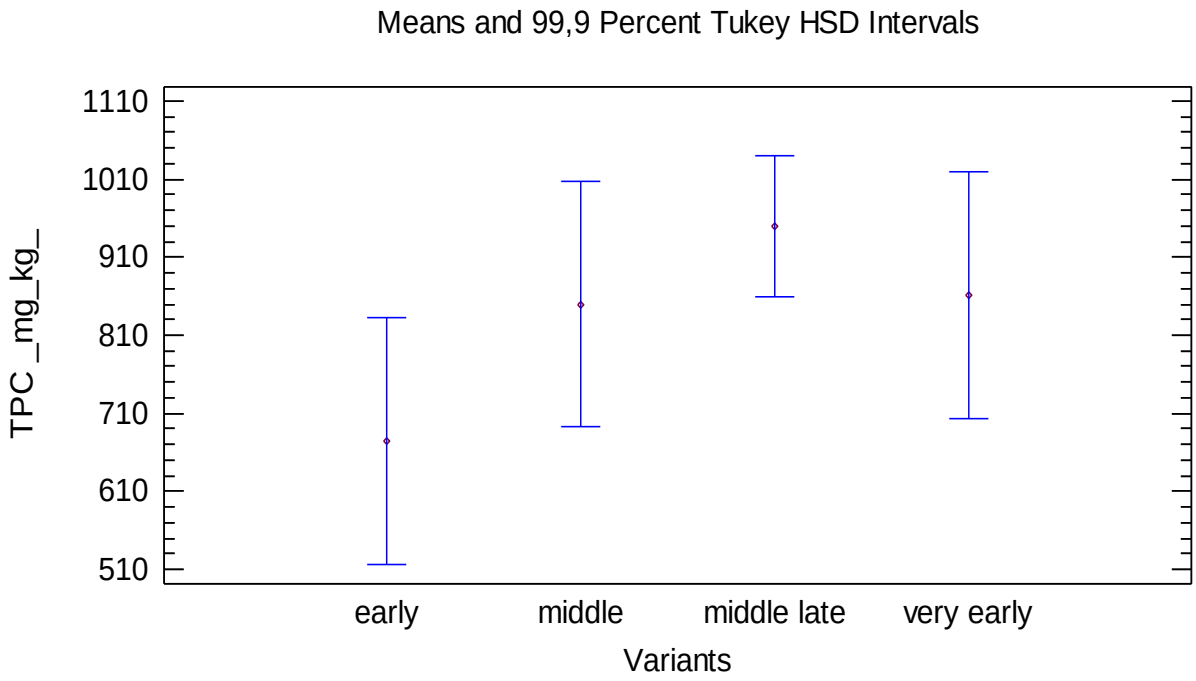

Figure 3 Dependence of total polyphenol content (TPC) on pea variety ripening.

Table 3 Statistically significant differences in total polyphenols content (TPC) of chosen pea varieties by Tukey HSD in ANOVA (Statgraphic).

Variety (mg/kg)	´Premium´(P)	´Exzeleus´(E)	´Ambassador´ (A)	´Utrio´ (U)	´Flavora´ (F)	´Jumbo´ (J)
P	–	±186.949***	±117.067***	±160.884***	±175.212***	±505.49***
E	±186.949***	–	±698816**	±148837	±117367	±318541***
A	±117.067***	±69.8816**	–	±84.7653***	±58.1448*	±388.423***
U	±160.884***	±14.8837	±84.7653***	–	±26.6204	±303.658***
F	±175.212***	±11.7367	±58.1448*	±26.6204	–	±330.278***
J	±505.49***	±318.541***	±388.423***	±303.658***	±330.278***	–

p <0.05 (*): statistically significant (at 95.0 % confidence level)
p <0.01 (**): high statistically significant (at 99.5 % confidence level)
p <0.001 (***): very high statistically significant (at 99.9% confidence level)

2.2 Statistical evaluation of antioxidant capacity differences significance within the frame of chosen varieties

Very high statistically significant differences (at 99.9% confidence level) were found in case of variety ´Flavora´ vs. all observed varieties as it is figured in table 4. Also in case of cople ´Exzeleus´ vs. ´Utrio´ was determined very high statistically significant differences.

´Flavora´ belongs to middle type of pea variety and this group obviously reached the highest value according the figure 4, where the varieties are arranged by ripening and statistically compared. According to Tukey test at 99.9% confidence level, there were found statistically significant differences in all compared couples except of ´early - middle late´ and ´early - very early´ which are not statistically different from the total antioxidant capacity point of view.

Following the both observed parameters, TPC and TAC, variety ´Flavora´ (as a member of middle ripening varieties) seems to be the most optimal from the human nutrition point of view.

Table 4 *Statistically significant differences at the 99.9% confidence level by Tukey HSD in ANOVA (Statgraphic) of antioxidant capacity (TAC %) in chosen varieties of peas* **.**

Variety (%)	´Premium´(P)	´Exzeleus´(E)	´Ambassador´ (A)	´Utrio´ (U)	´Flavora´ (F)	´Jumbo´ (J)
P	–	±1.273*	±0.112	±0.334	±5.048 ***	±0.089
E	±1.273*	–	±1.385 **	±1.607***	±6.321 ***	±1.184 *
A	±0.112	±1.385 **	–	±0.222	±4.936 ***	±0.201
U	±0.334	±1.607***	±0.222	–	±4.714 ***	±0.423
F	±5.048 ***	±6.321 ***	±4.936 ***	±4.714 ***	–	±5.137***
J	±0.089	±1.184 *	±0.201	±0.423	±5.137***	–

p <0.05 (*): statistically significant (at 95.0 % confidence level)
p <0.01 (**): high statistically significant (at 99.5 % confidence level)
p <0.001 (***): very high statistically significant (at 99.9% confidence level)

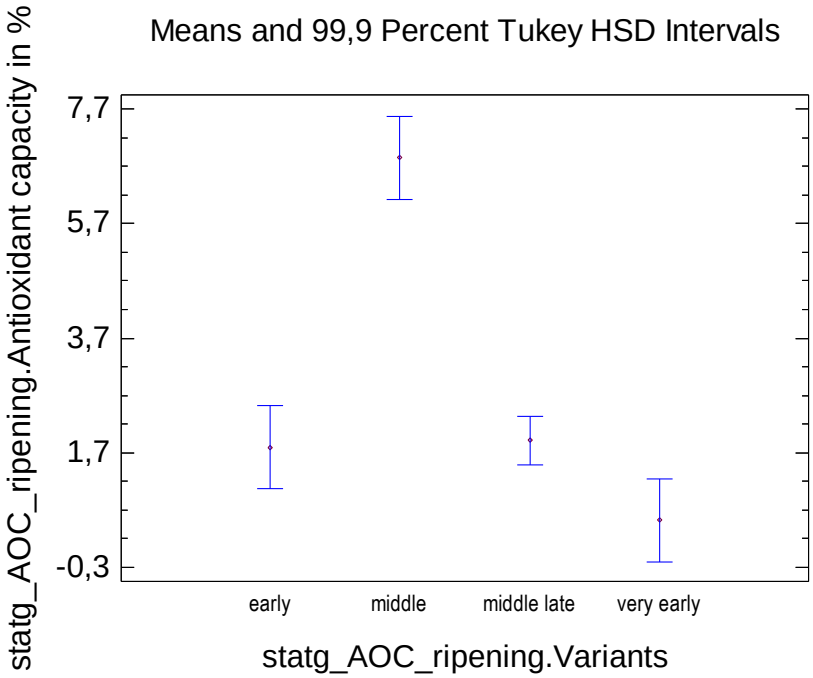

Figure 4 Dependence of total antioxidant capacity (TAC) on pea variety ripening.

CONCLUSION

The changes of total polyphenols content in dependence on chosen garden pea varieties were estimated in the article. Within the all observed varieties, when they were arranged by ripening, there was estimated significant difference in case of garden pea varieties ´early – middle late´. From dependence of total antioxidant capacity (TAC) on pea variety ripening, there were found statistically significant differences in all compared couples except of ´early – middle late´ and ´early – very early. The various varieties had significant influence on TPC and TAC according to used statistical analyzes, that is why there is strong recommendation of multi-annual results estimation according to submitted methodology.

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Contact address:

prof. RNDr. Alžbeta Hegedüsová, PhD., Slovak University of Agriculture in Nitra, Horticulture and Landscape Engineering Faculty, Department of Vegetable Production, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: alzbeta.hegedusova@uniag.sk.

Ing. Ivana Mezeyová, PhD., Slovak University of Agriculture in Nitra, Horticulture and Landscape Engineering Faculty, Department of Vegetable Production,

Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic, E-mail: ivana.mezeyova@uniag.sk.

Ing. Mária Timoracká, PhD., Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, Faculty of Biotechnology and Food Sciences, Department of Chemistry, 949 76 Nitra, Slovakia, maria.timoracka@uniag.sk.

Ing. Miroslav Šlosár, PhD., Slovak University of Agriculture in Nitra, Horticulture and Landscape Engineering Faculty, Department of Vegetable Production, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic, E-mail: miroslav.slosar@uniag.sk.

doc. Ing. Janette Musilová, PhD., Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, Faculty of Biotechnology and Food Sciences, Department of Chemistry, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: janette.musilova@uniag.sk.

doc. RNDr. Tünde Juríková, PhD., Constantine the Philosopher University in Nitra, Faculty of Central European Studies, Institute for Teacher Training, Nabrezie mladeze 91, 949 76 Nitra, Slovakia, E-mail: tjurikova@ukf.sk.