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Dynamics of polyphenolics formation in different plant parts and different growth phases of selected buckwheat cultivars

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ABSTRACT

The changes of dynamics of total polyphenolics formation in various anatomical parts (stems, leaves, flowers and seeds) of common buckwheat (*Fagopyrum esculentum* Moench.) during vegetation period were surveyed. Six cultivars were analysed: Pyra, Spacinska, Emka, Kasho, Jana C1 and Hrusowska. The content of total polyphenolics was evaluated in growth phase I. (formation of buds), in phase II. (at the beginning of flowering), in phase III. (full blossoming) and in phase IV. (full ripeness). The total polyphenolics content was assessed by using Folin-Ciocalteau assay spectrophotometrically on Shimadzu UV-1800. When evaluating all four anatomical parts of common buckwheat we can state that the flowers contained the highest concentration of total polyphenolics where the values were in range from 14.93 to 25.16 $mg.g^{-1}$. In last phase (IV. phase) where stems, leaves, and seeds were evaluated, buckwheat leaves exerted the highest content of these compounds, and the values were in range from 68.74 to 90.27 $mg.g^{-1}$. Maximal increase of total phenolics content was manifested in each variety in phase IV., i.e. at the end of the vegetation period. From the standpoint of the content of total polyphenolics in individual anatomical parts the cultivars Pyra, Hrusowska and Emka were the most suitable ones as functional foods.

Key words: total polyphenolics, buckwheat, anatomic parts

IZVLEČEK

RAZLIKE PRI NASTAJANJU POLIFENOLOV V DELIH RASTLIN IN FAZAH RASTI PRI IZBRANIH KULTIVARJIH AJDE

Proučevane so razlike v vsebnosti skupnih polifenolov v delih rastlin (stebla, listi, cvetovi in semena) pri navadni ajdi (*Fagopyrum esculentum* Moench.) med rastjo. Proučevanih je bilo šest kultivarjev: Pyra, Spacinska, Emka, Kasho, Jana C1 in Hrusowska. Koncentracija skupnih polifenolov je bila raziskana v rastni fazi I. (formiranje socvetij), v fazi II. (začetek cvetenja), v fazi III. (polno cvetenje) in v fazi IV. (polna zrelost). Koncentracija skupnih polifenolov je bila analizirana spektrofotometrično z uporabo reagenta Folin-Ciocalteau na aparaturi Shimadzu UV-1800. Najvišja koncentracija polifenolov je bila v cvetovih (14,93 do 25,16 $mg.g^{-1}$). V zrelosti (IV. faza) je bilo največ polifenolov v listih (68,74 do 90,27 $mg.g^{-1}$). Vsebnost polifenolov se je pri vseh kultivarjih povečala v fazi IV., to je na koncu rasti. Glede na vsebnost skupnih polifenolov v posameznih delih rastlin so kultivarji Pyra, Hrusowska in Emka najbolj primerni za uporabi pri pripravi funkcijskih živil.

Ključne besede: skupni polifenoli, ajda, deli rastlin

1 INTRODUCTION

Buckwheat (*Fagopyrum esculentum* Moench.) has been traditionally used as a human food source in the world. This crop was very popular in 17th-19th centuries in western countries, but later in 20th century was substituted by wheat (Cawoy et al., 2009). At present times buckwheat is precious crop throughout the world with regard to recently found nutritive traits. It has been the most versatile functional food of 21st century (Zhang and Chen, 2004) not only as the food or forage, but also as medicine and melliferous plant (Bonafaccia et al., 2003; Christa and Soral-Smietana, 2008; Tang et al., 2009). Nutritional studies have demonstrated potential

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benefits of buckwheat (Kreft et al., 1996). Many of health benefits of buckwheat have been attributed to its high level of polyphenol compounds, which exhibit antioxidant activity (Wijngaard and Arendt, 2006). Polyphenol compounds have been extensively researched in the last decade for health promoting properties such as their role in the prevention of degenerative diseases, which include cancer and cardiovascular diseases (Scalbert et al., 2005). Besides, many polyphenol compounds stimulate the immune system and slow aging processes (Chuah et al., 2008; Podsedek, 2007).

Buckwheat is rich in vitamins, especially those of the B group (Fabjan et al., 2003), and is also an important source of trace elements (Zn, Mn, Cu, Se) as well as macroelements (K, Na, Ca, Mg) (Stibilj et al., 2004). Buckwheat is a potentially important source of rutin, a natural flavonoid with antihyperglycemic, antihypertensive and antioxidative properties (Lee et al., 2007), too. Buckwheat is considered as important functional food (Kreft et al., 2006). Its leaves and young parts of the plant are consumed in some countries as a vegetable, that's why the dynamics polyphenol compounds formation in different growth phases and various anatomic parts was the objective of our work.

2 MATERIALS AND METHODS

Materials:
Six cultivars of common buckwheat (*Fagopyrum esculentum* Moench.) Pyra (P), Spacinska (S), Emka (E), Kasho (K), Jana C1 (J) and Hrusowska (H) were obtained from Plant Production Research Centre in Piešťany.

The content of total polyphenolics was evaluated in growth phase I. (formation of buds), in phase II. (at the beginning of flowering), in phase III. (full flowering) and in phase IV. (full ripeness).

Determination of total polyphenols content (TPC)
Folin-Ciocalteau reagent and gallic acid were purchased from Merck (Germany). Sodium carbonate, methanol, ethanol were obtained from Sigma (USA) and 2,2-diphenyl-1-picrylhydrazyl radical from Organics (USA).

The total polyphenol content was assessed by the method used by Lachman et al. (2003) employing the reduction of a phosphowolframate-phosphomolybdate complex to blue products by phenolic compounds. Briefly, an aliquot of the extract, blank or standard was placed in a 50 mL flask, where the Folin-Ciocalteu assay (2.5 mL) was added and the mixture was allowed to react for 3 minutes under continuous stirring before a solution of sodium carbonate (7.5 mL) was added and mixed thoroughly. The volume was then made up to 50 mL with distilled water and left standing at room temperature for 2 h. The absorbance was measured at 765 nm using Shimadzu UV-1800 spectrophotometer (Japan). Results were expressed as mg gallic acid equivalents (GAE) per kg fresh weight (FW).

Statistical analysis
Statistical processing of the results was carried out in program Statistica 6.0, where the significance of differences between monitored parameters in individual anatomical parts of common buckwheat plants with the T- test at $\alpha < 0.05$ were evaluated.

3 RESULTS

The rate of formation of polyphenol compounds during vegetation (4 taking of samples) in individual anatomic parts of common buckwheat (stems, leaves, flowers and seeds) were observed. In the phase I. (formation of buds) stems and leaves were analysed. When evaluating total content of polyphenolic compounds (TPC), leaves of each cultivar contained significantly higher amount of polyphenol compounds. In leaves of variety Pyra there was recorded 7.7-fold higher concentration of (TPC) in comparison to stems, namely 9.33 $mg.g^{-1}$.

In the phase II. also flowers were analysed. The highest concentration of TPC was found in flowers of each variety, followed by the leaves and the lowest level of polyphenol compounds was recorded in stems of common buckwheat. In flowers there was recorded in average 10-times higher concentration than in stems and the highest concentration of (TPC) was recorded again in variety Pyra (18.05 $mg.g^{-1}$). In the phase III. all anatomical parts (stems, leaves, flowers and seeds) were analysed. In this phase, on the basis of obtained results we could state that the total polyphenols contents in individual anatomic parts of common buckwheat were in followed order: flowers > leaves > seeds > stems. In leaves there were 10-11.5-fold higher values than in stems and the highest value was recorded in variety Pyra (22.83 $mg.g^{-1}$). All values were 14-fold higher in flowers in the phase III. when compared to tested stems and the variety Hruszowska was the richest one in total polyphenol compounds (25.16 $mg.g^{-1}$). The content of TPC in the seeds was recorded from 7.36 $mg.g^{-1}$ (Emka) to 15.48 $mg.g^{-1}$ (Pyra). In the last phase the stems, leaves and seeds were analysed. There were in average 26.7-fold higher concentrations of TPC in leaves in comparison to stems and the highest concentration was recorded again in variety Pyra a presented 90.27 $mg.g^{-1}$. On the basis of TPC we can state that anatomic parts in this phase had decreasing tendency as following: leaves >seeds > stems.

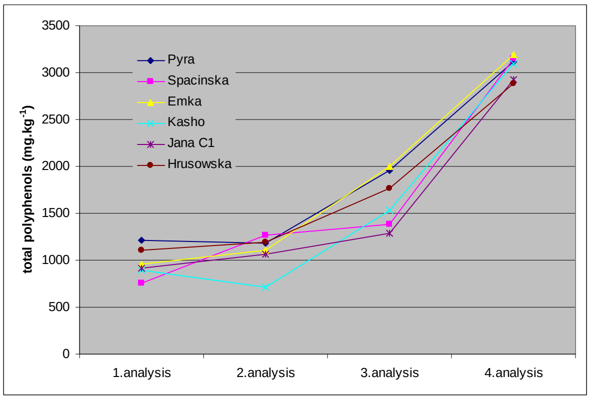

Figure 1. Content of total polyphenolics in stems

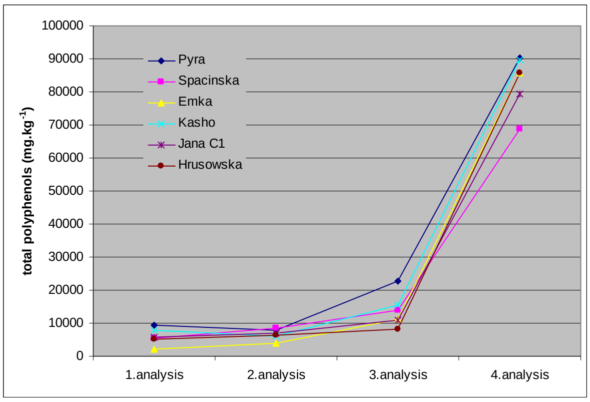

Figure 2. Content of total polyphenolics in leaves

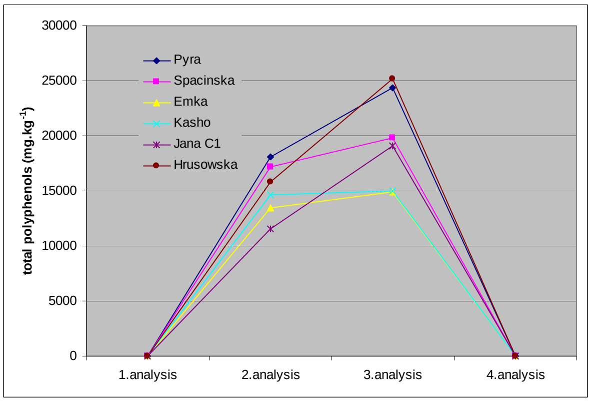

Figure 3. Content of total polyphenolics in flowers

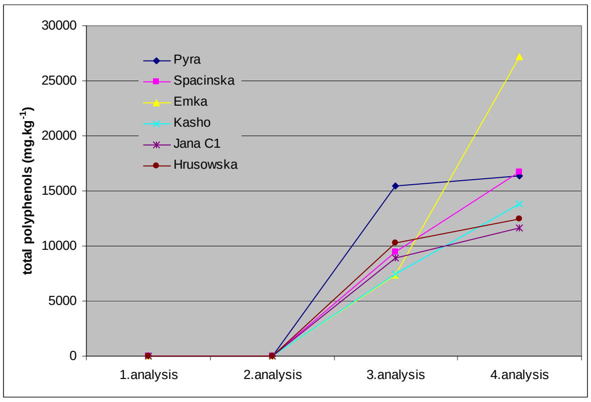

Figure 4. Content of total polyphenolics in seeds

When monitoring of the dynamics of polyphenol compound formation, there was maximal increase of TPC in stems of each variety in phase IV. and the highest increase (by 233 % in comparison to phase I) was observed in variety Spacinska. Similarly also in leaves of common buckwheat there was the highest increase of TPC in IV. phase and in variety Emka there was even 36-fold increase in comparison to phase I. When monitoring the dynamics of formation of TPC in flowers there was maximum increase in III. phase. This increase was in range from 15.2 to 65.7 % in individual cultivars in comparison to TPC in phase I. Also the seeds of common buckwheat are very important for the consumption, and thus they were investigated in phases III. and IV. The increase of TPC was recorded in IV. phase and the highest increase was in variety Emka that presented even 268 % increase in comparison to phase III. Statistical evaluation of obtained results are presented in Table 1.

Table1. Statistical evaluation of differences of total polyphenolics content in observed cultivars

			PYRA						SPACINSKA						EMKA						KASHO						JANA C1						HRUSOWSKA					
			III. phase				IV. phase		III. phase				IV. fáza		III. phase				IV. phase		III. phase				IV. phase		III. phase				IV. phase		III. phase				IV. phase	
			St	L	F	S	L	S	St	L	F	S	L	S	St	L	F	S	L	S	St	L	F	S	L	S	St	L	F	S	L	S	St	L	F	S	L	S
PYRA	III.phase	St	1						x						0						x						x						x					
		L		1						x						x						x						x						x				
		F			1						x						x						x						x						0			
		S				1						0						x						x						x						0		
	IV.phase	L					1						x						0						0						x						x	
		S						1						0						x						x						x						0
SPACINSKA	III.phase	St							1						x						x						0						x					
		L								1						x						0						x						x				
		F									1						x						x						0						x			
		S										1						0						0						0						0		
	IV.phase	L											1						x						x						x						x	
		S												1						x						x						x						0
EMKA	III.phase	St													1						x						x						x					
		L														1						x						x						x				
		F															1						0						x						x			
		S																1						0						x						0		
	IV.phase	L																	1						0						0						0	
		S																		1						x						x						0
KASHO	III.phase	St																			1						x						x					
		L																				1						x						x				
		F																					1						x						x			
		S																						1						x						0		
	IV.phase	L																							1						x						0	
		S																								1						x						0
JANA C1	III.phase	St																									1						x					
		L																										1						x				
		F																											1						x			
		S																												1						0		
	IV.phase	L																													1						x	
		S																														1						0
HRUSOWSKA	III.phase	St																															1					
		L																																1				
		F																																	1			
		S																																		1		
	IV.phase	L																																			1	
		S																																				1

St-stems, L-leaves, F-flowers. S-seeds, x- statistically significant differences, 0-statistically non significant differences

4 DISCUSSION AND CONCLUSION

The present report is a based on the evidence that polyphenols are the most abundant antioxidants in the diet. Experimental studies on animals or cultured human cell lines support a role of polyphenols from plants in the prevention of cardiovascular diseases (Truswell, 2002).

Buckwheat is one of the best grain sources of polyphenol compounds (Gallardo et al., 2006), where main polyphenols found are glycosides of the flavonol quercetin followed by glycosides of the flavones apigenin and luteolin (Dietrych-Szostak and Oleszek, 1999). Our results are in consistency with results of other authors (Kreft et al., 2006; Kalinova et al., 2006) who also referred about higher content of polyphenol compounds in flowers and leaves than in other anatomic parts of common buckwheat. Also Golisz et al. (2007) identified higher amount of phenolic compounds in flowers (9.14 mg. g^{-1}) and in leaves of common buckwheat (8.90 mg. g^{-1}) when compared to stems (2.46 mg .g^{-1}). Formation of polyphenol compounds is affected by various factors as well as variety and agro-technical conditions (Fabjan et al., 2003) and in formation of polyphenol compounds mainly stresses affect plant during vegetation (Gross, 2003).

The changes of polyphenol compounds formation dynamics during vegetation have been the subject of only few authors. Our results suggest an increase of polyphenol compounds content during vegetation in all tested varieties. Quantity and quality polyphenol compounds according to Hamilton et al. (2001) are strongly determined by genetic factors. Our results confirmed statistically significant differences among cultivars in phase I. when evaluating the content of total polyphenolics in stems as well as in leaves. Similarly statistically significant differences were confirmed in the content of total polyphenolics also in the growth phase II. among all cultivars. Different situation was recorded in phases III. and IV., where inter-varietal statistical significance in the content of total phenolics in individual anatomic parts of common buckwheat plants was not always confirmed. Kraus et al. (2003) also refers that the concentration of polyphenol compounds varies with plant phenology and season. With regard on the dynamics of polyphenol compounds, our results are in consistency with Kalinová and Dadáková (2009) who also found lower content of total polyphenolics at the beginning of vegetation period when compared to the end of vegetation. Content of polyphenol compounds depends on developing phase of tested buckwheat. In common buckwheat the rutin content in leaves increased with the age of the plant, with the maximum at the stage of maturity or at the stage of full flowering in case of dry weather conditions after flowering, and in stems the rutin content slightly decreased (Kalinová et al., 2006).

Our results suggest that flowers and leaves were the richest in total polyphenolics content among the analysed anatomic parts of common buckwheat, followed by the seeds and the lowest content of these health beneficial substances was obtained in stems.

When evaluating the dynamics of total polyphenolics formation the maximal increase of their content was observed in each variety in the phase IV., at the end of vegetation period.

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