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Cultivar and growth phases – the factors affecting antioxidant activity of buckwheat (*Fagopyrum esculentum* Moench.)

Janette MUSILOVÁ[1], Jaromír LACHMAN[2], Judita BYSTRICKÁ[3], Alena VOLLMANNOVÁ[4], Iveta ČIČOVÁ[5], Mária TIMORACKÁ[6]

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ABSTRACT

The aim of this study was to assess the influence of cultivar and growth phase on the antioxidant activity (AOA) changes in common buckwheat (*Fagopyrum esculentum* Moench), as well as the its distribution in different plant parts. During 4 growth phases (GP) (buds formation - I, beginning of flowering - II, full flowering - III, full maturity - IV) stems, leaves, flowers, seeds were collected sequentially from 6 buckwheat cultivars – 'Pyra', 'Spacinska', 'Kasho', 'Jana C1', 'Hrusowska', 'Emka'. The highest values of AOA were measured in flowers (GP III) in 'Jana C1' (93.17%) and the lowest value in stems (GP I) in 'Spacinska' (46.09%). The highest increase of AOA was observed in GP IV in stems in 'Pyra'. Differences were compared for statistical significance at the level $P < 0.05$.

Key words: buckwheat, cultivar, growth phase, plant part, antioxidant activity

IZVLEČEK

SORTA IN RAZVOJNE FAZE RASTLINE KOT DEJAVNIKI VPLIVA NA ANTIOKSIDATIVNO AKTIVNOST NAVADNE AJDE (*Fagopyrum esculentum* Moench.)

Namen te raziskave je bil oceniti vpliv sorte in razvojnih faz navadne ajde (*Fagopyrum esculentum* Moench) na antioksidativno aktivnost različnih organov rastline. V štirih razvojnih fazah (GP; tvorba popkov-I, začetek cvetenja-II, polno cvetenje- III, polna zrelost-IV) smo vzorčili stebla, liste, cvetove in semena pri šestih sortah navadne ajde ('Pyra', 'Spacinska', 'Kasho', 'Jana C1', 'Hrusowska', 'Emka'). Največja antioksidativna aktivnost (AOA) je bila izmerjena v cvetovih pri sorti 'Jana C1' (GP III, 93.17 %) in najmanjša v steblih pri sorti 'Spacinska' (GP I; 6.09%). Največje povečanje AOA je bilo izmerjeno v steblih pri sorti 'Pyra' v razvojni fazi GP IV. Statično ovrednotenje razlik je bilo opravljeno na ravni $P < 0.05$.

Ključne besede: navadna ajda, sorta, razvojne faze, organi rastline, antioksidativna aktivnost

[1] Assoc. Prof. Ing., Ph.D., Dept. of Chemistry, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra; Slovak Republic, e-mail: janette.musilova@uniag.sk

[2] Prof., Ph.D, Dept. of Chemistry, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic, e-mail: lachman@af.czu.cz

[3] Assoc. Prof. Ing., Ph.D, Dept. of Chemistry, FBFS, SUA in Nitra; Slovak Republic, e-mail: judita.bystricka@centrum.sk

[4] Prof., RNDr., Ph.D, Dept. of Chemistry, FBFS, SUA in Nitra; Slovak Republic, e-mail: alena.volmannova@uniag.sk

[5] Ing., Ph.D., Plant Production Research Center in Piešťany, Slovak Republic, e-mail: cicova@vurv.sk

[6] Ing., Ph.D., Dept. of Chemistry, FBFS, SUA in Nitra; Slovak Republic, e-mail: maria.timoracka@uniag.sk

1 INTRODUCTION

Buckwheat was one of the basic components of diet of our ancestors. In 17th - 19th century, was very popular in western countries, which was later replaced by wheat (Cawoy et al., 2009). Buckwheat currently serves as an alternative crop, replacing rice or potatoes, and is used as animal feed, pharmaceutical, and honey plant (Holasova et al., 2002; Christa and Soral-Śmietana, 2008; Tang et al., 2009). Agricultural value is attributed mainly to 9 varieties of common buckwheat (*Fagopyrum esculentum* Moench.) which is used more frequently and tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertner) which is grown mainly in the mountain areas.

Almost all parts of buckwheat are the source of many health-benefit components: vitamins, with a balanced amino acid composition, proteins (rich in arginine and lysine), microelements (Cu: 4.29 μg g^{-1}, Mn: 10.20 μg g^{-1}, Fe: 25.14 μg g^{-1}, Zn: 17.89 μg g^{-1}) and macroelements (K, Ca, Mg) (low content of N and high content of K is desirable to reduce the risk of certain diseases of people in developed world. The buckwheat flour contains 12.61% dry matter (DM) of proteins and 1.74% DM of total minerals (Krupa-Kozak et al., 2011). Buckwheat is also the important source of elements and phenolic compounds, which contribute to the antioxidant effect of buckwheat on the human organism.

Phenolic compounds in buckwheat include phenolic acids and flavonoids. In buckwheat, the content of ferulic acid and hydroxycinnamic acid is low. Bran-aleurone fraction of buckwheat contains bound syrignic, *p*-hydroxybenzoic, vanillic and *p*-coumaric acids. Zadernowski et al. (1992) have identified 20 and 14 phenolic acids in buckwheat groats and hulls, respectively. Of these, *p*-coumaric, vanillic, *p*-hydroxybenzoic and caffeic acids are the predominant phenolic acids in groats (4.6, 1.7, 1.7 and 1.3 mg 100 g^{-1} respectively); *p*-coumaric, vanillic, sinapic and gentisic acids are the major phenolic acids in the hulls (3.6, 1.65, 1.4 and 1.1 mg 100g^{-1} respectively) (Shahidi and Naczk, 2004).

A larger proportion of phenolic compounds in buckwheat are flavonoids. Although the flavonoids, in general, possess ideal structure for antioxidant activity, the differences in chemical structures of different flavonoids would affect their antioxidant activities. The synergism among the antioxidants in the mixture made the antioxidant activity, not only dependent on the concentration of antioxidant, but also on the structure and interaction among the antioxidants (Sun and Ho, 2005; Liu et al., 2008). The antioxidant activity of phenolic acids and their esters depends on the number of hydroxyl groups in the molecule; this will be strengthened by steric hindrance. The antioxidant effect of phenolic compounds was declare by authors (Ismail et al., 2004; Prakash et al., 2007; Faller and Fialho, 2009), while the antioxidant activity was found to be significantly correlated to the polyphenolic content, with a correlation coefficient of 0.624 ($P < 0.01$, $n = 17$) (Ikeda et al., 2001).

Although phenolic compounds and some of their derivatives are very efficient in preventing autooxidation, only a few phenolic compounds are currently allowed as food antioxidants. The major considerations for acceptability of such antioxidants are their activity and potential toxicity and /or carcinogenicity. The approved phenolic antioxidants have been extensively studied, but the toxicology of their degradation products still is not clear (Shahidi and Naczk, 2004).

The presented work is a part of a broader topics dealing with polyphenolic compounds with antioxidant effects in selected pseudocereals. One of the aims of which is discussed in this section is to study the influence of buckwheat cultivar on changes in antioxidant activity in different parts of the plant during its growth.

2 MATERIAL AND METHODS

2.1 Plant material

In the experimental work we investigated changes of antioxidant activity:
- in six cultivars of common buckwheat (*Fagopyrum esculentum* Moench.) ('Pyra', 'Spacinska', 'Kasho', 'Jana C1', 'Hrusowska', 'Emka'),
- during the four growth phases (GP I: making of buds, GP II: beginning of flowering, GP III: full flowering, GP IV: full maturity),
- in different parts of the plant (stem, leaf, flower, seed),
where:
- stems and leaves were collected at all four growth phases,
- flowers were collected in GP II and GP III,
- seeds were collected in GP III and GP IV.

The buckwheat cultivars were grown on land Plant Production Research Center in Piešťany.

2.2 Antioxidant activity (AOA)

AOA in different parts of the plant was determined using method based on radical reaction of 2.2-diphenyl-1-picrylhydrazyl (DPPH) according Brand-Williams et al. (1995). Absorbance was measured at 515.5 nm using a Shimadzu spectrophotometer UV-VIS 1800 at 23 °C and % inhibition DPPH indicating how is monitored component able to remove radical DPPH at the time calculated from the formula:

$$\% \text{ inh. DPPH} = [(A_{t0} - A_{t10})/A_{t10}].100;$$

where: A_{t0} - absorbance at time t = 0 min (solution DPPH)
A_{t10} - absorbance at time t = 10 min.

2.3 Statistical analysis

All analysis were run in quadruplicate. In the work the statistical program Statgraphics (multifactorial analysis of variance, LSD-test contrasts, $P < 0.05$) was used.

3 RESULTS AND DISCUSSION

Based on the results obtained from the analysis of six cultivars of buckwheat (*Fagopyrum esculentum* M.) it can be concluded that the AOA in GP I was highest in the stems 'Jana C1' and lowest one in 'Spacinska'. In contrast, in this cultivar the highest AOA in leaves was determined (Tab. 1).

Table 1: Antioxidant activity (%) determined in different parts of buckwheat plant during four growth phases

	'Pyra'				'Spacinska'				'Emka'			
GP	S	L	F	A	S	L	F	A	S	L	F	A
I	54.94	67.11	-	-	46.09	86.97	-	-	62.20	69.53	-	-
II	59.22	83.95	89.55	-	47.50	88.65	88.79	-	65.06	78.26	89.44	-
III	63.30	88.34	91.16	77.37	51.35	88.72	90.94	85.51	69.79	81.43	92.38	68.39
IV	84.17	89.66	-	87.47	60.64	89.83	-	87.33	88.36	89.25	-	88.86
IV/I	1.53	1.34			1.32	1.03			1.42	1.28		
	'Jana C1'				'Hrusowska'				'Kasho'			
GP	S	L	F	A	S	L	F	A	S	L	F	A
I	68.29	79.93	-	-	52.54	74.20	-	-	53.66	79.98	-	-
II	71.64	81.55	89.19	-	59.03	78.00	90.34	-	69.42	82.85	90.73	-
III	75.95	89.11	93.17	75.17	62.26	87.98	91.73	88.88	70.27	90.12	92.01	88.40
IV	80.82	91.29	-	88.94	66.73	88.94	-	90.47	76.71	91.55		89.21
IV/I	1.18	1.14			1.27	1.20			1.43	1.14		

Highly statistically significant differences AOA (*P*-value<0.01) among plant parts in GP I were confirmed; AOA significant difference (*P*-value<0.05) between 'Jana C1' and other cvs. were also confirmed, there are no significant differences in AOA among cultivars 'Pyra', 'Spacinska', 'Emka', 'Hrusowska' and 'Kasho' (Tab. 2).

Table 2: Analysis of Variance for AOA (GP I)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:cultivar	803.616	5	160.723	3.23	0.0150
B:plant part	4423.87	1	4423.87	88.89	0.0000
RESIDUAL	2040.45	41	49.7672		
TOTAL (CORRECTED)	7267.94	47			

In GP II in all investigated buckwheat cultivars AOA was increased in plant parts in order: stems < leaves < flowers, the AOA differences among plant parts are highly significant (*P*-value < 0.01, Tab. 3). There is no statistically significant difference (*P*-value > 0.05, Tab. 3) in AOA among cultivars in GP II.

Table 3: Analysis of Variance for AOA (GP II)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:cultivar	374.164	5	74.8327	1.76	0.1348
B:plant part	9856.36	2	4928.18	115.61	0.0000
RESIDUAL	2728.2	64	42.6282		
TOTAL (CORRECTED)	12958.7	71			

From the point of view of antioxidant activity of most utilised parts of buckwheat plant the GP III is the most important growth phase, providing stems, leaves, flowers and seeds. In this phase the highest AOA was determined in flowers across cultivars increased in the order: 'Spacinska' < 'Pyra' < 'Hrusowska' < 'Kasho' < 'Emka' < 'Jana C1' (Tab. 1). Highly significant differences of AOA among all parts of the plant (*P*-value < 0.01, Tab. 4) as well as among cultivars 'Emka', 'Jana C1', 'Emka', 'Kasho', 'Kasho', 'Pyra', 'Kasho' and 'Spacinska' (*P*-value < 0.05; Tab. 4) were confirmed.

Table 4: Analysis of Variance for AOA (GP III)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:cultivar	616.519	5	123.304	2.50	0.0368
B:plant part	9664.57	3	3221.52	65.21	0.0000
RESIDUAL	4297.91	87	49.4012		
TOTAL (CORRECTED)	14579.0	95			

In the GP IV we analysed stems, leaves and seeds of buckwheat. In all plant parts the maximum of AOA in this growth phase (Tab. 1) was determined. Highly statistically significant differences in AOA (P-value < 0.01, Tab. 5) among the parts of plants as well as among the cultivars (*P*-value < 0.05) 'Emka' and 'Hrusowska', 'Emka' and 'Spacinska', 'Hrusowska' and 'Jana C1', 'Hrusowska' and 'Pyra', 'Jana C1' and 'Spacinska', 'Kasho' and

'Spacinska', 'Pyra' and 'Spacinska' (Tab. 5) were confirmed.

Table 5: Analysis of Variance for AOA (GP IV)

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:cultivar	784.37	5	156.874	4.60	0.0012
B:plant part	2795.51	2	1397.75	41.02	0.0000
RESIDUAL	2180.77	64	34.0746		
TOTAL (CORRECTED)	5760.65	71			

Holasova et al. (2002) compared the AOA values in whole buckwheat seeds, dehulled buckwheat seeds, buckwheat straws, leaves and hulls. The leaves proved a higher than triple antioxidant activity compared with seeds, whereas the straws and hulls had a lower antioxidant activity than seeds. The above findings correspond to our results, when the highest AOA values were determined in all cultivars except 'Hrusowska' in flowers and leaves, then in seeds and stems (Tab. 1). Gorinstein et al. (2007) determined the AOA in different cereals and pseudocereals including buckwheat. The values of AOA determined by DPPH radical scavenging method in seeds are comparable to our results, ranging between 80.0 ± 7.0%. Brindzová et al. (2009) evaluated the AOA using DPPH test in fifteen cultivars of cereals and nine cultivars of pseudocereals and confirmed statistically significant differences ($P \leq 0.05$) between the investigated cultivars.

In common buckwheat, the polyphenolics (rutin, quercetin, cyanidin and others) in the groats might be an important factor that determines their colour properties. On the other hand, buckwheat has an abundance of polyphenolic compounds (flavonoids, catechins, vitamin P), which have a yellow colour (Ikeda et al., 2001).

The colour of peel is one of the cultivar sign of buckwheat. The relationship between the hull colour and antioxidant activity of the flour was analysed by Fujita et al. (2004) and they found, that the hull colour would not be consider to be useful estimating the antioxidant activity of the flour. The authors suggested to judge antioxidant effects of buckwheat by flour colour and not by the colour of peel. Sedej et al. (2010) presented, that strong antioxidant activity of buckwheat flour extracts might be attributed to the presence of polyphenols, especially rutin, as the main antioxidative component in buckwheat.

The largest increase in antioxidant activity in parts of buckwheat during different growth phases was found in 'Pyra'. AOA determined in *stems* in GP IV (AOA_{IV}) was 1.53 multiple higher than that in GP I (AOA_I) and about 32.97% higher than that in GP III (AOA_{III}) (Tab. 1). Even when evaluating this dependence the impact of cultivar was confirmed, e.g.: the biggest difference in AOA between the first and the second growth phase (Fig. 1) was determined in 'Kasho'. 'Pyra' the largest dynamics in AOA between GP I and GP IV in buckwheat leaves (AOA_{IV}/AOA_I = 1.34), as well as the largest increase between GP I and GP II (Δ = 25.9%) was observed. In 'Spacinska' was not even 1 % difference in AOA (Fig. 2) between GP II and GP III confirmed.

In flowers and *seeds*, which were collected only during two growth buckwheat phases, the determined AOA values were increased in most cultivars (Δ = 4.46% in flowers of 'Jana C1' and Δ = 29.93% in seeds of 'Emka') (Fig. 3, 4).

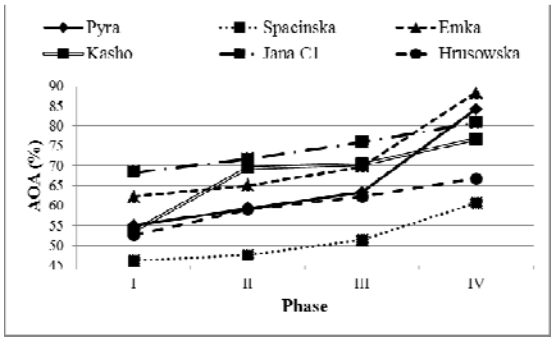

Figure 1: Dynamics of AOA (%) in stems during growth phases I – IV

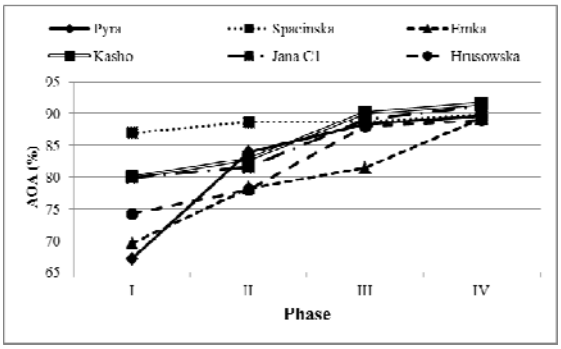

Figure 2: Dynamics of AOA (%) in leaves during growth phases I - IV

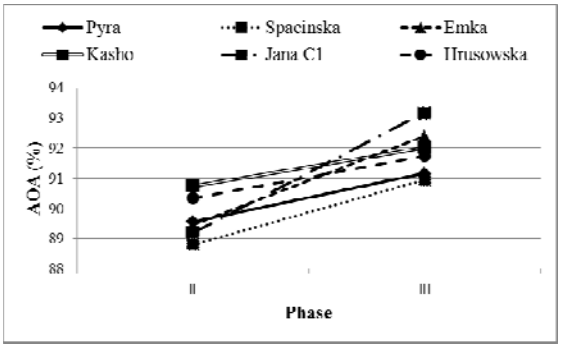

Figure 3: Dynamics of AOA (%) in flowers during growth phases II – III

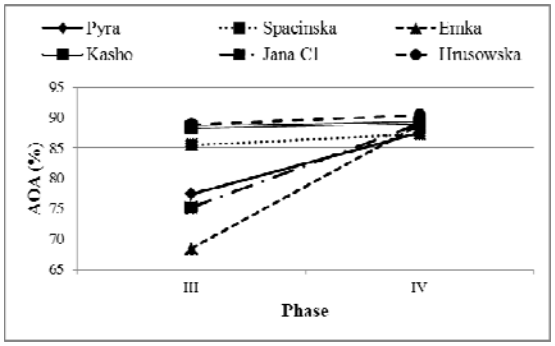

Figure 4: Dynamics of AOA (%) in seeds during growth phases III - IV

In all investigated cultivars highly statistically significant differences in AOA values between studied buckwheat plant parts (*P*-value < 0.01) (Tab. 6) were confirmed. With exception of 'Spacinska' (P-value < 0.05) there are also statistically high significant differences in AOA in all buckwheat cultivars between growth phases (*P*-value < 0.01) (Tab. 7).

Table 6: Multiple Range Tests for AOA by plant part (Method: 95.0 percent LSD)

	Pyra	Spacinska	Emka	Kasho	Jana C1	Hrusowska
plant part	HG	HG	HG	HG	HG	HG
stems	X	X	X	X	X	X
seeds	X	X	X	X	X	X
leaves	X	X X	X	X X	X	X
flowers	X	X	X	X	X	X

HG – Homogeneous Groups

Table 7: Multiple Range Tests for AOA by growth phase (Method: 95.0 percent LSD)

	Pyra	Spacinska	Emka	Kasho	Jana C1	Hrusowska
plant part	HG	HG	HG	HG	HG	HG
stems	X	X	X	X	X	X
seeds	X	X	X	X	X	X
leaves	X	X X	X	X X	X	X
flowers	X	X	X	X	X	X

HG – Homogeneous Groups

4 CONCLUSION

In six cultivars of common buckwheat we monitored changes in antioxidant activity, depending on the growth phase, as well as on the part of buckwheat plant. We have confirmed statistically significant differences in AOA among cultivars during plant development as well as among cultivars in different parts of the plant. Flowers harvested in GP III showed the highest AOA and measured values ranged from 90.94% (cv. Spacinska) to 93.17% ('Jana' C1). Seeds are the most frequently used buckwheat part plant in the food industry, which are used e.g. for the production of flour and meal. In GP IV (full maturity) the highest average AOA value was determined in seeds of 'Hrusowska' (90.47%) followed by 'Kasho' (89.21%), 'Jana C1' (88.94%), 'Emka' (88.86%), 'Pyra' (87.47%) and 'Spacinska' (87.33%).

Although buckwheat does not belong to the majority of agricultural crops, its use in the food industry has great perspectives. In addition, it contains a large number of bioactive substances, is a source of antioxidants, with a positive effect on the human organism. The use of buckwheat in food production - and not just seeds, but also other parts of the plant - can improve the nutritional value of foods, or to replace the synthetic antioxidants used as food additives by antioxidants from natural sources.

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