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Buckwheat cultivars — phenolic compounds profiles and antioxidant properties

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Abstract: Common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*Fagopyrum tataricum*) cultivars originating in different world countries were investigated and compared for their quantitative and qualitative abundance of phenolics and flavonoids. Moreover, the antioxidant properties were tested using two different methods. The total phenolic and total flavonoid content ranged from 0.897 to 4.226 mg GAE g^{-1} dw and from 0.238 to 4.626 mg rutin g^{-1} dw, respectively. Flavonoids — rutin, quercetin, and hydroxybenzoic acids — gallic, protocatechuic, vanillic and syringic were identified and quantified. Rutin was the most abundant flavonoid and protocatechuic acid was the most abundant phenolic acid in evaluated cultivars. All cultivars showed significant antiradical properties, but their chelating activity was weak. The German cultivar of tartary buckwheat Lifago had significantly higher phenolic content and better antioxidant properties than other cultivars. The content of rutin was 24 times higher and free radicals scavenging activity about 70 % higher than the average value of other cultivars.

Keywords: buckwheat, cultivars, flavonoids, phenolic acids, antioxidant activity

Introduction

Buckwheat (genus *Fagopyrum*) is an old traditional crop, and nowadays is very popular. Although buckwheat is classified as a pseudocereal, in contrast to cereals, it belongs to the class *Dicotyledonae*, family *Polygonaceae*. Cereals are assigned to the *Monocotyledonae*, family *Poaceae*. However, utility and chemical composition of buckwheat and cereal grain is similar. The genus *Fagopyrum* has about 15 species, among them, only common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*Fagopyrum tataricum*) are cultivated (Choi et al., 2013).

Buckwheat is a rich source of nutritive and bioactive components, including proteins with high content of essential amino acids (mainly lysine, threonin), dietary fibre, flavonoids, phenolic acids, B vitamins, tocopherols, tocotrienols, minerals (Jambrec et al., 2015; Qin et al., 2013). Unique is the presence of rutin (flavonol quercetin-3-*O*-rutinoside), that was not found in any other cereals. Rutin decreases the capillary fragility, reduces the risk of atherosclerosis, and has antiinflammatory, antimutagenic, anticarcinogenic, antihemorrhagic, antioxidative, hypotensive effects (Ahmed et al., 2014; Benso et al., 2016; Choi et al., 2014). Buckwheat proteins have relatively low digestibility, what is probably the reason of positive activities against obesity, constipation, colon tumour, mammary tumour, hypercholesterolaemia. The decreased digestibility of proteins may be caused by antinutritional factors (protease inhibitors, tannins, phytic acid) present in buckwheat (Yiming et al., 2015). Buckwheat does not contain gluten and therefore it may be used in gluten-free diets.

Because the content of nutritive and bioactive compounds in plants depends on various factors including cultivar, environmental and growth conditions, the aim of our study was to assess the profile of phenolic compounds and antioxidant activity of buckwheat cultivars originating in different countries of the world.

Material and methods

Materials and reagents

Twenty two varieties of *Fagopyrum esculentum* and two varieties of *Fagopyrum tataricum* were analysed. Samples were provided by the Gene Bank of the Research Institute of Plant Production (Piešťany, Slovak Republic). Crops, originating in different world countries (Table 1), were grown in locality Piešťany — Borovce (Slovak Republic).

Hexane (p.a.), ethyl acetate (p.a.), methanol (HPLC grade), acetonitrile (HPLC grade), acetic acid (p.a.) were purchased from Mikrochem, Pezinok, Slovak Republic. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, aluminum chloride, phenolic acids (gallic, caffeic, protocatechuic, vanillic, syringic, *p*-coumaric, ferulic) and flavonoids (rutin, quercetin) were purchased from Sigma- Aldrich, Steinheim, Germany. Ferrozine and Folin-Ciocalteu reagent were purchased from Merck, Darmstadt, Germany.

Sample preparation
The buckwheat samples were milled and passed through a 0.5 mm sieve. Consequently, they were defatted with hexane (1:5, two times, 1 h at room temperature), extracted with 65 % ethanol (1:20, three times, 1 h at 80 °C) according to Mikulajová et al. (2007). Aqueous phase was re-extracted with ethyl acetate (1:1, two times), concentrated to dryness and dissolved in 96 % ethanol for determination of antioxidant properties, total phenolic and total flavonoid content, and in methanol for phenolic compounds analysis by HPLC/DAD.

DPPH radical scavenging activity
Antiradical activity against DPPH was investigated by the method described by Yen and Chen (1995). The decrease of absorbance at 517 nm as result of DPPH radical decolouration, from purple to yellow colour, induced by present antioxidants was recorded. The antioxidant activity of tested sample was expressed as the amount of scavenged DPPH radicals per gram of buckwheat dry weight (mg DPPH g^{-1} dw).

Ferrous ion chelating activity
The ability of buckwheat samples to chelate ferrous ions was estimated by the method of Gülçin et al. (2003). Ferrozine can form red coloured complexes with Fe^{2+} that can be disturbed in the presence of chelating agents. A consequent decrease in red colour intensity at 562 nm is measured. In our research the percentage (%) inhibition of ferrozine-Fe^{2+} complex formation was calculated.

Total phenolic content
The total phenolic content of buckwheat was measured spectrophotometrically with Folin-Ciocalteu reagent (Yu et al., 2004). The intensity of developed blue colour was measured as A_{765}. The standard curve was prepared using gallic acid and the results were expressed in gallic acid equivalent per gram of buckwheat dry weight (mg GAE g^{-1} dw).

Total flavonoid content
The total flavonoid content of buckwheat was determined by spectrophotometric assay with aluminium chloride (Kreft et al., 2002). The absorbance of developed yellow colour at 420 nm was measured. The standard curve was prepared for rutin and the amount of total flavonoids was expressed in rutin equivalent per gram of buckwheat dry weight (mg rutin g^{-1} dw).

Phenolic compound analysis by HPLC/DAD
HPLC analysis was performed with Agilent 1200 Series HPLC system equipped with diode array detector (DAD) and Zorbax Eclipse XDB-C18 column (4.6 × 150 mm, 5 µm, Agilent Technologies, Santa Clara, California, USA). Gradient elution was carried out using the solvent system consisted of solvent A: water/acetic acid mixture adjusted to pH 2.8, and B: acetonitrile at the constant flow rate of 1 ml min^{-1}. The detection wavelength was 272 nm and 350 nm, respectively. Phenolic compounds identification was done by comparing with the standards of phenolic acids and flavonoids, and phenolics quantification was evaluated with ChemStation software 12.2 (Agilent Technologies, Santa Clara, California, USA).

Statistical analysis
Analyses were performed in quadruplicates. Obtained data were reported as means ± standard deviation. Results were subjected to correlation analysis and analysis of differences among mean values by Student test ($p < 0.05$).

Results and discussion

Phenolics analysis
Total phenolic content. The total content of phenolic compounds in all cultivars ranged from 0.897 to 4.226 mg GAE g^{-1} dw (Table 1). The mean phenolics content for the *Fagopyrum esculentum* cultivars was 1.148 mg GAE g^{-1} dw and for the *Fagopyrum tataricum* cultivars was 2.676 mg GAE g^{-1} dw. *Fagopyrum tataricum* cultivar Lifago showed markedly higher content of phenolics (4.226 mg GAE g^{-1} dw). The next evaluated cultivar of *Fagopyrum tataricum* Idel contained only third amount of total phenolics of cultivar Lifago (1.125 mg GAE g^{-1} dw). Winsor Royal, Emka and FAG 38/82 were the *Fagopyrum esculentum* cultivars with the highest phenolics content and Východoslovenská krajová the lowest one.
Total flavonoid content. The total flavonoid content in buckwheat cultivars ranged from 0.238 to 4.626 mg rutin g^{-1} dw (Table 1). The highest content of total flavonoids from *Fagopyrum esculentum* cultivars showed Emka, Hrusowska and FAG 29/79, and the lowest content was found in cultivar La Harpé. The mean value of total flavonoids for the *Fagopyrum esculentum* cultivars was 0.372 mg rutin g^{-1} dw, what is 12.4 times less than in cultivar Lifago.
Previous papers (Sedej et al., 2012; Kiprovski et al., 2015; Jambrec et al., 2015; Qin et al., 2010; Inglett et al., 2011) reported diverse content of total phenolics (0.6–5.4 mg GAE g^{-1} dw in *Fagopyrum esculentum;* 13.0 mg GAE g^{-1} dw in *Fagopyrum tataricum*), total flavonoids (0.07–3.8 mg rutin g^{-1} dw in *Fagopyrum*

Tab. 1. Characterization of buckwheat cultivars, their total phenolic content (mg GAE g^{-1} dw), total flavonoid content (mg rutin g^{-1} dw), DPPH radicals scavenging activity (mg DPPH g^{-1} dw) and chelating activity (%).

Accession number	Cultivar	Origin	TPC (mg GAE g^{-1} dw)	TFC (mg rutin g^{-1} dw)	DPPH assay (mg DPPH g^{-1} dw)	Chelating activity (%)
			Fagopyrum esculentum			
00008	Špačinská 1	SVK	0.968 ± 0.044	0.316 ± 0.014	10.665 ± 0.497	3.62 ± 0.24
00009	Východoslovenská krajová	SVK	0.897 ± 0.012	0.330 ± 0.014	5.980 ± 0.249	2.21 ± 0.08
00010	PY-EP1	SVK	1.076 ± 0.042	0.381 ± 0.009	10.204 ± 0.444	3.67 ± 0.25
00011	PY-EP2	SVK	1.191 ± 0.056	0.364 ± 0.002	10.039 ± 0.260	5.16 ± 0.24
00007	Pyra	CZE	0.974 ± 0.037	0.281 ± 0.007	10.952 ± 0.107	4.07 ± 0.24
00031	Jana C1	CZE	0.970 ± 0.030	0.341 ± 0.011	8.431 ± 0.257	7.59 ± 0.19
00023	Emka	POL	1.443 ± 0.059	0.507 ± 0.027	13.898 ± 0.559	5.33 ± 0.28
00032	Hruszowska	POL	1.262 ± 0.046	0.493 ± 0.032	13.690 ± 0.161	4.20 ± 0.14
00003	Bogatyr	RUS	0.984 ± 0.037	0.285 ± 0.015	7.714 ± 0.345	6.05 ± 0.45
00004	Ballada	RUS	1.052 ± 0.035	0.317 ± 0.005	9.265 ± 0.251	2.59 ± 0.07
00016	FAG 38/82	RUS	1.439 ± 0.044	0.445 ± 0.012	11.016 ± 0.307	2.60 ± 0.13
00026	Aiva	LVA	1.034 ± 0.024	0.322 ± 0.013	8.774 ± 0.151	0.99 ± 0.05
00025	Bamby	AUT	1.400 ± 0.061	0.426 ± 0.017	10.438 ± 0.144	5.32 ± 0.36
00022	Darja	SVN	1.029 ± 0.024	0.272 ± 0.011	8.233 ± 0.195	0.71 ± 0.03
00001	Alex	GER	1.153 ± 0.013	0.403 ± 0.003	10.208 ± 0.511	1.79 ± 0.03
00014	FAG 120/82	GER	1.274 ± 0.007	0.410 ± 0.002	12.645 ± 0.078	2.54 ± 0.10
00015	FAG 29/79	GER	1.220 ± 0.013	0.481 ± 0.018	8.934 ± 0.032	0.97 ± 0.04
00018	FAG 88/84	GER	1.141 ± 0.012	0.334 ± 0.003	5.712 ± 0.135	5.01 ± 0.27
00005	La Harpe	FRA	0.930 ± 0.020	0.238 ± 0.008	7.744 ± 0.226	2.18 ± 0.09
00013	St Jacut	FRA	1.319 ± 0.039	0.426 ± 0.014	10.878 ± 0.290	1.02 ± 0.05
00030	Kasho-02	JPN	1.051 ± 0.027	0.357 ± 0.016	9.885 ± 0.249	2.57 ± 0.03
00034	Winsor Royal	USA	1.456 ± 0.029	0.446 ± 0.012	14.866 ± 0.337	2.87 ± 0.20
			Fagopyrum tataricum			
00006	Lifago	GER	4.226 ± 0.038	4.626 ± 0.097	32.685 ± 0.516	6.04 ± 0.24
00002	Idel	RUS	1.125 ± 0.036	0.361 ± 0.004	9.161 ± 0.203	2.09 ± 0.13

TPC – total phenolic content, TFC – total flavonoid content

esculentum; 6.6—22.7 mg rutin g^{-1} dw in *Fagopyrum tataricum*).

Identification of phenolic compounds. Hydroxybenzoic acids — gallic, protocatechuic, vanillic and syringic, and flavonols — rutin, quercetin, were identified and quantified in tested buckwheat cultivars (Table 2).

Inglett at al. (2011), Guo et al. (2011), Sedej at al. (2012), Kiprovski et al. (2015) identified ferulic, chlorogenic, caffeic, *p*-coumaric, and sinapic acids in buckwheat seeds as well. Rutin was the most abundant phenolic compounds in grains (48.0—89.2 %), followed by protocatechuic acid (1.8—26.1 %), whereas vanillic acid was the least abundant (0.63—6.96 %). Percentages mentioned above represent proportion of particular compound from total phenolics content determined by HPLC/DAD analysis.

Cultivar Lifago had the highest rutin, flavonoid and phenolic acids contents (23.8-fold, 24.3-fold and 2.7-fold higher than average value of other cultivars). Cultivars FAG 29/79 and Emka contained the second largest amount of phenolics and flavonoids, as well (Fig. 1).

Recorded rutin content (0.06—0.30 mg g^{-1} dw) of investigated *Fagopyrum esculentum* cultivars is in accordance with previous findings of Kiprovski et al. (2015), and Sedej et al. (2012). Guo et al. (2011) reported rutin content in range from 5.2 to 14.5 mg g^{-1} dw of tartary buckwheat grown in China, what is more than amount determined in our study.

Antioxidant properties

Antioxidant properties of buckwheat cultivars were determined with two independent *in vitro* methods. DPPH method is focused on evaluation of ability to scavenge the stable free DPPH radicals by compounds in the sample that act as primary antioxidant. This method provides information

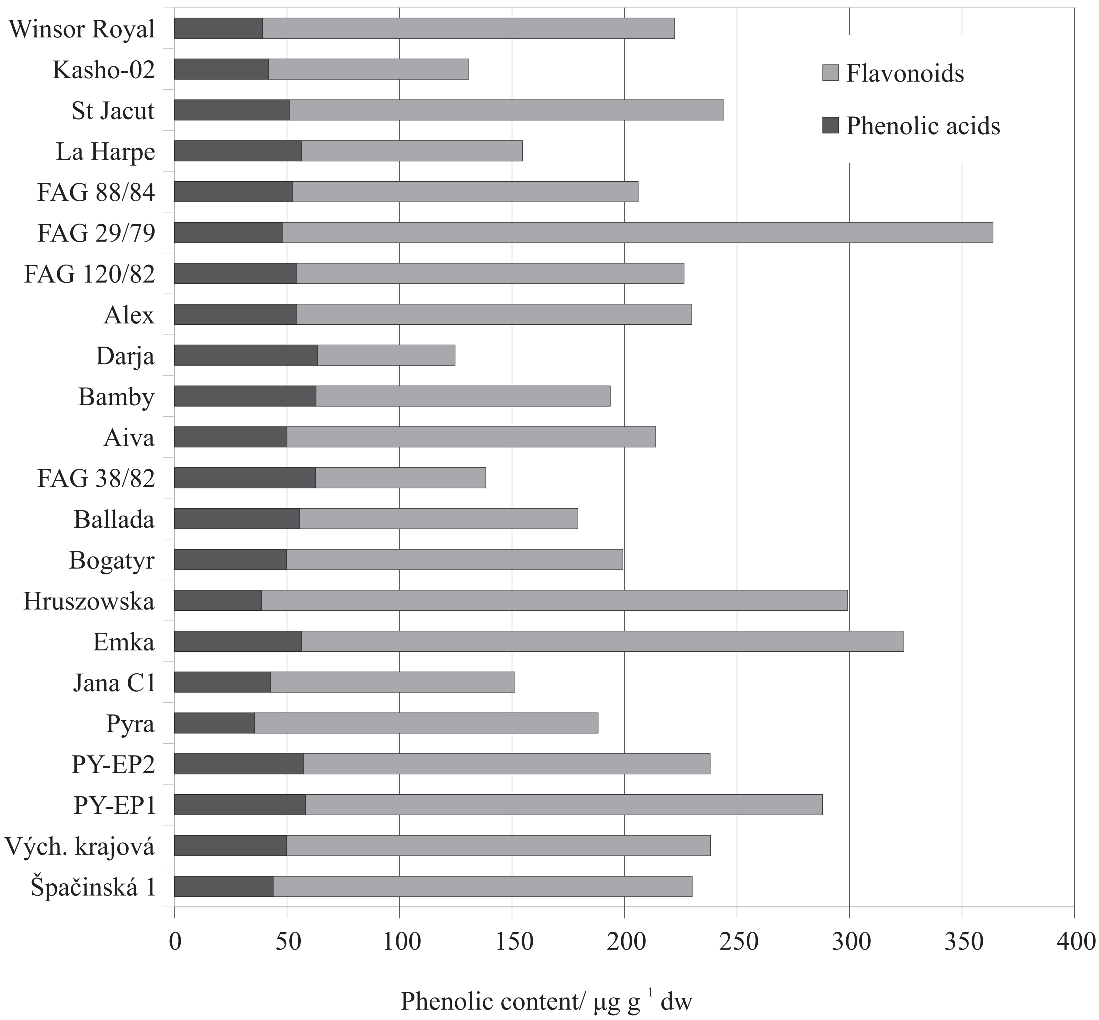

Fig. 1. Total content of phenolic acids and flavonoids (µg g^{-1} dw) in *Fagopyrum esculentum* cultivars determined by HPLC/DAD analysis.

about the total antioxidant capacity of a sample, and it is not specific for particular compounds. Method of chelating activity on Fe^{2+} measures metal chelating ability of compounds that have effect like secondary antioxidant. Transition metals (iron and copper) can participate in oxidative damage by catalysing the formation of reactive oxygen species. Stabilizing of transition metals by chelation can delay this process. Because the antioxidant compounds present in samples are chemically diverse, the application of independent methods allows to cover different aspects of antioxidant efficacy.

DPPH radical scavenging activity. All cultivars showed significant scavenging activity against DPPH radicals (ranged from 5.712 to 32.685 mg DPPH g^{-1} dw, Table 1). Cultivars with the highest phenolic content displayed the best antioxidant abilities, in descending order: Lifago, Winsor Royal and Emka. Cultivars Fag 88/84 and Východoslovenská krajová had the lowest activity. *Fagopyrum esculentum* cultivars scavenged 10.008 mg DPPH g^{-1} dw on average, what was 3.3-fold less than *Fagopyrum tataricum* cultivar Lifago.

Ferrous ion chelating activity. The Fe^{2+}-chelating activity of the tested cultivars was weak (ranged from 0.71 % to 7.59 %, Table 1). Cultivars Jana C1 (7.59 %), Bogatyr (6.05 %) and Lifago (6.04 %) exhibited the highest chelating effect. On the other hand, the lowest Fe^{2+}-chelating activity had cultivars Darja (0.71 %), FAG 29/79 (0.97 %) and Aiva (0.99 %). Apparently, chelation is not the major mechanism of buckwheats antioxidant ability, but it is important for the stabilisation of lipid matrices in the presence of traces of metals.

Correlation analysis showed a high correlation between the amount of phenolics and scavenging activity, what suggests that phenolic compounds, including flavonoids, dominantly contribute to antiradical properties of buckwheat. On the other hand, chelating properties are less pronounced, because a weak correlation between phenols content and chelating activity was found (Table 3). It can be concluded, that buckwheat grains contain

Tab. 2. The results of HPLC/DAD analysis of phenolics content (µg g^{-1} dw) of buckwheat cultivars.

Cultivar	Phenolic compounds (µg g^{-1} dw)					
	Gallic acid	Protocatechuic acid	Vanillic acid	Syringic acid	Rutin	Quercetin
			Fagopyrum esculentum			
Špačinská 1	6.27 ± 0.33	22.19 ± 0.52	3.97 ± 0.34	11.40 ± 0.31	159.13 ± 4.61	27.13 ± 0.84
Vých. krajová	6.21 ± 0.32	19.72 ± 0.71	10.74 ± 0.51	13.31 ± 0.47	181.52 ± 7.25	6.79 ± 0.18
PY-EP1	10.03 ± 0.27	27.99 ± 1.08	4.90 ± 0.26	15.34 ± 0.84	223.57 ± 2.36	6.24 ± 0.23
PY-EP2	9.96 ± 0.10	29.02 ± 1.26	4.31 ± 0.16	14.20 ± 0.17	171.68 ± 8.61	8.89 ± 0.36
Pyra	5.17 ± 0.52	16.23 ± 0.71	3.58 ± 0.16	10.59 ± 0.21	138.68 ± 5.61	14.03 ± 0.91
Jana C1	8.15 ± 0.22	20.58 ± 0.46	3.78 ± 0.10	10.58 ± 0.11	104.82 ± 2.74	3.72 ± 0.01
Emka	12.31 ± 0.52	24.31 ± 1.02	6.09 ± 0.11	13.82 ± 0.26	255.52 ± 9.14	12.16 ± 0.45
Hruszowska	8.54 ± 0.45	15.95 ± 0.46	4.18 ± 0.09	9.99 ± 0.41	259.55 ± 7.16	0.89 ± 0.107
Bogatyr	7.74 ± 0.44	25.67 ± 0.97	4.16 ± 010	12.24 ± 0.32	138.11 ± 4.30	11.43 ± 0.61
Ballada	9.25 ± 0.43	27.64 ± 1.01	6.23 ± 0.10	12.58 ± 0.56	116.34 ± 1.66	7.25 ± 0.34
FAG 38/82	11.66 ± 0.17	29.07 ± 0.71	8.65 ± 0.32	13.19 ± 0.22	68.58 ± 2.16	7.05 ± 0.20
Aiva	8.93 ± 0.24	24.84 ± 0.71	5.11 ± 0.20	11.17 ± 0.42	153.15 ± 8.22	10.73 ± 0.45
Bamby	13.21 ± 0.35	23.26 ± 0.43	12.09 ± 0.60	14.32 ± 0.26	124.38 ± 2.04	6.51 ± 0.27
Darja	10.97 ± 0.51	32.49 ± 0.65	4.62 ± 0.19	15.63 ± 0.12	59.93 ± 1.14	1.09 ± 0.01
Alex	10.23 ± 0.33	24.41 ± 0.61	6.61 ± 0.24	13.21 ± 0.60	164.72 ± 2.76	10.80 ± 0.23
FAG 120/82	11.72 ± 0.46	24.78 ± 1.06	5.71 ± 0.28	12.16 ± 0.44	163.79 ± 3.78	8.28 ± 0.34
FAG 29/79	10.95 ± 0.31	20.38 ± 0.54	4.31 ± 0.11	12.33 ± 0.55	304.45 ± 5.45	11.37 ± 0.60
FAG 88/84	9.02 ± 0.22	28.35 ± 0.64	5.46 ± 0.15	9.72 ± 0.36	144.38 ± 8.04	9.08 ± 0.27
La Harpe	10.10 ± 0.26	29.73 ± 1.14	1.88 ± 0.06	14.72 ± 0.71	93.49 ± 4.15	4.75 ± 0.08
St Jacut	9.98 ± 0.35	24.01 ± 0.82	4.42 ± 0.10	12.86 ± 0.40	183.59 ± 5.35	9.25 ± 0.33
Kasho-02	7.92 ± 0.21	13.37 ± 0.44	9.14 ± 0.45	11.37 ± 0.54	74.48 ± 2.35	14.47 ± 0.60
Winsor Royal	9.11 ± 0.12	14.30 ± 0.47	5.23 ± 0.31	10.46 ± 0.68	171.73 ± 5.25	11.52 ± 0.44
			Fagopyrum tataricum			
Lifago	26.05 ± 0.91	74.3 ± 2.1	26.46 ± 0.80	12.72 ± 0.30	3737.2 ± 50.2	313.50 ± 5.11
Idel	10.02 ± 0.51	40.0 ± 0.7	5.13 ± 0.17	14.50 ± 0.41	162.28 ± 5.23	11.87 ± 0.36

Tab. 3. Correlation coefficients between phenolic composition and antioxidant activity methods in buckwheat cultivars.

Parameter	DPPH assay	Chelating activity
Total phenolic content	0.948	0.295
Total flavonoid content	0.920	0.303
∑phenolics	0.907	0.299
∑flavonoids	0.908	0.301
∑phenolic acids	0.765	0.191
Gallic acid	0.838	0.205
Protocatechuic acid	0.691	0.186
Vanillic acid	0.779	0.258
Syringic acid	–0.045	–0.267
Rutin	0.908	0.301
Quercetin	0.901	0.300

∑phenolics, ∑flavonoids, ∑phenolic acids – sum of compounds determined by HPLC/DAD.

substances active mainly as primary antioxidants. Secondary antioxidant components are less abundant.

Conclusion

This study was designed in order to characterise 24 buckwheat cultivars in terms of both phenolic compounds profile, and antioxidant activity. Evaluated cultivars originated in different world countries and were cultivated in Slovakia. German tartary buckwheat cultivar Lifago was found to contain significantly higher amount of phenolic compounds, mainly rutin, and showed the highest antioxidant capacity among other tested cultivars. It was followed by Polish common buckwheat cultivar Emka. Slovak cultivars exhibited values close to average. The amount of phenolics and antioxidant properties related with it was dependent on a cultivar, so that we can conclude that an increase of bioactive compounds intake can be achieved by selection of suitable cultivars. This is important for development and production of functional foods.

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