

Antioxidant Properties and Flavonoid Composition as Quality Index of the Hulls and Groats from Common, Tartary and an Interspecific Hybrid of Buckwheat

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ABSTRACT

This paper presents a field program addressed to buckwheat genotypes with the aim to develop cultivars of high antioxidant capacity and high flavonoid concentration in the hulls and groats. The antioxidant capacity of groat and hull separated from buckwheat seed was evaluated against 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical cation (ABTS^{•+}) and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH[•]), and against the superoxide anion radical (O₂^{•-}) while the reducing capacity was measured directly by cyclic voltammetry (CV) method. The extent of variation in the content of rutin, quercetin and flavone C-glucosides in hull and groat was determined by high-performance liquid chromatography with diode array detection (HPLC-DAD). The applied analytical strategy for the determination of the antioxidant capacity created a useful quality parameter for buckwheat. The applied methods provided the same rank of the antioxidant capacity among hulls and groats however the real antioxidant capacity values differed according to the methods. The rank of values of the antioxidant capacity vs. applied methods was as follows: ABTS assay ≈ PCL assay > DPPH RSA ≈ CV assay. The rutin and flavone C-glucosides content in the groats from common buckwheat cultivars and the interspecific hybrid were lower than those noted in the hulls. In contrast, groat and hull from tartary buckwheat accession contained comparable high level of flavonoids. The extent of variation in the content of rutin, quercetin and flavone C-glucosides in the hull may be useful for researchers working on the resistance of buckwheat lines against pathogens and diseases whilst extent of variation in the flavonoid composition in groats should be important from the nutritional point of view. Therefore, flavonoid composition of the hulls and groats from different buckwheat genotypes may serve as a quality index.

Keywords: antioxidant capacity, buckwheat genotypes, flavonoids, groats, hulls

Abbreviations: AC, antioxidant capacity; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DPPH RSA, DPPH radical scavenging activity; PCL, photochemiluminescence; CV, cyclic voltammetry

INTRODUCTION

Buckwheat, frequently classified as a pseudocereal, is one of the minor crops cultivated by ethnic groups in developed and developing countries which is an integral part of their diet and culture (Przybylski and Gruczyńska 2009). There are many species of buckwheat in the world, and mainly nine species have agricultural meaning (Campbell 1997; Li and Zhang 2001). Generally, *Fagopyrum* has two groups of species: annual (*Fagopyrum esculentum* Moench, *Fagopyrum tataricum* L. and *Fagopyrum giganteum* Krotov) and perennial species (*Fagopyrum cymosum* Meissn, *Fagopyrum suffruticosum* Fr. Schmidt and *Fagopyrum ciliatum* Jaegt). Among these species, only common buckwheat (*F. esculentum*) and tartary buckwheat (*F. tataricum*) are commonly grown, the latter is mainly grown in some mountainous regions (Jiang *et al.* 2007).

The mature dry seed of buckwheat is a triangular fruit or achene. The hull (pericarp, fruit coat), the outer layer of the achene, is a hard fibrous structure that is usually dark brown or black in colour. Removal of hulls by impact dehulling releases whole groats. Common buckwheat has softer hull than its *F. tataricum* relative. Buckwheat seeds have taste harsh for *F. esculentum* and light bitter for *F. tataricum* (Steadman *et al.* 2001). The attempts to improve agronomic characters of the two cultivated species (quantity and quality of yield, resistance to environmental conditions, etc.) by the interspecific crosses have failed due to hybrid

sterility (Wang *et al.* 2002). The discovery of new, wild buckwheat species, including self-compatible buckwheat and species closely related to common buckwheat (*Fagopyrum homotropicum* Ohnishi), supplied new sources of germplasm for breeding programs (Zeller 2001). Hybridization supports the transfer of desirable traits to buckwheat crops to increase their tolerance for environmental stress factors and to improve crop quality (Campbell 2003). Buckwheat is presently considered as food component of high nutritional value (Li and Zhang 2001). Since it combines nutritionally valuable protein, lipid, dietary fiber and minerals with medically beneficial components, such as flavonoids, fagopyrin and sterols, it has been receiving increasing attention as a potential functional food (Steadman *et al.* 2000; Sensoy *et al.* 2005; Jiang *et al.* 2007; Christa and Soral-Śmietana 2008) and a source of antioxidant activity in functional foods (Holasova *et al.* 2002; Zielińska and Zieliński 2009). Buckwheat is known for its high rutin (quercetin 3-rutinoside) content which was first discovered in buckwheat in the 19th century. At the present time, buckwheat is still considered to be a major dietary source of rutin. However, there is a wide variation of rutin content in buckwheat seed depending on the species, variety and the environmental conditions under they are produced (Jiang *et al.* 2007; Brunori *et al.* 2009). Common buckwheat is reported to contain less rutin in the seed than tartary buckwheat (Fabjan *et al.* 2003; Kreft *et al.* 2006). Despite rutin, other flavonoids such as quercetin, myricetin hyperoside (quer-

cetin 3-*O*- β -D-galactoside), quercitrin (quercetin 3-*O*- α -L-rhamnoside), epicatechin and flavone C-glucosides were identified in buckwheat during last decade (Watanabe 1998; Fabjan *et al.* 2003; Jiang *et al.* 2007; Kalinova and Vrchoťova 2009). The main flavone C-glucosides present in buckwheat include orientin and homoorientin, a pair of isomeric compounds, and their 4'-deoxy analogues, namely vitexin and isovitexin (Dietrych-Szostak and Oleszek 1999; Zielińska *et al.* 2007a). In many cases, flavonoids show a greater efficacy as antioxidants in food systems on a mole-to-mole basis than the antioxidants vitamin C, vitamin E and β -carotene (Rice-Evans *et al.* 1996). The antioxidant activity of flavone C-glucosides and rutin evaluated with different chemical assays has been recently reported (Zielińska *et al.* 2010; Zielińska and Zieliński 2011).

The composition of flavonoids in buckwheat may vary both in hulls and groats (Oomah and Mazza 1996). Buckwheat groats are important for human consumption since processed flour and groats quality are the forms mostly used. Therefore, processors prefer the large seeded cultivars and the quality of groats is also of their interest.

Buckwheat is also one of the crops with allelopathic potential (Golisz *et al.* 2007). Rutin and other flavonoids are UV-B absorbing plant metabolites with the role to protect the seeds from the harmful effects of UV-B radiation and diseases (Fabjan *et al.* 2003). The protective site is related to the hulls and therefore the antioxidative properties and flavonoid composition of hulls may represent a quality index for different buckwheat genotypes.

This paper presents a field program addressed to differentiated buckwheat genotypes, with the aim to develop buckwheat with high antioxidant capacity and high flavonoid concentration in the hull and groat. The first objective of the work reported here was to evaluate the antioxidant capacity of the hulls and groats against stable, non-biological radicals such as 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonate) radical cation (ABTS^{•+}) and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH[•]), against the key reactive oxygen intermediate – superoxide anion radical (O₂^{•-}) and reducing capacity by direct electrochemical method - cyclic voltammetry (CV). The second aim was to determine the extent of variation of rutin, quercetin and flavone C-glucosides in hulls and groats of different buckwheat genotypes. Both aims addressed in this study were used to consider the antioxidant capacity and flavonoid composition of hulls and groats as new quality index of buckwheat genotypes.

MATERIALS AND METHODS

Chemicals

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), quercetin and rutin (quercetin-3-rutinoside) were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, U.S.A.). Methanol, acetonitrile, formic acid, acetic acid (supra-gradient) and sodium acetate were from Merck KGaA, Darmstadt, Germany. Photochemiluminescence analytical kit for measurement of the antioxidant capacity (kit No. 400.801) was from Analytik Jena AG (Jena, Germany). Orientin (3',4',5,7-tetrahydroxyflavone-8-glucoside), homoorientin (3',4',5,7-tetrahydroxy-flavone-6-glucoside), vitexin (4',5,7-trihydroxyflavone-8-glucoside) and isovitexin (4',5,7-trihydroxyflavone-8-glucoside) of HPLC-grade were obtained from Extrasynthese Co. Inc. (Lyon, France). All other reagents of reagent-grade quality were from POCh, Gliwice, Poland. Water was purified with a Mili-Q-system (Milipore, Bedford, USA). All solutions prepared for HPLC were passed through a 0.45 μ m nylon filter before use.

Plant materials

The common buckwheat cvs. 'Karmen', 'Anita', 'Volma', 'Smuglianka' (from the Belarusian plant breeding program) and cv. 'Vokiai' (originated from Lithuanian Institute of Agriculture

breeding program), a tartary buckwheat variety (local accession from Olsztyn region, Poland) and a new interspecific buckwheat hybrid cv. 'Teresa' were investigated in this study. The interspecific buckwheat hybrid *Fagopyrum homotropicum* Ohnishi \times *Fagopyrum esculentum* Moench, cv. 'Teresa', originated from the breeding program of the Institute of Plant Breeding, University of Technology Munich, Germany. The hybrid was produced by crossing the wild species with two common buckwheat cultivars – 'Hruszowska' and 'Sumchanka'. Samples of mature buckwheat seeds were collected from experimental fields in Bałcyny near Ostróda in Production-Experimental Station of the University of Warmia and Mazury in Olsztyn. For analysis, only mechanically and physiologically undamaged seeds were used. The seeds of each variety were manually separated into hulls and groats. The material was freeze-dried, milled into powder and then stored at -40°C in polyethylene bags. The dry matter content of buckwheat hulls and groats was determined.

Sample preparation

About 200 mg of powder from lyophilized buckwheat groat and hull samples were macerated with 1 mL of 80% methanol for 24 h in the dark at 4°C. Next, the mixture was vortexed for 30 s, sonicated for 30 s and centrifuged for 5 min (13,200 \times g at 4°C). That step was repeated again with the next volume of 80% methanol (1 mL) and supernatants were collected in 2 mL flask. Finally, all extracts were kept at -80°C prior to further analysis.

ABTS assay

The method described by Re *et al.* (1999) was used to determine the antioxidant activity with a minor modification described below. For measurements, the ABTS^{•+} solution was diluted with 80% methanol, respectively, to the absorbance of 0.70 \pm 0.02 at 734 nm. For the spectrophotometric assay, 1.48 mL of the ABTS^{•+} solution and 20 μ L of 80% methanol extracts of hulls and groats were mixed and absorbance was measured immediately after 6 min at 734 nm at 30°C. The ABTS^{•+} working solution was used as blank sample. The standard curve based on the reduction in absorbance of the ABTS^{•+} by Trolox standard solutions within the range of 0.1-2.5 mM was constructed ($y = 35.29x + 1.48$; $R^2 = 0.99$). The measurements were carried out using a temperature controlled spectrophotometer UV-160 IPC with CPS-Controller (Shimadzu, Japan) and the results were expressed as μ mol Trolox/g d.m.

DPPH RSA assay

DPPH radical scavenging activity (DPPH RSA) was determined in 80% methanol extracts of hulls and groats using a modified method of Brand-Williams *et al.* (1995). The Trolox standard solutions within the range of concentration 0.1 – 1.0 mM in 80% methanol were assayed under the same conditions, and the linear response of Trolox concentrations was used for standard curve construction ($y = 84.66x + 1.26$; $R^2 = 0.99$). DPPH[•] scavenging activity of the buckwheat hulls and groats samples was expressed in terms of Trolox equivalent antioxidant capacity on the basis of reduction in absorbance of the DPPH[•] solution by standards at 515 nm. The measurements were carried out using a temperature controlled spectrophotometer UV-160 IPC with CPS-Controller (Shimadzu, Japan) and results were expressed as μ mol Trolox/g d.m.

PCL assay

Photochemiluminescence (PCL) method was used to measure the antioxidant activity of the buckwheat material against superoxide anion radicals (O₂^{•-}). In the PCL assay the photochemical generation of free radicals is combined with the sensitive detection by using chemiluminescence (Popov and Lewin 1999). This reaction is induced by optical excitation of a photosensitizer S which results in the generation of the superoxide radicals (O₂^{•-}):



The free radicals are visualized with the chemiluminescent detection reagent luminol which works as photosensitizer as well

as oxygen radical detection reagent. This reaction takes place in the Photochem[®]. The light source was a mercury discharge lamp phosphorus coated with maximal excitation at $\lambda = 351$ nm. The detailed protocol was carried out as previously described (Zielińska *et al.* 2007b). Briefly, the antioxidant activity of 80% methanol extracts of buckwheat hulls and groats was determined using a commercial kit provided by the manufacturer. Exactly, 2.3 mL reagent 1 (methanol), 200 μ L reagent 2 (buffer solution), 25 μ L reagent 3 (photosensitizer) and 10 μ L buckwheat extract were mixed and measured with a Photochem[®] apparatus (Analytik Jena, Leipzig, Germany). These are standardized conditions, so the results are comparable to other assays. The antioxidant capacity was assayed by means of the area under the curve of hull and groat extracts. The results were expressed as μ mol Trolox/g d.m.

Cyclic voltammetric assay

A potentiostat/galvanostat G 750 (Gamry Ins., USA) was used for voltammetric experiments. A conventional three electrode system: (a) a 3 mm diameter glassy carbon working electrode (BAS MF-2012), (b) a Ag/AgCl electrode as reference electrode, and (c) a platinum as counter electrode was used. Cyclic voltammetric (CV) experiments were performed with 80% methanol extracts of hulls and groats mixed with 0.2 M sodium acetate-acetic buffer (pH 4.5 in 80% methanol) at ratio 1:1 (v/v) according to Cosio *et al.* (2006). The voltammetric experiments were performed at room temperature using apparatus cell of 200 μ L volume, to which analyzed extract mixed previously with the buffer solution was introduced. The cyclic voltammograms were acquired from 100 to 1200 mV at scanning rate of 100 mV s⁻¹. Prior to use, the surface of the glassy carbon electrode was carefully polished with 0.05 μ m alumina paste and ultrasonically rinsed in deionized water and after that washed with methanol. This procedure was repeated after each cycle. For the test purpose, the total charge below anodic wave curve of the voltammogram was calculated. The cyclic voltammograms of Trolox solutions within the concentration range of 0.1 – 2.5 mM was also acquired from 100 to 1200 mV, and then the linear response of Trolox concentrations ($y = 114.11x + 5.31$; $R^2 = 0.99$) was used to standard curve construction. The total charge under anodic wave of the background signal (solvent + supporting buffer) was subtracted from the total charge under anodic wave obtained for each extract and Trolox which were recorded from 100 to 1200 mV. The results were expressed as μ mol Trolox/g d.m.

Determination of flavonoids by HPLC-DAD

The 80% methanol extracts buckwheat hulls and groats were subjected to identification and quantitative analysis of flavonoids with the use of an HPLC system (Shimadzu, Kyoto, Japan), consisting of two pumps (LC-10 AD), DAD detector (SPD-M10A_{VP}) set at 330 nm, autosampler set to 20 μ L injection (SIL-10 AD_{VP}), column oven (CTO-10 AS_{VP}) and system controller (SIL-10 AD_{VP}) (HPLC-DAD). All chromatographic determinations were performed at 35°C with the flow rate of 0.8 mL/min on C18(2) Luna 5 μ m column, 4.6 \times 200 mm (Phenomenex, Torrance, CA, USA). The flavonoids were eluted in a gradient system composed of aqueous 4% formic acid (solvent A) and acetonitrile containing 4% of formic acid (solvent B). Gradients were as follows: 12-22-70-12-12% B at gradient time $t_G=0-9-22-40-45-50$ min. Rutin, quercetin, orientin, homoorientin, vitexin and isovitexin stock solutions were prepared in methanol at the concentration of 500, 500, 517, 477, 509 and 574 μ M, taking into account the purity of the standards. For quantitative analysis, calibration standards were prepared in duplicate at five concentrations within the range of 0.1 – 40 μ M of each compound. All data are the average of triplicate analyses.

Statistical analysis

The results are given as the means and the standard deviation of three independent experiments. Data were subjected to one-way analysis of variance (ANOVA) at a significance level of $P < 0.05$ with Statistica 7.1.30.0 software (Statsoft Inc., USA) for Windows using a PC-Pentium. The correlation analysis was performed and

the Pearson correlation coefficient was calculated.

RESULTS AND DISCUSSION

Antioxidant capacity (AC) of buckwheat hulls and groats determined against ABTS^{•+} and DPPH[•] radicals

The antioxidant capacity of hulls and groats manually separated from common buckwheat, interspecific hybrid and tartary buckwheat seeds determined against ABTS^{•+} and DPPH[•] radicals are shown in **Table 1** and **Table 2**, respectively. The results provided by ABTS assay for buckwheat hulls showed the highest antioxidant capacity of hull from tartary buckwheat accession while about three-fold lower value was noted for hull from *F. esculentum* \times *F. homotropicum* hybrid and almost six times lower for hulls from common buckwheat cultivars (**Table 1**). The antioxidant capacity of hulls originated from common buckwheat cultivars ranged from 6.78 to 11.23 μ mol Trolox/g d.m. The rank of the antioxidant capacity values of hull samples provided by the ABTS assay was as follows: tartary buckwheat accession > interspecific hybrid 'Teresa' > 'Smuglianka' \approx 'Karmen' > 'Vokiai' > 'Anita' > 'Volma'. A similar rank of antioxidant capacity of groats was noted as follows: tartary buckwheat accession > interspecific hybrid 'Teresa' > common buckwheat cultivars. The antioxidant capacity of groats from common buckwheat cultivars ranged from 12.61 to 18.02 μ mol Trolox/g d.m. Groats from common buckwheat cultivars showed higher antioxidant capacity by 33–139% when compared to those provided for the corresponding hulls whilst antioxidant capacity of groat from interspecific hybrid Teresa was higher only by 9%. In contrast, groat from tartary buckwheat local accession showed lower value by 19% than that of hull.

The antioxidant capacity of hulls and groats determined against DPPH[•] radicals confirmed the findings based on ABTS assay (**Table 2**). The same rank of the antioxidant capacity values of hull and groat samples was observed and generally the antioxidant capacity of groat from common buckwheat cultivars was higher by 3–92% in comparison to the respective values of hull. In contrary, the antioxidant

Table 1 Antioxidant capacity of buckwheat hulls and groats provided by ABTS assay (μ mol Trolox/g d.m.).

Buckwheat species	Buckwheat cultivar	Hull*	Groat*
<i>F. esculentum</i>	Karmen	11.11 \pm 0.08 c	18.02 \pm 0.11 e
	Anita	7.69 \pm 0.03 ab	15.36 \pm 0.28 c
	Volma	6.76 \pm 0.04 a	16.20 \pm 0.12 d
	Smuglianka	11.23 \pm 0.05 c	14.97 \pm 0.05 b
	Vokiai	8.14 \pm 0.33 b	12.61 \pm 0.13 a
<i>F. tataricum</i>	Local accession	51.44 \pm 2.23 e	41.67 \pm 0.49 g
<i>F. esculentum</i> \times	Teresa	18.97 \pm 0.32 d	20.63 \pm 0.25 f
<i>F. homotropicum</i>			

* Data expressed as means \pm standard deviations of three independent extractions (n = 3). Means in a column followed by different letters correspond to significant differences ($P < 0.05$).

Table 2 Antioxidant capacity of buckwheat hulls and groats provided by DPPH RSA (μ mol Trolox/g d.m.).

Buckwheat species	Buckwheat cultivar	Hull*	Groat*
<i>F. esculentum</i>	Karmen	6.01 \pm 1.30 b	7.67 \pm 1.18 b
	Anita	3.14 \pm 1.97 a	6.02 \pm 1.17 ac
	Volma	4.33 \pm 0.16 a	7.01 \pm 0.83 ab
	Smuglianka	6.60 \pm 0.89 b	6.81 \pm 1.19 ab
	Vokiai	3.93 \pm 1.21 a	5.24 \pm 0.61 c
<i>F. tataricum</i>	Local accession	36.21 \pm 0.93 d	36.01 \pm 0.68 d
<i>F. esculentum</i>	Teresa	13.36 \pm 1.34 c	7.24 \pm 1.75 ab
<i>F. homotropicum</i>			

* Data expressed as means \pm standard deviations of three independent extractions (n = 3). Means in a column followed by different letters correspond to significant differences ($P < 0.05$).

Table 3 Antioxidant capacity of buckwheat hulls and groats determined by PCL assays ($\mu\text{mol Trolox/g d.m.}$).

Buckwheat species	Buckwheat cultivar	Hull*	Groat*
<i>F. esculentum</i>	Karmen	12.02 \pm 1.44 a	17.01 \pm 0.58 a
	Anita	11.60 \pm 0.25 ab	18.12 \pm 0.18 b
	Volma	12.28 \pm 0.26 ac	17.48 \pm 0.50 ab
	Smuglianka	13.38 \pm 0.80 c	15.74 \pm 0.47 d
	Vokiai	10.76 \pm 1.01 b	12.68 \pm 0.85 c
<i>F. tataricum</i>	local accession	25.02 \pm 0.09 e	29.56 \pm 1.16 f
<i>F. esculentum</i> × <i>F. homotropicum</i>	Teresa	15.61 \pm 1.40 d	19.11 \pm 0.07 e

* Data expressed as means \pm standard deviations of three independent extractions (n = 3). Means in a column followed by different letters correspond to significant differences ($P < 0.05$).

capacity of groat from interspecific hybrid 'Teresa' showed lowered value by 85% than that of hull whilst the same values of antioxidant capacity were noted in hull and groat from tartary buckwheat accession. It is worthwhile to note that values of the antioxidant capacity of hulls and groats provided by the ABTS assay were almost two-fold higher than those provided by the DPPH assay. This relationship was observed previously in samples originating from buckwheat products after hydrothermal treatment and buckwheat sprouts were screened for their antioxidant capacity by these two assays (Zielińska *et al.* 2007a, 2007b). There was a strong positive correlation between the values of antioxidant capacity of hulls and groats determined against ABTS^{•+} and DPPH[•] radicals. A correlation coefficient (r) of 0.99 and 0.98 was obtained for the hull and groat samples, respectively.

Antioxidant capacity of buckwheat hulls and groats provided by the PCL method

In this study, the antioxidant capacity of hull and groat extracts from common buckwheat cultivars, interspecific hybrid and tartary buckwheat local accession represents their ability to scavenge O₂^{•-} radicals generated from luminol, a photosensitizer, when exposed to UV light (Popov and Lewin 1999). The results are compiled in Table 3. The antioxidant capacity of the samples provided by the PCL method was comparable with values obtained by the ABTS assay, thus confirming our previous observation made on different buckwheat products after thermal treatment (Zielińska *et al.* 2007a). The rank of the antioxidant capacity values of the hulls provided by the PCL assay was as follows: tartary buckwheat > interspecific hybrid 'Teresa' > 'Smuglianka' > 'Volma' \approx 'Karmen' > 'Vokiai'. A similar rank was noted for groat samples. As for the hull samples, tartary buckwheat showed 60% higher antioxidant capacity than the hybrid, and twice as high as the common buckwheat cultivars. The same relationship was observed for antioxidant capacity of groats. Moreover, the antioxidant capacity of groats was higher than those noted for hulls. In detail, groats from common buckwheat cultivars showed higher values by 35%, from tartary buckwheat by 18% and from hybrid 'Teresa' by 22% in comparison to the respective values of hulls provided by PCL assay. The values of antioxidant capacity provided by the PCL assay for hulls were highly correlated with those obtained by the ABTS and DPPH RSA assay (r = 0.98 and r = 0.97, respectively). A similar high correlation was found for the antioxidant capacity of groats (r = 0.78 and r = 0.93, respectively).

Reducing capacity of buckwheat hulls and groats determined by CV assay

In this study, we applied the CV for the rapid screening of the reducing capacity of hulls and groats originating from different buckwheat genotypes, and then for comparing the results with those obtained by ABTS, DPPH and PCL assays. The CV method has been recently shown to be con-

Table 4 Reducing capacity of buckwheat hulls and groats determined by cyclic voltammetry (CV) assay ($\mu\text{mol Trolox/g d.m.}$).

Buckwheat species	Buckwheat cultivar	Hull*	Groat*
<i>F. esculentum</i>	Karmen	5.45 \pm 0.87 ab	6.93 \pm 0.25 b
	Anita	4.98 \pm 0.21 a	6.20 \pm 0.50 a
	Volma	4.09 \pm 0.22 d	6.23 \pm 0.35 a
	Smuglianka	6.53 \pm 0.27 c	7.07 \pm 0.18 b
	Vokiai	5.65 \pm 0.33 ab	5.89 \pm 0.22 a
<i>F. tataricum</i>	local accession	11.76 \pm 0.80 e	21.86 \pm 0.60 c
<i>F. esculentum</i> × <i>F. homotropicum</i>	Teresa	6.19 \pm 0.28 bc	6.21 \pm 0.26 a

* Data expressed as means \pm standard deviations of three independent extractions (n = 3). Means in a column followed by different letters correspond to significant differences ($P < 0.05$).

venient for evaluating the reducing/antioxidant capacity of human plasma, animal tissues, edible plants, wines, different types of tea and coffee, buckwheat sprouts and buckwheat products after thermal treatment (Chevion *et al.* 2000; Kilmartin *et al.* 2001; Kilmartin and Hsu 2003; Zielińska *et al.* 2007a, 2007b) as well as for the determination of the reducing/antioxidant activity of flavonoids (Zielińska *et al.* 2010; Zielińska and Zieliński 2011).

The representative cyclic voltammograms of the hull and groat from common buckwheat cv. Volma recorded from -100 to +1300 mV at a scanning rate of 100 mV s⁻¹ is shown in Fig. 1. The observed anodic wave was broadened due to the response of several antioxidants with different oxidation potentials, including mainly polyphenols (Watanabe 1998; Holasaova *et al.* 2002; Fabjan *et al.* 2003; Zieliński *et al.* 2006; Jiang *et al.* 2007; Morishita *et al.* 2007; Ragubeer *et al.* 2010). Therefore, the area under the curve (AUC) was taken to reflect the total antioxidant capacity of the samples (Chevion *et al.* 2000; Ragubeer *et al.* 2010). The higher AUC indicated a higher antioxidant capacity of the investigated extracts. The results provided by CV method are compiled in Table 4. The antioxidant capacity of hull from tartary buckwheat was the highest, being almost two-fold higher than those values obtained for hull from interspecific hybrid 'Teresa' and common buckwheat cultivars. In the case of groats, tartary buckwheat showed about three-fold higher antioxidant capacity in comparison to groats from hybrid 'Teresa' and from common buckwheat cultivars. The applied CV method confirmed clearly the highest antioxidant capacity of hull and groat from tartary buckwheat as was shown with ABTS, DPPH RSA and PCL assays. However, results showed comparable levels of antioxidant capacity in hull and groats from interspecific hybrid 'Teresa' and common buckwheat cultivars which was not observed by three previously applied assays. The values of antioxidant capacity of hulls and groats showed the same level as those provided by DPPH RSA assays, thus confirming our previous finding related to the antioxidant capacity of thermally treated buckwheat evaluated by CV and DPPH RSA assay (Zielińska *et al.* 2007a). The values of antioxidant capacity of hulls and groats from common, tartary and hybrid species obtained by CV method were highly correlated with those obtained by ABTS assay (r = 0.97 and r = 0.97, respectively), by DPPH RSA assay (r = 0.96 and r = 1.00, respectively), and by PCL assay (r = 0.94 and r = 0.92, respectively). Therefore, the applied analytical strategy for the determination of the antioxidant capacity of hulls and groats, based on the four analytical assays offering different chemical principles, was a useful quality parameter of the hulls and groats from common, tartary and a new hybrid buckwheat varieties. The applied methods provided the same rank of the antioxidant capacity among hulls and groats however the real antioxidant capacity values were different. The rank of values of the antioxidant capacity vs. applied methods was as follows: ABTS assay \approx PCL assay \gg DPPH RSA \approx CV assay.

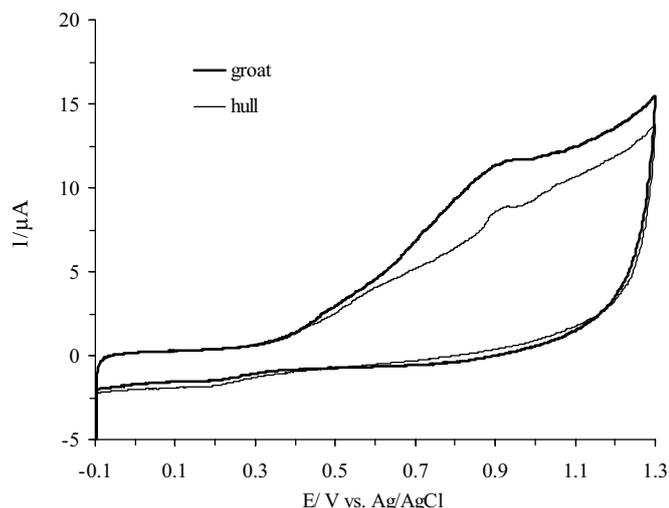
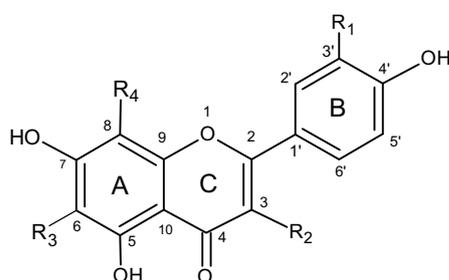


Fig. 1 The representative cyclic voltammograms of the hull and groat from common buckwheat cv. ‘Volma’. Operative conditions: concentration of each extract (100 mg/mL 80% methanol); solution, 80% methanol mixed with 0.2 M sodium acetate-acetic buffer (pH 4.5) at ratio 1: 1 (v/v); scan rate 100 mV s⁻¹.



Name	R ₁	R ₂	R ₃	R ₄
rutin	OH	rham-glu	H	H
quercetin	OH	OH	H	H
orientin	OH	H	H	glu
homoorientin	OH	H	glu	H
vitexin	H	H	H	glu
isovitexin	H	H	glu	H

glu: glucose; rham-glu: rhamnose-glucose

Fig. 2 Chemical structure of buckwheat flavonoids: Rutin, quercetin, orientin, homoorientin, vitexin and isovitexin.

Flavonoid composition and content in buckwheat hull and groat

Flavonoid composition and their content in buckwheat hull and groat extracts were determined using a high-performance liquid chromatographic (HPLC) separation method coupled with photodiode array (PDA) detection. Column chromatography on a C₁₈ support allowed us to separate rutin, quercetin and flavone C-glucosides both from hull and groat of each buckwheat genotypes. The chemical structures of rutin, quercetin and flavone C-glucosides (orientin, homoorientin, vitexin and isovitexin) are shown in **Fig. 2**, whereas a typical HPLC chromatogram of flavonoids recorded at 330 nm from hull and groat of common buckwheat cv. ‘Volma’ is shown in **Fig. 3A** and **Fig. 3B**, respectively.

The content of flavonoids in buckwheat hulls provided by HPLC-DAD is shown in **Table 5**. Rutin was the major buckwheat flavonoid and its content was the highest in the hull of the tartary buckwheat accession (847 μg/g d.m.), being almost three-fold higher than in *F. esculentum* × *F. homotropicum* hybrid and eighteen-fold higher than the average content in common buckwheat cultivars. The content of quercetin was up to 22 times lower when compared to rutin content, depending of buckwheat genotypes. The hull from common buckwheat and from interspecific hybrid

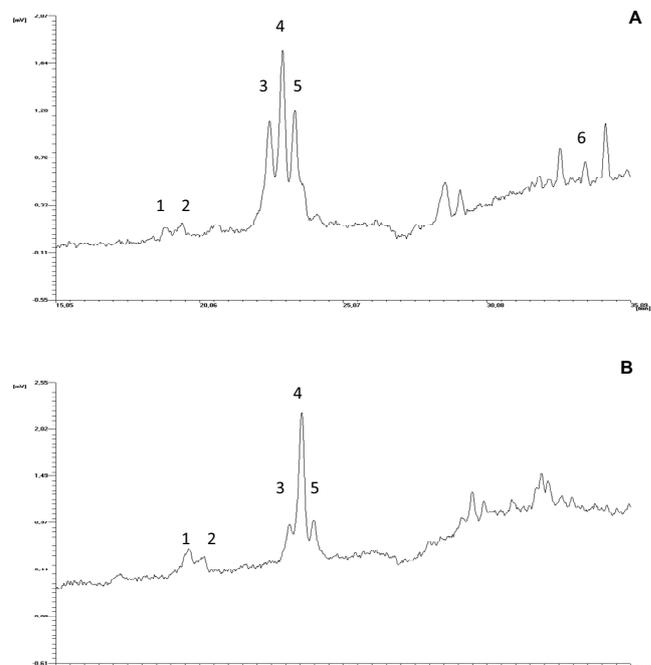


Fig. 3 Typical HPLC chromatograms of flavonoids recorded at 330 nm from (A) hull and (B) groat of common buckwheat cv. ‘Volma’. Peak labeled: (1) homoorientin, (2) orientin, (3) vitexin, (4) rutin, (5) isovitexin, (6) quercetin. The preparation of extracts injected into HPLC column was as described in ‘Materials and methods’.

‘Teresa’ contained also small quantity of flavone C-glucosides (orientin, homoorientin, vitexin and isovitexin) whilst only orientin was detected in hull from tartary buckwheat. Among flavone C-glucosides vitexin and isovitexin contents were about six-fold higher than content of orientin and homoorientin.

Rutin, quercetin and orientin formed about 95.3, 4.2 and 0.5% of total flavonoid content in hull from tartary buckwheat, whilst contribution of rutin, quercetin and flavone C-glucosides in the hull from hybrid ‘Teresa’ was 54.2, 2.7 and 43.1%, respectively (**Table 5**). There was a similarity in the contribution of flavonoids in the hulls of the hybrid and the common buckwheat cultivars. Rutin, quercetin and flavone C-glucosides formed an average of 34.6–58%, 3.4–8.9% and 43–54.8% of the total flavonoid content in the particular type of buckwheat. These findings showed significant differences in the content and composition of flavonoids in the hull from tartary buckwheat accession when compared to those noted in hulls from hybrid and common cultivars. Therefore, it can be concluded that the allelopathic potential (Golisz *et al.* 2007), protection against harmful effects of UV-B radiation and diseases (Fabjan *et al.* 2003) and antioxidant protective screen in tartary buckwheat seed is mainly formed by rutin whilst in hybrid and common buckwheat seeds it is formed both by rutin and flavone C-glucosides. The protective site related to the flavonoid composition of buckwheat hulls may represent a quality index of different varieties. Moreover, buckwheat hulls may in some areas of large scale production pose some problems and the question of how to utilize this waste material is still not fully resolved. The content of rutin, quercetin as well as total flavonoids was highly correlated with the antioxidant capacity provided by ABTS assay ($r = 0.99$, $r = 0.97$ and $r = 0.92$, respectively), by DPPH RSA assay ($r = 0.99$, $r = 0.96$ and $r = 0.93$, respectively), by PCL assay ($r = 0.97$, $r = 0.95$ and $r = 0.92$, respectively) and by CV assay ($r = 0.93$, $r = 0.90$ and $r = 0.85$, respectively). In contrary, no correlation was observed between antioxidant capacity of hulls and their flavone C-glucosides content.

The content of flavonoids in buckwheat groats is shown in **Table 6**. Rutin was the major buckwheat flavonoid with the highest content in tartary buckwheat (552 μg/g d.m.)

Table 5 Content of flavonoids in buckwheat hulls provided by HPLC-DAD ($\mu\text{g/g d.m.}$)*.

Buckwheat species	Buckwheat cultivar	Rutin	Quercetin	Flavone C-glucosides				Total content
				Orientin	Homoorientin	Vitexin	Isovitexin	
<i>F. esculentum</i>	Karmen	32.47 ± 0.03 b	4.30 ± 0.10 c	5.70 ± 0.08 b	4.73 ± 0.01 e	23.18 ± 0.01 d	23.56 ± 0.06 e	93.94 ± 0.21 a
	Anita	52.31 ± 0.44 a	9.73 ± 0.14 d	5.03 ± 0.03 a	4.50 ± 0.02 d	19.51 ± 0.04 c	17.70 ± 0.05 c	108.78 ± 0.42 d
	Volma	43.21 ± 0.11 c	4.92 ± 0.07 a	3.46 ± 0.01 c	2.15 ± 0.02 a	17.94 ± 0.10 a	15.77 ± 0.01 b	87.45 ± 0.05 b
	Smuglianka	53.39 ± 1.83 a	3.49 ± 0.01 b	4.98 ± 0.09 a	2.71 ± 0.01 b	18.06 ± 0.03 a	20.67 ± 0.02 d	103.30 ± 1.47 c
	Vokiai	54.06 ± 1.10 a	4.97 ± 0.09 a	5.70 ± 0.08 b	3.23 ± 0.02 c	17.45 ± 0.05 b	7.82 ± 0.02 a	93.23 ± 0.90 a
<i>F. tataricum</i>	Local accession	847.32 ± 3.30 e	37.75 ± 0.09 f	4.39 ± 0.04 d	n.d.	n.d.	n.d.	889.46 ± 2.38 e
	Teresa	330.42 ± 2.19 d	16.40 ± 0.06 e	33.42 ± 0.01 e	28.61 ± 0.06 f	119.53 ± 0.83 f	81.52 ± 0.10 f	609.90 ± 2.06 f

× *F. homotropicum*

n.d. – not detected

* Data expressed as means ± standard deviations of three independent extractions (n = 3). Means in a column followed by different letters correspond to significant differences ($P < 0.05$).**Table 6** Content of flavonoids in buckwheat groats provided by HPLC-DAD ($\mu\text{g/g d.m.}$)*.

Buckwheat species	Buckwheat cultivar	Rutin	Quercetin	Flavone C-glucosides				Total content
				Orientin	Homoorientin	Vitexin	Isovitexin	
<i>F. esculentum</i>	Karmen	22.28 ± 0.25 b	n.d.	3.72 ± 0.01 d	2.85 ± 0.01 c	2.72 ± 0.01 c	2.07 ± 0.01 d	33.64 ± 0.22 d
	Anita	20.22 ± 0.03 a	n.d.	2.26 ± 0.01 a	2.13 ± 0.02 b	2.92 ± 0.01 e	1.43 ± 0.01 b	28.96 ± 0.04 b
	Volma	23.36 ± 0.05 b	n.d.	3.30 ± 0.01 c	3.93 ± 0.01 a	3.45 ± 0.03 f	3.27 ± 0.01 f	37.31 ± 0.03 e
	Smuglianka	19.26 ± 0.01 a	n.d.	n.d.	n.d.	0.95 ± 0.01 a	1.08 ± 0.01 a	21.29 ± 0.02 a
	Vokiai	20.02 ± 0.16 a	n.d.	3.83 ± 0.01 e	3.39 ± 0.07 d	2.80 ± 0.02 d	1.68 ± 0.01 c	31.72 ± 0.06 c
<i>F. tataricum</i>	Local accession	551.78 ± 2.74 d	254.77 ± 0.86	47.68 ± 0.07 f	20.01 ± 0.10 e	n.d.	n.d.	874.24 ± 2.57 f
<i>F. esculentum</i>	Teresa	9.64 ± 0.01 c	n.d.	3.20 ± 0.01 b	3.89 ± 0.03 a	2.19 ± 0.01 b	2.58 ± 0.01 e	21.50 ± 0.03 a

× *F. homotropicum*

n.d. – not detected

* Data expressed as means ± standard deviations of three independent extractions (n = 3). Means in a column followed by different letters correspond to significant differences ($P < 0.05$).

The value obtained is 26-times higher than those obtained in common buckwheat cultivars and almost 60-times higher than that obtained in the interspecific hybrid, the difference being significant. In contrast to the flavonoid composition of the hulls (Table 5), quercetin was only found in groat from tartary buckwheat accession (Table 6). The rutin and quercetin accounted for 63 and 29% of the total flavonoid content in groats from the tartary buckwheat accession. Moreover, a significantly higher content of flavone C-glucosides, namely orientin (16-times higher) and homoorientin (6-times higher), were noted in groat from tartary buckwheat accession than in groats from common buckwheat cultivars and the interspecific hybrid. The flavone C-glucosides formed almost 8% of the total flavonoid content as vitexin and isovitexin were not detected. The rutin and flavone C-glucosides formed about 45 and 55% of total flavonoid content in the groat from hybrid Teresa (Table 6) whilst these compounds formed an average 62.6–69.8% (rutin) and 30.2–37.4.8% (flavone C-glucosides) of the total flavonoid content in the from common buckwheat cultivars. One exception was found for common buckwheat cv. 'Smuglianka', for which a high contribution of rutin (90.5%) was due to the very low content of vitexin and isovitexin, and lack of orientin and homoorientin (Table 6). These findings showed significant differences in the content and composition of flavonoids in the groat from tartary buckwheat when compared to those noted in groats from interspecific hybrid and common buckwheat cultivars. The protective site related to the flavonoid composition of buckwheat groats is less clear as a quality index of different genotypes, due to the surprising low content of flavonoids in groat from hybrid Teresa. The content of rutin, flavone C-glucosides as well as total flavonoids was highly correlated with antioxidant capacity provided by ABTS assay ($r = 0.96$, $r = 0.96$ and $r = 0.97$, respectively), by DPPH RSA assay ($r = 0.99$, $r = 0.98$ and $r = 0.99$, respectively), by PCL assay ($r = 0.91$, $r = 0.91$ and $r = 0.91$, respectively) and by CV assay ($r = 1.00$, $r = 0.97$ and $r = 1.00$, respectively).

It should be pointed out that the content of rutin and flavone C-glucosides in groats from common buckwheat cultivars and the interspecific hybrid were lower than those noted in the hulls. As a result, the total flavonoid contents were three-fold lower in common buckwheat cultivars and

28 times lower in the interspecific hybrid. In contrast, groat and hull from the tartary buckwheat accession contained comparable high levels of flavonoids. Therefore, the results obtained in this study may be useful for research related to breeding buckwheat lines (Öschlager *et al.* 2010), for herbal practitioners looking for new materials rich in polyphenols (Zielińska *et al.* 2009; Baumgertel *et al.* 2010) as well as for the food and pharmaceutical industry due to the described antyglycation activity of rutin, quercetin, flavone C-glucosides and their metabolites (Cervantes-Laurean *et al.* 2006; Peng *et al.* 2008; Pashikanti *et al.* 2010; Wronkowska *et al.* 2010).

CONCLUSIONS

The applied analytical strategy for the determination of the antioxidant capacity of hulls and groats, based on the four analytical assays offering different chemical principles, was a useful quality parameter of the hulls and groats from different buckwheat genotypes. The applied methods provided the same rank of the antioxidant capacity among hulls and groats however the real antioxidant capacity values were different. The rank of values of the antioxidant capacity vs. applied methods was as follows: ABTS assay \approx PCL assay \gg DPPH RSA \approx CV assay. The content of rutin and flavone C-glucosides in groats from common buckwheat cultivars and interspecific hybrid of buckwheat were lower than those noted in the hulls. In contrast, groat and hull from tartary buckwheat local accession contained comparable high levels of flavonoids. The extent of variation in the content of rutin, quercetin and flavone C-glucosides in buckwheat hulls may be useful for researchers working on the resistance of buckwheat lines against pathogens and diseases whilst extent of variation in the flavonoid composition in groats should be important from the nutritional point of view. Therefore, flavonoid composition of the hulls and groats from buckwheat genotypes may serve as a quality index. These are prerequisites for breeding buckwheat lines with optimized antioxidant/reducing power and flavonoid composition for various functional properties. The results indicate that some buckwheat cultivars have a potential role in the development of natural health products, either as functional foods or nutraceuticals.

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