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DETERMINATION OF THE ANTIOXIDANT ACTIVITY OF RUTIN AND ITS CONTRIBUTION TO THE ANTIOXIDANT CAPACITY OF DIVERSIFED BUCKWHEAT ORIGIN MATERIAL BY UPDATED ANALYTICAL STRATEGIES

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Key words: rutin, antioxidant activity, contribution to buckwheat antioxidant capacity, spectrophotometric methods, photochemiluminescence, cyclic voltammetry

The antioxidant activity of rutin and selected common buckwheat-originated materials, namely groat, hull, flour and sprouts were measured against stable, non-biological radicals such as 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical cation ($ABTS^{\bullet+}$) and 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) using a spectrophotometric assay, against the key reactive oxygen intermediate – superoxide anion radical ($O_2^{-\bullet}$) with a photochemiluminescence assay (PCL) while reducing capacity was determined with the cyclic voltammetry method (CV). The antioxidant activity was presented independently of the techniques used as TEAC values and then the contribution of rutin to the antioxidant capacity of buckwheat material was calculated. The order of the antioxidant activity of rutin provided by the updated analytical strategy was as follows: CV = DPPH RSA > PCL > ABTS RSA. Buckwheat groat and hull represented low antioxidant capacity samples whilst that of sprouts was ranked as high antioxidant capacity one. The results showed a low contribution of rutin to the antioxidant capacity of buckwheat groat and hull. The highest contribution of rutin to the antioxidant capacity was noted in buckwheat sprouts, especially those produced in light. The specificity of the rank of methods used as CV = DPPH RSA > ABTS RSA > PCL was concluded since the lowest antioxidant gap was provided with CV and DPPH RSA whilst the highest one with ABTS RSA and PCL.

INTRODUCTION

Flavonoids, a large group of plant polyphenol secondary metabolites, are widely distributed in medicinal plants, fruits, teas and health beverages [Manach *et al.,* 2004]. Interest in the flavonoids stems from their diversity, biological significance as secondary plant metabolites, their impact on essential plant growth, development, stress adaptation and defense. Besides the importance for the plant itself, flavonoids are part of the human diet. In consequence, commercial interest in these compounds as well as in flavonoid-rich plant sources is considerable. All of these aspects justify the intense interest in flavonoids which has been manifested over several decades [Robards & Antolovich, 1997; Walle, 2004].

Buckwheat refers to any member of the *Fagopyrum* family (*Polygonaceae*). There are many species of buckwheat world wide, and mainly nine species have agricultural meaning. 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*) is commonly grown, while *F. tartaricum* is grown in some mountainous region [Jiang *et al*., 2007].

The presence of rutin (quercetin-3-rhamnosyl glucoside), suggested as the main buckwheat flavonoid, was reported in buckwheat green parts [Oomah & Mazza, 1996]. Common buckwheat seeds (both groat and hull) and sprouts are an important source of rutin [Zielińska *et al*., 2007a,b]. In general, a rutin level is much lower in common buckwheat seeds than in other buckwheat species [Shevchuk, 1983]. Rutin and other flavonoids are UV-B absorbing plant metabolites in order to protect the seeds from the harmful effects of UV-B radiation and diseases [Fabjan *et al.,* 2003]. Various biological and pharmacological activities have been attributed to rutin. The most sound evidences of potentially-beneficial effect of rutin as well as buckwheat origin from *in vitro* studies and from experiments with animals. Rutin is known for its anti-inflammatory and vasoactive properties, as well as for its capability to diminish capillary permeability and to reduce the risk of arteriosclerosis, whereby reducing coronary heart disease, possibly through the diminishing of platelet aggregation [La Casa *et al.,* 2000; Jiang *et al.,* 2007]. There are also studies that show a dose-response effect of rutin in inhibiting low-density lipoprotein (LDL) peroxidation [Jiang *et al.,* 2007], and the antioxidant activity of rutin in Fenton reaction [Cailet *et al.,* 2007].

These properties are potentially beneficial in preventing diseases and protecting the stability of the genome. Rutin has also displayed protective effects against ethanol-induced

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gastric lesions [La Casa *et al.,* 2000], against DNA damage [Zhao *et al.,* 2003; Undeger *et al*., 2004]. It is also a protective agent against carcinogenesis [Webster *et al.,* 1996]. Rutin was suggested as one of the most potent natural inhibitors of advanced glycation endproducts (AGEs) accumulation in human body, suggesting that its anti-glycation activity may mainly be due to its radical scavenging activity [Peng *et al.,* 2008]. Up till now, only a few studies have been performed on humans in this respect. Probably, the first study was described by Griffith *et al.* [1944]. He *et al.* [1995] reported a hypocholesterolemic effect in humans after the intake of buckwheat products.

The antioxidant activity of rutin has been studied in various model systems [Miller & Rice-Evans, 1996; Rice-Evans *et al.,* 1996; Re *et al.,* 1999; Pietta, 2000; Aliaga & Lissi, 2004; Prior *et al.,* 2005; Balasundram *et al.,* 2006; Yang *et al.,* 2008], but no information exist in respect to the relative contribution of rutin to the antioxidant activity of buckwheat-originated material.

A great multiplicity of methods have been used to evaluate the antioxidant activity of polyphenols by using different techniques of inducing and catalyzing oxidation and measuring the end point of oxidation for foods and biological systems [Frankel & Finley, 2008; Magalhaes *et al*., 2008]. More recently, a highly attractive, convenient and sensitive voltammetric and photochemiluminescene approaches to study antioxidant activities have been reported [Cosio *et al.,* 2006; Besco *et al.,* 2007; Zielińska *et al.,* 2007a].

In this study, for the first time, the antioxidant activity of rutin was measured in parallel with buckwheat-originated material by means of spectrophotometric assays (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonate) Radical Scavenging Activity, ABTS RSA; 2,2-diphenyl-1-picrylhydrazyl Radical Scavenging Activity, DPPH RSA, photochemiluminescence (PCL) and cyclic voltammetry (CV) methods. The contribution of rutin to the antioxidant capacity of buckwheat material might be a tool for a better selection of buckwheat-based material for different applications in functional food formulations and then for preventing many diseases resulting from the increased intake of buckwheat-based products.

Therefore, an application of the determined antioxidant activity of rutin for the calculation of its contribution to the total antioxidant capacity of selected buckwheat samples such as groat, hull, flour and sprouts of common buckwheat was provided based on the updated analytical strategies.

MATERIALS AND METHODS

Reagents

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) 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. ACL (Antioxidant Capacity of Lipid-soluble substances) kit (no. 400.801) for the PCL assay was from Analytik Jena AG (Jena, Germany). All other reagents of reagent-grade quality were from POCh, Gliwice, Poland. Water was purified with a Mili-Q-system (Milipore, Bedford, USA).

Buckwheat-originated samples

Seeds were obtained from common buckwheat variety Volma (originated from Bialorus) which was sown on an experimental field in Bałcyny near Ostróda at the Production-Experimental Station of the University of Warmia and Mazury in Olsztyn. The groat and hull were manually separated from ripe seeds in the laboratory, and then immediately freeze-dried. The lyophilized material was milled into dust and stored at -40°C in polyethylene bags until analysed. The buckwheat flour "BIO" was provided from a healthy food store in Olsztyn, Poland. According to the producer's information, it was obtained from common buckwheat variety Kora, commonly cultivated in North Poland, as a product consistent with the ecological agriculture requirements. The flour was freeze-dried and stored at -40°C until analysed. The buckwheat sprouts were produced from common buckwheat variety Luba. The seeds for germination were provided by the Plant Breeding Station in Palikole, Poland. Whole buckwheat seeds (25 g) were soaked in 125 mL of distilled water at a room temperature and shaken every 30 min. After 12 h, water was drained off and the seeds were transferred to an incubator (Cliambic Cabinet, model Economic Deluxe EC00-065, Snijders Scientific b.v, Netherlands). Sprouting was carried out at a temperature of 25°C and humidity of 95%, with or without 24 h exposition to light. Buckwheat sprouts were harvested 10 days after seeding (DAS) and then lyophilized.

Rutin standard solutions

The chemical structure of rutin is shown in Figure 1. An appropriate amount of standard was dissolved in methanol and its concentration was confirmed by a UV measurement according to Wiczkowski *et al*. [2003] and Franke *et al.* [2004]. For the measurement of the antioxidant activity with the TEAC assay and cyclic voltammetry, exactly 500 μmol/L concentration of a rutin solution was prepared, whilst for the DPPH RSA and PCL assays the solution was prepared in methanol up to 150 μmol/L and 100 μmol/L, respectively.

Preparation of buckwheat-originated samples

About 100 mg of dried and pulverized buckwheat material were extracted with 1 mL of 80% (v/v) methanol by 30 s sonication. Next, the mixture was vortexed for 30 s, again sonicated and vortexed, and centrifuged for 5 min (5,000 x *g* at 4°C). That step was repeated 5 times and the resultant supernatants were collected into a 5-mL flask. For the cyclic voltammetric experiments, the extraction of buckwheat mate-

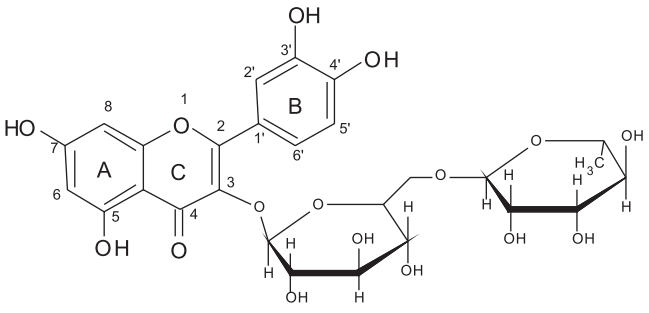

FIGURE 1. Chemical structure of rutin.

rial was carried out as follows: about 500 mg of dried and pulverized buckwheat samples were extracted with 2.5 mL of 80% methanol by 60 s sonication. Next, the mixture was vortexed for 60 s, again sonicated and centrifuged for 5 min (5,000 x *g*, 4°C). That step was repeated on the residue with next volume of 2.5 mL of the solvent. Supernatants were collected into 5-mL flask. Finally, all extracts were kept at -80°C prior to further analysis. The dry matter content of buckwheat groat, hull, flour "Bio", 10 DAS sprouts cultivated in dark and 10 DAS sprouts cultivated in light was 96.2, 97.4, 87.6, 20.7 and 11.4%, respectively. Separated extractions of each sample were performed in triplicate.

Measurement of the antioxidant activity of rutin and buckwheat-originated samples with the ABTS RSA assay

The method described by Re *et al.* [1999] was used to determine the antioxidant activity of rutin and buckwheat-originated material. The analytical strategy of ABTS RSA assay was based on the reduction in the absorbance of the $ABTS^{\bullet+}$ solution at 734 nm. For measurements, the $ABTS^{\bullet+}$ solution was diluted with 80% (v/v) methanol, respectively, to the absorbance of 0.70±0.02 at 734 nm. For the spectrophotometric assay, 1.48 mL of the $ABTS^{\bullet+}$ solution and 20 μL of rutin standard solution (500 μmol/L) or buckwheat extract (20 mg/mL) were mixed and absorbance was measured immediately after 6 min at 734 nm at 30(C. The $ABTS^{\bullet+}$ working solution was used as a blank sample. The standard curve based on the length of time of the lag phase *vs*. Trolox concentration was constructed within the range of 0.1-2.5 mmol/L of Trolox ($y=35.29x+3.75$; $R^2=0.99$). The measurements were carried using a temperature-controlled spectrophotometer UV--160 1PC with CPS-Controller (Shimadzu, Japan).

Determination of DPPH Radical Scavenging Activity (DPPH RSA) of rutin and buckwheat-originated samples

Measurement of DPPH RSA was based on the reduction in the absorbance of the $DPPH^{\bullet}$ solution at 515 nm. The $DPPH^{\bullet}$ scavenging activity was determined using rutin standard solution (150 μmol/L) or buckwheat extract (20 mg/mL) as described previously in details [Zielińska *et al.,* 2007b]. The Trolox standard solutions within the concentration range of 0.1–2.5 mmol/L in methanol were assayed under the same conditions, and the liner response of Trolox concentrations was used for standard curve construction ($y=84.66x+1.26$; $R^2=0.99$). Then $DPPH^{\bullet}$ scavenging activity of the buckwheat samples was expressed in terms of Trolox equivalent antioxidant capacity.

Determination of the antioxidant activity of rutin and buckwheat-originated samples with the photochemiluminescence method (PCL)

The photochemiluminescence assay has combined very fast photochemical excitation of radical generation with the highly sensitive luminometric detection. Because of the high sensitivity of the chemiluminescence of luminol, only nanomolar concentrations of non-enzymatic antioxidant substances are required to observe the photochemiluminesce effect and therefore the stock solution of rutin was further diluted with methanol according to the measurement requirements. The antioxidant activity of rutin (100 μmol/L) and buckwheat extracts (20 mg/mL) was evaluated by using the Photochem® device, and the PCL kit supplied by Analytik Jena AG. The principles of the assay have been described recently [Besco *et al*., 2007]. The antioxidant activity of rutin represents its ability to scavenge $O_2^{-\bullet}$ radicals generated from luminol, a photosensitizer, when exposed to UV light. The detailed protocol was carried out as previously described [Zielińska *et al.,* 2007b].

Measurement of the antioxidant activity of rutin and buckwheat-originated samples with the cyclic voltammetry (CV)

A potentiostat KSP system (Poland) was used for voltammetric experiments. A conventional three-electrode system: (a) a 3 mm diameter glassy carbon working electrode (BAS MF-2012), (b) an Ag/AgCl electrode as a reference electrode, and (c) a platinum as a counter electrode was used in the study. Cyclic voltammetric experiments were performed with rutin standard solutions (500 μmol/L) or buckwheat extracts (100 mg/mL) mixed with 0.2 mol/L sodium acetate-acetic buffer (pH 4.5 in 80% (v/v) methanol) at the ratio of 1:1 (v/v) according to Cosio *et al*. [2006]. The sodium acetate-acetic buffer acted also as a supporting electrolyte for the cyclic voltammetry measurements. The voltammetric experiments were performed at a room temperature using apparatus cell of 200 μL volume, to which the analyzed standard solution mixed previously with the buffer solution was introduced. The cyclic voltammograms were acquired in the range of -100 to +800 mV and from -100 to +1300 mV at a scanning rate of 100 mV s^{-1}. Prior to use, the surface of the glassy carbon electrode was carefully polished with 0.05 μm alumina paste and ultrasonically rinsed in deionized water and afterwards washed with methanol. This procedure was repeated after each cycle. For the test purpose, the total charge below the anodic wave curve of the voltammogram was calculated. The CV method is actually based on the correlation between the total charge below anodic wave of cyclic voltammograms and the antioxidant activity of the compound under investigation. The cyclic voltammograms of Trolox solutions within the concentration range of 0.1–2.5 mmol/L were also acquired in the range of -100 to +800 mV and from -100 to +1300 mV, and then the linear response of Trolox concentrations was used for respective standard curves construction ($y=82.59x+3.75$; $R^2=0.99$ and $y=121.13x+4.25$; $R^2=0.99$). They were next applied to express the antioxidant activity of rutin and antioxidant capacity of buckwheat-originated samples as Trolox equivalent antioxidant capacity. The total charge under the anodic wave of the background signal (solvent + supporting buffer) was subtracted from the total charge under the anodic wave obtained for each compound and Trolox which were recorded firstly within the range of -100 to +800 mV and secondly from -100 to +1300 mV.

Determination of rutin content in buckwheat material

The content of rutin in buckwheat-originated samples was determined with the HPLC as it was recently described by Zielińska *et al.* [2007a]. All solutions prepared for HPLC were filtered through a 0.45 μm nylon membrane before use.

Contribution of rutin to the antioxidant capacity of buckwheat samples

The contribution of rutin, based on its antioxidant activity provided by ABTS RSA, DPPH RSA, PCL and CV, to the antioxidant capacity of selected buckwheat samples determined with the above-listed methods, was calculated. To this end, it was taken into account that the content of rutin, determined with the HPLC method, was multiplied by its antioxidant activity provided by the applied methodology strategy. After that, the contribution was divided by the antioxidant capacity of buckwheat groat, hull, flour and sprouts, and finally expressed as percentage of contribution.

Statistical analysis

The results are given as the means and the standard deviation of three independent extractions (n=3). Statistical analysis was applied for the comparison of the means and then performed using Fischer LSD test and a significance level was set at $p<0.05$.

RESULTS AND DISCUSSION

The antioxidant activity of rutin determined with ABTS RSA, DPPH RSA, PCL and CV methods

In this study, the antioxidant activity of rutin was expressed as Trolox Equivalent Antioxidant Activity. A special focus was put on the CV experiments. In this case, the cyclic voltammogram of rutin was recorded in the range of -100 to +800 mV and from -100 to +1300 mV at a scanning rate of 100 mV s^{-1} as it was shown in Figure 2. The selection of these two ranges of the applied potentials was due to the clear discrimination of the electrochemical oxidation process corresponding to the oxidation of 3',4'-dihydroxyl moiety at ring B, and that noted at a higher potential corresponding to the oxidation of 5,7-dihydroxyl moiety at ring A of a rutin molecule. The recorded cyclic voltammogram in the range of -100 to +800 mV showed a well-defined reversible wave with oxidation peak potentials of 0.41 V (*vs.* Ag/AgCl). When cyclic voltammogram of rutin was recorded in the range of -100 to +1300 mV (Figure 3), the second oxidation peak occurred at potentials of 1.09 V, corresponding to an irreversible reaction which involves the 5,7-dihydroxy group. When the calculation of the antioxidant activity of rutin was based on the area under the anodic current waveform within the range from +100 to +800 mV provided for Trolox, then the antioxidant activity reflected only the activity corresponding to the catechol group in ring B and double bond between C-2 and C-3, conjugated with the 4-oxo group in ring C. In contrast, when calculation was made within the range from +100 to +1300 mV and then compared to Trolox (Figure 3), the antioxidant activity of rutin was almost three fold higher. Therefore, it was suggested that in order to evaluate the antioxidant activity of rutin with the CV method, the cyclic voltammograms need to be recorded up to +1300 mV.

The antioxidant activity of rutin provided with the ABTS RSA, DPPH RSA, PCL and CV methods is shown in Figure 4. The antioxidant activity of rutin against $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals was with an excellent agreement to that reported by Re *et al.* [1999] but was lower as compared to that determined by Rice-Evans *et al*. [1996] when $ABTS^{\bullet+}$ radical cation was formed by the interaction of ABTS with the ferrylmyoglobin radical species, generated by the activation of metmyoglobin with H_2O_2. The antioxidant activity of rutin was slightly lower than the theoretical calculated as 1.5 Trolox equivalent [Aliaga & Lissi, 2004]. This observation was supported by a recent study into a structure-activity relationship (SAR) of flavonoids [Balasundram *et al.,* 2006].

The ability of rutin to scavenge superoxide radicals was in agreement to that reported by Watanabe [2007] who showed that rutin scavenged $O_2^{-\bullet}$ produced in the xanthine/xanthine oxidase system in a dose-dependent manner. Our findings support also a very early investigation of $O_2^{-\bullet}$ scavenging properties of 38 flavonoids in a non-enzymatic system

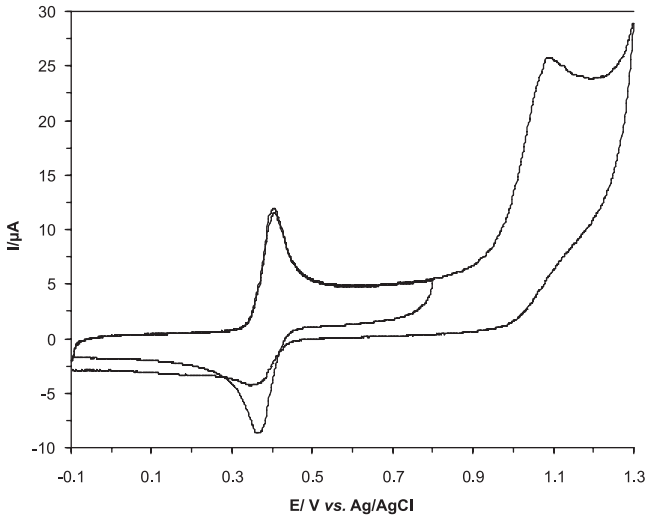

FIGURE 2. The cyclic voltammograms of 0.25 mmol/L of standard solution (final concentration) of rutin in 0.1 mol/L sodium acetate-acetic buffer (final concentration at pH 4.5) in 90% methanol recorded from -100 to +800 mV and from -100 to +1300 mV; scan rate 100 mV s^{-1}.

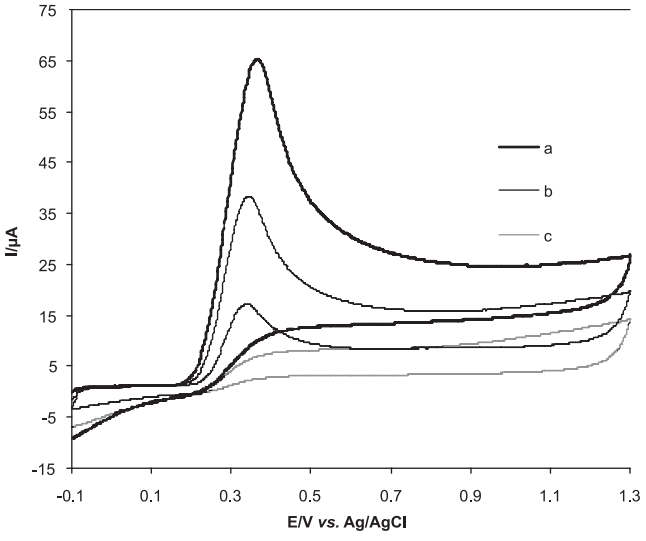

FIGURE 3. Selected cyclic voltammograms of (a) 2.5 mmol/L, (b) 1.25 mmol/L and (c) 0.5 mmol/L of Trolox solution (final concentration) in 0.1 mol/L sodium acetate-acetic buffer (final concentration at pH 4.5) in 90% methanol recorded from -100 to +1300 mV; scan rate 100 mV s^{-1}.

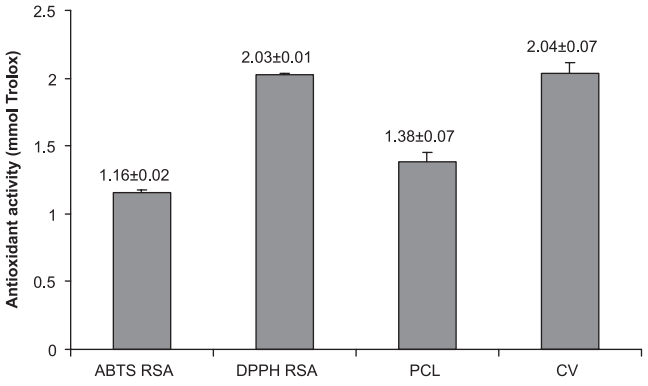

FIGURE 4. The antioxidant activity of rutin determined by ABTS RSA, DPPH RSA and PCL methods (n=6). Data are expressed as means ± standard deviation (n = 6).

isolated from *Sideritis mugronensis*, *Sideritis javalambrensis* and *Cayaponia tayuya* [Huguet *et al.,* 1990].

The antioxidant activity of rutin derived from CV experiments reflected the presence of the catechol group in ring B, double bond between C-2 and C-3, conjugated with the 4-oxo group in ring C and resorcinol group in ring A. It was clearly indicated that catechol group in ring B was more easily oxidizable than the resorcinol group in ring A of a rutin molecule. The provided electrochemical behavior of rutin was in agreement to the last report related to rutin and quercetin [Brett & Ghica, 2003; Aliaga & Lissi, 2004; Ghica & Brett, 2005; Timbola *et al.,* 2006; Zielińska *et al.,* 2008]. This finding was in agreement to the recent work by Blasco *et al.* [2005] in which differentiation of the antioxidant power of phenolic compounds was based on values of their oxidation potentials. The order of the antioxidant activity of rutin provided by the updated analytical strategy was as follows: CV = DPPH RSA > PCL > ABTS RSA. Therefore, it may also be suggested that the CV assay is an efficient tool for describing the antioxidant activity of rutin based on its redox properties. However, it should also be taken into account that variation in media, substrates, oxidants, evaluating indices and detecting methods can greatly influence the determination of the antioxidant activity of rutin in different systems, which may account for the data presented in this study.

Antioxidant capacity of buckwheat samples derived from ABTS RSA, DPPH RSA, PCL and CV assays

In this study, the antioxidant capacity of buckwheat samples was measured as the ability of 80% methanol extracts for their free radical scavenging activity against $ABTS^{\bullet+}$, $DPPH^{\bullet}$ and $O_2^{\bullet-}$, and for their reducing capacity by means of CV experiments within the range of +100 up to +1300 mV. The data are compiled in Table 1. The antioxidant capacity of buckwheat samples determined with CV and DPPH RSA methods was about twice lower when compared to ABTS RSA and PCL, respectively. Buckwheat groat, hull and flour represented low antioxidant capacity samples whilst that of sprouts was ranked as the high antioxidant capacity one. The sprouts showed a significantly higher capacity by 10 up to 50 times, depending on the assay, when compared to the antioxidant capacity of groats and hulls. The rank of the antioxidant capacity of buckwheat samples was PCL > ABTS RSA > DPPH RSA = CV.

Rutin content

The concentration of rutin was determined in an 80% (v/v) methanol extract of buckwheat material with the HPLC method and the results, after recalculation on dry matter, are presented in Table 2. The content of rutin was the highest in buckwheat sprouts followed by flour "BIO", hull and groat. The determined rutin content in groat and hull of com-

TABLE 1. Antioxidant capacity of buckwheat groat, hull and sprouts provided by ABTS RSA, DPPH RSA, PCL and CV assays (μmol Trolox/g dm)*.

Buckwheat material	ABTS RSA	DPPH RSA	PCL	CV
Groat	16.20 ± 0.09^{aB}	7.09 ± 0.59^{aA}	17.48 ± 0.36^{abC}	6.44 ± 0.37^{cA}
Hull	6.69 ± 0.14^{aB}	4.33 ± 0.11^{aA}	12.28 ± 0.19^{aC}	4.17 ± 0.26^{bA}
Flour "BIO"	26.89 ± 0.31^{bD}	13.06 ± 0.19^{aB}	20.77 ± 0.51^{bC}	2.37 ± 0.16^{aA}
10 DAS sprouts (dark)	197.71 ± 6.10^{cB}	203.15 ± 5.76^{bB}	287.20 ± 4.42^{cC}	56.56 ± 0.87^{dA}
10 DAS sprouts (light)	304.68 ± 0.32^{dB}	319.53 ± 4.45^{cC}	384.26 ± 7.13^{dD}	55.74 ± 0.20^{dA}

*Data expressed as means ± standard deviations of three independent extractions (n = 3). Means in a column followed by the different lower case letter correspond to significant differences ($p<0.05$). Means in the same raw followed by the different capital letter correspond to significant differences ($p<0.05$).

TABLE 2. The relative contribution of rutin to the antioxidant capacity of buckwheat samples (%).

Buckwheat material	Rutin content (μmol/g dm)	Assay/ total contribution (%)			
		ABTS RSA	DPPH RSA	PCL	CV
Groat	0.04 ± 0.01^{a}	0.28 ± 0.01^{aA}	1.12 ± 0.07^{aB}	0.30 ± 0.003^{aA}	1.22 ± 0.05^{bC}
Hull	0.07 ± 0.002^{ab}	1.23 ± 0.02^{cC}	3.31 ± 0.08^{bA}	0.79 ± 0.02^{bB}	3.47 ± 0.20^{cA}
Flour "BIO"	0.25 ± 0.002^{b}	1.09 ± 0.004^{bA}	3.91 ± 0.02^{cB}	1.67 ± 0.03^{cA}	21.77 ± 1.35^{aC}
10 DAS sprouts (dark)	6.27 ± 0.15^{c}	3.68 ± 0.03^{dB}	6.29 ± 0.34^{dC}	3.01 ± 0.03^{dA}	22.62 ± 0.21^{aD}
10 DAS sprouts (light)	12.51 ± 0.18^{d}	4.77 ± 0.09^{eA}	7.95 ± 0.02^{eB}	4.94 ± 0.02^{eA}	45.79 ± 0.52^{dC}

Data expressed as means ± standard deviations of three independent extractions (n = 3). Means in a column followed by the different lower case letter correspond to significant differences ($p<0.05$). Means in the same raw followed by capital letter correspond to significant differences ($p<0.05$).

mon buckwheat variety Volma was twice lower than that previously reported in buckwheat groats and hull of Kora variety originating from Poland [Zielińska *et al*., 2007b] but it was in accordance to the evidences that buckwheat germination provides an increased rutin content [Kim *et al.,* 2007]. Moreover, in this study common buckwheat variety Volma originating from East Europe was found to be a poor source of rutin when compared to other buckwheat species originating from Canada [Jiang *et al.,* 2007], Central Europe [Kreft *et al*., 2006] and Japan [Morishita *et al*., 2007].

The relative contribution of rutin to the antioxidant capacity of buckwheat samples

The relative contribution of rutin to the antioxidant capacity of buckwheat samples determined by means of ABTS RSA, DPPH RSA, PCL and CV is shown in Table 2. The highest contribution of rutin to the antioxidant capacity was found in relation to buckwheat sprouts, especially those produced in light. This finding indicates the importance of the germination conditions since buckwheat sprouts are currently recognised as a new vegetable. The results showed a low contribution of rutin to the antioxidant capacity of buckwheat groats (below 1.2%), whilst approximately a three-fold higher contribution was noted in hull and flour "BIO" (up to 3.9%). It was consistent with rutin content shown in Table 1, but the higher contribution found in hull did not reflect the antioxidant capacity as hull showed lower capacity than groat (Table 2). The latest finding should not be extended for buckwheat breeding lines, since in this study, the buckwheat represented one among other varieties originating from field experiment dedicated to the elaboration of buckwheat variety of a high nutritional value. Moreover, the study showed the importance of processing buckwheat into flour as this material had higher antioxidant capacity and relative contribution of rutin.

Our findings are in agreement with those report by Morishita *et al.* [2007] who showed also a low contribution of rutin to the antioxidant capacity of common buckwheat seeds (about 2%). They also found that the contribution of (-)-epicatechingallate was almost the same as that of rutin, whilst (-)-epicatechin was the major contributor of the antioxidant capacity and accounted for 13-15%, depending on buckwheat variety. The low percentage contribution of rutin to the antioxidant capacity of buckwheat material indicates that focus on rutin as the main flavonoid in buckwheat can be overestimated. It can be explained by the reported presence of catechins and condensed tannins in buckwheat material whose antioxidant activities have been reported to be higher than that of rutin [Watanabe *et al*., 1997; Watanabe, 1998; Morishita *et al.,* 2007; Karamać *et al*., 2007; Amarowicz, 2007] as well as other potent antioxidants such as quercetin, flavone *C*-glucosides and procyanidins [Tsimogiannis & Oreopoulu, 2006; Zielińska *et al*., 2007b; Watanabe, 2007].

CONCLUSIONS

In this study, rutin and buckwheat-originated material exhibited ability to scavenge different types of free radicals. The application of cyclic voltammetry showed dependence of the antioxidant activity of rutin on the first and second oxidation potentials and was found a useful tool for the evaluation of the antioxidant capacity of buckwheat material. The contribution of rutin to the antioxidant capacity of selected buckwheat samples was low, and it was concluded that focus on rutin as the main antioxidant in buckwheat can be overestimated. It was suggested that the most appropriate assays for the measurement of the antioxidant activity of rutin was CV followed by DPPHA RSA. It was due to the specificity of the rank of methods used as CV = DPPH RSA > ABTS RSA > PCL since the lowest antioxidant gap was provided by CV and DPPH RSA whilst the highest one by ABTS RSA and PCL. The term "antioxidant gap" represents those antioxidants that were not measured and was calculated as the difference between the antioxidant capacity of buckwheat material and the antioxidant capacity provided by rutin content and its specific antioxidant activity determined under each assay.

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