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EVALUATION OF PHOTOCHEMILUMINESCENT, SPECTROPHOTOMETRIC AND CYCLIC VOLTAMMETRY METHODS FOR THE MEASUREMENT OF THE ANTIOXIDANT CAPACITY: THE CASE OF ROOTS SEPARATED FROM BUCKWHEAT SPROUTS

Danuta Zielińska[1], Wiesław Wiczkowski[2], Mariusz K. Piskuła[2]

[1]Department of Chemistry, University of Warmia and Mazury in Olsztyn; [2]Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn

Key words: roots separated from buckwheat sprouts, light conditions, antioxidant capacity, chemiluminescence, spectrophotometric methods, cyclic voltammetry, phenolics profile

This paper describes the use of photochemiluminescence (PCL), spectrophotometric methods (TEAC, FCR reducing capacity) and cyclic voltammetry for the measurement of the antioxidant capacity of roots obtained from dark- and light-grown buckwheat sprouts. A 80% methanol was used for the preparation of extracts originated from roots separated from 6 and 8 DAS (days after seeding) buckwheat sprouts. The 8 days germination period was sufficient to obtain a good quality sprouts with completely removed pericarps and therefore the roots were collected within this period for the experiments. Comparison of the PCL with TEAC assays showed that these methods provided similar values of antioxidant capacity of the roots. Results showed that antioxidant capacity of roots separated from 6 and 8 days sprouts obtained under dark conditions was higher than that noted for roots separated from sprouts planted in light. The FCR reducing capacity values of roots originated from dark and light-grown sprouts were highly correlated with PCL ACL (r= 0.94 and r=1.00, respectively) and TEAC values (r= 0.98 and r=0.99, respectively). The antioxidant capacity of roots obtained from sprouts produced under dark and light conditions evaluated by PCL and TEAC assay gave almost four and three times higher values than that provided by cyclic experiments. In contrast, an excellent agreement was noted between data provided by FCR reducing capacity and antioxidant capacity obtained by cyclic voltammetric experiments indicating that only part of antioxidants present in the root extracts was able to be oxidized on a glassy carbon electrode. The phenolic compounds were the main antioxidants found in root extracts. It was suggested that antioxidant capacity of roots separated from sprouts may be a potential indicator of sprouts resistance against reactive oxygen intermediates resulting in healthy buckwheat sprouts for a consumer.

INTRODUCTION

Plant natural products have been known to exhibit various bioactivities such as antioxidative, antivirus, antibacterial and radical scavenging actions [Ng *et al*., 2000]. Moreover, a significant research have been conducted on the isolation, characterization and elucidation of the specific mode of action of phytotoxic natural products from allelopathic plants to design new, environmentally friendly herbicides [Iqbal *et al*., 2003]. The antivirus, antibacterial and phytotoxic actions are suggested to be associated with high antioxidant capacity of the source material [Moure *et al*., 2001]. An attractive material in this respect is buckwheat (*Fagopyrum esculentum*). Japanese farmers have known for some time that weeds were rather smaller in buckwheat fields than those in other cropped fields, suggesting that buckwheat is a successful competitor against weeds and that it has allelopathic effects on the growth of some weeds species [Tominga & Uezu, 1995]. Buckwheat has been described as containing secondary products with significant plant growth inhibitory activity, especially specifically long-chain fatty acids and phenolic compounds [Tsuzuki *et a*l., 1987]. Phenolic compounds have been widely reported to be highly potent inhibitory substances to seed germination and to seedling roots and shoot elongation [Yamada *et al.,* 1995]. These secondary metabolites can also be responsible for the resistance of buckwheat germ against fungi during germination [Yamada *et al*., 1995]. Therefore, a detailed and simple measurement of antioxidant capacity of buckwheat material is requested since recently Kim *et al.* [2001] have been the first to recommend the buckwheat sprouts as a new vegetable. Buckwheat sprouts, which are consumed as whole sprouts with roots, are more expensive than soybean sprouts and other types of sprouts, due to the higher price of buckwheat seeds and technical difficulties in buckwheat sprouts production. In order to extend the knowledge about production of sprouts, it was suggested that the antioxidant capacity of roots separated from ready-to eat buckwheat sprouts produced under different light conditions be checked. The hypothesis was that high antioxidant capacity of roots separated from sprouts may be a potential indicator of sprouts resistance against microbial sources as well as against reactive oxygen intermediates resulting in healthy buckwheat sprouts for consumer. Therefore, the aim of this study was (1) to determine antioxidant capacity of roots sep-

Author's address for correspondence: Danuta Zielińska, University of Warmia and Mazury in Olsztyn, Faculty of Environmental Management & Agriculture, Department of Chemistry, Plac Łódzki 4, 10-957 Olsztyn, Poland; e-mail: dziel@uwm.edu.pl

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arated from ready-to-eat buckwheat sprouts produced under different light conditions, and (2) to evaluate different assays of antioxidant capacity when applied for roots separated from ready-to eat buckwheat sprouts.

MATERIALS AND METHODS

Reagents

Rutin (quercetin-3-rutinoside), catechin, ferrulic acid, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, U.S.A.). Cyanidin-3-galactoside was obtained from Polyphenols Laboratories (Sandnes, Norway). Kits of chemicals for determination of ACW (Antioxidant Capacity of Water-soluble substances) and ACL (Antioxidant Capacity of Lipid-soluble substances) (kits no. 400.801) for photochemiluminescence (PCL) assay were purchased 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).

Seed germination

Buckwheat (*Fogopyrum esculentum*, variety Luba) was used for the germination. Whole buckwheat seeds (25 g) were soaked in 125 mL of distilled water at room temperature and shaken every 30 min. After 12 h the 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 6 and 8 days after seeding (DAS). The germination was carried out in triplicate. Roots were separated from 6 and 8 DAS sprouts by hand and after that were lyophilised.

Preparation of 80% methanol extracts

Approximately 100 mg of lyophilized whole buckwheat seeds and roots separated from 6 and 8 DAS sprouts were extracted with 1 mL of 80% methanol by 30 s sonication. Next, the mixture was vortexed for 30 s, again sonicated and vortexed, and centrifuged for 5 min (5 000 ×*g* at 4°C). That step was repeated 5 times and supernatants finally were collected in 5 mL flask. So obtained extracts were kept at -80°C prior to further analysis.

Determination of the antioxidant capacity of water (ACW) and lipid-soluble (ACL) compounds present in the extracts

The photochemiluminescence (PCL) assay was used to measure the antioxidant activity of buckwheat roots extracts with a Photochem® apparatus (Analytik Jena, Leipzig, Germany) against superoxide anion radicals generated from luminol, a photosensitizer, when exposed to UV light [Popov & Lewin, 1999]. The antioxidant activity of 80% methanol extracts of roots was measured using both 'ACW' and 'ACL' kits provided by the manufacturer designed to measure the antioxidant activity of hydrophilic and lipophillic compounds, respectively. Lag time (s) for the ACW assay, obtained from the PCLsoft® control and analysis software was used as the radical-scavenging activity and the antioxidant capacity calculated by comparison with a Trolox standard curve and then expressed as μmol Trolox/g of dry matter of roots. Antioxidant capacity using the ACL kit was monitored for 180 s and expressed as μmol Trolox/g d.m. The roots extracts were centrifuged (5 min at 16,000 ×*g*) prior to analysis. Antioxidant assay was carried out in triplicate for each sample, and 20 μL of the diluted extract (1:20, v/v) was sufficient to correspond within the standard curve.

Trolox Equivalent Antioxidant Capacity (TEAC) assay

TEAC was determined following a procedure described by Re *et al.* [1999] using a spectrophotometer UV-160 1PC (Shimadzu, Japan). The Trolox equivalent antioxidant capacity of 80% methanol root extracts was calculated, using Trolox standard curve, on the basis of a percentage increase of absorbance at 734 nm. Additionally, a Trolox equivalent antioxidant activity of rutin was determined parallel with the Trolox standard curve.

FCR reducing capacity assay

FCR reducing capacity assay by means of Folin-Ciocalteu's reagent (FCR) application was carried out according to Shahidi & Naczk [1991] using a spectrophotometer UV-160 1PC (Shimadzu, Japan). Data were reported as mg of rutin equivalents per gram of dry matter.

UV spectra of root extracts

Phenolic quality profile of 80% methanolic extracts was determined according to a procedure described by Oomah *et al*. [2005]. Briefly, the method consisted of adding 400 μL of sample with 600 μL of a solution of 2% HCl in 80% methanol in a micro quartz cuvette. The UV-VIS spectra were recorded from 250 to 600 nm with a spectrophotometer UV-160 1PC (Shimadzu, Japan) using catechin, ferulic acid, rutin and cyanidin-3-glucoside as quality standards. Standards were prepared in aqueous methanol 80% (v/v). The absorbance peak was checked at 280, 320, 360 and 520 nm.

Voltammetric experiments

The cyclic voltammetric experiments were performed using 80% methanol extracts mixed with 0.2 mol/L sodium acetate-acetic buffer (pH 4.5) at a ratio of 1:1 (v/v) according to Cosio *et al.* [2006]. The sodium acetate-acetic buffer acted as supporting electrolyte for the voltamperometric measurement. The measurements were carried out using a conventional three electrode system: (a) a 3 mm diameter glassy carbon working electrode (BAS MF-2012), (b) a Ag/AgCl electrode as reference one, and (c) a platinum electrode as counter electrode. The voltammetric experiments were performed at room temperature using voltammetric apparatus cell, to which the analysed buckwheat roots extract previously mixed with the supporting electrolyte was introduced. Exactly 100 μL of the roots extract and 100 μL of buffer were used in this respect. In order to avoid the diminishing of sensitivity of the working electrode, it was carefully polished with 0.05 μm alumina paste (Polishing alumina, BAS) and ultrasonically

rinsed in deionized water at the end of each cycle. After washing, the electrode was ready for further tests. The cyclic voltammograms were recorded by scanning the potential from -100 to +1300 mV. Cyclic voltammograms were acquired with potentiostat/galvanostat KSP (Poland) at a scanning rate of 100 mV s^{-1}. For the test purpose, the total charge below anodic wave curve of the voltammogram was recorded. This method is actually based on the correlation between the total charge below anodic wave of cyclic voltammograms and the antioxidant capacity of the sample and reference substance. The Trolox solution in 80% (v/v) methanol within the concentration range of 0.05–2.50 mmol/L was used and the results were expressed as μmol Trolox/ g d.m. The total charge under anodic wave of the background signal (solvent + supporting electrode) was subtracted from total charge under anodic wave obtained for each standard and sample measured.

Statistical analysis

The results are given as the means and the standard deviation of three independent experiments. Statistical analysis was performed using Student's t-test and significance level was set at $p<0.05$.

RESULTS AND DISCUSSION

Buckwheat roots from sprouts

The duration of germination period is very important in term of quality of buckwheat sprouts because the pericarp-remained sprouts are not only difficult to be removed, but also to become an obstacle in using them as fresh vegetables. After 8 days of germination the roots were separated from sprouts since the obtained sprouts did not contain their pericarps which were completely removed and therefore the roots were separated. The 8 days germination time applied in this work was in accordance with the report of Kim *et al.* [2004] who introduced the mass production system to produce buckwheat sprouts and the same period of germination was sufficient to obtain a good quality sprouts with almost completely removed pericarps. Roots separated from sprouts produced in dark were brown whereas those separated from sprouts produced in light showed bright-white roots (Figure 1).

Antioxidant capacity of water (ACW) and lipid-soluble (ACL) compounds

The antioxidant capacity of roots separated from buckwheat sprouts was evaluated by using the Photochem® device and the ACW and ACL kits supplied by Analytik Jena AG. The Photochem® device is the first system that can quantitate the antioxidant capacity of water- and lipid-soluble substances. It combines the very fast photochemical excitation of radical generation with the highly sensitive luminometric detection. Because of the high sensitivity of the PCL of luminol, only nanomolar concentrations of non-enzymatic antioxidant substances are required to the observance of PCL (*i.e.* lag $-lag_0$). The principles of the assay have been described recently [Besco *et al*., 2007]. In this study, the 80% methanol extracts were taken for ACW and ACL measurements with this system, and the results are expressed as Trolox equivalents. The same system was considered by Oomah *et al.* [2006] when different genotypes of lupin seeds were evaluated for their antioxidant capacity by ACW and ACL measurements.

The whole buckwheat seed taken for the germination appeared mainly antioxidant capacity formed by lipid-soluble compounds (ACL; 59.5 μmol Trolox/g d.m.) and only in a part by the water-soluble compounds (ACW; 2.5 μmol Trolox/g d.m.). Probably, it was the reason that extracts from roots separated from the sprouts exhibited very low ACW values below of the detection limit. Therefore, the only antioxidant capacity of 80% methanol-soluble compounds (ACL) of roots are reported in Table 1. A typical calibration curve of

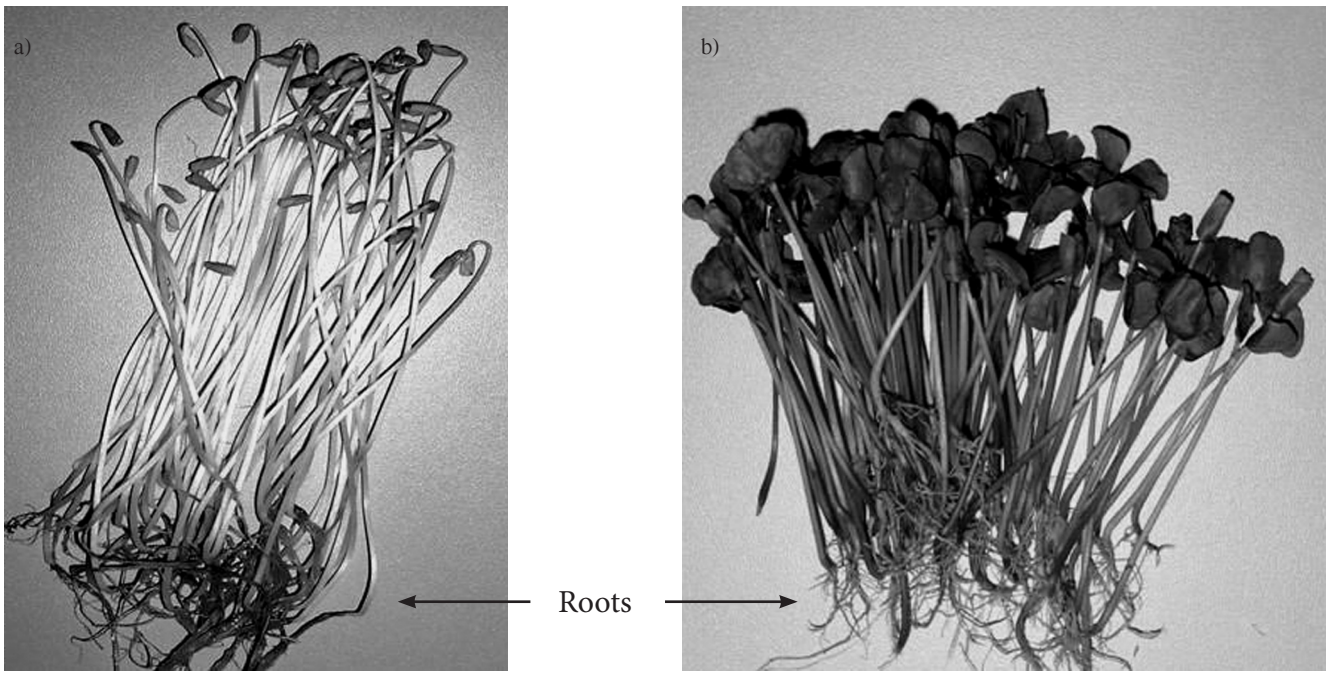

FIGURE 1. Roots separated from dark (a) and light-grown (b) 8 DAS sprouts.

Trolox for ACL calculation is shown on Figure 2. It was found that ACL values of roots separated from dark- and light--grown sprouts were almost six and about four times higher, respectively than that related to ungerminated buckwheat seeds. Moreover, roots separated from dark-grown 6 and 8 DAS sprouts showed higher ACL values by 52 and 31% than those obtained from light-grown sprouts. It was also found that roots from dark- and light-grown 6 DAS sprouts had higher ACL values by 30 and 12% when compared to those separated from dark- and light-grown 8 DAS sprouts. These findings clearly indicate for light conditions as an important factor affecting the ACL values of roots. On the other hand, it can be suggested that roots are a very rich source of 80% methanol-soluble compounds having antioxidant activity against superoxide anion radicals. Indeed, during the last decade it has emerged that joint exudation of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide and hydroxyl radicals) and peroxidase plays an important role in the defense system of plants against pathogenic organisms [Vera-Estrella *et al*., 1993; Scott-Craig *et al*., 1995; Bestwick *et a*l., 1998]. It has been shown that roots of intact plants are capable of releasing H_2O_2 into the surrounding medium, even in the absence of pathogen attack or other stress elicitors [Frahry & Schopfer, 1998]. The release of reactive oxygen intermediates and peroxidase was also reported in germinating radish seeds controlled by light and hormones [Schopfer *et al*., 2001]. Therefore, the data provided by PCL ACL method indicate for the importance of antioxidant capacity of roots originated from germinated buckwheat as a potential indicator of sprouts resistance against reactive oxygen intermediates which can be taken into account for sprouts technology improvement. Moreover, the Photochem® device was fully applicable for the evaluation of the antioxidant capacity originated from 80% methanol-soluble compounds (ACL) of roots separated from buckwheat sprouts.

TABLE 1. The antioxidant capacity of lipid-soluble (ACL) compounds of roots separated from buckwheat sprouts produced under different light conditions ($\overline{x}$ ± SD).

Material	ACL (μmol Trolox/g d.m.)	
	Dark conditions	Light conditions
Whole buckwheat seeds	59.51 ± 1.37[a]	59.51 ± 1.37[a]
Roots from 6 DAS sprouts	375.80 ± 26.21[b]	247.86 ± 10.21[b]
Roots from 8 DAS sprouts	288.73 ± 13.50[c]	221.22 ± 3.42[c]

Data expressed as mean ± standard deviation (n = 3). Means in a column followed by different letters are significantly different ($p \leq 0.05$).

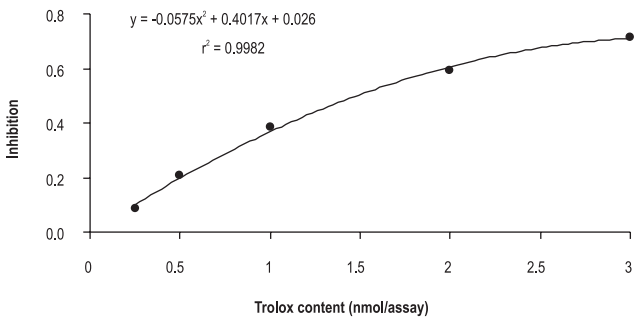

FIGURE 2. Calibration curve of Trolox in the calculation of Trolox equivalents for ACL measurements.

Trolox Equivalent Antioxidant Capacity (TEAC) and FCR reducing capacity

Recently, it was proposed that the following procedures and applications of three assays can be considered for standardization of antioxidant capacity measurements: the oxygen radical absorbance capacity (ORAC) assay, the Folin-Ciocalteu's method, and possibly the Trolox equivalent antioxidant capacity (TEAC) assay [Prior *et al*., 2005]. In this study, two out of the above listed spectrophotometric assays were taken for the evaluation of antioxidant capacity of roots separated from buckwheat sprouts. Therefore, the 80% methanol extracts of the ungerminated buckwheat seeds and roots from 6-8 DAS sprouts were examined for their free radical scavenging activity against $ABTS^{\bullet+}$ cation radical (TEAC), and for their reducing capacity by means of Folin-Ciocalteu's reagent (FCR) application.

It was found that TEAC values of roots separated from dark-grown 6 and 8 DAS sprouts were about nine and six times higher whilst those roots obtained from light-grown 6 and 8 DAS sprouts showed almost seven times higher TEAC values when compared to ungerminated whole buckwheat seeds (Table 2). Moreover, roots separated from dark-grown 6 DAS sprouts showed higher TEAC by 33% than those obtained from light-grown sprouts. In contrast, no difference in antioxidant capacity was found between roots separated from dark and light-grown 8 DAS sprouts. These findings clearly indicate for differential light conditions as an important factor affecting the TEAC values of roots separated from sprouts. It also indicates that the impact of light conditions is limited to the early phase of germination and roots elongation. On the other hand, it can be suggested that roots are a very rich source of 80% methanol-soluble compounds having antioxidant activity against ABTS cation radicals. In this study the provided TEAC values of roots separated from dark- and light-grown 6 and 8 DAS sprouts showed the similar values as ACL data provided by PCL assay. It indicates that spectrophotometric and photochemiluminescence (PCL) assays are both highly applicable for the determination of antioxidant capacity of roots. The results from TEAC assay obtained for whole buckwheat seeds, and roots separated from 6 and 8 dark and light-grown 6 and 8 DAS sprouts were correlated with those obtained by PCL ACL (r=0.99).

In this study, the reducing capacity of the buckwheat roots was measured by means of Folin-Ciocalteu's reagent (FCR) application. The Folin-Ciocalteu's reagent (FCR) actually measures reducing capacity of the sample whereas the same assay based on the reaction of FCR is usually recognized as "total phenolic assay". In this paper, in order to avoid misunderstanding on the actual meaning of "total phenolic contents", the "FCR reducing capacity" was used as suggested by Huang *et al*. [2006].

FCR reducing capacity of roots separated from dark--grown 6 and 8 DAS sprouts were about 12 and 6 times higher

TABLE 2. Trolox equivalent antioxidant capacity (TEAC) and FCR reducing capacity of roots separated from buckwheat sprouts produced under different light conditions ($\overline{x}$ ± SD).

Material	Dark conditions		Light conditions	
	TEAC (μmol Trolox/g d.m.)	FCR reducing capacity (mg of rutin equiv/g d.m.)	TEAC (μmol Trolox/g d.m.)	FCR reducing capacity (mg of rutin equiv/g d.m.)
Whole buckwheat seeds	41.55 ± 0.78[a]	10.99 ± 0.60[a] (43.2 ± 2.4)*	41.55 ± 0.78[a]	10.99 ± 0.60[a] (43.2 ± 2.4)*
Roots from 6 DAS sprouts	383.22 ± 34.55[b]	136.53 ± 2.29[b] (536.7 ± 9.0)*	287.93 ± 1.76[b]	114.93 ± 3.70[b] (451.8 ± 14.5)*
Roots from 8 DAS sprouts	242.39 ± 2.68[c]	64.40 ± 0.51[c] (253.2 ± 2.0)*	284.60 ± 4.24[b]	97.24 ± 2.37[c] (382.3 ± 9.3)*

Data expressed as mean ± standard deviation (n = 3). Means in a column followed by the differ letter are significantly different ($p \leq 0.05$). * Values indicated in the brackets show the converted FCR reducing capacity into antioxidant capacity expressed as μmol Trolox/g d.m. when TEAC of rutin equal to 2.4 mmol/L was used.

whilst those roots obtained from light-grown 6 and 8 DAS sprouts showed almost 10 times higher FCR values when compared to ungerminated whole buckwheat seeds (Table 2). Moreover, roots separated from dark-grown 6 DAS sprouts showed twice higher FCR when compared to those obtained from light-grown sprouts. The same trend, however in smaller extent, was noted for roots separated from light-grown 6 and 8 DAS sprouts. The FCR reducing capacity values of roots originated from dark and light-grown sprouts were highly correlated with ACL (r= 0.94 and r=1.00, respectively) and TEAC values (r= 0.98 and r=0.99, respectively).

In this work the determined TEAC of rutin showed value equal to 2.4 mmol/L of Trolox. This value was in agreement with that reported by Rice-Evans *et al.* [1996] and it was used to express FCR reducing capacity as antioxidant capacity (μmol Trolox/g d.m.) to make further comparison of data obtained from different assays. In this case, the antioxidant capacity values were higher to those provided by PCL ACL (Table 1) and TEAC assay (Table 2). However, one exception was found. It was the case of roots separated from dark-grown 8 DAS sprouts for which the antioxidant capacity determined by spectrophotometry and photochemiluminescence gave the value at a similar level.

Antioxidant capacity of sprouts derived from the voltammetric experiments

A cyclic voltammogram (CV tracing) provides information describing the integrated antioxidant capacity without the specific determination of the contribution of each individual component. It is based on the analysis of the anodic current (AC) waveform which is a function of the reductive potential of a given compound in the sample and/or a mixture of components. The total antioxidant capacity of the sample is a function combining two sets of parameters. The first parameter is the biological oxidation potential whereas the second parameter is the intensity of the anodic AC current (Ia), reflecting the concentration of the components. Recently, it has been proposed that the area under the AC wave (S; related to the total charge) is a better parameter reflecting the antioxidant capacity of the sample [Chevion *et al.,* 2000].

The cyclic voltammograms of the analysed 80% methanol extract of roots separated from buckwheat sprouts were recorded as shown in Figure 3 (whole buckwheat seeds and roots separated from 8 DAS buckwheat sprouts produced in dark and light conditions). The observed anodic wave was broadened due to the response of several antioxidants with different oxidation potentials as it was reported by Kim *et al.* [2004] and by Blasco *et al.* [2005]. In contrast, voltammograms obtained for the standard solutions of Trolox (0.05-2.50 mmol/L) showed well resolved peaks and a shoulder in the potential region up to 1.1 V. A typical CV tracing of different Trolox concentrations is shown in Figure 4. The total charge under the anodic current (AC) waveform, provided by CV computer software, was used to calculate antioxidant capacity of the sample, based on the function AC *vs*. set of Trolox solutions (Figure 5) as it was suggested by Chevion *et al.* [2000].

The results obtained confirmed in the term of quality the changes in antioxidant capacity of roots obtained from sprouts after 6 and 8 days of germination under dark and light conditions provided by PCL ACL, TEAC and FCR reducing capacity. Similarly to the previous results, 6 DAS roots separated from sprouts harvested in dark had higher antioxidant capacity by 52% than those originated from sprouts grown in light. In contrast, 8 DAS roots from dark conditions showed

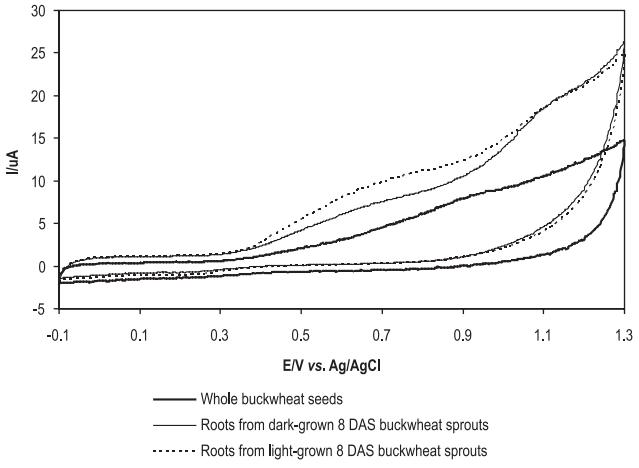

FIGURE 3. Cyclic voltammograms of the analysed extracts originated from 8 DAS roots separated from buckwheat sprouts obtained in dark and light conditions. Operative conditions: concentration of each extract (8 mg/mL 80% methanol); sample preparation: 80% methanol extract mixed with 0.2 mol/L sodium acetate-acetic buffer (pH 4.5) at a ratio of 1:1 (v/v); scan rate 100 mV s^{-1}.

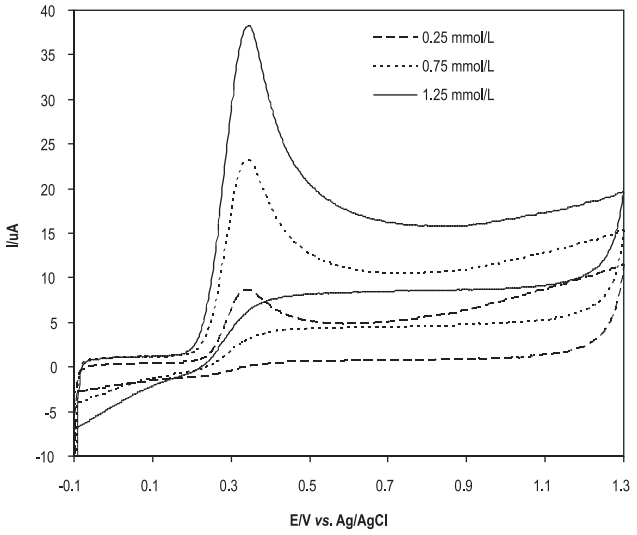

FIGURE 4. Selected cyclic voltammograms of Trolox concentration within the range of 0.05-2.5 mmol/L. Operative conditions: 80% methanol solution of the standard mixed with 0.2 mol/L sodium acetate-acetic buffer (pH 4.5) at a ratio of 1:1 (v/v); scan rate 100 mV s^{-1}.

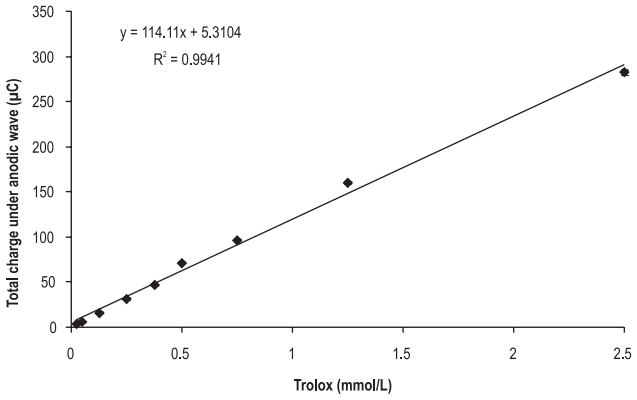

FIGURE 5. The dependency of the total charge under the anodic wave as a function of increasing concentration of Trolox (0.05-2.5 mmol/L).

about 37% lower antioxidant capacity when compared to those harvested in light (Table 3). This finding supports our previous conclusion based on PCL ACL data, TEAC values and FCR reducing capacity that impact of the light conditions on the formulation of root resistance against reactive oxygen intermediates was limited to the early phase of germination and roots elongation. On the other hand, it can be suggested that root extracts were the source of 80% methanol-soluble

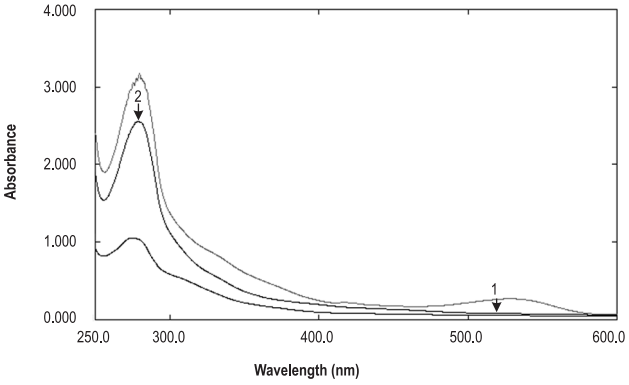

FIGURE 6. The UV-VIS spectra of extracts originated from whole buckwheat (lower spectra) and 8 DAS roots separated from buckwheat sprouts obtained in dark (middle spectra) and light conditions (upper spectra). Peak 1: λ=560 nm, peak 2: λ=280 nm. The UV spectra were recorded as described in "Material and methods".

compounds having ability to be oxidized on glassy carbon electrode. Comparison of the CV with PCL ACL and TEAC assays has shown that these methods yielded considerably different chemical information. The antioxidant capacity of roots obtained from sprouts produced under dark and light conditions evaluated by PCL ACL and TEAC assay gave almost four and three times higher values than those provided by cyclic experiments. In contrast, an excellent agreement was noted between data provided by FCR reducing capacity and antioxidant capacity obtained by cyclic voltammetric experiments.

The UV spectra of root extracts originated from dark- and light-grown 8 DAS sprouts indicated for phenolic compounds as the main antioxidant compounds (absorbance at 280 nm) (Figure 6). Moreover, presence of anthocyanins was confirmed in roots extracts (absorbance of cyanidin-3-glucoside at 520 nm) but not in buckwheat seed extract. Roots separated from light-grown 8 DAS sprouts contained more anthocyanins when compared to those obtained from dark-grown sprouts (Figure 6). These finding is in agreement with recent report of Kim *et al*. [2007] who isolated cyanidin 3-*O*-glucoside, cyanidin 3-*O*-rutinoside, cyanidin 3-*O*-galactoside and cyanidin 3-*O*-galactopyranosyl-rhamnoside from the sprouts of common buckwheat. It is possible that some of these compounds can be distributed within the roots. There are still many active fractions on which work is being done [Piskuła *et al*., 2006]. It is likely that all these fractions contribute toward the antioxidant capacity of roots originated from buckwheat sprouts.

TABLE 3. The antioxidant capacity of roots separated from buckwheat sprouts produced under different light conditions as measured by cyclic voltammetric method ($\bar{x}$ ± SD).

Material	Dark conditions		Light conditions	
	Total charge below anodic wave (µC)	Antioxidant capacity (µmol Trolox/g d.m.)	Total charge below anodic wave (µC)	Antioxidant capacity (µmol Trolox/g d.m.)
Whole buckwheat seeds	23.41 ± 1.20[a]	7.69 ± 0.51[a]	23.41 ± 1.20[a]	7.69 ± 0.51[a]
Roots from 6 DAS sprouts	222.44 ± 6.25[b]	91.86 ± 2.74[b]	145.94 ± 2.94[b]	60.27 ± 1.31[b]
Roots from 8 DAS sprouts	132.12 ± 1.61[c]	54.56 ± 0.73[c]	180.40 ± 3.09[c]	74.50 ± 1.39[c]

Data expressed as mean ± standard deviation (n = 3). Means in a column followed by the differ letter are significantly different ($p \leq 0.05$).

CONCLUSIONS

1. The antioxidant capacity of roots separated from 6 and 8 days sprouts obtained under dark conditions was higher than those noted for roots from sprouts produced under light.

2. The Photochem® device, two spectrophotometric assays – Trolox equivalent antioxidant capacity (TEAC) and FCR reducing capacity, and cyclic voltammetric experiments were fully applicable for the evaluation of the antioxidant capacity of roots separated from buckwheat sprouts.

3. Antioxidant capacity of roots separated from sprouts may be a potential indicator of sprouts resistance against microbial sources as well as against reactive oxygen intermediates resulting in healthy buckwheat sprouts for consumer. This conclusion was supported by our visual observations during early stage of germination, in which both sprouts with roots produced in light were more flexible for microbial development when compared to those produced in dark.

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OCENA METOD CHEMILUMINESCENCYJNYCH, SPEKTROFOTOMETRYCZNYCH I CYKLICZNEJ WOLTAMPEROMETRII DO POMIARU POJEMNOŚCI ANTYOKSYDACYJNEJ KORZONKÓW KIEŁKÓW GRYCZANYCH

Danuta Zielińska[1], Wiesław Wiczkowski[2], Mariusz K. Piskuła[2]

[1]Katedra Chemii, Uniwersytet Warmińsko-Mazurski w Olsztynie, [2]Instytut Rozrodu Zwierząt i Badań Żywności PAN w Olsztynie

W pracy badano pojemność przeciwutleniającą korzonków po oddzieleniu ich od 6 i 8 – dniowych kiełków gryczanych hodowanych w ciemności i w świetle. Do oceny pojemności antyoksydacyjnej zastosowano metody oparte na badaniu zdolności do wymiatania anionorodników ponadtlenkowych generowanych chemiluminescencyjnie, kationorodników ABTS (test TEAC) przez 80% metanolowe ekstrakty oraz metody wykorzystujące własności oksydatywno-redukcyjne związków bioaktywnych obecnych w ekstraktach (pojemność antyoksydacyjna mierzona metodą cyklicznej woltamperometrii oraz pojemność redukująca badana metodą spektrofotometryczną). Uzyskane rezultaty wykazały, że pojemność przeciwutleniająca korzonków pochodzących od 6 i 8 dniowych kiełków uzyskanych w ciemności była wyższa od pojemności przeciwutleniającej korzonków oddzielonych od 6 i 8 dniowych kiełków hodowanych w świetle (tab. 1-3). Pojemność antyoksydacyjna korzonków wyznaczona metodą chemiluminescencyjną (tab. 1) i testem TEAC (tab. 2) była na jednakowym poziomie, który był 3-4-krotnie wyższy od wyników uzyskanych metodą cyklicznej woltamperometrii (tab. 3). Stwierdzono ponadto, że wyniki pojemności redukującej korzonków uzyskanych z kiełków hodowanych w ciemności i w świetle były skorelowane z wynikami uzyskanymi metodą chemiluminescencyjną (r=0,94 i r=1,00) i testem TEAC (r=0,98 i r=0,99). Wysoką zgodność wykazały wyniki pojemności redukującej z rezultatami badań metodą cyklicznej woltamperometrii wskazując, że tylko część związków przeciwutleniających obecnych w ekstraktach z korzonków wykazywała zdolność do utleniania na elektrodzie węglowej. Na podstawie analizy widm UV stwierdzono, że obejmują one związki fenolowe oraz antocyjanidyny. Stwierdzono, że pojemność antyoksydacyjna korzonków uzyskanych z kiełków gryczanych może stanowić cenny wskaźnik odporności korzonków na działanie czynników mikrobiologicznych, grzybów oraz reaktywnych form tlenu powstających w czasie kiełkowania nasion.