J Am Oil Chem Soc (2010) 87:559–566
DOI 10.1007/s11746-009-1525-x

ORIGINAL PAPER

Antioxidant Activity of Tannin Fractions Isolated from Buckwheat Seeds and Groats

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Received: 9 September 2009 / Revised: 24 November 2009 / Accepted: 24 November 2009 / Published online: 15 December 2009

Abstract Phenolic compounds were extracted with 80% (v/v) aqueous acetone from buckwheat seeds and groats. Tannin fractions were obtained from the crude extracts by Sephadex LH-20 column chromatography. Total phenolic contents of isolated fractions from buckwheat seeds and groats were 477 and 371 mg catechin equiv/g, respectively. The analyzed samples were characterized by electrophoretic separations using capillary zone electrophoresis. Both fractions exhibited strong antioxidant activity. The tannin fraction of buckwheat seeds reduced 1,1-diphenyl-2-picrylhydrazyl radicals ($DPPH^{\cdot}$) and [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] diammonium salt radical cations ($ABTS^{\cdot+}$) stronger than the fraction of buckwheat groats. The EC_{50} values amounted to 0.019 and 0.020 mg while Trolox equivalent antioxidant capacity values were 4.06 and 3.55 mmol Trolox equiv/g for tannin fractions of buckwheat seeds and groats, respectively. Similarly, antioxidant activity of the tannin fraction of buckwheat seeds measured by photochemiluminescence assay, was higher than antioxidant activity noted for the fraction obtained from groats. However, both fractions inhibited oxidation to the same extent in applied lipid models: β-carotene-linoleic acid emulsion and L-α-lecithin liposomes. In β-carotene-linoleic acid emulsion system, 1 mg of tannin fractions exhibited similar antioxidant activity to 0.5 mg butylated hydroxyanisole (BHA).

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Keywords Buckwheat seeds · Buckwheat groats · Tannin fractions · Antioxidant activity · DPPH assay · ABTS assay · Photochemiluminescence · β-Carotene-linoleic acid emulsion · L-α-Lecithin liposomes

Introduction

Buckwheat belongs to the *Polygonaceae* family, but due to its cultivation method and utilization, it is usually grouped with the cereals. Along with amaranth and quinoa, buckwheat is considered a pseudo cereal. Common buckwheat (*Fagopyrum esculentum*) and Tartary buckwheat (*Fagopyrum tartaricum*) are two species commonly cultivated. Buckwheat seeds have a high nutritional value. Compared to other cereals, the amino acids in buckwheat proteins are well balanced and rich in lysine [1]. Furthermore, the high antioxidant potential distinguishes buckwheat seeds and meal from cereals [2]. Tocols (tocopherols and tocotrienols), phenolic compounds (phenolic acids, flavonols, flavones, flavan-3-ols and their oligomers), as well as reduced glutathione, inositol phosphates and melatonin are bioactives present in buckwheat [3–5]. Przybylski et al. [6] revealed that lipophilic components do not participate in the antioxidant capacity of buckwheat groats and seeds significantly; rather phenolic compounds dictate antioxidant potential.

Rutin is the predominant phenolic compound of buckwheat. The content of this flavonol in various morphological parts of buckwheat, as well as the changes in its content depending on the variety and technological processing of buckwheat seeds, have been extensively investigated [7, 8]. Jiang et al. [9] noted that the antioxidant capacity of buckwheat seeds of various cultivars is correlated with the rutin content. In contrast, Oomah and Mazza [10] found only a

weak correlation between antioxidant activity and the total flavonoids content, but a nonsignificant correlation with the rutin content of buckwheat. Due to those findings they suggested that components other than flavonoids were responsible for the measured antioxidant activity. Those components can include flavan-3-ols oligomers (condensed tannins). This group of compounds has not been as intensively studied as flavonols in buckwheat, but as early as 1977, Durkee [11] noted the presence of flavan-3,4-diols and soluble oligomeric condensed tannins based on pelargonidin and cyanidin structures in the bran-aleurone fraction of buckwheat seeds. Watanabe [12] isolated four flavan-3-ols monomers: (−)-epicatechin, (+)-catechin 7-*O*-*β*-D-glucopyranoside, (−)-epicatechin 3-*O*-*p*-hydroxybenzoate and (–)-epicatechin 3-*O*-(3,4-di-*O*-methyl) gallate from buckwheat groats. The antioxidant activity of those catechins was higher than that of rutin, and the total amount of isolated catechins exceeded the amount of rutin. Watanabe et al. [3] separated ethanolic extract from buckwheat hulls on a Sephadex LH-20 column into eight fractions. Two of them contained soluble condensed tannins with various degrees of polymerization along with low molecular weight compounds. Both fractions exhibited strong peroxyl radical-scavenging activity. Quettier-Deleu et al. [13] confirmed the presence of much higher amounts of flavan-3-ols and proanthocyanidins oligomers in buckwheat hulls and flour in comparison to the amount of flavonols (rutin, quercetin and hyperoside). Recently, Olschlager et al. [5] isolated other compounds belonging to galloylated flavan-3-ols, procyanidins and propelargonidins from buckwheat seeds.

Proanthodyanidins have been isolated from buckwheat hulls or whole seeds to date [3, 5]. Nevertheless, condensed tannins are also present in significant amounts in buckwheat groats [14]. Raw buckwheat groats are obtained by dehulling whole seeds. In Central and Eastern Europe roasted groats are popular—they are consumed after cooking with meat or vegetables.

In many studies, it has been stated that tannins are considerably more efficient antioxidants than low molecular weight phenolic compounds. Both proanthocyanidins and hydrolyzable tannins exhibited much higher antioxidant activity in $ABTS^{\cdot+}$ assay than catechin and methyl gallate [15]. $DPPH^{\cdot}$ scavenging effect of 51 tannins from oriental medical herbs was higher in comparison to majority of studied flavones, flavonols and phenolic acids [16]. Ariga and Hamano [17] revealed that most of the low molecular weight antioxidants reacted with one or two peroxyl radical per molecule, whereas dimeric procyanidins could trap eight peroxyl radicals per molecule. High antioxidant activity of tannins results from their structure: proximity of many aromatic rings and hydroxyl groups [15].

The aim of this study was to separate tannin fractions from buckwheat seeds and groats and to determine their antioxidant activity using assays, which involve various mechanisms of scavenging of free radicals (synthetic or generated during oxidation of lipid substrates) or reactive oxygen species.

Experimental Procedures

Materials

The plant materials investigated included buckwheat seeds and groats. Portion of 1 kg of the authenticated seeds (*F. esculentum* Moench var. Panda) were obtained from the Plant Breading Station in Olsztyn (Poland). Roasted buckwheat groats (500 g) was acquired at local market in Olsztyn.

Chemicals

All chemicals used were of analytical grade. Special reagents were Sephadex LH-20, (+)-catechin, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), *β*-carotene, linoleic acid, butylated hydroxyanisole (BHA), Tween 40, diethylenetriaminepentaacetic acid (DTPA), L-α-lecithin (type XVI-E from fresh egg yolk), *tert*-butylhydroquinone (TBHQ), 2,2′-azobis(2-methylpropionamidine) dihydrochlorid (AAPH) from Sigma-Aldrich Co. Ltd. (Poznań, Poland). Antioxidant capacity of lipid-soluble substance (ACL) kits for photochemiluminescence (PCL) assay were obtained from Analytic Jena AG (Jena, Germany). Reagents for capillary electrophoresis were purchased from Merck KGaA (Darmstadt, Germany). Folin-Ciocalteu's reagent (FCR) and remaining chemicals were acquired from the P.O.Ch. Company (Gliwice, Poland).

Separation of Tannin Fractions

Seeds and groats of buckwheat (250 g portions) were ground in a coffee mill (BSH Bosch & Siemens Hausgeräte GmbH, Munich, Germany). Phenolic compounds were extracted in triplicate (3 × 80 g) from ground material using 80% (v/v) acetone at a material-to-solvent ratio of 1:8 (w/v). The extraction was carried out three times for 15 min in thermostatic water bath (model 357, Elpan, Lubawa, Poland) heated to 60 °C. Acetone from combined supernatants was evaporated using a rotary evaporator (Rotavapor R-200, Büchi Labortechnik AG, Flawil, Switzerland) at 40 °C. The resultant aqueous residues were lyophilized for ∼48 h at −70 °C and 0.013 mbar (Lyph Lock 6 freeze dry system, Labconco

Co., Kansas City, MO). A solution of the crude phenolic extracts (2 g) in 20 mL of 95% (v/v) methanol was loaded into a chromatographic column (5 × 40 cm) filled with Sephadex LH-20 and equilibrated with 95% (v/v) ethanol. Low molecular weight phenolic compounds were eluted with 1 L of 95% (v/v) ethanol, and then the tannin fraction eluted with 600 mL of acetone:water (1:1 v/v) [18]. The organic solvent from the tannin fractions was evaporated and the water residue was removed during lyophilization. The tannin fractions obtained from three independent chromatographic separations were combined and stored at −20 °C till needed for further analyses.

Determination of Total Phenolic Content

The total phenolic content of the separated tannin fractions was determined by a colorimetric assay according to Naczk and Shahidi [19]. Briefly, 0.25 mL of methanolic solutions of tannin fractions was mixed with 0.25 mL of FCR (diluted 1:1 (v/v) with distilled water), 0.5 mL of a saturated solution of sodium carbonate and 4 mL of water. The mixtures were left to stand for 25 min at ambient temperature and then centrifuged (MPW-210, MPW Med. Instruments, Warsaw, Poland) for 5 min at 5000×g. Absorbance readings of supernatants were taken at $\lambda = 725$ nm using a spectrophotometer DU-7500 (Beckman Instruments Inc., Fullerton, CA, USA). (+)-Catechin within the concentration range of 0.02 to 0.14 mg/mL was used to obtain the standard curve ($r^2 = 0.996$). The results were expressed as mg of catechin equivalents per g of tannin fraction.

Capillary Zone Electrophoresis

Portions of the tannin fractions (2 mg) were dissolved in 2 mL of 100 mM boric buffer pH 8.5 and filtered through a syringe filter (0.45 μm). The samples were separated by capillary zone electrophoresis (CZE) using a Beckman P/ACE 5510 system (Beckman Instruments Inc., Fullerton, CA, USA) with a UV diode array detection set at 280 nm. An uncoated silica capillary tube (Beckman Instruments Inc.) with an inner diameter 50 μm and a length of 77 cm (the effective length was 70 cm) installed in a user-assembled cartridge was employed. Separations were carried out in the 100 mM boric buffer pH 8.5, at a constant voltage of 20 kV, polarity—negative to positive, using pressure injections (3 s) and a constant capillary temperature of 40 °C.

Scavenging of DPPH Radicals

Scavenging activity of tannin fractions against DPPH radicals was monitored according to the method described by Yen and Chen [20]. A 0.1 mL methanolic solution containing 0.01–0.05 mg of the tannin fraction was mixed with 0.25 mL of 0.1 mM methanolic solution of DPPH. Then, 2 mL of methanol was added. The mixture was vortexed vigorously (Vortex Genie2, Scientific Industries INC., Bohemia, NY, USA) and subjected to a 20-min quiescent period. The absorbance at 517 nm was recorded. The percentage of scavenged $DPPH^{\cdot}$ was calculated and plotted as a function of the concentration of tannin fractions (mg/assay). The EC_{50} value, defined as the amount of antioxidant (mg) necessary to inhibit the initial $DPPH^{\cdot}$ by 50%, was read from the plot.

Evaluation of Trolox Equivalent Antioxidant Capacity

The Trolox equivalent antioxidant capacity (TEAC) of buckwheat tannin fractions was determined using the ABTS radical cation decolorization assay [21]. $ABTS^{\cdot+}$ was prepared by the reaction of 192 mg ABTS and 33 mg potassium persulfate in 50 mL water. The mixture was stirred overnight in darkness at room temperature, afterwards the solution was diluted with methanol up to a final absorbance of 0.70 ± 0.02 at 734 nm. Then, 2-mL portions of $ABTS^{\cdot+}$ were vortexed with 20 μL of methanolic solution of the tannin fractions (0.2 mg/mL). Samples were incubated at 30 °C using a block heater (TH-24, Meditherm, Warsaw, Poland) and the absorbance was read at $\lambda = 734$ nm after 6 min exactly. The results were calculated using the standard curve for Trolox (0.2–1.6 μmol/mL, $r^2 = 0.997$) and expressed in mmol of Trolox equivalents per g of the tannin fraction.

PCL Assay

The PCL method, based on the photo-induced chemiluminescence of luminol, was carried out according to the method described by Popov and Lewin [22] using a PHOTOCHEM® device equipped with a PCLsoft® control and analysis software (Analytic Jena AG, Jena, Germany). The antioxidant capacities of the buckwheat tannin fractions were determined using ACL kits for the measurement of lipid/methanol-soluble compounds. Briefly, 2.3 mL of methanol (reagent 1), 200 μL of buffer solution (reagent 2), 25 μL of luminol (reagent 3) were mixed. Then, 20 μL of the methanolic solution containing the tannin fraction (0.2 ng) or standards (Trolox (0.5–3 nmol) and catechin (0.05–1 nmol)) was added. The presence of lipid-soluble antioxidants in the methanolic system resulted in a decrease of the integral of PCL intensity. The percentage of inhibition of chemiluminescence of luminol was used as a parameter for quantification. The results were expressed as mmol of Trolox and catechin equivalents per g of tannin fraction.

β-Carotene-Linoleic Acid Model System

The antioxidant activity of buckwheat tannin fractions was evaluated using the emulsion with β-carotene and linoleic acid [23]. The emulsion was formed by the dissolution of 5 mg of β-carotene in 5 mL of chloroform, followed by the addition of 40 mg of linoleic acid and 400 mg of Tween-40. Chloroform was evaporated by flushing with nitrogen and 80 mL of deionized water was added. The mixture was stirred in an ultrasonic bath (Ultron, Olsztyn, Poland). Methanolic solutions (0.2 mL) containing 1 mg of tannin fraction or 0.5 mg of BHA were added to a series of tubes with 5 mL of the prepared emulsion. Immediately, the zero-time absorbance at 470 nm was recorded. Samples were placed in a block heater at 50 °C and measurements of absorbance were continued at an interval of 15 min over a 120 min period. The results were expressed as percentages of unoxidized β-carotene over time. The percentage of unoxidized β-carotene was calculated as follows:

$$\%\ \text{unoxidized}\ \beta\text{-carotene} = A_t/A_{t=0} \times 100\%$$

where A_t was the absorbance at 470 nm at any time t; $A_{t=0}$ was the absorbance at 470 nm at time zero.

L-α-Lecithin Liposome Oxidation

Liposomes were prepared according to Azuma et al. [24]. The mixture of 9 mg L-α-lecithin/1 mL 10 mM Tris–HCl buffer, pH 7.4 containing 0.5 mM DTPA was passed 21 times through a polycarbonate membrane of 100 nm size using the LiposoFast-Basic system (Avestin Europe GmbH, Mannheim, Germany). The liposomal suspension (1 mL) was diluted with 0.95 mL of Tris–HCl buffer. Then, 1 mg of tannin fraction or 0.5 mg of TBHQ dissolved in 50 μL of methanol was added. The peroxidation was initiated by the addition of 50 μL 0.2 M AAPH and the reaction was allowed to proceed for 4 h at 60 °C in darkness. Liposome oxidation was monitored by determining the conjugated dienes formation according to Huang and Frankel [25]. Aliquots (100 μL) were taken at 1-h intervals and mixed with 2.5 mL of methanol. The absorbance was read at $\lambda = 234$ nm with methanol as a blank. The results were calculated as nmol of conjugated diene hydroperoxides formed per mg of L-α-lecithin by using the molar absorptivity coefficient $\varepsilon = 26{,}000\ \text{L cm}^{-1}\ \text{mol}^{-1}$. The results were plotted as the amount of conjugated dienes versus time.

Statistical Analysis

All analyses were run in three replicates. The mean values were compared using Student's t test of the GraphPad Prim5 program (GraphPad Software Inc., San Diego, CA, USA). Differences were considered significant when $P < 0.05$.

Results and Discusion

Characteristic of Tannin Fractions

The characterization of the tannin fractions from seeds and groats of buckwheat included the determination of the total phenolic contents using FCR and presentation of their CZE separation profiles.

The total phenolic content of the tannin fraction from buckwheat seeds was higher than that from buckwheat groats (Table 1). Constituents of tannin fractions, which reacted with FCR expressed as catechin equivalents amounted to 477 and 371 mg/g of fraction. These values are comparable with those obtained by other researchers for tannin fractions isolated using an analogical method from legume seeds, evening primrose as well as walnuts and hazelnuts kernels [26–28]. On the other hand, a much higher total phenolic content (927 mg catechin equiv/g) was noted in the tannin fraction from *Mallotus philippinensis* bark [29].

Due to the system of mobile phases used during the separation of the tannin fractions, practically all the sugars present in crude aqueous acetonic extracts were coeluted with low molecular weight phenolic compounds. That is the reason for a higher content of phenolic compounds in the tannin fraction than in the crude extract [27]. Similarly, the content of the phenolic compounds in crude extracts of seeds and groats of buckwheat noted in the literature [30–32] is lower than that noted for the tannin fractions in

Table 1 Total phenolic content, 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\cdot}$) scavenging activity and Trolox equivalent antioxidant capacity (TEAC) of buckwheat tannin fractions

Buckwheat tannin fraction	Total phenolics (mg catechin equiv/g)*	$DPPH^{\cdot}$ scavenging activity EC_{50} (mg)	TEAC (mmol Trolox equiv/g)*
Seeds	477 ± 11a	0.019	4.06 ± 0.14a
Groats	371 ± 10b	0.021	3.55 ± 0.09b

Data expressed as means ± standard deviations ($n = 3$). In the same column, means with different letter (a, b) differ significantly ($P < 0.05$)

* Results are expressed as equivalents (equiv) of standard per g of tannin fraction

this study (Table 1). Sun and Ho [31] revealed that the total phenolic content of acetonic extract of whole buckwheat seeds was 3.3 g catechin equiv/100 g of seeds and was even lower for methanolic, ethanolic, butanolic or ethyl acetate extracts. Whereas 80% (v/v) aqueous methanolic extract from buckwheat hulls and seeds contained 3,900 and 726 mg ferulic acid equiv/100 g of product, respectively [30].

The electrophoretic separations of the tannin fractions from seeds and groats of buckwheat obtained using CZE are shown in Fig. 1. There are two regions present in the electrophoretic profile of the tannin fraction of buckwheat groats: higher peaks with migration time 5–11 min and lower peaks appearing between 11 and 16 min of separation. In the electrophoretic profile of buckwheat seeds, peaks with a migration time 5–11 min are lower than in the electrophoregram of the tannin fraction from buckwheat groats, whereas the distribution and height of peaks with a migration time of 11–16 min is similar for both analyzed samples. During CZE, small molecules with large positive charge reach the cathode end of the capillary tube first, then larger cations with a smaller charge, neutral molecules, large anions with a small charge and lastly small anions with a large charge. On that basis, it can be stated that the tannin fraction of buckwheat groats contains more molecules with a positive charge in the conditions of separation (pH 8.5), especially cations with a high molecular weight, in comparison to the fraction of buckwheat seeds. Whereas both analyzed fractions are characterized by a similar profile of negatively charged molecules. The electrophoregrams of CZE separations allow us to conclude that the tannin fractions from seeds and groats of buckwheat are characterized by their containing much more diverse molecular weights and charges in comparison to constituents of fractions from edible nuts, which were analyzed in an analogical method by Karamać [28]. Primarily only negatively charged molecules were present in the tannin fraction of walnuts, while one wide peak with a retention time of 8–12 min was predominant in the electrophoregram from almond and hazelnut tannin fractions.

Scavenging Activity against Stable Free Radicals

Figure 2 depicts the results of the scavenging activity of the tannin fractions from the seeds and groats of buckwheat against DPPH$^{\cdot}$. The tannin fraction from seeds had a slightly higher antioxidant activity than the tannin fraction from groats ($P < 0.05$). The largest addition of the tannin fraction from seeds and groats used in the assay (0.05 mg/assay) caused scavenging of 93 and 89% of radicals, respectively. The amount of the tannin fraction necessary to inhibit the initial DPPH$^{\cdot}$ by 50% (EC_{50} value) was lower for tannins from seeds only by 0.002 mg in comparison to the fraction from groats (Table 1). The values of EC_{50} obtained

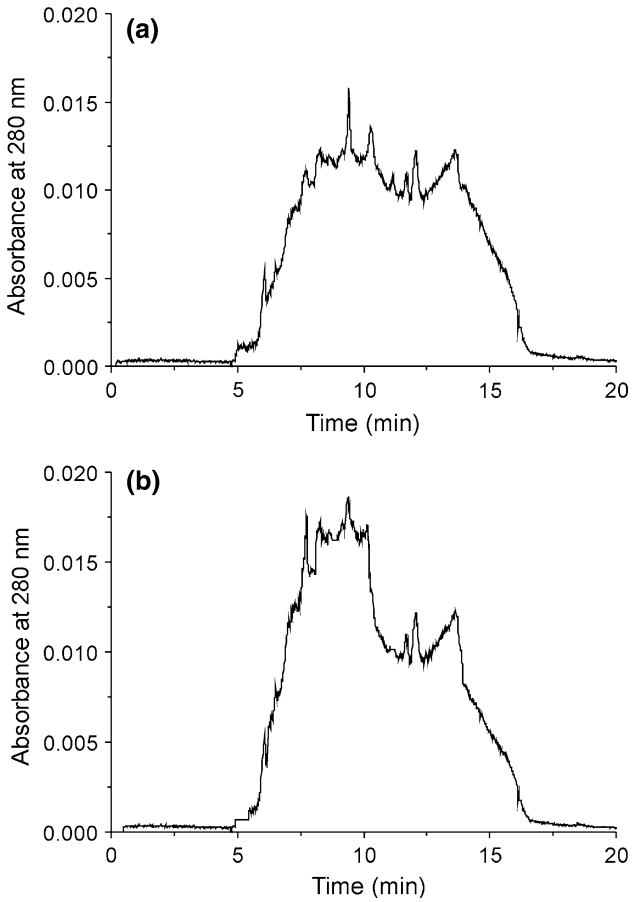

Fig. 1 Capillary zone electrophoresis (CZE) separation of tannin fractions from buckwheat seeds (**a**) and groats (**b**)

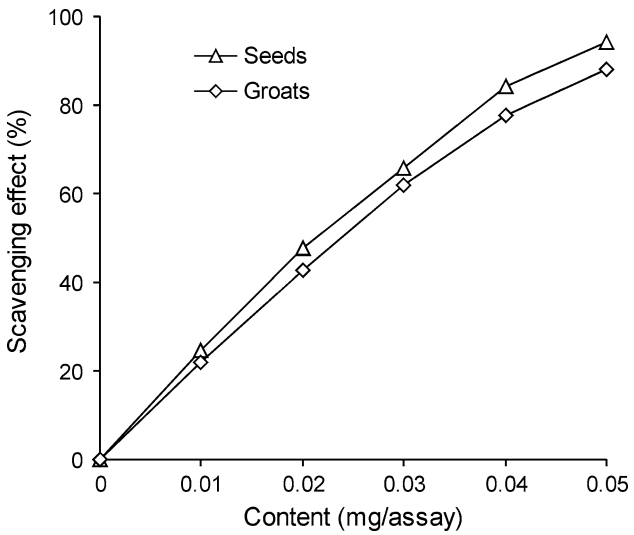

Fig. 2 Scavenging effect of buckwheat tannin fractions on 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH$^{\cdot}$)

are higher than those reported by Alasalvar et al. [33] and Karamać [28] for the tannin fractions of kernels and skins of hazelnuts as well as seeds of walnuts, which amounted to 0.013, 0.010 and 0.008 mg, respectively. Even a lower amount of the tannin fraction of bark of *M. philippinensis* (0.004 mg) was sufficient to scavenge half of $DPPH^{\cdot}$ [29]. On the other hand, values of EC_{50} noted in the present study were comparable to results obtained for the tannin fraction isolated from almonds [28].

The TEAC values of the tannin fractions of seeds and groats of buckwheat are listed in Table 1. The TEAC values for the fraction isolated from seeds was higher ($P < 0.05$) in comparison to the fraction from groats. Tannin fractions from hazelnuts and almonds obtained using Sephadex LH-20 column chromatography reduced ABTS radical cations to a smaller extent, and TEAC values amounted to 2.82 and 1.98 mmol Trolox/g, respectively [28]. Higher TEAC values (from 6.01 to 8.17 mmol Trolox/g) were noted for tannin fractions from vetch, the bark of *M. philippinensis* and hazelnut skins [27, 29, 33].

Scavenging Activity against Radicals Generated in PCL Assay

Antioxidant capacity of the lipid-soluble constituents of the tannin fractions of buckwheat seeds and groats measured by the PLC assay is shown in Table 2. The tannin fraction of seeds scavenged superoxide anion radicals better ($P < 0.05$) than the fraction isolated from groats, based on values obtained by PCL–ACL of 7.49 and 5.96 mmol Trolox equiv/g, respectively. These results were high and similar to those noted for the tannin fractions from walnuts and almonds [28], but also for acetonic extracts of blueberry and bearberry leaves, which are rich in hydrolyzable and condensed tannins [34]. Lower values from PCL–ACL were reported for aqueous acetonic and methanolic extracts of canola hulls, an ethanolic extract of evening primrose and its fractions, and also pure phenolic compounds with a low molecular weight, such as BHA, BHT, octyl gallate, propyl gallate and quercetin, to name a few [34–36].

Since condensed tannins, polymers of flavan-3-ols, are predominant constituents of the analyzed tannin fractions, the results of the PCL–ACL assay were additionally expressed as catechin equivalents (Table 2). Those results were much lower in comparison to the results expressed as Trolox equiv.

Table 2 Antioxidant activity of lipophilic compounds of buckwheat tannin fractions evaluated by the photochemiluminescence (PCL) assay

Buckwheat tannin fraction	Antioxidant capacity of lipid-soluble (ACL) compounds	
	(mmol Trolox equiv/g)*	(mmol catechin equiv/g)*
Seeds	7.49 ± 0.23a	1.67 ± 0.16a
Groats	5.96 ± 0.41b	1.24 ± 0.09b

Data are expressed as means ± standard deviations ($n = 3$). In the same column, means with different letters (a, b) differ significantly ($P < 0.05$)

* Results are expressed as equivalents (equiv) of the standard per g of tannin fraction

Antioxidant Activity Measured in Lipid Systems

The decrease in the percentage of unoxidized β-carotene in the presence of analyzed tannin fractions or BHA with the oxidation of β-carotene and linoleic acid is shown in Fig. 3. Both fractions showed high, not significantly different ($P > 0.05$) antioxidant activity. Portions of 1 mg of the tannin fractions were able to protect 95% β-carotene from oxidation. The antioxidant activity of BHA (0.5 mg) appeared to be only slightly higher after 120 min of processing. The results obtained by this assay for the tannin fraction from buckwheat seeds and groats were very high in comparison to results of other tannin fractions isolated using Sephadex LH-20 column chromatography from nuts, legume seeds, canola hulls and evening primrose [26, 28]. The comparison of antioxidant activity of methanolic extracts from buckwheat seeds leads to a different conclusion. The protecting activity of those extracts against oxidation of β-carotene and linoleic acid is much weaker than sunflower kernels and hulls, potatoes, wheat germs, and blueberries [30]. On the other hand, Sun and Ho [31] revealed, that extracts of buckwheat obtained using various organic solvents were weaker antioxidants than BHA, BHT and TBHQ in β-carotene-linoleic acid emulsion.

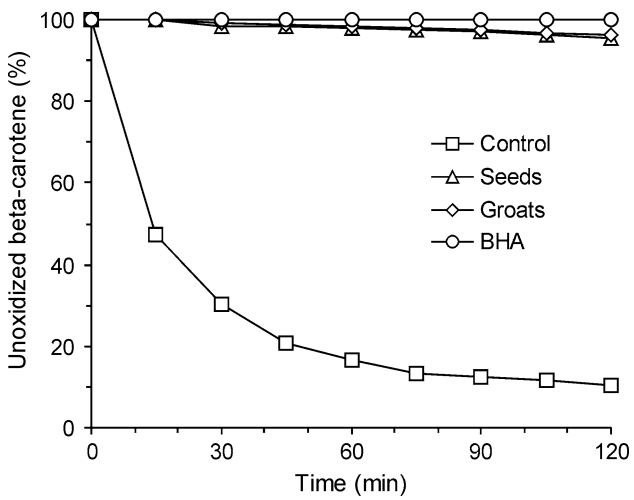

Fig. 3 Antioxidant activity of buckwheat tannin fractions and BHA in a β-carotene-linoleic acid model system

The antioxidant activity of the tannin fractions of seeds and groats of buckwheat in a L-α-lecithin liposome system, similar to a β-carotene and linoleic acid assay, were the same for both samples analyzed (Fig. 4). After 4 h of oxidation, the amount of conjugated dienes formed in the L-α-liposomes with addition of tannin fractions was 0.76 times smaller than in the control samples. After that time, the examined fractions protected L-α-lecithin liposomes weaker than synthetic antioxidant TBHQ. The amount of conjugated diene hydroperoxides measured in sample with TBHQ was 105 nmol/mg L-α-lecithin and was 0.72 times lower than that for samples with tannin fractions.

Conclusions

The comparison of antioxidant activity of tannin fractions from buckwheat with the literature data concerning the antioxidant activity of fractions isolated from other plants, leads to the conclusion that buckwheat fractions are strong antioxidants. The tests applying lipid models (β-carotene-linoleic acid emulsion and L-α-lecithin liposomes systems) revealed similar antioxidant activity of both analyzed tannin fractions. In contrast, $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ tests indicated a higher antioxidant activity of the tannin fraction from buckwheat seeds in comparison to the fraction isolated from groats. Possibly, slightly different constituents of the two fractions were responsible for these observations. The fraction from buckwheat seeds probably contained more constituents reacting with radicals through single electron transfer (ET) reactions. This chemical principle is the basis for $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ tests [37]. In assays with a β-carotene-linoleic acid emulsion and L-α-lecithin liposomes, antioxidants deactivated radicals mainly by hydrogen atom transfer (HAT) reactions. The tannin fraction from buckwheat seeds possesses higher antioxidant activity than the fraction from groats in tests based on the ET mechanism, which can be also concluded from the results of the reaction with FCR (an oxidation/reduction reaction) [38].

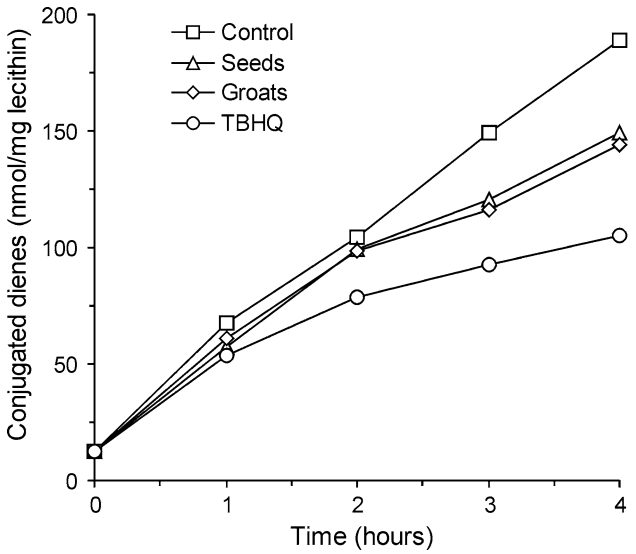

Fig. 4 Antioxidant activity of buckwheat tannin fractions and TBHQ in a L-α-lecithin liposomes system

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