DOI: 10.2428/ecea.2012.19(04)046 ECOL CHEM ENG A. 2012;19(4-5):441-449

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DETERMINATION OF TOTAL PHENOLIC COMPOUND CONTENT AND ANTIOXIDANT PROPERTIES OF EDIBLE BUCKWHEAT SPROUTS

POMIAR CAŁKOWITEJ ZAWARTOŚCI ZWIĄZKÓW FENOLOWYCH ORAZ WŁAŚCIWOŚCI ANTYOKSYDACYJNYCH JADALNYCH KIEŁKÓW GRYKI

Abstract: Consumption of buckwheat sprouts is strongly recommended because they have greater nutritional value than buckwheat seeds or products made from them. Sprouts are an excellent source of protein, amino acids, minerals, fibre, rutin and vitamins. Moreover, consumption of buckwheat sprouts is particularly recommended due to their high antioxidant activity and phenolic compound content. The aim of this study was to visually evaluate buckwheat sprouts based on differences in their morphological traits, to measure their total phenolic compound content and to determine total antioxidant capacity. Buckwheat seeds were moistened with distilled water and germinated in Petri dishes lined with filter paper in natural light conditions at 22 ^{o}C. Sprouts were collected on days 2–6 of the culture. Ethanol extracts were prepared from the sprouts. Total phenolic compound content was determined by spectrophotometry using Folin-Ciocalteau reagent as described by Pasko et al. Phenolic compound content was expressed in μM of gallic acid per 1 g of fresh mass. Three spectrophotometric methods were used to measure the total antioxidant capacity of buckwheat seedling extracts – the method described by Brand-Williams et al based on properties of DPPH, a method using the ABTS radical cation, and the FRAP method. Antioxidant content was expressed in μM of trolox per gram of fresh weight. Buckwheat sprouts were found to exhibit substantial antioxidant activity. The study showed that buckwheat sprouts are most suitable for eating on the fifth day of growth, when they have the best properties for consumption and the mean length of the seedling, measured from the root to the cotyledon, is 131.90 mm. The total phenolic compound content in the ethanol extracts of the buckwheat sprouts is highest on the third day – 3.40 μM of gallic acid per gram fresh weight. Antioxidant content measured by the various methods first increases, and then after the third day decreases slightly. The maximum TEAC per gram fresh weight was obtained on the third day: 73.56 μM of trolox with the ABTS method, 11.47 μM of trolox with the DPPH method and 128.99 μM of trolox with the FRAP method.

Keywords: buckwheat sprouts, total phenolic compound content, DPPH, ABTS, FRAP

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Introduction

Buckwheat belongs to the *Polygonaceae* family and is included among the "pseudocereals", i.e. plants which are not cereals but produce seeds rich in starch. The most popular species for cultivation are *Fagopyrum esculentum* Moench (grown in Poland, among other countries) and *Fagopyrum tartaricum* [1]. There has been increasing interest in buckwheat due to its health-promoting properties, including antineoplastic, antioxidant and antibacterial properties. Buckwheat is considered to be both an edible and a medicinal plant [2]. Rutin, which is used in the pharmaceutical industry, is obtained from its leaves and flowers. Rutin strengthens the capillaries of the circulatory system and has anti-inflammatory properties. Buckwheat is also used to produce vinegar, wine and tea [3]. Buckwheat grain does not contain gluten and is mainly processed for groats [1]. It is also used to produce flour from which pasta [4], bread and other products are produced. Buckwheat seeds are a good source of protein, containing more protein than rice or wheat [5]; moreover, they have high energy value and high content of unsaturated fatty acids (due to which antisclerotic activity is attributed to buckwheat) and are rich in microelements and vitamins. Buckwheat hulls obtained during production of groats are used to stuff pillows and mattresses [6]. Buckwheat is a valuable melliferous plant; buckwheat honey exhibits much higher antioxidant activity than acacia, linden, and multifloral honey, among others [7]. The main fatty acid in buckwheat seedlings is linoleic acid [8], while the main anthocyanin present in buckwheat sprouts is cyanidin 3-O-rutinoside (C3R) [9].

Buckwheat sprouts are an excellent source of protein, amino acids, minerals (Fe, Zn, Mn, Mg, Cu, Ca), fibre and rutin [2]. Moreover, the sprouts have higher nutritional value (more amino acids, minerals, protein, polyunsaturated fatty acids and crude fibres) than the seeds or seed products [10].

S.L. Kim et al demonstrated that the total amino acid content in buckwheat sprouts is about 28–38 % higher than in the seeds. Their research showed that buckwheat sprouts contain significantly more of such amino acids as aspartic acid, glutamic acid and lysine; however, the concentration of arginine and cysteine (amino acids containing sulphur) is lower than in the seeds [2].

This study presents results concerning the morphology and antioxidant content of edible buckwheat sprouts. Sprouts are a natural source of nutrients, fibre, vitamins and microelements. They contain significantly more concentrated nutrients than seeds and adults plants. During germination, high-molecular-weight reserve substances present in the seeds (proteins, carbohydrates and fats) are broken down into simple compounds that are easily assimilated by the human body. Furthermore, enzymes taking part in the breakdown of these substances facilitate the digestion of foods eaten with sprouts. Eating sprouts activates the immune system, corrects vitamin and mineral deficiencies, protects against many serious illnesses, and has antineoplastic effects.

The aim of the present study was to evaluate the morphology of buckwheat sprouts and to measure the total phenolic compound content and total antioxidant activity in ethanol extracts from buckwheat seedlings. Total phenolic compound content was determined by the method described by Singleton and Samuel-Raventos [11] and Pasko

et al [12] using the Folin-Ciocalteu reagent. Three spectrophotometric methods were used to measure antioxidant activity in the seedling extracts: the method of Brand-Williams et al using the synthetic DPPH radical [13], the method using the ABTS radical cation described by Re et al, modified by Bartosz [14, 15], and the FRAP method proposed by Benzie et al in 1996 [16]. Spectrometric measurements were recorded using a Helios Epsilon VIS apparatus. The results will make it possible to choose the optimal time for consumption of buckwheat sprouts.

Materials and methods

Materials

Chemicals

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent and 2,4,6-tris(2-pyridylo)-1,3,5-triazyne (TPTZ) were purchased from Sigma; trolox from Aldrich; potassium persulfate ($K_2S_2O_8$) from Sigma-Aldrich; ethanol, HCl, $FeCl_3 \cdot 6H_2O$ and $FeSO_4 \cdot 7H_2O$ from POCH. All chemicals and reagents were analytical grade or purest quality.

Plant material

Buckwheat seeds for the study were purchased from Dary Natury. The seeds were moistened with distilled water and germinated on Petri dishes lined with filter paper at 22 ^oC in natural light conditions [17]. The seeds were watered. One gram of seedlings without seed coats was collected each day from days 2 to 6 after sowing. The seedlings were homogenized with 10 cm^3 of ethanol and the homogenate was centrifuged. The extracts were frozen and stored for further testing.

Methods

Examination of the morphology of the germinating buckwheat seeds

The morphological characteristics determined in the buckwheat seedlings were length of the entire seedling, number of seedlings per gram fresh weight, and the mass of one seedling. Seedling length was measured with a ruler and the results were given in millimetres. The mass of the seedlings was measured on an analytical balance and expressed in grams. The results of the direct measurements were analysed statistically.

Determination of total phenolics compound content

The total content of phenolic compounds was determined by the spectrophotometric method using the Folin-Ciocalteu reagent, described by Singleton and Samuel-Raventos [11] and Pasko et al [12]. This method is based on the reduction properties of phenolic

compounds and involves measuring the absorbance of the complex resulting from the reaction of phenolic compounds with the Folin-Ciocalteu reagent. 0.3 cm^3 7 % Na_2CO_3, 0.15 cm^3 of Folin-Ciocalteu reagent and 2.9 cm^3 of water were added to 0.1 cm^3 of seedling extract. The absorbance of the reaction mixture was measured after 1 hour at a wavelength of 725 nm. Phenolic compound content was expressed as μM of gallus acid per 1 g fresh weight of seedlings.

Determination of DPPH radical scavenging activity

The antioxidant activity of the ethanol extracts from the seedlings was determined by the method using the synthetic radical DPPH (1,1-diphenyl-2-picrylhydrazyl). 50 mm^3 of the ethanol extract diluted 5-fold was added to 1500 mm^3 of an ethanol solution of DPPH. When DPPH reacts with an antioxidant, the stable DPPH radical takes on electrons from the antioxidant and loses its intense violet colour. The decrease in absorbance was measured in relation to the control sample (DPPH solution + ethanol) 30 minutes after the reaction was initiated, at a wavelength of 517 nm [13]. Antioxidant content was expressed as μM of trolox per 1 g fresh weight.

Determination of ABTS radical scavenging activity

The ABTS method for determining antioxidant activity is based on the reaction of the ABTS radical cation with antioxidants present in the extract, which is accompanied by a decrease in the intensity of the colour of the solution. A solution of ABTS radical cation produced beforehand by potassium persulfate oxidation of 2,2'-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) ammonium salt was added to 50 mm^3 of ethanol extract from the buckwheat seedlings, diluted 15-fold. Absorbance was measured 30 minutes after the reaction was initiated, at λ = 414 nm [14]. Antioxidant content was expressed as μM of trolox per 1 g fresh weight.

Determination of FRAP activity

In the FRAP method, the antioxidants contained in the extract reduce Fe^{3+}-TPTZ (*2,4,6-trypiridyl-s-trizine*) complex to Fe^{2+} ions. 1.500 mm^3 of a reaction mixture consisting of 10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3 \cdot 6H_2O$ and acetate buffer with pH 3.6, mixed in proportions of 1:1:10 (the FRAP reagent), heated to 37 ^oC, was added to 50 mm^3 of ethanol extract from the buckwheat seedlings, diluted 15-fold. Absorbance was measured after 15 minutes of incubation at 37 ^oC at a wavelength of 593 nm. A standard curve was prepared for $FeSO_4 \cdot 7H_2O$ [18]. The results were expressed in μM of iron ions reduced by the antioxidants contained in 1g fresh weight of seedlings.

All determinations were made in at least three independent replications.

Results and discussion

Buckwheat sprouts have a mild flavour and are slightly crunchy. They can be eaten as a fresh vegetable or in salads, or used for many other purposes, *eg* as material for producing natural vegetable juice [2]. Buckwheat sprouts grow rapidly; a root with a well-developed root-hair zone was observed on the second day of growth, and a shoot with well-developed cotyledons on the third day. Buckwheat sprouts attain a considerable size (Fig. 1); their average length is 49.83 mm on the third day of growth, and 161.27 mm by the sixth day. The average mass of one seedling also increases rapidly – 2.5 times between days 2 and 6 of growth (Fig. 2). The number of seedlings per 1g fresh weight decreases with the time of growth: 26.2 on the second day and 10.3 on the sixth. Buckwheat sprouts are best when eaten on days 4 and 5. It should be emphasized, however, that the root of the seedling grows very rapidly and on the sixth day accounts for about 3/4 of the length of the seedling. The edible part with better flavour is the shoot, and the root should be removed from seedlings over 6 days old.

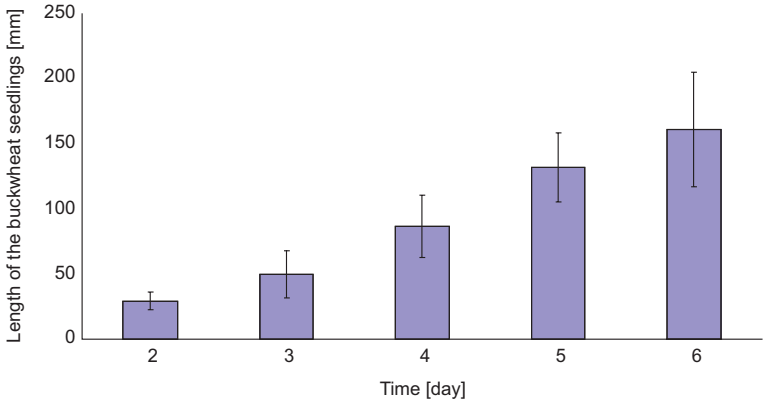

Fig. 1. Length of the buckwheat seedlings

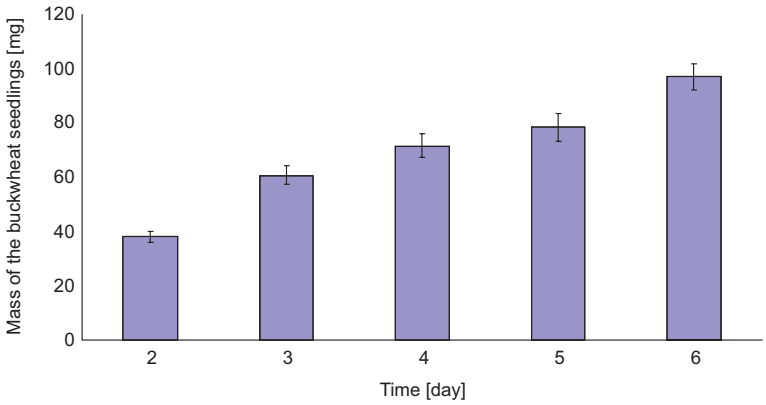

Fig. 2. Mass of the buckwheat seedlings

Determination of total antioxidant capacity and total phenolic compound content was begun after the second day of growth, when the sprouts had produced a radicle and most of them had an hypocotyl. Ethanol was used for the extracts due to the high content of phenolic compounds, particularly rutin. These compounds dissolve well in ethanol but very poorly in water [19].

Xu and Chang conducted a comparative study on the influence of the type of solvent on the phenolic compound content and antioxidant properties of a solution. The choice of solvent for the extraction depends on the type of substance being isolated, and different kinds of food require different kinds of solvents. When we use different solvents, we obtain different compositions of phenolic compounds and other antioxidants following extraction [20].

Phenolic compound content in the buckwheat seedlings ranged from 2.38 to 3.40 μM per gram fresh weight (Fig. 3). It was highest on the third day of growth, at 3.40 μM per gram fresh weight, and then the concentration decreased; on day 6 it was 2.38 μM per gram fresh weight, which is 85.3 % of the value for the 2-day-old seedlings. Buckwheat is very rich in phenolic compounds, which has been confirmed by other authors [21, 22]. The health-promoting effects of buckwheat in the human body are mainly due to its high rutin content. Rutin concentration in buckwheat depends on the organ of the plant and on the stage of development. S.L. Kim et al demonstrated that rutin concentration in buckwheat sprouts was 27 times higher than in the seeds; moreover, it increased each day after sowing. The authors showed that rutin concentration was highest on day 7, at 2933.7 mg/100 g DB [2]. Furthermore, Sharma et al noted the presence in buckwheat seedlings of such phenolic compounds as vitexin, isovitexin, orientin, isoorientin, quercetin and chlorogenic acid [22]. The amount of these phenolic compounds depends on the variety of buckwheat. The authors showed that rutin content is significantly higher in tartary buckwheat sprouts than in common buckwheat. Tartary buckwheat sprouts also contain more quercetin and chlorogenic acid.

Koyama et al found that the concentration and composition of phenolic compounds, particularly rutin, changes during germination of buckwheat; this observation has also been confirmed in the case of other plants: lupins [23] and lentils [24].

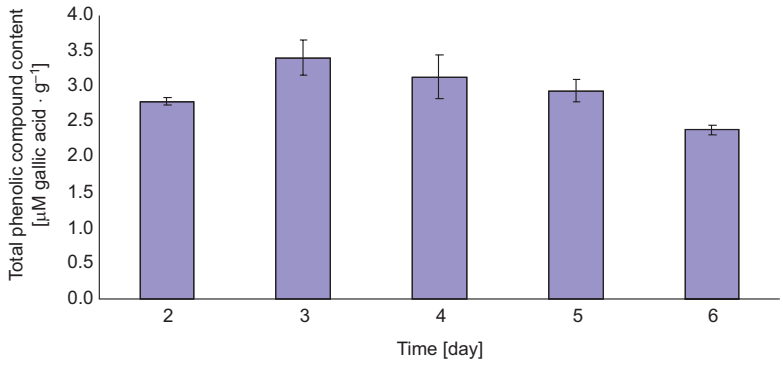

Fig. 3. Total content of phenolic compounds in the buckwheat seedlings

In lentils germinating in light, the concentration of trans-*p*-coumaric acid and trans-ferulic acid increases [24].

In the present study, three spectrophotometric methods were used to determine the antioxidant properties of the extracts. Each of these methods uses a coloured indicator substance: ABTS radical cation, DPPH radical or Fe^{3+}-TPTZ complex. When these substances react with the antioxidants contained in the sample, they lose their colour and the decrease in absorbance is proportional to the content of antioxidant compounds.

Total antioxidant capacity, as measured using the various methods, first increases and then begins to decrease slightly after the third day (Fig. 4). The maximum TEAC per gram of fresh weight was obtained on the third day of growth – 73.56 μM of trolox in the ABTS method, 11.47 μM of trolox in the DPPH method and 128.99 μM of trolox in the FRAP method. Due to the specificity of the reactions between the indicator substances and the antioxidants, different numerical values are obtained in the assays. If TEAC on the second day is given a value of 100 %, then on the third day the antioxidant content increases to about 125 % of the initial value in the ABTS method, 123 % in the DPPH method and 146 % in the FRAP method. On the sixth day, total antioxidant content decreases to about 88 % of the initial value in the ABTS method, 86 % in the DPPH method and 86.3 % in the FRAP method.

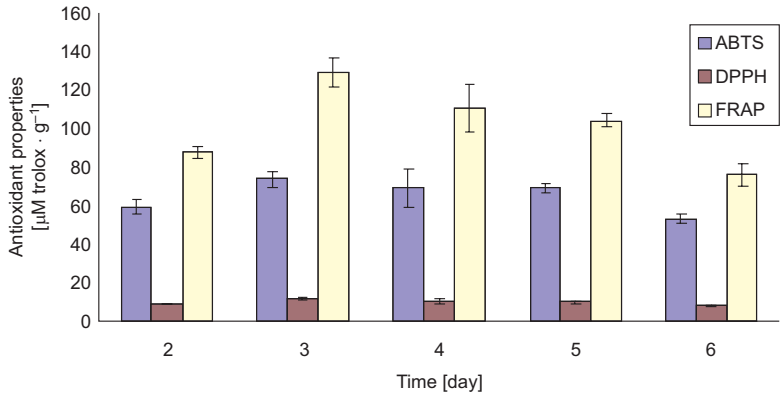

Fig. 4. Total antioxidant capacity of the buckwheat seedling extracts

Barton et al compared the results of measurements of antioxidant capacity obtained using different methods. According to the authors, the faster the reaction of the indicator substance, the higher the numerical values. They obtained the highest values using the FRAP method, intermediate values with the ABTS method, and the lowest values with the DPPH method [18].

A high correlation coefficient was noted between the results obtained using the three methods used to measure TEAC. The results also indicate a substantial correlation between the total phenolic compound content and the total antioxidant capacity of the extracts (Table 1), which may suggest that these compounds affect the antioxidant properties of sprouts. Other authors have also confirmed that the antioxidant properties of plant extracts are positively correlated with their phenolic compound content [20].

Table 1

Correlation coefficient between TEAC as determined by the methods used and the total content of phenolic compounds

	DPPH	FRAP	Total phenolic compound content
ABTS	0.9322	0.9604	0.9496
DPPH		0.9845	0.9947
FRAP			0.9813

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POMIAR CAŁKOWITEJ ZAWARTOŚCI ZWIĄZKÓW FENOLOWYCH ORAZ WŁAŚCIWOŚCI ANTYOKSYDACYJNYCH JADALNYCH KIEŁKÓW GRYKI

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Abstrakt: Spożywanie kiełków gryki jest szczególnie polecane, gdyż wykazują lepszą wartość odżywczą niż nasiona gryki czy produkty z nich powstałe. Kiełki są doskonałym źródłem białka, aminokwasów, minerałów, błonnika, rutyny oraz witamin. Ponadto spożywanie kiełków gryki jest polecane ze względu na ich znaczną aktywność antyoksydacyjną oraz zawartość związków fenolowych. Celem prezentowanej pracy była ocena wizualna kiełków gryki na podstawie różnic w cechach morfologicznych, pomiar całkowitej zawartości związków fenolowych oraz pomiar całkowitej zdolności antyoksydacyjnej.

Nasiona gryki zwilżone wodą destylowaną kiełkowały na szalkach Petriego wyłożonych bibułą filtracyjną, w naturalnych warunkach oświetlenia, w temperaturze 22 ^{o}C. Kiełki gryki zbierano od 2 do 6 dnia hodowli. Przygotowano etanolowe ekstrakty z siewek. Całkowitą zawartość związków fenolowych oznaczano metodą spektrofotometryczną z wykorzystaniem odczynnika Folina-Ciocalteau opisaną przez Paśko i współpr. Zawartość związków fenolowych wyrażono w μM kwas galusowego w przeliczeniu na 1 g świeżej masy. Do pomiaru całkowitej zdolności antyoksydacyjnej ekstraktów z siewek zastosowano metodę Brand-Wiliams i współpr. wykorzystującą właściwości DPPH, metodę ABTS oraz metodę FRAP. Zawartość antyoksydantów wyrażano w μM troloxu na 1 gram świeżej masy. Kiełki gryki wykazują znaczną aktywność antyoksydacyjną. Z przeprowadzonych badań wynika, że piąty dzień hodowli jest najbardziej odpowiednim dniem do spożycia tych kiełków. Kiełki mają w tym dniu najlepsze właściwości konsumpcyjne a średnia długość siewki mierzona od korzenia do liścieni wynosi 131.90 mm. Całkowita zawartość związków fenolowych w etanolowych ekstraktach z kiełków gryki jest w trzecim dniu największa i wynosi 3.40 μM kwasu galusowego w przeliczeniu na gram świeżej masy. Zawartość antyoksydantów mierzona różnymi metodami początkowo rośnie a po trzecim dniu hodowli nieznacznie spada. W trzecim dniu hodowli uzyskano maksymalna wartość w przeliczeniu na świeżą masę, wynosiła ona 73.56 μM troloxu w metodzie ABTS, 11.47 μM troloxu w metodzie DPPH i 128.99 μM troloxu w metodzie FRAP.

Słowa kluczowe: kiełki gryki, całkowita zawartość związków fenolowych, DPPH, ABTS, FRAP