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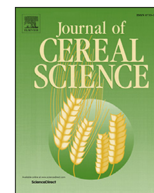

Basic chemical composition and bioactive compounds content in selected cultivars of buckwheat whole seeds, dehulled seeds and hulls

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A B S T R A C T

The objective of this study was to determine the basic chemical composition and the content of selected bioactive components in hulls, dehulled seeds and whole seeds of the selected cultivars/strains of buckwheat. In the tested material the content of ash, protein, fat, fatty acids, total carbohydrates, starch, dietary fibre, resistant starch, total polyphenols, profile of polyphenols and antioxidant activity was determined. We have found that buckwheat seeds were a rich source of protein and total carbohydrates. Dehulled seeds were also a source of resistant starch. Palmitic, oleic and linoleic acids were the dominant fatty acids in all samples. Analysis of extracts from whole seeds, dehulled seeds and hulls showed that there were twenty polyphenolic compounds in the tested material. The highest content of dietary fibre and total polyphenols as well as the highest antioxidant activity were found in the hulls and the lowest one in the dehulled seeds. Because of the rich composition, buckwheat can be used in the development of new food products especially for people on gluten free diet.

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1. Introduction

Recent studies have shown increasing interest in buckwheat (*Fagopyrum esculentum*), because of its high nutritional value and health beneficial properties (Bonafaccia et al., 2003; Stempińska et al., 2007; Stempińska and Soral-Śmietana, 2006; Verardo et al., 2010). In 2013 the buckwheat world production reached over 2.3 million tons and its cultivation area was over 2.2 million hectares (FAOSTAT, 2015). The main polysaccharide in buckwheat seeds is starch. Seeds are also a good source of dietary fibre, among other resistant starch (RS) (Stempińska et al., 2007; Stempińska and Soral-Śmietana, 2006). Other carbohydrates present in small amounts are dextrin as well as sucrose (Gąsiorowski, 2008). The content of protein in buckwheat seeds fluctuates from 8.5 to 19% and is dependent on the cultivar. Buckwheat protein has high biological value, which is corresponding to 92.3% of the biological value of egg protein (Gąsiorowski, 2008; Wronkowska and Haros, 2014). Buckwheat protein has balanced amino acid composition. Lysine, which is recognized as the first limiting amino acid in most cereals, is present in the buckwheat in a relatively large quantities (Gąsiorowski, 2008). Moreover, buckwheat is a gluten-free product (Khan et al., 2012; Sedej et al., 2011). It allows to use it for patients on gluten free diet, especially for these suffering from celiac disease. The buckwheat lipids (1.8–3.9% of seed) have the similar composition to those present in other cereals (Mazza, 1988; Gąsiorowski, 2008).

Beside of the basic components, buckwheat contains also several other heath beneficial components, for example polyphenolic compounds (Kiprovski et al., 2015; Verardo et al., 2010). Therefore buckwheat is a good source of nutrients and non-nutrient compounds and should be consumed more frequently. Based on above information it is reasonable to undertake research concerning the chemical composition of buckwheat, not only known cultivars, but especially the new strains too.

The objective of this research was assessment of basic chemical composition as well as concentration of selected bioactive compounds in selected cultivars and the new strains of whole seeds, dehulled seeds and hulls of buckwheat.

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2. Materials and methods

2.1. Buckwheat materials

The analyzed material were seeds of selected cultivars (*cv.*) (Kora and Panda) and strains (8/2008, 5/2006, 21/2002 and 9/2006) of buckwheat (*F. esculentum*). The seeds of buckwheat were obtained from the Department of Cultivation and Production in Palikije (Poland) harvested in 2011. The seeds were separated into following fractions: hulls and dehulled seeds. In order to obtain high precision of separation and analysis results, dehulled seeds and hulls have been separated manually. The whole seeds and fractions were crushed in an electric grinder (Tecator Foss, Hillerød, Sweden). The samples were stored at room temperature in the paper bags until analysis. The fractions of buckwheat were grinded directly before analysis of polyphenolic compounds and antioxidant activity.

2.2. Basic chemical composition

In all of samples the chemical composition was determined. Total proteins (proced. no 950.36), raw fat (proced. no 935.38), total dietary fibre (proced. no 991.43) and ash (proced. no 930.05) were measured according to the AOAC (2006) methods. The total carbohydrates content was calculated by the equation: total carbohydrates = 100-(protein + crude fat + ash) (Fortuna et al., 2003). The concentration of starch was determined by calcium chloride dissolution (ICC Standard, 1994). Resistant starch (RS) was measured by commercially available kit (cat no. K-RSTAR 09/14, Megazyme International Ireland, Wicklow, Ireland).

2.3. Composition of fatty acids

The composition of fatty acids was analyzed by gas chromatography after extraction of lipids from the whole seeds, dehulled seeds and hulls using the Folch's method (Folch et al., 1957). The free fatty acids were transformed into their respective methylated derivatives (methyl esters) in 14% (v/v) BF_3/Me–OH and extracted using hexane as fatty acids methyl esters and separated with the GC-17A-QP5050 GC–MS model (Shimadzu, Japan), using the capillary SP-2560 column (30 m x 0.25 mm x 0.25 μm; Supelco, Bellefonte, PA, USA). The carrier gas was helium with the flow rate of 5 ml/min. The temperature of column was kept at 60 °C for 5 min and then increased up to 220 °C (5 °C/min). This temperature was maintained for 23 min.

2.4. Extracts preparation

For the measurements of total polyphenols content and antioxidant activity, one gram of sample of buckwheat was weighed into an Erlenmeyer flask and then 40 ml of 0.08N hydrochloric acid in 80% methanol (POCH, Gliwice, Poland) was added. The mixtures were extracted at room temperature for 2 h by shaking (Elpan, water bath shaker type 357, Poland). Then the samples were centrifuged at 1500 rpm for 15 min (Centrifuge type MPW-340, Warsaw, Poland). The supernatants were decanted. The residues were re-extracted with 40 ml of 70% acetone (POCH, Gliwice, Poland) in the same conditions and centrifuged as mentioned above. Both supernatants were combined and stored at temperature −20 °C until analysis.

2.5. Determination of total polyphenols

The content of total polyphenols in extracts was estimated by the Folin-Ciocialteu reagent (Sigma–Aldrich, st. Louis, MO, USA) (Poli-Swain and Hillis, 1959). Extracts were diluted, where ratio of extracts to distilled water was 1:20. The reaction mixtures were prepared by mixing 5 ml of diluted extract, 0.5 ml of the Folin–Ciocalteu reagent and 0.25 ml of 25% sodium carbonate (POCH, Gliwice, Poland). The samples were left for 20 min. The absorbance was measured at 760 nm using the spectrophotometer (UV-1800, RayLeigh, Beijing Beifen-Ruili Analytical Instrument Co., Ltd., Beijing, China). Results were expressed as chlorogenic acid equivalents (CGA) in mg CGA per 100 g of sample.

2.6. High-performance liquid chromatography with electrochemical detection (HPLC/EC)

To isolate the phenolic fraction, the protocol reported by Van Hung and Morita (2008) was used. The chromatographic separation was performed on an Agilent HPLC 1100 series system (Agilent, Waldbronn, Germany), which was equipped with a degasser, a binary pump, an auto-sampler and a thermo-stated column compartment. The extracts of whole seeds, dehulled seeds and hulls were separated on a LiChrospher 60 RP-select B column (125 mm × 4.6 mm ID, 5 μm particle size) in combination with an appropriate guard column (4 mm × 4 mm; 5 μm particle size) (Merck, Germany). The column was thermo stated at 25 °C.

The mobile phase and gradient program were used as previously described by Verardo et al. (2010) with some modifications. A gradient elution was carried out using the following solvent system: mobile phase A, water/formic acid (99:1, v/v); mobile phase B, mobile phase A/acetonitrile (60:40, v/v). The gradient program was as follows: from 2% B to 6% B in 5 min, from 6% to 10% in 10 min, from 10% to 17% in 5 min, from 17% to 36% in 8 min, from 36% to 38.5% in 6 min, from 38.5% to 60% in 6 min, from 60% to 100% in 5 min and from 100% to 2% in 2 min. The flow rate was 0.5 mL/min; the injection volume was 20 μL.

Mass spectrometry analyses were accomplished on an Applied Biosystems MDS Sciex (Concord, Ontario, Canada) API 2000 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) interface. ESI ionization was performed in the positive ion mode. High purity nitrogen used as a sheath gas, was produced by a nitrogen generator. All experiments were carried out in the negative ion mode. The ion source parameters were as follows: ion spray voltage (IS): 4400 V; nebulizer gas (gas 1): 30 psi; turbo gas (gas 2): 10 psi; temperature of the heated nebulizer (TEM): 250 °C; curtain gas (CUR): 30 psi. Nitrogen (99.9%) from Peak NM20ZA was used as the curtain and collision gas. The ion path parameters for analyzed compounds were as follows: declustering potential (DP): 20 V; focusing potential (FP): 200 V; entrance potential (EP): 10 V; collision cell entrance potential (CEP): 0 V; collision cell exit potential (CXP): 2 V, respectively.

2.7. Determination of antioxidant activity

Extracts were also used to determine (spectrometrically) antioxidant activity by identifying the samples ability to extinguish $ABTS^{\bullet+}$ (2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) free radical (Re et al., 1999). The method involved colorimetric determination of the amount of the colored solution of $ABTS^{\bullet+}$ which was reduced by the antioxidants present in the analyzed product. The absorbance was measured at a wavelength 734 nm with a spectrometer (UV-1800, RayLeigh, Beijing Beifen-Ruili Analitical Instrument Co., Ltd., Beijing, China). Values obtained for each sample were compared to the concentration–response curve of the standard Trolox solution and expressed as micromoles of Trolox equivalent per gram of fresh weight (TEAC).

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