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Phenolic profile and antioxidant activity in selected seeds and sprouts

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ABSTRACT

The aim of this study was to investigate the effect of germination on the phenolic acids and flavonoids profile, as well as antioxidant activity (AA), in selected edible seeds of mung beans, radish, broccoli and sunflower. Germination increased the total phenolic (TP) and flavonoid (TF) levels, as well as the AA of the seeds, and influenced the profile of free and bound phenolic compounds. Among the samples, mung bean was characterised by lowest levels of TP and TF, as well as AA, evaluated using ABTS, DPPH and FRAP assays. Sunflower and radish sprouts were the most rich in phenolic compounds. Insignificant amounts of free phenolic acids were found in the free phenolic acid fraction; alkaline hydrolysis of the seeds and sprouts extracts provided the majority of the phenolic acids. The amounts of free and bound flavonoids were inconsiderable both for seeds and sprouts.

1. Introduction

Plant-based foods are a good source of nutrients, dietary fibre, mineral and phenolic compounds. Unfortunately, fresh fruits and vegetables are usually seasonal and therefore they are expensive out of season. Furthermore, most of the out-of-season crops are cultivated under artificial conditions, and then they are prematurely harvested and exported to other parts of the world. All these factors result in a decline in a nutrient value of the crops. An excellent alternative for plant foods are sprouts, which can be consumed in fresh form at all times of the year. Edible seeds and sprouts are a good source of antioxidants, such as: phenolic acids, flavonoids, trace elements and vitamins (Pasko et al., 2009). These plants qualify as a functional food, providing natural protection against heart disease and some forms of cancer. Common sprouting seeds include mung beans, radish, broccoli, sunflower, lentil, soybean, alfalfa, cabbage, wheat, rice, pea, amaranth, quinoa and others. In Poland the most popular seeds used for germination are mung bean, radish, broccoli and sunflower.

Germination of edible seeds to produce sprouts increases their nutritive value (Dueñas, Hernández, Estrella, & Fernández, 2009; Hung, Hatcher, & Barker, 2011; Martinez-Villaluenga et al., 2010). Several studies have reported higher levels of nutrients and lower contents of antinutrients in sprouts compared to the ungerminated seeds (Martinez-Villaluenga et al., 2010; Oloyo, 2004; Zieliński, Frias, Piskuła, Kozłowska, & Vidal-Valverde, 2005). However, information about free and bound phenolics in raw and sprouted seeds is scarce. The content and composition of bioactive compounds in the sprouts depends on many factors, e.g. climatic and agronomic conditions of growth, storage conditions of sprouts, level of their maturity, and also on their variety (Cevallos-Casals & Cisneros-Zevallos, 2010). Due to the fact that sprouts are considered as a cheap and relatively new source of functional foods it is necessary to determine and characterise bioactive compounds occurring in ready-to-eat germinated seeds.

The aim of this study was to investigate the effect of germination on the phenolic acids and flavonoids profile, as well as antioxidant activity, in the seeds and sprouts of mung beans, radish, broccoli and sunflower.

2. Materials and methods

2.1. Chemicals

Methanol, sodium carbonate, and potassium persulfate were purchased from POCh (Gliwice, Poland). Other reagents (aluminium chloride, sodium nitrate, sodium hydroxide, ascorbic acid and acetic acids, iron(III) chloride, hydrochloric acid, di-sodium EDTA, sodium acetate) were obtained from Chempur (Piekary Śląskie, Poland). Folin–Ciocalteau reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt) (ABTS), TPTZ (2,4,6-tri(2-pyridyl-*s*-triazine)) were purchased from Sigma–Aldrich Chemie (Steinheim, Germany). All standards of phenolic acids and flavonoids were from Sigma–Aldrich Chemie or Fluka Chemie AG (Buchs, Switzerland).

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2.2. Materials

Seeds of mung bean (*Vigna radiata* L. Wilczek), radish (*Raphanus sativus* L. var. 'Flamboyant 2'), broccoli (*Brassica oleracea* L. var. italica 'Ramoso Calabrese') were purchased from Diet Food (Warsaw, Poland), and sunflower (*Helianthus annuus* L.) seeds from KHNO POLAN Ltd (Krakow, Poland).

2.2.1. Seed germination

Seeds were sterilised for 1 min by immersion in ethanol. Then the seeds were steeped in deionised water in ratio 1:10 (m/v) for 12 h. After pouring off the soaking water, the seeds were spread on sterile stackable trays (three for one species) and were washed twice a day using deionised water to avoid microbial growth. Sunflower seeds were initially peeled of their shells.

Germination of the seeds was carried out at room temperature in the range of 22 ± 1 °C (12/12 h day/night). Sprouted seeds were harvested after 5 days of growth. The seeds and their sprouts were lyophilised with an Alpha 1–4 freeze-dry system (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany), ground in a knife mill Grindomix GM 200 (Retsch, Düsseldorf, Germany) and stored in the dark until further analyses.

2.3. Procedure of extraction

Methanol extraction was carried out according to the method of Khattak, Zeb, Bibi, Khalil, and Khattak (2007). The ground seeds (5 g) and sprouts (3 g) were extracted for 20 min by shaking with 20 ml of methanol (99.8%) in a screw-capped tube. Extractions were carried out three times. The combined extracts were centrifuged at 10 000 rpm for 10 min and the organic solvents were removed at 35 ± 3 °C using a rotary vacuum evaporator RVO 200A (INGOS Laboratory Instruments Ltd., Prague, Czech Republic). The obtained residue was dissolved in 10 ml of methanol (99.8%) and stored in a freezer until analysis. The extraction procedure was carried out in triplicate.

2.3.1. Determination of total phenolic content

Total phenolic content of methanolic extracts was assayed as described by Meda, Lamien, Romito, Millogo, and Nacoulma (2005), using Folin–Ciocalteau reagent with final reaction measurements carried out at 760 nm. A 0.1-ml aliquot of methanolic extract was diluted with 0.4 ml of deionised water, then the obtained solution was mixed with 2.5 ml of 0.2 M Folin–Ciocalteau reagent and 2 ml of 7.5% (m/v) sodium carbonate solution. After 2 h of incubation, the absorbance was measured against a blank, using UV/Vis spectrophotometer V-530 (Jasco, Tokyo, Japan). Total phenolic content was expressed as mg of gallic acid equivalents (GAE) per g dry matter (d.m.) of seeds and sprouts.

2.3.2. Determination of total flavonoids content

Total flavonoid content in seeds and sprouts was analysed by a spectrophotometric method described by Boateng, Verghese, Walker, and Ogutu (2008). The method is based on the reaction between the flavonoids and aluminium chloride, forming a yellow complex. Four millilitres of deionised water and 0.3 ml of sodium nitrate solution (15 g/100 ml) were added to 1 ml of appropriately diluted methanol extract. After that 0.3 ml of aluminium chloride methanolic solution (10 g/100 ml) and 4 ml of sodium hydroxide solution with concentration (4 g/100 ml) were added to the resulting solution and then the whole sample was diluted with deionised water to a final volume of 10 ml. The mixture was stirred and left to stand for 15 min and finally absorbance was measured at 510 nm. The total flavonoids content in the extracts was compared to the standard curve for quercetin solutions and expressed as mg of quercetin equivalents (QE) per g d.m. of seeds and sprouts.

2.3.3. Determination of ABTS cation radical-scavenging activity

Determination of ABTS cation radical-scavenging activity was based on the reduction of the ABTS cation radical (dissolved in phosphate buffered saline (PBS)) by methanolic extracts from seeds and sprouts according to the method of Martinez-Villaluenga et al. (2010). ABTS cation radical was obtained in the reaction of 2 mM phospate-buffered stock solution of 2,2′-azino-bis(3-ethylbenothiazoline-6-sulfonic acid) diammonium salt (ABTS) with potassium persulfate. The mixture was left to stand for 24 h, until the reaction was completed and then ABTS solution was dilluted by PBS to obtain an absorbance of 0.800 ± 0.03 at $\lambda = 734$ nm. Fifty microlitres of appropriately diluted methanolic extract of seeds or sprouts was mixed with 6 ml of the $ABTS^{\bullet+}$ solution and the absorbance of the resulting solution was measured after 15 min at 734 nm. Antioxidant activity was expressed as mg of Trolox equivalents per g d.m. of seeds and sprouts.

2.3.4. Determination of DPPH radical-scavenging activity

In order to determine DPPH radical-scavenging activity a method described by Moure et al. (2001) was used with minor modification. An aliquot of 3.9 ml of 0.1 mM DPPH radical in methanol was mixed with 0.1 ml of methanolic extract of the sample. After 60 min of incubation the absorbance of the sample was measured at 515 nm in a UV/Vis spectrophotometer (V-530; Jasco, Tokyo, Japan). The DPPH radical-scavenging activity in the extracts was expressed as mg of Trolox equivalents per g d.m. of seeds and sprouts.

2.3.5. Determination of ferric reducing antioxidant power (FRAP)

The ferric ion reducing activity of the methanol extracts was measured according to the method of Benzie and Strain (1996) with some modifications. At low pH, when a ferric-tripyridyltriazine (Fe^{III}-TPTZ) complex is reduced to the ferrous (Fe^{II}) form, an intense blue colour develops with an absorption maximum at 593 nm. To 3.3 ml of acetate buffer (pH 3.6) consisting of 18.5 ml 0.2 M CH_3COOH and 1.5 ml 0.2 M CH_3COONa, 0.33 ml 20 mM $FeCl_3$ and 0.33 ml 10 mM TPTZ (2,4,6-tri(2-pyridyl)-*s*-triazine) in 40 mM HCl were added. After 5 min of incubation at 37 °C, an aliquot of 0.33 ml of methanol extract was added to the mixture and the absorbance was measured at 593 nm after a further 15 min of incubation at 37 °C. Ferric reducing antioxidant power was expressed as mmol of Fe^{2+} per 100 g d.m. of seeds and sprouts.

2.3.6. Free phenolic acids and flavonoids

Free phenolic acids and flavonoids were analysed by high-performance liquid chromatography (HPLC; LaChrom, Merck-Hitachi, Tokyo, Japan) using UV detection, according to the method described by Socha et al. (2011) with minor modifications. The samples were separated on an ODS Hypersil column (250 × 4.6 μm × 5 μm, Thermo Fisher Scientific Inc., Waltham, MA) at a temperature of 30 °C. Phenolic acids, such as gallic, protocatechuic, syringic and vanillic were detected at 280 nm, and chlorogenic, ferulic, caffeic, *p*-coumaric and synapic acids at 320 nm. Flavonoids (quercetin, kaempferol, apigenin and luteolin) were monitored at 360 nm. Chromatographic separation was performed with gradient elution at a flow rate of 1 ml min^{-1} using two solvents: A – 2.5 g per 100 ml of acetic acid, and B – acetonitrile, as mobile phases. The chromatographic analysis was conducted as follows: for the first 10 min a linear gradient was applied, with mobile phase B increasing from 3% to 8%, followed by increase in phase B to 15%, 20%, 30% and 40% at 20, 30, 40 and 50 min, respectively. Finally the column was eluted isocratically for 10 min before the next injection. Before the chromatographic analyses, methanol extracts were filtered using 0.45 μm Millex-LCR syringe filters (PTFE), then purified using Hyper Sep C18 columns (500 mg, 6 ml; Polygen, Gliwice, Poland) and appropriately