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Comparison of phenolic profiles and antioxidant properties of European *Fagopyrum esculentum* cultivars

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ABSTRACT

The purpose of this study was to investigate composition and content of phenolic compounds in seeds of common buckwheat (*Fagopyrum esculentum* Moench) cultivars from Western, Central and Southeastern Europe grown in the Balkan area, and to compare them with cultivars from the Balkan. Mostly detected hydroxycinnamic acids in seeds of the investigated cultivars were caffeic and chlorogenic acid derivatives. More than ten different flavanols were detected in the investigated seeds, based on which all tested buckwheat cultivars were divided into two groups: those with high propelargonidins (epiafzelechin–epicatechin) and those with high procyanidins contents. 'Novosadska' had the highest level of phenolic acids, proanthocyanidins, flavones and most of the flavonols. However, 'Bosna 1' and 'Bosna 2' were highlighted with the greatest rutin content (up to 46 times higher than in other cultivars). All buckwheat cultivars had quite high antioxidant capacity (more than 80% of neutralized radicals), yet, 'Novosadska', 'Godijevo', 'Spacinska 1' and 'Bamby' excelled.

1. Introduction

Buckwheat (*Fagopyrum esculentum* Moench) represents an important functional food, cover crop and pseudocereal. Buckwheat is a rich source of vitamins, essential amino acids and phenolic compounds, which are responsible for many of the health benefits and antioxidant properties of this plant (Holasova et al., 2002). Buckwheat grains have been well known as a source of phenolic compounds: quercetin- and kaempferol-3-glycoside, as well as a trace quantity of flavonol triglycoside (Watanabe, Ohshita, & Tsushida, 1997). In particular, it is an important industrial source of rutin (quercetin-3-rutinoside), a flavonol glycoside that protects plants from ultraviolet (UV) radiation and diseases (Hinneburg & Neubert, 2004; Kalinova, Triska, & Vrchotova, 2006).

Due to significant nutritional or medicinal benefits of buckwheat flour, buckwheat is mainly grown for the production of seeds (Wijngaard & Arendt, 2006). According to Sedej, Mandic, Sakac, Misan, and Tumbas (2010), whole grain buckwheat flour possess better radical scavenging abilities (DPPH$^{\cdot}$, $^{\cdot}$OH, and $O_2^{\cdot-}$ radicals), as well as better reducing activity (β-carotene bleaching method), and chelating activity on Fe^{2+} than flour from dehulled seeds. The same authors investigated buckwheat seed fractions and further concluded that buckwheat hull is superior in antioxidant compounds content and antioxidant ability, mostly due to high rutin content in hull (Sedej et al., 2012).

Buckwheat is grown in many countries in Asia, Europe, South Africa, Canada, USA, Brazil, and in certain other countries around

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the world. Its presence is firstly documented in Finland 5300 BCE and in the Balkans 4000 BCE (Stanic, 2011). The concentration of natural antioxidants may show strong variation depending on several factors including variety, location, and environmental conditions (Dietrych-Szostak & Oleszek, 1999). Due to the fact that the Balkan area represents one of the oldest habitats of *F. esculentum*, the aim of this paper was to analyze in detail phenolic profiles and antioxidant activities of *F. esculentum* cultivars from Western, Central and Southeastern Europe grown in the Balkan area and to compare them with the Balkan buckwheat cultivars.

2. Material and methods

2.1. Plant material

Buckwheat seeds (*F. esculentum* Moench), which represent the collection (Institute of field and vegetable crops, Novi Sad, Serbia) of different buckwheat cultivars from: Serbia ('Novosadska'), Slovenia ('Darja', 'Prekmurska' and 'Cebelica'), Bosnia and Herzegovina ('Bosna 1' and 'Bosna 2'), Montenegro ('Godijevo' and 'Lokve'), Austria ('Bamby'), Czech Republic ('Ceska'), France ('La Harpe'), Slovakia ('Spacinska 1') were used as plant material for this research. Seeds were ground to a fine powder in liquid nitrogen with cooled mortar and pestle. Obtained seeds powder was used as plant material in this experiment.

2.2. Extraction of phenolic compounds

The extraction of seed samples for phenolic analysis was performed as described by Mikulic-Petkovsek, Slatnar, Stampar, and Veberic (2012), with some modifications. Seed powder 0.3 g, were extracted with 5 mL methanol containing 1% (w/v) 2,6-di-tert-butyl-4-methylphenol (BHT) in a cooled ultrasonic bath for 1 h. BHT was added to the samples to prevent oxidation. After extraction, the seeds extracts were centrifuged for 10 min at 10,000 rpm. Each supernatant was filtered through the Chromafil AO-20/25 polyamide filter produced by Macherey-Nagel (Düren, Germany) and transferred to a vial prior to injection into the HPLC (high performance liquid chromatography) system.

2.3. Extraction for total contents of phenolic compounds and antioxidant ability (scavenging) tests

As for spectrophotometric analysis, 10 mL MeOH:H_2O:CH_3COOH (140:50:10) were added to 0.2 g of seed powder (1/50, w/v). The extracts were rapidly vacuum-filtered through a sintered glass funnel and kept refrigerated until assayed.

2.4. Determination of individual phenolic compounds using HPLC-DAD-MS analysis

Phenolic compounds were analyzed on a Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, USA) with a diode array detector at 280 nm (cinnamic acid derivatives and flavanols) and 350 nm (flavonols and flavones). Spectra of the compounds were recorded between 200 and 600 nm. The column was a Gemini C_{18} (150 × 4.6 mm 3 μm; Phenomenex, Torrance, USA) operated at 25 °C. The elution solvents were aqueous 0.1% formic acid in twice distilled water (A) and 0.1% formic acid in acetonitrile (B). Samples were eluted according to the linear gradient from 5% to 20% B in the first 15 min, followed by a linear gradient from 20% to 30% B for 5 min, then an isocratic mixture for 5 min, followed by a linear gradient from 30% to 90% B for 5 min, and then an isocratic mixture for 15 min before returning to the initial conditions (Wang, Zheng, & Galletta, 2002). The injection amount was 20 μL and flow rate was 0.6 mL min^{-1}. All phenolic compounds presented in our results were identified by an HPLC-Finnigan MS detector and an LCQ Deca XP MAX (Thermo Finigan, San Jose, CA) instrument with electrospray interface (ESI) operating in negative ion mode. The analyses were carried out using full scan data-dependent MS^n scanning from *m/z* 110–1500. Column and chromatographic conditions were identical to those used for the HPLC–DAD analyses. The injection volume was 10 μl and the flow rate maintained at 0.6 mL min^{-1}. The capillary temperature was 250 °C, the sheath gas and auxiliary gas were 60 and 15 units, respectively; the source voltage was 3 kV and normalized collision energy was between 20% and 35%. Spectral data were elaborated using the Excalibur software (Thermo Scientific). The identification of compounds was confirmed by comparing retention times and their spectra, as well as by adding the standard solution to the sample and by fragmentation (Tables 1 and 2).

Concentrations of phenolic compounds were calculated from peak areas of the sample and the corresponding standards and expressed in mg 100 g^{-1} dry weight (DW) of seeds. For compounds lacking standards, quantification was carried out using similar compounds as standards. Thus, caffeoyldihexose, caffeic acid-hexoside, caffeic acid-pentoside and unknown phenol 692 were quantified in equivalents of caffeic acid; B type procyanidin dimer, procyanidin trimer, procyanidin dimer monogalate, procyanidin tetramer and procyanidin B2 dimetilygallate in equivalents of procyanidin B2, epiafzelechin–epicatechin in equivalents of epicatechin; isoorientin, orientin, quercetin-hexoside gallate, vitexin in equivalents of quercetin 3-galactoside; kaempferol-3-rutinoside and unknown C-glycoside derivate in equivalents of quercetin 3-glucoside.

Table 1
Characterization of hydroxycinnamic acids and flavanols from buckwheat seeds (UV max 280).

$[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification
503	341, 179	Caffeoyldihexose
353	191, 179, 135, 173	3-Caffeoylquinic acid (neochlorogenic acid)
341	179	Caffeic acid-hexoside
311	179	Caffeic acid-pentoside
353	191, 179, 135	5-Caffeoylquinic acid (chlorogenic acid)
692	530, 311, 350	692 unknown hexoside
289	245, 205, 231, 179	Catechin
577	425, 407, 289	Procyanidin dimer (catechin-catechin)
289	245, 205, 231, 109	Epicatechin
865	695, 577, 451, 425, 407, 289	Procyanidin trimer 1
561	289, 407, 435, 543, 329	Epiafzelechin-epicatechin
1153	865, 577, 407, 423, 405	Procyanidin tetramer isomer (4 epicatechin units)
729	407, 559, 451, 577, 441, 289	Procyanidin dimer monogalate
695	543, 451, 289, 243	Procyanidin trimer 2 (3 epicatechin units)
849	577, 559, 451, 425, 407, 289	
741	469, 605, 615, 587, 467, 271	Procyanidin trimer 3 [epiafzelechin-(4–8)-epicatechin-o-(3,4-dimethyl)-gallate]
757	605, 587, 631, 469, 437	Procyanidin b2 dimetilygallate