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Potentially bioaccessible phenolics, antioxidant activity and nutritional quality of young buckwheat sprouts affected by elicitation and elicitation supported by phenylpropanoid pathway precursor feeding

Michał Świeca

Department of Biochemistry and Food Chemistry, University of Life Sciences, Skromna Str. 8, 20-704 Lublin, Poland

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ABSTRACT

This paper presents the study on impact of elicitation and the phenylpropanoid pathway feeding on the nutritional quality, the potentially bioaccessible phenolics and the antioxidant capacity of young buckwheat sprouts. Phenolics content was increased by elicitation and feeding with tyrosine and shikimic acid – an elevation of 30% and 17%, respectively. Antioxidant capacity was improved by feeding with tyrosine – an increase of 16.7% and 17.1% in both untreated and treated sprouts, respectively. The highest protein digestibility was determined for the control sprouts and those obtained after tyrosine feeding. The lowest starch digestibility was found for elicited sprouts obtained from seeds fed with tyrosine (a decrease by 52%). An increase of expected glycemic index by 38% was determined for elicited sprouts obtained after phenylalanine feeding. Starch and protein digestibility were negatively correlated with total phenolics ($r = -0.55$ and -0.58, respectively), however starch digestibility was also affected by resistant starch content.

1. Introduction

Nowadays, consumers expect more from food products besides nutritional quality, they are looking for an added value associated with its potential bioactivity. Buckwheat seems to meet these requirements being characterized by high quality proteins, starch, vitamins (e.g. B_1, B_2 and B_3), minerals (iron, zinc and selenium) and a high content of flavonoids (phenolics with well-documented antioxidant, anticancer and anti-inflammatory activity) (Ahmed et al., 2013; Christa & Soral-Smietana, 2008; Guo & Yao, 2006; Kim, Kim, & Park, 2004; Ren & Sun, 2014). Buckwheat is consumed in a processed (milled to flour or cooked grouts), as well as in low-processed form (sprouts or leaves). Buckwheat sprouts are consumed as "young" sprouts (sprouted 2–4 days) and leafy vegetable (sprouted until the appearance of leaves and consumed mainly in salad) (Kim et al., 2004). "Young" buckwheat sprouts are characterized by high nutritional value but contains much lower amounts of flavonoids that have the positive effect on cardiovascular system and body immunity (Carocho & Ferreira, 2013; Liu, Chen, Yang, & Chiang, 2008). Food quality can be improved at each step of its production and distribution. The bioactives content in low-processed food may be supplemented by enrichment with exogenous components and/or by modification of its production, for example changes in germination or storage conditions (Świeca, Seczyk, & Gawlik-Dziki, 2014; Tomás-Barberán, & Espín, 2001). Sprouting is an effective and cheap tool by which the food's nutrients digestibility and vitamin content can be increased. Sometimes, however, germination significantly decreases (e.g., color-coating legumes) the level of bioactive phytochemicals, including polyphenols. It may lead to undesirable changes in the bioactivity of sprouts (Fernandez-Orozco et al., 2008; Świeca, Gawlik-Dziki, Kowalczyk, & Złotek, 2012). Sprouting of buckwheat significantly elevates the level of flavonoids and decreases the nutrient content (Ikeda, Arioka, Fujii, Kusano, & Oku, 1984; Kim et al., 2004), thus there is a need to look for technologies increasing the phenolics content during early germination. It has been shown that elicitation and elicitation supported by precursor feeding may enhance the nutraceutical quality of sprouts by induction of synthesis (overproduction) of low-molecular weight antioxidant e.g. phenolics (Baenas, García-Viguera, & Moreno, 2014; Pérez-Balibrea, Moreno, & García-Viguera, 2011; Świeca et al., 2014). As a response to variable environmental conditions, as well as elicitors plants modify their metabolism, adjusting to existing conditions which allow the modification of the composition and activity of food. Phenolics are primarily produced through the pentose phosphate, shikimate, and phenylpropanoid pathways (Świeca et al., 2014).

E-mail address: michal.swieca@up.lublin.pl

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Elicitors increase the activity of key enzymes of this pathway (tyrosine ammonia-lyase and phenylalanine ammonia-lyase) and the effectiveness of phenolics synthesis may be additionally enhanced by enrichment of culture with precursors such as shikimic acid, phenylalanine and tyrosine (Świeca et al., 2014; Baenas et al., 2014; Pérez-Balibrea et al., 2011). Willow bark extract (an elicitor selected for this study) is a rich source of salicylic acid and its derivatives, which act as plant hormones inducing stress response. Willow bark extract induces *inter alia* the phenylpropanoid pathway, what usually translates to a subsequent increase in antioxidant capacity (Yuan & Lin, 2008).

The nutritional and nutraceutical quality the food of plant-origin is mainly determined by nutrients and bioactive phytochemicals content, their bioaccessibility and bioavailability, as well as interactions between them (Fernández-García, Carvajal-Lérida, & Pérez-Gálvez, 2009; Guo, Yao, & Chen, 2007; Świeca, Gawlik-Dziki, Dziki, Baraniak, & Czyz, 2013). Unfortunately, evaluation of food quality is usually performed based only on the result of "chemical extracts", which usually differ significantly from those found for samples obtained after digestion (potential bioaccessibility). The digestive tract acts as an effective extractor releasing phenolics from glycosides and complexes with food matrix (cell walls, protein and starch). In the light of this *in vitro* digestion models should be widely used for studying the qualitative-quantitative and structural changes, as well as digestibility of food components from natural components.

The aim of the study was to evaluate the impact of elicitation and feeding with the phenylpropanoid pathway precursor on the nutritional quality, content of potentially bioaccessible phenolics and antioxidant capacity of sprouts.

2. Materials and methods

2.1. Chemicals

Ferrozine (3-(2-pyridyl)-5,6-bis-(4-phenyl-sulphonic acid)-1,2,4-triazine), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), α-amylase, pancreatin, pepsin, bile extract, linoleic acid, ammonium thiocyanate, thiobarbituric acid, polyvinylpyrrolidone, shikimic acid, L-phenylalanine, L-tyrosine, Tween-20 and haemoglobin were purchased from Sigma–Aldrich company (Poznan, Poland). All others chemicals were of analytical grade.

2.2. Materials

Dormant seeds (S), as well as buckwheat sprouts were studied. Buckwheat seeds were purchased from PNOS S.A. in Ozarów Mazowiecki, Poland. Seeds were sterilized in 1% (v/v) sodium hypochloride for 10 min, then drained and washed with distilled water until they reached neutral pH. After that, the seeds were placed in distilled water (C, control and E, elicitor treatment) or phenolic precursor solution (0.1 mM shikimic acid – Sh and Sh + E; 0.1 mM L-phenylalanine – Phe and Phe + E; 0.1 mM l-tyrosine -Tyr and Tyr+UV) and soaked for 4 h at 25 °C. Seeds were germinated in dark for 3 days in a growth chamber on Petri dishes (ϕ 125 mm) lined with absorbent paper (approximately 400 seeds per dish). Seedlings were watered daily with 5 mL of Milli-Q water. For elicitor treatment (E, Sh+E, Phe+E and Tyr+E), 1-day-old sprouts were watered with 5 ml of 0.01% infusion of *Salix myrsinifolia* bark. After that, the plates were covered and sprouts were germinated under control conditions. Condition of seeds and sprouts treatments were selected according to previous screening tests (data not shown) concerning the selection of the optimal concentrations of precursors and elicitor. The sprouts were freeze dried, ground using a cutter mill (particles below 0.2 mm) and used for further analysis.

2.3. Digestion in vitro

In vitro digestion was performed as described previously Świeca, Baraniak, and Gawlik-Dziki (2013). For simulated mastication and gastrointestinal digestion, germinated buckwheat sprouts (500 mg of lyophilized sprouts) were homogenized in 3.5 mL of simulated salivary fluid (2.38 g Na_2HPO_4, 0.19 g KH_2PO_4 and 8 g NaCl, 200U α-amylase (E.C. 3.2.1.1 in 1 l H_2O, pH – 6.75) and shaken for 10 min at 37 °C. Next, the samples were adjusted to pH 1.2 with HCl (5 mM), suspended in 1.25 mL of simulated gastric fluid (300 U/mL of pepsin A, EC 3.4.23.1 in 0.03 M HCl, pH 1.2) and shaken for 120 min. at 37 °C. After simulated gastric digestion, samples were adjusted to pH 6 with 0.1 M $NaHCO_3$ and suspended in simulated intestinal juice (0.05 g of pancreatin (4 × USP) and 0.3 g of bile extract in 2.0 mL 0.1 M $NaHCO_3$. Next the samples were adjusted to pH 7 with 1 M NaOH. Finally 1.25 mL of 120 mM NaCl and 5 mM KCl was added to the sample. The prepared samples underwent *in vitro* intestinal digestion for 120 min.

2.4. Phenolics analysis

2.4.1. Total phenolics content

The amount of total phenolics was determined using Folin-Ciocalteau reagent (Singleton, Orthofer, & Lamuela-Raventos, 1974). To 0.5 mL of the extract obtained after digestion *in vitro*, 0.5 mL H_2O, 2 mL Folin–Ciocalteau reagent (1:5 H_2O) were added, and after 3 min, 10 mL of 10% Na_2CO_3 and the contents were mixed and allowed to stand for 30 min. Absorbance at 725 nm was measured. The amount of total phenolics was expressed as a gallic acid equivalent (GAE) in mg per g of sprouts dry mass (d.m.).

2.4.2. Total flavonoid content

Total flavonoid content was determined according to the method described by Lamaison and Carnet (1990). One milliliter of the extract obtained after digestion *in vitro* was mixed with 1 mL of 2% $AlCl_3 \times 6H_2O$ solution and incubated at room temperature for 10 min. Thereafter, absorbance at 430 nm was measured. Total flavonoids content was expressed as a quercetin equivalent (QE) in mg per g of sprouts dry mass (d.m.).

2.4.3. Condensed tannins content

Condensed tannin content was determined according to the method described by Sun, Ricardo-da-Silva, and Spranger (1998). To 0.2 mL of the extract obtained after digestion *in vitro*, 1 mL of freshly prepared vanillin reagent (1% (w/v) vanillin in glacial acetic acid:HCl solution (92:8)) was added. After incubation for 20 min at 30 °C, the absorbance was measured at 510 nm. Condensed tannin content was expressed as a (+)-catechin equivalent (CE) in mg per g of sprouts dry mass (d.m.).

2.5. Antioxidant activities

2.5.1. Antiradical activity (ABTS)

The experiments were carried out using the ABTS decolorization assay (Re et al., 1999). The ABTS radical cation ($ABTS^{+\cdot}$) was produced by reacting 7 mM stock solution of ABTS with 2.45 mM potassium persulphate (final concentration) and allowing the mixture to stand in the dark for at least 6 h at room temperature prior to use. The $ABTS^{+\cdot}$ solution was diluted to an absorbance of 0.7 ± 0.05 at 734 nm (Lambda 40 UV–Vis spectrophotometer, Perkin Elmer Inc. Waltham, USA). Then, 40 μL of the extract obtained after digestion *in vitro* were added to 1.8 mL of $ABTS^{+\cdot}$