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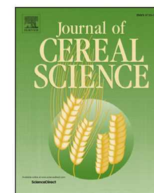

Influence of sprouting and elicitation on phenolic acids profile and antioxidant activity of wheat seedlings

Urszula Gawlik-Dziki [a], [*], Dariusz Dziki [b], Renata Nowak [c], Michał Świeca [a], Marta Olech [c], Wioletta Pietrzak [c]

[a] Department of Biochemistry and Food Chemistry, University of Life Sciences, Skromna Str. 8, 20-704, Lublin, Poland
[b] Thermal Engineering Department, University of Life Sciences, Doświadczalna Str. 44, 20-280, Lublin, Poland
[c] Chair and Department of Pharmaceutical Botany, Medical University of Lublin, Chodźki Str. 1, 20-093, Lublin, Poland

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ABSTRACT

Influence of germination and elicitation on nutraceutical potential of sprouted wheat was investigated. The seeds of three Polish winter wheat cultivars were elicited with 0.1% (w/v) of *Saccharomyces cerevisiae* (SC) extract, 0.1% (v/v) *Salix daphnoides* (SD) bark extract and their combinations (SC/SD 1:1). Seeds were germinated for 4 day at 20 °C in darkness. For phenolic acids determination LC-ESI-MS/MS analysis was used. Antiradical ability, chelating power, reducing power and ability to prevent lipids against oxidation were determined. Bioaccessibility of antioxidative compounds was estimated using *in vitro* digestion method. Eleven phenolic acids (protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, syryngic, *p*-coumaric, *cis*- and *trans*-ferulic, salicylic, *trans*- and *cis*-synapinic) were identified. Sprouting increased *p*-hydroxybenzoic, syryngic and *p*-coumaric acids level. The most effective elicitor for improving antioxidant activity of potentially bioaccessible phytochemicals was SD. Especially high effect was obtained for antiradical activity, chelating power and ability to lipid prevention.
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1. Introduction

Damages of cells and tissues induced by reactive oxygen species (ROS) is related to the etiology of chronic diseases. Endogenous antioxidant defenses are not always sufficient to completely counteract ROS (Poljšak et al., 2011). Thus, diet-derived antioxidants, including phenolic compounds, appear to be particularly important in protecting against chronic diseases by improving antioxidant status (Poljšak et al., 2011).

Wheat bread is the most popular staple food in the world. Wheat (*Triticum aestivum*) is by far the most important crop (with a total global production of about 600–700 million tons) for bread making because of its supreme baking performance in comparison with all other cereals (Adom et al., 2003). The main determinants of the health-promoting effect of breads are genetic factors and the type of wheat and flour used for bread-making (Adom et al., 2003). The main antioxidants in cereal grains are phenolic acids, which seem to have the greatest health-promoting potential as a result of their scavenging free radicals, inhibition of lipid peroxidation, and thus their anticancer activity (Gunenc et al., 2013). Bread made with low extraction wheat flour is a food with a low antioxidant capacity (Gunenc et al., 2013). Wholemeal wheat bread has a higher antioxidant capacity than corresponding patent wheat flour-based breads (Dziki et al., 2014).

Despite wheat breads possess some antioxidant potential, its fortification is reasonable in order to improve the antioxidants level in the common diet. An interesting future trend can be the supplementation of wheat bread in flour from sprouted cereals and use this raw material in food technology for making valuable health products, such as noodles, pasta, laddu, unleavened bread, porridge and gruels for newborns (Shingare and Thorat, 2013).

Germination of seeds has been shown to be a successful strategy to increase the content of bioactive compounds such as phenolic antioxidants (Koehler et al., 2007). Due to the lack of public acceptance of transgenic food, elicitation has recently become a popular way to enhance the quality of edible plants, especially in relation to their health-promoting phytochemicals. Modification of chemical composition and thus, bioactivity of sprouts by elicitors is cheaper and socially acceptable. Several authors have applied exogenous elicitors to stimulate the biosynthesis of bioactive compounds (Złotek et al., 2014).

Inducing factors include abiotic elicitors (metal ions and

* Corresponding author.
E-mail address: urszula.gawlik@up.lublin.pl (U. Gawlik-Dziki).

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inorganic compounds), chilling, biotic elicitors (derived from fungi, bacteria, viruses or herbivores), plant cell wall components, and plant hormones (e.g. salicylic acid and jasmonic acid) (Zhao et al., 2005; Swieca et al., 2014). So far, infusions of yeast (a component of fungal cell wall) and willow bark (a source of salicylic acid) are known to be effective inducers of antioxidant synthesis in broccoli (Gawlik-Dziki et al., 2013). Stress conditions significantly disturb the redox homeostasis of the organism, which usually results in increasing levels of antioxidants, for example phenolics and vitamins (Gawlik-Dziki et al., 2013).

Our previous study clearly showed that taking into account both biological and microbiological purity, the best quality of sprouted seeds were obtained after germination during 4 day at 20 °C and elicited by *Salix daphnoides* (SD) bark extract (Dziki et al., 2015) and combination of SD and *Saccacharomyces cerevisiae* (SC) extracts (Świeca and Dziki, 2015). The cited studies concern only chemically extractable antioxidants and various extraction systems. However, from the consumer point of view, the most significant things, often overlooked in the design of fortified foods, are the real value of the nutraceutical foods. The biological properties of antioxidants may depend on their release from the food matrix during the digestion process (bioaccessibility) and may differ quantitatively and qualitatively from those produced by the chemical extraction (Serrano et al., 2007).

Thus, the presented paper is a continuation and extension of our earlier research, and its goal is to determine the influence of germination and/or elicitation on phenolic acids profile and antioxidant potential of potentially bioaccessible compounds, compared to those obtained after chemical extraction, and determine whether the resulting product - flour from sprouted wheat seeds can be a valuable raw material for functional food and/or food supplements production.

2. Materials and methods

2.1. Chemicals

Standards of gallic, protocatechuic, 3-OH-benzoic, 4-OH-benzoic, vanillic, syringic, *p*-coumaric, ferulic, salicylic, 3-OH-cinnamic acid were purchased from Sigma–Aldrich Fine Chemicals (St. Louis, MO, USA). Gentisic, sinapic, veratric acids were from ChromaDex (Irvine, USA). Caffeic acid and LC grade methanol (MeOH) was purchased from Avantor Performance Materials (Gliwice, Poland). LC grade water was prepared using a Millipore Direct-Q3 purification system (Bedford, MA, USA).

2.2. Plant material

Investigations were carried out on three Polish winter wheat cultivars (*Triticum aestivum*, ssp. *vulgare*): Bogatka, Mulan and Muszelka from the field experiment conducted in 2013 at experimental station belonging to the Lublin Agricultural Advisory Center in Końskowola. Before germination, seeds were sterilized in 1% (v/v) sodium hypohloride for 10 min, drained and washed with distilled water until they reached a neutral pH. The seeds were placed in distilled water (control) and in distilled water containing 0.1% (w/v) of *Saccacharomyces cerevisiae* (SC) solution, 0.1% (v/v). For elicitor preparation instant yeast were dissolved in distilled water at concentration of 0.1% (w/v) and autoclaved. Another part of seeds was elicited with *Salix daphnoides* (SD) bark extract (bark of *S. daphnoides*) obtained from ecological farm, Poland were dried and extracted with boiling water at concentration of 0.1% (w/v), and their combinations (SC/SD 1:1) for 6 h at 25 °C.

Seeds were germinated for 4 days in a controlled incubator (ICH 256, Memmetr, Germany, Duesseldorf) at 20 °C in darkness. Seedlings were dried at 60 °C to the moisture level 12% (wet basis) and pulverized in laboratory mill.

2.3. LC-ESI-MS/MS analysis of phenolic acids

2.3.1. Extraction of plant material

Plant material (2 g) was placed in the stainless-steel cell of Dionex (Sunnyvale, CA, USA) ASE 100 accelerated solvent extractor. The extraction conditions were optimized, giving the best parameters of extraction for: methanol concentration 80%, temperature: 80 °C. Three cycles (for 15 min) of extraction were performed (Pietrzak et al., 2014). The obtained methanolic extracts were combined and concentrated under reduced pressure, then dissolved in 10 ml of 80% methanol in volumetric flask.

2.3.2. Sample preparation

Five mL of extract was diluted with 5 ml of water and applied to Sep-Pak C18 Cartridges (500 mg, Waters, USA) previously activated with 10 ml of methanol and 10 mL of water. The cartridge was eluted with 10 mL of 80% methanol. The combined eluates were concentrated under reduced pressure, then dissolved in 2 ml of 50% methanol in volumetric flask. Prior to further analysis extracts were filtered again through 45 μm nylon filters.

2.3.3. LC-ESI-MS/MS conditions of analysis of phenolic acids

Phenolic acid contents were determined by reversed-phase high-performance liquid chromatography and electrospray ionization mass spectrometry (LC-ESI-MS/MS) according to method previously described (Nowacka et al., 2014) with slight modifications (capillary temperature used for ESI was changed from 600 °C to 620 °C).

Agilent 1200 Series HPLC system (Agilent Technologies, USA) equipped with a binary gradient solvent pump, a degasser, an autosampler and column oven connected to 3200 QTRAP Mass spectrometer (AB Sciex, USA) was used. Chromatographic separations were carried out at 25 °C, on a Zorbax SB-C18 column (2.1 × 50 mm, 1.8-μm particle size; Agilent Technologies, USA) with a mobile phase consisting of water containing 0.1% HCOOH (solvent A) and methanol containing 0.1% HCOOH (solvent B), using 3 μL injections. The flow rate was 400 $\mu L\ min^{-1}$ and the gradient was as follows: 0–1 min – 5% B; 2–4 min – 20% B; 8–9.5 min – 70% B; 11.5–15 min – 5% B.

The QTRAP-MS system was equipped with electrospray ionisation source (ESI) operated in the negative-ion mode. ESI worked at the following conditions: capillary temperature 400 °C, curtain gas at 30 psi, nebulizer gas at 60 psi, negative ionisation mode source voltage −4500 V. Nitrogen was used as curtain and collision gas. For each compound the optimum conditions of Multiple Reaction Mode (MRM) were determined in the infusion mode. The data was acquired and processed using Analyst 1.5 software (AB Sciex, USA). Triplicate injections were made for each standard solution and sample. The analytes were identified by comparing retention time and m/z values obtained by MS and MS^2 with the mass spectra from corresponding standards tested under the same conditions. The calibration curves obtained in MRM mode were used for quantification of all analytes. The identified phenolic acids were quantified on the basis of their peak areas and comparison with a calibration curve obtained with the corresponding standards. Linearity ranges for calibration curves were specified.

The limits of detection (LOD) and quantification (LOQ) for phenolic compounds were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations.

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