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Antioxidant properties of wheat and rye bran extracts obtained by pressurized liquid extraction with different solvents

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ABSTRACT

Rye and wheat brans are valuable sources of bioactive compounds, which could be used for the development and commercialization of high added value functional ingredients such as dietary antioxidants. The aim of this study was to evaluate antioxidant potential of rye and wheat bran using different polarity solvents. Cereal brans were ground to four different particle size fractions and extracted at 10.3 MPa pressure and 80 °C temperature by consecutive application of hexane, acetone and methanol:water (80:20%). The highest extract yield was obtained from rye bran using methanol-water; particle size in most cases had a significant effect. Antioxidant potential of extracts was assessed by $ABTS^{+\bullet}$, $DPPH^{\bullet}$ scavenging, ORAC and total phenols content (TPC) assays. Extraction solvent had a major influence on TPC and antioxidant activity of the extracts. The most active extracts were obtained using methanol:-water; rye bran extracts, in general, were stronger antioxidants than wheat bran extracts. For the majority of assays, reduction of particle size resulted in higher antioxidant activity values. However, $ABTS^{+\bullet}$ scavenging was found to decrease by decreasing particle size of rye bran used for extraction.

1. Introduction

There is a growing demand for healthy food products, which may be produced by adding valuable ingredients to traditional foods. Such ingredients may be obtained from various plant origin sources, including their processing waste and by-products. Cereal bran is often discarded during flour milling and used for animal feed as a by-product. The bran contains various valuable compounds and therefore it could be used more efficiently, for instance, for enriching food products with antioxidatively active ingredients. However, until now, the majority of studies have been focused on phytochemicals from such sources as vegetables, fruits, and spices, while cereals also accumulate valuable constituents, which might be used in the development and production of healthy dietary ingredients (Liu, 2007). Phenolic acids and other antioxidants, which are abundant in cereals, may be more efficiently used as free radical scavengers as well as reducing and chelating agents of metal ions (Zielinski and Kozlova, 2000).

Numerous studies have shown that the highest concentrations of valuable phytochemicals are presented in the bran/germ fraction of whole wheat grain. For instance, the bran/germ fraction in whole grain wheat flour contributed 83% of total phenolic content, 79% of total flavonoid content, 78% of total zeaxanthin, 51% of total lutein, and 42% of total β-cryptoxanthin (Liu, 2007; Adom et al., 2005). Similar findings were reported in another study (Žilic et al., 2012) where the bran fraction contained significantly higher concentrations of phenolic acids, flavonoids and yellow pigments. However, only a small amount of low molecular weight wheat bran antioxidants are present in a free form. Most of them are bound by ester-links with cell wall components such as arabinoxylans and lignin and therefore are not soluble in the commonly used solvents (Liyana-Pathirana and Shadidi, 2006). Alkylresorcinols (AR) are another specific group of phenolic compounds in wheat and rye (Ross et al., 2004; Bondia-Pons et al., 2009). ARs are amphiphilic 1,3-dihydroxybenzene derivatives with odd numbered alk(en)yl chain (17–25 carbon atoms) at the 5th position of the benzene ring. Different cereals contain individual mixtures of C17:0-C25:0 AR homologues present in various proportions (Annica et al., 2010). Whole grain and bran products of wheat and rye contain the

Abbreviations: ABTS, 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid); $DPPH^{\bullet}$, 2,2-diphenyl-1-picrylhydrazyl radical; DW, Dry weight; GAE, Gallic acid equivalents; LSD, Fisher's least significant difference; ORAC, Oxygen radical absorbance capacity; PLE, pressurized liquid extraction; RSC, Radical scavenging capacity; TEAC, Trolox equivalent antioxidant capacity; Trolox, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid; TPC, Total phenolic content.

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highest amounts of these compounds, whereas refined flour or its products are stripped of these constituents (Mattila et al., 2005; Ross and Kochhar, 2009). Therefore, the content of ARs can be used as a marker for whole grain content in wheat and rye products (Chen et al., 2004).

High value components may be extracted from cereal brans by using conventional and high pressure extraction techniques such as pressurized liquid extraction (PLE) and supercritical fluid extraction. The main advantage of the latter methods is that, by a proper selection of solvent and process parameters, it is possible to improve extraction efficiency and speed, while using less solvent. However, PLE equipment is quite expensive, it should be produced from corrosive-resistant materials, and when high temperatures are applied, some thermally sensitive bioactive compounds may degrade (Herrero et al., 2013). For instance, ferulic acid and vanillin were extracted from flax shives, wheat and corn brans using pressurized solvents (0.5 M NaOH, water, ethanol, ammonia) (Anvar et al., 2009). PLE-LC-MS was developed as a new method for determination of tocols in wheat and rye (Bustamante-Rangel et al., 2007). PLE was also used to extract *Fusarium* toxin zearalenone from wheat and corn, ochratoxin A from breakfast and baby cereals (Pallaroni and Von Holst, 2003; Pérez-Torrado et al., 2010; Zinedine et al., 2010) and polyphenols from sorghum (Barros et al., 2013). Elaborated extraction conditions were efficient in extracting similar amount of phenols and 12% more antioxidants from black sorghum compared to traditional methods using aqueous acetone and acidified methanol.

To the best of our knowledge, PLE has not been evaluated until now for the preparation of extracts possessing antioxidant capacity from rye and wheat brans. Moreover, the studies on rye bran extraction are remarkably scarcer than those on wheat bran. Therefore, the aim of this study was to evaluate the antioxidative capacity of different fractions extracted from wheat and rye bran by using different polarity solvents. Different particle size wheat and bran fractions were used for the extraction by PLE. The efficiency of the extraction procedures was assessed, applying a systematic evaluation of antioxidant potential by measuring extract properties using several assays.

2. Experimental

2.1. Solvents and chemicals

Hexane, acetone and methanol were obtained from Chempur (Piekary Śląskie, Poland), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azino-bis(3ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt, 2,2-azobis-(2-amidino-propane) dihydrochloride (AAPH), Folin-Ciocalteu phenol reagent (2 M), 3,4,5-trihydroxybenzoic acid (gallic acid), 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) were purchased from Sigma–Aldrich (St. Louis, MO, USA). 2,4,6-Tri(2-pyridyl)-s-triazine, fluorescein (acid-free) were obtained from Fluka Analytical (Steinheim, Switzerland).

2.2. Wheat and rye bran extraction

Sowing rye (*Secale cereale* L.) and plain wheat (*Triticum aestivum* L.) brans were donated by AB "Kauno Grūdai"(Kaunas Lithuania) and stored in bags at <20 °C in a dry ventilated room. Before extraction, bran was ground in an ultra-centrifugal rotor mill Retsch ZM200 (Retsch GmbH, Haan, Germany) and separated by different hole size sieves into fractions according to particle size: >1 mm, 1–0.5 mm, 0.5–0.23 mm, <0.23 mm. Soxhlet extraction was performed in an automated extractor (Behr Labor-Technik, Düsseldorf, Germany) as a standard technique (AOAC reference method, 1995) using hexane and acetone. Twenty grams of ground brans were loaded into a cellulose thimble and inserted into an inner tube of the apparatus. The rate of extraction was 1 cycle per 5 min. The Soxhlet extraction residue was re-extracted with methanol:water (80:20%) at 25 °C for 90 min in closed vials using a shaker. All extractions were performed one after another with the same bran sample after evaporating previous solvent. PLE was performed in an accelerated solvent extraction apparatus ASE 350 (Dionex, Sunnyvale, CA, USA) for each sample, consecutively applying different polarity solvents, namely hexane, acetone and the mixture of methanol:water (80:20%), further referred to as methanol. The samples consisting of 20 g ground bran were mixed with 2 g of diatomaceous earth and placed in a 66 mL Dionex stainless-steel cell (2.9 cm diameter). The cells were equipped with a stainless steel frit and cellulose filter at the ends of the cell to avoid solid particles in the collection vial. The following conditions were used for the extraction: the cells were preheated 5 min to ensure that the samples reached thermal equilibrium at 10.3 MPa pressure and 80 °C temperature before static extraction with 3 cycles extraction (total time – 15 min). A flush volume of 100% of the cell was used and finally the cell was purged for 60 s with nitrogen to collect the extract in the collection vial. Organic solvents were removed from the extracts in a rotary vacuum evaporator (Büchi, Flawil, Switzerland) at 40 °C, while water was removed by freeze drying. Dry extracts were weighed by analytical balances. Extractions were replicated three times.

2.3. Determination of antioxidant activity indicators

2.3.1. Total phenolic content (TPC)

The TPC was determined in rye and wheat bran extracts using the method of Singleton and Rossi (1965) with slight modifications. Ten μL of appropriate dilutions of the extracts or gallic acid solutions were oxidized with 190 μL Folin-Ciocalteau's reagent solution in deionized water (1:13). The reagents were mixed, allowed to stand for 3 min and then neutralized with 100 μL of 7% Na_2CO_3. The mixture was vortexed for 90 min and the absorbance was measured at 765 nm in the FLUOstar Omega reader (BMG Labtech, Offenburg, Germany). The TPC was calculated using the gallic acid calibration curve and expressed in mg gallic acid equivalents per g extract (mg GAE/g) and per g of bran dry weight (DW).

2.3.2. $ABTS^{\bullet+}$ scavenging

The Trolox equivalent antioxidant capacity (TEAC) assay was used to determine radical scavenging capacity (RSC) of bran extracts as described elsewhere (Re et al., 1999) with slight modifications. The working solution of $ABTS^{\bullet+}$ was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulphate; two stock solutions were mixed in equal quantities and kept for 14–16 h. The working solution further was diluted with a PBS (phosphate buffer solution) to obtain an absorbance of 0.80 ± 0.03 at 734 nm. Bran extracts or Trolox solutions (3 μL) were reacted with 300 μL of $ABTS^{\bullet+}$ solution for 30 min and the absorbance was read at 734 nm in a FLUOstar Omega reader. Trolox solutions (50–2500 μM/L) were used for calibration. The percentage RSC of $ABTS^{\bullet+}$ was calculated by the formula: $[(Abs_{control} - Abs_{sample})/(Abs_{control})] \times 100$, where $Abs_{control}$ and Abs_{sample} are the absorbances of $ABTS^{\bullet+}$ in the control mixture with methanol and extract, respectively. The TEAC values were calculated from the calibration curve and the RSC values were expressed in μmol Trolox equivalents (TE) per g extract (μM TE/g) and g of bran DW.

2.3.3. DPPH$^{\bullet}$ scavenging

The DPPH$^{\bullet}$ assay is based on radical scavenging by the antioxidant, which results in a decrease in absorbance at 515 nm (Brand-