Bulgarian Chemical Communications, Volume 51, Special Issue A (pp. 75- 78) 2019

Antioxidant potential of wheat bran phenolics extracted with organic solvents

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Received January 22, 2019; Revised March 5, 2019

Wheat bran as a byproduct cannot only be a food additive but also a source of antioxidants that can be easily extracted. To investigate its antioxidant potential, wheat bran was extracted using aqueous solution (80%, v/v) of acetone, methanol and ethanol. Total phenolics content was determined by the reaction with Folin-Ciocalteu's phenol reagent and antioxidant properties were compared using TEAC, FRAP, DPPH, ACL, and ACW assays. UV-spectrum comparison, TLC and RP-HPLC-DAD were applied to highlight differences among extracts. Extraction yield varied from 10.4 to 13.6%, total phenolics content varied from 16.4 to 20.2 mg/g extract, TEAC from 0.17 to 0.25 mmol Trolox/g extract, FRAP values ranged between 0.183 and 0.242 mmol Fe^{2+}/g extract, and IC_{50} values for DPPH were recorded in the range from 1.29 to 1.70 mg extract/ml. Values for PCL ranged from 0.116 to 0.168 nmol Trolox/g extract for ACL and from 50.5 to 55.7 mmol vit. C/g extract for ACW. Chromatographic separation did not reveal any major differences among phenolic constituents.

Keywords: Wheat bran; Antioxidant potential; Organic solvents; Phenolic compounds; HPLC; TLC

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a significant ingredient of the human diet. It is used worldwide to produce various of products such as bread, noodles, and cakes. It is an important source of energy and proteins.

Many of the wheat-based foods are produced from white flour. High consumption of whole grain products is typical of diets rich in a broad spectrum of nutrients, e.g. the Mediterranean diet [1].

Most of the bioactives of the whole grain are located in the bran or outer layers of the grain. Fiber is abundant in bran and as such bran can be a good source of bioactives [2]. Wheat bran is an especially good source of non-phenolic compounds with antioxidant activity like arabinoxylans, which are part of dietary fiber. Also numerous phenolics are located in the outer parts of cereals. Due to the increasing awareness of consumers and to the increasing interest in products made from whole grain or with bran addition, wheat bran has become a significant part of human diet. The high health beneficial potential of bran and wheat bran in particular resulted in many attempts to investigate the possibilities of incorporating bran in foods traditionally produced without bran and to examine their properties [3-8].

Phenolic compounds that can be found in bran may exhibit a positive effect when included into the diet. Research on mice with induced type 2 diabetes demonstrated that inclusion of soybean bran exhibited potential benefit in glycemic control [4]. Phenolics from wheat bran were also associated with antitumor activity [9].

In phytochemistry, mixtures of water with organic solvents such as methanol, ethanol, acetone, propanol, dimethylformamide, ethyl acetate, and propanol are used to extract phenolic compounds from plant material [10].

The aim of this study was to investigate the effect of the solvent on the antioxidant activity of the obtained extracts of wheat bran.

MATERIALS AND METHODS

Chemicals

Methanol and acetonitrile were acquired from the P.O.Ch. Company (Gliwice, Poland). Ferrous chloride, sodium persulfate, the Folin-Ciocalteu's phenol reagent, 2,2′-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ), sulphuric acid, and 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) were obtained from Sigma (Poznań, Poland). Kits for ACL and ACW were obtained from Analytic Jena (Jena, Germany).

Material

Material for this study was supplied by a commercial bakery located in Germany

Extraction

Material (10 g) was extracted using 80% (v/v) acetone, methanol and ethanol at 70°C for 15 min in 1:10 (w/v) solid material to solvent ratio [11]. After cooling, the supernatant was decanted, the residue was re-extracted twice, and supernatants

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were combined. The organic solvent was removed under vacuum (Büchi Rotavapor R-200, Büchi Labortechnik AG, Flawil, Switzerland) at 40°C and the water residue was freeze-dried (Freezone 6, model 77530, Labconco Co., Kansas City, MO, USA). Crude extract was stored under -20 °C until further analyzed.

Total phenolics content (TPC)

The content of total phenolics was investigated using the Folin and Ciocalteu's phenol reagent [11]. Absorbance in this and other spectrophotometric assays was measured using a DU-7500 spectrophotometer (Beckman Instruments, Fullerton, CA, USA). Results were calculated using (+)-catechin calibration curve per 1 g of extract.

TEAC assay

Antiradical activity against $ABTS^{\bullet+}$ was determined as Trolox equivalent antioxidant capacity (TEAC) and investigated according to Re *et al*. [12]. Results were expressed as mmol of Trolox equivalents per 1 g of extract.

FRAP assay

Ferric reducing antioxidant power (FRAP) was investigated according to Benzie *et al.* [13]. The results were expressed as µmol of Fe^{2+} per g of extract.

DPPH assay

Antioxidant capacity against $DPPH^{\bullet}$ was determined using method of Yen and Chen [14]. The results were expressed as EC_{50}. This value informs about the amount of extract which is required to scavenge 50% of the radicals present in the reaction mixture.

PCL assay

Photochemiluminescence assay was performed according to Popov and Lewin [15,16]. Measurements were performed by Photochem system with PCL software (Analytik Jena). Trolox for ACL (lipid-soluble antioxidative capacity) and vitamin C for ACW (water-soluble antioxidative capacity) were used to plot the calibration curve. The results were expressed as nmol of Trolox or vitamin C equivalents per g of extract.

RP-HPLC-DAD

Before HPLC analysis, twenty mg of the extract was dissolved in 2 ml of 80% (v/v) methanol and filtrated through CHROMAFIL Xtra PET-45/25 (0.45 µm) filter (Macherey-Nagel GmbH, Düren, Germany).

Phenolic profile was determined using an HPLC system (Shimadzu Corp., Kyoto, Japan) consisting of a CTO-20AC column oven, two LC-30AD pumps, a CBM-20A system controller, an SIL-30AC autosampler, and an SPD-M30A photodiode array detector. A volume of 10 µl of filtered sample was injected onto a Luna C18(2) column (150 × 4.6 mm, 3 µm, Phenomenex, Torrance, CA, USA). Gradient elution of acetonitrile-water-trifluoroacetic acid (5:95:0.1, v/v/v) (solvent A) and acetonitrile-trifluoroacetic acid (100:0.1, v/v/v) (solvent B) with a flow rate of 1 mL/min was used. Solvent B was increased from 0 to 25% from 0-5 min, 25-35% from 5 to 7 min, 35-42% from 7 to 11 min, decreased and maintained at 0 from 11.2 to 14 min. The diode array detection was scanned over a wavelength range of 200 to 400 nm.

Thin layer chromatography

Thin layer chromatography (TLC) on silica gel plates (TLC silica gel 60 F_{254}, Merck, Darmastadt, Germany) with chloroform/methanol/water (65:35:10, v/v/v, lower phase) as a mobile phase was used to analyze the three extracts. The spots on the plate were visualized by spraying with 5% sulfuric acid and heating 100°C for 5 min [17].

Statistical analysis

All analyses were done in triplicate. The results were reported as mean values ± standard deviation. Differences among extracts were determined using ANOVA and Tukey's test. SPSS 22 version (SPSS Inc., Chicago, IL, USA) was used in this research for statistical analysis.

Table 1. Extraction yield and antioxidant potential of wheat bran extracts obtained with different aqueous organic solvents.

Results	Solvent used for extraction		
	80% methanol	80% ethanol	80% acetone
Extraction yield (%)	13.6 ± 0.14^{a}	13.4 ± 0.121^{a}	10.4 ± 0.261^{b}
TPC (mg/g)	17.3 ± 0.055^{b}	16.4 ± 0.528^{c}	20.2 ± 0.078^{a}
ABTS (mmol Trolox/g)	0.180 ± 0.006^{c}	0.173 ± 0.003^{b}	0.249 ± 0.008^{a}
FRAP (mmol Fe^{2+}/g)	0.183 ± 0.004^{c}	0.194 ± 0.001^{b}	0.242 ± 0.003^{a}
DPPH (EC_{50} (mg/ml))	1.81 ± 0.03^{a}	1.70 ± 0.04^{b}	1.29 ± 0.05^{c}
ACL (mmol Trolox/g)	0.123 ± 0.010^{b}	0.116 ± 0.006^{c}	0.168 ± 0.010^{a}
ACW (mmol vit. C/g)	0.055 ± 0.002^{a}	0.051 ± 0.002^{a}	0.058 ± 0.004^{a}

*) Means in the same row with different letters are significantly different ($P<0.05$)

RESULTS AND DISCUSSION

Extraction yields for 80% methanol and 80% ethanol were similar, i.e. 13.6 and 13.4%, respectively. Lower amount of extract (10.4%) was obtained using 80% acetone (Table 1).The highest value for TPC was noted for the acetonic extract (20.2 mg/g) and the lowest for the ethanolic one (164 mg/g). In the case of the antioxidant activities of extracts, their values were obtained mostly in a similar order. The highest value for TEAC was recorded for the acetonic extract (0.25 Trolox mmol/g), as well as for FRAP (242 μmol Fe^{2+}/g), DPPH (EC_{50} = 1.29 mg/ml) and ACL (0.168 mmol Trolox/g). Methanolic and ethanolic extracts exhibited similar ($P < 0.05$) results of TEAC and ACL. The methanolic extract was characterized by the weakest activities assayed using FRAP (0.183 mmol Fe^{2+}/g) and DPPH (IC_{50} = 1.81 mg/ml). The highest results achieved for the ~~of~~ acetonic extract were due to the lower extractability of 80% acetone compared with 80% methanol and 80% ethanol [17].

Smuda *et al*. [18] obtained similar results. Acetone extracted the lowest and methanol the highest number of total phenolics, which is in accordance with our findings when comparing our results multiplied by extraction yield. However, these authors obtained higher concentration of total phenolics and described significant differences in antioxidant activity among alcoholic and acetonic extracts. Nevertheless, they did not specify the exact concentrations of solvents used. Other authors reported similar levels of total phenolics [19,20]

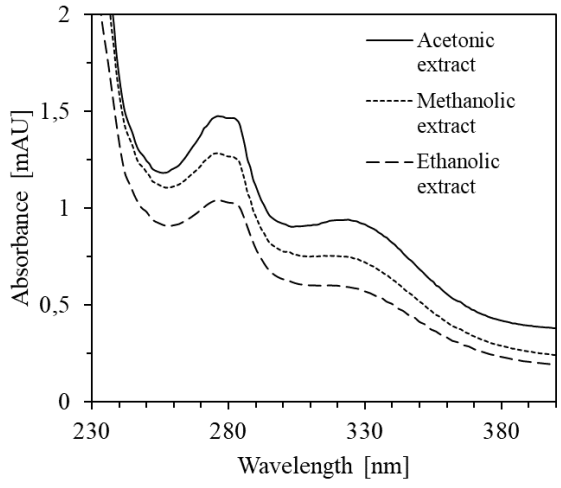

Figure 1. UV spectrum comparison for acetonic, methanolic, and ethanolic extracts

The UV spectrum of extracts is presented in Fig. 1. Two absorption maxima were detected at wavelengths of 277 and 323 nm. They might indicate the presence of phenolic acids. Hydroxybenzoic acid derivatives are reported to exhibit maxima in the range from 200 to 290 nm [20]. Hydroxycinnamic acid derivatives, flavones, and flavonols exhibit absorbtion maxima in the range from 305 to 360 nm [21-23]. Fig. 2 presents a TLC chromatogram. There are no major differences among the samples. Extracts were also analyzed using RP-HPLC-DAD. Phenolic fingerprint for 280 nm wavelength is presented in Fig. 3. Chromatograms were almost identical and there were no differences detected in concentrations of individual compounds. Analysis of their UV spectrum revealed that phenolic constituents belonged to phenolic acids, both hydroxybenzoic and hydroxycinnamic acids derivatives, but revealed no flavonoids. Other authors reported the presence of phenolic acids and flavonoids in whole grains as well as in bran [24-26], but the overall content of flavonoids in common wheat bran extracted with acetone or alcohol is below 0.2-0.71 mg/g of bran [20,26].

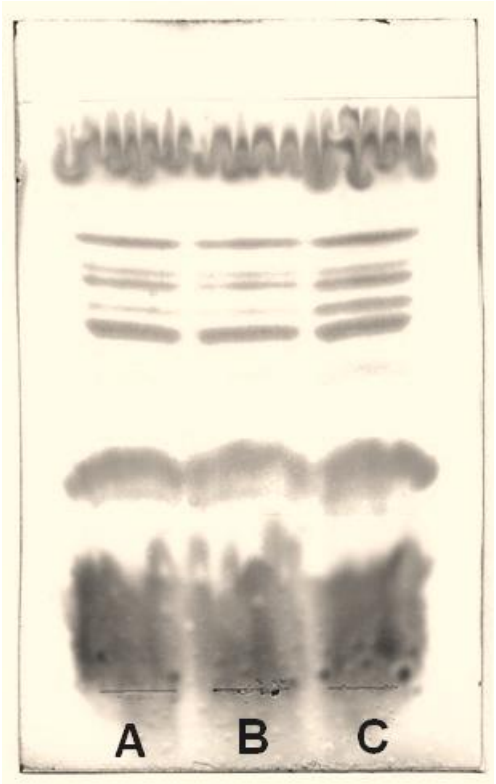

Figure 2. TLC separation for ethanolic (E), methanolic (M), and acetonic (A) extracts.

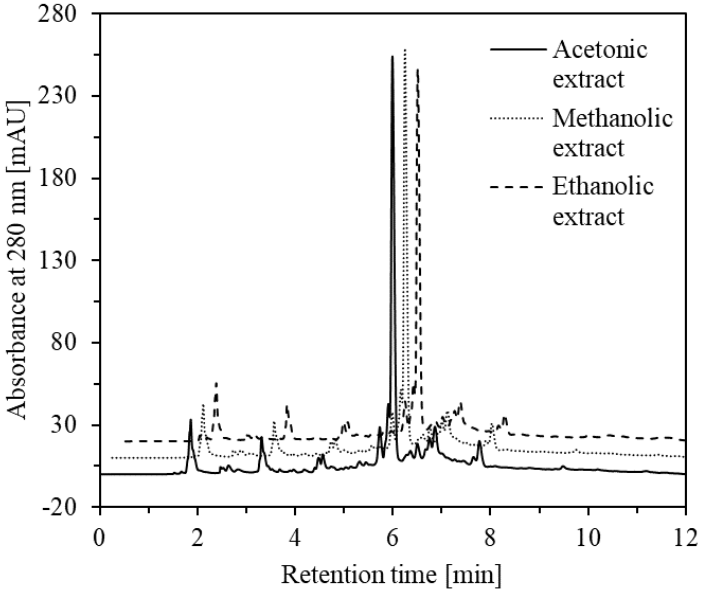

Figure 3. RP-HPLC-DAD chromatograms of acetonic, methanolic, and ethanolic extracts.

CONCLUSIONS

The acetonic extract was characterized by a significantly higher content of total phenolics and antioxidant activity than the extracts obtained using 80% methanol and 80% ethanol. All the obtained extracts were characterized by a high antioxidant activity assayed by ABTS, FRAP, DPPH, ACL, and ACW methods.

Acknowledgements: *The study was financed by The National Centre for Research and Development, project entitled:"JPI HDHL Food Processing for Health - Food Fermentations for Purpose: Health Promotion and Biopreservation".*

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