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Effect of heat treatment on the antioxidant capacity of dry wheat bran

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The aim of the study was to investigate antioxidant ~~the~~ capacity of phenolic compounds from wheat bran heated at 120ºC for 10 min (1WH), at 120ºC for 40 min (2WH), at 140ºC for 10 min (3WH), at 140ºC for 40 min (4WH), and at 160ºC for 10 min (5WH). The extract of the untreated bran served as a control. Wheat bran was extracted using 80% methanol (v/v) at 70°C for 15 minutes in triplicate. The antioxidant activity of the extracts was investigated using ABTS, FRAP, and DPPH methods. The contents of total phenolic compounds in the extracts were determined using a Folin-Ciocalteu's phenol reagent. The profile of phenolic compounds was determined using an HPLC method.

The sample 5WH was characterized by the highest content of total phenolics content of (3.02 mg/g bran), whereas the lowest total phenolics content was noted for 1WH and 2WH samples (2.10 and 2.11 mg/g bran). The effect of heating on the results of ABTS and FRAP assays was not strong. Heat processing increased the antiradical potential of bran against DPPH radical. This effect could be caused by Super Heated Steam during heat treatment. The effect of heat treatment on the profile of phenolic compounds in wheat bran was confirmed using the HPLC analysis.

Keywords: Wheat bran; Phenolic compounds; Heating; Antioxidant activity

INTRODUCTION

Cereals are major constituent of a human diet, being the important source of proteins and energy, particularly in developing countries. Cereals and their products are rich in antioxidant phytochemicals that make them ideal for developing functional foods and ingredients [1]. Cereals and their by-products, like bran, exhibit antioxidative, antimutagenic, and anticarcinogenic activities [2]. These wastes are also a good source of dietary fibers [3] and bioactive compounds like phenolic acids, pigments, flavonoids, tannins, and vitamins. Hence, the production of value added products such as food additives and supplements from food processing wastes has gained worldwide attention [4]. The valorizations of wastes are not only economical but also environmentally benign [5].

The aim of this study was to investigate the effect of Super Heated Steam treatment on the antioxidant capacity of phenolic compounds from wheat bran.

EXPERIMENTAL

Chemicals

Methanol and acetonitrile were acquired from the P.O.Ch. Company (Gliwice, Poland). Ferrous chloride, sodium persulfate, the Folin-Ciocalteau phenol reagent, 2,2′-diphenyl-1-picrylhydrazyl (DPPH), (+)-catechin, ferulic acid, *p*-coumaric acid, rutin 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTS), and 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) were obtained from Sigma (Poznań, Poland).

Material

Wheat bran supplied by Ernst Böcker GmbH & Co. (Minden, Germany), were treated by Super Heated Steam (SHS) in a pilot apparatus designed and manufactures by TNO (Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek - Netherlands Organisation for Applied Scientific Research). Samples (89% dry matter) were treated at different combination of temperature: at 120ºC for 10 min (sample 1WH), at 120ºC for 40 min (2WH), at 140ºC for 10 min (3WH), at 140ºC for 40 min (4WH), and at 160ºC for 10 min (5WH). The extract of the untreated bran served as a control (WH). After the SHS treatment, samples were freeze dried for further analysis.

Extraction

Material was extracted using 80% (v/v) methanol at 70°C for 15 min, at 1:10 (w/v) solid material to solvent ratio [6]. After cooling, the supernatant was decanted, the residue was re-extracted twice, and supernatants were combined.

Total phenolics content

The content of total phenolic compounds in the examined extracts was investigated using Folin and Ciocalteu's phenol reagent [7]. (+)-Catechin was used as a standard in this research. The results were expressed as mg catechin equivalents (CE)/g bran.

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ABTS assay

The antiradical activity against ABTS•+ was determined as Trolox equivalent antioxidant capacity (TEAC) and investigated according to Re *et al*. [8]. Results were expressed as μmol Trolox equivalents per g of bran.

FRAP assay

The ferric reducing antioxidant power was determined according to the method described by Benzie at al. [9]. Results were expressed as μmol of Fe^{2+} per g of bran.

DPPH assay

The antiradical activity against DPPH radical was tested using method described by Yen and Chen [10]. Results were expressed as μmol of Trolox equivalents per g of bran.

HPLC analysis

An HPLC system (Shimadzu Corp., Kyoto, Japan) consisting of a CTO-20AC column oven, two LC-30AD pumps, an CBM-20A system controller, an SIL-30AC autosampler, and an SPD-M30A photodiode array detector was used to separate the phenolic compounds in the samples. The filtered sample (10 μL) 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 60% from 0-18 min, decreased and maintained at 0 from 18.20-20.0 min [11]. The diode array detection was performed by scanning over a wavelength range from 200 to 400 nm. The wavelength of 280 nm was selected for quantification of the major phenolic constituents. The quantification was based on calibration curves of (+)-catechin and benzoic acid.

Statistical analysis

All analyses were triplicated. The results were reported a 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.

RESULTS AND DISCUSSION

The effect of SHS treatment on the content of total phenolics in wheat bran was noted when the process was conducted at 140°C for 40 min (4WH), and at 160°C for 10 min (5WH). The content of total phenolics in samples 4WH and 5WH were 5.43 and 3.02 mg/g, respectively. The content of total phenolics in untreated bran was of 2.17 mg/g (Table 1).

Table 1. Effect of bran heating on the content of total phenolics and results of ABTS, FRAP, and DPPH assays.

Material	Total phenolics (mg/g)	ABTS assay (μmol Trolox/g)	FRAP assay (μmol Fe^{2+}/g)	DPPH assay (μmol Trolox/g)
WH untreated	2.17 ± 0.03^{dc}	23.4 ± 0.6^{c}	17.8 ± 0.3^{d}	3.42 ± 0.01^{e}
1WH	2.10 ± 0.01^{e}	24.0 ± 0.8^{c}	21.2 ± 0.2^{c}	5.58 ± 0.02^{d}
2WH	2.11 ± 0.01^{de}	24.0 ± 0.4^{c}	21.5 ± 0.4^{c}	6.28 ± 0.04^{c}
3WH	2.25 ± 0.07^{c}	23.0 ± 0.6^{c}	22.1 ± 0.2^{c}	6.55 ± 0.19^{c}
4WH	2.43 ± 0.07^{b}	25.3 ± 0.6^{b}	24.9 ± 0.2^{b}	7.27 ± 0.25^{b}
5WH	3.02 ± 0.06^{a}	29.9 ± 0.5^{a}	34.3 ± 0.5^{a}	8.55 ± 0.11^{a}

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

Samples 4WH and 5WH exhibited the highest results of ABTS (25.3 and 29.9 μmol Trolox/g, respectively), FRAP (24.9 and 34.3 μmol Fe^{2+}/g, respectively), and DPPH (7.27 and 8.55 μmol Trolox/g, respectively) assays (Table 1). The high results of antioxidant assays of samples 4WH and 5WH can be caused by the formation of Maillard reaction products. Antioxidant properties of these compounds was reported by numerous authors [12].

The presence of 9 main phenolic compounds in the extracts of wheat bran was confirmed with the HPLC method (Fig. 1). Compounds 1-4 and 6-8 showed UV spectra with maxima of absorption between 253 and 282 nm. UV spectra of compound 5 and 9 exhibited maxima of absorption at 300 and 326 nm (Fig. 2). Most likely, these compounds were derivatives of *p*-coumaric and ferulic acid [13].

The content of individual phenolic compounds in wheat bran is reported in Table 2. Worth of emphasizing is a fact that some of these compounds (2, 3, 4, 9) were determined only in the SHS-treated bran. Thermal process increased the extraction availability of these compounds or liberated them from cell walls [14]. A low content of compound 7 in sample WH4 and WH5 confirms that it is thermally instable.

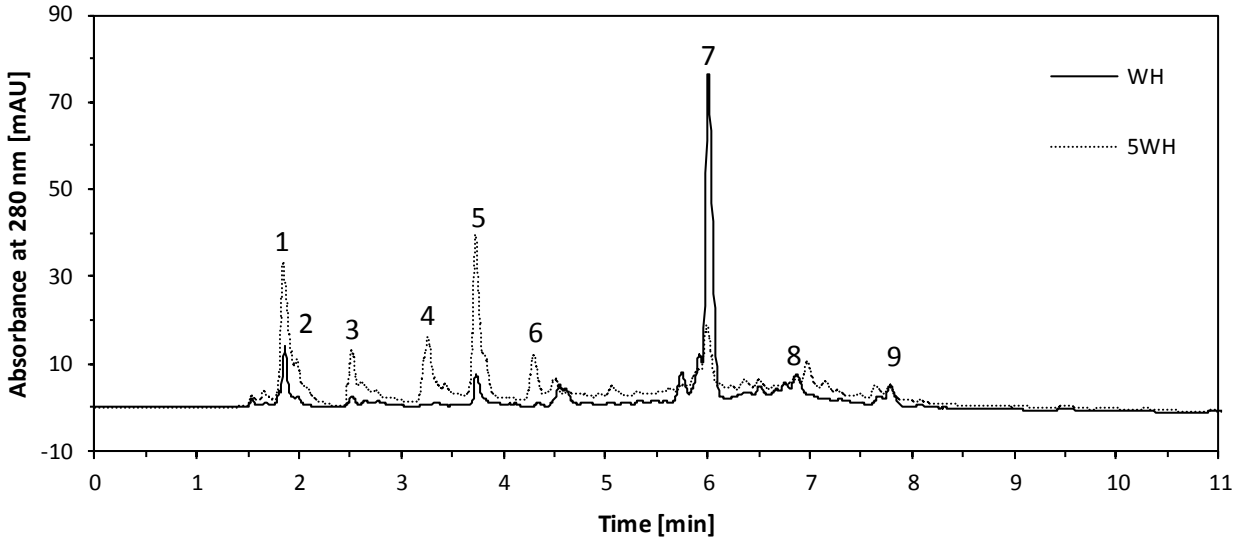

Fig. 1. HPLC chromatograms of phenolic compounds of wheat bran.

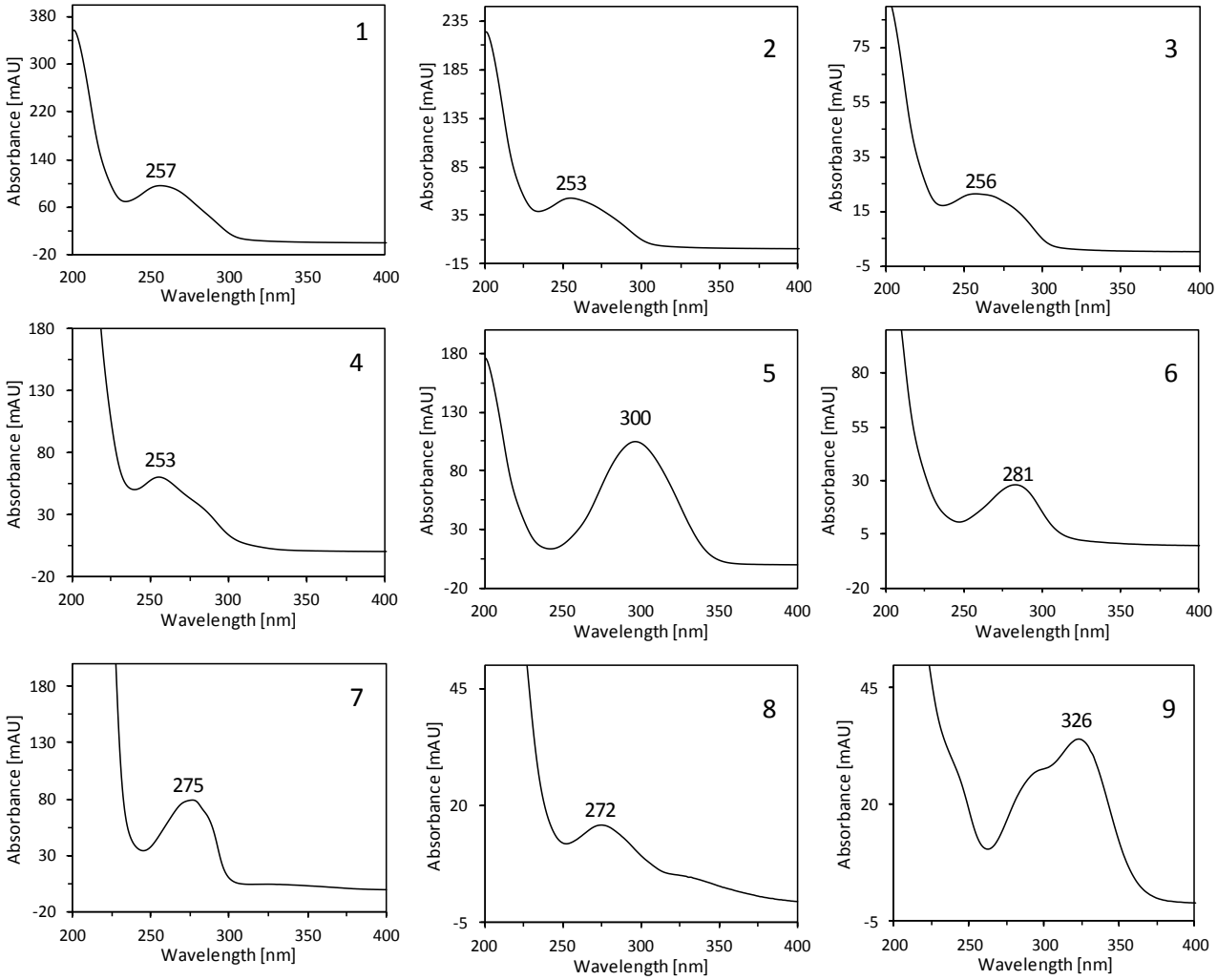

Fig. 2. UV-DAD spectra of individual phenolic compounds of wheat bran.

Table 2. Content of individual phenolic compounds in wheat bran (mg/g).

Compound	Material					
	WH untreated	1WH	2WH	3WH	4WH	5WH
1	0.082 ± 0.004^{f}	0.113 ± 0.006^{e}	0.131 ± 0.007^{d}	0.152 ± 0.008^{c}	0.219 ± 0.011^{b}	0.261 ± 0.013^{a}
2	-	-	-	-	0.083 ± 0.004	0.120 ± 0.006
3	-	0.018 ± 0.001^{e}	0.027 ± 0.001^{d}	0.031 ± 0.002^{c}	0.063 ± 0.003^{b}	0.084 ± 0.004^{a}
4	-	0.065 ± 0.003	0.072 ± 0.004^{d}	0.083 ± 0.004^{c}	$0.133\ 0.007^{b}$	0.163 ± 0.008^{a}
5	0.025 ± 0.001^{c}	-	-	0.015 ± 0.001^{d}	0.037 ± 0.002^{b}	0.165 ± 0.008^{a}
6	-	0.137 ± 0.007^{b}	-	0.036 ± 0.002^{c}	0.124 ± 0.006^{b}	0.392 ± 0.020^{a}
7	0.600 ± 0.030^{b}	0.581 ± 0.029^{b}	0.726 ± 0.036^{a}	0.637 ± 0.032^{b}	0.292 ± 0.015^{c}	0.273 ± 0.014^{c}
8	0.032 ± 0.002^{d}	-	0.141 ± 0.007^{c}	0.196 ± 0.010^{b}	0.203 ± 0.010^{b}	0.389 ± 0.019^{a}
9	-	0.048 ± 0.002^{a}	0.044 ± 0.002^{a}	0.044 ± 0.002^{a}	0.029 ± 0.001^{c}	0.036 ± 0.002^{b}

Means in the same row with different letters are significantly different ($P<0.05$). Compound 5 expressed as *p*-coumaric acid equivalents, compound 9 expressed as ferulic acid equivalents, other compounds expressed as catechin equivalents.

CONCLUSION

Wheat bran is a rich source of phenolic compounds with a high antioxidant activity. This activity is affected by the Super Heated Steam process. Most likely, phenolic compounds are liberated from plant cell walls at high temperatures.

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