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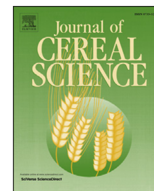

Effects of microfluidization on antioxidant properties of wheat bran

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ABSTRACT

Phenolic compounds present in native wheat bran are majorly bound to polysaccharides and entrapped in the fibre matrix. Recently, it was demonstrated that the microfluidization process could significantly improve physicochemical properties of wheat bran due to particle size reduction and microstructure modification. The current study provides further evidence that the process also significantly increased the contents of surface-reactive, alkaline and acid hydrolysable phenolics by 280%, 60% and 20%, respectively, after a total of 8 passes through the IC_{200} and IC_{87} chambers. Accordingly, the associated antioxidant capacity increased with increase in the extent of the treatment. However, there was a decrease in solvent extractable phenolic contents due to their dispersion in water and loss during the treatment. It is also worth noting that the residues after alkaline and acid hydrolysis still contained a high content of surface-reactive phenolics, which might indicate a significant underestimation of the total phenolic content and antioxidant capacity of wheat bran when using the conventional method based on solvent extraction and alkaline and/or acid hydrolysis.

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1. Introduction

It has been demonstrated that wheat grain is rich in phenolic acids which can be divided into two categories: benzoic acid derivatives such as gallic, vanillic, syringic and *p*-hydroxybenzoic acids, and cinnamic acid derivatives including ferulic, *p*-coumaric and caffeic acids (Kim et al., 2006; Yu and Cheng, 2007). These phenolic acids have strong antioxidant activity which may modulate cellular oxidative status and prevent biologically important molecules such as DNA, proteins, and membrane lipids from oxidative damage (Yu et al., 2002). Therefore, they are believed to significantly contribute to the health benefits of whole wheat consumption observed in numerous epidemiological studies (Willcox et al., 2004).

Ferulic acid is the most abundant phenolic acid, representing up to 90% of total phenolic acids present in wheat grain (Adom and Liu, 2002) and is mainly concentrated in the bran fraction (Zhou et al., 2004). In the bran, ferulic acid exists in free, soluble conjugated and insoluble bound forms, the latter one accounting for about 99% (Adom and Liu, 2002). Free and some conjugated ferulic acid is thought to be readily available for absorption in the human small or large intestines; however, those in the bound form can only be absorbed after being liberated from indigestible polysaccharides to which they are covalently bound (Mateo et al., 2009).

An *in vitro* study showed that the action of β-glucuronidases and esterases of gut microflora located mainly in the large intestine could result in the release of phenolic compounds from fibre matrix in cereal-products (Poquet et al., 2008). The liberated antioxidants remaining in the colonic lumen can counteract free radicals which are believed to be involved in the etiology of colorectal cancer. On the other hand, recent studies have highlighted that the antioxidant functional groups, which are bound to the insoluble fractions of various food components, are reactive and thus may exert an antioxidant activity by quenching free radicals present in the solvent matrix through a surface reaction phenomenon (Serpen et al., 2007). This, together with the released antioxidant phenolic acids, might act along the whole length of the digestive tract by trapping oxidative compounds.

As has been suggested, the structural network formed by the dietary fibre matrix may physically block the components entrapped in the fibre complex from interaction with other molecules in the gastrointestinal tract (Nystrom et al., 2007). This may hinder the release of the bound phenolic compounds and limit their antioxidant reactivity as well. In a recent study, we found that the microfluidization process could substantially increase the surface area of wheat bran particles by reducing their particle size,

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loosening their microstructure, and even creating pores or cavities inside them (Wang et al., 2012). Therefore, it was speculated that this process might facilitate the release of bound phenolic compounds from fibre matrix or expose more antioxidant functional groups to the surrounding environment.

The objective of this study was to assess the effectiveness of the microfluidization process in improving the antioxidant capacity of bound phenolic compounds in wheat bran, and to test if microfluidization can increase the contents of surface-reactive, solvent extractable, alkaline and acid hydrolysable phenolic compounds.

2. Materials and methods

2.1. Materials and chemicals

Coarse wheat bran was obtained from ConAgra Foods (Omaha, NE, USA) and pretreated by following the procedure described in our previous report (Wang et al., 2012).

The compounds 2,2′-azino-bis[3-ethylbenz-thiazoline-6-sulphonate (ABTS), 2,2-diphenyl -1-picrylhydrazyl (DPPH), 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4′′-disulfonic acid monosodium salt (Ferrozine), Folin–Ciocalteu's phenol reagent, potassium ferricyanide ($K_3Fe(CN)_6$), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA-$Na_2 \cdot 2H_2O$), Iron (II) chloride, potassium persulfate, ferric chloride ($FeCl_3$) and L-ascorbic acid were purchased from Sigma–Aldrich (St. Louis, MO, USA). Gallic acid was obtained from Acros Organics (New Jersey, USA). All the solvents used were of HPLC grade, and other chemicals and reagents were of analytical grade.

2.2. Processing of wheat bran by microfluidization

An M-110P Microfluidizer Processor (Microfluidics, Newton, MA, USA) was used for the processing. Ground wheat bran was dispersed in distilled water at a ratio of wheat bran: water 1:50 (wt/wt). The suspension was then processed in a Microfluidizer with two sizes of interaction chambers (200 μm (IC_{200}) and 87 μm (IC_{87}) in diameter) at room temperature. Processing pressures were 159 MPa and 172 MPa for IC_{200} and IC_{87}, respectively. Interaction chambers used in this study had a "Z" shape. Bran suspensions were first processed through the IC_{200} chamber for 1–3 passes. One pass meant the sample went through the machine one time. All those samples further processed with IC_{87} were pre-processed by an IC_{200} for three passes. The processed bran samples were collected by centrifugation and freeze dried. The moisture content of the freeze-dried samples was determined according to the standard method 44-15A (AACC International, 2000) and the values ranged from 5.4 to 6.6%. Dry samples were sealed in air-tight glass containers and stored at −30 C for analysis. All tests were duplicated.

2.3. Extraction of phenolic compounds

2.3.1. Solvent extractable phenolic compounds

A reported method (Zhou and Yu, 2004) with some modifications was used for the extraction. This protocol was shown to be effective in extracting free phenolic compounds from cereal bran. Briefly, 1 g untreated or microfluidized bran samples was extracted three times with 20 mL of 50% acetone for 20 min with shaking. Supernatants were collected by centrifugation at 8000 *g* for 10 min, evaporated in vacuum, reconstituted in 10 mL of DMSO/methanol (50:50, v/v) and stored at −30 c until analysis.

2.3.2. Alkaline and acid hydrolysable phenolic compounds

The residue after the solvent extraction was air-dried for 12 h and hydrolysed with 2 M NaOH at room temperature for 4 h with shaking under nitrogen. The resulting hydrolysate was acidified to pH 2 with 6 M HCl and centrifuged. The bran residue obtained was air dried for 12 h and hydrolysed with 6 M HCl at 95 °C for 1 h. The value of pH for the resulting hydrolysate was then adjusted to 2 and centrifuged. The final bran residue obtained was air dried for other analyses. Each supernatant from alkaline and acid hydrolysis was extracted with hexane five times to remove lipid contaminants. The liberated phenolic compounds were then extracted with ethyl acetate six times and subsequently evaporated to dryness. The extracts were reconstituted in 10 mL of DMSO/methanol (50:50, v/v) and stored at −30 c until analysis.

2.4. Determination of total phenolic content of wheat bran

2.4.1. Direct procedure for ground raw, microfluidized wheat bran and the residue after alkaline and acid hydrolysis

The content of surface-reactive phenolic compounds of ground raw, microfluidized wheat bran and the residue after alkaline and acid hydrolysis was determined by a direct procedure (Serpen et al., 2007) modified from the Folin–Ciocalteau method (Singleton et al., 1999). Briefly, 10 mg powdered wheat bran sample was mixed with 2.5 mL of Folin–Ciocalteu reagent (10%, wt/wt, in distilled water) in a centrifuge tube. After 5 min, 2 mL of sodium carbonate aqueous solution (7.5%, wt/wt) was added. The mixture was then incubated for 2 h at room temperature, being vortexed several times during the incubation. The mixture was centrifuged at 10,000 rpm for 15 min. The supernatant was collected and absorbance at 725 nm was measured using a Shimadzu spectrophotometer (model 2500). A standard curve with serial gallic acid solutions was used for calibration. Results were expressed as mg of gallic acid equivalents (GAE) per gram of dry weight (d.w.). Additional dilution was made with cellulose when the absorbance was over the linear range of the standard curve.

2.4.2. Contents of solvent extractable, alkaline and acid hydrolysable phenolic compounds

The phenolic content of the extracts and hydrolysates was quantified according to the modified Folin–Ciocalteau method (Singleton et al., 1999) as reported in a previous study (Wang et al., 2009). Results were expressed as mg of gallic acid equivalents (GAE) per gram of dry weight (d.w.).

2.5. Determination of ABTS radical scavenging activity

ABTS radical scavenging activity was measured following a reported procedure (Serpen et al., 2007) with some modifications. The ABTS reagent was prepared by incubating 7 mmol/L ABTS aqueous solution with 2.45 mmol/L potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The solution was further diluted with a mixture of ethanol:water (50:50, v/v) to obtain an absorbance of 0.70 ± 0.02 at 734 nm. Ten mg of wheat bran sample was mixed with 6 mL ABTS reagent. The mixture was vortexed periodically throughout the 60 min incubation period to facilitate the surface reaction between the bran and the ABTS reagent. After centrifugation at 10,000 *g* for 5 min, the absorbance of the optically clear supernatant was recorded at 734 nm using a Shimadzu spectrophotometer (model 2500). A calibration curve was prepared with different concentrations of Trolox (ranging from 0 to 0.2 mmol/L) and the results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC, μmol Trolox equivalents/g d.w.). Additional dilution was made with cellulose when the absorbance was over the linear range of the standard curve.

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