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Original Research Article

Phytochemical composition and antiproliferative activities of bran fraction of ten Maryland-grown soft winter wheat cultivars: Comparison of different radical scavenging assays

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ABSTRACT

Phytochemicals (ferulic acid, tocopherols, and carotenoids) composition and antiproliferative activities of bran samples of the 10 soft winter wheat varieties grown in Maryland were investigated. All extracts were assayed for total phenolic content and free radical scavenging capacities by multiple colorimetric assays along with cellular antioxidant activity (CAA) and antiproliferative activity. Ferulic acid was the predominant phenolic acid in all 10 wheat bran samples with concentration ranging between 1.1 and 1.7 mg/g. The concentrations of lutein, zeaxanthin, and β-carotene ranged between 1.0–1.4, 0.2–0.3, and 0.1–0.2 μg/g, respectively. Significant amount of α-tocopherol (2.3–5.3 μg/g) was quantified in all bran samples along with minor quantity of δ-tocopherol (~0.1 μg/g). No significant correlation between ferulic acid, tocopherol and carotenoid content and in vitro antioxidant radical scavenging assays or total phenolic content was observed. The Jamestown wheat bran demonstrated significant antiproliferative activities against both HT-29 and Caco-2 colon cancer cells at concentration of 50 mg bran equivalent (BE)/mL.

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1. Introduction

Wheat and its products are an important part of the human diet. Wheat is the third most important field crop in both planted acreage and gross farm receipts, behind corn and soybeans in US (USDA, 2012). It is one of most important agricultural product commodities consumed globally. Wheat bran, a byproduct of the flour milling industry, is an important, inexpensive and readily available source of dietary fiber. It has primarily been used as animal feed, but wheat and other cereals bran have gained importance in various food product formulations and dietary supplement markets due to high fiber and bioactive constituents (Doty, 2012). This has been attributed to the recent epidemiological studies with whole grain foods which suggest that whole grains provide health-promoting protective effects against certain types of cancers, cardiovascular diseases and type-2-diabetes (de Munter et al., 2007; Mellen et al., 2009; Schatzkin et al., 2007). Most of the health beneficial effects of the whole grains are due to bioactive phytochemicals, vitamins, minerals, and fiber present in high concentration in the bran fraction of the grain.

The bran fraction constitutes approximately 15–20% of dry grain weight. It usually comprises the outermost portion of the grain composed of several layers (pericarp, testa, and hyaline) that are characterized by distinct structures and composition. The inner layer is composed of aleurone cells, and it constitutes approximately 6–7% of the bran. The percent values for bran fractions vary with the type of wheat cultivar (Hemery et al., 2012).

There have been large numbers of peer-reviewed publications on antioxidant capacity of wheat bran fraction in recent years.

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Significant antioxidant capacity and phenolic compounds have been detected in wheat, wheat bran and wheat-based products (Liyana-Pathirana and Shahidi, 2007; Moore et al., 2005, 2006a; Zhou et al., 2004a,b, 2005). In addition, phenolic acids exist in both soluble and insoluble bound forms in wheat grains, and around 90% of total phenolic acids are in insoluble forms, tightly bound to cell wall polymers. Thus, it is difficult to extract all the forms of phenolic compounds that are responsible for antioxidant capacities from wheat grains. Moreover, the wide variations in the reported antioxidant capacity values of these compounds stems from the differences in procedures used for the assay of antioxidants and the methodologies used for extraction of antioxidants (Luthria, 2006). Recently, researchers have developed a new procedure to measure the antioxidant capacities of insoluble bound phenolic acids in foods and cereal grains (Celik et al., 2013; Gokmen et al., 2009; Serpen et al., 2008; Tufan et al., 2013). Apak et al. (2007) utilized the QUENCHER-CUPRAC method for assaying total antioxidant capacity, whereas Serpen et al. (2007) measured antioxidant capacity in the insoluble portion of the food by QUENCHER procedure using $ABTS^{\bullet+}$ or $DPPH^{\bullet}$.

In previous publications by Zhou et al. (2004b), the authors described phytochemicals and antioxidant properties of 7 wheat varieties from 4 countries. In another study the same group carried out antioxidant capacity and phytochemical analysis of hard red winter wheat varieties (Zhou et al., 2004a). In a very recently published study, the authors reported phytochemicals composition, antioxidant activities, and antiproliferative activities of 10 wheat flour samples (Lv et al., 2012). In a continuation of our research on wheat, we report here a systematic comparison of 3 classes of phytochemicals (phenolic acids, tocopherols, and carotenoids) from bran fraction of 10 soft red winter wheat (*Triticum aestivum* L.) varieties commonly cultivated in the mid-Atlantic region of the United States. In this study, we examine the scavenging activities against hydroxyl ($HO^{\bullet}$), 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), 2,2′azinobi-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$), and peroxyl radicals along with total phenolic content by commonly used Folin-Ciocalteu assay. In addition, reduction of oxidative stress in human liver cancer Hep G2/C3A cells, and antiproliferative activities in HT-29 and Caco-2 human colon cancer cells of soft winter wheat bran samples are also investigated.

2. Materials and methods

2.1. Wheat samples

Ten soft red winter wheat (*Triticum aestivum* L.) varieties, SS520, SSMPV57, SS5205, USG3555, USG3665, USG3315, Branson, Shirley, Jamestown and Chesapeake, representing a sample of elite commercial cultivars currently grown in the mid-Atlantic region of the United States, were grown in the field at Clarksville (MD, USA) in yield trial plots 4 m long × 1 m wide at a density of approximately 350,000 plants ha^{-1}. Plots were planted following a crop of corn on October 2010. Plots were fertilized with a fall application of 16 kg ha^{-1} of nitrogen, 40 kg ha^{-1} of phosphorus and 80 kg ha^{-1} of potassium. Additionally, 30–80 kg ha^{-1} of nitrogen was applied in March or April 2011. Grain from the field plots was mechanically harvested, threshed and cleaned of debris prior to laboratory testing.

2.2. Preparation and extraction of wheat bran

Each wheat sample was ground to a particle size of 40-mesh using a handheld coffee grinder and separated into flour and bran fraction. The bran yield was about 17.0–22.2%. The milled bran samples were kept in a −20 °C freezer in airtight containers until analysis. The assay for the extraction of antioxidants was conducted according to a previously reported laboratory procedure (Moore et al., 2006a). First, 0.5 g of ground wheat bran was extracted with 5 mL of 50% acetone in a screw-capped tube in the dark at ambient temperature for 24 h. The supernatants were collected by centrifugation and stored under nitrogen in the dark at low temperature until further analysis. The acetone extracts were used for estimating total phenolic content (TPC), relative $DPPH^{\bullet}$ scavenging capacity (RDSC), oxygen radical absorbing capacity (ORAC), hydroxyl radical scavenging capacity (HOSC), and $ABTS^{\bullet+}$ scavenging capacity. For each type of analysis 3 independent samples were analyzed in 4 analytical replicates.

2.3. Chemicals and reagents

Disodium ethylenediaminetetraacetate (EDTA), 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$), fluorescein (FL), lauryl sulfate sodium salt, sodium hydroxide, ethyl ether, ethyl acetate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), tocopherols (α-, δ-, and γ-), ascorbic acid, β-carotene, 2′,7′-dichlorofluorescein diacetate (DCFHDA), fetal bovine serum, hepes (pH 7.4), L-glutamine, insulin, hydrocortisone, antibiotic–antimycotic and gentamicin were purchased from Sigma–Aldrich (St. Louis, MO, USA). Iron (III) chloride, 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), $2NH_4$ (ABTS chromophore, diammonium salt), and thirty percent ACS-grade hydrogen peroxide were obtained from Fisher Scientific (Fair Lawn, NJ, USA). 2,2′-Azinobis (2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals (Richmond, VA, USA). Ultrapure water was used for all experiments, which was prepared by an ELGA Purelab ultra Genetic polishing system with <5 ppb TOC and resistivity of 18.2 mΩ (Lowell, MA, USA). Human hepatoma cell line Hep G2/C3A, human colorectal cell lines HT-29 and Caco-2 were obtained from American Type Culture Collection (ATCC). All cell culture medium components were purchased from Invitrogen (Grand Island, NY, USA). All other chemicals and solvents were of analytical grade and were used directly without further purification.

2.4. Measurement of TPC

The TPC of wheat bran was determined according to a laboratory procedure described previously (Yu et al., 2002). In general, the final reaction mixture contained 50 μL wheat bran extract, 250 μL of the Folin-Ciocalteu reagent, 750 μL of 20% sodium carbonate, and 3 mL ultrapure water. Gallic acid was used as the standard. After 2 h of reaction at ambient temperature in the dark, absorbance was read at 765 nm Results were expressed as mg of gallic acid equivalent (GAE) per gram of wheat bran on a dry weight basis.

2.5. Quantification of ferulic acid in wheat bran

Each wheat bran sample was analyzed for its ferulic acid soluble free and conjugated and insoluble bound according to the laboratory method described by Moore et al. (2005). Ground wheat bran was extracted with acetone/methanol/water (7:7:6, v/v/v) first to obtain the soluble supernatant and residue. The residue was hydrolyzed with sodium hydroxide, and then extracted with ethyl ether and ethyl acetate (1:1, v/v) for analysis of insoluble bound ferulic acid. Soluble, free, and conjugated ferulic acid in the supernatant was separated under acidic conditions (pH = 2). Ferulic acid was extracted with ethyl ether and ethyl acetate (1:1, v/v). After evaporating the organic phase under nitrogen, residue was re-dissolved in methanol and filtered through a 0.20 μm membrane filter. The filtered extract was analyzed for ferulic acid quantification by HPLC analysis. Briefly, the elution program was as follows: mobile phase A consisted of acetic acid/H_2O (2:98, v/v) and mobile phase B consisted of acetic acid/acetonitrile/H_2O (2:30:68, v/v/v). Elution