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Phytochemical profiles and antioxidant activity of brown rice varieties

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ABSTRACT

The phytochemical content and antioxidant activity of eight varieties of brown rice (BR) are reported. The total phenolic contents of BR ranged from 72.45 to 120.13 mg of gallic acid equiv./100 g. The phenolics from bound fraction contributed 40.6–50.2% of the total phenolic content. The total flavonoid contents of BR ranged from 75.90 to 112.03 mg catechin equiv./100 g. The flavonoids from the bound fraction contributed 26.9–48.2% of total flavonoids. *Trans*-ferulic acid was the predominant phenolic acid in BR. Total *trans*-ferulic acid content ranged from 161.42 to 374.81 μg/100 g. The percentage of *trans*-ferulic acid in bound fraction ranged from 96.4% to 99.2%. Only α- and γ-tocopherols and -tocotrienols were detected in BR with α-tocopherol and γ-tocotrienol being the predominant. The total peroxyl radical scavenging capacity (PSC) of BR ranged from 18.29 to 40.33 mg vitamin C equiv./100 g. The bound fraction contributed 67.2–77.2% of total PSC.

1. Introduction

Regular consumption of whole grains has been correlated with reduced risk of developing various chronic diseases, including cardiovascular disease, type 2 diabetes, obesity, and cancer (Okarter & Liu, 2010; Xi & Liu, 2016). The bioactive phytochemicals in whole grains have been proposed to account for the health benefits of whole grain consumption (Liu, 2007; Okarter & Liu, 2010). Phenolic acids are a class of the most common phytochemicals in whole grains. Phenolic acids can be divided into two major subgroups: *p*-hydroxybenzoic acid and *p*-hydroxycinnamic acid derivatives. *P*-hydroxybenzoic acid derivatives include *p*-hydroxybenzoic acid, protocatechuic acid, vannilic acid (VA), syringic acid (SYA), and gallic acid. *P*-hydroxycinnamic acid derivatives, which includes ferulic acid (FA), *p*-coumaric acid (*p*-COA), sinapic acid (SIA), caffeic acid (CFA), and chlorogenic acid (CHA), are the predominant phenolic acids found in grains (Guo & Beta, 2013; Okarter, Liu, Sorrells, & Liu, 2010). They mainly occur as bound forms, covalently linked to cell wall structural components such as cellulose, hemicellulose, lignin, pectin, and proteins through ester or ether bonds (Adom & Liu, 2002; Liu, 2007).

Rice (*Oryza sativa* L.) is the most important grain crop worldwide. It serves as a staple food for approximately half of the world's

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population. Rice is the grain with the second highest production after maize in the world (Goufo & Trindade, 2014). Harvested rice, known as paddy or rough rice, needs to be processed before being consumed by humans. The process starts with removing the inedible husk that covers the grain, thereby producing whole grain rice. Thereafter, the milling process strips the bran layer and germ from whole grain rice, turning whole grain rice into white rice. Final refining steps may include polishing and grading. White rice has a lower nutritional value when compared to whole grain rice, due to the loss of vitamins, minerals, phenolics, fibers, and other bioactive compounds (Liu, 2007). According to the husked grain color, whole grain rice varieties can be subdivided into non-pigmented rice varieties (BR varieties) and pigmented rice varieties (purple, black, and red rice varieties).

Excess free radicals can cause oxidative damage to all types of biomolecules including DNA, proteins, and lipids that have been associated with many chronic diseases (Willcox, Ash, & Catignani, 2004). The scavenging of free radical by the phytochemicals from dietary whole grain consumption may be a mechanism by which whole grain have prevention effect of chronic diseases (Liu, 2007). Peroxyl radical, which commonly exit in human body, is oxygen-centered radical formed during the breakdown of organic peroxides. The peroxyl radical scavenging capacity (PSC) assay, which was established by Adom and Liu (2005), was an accurate, precise, and reproducible approach for assessing the antioxidant activities of pure compounds and food extracts. The PSC of free and bound fractions of grains (corn, wheat, oat, and rice), diverse corn, foxtail millet, and adlay varieties were reported before (Adom & Liu, 2005; de la Parra, Serna Saldivar, & Liu, 2007; Wang, Chen, Xie, Ju, & Liu, 2013; Zhang & Liu, 2015). However, the PSC of diverse BR varieties was not reported previously.

Several studies have reported the phytochemical content and antioxidant activity of wild rice varieties, different colored rice varieties, and black rice varieties (Min, Gu, McClung, Bergman, & Chen, 2012; Qiu, Liu, & Beta, 2010; Shao, Xu, Sun, Bao, & Beta, 2014; Zhang, Shao, Bao, & Beta, 2015). Qiu et al. (2010) reported the total phenolic content (TPC), total antioxidant activity (TAA) and phenolic acids composition of soluble and insoluble phenolics of wild rice varieties in Canada. Shao et al. (2014) identified and quantified the phenolic acids and anthocyanins in bran, embryo and endosperm of rice with different bran color in China. Zhang et al. (2015) determined the phenolic compounds and TAA of breeding lines between white and black rice in China. Min et al. (2012) analyzed the free and bound TPC, TAA, and profiles of phenolics in whole grain rice of different bran color. However, phytochemical content and TAA of free and bound fractions of diverse BR varieties were rare. Therefore, a complete study of different varieties of BR including both free and bound phytochemicals and TAA are needed.

The investigated BR varieties in present study are commercially grown rice varieties planted in northern (Longjing25, Longjing26, Sonjing16), central (Zhongjiazao17, Fenghuazhan, Huanghuazhan, Tianyouhuazhan), and southern (Wuyou308) areas of China. The objectives of this study were (1) to determine the TPC, total flavonoid contents (TFC) of free and bound fractions of eight BR varieties; (2) to determine the TAA of free and bound fractions of eight BR varieties using PSC assay; and (3) to identify and quantify the free and bound phenolics in different varieties of common BR.

2. Materials and methods

2.1. Materials

Acetone, methanol (MeOH), ethanol (EtOH), 95% EtOH, ethyl acetate, hydrochloric acid (HCl), sodium hydroxide (NaOH), potassium hydroxide (KOH), sodium carbonate (Na_2CO_3), sodium chloride (NaCl), potassium phosphate monobasic (KH_2PO_4), and potassium phosphate dibasic (K_2HPO_4) were purchased from Mallinckrodt Chemicals (Phillipsburg, NJ, USA). Folin–Ciocalteu reagent, *trans*-FA, CHA, VA, CFA, *p*-COA, SYA, SIA, sodium borohydride ($NaBH_4$), chloranil, vanillin, acetic acid, catechin (CA) hydrate, quercetin (QU), kaempferol (KA), pyrogallol, trifluoroacetic acid, ascorbic acid, 2′,7′-dichlorofluorescein-diacetate (DCFH-DA), were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Vitamin E isomers (α-tocopherol, γ-tocopherol, α-tocotrienol, γ-tocotrienol) were purchased from Calbiochem (Darmstadt, Germany). Tetrahydrofuran (THF), NaEDTA, sodium sulphate, hexane and aluminum chloride ($AlCl_3$) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). 2,2′-azobis (2-amidinopropane) dihydrochloride (ABAP) was purchased from Wako Chemicals (Richmond, VA, USA). Gallic acid was purchased from ICN Biomedical Inc. (Aurora, OH, USA).

2.2. Methods

2.2.1. BR samples and sample preparation

Eight Chinese BR (*Oryza sativa* L.) varieties used in present study are described in Table 1. Longjing25, Longjing26, Sonjing16 were obtained from Rice Research Institute, Heilongjiang Academy of Land Reclamation Sciences (Heilongjiang Province, China), other BR varieties were supplied by Jiangxi Keyuan Seed Limited Company (Jiangxi Province, China). Rice was dehulled using a THU Satake rice machine (Satake Engineering Co., Tokyo, Japan). After dehulling, whole-grain BR was ground to a fine powder by a DFY-200 laboratory mill (Linda machinery, Zhejiang, China) and sieved (100 mesh) to a uniform size. Samples were stored in sealed polyethylene bags at −80 °C until further analysis.

2.2.2. Extraction of free phenolics

Free phenolics of BR samples were extracted using a modified method reported previously from our laboratory (Adom & Liu, 2002; Chu & Liu, 2005; de la Parra et al., 2007). Briefly, 2.0 g of each sample was blended with 50 mL of 80% chilled acetone for 10 min followed by centrifugation at 2500*g* for 10 min. The supernatant was removed and residue was re-extracted twice. The supernatants were pooled, evaporated in a rotary evaporator at 45 °C to dryness, and reconstituted to 10 mL with distilled water. Each sample was extracted in triplicate and extracts were stored at −40 °C until analysis.

2.2.3. Extraction of bound phenolics

Bound phenolics of BR samples were extracted using the method reported previously by our laboratory with modification (Adom & Liu, 2002; Kremer Faller, Fialho, & Liu, 2012; Zhang & Liu, 2015). The residues obtained after the extraction of free phenolics were digested with 20 mL of 2 M NaOH for 1 h while shaking under nitrogen at room temperature. Then, the mixture was acidified with concentrated HCl to pH 2.0 and was extracted using hexane to remove lipids. Remaining mixture was extracted with ethyl acetate for five times. The ethyl acetate extracts were evaporated to dryness at 45 °C and reconstituted with 10 mL distilled water. The final extracts were stored at −40 °C until analysis.

2.2.4. Determination of TPC

The TPCs in both free and bound fractions (Free-TPC and Bound-TPC, respectively) were measured using the Folin-Ciocalteu colorimetric method reported by Adom, Sorrells, and Liu (2005). Briefly, the sample extracts were reacted with Folin-Ciocalteu reagent as described previously. Then the mixture was alcalinized with Na_2CO_3. The absorbance of the mixture was measured at 760 nm after 90 min using an MRX Microplate Reader (Dybex Technologies Inc.,