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Bound phenolic compounds and antioxidant properties of whole grain and bran of white, red and black rice

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ABSTRACT

Total phenolic content (TPC), individual phenolic acid and antioxidant capacity of whole grain and bran fraction 18 rices with different bran color were investigated. The levels of TPC in bound fractions were significantly higher than those in the free fractions either in the whole grains or brans. The main bound phenolic acids in white rice samples were ferulic acid, *p*-coumaric acid, and isoferulic acid, and in pigmented rice samples were ferulic acid, *p*-coumaric acid, and vanillic acid. The protocatechuic acid and 2,5-dihydroxybenzoic acid were not detected in white samples. The content of gallic acid, protocatechuic acid, 2,5-dihydroxybenzoic acid, ferulic acid, sinapic acid had significantly positive correlations with TPC and antioxidant capacity. This study found much wider diversity in the phenolics and antioxidant capacity in the whole grain and brans of rice, and will provide new opportunities to further improvement of rice with enhanced levels of the phytochemicals.

1. Introduction

Rice is a widely consumed staple food, providing energy and nutrients for more than half of the world's population, particularly in Asia. Recently, pigmented rice varieties have received increased attention from consumers for their high bioactive compounds, presenting antioxidant, anti-inflammatory and other health benefits (Alves et al., 2016). These compounds include phenolics, tocols, sterol derivatives, among others (Sumczynski, Kotásková, Družbíková, & Mlček, 2016). It is also well known that these compounds are primarily located in the bran layer of rice grain, which is regarded as rice by-products (Verardo et al., 2016).

Polyphenols, such as phenolic acids, anthocyanins, and proanthocyanidins, have been reported as the major antioxidants in rice (Min, McClung, & Chen, 2011). Generally, white rice contains mainly phenolic acids, red rice is characterized by the presence of procyanidins, whereas black rice is characterized by the presence of anthocyanins (Finocchiaro, Ferrari, & Gianinetti, 2010; Zaupa, Calani, Del Rio, Brighenti, & Pellegrini, 2015; Zhang, Shao, Bao, & Beta, 2015). Phenolic acids can be classified as free, esterified and insoluble-bound forms (Adom & Liu, 2002; Liyana-Pathirana & Shahidi, 2006; Naczk & Shahidi, 1989; Sosulski, Krygier, & Hogge, 1982). The distribution of phenolic acids exhibits varietal differences, and rice bran has the highest total phenolic content (TPC) among four different fractions of whole rice grain (Shao, Xu, Sun, Bao, & Beta, 2014a; Ti, Li, et al., 2014). Overall, ferulic, *p*-coumaric, isoferulic, syringic, vanillic, sinapic, caffeic, *p*-hydroxybenzoic, and protocatechuic acid are present in the whole rice grain, of which ferulic acid is the most abundant phenolic acid (Shao & Bao, 2015; Shao et al., 2014a; Sosulski et al., 1982; Zaupa et al., 2015; Zhang et al., 2015) in insoluble-bound fraction. The phenolic contents were positively correlated with the antioxidant capacity (Paiva et al., 2016; Shen, Jin, Xiao, Lu, & Bao, 2009). Anthocyanins are a group of reddish to purple flavonoids that exist in black rice and other pigmented cereal grains (Abdel-Aal, Young, & Rabalski, 2006; Bellido & Beta, 2009; Hosseinian, Li, & Beta, 2008; Jang & Xu, 2009). In black rice, anthocyanins accumulate in the outer layers as free forms (Zhang, Zhang, Zhang, & Liu, 2010), and cyanidin-3-*O*-glucoside and peonidin-3-*O*-glucoside have been identified in black rice bran as the main anthocyanin components (Shao, Xu, Sun, Bao, & Beta, 2014 b).

Currently, identification and characterization of bioactive compounds are among the hottest themes in the research field of rice nutritional quality. Many reports have focused on the determination of phenolic acids and anthocyanins and their antioxidant activity (Min, Gu, McClung, Bergman, & Chen, 2012; Min et al., 2011; Shao & Bao, 2015; Shao et al., 2014a; Sosulski et al., 1982; Sumczynski et al., 2016; Ti, Li, et al., 2014; Ti, Zhang, et al., 2014; Zhang et al., 2015). However,

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the differences in bound phenolics and their relation to the antioxidant activity have not been well understood partially due to genotypic diversity. Detailed investigations of the polyphenols, phenolic acids and their relation to antioxidant activities may promote the development of rice-based functional foods.

The objectives of this research were: (1) to evaluate and compare the composition and levels of bound phenolic acids of whole grains and brans from 7 white rice, 4 red rice and 7 black rice samples; (2) to investigate the antioxidant activities of free and bound fractions using DPPH and ABTS radicals methods; (3) to assess the correlations between individual bound phenolic acids and total phenolic content or antioxidant capacity.

2. Materials and methods

2.1. Samples and sample preparation

A total of 18 rice accessions (breeding lines) including seven white rice, four red rice and seven black rice, were employed in this study. All the rice was cultivated and harvested in Hangzhou China, in 2016. After sun drying to a moisture content of 10–11%, grains were stored under darkness at 4 °C. Prior to analysis, grains were dehusked on a Satake Rice Machine (Satake Co., Tokyo, Japan) to obtain whole grain rice (brown rice). Any broken kernels were removed. The whole grains were polished on a Satake mill (Satake Corp., Tokyo, Japan) to collect the brans. The whole grains and brans were ground and passed through a 100 mesh sieve on a Cyclone sample mill (UDY Corporation, Fort Collins, CO).

2.2. Chemicals

Folin-Ciocalteu reagent, phenolic acid standards, Trolox, DPPH, ABTS were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Potassium persulfate and HPLC grade methanol were purchased from Merck (Darmstadt, Germany). Hexanes and ethyl acetate were purchased from Tedia (Fairfield, USA). Hydrochloric acid, sodium hydroxide, sodium carbonate, Sodium sulfate, potassium chloride, and sodium acetate were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). HPLC grade acetic acid was purchased from Macklin (Shanghai, China).

2.3. Extraction of free fractions

Extraction of free phenolics was accomplished depending on a modification of the methods reported by Shao et al. (2014a). Briefly, rice flour (1.0 g) was defatted with hexanes (10 mL) prior to extraction twice using 80% methanol (40 mL). Each time, the mixture was kept on a mechanical shaker (Multi-speed oscillator HY-8, Changzhou Guohua Electric Appliance Co., Ltd, Jiangsu, China) for 30 min at room temperature. The mixture was subsequently centrifuged (TGL-20B high-speed desktop centrifuge, Shanghai Anting Scientific Instrument Factory, Shanghai, China) at 4000 × *g* for 10 min at room temperature. The supernatants were gathered and combined. After adjusting the pH to about 1.5–2.0, the supernatant was concentrated using a rotary evaporator (IKA RV10 digital V, Staufen, Germany) at 40 °C. The concentrated free fraction was extracted three times using ethyl acetate (60 mL). The ethyl acetate extracts were pooled and rotary evaporated at 40 °C. The dried extracts were dissolved in 50% methanol (5 mL) and used as crude free phenolic extracts.

2.4. Extraction of bound fractions

The solid residue after the extraction of soluble phenolics was washed with distilled water (Zhang et al., 2010) and then digested with 4 M NaOH (20 mL) at room temperature on a shaker (Multi-speed oscillator HY-8, Changzhou Guohua Electric Appliance Co., Ltd, Jiangsu, China) for 2 h. The mixture was then adjusted to pH 1.5–2.0 with concentrated HCl and then extracted with 60 mL of ethyl acetate for three times. The combined ethyl acetate fractions were evaporated to dryness at 40 °C and dissolved in 5 mL of 50% methanol. The extracts were used as bound fractions on analysis of phenolic compounds (Shao et al., 2014 b).

2.5. Extraction of anthocyanins

Briefly, 0.5 g rice flour was extracted three times with 15 mL methanol: 1 M HCl (85:15, v/v) using a shaker under dark conditions, and then centrifuged at 4000 × *g* (TGL-20B high-speed desktop centrifuge, Shanghai Anting Scientific Instrument Factory, Shanghai, China) for 15 min at room temperature. The clear supernatants were anthocyanin extracts (Shao et al., 2014 b).

2.6. Determination of color parameters of flours

The color parameters of flours of whole grain and bran samples were measured with an NS800 spectrophotometer (Shenzhen 3nh Technology Co., Ltd, Shenzhen, China). The color was expressed using L^*, a^*, and b^* the space coordinates. L^* indicates lightness (100 = white and 0 = black). a^* indicates redness-greenness and b^* indicates yellowness-blueness. Besides, the chroma (C) value indicates color intensity or saturation, calculated as $C = (a^{*2} + b^{*2})^{1/2}$, and the Hue angle was calculated as $H^o = \tan^{-1}(b^*/a^*)$ (Shen et al., 2009).

2.7. Determination of total phenolic content (TPC)

The total phenolic content was determined by the Folin-Ciocalteu colorimetric method with minor modification (Shao et al., 2014a). Briefly, 200 μL of appropriately diluted crude extracts or standard solutions were reacted with 1.5 mL of 10-fold diluted Folin-Ciocalteu reagent, which was freshly made. After 5 min, the mixture was neutralized with 1.5 mL saturated sodium carbonate (75 g/L), the absorbance was measured at 725 nm using a Shimadzu UV-2550 spectrometer (Shimadzu Inc., Kyoto, Japan) after 2 h of reaction at room temperature in the dark. Gallic acid was used as the standard. The total phenolic content was expressed as milligrams of gallic acid equivalent (mg GAE) per gram of rice flour.

2.8. Determination of DPPH radical scavenging activity

The DPPH assay (Brand-Williams, Cuvelier, & Berset, 1995) was carried out with slight modification (Shao et al., 2014a). Briefly, 100 μmol/L of DPPH radical solution was prepared in methanol. Appropriately diluted crude extracts or standards (100 μL) were added to 1.5 mL DPPH solution. After 30 min of incubation at room temperature in the dark, the absorbance at 517 nm was measured. The DPPH scavenging activity (%) of both samples and standard (Trolox) was calculated as follows:

$$\text{DPPH\%} = (1 - A_{sample}/A_{control}) \times 100\%$$

DPPH radical scavenging activities of crude extracts were expressed as μM of Trolox equivalents (TE) per gram of rice flour using a standard curve of Trolox.

2.9. Determination of radical cation $ABTS^+$ scavenging activity

Total antioxidant capacity of rice extracts was carried out using a spectrophotometer by the improved 2,2-azino-bis-(3-ehylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) radical cation method as described (Re et al., 1999; Shen et al., 2009). $ABTS^{\cdot +}$ radical cation was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate at room temperature in dark for 16 h. The $ABTS^{\cdot +}$ solution was diluted with 80% ethanol to an absorbance around 0.700 at 734 nm.