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Bioactive compounds and antioxidative activity of colored rice bran

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ABSTRACT

The profiles of bioactive compounds (including phenolics and flavonoids in free and bound fractions, anthocyanins, proanthocyanidins, vitamin E, and γ-oryzanol) of outer and inner rice bran from six colored rice samples collected from local markets were investigated. Proanthocyanidins could only be detected in red rice bran but not in black rice bran. The free fraction of the extracts dominated the total phenolics (72–92%) and the total flavonoids (72–96%) of colored rice bran. Most of the phenolic acids (83–97%) in colored rice bran were present in the bound form. Protocatechualdehyde was identified for the first time in the bound fraction of red rice bran by high performance liquid chromatography-photodiode array/electrospray ionization tandem mass spectrometry. The antioxidative activities of the free fraction of the colored rice bran were attributed to the proanthocyanidins in red colored rice and anthocyanins in black rice, while that of the bound fraction was mainly due to the phenolic acids.

1. Introduction

Rice (*Oryza sativa* L.) is the most important cultured crop in Asia that has been used as a staple food for more than half of the population in the world. Some special rice cultivars have pigments in their pericarp and seed coat which exhibit colors of the pigments on the surface. These rice cultivars with unusual colors, such as red and black, are known as colored rice or pigmented rice. Although the colored rice has been considered as a nutritious food for weak people in traditional Chinese medicine, the breeding and cultivation of colored rice was discouraged and prohibited since early 19th century in Taiwan due to the agricultural policy of rice variety improvement [1].

Anthocyanins (ACNs) and proanthocyanidins (PA)/condensed tannins are common pigments in black rice and red rice, respectively [2–4]. In addition, the health beneficial components of common rice, including sterols, γ-oryzanol, tocopherols, tocotrienols, and phenolic compounds, can be found in colored rice bran as well [5,6]. The major phenolic acids in rice include ferulic acid, *p*-coumaric acid, and diferulate, which exist especially in the outer

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layer of grains such as pericarp and aleurone [7]. Both types, bound and free form, of phenolics can be found in rice bran. The free-form phenolics can be simply extracted with methanol or other aqueous-organic solvents. The bound-form phenolics are bound with cell wall polysaccharides and lignins through ester or ether linkages, which need to be hydrolyzed with alkaline or acid for releasing [4,8].

The beneficial effects of whole grains (including colored rice) consumption have been widely reported in this decade. The antioxidative activity, body fat reduction, anti-inflammatory, cardioprotective, and antiatherogenic effects of colored rice have been proved *in vivo* tests [9–12]. In Taiwan, the cultivation and consumption of colored rice are getting popular for local farmers and people for health concerns. In this study, six commercially-available colored rice (3 black and 3 red) in local markets were studied. The objective of this study is to establish the profiles of bioactive compounds (including phenolics and flavonoids in free and bound fractions, ACNs, PA, vitamin E, and γ-oryzanol) of outer and inner rice bran from these six colored rice. The information revealed in this study can provide the distribution and contents of bioactive compounds in colored rice bran that are important for the applications of colored rice.

2. Materials and methods

2.1. Rice bran

Colored rice bran from six rice samples collected from the local markets were obtained by polishing the colored rice using a laboratory rice mill (VP-31T, Yamamoto Co. Ltd., Tendu, Japan) with the setting of 5 for flow. Four of the six colored rice, including two black and two red rice, were domestically grown. Two colored rice grown in Eastern Taiwan, Taibalang black waxy rice (HB) and Taibalang red waxy rice (HR), were purchased from Hualien KuangFeng Farmer's Association (Hualien, Taiwan) and another one grown in Eastern Taiwan, Guangfu red rice (GR), was purchased from a local farmer (Hualien, Taiwan). A black rice (WB) cultivated in western Taiwan was provided by Yeedon Enterprise Co. Ltd. (Taipei, Taiwan). Two imported rice samples were from Thailand, one black rice (TB) and one red rice (TR), and provided by Yeedon Enterprise Co. Ltd. Rice bran collected from the first pass and from the second to forth passes of the polishing process were defined as RB-1st (outer bran) and RB-2nd (inner bran), respectively. The bran samples were stored at 4°C until analyses.

2.2. Proximate composition analysis

The contents of moisture, crude lipid, crude protein, ash, and dietary fiber of colored rice bran were analyzed according to American Association of Cereal Chemists international approved methods 44-15A, 30-25, 46-11A, 08-01, and 32-07, respectively [13].

2.3. Determination of total phenolics and total flavonoids

2.3.1. Extraction and fractionation

Free and bound phenolics were extracted and fractionated basically following the methods of Dvořáková et al [8] and Lin and Lai [4] with some modifications. One hundred milligrams of rice bran were extracted with 2 mL of cold 80% ethanol for 10 minutes at room temperature with continuous stirring. After centrifugation (10,000 *g*, 10 minutes, 4°C), the supernatant and the precipitate were separated by decantation. The precipitate was extracted again as described above one more time. The supernatants were combined, brought to a total volume of 5 mL with 80% ethanol, and defined as crude free phenolics extract (CFPE) which were later used for the analyses of total phenolics (TP), total flavonoids (TF), and antioxidative activity.

Four milliliters of the CFPE was evaporated with a rotary evaporator at 40°C to eliminate ethanol and then adjusted to pH 2 with 1N HCl. The acidified CFPE was extracted with ethyl acetate (v/v = 1:1) three times. The ethyl acetate extract (organic layer) was collected and dehydrated with anhydrous sodium sulfate. After filtration with a Whatman Number 2 filter paper, the extract was brought to dryness with a rotary evaporator and redissolved in 0.5 mL of 80% ethanol. This extract was defined as free phenolic acids extract (FPAE) of which the specific phenolic acid was analyzed with high performance liquid chromatography (HPLC).

The residue of rice bran left after extraction with 80% ethanol in the beginning was hydrolyzed with 20 mL of 2N NaOH and stirred for 4 hours at room temperature under nitrogen. The solution was acidified to pH 5 with 6N HCl and mixed with 95% ethanol (v/v = 1:4) to precipitate polysaccharides overnight. After centrifugation (10,000 *g*, 10 minutes, 4°C), the supernatant was collected and evaporated to remove ethanol. The concentrated solution was adjusted to pH 2 with 1N HCl and then extracted with ethyl acetate by the same procedure as extraction of FPAE. The dried extract was redissolved with 1 mL of 80% ethanol, and this fraction was called bound phenolics extract (BPE).

2.3.2. Determination of TP

The amounts of TP in CFPE were determined using the Folin–Ciocalteu colorimetric method. The appropriate diluted extract (50 μL) was mixed with 1 mL of 2% sodium carbonate, then 100 μL of 50% Folin–Ciocalteu reagent was added in 2 minutes later. The mixed solution was incubated at room temperature for 30 minutes in the dark. The absorbance at 750 nm was measured using a spectrophotometer (UA-160A, Shimadzu, Kyoto, Japan) and the TP of CFPE were expressed as ferulic acid equivalent [mg FAE/g dry matter (DM)].

2.3.3. Determination of TF

For the assay of TF, the appropriate diluted extract (100 μL) was mixed with 400 μL of distilled water and 30 μL of 5% sodium nitrite, and then reacted with 30 μL of 10% aluminum chloride. After adding 1N NaOH (400 μL), 240 μL of distilled water was added. The absorbance at 510 nm of the well-mixed mixture was measured. TF was calibrated with a standard