7580 J. Agric. Food Chem. 2010, 58, 7580–7587
DOI:10.1021/jf1007665

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Phenolic Profiles and Antioxidant Activity of Black Rice Bran of Different Commercially Available Varieties

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Increased consumption of whole grains has been associated with reduced risk of developing major chronic diseases. These health benefits have been attributed in part to their unique phytochemicals. Previous studies on black rice mainly focused on anthocyanins. Little is known about the phytochemical profiles and antioxidant activities of different black rice varieties. The objective of this study was to determine the phytochemical profiles and antioxidant activity of rice bran samples from 12 diverse varieties of black rice. The free, bound, and total phenolic contents of black rice bran samples ranged from 2086 to 7043, from 221.2 to 382.7, and from 2365 to 7367 mg of gallic acid equiv/100 g of dry weight (DW), respectively. The percentage contribution of free phenolics to the total ranged from 88.2 to 95.6%. The average values of free, bound, and total phenolic contents of black rice bran were 8, 1.5, and 6 times higher than those of white rice bran, respectively ($p < 0.05$). The free, bound, and total flavonoid contents of black rice bran samples ranged from 3462 to 12061, from 126.7 to 386.9, and from 3596 to 12448 mg of catechin equiv/100 g of DW, respectively. The percentage contribution of free flavonoids to the total ranged from 96.3 to 97.6%. The average values of free, bound, and total flavonoid contents of black rice bran were 7.4, 1.9, and 6.7 times higher than those of white rice bran, respectively ($p < 0.05$). The free, bound, and total anthocyanin contents of black rice bran samples ranged from 1227 to 5096, from 4.89 to 8.23, and from 1231 to 5101 mg of cyanidin-3-glucoside equiv/100 g of DW, respectively. The percentage contribution of free anthocyanins to the total ranged from 99.5 to 99.9%. Cyanidin-3-glucoside, cyanidin-3-rutinoside, and peonidin-3-glucoside were detected in black rice bran samples and ranged from 736.6 to 2557, from 22.70 to 96.62, and from 100.7 to 534.2 mg/100 g of DW, respectively. The free, bound, and total antioxidant activities of black rice bran samples ranged from 476.9 to 180, from 47.91 to 79.48, and from 537.5 to 1876 μmol of Trolox equiv/g of DW, respectively. The percentage contribution of free antioxidant activity to the total ranged from 88.7 to 96.0%. The average values of free, bound, and total antioxidant activity of black rice bran were more than 8, 1.5, and 6 times higher than those of white rice bran, respectively ($p < 0.05$). The total antioxidant activity of black rice bran was correlated to the content of total phenolics, total flavonoids, and total anthocyanins and also was significantly correlated to the contents of cyanidin-3-glucoside, cyanidin-3-rutinoside, and peonidin-3-glucoside. These results indicate that there are significant differences in phytochemical content and antioxidant activity among the different black rice varieties. Black rice bran has higher content of phenolics, flavonoids, and anthocyanins and has higher antioxidant activity when compared to white rice bran. Interestingly, the phenolics, flavonoids, and anthocyanins of black rice bran are mainly present in free form. Knowing the phytochemical profile and antioxidant activity of black rice bran gives insights to its potential application to promote health.

KEYWORDS: Black rice; rice bran; antioxidant activity; phenolics; flavonoids; anthocyanins

INTRODUCTION

Epidemiological studies have shown that increased consumption of whole grains and their products is associated with reduced risk of developing chronic diseases, such as cardiovascular disease, type II diabetes, obesity, and cancer (*1*). These health benefits have been attributed in part to the unique phytochemical content of whole grains (*2*). However, the phytochemicals of whole grains have not received as much attention as those in fruits and vegetables because many previous studies have underestimated grain phytochemicals (*2*).

Phytochemicals are defined as the bioactive non-nutrient plant compounds found in fruits, vegetables, whole grains, and other plant foods and are classified as carotenoids, phenolics, alkaloids,

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 Published on Web 06/03/2010

nitrogen-containing compounds, and organosulfur compounds (*3*). The whole grain phytochemicals include carotenoids (lutein, zeaxanthin, β-cryptoxanthin, and β-carotene), phenolics, and vitamin E. Phenolics include phenolic acids (p-coumaric, caffeic, ferulic, vanillic, and syringic acids) and flavonoids (flavonols, flavones, catechins, and anthocyanins). Whole grain phenolics have potent antioxidant activity and are able to scavenge free radicals that may increase oxidative stress and potentially damage large biological molecules, such as lipids, proteins, and DNA (*2*).

Rice (*Oryza sativa* L.) is one of the world's most important crops, providing a staple food for more than 50% of the world's population. Rice has two major subspecies, *Oryza sativa* L. *japonica*, primarily consumed in Southeast Asia, northern China, Japan, and the United States, and *Oryza sativa* L. *indica*, which is mainly consumed in India, southern China, and Southeast Asia. Traditionally, rice is typically consumed as polished white rice with the husk, bran, and germ fractions removed. Rice bran contains high amounts of fiber and bioactive phytochemicals, such as tocopherols, tocotrienols, oryzanols, vitamin B complex, and phenolic compounds. Most of these phytochemicals are recognized as bioactive compounds improving human health and well-being. Their vital functions include scavenging free radicals, antioxidant activity, enhancement of immune systems, and reduction of the risk of developing cancer and heart disease (*2*, *4*−*6*). In addition, the phytochemicals are responsible for the color of rice. These compounds are usually concentrated in the pericarp and testa, or the bran part of the rice kernel, resulting in rice with different colors, red, purple, and black.

Black rice is a good source of fiber, minerals, and phytochemicals besides basic nutrients (*7*, *8*). A number of black rice cultivars are grown in Asia, especially in China. As consumers' health awareness increases and their food patterns change, research and development of special foods such as black rice, colored corn, and soybean has received more attention. Traditional black foods, particularly black rice, have been favored by the Chinese for a long time. The new processed foods with black rice materials are growing rapidly and drawing attention from consumers. In China and many Asian countries, glutinous black rice is commonly consumed in a sweetened form, or dessert, not used as the main meal. Nonglutinous black rice varieties are mainly used as staple food to replace white rice. The popularity of black rice is partially due to the distinct compositions of phytochemicals, especially flavonoids and anthocyanins, which have been shown to have beneficial effects in the prevention of chronic diseases associated with oxidative stress.

Previous studies have reported the phytochemical content and antioxidant activity of whole grain white rice (*9*−*12*). Several studies also reported the phytochemicals in and antioxidant activity of rice bran (*13*, *14*). However, none of these studies reported the bound phytochemicals and the contributions of free and bound fractions to the total phenolics and antioxidant activity. Adom and Liu reported that the phenolic content of whole grains had been underestimated in the literature without determination of bound phenolics linked to the cell wall materials (*9*). Compared to white rice, previous studies on black rice mainly focused on anthocyanins, not on phytochemical profiles, and limited studies reported the phenolic content and antioxidant activity of black rice (*15*−*17*). Again, these studies did not report the bound phytochemicals. Obviously, their phenolic content and antioxidant activity were underestimated without inclusion of the bound fraction as described previously (*2*, *9*). In addition, no studies emphasized subspecies, types, and varieties of black rice in the previous literature. Therefore, more complete analyses of phytochemical profiles and antioxidant activity of diverse varieties of black rice are needed. The objectives of this study were (1) to determine the phytochemical profiles, including both free and bound, and antioxidant activity of rice bran samples from 12 diverse varieties of black rice; (2) to compare the differences of phytochmicals and antioxidant activity between black and white rice varieties; and (3) to investigate the correlations among total phenolics, flavonoids, anthocyanins, and antioxidant activity of samples tested.

MATERIALS AND METHODS

Chemicals and Reagents. Methanol (MeOH), ethanol (EtOH), hexanes, ethyl acetate, hydrochloric acid (HCl), acetic acid (HAC), potassium chloride (KCl), sodium acetate (NaAC), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), potassium phosphate monobasic (KH_2PO_4), and potassium phosphate dibasic (K_2HPO_4) were of analytical grade and were purchased from Mallinckrodt Chemicals (Phillipsburg, NJ). 2′,7′-Dichlorofluorescin diacetate (DCFH-DA), fluorescein disodium salt, apigenin, sodium borohydride ($NaBH_4$, reagent grade), chloranil (analytical grade), vanillin (analytical grade), and catechin hydrate were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). Tetrahydrofuran (THF, analytical grade) and aluminum chloride ($AlCl_3 \cdot 6H_2O$, analytical grade) were purchased from Fisher Scientific (Fair Lawn, NJ). Folin−Ciocalteu reagent, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), trifluoroacetic acid (TFA, chromatographic grade), and acetonitrile (chromatographic grade) were purchased from Sigma. Gallic acid was purchased from ICN Biomedicals, Inc. (Aurora, OH). 2,2′-Azobis(2-amidinopropane) dihydrochloride (ABAP) was purchased from Wako Chemicals USA, Inc. (Richmond, VA). Cyanidin 3-glucoside (Cy-3-G), cyanidin 3-rutinoside (Cy-3-R), and peonidin 3-glucoside (Pe-3-G) were purchased from Polyphenols Laboratories (Sandnes, Norway).

Grain Samples and Sample Preparation. Rice samples including 12 black rice varieties and 2 white rice varieties used in this study were obtained from the Bio-Technical Research Institute and Rice research Institute of Guangdong Academy of Agricultural Sciences and the Shanxi Province Rice Research Institute, China, respectively (**Table 1**). All of the rice varieties were commercially available. The 12 black rice samples can be divided into two subspecies, *indica* (7 varieties) and *japonica* (5 varieties), or into two types, glutinous (6 varieties) and nonglutinous (6 varieties). The rice varieties used in this study were grown in the Experimental Station of Guangdong Academy of Agricultural Sciences in 2008. They were sown in late March, transplanted on April 30, and harvested in mid-July. Rice grains were air-dried until their moisture content was reduced to approximately 13% and stored at room temperature for 3 months. Then they were dehusked and polished on a Satake Rice Machine (Satake Co., Japan) to obtain approximately 10% (w/w) of fresh rice bran. The bran samples were sieved by passing through a 60-mesh sieve on a Cyclone Sample Mill (UDY Corp., Fort Collins, CO). Each sample was stored at −40 °C until analysis. The moisture content of all samples was determined using the oven-drying method at 105 °C for 16 h. The percent weight of rice bran fraction to the whole grain rice is presented in **Table 1**.

Extraction of Free Phenolic Compounds. Free phenolic compounds of rice bran samples were extracted using the method previously reported by our laboratory (*18*, *19*). Briefly, 0.5 g of rice bran flour was blended with 50 mL of chilled acidified methanol (95% methanol and 1 M HCl 85:15, v/v). The mixture was then centrifuged at 2500*g* for 10 min. The supernatant was removed, and the remaining pellet was again extracted with 50 mL of chilled acidified methanol. The supernatants were pooled and made up to a final volume of 100 mL with chilled acidified methanol. The extracts were stored at −40 °C until analysis.

Extraction of Bound Phenolic Compounds. Bound phenolics of rice bran samples were extracted using the method previously reported by our laboratory (*2*, *9*, *18*, *19*). Briefly, bound phenolics were extracted from the residue from the free phenolic extraction. The residue was first digested with 20 mL of 2 M sodium hydroxide at room temperature for 1 h while shaking under nitrogen. The mixture was then neutralized with concentrated hydrochloric acid. Hexanes were used to extract lipids in the mixture. The remaining mixture was then extracted five times with ethyl acetate. The ethyl acetate fractions were pooled and evaporated to dryness. The bound phenolics were reconstituted in acidified methanol (95% methanol and 1 M HCl 85:15, v/v) and stored at −40 °C until analysis.

Table 1. Description of Rice Bran Samples

sample	variety name	bran color	type	% of bran to brown rice (g/100 g)
1	Heizhenzhu	black	*japonica*, nonglutinous	10.46
2	Heixian 3	black	*indica*, nonglutinous	10.32
3	Longjin 01	black	*japonica*, nonglutinous	10.12
4	Qindao 2	black	*japonica*, glutinous	9.92
5	Heijing 72	black	*japonica*, nonglutinous	10.41
6	Heiyounian 97	black	*indica*, nonglutinous	10.04
7	Heifengnuo	black	*indica*, glutinous	10.22
8	Heisuai	black	*indica*, glutinous	10.43
9	Heinianmi 5	black	*indica*, nonglutinous	9.96
10	Heijing 04	black	*japonica*, glutinous	10.13
11	Yunxiangnuo	black	*indica*, glutinous	10.16
12	Heinuo 9933	black	*indica*, glutinous	10.36
13	Guinongzhan	white	*indica*, nonglutinous	10.11
14	Huidao 7	white	*japonica*, nonglutinous	10.23

Determination of Total Phenolic Content. The total phenolic content of each rice bran sample was determined using the colorimetric method described by Singleton et al. (*20*) and modified in our laboratory (*21*, *22*). Briefly, extracts were reacted with Folin−Ciocalteu reagent and then neutralized with sodium carbonate. After 90 min, the absorbance of the resulting solution was measured at 760 nm. Gallic acid was used as the standard, and total phenolic content was expressed as milligrams of gallic acid equivalents per 100 g of dry weight (DW) of sample.

Determination of Total Flavonoid Content. The total flavonoid content of each rice bran sample was determined using the sodium borohydride/chloranil-based assay developed by our laboratory (*23*). Briefly, 4 mL extracts of tested samples were added into test tubes (15 × 150 mm), dried to dryness under nitrogen gas, and reconstituted in 1 mL of terahydrofuran/ethanol (THF/EtOH, 1:1, v/v). Catechin standards (0.1−10.0 mM) were prepared fresh before use in 1 mL of THF/EtOH (1:1, v/v). Then 1 mL of 50 mM $NaBH_4$ solution and 0.5 mL of 74.6 mM $AlCl_3$ solution were added into each test tube with 1 mL of sample solution or 1 mL of catechin standard solution. Then the test tubes were shaken in an orbital shaker at room temperature for 30 min. An additional 0.5 mL of 50.0 mM $NaBH_4$ solution was added into each test tube with shaking continued for another 30 min at room temperature. Then, 2.0 mL of cold 0.8 M acetic acid solution was added into each test tube, and the solutions were kept in the dark for 15 min after a thorough mix. Then 1 mL of 20.0 mM chloranil was added into each tube, which was heated at 95 °C with shaking for 60 min. The reaction solutions were cooled using tap water, and the final volume was brought to 4 mL using methanol. Then, 1 mL of 1052 mM vanillin was added into each tube and mixed. Then 2 mL of 12 M HCl was added to each tube, and the reaction solutions were kept in the dark for 15 min after a thorough mix. Aliquots of final reaction solutions (200 μL) were added into each well of a 96-well plate, and the absorbances were measured at 490 nm using a MRX Microplate Reader with Revelation workstation (Dybex Technologies, Inc., Chantilly, VA). Total flavonoid content was expressed as milligrams of catechin equivalents per 100 g of DW of sample. Data were reported as mean ± SD for at least three replicates.

Determination of Total Anthocyanin Content. Total anthocyanin contents of the rice bran were measured using a spectophotometric pH differential protocol (*24*) and modified in our laboratory (*25*). The rice bran extracts were mixed thoroughly with 0.025 M potassium chloride (pH 1) buffer. The absorbance of the mixture was then measured at 515 and 700 nm against distilled water blank. The rice bran extracts were then combined similarly with sodium acetate buffer (pH 4.5), and the absorbance of these solutions was measured at the same wavelengths. The anthocyanin content was calculated as

$$\text{total anthocyanins (mg/100 g of DW of sample)} = A \times \text{MW} \times \text{DF} \times 1000/(\varepsilon \times \text{C})$$

where A is absorbance = $(A_{515} - A_{700})_{\text{pH } 1.0} - (A_{515} - A_{700})_{\text{pH } 4.5}$; MW is molecular weight for Cy-3-G = 449.2; ε is the molar absorptivity of Cy-3-G = 26900; C is the concentration of the buffer in mg/mL; and DF is the dilution factor (0.1 mL of free phenolic extract sample was diluted to 10 mL, DF = 100; 2 mL of bound phenolic extract was diluted to 10 mL, DF = 5). Anthocyanin content was expressed as milligrams of Cy-3-G equivalents per 100 g of DW of sample for triplicate extracts. The percent recoveries for cyanidin-3-glucoside in spiked black rice bran and white rice bran samples were 97.5 ± 0.82 and 102.4 ± 0.74, respectively.

Determination of Anthocyanin Composition. The anthocyanin composition and individual content of black rice bran samples were determined using a HPLC method described by Bellido and Beta (*26*) with modifications. Briefly, the sample was analyzed using a 2695 HPLC system (Waters Corp., Milford, MA) equipped with a model 996 photodiode array detector and model 717 plus autosampler (Waters Corp.). Empower 2 software was used to acquire and analyze experimental chromatographic data. Separation of anthocyanins was accomplished on a C18 column (150 mm × 3 mm i.d., 3 μm, Phenomenex, Torrance, CA). The column temperature was maintained at 35 °C by a Waters temperature control module. The mobile phase consisted of 4.5% formic acid in water (solvent A) and 100% methanol (solvent B). The following gradient was used: solvent B, 0 min, 10%; 30 min, 25%; 34 min, 33%; 42 min, 90%; and 45−50 min, 10%. Other chromatographic conditions included a constant flow rate of 0.4 mL/min, an injection volume of 10 μL, and a run time of 50 min. The chromatogram obtained at a wavelength of 520 nm was used to quantify the anthocyanins. The retention time (RT), percentage peak area under the curve, and spectroscopic data of anthocyanin standards (Cy-3-G, Cy-3-R, and Pe-3-G) were used to identify the quantity of anthocyanins present in the samples. The recoveries of Cy-3-G, Cy-3-R, and Pe-3-G in the spiked samples were 95.8, 97.4, and 97.3%, respectively. The concentrations of each anthocyanin were calculated on the basis of its standard curve. Data were reported as mean ± SD for at least three replicates.

Determination of Antioxidant Activity. The antioxidant activity was determined using the oxygen radical absorbance capacity (ORAC) assay described by Huang et al. (*27*) and modified in our laboratory (*28*). Briefly, rice bran extract dilutions were prepared with 75 mM phosphate buffer (pH 7.4). The assay was performed in black-walled 96-well plates (Corning Scientific, Corning, NY). The outside wells of the plate were not used as there was much more variation from them than from the inner wells. Each well contained 20 μL of extracts or 20 μL of Trolox standard (range = 6.25−50 μM) and 200 μL of fluoroscein (final concentration = 0.96 μM), which were incubated at 37 °C for 20 min. After incubation, 20 μL of 119 mM ABAP was added to each well. Fluorescence intensity was measured using a Fluoroskan Ascent FL plate-reader (Thermo Labsystems, Franklin, MA) at excitation of 485 nm and emission of 520 nm for 35 cycles every 5 min. ORAC was expressed as micromoles of Trolox equivalents per gram of DW.

Statistical Analysis. Data were reported as mean ± standard deviation (SD) for triplicate determinations of each sample. ANOVA and Tukey's comparison test were performed using Minitab Statistical Software version 15 (State College, PA) to identify differences between values. Statistical significance was defined to be at a level of $p < 0.05$.

RESULTS

Total Phenolic Content. The free and bound phenolic contents of rice bran and the percentage contribution of each fraction to the total of different genotype samples are presented in **Table 2**, expressed as milligrams of gallic acid equivalent per 100 g of sample on a dry weight basis. The free phenolic content of 12 black rice bran samples ranged from 2086 (Heinuo 9933) to 7043 (Heizhenzhu) mg of gallic acid equiv/100 g of DW. The percentage contribution of free fractions to the total ranged from 88.2 (Heinuo 9933) to 95.6% (Heizhenzhu). The bound phenolic content ranged from 221.2 (Heijing 04) to 382.7 (Heijing 72) mg of gallic acid equiv/100 g of DW. The percentage contribution of bound fractions to the total ranged from 4.4 (Heizhenzhu) to 11.8% (Heinuo 9933). The total phenolic content ranged from 2365 to 7367 mg of gallic acid equiv/100 g of DW. The coefficient of variation (CVs) of phenolic content in black rice varieties was 30.1%, indicating that there were significant genotype differences among black rice varieties. Heizhenzhu had the highest total phenolic content among the 12 black rice varieties, followed by Heixian 3, Longjin 01, Qindao 2, Heijing 72, and Heiyounian 97. The total phenolic content of Heiyounian 97 was not significantly different from those of Heifengnuo, Heisuai, and Heinianmi 5, but significantly different from that of Heijing 04. Yunxiangnuo had relatively low total phenolic content, and Heinuo 9933 had the lowest total phenolic content among the 12 black rice varieties.

Table 2. Total Phenolic Content of Rice Bran and Percentage Contribution of Free and Bound Fractions to the Total Phenolics

variety name	phenolics (mg of gallic acid equiv/100 g of DW) free	bound	total
Heizhenzhu	7043 ± 45 i[a] (95.6)[b]	324.1 ± 15.1 d (4.4)	7367 ± 60 j
Heixian 3	5108 ± 44 h (93.7)	343.7 ± 15.2 de (6.3)	5452 ± 51 i
Longjin 01	4420 ± 84 g (92.8)	345.6 ± 5.8 de (7.2)	4766 ± 88 h
Qindao 2	4310 ± 98 g (95.1)	223.4 ± 10.0 b (4.9)	4533 ± 91 g
Heijing 72	3968 ± 86 f (91.2)	382.7 ± 11.3 f (8.8)	4350 ± 75 f
Heiyounian 97	3531 ± 28 de (94.0)	227.5 ± 5.9 b (6.0)	3758 ± 22 e
Heifengnuo	3467 ± 49 de(93.0)	263.1 ± 3.1 c (7.0)	3730 ± 51 de
Heisuai	3347 ± 31 d (91.0)	331.0 ± 11.5 de (9.0)	3678 ± 42 de
Heinianmi 5	3321 ± 83 d (91.1)	322.9 ± 14.1 d (8.9)	3643 ± 94 de
Heijing 04	3354 ± 56 d (93.8)	221.2 ± 4.1 b (6.2)	3575 ± 54 d
Yunxiangnuo	2923 ± 12 c (89.0)	361.6 ± 7.4 ef (11.0)	3285 ± 13 c
Heinuo 9933	2086 ± 15 b (88.2)	279.2 ± 19.1 c (11.8)	2365 ± 6 b
Guinongzhan	530.2 ± 10.6 a (73.8)	188.5 ± 7.1 a (26.2)	718.6 ± 13.7 a
Huidao 7	441.0 ± 19.3 a (67.4)	213.1 ± 5.6 a (32.6)	654.0 ± 24.1 a

[a] Values with no letters in common in each column are significantly different ($p < 0.05$). [b] Values in parentheses indicate percentage contribution to the total.

In comparisons of total phenolic content of black rice bran versus white rice bran (**Table 3**), the average values of free, bound, and total phenolic contents of black rice bran were 3906, 302.2, and 4208 mg of gallic acid equiv/100 g of DW, respectively, which were 8, 1.5, and 6 times higher, respectively, than those of white rice bran (485.6, 200.8, and 686.3 mg of gallic acid equiv/100 g of DW, respectively). Those results indicated that total phenolic content of black rice bran was significantly higher than that of white rice bran ($p < 0.05$).

The 12 black rice varieties can also be divided into two subspecies, *indica* and *japonica*, or two types, glutinous and nonglutinous. In the comparison between *indica* and *japonica*, the average values of free and total phenolic contents of *japonica* were 4619 and 4918 mg of gallic acid equiv/100 g of DW, respectively, which were 36.0 and 32.8% higher ($p < 0.05$) than those of *indica*, respectively. In the comparison between glutinous and nonglutinous types, the average values of free and total phenolic contents of nonglutinous type were 4565 and 4890 mg of gallic acid equiv/100 g of DW, respectively, which were 40.6 and 38.6% higher ($p < 0.05$) than those of glutinous, respectively (**Table 3**). This may be due to the genetic differences among various subspecies and types.

Total Flavonoid Content. The free and bound flavonoid contents of rice bran and the percentage contribution of each fraction to the total of different samples are presented in **Table 4**, expressed as milligrams of catechin equivalents per 100 g of sample on a dry weight basis. The free flavonoid content of 12 black rice bran samples ranged from 3462 (Heinuo 9933) to 12061 (Heizhenzhu) mg of catechin equiv/100 g of DW. The percentage contribution of free flavonoids to the total ranged from 96.3 (Heinuo 9933) to 97.6% (Yunxiangnuo). The bound flavonoid content ranged from 126.7 (Yunxiangnuo) to 386.9 (Heizhenzhu) mg of catechin equiv/100 g of DW. The bound fraction accounted for 2.4 (Yunxiangnuo) to 3.7% (Heinuo 9933) of the total flavonoids. The total flavonoid content ranged from 3596 to 12448 mg of catechin equiv/100 g of DW. The CVs of total flavonoid contents of different black rice varieties were 31.5%, indicating that there

Table 3. Comparison of Phytochemical Content and ORAC Values in Different Rice Subspecies and Types (Mean ± SD)

		white/black	*indica*/*japonica*	glutinous/nonglutinous
phenolics (mg of gallic acid equiv/100 g of DW)	free	485.6 ± 63.1/3906 ± 1254[b]	3397 ± 904/4619 ± 1417[a]	3248 ± 728/4565 ± 1373[a]
	bound	200.8 ± 17.4/302.2 ± 57.0[a]	304.1 ± 48.5/299.4 ± 73.5	279.9 ± 56.9/324.4 ± 52.2
	total	686.3 ± 45.7/4208 ± 1267[b]	3702 ± 916/4918 ± 1440[a]	3528 ± 705.3/4890 ± 1385[a]
flavonoids (mg of catechin equiv/100 g of DW)	free	1012 ± 221/7444 ± 2339[b]	6735 ± 2009/8435 ± 2623	5605 ± 1279/9282 ± 1513[b]
	bound	128.4 ± 31.2/244.5 ± 82.0[a]	221.1 ± 76.0/277.3 ± 86.8	179.0 ± 41.4/310.0 ± 52.8[b]
	total	1141 ± 252.2/7688 ± 2419[b]	6956 ± 2082/8713 ± 2709	5784 ± 1315/9592 ± 1562[b]
anthocyanins (mg of cyanidin-3-glucoside equiv/100 g of DW)	free	18.29 ± 21.51/2715 ± 991[b]	2324 ± 720/3262 ± 1132	2180 ± 715/3250 ± 981[a]
	bound	0.48 ± 0.10/6.22 ± 1.16[b]	6.37 ± 1.35/6.01 ± 0.93	6.02 ± 1.26/6.41 ± 1.13
	total	18.78 ± 21.61/2721 ± 991[b]	2331 ± 720/3268 ± 1132	2186 ± 715/3257 ± 980[a]
Cy-3-G (mg/100 g of DW)	free		1440 ± 471/2093 ± 412[a]	1444 ± 473/1979 ± 505[a]
Cy-3-R (mg/100 g of DW)	free		72.50 ± 25.06/84.92 ± 11.39	68.05 ± 24.78/87.30 ± 10.52
Pe-3-G (mg/100 g of DW)	free		308.5 ± 154.4/363.7 ± 67.6	302.2 ± 140.4/360.7 ± 110.8
ORAC value (μmol of Trolox equiv/g of DW)	free	119.1 ± 24.5/989.9 ± 350.7[b]	857.4 ± 284.2/1175 ± 379[a]	797.9 ± 217.5/1182 ± 367[a]
	bound	42.58 ± 3.38/65.45 ± 11.74[a]	65.86 ± 10.42/64.88 ± 14.68	59.86 ± 9.85/71.04 ± 11.45[a]
	total	161.6 ± 21.1/1055 ± 356[b]	923.3 ± 289.3/1240 ± 387[a]	857.3 ± 213.8/1253 ± 373[a]

[a] Differences of black vs white, India vs Japonica, or glutinous/nonglutinous are significant at 0.05 level ($p < 0.05$), respectively. [b] Differences is significant at 0.01 level ($p < 0.01$), respectively.

Table 4. Total Flavonoid Content of Rice Bran and Percentage Contribution of Free and Bound Fractions to the Total Flavonoids

	flavonoids (mg of catechin equiv/100 g of DW)		
variety name	free	bound	total
Heizhenzhu	12061 ± 331 i[a] (96.9)[b]	386.9 ± 21.4 h (3.1)	12448 ± 327 i
Longjin 01	9882 ± 2261 h (96.6)	347.7 ± 9.6 gh (3.4)	10229 ± 235 h
Heiyounian 97	8862 ± 313 g (96.6)	314.9 ± 20.3 g (3.4)	9177 ± 294 g
Heixian 3	8651 ± 542 fg (96.6)	304.8 ± 24.2 g (3.4)	8956 ± 552 fg
Heijing 72	8309 ± 416 fg (97.0)	253.9 ± 17.2 f (3.0)	85620 ± 433 fg
Heinianmi 5	7927 ± 302 ef (96.9)	251.9 ± 21.0 f (3.1)	8179 ± 290 ef
Heisuai	7320 ± 237 e (97.0)	229.2 ± 7.2 ef (3.0)	7549 ± 242 e
Heijing 04	6117 ± 20 d (96.6)	212.1 ± 15.4 def (3.4)	6329 ± 8 d
Heifengnuo	5847 ± 167 cd (96.9)	187.2 ± 5.3 cde (3.1)	6034 ± 172 d
Qindao 2	5809 ± 241 cd (96.9)	185.8 ± 13.0 cd (3.1)	5995 ± 230 cd
Yunxiangnuo	5075 ± 140 c (97.6)	126.7 ± 5.0 ab (2.4)	5201 ± 136 c
Heinuo 9933	3462 ± 199 b (96.3)	133.1 ± 7.3 ab (3.7)	3596 ± 2020 b
Guinongzhan	1168 ± 44 a (88.6)	150.4 ± 6.3 bc (11.4)	1319 ± 48 a
Huidao 7	855.9 ± 86.6 a (89.0)	106.3 ± 8.2 a (11.0)	962 ± 94.9 a

[a] Values with no letters in common in each column are significantly different ($p < 0.05$). [b] Values in parentheses indicate percentage contribution to the total.

Table 5. Total Anthocyanin Content of Rice Bran and Percentage Contribution of Free and Bound Fractions to the Total Anthocyanins

	anthocyanins (mg of Cy-3-G equiv/100 g of DW)		
variety name	free	bound	total
Longjin 01	5096 ± 79 i[a] (99.9)[b]	5.13 ± 0.15 b (0.1)	5101 ± 79 i
Heizhenzhu	3443 ± 62 h (99.8)	6.54 ± 0.45 e (0.2)	3450 ± 61 h
Heisuai	3370 ± 29 h (99.8)	6.10 ± 0.34 cde (0.2)	3376 ± 28 h
Heijing 72	3093 ± 80 g (99.8)	6.94 ± 0.44 e (0.2)	3040 ± 80 g
Heixian 3	2952 ± 16 g (99.8)	6.36 ± 0.27 de (0.2)	2959 ± 16 g
Heiyounian 97	2496 ± 10 f (99.7)	8.20 ± 0.48 f (0.3)	2504 ± 11 f
Heinianmi 5	2423 ± 89 ef (99.8)	5.30 ± 0.41 bc (0.2)	2428 ± 90 ef
Heijing 04	2369 ± 50 ef (99.8)	4.89 ± 0.07 b (0.2)	2374 ± 50 ef
Qindao 2	2310 ± 50 e (99.7)	6.52 ± 0.10 e (0.3)	2316 ± 50 e
Yunxiangnuo	2006 ± 19 d (99.7)	5.54 ± 0.27 bcd (0.3)	2011 ± 19 d
Heifengnuo	1798 ± 6 c (99.5)	8.23 ± 0.09 f (0.5)	1806 ± 6 c
Heinuo 9933	1227 ± 21 b (99.6)	4.86 ± 0.17 b (0.4)	1231 ± 22 b
Guinongzhan	33.50 ± 0.76 a (98.4)	0.55 ± 0.02 a (1.6)	34.06 ± 0.75 a
Huidao 7	3.09 ± 0.37 a (98.7)	0.41 ± 0.03 a (1.3)	3.50 ± 0.40 a

[a] Values with no letters in common in each column are significantly different ($p < 0.05$). [b] Values in parentheses indicate percentage contribution to the total.

were significant genotype differences in flavonoid content among the black rice varieties. Heizhenzhu variety had the highest total flavonoid content among the 12 black rice varieties, followed by Longjin 01, Heiyounian 97 = Heixian3 = Heijing 72, Heinianmi 5 = Heisuai, Heijing 04 = Heifengnuo, Qindao 2, and Yunxiangnuo. Heinuo 9933 had the lowest flavonoid content among the varieties tested. The order of flavonoid content was similar to the ranking of the total phenolic content.

Similar to the phenolic content, the average values of free, bound, and total flavonoid contents of black rice bran were 7444, 244.5, and 7688 mg of catechin equiv/100 g of DW, respectively, which were 7.4, 1.9, and 6.7 times higher than those of corresponding fractions of white rice bran (1012, 128.4, and 1141 mg of catechin equiv/100 g of DW, respectively) (**Table 3**). This indicates that the flavonoid content of black rice bran is significantly higher than that of white rice bran ($p < 0.05$). In comparison of the flavonoid content in the different black rice subspecies, the average values of free, bound, and total flavonoid contents of *japonica* were 25.2, 25.4, and 25.3% higher than those of *indica*, respectively, but not significantly ($p > 0.05$). However, the average values of free, bound, and total flavonoid contents of nonglutinous type were 65.6, 73.2, and 65.8% higher than those of glutinous type ($p < 0.01$), respectively (**Table 3**).

Total Anthocyanin Content. The free and bound anthocyanin contents of rice bran and the percentage contribution of each fraction to the total of different samples are presented in **Table 5**, expressed as milligrams of Cy-3-G equivalents per 100 g of DW. The free anthocyanin content ranged from 1227 (Heinuo 9933) to 5096 mg of Cy-3-G equiv/100 g DW. The percentage contribution of free anthocyanins to the total in all black rice varieties tested was > 99.5%, indicating that the free anthocyanins are the major fraction of total anthocyanins in black rice bran. The bound anthocyanin content of black rice bran ranged from 4.86 (Heinuo 9933) to 8.23 (Heifengnuo) mg of Cy-3-G equiv/100 g of DW. The percentage contribution of bound anthocyanins to the total was < 0.5% in all black rice varieties tested. The total anthocyanin content of black rice bran ranged from 1231 to 5101 mg of Cy-3-G equiv/100 g of DW. The CVs were 36.4%, indicating that there were significant genotype differences in anthocyanin content among black rice varieties. Briefly, Longjin 01 had the highest total anthocyanin content among the 12 black rice varieties, followed by Heizhenzhu = Heisuai, Heijing 72 = Heixian 3, Heiyounian 97 = Heinianmi 5 = Heijing 04, and Qindao 2. The remaining varieties in order were Yunxiangnuo > Heifengnuo > Heinuo 9933. The order of total anthocyanin content was very similar to the ranking of the total flavonoid content.

The average values of free, bound, and total anthocyanins of black rice bran were 2715, 6.22, and 2721 mg of Cy-3-G equiv/100 g of DW, respectively, which were 148, 13, and 145 times higher than those of corresponding fractions of white rice bran (18.29, 0.48, and 18.78 mg of Cy-3-G equiv/100 g of DW, respectively) (**Table 3**). This indicates that the anthocyanin content of black rice bran is significantly higher than that of white rice bran ($p < 0.01$). The average values of free and total anthocyanins of *japonica* were 40.4 and 40.2% higher than those of *indica*, respectively, although not significantly ($p > 0.05$). The average values of free and total anthocyanin contents of nonglutinous were 49.1 and 48.9% higher than those of glutinous ($p < 0.05$), respectively (**Table 3**).

Anthocyanin Composition. The Cy-3-G, Cy-3-R, and Pe-3-G contents of black rice bran of different samples are presented in **Table 6**. The Cy-3-G, Cy-3-R, and Pe-3-G contents of the 12 black rice varieties ranged from 736.6 to 2557, from 22.70 to 96.62, and from 100.7 to 534.2 mg/100 g of DW, respectively, with average values of 1712, 77.68, and 331.5 mg/100 g of DW, respectively. The average value of Cy-3-G content is 22 and 5 times higher than those of Cy-3-R and Pe-3-G contents, respectively. Heizhenzhu and Longjin 01 had the highest Cy-3-G content among the 12 black rice varieties, followed by Heijing 72, Heisuai, and Heixian 3. The remaining varieties in order were Qindao 2 > Heijing 04 = Heinianmi 5 > Heiyounian 97 = Yunxiangnuo = Heifengnuo > Heinuo 9933. The order of Cy-3-G was similar to those of total anthocyanins, total phenolics, and total flavonoids. The 12 black rice varieties in order of Cy-3-R content were Longjin 01 = Heizhenzhu = Heinianmi 5 = Heisuai = Heixian 3 = Qindao 2 > Heijing 72 = Heiyounian 97 > Heijing 04 = Heinuo 9933 > Yunxiangnuo. Heixian 3 and Heisuai had the highest Pe-3-G contents among the 12 black rice varieties, and Yunxiangnuo had the lowest content. The remaining varieties in order of Pe-3-G content were Qindao 2 = Heizhenzhu = Heijing 72 > Longjin 01 > Heiyounian 97 > Heijing 04 = Heifengnuo = Heinuo 9933 > Heinianmi 5.

The average value of Cy-3-G content of *japonica* subspecies was 45.3% higher than that of *indica* ($p < 0.05$), and the average value of Cy-3-G content of nonglutinous type was 37% higher than that of glutinous type ($p < 0.05$; **Table 3**). There were no significant differences in Cy-3-R and Pe-3-G contents between

Table 6. Profiles of Anthocyanins of Rice Bran Samples

variety name	anthocyanins (mg/100 g of DW)		
	Cy-3-G	Cy-3-R	Pe-3-G
Heizhenzhu	2557 ± 612 f[a]	95.71 ± 5.63 d	422.6 ± 24.2 e
Longjin 01	2440 ± 56 f	96.62 ± 5.03 d	321.5 ± 10.1 d
Heijing 72	2061 ± 58 e	74.46 ± 3.54 bc	381.4 ± 15.2 e
Heisuai	2046 ± 58 e	93.27 ± 4.30 d	498.4 ± 12.0 f
Heixian 3	2032 ± 72 e	88.71 ± 5.59 d	534.2 ± 33.4 f
Qindao 2	1840 ± 85 d	85.35 ± 4.35 cd	424.2 ± 22.2 e
Heijing 04	1565 ± 89 c	72.44 ± 3.66 b	268.7 ± 9.7 bc
Heinianmi 5	1525 ± 54 c	94.37 ± 5.95 d	221.5 ± 10.8 b
Heiyounian 97	1261 ± 55 b	73.93 ± 4.28 bc	283.0 ± 7.1 cd
Yunxiangnuo	1251 ± 57 b	22.70 ± 1.13 a	100.7 ± 6.3 a
Heifengnuo	1226 ± 66 b	72.63 ± 4.25 bc	260.4 ± 15.3 bc
Heinuo 9933	736.6 ± 55.3 a	61.92 ± 2.23 b	261.1 ± 12.4 bc

[a] Values with no letters in common in each column are significantly different ($p < 0.05$).

Table 7. Total Antioxidant Activity of Rice Bran and the Percentage Contribution of Each Fraction to the Total

variety name	ORAC (μmol of Trolox equiv/g of DW)		
	free	bound	total
Heizhenzhu	1801.3 ± 181.4 h[a] (96.0)[b]	74.68 ± 1.62 fg (4.0)	1876 ± 180 g
Heixian 3	1400.7 ± 63.1 g (94.6)	79.48 ± 7.38 g (5.4)	1480 ± 70 f
Heijing 72	1134.6 ± 63.6 f (93.5)	78.72 ± 0.80 fg (6.5)	1213 ± 63 e
Qindao 2	1104.8 ± 80.3 f (95.8)	47.91 ± 4.36 ab (4.2)	1153 ± 78 e
Longjin 01	1064.6 ± 71.2 ef (93.6)	73.02 ± 1.80 efg (6.4)	1138 ± 70 e
Heisuai	955.5 ± 69.1 def (93.5)	66.70 ± 1.07 def (6.5)	1022 ± 70 de
Heinianmi 5	838.9 ± 85.2 cd (92.1)	71.84 ± 5.85 efg (7.9)	910.8 ± 82.3 cd
Heiyounian 97	851.6 ± 76.7 cde (94.6)	48.52 ± 5.27 abc (5.4)	900.2 ± 72.2 cd
Heifengnuo	797.7 ± 62.3 cd (93.0)	59.85 ± 3.51 bcd (7.0)	857.5 ± 59.4 cd
Heijing 04	771.8 ± 46.3 cd (93.9)	50.05 ± 4.14 abc (6.1)	821.9 ± 49.3 bcd
Yunxiangnuo	680.7 ± 26.8 bc (90.2)	73.96 ± 6.08 fg (9.8)	754.7 ± 23.0 bc
Heinuo 9933	476.9 ± 33.3 b (88.7)	60.68 ± 2.82 cde (11.3)	537.5 ± 35.6 b
Guinongzhan	136.4 ± 6.3 a (77.2)	40.19 ± 4.23 a (22.8)	176.6 ± 9.7 a
Huidao 7	101.7 ± 10.1 a (69.3)	44.97 ± 2.68 a (30.7)	146.7 ± 10.4 a

[a] Values with no letters in common in each column are significantly different ($p < 0.05$). [b] Values in parentheses indicate percentage contribution to the total.

indica and *japonica* subspecies or between glutinous and nonglutinous types ($p > 0.05$; **Table 3**).

Total Antioxidant Activity. The free and bound antioxidant activities of rice bran and the percentage contribution of each fraction to the total of different rice bran samples are presented in **Table 7**, expressed as ORAC values (μmol of Trolox equiv/g of DW). The free ORAC values ranged from 476.9 (Heinuo 9933) to 1801(Heizhenzhu) μmol of Trolox equiv/g of DW. The percentage contribution of free ORAC values to the total ranged from 88.7 (Heinuo 9933) to 96.0% (Heizhenzhu). The bound ORAC values ranged from 47.91 (Qindao) to 79.48 (Heixian 3) μmol of Trolox equiv/g of DW. The percentage contribution of bound ORAC values to the total ranged from 4.0 (Heizhenzhu) to 11.3% (Heinuo 9933). The total ORAC values ranged from 537.5 to 1876 μmol of Trolox equiv/g of DW. Heizhenzhu had the highest antioxidant activity, whereas Heinuo 9933 had the lowest antioxidant activity among the 12 black rice varieties tested. The remaining 10 varieties in order of antioxidant activity were Heixian 3 > Heijing 72 = Qindao 2 = Longjin 01 = Heisuai = Heiyounian 97 = Heinianmi 5 > Heifengnuo = Heijing 04 > Yunxiangnuo > Heinuo 9933. This order was consistent with those of total phenolics and total flavonoids. The average values of free, bound, and total antioxidant activities of black rice bran were 989.9, 65.45, and 1055 μmol of Trolox equiv/g of DW, respectively, which were 8, 1.5, and 6 times higher than those of corresponding fractions of white rice bran (119.1, 42.58, and 161.6 μmol of Trolox equiv/g of DW, respectively). This indicates that the total antioxidant activity of black rice bran was significantly higher than that of white rice bran ($p < 0.05$). The average values of free and total antioxidant activities of *japonica* subspecies were 37.0 and 34.3% higher than those of *indica*, respectively, ($p < 0.05$; **Table 3**). The average values of free and total antioxidant activities of nonglutinous type were 48.1 and 46.2% higher than those of glutinous, respectively ($p < 0.05$; **Table 3**).

DISCUSSION

Phenolic Profiles of Black Rice Bran. The results presented in this study indicated that the total phenolics of black rice bran ranged from 2365 to 7367 mg of gallic acid equiv/100 g of DW among the 12 black rice varieties analyzed (**Table 2**). However, in previous studies the total phenolic content of five long-grained white rice bran from commercial available cultivars in Thailand was reported in the range of 220−320 mg of gallic acid equiv/100 g of DW (*12*), and the phenolic content of five commercially available varieties of white rice bran in Pakistan ranged from 251 to 359 mg of gallic acid equiv/100 g of DW (*13*). Jang et al. separated black rice bran into two fractions (outer and inner layers) and reported that the phenolic contents of the outer and inner layer bran were 113.9 and 489.1 μg of catechin equiv/g of DW, respectively (*17*). Shen et al. reported that the total phenolic content of black rice grains of six varieties ranged from 841 to 1245 mg of gallic acid equiv/100 g of DW, with an average of 1056 mg of gallic acid equiv/100 g of DW (*29*). These values of total phenolics of rice bran reported in the literature were significantly lower than those of black rice bran reported in the present study. This is mainly due to the differences in the methodology used for grain phytochemical analyses, and the previous studies did not include the determination of bound phytochemicals. Our group developed a methodology to determine the complete phenolic profiles of whole grains (*9*). This method identifies and quantifies the free and bound forms of phenolics in corn, wheat, oat, and rice. We reported that the major portion of phytochemicals in the grains was present in the bound form (85% in corn, 76% in wheat, 75% in oat, and 60% in rice), suggesting that the total phytochemical contents of grains have been commonly underestimated because traditional methodologies do not include bound phenolics (*9*). Obviously, the previous studies did not determine the bound phytochemicals, so their total phenolic values were underestimated when compared to our current results.

The results in this study showed that the free phenolic contribution to the total ranged from 88.2 to 95.6% among the 12 black rice varieties tested, all > 85% (**Table 2**). These results were different from those in previous studies in which the bound phenolic fraction of whole brown rice grain contributed > 60% to the total (*9*). This may be due to (1) black rice being rich in anthocyanins when compared to white rice and (2) differences in the chemical compositions of rice bran and whole brown rice grain. Whole rice grain (brown rice) is composed of starchy endosperm, germ, and bran (including aleurone). Rice bran (including germ) is a rich source of fiber, vitamins, minerals, and phytochemicals. The phytochemicals mainly exist as glycosides linked to various sugar moieties or as other complexes linked to organic acids, amines, lipids, carbohydrates, and other phenols. They are commonly present in the bound form and are typically components of complex structures such as lignins and hydrolyzable tannins and linked to cell wall structural components such as cellulose, lignin, and proteins through ester bonds. Phenolic compounds from the bound fraction of whole grain are particularly interesting because the bound phytochemicals, mainly in the β-glycoside conjugations, cannot be digested by human enzymes and could survive both the stomach and small intestine digestions, possibly reaching the colon. The colonic microflora may release the bound phytochemicals through fermentation and thus provide site-specific health benefits in the colon and possibly other tissues after absorption (*2*, *9*).

The total flavonoid contents of the 12 black rice varieties were quantified using the new sodium borohydride/choranil (SBC)-based assay, and their contents ranged from 3596 to 12448 mg of catechin equiv/100 g of DW (**Table 4**). These results are significantly higher than those reported in the previous studies using the aluminum chloride colormetric method (*10*, *12*). Gorinstein et al. reported that the total flavonoid content of rice bran was about 185 mg of catechin equiv/100 g of DW (*10*). Chotimarkorn et al. reported that the total flavonoid content of rice bran from five Thai cultivars was in the range of 30−100 mg of catechin equiv/100 g of DW (*12*). Adom et al. reported that the flavonoid content of bran/germ fractions in five tested wheat varieties ranged from 214.6 to 272.6 mg of catechin equiv/100 g of DW (*21*). However, all of these studies reported flavonoid content determined by the aluminum chloride colorimetric method, which measured only partial flavonoids, not total, and this assay should not be used for total flavonoid analysis (*23*). The SBC assay was designed to quantify total flavonoids including all subgroups of flavones, flavonols, flavonones, flavononols, isoflavonoids, flavanols, and anthocyanins (*23*).

The total anthocyanin contents of the 12 black rice varieties ranged from 1231 to 5101 mg of Cy-3-G equiv/100 g of DW (**Table 5**). The percentage contribution of free fraction to the total anthocyanins in black rice bran was > 99.5%. The Cy-3-G, Cy-3-R, and Pe-3-G contents ranged from 736.6 to 2557, from 22.70 to 96.62, and from 100.7 to 534.2 mg/100 g of DW, respectively. Their average values were 1712, 77.68, and 331.5 mg/100 g of DW, respectively. The mean of Cy-3-G content is 22 and 5 times higher than that of Cy-3-R and Pe-3-G, respectively (**Table 6**). This result is similar to the early studies that Cy-3-G was the major anthocyanin in black rice grains, followed by Pe-3-G (*30*−*32*).

There were significant correlations between free phenolics and free flavonoids ($r = 0.8323$, $p < 0.01$) and between total phenolics and total flavonoids ($r = 0.8376$, $p < 0.01$). Similar correlations were observed between free anthocyanin content and free flavonoid content ($r = 0.7624$, $p < 0.01$) and between total anthocyanin content and total flavonoid content ($r = 0.7633$, $p < 0.01$). Cy-3-G content was significantly correlated to the content of free anthocyanins ($r = 0.8686$, $p < 0.01$), free phenolics ($r = 0.8127$, $p < 0.01$), and free flavonoids ($r = 0.8094$, $p < 0.01$) of black rice bran, respectively. Cy-3-R content was significantly correlated to the content of free anthocyanins ($r = 0.5818$, $p < 0.05$) and free flavonoids ($r = 0.6300$, $p < 0.05$) of black rice bran, respectively.

Antioxidant Activity of Black Rice Bran. The total ORAC values of the 12 diverse varieties of black rice bran ranged from 476.9 to 1801 μmol of Trolox equiv/g of DW in the free fraction, from 47.91 to 79.48 μmol of Trolox equiv/g of DW in the bound fraction, and from 537.5 to 1876 μmol of Trolox equiv/g of DW in total. The percentage contribution of the free fraction to the total ranged from 88.7 to 96.0%. The average values of free, bound, and total ORAC of black rice bran were 989.9, 65.45, and 1055 μmol of Trolox equiv/g of DW, respectively (**Table 7**), which were more than 8, 1.5, and 6 times higher than those of white rice bran, respectively (119.1, 42.58, and 161.6 μmol of Trolox equiv/g of DW). This indicates that black rice bran has higher antioxidant activity than white rice bran. The antioxidant activity of black rice bran extract has been determined using previously reported antioxidant activity assays including inhibition of peroxidation of linoleic acid, inhibition of peroxidation of rabbit lipid erythrocyte membranes, reduction of potassium ferricyanide, and scavenging of superoxide anions and hydroxyl radicals (*31*). Although these results showed that the extracts of black rice had higher antioxidant activity than those of white rice, it is difficult to compare their results to the antioxidant activity reported here using the ORAC assay.

There were significant correlations between ORAC values and total phenolics ($r = 0.9810$, $p < 0.01$) and between ORAC values and total flavonoids ($r = 0.8281$, $p < 0.01$). ORAC values also significantly correlated to the total anthocyanin content ($r = 0.5763$, $p < 0.05$). It is interesting to note that the correlations between ORAC and total phenolics/flavonoids are higher than those of total anthocyanins. This result is different from the previous studies that antioxidant activity of black rice was most significantly ($p < 0.01$) related to anthocyanin content in the FRAP assay (*33*). Significant correlations existed between the ORAC value of black rice bran and three individual anthocyanins, Cy-3-G, Cy-3-R, and Pe-3-G, with correlation coefficients of 0.8499 ($p < 0.01$), 0.5764 ($p < 0.05$), and 0.6810 ($p < 0.05$), respectively. These results were similar to our previous reports that Cy-3-G was the predominant phytochemical responsible for the antioxidant activity of black rice (*33*).

Potential Application of Black Rice Bran. Recently, more and more nonglutinous black rice varieties have been developed and gained increasing popularity as staple food to replace white rice. Here we show black rice bran has a high content of phenolics and flavonoids and has potent antioxidant activity. There are significant differences in phytochemical content and antioxidant activity among the 12 diverse black rice varieties. This opens a door for scientists to breed new varieties with higher nutritional values and for the food industry to develop new products to compete for today's functional food markets. Black rice bran is rich in anthocyanins, which can be used as food colorants in a wide variety of foods.

As consumers become more concerned about the safety of artificial colorants, the trend of using natural colorants in food products has increased intensively. The black rice bran with highly concentrated anthocyanins has potential to replace black, purple, or red artificial colorants in foods and dietary supplements. Black rice bran, with low moisture content and low cost, has great potential to be a new source of natural pigments for applications in the food and pharmaceutical industries.

In summary, black rice bran has higher contents of phenolics, flavonoids, and anthocyanins and has higher antioxidant activity when compared to white rice bran. The phenolics, flavonoids, and anthocyanins of black rice bran are mainly present in free form. Cy-3-G was the major anthocyanin in black rice grains, followed by Pe-3-G and Cy-3-R. There are significant differences in phytochemical content and antioxidant activity among the different black rice varieties. Total antioxidant activity determined using the ORAC assay is significantly correlated to the content of total phenolics, total flavonoids, and anthocyanins, respectively. Black rice bran has the potential to be used as a functional ingredient and natural colorant for food and pharmaceutical applications.

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Received for review February 24, 2010. Revised manuscript received May 17, 2010. Accepted May 17, 2010.