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ARTICLE

Antioxidant and α-Glucosidase Inhibitory Activity of Colored Grains in China

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Colored grains including red, purple, and black rice, purple corn, black barley, and black soybean contain anthocyanins. The present study was designed to (i) identify and quantify the individual anthocyanins and measure the total phenolic content (TPC), (ii) evaluate the antioxidant and α-glucosidase inhibitory activity, and (iii) correlate the TPC with total antioxidant activity and α-glucosidase inhibitory potency in these colored grains. The TPC was measured using a Folin−Ciocalteu assay, while the total antioxidant activity was determined by a method based on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity. Among all of the studied colored grains, black rice possessed the highest TPC, which was 86 times greater than that of red rice. In addition, black rice had the highest total anthocyanin contents and α-glucosidase inhibitory activity. A significant positive correlation of the antioxidant activity and α-glucosidase inhibitory activity with total anthocyanin content and TPC was observed in this study. It is concluded that black rice possesses the highest antioxidant activity and α-glucosidase inhibitory among all of the colored grains tested and can be further explored as a functional food.

KEYWORDS: Anthocyanins; antioxidant; α-glucosidase inhibitory

INTRODUCTION

Interest in glucosidase inhibitors is growing because it has implication in the management of diabetes mellitus (DM). DM is a serious metabolic disorder that affects approximately 4% of the population worldwide and is expected to increase to 5.4% in 2025 (*1*). Grains and cereals are generally recommended for diabetic patients to control their blood glucose level (*2*, *3*). Acting as a key enzyme for carbohydrate digestion, intestinal α-glucosidase is one of the glucosidases located at the epithelium of the small intestine. α-Glucosidase has been recognized as a therapeutic target for modulation of postprandial hyperglycemia, which is the earliest metabolic abnormality to occur in type-2 DM (*4*, *5*). The inhibition on intestinal α-glucosidases would delay the digestion and absorption of carbohydrates and, consequently, suppress the postprandial hyperglycemia (*6*).

Antioxidants refer to compounds possessing free-radical-scavenging activity, transition-metal-chelating activity, and/or singlet-oxygen-quenching capacity (*7*, *8*). Accumulated evidence has suggested that diabetic patients are under oxidative stress, with an imbalance between the free-radical-generating and radical-scavenging capacities. The increased free-radical production and reduced antioxidant defense may partially mediate the initiation and progression of diabetes-associated complications (*9*, *10*). Colored grains are rich in pigments called anthocyanins. Among these anthocyanins, cyanidin-3-glucoside has an antioxidant activity that is 3.5 times stronger than Trolox (vitamin E analogue) (*11*). A structure−activity relationship study has revealed that the antioxidant activity of anthocyanidins is dependent upon positions of hydroxylation and glycosylation (*11*).

Despite some research on anthocyanins in some colored grains, a systematic comparison on their relative abundance and antioxidant and glucosidase-inhibiting activity is lacking. The present study was therefore carried out to (i) identify and quantify the individual anthocyanins and total phenolic content (TPC) in red, purple, and black rice, purple corn, black barley, and black soybean and (ii) assess their relative antioxidant and α-glucosidase inhibitory activities.

MATERIALS AND METHODS

Materials. Red, purple, and black rice, purple corn, and black barley were provided by the Chinese National Genebank (Beijing, China). Individual anthocyanin standards (cyanidin-3,5-diglucoside, cyanidin-3-glucoside, cyanidin-3-arabinoside, delphindin-3-glucoside, malvidin-3-glucoside, petunidin-3-glucoside, peonidin-3-arabinoside, peonidin-3-glucoside, and peonidin-3-galactoside) (**Figure 1**) were obtained from polyphenols (Sandnes, Norway). Standards of gallic acid, Trolox, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, Folin−Ciocalteu phenolic reagent, and rat intestinal acetone powder were purchased from Sigma-Aldrich (St. Louis, MO). Ethanol and trifluoroacetic acid (TFA) were obtained from Beijing Chemical Reagent (Bejing, China).

Extraction. All samples were dried at 40 °C, ground in a laboratory mill, and passed through a 80-mesh screen sieve. Extractions were carried out according to the method previously described, with slight modifications (*12*). Briefly, 10 g of sample was extracted twice in 100 mL of ethanol acidified with 1.0 N HCl (85:15, v/v) for 2 h at room temperature. After

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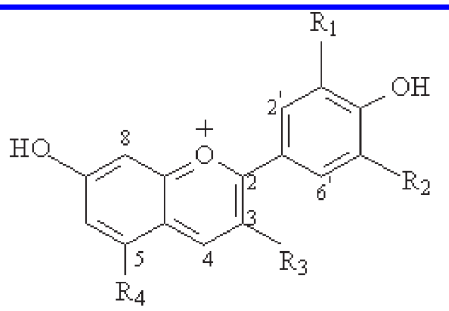

Anthocyanin	R_1	R_2	R_3	R_4
Cyanidin-3,5-diglucoside	OH	H	O- β -D-glucose	O- β -D-glucose
Cyanidin-3-glucoside	OH	H	O- β -D-glucose	OH
Cyanidin-3-arabinoside	OH	H	O- β -D-arabinose	OH
Delphindin-3-glucoside	OH	OH	O- β -D-glucose	OH
Malvidin-3-glucoside	OCH_3	OCH_3	O- β -D-glucose	OH
Petunidin-3-glucoside	OCH_3	OH	O- β -D-glucose	OH
Peonidin-3-arabinoside	OCH_3	H	O- β -D-arabinose	OH
Peonidin-3-glucoside	OCH_3	H	O- β -D-glucose	OH
Peonidin-3-galactoside	OCH_3	H	O-α-D-galactose	OH

Figure 1. Chemical structures of anthocyanins in colored grains.

vacuum filtration at 50 °C, the supernatants were combined and concentrated to $^1/_3$ volume under a reduced pressure in a rotary evaporator. The resultant extracts were then stored at 4 °C until analysis.

Determination of TPC. TPC was measured using the Folin−Ciocalteu method described previously by Zhou et al. (*13*) and modified by Fang et al. (*14*). Briefly, 50 μL of the extract was mixed in 5 mL of distilled deionized water followed by the addition of 500 μL of Folin−Ciocalteu reagent (1 M) and 500 μL of Na_2CO_3 (20%, w/v). The mixture was thoroughly mixed and allowed to stand for 60 min at room temperature before the absorbance was measured at 765 nm (Bio-Rad Smart Spec Plus spectrophotometer, Hercules, CA). Quantification was performed with respect to the standard curve of gallic acid. The results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of dry weight (dw). All determinations were performed in triplicates.

Determination of Total Anthocyanins. Quantification of anthocynins was carried out as previously described by Giusti et al. (*15*). Samples were dissolved in 0.025 M potassium chloride solution (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5), and the absorbance was measured at 510 and 700 nm in a BioRad Smart Spec Plus spectrophotometer. Data were expressed as milligrams of anthocyanins per 100 g of fresh weight of seed powder using a molar extinction coefficient of 26900, a molecular weight of 449, and an absorbance of $A = [(A_{510}-A_{700})\ \text{pH}\ 1.0 - (A_{510}-A_{700})\ \text{pH}\ 4.5]$.

High-Performance Liquid Chromatography (HPLC) Analysis of Individual Anthocyanidins. A HPLC system equipped with two Shimadzu LC-20A pumps, a Shimadzu LC-20 autosampler, a SPD-20A UV/vis detector, and an Alltima C18 column (4.6 × 250 mm, Metachem Technologies, Inc., Torrance, CA) was used. The wavelength of the UV detector was set at 520 nm. The mobile phase was a mixture of solvent A (HPLC water containing 0.1% TFA) and solvent B (acetonitrile containing 0.1% TFA). The elution started with 5% B with a linear gradient to 25% B in 38 min and then to 90% B from 38 to 55 min. The flow rate was set at 1.0 mL/min, and the injection volume was 10 μL. Each anthocyanidin was quantified according to its calibration curve.

Evaluation of the Total Antioxidant Activity Using the DPPH Method. The DPPH radical-scavenging activity was determined using the method reported by Yen and Chen (*16*). DPPH (100 μM) was dissolved in 96% ethanol. The extract was dissolved in ethanol in a ratio of 1:3. The DPPH solution (1 mL) was mixed with 1 mL of the extract solution. The mixture was shaken and allowed to stand at room temperature in the dark for 10 min. The decrease in absorbance of the resulting solution was monitored at 517 nm after 10 min. The results were corrected for dilution and expressed in micromolar Trolox equivalents (TE) per 100 g of dw. All determinations were performed in triplicates ($n = 3$).

Measurement of α-Glucosidase Inhibitory Activity. The α-glucosidase inhibitory activity was determined as described previously, with some slight modification (*17*). α-Glucosidase (1 unit/mL) activity inhibition was assayed using 50 μL of extracts with varying concentrations incubated with 100 μL of 0.1 M phosphate buffer (pH 7.0) in 96-well plates at 37 °C for 10 min. After preincubation, 50 μL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 7.0) was added to each well at varying time intervals. The reaction mixtures were incubated at 37 °C for 5 min. The absorbance readings were recorded at 490 nm on a microplate reader before and after incubation (BioRad, IMAX, Hercules, CA). The results were expressed as a percentage of α-glucosidase inhibition and calculated according to the following equation: percent inhibition $= \text{Abs}^{\text{control}} - \text{Abs}^{\text{extract}} \times 100/\text{Abs}^{\text{control}}$. IC_{50} is defined as the concentration of grain extracts required to inhibit 50% of the enzyme activity.

Statistical Analysis. All values were expressed as mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA) followed by posthoc Dunnett's *t* test. Differences with $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Individual Anthocyanidins. We used HPLC to quantify the individual anthocyanins in these colored grains. **Table 1** showed

Table 1. Average Concentration of Antrocyanins in Red, Purple, and Black Rice, Purple Corn, Black Barley, Black Soybean, and Black Soybean Coat (mg/100 g)[a]

	red rice	purple rice	black rice	purple corn	black barley	black soybean	black soybean coat
cyanidin-3,5-diglucoside	nd	nd	nd	nd	nd	nd	nd
cyanidin-3-glucoside	1.50 ± 0.13	148.83 ± 7.88	631.01 ± 13.08	20.18 ± 1.38	2.52 ± 0.71	21.29 ± 2.25	199.26 ± 5.49
cyanidin-3-arabinoside	nd	nd	nd	nd	nd	nd	nd
delphindin-3-glucoside	nd	8.38 ± 0.26	71.03 ± 1.06	nd	2.13 ± 0.22	41.35 ± 3.01	365.90 ± 11.20
malvidin-3-glucoside	nd	nd	nd	7.48 ± 0.11	nd	1.26 ± 0.19	4.78 ± 0.23
petunidin-3-glucoside	nd	20.16 ± 1.33	90.04 ± 4.15	nd	28.57 ± 1.64	7.21 ± 0.34	62.32 ± 5.26
peonidin-3-arabinoside	nd	nd	nd	24.82 ± 1.76	nd	nd	nd
peonidin-3-glucoside	nd	82.09 ± 5.13	362.87 ± 21.08	29.61 ± 2.89	nd	2.92 ± 0.14	22.55 ± 0.98
peonidin-3-galactoside	nd	nd	nd	2.67 ± 0.23	nd	nd	nd

[a] nd = not detected. Data are expressed as mean ± SD of triplicate samples.

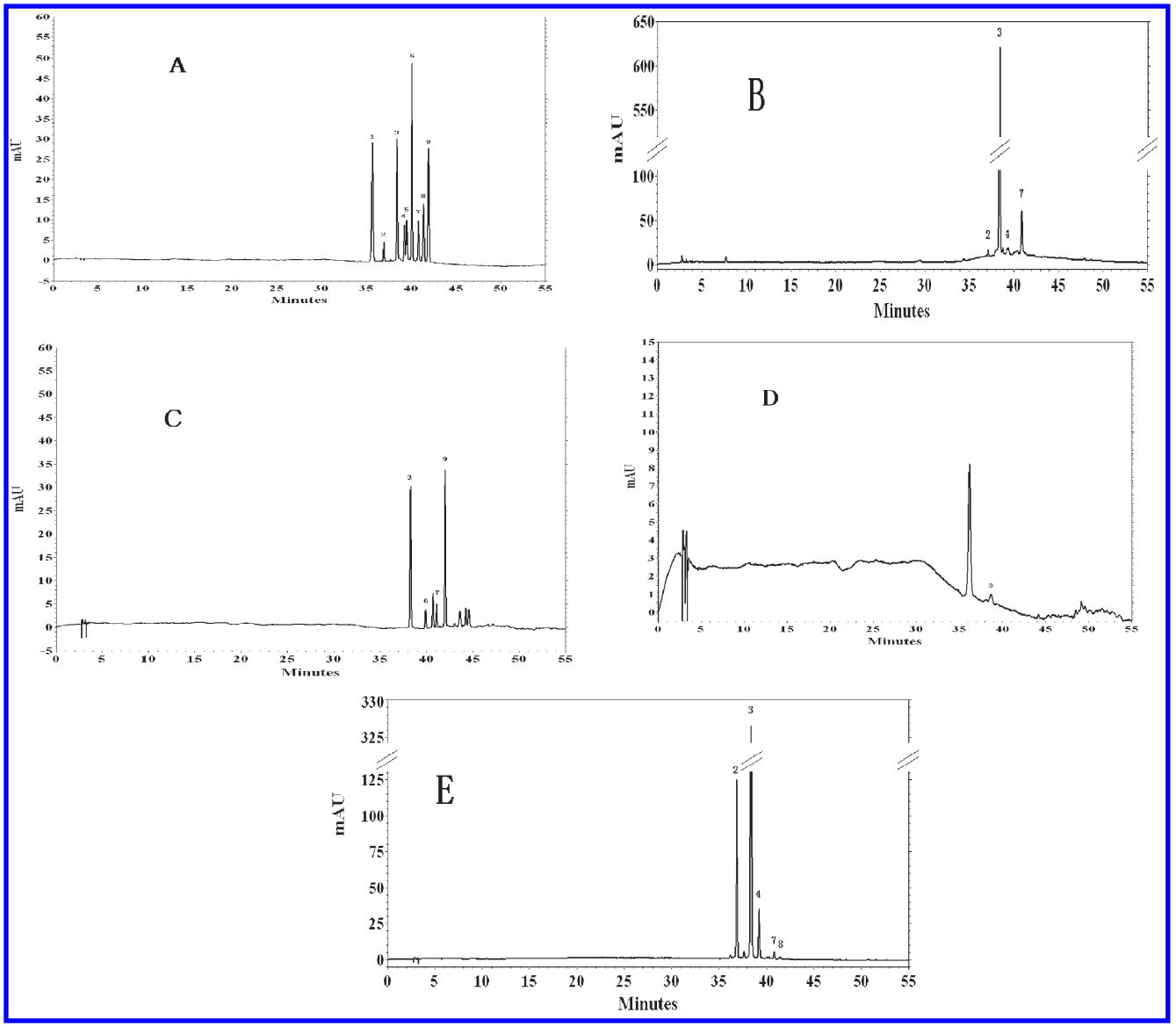

Figure 2. (A) HPLC chromatogram of anthocyanins standard. (B) HPLC chromatogram of anthocyanins from black rice. (C) HPLC chromatogram of anthocyanins from purple corn. (D) HPLC chromatogram of anthocyanins from black barley. (E) HPLC chromatogram of anthocyanins from black soybean coat. Peak 1, cyanidin-3,5-diglucoside; peak 2, delphindin-3-glucoside; peak 3, cyanidin-3-glucoside; peak 4, petunidin-3-glucoside; peak 5, cyanidin-3-arabinoside; peak 6, peonidin-3-galactoside; peak 7, peonidin-3-glucoside; peak 8, malvidin-3-glucoside; peak 9, peonidin-3-arabinoside.

the contents of individual anthocyanidins in red, purple, and black rice, purple corn, black barley, black soybean, and black soybean seed coat. **Figure 2** showed typical HPLC chromatograms of anthocyanin profiles in these colored grains. It was noticed that red rice contained only cyaniding-3-glucoside, while purple and black rice contained four types of anthocyanins, namely, cyaniding-3-glucoside, delphindin-3-glucoside, petunidin-3-glucoside, and peonidin-3-glucoside. Black barley had three species of anthocyanins, including cyanidin-3-glucoside, delphindin-3-glucoside, and petunidin-3-glucoside, while black soybean coat contained

Table 2. TPC, TAC, and Anti-DPPH Radical Activity of Red, Purple, and Black Rice, Purple Corn, Black Barley, Black Soybean, and Black Soybean Coat[a]

	TPC	TAC	DPPH
red rice	0.10 ± 0.01 d	0.05 ± 0.01 f	1.63 ± 0.15 c
purple rice	4.62 ± 0.18 b	1.22 ± 0.08 c	30.92 ± 1.58 b
black rice	8.58 ± 0.56 a	3.83 ± 0.04 a	73.47 ± 4.63 a
purple corn	1.11 ± 0.09 c	0.31 ± 0.01 d	1.68 ± 0.19 c
black barley	0.46 ± 0.04 cd	0.27 ± 0.05 de	2.21 ± 0.37 c
black soybean	0.75 ± 0.06 cd	0.19 ± 0.02 e	4.59 ± 0.27 c
black soybean coat	5.26 ± 0.42 b	1.63 ± 0.03 b	13.94 ± 4.86 b

[a] Data are expressed as mean ± SD of triplicate samples. TPC was expressed as grams of GAE/100 g. TAC was expressed as milligrams of anthocyanin/gram. The anti-DPPH capacity was expressed as micromolar TE/gram. Values in the same column sharing different letters are expressed as significantly different ($p < 0.05$).

Table 3. α-Glucosidase Inhibitory Activity of Red, Purple, and Black Rice, Purple Corn, Black Barley, Black Soybean, and Black Soybean Coat[a]

	IC_{50}
red rice	>1000
purple rice	475.14 ± 25.46
black rice	13.56 ± 1.2
purple corn	833.33 ± 56.31
black barley	>1000
black soybean	>1000
black soybean coat	111.11 ± 21.24

[a] IC_{50} was expressed as mg/mL.

five species of anthocyanins, namely, cyanidin-3-glucoside, delphindin-3-glucoside, malvidin-3-glucoside, petunidin-3-glucoside, and peonidin-3-glucoside.

TPC and Total Anthocyanins Content (TAC). TPC measured by the Folin−Ciocalteu method varied widely in colored grains. Phenolic compounds are considered as the major compounds that contribute to the total antioxidant activities of the grains (*18*). In the present study, black rice, with an average of 8.58 ± 0.56 g of GAE/100 g, was found to possess the highest TPC among all of the studied colored grains and had GAE 86 times greater than that of red rice (0.10 ± 0.01 g of GAE/100 g). Purple rice (4.62 g of GAE/100 g) also had a high level of phenolics. Black soybean coat had an average of 5.26 g of GAE/100 g, which was significantly higher than black soybean. It is known that the phenolic compounds are mainly present in the seed coats (*19*).

TAC varied significantly among black, purple, and red grains (**Table 2**). Significant differences in the concentrations of TAC were previously reported among black, brown, and red sorghum (*20*), as well as among blue, pink, purple, and red rice (*12*). In the present study, black rice had the highest TAC, followed by black soybean coat and purple corn (**Table 2**). Astadi et al. (*21*) reported a higher level of TAC in black soybean. At present, most of the purple corn is used in ornamentation for its colorful appearance and only a small amount is being used in making naturally colored tortillas (*12*). In contrast, red rice had a very low concentration of TAC, because only a small cyanidin-3-glucoside peak was detected under the present conditions.

Antioxidant Activity. The antioxidant activities of colored grain extracts were evaluated by measuring their DPPH radical-scavenging activities. All of the extracts exhibited strong antioxidant activities (**Table 2**). Among the tested samples, black rice had the greatest DPPH free-radical-scavenging capacity (73.47 μM TE/g), whereas red rice had the least DPPH free-radical-scavenging capacity (1.68 μM TE/g). In this research, the DPPH scavenging activity of purple rice was higher than that in black soybean seed coat. However, the levels of TPC and TAC in purple rice were lower than that in black soybean seed coat. Brown et al. (*20*) once reported that anthocyanins contributed mainly to total TPC and antioxidant activity. However, our results did not support this claim. The possible reason is that anthocyanins content in black soybean seed coat is perhaps to have a color interference with the DPPH radical, leading to underestimation of its antioxidant activity (*21*). Thus, the anthocyanins levels in colored grains do not necessarily correspond to their DPPH scavenging capacity (*22*).

Table 4. Correlation Coefficient of Total Phenolic Acids and Total Anthocyanins to DPPH and α-Glucosidase Inhibition Assays

	DPPH	α-glucosidase inhibition
TPC	0.916[a]	−0.929
TAC	0.958[a]	−0.856

[a] Correlation is significant at $p < 0.01$ level (two-tailed).

α-Glucosidase Inhibition Activities. To determine the α-glucosidase inhibition ability of colored grains *in vitro*, we calculated the IC_{50} values (**Table 3**). The black rice was the most active (IC_{50} of 13.56 mg/mL), followed by the black soybean seed coat (IC_{50} of 111.11 mg/mL). The IC_{50} values in red rice, black barley, and black soybean were all higher than 1000 μg. To explain, it is known that certain polyphenols, such as anthocyanins, can directly induce secretion of insulin from pancreatic cells in *ex vivo* assays (*23*). Similar to acarbose, anthocyanin could act as a competitive α-glucosidase inhibitor because of the structural similarity between the normal substrate maltose and the glucosyl group, which is β-linked to the anthocyanin (*24*).

Correlation of TPC and TAC with DPPH and α-Glucosidase Inhibition Activities. Correlation coefficients for TPC and TAC with the DPPH assay and α-glucosidase inhibition activities were shown in **Table 4**. Zhou et al. (*13*) have demonstrated a high correlation between the content of total phenolic compounds and their antioxidant capacity. The results (**Table 4**) obtained in our study showed that TPC and TAC significantly correlate with the DPPH assay ($p < 0.01$). Except for red rice, black barley, and black soybean, TPC and TAC positively correlate with α-glucosidase inhibition activities. In conclusion, black rice appeared to possess the most active antioxidant activity and α-glucosidase inhibitory activity among all of the colored grains tested and should be explored further as a functional food.

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