Appl Biol Chem (2018) 61(1):49–60
https://doi.org/10.1007/s13765-017-0335-3

Online ISSN 2468-0842
Print ISSN 2468-0834

ARTICLE

Effect of cultivars and milling degrees on free and bound phenolic profiles and antioxidant activity of black rice

Sehun Choi[1] · Han-Seok Seo[2] · Kwang Rag Lee[3] · Sunghee Lee[3] · Jihyun Lee[1]

Received: 3 November 2017 / Accepted: 24 November 2017 / Published online: 2 December 2017
© The Korean Society for Applied Biological Chemistry 2017

Abstract Six black rice cultivars (Heukjinju, Sintoheugmi, Heukhyangchal 1, Bosukheukchal, Sinnongheukchal, and Josengheukchal) and varying milling degrees (step 0, 0%; step 1, 4.2%; and step 2, 10.5%, w/w) were used to evaluate the effects of cultivars and milling degrees of black rice (*Oryza sativa* L.) on the total phenolic contents (TPC), total flavonoid contents (TFC), antioxidant activity (2,2-diphenyl-1-picrylhydrazyl free radical assay), and phenolic composition in free and bound phenolic fractions. Unpolished (step 0) Sintoheugmi showed significantly higher TPC, TFC, antioxidant activity, phenolic acid levels, and anthocyanin levels than other unpolished cultivars ($p < 0.05$). As milling degree increased, TPC, TFC, antioxidant activity, phenolic acid levels, and anthocyanin levels decreased significantly ($p < 0.05$). TPC, TFC, and antioxidant activity were significantly higher in free phenolic fractions than bound phenolic fractions of black rice extracts, regardless of cultivars ($p < 0.05$). The major phenolic acid was ferulic acid, and the major anthocyanin found in free phenolic fractions in black rice samples was cyanidin-3-O-glucoside. The sum of individual phenolic acid levels (255.2 ± 0.0 μg/g) and the sum of anthocyanins levels (831.4 ± 0.3 μg/g) were significantly higher in Sintoheugmi black rice than in the other cultivars for step 0 (unpolished rice) ($p < 0.05$). For step 1 and step 2, Heukjinju black rice contained significantly higher sum of phenolic acid levels and sum of anthocyanin levels than the other cultivars ($p < 0.05$). For use as a better functional ingredient, it is, therefore, important to consider different milling degrees together with different black rice cultivars having the highest antioxidant component.

Keywords Anthocyanin · Antioxidant · Black rice · Cyanidin · Flavonoid · High-performance liquid chromatography · Milling · Phenolic acid

✉ Jihyun Lee
jihlee@cau.ac.kr

[1] Department of Food Science and Technology, Chung-Ang University, Anseong 17546, Republic of Korea

[2] Department of Food Science, University of Arkansas, Fayetteville, AR 72704, USA

[3] Prepared Food Development Team, R&D center, Nongshim, Seoul 07057, Republic of Korea

Introduction

Specialty rice, such as pigmented rice and aromatic rice, have been developed in response to increases in income levels due to social development. Black rice is produced in some parts of southern Asia, China, and Korea, and the production of this pigmented rice has greatly increased farm income. Recently, black rice species have received attention from researchers and the food industry because they are potential sources of anthocyanins. In previous studies, the total phenolic contents (TPC) and total flavonoid contents (TFC) of black rice cultivars were significantly higher than those of non-pigmented, green, and red rice cultivars [1, 2]. Wu et al. [3]. reported that the anthocyanin levels in black rice were higher than those in other available anthocyanin sources. The main anthocyanin in black rice is cyanidin-3-O-glucoside, which is followed by a minor proportion of the anthocyanin peonidin-3-O-glucoside. These anthocyanins are reported to have antioxidative, anticancer, and anti-inflammatory activities [4]. Anthocyanins may be metabolized into phenolic acids such as protocatechuic acids after consumption [5].

Anthocyanin in plants may play an antioxidant role against reactive oxygen species that result from external stresses such as ultraviolet, pathogens, and some microbes [6]. Additionally, black rice is a particularly economically important rice species that contains many beneficial compounds, such as other polyphenolics, γ-oryzanol, vitamin E, and phytic acid [7].

Black rice has a characteristically dark purple and black color from the anthocyanin pigments, which are mostly located in the aleurone and pericarp layers of the rice bran. Additionally, black rice contains phenolic acids in free and/or bound forms [8]. Free phenolic acids are absorbed in the human stomach and small intestine and can protect against oxidation of low-density lipoprotein and liposomes [9]. Insoluble, bound phenolic acids are partly digested in small intestine by enzymes, and some are digested in the colon by the colonic microflora [10]. Bound phenolics of black rice were not investigated in many studies on phenolic composition of black rice samples [11, 12].

There is little information on the varietal differences of phenolic composition in black rice. Also, different extraction solvents were used in different studies [8, 11]; thus, it is difficult to compare phenolic contents of different black rice varieties studied in previous studies. More importantly, there is little information on the changes of phenolic composition during milling process. Generally, rice is consumed in the form of rice flour and kernel after milling. The quality of raw rice before processing significantly affects the quality of rice after processing [13]. The degree of removal of rice bran during the milling process is closely related to milling yield and rice quality and is affected by the degree of milling, i.e., the ratio of the weight of removed rice bran to the weight of unpolished rice. Different milling degrees of rice are used for different purposes. Usually, textural properties of milled rice are favorable; however, consumption of milled rice may increase blood glucose levels, when measured by glycemic index, rapidly than consumption of the same amount of unmilled rice. Thus, patients with diabetes are recommended to consume un-milled rice. Also, when the rice bran and germ are completely removed, anthocyanin compounds may be also removed.

In this study, we evaluated the effect of cultivars and milling degrees in black rice on the TFC, TPC, antioxidant activity (2,2-diphenyl-1-picrylhydrazyl free radical assay (DPPH)), and phenolic composition, including free and bound phenolic acids and anthocyanins.

Materials and methods

Chemicals and reagents

Cyanidin-3-O-glucoside and peonidin-3-O-glucoside were purchased from Extrasynthese (Lyon, France). Phenolic acid standards (i.e., gallic acid, syringic acid, vanillic acid, *p*-coumaric acid, and ferulic acid) and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Rice samples and preparation

Six black rice (*Oryza sativa* L.) cultivars (Bosukheukchal, Heukhyangchal 1, Heukjinju, Josengheukchal, Sinnongheukchal, and Sintoheugmi) were used in this study. The Heukjinju cultivar was harvested in October 2016 at a local farm in Jindo, Jeollanam-do, Republic of Korea. All the other cultivars were harvested in October 2016 from a local farm in Hoengseong-gun, Gangwon-do, Republic of Korea. Before distribution, the grains were dried to a moisture content of about 12–13%. The rough rice samples were then placed in a sack in dark storage at room temperature (21–23 °C) for 1–2 mon before dehulling on a Satake Rice Huller (Satake, Tokyo, Japan). After dehulling, an FS-2000 rice polisher (Misul, Seoul, Korea) was used to mill the unpolished rice to three different milling degrees (step 0, 0%; step 1, 4.2%; and step 2, 10.5%, w/w). The milling degree of step 0 is typical brown rice level, and that of step 2 is typical white rice level.

Colorimetric measurements

The color of the black rice powders varied in the three different milling degrees, and the color of the samples was measured using a colorimeter of HunterLab model UltraScan PRO (Reston, VA, USA). Color characteristics were indicated by Hunter's values: L^* (whiteness), a^* (redness), and b^* (yellowness). The calibration of the colorimeter was conducted using a standard white tile ($L^* = 100.03$, $a^* = 0.09$, $b^* = 0.15$). Samples were prepared in triplicate ($n = 3$). The total color difference (ΔE^*) between black rice samples of the same cultivar treated with steps 1 and 2 and the unpolished samples of step 0 were calculated using the following equation:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}.$$

Extraction of free and bound phenolics

The free and bound phenolics in the black rice samples were extracted sequentially. The first extraction used 80% methanol for free phenolic extraction, and the second

extraction employed alkaline and acid hydrolysis for bound phenolic extraction [8]. The methodologies used are as follows: a sample of 100 g of black rice kernels was ground and passed through an 18-mesh sieve. The powder [0.05 g (± 1%)] was mixed with 8 mL 80% methanol, and the mixture was sonicated at 35 °C for 1 h. The supernatant was centrifuged at 4321 g for 25 min, and the extraction was performed again. The pH of combined supernatants, i.e., the free phenolic fraction, was adjusted to 4.5–5.5 using 6 M HCl.

After washing the residue with water, the residues were sonicated with 20 mL of 4 M NaOH for 2 h and the supernatant was collected. The sonication process was conducted again with the remaining residues. The combined supernatants were adjusted to 4.5–5.5 using 6 M HCl and then centrifuged at 4321 g for 25 min. The supernatants were used in the bound phenolic analysis.

The free and bound fractions were filtered through a 0.22-μm PTFE membrane filter (Merck, Darmstadt, Germany) before high-performance liquid chromatography (HPLC) analysis.

Determination of TPC and TFC

The TPC and TFC of diluted free and bound extracts of black rice samples were determined [14]. The TPC was expressed as milligrams of gallic acid equivalent per gram of the sample (mg GAE/g), and TFC was expressed as milligrams of quercetin aglycone equivalent per gram of the sample (mg QE/g).

Determination of antioxidant activity

The antioxidant activity of diluted free and bound extracts of black rice samples was determined using DPPH radical scavenging activity assays [8, 15]. The total antioxidant activity of the DPPH assays was expressed as milligrams of Trolox equivalent per gram of the sample (mg TE/g).

Determination of phenolic acids and anthocyanins using HPLC

The phenolic composition of free and bound extracts of black rice samples was determined using a HPLC system (Agilent 1260 Infinity II LC system, Agilent, Santa Clara, CA, USA) equipped with Agilent 1200 series VWD detector. The phenolic acid and anthocyanin profiles were determined according to Kim and Lee's method [16]. Phenolic compounds were separated on a Zorbax Eclipse XDB-C18 (4.6 × 250 mm, 5 μm, Agilent) column after loading 20 μL of injection volume. The mobile phases consisted of A (1% TFA in water solution) and B (1% TFA in acetonitrile solution) at the flow rate of 1.0 mL/min. The solvent gradient was programmed as follows: 0–6.5 min, 10–12% B; 6.5–10.5 min, 12–13% B; 10.5–33 min, 13–17% B; 33–60 min, 17–65% B; 60–70 min, 65–95% B. The hydroxyl benzoic phenolic acids (gallic acid, syringic acid, and vanillic acid), hydroxycinnamic acids (*p*-coumaric acid and ferulic acid), and anthocyanins (cyanidin-3-O-glucoside and peonidin-3-O-glucoside) were detected at 280, 320, and 520 nm, respectively. The quantification linear ranges of all phenolic acids and anthocyanins were between 0.05 and 10 μg/mL, with an R^2 value of over 0.9984.

Statistical analysis

Data analysis was performed with SPSS statistical software version 23 (SPSS, Inc., Chicago, IL, USA). The results were reported as mean ± standard deviation. A multivariate analysis of variance (MANOVA) at $p < 0.05$ was used to determine the effect of cultivar differences, milling degrees, and cultivar–milling degree interactions of black rice on all results of the analysis. The significant differences among black rice cultivars in the same milling degree and among different milling degrees in the same cultivar were determined using one-way ANOVA followed by Duncan's multiple range test at $p < 0.05$. The correlation analysis among phenolic acids, anthocyanins, color parameters, TPC, TFC, and antioxidant activities was performed with Pearson's correlation test at $p < 0.05$. Standardized principal component analysis (PCA) was performed using XLSTAT software (XLSTAT, ver. 2017.03, Microsoft Excel Add-in software, New York, NY, USA) to visualize clustering formation of cultivars varying in different milling degrees and the relationship between antioxidants and the differently treated black rice samples.

Results and discussion

Color of black rice samples

As milling degree increased, the L^*, a^*, and b^* values significantly increased ($p < 0.05$), regardless of black rice cultivars (Table 1). These results concur with those of a precious study, in which the L^* significantly increased with milling degree [17]. The L^* value for Heukhyangchal 1 was higher than for the other cultivars for step 0 and step 2 ($p < 0.05$), and the L^* value for Bosukheukchal was lower than for the other cultivars for all milling degrees ($p < 0.05$).

For step 2, the a^* value for Sintoheugmi was higher than for other black rice cultivars and the a^* value for Heukhyangchal 1 was lower than for other black rice cultivars ($p < 0.05$). Because unpolished black rice (i.e., step 0) has

Table 1 Hunter's color values (L^*: whiteness, a^*: redness, b^*: yellowness) of black rice cultivars varying in milling degrees

Cultivar	Milling degree	Color values			
		L^*	a^*	b^*	ΔE^*
Bosukheukchal	Step 0	39.45 ± 0.04aA	0.48 ± 0.08aA	− 1.00 ± 0.05aA	
	Step 1	41.17 ± 0.02aB	1.87 ± 0.05cdB	− 0.79 ± 0.04aB	2.2
	Step 2	53.39 ± 0.04aC	3.18 ± 0.03eC	0.81 ± 0.03bC	14
Heukhyangchal 1	Step 0	42.50 ± 0.01fA	0.96 ± 0.05cA	− 0.47 ± 0.02dA	
	Step 1	42.54 ± 0.02cA	1.97 ± 0.02dB	− 0.08 ± 0.04cB	1.1
	Step 2	69.22 ± 0.03fB	2.07 ± 0.06aC	3.80 ± 0.02fC	27
Heukjinju	Step 0	41.41 ± 0.02dA	0.55 ± 0.08abA	− 0.64 ± 0.07cA	
	Step 1	41.86 ± 0.02cB	1.36 ± 0.02aB	− 0.51 ± 0.04bB	0.9
	Step 2	58.60 ± 0.02bC	2.73 ± 0.05cC	1.60 ± 0.02cC	17
Josengheukchal	Step 0	41.46 ± 0.44eA	1.36 ± 0.06dA	− 0.42 ± 0.06dA	
	Step 1	44.16 ± 0.10fB	2.51 ± 0.03eB	0.71 ± 0.04dB	3.1
	Step 2	62.19 ± 0.06eC	3.06 ± 0.01dC	2.67 ± 0.06eC	21
Sinnongheukchal	Step 0	40.74 ± 0.04cA	0.56 ± 0.09abA	− 0.76 ± 0.05bA	
	Step 1	41.39 ± 0.03bB	1.78 ± 0.17bcB	− 0.60 ± 0.09bB	1.4
	Step 2	61.95 ± 0.08dC	2.30 ± 0.06bC	0.58 ± 0.07aC	21
Sintoheugmi	Step 0	39.68 ± 0.00bA	0.66 ± 0.04bA	− 0.45 ± 0.03dB	
	Step 1	42.86 ± 0.01eB	1.71 ± 0.03bB	− 0.82 ± 0.06aA	3.4
	Step 2	60.34 ± 0.02cC	4.53 ± 0.01fC	1.94 ± 0.03dC	21
Cultivar effect		***	***	***	
Degree of milling effect		***	***	***	
Cultivar × degree of milling		***	***	***	

Values are presented as mean ± standard deviation. Values followed by different lowercase letters in different cultivars for the same milling degree are significantly different ($p < 0.05$). Values followed by different capital letters with each column in the same cultivar for different milling degrees are significantly different ($p < 0.05$). Meanings of ΔE^* values were obtained from https://www.hunterlab.com/ and are as follows, ΔE^* value 0–1: a normally invisible difference; ΔE^* value 1–2: very small difference, only obvious to a trained eye; ΔE^* value 2–3.5: medium difference, obvious to an untrained eye; ΔE^* value > 3.5: obvious difference

black and dark purple bran layers, the L^* and a^* values were lower than for polished rice (i.e., step 1 and step 2). While bran layers are removed during milling, the purple color pigments in the aleurone and pericarp layers of the bran are removed, resulting in increased L^* and a^* values.

Based on ΔE^* values, there was an obvious difference between step 0 and step 2, regardless of cultivars. There were, however, smaller differences between step 0 and step 1 than between step 0 and step 2. The Heukjinju cultivar especially showed invisible difference (a ΔE^* of 0.9) between step 0 and step 2. The ΔE^* varied as milling degree increased for different cultivars. The different ΔE^* levels may have been caused by different thickness levels of the rice bran layer for the different cultivars.

Total phenolic content and total flavonoid content

The TPC and TFC of free and bound phenolic fractions of black rice samples are shown in Table 2. The MANOVA results of TPC and TFC showed significant differences in different cultivars and milling degrees ($p < 0.001$). There were interactions between cultivars and milling degrees ($p < 0.001$).

The TPC of free and bound fractions in unpolished rice (step 0) were 2.10–3.74 and 0.26–0.79 GAE mg/g, respectively. The TPC of the total fraction are the sum of TPC of free and bound fractions. Free phenolics were dominant in TPC for all black rice cultivars varying in milling degrees. The Sintoheugmi cultivar contained significantly higher TPC of total fraction than the other cultivars, followed by Heukjinju > Bosukheukchal > Sinnongheukchal > Heukhyangchal 1 > Josengheukchal for step 0 ($p < 0.05$). The TPC of free and bound fractions significantly decreased as milling degree increased in all cultivars ($p < 0.05$). The TPC, however, decreased at different rates for the different cultivars and milling degrees. The cultivar, Heukjinju, for example, had significantly higher TPC of total fraction (2.97 ± 0.02 GAE mg/g) than the other cultivars for step 1 ($p < 0.05$). The ΔE^* was also

Table 2 Total polyphenol and total flavonoid contents in free and bound phenolic fractions of black rice cultivars varying in milling degrees

Cultivar	Milling degree	Total polyphenol contents (mg GAE/g)			Total flavonoid contents (mg QE/g)		
		Free	Bound	Total	Free	Bound	Total
Bosukheukchal	Step 0	2.94 ± 0.09dC	0.49 ± 0.04bC	3.43 ± 0.10dC	0.42 ± 0.04cC	0.14 ± 0.01bcB	0.56 ± 0.03bC
	Step 1	1.78 ± 0.07aB	0.33 ± 0.03cB	2.12 ± 0.09aB	0.33 ± 0.03bB	0.04 ± 0.01aA	0.38 ± 0.02bB
	Step 2	0.90 ± 0.01bA	0.14 ± 0.06aA	1.04 ± 0.05aA	0.25 ± 0.02bA	0.04 ± 0.01bA	0.29 ± 0.03bA
Heukhyangchal 1	Step 0	2.72 ± 0.07cC	0.26 ± 0.04aB	2.98 ± 0.04bC	0.25 ± 0.02abC	0.10 ± 0.00abB	0.35 ± 0.01aC
	Step 1	2.57 ± 0.05dB	0.13 ± 0.01aA	2.70 ± 0.03cB	0.17 ± 0.02aB	0.04 ± 0.02aA	0.21 ± 0.02aB
	Step 2	1.51 ± 0.05dA	0.09 ± 0.03aA	1.60 ± 0.08cA	0.05 ± 0.03aA	0.02 ± 0.01aA	0.08 ± 0.02aA
Heukjinju	Step 0	2.97 ± 0.05dC	0.75 ± 0.06cC	3.72 ± 0.09eC	0.60 ± 0.04dB	0.16 ± 0.02cB	0.76 ± 0.06cB
	Step 1	2.72 ± 0.04eB	0.26 ± 0.02bB	2.97 ± 0.02eB	0.36 ± 0.06bA	0.14 ± 0.00cB	0.50 ± 0.06cA
	Step 2	1.78 ± 0.06eA	0.11 ± 0.04aA	1.90 ± 0.03dA	0.35 ± 0.04cA	0.07 ± 0.01cA	0.42 ± 0.05cA
Josengheukchal	Step 0	2.10 ± 0.04aC	0.55 ± 0.04bB	2.65 ± 0.08aC	0.20 ± 0.01aC	0.09 ± 0.06aB	0.29 ± 0.04aC
	Step 1	1.77 ± 0.00aB	0.49 ± 0.04dB	2.25 ± 0.03bB	0.16 ± 0.02aB	0.02 ± 0.01aAB	0.19 ± 0.02aB
	Step 2	1.27 ± 0.04cA	0.27 ± 0.05bA	1.54 ± 0.08cA	0.05 ± 0.01aA	n.d.	0.05 ± 0.01aA
Sinnongheukchal	Step 0	2.42 ± 0.03bC	0.75 ± 0.05cC	3.17 ± 0.08cC	0.34 ± 0.01bcC	0.17 ± 0.01cC	0.51 ± 0.03bC
	Step 1	2.10 ± 0.03bB	0.67 ± 0.01eB	2.77 ± 0.02cdB	0.30 ± 0.01bB	0.10 ± 0.02bB	0.41 ± 0.03bB
	Step 2	1.23 ± 0.03cA	0.57 ± 0.00cA	1.80 ± 0.03dA	0.26 ± 0.01bA	0.02 ± 0.01aA	0.29 ± 0.02bA
Sintoheugmi	Step 0	3.74 ± 0.05eC	0.79 ± 0.01cC	4.53 ± 0.06fC	0.83 ± 0.14eB	0.22 ± 0.02dC	1.05 ± 0.14dB
	Step 1	2.20 ± 0.05cB	0.64 ± 0.05eB	2.83 ± 0.01dB	0.43 ± 0.03cA	0.13 ± 0.01cB	0.56 ± 0.02cA
	Step 2	0.71 ± 0.03aA	0.52 ± 0.04cA	1.23 ± 0.07bA	0.39 ± 0.02cA	0.07 ± 0.01cA	0.45 ± 0.01cA
Effect of cultivar		***	***	***	***	***	***
Effect if degree of milling		***	***	***	***	***	***
Cultivar × degree of milling		***	***	***	***	***	***

Values are presented as mean ± standard deviation. Values followed by different lowercase letters in different cultivars for the same milling degree are significantly different ($p < 0.05$). Values followed by different capital letters with each column in the same cultivar for different milling degrees are significantly different ($p < 0.05$). *** means significant differences at $p < 0.001$. n.d. denotes: not detected

minimal in the Heukjinju cultivar when milled from step 0 to step 1. The purple and black color pigments and anthocyanin loss may, therefore, be minimal in the Heukjinju cultivar. For step 2, the cultivars Heukjinju and Sinnongheukchal showed significantly higher TPC than the other cultivars ($p < 0.05$).

Flavonoids have nitric oxide and oxygen scavenging activities [18]. The TFC was higher in free fraction than in bound fraction in all cultivars. For step 0, the TFC showed 0.20–0.83 and 0.09–0.22 QE mg/g in free and bound fractions, respectively. The TFC was not detected in bound fraction in step 2 for the Josengheukchal cultivar. The TFC of total fraction is the sum of TFC of free and bound fractions. For step 0, the Sintoheugmi cultivar showed a higher TFC of total fraction (1.05 ± 0.02 QE mg/g) than the other cultivars ($p < 0.05$), followed by Heukjinju > Bosukheukchal > Sinnongheukchal > Heukhyangchal 1 > Josengheukchal. This pattern of results is similar to that seen for the TPC. The Sintoheugmi cultivar contained significantly higher free (0.83 ± 0.14 QE) and bound (0.22 ± 0.02 QE) flavonoids than the other cultivars ($p < 0.05$). For step 1, the Sintoheugmi and Heukjinju cultivars contained significantly higher TFC (0.50–0.56 QE mg/g) than other black rice cultivars (0.19–0.41 QE mg/g) ($p < 0.05$).

DPPH radical scavenging activity

The DPPH radical scavenging activity results of black rice samples are shown in Table 3. The DPPH radical scavenging activity of free phenolic fraction was higher than that of bound phenolic fraction, as was similarly seen in the TPC and TFC results. DPPH radical scavenging activity of free and bound phenolic extracts was significantly different as milling ($p < 0.05$). For step 0, the DPPH radical scavenging activity of free and bound phenolic fractions was 6.31–9.36 and 1.17–1.49 TE mg/g, respectively. For step 2, the DPPH radical scavenging activity of free and bound phenolic fractions was 4.29–6.54 and 0.66–1.02 TE mg/g, respectively. The DPPH radical scavenging activity of total

Table 3 Antioxidant activity (DPPH radical scavenging activity) in free and bound phenolic fractions of black rice cultivars varying in milling degrees

Cultivar	Milling degree	DPPH (mg TE/g)		
		Free	Bound	Total
Bosukheukchal	Step 0	7.84 ± 0.03dC	1.40 ± 0.01bcC	9.24 ± 0.03dC
	Step 1	7.30 ± 0.02dB	1.29 ± 0.00cB	8.59 ± 0.02dB
	Step 2	4.29 ± 0.26aA	0.79 ± 0.08bA	5.07 ± 0.25aA
Heukhyangchal 1	Step 0	6.98 ± 0.10bC	1.25 ± 0.07aC	8.23 ± 0.16bC
	Step 1	6.42 ± 0.04bB	1.07 ± 0.01aB	7.49 ± 0.04bB
	Step 2	5.31 ± 0.22bA	0.66 ± 0.04aA	5.98 ± 0.22bA
Heukjinju	Step 0	8.17 ± 0.18eC	1.49 ± 0.08cC	9.65 ± 0.27eC
	Step 1	7.18 ± 0.17cdB	1.27 ± 0.03cB	8.45 ± 0.20cdB
	Step 2	6.54 ± 0.13cA	1.02 ± 0.04cA	7.55 ± 0.10dA
Josengheukchal	Step 0	6.31 ± 0.17aC	1.17 ± 0.04aB	7.48 ± 0.19aC
	Step 1	5.88 ± 0.05aB	1.07 ± 0.08aAB	6.95 ± 0.11aB
	Step 2	5.42 ± 0.02bA	0.99 ± 0.02cA	6.40 ± 0.01cA
Sinnongheukchal	Step 0	7.44 ± 0.11cC	1.39 ± 0.02bC	8.83 ± 0.13cC
	Step 1	6.91 ± 0.32cB	1.27 ± 0.03cB	8.19 ± 0.33cB
	Step 2	6.41 ± 0.15cA	1.02 ± 0.01cA	7.43 ± 0.14dA
Sintoheugmi	Step 0	9.37 ± 0.12fC	1.40 ± 0.04bcC	10.76 ± 0.13fC
	Step 1	7.60 ± 0.16eB	1.18 ± 0.04bB	8.78 ± 0.20cdB
	Step 2	5.56 ± 0.18bA	0.67 ± 0.06aA	6.23 ± 0.12bcA
Effect of cultivar		***	***	***
Effect of degree of milling		***	***	***
Cultivar × degree of milling		***	***	***

Values are presented as mean ± standard deviation. Values followed by different lowercase letters in different cultivars for the same milling degree are significantly different ($p < 0.05$). Values followed by different capital letters with each column in the same cultivar for different milling degrees are significantly different ($p < 0.05$). *, **, *** means significant differences at $p < 0.05$, 0.01, and 0.001, respectively

phenolic extracts of the Sintoheugmi cultivar was higher than the other cultivars for step 0 ($p < 0.05$), followed by Heukjinju > Bosukheukchal > Sinnongheukchal > Heukhyangchal 1 > Josengheukchal ($p < 0.05$), similar to the results of the TPC and TFC. There was, however, no significant difference in the TFC in Bosukheukchal, Heukjinju, and Sintoheugmi for step 1 ($p < 0.05$). For step 2, the Heukjinju cultivar showed the highest DPPH radical scavenging activity among the six black rice cultivars ($p < 0.05$).

Determination of individual phenolic acids and anthocyanins in free and bound phenolic fractions

A typical HPLC chromatogram for analysis of free and bound phenolics is shown in Fig. 1. The quantification of five phenolic acids (gallic acid, syringic acid, vanillic acid, *p*-coumaric acid, and ferulic acid) and two anthocyanins (cyanidin-3-O-glucoside and peonidin-3-O-glucoside) in black rice samples was performed using authentic standards. The free and bound phenolic acid compositions in black rice samples varied according to the three different milling degrees (step 0, 1, and 2), as shown in Table 4. The effect of cultivar, the effect of degree of milling, and the two-way interactions between cultivar and degree of milling on phenolic acid and anthocyanin levels were confirmed by the MANOVA ($p < 0.001$) test.

In all black rice cultivars, ferulic acid (59–75% of the total phenolic acids) and cyanidin-3-O-glucoside levels (93–98% of the total anthocyanins) were predominant. In all cultivars, the levels of phenolic acid and anthocyanins decreased as the milling degree increased ($p < 0.05$). The unpolished black rice (i.e., step 0) contained the highest total phenolic acid and anthocyanin levels for all black rice cultivars, as was noted before in a previous study [8]. The average total phenolic acid levels of all investigated cultivars were 216.3 μg/g in step 0, 152.1 μg/g in step 1, and 99.1 μg/g in step 2. The average total anthocyanin levels of all investigated cultivars were 494.4 μg/g in step 0, 385.1 μg/g in step 1, and 199.9 μg/g in step 2. The levels of ferulic acid ranged between 117.3 and 182.7 μg/g in step 0, 73.9 and 141.2 μg/g in step 1, and 37.0 and 122.2 μg/g in step 2. These values are similar to those reported by

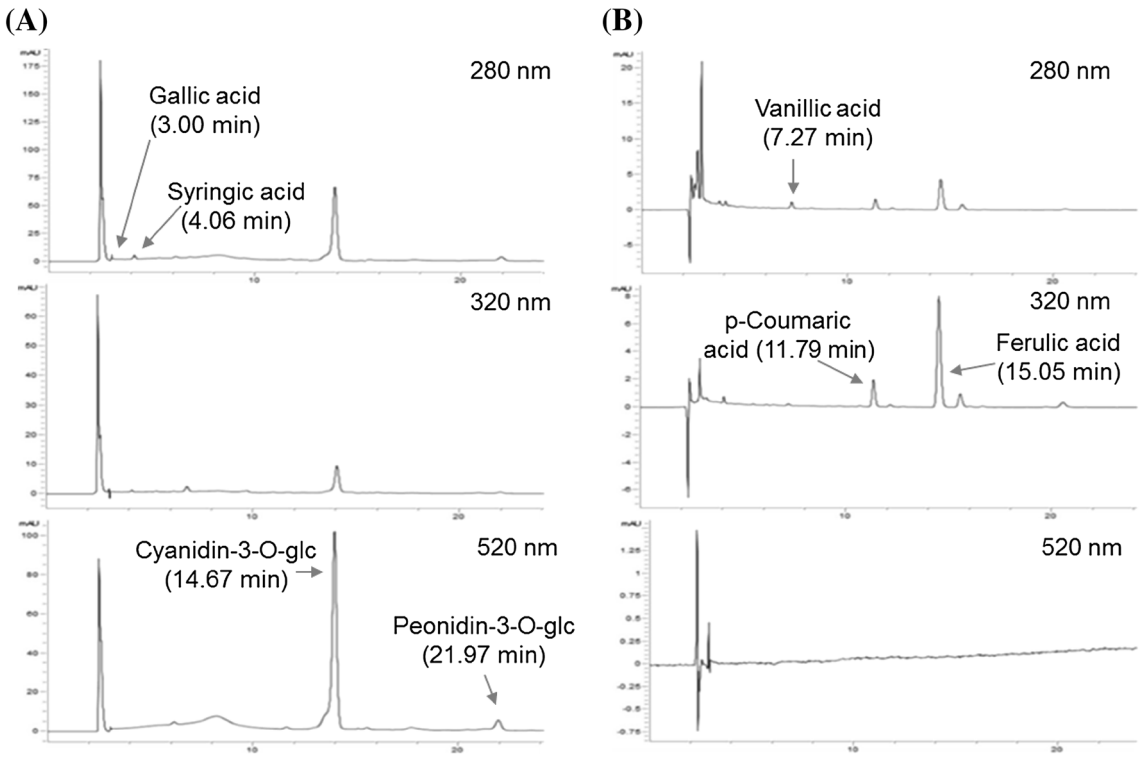

Fig. 1 HPLC chromatogram for determination of phenolic acids and anthocyanins in free (A) and bound (B) phenolic fractions of black rice (cv. Sintoheugmi)

Sumczynski et al. (2016), which ranged between 162.9 and 237.0 μg/g [8]. The levels of gallic acid ranged between 3.3 and 9.8 μg/g in step 0, 0.7 and 7.1 μg/g in step 1, and 0.3 and 1.9 μg/g in step 2. The levels of syringic acid ranged between 17.5 and 29.3 μg/g in step 0, 11.6 and 23.0 μg/g in step 1, and 1.2 and 19.2 μg/g in step 2. The levels of vanillic acid ranged between 6.4 and 17.9 μg/g in step 0, trace level (detected but below LOQ) and 8.7 μg/g in step 1, and not detected and 5.4 μg/g in step 2. The levels of *p*-coumaric acid ranged between 23.4 and 38.1 μg/g in step 0, 19.6–26.1 μg/g in step 1, and 16.8–19.5 μg/g in step 2. In another study, the levels of gallic acid, syringic acid, vanillic acid, and *p*-coumaric acid in black rice ranged 12.9–23.4, 1.7–3.5, 17.3–54.4, and 3.9–50.6 μg/g, respectively [8]. The levels of cyanidin-3-O-glucoside ranged between 258.2 and 809.4 μg/g in step 0, 190.3 and 490.9 μg/g in step 1, and 72.2 and 320.0 μg/g in step 2. The levels of cyanidin-3-O-glucoside concurred with previously reported values (i.e., 515 μg/g), and anthocyanins were found only in free phenolic fraction of black rice [19]. It is, however, difficult to compare these data with other studies to show whether tested black cultivars in this study had higher levels of phenolic acids than other black rice varieties in previous studies because most prior studies did not report on milling degrees [8], and some studies used different extraction solvents [20].

Of the tested cultivars, Sintoheugmi contained the highest levels of total phenolic acids (255.2 μg/g) and anthocyanins (831.4 μg/g) in step 0, followed by Heukjinju > Bosukheukchal > Heukhyangchal > Sinnongheukchal > Josengheukchal, similar to the previous antioxidant activity assay. For step 1 and step 2, Heukjinju contained the highest levels of total phenolic acids (198.4 and 161.9 μg/g in step 1 and step 2, respectively) and anthocyanins (500.6 and 326.1 μg/g in step 1 and step 2, respectively). These data are similar to the TPC, TFC, and DPPH assay results. Although unpolished Sintoheugmi black rice (step 0) contained significantly higher ferulic acid levels than other unpolished black rice cultivars ($p < 0.05$), polished Sintoheugmi (step 2) had significantly lower ferulic acid levels than the other cultivars ($p < 0.05$). In step 1, gallic acid and syringic acid levels (7.1 ± 0.8 and 23.0 ± 0.2 μg/g, respectively) in Heukjinju rice were significantly higher than those of the other cultivars ($p < 0.05$). In contrast, Sintoheugmi contained significantly lower levels of gallic acid and syringic acid than the other cultivars in step 1 ($p < 0.05$). Unpolished Sintoheugmi black rice (step 0) contained the highest cyanidin-3-O-glucoside levels (809.4 μg/g) with 1.5-fold higher levels than the second-highest cyanidin-3-O-glucoside cultivar, Heukjinju (544.9 μg/g), followed by

Table 4 Total and free phenolic acid and anthocyanin contents of black rice cultivars varying in milling degrees

Sample	Milling degree	Phenolic acid content (μg/g)						Anthocyanins (μg/g)		
		Gallic acid	Syringic acid	Vanillic acid	*p*-Coumaric acid	Ferulic acid	Sum	Cyanidin-3-O-glucoside	Peonidin-3-O-glucoside	Sum
Bosukheukchal	Step 0	9.8 ± 0.1cC (9.8)	29.3 ± 0.5dC (29.3)	13.8 ± 0.2eB (t.r.)	33.5 ± 0.1cC (10.6)	128.5 ± 0.8bcC (36.1)	214.9 ± 1.5cC (85.8)	487.5 ± 19.4dB (487.5)	11.6 ± 0.1cB (11.6)	499.1 ± 19.3dB (499.0)
	Step 1	1.8 ± 0.1bB (1.8)	17.2 ± 0.2cB (17.2)	8.7 ± 0.1eA (t.r.)	19.8 ± 0.1aB (t.r.)	78.7 ± 0.8bB (19.9)	126.2 ± 0.5bB (38.9)	466.1 ± 6.3dB (466.1)	11.3 ± 0.1dB (11.3)	477.5 ± 6.2dB (477.5)
	Step 2	0.8 ± 0.3bA (0.8)	12.0 ± 0.2cA (12.0)	n.d.	16.8 ± 0.1aA (t.r.)	42.9 ± 0.4bA (9.2)	72.5 ± 0.6bA (21.9)	295.7 ± 0.5eA (295.7)	7.0 ± 0.2eA (7.0)	302.7 ± 0.7eA (302.7)
Heukhyangchal 1	Step 0	6.9 ± 0.2bC (6.9)	22.9 ± 0.8bC (22.9)	8.8 ± 0.2cB (t.r.)	38.1 ± 0.2fC (10.7)	131.3 ± 1.1cC (41.6)	208.0 ± 1.3bC (82.1)	258.2 ± 1.3aC (258.2)	7.0 ± 0.1aB (7.0)	265.2 ± 1.2aC (265.2)
	Step 1	4.2 ± 0.1dB (4.2)	18.8 ± 0.4dB (18.8)	5.3 ± 0.2bA (t.r.)	26.1 ± 0.1dB (t.r.)	120.9 ± 0.1eB (37.7)	175.2 ± 0.7eB (60.7)	240.8 ± 0.9bB (240.8)	6.8 ± 0.1aB (6.8)	247.7 ± 1.0bB (247.7)
	Step 2	1.6 ± 0.1cdA (1.6)	10.2 ± 0.3bA (10.2)	n.d.	19.3 ± 0.1bA (t.r.)	62.9 ± 1.8dA (20.6)	93.9 ± 2.2cA (32.3)	184.5 ± 1.8cA (184.5)	5.3 ± 0.1bA (5.3)	189.9 ± 1.9cA (189.9)
Heukjinju	Step 0	9.6 ± 0.3cC (9.6)	28.0 ± 1.2cdC (28.0)	8.1 ± 0.1bC (t.r.)	31.3 ± 0.2bC (10.7)	158.1 ± 1.1dC (59.4)	235.0 ± 1.7dC (107.6)	544.9 ± 1.7eC (544.9)	11.0 ± 0.2bcC (11.0)	555.9 ± 1.9eC (555.9)
	Step 1	7.1 ± 0.8fB (7.1)	23.0 ± 0.2eB (23.0)	6.7 ± 0.2cB (t.r.)	20.4 ± 0.1bB (t.r.)	141.2 ± 1.0fB (51.1)	198.4 ± 0.3fB (81.2)	490.9 ± 8.4eB (490.9)	9.6 ± 0.1cB (9.6)	500.6 ± 8.2eB (500.6)
	Step 2	1.9 ± 0.1dA (1.9)	13.2 ± 0.5dA (13.2)	5.4 ± 0.6A (t.r.)	19.2 ± 0.1bA (t.r.)	122.2 ± 1.1fA (33.6)	161.9 ± 2.3fA (48.7)	320.0 ± 0.8fA (320.0)	6.2 ± 0.1dA (6.2)	326.1 ± 0.7fA (326.1)
Josengheukchal	Step 0	3.3 ± 0.2aB (3.3)	17.5 ± 0.4aC (17.5)	17.9 ± 0.1fC (t.r.)	23.4 ± 0.1aC (t.r.)	117.3 ± 1.2aC (25.5)	179.3 ± 0.7aC (46.3)	356.1 ± 2.5bC (356.1)	22.5 ± 0.5dC (22.5)	378.6 ± 3.0bC (378.6)
	Step 1	3.1 ± 0.1cB (3.1)	14.7 ± 0.5bB (14.7)	8.1 ± 0.2dB (t.r.)	21.7 ± 0.2cB (t.r.)	94.4 ± 1.9cB (20.4)	141.9 ± 2.6cB (38.2)	190.3 ± 4.6aB (190.3)	12.9 ± 0.1eB (12.9)	203.2 ± 4.7aB (203.2)
	Step 2	1.5 ± 0.1cA (1.5)	9.6 ± 0.6bA (9.6)	5.4 ± 0.1A (t.r.)	19.5 ± 0.1bA (t.r.)	72.8 ± 1.0eA (17.1)	108.9 ± 1.9eA (28.3)	72.2 ± 1.1aA (72.2)	5.7 ± 0.2cA (5.7)	77.9 ± 1.3aA (77.9)
Sinnongheukchal	Step 0	6.6 ± 0.2bC (6.6)	25.4 ± 0.7cC (25.4)	9.6 ± 0.1d (t.r.)	36.1 ± 0.2eB (10.6)	127.5 ± 2.9bC (44.3)	205.2 ± 3.4bC (87.0)	426.2 ± 0.7cC (426.2)	10.0 ± 0.1bC (10.0)	436.2 ± 0.6cC (436.2)
	Step 1	5.6 ± 0.3eB (5.6)	22.4 ± 0.5eB (22.4)	t.r.	19.6 ± 0.1aA (t.r.)	73.9 ± 1.3aB (23.4)	121.5 ± 1.0aB (51.4)	379.8 ± 2.3cB (379.8)	8.9 ± 0.2bB (8.9)	388.7 ± 2.4cB (388.7)
	Step 2	1.0 ± 0.1bA (1.0)	19.2 ± 0.9eA (19.2)	n.d.	19.2 ± 0.2bA (t.r.)	60.0 ± 0.3cA (20.4)	99.5 ± 0.7dA (40.7)	200.3 ± 1.0dA (200.3)	5.3 ± 0.1bA (5.3)	205.6 ± 1.1dA (205.6)

Table 4 continued

Sample	Milling degree	Phenolic acid content (μg/g)						Anthocyanins (μg/g)		
		Gallic acid	Syringic acid	Vanillic acid	*p*-Coumaric acid	Ferulic acid	Sum	Cyanidin-3-O-glucoside	Peonidin-3-O-glucoside	Sum
Sintoheugmi	Step 0	7.3 ± 0.1bC	23.7 ± 1.0bcC	6.4 ± 0.1aB	35.2 ± 0.3dC	182.7 ± 0.7eC	255.2 ± 0.0eC	809.4 ± 1.4fC	22.0 ± 1.1dC	831.4 ± 0.3fC
		(7.3)	(23.7)	(t.r.)	(10.6)	(57.0)	(98.6)	(809.4)	(22.0)	(831.4)
	Step 1	0.7 ± 0.0aB	11.6 ± 0.1aB	4.9 ± 0.0aA	20.3 ± 0.1bB	111.9 ± 0.4dB	149.2 ± 0.3dB	480.5 ± 2.0dB	12.6 ± 0.3eB	493.0 ± 2.3eB
		(0.7)	(11.6)	(t.r.)	(t.r.)	(34.3)	(46.5)	(480.5)	(12.6)	(493.0)
	Step 2	0.3 ± 0.1aA	1.2 ± 0.0aA	n.d.	19.5 ± 0.1bA	37.0 ± 0.9aA	58.0 ± 1.1aA	93.6 ± 1.2bA	3.3 ± 0.1aA	96.9 ± 1.1bA
		(0.3)	(1.2)		(t.r.)	(11.1)	(12.6)	(93.6)	(3.3)	(96.9)
Effect of cultivar		***	***	***	***	***	***	***	***	***
Effect of degree of milling		***	***	***	***	***	***	***	***	***
Cultivar × degree of milling		***	***	***	***	***	***	***	***	***

Total phenolic compounds are the sum of free plus bound phenolic compounds. Values are presented as mean ± standard deviation. Values followed by different lowercase letters in different cultivars for the same milling degree are significantly different ($p < 0.05$). Values followed by different capital letters with each column in the same cultivar for different milling degrees are significantly different ($p < 0.05$). *** means significant differences at $p < 0.001$. n.d and t.r. denote "not detected" and "detected at trace level (detected but below LOQ)"

Heukjinju > Bosukheukchal > Sinnongheukchal > Josengheukchal > Heukhyangchal 1 ($p < 0.05$). Partially polished black rice (step 1) of Sintoheugmi and Heukjinju cultivars, however, contained similar levels of cyanidin-3-O-glucoside (480.5 vs. 490.9 μg/g, respectively). Polished Sintoheugmi black rice (step 2) had significantly lower levels of cyanidin-3-O-glucoside (93.6 μg/g) than polished Heukjinju black rice (320.0 μg/g) ($p < 0.05$). The Heukjinju cultivar showed a minimal ΔE^* as a result of milling from step 0 to step 1. The anthocyanin results concurred with the trend of the ΔE^* results, shown in Table 1. These results show that the distribution of phenolics in black rice is not uniform for all cultivars and that, for example, it is likely that phenolic compounds are mainly present in the bran layer in the Sintoheugmi cultivar compared to Heukjinju cultivar, in which the phenolic compounds are evenly present in all layers. The degree of milling may, therefore, have a bigger impact on black rice cultivars with high phenolic contents in the bran layers, such as the Sintoheugmi cultivar. This observation is important because different milling degrees are used for different purposes of processing (e.g., cooking) of black rice. If black rice is used as a source of anthocyanin pigments, then unpolished Sintoheugmi or a coproduct (i.e., bran layer) of Sintoheugmi may be used as a source of the cyanidin-3-O-glucoside anthocyanin.

The predominant phenolic acid and anthocyanin (i.e., ferulic acid and cyanidin-3-O-glucoside) levels were significantly higher in Sintoheugmi (step 0) and Heukjinju (step 1–2) black rice cultivars than other black rice cultivars ($p < 0.05$). Some black rice cultivars, however, contained higher levels of other phenolic acids than the Sintoheugmi and Heukjinju cultivars. For example, the Josengheukchal cultivar contained significantly higher levels of vanillic acid (17.9 μg/g in step 0) than Sintoheugmi and Heukjinju cultivars (6.4–8.1 μg/g in step 0) ($p < 0.05$).

Gallic acid, syringic acid, vanillic acid, *p*-coumaric acid, and ferulic acid were found in step 0 and step 1 of all black rice cultivars, and vanillic acid was not found in Sintoheugmi, Sinnongheukchal, Bosukheukchal, and Heukhyangchal 1 cultivars after the step 2 milling degree.

In general, the sum of bound phenolic acid concentrations was higher (118.3–156.6 μg/g in the step 0, 70.1–117.2 μg/g in the step 1, and 45.4–113.1 μg/g in the step 2 black rice) than that of free phenolic acid concentrations (46.3–107.6 μg/g in the step 0, 38.2–81.2 μg/g in the step 1, and 12.6–48.7 μg/g in the step 2 black rice), as has also been shown in a previous study [21]. The major phenolic acids in free fraction were ferulic acid and syringic acid, and the major phenolic acids in bound fraction were ferulic acid and *p*-coumaric acid for all black rice samples, regardless of milling degrees. In black rice,

Table 5 Correlation coefficients between color values, TPC, TFC, DPPH, and phenolic compounds in free and bound phenolic fractions of black rice samples

	L^*	a^*	b*	ΔE	TPC	TFC	DPPH
Free phenolics							
Gallic acid	− .668**	− .818***	− .593**	− .695**	.848***	.499*	.693**
Syringic acid	− .699**	− .903***	− .729**	− .725**	.806***	.426	.705**
p-coumaric acid	− .472*	− .725**	− .451	− .489*	.706**	.579*	.662**
Ferulic acid	− .584*	− .790***	− .543*	− .622**	.910***	.653**	.825***
Cyanidin-3-O-glucoside	− .686**	− .727**	− .685**	− .662**	.795***	.839***	.855***
Peonidin-3-O-glucoside	− .631**	− .552*	− .525*	− .626**	.586*	.473*	.556*
Bound phenolics							
Vanillic acid	− .619**	− .596**	− .518*	− .646**	.215	.122	.609**
p-coumaric acid	− .569*	− .601**	− .460	− .618**	.202	.156	.500*
Ferulic acid	− .672**	− .764***	− .559*	− .709**	.304	.563*	.761***

*, **, *** indicate the significant levels (p) at 0.05, 0.01, and 0.001, respectively. TPC, TFC, and DPPH stand for total phenolic contents, total flavonoid contents, and antioxidant activity (DPPH radical scavenging activity), respectively

phenolic acids such as ferulic acid are mainly present as a bound form esterified to macromolecules, and the linkages between the two are hydrolyzed by intestinal enzymes [10]. Ferulic acid was present in exclusively bound forms in all cultivars, concurring with the findings of a previous study [8]. Among the phenolic acids, gallic acid and syringic acid were only found in free phenolic fraction. Vanillic, *p*-coumaric, and ferulic acid concentrations were higher in bound fraction than in free fraction. No anthocyanins were detected in bound fractions. These results are similar to the previously reported study [21] on free and bound phenolic contents in black rice samples.

Correlation among color parameters, antioxidant activity, and phenolic compounds

The correlations among the individual phenolic compounds to Hunter color parameters TPC, TFC, DPPH radical scavenging activity in free and bound phenolic fractions are shown in Table 5. All phenolic compounds in free phenolic fraction of black rice showed significantly negative correlation with L^*, a^*, b^*, and ΔE ($p < 0.05$) and significantly positive correlation with TPC, TFC, and DPPH ($p < 0.05$) with exception of syringic acid and TFC ($p > 0.05$) (Table 5). Cyanidin-3-O-glucoside and peonidin-3-O-glucoside, the most abundant anthocyanin pigments in black rice [22], were significantly negatively correlated with ΔE^* ($r = -0.662$ and $r = -0.626$, respectively) ($p < 0.05$). Most bound vanillic, *p*-coumaric, and ferulic acids showed significantly negative correlation with L^*, a^*, and ΔE ($p < 0.05$). Free ferulic acid showed high positive correlation with TPC ($r = 0.910$) and DPPH ($r = 0.825$) ($p < 0.001$). Cyanidin-3-O-glucoside had a significantly high positive correlation with all TPC, TFC, DPPH values ($r = 0.795$–0.855) ($p < 0.001$).

Bound vanillic, *p*-coumaric, and ferulic acids showed significantly negative correlation ($r = -0.646 \sim -0.709$) with ΔE^* ($p < 0.05$). Bound ferulic acid levels were significantly positively correlated with TFC ($r = 0.563$) and DPPH radical scavenging activity ($r = 0.761$) ($p < 0.05$). The DPPH radical scavenging activity showed significantly ($p < 0.05$) positive correlation with bound vanillic ($r = 0.609$), *p*-coumaric ($r = 0.500$), and ferulic acid ($r = 0.761$) ($p < 0.05$). There was no correlation between bound phenolic acids and TPC ($p > 0.05$). It could be suggested that individual phenolic compounds present in free and bound phenolic fractions demonstrate their antioxidant ability differently. These individual phenolic compounds may act individually, synergistically, or antagonistically. The antioxidant mechanisms of the individual phenolic compounds need to be clarified through further studies.

Principal component analysis

The PCAs, i.e., PC1 and PC2, were performed on TPC, TFC, DPPH, and individual phenolic compounds in free and bound phenolic fractions (Fig. 2). The PC1 explained approximately 54.27% of the total variance, in which step 0 black rice samples were on the right side and step 2 black rice samples were on the left side. Partially milled rice (step 1) was located between the two steps. PC2 explained approximately 21.09% of the total variance. Step 1 of Heukjinju (HJ1) was located especially close to step 0 of the black rice samples, and this may be explained by high levels of phenolic compounds in HJ1. The PCA loading

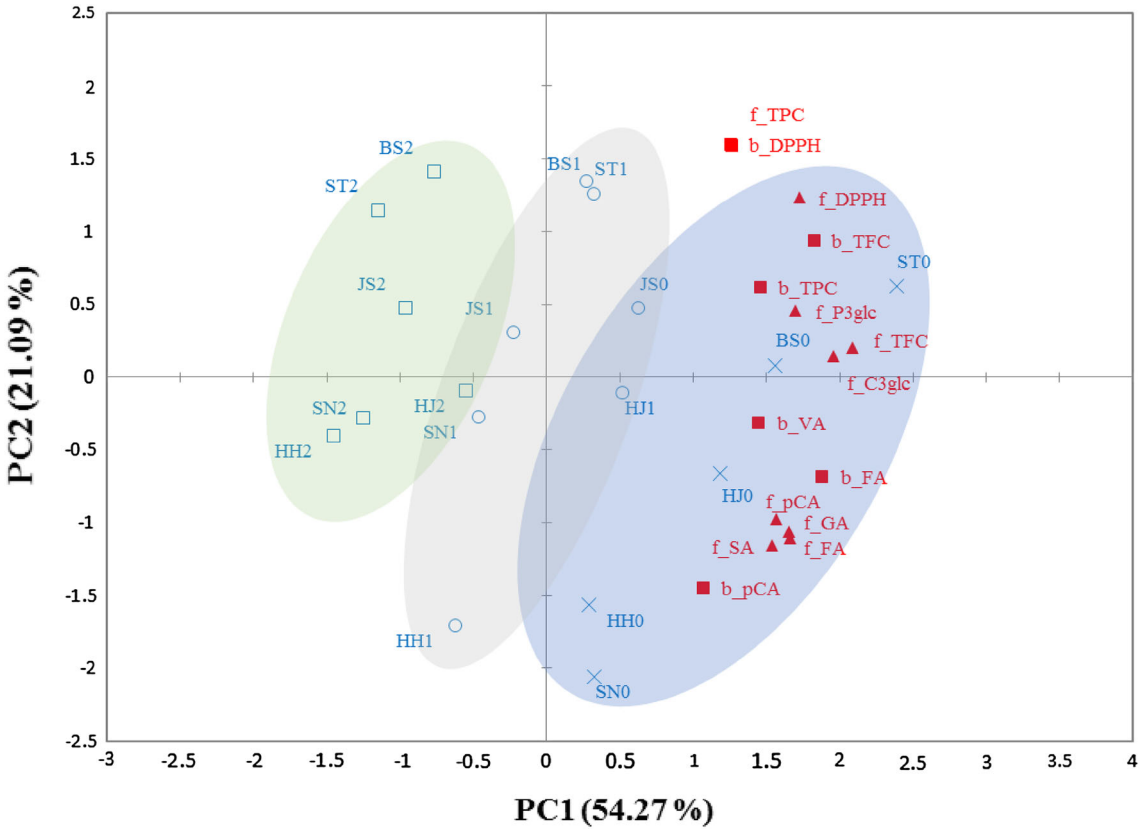

Fig. 2 Principal component analysis (PCA) plots (PC1 and PC2) of free and bound phenolics and antioxidant activity of black rice cultivars varying in milling degree. The sample labels are as follows: step 0 (0), step 1 (1), step 2 (2), Bosukheukchal (BS), Heukhyangchal 1 (HH), Heukjinju (HJ), Josengheukchal (JS), Sinnongheukchal (SN), Sintoheugmi (ST), gallic acid (GA), syringic acid (SA), *p*-coumaric acid (pCA), ferulic acid (FA), peonidin-3-O-glucoside (P3glc), cyanidin-3-O-glucoside (C3glc) in free phenolic fraction (*f*) and bound phenolic fraction (*b*)

plot indicated that there were high levels of antioxidant activity, TPC, TFC, and individual phenolic compounds in the step 0 samples.

All antioxidant activities and individual phenolic compounds showed positive values for PC1, indicating a strong correlation with unpolished rice. Cyanidin-3-O-glucoside is located close to the TFC of free phenolics extracts, suggesting that the anthocyanin pigment, which is one type of flavonoid, is correlated with the TFC. Along PC1, antioxidant activities were on the higher side, whereas individual phenolic compounds were relatively lower in position.

In summary, the color parameters, TPC, TFC, antioxidant activity, and phenolic compositions were affected by the cultivar and milling degree of black rice. As the milling degree increased, the TPC, TFC, antioxidant activity, individual phenolic acid levels, and individual anthocyanin levels decreased significantly ($p < 0.05$). The decrease rate, however, was dependent on the cultivar of black rice. For example, unpolished Sintoheugmi (step 0) contained significantly higher amounts of phenolic acid and anthocyanins than other black rice cultivars ($p < 0.05$); however, as the milling degree increased (i.e., step 1 and 2), these levels decreased dramatically and the Heukjinju black rice cultivar was shown to have a significantly higher sum of phenolic acid and anthocyanins than other black rice cultivars ($p < 0.05$). It is, therefore, important to consider different milling degrees together with different black rice cultivars having the highest antioxidant component to obtain the better functional ingredient.

Acknowledgments This work was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry (IPET) through the High Value-added Food Technology Development Program. This study was funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) (316059-02). And this research was supported by the Chung-Ang University Research Scholarship Grants in 2017.

References

1. Ko M, Choi H, Han B, Yoo S, Kim H, Choi S, Hur N, Kim C, Kim B, Baik M (2011) Antioxidative components and antioxidative capacity of brown and black rices. Food Eng Prog 15:195–202
2. Kim DJ, Oh SK, Yoon MR, Chun A, Hong HC, Lee JS, Kim YK (2010) Antioxidant compounds and antioxidant activities of the 70% ethanol extracts from brown and milled rice by cultivar. J Korean Soc Food Sci Nutr 39:467–473
3. Wu X, Beecher G, Holden JM, Haytowitz BD, Gebhardt SE, Prior RL (2006) Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. J Agric Food Chem 54:4069–4075
4. Hu C, Zawistowski J, Ling W, Kitts DD (2003) Black rice (*Oryza sativa* L. *indica*) pigmented fraction suppresses both reactive oxygen species and nitric oxide in chemical and biological model systems. J Agric Food Chem 51:5271–5277
5. Wang D, Wei X, Yan X, Jin T, Ling W (2010) Protocatechuic acid, a metabolite of anthocyanins, inhibits monocyte adhesion and reduces atherosclerosis in apolipoprotein E deficient mice. J Agric Food Chem 58:12722–12728
6. Gechev TS, Van Breusegem F, Stone JM, Denev I, Laloi C (2006) Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. BioEssays 28:1091–1101
7. Kong S, Lee J (2010) Antioxidants in milling fractions of black rice cultivars. Food Chem 120:278–281
8. Sumczynski D, Kotásková E, Družbíková H, Mlček J (2016) Determination of contents and antioxidant activity of free and bound phenolics compounds and in vitro digestibility of commercial black and red rice (*Oryza sativa* L.) varieties. Food Chem 211:339–346
9. Min B, Gu L, McClung AM, Bergman CJ, Chen MH (2012) Free and bound total phenolic concentrations, antioxidant capacities, and profiles of proanthocyanidins and anthocyanins in whole grain rice (*Oryza sativa* L.) of different bran colours. Food Chem 133:715–722
10. SauraCalixto F, Serrano J, Goñi I (2007) Intake and bioaccessibility of total polyphenols in a whole diet. Food Chem 101:492–501
11. Chung H, Shin J (2007) Characterization of antioxidant alkaloids and phenolic acids from anthocyanin-pigmented rice (*Oryza sativa* cv. *Heugjinjubyeo*). Food Chem 104:1670–1677
12. Jang S, Xu Z (2009) Lipophilic and hydrophilic antioxidants and their antioxidant activities in purple rice bran. J Agric Food Chem 57:858–862
13. Juliano BO (1979) The chemical basis of rice grain quality. In: Proceedings of the workshop on chemical aspects of rice grain quality. International Rice Research Institute, Los Banos, Laguna, pp 69–90
14. Shen Y, Jin L, Xiao P, Lu Y, Bao J (2009) Total phenolics, flavonoids, antioxidant capacity in rice grain and their relations to grain color, size and weight. J Cereal Sci 49:106–111
15. Cho M, Ko SB, Kim JM, Lee OH, Lee DW, Kim JY (2016) Influence of extraction conditions on antioxidant activities and catechin content from bark of *Ulmus pumila* L. Appl Biol Chem 59:329–336
16. Kim I, Lee J (2017) Comparison of different extraction solvents and sonication times for characterization of antioxidant activity and polyphenol composition in mulberry (*Morus alba* L.). Appl Biol Chem 60:509–517
17. Lamberts L, De Bie E, Vandeputte GE, Veraverbeke WS, Derycke V, De Man W, Delcour JA (2007) Effect of milling on colour and nutritional properties of rice. Food Chem 100:1496–1503
18. Jovanovic SV, Steenken S, Simic MG, Hara Y (1998) Antioxidant properties of flavonoids: reduction potentials and electron transfer reactions of flavonoids radicals. In: RiceEvans C, Packer L (eds) Flavonoids in health and disease. Marcel Dekker, New York, pp 137–161
19. Shao Y, Xy F, Sun X, Bao J, Beta T (2014) Identification and quantification of phenolic acids and anthocyanins as antioxidants in bran, embryo and endosperm of white, red and black rice kernels (*Oryza sativa* L.). J Cereal Sci 59:211–218
20. Bhat FM, Riar CS (2017) Extraction, identification and assessment of antioxidative compounds of bran extracts of traditional rice cultivars: an analytical approach. Food Chem 237:264–274
21. Ti H, Zhang R, Zhang M, Wei Z, Chi J, Deng Y, Zhang Y (2015) Effect of extrusion on phytochemical profiles in milled fractions of black rice. Food Chem 178:186–194
22. Pereira-Caro G, Cros G, Yokota T, Crozier A (2013) Phytochemical profiles of black, red, brown, and white rice from the Camargue region of France. J Agric Food Chem 61:7976–7986