Note

Effect of Germination on the Antioxidant Capacity of Pigmented Rice (*Oryza sativa* L. cv. Superjami and Superhongmi)

Soo Im CHUNG*, Lara Marie Pangan LO and Mi Young KANG

Department of Food Science and Nutrition, Brain Korea 21 Plus, Kyungpook National University, 1370 Sankyuk-dong, Daegu, 702-701, Republic of Korea

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The effect of germination on the antioxidant capacity of pigmented and non-pigmented rice was investigated. The blackish purple Superjami, reddish-brown Superhongmi, and ordinary non-pigmented brown rice were germinated for 72 h. The germinated rice grains were extracted with 80% methanol and their antioxidant activities and compounds were analyzed. Germination substantially increased the free radical scavenging activity, reducing power, ferrous chelating ability, and superoxide dismutase activity in all the rice samples. The anthocyanin, tannin, phenolic, phytic acid, tocopherol, and tocotrienol contents were markedly higher in germinated rice compared to those of the non-germinated ones. The pigmented varieties exhibited greater antioxidant capacity and higher amounts of antioxidant compounds than the non-pigmented brown rice in both germinated and non-germinated forms. Superjami showed higher antioxidant activities and anthocyanin, phenolic, tocopherol, and tocotrienol contents than Superhongmi. These findings illustrate that germination could increase the antioxidant compounds and enhance the antioxidant capacity of pigmented rice which may be useful as functional foods.

Keywords: pigmented rice, germination, antioxidant activity, antioxidant compounds

Introduction

Germination has been shown to improve the nutritional value and increase the amount of bioactive compounds in various cereal grains including rice, oat, wheat, and barley (Hubner and Arendt, 2013). It has also been reported that germination could improve the texture and eating quality of brown rice (Wu *et al.*, 2013b). This process involves soaking of the grains in water for a few days to induce slight germination. The rice is considered germinated when the primary root is already visible (Moongngarm and Khomphiphatkul, 2011). A number of studies have shown that germinated rice possesses various pharmacological properties, such as antidiabetic, antihyperlipidemia, antioxidant, and anticancer (Mohd Esa *et al.*, 2013; Patil and Khan, 2011; Wu *et al.*, 2013b). Biochemical changes occur during germination of rice which causes softening of the endosperm and an increase in nutrient

bioavailability (Islam and Becerra, 2012; Patil and Khan, 2011).

Pigmented rice varieties are rice grains with colored pericarp and have been shown to have higher amounts of nutrients and greater antioxidant capacity than ordinary non-pigmented rice (Laokdiluk *et al.*, 2011; Kang *et al.*, 2013). A study conducted by Nam *et al.* (2005) revealed that extracts from pigmented rice brans have anticancer and antimutagenic properties. Moreover, consumption of pigmented rice has been associated with reduced risk of developing hyperlipidemia and cardiovascular disease (Ling *et al.*, 2001). Due to the high antioxidant potential and functional properties of pigmented rice, new lines of this variety with enhanced biofunctional properties are continuously being developed and produced. In Korea, new pigmented rice varieties, Superjami and Superhongmi, have been recently developed through conventional breeding. Superjami, a blackish purple rice,

*To whom correspondence should be addressed.

E-mail: zizibe0312@nate.com

contains high amounts of cyanidin-3-glucoside, an anthocyanin that has strong antioxidant capacity (Kwon *et al.*, 2011). Superhongmi, a reddish brown rice, contains acetylated procyanidins that have free radical scavenging activity (Seo *et al.*, 2011).

With the growing health problems and rapidly increasing incidences of metabolic diseases worldwide, natural foods with strong health-promoting properties are greatly needed. While a number of studies have been conducted on the antioxidant capacity of pigmented rice and physiological functions of germinated brown rice (Kang *et al.*, 2013; Lin *et al.*, 2015; Mohd Esa *et al.*, 2013; Shen *et al.*, 2009; Wu *et al.*, 2013b), there were limited reports on the functional properties of germinated pigmented rice (Jiapong *et al.*, 2011; Sutharut and Sudarat, 2012; Umnajkitikorn *et al.*, 2013). The present study was carried out to determine the effect of germination on the antioxidant activity of pigmented rice Superjami and Superhongmi, in comparison to that of the ordinary non-pigmented brown rice. The antioxidant components, such as anthocyanin, tannin, phenolic compounds, phytic acid, tocopherol, and tocotrienol, of the germinated and non-germinated rice samples were also analyzed.

Materials and Methods

Rice samples and chemicals Whole grain pigmented rice, Superjami and Superhongmi, and ordinary non-pigmented brown rice Hwacheong were obtained from the Department of Agricultural Science, Korea National Open University. They were grown from May to October 2014 under the required cultivation conditions. All chemicals used in this study are of analytical grade and purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Germination of rice samples The rice grains were germinated following the methods described by Wu *et al.* (2013a) with slight modifications. Briefly, 2 sets of 60 g grains from each rice sample were washed twice with distilled water and 70% ethanol. The first set was placed evenly in a tray laid with paper towel and dried in an oven at 50°C for 2 h to lower the moisture content. The dried rice grains, which served as the control or non-germinated samples, were ground and pulverized using a grinding machine (HMF-3250S, Hanil Electronics, Seoul, South Korea), packed in hermetically sealed Ziploc plastic bags, and stored at -20°C until further analysis. The second set of rice grains was placed evenly in a tray overlaid with cotton pads and cheesecloth and enough water was added until the grains were soaked. The whole tray was covered with a clean transparent plastic wrap with holes to accommodate proper moisture condition and incubated at 30°C. The rice grains were checked every 24 h to ensure there was no foul odor and fungal growth. After 72 h, the germinated rice grains were dried, pulverized, and stored using the same method described above for the non-germinated grains. The 72-h germination period was selected as the optimum time for rice germination based on the

results of our preliminary investigation. Our initial observation revealed that the antioxidant activity and the amount of antioxidant components increased with germination time (data not shown), however, fungi developed in the germinated rice after 72 h.

Preparation of methanolic extracts from germinated and non-germinated rice Methanolic extracts from the rice samples were prepared using the method of Dutta *et al.* (2012). Briefly, the rice powder (3 g) was mixed with 80% methanol (30 mL) and the mixture was subjected to overnight shaking at 25°C using a twister machine (VS-96TW, Vision Scientific Co., Ltd., Daejeon, South Korea), followed by centrifugation at 10,000 rpm for 10 min. The extracts were then filtered using a 0.45 µm pore size syringe driven filter (Chromdisc, E.Chrom Science Inc., Daegu, South Korea) and stored at -20°C until further analysis.

Determination of antioxidant activities of the methanolic extracts

1,1-diphenyl-2-picrylhydrazyl (DPPH)-radical scavenging activity The DPPH-radical scavenging activity of the methanolic extracts was measured using the method of Chakuton *et al.* (2012). Briefly, four different concentrations of the methanolic extract (0.5, 1, 5, and 10 mg/mL) were prepared and an aliquot of 200 µL each was added to 1.8 mL of DPPH solution in absolute ethanol. The mixture was left at room temperature in the dark for 1 h. The absorbance at 515 nm was measured and the scavenging activity was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [1 - (A_{\text{sample}}/A_{\text{blank}})] \times 100 \quad \dots \text{Eq. 1}$$

The effective concentration at which 50% (EC₅₀) of the DPPH radicals are scavenged was calculated by interpolation from linear regression analysis. Butylated hydroxyanisole (BHA) was used as standard.

2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid)[ABTS] radical scavenging activity The ABTS radical scavenging activity was determined according to the method of Privita-Edwina *et al.* (2014) with slight modifications. The ABTS was dissolved in distilled water (7.4 mM) and mixed with potassium persulfate solution (2.6 mM) in equal amounts. The solution was allowed to stand at ambient temperature in the dark for 16-24 h. Absorbance of the resulting solution was between 1.4 to 1.5 readings at 734 nm prior to use. The methanolic extract (500 µL) was added to 1.0 mL of ABTS solution and the mixture was allowed to stand for 30 min. The absorbance was measured at 734 nm. Ascorbic acid was used as standard and the results were expressed as mg of ascorbic acid equivalent (AAeq) per 100 g of extract.

Hydroxyl radical scavenging activity The hydroxyl radical scavenging activity of the methanolic extracts was determined according to the method of Privita-Edwina *et al.* (2014). Briefly, 200 µL of 10 mM H₂O₂ was added to the reaction mixture consisting of 200 µL of 10 mM FeSO₄/ EDTA solution, 200 µL of 10 mM 2-deoxyribose, 1 mL of 0.1 M sodium phosphate buffer (pH 7.4) and 200 µL of the methanolic extracts (0.5, 1, 5, and 10 mg/

mL). The mixture was incubated at 40°C for 2 h and added with 1 mL of 2.8% trichloroacetic acid (TCA) and 1 mL of 0.67% thiobarbituric acid. The mixture was heated to 100°C for 10 min. The absorbance was measured at 532 nm and the scavenging activity was calculated as follows:

$$\text{Scavenging activity (\%)} = [1 - (A_{\text{sample}}/A_{\text{blank}})] \times 100 \quad \dots\dots\text{Eq. 2}$$

The EC₅₀ of the scavenging activity was calculated using linear regression analysis and ascorbic acid was used as standard for comparison.

Iron (Fe²⁺) chelating ability The ability of the methanolic extracts to chelate metals was determined using the method of Sudan *et al.* (2014). Four different concentrations of the extracts (0.5, 1, 5, and 10 mg/mL) were prepared and an aliquot of 100 µL was added to 50 µL of 2 mM FeCl₂. Subsequently, 100 µL ferrozine and 1.7 mL of 95% ethanol were added and the mixture was left to stand at room temperature for 10 min. The absorbance of the resulting solution was measured at 562 nm and the iron chelating ability was calculated using the following equation:

$$\text{Chelating ability (\%)} = [1 - (A_{\text{sample}}/A_{\text{blank}})] \times 100 \quad \dots\dots\text{Eq. 3}$$

EDTA was used as standard and the EC₅₀ value was calculated using linear regression analysis.

Reducing power The reducing power of the methanolic extracts was determined using the method of Mau *et al.* (2003) with some modifications. The extract (500 µL) was mixed with 800 µL of 0.2 M phosphate buffer (pH 6.6) and 500 µL of 5 mM K₂FeCN₆. The mixture was incubated in water bath at 50°C for 20 min to reduce the ferricyanide to ferrocyanide. It was added with 10% TCA (500 µL) and centrifuged at 5000 rpm for 10 min. The supernatant was added with 500 µL of distilled water and 100 µL of FeCl₃. The mixture was shaken vigorously and the absorbance was measured at 700 nm. A higher absorbance indicates a higher reducing power. The EC₅₀, effective concentration at which the absorbance was 0.5, was obtained by linear regression analysis. Ascorbic acid was used as standard.

Superoxide dismutase (SOD)-like activity To assess the capability of the methanolic extracts to scavenge superoxide anions, superoxide dismutase assay was performed following the method of Marklund and Marklund (1974) with some modifications. The reaction mixture, consisting of 1 mL of 50 mM Tris-HCl/10 mM EDTA buffer (pH 8.5), 60 µL of 7.2 mM pyrogallol, and 60 µL of the methanolic extract, was incubated at 25°C for 10 min. The mixture was added with 30 µL of 1 N HCl and the absorbance was measured at 420 nm. The SOD-like activity was calculated using the following equation:

$$\text{SOD-like activity (\%)} = [1 - (S_{\text{abs}} - B_{\text{abs}}) / C_{\text{abs}}] \times 100 \quad \dots\dots\text{Eq. 4}$$

where S_{abs} was the absorbance of the sample solution, B_{abs} was the absorbance of the reaction mixture containing buffer instead of pyrogallol, and C_{abs} was the absorbance of the reaction mixture

containing buffer instead of the methanolic extract.

Determination of total anthocyanin content The total anthocyanin content of the methanolic extract was determined using pH-differential method (Chakuton *et al.* 2012). Briefly, the extract was diluted in 0.025 M KCl buffer (pH 1.0) and placed in the dark at room temperature for 30 min. The absorbance was measured at 520 nm and 700 nm. The methanolic extract was mixed with 0.4 M sodium acetate buffer (pH 4.5) and the absorbance was measured again at 520 nm and 700 nm. The results were calculated using malvidin-3-O-glucoside equation:

$$\text{Total anthocyanin content (mg malvidin / 100 g rice)} = (A \times \text{MW} \times \text{DF} \times 1000) / (\epsilon \times 1) \quad \dots\dots\text{Eq. 5}$$

where A (absorbance) = (A_{520 nm} - A_{700 nm}) pH 1.0 - (A_{520 nm} - A_{700 nm}) pH 4.5, MW = molecular weight of malvidin-3-O-glucoside (493.5 g/mol), DF = dilution factor for sample (0.4 mL), and ε = molar extinction coefficient (28,000).

Determination of tannin content Colorimetric estimation of the tannin content based on the blue color formed by the reduction of phosphotungstomolybdic acid by tannin-like compounds in alkaline medium was determined following the method described by Padma *et al.* (2013). The methanolic extract (200 µL) was mixed with 1.5 mL distilled water, then Folin-Denis reagent (100 µL) and Na₂CO₃ (200 µL) were added. The absorbance of the resulting solution was measured at 700 nm. The total tannic acid content was expressed as mg of tannic acid equivalent (TAE)/g of extract.

Determination of total phenolic content The total phenolic content of the methanolic extracts was measured using the Folin-Ciocalteu colorimetric method (Velioglu *et al.*, 1998). The extract (100 µL) was mixed with 2 mL of 2% Na₂CO₃. After 3 min, the mixture was then added with 50% Folin-Ciocalteu's reagent (100 µL). The absorbance was measured at 750 nm and the results were expressed as mg of gallic acid equivalents (GAE)/100 g of extract.

Determination of phytic acid content The phytic acid contents of the germinated and non-germinated grains were determined spectrophotometrically based on the method of Chakuton *et al.* (2012) with some modifications. Briefly, the rice powder (1.5 g) was extracted with 0.1 M HCl (10 mL) by continuous shaking at 200 rpm for 24 h. The mixture was centrifuged at 3000 rpm for 20 min. The supernatant (500 µL) was mixed with 1% FeCl₃ solution, heated at 100°C in a water bath, and then allowed to cool at ambient temperature until precipitate developed. The mixture was centrifuged at 5000 rpm for 10 min and the supernatant (100 µL) was mixed with 2,2' bipyridine solution. The solution was transferred to a 96-well culture plate and incubated for 5 min. The absorbance was measured at 519 nm. The method was calibrated with standard phytic acid solutions and the results were expressed as mg phytic acid equivalent/100 g of rice.

Determination of tocopherol and tocotrienol contents The

Table 1. Free radical scavenging activities of methanolic extracts from germinated and non-germinated pigmented rice.

Antioxidant activity	Rice sample	Non-germinated	Germinated
DPPH radical scavenging activity (%)	Brown rice	26.15 ± 1.74 ^a	63.42 ± 3.52 ^{a*}
	Superjami	59.84 ± 0.57 ^c	84.30 ± 0.74 ^{c*}
	Superhongmi	31.58 ± 1.57 ^b	67.04 ± 2.21 ^{b*}
	BHA	92.61 ± 1.47 ^d	92.61 ± 1.47 ^d
ABTS radical scavenging activity (mg AAeq/100 g extract)	Brown rice	15.83 ± 1.95 ^a	65.96 ± 1.57 ^{a*}
	Superjami	108.75 ± 1.80 ^c	143.63 ± 2.38 ^{c*}
	Superhongmi	58.92 ± 1.35 ^b	118.13 ± 3.36 ^{b*}
Hydroxyl radical scavenging activity (%)	Brown rice	44.05 ± 0.54 ^a	56.54 ± 0.71 ^{a*}
	Superjami	48.57 ± 0.71 ^b	65.75 ± 0.71 ^{c*}
	Superhongmi	47.86 ± 0.68 ^b	62.27 ± 0.57 ^{b*}
	Ascorbic acid	87.57 ± 1.25 ^c	87.57 ± 1.25 ^d

Values are means ± SD ($n = 3$). Means in the same column with different letters are significantly different at $P < 0.05$. * indicates significant difference ($P < 0.05$) between germinated and non-germinated samples.

Table 2. Iron (Fe^{2+}) chelating ability, reducing power, and SOD-like activity of methanolic extracts from germinated and non-germinated pigmented rice.

Antioxidant activity	Rice sample	Non-germinated	Germinated
Fe^{2+} chelating ability (%)	Brown rice	66.78 ± 1.71 ^a	81.00 ± 1.08 ^{a*}
	Superjami	83.97 ± 0.43 ^c	88.82 ± 0.53 ^{c*}
	Superhongmi	79.37 ± 0.42 ^b	86.73 ± 0.52 ^{b*}
	EDTA	95.44 ± 0.09 ^d	95.44 ± 0.09 ^d
Reducing power (O.D. at 700 nm)	Brown rice	0.25 ± 0.00 ^a	0.48 ± 0.01 ^{a*}
	Superjami	0.50 ± 0.01 ^c	0.82 ± 0.01 ^{c*}
	Superhongmi	0.35 ± 0.01 ^b	0.59 ± 0.01 ^{b*}
	Ascorbic acid	0.83 ± 0.01 ^d	0.83 ± 0.01 ^d
SOD-like activity (%)	Brown rice	5.10 ± 0.95 ^a	40.71 ± 2.57 ^{a*}
	Superjami	10.35 ± 0.80 ^c	77.90 ± 2.58 ^{c*}
	Superhongmi	7.65 ± 0.55 ^b	46.27 ± 0.36 ^{b*}

Values are means ± SD ($n = 3$). Means in the same column with different letters are significantly different at $P < 0.05$. * indicates significant difference ($P < 0.05$) between germinated and non-germinated samples.

tocopherol (α , β , and γ) and tocotrienol (α , β , and δ) contents of the methanolic extracts were determined according to the method described by Jeng *et al.* (2011). The extract was filtered through a 0.45 μM filter membrane and analyzed using HPLC system equipped with fluorescence detector (λ_{ex} 298 nm, λ_{em} 328 nm) and C18 analytical column (4.6 x 150 mm, 5 μm). The mobile phase was acetonitrile and methanol (60:40) with 1 mL/min flow rate. The data were calibrated using standard tocopherol and tocotrienol set kits.

Statistical analysis Data were analyzed using one-way ANOVA (Statistical Package for Social Sciences software program version 22.0, SPSS Inc., Chicago, IL, USA) and the values were reported as mean ± standard deviation (SD). The difference between the means was assessed using Tukey's test and independent student t-test. Statistical significance was considered at $p < 0.05$.

Results

Free radical scavenging activities Germination significantly increased the DPPH, ABTS, and hydroxyl radicals scavenging activities of all the rice samples (Table 1). In both non-germinated and germinated conditions, Superjami exhibited the highest DPPH and ABTS scavenging activities, followed by Superhongmi, then the ordinary non-pigmented brown rice Hwacheong. The hydroxyl radical scavenging activity was also significantly higher in both pigmented rice methanolic extracts than that of the brown rice methanolic extract.

Ferrous ion chelating ability, reducing power, and SOD-like activity The pigmented rice samples showed significantly higher Fe^{2+} chelating ability, reducing power, and SOD-like activity than the non-pigmented rice (Table 2). The antioxidant activities markedly increased after germination for 72 h in all rice samples. The Superjami exhibited the highest Fe^{2+} chelating ability, reducing power, and SOD-like activity in both germinated and non-

Table 3. EC₅₀ values of germinated and non-germinated pigmented rice methanolic extracts on DPPH and hydroxyl radicals scavenging assays, ferrous ion chelating activity, and reducing power.

Antioxidant assay	Rice sample	Non-germinated	Germinated
DPPH radical scavenging (mg/mL)	Brown rice	20.58 ± 1.52 ^c	14.27 ± 0.47 ^{d*}
	Superjami	7.07 ± 0.36 ^b	3.81 ± 0.15 ^{b*}
	Superhongmi	17.93 ± 1.59 ^c	6.80 ± 0.61 ^{c*}
	BHA	0.27 ± 0.08 ^a	0.27 ± 0.08 ^a
Hydroxyl radical scavenging (mg/mL)	Brown rice	13.65 ± 0.61 ^c	7.65 ± 0.24 ^{c*}
	Superjami	9.35 ± 0.16 ^b	5.19 ± 0.08 ^{b*}
	Superhongmi	9.98 ± 0.17 ^b	5.37 ± 0.06 ^{b*}
	Ascorbic acid	2.35 ± 0.04 ^a	2.35 ± 0.04 ^a
Fe ²⁺ chelating activity (mg/mL)	Brown rice	4.72 ± 0.03 ^d	3.47 ± 0.06 ^{c*}
	Superjami	3.79 ± 0.15 ^b	3.12 ± 0.06 ^{b*}
	Superhongmi	4.19 ± 0.04 ^c	3.19 ± 0.05 ^{b*}
	EDTA	0.49 ± 0.01 ^a	0.49 ± 0.01 ^a
Reducing power (mg/mL)	Brown rice	26.26 ± 1.00 ^d	11.64 ± 0.19 ^{d*}
	Superjami	6.07 ± 1.21 ^b	1.76 ± 0.55 ^{b*}
	Superhongmi	11.15 ± 1.96 ^c	4.69 ± 0.41 ^{c*}
	Ascorbic acid	1.27 ± 0.08 ^a	1.27 ± 0.08 ^a

Values are means ± SD ($n = 3$). Means in the same column with different letters are significantly different at $P < 0.05$. * indicates significant difference ($P < 0.05$) between germinated and non-germinated samples.

germinated forms.

EC₅₀ values on antioxidant assays The EC₅₀ values on DPPH scavenging activity, Fe²⁺ chelating ability, and reducing power were lowest in germinated and non-germinated Superjami methanolic extracts among the rice samples analyzed (Table 3). The extracts from pigmented varieties showed significantly lower EC₅₀ values on hydroxyl radical scavenging activity than that of brown rice. All rice samples exhibited a considerable decrease in EC₅₀ values after germination.

Antioxidant components The anthocyanin was not detected in non-germinated brown rice and Superhongmi, but was present in germinated Superhongmi (Table 4). Furthermore, the total anthocyanin content in Superjami significantly increased after germination. Superhongmi showed considerably higher tannin content than Superjami. A marked increase in the tannin content was observed in both pigmented rice samples after germination. Tannin was not detected in the brown rice sample in both non-germinated and germinated forms. Germination significantly increased the total phenolic content in all rice samples. Superjami exhibited the highest total phenolic content, followed by Superhongmi, then the non-pigmented brown rice. Similarly, the phytic acid, tocopherol, and tocotrienol contents significantly increased after germination in all rice samples. Superjami and Superhongmi showed the highest amount of phytic acid in non-germinated and germinated forms, respectively. The tocopherol content was highest in Superjami and lowest in Superhongmi. The tocotrienol content, on the other hand, was highest in Superjami, followed by Superhongmi, then the non-pigmented brown rice.

Discussion

In the present study, the effect of germination for 72 h on the antioxidant capacity of blackish purple rice Superjami and reddish brown rice Superhongmi, in comparison to that of non-pigmented ordinary brown rice Hwacheong, was determined. Results showed that germination markedly increased the DPPH, ABTS, and hydroxyl radicals scavenging activities, Fe²⁺ chelating ability, reducing power, and SOD-like activity in all the rice samples. Moreover, the total anthocyanin, tannin, total phenolic, phytic acid, tocopherol, and tocotrienol contents were significantly higher in germinated rice compared to those of non-germinated ones. The EC₅₀ values on DPPH and hydroxyl radical assays, Fe²⁺ chelating activity, and reducing power significantly decreased in all rice samples after germination. All rice samples, whether germinated or not, showed lower antioxidant capacity than the standard antioxidants BHA, EDTA, and ascorbic acid. Extracts from Superjami exhibited the lowest EC₅₀ values and highest amount of antioxidant compounds, followed by that of Superhongmi, then brown rice. A past study on germinated rice also revealed that germination significantly enhanced the DPPH radical scavenging activity and reducing power in non-pigmented brown rice (Lin *et al.*, 2015). Sutharut and Sudarat (2012) also observed an increase in the reducing power of pigmented rice after germination.

Since the antioxidant capacity of a food or plant extract differs depending on the type of oxidant involved in the analysis, it is essential to use a variety of assays to fully evaluate the antioxidant potential of the sample extract (MacDonald-Wicks *et al.*, 2006). Antioxidants are classified into primary or chain-breaking antioxidants, which scavenge free radicals and convert them into

Table 4. Antioxidant components in germinated and non-germinated pigmented rice.

Antioxidant component	Rice sample	Non-germinated	Germinated
Total anthocyanin content (mg malvidin/100 g extract)	Brown rice	ND	ND
	Superjami	409.34 ± 8.69	729.22 ± 9.11 ^{b*}
	Superhongmi	ND	18.36 ± 2.85 ^a
Tannin content (mg TAE/g extract)	Brown rice	ND	ND
	Superjami	152.14 ± 9.61 ^a	1752.36 ± 11.84 ^{a*}
	Superhongmi	1374.57 ± 8.25 ^b	3654.54 ± 10.21 ^{b*}
Total phenolic content (mg GAE/100 g extract)	Brown rice	162.21 ± 2.25 ^a	170.01 ± 5.02 ^a
	Superjami	242.11 ± 4.72 ^c	476.42 ± 2.98 ^{c*}
	Superhongmi	209.21 ± 3.35 ^b	343.01 ± 3.98 ^{b*}
Phytic acid content (mg/100 g rice)	Brown rice	1.18 ± 0.05 ^a	3.08 ± 0.04 ^{a*}
	Superjami	2.45 ± 0.16 ^c	5.40 ± 0.03 ^{b*}
	Superhongmi	2.04 ± 0.03 ^b	6.05 ± 0.08 ^{c*}
Tocopherol (α , β , γ) content (μ g/100 g rice)	Brown rice	25.01 ± 2.40 ^b	77.64 ± 2.08 ^{b*}
	Superjami	48.20 ± 6.75 ^c	114.59 ± 5.74 ^{c*}
	Superhongmi	5.19 ± 6.05 ^a	17.25 ± 0.32 ^{a*}
Tocotrienol (α , β , δ) content (μ g/100 g rice)	Brown rice	53.54 ± 7.23 ^a	199.69 ± 4.21 ^{a*}
	Superjami	156.44 ± 8.10 ^c	305.37 ± 6.50 ^{c*}
	Superhongmi	126.84 ± 6.40 ^b	267.26 ± 5.38 ^{b*}

Values are means ± SD ($n = 3$). Means in the same column with different letters are significantly different at $P < 0.05$. * indicates significant difference ($P < 0.05$) between germinated and non-germinated samples. ND, not detected.

stable molecules, and secondary or preventive antioxidants, which suppress radical formation and act as oxygen scavengers or chelators for catalyst metal ions (Wanasundra and Shahidi, 2005). The DPPH and ABTS radical scavenging assays measure the ability of the sample extract to donate an electron or hydrogen to stable free radicals DPPH and ABTS (Moon and Shibamoto, 2009). The hydroxyl scavenging assay determines the ability of the extract to scavenge hydroxyl radicals, the most reactive among the reactive oxygen species, generated via the Fenton reaction (Lee *et al.*, 2004). The Fe^{2+} chelating ability assay, which measures the capacity of the compounds present in sample to compete with ferrozine for ferrous ion, is commonly used to assess the secondary antioxidant activity of the sample extract (Vladimir-Knezevic *et al.*, 2011). The reducing power assay measures the capacity of the sample extract to donate electron to Fe^{3+} /ferricyanide complex, converting it to its ferrous form (Niki, 2010). The SOD-like activity assay measures the ability of the sample extract to catalyze the conversion of superoxide radicals into hydrogen peroxides, providing a defense mechanism against oxidative damage (Nagami *et al.*, 2004). The relatively high free radical scavenging activities, Fe^{2+} chelating ability, reducing power, and SOD-like activity observed in Superjami and Superhongmi methanolic extracts, particularly those from germinated grains, suggest that these pigmented rice varieties have strong primary and secondary antioxidant potential.

The substantial increase in the antioxidant activities of

germinated rice methanolic extracts may have been possibly due to the significant increase in the total phenolic, anthocyanin, tannin, phytic acid, tocopherol, and tocotrienol contents during germination of the grains. The anthocyanins, tannins, phenolics, phytic acid, tocopherols, and tocotrienols are natural antioxidant compounds that can scavenge free radicals and inhibit the formation of reactive oxygen species, thus preventing oxidative damage (Dai and Mumper, 2010; Goufo and Trindade, 2014; Ichikawa *et al.*, 2001; Zhao *et al.*, 2011). Several studies have shown that the antioxidant capacity of rice has a positive correlation with its phenolic content (Goffman and Bergman, 2004; Jin *et al.*, 2009; Shen *et al.*, 2009). Tian *et al.* (2004) previously reported that germination significantly increased the amount of phenolic acids in brown rice. Similarly, the total anthocyanin content has been found to increase in black rice after germination (Sutharut and Sudarat, 2012). Kaukorvirta-Norja *et al.* (2004) accounted that during germination, the cell walls surrounding various compounds are broken down and the free and bound phenolics are released leading to increased phenolic content in germinated rice. Furthermore, the dormant enzymes are activated to break down large molecular substances during germination, causing an increase in the nutrients and generation of bioactive components in rice (Moongngarm and Saeung, 2010). Pigmented rice are known to possess higher antioxidant capacity than ordinary non-pigmented varieties. Results of the present study demonstrate that germination for 72 h could further enhance the antioxidant

activity and increase the amount of antioxidant compounds in pigmented rice.

Conclusion

Germination markedly increased the DPPH, ABTS, and hydroxyl radicals scavenging activities, ferrous ion chelating ability, reducing power, and SOD-like activity in both pigmented and non-pigmented rice samples. This increase in the antioxidant activity is possibly due to the substantial increase in the levels of anthocyanin, tannin, phenolic compounds, phytic acid, tocopherol, and tocotrienol in rice after germination. Extracts from pigmented rice, particularly Superjami, exhibited greater antioxidant activity and higher amounts of antioxidant components than that of the non-pigmented brown rice. The results illustrate that germination for 72 h can be a useful method in enhancing the antioxidant capacity of pigmented rice. Germinated Superjami and Superhongmi may be beneficial as functional foods with strong antioxidant potential.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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