Antioxidant Capacity of Giant Embryo Rice Seonong 17 and Keunnunjami

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***Abstract*—The antioxidant capacity of giant embryo brown rice Seonong 17 and Keunnunjami, in comparison with that of the normal embryo brown rice, was investigated. The rice grains were extracted with 70% ethanol and their antioxidant activity and components were analyzed. Compared with the normal brown rice, the giant embryo rice samples showed higher free radical scavenging, reducing power, ferrous chelating, xanthine oxidase inhibitory, and superoxide dismutase-like activities. Between the two giant embryo rice samples, Keunnunjami rice exhibited greater antioxidant activity than Seonong 17 rice. The total phenolic and total flavonoid contents were highest in Keunnunjami and lowest in normal embryo rice, indicating that these functional components are related to the high antioxidant activity observed in Keunnunjami. These findings provide significant information on the antioxidant potential of Seonong 17 and Keunnunjammi giant embryo rice cultivars. Keunnunjami, in particular, could be a good source of natural antioxidants and may be beneficial as a functional biomaterial in the development of food products with strong antioxidant capacity.**

***Index Terms*—antioxidant activity, giant embryo rice, Keunnunjami, Seonong 17**

I. Introduction

With the growing market demand for agricultural crops with enhanced functional properties, rice breeders and research scientists across the globe have focused on developing new rice cultivars with increased nutritional value. In the past years, a number of functional rice grains with health-promoting properties have been developed and produced such as pigmented rice, giant embryo rice, and high-amylose rice [1]-[3]. The giant embryo rice, in particular, is a mutant rice that has an enlarged embryo and possesses higher protein, mineral, and vitamin contents than the normal embryo rice [4], [5]. It was also found to have high amounts of gamma-oryzanol and gamma-aminobutyric acid, a compound known for its anticancer, hypolipidemic and antihypertensive effects [4], [6].

Keunnunjami, a pigmented (blackish purple) giant embryo rice, has been recently developed in Korea. It was bred from a three-way cross between Heugjinjubyeo, Suwon 425, and EM76 cultivars and was found to contain a high amount of cyanidin 3-glucoside, an anthocyanin compound widely present in plants and has antioxidant property [7]. Seonong 17, on the other hand, is a non-pigmented rice with an enlarged embryo developed from Hwachungbyeo rice, a Korean japonica cultivar. Little is still known on the nutritional functions of these two giant embryo rice mutants. Knowledge on their nutritional properties is essential for a better understanding of their potential uses and applications.

Previous reports indicated that giant embryo rice has higher antioxidant activity compared with the normal embryo rice [8]. Pigmented rice has been also shown to have greater reducing power and antioxidant capacity than ordinary white rice [9], [10]. With the rapidly increasing incidence of various metabolic disorders worldwide and the rising cost of health care, functional foods with strong antioxidant potential are greatly needed. The antioxidative properties of Seonong 17 and Keunnunjami giant embryo rice have not been investigated yet. Hence, this study was carried out to analyze their antioxidant activity, in comparison with that of the ordinary normal embryo brown rice.

II. Materials and Methods

A. Materials and Chemicals

The giant embryo brown rice samples Seonong 17 and Keunnunjami were obtained from Plant Molecular Breeding Center (Seoul, Korea) and Department of Agricultural Science, Korea National Open University (Seoul, Korea), respectively. Seonong 17 was grown in June-October 2011 while Keunnunjami was grown in May-September 2011. The rice grains were stored at -4 ℃ with 30% relative humidity. The ordinary normal embryo brown rice was purchased from a local market in Daegu, Korea. The chemicals were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

B. Preparation of Ethanolic Extracts from the Rice Samples

The rice grains were ground into powder and extracted three times with 70% ethanol, with a solvent to solid material ratio of 3:1 (15mL ethanol: 5g rice). The extracts were filtered using Whatman no. 2 filter paper to remove any debris. The filtrates were concentrated using a rotary evaporator (Eyela N-1000, Tokyo, Japan) and then

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lyophilized. The samples were stored at -80 ℃ deep-freezer. The extracts were dissolved in 0.1g/mL dimethyl sulfoxide prior to use.

C. Determination of Free Radical Scavenging Ability

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the sample extracts was measured based on the method of [11]. Briefly, the sample was mixed with DPPH solution and shaken vigorously and left to stand for 30 min in the dark at room temperature. The absorbance of the mixture was measured at 517nm and the scavenging activity was calculated as follows:

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

The 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) radical scavenging activity was determined based on the oxidation of ABTS by potassium persulfate [12]. The ABTS was dissolved in phosphate buffered saline and mixed with potassium perfulfate solution. The mixture was allowed to stand in the dark for 12-16 hr at room temperature to assure the complete formation of stable ABTS radical. The radical cation solution was then mixed with the sample extract and the absorbance at 734nm was measured at 1-min interval for 4 min. Ascorbic Acid (AA) was used as the standard solution and the results were expressed as AA equivalents (μg AAeq/g).

The hydroxyl radical scavenging activity was determined using the 2-deoxyribose oxidation method by [13]. The rice extract (0.2mL) was mixed with 10mM $FeSO_4$/EDTA (0.2mL), 10mM 2-deoxyribose (0.2mL), 100mM phosphate buffer (1.2mL, pH 7.4), and 10mM H_2O_2 (0.2mL) and incubated at 37 ℃ for 2 hr. The reaction was stopped by the addition of 2.8% trichloroacetic acid (1mL) and 0.8% thiobarbituric acid (1mL). The mixture was heated in water bath (100 ℃) for 10 min. After cooling, the absorbance was measured at 532nm and the scavenging activity was calculated as follows:

$$\text{Hydroxyl scavenging activity (\%)} = [1-(A_{sample})/A_{blank}]\times 100 \quad (2)$$

D. Determination of Ferrycyanide Reducing Power

The reducing power of the rice extracts was determined based on the method of [14]. The extract (500μL) was mixed with 2M phosphate buffer (5.0mL, pH 6.6) and 1% potassium ferricyanide (5mL). The mixture was incubated at 50 ℃ for 20 min, added with 10% trichloroacetic acid (5mL), and centrifuged at 650×*g* for 10 min. The supernatant (5mL) was mixed with distilled water (5mL) and 0.1% ferric chloride (1mL). The absorbance was measured at 700 nm. Higher absorbance indicates higher reducing power.

E. Measurement of Fe^{2+}-Chelating Ability

The Fe^{2+}-chelating ability was determined according to the method described by [15]. The Fe^{2+} level was monitored by measuring the formation of the ferrous ion-ferrozine complex. The sample extract (1.0mL) was mixed with 2mM $FeCl_2$ (0.1mL) and 5mM ferrozine (0.2mL) and the mixture was left to stand at room temperature for 10 min. The absorbance of the solution was measured at 562nm. A lower absorbance indicates a stronger Fe^{2+}-chelating ability. The ability to chelate the ferrous ion was calculated as follows:

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

F. Analysis of Xanthine Oxidase (XOD) Inhibitory Activity

The XOD inhibitory activity was analyzed using the method of [16]. The rice extracts (100μL) were mixed with 2mM xanthine, 0.1M potassium phosphate buffer (pH 7.5), and 0.2 unit/mL xanthine oxidase. The mixture was incubated at 37 ℃ for 5 min and then added with 1N HCl. The absorbance was measured at 292nm.

G. Measurement of Superoxide Dismutase (SOD)-Like Activity

The SOD-like activity was determined based on the method of [17]. The reaction mixture consisted of 100μL of the sample extract, 3mL of Tris-HCl buffer (50mM trisamino-Methane, 10mM EDTA, pH 8.5), and 200μL of 7.2mM pyrogallol. It was incubated in a water bath at 25 ℃ for 10 min and then mixed with 100μL of 1 N HCl to stop the reaction. The absorbance was measured at 420nm and the SOD-like activity was calculated as follows:

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

H. Determination of Total Phenolic and Total Flavonoid Contents

The total phenolic content of the rice extracts was measured using the Folin-Ciocalteu colorimetric method [18]. The sample extract (100μL) was mixed with 2% Na_2CO_3 (2mL). After 3 min, the mixture was then added with of 50% Folin-Ciocalteu's reagent (100μL). The absorbance was measured at 750nm after 1 hr and the results were expressed as gallic acid equivalents (mg of gallic acid/100g of rice).

The total flavonoid content was determined using colorimetric method of [19]. The extract (100μL) was mixed with distilled water (1.25mL) and 5% $NaNO_2$ solution (75μL). After 5min, 150μL of 10% AlCl3•6H2O was added to the mixture. After 6 min, 1M NaOH (500μL) and distilled water (425μL) were added. After 15 min, the absorbance was measured at 510nm and the results were expressed as (-)-epicatechin equivalents (mg of epicatechin/100g of rice).

I. Statistical Analysis

All experimental data were recorded as mean ± Standard Error (SE) of at least triplicate measurements. Means were compared using Tukey's test at $p<0.05$ using Statistical Package for Social Science software program version 19.0 (SPSS Inc., Chicago, IL).

III. Results

A. Free Radical Scavenging Activity

The Keunnunjami rice extract showed substantially higher DPPH and ABTS scavenging activities compared with the normal brown rice and Seonong 17 extracts (Table I). The hydroxyl radical scavenging activity was highest in Seonong 17, followed by Keunnunjami.

TABLE I. Free Radical Scavenging Ability of Giant Embryo Rice Extracts

Cultivar	DPPH radical (%)[1]	ABTS radical (μg AAeq/g)[2]	Hydroxyl radical (%)
Brown rice	36.10 ±0.85[a]	67.54 ±1.28[a]	40.68 ±1.76[a]
Seonong 17	64.43 ±1.96[b]	81.71 ±0.79[a]	59.35 ±1.24[c]
Keunnunjami	71.62 ±1.11[c]	360.74 ±0.47[b]	52.36 ±0.83[b]

[1]Values are means ± SE (n=3). Means in the same column not sharing a common superscript are significantly different at $p<0.05$.
[2]AAeq = ascorbic acid equivalent.

B. Ferricyanide Reducing Power and Ferrous Ion Chelating Ability

The ferrycyanide reducing power was significantly higher in Keunnujami than those of the other samples (Table II). On the other hand, the Fe^{2+} chelating ability was highest in Seonong 17 rice.

TABLE II. Ferrycyanide Reducing Power and Ferrous Ion Chelating Ability of Giant Embryo Rice Extracts

Cultivar	Reducing power (O.D. at 700 nm)[1]	Fe^{2+} chelating ability (%)
Brown rice	0.17 ±0.00[a]	64.98 ±1.00[a]
Seonong 17	0.20 ±0.00[a]	68.80 ±0.55[b]
Keunnunjami	0.28 ±0.47[b]	61.68 ±0.87[a]

[1]Values are means ± SE (n=3). Means in the same column not sharing a common superscript are significantly different at $p<0.05$.

C. XOD Inhibitory and SOD-Like Activities

The XOD inhibitory and SOD-like activities were highest in Keunnunjami, followed by Seonong 17, and then the normal embryo brown rice (Table III).

TABLE III. XOD Inhibitory Activity and SOD-Like Activity of Giant Embryo Rice Extracts.

Cultivar	XOD inhibitory activity (%)[1]	SOD-like activity (%)
Brown rice	50.50 ±0.42[a]	16.70 ±0.75[a]
Seonong 17	54.72 ±0.82[b]	18.22 ±0.78[b]
Keunnunjami	61.70 ±0.78[c]	22.45 ±0.45[c]

[1]Values are means ± SE (n=3). Means in the same column not sharing a common superscript are significantly different at $p<0.05$.

D. Total Phenolic and Total Flavonoid Contents

The Keunnunjami rice extract exhibited the highest total phenolic and total flavonoid contents among the rice samples analyzed (Table IV). The normal brown rice, on the other hand, showed the lowest phenolic and flavonoid contents.

TABLE IV. Total Phenolic and Flavonoid Contents of Giant Embryo Rice Extracts

Cultivar	Total phenolic content (mg/100 g)[1]	Total flavonoid content (mg/100 g)
Brown rice	44.81 ±0.83[a]	9.48 ±0.19[a]
Seonong 17	147.50 ±1.15[b]	11.58 ±0.22[a]
Keunnunjami	223.01 ±2.21[c]	67.65 ±0.24[b]

[1]Values are means ± SE (n=3). Means in the same column not sharing a common superscript are significantly different at $p<0.05$.

IV. Discussion

The antioxidant capacity of a particular food or compound differs with the different oxidants it is measured against a variety of antioxidant assays must be employed in order to fully assess the antioxidant potential of a given sample. Antioxidants can be classified into primary (chain-breaking) antioxidants, which scavenge free radicals and transforms them into stable molecules, and secondary (preventive) antioxidants, which inhibit the formation of radicals and act as oxygen scavengers or chelators for catalyst metal ions [20]. In the present study, the antioxidant capacity of the ethanolic extracts from giant embryo rice Seonong 17 and Keunnunjami, in comparison with that of the normal brown rice, were analyzed using seven different approaches (DPPH, ABTS, and hydroxyl radicals scavenging activities, reducing power, Fe^{2+} chelating ability, XOD inhibitory activity, and SOD-like activity).

The DPPH and ABTS radical scavenging assays are widely used in determining the antioxidant activity of plant extracts and bioactive compounds. They measure the ability of the sample extract to donate an electron or hydrogen to stable free radicals DPPH and ABTS, resulting in the discoloration of the assay solutions [21]. Among the reactive oxygen species, the hydroxyl radical is considered the most reactive and induces severe biological damage [22]. The radical is generated via the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + OH^-$) and reacts with deoxyribose to produce thiobarbituric reactive substances (TBARS). The hydroxyl quenchers decrease TBARS production by competing with deoxyribose for hydroxyl radicals [23]. The ferrycyanide reducing power assay measures the capacity of the sample extract to donate electron to Fe^{3+}/ferricyanide complex, converting it to its ferrous form [24]. Higher reducing capacity indicates higher antioxidant activity. Compounds that have reducing power could act as both primary and secondary antioxidants because they can also reduce the oxidized intermediates of lipid peroxidation process [25]. The ferrous iron chelating ability assay is widely used to measure the secondary antioxidant activity of the sample extract. The antioxidants present in the sample compete with ferrozine for ferrous iron, resulting in a decrease in the red color of the iron-ferrozine complex [26]. The radical chain reaction is initiated by iron and other transition metals which act as catalysts for the first free radical generation, which is then inhibited by chelating agents through stabilization of the transition metals [27]. The XOD inhibitory activity assay measures the ability of

the sample extract to inhibit xanthine oxidase, a flavoportein enzyme that catalyzes the oxidation of hypoxanthine to xanthine and generates superoxide radicals [28]. Extracts with strong inhibitory effect against the enzyme reduces oxidative stress through indirect antioxidant action [29]. The SOD-like activity assay measures the capacity of the sample extract to convert superoxide radicals into hydrogen peroxide [30].

Results of the present study showed that the giant embryo rice Seonong 17 and Keunnunjami have markedly higher free radical scavenging activity, reducing power, Fe^{2+} chelating ability, XOD inhibitory activity, and SOD-like activity than the normal embryo brown rice. "Reference [8]" also reported that giant embryo rice has greater radical scavenging activity compared with the normal embryo rice. Between the two giant embryo rice samples, the Keunnunjami rice exhibited stronger antioxidant activities than Seonong 17. Keunnunjami is a pigmented rice with a blackish purple pericarp while Seonong 17 is non-pigmented. Studies in the past have shown that pigmented varieties have greater radical scavenging activity than the normal white rice [10], [31]. Rice bran extracts from pigmented varieties were also found to have higher reducing power than those from non-pigmented ones [9], [32]. The strong antioxidant capacity exhibited by Keunnunjami is likely due to its high amounts of total phenolic and total flavonoid contents. Polyphenols and flavonoids are natural antioxidant compounds that are widely distributed in plants and have protective roles against oxidative stress-related disorders by scavenging free radicals and suppressing the formation of reactive oxygen species [21], [24]. Keunnunjami rice has been reported to have high cyanidin-3-glucoside content [7], an anthocyanin compound predominantly present in pigmented rice varieties and is a natural antioxidant [2], [33]. The antioxidant capacity of rice has been shown to be positively correlated to its phenolic and flavonoid contents [31], [34]. Thus, the strong antioxidant capacity exhibited by Keunnunjami relative to Seonong 17 and normal embryo brown rice is probably due to its substantial amount of total phenolic and total flavonoid contents.

Taken together, the antioxidant activity was highest in Keunnunjami, followed by Seonong 17, and then normal embryo brown rice. The same trend was also observed on the total phenolic and total flavonoid contents of the rice samples, indicating that these functional components may be related to the antioxidant activity of Keunnunjami and Seonong 17. Further studies are needed to determine the specific compounds responsible for the antioxidant activities in these rice mutants. The giant embryo rice samples, particularly Keunnunjami, could be a good source of natural antioxidants and may be useful in the development of functional food with strong antioxidative properties.

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