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Article

Comparative Analysis of Physicochemicals and Antioxidative Properties of New Giant Embryo Mutant, YR23517Acp79, in Rice (*Oryza sativa* L.)

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Nutritional and physicochemical properties of new giant embryo mutant rice (YR23517Acp79, YR) were analyzed. YR exhibited increased total protein (9.3±0.3%), lipid (3.7±1.1%), amino acid (663.28±1.9 mg/g), and mineral contents (Ca=284.0±6.2, Mg=1417.5±13.6 mg/kg). In YR brown rice four major physicochemicals, including gamma-aminobutyric acid (brown rice= 0.46±0.014 mg/g), γ-oryzanol (0.43±0.021 mg/g), vitamin B_1 (6.42±0.3 mg/kg), and tocopherols (alpha= 2.68±0.1, beta=0.11±0.01, gamma=0.05±0.001 mg/100 g) increased in comparison to reported giant embryo (Keunnunbyeo, KB) and normal embryo rice (Ilmibyeo, IB). YR showed higher scavenging activities against 1,1-diphenyl-2-picrylhydrazyl (0.2 g/mL=57.1±2.25) and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (0.2 g/mL=50.2±1.45) radicals but also inhibited lipopolysaccharide-induced nitric oxide production without cytotoxicity. These results indicate YR is a high quality functional rice due to its high nutrition content and antioxidant effects of physicochemicals.

Key words: antioxidant, gamma amino butyric acid, giant embryo brown rice, physicochemical

Rice (*Oryza sativa* L.) is the most important cereal crop in many parts of the world [Zhou *et al*., 2002]. The nutrient contents and functional metabolites in rice could be improved by biofortification through cultivar selection and breeding, which is an important approach to improving both the quantity and quality of rice-related food materials [Champagne *et al*., 2004]. Recently, giant embryo brown rice has become increasingly popular as it contains high nutrient contents and physicochemical traits compared to normal embryo brown rice [Zhang *et al*., 2005]. In particular, giant embryo brown rice has been reported to enhance γ-aminobutyric acid (GABA) content, which is a very useful component for the control high blood pressure and reduction of cholesterol in the blood [Wang and Oram, 2002], as a tranquilizer, and regulation of neuronal excitability [Saikusa *et al*., 1994].

It is widely recognized that free radical-induced oxidative stress is the essential cause for many human disease such as DNA damage, cancer, atherosclerosis, ageing, and inflammatory disorder [Hodzic *et al*., 2009]. Giant brown rice was reported a rich source of natural antioxidants including phenolic acid, flavonoid, tocopherol, and sterol compounds [Lee *et al*., 2007a]. Therefore, many rice breeding and functional metabolite researchers have shown increased interest in developing new giant embryo rice cultivars.

In a previous report, we reported that new giant embryo mutant rice (YR23517Acp79, YR) possesses new morphological traits as well as a high amount of nutrients [Park *et al*., 2009]. YR is appealing, not only because it represents a genetic basis for improving breeding of giant embryo rice, but also because it has increased nutritional quality. Choi *et al.* [2006a], reported that the giant embryo brown rice (Keunnunbyeo, KB) has higher lipid, free sugar, and mineral contents compared to normal embryo brown rice (Ilmibyeo, IB). However, YR, which has a higher total physicochemicals and nutrient content

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than that of reported KB, has not yet been investigated.

In the present study, the nutrient contents and physicochemical properties of YR brown rice are reported for the first time. Furthermore, the antioxidative effects of YR rice under various extract conditions and antioxidant assay systems without cytotoxicity were investigated. The newly measured physicochemical properties, nutrient contents, and biological activity of YR rice can be used to enhance health promotion and develop new functional rice.

Materials and Methods

Plant materials. Brown rice with new giant embryo rice (YR23517Acp79, YR), brown rice with giant embryo rice (KB), and normal embryo brown rice (IB) were grown at the National Institute of Crop Science (NICS), Rural Development Administration (RDA), Miryang, Korea during the 2009 growing season. All rice seeds were stored at 4°C and dehusked using a rice sheller (SY88-TH, Ssangyong Ltd, Incheon, Korea) for preparation of brown rice.

Reagents. GABA, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-Azino-*bis*-(3-ethylbenzthia-zoline-6-sulfonic acid) (ABTS), Quercetin, Gallic acid, Folin-Ciocalteu, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolidium bromide], and LPS (lipopolysaccharide) were purchased from Sigma (St. Louis, MO). Griess reagent kit was obtained from Promega (Madison, WI). All other reagents and solvents were of analytical grade.

Instruments. Extracts of grounded giant and normal embryo brown rice were centrifuged using a Vision apparatus (VS-30000MT, Vision, Bucheon, Korea). Morphological properties were observed by a scanning electron microscope (Hitachi-3000, Tokyo, Japan). Spectrophotometric experiments were measured using a Molecular Devices spectrophotometer (VersaMax, Sunnyvale, CA). Total protein contents of samples were measured according to the Kjeldahl method using a Kjeltec Auto Anlayzer. Lipid contents were measured by the Soxhlet method using the Buchi B-811 extraction system (Flawil, Switzerland) [Juliano, 1985]. The filtrate was evaporated in the round-bottom flask of a rotary evaporator (RE. IKA RV05basic 1B, Staufen, Germany) and gas chromatography-flame ionization detector (GC-FID) system (Agilent, Wilmington, DE) for fatty acid analysis. Total protein contents were measured by Kjeltec 8460 (FOSS, Hillerod, Denmark). Mineral composition and contents were determined by Inductively Coupled Plasma (ICP) (ELAN DRC II, Perkin Elmer, Shelton, CT). Total amino acid, GABA, tocopherol, and vitamin B_1 contents were determined by Ultra performance liquid chromatography (LC) and AccQtag Ultra system (Waters, Milford, MA). Free sugar contents were analyzed using a binary gradient high performance liquid chromatography (HPLC) pump (Waters 1529), by fluorescence detector (Waters 2475), and a refractive index detector (Waters 2414).

Extraction of brown rice. Giant embryo (YR, KB) and normal embryo (IB) rice were pulverized (100 mesh) using a grinder, and 1 kg of each powdered sample was sequentially extracted with ethyl acetate (EtOAc) (5 L), 70% ethanol (EtOH) (5 L), and methanol (MeOH) (5 L) for 5 days at room temperature. YR extracts were obtained from EtOAc, 70% EtOH, and MeOH fractions at 86.2, 115.7, 128.3 g, respectively. The KB extracts were obtained from EtOAc-(82.1 g), 70% EtOH-(103.8 g) and MeOH-(112.5 g) fractions. IB extracts were obtained from EtOAc-(81.6 g), 70% EtOH-(108.2 g) and MeOH-(114.0 g). All solvent extracts were calculated in mg/mL (ppm).

Scanning electron microscopy. YR, KB, and IB brown rice were fractured in the midregion using a razor blade by applying pressure to the top of the grain. The fractured giant and normal embryo brown rice grains and starch granular structures were examined using an environmental scanning electron microscope operated at 5.0 kV.

Analyses of protein, lipid, and amylose. YR, KB, and IB brown rice were pulverized (100 mesh) using a grinder. Two grams of pulverized seeds was added to 200 mL of *n*-hexane in an extraction thimble and boiled for 2 h at 110°C. After cooling to room temperature in a desiccator, the extracted oil was weighed. Total lipid contents were determined on a dry matter basis of barnyard millet grains. The amylose content was estimated by colorimetric analysis [Juliano, 1985]. Briefly, a paste was mixed for 15 min in a 100-mL flask using 500 mg of barnyard millet species grain in boiling water combined with 1 mL of 95% EtOH and 9 mL of 1 N NaOH. After cooling to room temperature, 100 mL of distilled water were added, and a 5-mL aliquot was transferred to a new flask. Subsequently, 1 mL of 1 N acetic acid and 2 mL of 2% I_2-KI solution were added, and the volume was raised up to 100 mL with distilled water. Absorbance was measured at 620 nm using a spectrophotometer.

Determination of Fatty acid. Each sample (500 mg) was placed in a test tube, and 10 mL of diethyl ether was added. The samples were placed in a shaking incubator (100 rpm) at 50°C for 2 days. The supernatant was transferred into a new test tube and then evaporated. The extracted oil (0.15 g) from each sample was placed in a vial, and 5 mL of methylation solution (H_2SO_4 :MeOH:

Toluene, 1:20:10, v/v) was added. The vial was then heated in a water bath (150°C) for 1 h and allowed to cool at room temperature. Five milliliters of distilled water was then added and shaken vigorously. The mixture was separated into two layers, and the upper layer was removed by a Pasteur pipette and dried using anhydrous Na_2SO_4 for 10 min. Subsequently, 1 mL of the removed sample solution was directly injected into the GC using an automatic sampler (Aglient 7683B). An Agilent 7890A gas chromatograph equipped with a flame ionization detector (FID) and high polarity-free fatty acid phase (HP-FFAP) capillary column (Agilent) was used. The oven temperature was raised from 150 to 230°C at a constant rate of 2.5°C/min. The injector and detector temperatures were kept at 250 and 230°C, respectively. The carrier gas was nitrogen at a flow rate of 1 mL/min, and the split ratio at the injector port was 50:1.

Analyses of GABA and free amino acid contents. Distilled water (2 mL) was added to the pulverized giant and normal brown rice (500 mg). The vapor-extracted samples were then dried, cooled, and centrifuged at 13,000 g for 10 min at 4°C. The supernatant was measured for concentrations of GABA and amino acids by the AccQ · Tag UPLC detection system (Waters). The sample was ultra-filtered using a SmartPor Syringe Filter, 25 mm, 0.2 μm (Woongki Ltd., Seoul, Korea). Once the filtrate had been diluted to a suitable concentration, fluorescence derivatization was performed following the Accq · Tag manufacturer's instruction. A 20-μL aliquot of sample solution, 60 μL of AccQ-fluor borate buffer, and 20 mL of AccQ-fluor reagent were mixed together, and the mixture was incubated for 5 min at 55°C. Separation of derivatized amino acids and GABA were performed on an AccQ · Tag Ultra column (2.1×100 mm, 1.7 mm, Waters) by gradient elution at 30°C. Two eluents were used: A (AccQ · Tag Ultra Eluent A, Waters), B (AccQ · Tag Ultra Eluent B, Waters). The samples were injected (0.5 μL injection volume) onto the column and eluted at 37°C at a flow rate of 0.4 mL/min according to the following gradient: initial 0% B; 0.54 min/0.1% B; 5.74 min/9.1% B; 7.74 min/21.2%B; 8.64 min/59.6% B; 9.50 min/0.1% B. The derivatized amino acids and GABA were detected by a photodiode array (PDA) eλ detector (Waters). The chromatography data was analyzed using Empower 2 software (Waters). To determine the concentrations of total amino acids and GABA, a standard solution containing known concentrations of GABA and total amino acid were analyzed with samples in every series of the analysis [Lee *et al*., 2007c].

Mineral content analysis. Exactly 1 g of each brown rice grain powder was digested with 5 mL of concentrated nitric acid at 100-150°C. The residual acid was evaporated, and 30 mL of distilled water was added, followed by analysis using an inductively coupled plasma (ICP) instrument. The amount of each trace element was measured based on the standard curves of Ca, Mg, and Fe as standard minerals.

Measurement of γ-oryzanol. Variations in γ-oryzanol content between giant embryo (YR, KB) and normal embryo (IB) brown rice were analyzed according to the method of Oh *et al*. [2009] with slight modification. One gram of each brown rice grain powder was extracted with 10 mL of propanol for 18 h at room temperature. The mixture was then filtered through a 0.45-μm syringe filter (Millipore MSI, Westboro, MA). HPLC analysis was performed using the Waters 1529 system with an octadecyle saline (ODS) column (4.6×150 mm, 3.5 μm BEH Technology, Milford, MA) at an absorbance of 330 nm, oven temperature at 30°C, and 20 μL injection. Mobil phase was H_2O/CH_3CN, isocratic at 15:85 (v/v) at a flow rate of 1.65 mL/min. γ-oryzanol was measured based on retention time and calculated by comparing the peak areas of the standard sample calibration curve.

Analyses of tocopherols and vitamin B_1 contents. A 500-mg rice powder sample was suspended in 20 mL of extraction solvent (MeOH/EtOAc/petroleum ether, 1:1:1, v/v), after which 1 mL of internal standard (α-tocopherol acetate) solution was added and mixed for 30 s. After cooling for 10 min, extracts were filtered three times through a filter paper (Whatman No. 4) and concentrated to 60 mL. Subsequently, 1.5 mL of saturated KOH were added to the filtrate and incubated for 18 h. After filtration, the sample was analyzed by HPLC (Waters 1529 system) under optimized conditions: (YMC-pack Silica, 4.6×150 mm, 5 μm, Waters), fluorescence detection (Waters 2475, excitation at 292 nm, emission at 327 nm) [Choi *et al*., 2006a]. The rice powder sample (500 mg) was suspended in 10 mL of extraction solvent (dilute with water) for 3 h with sonication. The extracts were then filtered three times through filter paper (Whatman No. 4) and analyzed by HPLC (Waters 1529 system) under optimized conditions; (Zorbax SB-C8, 4.6×150 mm, Agilent), UV/Vis wavelength at 245 nm [Capanoglu *et al*., 2008].

Determination of total phenolic content. Total phenolic content in each of giant embryo (YR, KB) and normal embryo (IB) rice were measured according to the modified Folin-Denis method [Choi *et al*., 2006b]. Briefly, Folin-Ciocalteu reagent (0.5 mL) and 10% Na_2CO_3 solution (0.5 mL) was added to each extract (1 mL) and mixed vigorously. After 5 min, the absorbance was measured at 724 nm. The total phenolic content of each extract was calculated in mg of gallic acid equivalents (mg GAE/g extract).

DPPH radical and trolox equivalent antioxidant capacity (TEAC) scavenging activities. Antioxidant activities of giant embryo (YR, KB) and normal embryo (IB) brown rice crude extracts were measured based on the scavenging activity of stable DPPH free radical following the method described by Lee *et al*. [2007b]. Various concentrations of crude extracts were added to 0.15 mM DPPH ethanol solution. The reaction mixture was then shaken for 30 min, after which the absorbance was measured at 517 nm. TEAC assay is based on the relative ability of antioxidants to scavenge $ABTS^{\bullet+}$ radical cation compared to a standard (Trolox) [Re *et al*., 1999]. Radical cation was prepared by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate. The reaction mixture was maintained for 6-8 h to achieve thorough mixing and the absorbance reached a stable value. $ABTS^{\bullet+}$ solution was diluted with ethanol, and the absorbance was read at 734 nm. Then, 0.9 mL of $ABTS^{\bullet+}$ solution and 0.1 mL of crude extracts solution were mixed together for 30 s, and the absorbance was measured immediately after 1 min at 734 nm. Antioxidant activity of each compound was calculated by determining the decrease in absorbance at different concentrations.

Cell culture and viability assay. Raw 264.7 cells (murine macrophage cell line) were obtained from American Type Culture Collection (Manassas, VA) and were grown in Dulbecco's modified minimum essential medium (DMEM) (Invitrogen, NY) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen, CA), L-glutamine (2.5 mM), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (40 mM; Biopure, Cambridge, CA), and antibiotics [penicillin (50 U/mL) and streptomycin (50 g/mL)] (Gibco, Invitrogen, Carlsbad, CA)] at 37°C in a 5% CO_2 humidified atmosphere [Ando *et al*., 1993]. After treatment with rice extracts, cells were assayed for growth activity by the MTT (Sigma) based colorimetric method as previously described [Seo *et al*., 2010]. Briefly, cells were seeded at densities of 5×10^3 cells/well in 96-well culture plates. Cells were treated with rice extracts for 72 h. The attached cells were incubated with MTT (0.5 mg/mL, 3 h) and subsequently solubilized in dimethyl sulfoxide (DMSO). The absorbance at 550 nm was then measured using a microplate reader.

Measurement of nitric oxide (NO) scavenging activity. Raw 264.7 cells (4×10^5 cells/well) in a 24-well plate were treated with or without a 70% ethanol rice extraction mixture (0.2 mg/mL) for 3 h, followed by incubation with 1 μg/mL of LPS for 24 h. The amount of nitrite was determined based on the amount that was measured in the Raw 264.7 cell culture supernatant using the Griess reagent system according to the manufacturer's instructions (Promega) [Chung *et al*., 1997].

Statistical analysis and curve fitting. All measurements were repeated three times, and the results were reported as the mean ± SD. Data analysis and curve fitting were performed using the Sigma Plot program 2001 (Systat Software Inc., Chicago, IL).

Results and Discussion

Images of new giant embryo brown rice (YR23517Acp79: YR), reported giant embryo brown rice (KB), and normal embryo brown rice (IB) are shown in Fig. 1. The photo of YR rice (A) clearly shows enlarged embryos compared to those of KB (B) and IB (C) rice. The single embryo weights of YR, KB, and IB rice were 2.48±0.26, 1.76±0.18, and 1.18±0.22 mg, respectively. On the other hand, the single-grain weight of YR (14.78±1.25) rice was lighter than those of KB (22.16±1.58) and IB (21.78±1.55) rice (Table 2). Furthermore, the embryo volume of YR rice was approximately 3 and 2 times larger compared to those of IB and KB rice. The contents of GABA in YR rice were 2-4 times higher than those of KB and IB rice. The amount of GABA in YR rice was proportional to an embryo size (Table 6). Therefore, TY rice has a good potential for functional rice in terms of GABA.

Morphological properties. Scanning electron micrographs of starch granules from giant embryo (YR, KB) and normal embryo (IB) rice are shown in Fig. 2. The endosperm region is composed of cell wall substances

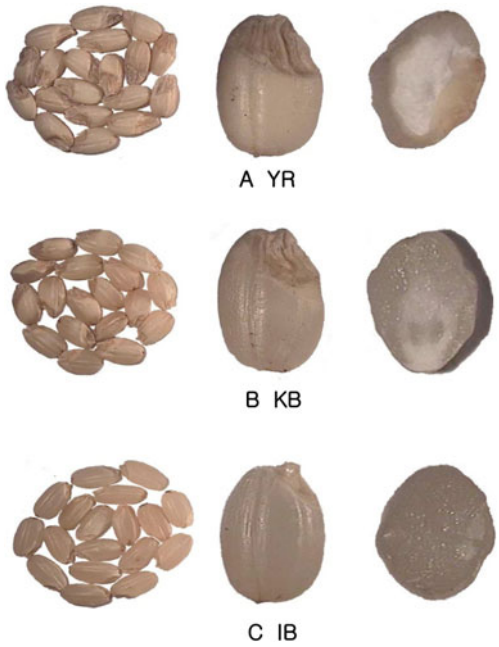

Fig. 1. Cross-sectional micrographs of whole grain (left, middle) giant embryo (YR, KB), and normal embryo (IB) brown rice. A, YR; B, KB; C, IB.

and starch granules, which cluster into amyloplasts or compound starch granules. Normally, non-glutinous rice starch granules display large polyhedral and irregularly-shaped granules [Kang *et al*., 2006]. On the other hand, glutinous rice starch is comprised of polygonal-shaped granules with a non-porous and smooth surface [Laovachirasuwan *et al*., 2010]. Therefore, starch granules in YR rice, which shows glutinous traits, have a smaller crystal and polygonal structure than those of other non-glutinous rice granules (KB, IB). These variations in starch granule morphology may be due to biological origin and physiology. Furthermore, variations in amylose and amylopectin contents of giant embryo (YR, KB) and normal embryo (IB) rice could affect starch granule size and shape.

Protein, lipid, and amylose contents. Proteins and lipids are important nutritional sources in cereals [Zhou *et al*., 2002]. The protein, lipid, and amylose contents of giant embryo (YR, KB) and normal embryo (IB) brown rice are shown in Table 1. YR rice contained the highest average protein (9.3±0.3) and lipid (3.7±0.4) contents. The amylase content of rice is the critical factor of estimating rice quality in terms of cooking and pasting properties. As determined by the Juliano method [Laovachirasuwan *et al*., 2010], amylose contents of giant embryo (YR, KB) and normal embryo (IB) brown rice were 5.5±0.6 (YR), 14.7±0.8 (KB), and 16.5±0.5% (IB). Consequently, YR rice has a good potential to improve the processing property of a glutinous traditional Asian dessert.

Table 1. Comparison of the protein, lipid, and amylose contents of giant embryo (YR, KB) and normal embryo (IB) brown rice[1)]

Rice varieties	Compositions (%)		
	Protein[1)]	Lipid[1)]	Amylose[1)]
YR	9.3±0.3	3.7±0.4	5.5±0.6
KB	8.1±0.1	3.3±0.2	14.7±0.8
IB	7.4±0.2	2.3±0.1	16.5±0.5

[1)]The values indicate the mean ± SD (n=3) of each sample.

Analysis of fatty acid composition. The lipid content of some plants is regarded as a useful quality due to the presence of unsaturated fatty acids, such as oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids [Yoshida *et al*., 2011]. These unsaturated fatty acids possess many biological activities, such as reduction of cholesterol levels in the human blood [Wang and Oram, 2002] and enhancement of long-term potentiation [Honore *et al*., 1994]. These three unsaturated fatty acids accounted for more than 75% of the total fatty acid contents in giant embryo (YR, KB) and normal embryo (IB) brown rice (Table 3). YR brown rice exhibited a linoleic acid content of 38.3±2.8%, which was significantly higher than those of the other rice varieties. Kang *et al.* [2010] reported that oleic and linoleic acids were the main fatty acids in Korean glutinous rice varieties. In the case of giant embryo (YR) rice, the contents of oleic acid (C18:1) and

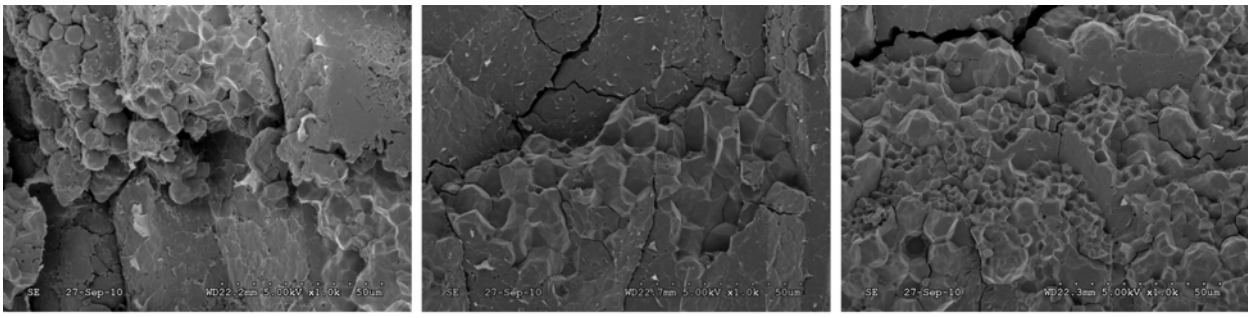

Fig. 2. Scanning electron micrographs of cross-sectioned giant embryo (YR, KB) and normal embryo (IB) brown rice kernel endosperm. A, YR; B, KB; C, IB.

Table 2. Comparison of the embryo weight of giant embryo (YR, KB) and normal embryo (IB) brown rice

Rice varieties	Single grain weight (mg)	Single embryo weight (mg)	Ratio of the embryo to single grain (%)
YR	14.78±1.25	2.48±0.26	16.78±0.34
KB	22.16±1.58	1.76±0.18	7.94±0.23
IB	21.78±1.55	1.18±0.22	5.41±0.48

The values indicate the mean ± SD (n=3) of each sample.

linoleic acid (C18:2) were 41.5±4.5 and 38.3±2.8%, respectively, indicating YR rice contained higher protein and lipid contents than those of normal brown rice.

Analysis of amino acid composition. Protein is the most abundant component in crop grain, and the nutritional quality of a protein depends on its amino acid composition and digestibility [Kang *et al.*, 2010]. The amino acid compositions of giant embryo (YR, KB) and normal embryo (IB) brown rice are presented in Table 4. Glutamic acid (Glu) was the most abundant amino acid in all rice grain varieties with concentrations ranging between 217.41±1.5 - 220.69±1.5 mg/g, followed by asparatic acid, alanine, and tyrosine, with concentrations of 116.52±2.1 - 132.81±1.4, 30.83±2.0 - 34.17±0.7, and 19.15±1.4 - 31.84±2.8 mg/g, respectively. On the other hand, cysteine and leucine were the least abundant amino acids. YR rice contained 663.28±1.9 mg/g of total amino acids, which was the highest value among the giant embryo rice samples. The above results indicate that YR rice variety has superior nutritional quality compared with other rice grains in terms of amino acid composition.

Tocopherol, vitamin B_1, and mineral contents. Tocopherols are a class of bioactive compounds that possess vitamin E. Tocopherols consist of alpha, beta, gamma, and delta forms, which are determined by the number and positions of methyl groups on the chromanol ring moiety. In particular, α-tocopherol is the main form found in supplements and in brown rice grains. It reportedly possesses many biological activities including antioxidative effects and prevention of Alzheimer's disease. [Frank and Gupta, 2005]. Tocopherol contents of giant embryo (YR, KB) and normal embryo (IB) brown

Table 3. Comparision for fatty acid of new giant embryo (YR, KB) and normal embryo (IB) brown rice

Rice varieties	Fatty acid content (%)[1)]						
	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)	Linolenic acid (C18:3)	SF*	USF**
YR	16.4±1.7	2.1±0.2	41.5±4.5	38.3±2.8	1.6±0.2	21.5±1.5	79.8±5.3
KB	18.4±2.1	4.6±0.6	42.7±5.1	33.0±5.9	1.3±0.2	23.0±2.3	75.7±5.6
IB	18.1±1.1	1.8±0.4	42.3±2.8	36.6±4.1	1.3±0.2	19.9±2.1	78.9±6.2

[1)]The values indicate the means of three replications of the experiment fatty acid content of each sample (mean ± SD, n=3)
*SF: saturated fatty acid, **USF: unsaturated fatty acid.

Table 4. Amino acid contents of giant embryo (YR, KB) and normal embryo (IB) brown rice[1)]

Amino acid	Amino acid contents (mg/100 g)		
	YR23517Acp79 (YR)	Keunnunbyeo (KB)	Ilmibyeo (IB)
Asp	132.81±1.4 (20.01%)	118.21±1.7 (19.34%)	116.52±2.1 (19.50%)
Thre	19.67±1.9 (2.96%)	16.56±1.6 (2.71%)	14.16±2.2 (2.37%)
Ser	15.76±2.1 (2.38%)	13.40±2.1 (2.19%)	11.92±1.2 (2.00%)
Glu	220.69±1.5 (33.27%)	218.89±2.2 (35.82%)	217.41±1.5 (36.38%)
Pro	28.68±1.1 (4.33%)	26.34±1.8 (4.31%)	27.27±1.4 (4.57%)
Gly	22.22±0.8 (3.35%)	20.45±2.1 (3.35%)	19.03±2.1 (3.18%)
Ala	34.17±0.7 (5.16%)	32.17±2.9 (5.26%)	30.83±2.0 (5.16%)
Cys	5.08±1.8 (0.77%)	1.70±1.8 (0.28%)	1.72±1.7 (0.29%)
Val	17.16±2.1 (2.59%)	15.32±1.2 (2.51%)	13.79±0.9 (2.31%)
Met	11.09±1.5 (1.67%)	14.69±1.5 (2.40%)	18.91±0.8 (3.16%)
Ile	9.69±2.4 (1.46%)	6.54±2.8 (1.07%)	15.53±0.8 (2.60%)
Leu	8.37±4.5 (1.26%)	6.88±1.7 (1.13%)	15.66±0.5 (2.62%)
Tyr	31.84±2.8 (4.81%)	29.19±1.6 (4.77%)	19.15±1.4 (3.20%)
Phe	24.40±2.1 (3.68%)	21.55±0.8 (3.53%)	19.16±1.8 (3.20%)
His	27.88±1.7 (4.20%)	22.69±0.8 (3.71%)	19.14±1.7 (3.20%)
Lys	25.89±1.5 (3.90%)	21.60±2.7 (3.53%)	17.62±0.6 (2.95%)
Arg	27.88±2.2 (4.20%)	24.97±1.5 (4.09%)	19.80±2.1 (3.31%)
Total	663.28±1.9 (100%)	611.15±1.8 (100%)	597.62±1.5 (100%)

[1)]The values indicate the means ± SD (n=3) of each sample. Means followed by different superscripts within the column are significantly different (p <0.05).

rice are shown in Table 5. YR rice contained 2.84±0.05, 2.68±0.1, and 0.11±0.001 mg/100 g total tocopherols, α-tocopherol, and β-tocopherol, which were about 2.7, 2.8, and 5.1 times higher than those of other rice varieties, respectively. It is well-known that vitamin B_1 (thiamine) is required for prevention of alcoholic brain disease [Martin *et al*., 2003]. YR rice also contained a vitamin B_1 content of 6.42±0.3 mg/kg, which was significantly higher than those of other rice varieties. The mineral contents of Ca, Fe, and Mg in each rice grain varieties are summarized in Table 5. The mean content values of giant embryo (YR, KB) and normal embryo (IB) brown rice were Mg > Ca > Fe, and these were the most abundant minerals found in this rice. YR rice contained the highest average contents of Mg (1417.5±13.6 mg/kg) and Fe (22.6±0.6 mg/kg), whereas IR rice contained the lowest average amounts of Ca (164.0±5.2 mg/kg) and Fe (30.3±0.8 mg/kg). It is widely recognized that giant embryo is enriched with tocopherols, minerals and GABA in pre-germinated seeds. Based on the results, YR giant embryo brown rice could enhance total tocopherols, mineral, vitamin B_1 contents, because it has the largest embryo size.

Changes in GABA during germination and γ-oryzanol contents. GABA is reportedly the main inhibitory neurotransmitter in the human nervous system and plays a role in regulating neuronal excitability, blood pressure [Watanabe *et al*., 2002]. Moreover, GABA is enhanced in germinated brown rice in comparison with non-germinated brown rice. To determine the effects of various germinated conditions, GABA contents in giant embryo (YR, KB) and normal embryo (IB) brown rice were analyzed, and the results are shown in Table 6. Among the three rice varieties, YR rice exhibited the highest GABA content and variation (0.46±0.014 → 2.95±0.011 mg/g, 2.2~4.8 times) during germination compared to other rice varieties. These results suggest that GABA was significantly influenced by germination conditions, such as rice germination time and length. In addition, these results indicated that germination process can increase GABA concentrations in brown rice seeds, possibly because the plant germination induces metabolic change in seeds, and some secondary metabolites were broken down after hydration, which result in an accumulation of a soluble nitrogen compounds such as GABA. γ-Oryzanol is mainly comprised of a mixture of substances derived from rice bran oil, including sterols and ferulic acid. It is widely used as an antioxidant supplement as well as for reducing cholesterol levels and preventing blood hypertension [Juliano *et al*., 2005]. Regarding the above-mentioned health benefits, many researchers have concentrated on producing γ-oryzanol-rich rice materials. YR rice had the highest total γ-oryzanol content (0.43±0.021 mg/g) compared to other rice varieties (KB: 0.32±0.028, IB: 0.23±0.031 mg/g) (Table 6).

Radical-scavenging activity. DPPH- and ABTS-radical scavenging tests are commonly used to determine the total antioxidant status of various specimens based on their reproducibility and ease of quality control. To determine the antioxidant activities of giant embryo (YR, KB) and normal embryo (IB) brown rice, extractions with EtOAc, 70% EtOH, and MeOH were carried out. Table 7 shows the DPPH- and ABTS-radical scavenging activities of various rice crude extracts. The EtOAc fraction showed potent DPPH- and ABTS-radical scavenging

Table 5. Vitamin B_1, tocopherols, and minerals of giant embryo (YR, KB) and normal embryo (IB) brown rice[1)]

Rice varieties	Vitamin B_1 (mg/kg)	Tocopherol (mg/100 g)				Minerals (mg/kg)		
		α-tocopherol	β-tocopherol	γ-tocopherol	Total[2)]	Ca	Mg	Fe
YR	6.42±0.3	2.68±0.1	0.11±0.01	0.05±0.001	2.84±0.05	284.0±6.2	1417.5±13.6	22.6±0.6
KB	4.82±0.2	0.98±0.05	0.02±0.02	0.02±0.001	1.02±0.03	167.1±4.8	1294.0±11.8	29.6±1.1
IB	4.87±0.2	0.95±0.04	0.05±0.01	0.09±0.001	1.09±0.02	164.0±5.2	875.0±14.3	30.3±0.8

[1)]The values indicate the mean ± SD (n=3) of each sample
[2)]α-tocopherol+β-tocopherol+γ-tocopherol.

Table 6. Variation of GABA and γ-oryzanol content of giant embryo (YR, KB) and normal embryo (IB) brown rice[1)]

Rice varieties	GABA (mg/g)			γ-oryzanol (mg/g)
	Brown rice	Germinated rice (48 h)	Germinated rice (1 cm)	
YR	0.46±0.014	1.76±0.021	2.95±0.011	0.43±0.021
KB	0.22±0.008	0.87±0.017	1.12±0.009	0.32±0.028
IB	0.04±0.003	0.44±0.009	0.57±0.006	0.23±0.031

[1)]The values indicate the mean ± SD (n=3) of each sample.

Table 7. Antioxidant effects of solvent fractions from giant embryo (YR, KB) and normal embryo (IB) brown rice[1)]

Rice extracts	Total phenolic contents (mg GA[2)]/g)	DPPH radical scavenging assay[3)] (%)			ABTS radical scavenging assay[3)] (%)		
		EtOAC	70% EtOH	MeOH	EtOAC	70% EtOH	MeOH
YR	1.94±0.03	57.1±2.2[1)]	44.3±1.5	41.2±1.5	50.2±1.4	45.2±1.6	41.7±1.3
KB	1.52±0.02	51.9±1.9	32.9±1.7	40.4±1.8	24.5±1.2	38.2±1.8	31.2±1.4
IB	0.62±0.02	42.2±1.7	34.8±1.6	35.2±1.5	16.8±1.8	32.2±1.6	38.5±1.5

[1)]Effective activity indicate the mean ± SD (n=3) of each sample
[2)]Gallic acid (GA) was used as a standard for measuring of the total phenolic content.
[3)]Concentration of all rice extracts were treated 0.2 mg/mL

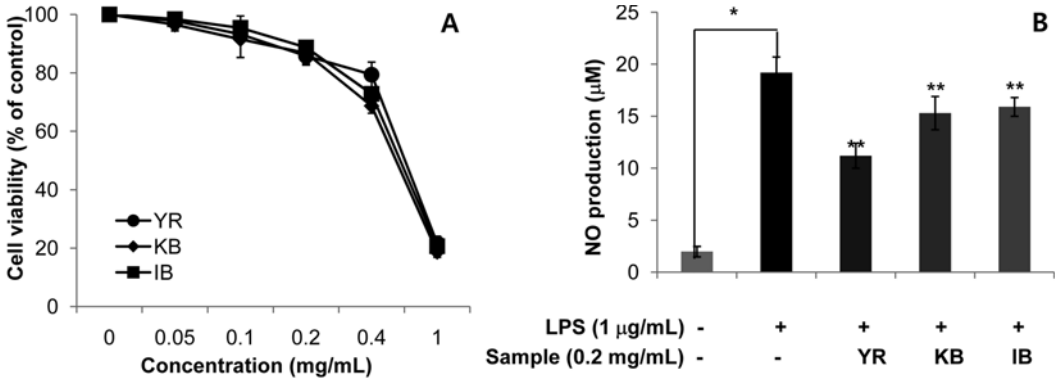

Fig. 3. NO scavenging activity of giant embryo (YR, KB) and normal embryo (IB) brown rice. (A) Cell viabilities of the methanol extracts in different rice cultivars; (B) The productions of LPS-induced nitric oxide from the methanol extracts of different rice cultivars.

activities of 57.1±2.25 and 50.2±1.45 at 0.2 mg/mL, whereas the MeOH fraction showed the lowest DPPH- and ABTS-radical scavenging activities 41.2±1.58 and 41.7±1.33 at 0.2 mg/mL, respectively. In the two antioxidant assays, the EtOAc fraction of YR rice exhibited the strongest free radical scavenging activities against DPPH and ABTS compared to the other brown rice crude extracts.

NO production inhibitory activity. Nitric oxide (NO) is a reactive metabolite generated by nitric oxide synthase (NOS). Activated macrophases release NO, a toxic radical species that causes DNA mutation, cell apoptosis, and leads to metabolic diseases such as cancer and cardiovascular disease [Hsu *et al*., 2010]. To evaluate whether or not giant embryo (YR, KB) and normal embryo (IB) brown rice could inhibit LPS-induced NO generation in RAW 64.7 cells, we investigated NO production using Griess reaction assay. It is well known that LPS upregulates ROS and NO production through inducible nitric oxide synthase (iNOS) induction [Han *et al*., 2010]. As shown in Fig. 3, exposure of the cells to 1 g/mL of LPS increased the NO content (19.2±1.2 μM) of the cells by more than 8 fold. However, upon treatment with the giant embryo (YR, KB) and normal embryo (IB) brown rice extracts, the NO content of the LPS stimulated-cells was reduced. In particular, YR rice extract showed the highest inhibition of LPS-induced NO production (11.8±1.1 μM) with having low cytotoxicity. It has recently been reported that phenolic compounds have a greater nitrate scavening effect in many rice cultivars [Rao *et al*., 2010]. YR brown rice extracts exhibited the highest polyphenolic content compared to other rice varieties.

In the present study, new giant embryo rice (YR) with wild type normal and giant embryo rice (IB, KB) were selected for comparative nutrient content analysis and measurement of antioxidant activities. YR rice is considered as a latent trait for quality enhancement in rice research as it has high quality components compared to other normal embryo rice grain varieties [Lee *et al*., 2007c]. Compared to IB rice, obvious increases in protein, lipid, fatty acid, vitamin B_1, vitamin E, mineral, and total amino acid contents were detected in YR rice. Increases in mineral elements such as Ca and Mg and essential amino acids would help solve malnutrition problems. YR rice contained approximately 2-3 fold higher GABA content (Table 4) compared to those of KB and IB rice. Furthermore, YR rice also showed increased γ-oryzanol contents of about 1.5-2 fold (Table 4) compared to those of KB and IB rice. Evaluation of the

antioxidant capacity of functional quality rice is gaining more attention since it was found that phytochemicals are effective antioxidants. Amongst giant embryo (YR, KB) and normal embryo (IB) brown rice, YR extracts were the most effective in free radical scavenging of DPPH and ABTS. Indeed, antioxidative activities of brown rice-related compounds have been reported. YR rice has about a 3 fold higher ratio of embryo to single grain compared to normal rice and approximately 2 fold higher ratio compared to previously reported Keunnun (KB) rice. These results suggest that new giant embryo mutant rice (YR) possesses more antioxidative effects and health benefits due to its high nutrition components, γ-oryzanol, vitamin E, and GABA. Therefore, YR rice could be used in the new functional food industry, and consumption of these products could afford health benefits to rice consumers.

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