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Determination of Optimal Harvest Time of Chuchung Variety Green Rice® (*Oryza sativa* L.) with High Contents of GABA, γ-Oryzanol, and α-Tocopherol

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ABSTRACT: In our previous study, an early-maturing variety of rice (*Oryza sativa* L.), Jinbu can have feature with unique green color, various phytochemicals as well as nutritive components by the optimal early harvesting, called Green Rice® (GR). The aims of the present field experiments were to evaluate the changes in the weight of 1,000 kernels, yield, and contents of proximate and bioactive compounds in Chuchung, a mid-late maturing variety, during the pre-harvest maturation of rough rice and to research the appropriate harvest time and potent bioactivity of Chuchung GR. The weights of 1,000 kernels of Chuchung GR dramatically increased until 27 days after heading (DAH). The yields of Chuchung GR declined after 27 DAH and significantly declined to 0.0% after 45 DAH. The caloric value and total mineral contents were higher in the GR than in the full ripe stage, the brown rice (BR). In the GR, the contents of bioactive compounds, such as γ-aminobutyric acid, γ-oryzanol, and α-tocopherol, were much higher ($P<0.05$) than those in the BR, specifically during 24～27 DAH. Therefore, bioactive Chuchung GR can be produced with a reasonable yield at 24～27 DAH and it could be useful for applications in various nutritive and functional food products.

Keywords: Green Rice®, γ-aminobutyric acid, γ-oryzanol, α-tocopherol

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for over half of the world's population (1). The rice quality depends on the time of harvesting as significant changes in bioactive compound contents occur during the grain development and maturation period (2,3). In general, the ideal harvesting time of rice is determined based on both, the head rice yield and the physicochemical and nutritional characteristics such as moisture content, protein content, milling suitability, and taste (4-6). The average rice crop in Korea is ready for harvest at 40～50 days after heading (DAH) in terms of taste and at 45～55 DAH in terms of its milling and physicochemical characteristics (7). If harvested too early, the head rice yield is lower, and the moisture, protein, and amylose contents becomes high (8-10).

In some areas, however, immature rice is used for many rice products because of its nutritional and physicochemical properties (2). Evidence indicates that developing grains have higher contents of phytochemicals such as oryzanols, tocopherols, and phenolic compounds, than do fully mature grains (2,3). It is thus important to determine the changes in these physicochemical characteristics during the pre-harvest maturation period.

We described certain immature rice as Green Rice® (GR). GR is rice (*Oryza sativa* L.) that is harvested approximately 14～30 days before the transition from the light-green ripe stage to the full ripe stage, which is known as brown rice (BR). Results from our previous study suggested that the optimum harvest time of Jinbu GR, an early-maturing variety, would be 23 DAH based on the yield, color intensity, proximate, and minerals contents (11). However, little is known about Chuchung GR, a mid-late maturing variety.

The objectives of this study were to investigate the changes of bioactive compounds as well as proximate contents and mineral contents of GR and BR, and to determine the appropriate harvest time of Chuchung GR by measuring the weight of 1,000 kernels at different

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DAH.

MATERIALS AND METHODS

Rice varieties

The rice samples used in this study, Chuchung and Jinbu, are representative varieties of short grains in Korea and were obtained from the Korean seed and variety service (Gyeonggi, Korea). Chuchung and Jinbu are varieties of mid-late-maturing and early-maturing, respectively.

Sampling

The field experiments of Chuchung were conducted in 2004 and 2005 and Jinbu in 2004 at a certified farm in Gyeonggi, Korea, with natural illumination. The mean temperatures in 2004 and 2005 were 21.03°C and 21.13 °C, respectively with 1,217.0 mm and 1,427.7 mm of annual precipitation. The seedlings were grown in seedling boxes for 25 days and then transplanted into the experimental paddy (30×14 cm) by hand on June 1st of each year. The rice samples of 30 kg were harvested each time directly from the field by hand. The samples of Chuchung were harvested 8 times from 17 DAH to 45 DAH in 2004 and 5 times from 21 DAH to 45 DAH in 2005. The samples of Jinbu were harvested 6 times from 20 DAH to 42 DAH in 2004. The grain samples were dried to 15% moisture content by ambient air with a drying ratio of 0.5%/h. The dried paddy grains were de-hulled with a test husker (Satake Engineering Co., Hiroshima, Japan).

The yield of GR

The total yield of GR was measured as the ratio of GR among the initial grain sample of 200 g, and the means were compared using Duncan's multiple range test. GR was sorted manually, and the weights of 1,000 kernels of BR and GR were weighed 3 times by an electronic balance (Sartorius, Goettingen, Germany) with accuracy of 0.01 g.

Proximate and mineral analyses

The de-hulled grain samples were ground to pass through a 100-mesh screen. The ground rice powder was then used for proximate, mineral and bioactive compound analyses. The moisture content was measured by drying the grains in an oven at 105°C for 12 h, and the ash content was measured by the dry ashing method at 550°C for 5 h. The crude fat was measured using a Soxhlet apparatus with ether as the solvent. The crude protein was assessed by nitrogen determination (N×5.95) using the Kjeldahl method, and the carbohydrate content was determined using the formula difference method. All methods are described in the AOAC guidelines (12).

The mineral contents, i.e., potassium, magnesium, calcium, and sodium, were determined using inductively coupled plasma (ICP) spectrometry (Horiba Jobin Yvon Inc., Edison, NJ, USA) as described by Itani et al. (13). Briefly, the samples were wet digested and transferred to a 100 mL volumetric flask, distilled water was added to 100 mL, and the contents were filtered and analyzed using ICP for the estimation of each element.

Bioactive compounds analyses

The γ-aminobutyric acid (GABA) was content was determined using the procedures of Baum et al. (14) with some modifications. Ground rice powder (200 mg) was added to 800 μL of a mixture of methanol : chloroform : water=(12:5:3, v/v/v), and the solution was vortexed and centrifuged at 12,000 *g* at 4°C for 15 min. The supernatant was collected in a flask, and the residue was extracted again with a chloroform : water (3:5, v/v) solution (800 μL). The second supernatant was combined with the first supernatant. The collected sample was freeze-dried and re-dissolved in water. Then the sample was passed through 0.45 μm filters and analyzed using an amino acid analysis system (Waters, Milford, MA, USA) after the derivatization with 6-aminoquinonly-*N*-hydrocysuccinimidyl carbonate, as described by Cohen and Michaud (15).

γ-Oryzanol and tocopherols were extracted and analyzed based on the method reported by Heinemann et al. (16) with some modifications. Rice powder (5 g) was extracted with 50 mL of dichloromethane : methanol (2:1, v/v) at 40°C for 20 min by ultrasonic cleaner (Kodo, Seoul, Korea) and then filtered. Samples were extracted 3 times using this process, but the amount of solvent was 30 mL the 2nd time and 20 mL the last time. The sum of extracts was evaporated using a rotary vacuum evaporator (Tokyo Rikakikai Co., Tokyo, Japan) at 40°C. Residual materials were then dissolved with 1 mL methanol at 40°C for 5 min by ultrasonic and filtered 2 times using a syringe filter (Millipore, Billerica, MA, USA). The concentration of γ-oryzanol was determined spectrophotometrically by determining UV absorbance at 315 nm in a 10 mm cell of the solution in chloroform in a JASCO UV-900 instrument (JASCO Corporation, Tokyo, Japan), followed by calculations using the specific extinction coefficient 358.9 (17,18). The analytical reference standard for γ-oryzanol was obtained from Il-Dong Pharmaceutical Company (Seoul, Korea).

The tocopherols in the rice sample were purified using normal-phase high-performance liquid chromatography (HPLC) with a SUPELCOSIL™ LC-Si HPLC column, (250×4.6 mm; Supelco Inc., Bellefonte, PA, USA) (19). The mobile phase consisted of 99% hexane, 0.5% ethyl acetate, and 0.5% acetic acid. The flow rate was 1.8 mL/min. The excitation and emission wavelengths of the fluorescence detector were set at 290 and 330 nm, respectively.

The total flavonoid content was determined with a procedure using 10% diethylene glycol (20). The 50% aqueous methanol rice extract rice infusion mixture (100 μL) was homogenized and reacted at 37°C for 60 min, and the absorbance at 420 nm was measured. The total flavonoid content was expressed as naringin equivalents.

To extract the phenolic acid fraction, the rice bran was defatted using *n*-hexane and subjected to extraction using the method described by Krzysztof et al. (21). In brief, 500 g of defatted rice bran was suspended in 1 N NaOH at room temperature under a nitrogen atmosphere. The suspension was acidified to pH 1.5∼2.5 by the gradual addition of HCl and extracted 3 times with acetate. The ethyl acetate fraction was evaporated at 37°C and analyzed using an HPLC instrument (JASCO Corporation) equipped with a UV detector and a μ-Bondapack C_{18} column (3.9×300 mm internal diameter; Waters). The mobile phases were 0.05% phosphoric acid (solvent A) and methanol (solvent B), and the percentage of solvent B was increased in a gradient from 2 to 50% over a 45 min elution. The chromatography was performed at a flow rate of 0.5 mL/min, and the injection volume was 20 μL. The absorbance at 280 nm was determined.

The total phenolic content of the extracts was determined according to the Folin-Ciocalteu method (22). The ethyl acetate fraction (200 μL) was mixed with 1.0 mL of 50% Folin-Ciocalteu reagent and 0.8 mL of 2% Na_2CO_3 solution and then allowed to stand for 30 min at room temperature. The absorbance at 760 nm was measured, and the total phenolic levels were expressed as catechin equivalents.

Statistical analysis

Each experiment comprised 3 replicates. The statistical analysis was performed using SPSS (version 18.0, SPSS Inc., Chicago, IL, USA). The differences between individual rice samples were assessed using one-way ANOVA. All of the data are expressed as the mean±SD, and the differences between the samples were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The changes in 1,000 kernels weight and GR yield in maturing Chuchung

Fig. 1 shows the changes in the weight of 1,000 kernels according to rice varieties and years. The weight of 1,000 kernels of Chuchung in 2004 was 14.17 g on the 17 DAH and increased dramatically to 18.92 g on the 21 DAH, but hereafter it slowly increased. The weight of 1,000 kernels of Chuchung in 2005 was 21.23 g on the 24 DAH, and it gently increased to 21.77 g on the 27 DAH. In the case of Jinbu, an early-maturing variety, the weight of 1,000 kernels was 17.68 g on the 20 DAH and increased dramatically to 22.44 g on the 30 DAH, but it gently increased on the 40 DAH.

Fig. 2 shows the changes in the yield of GR according to rice varieties and years. The yield of Chuchung GR in 2004 was in the range of 98.99∼83.84% from the 17 DAH to 26 DAH, and after 31 DAH, it dramatically decreased. In 2005, the yield of Chuchung GR was 80.6% on the 24 DAH, 76.9% on the 27 DAH, and it rapidly decreased hereafter. For the Jinbu variety, the yield of GR rapidly decreased from 82.67% on the 23 DAH to 53.2% on the 26 DAH.

In our preliminary experiment with the rice samples produced in 2004 and 2005, the weight of 1,000 kernels, and the yield of GR of the mid-late-maturing variety of Chuchung were somewhat lower than those of the early-maturing variety of Jinbu. In both varieties, the weight of 1,000 kernels increased, and the yield of GR decreased with increasing DAH. In the 2004 study, the

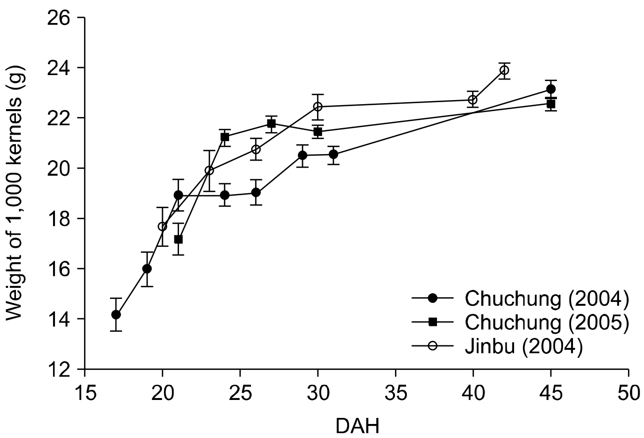

Fig. 1. The changes of the weight of 1,000 kernels for Chuchung and Jinbu rice according to days after heading (DAH).

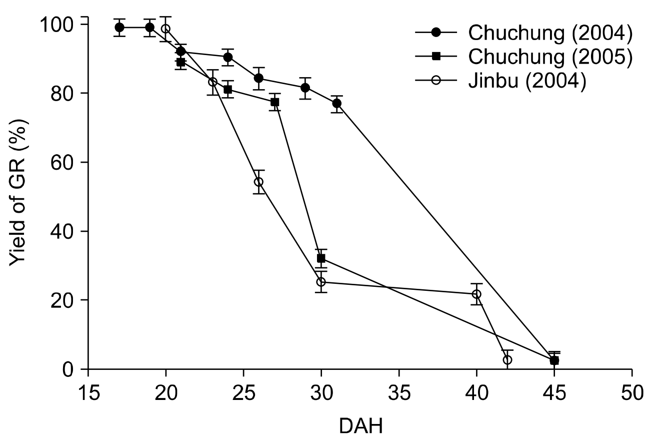

Fig. 2. The changes of the yield of Green Rice® (GR) for Chuchung and Jinbu rice according to days after heading (DAH).

yield of GR was over 98.9% until the 19 DAH and decreased to 90.2% and 81.0% on the 25 and 29 DAH, respectively (data not shown). The weight of 1,000 kernels was 12.5 g on the 15 DAH and 16.0 g on the 19 DAH (data not shown), suggesting a relatively large increase during the early period after heading. The weight increased relatively slowly thereafter until the 31 DAH. Also, the yield of Chuchung GR dramatically decreased after 27 DAH, and the weight of 1,000 kernels rapidly increased until 24 DAH. Based on these data, the optimal range of harvesting time for Chuchung GR is on the 24~27 DAH, approximately 2~3 d later than that for the early-maturing variety, Jinbu (22~24 DAH) (24). Lin and Lai (2) reported that immature grains from *Japonica* rice cultivated in a phytotron are suitable for harvest between the 15 and 18 DAH for food applications. However, the yield of the rice grains is also important for practical use, and the environmental conditions under which the rice is grown should be considered to determine the suitable harvest time of the grains.

The changes in the proximate and mineral contents in maturing Chuchung

Table 1 shows the results of the proximate composition analyses of the Chuchung GR according to the DAH. The carbohydrate content decreased over time after heading and was lowest in the ripe BR. The crude fat content was somewhat higher in the GR than in the ripe BR, as highest as 2.91% on the 24 DAH ($P<0.05$). The crude protein content gradually decreased during grain development and was lowest on the 27 DAH but increased on the 45 DAH. The crude ash content did not show any obvious trend according to DAH. The caloric value of the Chuchung rice tended to decrease with increasing DAH and was lowest in the ripe Chuchung BR harvested on the 45 DAH with no significance. The caloric value of Chuchung rice ranging from 348.98 to 362.44 kcal/100 g was somewhat lower than the 364 kcal/100 g for the ripe Jinbu BR but these were similar to the 350 kcal/100 g for ordinary BR, based on the Korean Standard Food Composition Table (23).

The mineral contents in the grains of Chuchung rice during maturation are also shown in Table 1. It was somewhat higher in the Chuchung GR than in the ripe BR and decreased with increasing DAH. Although the data were not presented in this report, the Chuchung variety harvested on the 17 DAH showed markedly higher contents of Ca (21.29±0.02 mg/100 g, $P<0.05$), Fe (1.84±0.04 mg/100 g), Mg (114.38±1.13 mg/100 g), and K (371.63±2.90 mg/100 g, $P<0.05$) than the ripe BR, and its total mineral content was also significantly higher than that of the ripe BR (606.19±1.73 mg/100 g) ($P<0.05$). The Mg/K ratio of the GR was highest in the sample harvested on the 21 DAH (0.44±0.22) but was generally lower than that of the ripe BR and also lower than that of the early-maturing rice Jinbu GR (0.54±0.09) (11). This value is far lower than that of the Japanese variety Koshihikari (0.78), which is known to receive high sensory (taste) scores (13). In general, the mineral contents are affected by multiple factors, including the nutrients contained in the soil, fertilization, and plant species (24); therefore, these factors need to be

Table 1. Changes on the proximate and mineral contents in Chuchung rice according to DAH

Contents	DAH			
	Green Rice®			Brown rice
	21st	24th	27th	45th
Proximate contents (%)				
Carbohydrate	78.14±6.01[a]	77.33±2.93[a]	76.84±5.93[a]	76.32±4.22[a]
Crude fat	2.76±0.14[ab]	2.91±0.05[a]	2.56±0.16[b]	2.06±0.80[c]
Crude protein	6.26±0.93[a]	6.00±0.03[a]	5.51±0.72[a]	6.29±0.21[a]
Crude ash	1.13±0.30[a]	1.18±0.11[a]	1.12±0.23[a]	1.08±0.03[a]
Caloric value (kcal/100 g)	362.44±4.30[a]	359.51±1.13[a]	352.44±2.93[a]	348.98±2.15[a]
Mineral contents (mg/100 g)				
Mg	112.52±3.93[a]	110.1±2.11[a]	99.55±0.23[a]	111.81±5.91[a]
K	258.37±1.73[ab]	253.25±3.22[ab]	261.65±0.43[a]	205.31±2.15[b]
Ca	14.87±0.43[a]	14.85±0.63[a]	12.52±0.12[a]	11.20±0.53[a]
P	277.96±2.92[a]	274.92±2.13[a]	246.33±0.94[a]	267.38±2.36[a]
Na	12.42±0.37[ab]	7.32±0.42[c]	15.51±0.03[a]	9.76±0.23[b]
Fe	0.75±0.01[ab]	0.91±0.02[a]	0.62±0.02[b]	0.73±0.01[ab]
Mg/K	0.44±0.22[a]	0.43±0.38[a]	0.38±0.21[a]	0.54±0.32[a]
Total minerals (mg/100 g)	675.84±5.93[a]	661.34±2.93[a]	636.18±1.95[ab]	606.19±1.73[b]

DAH: days after heading.
The results are the mean±SD of triplicate measurements. The values in rows denoted by the same letters (a-c) are not significantly different ($P<0.05$).

considered when the yield and harvest time of the GR are determined according to the rice variety.

The changes in the bioactive compounds in maturing Chuchung

The contents of bioactive compounds in the GR were significantly higher than those in the BR, as shown in Fig. 3 and Table 2. Specifically, the GABA content was the highest on the 27 DAH, nearly 17 times as high as that in the BR on the 45 DAH. GABA is a free amino acid that is primarily produced by the decarboxylation of L-glutamic acid, which is catalyzed by the enzyme glutamate decarboxylase (25). The concentration of GABA and the concentration of L-glutamic acid in rice are thus negatively correlated (26). In this study, the GABA content in the rice grains increased at the pre-harvest maturation stages and then decreased markedly at the mature stage

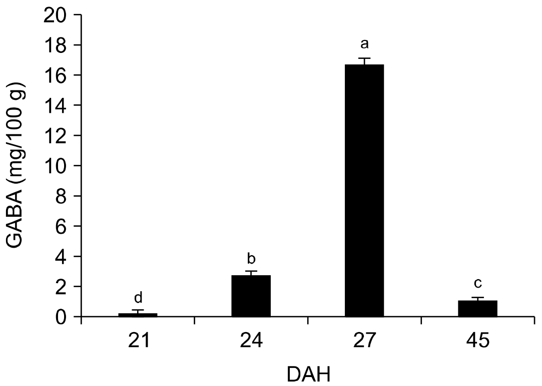

Fig. 3. The changes in the γ-aminobutyric acid (GABA) content of maturing Chuchung rice according to days after heading (DAH). The results are the mean±SD of triplicate measurements. Values with different letters (a-d) on the bars are significantly different at *P*<0.05, as analyzed by Duncan's multiple range test.

in the Chuchung variety. The GABA content was the highest on the 27 DAH, nearly 17 times as high as that in the Chuchung BR on the 45 DAH, the stage at which the total protein and L-glutamic acid contents were the lowest. The L-glutamic acid content was lowest in the premature grain, ranging from 1,076.3 mg/g to 908.5 mg/g but increased to 1,096.6 mg/g in the mature stage (data not shown). The L-glutamic acid content was lowest in the premature grain, ranging from 1,076.3 mg/g to 908.5 mg/g but increased to 1,096.6 mg/g in the mature stage (data not shown). Moreover, the content of GABA (16.7 mg/100 g) on the 27 DAH was much higher than that of the intact BR (4.1 mg/100 g) or germinated BR, which ranged from 8.8 to 10.1 mg/100 g, though these values can vary according to the cultivar or variety (27). Although the presence of GABA in plants has been known for nearly a half century, the role of GABA in plants remains undefined. Studies suggest, however, that GABA levels in plants are enhanced during high stress conditions, such as mechanical stimulation, hypoxia, cytosolic acidification, drought, and darkness (27,28). As plant extracts containing high concentrations of GABA are effective in regulating blood pressure and can affect neurotransmission and cancer, previous studies have investigated methods to increase the GABA concentration in plants through soaking and gaseous treatments, high pressure treatment, or the addition of specific chemicals, such as chitosan/glutamic acid (26-28). Our results showed that rice can be a good plant source of GABA if harvested at the proper time.

The γ-oryzanol content in the GR, ranging from 38 to 47 mg/100 g, was highest on the 21 DAH and gradually ($P<0.05$) decreased to 34 mg/100 g on the 45 DAH. γ-Oryzanol, a mixture of lipophilic phytosterols that are

Table 2. The changes of bioactive compounds in Chuchung rice according to DAH

Contents (mg/100 g)	DAH			
	Green Rice®			Brown rice
	21st	24th	27th	45th
γ-Oryzanol	47.11±2.52^{a}	40.26±2.00^{ab}	38.45±1.53^{b}	34.12±1.00^{c}
Total tocopherols	1.68±0.17^{a}	1.65±0.07^{a}	1.53±0.05^{a}	1.22±0.03^{b}
α	1.40±0.15^{a}	1.38±0.03^{a}	1.29±0.03^{a}	1.08±0.02^{b}
β and γ	0.20±0.02^{a}	0.21±0.02^{a}	0.19±0.02^{a}	0.10±0.00^{a}
δ	0.06±0.00^{a}	0.06±0.01^{a}	0.05±0.00^{a}	0.03±0.01^{a}
Total flavonoids	14.12±0.20^{a}	9.64±0.02^{b}	9.01±0.03^{b}	10.54±0.60^{ab}
Total phenolics	80.46±0.50^{b}	91.67±0.70^{ab}	60.32±0.12^{c}	95.00±0.72^{a}
Ferulic acid	32.65±0.11^{a}	25.90±0.40^{a}	20.26±0.06^{a}	26.91±0.03^{a}
p-Coumaric acid	18.60±0.20^{a}	14.70±0.02^{a}	11.80±0.05^{a}	15.32±0.04^{a}
Sinapinic acid	6.00±0.10^{a}	5.41±0.03^{a}	1.77±0.02^{a}	4.02±0.02^{a}
Benzoic acid	1.62±0.08^{a}	1.32±0.05^{a}	1.06±0.02^{a}	3.51±0.04^{a}
m-Hydroxybenzoic acid	1.09±0.01^{a}	0.81±0.02^{a}	0.76±0.08^{a}	0.74±0.03^{a}

DAH: days after heading.
The results are the mean±SD of triplicate measurements. The values in rows denoted by the same letters (a-d) are not significantly different ($P<0.05$).

composed of triterpene alcohols or sterols and a ferulic acid ester, exhibits antioxidative activity and a cholesterol-lowering effect (29). In this study, the γ-oryzanol content in the GR, ranging from 38 to 47 mg/100 g, was highest on the 21 DAH and gradually decreased to 34 mg/100 g on the 45 DAH. These results are slightly higher than those reported by Lin and Lai (2), who found that the average content of γ-oryzanol in immature *Japonica* grains was from 32.8 to 27.7 mg/100 g on the 15 and 18 DAH, respectively. This difference might be due to the degree of grain development, and other factors including the filling period needed for grain fullness, which depends on the growth temperatures, and fertilizers among others.

During the pre-harvest period, the α-tocopherol content ranged from 1.29 to 1.40 mg/100 g, and total tocopherols ranged from 1.53 to 1.68 mg/100 g, which were significantly ($P<0.05$) higher than those in the BR (1.08 mg/100 g for the α-tocopherol and 1.22 mg/100 g for the total tocopherols) as shown in Table 2. These results are within the previously reported range (2).

The total flavonoid had the highest concentration (14.12 mg/100 g) on the 21 DAH ($P<0.05$) and then sharply decreased by 9.01 mg/100 g on the 27 DAH in the GR and by 10.54 mg/100 g on the 45 DAH in the BR (Table 2). Total flavonoids, total phenolics, and ferulic acid were not significantly different during the maturation of Chuchung rice. Similar patterns have previously been reported during rice grain development (2). Mckeehen et al. (30) suggested that the ferulic acid content during wheat development was dependent on several factors: the rate of endosperm development relative to the bran synthesis rate during grain filling, the increase in linkage formation between phenolics and the cell wall materials, and a decrease in the activity of phenylalanine ammonia-lyase, which catalyzes the convertsion of phenylalanine to cinnamic acid.

This study investigated the changes in the yield of GR, and bioactive compounds during the pre-harvest maturation of *Japonica* rice, and the optimal harvest time of GR for the mid-late-maturing Chuchung variety in Gyeonggi, Korea to produce a reasonable yield, and to increase bioactive compound contents. The weight of 1,000 kernels for the Chuchung was the highest on the 27 DAH. The changes in the proximate composition and mineral contents of the Chuchung GR were not significant during the pre-harvest period. However, higher contents in GABA, γ-oryzanol, and α-tocopherol contents were found in the pre-harvest maturation of the rice grains. Accordingly, the ideal harvest time for Chuchung GR in the Gyeonggi is on the 24∼27 DAH; the rice at this stage could be a promising medicinal food. This demonstrates that targeted harvesting can be performed for the functional components of immature rice grains comparing to their completely matured grains.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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