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Changes in Physicochemical Properties and Antioxidant Activities of Brown Rice (*Oryza sativa* L.) throughout Germination

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Abstract

The objective of this research was to investigate the changes in the contents of physicochemical properties including γ-aminobutyric acid (GABA), total dietary fiber (TDF), amylose, protein, and fat content in brown rice through germination for 2 different years. Total phenolic contents and antioxidant activities of DPPH and ABTS radical scavenging capacities were also determined in different solvent extracts. For the physicochemical properties, GABA, TDF, protein, and fat content increased, whereas amylose levels decreased. Specially, GABA and TDF levels showed the greatest variations among cultivars and harvest years. Total phenolic content and antioxidant activity significantly increased. The average total phenolic content at a concentration of 0.5 mg/mL in different extract solvents occurred in this order: methanol>ethylacetate>chloroform>hexane extracts. Additionally, 'Keunnun' exhibited the highest GABA levels, highest total phenolic content, and highest antioxidant activity after germination, with increases of approximately 3.7, 2.0, and 1.9 times, respectively, compared to levels before germination. These results suggest that, because of its high physicochemical contents and strong radical scavenging activities, germinated brown rice can be used as beneficial supplement.

Key words: brown rice, physicochemical, germination, γ-aminobutyric acid, total dietary fiber, antioxidant activity, total phenolic content

INTRODUCTION

It is well established that changes in the functional components of plants are associated with processing technologies, which includes allowing the plants to germinate or not (1). Notably, germination results in more significant changes in the improvement of digestibility and physiological functions than other biochemical processes (2,3). There is considerable interest in studying natural products to find sources of healthy and functional foods (4). Among these natural products, cereals have shown themselves to be some of the most important in terms of lowering the risks of some human diseases (5). In germinated cereals, the activated hydrolytic enzymes decompose polysaccharides and proteins, which leads to an increase in oligosaccharides and amino acids (6). Moreover, the decomposition of higher-weight molecular materials leads to the generation of biofunctional components (7). There are many reports on the changes in nutritional components, and the improvement of functional, as well as nutritional, values of rice during germination (6). However, there is no sufficient information on various nutritional components in germinated brown rice (*Oryza sativa* L.). Some of the bioactive components in brown rice include γ-aminobutyric acid (GABA) and γ-oryzanol, which are synthesized during germination (8), as well as total dietary fiber (TDF), amylase, proteins and fats. GABA is known to be one of the major inhibitory neurotransmitters in the sympathetic nervous system and to play a role in cardiovascular function (9). TDF has received attention due to its apparent beneficial effects on glucose as well as lipid metabolism and bacterial metabolic activity (10). Amylose is related to water absorption, volume expansion, cohesiveness, tenderness, and separability of the cooked grains (11).

In the present study, we evaluated changes in these five physicochemical properties, such as GABA, TDF, amylose, protein, and fat from 10 brown rice cultivars throughout germination. Moreover, total phenolic contents and antioxidant activities, including DPPH and ABTS radical scavenging methods, were evaluated with different solvent extracts.

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MATERIALS AND METHODS

Plant material

Brown rice cultivars including 'Ilpum', 'Saechucheongbyeo', 'Samkwang', 'Seolgaeng', 'Hongjinju', 'Jeogjinju', 'Heugkwang', 'Jinbuchal', 'Keunnun', and 'Hwaseonchal', developed by the National Institute of Crop Science (NICS), Rural Development Administration (RDA) were selected for this study. These cultivars were grown at the experimental field of NICS, RDA, Suwon, Korea and harvested in 2007 and 2008. All samples were stored in a refrigerator at 4°C for one month until analysis.

Reagents

GABA, α-amylase, protease, amyloglucosidase and amylose were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents and solvents were of the highest laboratory grade (Sigma-Aldrich, St. Louis, MO, USA) and used without further purification.

Brown rice germination

Germination was carried out as previously described (12). Brown rice (5 g) was decontaminated with 1% sodium hypochlorite solution (50 mL), and then soaked in distilled water for 4 hr at 25°C. After draining the distilled water, the soaked brown rice was germinated under dark conditions in an incubator by layering over a moist filter paper, with continuous watering by capillary for 3 days.

Measurement of GABA content

GABA was determined by a Beckman amino acid analyzer (Beckman 6300, Beckman Co., Ltd, USA). The sample preparation for GABA analysis was measured according to a previously reported method with slight modifications (13). The pulverized brown rice (0.5 g) was diluted with 10 mL of 3% trichloroacetic acid solution. The sample solution was left at 25°C for 2 hr, and then centrifuged at 5,000 × *g* for 10 min. The supernatant was filtered with 0.45-μm syringe filter (MSI; Millipore, Westboro, MA, USA) prior to amino acid analysis.

Measurement of total dietary fiber (TDF) content

For the determination of TDF content, the Association of Official Analytical Chemists (AOAC) enzymatic-gravimetric method 991.43 was used (14). Briefly, the pulverized rice (1 g) was gelatinized with heat stable α-amylase in boiling water bath. After gelatinization, the sample was digested with protease and amyloglucosidase to remove starch and protein. Insoluble dietary fiber was filtered. The soluble dietary fiber in the filtrate was precipitated with 95% ethanol. TDF was calculated as the sum of insoluble and soluble dietary fibers.

Measurement of amylose content

Amylose was measured on a Beckman DU 650 Spectrophotometer (Beckman Coulter, USA). Amylose measurement was performed using iodine colorimetric methods, as previously reported (15). Briefly, the pulverized brown rice (100 mg) was placed in a 100 mL flask, then ethanol (1 mL) and 1 M NaOH (9 mL) were added. After stirring for 10 min in boiling water, the mixture was cooled to 25°C and the suspension was diluted to 100 mL by distilled water. And then, 1 mL of 1 M acetic acid and 2 mL of iodine reagent were added to the diluted solution. After stirring of the mixture solution for 20 min, the absorbance was measured at 620 nm using aforementioned UV-spectrophotometer. Amylose content was determined using the standard curves at the various concentrations of a pure amylose (16).

Measurement of protein content

The protein content of brown rice was determined according to the Kjeldahl procedure (17). The sample powder (0.2 g) was digested by a Buchi B-435 digestion system and Buchi B-412 scrubber with 20 mL of H_2SO_4 and 3.0 g of catalyst ($CuSO_4 : K_2SO_4 = 1:9$). Nitrogen content was calculated by Buchi B-399 auto-Kjeldahl system and then converted to protein by multiplication on 6.25.

Measurement of crude fat content

Fat was measured by the Soxhlet method using the Buchi B-811 extraction system (18). 200 mL of *n*-hexane was added to the powdered brown rice (2 g) in an extraction thimble and boiled for 2 hr at 105°C. After cooling, the extracted fat was weighed. Total fat content was determined on a dry matter basis.

Measurement of total phenolic content

The total phenolic content of sample extract was determined using Folin-Ciocalteau method (19). Briefly, each sample (500 μL) was added to 250 μL 2 N Folin-Ciocalteau reagent. After 5 min, 500 μL of 7% Na_2CO_3 solution was added with mixing. After 1 hr at room temperature, the absorbance at 750 nm (Beckman Coulter DU650, USA) was measured. The standard curve for total phenolic content was made using gallic acid solutions (0, 50, 100, 200, 500 mg/L) and the result was expressed as mg gallic acid equivalents (GAE/g) extract.

Measurement of DPPH radical scavenging activity

The antioxidant activity of the extract was measured on the basis of the scavenging activity of the stable DPPH free radical following the method described by Braca et al. (20). Various concentrations of extract were added to a concentration of 0.15 mM in EtOH, and the mixture was shaken vigorously. Absorbance at 517 nm (Beckman Coulter DU650, USA) was determined after 30 min, and

the radical scavenging effect was calculated as $[A_c - A_t/A_c] \times 100$, where A_t and A_c were the absorbance of samples with and without sample extracts, respectively.

Measurement of ABTS radical scavenging activity

This assay was based on the ability of different substances to scavenge the $ABTS^{\cdot+}$ radical cation in comparison to a standard (Trolox). $ABTS^{\cdot+}$ was dissolved in EtOH to make a concentration of 7 mM. This radical cation was produced by reacting the ABTS stock solution with 2.45 mM potassium persulfate and leaving the mixture for 10～14 hr until the reaction was complete and the absorbance was stable. The $ABTS^{\cdot+}$ stock solution was diluted in ethanol to an absorbance of 0.70 at 734 nm (Beckman Coulter DU650, USA) for measurement. After the addition of 0.9 mL of diluted $ABTS^{\cdot+}$ to 0.1 mL of sample, the absorbance was taken 1 min after the initial mixing (21). This scavenging activity (%) was expressed as a percentage using the following formula: $ABTS^{\cdot+}$ radical scavenging activity (%) = [(absorbance of control – absorbance of sample)/ absorbance of control] × 100

Statistical analysis

All measurements were repeated 3 times and the results were presented as the mean ± standard deviation (SD). Results were analyzed by using Sigma Plot 2001 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Changes in GABA content through germination

It is well established that enzymatic activity and functional components increase in cereal through the process of germination (8). Thus, the cereal's functional quality can be improved using germination as part of the processing method (6). Among changes in the functional components, the increase of GABA is noteworthy, due to the activation of glutamate decarboxylase, which converts glutamate to GABA (2). Changes in GABA content of 10 brown rice cultivars through germination for 2 different years are summarized in Table 1. Also, peaks of amino acid levels in brown rice were determined, as shown in Fig. 1. The GABA peak was identified by comparing retention time with amino acid standard (t_R = 83.0 min). GABA exhibited significant differences among cultivars and harvest years. Total average GABA content of 10 brown rice cultivars was 163.1 mg/100 g in 2007 and 229.0 mg/100 g in 2008. After germination, total average GABA content was 618.4 mg/100 g in 2007 and 901.7 mg/ 100 g in 2008. The GABA content in germinated brown rice increased approximately 4 times in comparison with that of non-germinated one. Among the 2007 cultivars before germination, the highest GABA content was 523.0 ± 13.3 mg/100 g in 'Heugkwang', while the levels in 'Samkwang', 'Jeogjinju', and 'Jinbuchal' were so low, they were undetectable (Table 1). However, 'Samkwang' (538.8 ± 10.5 mg/100 g), 'Jeogjinju' (488.1 ± 7.4 mg/100 g), and 'Jinbuchal' (644.9 ± 8.3 mg/100 g) showed significant increase after germination. Moreover, 'Ilpum' showed the lowest GABA contents (100.7 ± 4.8 →138.9 ± 2.9 mg/100 g) through germination (Table 1) and changes in peaks were shown in Fig. 1A and 1B. Moreover, the GABA content of 'Keunnun' increased 3.7 times after germination (413.6 ± 8.8→1,524.0 ± 21.0 mg/100 g), in agreement with a report by Lee et al. for rough rice (22). Due to the decomposition of polymers in brown rice, germination leads to the generation of biofunctional components and to improvement of organoleptic qualities (22). In 2008 cultivars, the GABA levels of 'Heugkwang' (316.2 ± 4.2→1336.0 ± 15.7; 4.2 times), 'Jinbuchal' (197.4 ± 2.7→1083.0 ± 14.2; 5.5

Table 1. Changes in GABA and TDF contents of brown rice cultivars through germination

Cultivar	GABA[1] content (mg/100 g)[3]				TDF[2] content (%)[3]			
	2007[4]		2008		2007		2008	
	BG[5]	AG[6]	BG	AG	BG	AG	BG	AG
Ilpum	100.7 ± 4.8^{e}	138.9 ± 2.9^{h}	105.1 ± 2.4^{f}	463.2 ± 3.2^{g}	16.0 ± 1.0^{b}	26.7 ± 1.5^{c}	15.9 ± 0.4^{b}	24.6 ± 1.3^{c}
Saechucheong	110.7 ± 4.3^{e}	236.7 ± 5.3^{g}	145.8 ± 2.9^{e}	668.7 ± 7.4^{f}	15.3 ± 0.7^{bc}	20.5 ± 0.8^{e}	15.7 ± 0.7^{b}	26.8 ± 1.6^{b}
Samkwang	151.2 ± 4.5^{d}	538.8 ± 10.5^{d}	100.5 ± 2.5^{f}	377.1 ± 2.9^{h}	15.2 ± 0.9^{bc}	24.7 ± 1.0^{d}	16.5 ± 1.1^{b}	24.8 ± 0.5^{c}
Seolgaeng	251.3 ± 9.6^{c}	567.1 ± 12.8^{d}	204.3 ± 5.1^{d}	685.2 ± 9.7^{f}	17.0 ± 1.2^{b}	26.3 ± 0.6^{c}	16.4 ± 0.6^{b}	26.9 ± 0.9^{b}
Hongjinju	100.8 ± 3.7^{e}	511.4 ± 9.7^{e}	226.2 ± 3.0^{cd}	948.9 ± 10.3^{d}	18.1 ± 0.6^{a}	28.3 ± 0.7^{b}	18.4 ± 0.2^{a}	22.7 ± 1.2^{d}
Jeogjinju	268.4 ± 5.2^{c}	488.1 ± 7.4^{f}	235.2 ± 4.6^{c}	758.1 ± 8.6^{e}	12.0 ± 0.4^{c}	37.5 ± 1.6^{a}	14.5 ± 0.3^{c}	36.5 ± 1.7^{a}
Heugkwang	523.0 ± 13.3^{a}	849.0 ± 19.3^{b}	316.2 ± 4.2^{b}	1,336.0 ± 19.7^{b}	17.1 ± 0.9^{b}	25.2 ± 1.1^{d}	18.8 ± 0.7^{a}	26.8 ± 0.7^{b}
Jinbuchal	163.8 ± 3.9^{d}	644.9 ± 8.3^{c}	197.4 ± 2.7^{d}	1,083.0 ± 14.2^{c}	16.7 ± 0.6^{b}	25.0 ± 1.1^{d}	18.6 ± 1.1^{a}	27.7 ± 0.5^{b}
Keunnun	413.6 ± 8.8^{b}	1,524.0 ± 21.0^{a}	528.6 ± 5.7^{a}	1,942.0 ± 26.3^{a}	16.0 ± 0.5^{b}	26.8 ± 0.9^{c}	17.9 ± 0.6^{a}	27.0 ± 0.9^{b}
Hwaseonchal	130.4 ± 3.1^{d}	684.7 ± 7.2^{c}	231.0 ± 4.6^{c}	754.5 ± 7.4^{e}	16.0 ± 0.5^{b}	26.1 ± 1.4^{c}	16.2 ± 1.0^{b}	24.2 ± 0.5^{c}

[1]GABA: γ-aminobutyric acid. [2]TDF: total dietary fiber. [3]The values indicate the mean ± SD (n=3) of the experiment for GABA and TDF contents of each cultivar. [4]Harvest year. [5]BG: before germination. [6]AG: after germination.
The different superscripts (a-h) in the same column mean significantly different at $p<0.05$.

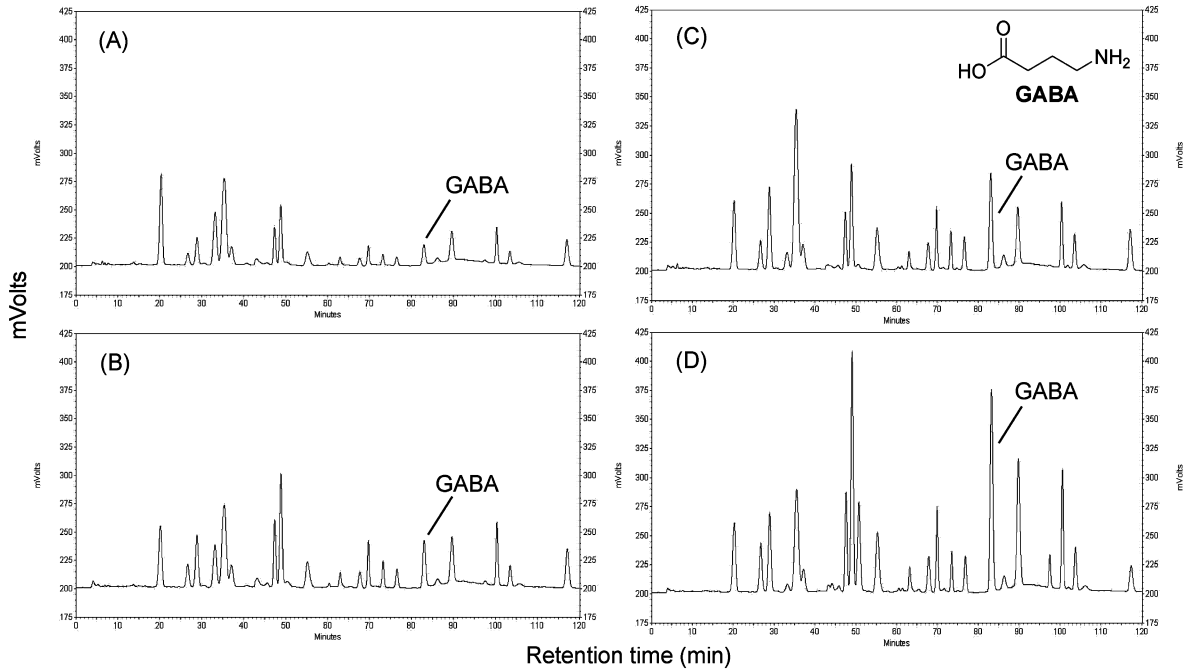

Fig. 1. Amino acid peaks of brown rice cultivars containing the highest and the lowest GABA contents during germination. (A) Ilpum before germination (2007), (B) Ilpum after germination (2007), (C) 'Keunnun' before germination (2008), (D) 'Keunnun' after germination (2008).

times), and 'Keunnun' (528.6±5.7→1942.0±26.3; 3.7 times) exhibited higher increases than other cultivars after germination. Brown rice 'Keunnun' also showed the highest GABA content in 2008 and its amino acid peaks were shown in Fig. 1C and 1D. This increase is mainly due to its large embryo, where most physiological activities take place (22). Thus, the germinated 'Keunnun' has the potential to be used as healthy food source. It can be concluded that GABA content was significantly affected by germination, cultivar, and harvest year. The evaluation of GABA in germinated brown rice is important when looking to enhance the dietary supplement's effect on human health, because GABA is responsible for various biological activities (9).

Changes in TDF content through germination

TDF has received great attention due to potential beneficial effects on glucose, as well as lipid metabolism, and its ability to reduce the risk of colorectal cancer (11). Recently, it was reported that TDF contents in rough rice and buckwheat increased through germination (22,23). However, the changes of TDF contents have not been studied with different brown rice cultivars. As shown in Table 1, TDF showed significant differences among cultivars and harvest years through germination. TDF contents ranged from 12.0 to 18.1% before germination and from 20.5 to 37.5% after germination in the 2007 harvest year. In 2008, TDF contents were in the ranges of 14.5～18.8% before germination and 22.7～36.5% after germination. Based on the above results, TDF was observed to increase approximately 2 times through germination. These results are in agreement with the report about TDF levels in buckwheat (23). Thus, the increase of TDF through germination may be related to the increase of pectic substances in the middle lamella, as previously reported (22). Moreover, since the beneficial effects TDF promote laxative effects, lower blood cholesterol, and product glucose attenuation (11), the germinated brown rice has the potential to be used as a healthy and functional food ingredient (22). Especially, 'Jeogjinju' exhibited the highest content and increase (2007: 12.0→37.5% and 2008: 14.5→36.5%) among cultivars for the 2 years of this study. This cultivar may have the highest quality of all the cultivars following germination.

Changes in amylose, protein, and fat contents through germination

It is well established that amylose levels are normally observed to be about 17～20% in rice and are related to water absorption, flutiness, cohesiveness, and tenderness (24). Thus, analysis of this component is important source for development of high quality rice (2). Protein and fat are also important nutritional components (25). Many researchers have focused on their contents (26,27), but there has been no report on the changes of contents through germination. The effects of germination on amylose, protein, and fat contents in harvested brown rice were evaluated for 2 years and their results are shown

in Table 2. The amylose contents before germination ranged from 6.9 to 22.7% in 2007 and ranged from 5.8 to 18.4% in 2008. After germination, their contents slightly decreased by comparison with nongerminated brown rice. In other words, the amylose concentrations of all the cultivars ranged from 6.5 to 21.2% in 2007, and ranged from 5.6 to 17.1% in 2010.

Although 10 brown rice cultivars had different amylose contents over the two year experimental period, no significant differences were found except in 'Jinbuchal'. After germination, 'Saechucheong' showed the highest amylose contents (2007: 22.7→21.1% and 2008: 18.4→17.9%), while 'Jinbuchal' exhibited the lowest (2007: 6.9→6.5% and 2008: 5.8→5.6%). Generally, higher amylose content in rice means lower eating quality (28). Therefore, our results suggest that the germinated brown rice likely has higher eating quality. Based on the above results, 'Jinbuchal' is more important source than other cultivars owing to its lowest amylose content. Also, this cultivar may contribute to the enhancement of the health benefits in processed and functional foods. This is the first documented evidence that amylose levels are slightly reduced in germinated brown rice. Up to now, although many studies reported protein and fat contents in rice, there has been no report on the changes of their contents in germinated brown rice cultivars. As shown in Table 3, all cultivars had different protein and fat contents, but no significant differences were observed in their contents through germination. On the basis of cultivars used for this study, total average protein content before germination showed 7.4%, while the content after germination exhibited 7.8%. Our results were consistent with japonica rice varieties with approximately 7.0% protein. Total average fat content of pregerminated rice was 2.6% and the germinated brown rice showed 3.0%. There was also little difference in protein content among cultivars and harvest years. The above results lead to the conclusion that protein and fat may not be key factors in determining the quality of the rice, especially when compared to GABA and TDF levels through germination.

Changes in total phenolic content through germination

It is well established that the contents of phenolic compounds in natural materials are well correlated with antioxidant activities (29). For this reason, many researchers have been focused on total phenolic content and antioxidant activity of natural food sources. Although the effects storage on phenolic content in rice has been previously studied (30), to our knowledge, there has been no report on the changes of total phenolic content through germination. The average total phenolic contents on various solvents of 10 brown rice cultivars at the concen-

Table 2. Changes in amylose, protein, and oil contents of brown rice cultivars through germination

Cultivar	Amylose content (%)[1]				Protein content (%)[1]				Fat content (%)[1]			
	2007[2]		2008		2007		2008		2007		2008	
	BG[3]	AG[4]	BG	AG	BG	AG	BG	AG	BG	AG	BG	AG
Ilpum	22.5±0.7^{a}	21.3±0.6^{a}	17.8±0.6^{a}	16.2±0.2^{b}	7.1±0.9^{b}	7.6±0.3^{b}	7.0±0.1^{b}	7.4±0.0^{b}	2.6±0.0^{a}	2.6±0.1^{a}	2.6±0.1^{a}	2.9±0.2^{a}
Saechucheong	22.7±0.9^{a}	21.1±1.1^{a}	18.4±0.5^{a}	17.9±0.6^{a}	6.7±0.2^{b}	7.1±0.1^{b}	6.9±0.5^{b}	7.5±0.2^{b}	2.5±0.0^{a}	2.8±0.1^{a}	2.5±0.0^{a}	2.9±0.2^{a}
Samkwang	22.3±1.0^{a}	20.2±0.5^{ab}	16.9±0.3^{b}	16.8±0.2^{ab}	6.7±0.4^{b}	6.9±0.0^{b}	6.8±0.4^{b}	7.2±0.2^{b}	2.7±0.2^{a}	2.8±0.3^{a}	2.6±0.1^{a}	3.2±0.3^{a}
Seolgaeng	22.0±0.4^{a}	20.3±0.3^{ab}	17.2±0.5^{b}	17.0±0.3^{ab}	7.2±0.5^{b}	7.6±0.3^{b}	6.4±0.2^{b}	6.9±0.0^{b}	2.6±0.1^{a}	2.9±0.5^{a}	2.8±0.3^{a}	3.3±0.3^{a}
Hongjinju	21.1±0.7^{b}	19.2±0.7^{b}	17.1±0.7^{b}	16.0±0.2^{b}	7.1±0.2^{b}	7.7±0.3^{b}	7.1±0.9^{b}	7.2±0.5^{b}	2.4±0.1^{a}	2.9±0.6^{a}	2.5±0.0^{a}	2.9±0.1^{a}
Jeogjinju	20.8±0.5^{b}	19.7±0.5^{b}	16.9±0.2^{b}	16.3±0.7^{b}	7.4±0.8^{b}	7.8±0.5^{b}	7.3±0.4^{b}	7.8±0.9^{b}	2.1±0.0^{a}	2.8±0.2^{a}	2.4±0.2^{a}	2.8±0.1^{a}
Heugkwang	18.9±0.5^{c}	19.1±0.7^{b}	16.2±1.1^{b}	16.2±0.3^{b}	7.5±0.7^{b}	7.9±0.1^{b}	8.0±0.7^{b}	8.6±0.8^{ab}	2.8±0.4^{a}	3.0±0.3^{a}	2.7±0.1^{a}	3.2±0.2^{a}
Jinbuchal	6.9±0.2^{e}	6.5±0.3^{d}	5.8±0.0^{d}	5.6±0.1^{d}	8.8±1.0^{a}	9.1±0.3^{a}	9.1±0.2^{a}	9.2±1.0^{a}	2.7±0.2^{a}	3.1±0.4^{a}	2.7±0.1^{a}	3.1±0.4^{a}
Keumnun	20.6±0.4^{b}	18.5±0.9^{b}	14.9±0.4^{c}	14.6±0.2^{c}	7.6±0.2^{b}	7.8±0.0^{b}	8.3±0.4^{b}	7.5±0.1^{b}	2.6±0.2^{a}	2.9±0.0^{a}	2.6±0.0^{a}	3.0±0.7^{a}
Hwaseonchal	7.9±0.5^{d}	7.8±0.3^{c}	6.1±0.2^{d}	5.6±0.0^{d}	7.4±0.0^{b}	7.9±0.2^{b}	7.9±0.2^{b}	8.4±0.1^{ab}	2.6±0.1^{a}	2.8±0.3^{a}	2.5±0.1^{a}	2.9±0.2^{a}

[1]The values indicate the mean±SD (n=3) of the experiment for amylose, protein, and oil contents of each cultivar.
[2]Harvest year. [3]BG: before germination. [4]AG: after germination.
The different superscripts (a-e) in the same column mean significantly different at $p<0.05$.

Fig. 2. Average total phenolic contents in different extraction solvents at the concentration of 0.5 mg/mL from 10 brown rice cultivars. BG: before germination, AG: after germination.

tration of 0.5 mg/mL through germination are shown in Fig. 2. In germinated brown rice, total phenolic content significantly increased. The methanol fraction showed the highest average content (1.4→2.1 mg GAE/g), followed by ethylacetate fraction (0.7→1.0 mg GAE/g), and chloroform fraction (0.3→0.5 mg GAE/g), while the hexane fraction exhibited the lowest content (0.1→0.1 mg GAE/g). Moreover, using a methanol extraction solvent, the germinated brown rice significantly increased in all cultivars (Table 3). Most notably, 'Keunnun' showed a maximum increase in total phenolic content (2007: 1.9±0.3→3.1±0.3 mg GAE/g, 2008: 1.6±0.2 →3.2±0.4 mg GAE /g) for 2 years, which might prove to be a very important concern regarding quality. These results suggest that the increase in total phenolic content through germination may be attributed to the bound phenolic compounds becoming free by the action of enhanced hydrolytic enzyme activity (31). In this work, changes in total phenolic content of brown rice cultivars through germination were investigated for the first time.

Changes in DPPH and ABTS radical scavenging activities through germination

The DPPH and ABTS radicals have been commonly used to measure antioxidative status (32). Especially, DPPH radical scavenging activity could be used to evaluate antioxidant activity in a relatively short time compared to other methods. Changes in the radical scavenging activities of various brown rice cultivars were determined by comparing the percentage inhibition of DPPH and ABTS radical formation by extracts of each cultivar, BHT, and Trolox. As shown in Fig. 3, the highest DPPH and ABTS radical scavenging activities showed from the methanol extract (DPPH: 42.7→58.1%, ABTS: 48.4→62.9%), followed by ethylacetate (DPPH:

Table 3. Changes in total phenolic content and radical scavenging activity of brown rice cultivars through germination

Cultivar	Total phenolic content (mg GAE/g)[1]				Radical scavenging activity (%)[1]							
					DPPH				ABTS			
	2007[2]		2008		2007		2008		2007		2008	
	BG[3]	AG[4]	BG	AG	BG	AG	BG	AG	BG	AG	BG	AG
Ilpum	1.6±0.2^{a}	2.1±0.3^{b}	1.5±0.0^{a}	2.0±0.2^{e}	44.7±2.3^{b}	56.7±2.5^{e}	40.1±1.4^{c}	53.9±2.1^{d}	48.1±2.8^{b}	65.3±2.9^{e}	51.2±2.0^{b}	70.3±3.0^{b}
Saechucheong	1.0±0.1^{b}	1.6±0.2^{c}	1.3±0.2^{b}	1.8±0.1^{c}	36.5±1.7^{c}	47.3±2.0^{e}	38.1±2.3^{c}	46.5±1.4^{e}	45.9±1.7^{c}	55.1±2.2^{e}	49.6±1.4^{c}	59.1±1.7^{e}
Samkwang	1.3±0.2^{ab}	1.8±0.1^{c}	1.1±0.1^{b}	1.9±0.2^{e}	42.5±1.5^{b}	54.6±2.7^{c}	46.3±2.0^{a}	58.1±2.6^{c}	50.4±2.2^{b}	63.8±1.7^{c}	53.1±1.6^{b}	60.7±2.4^{e}
Seolgaeng	0.7±0.1^{b}	1.6±0.1^{c}	0.9±0.0^{b}	1.7±0.1^{c}	33.1±1.4^{c}	43.5±0.9^{e}	39.4±0.8^{c}	52.0±1.8^{d}	41.2±1.7^{e}	48.9±1.8^{f}	40.5±1.9^{f}	51.3±1.3^{g}
Hongjinju	1.6±0.3^{a}	2.3±0.2^{b}	1.6±0.2^{a}	2.3±0.2^{b}	45.8±1.2^{b}	51.6±2.3^{d}	40.7±2.4^{c}	48.6±1.7^{de}	43.6±2.5^{d}	53.4±2.2^{e}	45.4±1.4^{e}	54.2±0.9^{f}
Jeogjinju	1.4±0.1^{ab}	2.1±0.1^{b}	1.7±0.1^{a}	2.2±0.3^{b}	43.1±1.3^{b}	58.1±1.7^{c}	49.8±2.0^{a}	57.6±2.9^{c}	48.8±1.6^{b}	60.1±2.3^{d}	53.7±2.6^{b}	68.9±2.0^{b}
Heugkwang	1.2±0.1^{ab}	1.9±0.3^{c}	1.1±0.0^{b}	1.8±0.2^{c}	40.3±1.2^{b}	50.4±2.3^{d}	43.3±3.1^{b}	62.7±1.6^{b}	43.5±1.9^{d}	49.7±2.0^{f}	47.1±1.8^{d}	53.7±1.6^{f}
Jinbuchal	1.5±0.2^{a}	2.4±0.2^{b}	1.2±0.1^{b}	1.7±0.2^{c}	43.5±1.9^{b}	60.4±3.1^{b}	38.6±0.8^{c}	45.9±1.5^{e}	49.7±2.7^{b}	63.4±1.7^{c}	52.1±1.6^{b}	62.5±2.3^{d}
Keunnun	1.9±0.3^{a}	3.1±0.3^{a}	1.6±0.2^{a}	3.2±0.4^{a}	50.4±1.3^{a}	94.7±3.7^{a}	47.1±2.4^{a}	93.1±3.7^{a}	56.3±2.3^{a}	98.1±4.3^{a}	59.1±2.1^{a}	98.9±4.7^{a}
Hwaseonchal	1.7±0.1^{a}	2.3±0.2^{b}	1.7±0.1^{a}	2.2±0.3^{b}	49.6±1.9^{a}	63.9±2.3^{b}	40.5±1.1^{c}	49.7±2.3^{de}	56.8±1.5^{a}	71.3±2.5^{b}	47.9±1.7^{d}	65.5±2.2^{c}

[1]The values indicate the mean±SD (n=3) of the experiment for total phenolic content and radical scavenging activity of each cultivar and sample concentration was 0.5 mg/mL. [2]Harvest year. [3]BG: before germination. [4]AG: after germination.
The different superscripts (a-g) in the same column mean significantly different at $p<0.05$.

Fig. 3. Average DPPH and ABTS radical scavenging activities in different extraction solvents at the concentration of 0.5 mg/mL from 10 brown rice cultivars. BG: before germination, AG: after germination.

29.5→38.7%, ABTS: 35.7→47.0%), chloroform (DPPH: 21.1→29.2%, ABTS: 22.6→33.2%), and hexane extracts (DPPH: 7.5→7.9%, ABTS: 10.5→12.1%). The radical scavenging activities in methanol extracts of germinated brown rice cultivars significantly increased and changes in their activities at the concentration of 0.5 mg/mL are shown in Table 3. The methanol extracts of all cultivars showed higher radical scavenging activities than those of DPPH when reacted with the ABTS radical. This result was in agreement with a report by Jeong et al. (33). In this study, the highest DPPH and ABTS radical scavenging activities were exhibited by 'Keunnun' and their activities exhibited 40% more increased than those of nongerminated (DPPH: 2007; 50.4→94.7%, 2008; 47.1 →93.1% and ABTS: 2007; 56.3→98.1%, 2008; 59.1→ 98.9%). It is thought that 'Keunnun' is better in the quality of antioxidant activity than other cultivars through germination. Although the methanol extracts of all cultivars showed lower radical scavenging activities than those of positive controls such as BHT and Trolox, these extracts might contain antioxidant compounds which were able to react aggressively with free radicals. Thus, our results suggest that the methanol extract of germinated brown rice has potent free radical scavengers.

CONCLUSION

This work has shown the changes of physicochemical properties including GABA, TDF, amylose, protein, and fat from brown rice cultivars through germination. Moreover, total phenolic contents and the scavenging activities of DPPH and ABTS radicals in the methanol extract of all cultivars were investigated. In the physicochemical studies, GABA and TDF contents markedly increased, while the remaining components showed slight variations. Total phenolic contents and antioxidant activities also significantly increased in germinated brown rice cultivars. Notably, 'Keunnun' may be a very important material owing to its high GABA, TDF, and total phenolic contents, as well as its antioxidant activity. Thus, our results may provide potential information for development of processed and nutraceutical foods from certain brown rice cultivars.

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