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Antioxidant Activities of Ethanol Extracts from Germinated Specialty Rough Rice

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Abstract To examine the possibility of using rough rice as a health-functional food, we investigated its changes in biological activity with germination. Antioxidant activities of the 70% ethanolic extracts of 'Goami2', 'Keunnunbeyo', and 'Heugkwangbeyo' were studied in comparison with those of ungerminated rough rice. The phytic acid level in rough rice decreased on germination, while the level of phenolic compounds increased. Reducing power increased in a dose-dependent manner and the germinated rough rice tended to have enhanced reducing power. Among the rough rice cultivars, the germinated 'Heugkwang' rough rice tended to be the most effective, with scavenging activities for the DPPH, superoxide, and hydroxyl radicals that were 1.6-, 1.3-, and 1.6-fold greater than those of the ungerminated 'Heugkwang' rough rice, respectively. We also found that the germination process increased antioxidant activity in all the rough rice cultivars, where activity was greatest for the 'Heugkwang' rough rice cultivar.

Keywords: germinated rough rice, phytic acid, antioxidant activity

Introduction

Active oxygen and nitrogen species can induce damage to the human body, and the over production of various forms of activated oxygen species such as oxygen radicals and non-free radical species is considered to be the main contributor to oxidative stress (1). These oxygen radicals can induce oxidative damage to biomolecules such as carbohydrates, proteins, lipids, and DNA (2, 3), thus accelerating aging, cancer, cardiovascular diseases, neurodegenerative diseases, and inflammation (4). Phytochemicals are bioactive substances of plants that have been associated with the protection of human health against chronic degenerative diseases (5). The major groups of phytochemicals that contribute to the total antioxidant capacity (TAC) of plant foods include polyphenols, carotenoids, and traditional antioxidant vitamins like vitamin C and E (6). Thus, it is important to increase antioxidant intake in the diet, and to search for natural antioxidant sources among plants to use as food additives. Cereals have been known to contain a high amount of hydroxycinnamic acid (HCA) derivatives that render potential health benefits (7). The current study tested the appropriateness of various types of functional rice as healthy cultivars. Specialized rice products, such as 'Goami2', 'Keunnunbeyo', and 'Heugkwangbeyo' have been produced and sold in Korea. In germinated cereal grains, activated hydrolytic enzymes decompose starch, non-starch polysaccharides, and proteins, which lead to an increase of oligosaccharides, and amino acids in barley (8), wheat (9), and oats (10). The decomposition of high molecular weight polymers during germination also leads to the generation of bio-functional substances, as well as improvements in organoleptic qualities due to the softening of texture and increase in flavor for barley (11), finger millet (12), oats (13), and rye (14). In addition, new compounds such as γ-aminobutyric acid (GABA), γ-oryzanol, and useful amino acids are synthesized during germination (15). Legumes, consumed after processing and germination are the most economical foods, and germination causes important changes in the biochemical, nutritional, and sensory characteristics of legumes. The nutritional value of legumes is enhanced through increases in essential amino acids, protein digestibility, amino acid availability, and certain vitamins, including thiamin, riboflavin, niacin, and ascorbic acid (16). However, physical damage to the embryo during the process of producing brown rice affects germination. In addition, if unhulled rice is pounded to produce brown rice, the embryo is not protected by the hull, thus exposing it to air and allowing oxidation to occur. Furthermore, because related enzymes are activated and hydrolysis takes place, it loses its germination ability (17, 18). In particular, hydrolysis works on the ester bond of fat and produces free fatty acids in a process involving various enzymes; for example, lipoxidase and lipase, which produce the smell of old rice and increase acidity (18). Therefore, to examine the possibility of using germinated specialty rough rice as a health-functional food, 'Goami2', 'Keunnun', and 'Heugkwang' rough rice were extracted with ethanol-water (70:30, v/v) and evaluated for their phytic acid contents, phenolic compounds, reducing powers, electron-donating abilities to the DPPH radical, and their capacities to scavenge hydroxyl and superoxide radicals.

Materials and Methods

Materials The 'Goami2', 'Keunnun', and 'Heugkwang' rough rice cultivars were grown at the National Institute of

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Crop Science, Rural Development Administration, Suwon, Korea during the 2006 growing season. Folin and Ciocalteu's phenol reagent and other chemicals were purchased from Sigma (St. Louis, MO, USA).

Sample preparation The rough rice was soaked in water for 3 days at room temperature, and the soaking water was changed every 24 hr. After 3 days of germination, the rough rice was dried at 60°C for 24 hr and then ground. Next, the germinated rough rice was extracted with 70% ethanol. The extracts were then filtered through Whatman No. 2 paper to remove any debris. The filtrates were concentrated using rotary evaporation (N-1000; Eyela, Tokyo, Japan) and then lyophilized. The extracts were dissolved in dimethyl sulfoxide (DMSO). For the phytic acid content analysis, the rice was ground into a fine powder with a blender (Food processor; J World Tech Co., Seoul, Korea). The powder was then passed through a 100-mesh sieve.

Phytic acid content The amount of phytic acid in the rough rice was measured using a UV visible spectrophotometer at a wavelength of 500 nm, according to the modified method of Haung *et al.* (19). Na-phytic acid was used as a reference, and the phytic acid level was calculated based on a standard curve.

Total phenolic compounds The amounts of total phenolic compounds in the rough rice were measured using a UV-visible spectrophotometer (DU-650; Beckman Coulter, Fullerton, CA, USA) at a wavelength of 700 nm, according to the modified Folin-Ciocalteu method (20). The gallic acid level was used as a reference, and the phenolic compound level was calculated based on a standard curve.

Reducing power assay The reducing power of the rough rice was measured using a UV-visible spectrophotometer at a wavelength of 700 nm according to the method of Oyaizu (21).

DPPH radical scavenging activity The scavenging activity for the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was evaluated using the method of Yen *et al.* (22), at a wavelength of 517 nm with a UV-visible spectrophotometer. The radical scavenging activity was calculated using the following equation:

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

Hydroxyl radical scavenging activity The scavenging activity for the hydroxyl radical was evaluated using the method of Halliwell *et al.* (23) at a wavelength of 520 nm with a UV-visible spectrophotometer. The radical scavenging activity was calculated using the following equation:

$$\text{Hydroxyl radical scavenging activity (\%)} = \{1 - (A_{bs} - A_{bo}) / (A_{bc} - A_{bo})\} \times 100$$

where A_{bo} is the absorbance at 520 nm with no treatment, A_{bc} is the absorbance of the treated control at 520 nm, and A_{bs} is the absorbance of the treated sample at 520 nm.

Superoxide radical scavenging activity The scavenging activity for the superoxide radical was evaluated using the following equation at a wavelength of 560 nm according to the xanthine–xanthine oxidase method (24):

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

Statistical analysis Statistical analysis was carried out using SPSS (version 11.5; SPSS Inc., Chicago, IL, USA). The results were expressed as means ± standard deviations (SD). Student's *t*-test for unpaired data was used in all parameters measured to determine the significance of the changes in the ungerminated and germinated treatments.

Results and Discussion

Extraction yields The extraction yields were expressed in terms of the solid content in the dried product per soluble solid content in the rough rice used on a dry basis. Table 1 shows the extractions yields of the 70% ethanol extracts from the 'Goami2', 'Keunnun', and 'Heugkwang' rough rice. The values significantly increased from 3.42, 4.50, and 3.02% before germination to 7.11, 7.18, and 5.58 % after germination, respectively. The extraction yields increases during germination because soluble starch increases with starch hydrolysis. Water with ethanol was selected as the extraction solvent since both are commonly used in the food industry in a variety of ways, and are more highly stable in the human body than any other solvents. The extraction yield is highly valued because a low extraction yield means a lower productivity despite high antioxidation. Therefore, 70% ethanol was used as the extraction solvent for this study. Rice that is germinated under optimal conditions for moisture, temperature, and oxygen will grow into 0.5- to 1-mm-tall sprouts called germinated rice (25). Since it takes 3 days to grow 0.1-0.5 mm sprouts, this research used a germination period of 3 days.

Phytic acid contents Phytic acid is the primary compound used for storing phosphorus in most seeds and cereal grains, and may account for more than 70% of the total phosphorus. Phytic acid is hydrolyzed enzymatically

Table 1. Yields of 70% ethanol extracts of rough rice[1)]

Experiments		Extract yield (% dry rough rice)
'Goami2' rough rice	Ungermination	3.42±0.21
	Germination	7.11±0.11**
'Keunnun' rough rice	Ungermination	3.50±0.04
	Germination	7.18±0.61**
'Heugkwang' rough rice	Ungermination	3.02±0.12
	Germination	5.58±0.28**

[1)]Results were expressed as the average of triplicate samples with mean ±SD. $^{**}p<0.01$ indicates significant differences between groups by Students's *t*-test.

by phytases, or broken down chemically into other inositol phosphates such as inositol pentaphosphate (IP5), inositol tetraphosphate (IP4), inositol triphosphate (IP3), and possibly inositol di- and monophosphates during storage, fermentation, germination, food processing, and digestion in the human gut (26). Only IP6 and IP5 have a negative effect on the bioavailability of minerals; the other hydrolytic products formed have a poor capacity to bind minerals, or the complexes formed are even more soluble (26). Figure 1 shows the phytic acid contents (mg/g) of the rough rice. The phytic acid contents of the 'Goami2', 'Keunnun', and 'Heugkwang' before germination were 6.08, 9.87, and 9.08 mg/g, respectively, and their contents after germination were lowered to 5.38, 6.38, and 6.23 mg/g, respectively. The phytic acid contents were significantly higher in the ungerminated rough rice. During the germination of the rough rice, the amount of phytic acid decreased in response to increases in phytic activity. This activity is involved with the conversion of phytic acid to myoinositol and phosphoric acid. Therefore, phosphorus, which is decomposed from phytic acid during germination, not only plays a vital role in energy transfer and metabolic regulation, but is also an important macromolecular constituent of molecules such as phospholipids, proteins, and nucleic acids (27, 28)

Phenolic compounds Phenolic compounds occur universally in the plant kingdom as secondary metabolic products. They contain a phenolic hydroxyl group, which has an antioxidative effect through interactions with its phenol ring and its resonance stabilization effect (29-31). Figure 2 shows the total phenolic contents of the 70% EtOH rough rice extracts. The total phenolic contents in the 'Goami2', 'Keunnun', and 'Heugkwang' rough rice increased from 2.6, 2.1, and 4.9 mg/g before germination, to 3.3, 3.1, and 7.9 mg/g after germination, respectively. The elevation in phenolic compounds in the germinated rough rice can be explained by an increase in the amount of free forms from alkaline hydrolysis, due to dismantling of the cell wall during germination. Therefore, hydrolyzable phenolic compounds produced during germination can potentially reduce mutations and improve human health.

Reducing power The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. A higher absorbance indicates a higher ferric reducing power (32-34). Figure 3 shows the reducing powers of the 70% EtOH rough rice extracts as a function of their concentration. Reducing power increased in a dose-dependent manner, reaching a maximum at ca. 20 mg/mL. The reducing power of the 'Goami2', 'Keunnun', and 'Heugkwang' rough rice at 5 mg/mL increased from

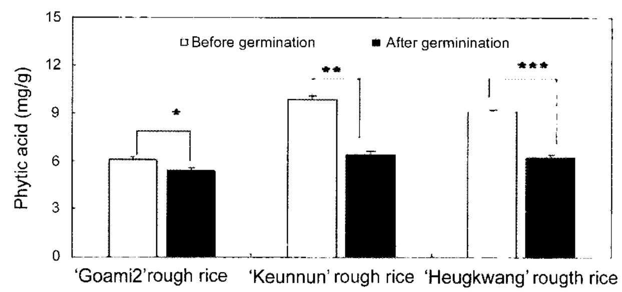

Fig. 1. Phytic acid content (mg/g) from rough rice. Results were expressed as the average of triplicate samples with mean±SD. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ indicates significant differences between groups by Students's t-test.

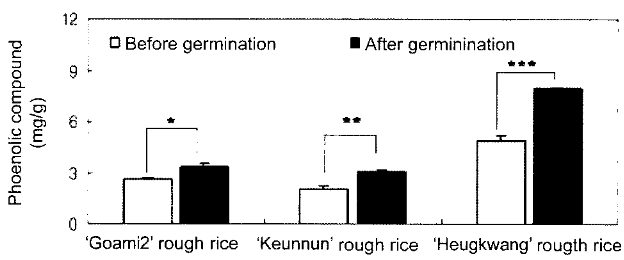

Fig. 2. Total phenolic content (mg/g) of 70% EtOH extracts from rough rice. Results were expressed as the average of triplicate samples with mean±SD. *$p<0.05$, **$p<0.01$, ***$p<0.001$ indicates significant differences between groups by Students's t-test.

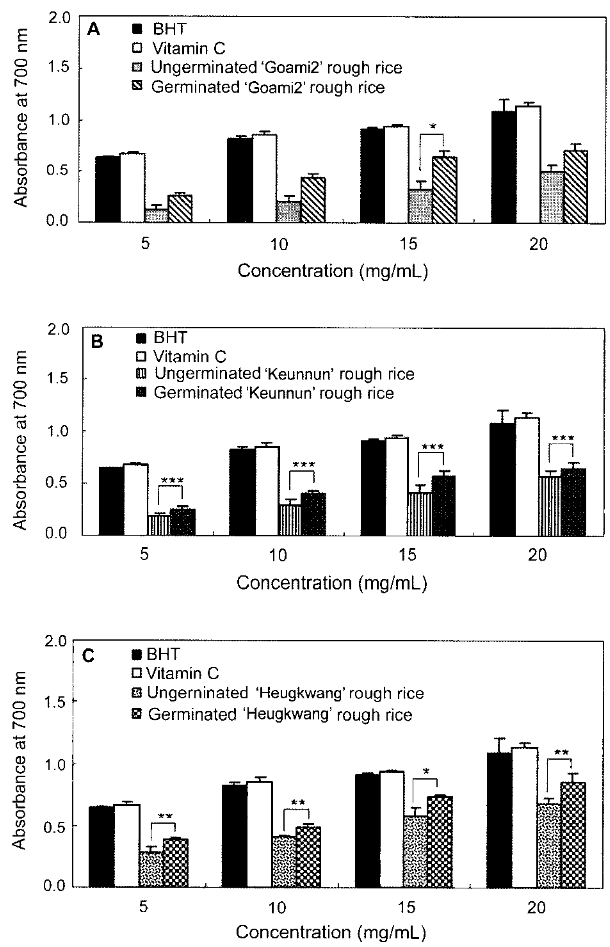

Fig. 3. Reducing power of 70% EtOH extracts from rough rice. Results were expressed as the average of triplicate samples with mean±SD. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ indicates significant differences between groups by Students's t-test.

0.18, 0.12, and 0.28 before germination, to 0.25, 0.26, and 0.33 after germination, respectively. At 20 mg/mL, the respective increase was from 0.56, 0.50, and 0.67 before germination, to 0.64, 0.71, and 0.72 after germination. Vitamin C and butylated hydroxytoluene (BHT) had excellent reducing powers of 0.67 and 0.64 at 5 mg/mL, respectively, and 1.13 and 1.08 at 20 mg/mL, respectively. With regards to reducing power, higher reducing activities can be attributed to higher amounts of polyphenolics, and the reducing capacity of a compound may reflect its antioxidant potential.

Free radical scavenging activity of DPPH The decrease in the absorbance of the DPPH radical caused by antioxidants is due to the scavenging of the radicals by hydrogen donation; this is a visible change from purple to yellow. The free radical scavenging activities of the 70% EtOH extracts of the ungerminated and germinated rough rice, along with the reference standards BHT and vitamin C, were determined using DPPH. The results are shown in Fig. 4. At 5 mg/mL, the ungerminated rough rice of 'Goami2', 'Keunnun', and 'Heugkwang' scavenged 22.6, 26.3, and 32.5% of the DPPH radicals, respectively. The respective rates for the germinated rough rice were 35.5, 36.1, and 52.6%, respectively. At 0.5 mg/mL, the scavenging values for the ungerminated rice were 16.4, 18.9, and 27.1%, respectively. For the germinated rough rice, the respective values were 22.7, 24.8, and 32.4%. The DPPH radical scavenging activities of the 70% EtOH rough rice extracts increased in a dose-dependent manner from the 0.5 to 5 mg/mL concentration. The greatest activity occurred in samples from the germinated 'Heugkwang' rough rice. In general, the polyphenol concentrations were positively correlated with antioxidant activity due to their hydrogen-donating abilities. Near linear correlations between DPPH radical scavenging activities and polyphenolic compound concentrations in various fruits and vegetables have been reported (35). These reports suggest that the radical scavenging capacities of extracts are mostly affected by the presence and position of the phenolic hydroxyl groups. The radical scavenging activity of phenolic compounds depends on their molecular structure, that is, on the availability of phenolic hydrogen and the possibility of stabilizing the resulting phenoxyl radicals that are formed by hydrogen donation.

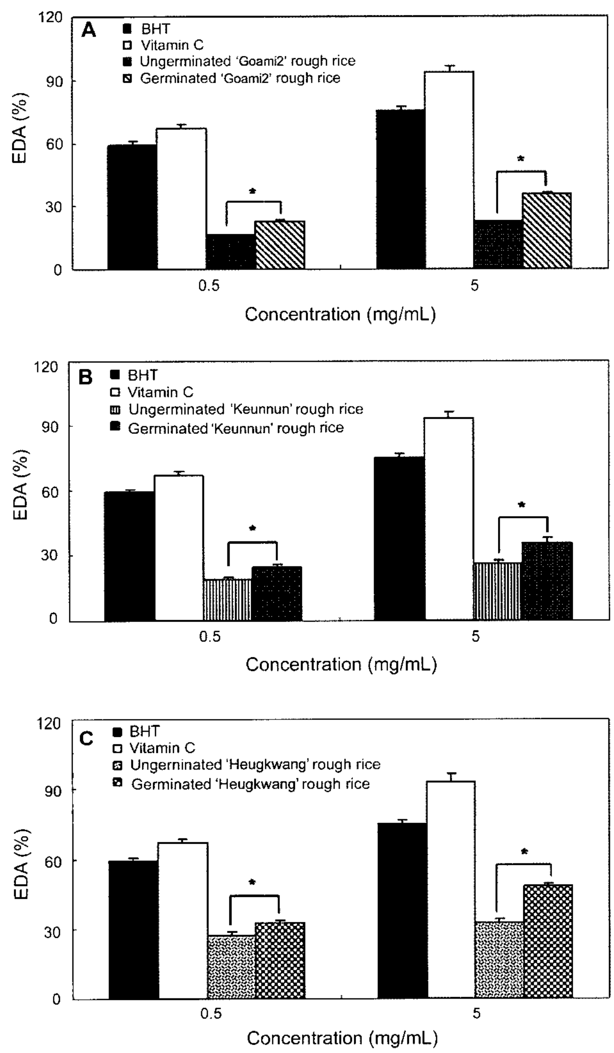

Fig. 4. Scavenging ability of 70% EtOH extracts from rough rice on DPPH radicals. Results were expressed as the average of triplicate samples with mean±SD. $*p<0.05$ indicates significant differences between groups by Students's *t*-test.

Superoxide radical scavenging activity Reactive oxygen species, including free radicals such as the superoxide radical and hydroxyl radical, and non-free-radical species such as H_2O_2 and singlet oxygen, play key roles in the oxidation process, which is considered one of the initial developmental steps of many chronic diseases (36). The superoxide radical scavenging activities of the 70% EtOH extracts from rough rice were determined using the xanthine–xanthine oxidase system. Figure 5 shows the percent inhibition of the superoxide anion radicals generated by adding 0.5-5 mg/mL of the 70% EtOH extracts. The superoxide radical scavenging activities of the rough rice extracts increased in dose-dependent manners from the 0.5 to 5 mg/mL concentration. From 0.5 to 5 mg/mL, the abilities of the 70% EtOH extracts from the ungerminated 'Goami2', 'Keunnun', and 'Heugkwang' rough rice to scavenge superoxide radicals ranged 17.5-25.4, 18.4-29.0, and 22.9-32.9%, respectively, whereas at the same concentrations, the abilities of the germinated rough rice extracts ranged 28.0-38.1, 26.8-41.6, and 44.1-55.87%, respectively. The superoxide radical scavenging abilities of the germinated rough rice were significantly higher than those of the ungerminated rough rice. The highest scavenging ability occurred with the extracts from the germinated 'Heugkwang' rough rice. Superoxide radicals indirectly initiate lipid oxidation because superoxide and hydrogen peroxide serve as precursors to singlet oxygen and hydroxyl radicals. Hence, the superoxide radical scavenging ability of the germinated rough rice shown in this study suggests that germinated rough rice has beneficial effects for decreasing the toxicity of superoxide radicals

Hydroxyl radical scavenging activity The hydroxyl radical is an extremely reactive free radical formed in

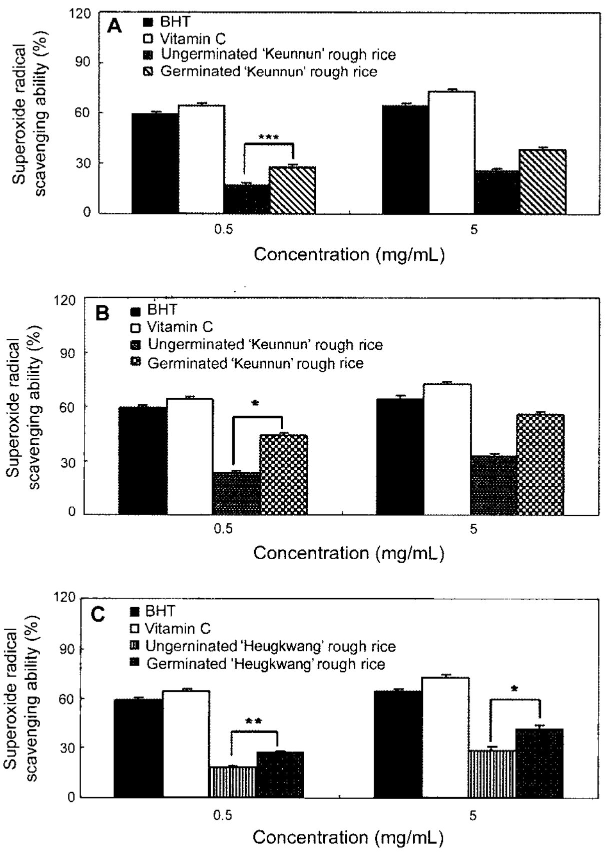

Fig. 5. Scavenging ability of 70% EtOH extracts from rough rice on superoxide radical. Results were expressed as the average of triplicate samples with mean±SD. *p<0.05, **p<0.01, and ***p<0.001 indicates significant differences between groups by Students's t-test.

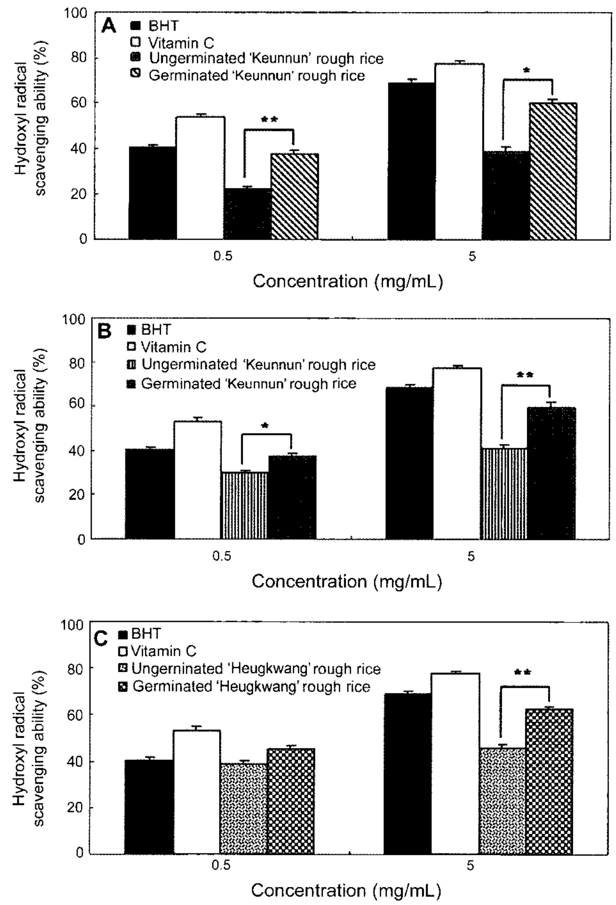

Fig. 6. Scavenging ability of 70% EtOH extracts from rough rice on hydroxyl radical. Results were expressed as the average of triplicate samples with mean±SD. *p<0.05 and **p<0.01 indicates significant differences between groups by Students's t-test.

biological systems, and has been implicated as a highly damaging species in free radical pathology, capable of damaging the biomolecules of living cells. The hydroxyl radical has the capacity to break DNA strands, which contributes to carcinogenesis, mutagenesis, and cytotoxicity. In addition, this radical species is thought to be one of the quick initiators of the lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids (37, 38). We investigated the hydroxyl radical scavenging activity of the 70% EtOH rough rice extracts using the Fenton reaction (Fig. 5). The hydroxyl radical scavenging activities of the rough rice extracts increased in a dose-dependent manner from the 0.5 to 5 mg/mL concentration. Over this concentration range, the scavenging abilities of the 70% EtOH extracts of the ungerminated *Goami2,* 'Keunnun', and 'Heugkwang' rough rice ranged 22.2-38.9, 29.6-41.8, and 38.5-45.7%, respectively, whereas the scavenging abilities of the germinated rough rice extracts ranged 37.8-60.2, 37.4-59.6, and 45.2-62.5%, respectively. The hydroxyl radical scavenging abilities of the germinated rough rice were higher than those of the ungerminated rough rice. The results of this examination indicate that the germinated rough rice had a noticeable effect for scavenging hydroxyl radicals; in particular, the germinated 'Heugkwang' rough rice has a high potential to ameliorate oxidative stress. Therefore, our research confirms that the rough rice germination process results in higher radical scavenging and antioxidant activities than those found in ungerminated rough rice. The present study suggests that germinated rough rice extracts are useful nutritional antioxidants for the nutraceutical industry. But further studies are needed to isolate and identify the antioxidant components within germinated rough rice.

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