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Effect of Germinated Brown Rice Concentrate on Free Amino Acid Levels and Antioxidant and Nitrite Scavenging Activity in *Kimchi*

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Abstract In this study, we investigated the effect of adding a 1, 3, or 5% solution of germinated brown rice concentrate (GBRC) to fermented *kimchi*. During fermentation, the concentration of free amino acids and essential amino acids increased with increasing concentrations of GBRC. In particular, higher levels of free amino acids were associated with a sweet taste compared with controls. The γ-aminobutyric acid (GABA) content of *kimchi* containing the 5% GBRC solution was 3 times higher than that of controls. The total phenolic compound content (130 mg%) did not change significantly in the control group, but increased in 10 mg% increments as the GBRC concentration rose from 1 to 3 to 5%. 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging activity and superoxide radical scavenging activity also increased with the GBRC concentration, with maximum activity during the ripe stage with GBRC measured at 79 to 82% compared with controls (30 to 71%). The nitrite scavenging activity was 10% higher with GBRC compared with controls and was highest when the pH was 1.2. These results showed that the addition of GBRC is effective in improving the function of *kimchi*.

Keywords: germinated brown rice, *kimchi*, free amino acid, antioxidant, nitrite scavenging activity

Introduction

Kimchi is a traditional Korean fermented vegetable dish (1) that is low in calories, carbohydrates, and fat. It also contains an abundance of organic acids and lactic acid producing bacteria that are produced during fermentation and provide several antioxidant effects. It also reduces the risk for anemia, hypertension, and cancer through the activity of such components as dietary fiber, vitamin C, beta carotene, and phenolic compounds (1, 2). Because *kimchi* is now consumed globally, many studies have been carried out in an attempt to boost the beneficial effects of *kimchi* using various substances, including chitosan (3), calcium (4), green tea and pumpkin powder (5), herbal medicines (6), xylitol (7), and the fungus *Flammulina velutipes* (8). However, the commercialization of these products has been a very slow process. Another food that has gained the attention of food scientists because of its beneficial effects on health is germinated brown rice. This product lowers the risk for cardiovascular and cerebrovascular disease through the activity of several of its components, including vitamins, dietary fiber, minerals (including calcium and phosphorus), superoxide dismutase (SOD), γ-aminobutyric acid (GABA), γ-orizanol, and arabinoxylan (9). The component of germinated brown rice that has attracted the most interest is GABA, a non protein amino acid that is produced in the brain and promotes brain functions by increasing acetylcholine levels and lowering blood pressure by modulating prolactin and growth hormone secretion (10, 11). The SOD concentration in brown rice increases during the germination process and catalyzes the processes that neutralize excess activated oxygen in the body (12). It is known that whole grains reduce the risk for cancer and heart disease through the effects of such components as dietary fiber, polyphenol, and lignan (13). To determine the optimum conditions for producing the most beneficial *kimchi* possible, we examined the effect of fermenting *kimchi* with a germinated brown rice concentrate (GBRC) on GABA levels and the antioxidative and nitrite scavenging effects of this food.

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Materials and Methods

Materials The GBRC used in this study was donated by Keimyung Foodex (Daegu, Korea). It contained solid components (61%) and GABA 1.5 mg/mL at 55 Brix with a pH of 6.18. All of the ingredients used to prepare *kimchi* in this study were supplied by the Bongwoori Company, Ltd. (Seoul, Korea). These included Chinese cabbages, each weighing approximately 2 kg, which were cultivated in the Hanam area in South Cholla Province of Korea. The other ingredients included radishes, red pepper powder, garlic, onion, scallion, sweet rice powder gruel, sugar (Samyang Company, Seoul, Korea), salt (sun-dried), fermented anchovy sauce (Buan, Chonbuk, Korea), and fermented shrimp (Buan).

***Kimchi* preparation and fermentation** After the outer layer of the cabbage was removed, the cabbage was cut into 4 pieces, which were soaked for 20 hr in a 1.5 to 2.0 fold volume of 10%(w/v) brine at room temperature (10-20°C), then washed 3 times with tap water, and dried for 3 hours. The final salt concentration in the cabbage was 2.0± 0.2% (Table 1). GBRC was added to 500 g of the pickled cabbage in concentrations of 1, 3, and 5%(w/w), and this mixture was then added to the *kimchi*. The *kimchi* was prepared 3 batches and sealed in a polyethylene bag that was kept at a temperature of 10°C for 21 days. The fermented *kimchi* was homogenized in a mixer (MC-811C; Samsung Company). The homo-genate was filtered through Whatman No. 3 filter paper, and the resulting

Table 1. Ingredient used to prepare *kimchi* in this study

Ingredients	Quantity (g)
Salted Chinese cabbage	500.0
Radish	43.5
Red pepper powder	21.8
Garlic	10.9
Ginger	4.2
Onion	6.4
Green onion	13.1
Fermented anchovy sauce	15.7
Fermented shrimp	8.7
Glutinous rice paste	15.6
Sugar	9.5
Salt	1.6

filtrate was used for analysis.

Free amino acids The amount of free amino acids in the *kimchi* was determined using an automatic amino acid analyzer (Biochrom 30; Biochrom, Cambridge, UK) according to the pretreatment method reported by Oh *et al.* (14). This analysis was carried out in a lithium citrate buffer solution at a rate of 0.33 mL/min and a ninhydrin flow rate of 0.33 mL/min at a column temperature of 37°C and a 40 mL injection volume.

Total phenol compound The total level of phenol compounds in the *kimchi* filtrate was measured using an UV-visible spectrophotometer (UV-1601; Shimadzu, Kyoto, Japan) at a wavelength of 700 nm, according to the Folin-Denis method (15). The tannic acid level was used as a reference, and the phenol compound level was calculated based on a standard curve.

DPPH free radical scavenging activity The scavenging activity of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was evaluated using the Blois method (16) at a wavelength of 517 nm in a UV-visible spectrophotometer (UV-1601; Shimadzu). The radical activity was calculated using the following equation:

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

Superoxide radical scavenging activity The scavenging activity of the superoxide radical ($-O_2^-$) was evaluated using the following equation at a wavelength of 550 nm according to the xanthine-xanthine oxidase cytochrome C conversion method (17):

$$-O_2^- \text{ scavenging activity}(\%)=\left(1-\frac{A_{sample}}{A_{blank}}\right)\times 100$$

Nitrite scavenging effect The nitrite scavenging effect and nitrite-scavenging rate (%) was evaluated using a UV-visible spectrophotometer (UV-1601; Shimadzu) at a wavelength of 520 nm according to the method reported by Noh *et al.* (18). A blank was prepared by adding 0.4 mL distilled water instead of the Griess reagent. The nitrite scavenging effect was determined on the basis of the following formula:

$$\text{Nitrite scavenging effect}(\%)=\left(1-\frac{A-C}{B}\right)\times 100$$

where, A = absorbance of sample during a reaction with 1 mM $NaNO_2$, B = absorbance of $NaNO_2$, C = absorbance of sample.

Statistical analyses Each experiment was repeated 3 times, and the results were expressed as the mean±SD for the 3 experiments. However, the free amino acid content was measured only once. Significance was determined at 5% based on an ANOVA analysis and a Duncan's multiple range tests.

Results and Discussion

Changes in the amount of free amino acids Changes in the level of free amino acids in the *kimchi* samples were determined immediately after each batch of *kimchi* was prepared, at the optimum stage of maturity (9 days after preparation) and during the over-fermented stage (21 days after preparation) (19). The concentration of total free amino acids increased with increasing amounts of GBRC and with fermentation time (Table 2). In particular, threonine and GABA levels were 3 times higher in the *kimchi* containing 5% GBRC than in the control group. The concentration of free amino acids that confer a sweet taste (threonine, serine, glycine, and alanine) and those conferring a bitter taste (valine, leucine, and phenylalanine) increased with increasing GBRC levels. The methionine level remained unchanged and the arginine level decreased slightly. The level of glutamic acid, which confers a sour taste decreased in a dose dependent manner. The aspartic acid concentration was lower than in the control group in the early stages with the addition of low concentrations of GBRC, but was similar in all groups as fermentation progressed. The concentrations of the essential amino acids increased with increasing GBRC levels throughout fermentation. Oh *et al.* (20) reported that as brown rice germinates, its concentration of free amino acids including GABA, glycine, alanine, valine, methionine, leucine, and lysine increases. Moreover, they reported that it was possible to prepare *kimchi* with a high concentration of essential amino acids by adding GBRC, which would increase the level of amino acids during fermentation. The amount of essential amino acids in 50 g of *kimchi* prepared with GBRC was 6 mg, which is the daily amount recommended by the World Health Organization (21). These results suggest that GBRC might be effective in increasing the functional properties of *kimchi.*

Changes in total phenol compounds Phenol compounds are prevalent as secondary metabolites in plants. They exist as various structures and molecular weights and are related to the innate flavor of food. They contain the

Table 2. Changes in free amino acids levels in *kimchi* with various concentrations of germinated brown rice concentrate (GBRC) fermented at 10°C

Free amino acid (mg/100 g of *kimchi*)	GBRC concentration (days)											
	Control group			1%			3%			5%		
	(0)	(9)	(21)	(0)	(9)	(21)	(0)	(9)	(21)	(0)	(9)	(21)
Taurine	5.01	5.20	5.45	5.11	5.33	5.13	5.06	4.49	4.89	5.91	4.76	4.68
Urea	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	27.09	10.53
Aspartic acid	13.57	15.94	16.25	10.01	12.15	17.54	8.19	9.38	18.55	2.54	6.54	19.59
Threonine	11.04	14.88	16.57	18.56	20.19	21.65	25.54	23.18	20.16	48.01	48.52	49.36
Serine	19.51	20.43	20.14	24.33	25.68	26.06	28.45	29.13	29.53	31.01	30.46	32.85
Glutamic acid	112.85	113.01	113.58	107.41	108.12	109.25	100.65	102.02	101.64	85.49	86.47	86.01
Sarcosine	ND[1)]	ND	ND	ND	ND	4.35	ND	ND	4.57	ND	ND	3.98
α-Aminoadipic acid	3.28	6.48	6.54	5.43	6.99	7.02	5.68	4.97	6.85	1.28	1.11	5.32
Proline	50.18	70.33	75.63	60.43	67.68	74.16	61.09	53.78	75.16	65.11	52.89	74.05
Glycine	14.00	14.15	15.06	15.01	16.91	17.54	17.61	18.38	18.93	18.65	21.75	23.01
Alanine	89.47	99.65	98.50	100.31	102.17	99.12	115.41	122.84	105.67	118.88	128.90	120.06
Citrulline	1.51	1.61	1.97	1.83	2.79	2.88	2.47	3.11	3.64	1.45	1.76	2.63
α-Amino-n-butyric acid	4.57	3.00	2.98	4.95	3.20	3.33	4.53	4.19	4.58	3.87	2.86	3.63
Valine	29.86	31.08	32.21	28.43	29.97	33.52	30.18	27.40	34.02	35.84	36.95	40.45
Cystine	5.17	8.70	8.75	5.92	9.98	9.99	6.36	9.41	9.68	7.14	8.15	9.14
Methionine	4.01	4.42	4.10	4.26	4.33	4.26	4.31	4.23	4.58	4.08	3.79	3.98
Cystathionine	1.17	1.85	1.92	1.68	2.48	2.49	1.79	1.78	2.25	1.84	1.85	2.07
Isoleucine	14.02	16.58	16.86	14.58	15.81	16.25	17.65	20.64	20.05	17.59	17.62	18.96
Leucine	20.72	22.44	23.54	21.11	22.08	23.67	20.98	22.00	23.46	25.57	25.69	26.98
Tyrosine	8.11	9.78	13.57	8.17	9.68	14.65	9.61	10.65	15.01	8.54	8.76	14.89
β-Alanine	2.16	3.42	3.53	2.58	3.43	3.51	2.59	3.53	3.48	3.03	3.21	3.41
Phenylalanine	12.17	15.22	16.38	13.06	14.78	15.28	13.54	14.37	15.94	12.96	13.81	14.86
α-Amino-n-butyric acid	8.80	8.82	8.81	13.76	13.74	13.76	18.05	18.04	18.00	24.35	24.33	24.36
Ethanolamine	5.35	5.22	5.64	5.39	5.33	5.38	5.49	5.53	5.68	5.36	5.42	5.81
δ-Hydroxylysine	ND	ND	1.04	ND	ND	0.54	ND	ND	0.74	ND	0.34	0.86
Ornithine	30.38	35.72	40.89	31.57	35.89	41.11	26.08	27.70	40.81	29.94	25.03	41.35
Lysine	10.31	22.40	38.69	11.62	22.11	39.84	14.81	21.35	38.54	18.62	19.80	39.06
1-Methyl-l-histidine	1.14	3.48	3.21	1.26	2.72	3.28	1.89	4.12	4.03	2.03	2.66	3.65
Histidine	7.58	7.83	7.58	7.13	7.59	7.84	7.84	8.26	8.48	7.01	6.56	6.96
Arginine	7.34	6.46	5.84	6.95	6.26	6.10	7.01	6.11	6.12	7.54	4.57	4.85
TA[2)]	493.28	568.13	605.23	530.85	577.39	629.50	562.86	580.59	645.04	593.64	622.64	700.34
EA[3)]	102.13	127.02	148.35	111.62	129.26	154.47	127.01	133.18	156.75	162.67	166.17	196.65

[1)]ND: not detected.
[2)]TA: total amino acid.
[3)]EA: essential amino acid.

phenolic hydroxyl group, which has an antioxidative effect through interactions with its phenol ring and has a resonance stabilization effect. Phenol compounds easily bind with proteins and induce various physiologic activities in microbes that retard microbial growth (22). The total phenol compound content of *kimchi* prepared in this study was determined immediately after its preparation, during the optimum stage of maturity and in the over-fermented stage. The total phenol content immediately after preparation with 1, 3, and 5% GBRC was 138.48, 144.47, and 148.35 mg%, respectively, compared with 131.17 mg% for controls (Fig. 1). During the optimum stage of *kimchi* maturity, the total phenol content increased significantly with increasing concentrations of

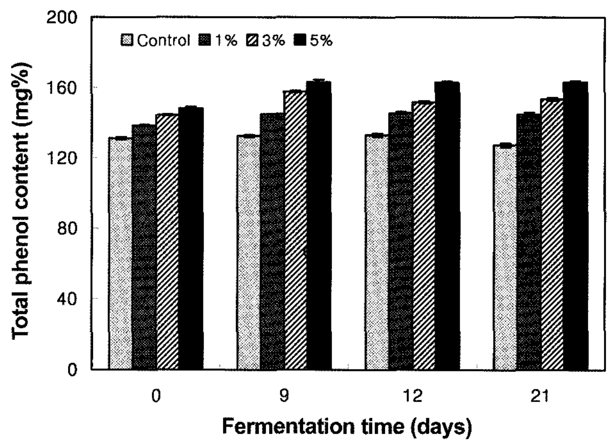

Fig. 1. Changes in total phenol content in *kimchi* extract containing various concentrations of germinated brown rice concentrate fermented at 10°C. Values are expressed as the mean±SD (n=3).

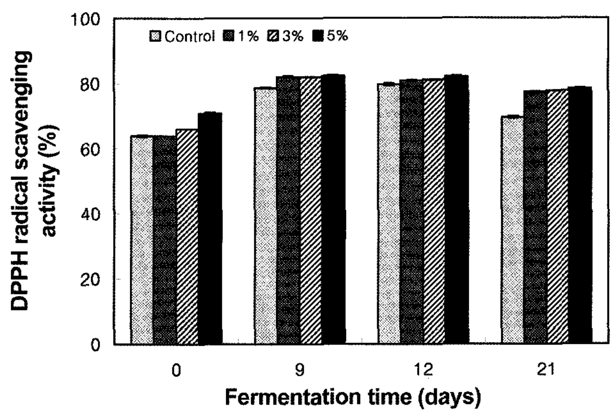

Fig. 2. Changes in DPPH free radical scavenging activity in *kimchi* extract containing various concentrations of germinated brown rice concentrate fermented at 10°C. Values are expressed as the mean±SD (n=3).

GBRC (144.95, 157.77, and 162.98 mg% with 1, 3, and 5% GBRC, respectively). The total phenol content was constant during the over-fermented stage; this finding is similar to that reported by Hwang *et al.* (23), in which the total amount of phenol compounds was high in mustard leaf *kimchi* after 6 days of fermentation compared with the amount detected immediately after its preparation. This increase was due to the presence of phenolic acids such as ρ-coumaric and ferulic acids and their interactions with microbes that produced ethyl or vinyl derivatives of phenol with antioxidative activity. Uda *et al.* (24) suggested that the amount of phenol compounds increases with fermentation time.

Changes in DPPH free radical scavenging activity DPPH is a stable free radical that has been used to estimate the free radical scavenging activity of antioxidants (25). When DPPH is scavenged by an antioxidant and transformed into DPPH-H, the color of the solution turns from purple to yellow; the degree of change can be detected by the degree of decay in absorbance at 517 nm (26). Free radical scavenging activity was measured in the *kimchi* immediately after its preparation, at the optimum stage of maturity (9-12 days after preparation), and at the over-fermented stage (21 days after preparation). Free radical scavenging activity immediately after preparation was similar in the control group and in the 1% GBRC group at 63.90 and 63.85%, respectively (Fig. 2). It was somewhat higher in the 3 and 5% GBRC groups (66.03 and 70.77%, respectively) and increased with increasing fermentation time. Free radical scavenging activity decreased to 69.53% in the control group in the over-fermented stage, but raised to the range of 77 to 79% in the GBRC groups. This finding is similar to that reported by Park *et al.* (5) in which DPPH free radical scavenging activity was higher in mustard leaf *kimchi* prepared by adding green tea and pumpkin powder at the optimum stage of maturity.

Changes in superoxide radical scavenging activity The superoxide radical ($-O_2^-$), which is formed by xanthine oxidase, transforms ferricytochrome c into ferrocytochrome c. This radical can be detected by an increase in absorbance at 550 nm (27, 28). The superoxide radical scavenging activity in our *kimchi* samples was investigated immediately after preparation, during the optimum stage of maturity (9-12 days after preparation) and the overfermented stage (21 days after preparation). Activity increased with fermentation time and thus, was highest during the over-fermented stage (Fig. 3). The $-O_2^-$ activity was 1. 5 times higher in the *kimchi* containing GBRC than in the control group and decreased during the over-fermentation stage. This suggests that differences in antioxidative activity are related to the products produced during *kimchi* fermentation. During the fermentation period, $-O_2^-$ activity was low in the control group (9-20%), but increased with the GBRC concentration to the range of 27 to 71%. Kim *et al.* (29) reported that the $-O_2^-$ activity was high 85.3 and 63.5%, respectively, in the hot water and ethanol extracts of green tea. Hong *et al.* (30) suggested that it was high (56-65%) in a fermented pine needle extract.

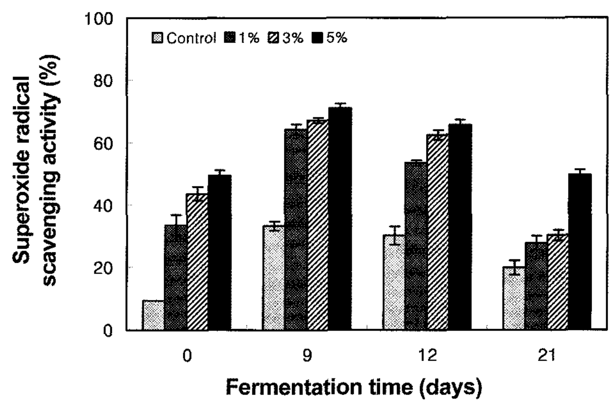

Fig. 3. Changes in superoxide radical scavenging activity in *kimchi* extract containing various concentrations of germinated brown rice concentrate fermented at 10°C. Values are expressed as the mean±SD (n=3).

Table 3. Changes in nitrite scavenging activity in *kimchi* extract containing various concentrations of germinated brown rice concentrate fermented at 10°C (unit: %)

pH	GBRC Conc.(%) \ Days	Nitrite scavenging ability[1)] 0	9	12	21
1.2	Control	57.94±1.14[c2)]	71.54±2.58[b]	73.35±0.57[c]	74.88±8.14[b]
	1	61.98±0.34[b]	88.22±1.41[a]	90.15±0.39[a]	93.84±2.71[a]
	3	61.71±0.53[b]	88.16±0.49[a]	88.82±0.15[b]	92.61±5.35[a]
	5	64.43±0.55[a]	86.63±0.76[a]	90.31±0.49[a]	92.12±2.78[a]
3.0	Control	44.79±1.64[b]	56.26±0.65[c]	59.24±0.69[c]	71.64±0.42[a]
	1	50.60±1.45[a]	65.01±1.15[a]	68.11±0.92[a]	77.51±2.24[a]
	3	49.74±1.77[a]	62.32±1.41[b]	65.62±1.67[b]	81.83±3.58[a]
	5	49.29±1.13[a]	61.02±0.71[b]	67.58±1.32[ab]	83.21±11.70[a]
4.2	Control	17.44±2.33[b]	34.68±0.61[d]	39.98±0.68[c]	51.74±1.86[b]
	1	22.98±0.95[a]	41.35±0.62[a]	49.46±1.20[a]	56.54±4.81[b]
	3	22.96±0.82[a]	38.21±1.02[c]	47.84±0.22[b]	70.38±0.67[a]
	5	23.31±1.44[b]	39.97±0.19[b]	46.89±0.83[b]	71.38±2.96[a]
6.0	Control	3.19±0.99[b]	19.07±0.71[b]	30.52±1.29[b]	40.86±0.60[b]
	1	4.51±0.48[ab]	24.79±0.66[a]	40.31±0.61[a]	47.93±3.85[a]
	3	5.19±0.96[a]	24.22±0.34[a]	39.34±0.46[a]	45.97±1.66[ab]
	5	5.29±0.35[a]	24.52±0.47[a]	39.42±1.21[a]	51.47±4.89[a]

[1)]Mean without letters is not significant ($p<0.05$); Values are expressed as the mean ± SD (n=3).
[2)]Mean values with the same superscript in each column are not significant ($p<0.05$).

Changes in nitrite scavenging effect Nitrite reacts with 2nd and 3rd grade amines in protein-rich foods, medicines, and residual pesticides to form nitrosamine. It is also present in large quantities in meat colors and both leafy and root vegetables. Nitrosamine converts to diazoalkane ($C_nH_{2n}N_2$, an alkaline nucleic acid), proteins, and intracellular components, which can increase the risk for cancer. Nitrite is toxic; the consumption of excess amounts of nitrite over time results in the oxidization of hemoglobin, which can lead to methemoglobinemia (31). The nitrite scavenging effect in cabbage *kimchi* in a range of acidic conditions (pH 1.2, 3.0, 4.2, or 6.0) was investigated immediately after preparation, during the optimum stage of maturity (9-12 days after preparation), and at the over-fermented stage (21 days after preparation). The scavenging effect was highest at a pH of 1.2 (Table 3). The scavenging rates were 57.94, 61.98, 61.71, and 64.43% immediately after preparation in the control group and in the 1, 3, and 5%(w/w) GBRC groups, respectively, which suggests that the scavenging effect increases with the GBRC concentration. The nitrite scavenging effect also increased with the length of fermentation time. Nitrite scavenging rates 12 days after *kimchi* preparation were 90.15, 88.82, and 90.31% in the 1, 3, and 5%(w/w) GBRC groups, respectively; these rates were significantly higher than the rate of 73.35% observed in the control group ($p<0.05$). The rate remained high during the over-fermented stage, but decreased with increasing pH. The nitrite scavenging effect was not proportional to the amount of GBRC added, however. It was higher before and after fermentation than in the control group. The fact that the nitrite scavenging effect was high at pH 1.2 suggests that nitrosamine production can be inhibited *in vivo*. This finding is similar to that reported by Kang *et al.* (32) who found that the nitrite scavenging effect in a pine needle extract was high at pH values lower than 3 and low at a pH of 6. Based on these results, we conclude that the addition of GBRC is effective in improving the function of *kimchi.*

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