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Changes in the Chemical and Functional Components of Korean Rough Rice Before and After Germination

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Abstract This study investigated changes in the chemical and functional components of germinated rough rice for the development of functional foods. The chemical components that were determined for 'Ilpum', 'Goami2', 'Keunnun', and 'Heugkwang' rough rice cultivars included dietary fiber, free sugars, free amino acids, and functional components such as tocopherols and γ-oryzanol. The crude protein, fat, total dietary fiber, and free sugar contents of the rough rice increased significantly after germination. The essential amino acid content was particularly increased. After the germination of the 'Ilpum', 'Goami2', 'Keunnun', and 'Heugkwang' varieties, the following increases were found: γ-aminobutyric acid increased 2.4, 2.5, 6.1, and 3.4 times, respectively; α-tocopherol, α-tocotrienols, and γ-tocotrienols increased significantly; and γ-oryzanol content increased 0.8, 1.1, 1.5, and 1.2 times, respectively. Thus, germinated rough rice has the potential to be used as a healthy and functional food ingredient.

Keywords: chemical component, γ-aminobutyric acid, γ-oryzanol, germinated rough rice, tocopherol

Introduction

Germination is a processing method by which the quality of a cereal can be improved for both digestibility and physiological function (1). During germination, enzymatic activity and bioactive compounds increase within the seed because metabolic activity releases the energy required for germination from stored carbohydrates, lipids, and proteins. New compounds such as γ-aminobutyric acid (GABA), γ-oryzanol, and useful amino acids are also synthesized during germination (2, 3).

Dietary fiber has beneficial effects on bowel transit time, affects glucose and lipid metabolism, reduces the risk of colorectal cancer, stimulates bacterial metabolic activity, detoxifies the colon luminal content, and helps to maintain the equilibrium of the colon ecosystem as well as the integrity of the intestinal mucosa by acting as a prebiotic (4, 5). Dietary fiber fits the definition of a functional food in that it can affect one or more targeted function in the body in a positive manner (5).

Tocopherols are regarded as intracellular antioxidants because they inhibit the peroxidation of polyunsaturated fatty acids in biological membranes (6). γ-Oryzanol, which is 13 to 20 times (w/w) higher in rice bran than total tocopherols and tocotrienols, reportedly decreases animal serum cholesterol levels, has anti-inflammatory activity, and can inhibit cholesterol oxidation *in vitro* (7). GABA functions as a major inhibitory neurotransmitter, plays a role in sensing pain and anxiety, and is involved in regulating cardiovascular function, including blood pressure and heart rate (8). GABA also potentiates insulin secretion in the pancreas (9-11).

Rough rice is rice that has not been processed, and thus possesses the hull and bran, which are removed during the threshing and polishing of rough rice. There have been active efforts within the food and food engineering industries to find products from waste byproducts that contain functional ingredients with physiological and nutritional value, but research on the physiological activators within rough rice is still lacking. We are committed to the development of functional food supplements from rough rice. Accordingly, to measure the chemical and functional compositions of germinated Korean rough rice, we have undertaken research into the dietary fiber, free sugars, free amino acids, tocopherols, and γ-oryzanol of germinated rough rice, to provide basic data in order to enhance its usage value.

Materials and Methods

Materials The 'Ilpum', 'Goami2', 'Keunnun', and 'Heugkwang' rough rice cultivars were grown at the National Institute of Crop Science, Rural Development Administration, Suwon, Korea, during the 2006 growing season.

Sample preparation The rough rice was soaked in water at 15°C, with water changes every 24 hr. Three days after germination, the rough rice was dried at 60°C for 24 hr and then ground in a food processor (J World Tech, Korea). The powdered rice samples were then passed through a 100-mesh sieve, and analyzed for their approximate free sugar, vitamin E, and γ-oryzanol contents.

Analysis of overall composition The AOAC standard method (12) was used for the determination of crude protein, fat, and ash. Crude protein content was measured by Kjeldahl method. Crude ash and fat were incineration

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at 550°C in an electric furance (F-2F; Kong Soung Co., Seoul, Korea) and soxhelt (Dong Ha-Tech., Seoul, Korea). The total dietary fiber content was measured using a dietary fiber assay kit (Sigma, St. Louis, MO, USA), according to Prosky's method (13)

Analysis of free sugars Free sugars were analyzed by extracting 5 g of the homogenized sample in 20 mL of water, which was then filtered through a 0.45-μm membrane and injected into an HPLC (Waters 2695; Waters, New Castle, DE, USA). The analysis conditions followed the method of Park *et al.* (14). Here we used a carbohydrate analysis column (4.6×150 mm; Waters), RI detector (Waters 2414; Waters), and acetonitrile:water 75:25 (v/v) mobile phase at a flow rate of 1 mL/min.

Analysis of free amino acids The amount of free amino acids in the rough rice powder before and after germination was determined using an automatic amino acid analyzer (Biochrom 30; Biochrom, Cambridge, UK), according to the pretreatment method reported by Oh (15). The analysis was carried out in a lithium citrate buffer and a ninhydrin at a flow rate of 0.33 mL/min, with a column temperature of 37°C and a 40-mL injection volume.

Analysis of vitamin E The vitamin E contents of the rough rice methanolic extracts were determined according to the procedure described by Lee *et al.* (16), with some modifications. In brief, an aliquot of each methanolic extract was evaporated under nitrogen gas. The residues were redissolved in *n*-hexane, filtered, and analyzed by normal phase HPLC (Younglin Ins., Seoul, Korea). Tocopherol and tocotrienol analysis was performed on an LiChrosphere-Diol 100 column (4.0×250 mm, i.d. 5 μm) using a hexane :isopropanol mobile phase of of 98.7:1.3 (v/v) at a flow rate of 1 mL/min. Peaks were detected by fluorescence using an excitation wavelength of 290 nm and an emission wavelength of 330 nm.

Analysis of γ-oryzanol γ-Oryzanol was analyzed using HPLC (Thermo Separation Products, San Jose, CA, USA) with a UV detector at 325 nm. The methanolic extracts were evaporated under nitrogen gas. The residues were redissolved in *n*-hexane, filtered, and analyzed by HPLC. The sample extracts were separated on a Nova-Pak C18 column (3.9×150 mm; Waters) using a modified version of the Rogers *et al.* (17) method. The extraction studies were performed using initial mobile phase conditions of 50% MeOH, 40% acetonitrile, 5% water, and 5% dichloromethane, at a flow rate of 1.0 mL/min for 5 min. The mobile phase was changed linearly to methanol, acetonitrile, water, and dichloromethane at a ratio of 45:45:5:5 (v/v/v) over the next 10 min. After 30 min, the mobile phase was changed linearly to a ratio of 40:45:5:10 (v/v/v) and held there for 60 min before returning to the initial conditions.

Statistical analysis Statistical analysis was carried out using SPSS version 11.5 (SPSS Inc., Chicago, IL, USA). The results are expressed as means ± standard deviations. Student's *t*-tests for unpaired data were used for all measured parameters to determine the significance of the changes before and after germination.

Results and Discussion

Composition Table 1 shows the changes in overall composition that we determined before and after germination. The crude protein and crude fat contents increased significantly after germination, probably because of the biosynthesis of new compounds during germination. These results agree with research reported for soybeans (18), mung beans (19), and germinated brown rice (20). After germination, the total dietary fiber content of the rough rice increased 1.6, 1.2, 1.1, and 1.9 times in the 'Ilpum', 'Goami2', 'Keunnun', and 'Heugkwang' cultivars, respectively. This tendency for an increase in dietary fiber after germination agrees with reports for buckwheat (21) and soybeans (18). Increases in dietary fiber result from the formation of primary cell walls, through an increase in pectic substances in the middle lamella. Total dietary fiber promotes beneficial physiological effects by acting as a laxative, lowering blood cholesterol, and producing glucose attenuation (5, 6).

Free sugar In the free sugar analysis of the rough rice by HPLC (Table 2), monosaccharides such as glucose and fructose, and disaccharides such as maltose, were found before and after germination. The free sugar content of the rough rice increased after germination; glucose showed the highest increase from 0.278−0.489 g/100 g to 1.307−

Table 1. Changes in the overall composition of rough rice before (BG) and after (AG) germination

		Overall compositions[1] (%)			
		Crude ash	Crude lipid	Crude protein	Total dietary fiber
'Ilpum' rough rice	BG	4.56±0.19	2.08±0.10	6.08±0.12	11.9±1.04
	AG	5.02±0.31	2.68±0.11*	6.75±0.03*	19.2±0.30*
'Goami2' rough rice	BG	5.30±0.09	2.63±0.33	7.05±0.07	20.6±0.86
	AG	5.51±0.06	3.47±0.03	7.37±0.03*	26.7±0.49*
'Keunnun' rough rice	BG	4.97±0.02	2.36±0.03	6.19±0.12	19.61±0.41
	AG	5.27±0.04*	3.28±0.05**	7.05±0.93*	22.1±0.24*
'Heugkwang' rough rice	BG	4.70±0.28	2.09±0.08	6.85±0.07	13.2±0.61
	AG	4.75±0.07	3.00±0.11*	7.04±0.07**	25.3±0.42**

[1]Results are expressed as the average of triplicate samples with mean±SD ;*p<0.05 and **p<0.01 by Students's t-test.

Table 2. Changes in free sugar content of rough rice before (BG) and after (AG) germination

		Free sugar[1)] (g/100g)			
		Glucose	Fructose	Maltose	Total free sugar
'Ilpum' rough rice	BG	0.278±0.054	0.201±0.052	0.018±0.031	0.498±0.075
	AG	1.691±0.180***	0.767±0.028***	0.530±0.308*	2.990±0.496**
'Goami2' rough rice	BG	0.489±0.215	0.225±0.021	0.078±0.006	0.810±0.409
	AG	1.563±0.082**	0.266±0.016	0.351±0.180*	2.190±0.244*
'Keunnun' rough rice	BG	0.499±0.112	0.319±0.066	0.030±0.060	0.854±0.116
	AG	1.611±0.134***	0.707±0.081**	0.325±0.142*	2.647±0.263***
'Heugkwang' rough rice	BG	0.395±0.180	0.273±0.165	0.050±0.095	0.723±0.249
	AG	1.307±0.216**	0.696±0.128*	0.374±0.139*	2.381±0.251**

[1)]Results are expressed as the average of triplicate samples with mean±SD; *p<0.05, **p<0.01, and ***p<0.001 by Students's t-test.

1.691 g/100 g. Total free sugars increased by 4 or 9 times after germination. However, other studies have provided contradictory reports. For example, some studies have shown increases in free sugars as a consequence of starch hydrolysis, whereas others have shown decreases in reducing and total sugars (22). The increases we found in maltose, glucose, and fructose after germination were similar to the reports of Kwon (23). The free sugar content after germination increased significantly, which could have been caused by starch hydrolysis.

Free amino acids The essential amino acid content

Table 3. Change in free amino acid content of rough rice before (BG) and after (AG) germination

Free amino acid (mg/100 g)	'Ilpum' rough rice		'Goami2' rough rice		'Keunnun' rough rice		'Heugkwang' rough rice	
	BG	AG	BG	AG	BG	AG	BG	AG
Phosphoserine	19.48	34.85	32.39	40.67	23.99	34.82	16.34	28.49
Taurine	18.96	29.50	27.47	43.71	21.94	38.49	18.04	39.17
Urea	223.21	0.00	2853.8	0.00	2588.7	0.00	1786.8	0.00
Aspartic acid	43.94	116.85	73.38	171.09	73.68	147.68	44.92	136.91
Threonine	6.84	83.52	20.97	196.50	10.77	189.81	5.49	113.89
Serine	15.14	138.62	47.87	384.15	26.52	319.97	21.07	184.73
Glutamic acid	60.92	117.53	76.34	172.98	74.99	319.20	42.03	216.11
α-Amino adipic acid	26.80	12.01	32.00	0.00	13.30	9.23	40.74	0.00
Glycine	12.68	59.75	38.56	114.71	31.22	113.59	19.04	68.99
Alanine	28.29	154.95	101.40	277.87	100.18	451.65	50.76	205.51
Valine	22.02	40.28	31.23	58.18	28.68	172.80	20.81	43.61
Cysthin	44.50	189.50	195.29	159.63	188.91	13.07	47.20	209.79
Cysthiein	27.09	118.93	155.80	78.77	144.94	68.23	24.43	199.23
Isoleucine	0.00	0.00	0.00	38.95	0.00	21.73	0.00	0.00
Phenylalanine	0.00	30.30	19.83	24.54	0.00	21.68	16.40	39.30
β-Alanine	0.00	22.60	0.00	49.05	0.00	41.01	0.00	29.43
γ-Amino butyric acid	31.59	78.46	52.29	145.78	81.53	509.07	51.29	178.59
$EOHNH_2$	5.72	17.97	21.66	19.43	16.53	27.22	14.18	19.16
NH_3	119.01	28.28	0.00	81.45	16.49	45.98	17.40	45.69
Hydroxylysine	48.31	27.15	60.20	57.74	61.16	25.81	57.86	59.21
Lysine	0.00	41.07	14.27	93.56	0.00	185.12	0.00	54.10
Histidine	0.00	0.00	0.00	21.49	0.00	58.06	0.00	15.26
Carnosine	248.90	69.71	159.44	327.07	163.22	229.77	103.81	278.61
Arginine	0.00	12.92	0.00	45.02	0.00	82.34	0.00	15.69
Proline	0.00	0.00	42.08	26.85	26.32	78.88	342.85	0.00
Total amino acid	1000.4	1424.8	4156.3	2629.2	3693.1	3205.2	2741.5	2181.5
Essential amino acid[1)]	28.9	230.7	86.3	478.2	39.5	731.5	42.7	281.5

[1)]Essential amino acids: threonine+valine+isoleucine+phenylalanine+lysine+histidine+arginine.

increased after germination (Table 3). Some essential amino acids that are necessary for growth in children (e.g., histidine and arginine) were generated after germination; and lysine, which is the first limiting amino acid in rice, also increased. Furthermore, the free amino acids that confer a sweet taste (i.e., threonine, serine, glycine, and alanine) as well as the flavor enhancers (i.e., taurine, glutamic acid, and aspartic acid) increased after germination. The level of GABA in the 'Ilpum', 'Goami2', 'Keunnun', and 'Heugkwang' rough rice increased 2.4, 2.5, 6.1, and 3.4 times, respectively, after germination. In particular, GABA increased greatly in the 'Keunnun' rough rice, possibly due to its large embryo, where most physiological activity takes place. Lee and Chang (24). reported that GABA content increased in germinated 'Keunnunbyeo' rice. In germinated cereal grains, hydrolytic enzymes are activated and decompose starch, non-starch polysaccharides, and amino acids. The decomposition of high molecular weight polymers during germination leads to the generation of bio-functional substances, and to improvements in organoleptic qualities due to the softening of texture and increase of flavor in cereal grains (25).

Vitamin E Natural vitamin E consists of 4 tocopherols and 4 tocotrienol homologs, including α, β, γ, δ, that all have antioxidant, anticancer, and cholesterol-lowering activity (26). α-Tocopherol content increased significantly after germination, as did the α- and γ-tocotrienol contents (Table 4). However, we did not detect β- and δ-tocotrienols before or after germination (Table 4). Plaza *et al.* (27) reported that the α-tocopherol content in soybean sprouts increased 6-fold compared to the content of the raw seed. Similar results were reported by *Marero et al.* (28) during soybean germination. Cereal grains are important dietary sources of tocopherols. The tocopherol isomer, α-tocopherol, has the strongest vitamin E activity and the greatest reactivity against singlet oxygen species. Tocotrienols reportedly inhibit cholesterol synthesis, lower serum cholesterol levels in animal models, and suppress tumor cell proliferation; and the γ- and δ-homologs have greater potency than the α-homolog (24). Oxidative stress is related to diabetes and diabetic complications, and fat-soluble vitamins such as vitamins A and E diminish the lipid content of blood plasma in patients with non-insulin-dependent diabetes mellitus.

γ-Oryzanol Rice bran is removed from the starchy endosperm during the rice milling process. Initially, γ-oryzanol was thought to be a single compound, but it's now known to be a mixture of at least 10 phytosteryl ferulates. Cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, and campesteryl ferulate have been identified as the major components, accounting for 80% of the γ-oryzanol in rice bran oil (8, 29). The γ-oryzanol contents of the 'Ilpum', 'Goami2', 'Keunnun', and 'Heugkwang' rough rice varieties increased 0.8, 1.1, 1.5, and 1.2 times, respectively, after germination (Table 5). In 'Keunnun' rough rice, it is thought that the increase in γ-oryzanol occurs in the germinating embryo. γ-Oryzanol is reported to possess health benefits such as the improvement of plasma lipid patterns, the reduction of total plasma cholesterol, increases in high density lipoprotein-cholesterol, and the inhibition of platelet aggregation (30). However, most research has been performed on experimental animals; thus, it is necessary to confirm if the beneficial effects of rough rice occur in humans.

In this study, the effects of germination were found according to the increased contents of dietary fiber, vitamin E, GABA, and γ-oryzanol, suggesting the high potential

Table 5. Change in the γ-oryzanol content of rough rice before (BG) and after (AG) germination

		γ-oryzanol content[1)] (mg/g)
'Ilpum' rough rice	BG	4.81±0.21
	AG	5.55±0.24
'Goami2' rough rice	BG	5.81±0.48
	AG	6.54±0.46
'Keunnun' rough rice	BG	4.85±0.43
	AG	7.36±1.69***
'Heugkwang' rough rice	BG	3.48±0.54
	AG	6.56±0.46*

[1)]Results are expressed as the average of triplicate samples with mean ±SD; *$p<0.05$ and ***$p<0.001$ by Students's *t*-test.

Table 4. Change in vitamin E content of rough rice before (BG) and after (AG) germination[1)]

		Tocopherol (T) (mg/100 g)				Tocotrienol (T_3) (mg/100 g)				α-TE[2)] (mg/100 g)
		α	β	γ	δ	α	β	γ	δ	
'Ilpum' rough rice	BG	0.14±0.01	0.05±0.07	3.19±0.01		0.13±0.02	-	0.03±0.01	-	0.38±0.04
	AG	0.64±0.01**	0.06±0.07	3.12±0.04		0.96±0.05**	-	0.38±0.16	-	1.95±0.04**
'Goami2' rough rice	BG	0.46±0.04	0.27±0.02	3.58±0.01	-	0.66±0.36	-	0.03±0.02	-	1.41±0.51
	AG	0.66±0.03*	0.48±0.04**	3.16±0.04	-	1.23±0.69*	-	0.57±0.04**	-	1.48±0.50
'Keunnun' rough rice	BG	0.56±0.04	-[3)]	1.02±0.11	-	0.16±0.09	-	0.04±0.38	-	0.52±0.02
	AG	0.75±0.04	-	0.67±0.03	-	0.28±0.14	-	0.06±0.62	-	1.27±0.02**
'Heugkwang' rough rice	BG	0.48±0.02	0.04±0.07	2.97±1.51	-	0.58±0.02	-	0.34±0.03	-	0.97±0.25
	AG	0.72±0.02*	0.18±0.02*	2.95±1.54	-	1.09±0.07*	-	0.46±0.05	-	1.43±0.12*

[1)]Results are expressed as the average of triplicate samples with mean±SD; *$p<0.05$ and **$p<0.01$ by Students's *t*-test.
[2)]α-Tocopherol equivalent.
[3)]Not detected.

use of germinated rough rice for beneficial purposes. The increases in dietary fiber, vitamin E, GABA, and γ-oryzanol after germination indicate that germinated rough rice is a useful food supplement for the prevention and improvement of lifestyle-induced diseases.

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