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RESEARCH NOTE

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Effects of Germination on Fatty Acid and Free Amino Acid Profiles of Brown Rice ‘Keunnun’

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Abstract The effect of germination on hydration and germination properties, and on the changes of fatty acids and amino acids profiles of a brown rice ‘Keunnun’ (KN) with a large embryo was compared to ‘Ilpumbyeo’ (IP) with a normal embryo. A rapid germination up to 24 hr was observed in both brown rice cultivars, afterward decreased with germination time. At 60 hr, the KN (86.0±4.24%) showed slightly lower germination capability than the IP (97.0±1.41%). Lower water uptake during germination was also found in the KN (1.22±0.02 g) compared to the IP (1.59±0.05 g). Major fatty acids were palmitic acid, oleic acid, and linoleic acid accounting for more than 95% of total fatty acids. The most abundant amino acid in both types was oleic acid, which was decreased during germination, whereas palmitic and linoleic acids were increased. Eight amino acids were detected, and a remarkable increase in γ-amino butyric acid (GABA) during germination was observed. The KN was characterized with higher tasty amino acids of aspartic acid, glutamic acid, glycine, and alanine.

Keywords: brown rice, enlarged embryo, germination, fatty acid, free amino acid

Introduction

Brown rice grains contain higher nutritional components, which are mainly existed in germ and bran layers. A brown rice ‘Keunnun’ (KN) with a large embryo was developed by traditional plant-breeding technique at the National Institute of Crop Science, Rural Development Administration (RDA) in Korea. In the previous study (1), the embryo of KN was observed approximately 2.5-3.0 folds larger than that of ordinary brown rice ‘Ilpumbyeo’ (IP). Although a KN has been cultivated within some limited area due to slightly higher retail cost, the brown rice is favored to consumers who are aware of good nutrition of foods.

The hard texture of cooked brown rice is one of problems despite of the higher contents of biofunctional components. Recently, a germinated brown rice has attracted public attention as germination utilizes the physiologically active substances that are present in bran, and soaking process improves the texture of brown rice (2). In addition, nutrients in the seed become easier to digest and absorb during germination (3). Germination of brown rice decomposes high molecular weight polymers and leads to the generation of biofunctional substances, improving organoleptic qualities due to softening texture and increasing flavor (4,5). Chemical constituents in germinated brown rice are vitamins, minerals, dietary fibers, plant lipid, and effective components such as phytic acid and ferulic acid (6,7). Free amino acids play an importance role in tastes of brown rice, as well. According to the research on lipid content and fatty acid compositions of brown rice, they vary by type of brown rice such as Indica and Japonica type (8), and cropping year (9) and season (10). Thus, this paper was conducted to examine the effect of germination on fatty acid compositions and free amino acid profiles of brown rice KN, and also to observe the hydration and germination properties.

Materials and Methods

Rice plants Brown rice ‘Keunnun’ (KN) and ‘Ilpumbyeo’ (IP) were grown at the National Institute of Crop Science, RDA, Suwon, Korea during the 2006 growing season. Rice paddies stored at 15°C were dehusked using a rice sheller (Model SY88-TH; Ssangyong Ltd., Incheon, Korea) for brown rice production.

Hydration property during germination Hydration property was measured by the amount of water uptake according to the method of Miyoshi and Sato (11) with slight modification. The 100 kernels of brown rice were placed on 2 layers of filter paper (Whatman® No. 2) in a petri dish (9-cm in diameter) that contained 10 mL of deionized water. Water uptake was measured after draining extra water completely, and average values were determined in triplicate measurements.

Germination property The 50 g of brown rice was placed in a perforated nylon bag, and soaked in distilled water for 4 hr at room temperature. Germination procedure was followed by the method of Choi *et al.* (12) with slight modification. After draining the distilled water, the soaked brown rice was germinated in a chamber at 30°C equipped with a water sprinkler to provide fresh water every 6 hr. Germinated brown rice seeds, which were determined by emerging its radicle at least 1.0 mm in length, were sampled at 12, 24, 36, 48, and 60 hr. Germination capability was determined by counting the number of germinated brown rice out of 100 rice seeds and expressed of percentile (%) of seeds. The average was determined with

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duplicate measurements.

Fatty acids compositions Fatty acids were separated by the method by Rafael and Mancha (13). The 0.5 g of freeze-dried brown rice powder was heated with a reagent of methanol:heptane:benzene:2,2-dimethoxypropane:H_2SO_4 (37:36:20:5:2, v/v). Digestion and lipid transmethylation took place simultaneously at 80°C. After cooling at room temperature, the upper phases containing fatty acids methyl esters (FAMEs) were prepared for the capillary gas chromatography (GC) analysis. A GC system (HP 6890; Agilent, Santa Clara, CA, USA) was equipped with a flame ionization detector (FID) and a HP-Innowax capillary (cross-linked polyethylene glycol) column (0.25 mm ×30 m). The initial column temperature was set at 150°C, and heated up to 280°C at a heating rate of 4°C/min. The carrier gas was nitrogen at a flow rate of 10 mL/min (14). The temperature of inlet and detector were constantly retained at 250 and 300°C, respectively. Standard FAME mixture (C14-22) obtained from Supelco (Sigma-Aldrich®; Bellefonte, PA, USA) was separated under the identical conditions for the fatty acid compounds.

Free amino acids profiles Germinated brown rice was freeze-dried (Medel KR/PVTFD 10A; Ilsin Lab Co., Ltd., Yangju, Korea) at the condenser temperature of −84°C and vacuum of 665.60 Pa. The dried brown rice was ground into fine particle (100 mesh) for chemical analysis using a grinding mill (Cyclotec™, 1093 sample mill; Foss Tecator Co., Hillerod, Denmark). The 1.0 g of germinated brown rice flour was diluted in 10 mL of 3% trichloroacetic acid solution, left at room temperature for 1 hr, and centrifuged at 19,319×g for 15 min. The collected supernatant was filtered through a Millipore 0.45-syringe filter. Amino acids were separated using an L-8800 high-speed amino acid autoanalyzer (Hitachinaka; Hitachi, Tokyo, Japan). Each filtered sample solution of 10 μL was injected and the analysis was carried out by ninhydrin reagent analysis set (Wako Chemical Inc., Osaka, Japan). The mixture of amino acids standard solution type AN-II and type B (Wako Chemical Inc.) were used.

Results and Discussion

Hydration and germination properties It was observed that higher water uptake at 12 hr soaking was found in KN (0.82±0.04 g), and compared to IP (0.71±0.02 g). At 24 hr, water uptake was similar in both rice cultivars, but hydration capability of IP increased over KN since 24 hr, resulting in lower hydration property of KN (1.22±0.03 g) than IP (1.59±0.05 g) at 60 hr hydration. On the other hand, a rapid germination occurred in KN (40.0%) at 12 hr germination, whereas lower in IP (12.5%), reflecting that higher water uptake in KN at the early stage (12 hr) could affect the rapid germination. But, as germination time approached close to 24 hr, the rate of germination in IP (85.5%) exceeded to KN (75.0%), resulting in lower germination of KN (86.0%) than IP (97.0%). It was assumed that the observed hydration and germination properties in KN and IP would be due to the different rice cultivars. Kozlowski (15) reports that the germination capability can be influenced by rice cultivar, storage conditions after harvesting, and diverse environmental variables.

Effects of germination on fatty acid compositions The major fatty acid profiles in KN and IP were mainly palmitic (16:0), oleic (18:1), and linoleic acids (18:2), comprising more than 95% of the total fatty acid compositions (Table 1). No detectable stearic (18:0) and linolenic acids (18:3) were identified. In raw brown rice, the fatty acids compositions of KN, as compared with IP, were higher in oleic acid (42.15 for KN vs. 36.85 for IP), whereas lower in palmitic acid (21.20 for KN vs. 22.15 for IP) and linoleic acid (36.65 for KN vs. 38.85 for IP). Although fatty acid contents were slightly varied with germination time, germination treatment decreased oleic acid, but increased linoleic and palmitic acids in both types. Total saturated fatty acids (SFA, palmitic+stearic acids) and unsaturated fatty acids (USFA, oleic+linoleic+ linolenic acids) were determined by individual fatty acid content. Generally, the SFA increased, but the USFA slightly decreased with germination time, showing approximately 3

Table 1. Fatty acid profiles of brown rice 'Keunnun' and 'Ilpumbyeo' during germination

Cultivar	Germination time (hr)	Composition (%)					SFA[1)]	USFA[2)]
		C16:0	C18:0	C18:1	C18:2	C18:3		
'Keunnun'	0	21.20	-	42.15	36.65	-	21.20	78.80
	12	25.30	-	32.80	41.85	-	25.30	74.65
	24	24.10	-	38.10	37.80	-	24.10	75.90
	36	28.45	-	32.15	39.40	-	28.45	71.55
	48	25.45	-	36.60	37.10	0.80	25.45	74.50
	60	24.95	1.60	34.65	37.65	1.10	26.55	73.40
'Ilpumbyeo'	0	22.15	0.40	36.85	38.85	1. 25	22.55	76.95
	12	31.40	-	23.65	45.05	-	31.40	68.70
	24	31.25	-	23.50	42.00	2.45	31.25	67.95
	36	30.85	-	25.40	43.80	-	30.85	69.20
	48	29.80	-	27.55	42.65	-	29.80	70.20
	60	30.50	-	26.30	43.25	-	30.50	69.55

[1)]SFA, saturated fatty acid and; [2)]USFA, unsaturated fatty acid.

Table 2. Free amino acid compositions in brown rice 'Keunnun' and 'Ilpumbyeo' during germination

Cultivar	Germination time (hr)	Free amino acid (mg/100 g)								
		Aspartic acid	Serine	Glutamic acid	Glycine	Alanine	Cystine	GABA	Hydroxy-lysine	Total
'Keunnun'	0	23.05	6.73	30.58	2.43	5.52	11.37	11.83	6.09	97.60
	12	5.91	3.51	16.69	3.01	18.49	5.99	21.83	5.37	80.81
	24	2.87	3.33	10.86	3.12	23.88	2.26	33.32	6.52	86.15
	36	2.86	2.97	6.90	2.94	17.69	7.52	30.73	5.73	77.33
	48	2.85	6.98	13.53	3.45	32.47	3.02	67.05	3.43	132.78
	60	3.12	6.84	19.24	5.14	35.10	9.58	81.25	3.29	163.56
'Ilpumbyeo'	0	6.52	3.16	10.46	2.82	9.95	9.19	6.27	5.59	53.96
	12	3.21	-	4.65	1.22	3.23	7.49	5.59	5.83	31.22
	24	-	1.28	4.39	1.34	4.49	8.02	10.84	5.38	35.73
	36	-	1.27	3.58	1.48	4.90	9.79	13.64	5.45	40.11
	48	-	3.70	5.26	2.39	10.33	7.17	24.64	6.22	59.70
	60	-	3.25	4.36	2.72	11.18	14.51	30.37	6.71	73.10

fold higher USFA than SFA in both cultivars. At 60 hr germination, the amount of SFA and USFA was 26.55 and 73.40% in KN, and 30.50 and 69.55% in IP, respectively.

Effects of germination on free amino acid profiles Eight amino acids were detected in both rice cultivars (Table 2). These include aspartic acid (Asp), serine (Ser), glutamic acid (Glu), glycine (Gly), alanine (Ala), cystine (Cys), γ-amino butyric acid (GABA), and hydroxyl-lysine (Hylys). Before germination, among 8 amino acids, 7 amino acids except alanine were higher in KN compared to those in IP. Most abundant amino acids were Glu (30.58 mg/100 g) followed by Asp (23.05) and GABA (11.83) in KN, whereas Glu (10.46), Ala (9.95), and Cys (9.19) in IP. Germination treatment affects the changes of various amino acids, showing a significant decrease in Asp and Glu, but an increase in Ala and GABA. At 60 hr germination, a remarkable increase in GABA was found in KN (81.25 mg/100 g) compared to IP (30.37 mg/100 g). Saikusa *et al.* (16) reports that GABA had a glutamate-like taste accompanied by sweetness. In addition, research on eating quality of rice reports that aspartic acid, glutamic acid, glycine, and alanine are classified as the important flavor-enhancing or tasty amino acids (17). Thus, the higher contents of aspartic acid, glutamic acid, and GABA in KN can affect positively the taste of cooked brown rice compared to IP. A clinical study reports that the symptoms of menopause or mental disorder are improved by taking 26.4 mg of GABA/day through the diet including defatted rice germ rich in GABA (18). They also suggests that eating less than 50 g of germinated brown rice prepared by soaking for more than 72 hr can provide effective amount of GABA.

The total amino acid (TAA) contents, which are determined by individual amino acid, were found approximately twice higher in KN (97.60 mg/100 g) compared to IP (53.96 mg/100 g). Germination increased the TAA contents up to 163.56 in KN, but 73.10 in IP. This result suggests that germination treatment has a positive effect on the amino acid profiles, reflecting that KN could have a potentiality as a nutritional brown rice. Despite of improving texture as well as some health benefits of germinated brown rice, concerns on germination procedures also have been arisen. As germinated brown rice is rich in nutritional components, it is easily infested by microorganisms, and also produces off-flavors during germination (17,19). Thus, germination procedure has to be conducted very carefully in terms of food safety and sanitation.

Germinated brown rice would be a valuable process because of improving eating quality of cooked brown rice and containing more biofunctional components. In particular, KN would have a potentiality as a health-promoting cereal crop due to enlarged embryo. But, the lower germination capability of KN should be improved to promote the production of KN, thus enhancing consumption of the functional rice.

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