Food Sci. Biotechnol. Vol. 18, No. 2, pp. 402 ~ 406 (2009)

Food Science and Biotechnology

Changes in Enzyme Activities of Rice with Respect to the Viviparity and the Duration of Lodging Time

Tai-Jeong Hwang, Won-Jong Lee[1], Jin-Chul Shin[2], Chul-Won Lee[3], and Suk-Shin Kim*

Department of Food Science and Nutrition, The Catholic University of Korea, Bucheon, Gyeonggi 422-743, Korea
[1]*Department of Food Science, Kangnung National University, Gangneung, Gangwon 210-702, Korea*
[2]*National Crop Experiment Station, Rural Development Administration, Suwon, Gyeonggi 441-100, Korea*
[3]*Defense Agency for Technology and Quality, Cheongryang PO Box 276, Seoul 130-650, Korea*

Abstract The Japonica cultivar 'Janganbyeo' was cultivated and about half of paddy field was totally lodged at 30 days after heading. Both lodged paddy and unlodged paddy were harvested at intervals. The lodged paddy and brown rice did not show any signs of viviparity until the 4th day of lodging. The activities of α-amylase, diastase, and lipase of lodged paddy and brown rice began to increase on the 2nd day of lodging and then increased slowly afterwards. The activities of protease and phytase of lodged paddy and brown rice reached the maximum values on the 2nd day and then decreased until the final day of lodging. In contrast, unlodged paddy and brown rice showed relatively small increase in viviparity and in activities of α-amylase, diastase, protease, lipase, and phytase. Especially, activities of diastase and lipase were observed even in unlodged paddy and brown rice.

Keywords: rice, viviparity, lodging, enzyme activity

Introduction

Germination has been reported to improve the nutritional quality of cereals by enhancing the degree of digestibility, physiological function, and the essential nutrients, and by suppressing the action of anti-nutrients (1). In case of rice germination, both the activities of enzymes such as phytase and the bioactive compounds such as gamma-aminobutyric acid (GABA) increase due to the metabolic needs during germination. Thus, the germinated rice has often been used as an enzyme food or its ingredient because it is rich in enzymes and bioactive compounds (2,3). In general, the enzyme food is considered to prevent the chronic diseases related to the immune function damage, hypertension, cancer, fatigue-related disorders, or the liver disorders (4).

In the mean while, typhoon, a tropical cyclone with strong wind and heavy rain has been one of the natural hazards, which can cause lodging of rice in Asia either tropical or temperate (5,6). Especially, the Korean peninsula which is one of the geographically passing zones of typhoon often experiences typhoon attacks and crop damages every year. Furthermore, more than half of the typhoons come in August or September when the rice paddy is on its ripening stage. In such case, rice paddy is easily lodged and the lodged rice often shows the viviparity, a kind of germination on the panicle (7,8). In general, the occurrence of viviparity is considered to be harmful owing to the yield loss and the quality deterioration of rice; however, the viviparity of rice may also be beneficial due to the active enzymes and the functional components desirable to health.

*Corresponding author: Tel: +82-2-2164-4316; Fax: +82-2-2164-4316
E-mail: kimsukshin@catholic.ac.kr
Received August 30, 2008; Revised October 22, 2008;
Accepted November 3, 2008

Recently, several research works regarding the germination of rice have been reported with respect to the functional components such as GABA (9,10), ferulic acid, and arabinoxylan (11), and antioxidative activities (12). In addition, a few literature reports have dealt with the enzymes of germinated rice (3,13). However, no reports have focused on the enzyme activity changes of rice with respect to the viviparity and the duration of lodging time.

The specific objective of this study, to use the lodged rice showing viviparity as a high-valued food item, was to determine the changes in enzyme activity of paddy and brown rice with respect to the viviparity and the duration of lodging time and to compare the changes with those of unlodged rice. The enzymes involved here were α-amylase, diastase, protease, lipase, and phytase.

In this study, a Japonica cultivar 'Janganbyeo' was used. The first reason is that it is a medium maturing variety which often experiences lodging during the ripening stage by typhoon attacks in August or September. The second reason is that it is relatively tolerant to lodging and suitable for cultivating in the central field of South Korea (14).

Materials and Methods

Materials Both lodged and unlodged samples in the form of paddy and brown rice were suuplied by the National Crop Experiment Station (NCES), Hwaseong, Gyeonggi, Korea. According to NCES, a Japonica rice cultivar 'Janganbyeo' was cultivated under basal fertilizer conditions, and about half of paddy field was totally lodged at 30 days after heading. Both lodged and unlodged paddy were harvested at intervals of 0, 2, 4, 6, 8, 11, 13, 15, 20, and 23 days, and solar dried to the 15% moisture content. The dried paddy was dehulled to brown rice using a rice huller (Satake, THU35A; Stake Corp., Hiroshima, Japan).

Measurement of viviparity of rice One-hundred rice grains were randomly picked up and examined by naked eyes if they have any signs of viviparous germination.

Measurement of α-amylase activity of paddy and brown rice The α-amylase activity was determined by AACC method 22-01 (15) as follows. Finely ground rice (5 g) was extracted using 100 mL 0.5% NaCl solution. The malt infusion (10 mL) was diluted to 100 mL with 0.5% NaCl solution at 30°C. Then the malt infusion and buffered dextrin were mixed. At an appropriate time interval the hydrolyzing mixture was mixed with 5 mL dilute I_2 solution at 20°C, and the resulting color was compared with a standard color. The α-amylase activity was expressed as a SKB α-amylase unit.

Measurement of diastase activity of paddy and brown rice The diastase activity was determined by AACC method 22-16 (15) as follows. Finely ground rice (25 g) was extracted with 500 mL distilled water at ambient temperature (15-25°C) for 2.5 hr. The enzyme extract was mixed with 100 mL buffered starch solution. After 30 min reaction time, 10 mL of 0.05 N ferrycianide solution was added to 5 mL digested starch solution. Then the mixture was titrated with 0.05 N thiosulfate solution to a complete disappearance of blue color. The diastase activity was expressed as °L based on the mL's of thiosulfate solution required for the titration.

Measurement of protease activity of paddy and brown rice The protease activity was determined by the AACC method 22-61 (15) as follows. Finely ground sample (20 g) was extracted using 100 mL acetate buffer solution (pH 4.7) at 40°C for 1 hr. The enzyme solution was added to 10 mL denatured hemoglobin solution at 40°C. After 30 min of reaction 10 mL of 30% trichloroacetic acid (TCA) acid solution was added, shaken vigorously, and filtered. The absorbance of filtrate was read at 275 nm. Protease activity was expressed as HUT/g.

Measurement of lipase activity of paddy and brown rice The lipase activity was measured using a dough method (16). Ground rice sample was defatted with petroleum ether. Then 98 mL triolein and 330 mL buffer solution (pH 7.5) were added. The dough was mixed thoroughly with a glass rod and incubated at 37°C for 60 min. The reaction was stopped by adding 100 mL of 1 N HCl. The oleic acid that had been hydrolyzed from the triolein was extracted into 5 mL isooctane and measured spectrophotometrically. The absorbance at 715 nm was read and compared with that of the oleic acid standard. Lipase activity was expressed as μmol of oleic acid/hr/g (17).

Measurement of phytase activity of paddy and brown rice The phytase activity was measured by incubating the enzyme extract with sodium phytate at pH 5.2 and 47°C for 1 hr (18). The proportions of enzyme extract, sodium phytate, and buffer solution in the reaction mixture were equal to the proportions used by Lolas and Markakis (19). The final concentration of buffer solution and phytate were 0.1 M and 1 mM, respectively. After incubation, 4 mL of 2 M HCl was added to stop the enzymatic reactions. The phytase activity was expressed as a degraded inositol hexaphosphate/min.

Statistical analysis The analysis of statistical differences ($p<0.05$) was performed using the statistical analysis program of SigmaPlot for Windows version 9.00 (Systat Software, Inc., Richmond, CA, USA).

Results and Discussion

Viviparity changes of paddy and brown rice during lodging The viviparity (%) of paddy and brown rice of 'Janganbyeo' either lodged or unlodged with respect to the duration of lodging time are shown in Fig. 1. In case of paddy, the signs of viviparity of lodged sample was not observed until the 4th day of lodging, and afterwards the viviparity rapidly increased to 13.7% on the 8th day. The average viviparity of lodged sample from the 8th day to the 23rd day was 13.0%. In contrast, unlodged sample did not show any signs of viviparity. In case of brown rice, the sign of viviparity of lodged sample was not observed until the 4th day of lodging. On the 6th day the viviparity increased to 33.1% and afterwards decreased with time down to 18.6%. The average viviparity (%) was 25.4% from the 6th day to the 23rd day. In contrast, unlodged brown rice showed no remarkable signs of viviparity with the average of 0.5% (0.04-0.99%).

Our results of viviparity seemed similar to those reported by Ju *et al*. (7). They reported the viviparity (%) values of 'Janganbyeo' paddy with different days after heading (25, 35, and 45 days) were 0.2, 2.4, and 6.2% after 4 days of lodging, 3.0, 15.8, and 12.9% after 8 days of lodging, and 11.9, 40.9, and 31.4% after 12 days of lodging, respectively. Some discrepancy might be due to the differences in the cultivating conditions of the 2 lodging experiments. In addition, the higher viviparity of brown rice than paddy may be due to the easy observation of the sign of viviparity on the brown rice kernel naked without husk.

α-Amylase activity changes of paddy and brown rice during lodging The changes in α-amylase activity of

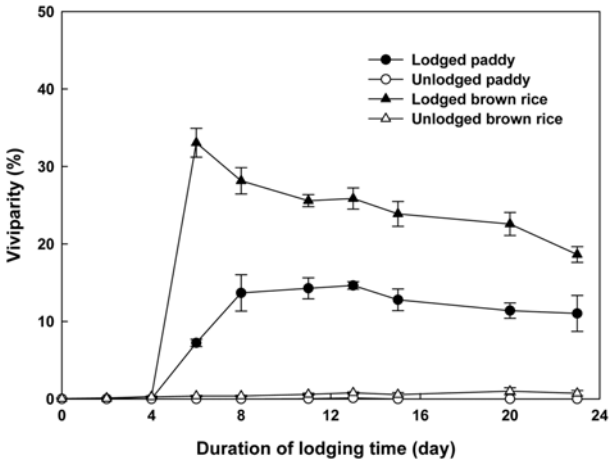

Fig. 1. Changes in viviparity (%) of paddy and brown rice of 'Janganbyeo' either lodged or unlodged with respect to the duration of lodging time.

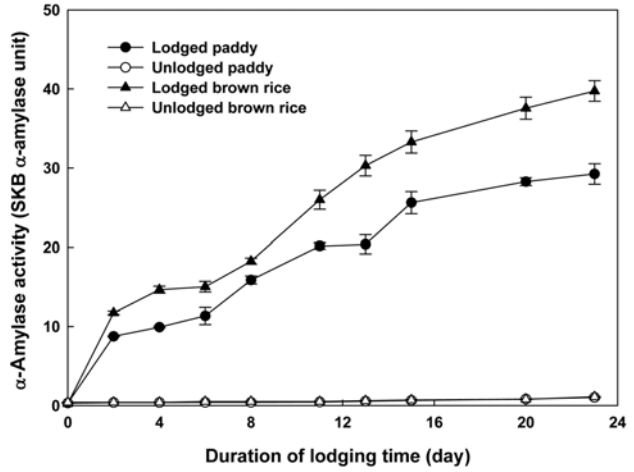

Fig. 2. Changes in α-amylase activity of paddy and brown rice of 'Janganbyeo' either lodged or unlodged with respect to the duration of lodging time.

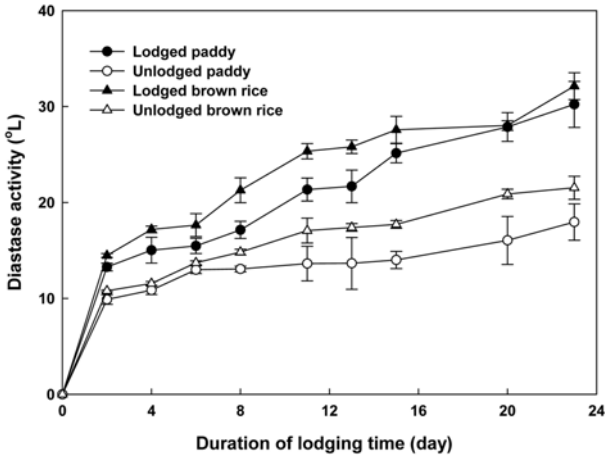

Fig. 3. Changes in diastase activity of paddy and brown rice of 'Janganbyeo' either lodged or unlodged with respect to the duration of lodging time.

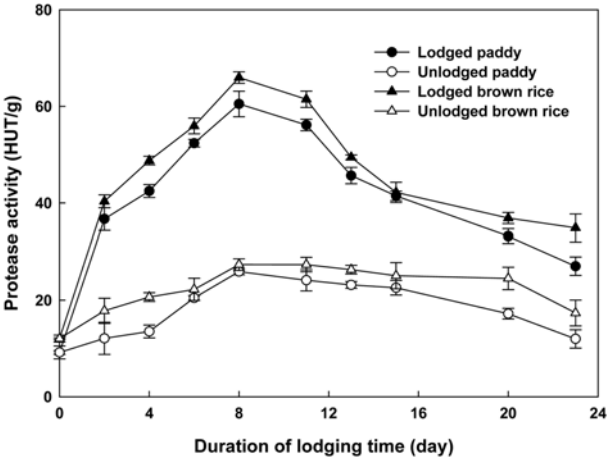

Fig. 4. Changes in protease activity of paddy and brown rice of 'Janganbyeo' either lodged or unlodged with respect to the duration of lodging time.

lodged or unlodged rice of 'Janganbyeo' in the form of paddy and brown rice with respect to the duration of lodging time are shown in Fig. 2. Unlodged paddy and brown rice did not show any remarkable α-amylase activity; in contrast, α-amylase activity of lodged paddy and brown rice increased after 2 days lodging. As shown in Fig. 1, the signs of viviparity of paddy and brown rice were scarecely observed on the 2nd day; however, sprouting must have been prepared inside of each kernel even before the 2nd day. In cereals, generally, α-amylase activity may be detected during the early germination. The α-amylase activity on the 2nd day seemed essential for providing energy and carbon skeletons to the embryo through the respiratory breakdown of utilizable substrates.

The α-amylase activity of lodged paddy increased from 0.3 SKB α-amylase unit on the 0 day of lodging to 8.8 on the 2nd day, 15.9 on the 8th day, 25.6 on the 15th day, and 29.2 SKB α-amylase unit on the 23rd day of lodging. The increasing rate of α-amylase activity was the highest (8.8 SKB α-amylase unit/day) on the 2nd day of lodging. In case of lodged brown rice, the α-amylase activity increased from 0.3 SKB α-amylase unit on the 0 day of lodging to 11.7 on the 2nd day, 18.3 on the 8th day, 33.3 on the 15th day, and 39.7 SKB α-amylase unit on the 23rd day of lodging. The increasing rate of α-amylase activity was the highest (11.7 SKB α-amylase unit/day) on the 2nd day of lodging. Our result was different from the report of Kim *et al.* (20) who reported that α-amylase activities decreased from 8 days after seeding and also from the report of Sen *et al.* (21) who reported that α-amylase activity reached a maximum by the 7th day of germination. Our result is also different from the report that the 2nd day α-amylase activity of lodged brown rice was higher than that of germinated brown rice at 25°C (22). The possible reason for the differences may be due to the difference between viviparous germination and normal germination. In addition, the overall α-amylase activity of lodged brown rice was higher than that of lodged paddy. This may be due to the fact that dehusking may stimulate germination of rice (23) and that the solid content in the same amount of ground sample is higher for brown rice than for paddy.

Diastase activity changes of paddy and brown rice during lodging Diastase activity (Fig. 3) was measured as the amount of reducing sugar produced by both α-amylase and β-amylase. Diastase activity was present even in unlodged paddy and brown rice, although its levels in unlodged samples were much lower than those in lodged ones. The diastase activity of lodged paddy increased to 13.3°L on the 2nd day of lodging, 17.2 on the 8th day, 25.1 on the 15th day, and 30.2°L on the 23rd day of lodging. In case of lodged brown rice, the diastase activity increased to 14.5°L on the 2nd day of lodging, 21.3 on the 8th day, 27.6 on the 15th day, and 32.1°L on the 23rd day of lodging, respectively. Like the case of α-amylase activity, the overall diastase activity of lodged brown rice was higher than that of lodged paddy. In addition, the maximum diastase activities of lodged paddy and brown rice were lower than those of germinated brown rice freeze dried (22). The difference seemed mainly due to the differences in moisture content of the 2 cases. Both the lodged paddy and brown rice showed sudden increase in diastase activity on the 2nd day of lodging. The β-amylase activity is usually

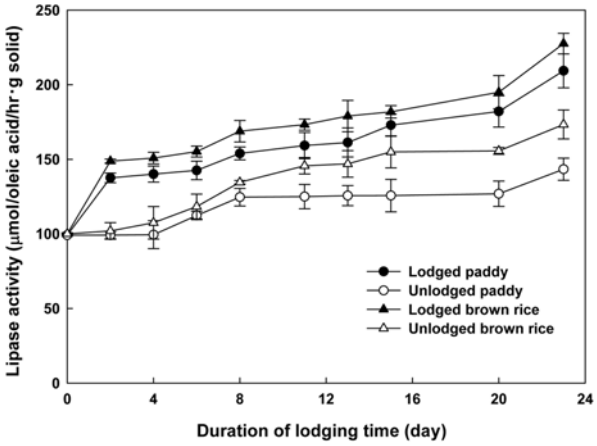

Fig. 5. Changes in lipase activity of paddy and brown rice of 'Janganbyeo' either lodged or unlodged with respect to the duration of lodging time.

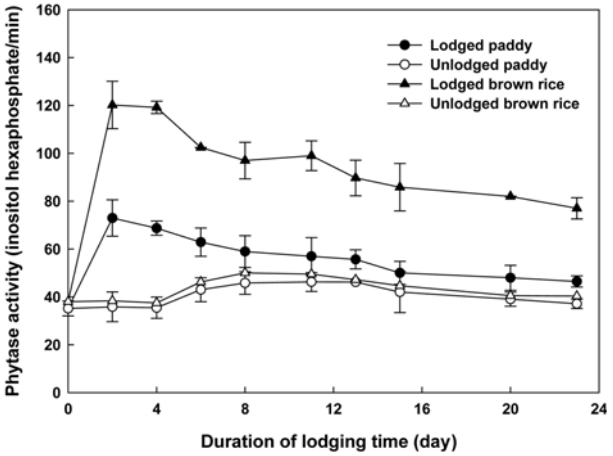

Fig. 6. Changes in phytase activity of paddy and brown rice of 'Janganbyeo' either lodged or unlodged with respect to the duration of lodging time.

a reliable indicator of the germination ability of seed stocks and of their vigor during germination (24). Therefore, the diastase activity of unlodged paddy and brown rice seemed mainly affected by the α-amylase activity.

Protease activity changes of paddy and brown rice during lodging The changes in protease activity of lodged or unlodged rice in the form of paddy and brown rice with respect to the duration of lodging time are shown in Fig. 4. The protease activity of lodged paddy increased from 9.2 HUT/g on the 0 day of lodging to 60.5 HUT/g on the 8th day, and then the activity decreased down to 27.0 HUT/g on the 23rd day of lodging. In case of lodged brown rice, the protease activity also increased from 12.0 HUT/g on the 0 day of lodging to 66.0 on the 10th day, and then decreased down to 34.9 HUT/g on the 23rd day of lodging. In the meanwhile, unlodged paddy and brown rice showed a small increase on the 8th day of lodging, and then showed a slight decrease afterwards. The overall protease activities of lodged paddy and brown rice were higher than those of unlodged paddy and brown rice. On the contrary, the maximum protease activities of lodged paddy and brown rice were lower than those of germinated brown rice freeze dried (22). The difference seemed mainly due to the differences in moisture content of the 2 cases as aforementioned.

Lipase activity changes of paddy and brown rice during lodging The changes of lipase activity of lodged or unlodged rice in the form of paddy and brown rice with respect to the duration of lodging time are shown in Fig. 5. The initial lipase activity of lodged paddy was 99.2 μmol oleic acid/hr·g solid on the 0 day of lodging, and then the activity increased to 137.6 on the 2nd day, 153.9 on the 8th day, 172.9 on the 15th day, and 209.3 mmol oleic acid/hr·g solid on the 23rd day of lodging. The lipase activity of unlodged paddy changed little until the 4th day of lodging, and after then the activity of paddy increased from 99.5 on the 4th day of lodging to 143.4 mmol oleic acid/hr·g solid on the 23rd day of lodging.

In case of lodged brown rice, the lipase activity increased from 100.11 μmol oleic acid/hr·g solid on the 0 day of lodging to 148.8 on the 2nd day, 163.8 on the 8th day, 181.8 on the 15th day, and 227.6 mmol oleic acid/hr·g solid on the 23rd day of lodging. The lipase activity of unlodged brown rice changed little until the 4th day of lodging, and after then the activity of paddy increased from 107.5 on the 4th day of lodging to 173.4 μmol oleic acid/hr·g solid on the 23rd day of lodging. Overall lipase activities of lodged paddy and brown rice were higher than those of unlodged paddy and brown rice. The lipase activity of the final day was in the order of lodged brown rice, lodged paddy, unlodged brown rice, and unlodged paddy.

.

Phytase activity changes of paddy and brown rice during lodging Phytase, myo-inositol hexaphosphoric acid phosphohydrolase, hydrolyzes phosphate linkages of phytic acid to release orthophosphate and myo-inositol (25, 26). An increase in phytase activity was observed during lodging (Fig. 6). The phytase activities of unlodged paddy and brown rice were 35.2 and 38.0 inositol hexaphosphate/min, respectively. The phytase activities did not show any significant changes during lodging. However, a remarkable increase in phytase activity was observed in case of lodged paddy and brown rice. The phytase activity of lodged paddy and brown rice increased to the maximum value on the 2nd day of lodging, and then showed a continuous decrease. The phytase activity of the lodged brown rice on the 2nd day was about 2 times higher than that of lodged paddy. In most plant materials, a large amount of phosphorus is present in the form of phytate. The phytate is a complex salt of calcium or magnesium with myo-inositol and is considered as the primary storage form of phosphorus and inositol in most seeds. Phosphorus is a vital compound for seed/grain development and successful seeding growth. The phosphorus in phytate form is either unavailable to or poorly utilized by monogastric animal and humans, because of the lack of the enzyme phytase required to hydrolyze the phytate and release the phosphorus (27).

The lodged paddy and brown rice did not show any signs of viviparity until the 4th day of lodging. The activities of α-amylase, diastase, and lipase of lodged paddy and brown rice began to increase on the 2nd day of lodging and then increased slowly afterwards. The

activities of protease and phytase of lodged paddy and brown rice reached the maximum values on the 2nd day and then decreased until the final day of lodging. In contrast, unlodged paddy and brown rice showed relatively small increase in viviparity, and in activities of α-amylase, diastase, protease, lipase, and phytase. Especially, the activities of diastase and lipase were observed even in unlodged paddy and brown rice. It can be concluded that the paddy and brown rice lodged for 2-8 days can be used as an enzyme food or its ingredient owing to their high enzyme activities.

Acknowledgments

This study was supported by the Research Fund, 2008 of The Catholic University of Korea.

References

1. Chavan JK, Kadam SS. Nutritional improvement of cereals by sprouting. Crit. Rev. Food Sci. 28: 401-437 (1989)
2. Lim KT, Chung JH, Hong JH, Kim JH, Lee ET, Im AL. Effect of air-phase germination with anion radiation and water-spraying on germination ratio, sprout growth, and GABA contents of germinated brown rice. Agri. Biosys. Eng. 7: 42-47 (2006)
3. Kim KS, Jang HD. Effects of chitosan and lactic acid on enzymatic activities and bioactive compounds during germination of black rice. J. Food Sci. Nutr. 9: 199-205 (2004)
4. Lee GS, Choi JY, Ko HS, Lee BST, Yu Y, Jeong CW, Park HG, Kim MK, Ryu JH, Jung IK, Cheong JH. Stress-reducing effects of brown rice *koji*. Food Sci. Biotechnol. 15: 63-69 (2006)
5. Kang YS. Wind and flooding damages of rice plants in Korea. Korean J. Crop Sci. 34: S45-S65 (1989)
6. Yamamoto H, Iwaya K. Influence of typhoon on the paddy rice production in Kyushu and Yamaguchi districts in 2004 and salty wind damage of rice caused by typhoon 0418 (Songda). Jpn. J. Crop Sci. 75: 535-541 (2006)
7. Ju YC, Han SW, Cho YC, Park KY. Effects of submerged condition, temperature, and ripening stages on viviparous germination of rice. Korean J. Crop Sci. 45: 20-25 (2000)
8. Ko JC, Kim BK, Lee KS, Choi WY, Choi HR, Cho EA, Yun SJ. Varietal difference in enzyme activities during preharvest germination of rice. Korean J. Crop Sci. 50: 378-383 (2005)
9. Komatsuzaki N, Tsukahara K, Toyoshima H, Suzuki T, Shimazu N, Kimura T. Effect of soaking and gaseous treatment on GABA content in germinated brown rice. J. Food Eng. 78: 556-560 (2007)
10. Choi HD, Park YK, Kim YS, Chung CH, Park YD. Effect of pretreatment condition on γ-aminobutyric acid content of brown rice and germinated brown rice. Korean J. Food Sci. Technol. 36: 761-764 (2004)
11. Rao RSP, Muralikrishna G. Water soluble feruloyl arabinoxylans from rice and ragi: Changes upon malting and their consequence on antioxidant activity. Phytochemistry 67: 91-99 (2006)
12. Kang BR, Park M, Lee HS. Germination dependency of antioxidative activities in brown rice. J. Korean Soc. Food Sci. Nutr. 35: 389-394 (2006)
13. Kim SS, Kim SY, Lee WJ. Microwave vacuum drying of germinated brown rice as a potential raw material for enzyme food. Korean J. Food Sci. Technol. 30: 1107-1113 (1998)
14. Park JS, Lee WW, Ju YC, Kim YH. Field lodging degree of rice varieties according to nitrogen application rate. Korean J. Crop Sci. 47: 226-235 (2002)
15. AACC. Approved Method of the AACC. 8th ed. Method 22-61. American Association of Cereal Chemists, St. Paul, MN, USA (1983)
16. Peterson DM. Lipase activity and lipid metabolism during oat malting. Cereal Chem. 76: 158-163 (1999)
17. Kwon DY, Rhee JS. A simple and rapid colorimetric method for determination of free fatty acids for lipase assay. J. Am. Oil Chem. Soc. 63: 84-92 (1986)
18. Rutgersson A, Bergman EL, Lingnert H, Sandberg AS. Optimization of temperature, time, and lactic acid concentration to inactivate lipoxygenase and lipase and preserve phytase activity in barley during soaking. Cereal Chem. 74: 727-732 (1997)
19. Lolas GM, Markakis P. The phytase of anvy beans (*Phaseolus vulgaris*). J. Food Sci. 42: 1094-1106 (1977)
20. Kim SL, Son YK, Son JR, Hur HS. Effects of germination condition and drying methods on physicochemical properties of sprouted brown rice. Korean J. Crop Sci. 46: 221-228 (2001)
21. Sen K, Choudhuri MM, Ghosh B. Changes in polyamine contents during development and germination of rice seeds. Phytochemistry 20: 631-633 (1981)
22. Kim SS, Kim SY, Lee WJ. Characteristics of germinated colored rice as a potential raw material for *sikhe*. Korean J. Food Sci. Technol. 30: 1092-1096 (1998)
23. Kazumitsu M, Tadashi S, Norindo T. Differences in the effects of dehusking during fermentation of seeds on the germination of seeds of indica and japonica rice (*Oryza sativa* L.). Ann. Bot. -London 77: 599-604 (1996)
24. Somen N, Gitali D, Swati S. Beta-amylase activity as an index for germination potential in rice. Ann. Bot. -London 75: 463-467 (1995)
25. Tetsushi Y, Kunisuke T, Zenzaburo K. Phytase activity associated with isolated aleurone particles of rice grains. Agr. Biol. Chem.-Tokyo 39: 289-290 (1975)
26. Burbano C, Muzquiz M, Osagie A, Ayet G, Cuadrado C. Determination of phytate and lower inositol phosphates in spanish legumes by HPLC methodology. Food Chem. 52: 321-325 (1995)
27. Carmen C. Effect of several germination conditions on total P, phytate P, phytase, and acid phosphatase activities and inositol phosphate esters in rye and barley. J. Agr. Food Chem. 49: 3208-3215 (2001)