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Effects of Chitosan and Lactic Acid on Enzymatic Activities and Bioactive Compounds during Germination of Black Rice

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Abstract

The effect of chitosan on enzymatic activities and on bioactive compounds was characterized during germination at 25°C for 7 days to search for a method to produce a germinated black rice. The germination rate was reduced by the addition of lactate and chitosan. The rotting rate was greatly decreased by chitosan, suggesting that the addition of chitosan into a germination solution might be an effective method for controlling fungal contamination during the germination of cereals. The addition of 100 and 200 ppm chitosan increased α-amylase activity after 7 days by up to 152% and 197%, respectively. The activities of β-amylase and β-glucosidase were lower with 200 ppm chitosan than in distilled water and 100 ppm lactate. The amount of total soluble phenolics and total flavonoids decreased rapidly for four days and thereafter remained constant until the seventh day. The antioxidant activity of germinated black rice, in terms of hydrogen-donating activity, increased slowly and did not correspond to the changes of total soluble phenolics and total flavonoids. The amount of phytic acid was reduced by the addition of 200 ppm chitosan compared to distilled water, indicating that chitosan could be used as an elicitor for the increase of phytase activity during the germination of black rice.

Key words: black rice, chitosan, enzyme activity, total soluble phenolics

INTRODUCTION

Rice (*Oryza sativa* L.) has a long history as an important staple cereal in Korea. In recent years, the consumption of rice in Korea has been rapidly decreasing due to increasingly westernized eating habits. But colored rices such as black rice, red rice and brown rice remain popular because of their various physiological functions (1). Korean black rice (*Oryza sativa* var.) is known to have originated from Southern China and began to be cultivated in 1990's. Some varieties are now being cultivated in several regions of Korea, including Jindo. The major varieties of black rices consumed in Korea are Suwon 415, Iksan 427 and Sanghaehyangbyeol (2).

Recent studies have shown that black rice has better properties for color and flavor and is richer in nutrients such as proteins, lipids, vitamins and minerals than polished rice (1). There are reports on black rice describing chemical composition (1), texture changes after cooking (3), characteristics of black rice Sikhe (4), microwave vacuum drying (5), pigments (6-8), pasting, and nutritional characteristics (2). The physiological properties of black rice include antioxidant activity (9-10), hepatoprotective activity (11), antimutagenic and anticarcinogenic activity (12). Those biological functions appear to be related very closely with anthocyanin.

Germination is one of processing methods by which the quality of cereal can be improved for digestibility and physiological functions. During the germination of black rice, enzymatic activities and bioactive compounds may be increased due to the metabolic activity for the production of energy from stored carbohydrates, lipids and proteins. For these reasons, germinated black rice has been used as a source of enzymes and bioactive compounds in the formulation of healthy foods such as *Sangsik*, which is a recently popularized alternative meal in Korea. In the production of germinated black rice, microbial contamination with fungi is a constant food safety challenge because the temperature and humidity for germination is also ideal for growth of fungi. According to the result of Oh and Choi (13), the addition of 100 ppm chitosan effectively suppresses the growth of fungi and increases the production of γ-aminobutyric acid (GABA) during the germination of brown rice for 3 days, compared to distilled water, suggesting that chitosan can be used as an antifungal agent and an elicitor of the production of bioactive compounds during the

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germination of cereal.

The objective of this study was to characterize the efficacy of chitosan as an antimicrobial agent and an elicitor of bioactive components during the germination of black rice by analyzing the characteristics of germinated black rice. Changes in enzymatic activities and bioactive compounds such as total soluble phenolics, total flavonoids and phytic acid were monitored.

MATERIALS AND METHODS

Materials and reagents

Black rice (Suwon 415) was purchased from Yeonmu Nonghyup (Yeonmu, Korea) immediately after polishing and used within two weeks. α-Amylase and β-amylase assay kits were obtained from megazyme international Ltd. (Wicklow, Ireland). Ammonium sulfate, ρ-nitrophenyl-β-D-glucopyranoside, sodium tetraborate, Folin-Ciocalteu reagent, gallic acid, quercetin, 1,1-diphenyl-2-picryl-hydryl (DPPH) 2,2′-biphyridine and phytic acid (dodecasodium salt hydrate) were purchased from Sigma Co. (St. Louis, USA). Chitosan (97% deacetylated) was obtained from Dong-Myong Co. (Seoul, Korea). The other reagents were of commercially available special grade.

Germination of black rice

Black rice was submerged in distilled water for 12 hr. About 400 black rice grains were put into petri-dishes containing 25 mL of germination solution and germinated for 7 days in a cold chamber maintained at a temperature of 25°C and a relative humidity of 75%; germination solution was replaced each day. Four kinds of germination solutions: distilled water, 100 ppm lactic acid, 100 ppm and 200 ppm chitosan were used. Chitosan was solubilized in 5% lactic acid and diluted with distilled water to make 100 or 200 ppm concentrations. Each day 4 petri-dishes containing different germination solutions were removed and kept at -60°C until analysis of enzymatic activity and bioactive compounds.

α-Amylase assay

α-Amylase activity was analyzed with an α-amylase assay kit according to the method of McCleary and Sheehan (14). A half gram of sample was milled with a hand mixer for 30 sec and placed into a 100 mL volumetric flask and extracted with 1% sodium chloride and 0.02% calcium chloride solution containing 0.02% sodium azide for 15 min at room temperature. After centrifugation at 1,000×g for 10 min, the supernatant was used as an extracted solution of enzyme. Both 0.2 mL of substrate solutions containing non-reducing end blocked ρ-nitrophenyl maltoheptaoside (BPNPG7) in the presence of excess levels of α-glucosidase and 0.2 mL of diluted extracted solution of enzyme which was activated at 40°C for 5 min were reacted at 40°C for 10 min and then terminated by adding of 3 mL of 20% tri-sodium phosphate solution and mixing with a vortex mixer. The amount of ρ-nitrophenol produced by the enzyme was measured by reading the absorbance at 410 nm with a spectrophotometer (Shimadzu Inc., Kyoto, Japan). One unit of enzyme activity was defined as the amount of enzyme required to produce 1 μmole of ρ-nitrophenol per min and expressed as Ceralpha units (CU). Measurement was done in triplicate and the data was expressed as mean±standard deviation.

β-Amylase assay

β-Amylase activity was analyzed with a β-amylase assay kit according to the method of McCleary and Codd (15). Extracted solution of enzyme was prepared by mixing 0.5 g of sample milled with a hand mixer for 30 sec and 5 mL of 50 mM Trizma base buffer (pH 8.0) for 1 hr with vortex mixer every 10 min. After centrifugation at 1,000×g for 10 min, the supernatant was used as an extracted enzyme solution. Both 0.2 mL substrate solutions containing ρ-nitrophenyl maltopentaoside (PNPG5) and 0.2 mL of extracted solution of enzyme which was diluted 1,250 times and activated at 40°C for 5 min were reacted at 40°C for 10 min and terminated by adding of 3 mL of 1% Trizma base solution and mixing with a vortex mixer. The amount of ρ-nitrophenol produced by enzyme was measured by reading the absorbance at 410 nm with the spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme to produce 1 μmole of ρ-nitrophenol from PNPG5 for 1 min and expressed as Betamyl units (BU). Measurements were performed in triplicate and the data was expressed as mean±standard deviation.

β-Glucosidase assay

The enzyme was extracted by the method of Kim and Yoon (16). Twenty grams of sample milled with a hand mixer for 30 sec were added to 200 mL of 0.2 M phosphate buffer (pH 7.0), sealed and extracted by stirring in cold chamber at 4°C. Ammonium sulfate was slowly added to the supernatant after centrifugation at 8,000×g for 30 min to bring it to 60%. After protein was precipitated by standing for 24 hr, the precipitate obtained from centrifugation at 10,000×g for 40 min was solubilized with 20 mL of 0.2 M phosphate buffer. The supernatant from centrifugation at 8,000×g for 30 min was used as extracted solution of enzyme. β-Glucosidase activity was estimated by the method of Peralta et al. (17). Both 0.2 mL of substrate solution containing 50 mM ρ-nitrophenyl-β-D-glucopyranoside and 0.2 mL of extracted solution of enzyme which was activated at

50°C for 5 min were reacted at 50°C for 10 min and the reaction was stopped by adding 1 mL of saturated sodium tetraborate solution. The amount of ρ-nitrophenol was measured by reading the absorbance at 405 nm with a spectrophotometer. One unit of enzyme activity was defined as the amount of β-glucosidase required to produce 1 μmole of ρ-nitrophenol per min. Measurements were performed in triplicate and the data was expressed as mean±standard deviation.

Total soluble phenolics assay

Total soluble phenolics were determined according to the method of Chandler and Dodds (18) based on that of Singleton and Rossi (19). A solution of black rice extract was prepared by incubating 0.2 g of sample milled with a hand mixer for 30 sec in 10 mL of 95% ethanol for 48 hr at -60°C and centrifuging at 8,000×g for 20 min. One mililiter of extracted solution was transferred to a test tube and mixed well with 1 mL of 95% ethanol and 5 mL of distilled water. A half milliliter of 50% Folin-Ciocalteu reagent was added to each sample and mixed. After 5 min, 1 mL of 5% Na_2CO_3 was added. The reaction mixture was mixed and allowed to stand for 60 min. The absorbance was read at 725 nm using a spectrophotometer with 95% ethanol as a blank. A standard curve was established using various concentrations of gallic acid in 95% ethanol. Absorbance values were converted to milligrams of total soluble phenolics per gram of extract.

Total flavonoid assay

Total flavonoid was measured using the method of Moreno et al. (20). Extracted solution prepared for the total soluble phenolics assay was also used as a test sample for total flavonoids. 0.1 mL of extracted solution was added to 0.9 mL of 80% ethanol. A half milliliter of that solution was mixed with 0.1 mL of 10% aluminum nitrate, 0.1 mL of 1 M potassium acetate and 4.3 mL of 80% ethanol, and allowed to stand for 40 min at room temperature. The absorbance was read at 415 nm using spectrophotometer. A standard curve was established using a various concentrations of quercetin. Absorbance values were converted to total flavonoids which were expressed as milligrams of quercetin equivalent per gram of extract.

Scavenging capacity of DPPH radical

Hydrogen-donating capacity was measured by the method of Chen et al. (21) with some modifications. Extracted solution prepared for total soluble phenolics assay was used as the test sample. Forty mL of different concentrations of test sample were mixed with 160 μL of 200 μm DPPH in ethanol, transferred into microplate wells and allowed to stand for 30 min at 37°C. Absorbance of the resulting solution was measured at 517 nm using an ELISA reader (Tecan Austria, Salzburg, Austria). Hydrogen-donating activity was expressed as inhibition percentage:

$$\text{Inhibition } (\%) = \frac{(A_{\text{control}} - A_{\text{sample}})}{(A_{\text{control}} - A_{\text{blank}})} \times 100$$

where A_{control} is the absorbance of the control, A_{sample} is the absorbance in the presence of sample and A_{blank} is the absorbance in the presence of ethanol.

Phytic acid assay

Phytic acid was measured by the method of Haug and Lanrsch (22). Samples milled with a hand mixer for 30 sec were added to 20 mL of 0.2 N HCl and stirred at room temperature for 4 hr. After centrifugation at 3,000×g for 30 min, the supernatant was used for the analysis of phytic acid. A half mL aliquot of extracted solution and 1 mL of 0.4 mM ferric solution were added into a tightly capped test tube. The mixture was reacted in boiling water bath for 30 min and cooled in ice water. Afterwards, 2 mL of 2,2′-biphyridine solution was added and mixed with a vortex mixer, the absorbance at 519 nm was read. A standard curve was made with phytic acid (dodecasodium salt hydrate) and used to calculate the amount of phytic acid in sample.

Statistical analysis

All results were expressed in mean±SD. Statistical analyses were done using SPSS software. Significance of differences among groups was verified with one-way ANOVA followed by Duncan's multiple range test. $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Characteristics of the germination of black rice

To produce the germinated black rice containing bioactive compounds, black rice was germinated at 25°C, which is known to be an optimum temperature, for seven days (23). The germination rate and the rotting rate were measured to examine the effect of chitosan on the germination of black rice and were shown in Fig. 1 and 2. Black rice began to germinate on the second day and continued to do so until the seventh day. The addition of 100 ppm lactate and 100 or 200 ppm chitosan delayed the germination of black rice compared to control. When 100 ppm lactate solution was compared with distilled water, used as a control, the germination rates with 100 ppm lactate and distilled water were 64.4% and 83.2% respectively at the seventh day. The germination rate of 100 and 200 ppm chitosan were 62.6% and 55.9%,

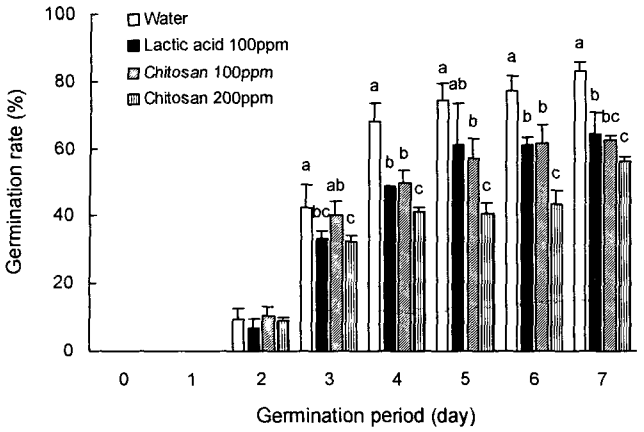

Fig. 1. Germination rate of black rice germinated with chitosan. Each dish contained approximately 400 black rice grains. Data are presented as means±SD (n=3). Different letters indicate significant differences from one another ($p<0.05$).

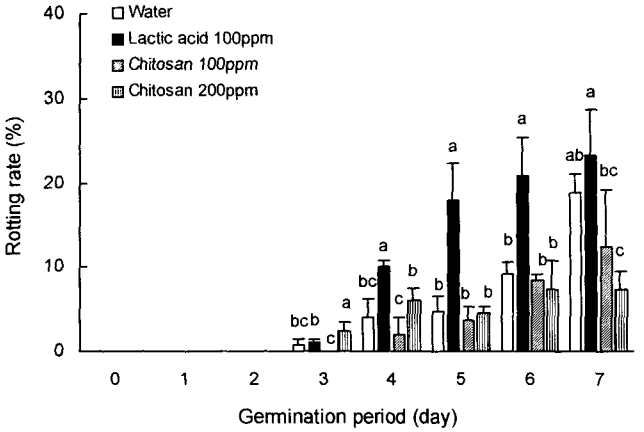

Fig. 2. Changes of rotting rate during the germination of black rice. Each dish contained approximately 400 black rice grains. Data are presented as means±SD (n=3). Different letters indicate significant differences from one another ($p<0.05$).

respectively. This result suggests that lactate and chitosan might reduce the metabolic activity of black rice to decrease the germination rate during germination.

To produce the germinated cereals as a component of *Sangsik* (a popular meal replacement food in Korea), the contamination of microorganisms such as fungi needs to be effectively controlled. The rotting rate of black rice caused by fungi is shown in Fig. 2. The addition of 100 ppm lactate increased the rotting rate after the fourth day, but 100 and 200 ppm chitosan reduced it significantly compared with control ($p<0.05$). The increased rotting rate by lactate might be due to the utilization of lactate by contaminated microorganisms for growth and is contrary to the result of Oh and Choi (13) that 100 ppm lactate reduced the rotting rate of brown rice. On the other hand, by the seventh day the addition of 200 ppm chitosan reduced the rotting rate compared to distilled water by more than 50%. The decreased rotting rate is consistent with the results of Oh and Choi (13) who demonstrated that treatment with 100 ppm chitosan reduced the rotting rate of germinated brown rice; They proposed that the addition of chitosan into a germination solution is an effective method for controlling the contamination of fungi during germination of cereals such as brown and black rices.

Considering the effects of chitosan on the germination and rotting rates of black rice, even though the treatment of chitosan slows the germination rate, it may successfully control the contamination of mold, a serious problem in the production of germinated black rice.

Changes in enzymatic activities during germination

In the production of *Sangsik*, germinated cereals are generally used as a source of several enzymes, especially amylase. In this study, the effects of lactate and chitosan on the α-amylase and β-amylase activities of black rice were investigated during the germination of black rice. The changes in α-amylase and β-amylase activities are shown in Fig. 3 and 4, respectively. The addition of 100 ppm lactate increased the α-amylase activity only on the forth day and seventh day compared to distilled water. The additions of 100 and 200 ppm chitosan increased the activity 152% and 197%, respectively by the seventh day, suggesting that chitosan can play a role as an elicitor for increasing α-amylase activity in germinated black rice. β-Amylase activity during the germination of black rice was not significantly changed by the addition of 100 ppm lactate compared to distilled water ($p<0.05$). Chitosan, however, at 100 ppm increased the α-amylase activity of germinated black rice only on the second day, and 200 ppm chitosan significantly decreased it from the second day onward ($p<0.05$). These results suggest that 200 ppm chitosan might suppress the production of β-

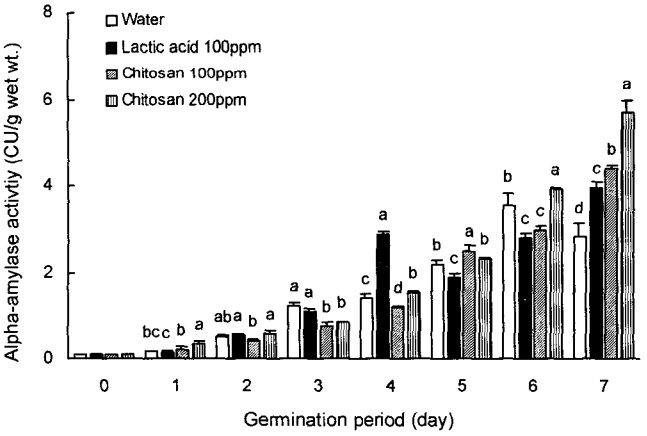

Fig. 3. Changes in α-amylase activity during the germination of black rice. Data are presented as means±SD (n=3). Different letters indicate significant differences from one another ($p<0.05$).

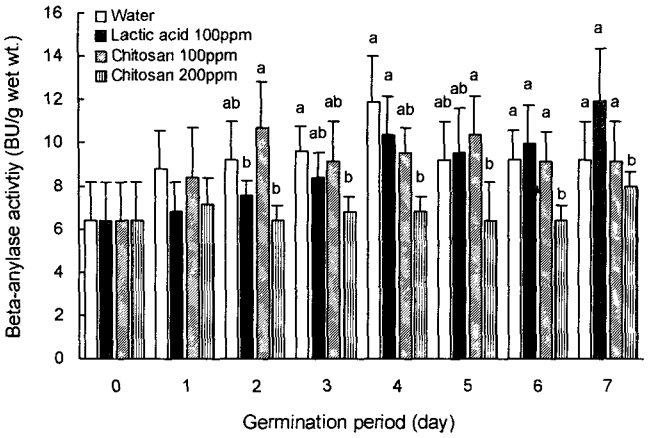

Fig. 4. Changes in β-amylase activity during the germination of black rice. Data are presented as means±SD (n=3). Different letters indicate significant differences from one another ($p<0.05$).

amylase during the germination of black rice or be a weak inhibitor for β-amylase activity in germinated black rice.

β-Glucosidase (β-D-glucoside glucohydrolase) is an enzyme that catalyzes the hydrolysis of glycosidic linkages in aryl and alkyl-D-glucosides as well as glycosides containing only carbohydrate residues (24). β-Glucosidase can hydrolyze phenolic glycosides to release free phenolics and carbohydrate. The change in ß-glucosidase activity during the germination of black rice is shown in Fig. 5. In rice germinated with distilleds water as a control, β-glucosidase activity was increased two fold after 7 days of germination. The additions of 100 ppm lactate and 100 and 200 ppm chitosan significantly slowed the increase of β-glucosidase activity from day 4 of germination, compared to distilled water ($p<0.05$). β-Glucosidase activity in black rice fermented with 200 ppm

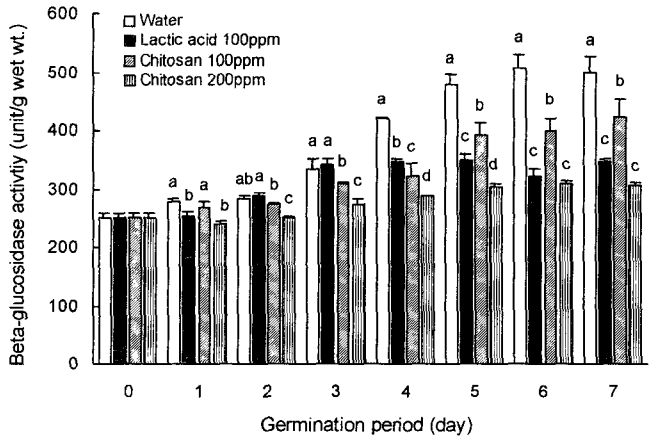

Fig. 5. Changes in β-glucosidase activity during the germination of black rice. Data are presented as means±SD (n=3). Different letters indicate significant differences from one another ($p<0.05$).

chitosan was only 61% of that of distilled water by the seventh day, indicating that lactate and chitosan can have a negative effect on the increase in β-glucosidase activity during the germination of black rice.

Changes in total soluble phenolics, total flavonoids and antioxidant activity

Soluble phenolics consisting of phenolic acid, flavonoids and anthocyanins play important roles in plant cells, tissues, and organs. They are known to be involved in growth regulation and in the process of differentiation and organogenesis. Recent studies have shown that phenolic compounds are involved in plant development during seed germination, plant-microbe recognition and signal transduction (25).

According to Fig. 6, total soluble phenolics rapidly decreased until the fourth day, and thereafter remained constant during the germination of black rice. The rapid decrease in total soluble phenolics is thought to result from the solubilization of anthocyanin or lignin pigments from black rice into the germination solution. After the first day of germination, the amount of total soluble phenolics in 100 and 200 ppm chitosan was higher than that in distilled water because of the role of chitosan as an elicitor for the formation of soluble phenolics during the germination of black rice. The amount of total flavonoids was about 10% of total soluble phenolics, and decreased at the similar rate to total soluble phenolics during germination due to the solubilization of some types of flavonoids or anthocyanins (Fig. 6).

The antioxidant activity of phenolic extracts in terms of the scavenging activity of DPPH radical during the germination of black rice increased slowly (Fig. 7), but did not correspond to the changes in total soluble phenolics and total flavonoids. The 200 ppm chitosan showed the highest antioxidant activity of the four treatments from the first day to the third day, and thereafter had similar levels of antioxidant activity as the black rice with 100 ppm lactate. These results suggest the possiblity that the major component in the phenolic extract contributing to the antioxidant activity of germinated black rice may be not flavonoids or anthocyanin pigments, which are released into germination solution, but other kinds of phenolics such as phenolic acids.

Changes in phytic acid

Phytic acid is known to be an antinutritional factor due to its ability to chelate divalent cations such as zinc, magnesium, calcium and iron to make them nutritionally unavailable (26). It has been reported that during the germination of seeds, the amount of phytic acid decreases in response to increases in phytase activity, which is involved in the conversion of phytic acid to myo-

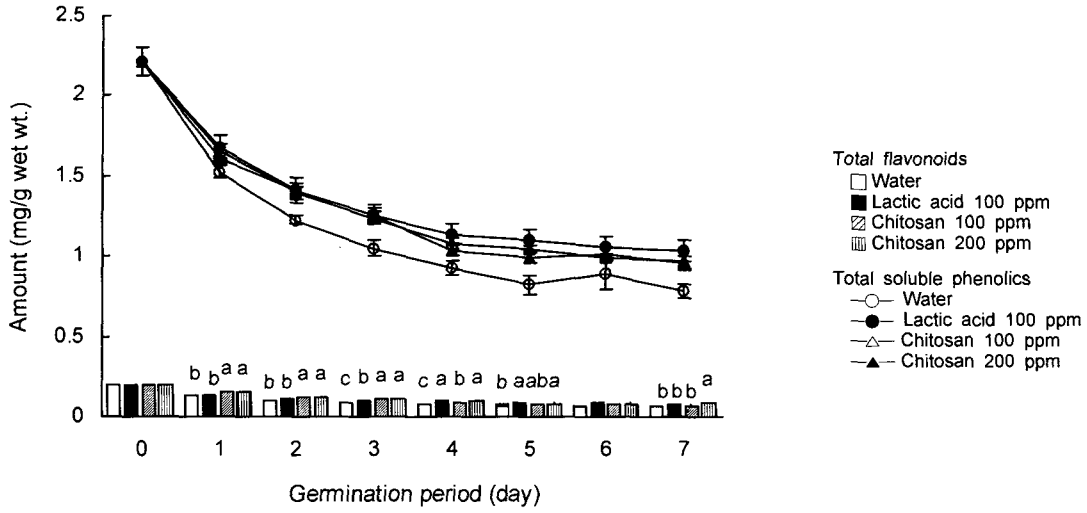

Fig. 6. Changes in total soluble phenolics and total flavonoids during the germination of black rice. Data are presented as means±SD (n=3). Different letters indicate significant differences from one another ($p<0.05$).

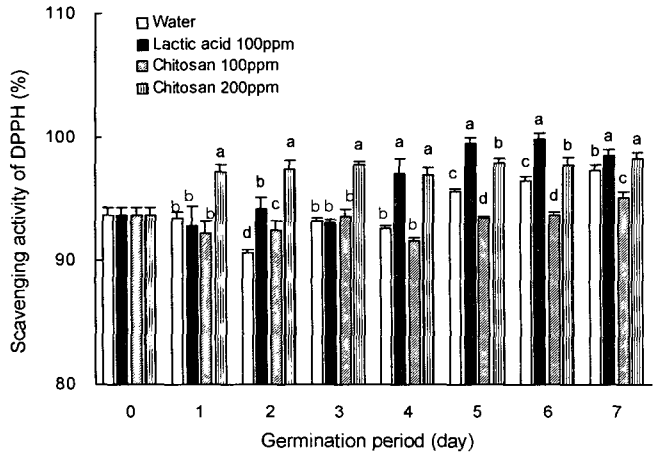

Fig. 7. Changes in antioxidant activity during the germination of black rice. Data are presented as means±SD (n=3). Different letters indicate significant differences from one another ($p<0.05$).

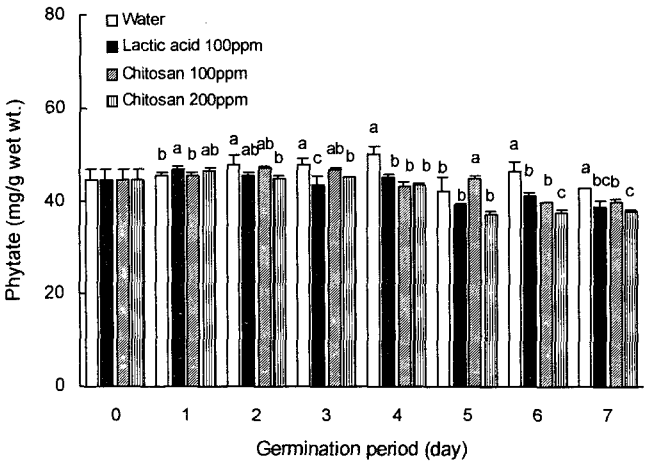

Fig. 8. Changes in phytate during the germination of black rice. Data are presented as means±SD (n=3). Different letters indicate significant differences from one another ($p<0.05$).

inositol and phosphoric acid (27).

The change in the amount of phytic acid during the germination of black rice is shown in Fig. 8. The addition of 100 ppm lactate and 100 and 200 ppm chitosan reduced the amount of phytic acid after the forth day, except for 100 ppm chitosan at the fifth day, compared with distilled water. These results suggest that chitosan could be used as an elicitor in the production of phytase hydrolyzing phytic acid during the germination of black rice.

In conclusion, the addition of chitosan into the germination solution significantly decreased the contamination of fungi during the germination of black rice although it reduced the germination rate, compared with distilled water. Chitosan increased α-amylase activity, decreased α-glucosidase activity and phytic acid, and slowed the increase of β-amylase activity. The antioxidant activity was slightly increased slightly by the addition of chitosan, while the amount of total soluble phenolics and total flavonoids were reduced dramatically. Therefore, chitosan can be used as an antimicrobial agent against fungi and as an elicitor for increasing α-amylase and antioxidant activities and to decrease phytic acid in germinated black rice.

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REFERENCES

1. Ha TY, Park SH, Lee CH, Lee SH. 1999. Chemical composition of pigmented rice varieties. *Korean J Food Sci Technol* 31: 336-341.
2. Chung HJ, Lim ST. 1999. Pasting and Nutritional charac-

teristics of black rices harvested in Korea. *J Food Sci Nutr* 4: 231-235.
3. Kim DW, Eun JB, Rhee CO. 1998. Cooking conditions and texturing changes of cooked rice added with black rice. *Korean J Food Sci Technol* 30: 562-568.
4. Kim SS, Kim SY, Lee WJ. 1998. Characteristics of germinated colored rice as a potential raw material of Sikhe. *Korean J Food Sci Technol* 30: 1092-1096.
5. Kim SS, Kim SY, Noh BS, Chang KS. 1999. Microwave vacuum drying of germinated colored rice as an enzymatic health food. *Korean J Food Sci Technol* 31: 619-624.
6. Choi HC, Oh SK. 1996. Diversity and function of pigments in colored rice. *Korean J Crop Sci* 41: 1-9.
7. Yoon JM, Hahn TR, Yoon HH. 1998. Effect of copigmentation on the stability of anthocyanins from a Korean pigmented rice variety. *Korean J Food Sci Technol* 30: 733-738.
8. Choi SW, Kang WW, Osawa T. 1994. Isolation and identification of anthocyanin pigments in black rice. *Foods Biotechnol* 3: 131-136.
9. Choi SW, Kang WW, Osawa T, Kawakishi S. 1994. Antioxidative activity of crysanthemin in black rice hulls. *Foods Biotechnol* 3: 233-237.
10. Chung YA, Lee JK. 2003. Antioxidative properties of phenolic compounds extracted from black rice. *J Korean Soc Food Sci Nutr* 32: 948-951.
11. Kwak TS, Park HJ, Jung WT, Choi JW. 1999. Antioxidative and hepatoprotective activity of colored-, scented and Korean native rice varieties based on different layers. *J Korean Soc Food Sci Nutr* 28: 191-198.
12. Nam SH, Kang MY. 1997. *In vitro* inhibitory effect of colored rice bran extracts carcinogenity. *Agri Chem Biotechnol* 40: 307-312.
13. Oh SH, Choi WG. 2000. Production of the quality germinated brown rices containing high γ -aminobutyric acid by chitosan application. *Korean J Biotechnol Bioeng* 15: 615-620.
14. McCleary BV, Sheehan H. 1987. Measurement of cereal α -amylase: A new procedure. *J Cereal Sci* 6: 237-251.
15. McCleary BV, Codd R. 1989. Measurement of β-amylase in cereal flours and commercial enzyme preparation. *J Cereal Sci* 9: 17-33.
16. Kim JS, Yoon S. 1999. Isoflavone contents and β-glucosidase activities of soybeans, Meju, and Doenjang. *Korean J Food Sci Technol* 31: 1405-1409.
17. Peralta RM, Kadowaki MK, Terenzi HF, Jorge JA. 1997. A highly thermostable β-glucosidase activity from the thermophilic fungus *Humicols grisea* var. *thermoidea*: purification and biochemical characterization. *FEMS Microbiology Lett* 146: 291-295.
18. Chandler SF, Dodds JH. 1983. The effect of phosphate, nitrogen, and sucrose on the production of phenolics and solasidine in callus cultures of *Solanum laciniatum*. *Plant Cell Rep* 2: 105-108.
19. Singleton VL, Rossi JA. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid. *Am J Enol Vitric* 149: 144-158.
20. Moreno MIN, Isla MI, Sampietro AS, Vattuone MA. 2000. Comparison of the free radical-scavenging activity of propolis from several regions of argentin. *J Ethnopharmacol* 71: 109-114.
21. Chen HM, Muramoto K, Yamauchi F, Fujimoto K, Nokihara K. 1998. Antioxidative properties of histidine-containing peptides designed from peptide fragments found in the digests of a soybean protein. *J Agric Food Chem* 46: 49-53.
22. Haug W, Lanrsch HJ. 1983. Sensitive method for the rapid determination of phytate in cereals and cereal products. *J Sci Food Agri* 34: 1423-1426.
23. Kim SL, Son YK, Son JR, Hur HS. 2001. Effect of germinated and drying methods on physicochemical properties of sprouted brown rice. *Korean J Crop Sci* 46: 221-228.
24. Yan TR, Lin YH, Lin CL. 1998. Purification and characterization of an extracellular β-glucosidase II with high hydrolysis and transglucosylation activities from *Aspergillus niger*. *J Agric Food Chem* 46: 431-437.
25. Pieta PG. 2000. Flavonoid as antioxidants. *J Nat Prod* 63: 1035-1042.
26. Zhow JR, Erdman JW. 1995. Phytic acid in health and disease. *Crit Rev Food Sci Nutr* 35: 495-504.
27. Kyriakidis N, Galiotou-Panayotou M, Stavropoulou A, Athanasopoulos P. 1998. Increase in phytase activity and decrease in phytate during germination of four common legumes. *Biotechnol Lett* 20: 475-478.

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