สภาวะที่เหมาะสมในการงอกต่อสารประกอบชีวภาพในข้าวงอก

Optimization of Germination Conditions on Bioactive Compounds in Germinated Rice

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บทคัดย่อ

ข้าวงอกเป็นอาหารสุขภาพที่เป็นที่รู้จักอย่างแพร่หลายเนื่องจากมีสารชีวภาพที่เป็นประโยชน์ในปริมาณสูง ในการศึกษานี้ได้ศึกษาผลของสภาวะการแช่เมล็ดข้าวและการงอกต่อปริมาณสารประกอบฟีนอลิค ฟลาโวนอยด์ แกมม่าอะมิโนบิวทิริคเอซิค (กาบ้า) และกิจกรรมการต้านอนุมูลอิสระของข้าวงอก (ขาวดอกมะลิ 105) หลังจากแช่ข้าวในน้ำกลั่นและนำเมล็ดข้าวไปบ่มให้เมล็ดงอก จากการวิเคราะห์ปริมาณสารประกอบฟลาโวนอยด์ของข้าวงอกที่ระยะเวลา 12 ชั่วโมงมีปริมาณสูงสุดเทียบเท่า 247.60 มิลลิกรัม กรดแกลลิคต่อ 100 กรัมน้ำหนักแห้ง ปริมาณสารประกอบฟีนอลิคทั้งหมดของข้าวงอกที่ระยะเวลา 12 ชั่วโมงมีปริมาณสูงสุดเทียบเท่า 44.64 มิลลิกรัม เคอวซิทินต่อ100กรัมน้ำหนักแห้ง การวิเคราะห์คุณสมบัติการทำปฏิกิริยากับอนุมูล (IC_{50})ใช้สารประกอบ1,1-Diphenyl-2-picryl hydrazyl (DPPH) และ 2,2´-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) พบว่าข้าวงอกที่ระยะเวลา 12 ชั่วโมงมีกิจกรรมสูงสุดเท่ากับ 447.94 มิลลิกรัมต่อลิตร และ 44.14 มิลลิกรัมต่อลิตรตามลำดับ ที่ระยะเวลา 24 ชั่วโมงของการงอกปริมาณกาบ้ามีค่าสูงสุดเท่ากับ 16.19 มิลลิกรัมต่อ100 กรัมน้ำหนักแห้ง การศึกษานี้พบว่าสภาวะที่เหมาะสมของการผลิตฟีนอลิค ฟลาโวนอยด์ และคุณสมบัติต้านอนุมูลอิสระในข้าวงอกที่แช่ไว้ที่ 25 องศาเซลเซียสเป็นระยะเวลา 24 ชั่วโมงและทำการงอกที่ 35 องศาเซลเซียสระยะเวลา 12 ชั่วโมงในขณะที่อุณหภูมิที่เหมาะสมที่ผลิตกาบ้าสูงสุดเท่ากับ 25 องศาเซลเซียสสำหรับการแช่น้ำเป็นระยะเวลา 24 ชั่วโมง และอุณหภูมิที่เหมาะสมสำหรับการงอกที่ 35 องศาเซลเซียสระยะเวลา 24 ชั่วโมง จากการศึกษานี้แสดงให้เห็นภาวะที่เหมาะสมของข้าวงอกคือการน้ำไว้ที่อุณหภูมิ 25 องศาเซลเซียสเป็นระยะเวลา 24 ชั่วโมงและทำการงอกที่ 35 องศาเซลเซียสระยะเวลา 12 ชั่วโมงซึ่งใช้เวลาสั้นจึงน่าจะลดค่าใช้จ่ายของการผลิตได้

คำสำคัญ: ข้าว การงอก สารประกอบชีวภาพ

Abstract

Germinated rice is a well-known functional food due to its high content of bioactive components. In this study, effects of soaking conditions and germination conditions on phenolic, flavonoid, gamma amino butyric acid (GABA) contents and antioxidant activity of germinated paddy rice (Khao Dok Mali 105) were investigated. The rice was soaked in distilled water followed by germinating. The total phenolic content of germinated rice at 12 h showed the highest level of 247.60 mg gallic acid equivalent /100 g dry weight. The flavonoid content of germinated rice at 12 h showed the highest level of 44.64 quercetin equivalent/ 100 g dry weight. 1,1-Diphenyl-2-picryl hydrazyl (DPPH) and 2,2´-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were used to determine the radical scavenging activity (IC_{50}), germinated rice at 12 h was highest of 447.94 mg/L and 44.14 mg/L, respectively. At 24 h of germination, the GABA content was highest of 16.19 mg/100 g dry weight. This study found that the optimal conditions for highest phenolic, flavonoid and antioxidant activity productions in germinated rice were 25 °C for soaking for 24 h and 35 °C

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for germination for 12 h, while the optimal temperatures for highest GABA production were 25 °C for soaking for 24 h and 35 °C for germination for 24 h. Optimizing process of were soaking rice grain at 25 °C for 24 h and 35 °C for germination for 12 h germinated rice could reduce cost of production.

Keywords: rice, germination, bioactive compound

Introduction

Rice is the largest cereal crop in the world. It is also the main staple food for world populations.[1] Rice components have several roles in disease prevention. It contains essential nutrient, dietary fiber, oil and hypoallergenic protein.[2] Germinated rice has been of interest throughout Asian countries as it contains high amounts of bioactive compounds such as γ-aminobutyric acid (GABA), γ-oryzanol, and dietary fiber. Germinated rice grain offers considerable benefits. Germinated rice contains high amounts of ferulic acid,[3] α-tocopherols[4] and phenolics[5] which have potent antioxidant and free radical scavenging properties.[6] Phenolic compound in rice such as ferulic acid has the capability to prevent the build-up of superoxide, controlling the aggregation of blood platelets and cholesterol-lowering properties as well as for their antioxidant capacity.[7] Additionally, the germination of rice releases its bound minerals, making them more absorbable by the body, and the rice is more tender and tastier. Seed germination is a complex process involving biochemical and physical activities[4] Hydrolytic enzymes are activated after water absorption and degrade large molecules of reserve compounds in the endosperm including biopolymers, carbohydrates, and polypeptides, to small biomolecules in germinated seeds.[8] Apart from nutrition level changes, germination also generates antioxidant compounds including phenolic and flavonoid contents.[5] In the plant, the GABA biosynthesis pathway is accomplished by GABA shunt and polyamine degradation.[9] GABA is a metabolic end product and is primarily produced by the decarboxylation of L-glutamic acid (L-Glu), catalyzed by glutamate decarboxylase (GAD, EC 4.1.1.15)[10] and the activity of GAD shows a high correlation with the germination ratio. The accumulation of GABA is related to the activity of GAD and substrate concentration of L-Glu. GABA is a well-known non-protein amino acid which exists widely in both plants and animals. It is a major inhibitory neurotransmitter in the cerebrospinal fluid of mammals.[11] Several health benefits of GABA have been reported e.g. lower blood pressure and blood cholesterol, greater kidney and liver activity, inhibition of cancer cell proliferation[12] and stimulation of cancer cell apoptosis. The optimization of the germination conditions could be applied for the production of GABA-rich products for promoting of the consumption to use as supplementary food. Rice cultivar Khao Dawk Mali 105 is a local rice cultivar which cultivated in Ayutthaya province in Thailand. Rice cultivar Khao Dawk Mali 105 is a long grain non-waxy Thai rice cultivar. Though many studies have been done on improving GABA production in germinated rice grain, the relationship between bioactive compounds and GABA Production on the optimal conditions in rice is still not known. This study aimed to investigate the effect of soaking, optimization of germination conditions of rice grain cultivar Khao Dawk Mali 105 and evaluating GABA production and its bio-active compounds.

Material and Methods

Rice sample

Ten kilograms of the paddy rice Khao Dawk Mali 105 were provided by the Rice Research Center, Ayutthaya province, Thailand.

Germination procedure

The experiment was performed by soaking 1 kg of rice seed in a 50 L tank using distillated water with the grain-to-water ratio of 1:10 (w/v) at 30±1°C for 24 hours. Non-soaked rice seed was used as a control sample. Germinated rice grains were incubated at 35°C and they were collected at 0, 12, 24, 36 and 48 hours, respectively. The germinated rice grains after attainment of the required germination period were dried at 55°C in hot air oven until the moisture contents were below 14%. All samples were stored at -20°C until analyses. In this study, unsoaked rice seed was used as control.

Extraction of unsoaked rice and germinated rice

The extraction of unsoaked rice (control) and germinated rice was performed using a modified version of the method described by Sutharut and Sudarat.[4] A 5 g portion of each of the rice seed samples was extracted with 75 ml of methanol at room temperature for 12 h (repeated three times) and then was filtered. The residue was evaporated at 50°C. The residue was weighted and stored at -20°C until analysis.

Total phenolic content (TPC)

The TPC was determined using a modified Folin–Ciocalteu method.[13] Each test sample (250 µl) was added to a test tube that contained 6.0 ml of distilled water. After vortexing the tubes, 500 µl of Folin–Ciocalteu's phenol reagent was added to each tube. The tubes were vortexed and 2 min later, 2.0 ml of 15% Na_2CO_3 was added to each tube. Thereafter, the absorbance of each sample was measured against a blank at 750 nm. A calibration curve was constructed using gallic acid as a standard. The TPC is expressed as milligrams of gallic acid per 100 grams dry weight.

Total flavonoid content

The total flavonoid content was determined using a modified version of the method described by Zhishen *et al*.[14] Each test sample (250 µl) and 1.25 ml of water were added then 75 µl of 5% $NaNO_2$, 150 µl of 10% $AlCl_3$ was added. After 6 min 0.5 ml of 1 M NaOH was added. The absorbance was measured at 510 nm. A calibration curve was constructed using quercetin as a standard. The total flavonoid content is expressed as milligrams of quercetin per 100 grams dry weight

Quantification of GABA content

GABA content was determined using a modified version of the method described by Karladee and Suriyong.[15] Each test sample (200 µl) was added to a test tube that contained 200 µl of 0.2 M borate buffer. One milliliter of 6% phenol was added to each tube. The tubes were vortexed and cooled in ice bath for 5 min. Later, 0.4 ml of sodium hyper chloride was added to each tube. The tubes were vortexed for 1 min and cooled in ice bath for 5 min, then incubated in boiling water bath for 10 min and then allowed to cool at room temperature. Thereafter, the absorbance of each sample was measured against a blank at 630 nm. A calibration curve was constructed using GABA as a standard. The GABA content is expressed as milligrams per 100 grams dry weight

Free-radical-scavenging activity

Antioxidant activities of the extracts were measured based on the scavenging of the stable free radical, 1,1-diphenyl-2-picrylhydrzyl (DPPH).[16] A sample of each extract in methanol, was added to 2 mL of DPPH solution. After 30 min, the absorbance was measured at of 517 nm. The DPPH radical-scavenging activity was calculated according to the following: % of DPPH scavenging activity = {1- (AbS/AbC)} x 100, where AbC was the absorbance of the control and AbS was the absorbance in the presence of the test compound. IC_{50} is the effective concentration in mg extract/mL which inhibits the DPPH activity by 50%. 1,1- diphenyl-2-picrylhydrazyl, butylated hydroxyanisole (BHA) was used as a control. Radical cation scavenging capacity was examined against $ABTS^{•+}$generated by the chemical method. The absorbance of the reaction mixture was measured at 734 nm and the BHT equivalent was calculated using a standard curve prepared with BHT.[17]

Experimental design and data analysis

The experiments were set up in a completely randomized design and repeated twice. The results were presented as the average of the repeated experiments by pooling individual data. One Way ANOVA and Tukey's Multiple Range Tests (P<0.05) were performed to determine significant differences among the means of the treatments using SPSS version. Simple linear regression was used to estimate the correlation between the total phenolic content or total flavonoid content and antioxidant capacity from both DPPH and ABTS radical scavenging assays.

Result and Discussion

Total phenolic, flavonoid and GABA contents

After being soaked in distillated water at 30°C for 24 hours (0 h), total phenolic content of Khao Dawk

Mali 105 rice seeds were significantly higher than that of the control (61.43 mg/100 g dry weight). Total phenolic content of the control increased with germination time and had the highest peak at hour 12 (Figure 1). The total phenolic content of germinated seeds on hour 12 was 1.73 times higher than hour 0. The amount of total phenolic compounds increased by 1.52 times from hour 0 (initial time) compared with that of the control.

Total flavonoid content

After being soaked in distillated water at 25°C for 24 hours (0 h) (initial time), total phenolic content of Khao Dawk Mali 105 rice seeds were significantly higher than that of the control (2.67 mg/100 g). Total flavonoid content of the control increased with germination time and had the highest peak at hour 12 (Figure 1b). The total flavonoid content of germinated seeds on hour 12 was 5.25 times more than hour 0 (initial time). The amount of total flavonoid compounds increased by 1.89 times from hour 0 compared with that of the control.

After being soaked in distillated water at 30°C for 24 hours (0 h) (initial time), GABA content of Khao Dawk Mali 105rice seeds were significantly higher than that of the control (3.22 mg/100 g). Total flavonoid content of the control increased with germination time and had the highest peak at hour 24 (Figure 1c). The GABA content of germinated seeds on hour 24 was 1.92 times more than hour 0 (initial time). The amount of GABA increased by 2.03 times from hour 0 (initial time) compared with that of the control.

Antioxidant capacity

As shown in Figure 2a and Figure 2b, antioxidant capacities of Khao Dawk Mali 105 rice seeds determined by DPPH and ABTS radical scavenging assays were different during germination. The seeds soaked in distillated water had significantly higher antioxidant capacities than the control. When seeds were germinated, antioxidant capacities of the control gradually increased with germination time and reached the maximum at hour 12 in which the antioxidant capacities were 1.52 and 1.79 times higher than those of hour 0 for DPPH and ABTS radical scavenging assays, respectively. The antioxidant capacities for DPPH and ABTS radical scavenging assays increased by 1.37 and 1.53 times from hour 0 compared with that of the control, respectively. The antioxidant capacities of seeds germinated increased with germination time, an hour 12 provided highest antioxidant activity in all

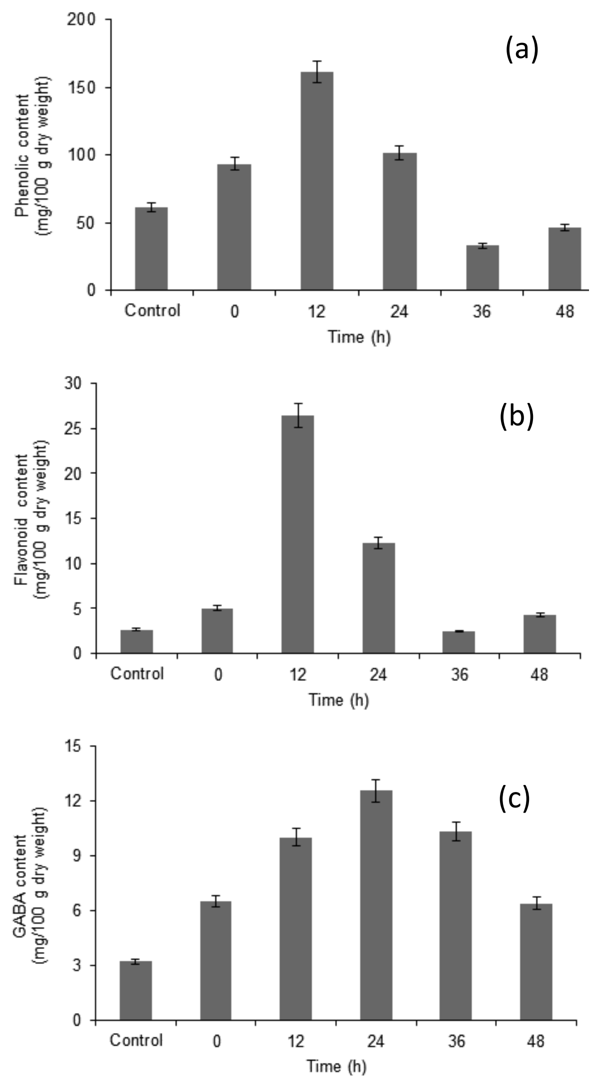

Figure 1 Phenolic (a), Flavonoid (b) and GABA (c) contents in rice seed during germination at 35ºC.

germination time as compared to those of hour 0. Among the germination time used in this study, hour 12 was found to be the optimal germination time enhancing antioxidant capacities. It produced the maximal antioxidant capacity with significant differences in every germination time compared with the control. Although, ABTS radical scavenging assay showed higher values of antioxidant capacities than those of using DPPH radical scavenging assay, these two processes showed the same tendency of outcomes.

This study showed that phenolic, flavonoid, GABA contents and antioxidant capacity of Khao Dawk Mali 105 rice seeds significantly increased after being soaked in water and during germination as compared to that of the control. This suggests that water activates the bioactive compounds and antioxidative systems after the seeds absorbed water and during germination.

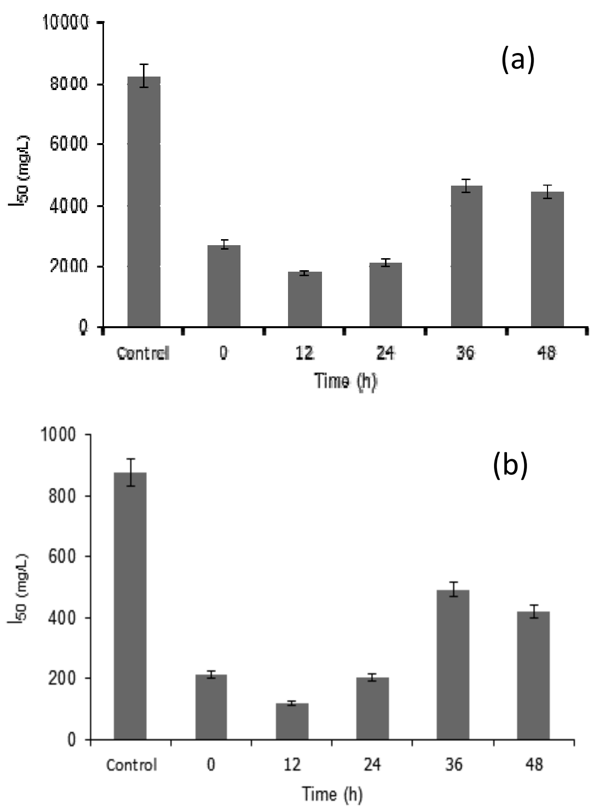

Figure 2 IC_{50} for DPPH (a) and ATBS (b) radical scavenging of rice seed during germination at 35°C.

In germinated seeds, hydrolytic enzymes are activated and decompose biopolymers and biomolecules. The decomposition of biopolymers and biomolecules during germination generates bioactive compounds, and improves in organoleptic qualities due to the softening of texture and increase of flavor in seeds. So, the appropriated amount of water uptake of the rice seed during soaking directly affected quality of the germinated rice seed. In addition, the amino acid in rice seed being used as storage proteins, which are degraded by water, converted to amides and transported to the growing parts of the rice seedling.[18] Increasing GABA content in rice seed after soaking in water was due to the synthesis of glutamic acid and glutamate decarboxylase (GAD) activation, which converts glutamate to GABA[19] The soaking prior before to germination take part in enhancement of the residual GABA content with activating GAD from the hypoxia condition due to the limited availability of oxygen.[20] The GABA content may increase rapidly in plant tissues in response to hypoxia. In addition, GAD activity was a more reliable index for the viability of rice.[20] In this study, the rice seed was soaked for the optimal soaking time of 24 hours, which attained the saturation. It was found that after soaking for 24 hours, the GABA content of rice seed ranged from 6.53-12.56 mg/100 g (Figure 2a). These values were higher than that of rice seed. This result indicates that soaking contributes to the increase in GABA content as similar reports by Saikusa *et al*., (1994),[21] where water soaking increased GABA contents. Different cultivars had different characteristics of water absorption. Hirunpong and Tungjaroenchai[22] found that the moisture up take of brown rice, during soaking at 35°C were 29.01, 29.64 and 31.04% at time 2, 3, and 3 hours, respectively, was the optimal soaking time. Rice cultivars and moisture contents of rice therefore affected the germination and production of GABA. GABA content in germinated rice seed increased 2.03-3.9 times, as compared to the un-germinated samples. In addition, different GABA content among rice cultivars might be come from varying GAD accumulation. However, most of rice seeds from different rice varieties revealed high quantity of GABA content during incubation. It was noticed that GABA accumulation in rice seed proceeded rapidly at an early stage of incubation, accompanied by the parallel loss of glutamate concentration. It suggested that a supply of glutamate would help to accumulate more GABA during rice germ soaking in water.[21] In addition, air was reported to be effect on GABA content during incubation. It was found that higher air feed rate, higher GABA accumulation was attained. It was reported that GABA content in japonica rice seed increased greatly during soaking in water.[21] Increasing GABA content in water soaked was also found in this experiment. Soaking time and rice varieties affected the GABA content of the seeds.[23] So, the enrich GABA condition should be studied more in details as well as the use of enrich GABA rice seed for some foods products preparation.

Varanyanond *et al*.[23] found that water soaking could enrich GABA content in the germ. GABA content of germ was 186.2 mg/kg of germ Khao Dawk Mali 105. The amount of GABA content in soaked germ was more than 1.5 times compared with GABA content of the unsoaked germ. In this study, the amount of high GABA contents found in soaked rice seed and germinated rice at 24 h were more than 2.25 and 4.3 times compared with GABA content of the unsoaked rice seed, respectively.

The antioxidant capacities of phenolics have been reported by donating electrons or hydrogen atoms from their hydroxyl and carboxyl groups and also inactivating lipid free radicals and preventing decomposition of hydroperoxides into free radicals.[24,25] It was found that total phenolics had positive correlation with antioxidant capacities by DPPH (with R^2=0.904) and ABTS radical scavenging assays (with R^2=0.880). The total flavonoids had positive correlation with antioxidant capacities by DPPH (with R^2=0.625) and ABTS radical scavenging assays (with R^2=0.611) (data not shown). This indicates the role of phenolic and flavonoid compounds as free radical scavengers in germinated rice seeds. The GABA contents in germinated rice seeds had no correlation with antioxidant capacities by DPPH and ABTS radical scavenging assays and it had also no correlation with phenolics and flavonoid contents.

In this study, it was found that total phenolics had positive correlation with antioxidant capacities by DPPH (with R^2=0.904) and ABTS radical scavenging assays (with R^2=0.880) higher than that of total flavonoids. During germination, the maximum phenolics and flavonoids in germinated rice were lesser germination time (12 h) than that of GABA (24 h). After12 h germination, phenolics and flavonoids in germinated rice were minimum contents at 36 h and began to increase after 48 h of germination while GABA content was gradually decrease till 48 h of germination. It indicated that GABA promoted growth in germination at first period of germination of rice seed.

Conclusions

Bioactive components and GABA content in rice grain could be increased by optimization of soaking and germination time and conditions. The results showed basis for up-scale GABA-enhanced rice grain. The results showed an increase in GABA production in the short time of germination at the optimal time and temperature of soaking and germination conditions. However, it is necessary to further study the metabolic pathways for high GABA production in rice grain. So, enhancement of the quantities of bioactive compounds in the rice grain provides a better functional food for achieving human health benefits and it also improves the flavor and taste.

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