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Changes of bioactive components in germinated paddy rice (*Oryza sativa* L.)

[1]Jirapa, K., [1]Jarae, Y., [1]Phanee, R. and [2,*]Jirasak, K.

[1]*Department of Chemistry, Faculty of Science, King Mongkut's University of Technology Thonburi, Bangkok 10140, Thailand*
[2]*Division of Biochemical Technology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok 10150, Thailand*

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Abstract

Effects of soaking conditions and germination conditions on phenolic, flavonoid, gamma amino butyric acid (GABA) contents and antioxidant activity of five germinated paddy rice cultivars were investigated. The rices were soaked in distilled water pH 7.0 at room temperature (30°C) for 24 h followed by germinating in aerobic incubator at 35°C for 12, 24, 36 and 48 hours. In this study, the total phenolic and contents were determined by Folin-Ciocalteu reagent and aluminium trichloride, respectively, germinated rice at 12 h showed the highest levels. 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 were highest. The phenolic and flavonoid contents including antioxidant activities by DPPH and ABTS of the germinated rice at 12 h were averagely about 2.96, 7.93, 3.64 and 5.52 times higher than in paddy rice, respectively. GABA content increased consistent with germination time. At 24 h of germination, the GABA contents were highest, which were averagely about 4.12 times higher than in paddy rices. After 24 h of germination, phenolic and flavonoid contents and antioxidant activities in all rice cultivars were decreased while GABA concentrations were decreased after 36 h of germination. The results showed high significant correlations between IC_{50} and phenolic content and between phenolic and flavonoid contents during germination. However, GABA content had no correlation with phenolic and flavonoid contents. From the results, accumulation and disappearance of these bioactive compounds in rice produced by germination method suggested that germination condition could improve nutrient levels and support effective uses of germinated rice grains for consumption for pharmaceutical application.

Introduction

Food antioxidants such as flavonoids and other phenolic compounds might also play a significant role as physiological and dietary antioxidants, thereby augmenting the body's natural resistance to oxidative damage (Shahidi, 2000). Natural antioxidants are known to exhibit a wide range of biological effects including antibacterial, antiviral, antiinflammatory, antiallergic, antithrombotic and vasodilatory activities. In fact, a fundamental property important for life is the antioxidant activity and this property may give rise to anticarcinogenicity, antimutagenicity and antiaging activity, among others (Cook and Samman, 1996). Antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods and to extend their shelf-life (Djeridane *et al.*, 1997). Phenolic acids are secondary metabolites widely distributed in the plant kingdom and are second only to flavonoids in terms of their dominance, suggesting that naturally occurring. Intake of dietary antioxidant such as polyphenolics has been considered as an important approach in the prevention of those chronic diseases, which maybe attribute to the antioxidant activity of polyphenols.

Rice is the largest cereal crop in the world. It is also the main staple food for world populations. Rice components have several roles disease prevention. It contains essential nutrient, dietary fiber, oil and hypoallergenic protein (Mori *et al.*, 1999). Germinated rice has been of interest throughout Asian countries (Moongngarm and Saetung, 2010) as it contains high amounts of bioactive compounds such as γ-aminobutyric acid (GABA), γ oryzanol, and dietary fiber (Moongngarm and Saetung, 2010). Germinated rice grain offers considerable benefits. Germinated rice contains high amounts of ferulic acid (Tian *et al.*, 2004), α-tocopherols (Moongngarm and

*Corresponding author.
Email: *jirasakkong@gmail.com*

Saetung, 2010) and phenolics (Sutharut and Sudarat, 2012) which have potent antioxidant and free radical scavenging properties (Gill and Tuteja, 2010). Phenolic compound in rice (Tian *et al*., 2005) such as ferulic acid has the capability to prevent the build-up of superoxide, controlling the aggregation of blood platelets (Kayahara, 2004) and cholesterol-lowering properties as well as for their antioxidant capacity (Nystrom *et al*., 2007). Additionally, the germination of rice frees its bound minerals, making them more absorbable by the body and the rice tendered and tastier (Kayahara, 2004). In the plant, the GABA biosynthesis pathway is accomplished by GABA shunt and polyamine degradation (Barry *et al*., 1999). 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) (Bouche *et al*., 2003) 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 (Bown and Shelp, 1997).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 (Liao *et al.*, 2013). Several health benefits of GABA have been reported (Imam *et al*., 2012), e.g. lower blood pressure (Matsuo *et al*., 2012) and blood cholesterol (Usuki *et al*., 2011), greater kidney and liver activity (Kim *et al.*, 2004), inhibition of cancer cell proliferation (Al-Wadei *et al.*, 2011) and stimulation of cancer cell apoptosis.The application of the germinations condition will be promising for the development of GABA-rich products and the promoting of the consumption of traditional rice.

Pong Ell is the unique rice cultivar grown only in Ayuthaya province while Luang Thong and Jek Chuey are the unique rice cultivars grown only in Saraburi province in Thailand. These rice cultivars are local rice cultivars. They are long grain non-waxy Thai rice cultivar. Though many studies have been done on improving bioactives compounds and GABA production in germinated rice grains, information on the gemination conditions for bioactives compounds and GABA production in these rice cultivars are still not reported. Phathum Thani 1which is grown in Phathum Thani province is hybrid rice between rice cultivar BKNA6-18-3-2 and PTT85061-86-3-2-1. These four rice varieties have disease resistance, pest resistance with high yield product per rai (650-774 kg/rai) and they might have high bioactive compounds that are beneficial for health and believed to have medicinal property. Khao Dawk Mali 105 (KDML105) variety has had a distinguished characteristic; aromatic, soft, and delicious. Even though this rice is popular for consumption, the rice production is limited as yields per rai is not high, averaging 360 kg/rai and is only suitable for certain areas. The aim of this study is to investigate the change of the levels of bioactive compounds in rice grain and germinated rice during germination made with different varieties, in different production areas in Thailand and in different generation time and to compare the differences between the bioactive compounds in these rices with three novel rice varieties. The relationships between bioactive compounds production during germination is also reported.

Materials and Methods

Rice sample

Paddy rice Pong Ell was provided by the Rice Research Center, Ayutthaya province. Paddy rice cv. Luang Thong and cv. Jek Chuey1 were provided by the Rice Research Center, Saraburi province, Thailand. Paddy rice cv. Khao Dawk Mali 105 (KDML 105) and cv. Prathum Thani 1were provided by the Rice Research Center, Prathum Thani 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 non-germinated 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 (2012). 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 (Singleton and Rossi, 1965). 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*. (1999). 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 (2012). 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) (Shimada *et al*., 1992). 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}DPPH is the effective concentration in mg extract/mL which inhibits the DPPH activity by 50%. Butylated hydroxyanisole (BHA) was used as a control. For 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid or ABTS assay, radical cation scavenging capacity was examined against $ABTS^{\bullet+}$generated by the chemical method. The absorbance of the reaction mixture was measured at 734 nm and the BHA equivalent was calculated using a standard curve prepared with BHA (Rea *et al.*, 1999). IC_{50}ABTS is the effective concentration in mg extract/mL which inhibits the ABTS activity by 50%.

Experimental design and data analysis

The experiments were set up in a completely randomized design. Triplicate determination was performed. 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.

Results

Total phenolic content

After being soaked in distillated water at 30ºC for 24 hours (0 h), total phenolic content of rice seeds were significantly higher than that of the control. Total phenolic content of the control increased with germination time and had the highest phenolic content at hour 12 (Figure 1). The total phenolic content of germinated seeds on hour 12 was 1.51-2.11times more than hour 0. The amount of total phenolic compounds increased by 2.45-3.31times from hour 0 compared with that of the control.

Total flavonoid content

After being soaked in distillated water at 25ºC for 24 hours (0 h), total flavonoid content of rice seeds were significantly higher than that of the control. Total flavonoid content of the control increased with germination time and had the highest peak at hour 12 (Figure 2). The total flavonoid content of germinated seeds on hour 12 was 1.58-2.03 times more than hour 0. The amount of total flavonoid compounds increased by 4.13-10.09 times from hour 0 compared with that of the control.

GABA content

After being soaked in distillated water at 30ºC for 24 hours (0 h), GABA content of rice seeds

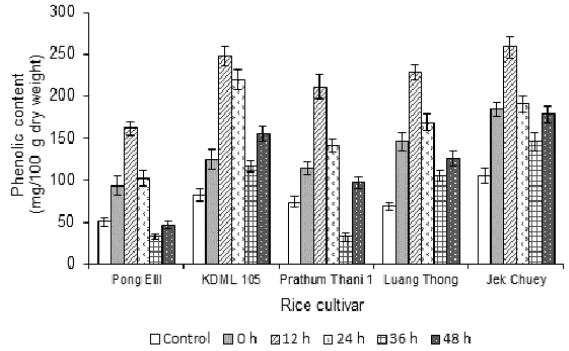

Figure 1. Phenolic content in rice seeds during germination at 35ºC

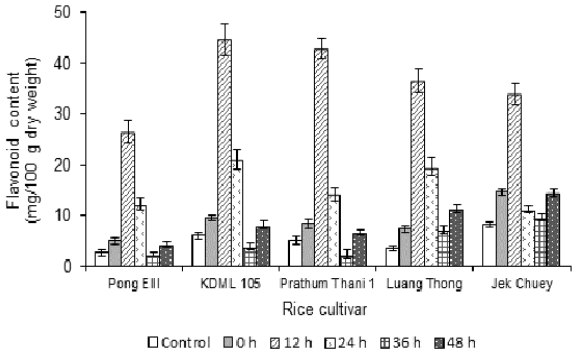

Figure 2. Flavonoid content in rice seeds during germination at 35ºC

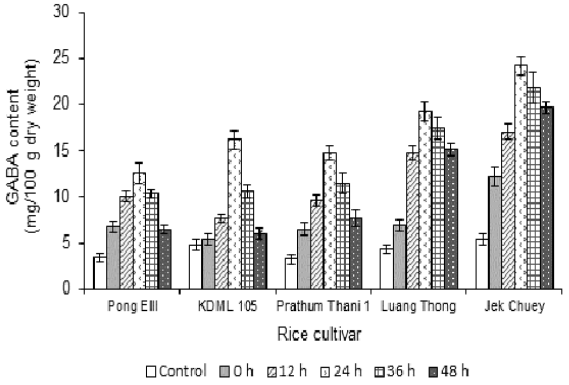

Figure 3. GABA content in rice seeds during germination at 35ºC

were significantly higher than that of the control. Total flavonoid content of the control increased with germination time and had the highest peak at hour 24 (Figure 3). The GABA content of germinated seeds on hour 24 was 1.14-2.26 times more than hour 0. The amount of GABA increased by 3.67-4.56 times from hour 0 compared with that of the control.

Antioxidant capacity

As shown in Figure 4 and Figure 5, antioxidant capacities of 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 1.18-1.83 and 1.65-3.35 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 1.79-5.15 and 3.03-7.99 times from hour 0 compared with that of the controls, respectively. The antioxidant capacities of seeds germinated increased with germination time, an hour 12 provided the highest antioxidant capacities in all 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.

Seed germination is a complex process involving biochemical and physical activities (Moongngarm and Saetung, 2010; Xu *et al.*, 2011). 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 (Ohtsubo *et al*., 2005, Saman *et al.*, 2008). Apart from nutrition level changes, germination also generates antioxidant compounds including phenolic and flavonoid contents (Tian *et al.*, 2004; Sutharut and Sudarat, 2012). This study showed that phenolic, flavonoid, GABA contents and antioxidant capacity of different 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.

From Table 1, the phenolic and flavonoid contents including antioxidant activities by DPPH and ABTS of the germinated rice at 12 h were averagely about 2.96, 7.93, 3.64 and 5.52 times higher than in paddy rice, respectively. GABA content increased consistent with germination time. At 24 h of germination, the GABA contents were highest, which were averagely about 4.12 times higher than in paddy rices.

The correlation between flavonoid and phenolic contents of germinated rice had a correlation coefficient of R^2=0.7377 while the correlation

Table 1. Phenolic, flavonoid and GABA contents (mg/100 g dry weight) and antioxidant activity (I_{50DPPH} and I_{50ABTS} (mg/L)) in different rice grain cultivars during germination at 35ºC

Sample	Pong Ell	KDML 105	Phathumt Thani 1	Luang Thong	Jek Chuey
Phenolic					
Control	50.34±5.02^{aA}	82.72±7.22^{aB}	74.04±7.12^{aBC}	69.16±5.23^{aC}	105.19±9.26^{aD}
0 h	93.28±11.56^{bA}	124.60±12.07^{bB}	114.52±7.42^{bC}	146.04±11.52^{bD}	184.24±8.47^{bE}
12 h	161.64±8.22^{cA}	247.50±11.46^{cB}	210.74±14.62^{cC}	229.26±9.26^{cCD}	258.22±12.36^{cE}
24 h	101.82±9.04^{dA}	220.03±12.32^{dB}	141.01±8.24^{dC}	168.92±10.52^{dD}	190.45±9.12^{bE}
36 h	32.89±3.23^{eA}	116.42±7.56^{eB}	32.09±5.32^{eA}	104.40±6.28^{eB}	146.26±10.23^{dC}
48 h	46.70±4.14^{fA}	154.51±9.42^{fB}	97.36±7.46^{fC}	126.11±8.42^{fD}	178.20±9.18^{eE}
Flavonoid					
Control	2.72±0.40^{aA}	6.07±0.61^{aB}	5.14±0.69^{aB}	3.62±0.45^{aA}	8.24±0.48^{aC}
0 h	5.07±0.60^{bA}	9.59±0.51^{bB}	8.42±0.89^{bBC}	7.36±0.62^{bC}	14.64±0.59^{bD}
12 h	26.46±2.20^{cA}	44.64±3.05^{cB}	42.91±1.87^{cB}	36.52±2.32^{cD}	34.02±2.04^{cD}
24 h	12.23±1.08^{dA}	21.09±1.95^{dB}	14.22±1.42^{dA}	19.64±1.76^{dB}	11.26±1.49^{bdA}
36 h	2.41±0.39^{aA}	4.02±0.68^{eA}	2.47±0.86^{eA}	7.24±0.62^{bB}	9.68±0.70^{dC}
48 h	4.42±0.47^{bA}	8.20±0.73^{bB}	6.78±0.55^{aB}	11.40±0.84^{eC}	14.46±0.76^{bD}
GABA					
Control	3.42±0.46^{aA}	4.76±0.64^{aABC}	3.22±0.49^{aA}	4.28±0.44^{aB}	5.39±0.62^{aC}
0 h	6.73±0.60^{bAC}	5.42±0.62^{aA}	6.51±0.74b^{AC}	6.92±0.63^{bC}	12.16±1.05^{bD}
12 h	10.03±0.51^{cA}	7.67±0.54^{bB}	9.62±0.66^{cA}	14.76±0.72^{cC}	17.02±0.82^{cE}
24 h	12.56±0.68^{dA}	16.19±1.02^{cB}	14.67±0.74^{dB}	19.28±1.24^{dC}	24.14±1.06^{dD}
36 h	10.34±0.49^{cA}	10.64±0.68^{dA}	11.45±0.65^{cA}	17.45±1.02^{dB}	21.82±1.20^{deC}
48 h	6.41±0.47^{bAB}	5.97±0.53^{aA}	7.64±0.92^{bB}	15.22±0.62^{cC}	19.64±0.71^{eD}
IC_{50DPPH}					
Control	3205.72±179.62^{aA}	2127.91±94.32^{aB}	2764.02±203.02^{aCD}	2924.06±125.64^{aC}	2428.64±219.22^{aD}
0 h	2716.59±121.44^{bA}	1284.62±68.25^{bB}	1514.27±128.74^{bC}	1686.42±117.06^{bC}	1432.16±102.46^{bC}
12 h	1791.62±149.76^{cA}	447.94±45.22^{cB}	536.51±56.32^{cB}	864.12±73.12^{cC}	772.42±62.22^{cC}
24 h	2140.54±114.52^{dA}	535.46±54.14^{dB}	1202.72±72.22^{dC}	1224.04±102.74^{dC}	867.05±35.02^{dD}
36 h	3040.16±124.84^{eA}	1495.26±49.28^{eB}	2426.14±176.71^{eC}	2460.19±124.32^{eC}	1896.24±92.16^{eD}
48 h	2424.42±152.02^{fA}	1446.72±89.62^{eB}	1651.36±106.02^{bB}	2326.03±143.46^{eA}	1619.64±67.35^{fB}
IC_{50ABTS}					
Control	352.78±14.32^{aA}	240.54±21.92^{aB}	320.06±11.42^{aC}	302.26±15.74^{aA}	291.16±11.42^{aD}
0 h	214.16±12.76^{bA}	92.09±9.06^{bB}	172.82±12.73^{bC}	146.22±12.32^{bD}	87.02±8.74^{bB}
12 h	116.52±14.64^{cA}	44.14±5.32^{cB}	59.54±6.02^{cC}	52.36±7.52^{cC}	36.42±3.95^{cD}
24 h	203.64±19.51^{bA}	62.09±9.74^{dB}	102.17±10.84^{dC}	121.87±11.76^{dC}	82.64±7.62^{bD}
36 h	352.82±24.36^{dA}	148.25±8.26^{eB}	259.42±14.62^{eC}	289.04±23.12^{eC}	207.36±11.04^{dD}
48 h	296.26±15.22^{eA}	122.41±9.63^{B}	224.62±14.16^{fC}	241.82±12.64^{fC}	157.98±10.32^{eD}

Mean values and standard deviations with different letters (a, b, c) in the same column indicate significant differences (P<0.05) during germination, and different letters (A, B, C) in the same row indicate significant differences (P<0.05) among rice cultivars.

between IC_{50} (IC_{50DPPH} and IC_{50ABTS}) and phenolic content of germinated rice had correlation coefficient of R^2=0.7982 and R^2=0. 8251, respectively. This study suggests that most of the antioxidant activity accessions results from the contribution of phenolic compounds. Also, it might be concluded that antioxidant activities are not limited to phenolics. It might be also come from the other antioxidant secondary metabolites, such as volatile oils, flavonoids and vitamins. The antioxidant capacity of phenolics is mainly due to their redox properties, which act as reducing agents, singlet oxygen quenchers and hydrogen donors.

Discussion

Increasing GABA content in rice seed after soaking in water was due to the synthesis of glutamic acid by glutamate decarboxylase (GAD). 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. The result in Figure 3 showed a similar trend after 12 hours, which is in accordance to the report by Benjamasuttikul and Naivikul, (2007). The appropriated amount of water uptake of the rice seed during soaking directly affected quality of the germinated rice seed. Different cultivars had different characteristics of water absorption. Hirunpong and Tungjaroenchai (2008) 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. In this study, the rice seed was soaked for 24 hours, which attained the saturation point, was the optimal soaking time. After soaking for 24 hours, the GABA content of rice seed in all rice cultivars (Figure 3, Table 1). These values were higher than that of control rice seed. This result indicates that soaking contributes to the increase in GABA content as similar reports by Saikusa *et al*., (1994), where water soaking increased GABA contents. The increase in GABA content during soaking may be come from glutamate decarboxylase (GAD) activation, which converts glutamate to GABA (Komatsuzaki *et al.* 2007). Soaking could lead to hypoxia due to the limited availability of oxygen for the grain (Dewar *et al.*, 1997) and GABA content may increase rapidly in

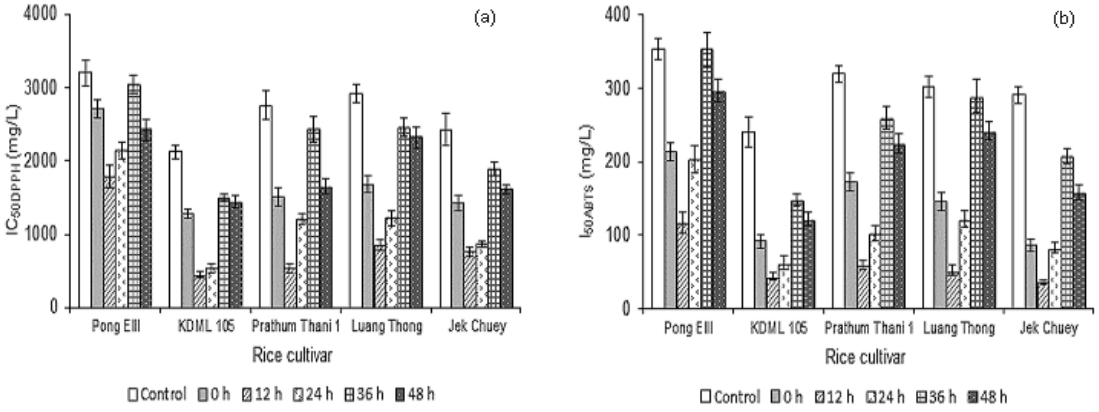

Figure 4. Comparison between IC_{50} for DPPH radical scavenging (a) and IC_{50} for ATBS radical scavenging (b) of rice seeds during germination at 35ºC

plant tissues in response to hypoxia (Crawford *et al*., 1994). In addition, GAD activity was a more reliable index for the viability of rice (Bautista *et al*., 1964). Different GABA content among rice cultivars might be come from varying GAD accumulation. The soaking prior before germination take part in enhancement of the residual GABA content with activating GAD from the hypoxia condition. Rice cultivars and moisture contents of rice therefore affected the germination and production of GABA. GABA content in germinated rice seed increased, as compared to the unsoaked samples. 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.

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 (Saikusa *et al*. 1994). Besides, air was reported to be effect during incubation: more air entered into the procedure of GABA formulation, higher accumulation of GABA will be attained. It was reported that GABA content in japonica rice seed increased greatly during soaking in water (Saikusa *et al*., 1994). Increasing GABA content in water soaked, was also found in this experiment. Soaking time and rice varieties affected the GABA content of the seeds. 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.

DPPH radical scavenging assay and ABTS radical scavenging assay. These methods are popular due to their high speed and sensitivity. However, it is essential to use more than one method to evaluate antioxidant capacity of plant materials because of the complex nature of phytochemicals. Antioxidant activity assays– ABTS and DPPH – with different ability of using stable radicals to react with antioxidants, ABTS assay was found to be more sensitive for the determination of the antioxidant activity for germinated rice. The present study also showed higher average antioxidant activity assay values for ABTS in comparison with DPPH (Table 1). Bondet *et al.* (1997) found that most phenolic antioxidants react slowly with DPPH, reaching a steady state in 1-6 h or longer. The ABTS has the extra flexibility in that it can be used at different pH levels (unlike DPPH, which is sensitive to acidic pH). The result is compatible with antioxidant capacities that were found that DPPH was lower antioxidant capacities than ABTS radical scavenging (Floegel *et al*., 2011).

In the present study, It was found that total phenolics had positive correlation with antioxidant capacities by DPPH (with R^2= 0.7982) and ABTS radical scavenging assays (with R^2= 0.8251). In addition, there is high correlation (R^2 = 0.9012) between ABTS and DPPH and it suggested that both the methods have similar predictive capacity for free radical scavenging for germinated rice. 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 (Michalak, 2006). The total flavonoids had positive correlation with antioxidant capacities by DPPH (with R^2= 0.5646) and ABTS radical scavenging assays (with R^2= 0.5514). 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 and ABTS radical scavenging assays 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 of 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 increase by optimization of soaking and germination time and conditions. The low content of bioactive components and GABA were increase Optimization of soaking conditions could increase bioactive components and GABA content in rice grain. The results showed basis for up-scale GABA-enhanced rice grain. The results showed increment of 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. Improvement quantity of bioactive rice would not only provide a better functional food for achieving human health benefits, but also improves the taste. Optimizing process of rice grain germination could also reduce cost of production.

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