Kasetsart J. (Nat. Sci.) 44 : 912 - 923 (2010)

The Effect of Germination on GABA Content, Chemical Composition, Total Phenolics Content and Antioxidant Capacity of Thai Waxy Paddy Rice

Suchada Maisont and Woatthichai Narkrugsa*

ABSTRACT

The changes were studied in the GABA content (gamma aminobutyric acid), chemical composition, total phenolics content and antioxidant capacity from the germination process of Thai waxy paddy rice (RD6). The paddy rice was soaked to about 33% wet basis moisture content and then germinated in the cabinet incubator (28-30°C) for 0, 12, 24, 36, 48 and 60 h. Embryos were cut to determine the GABA content, and the germinated paddy rice was dried at 50°C using a tray dryer, dehusked and ground for analysis. It was found that the germination process significantly ($p<0.05$) increased GABA, dietary fiber, total phenolics content and antioxidant capacity, while the fat, protein, starch and amylose content trended to decrease slightly. There was little change in the early stage of germination and then a marked increase after 36 h. For the GABA content after soaking, it gradually increased from 80 to 220 mg/100 g embryo fresh weight from 12 to 60 h. Based on the results, germinated paddy rice can be considered as an alternative source of GABA and of dietary fiber content with high total phenolics content and high antioxidant capacity.

Keywords: germination, GABA, malted rice, antioxidant, paddy rice

INTRODUCTION

Germination is the most common and effective process to improve the quality of cereal grain. Soaking is the first step in water penetration, which transforms the inactive tissue into living tissue. In this step, the grain's metabolism is activated in preparation for germination. During germination, the grain nutrient reserves degrade and are used for respiration and the synthesis of new cells that form the developing embryo, causing changes in the nutritional and biochemical composition (Bamforth and Barclay, 1993). With brown rice, during soaking and germination, increases in the gamma aminobutyric acid (GABA) content are the most popular (Saikusa *et al*., 1994; Shoichi and Ishikawa, 2004; Ohtsubo *et al*., 2005; Choi *et al*., 2006; Komatsuzaki *et al*., 2007; Watchraparpaiboon *et al*., 2007). GABA is a gamma amino acid transmitter that can inhibit the activity of nerves in the central nervous system. Many researchers have reported that GABA has several physiological functions, such as; acting as an inhibitory neurotransmitter in the central nervous system (Jakobs *et al*., 1993; Kayahara and Tsukahara, 2000); acting as an antihypertensive (Hayakawa *et al*., 2002); reducing plasma cholesterol levels (Miura *et al*., 2006); improving

Faculty of Agro Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand.
* Corresponding author, e-mail: knwoaith@kmitl.ac.th, smaisont@hotmail.com

Received date : 07/01/10 Accepted date : 24/05/10

blood glucose levels in diabetics (Ito *et al*., 2005; Seiki *et al*., 2005); and preventing chronic alcohol-related disease (Oh *et al*., 2003). Brown rice is also well-known as being rich in vitamins B and E, γ-oryzanols, dietary fiber and phenolic compounds (Champagne *et al*., 2004; Butsat and Siriamornpun, 2010). However, brown rice is not favorite to consumers, because of its hardness and dark color. Soaking and germination can make brown rice soften and activate bioactive compounds, which can induce consumers to have an increased interest in germinated brown rice. However, these processes involved several factors (Saikusa *et al*., 1994; Ohtsubo *et al*., 2005; Komatsuzaki *et al*., 2007).

In plant tissue, the GABA content is rather low (ranging from 0.03 to 2.00 mmol fresh weight) but it increases several times under certain conditions, such as anoxia, cytosolic acidification, cold shock, mechanical stimulation, water stress and plant development (Bown and Shelp, 1997). Previous studies reported that increases in the GABA content in brown rice was correlated with the cultivar (Varanyanond *et al*., 2005), harvest year (Shoichi and Ishikawa, 2004), soaking conditions (Sunte *et al*., 2007; Watchraparpaiboon *et al*., 2007) and germination conditions (Ohtsubo *et al*., 2005; Komatsuzaki *et al*., 2007). Soaking and germination could be considered as options to add value to paddy rice, which is a raw material tending to undergo increased developmental interest, but for which there is a lack of information, especially on GABA accumulation. Therefore, the purpose of the current experiment was to study the effect of the germination process on the GABA content, chemical composition, total phenolics content and antioxidant capacity of waxy paddy rice. Value-adding to paddy rice could then include using germinated paddy rice in various food industries.

MATERIALS AND METHODS

Materials

The variety of Thai waxy paddy rice (RD6) used in this study was harvested in September, 2007 from the Ubon Ratchatani Rice Research Center, Ubon Ratchatani province. The paddy rice was cleaned, dried and stored in plastic bags at 12-15°C, for three months before the germination study. The moisture content of the paddy rice was 12.68 % on a wet basis.

Chemicals

Standard gamma-aminobutyric acid (GABA), Folin-Ciocalteu phenol reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl), TPTZ (2,4,6-tripyridyl-S-triazine), gallic acid, Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid), sodium carbonate, ferric chloride and acetonitrile were purchased from Sigma-Aldrich Chemical Co. USA. Other common reagents used were analytical grade.

Measurement of hydration characteristics of paddy rice, husk and brown rice

The paddy rice grain is composed of two major physical components, the brown rice grain and the husk. During soaking, the moisture content of the paddy rice may not indicate the true moisture content of the brown rice component. Thus, the moisture content in each component (the paddy rice, brown rice and husk) was measured according to the method of Abhay and Gupta (2006), with some modifications. The paddy rice (30 g) was soaked in water (1:1.5 w/w) at room temperature (28-30°C). Samples were removed every 5 h. Each paddy rice sample was drained for 1 min and blotted with tissue paper two to three times to remove the surface water. After blotting, the husk was separated manually with a razor blade. Then the paddy rice, husk and brown rice moisture content were determined by AOAC (1990).

Soaking and germination process

The germination process of paddy rice was prepared according to the method of Puangwerakul (2007), with some modifications. Paddy rice (300 g) was soaked with water (1:1.5w/w), which the water changed every 12 h and then drained. Each paddy rice sample was packed into separate plastic boxes on a layer of tissue paper and left to germinate in the cabinet incubator (28-30°C) with relative humidity at 94-96% for 0, 12, 24, 36, 48 and 60 h.

Structure of paddy rice and germinated paddy rice

The embryo and aleuron layer of the paddy rice and germinated paddy rice were determined by transversely cutting dry grains at the center with a sharp razor blade. Samples were fixed on a stub with the help of double-side adhesive tape and sputter-coated with gold in a vacuum chamber. The samples were viewed with a scanning electron microscope (JEOL, JSM 6301F) under vacuum conditions at an accelerating voltage of 15 kV, by modifying the method of Mariotti *et al*. (2006).

GABA content measurement

The GABA content was measured with high performance liquid chromatography (HPLC). The system consisted of an Alliance HPLC 2695 unit with heater, a Hypersil Gold column C18, control temperature 35±1°C, a multi λ fluorescence detector (EX:250,EM:395 nm), The mobile phase consisted of acetonitrile:sodium acetate buffer with pH 4.95 (60:40) and a flow rate of 1 mL/min. Each paddy rice embryo was carefully cut with a razor blade to separate the embryo (Liu *et al*., 2005). The fresh germ was weighed to the nearest 100 mg. Then 5 mL of 6 N HCL was added and placed on a heating block at 110°C for 22 h. An internal standard of hydrolysate was added and diluted with deionized water. The filtrate was mixed with AccQ-fluor derivatization buffer and AccQ-fluor reagent for derivatization, after which sample volumes of 5 mL were injected into the HPLC unit (Liu *et al*., 1995).

Chemical compositions analysis

Germinated paddy rice was dried at 50±2°C in a tray- dryer until the moisture content decreased to 8-10 % on a wet basis, dehulled and ground using a Retsch Ultra centrifugal mill with a mesh sieve screen (0.12 mm). The moisture, protein and fat content and ash samples were determined according to AOAC (1990).The dietary fiber content was determined according to AOAC (2000). The content of starch was determined using the enzymatic glucoamylase method AACC (1990).

Phenolics extraction

After periods of 0, 12, 24, 36, 48 and 60 h, germinated paddy rice flour samples (approximately 2 g dry matter) were extracted with 20 mL of 80% ethanol for 30 min at room temperature and then centrifuged with a benchtop centrifuge (Allegra X-12R, Beckman Coulter, Inc. USA) at 6,000 rpm and 4°C for 30 min. After centrifugation, the supernatant was separated from the residue and stored at -4°C for further analysis (Ragaee *et al*., 2006).

Total phenolics content determination

The total phenolics content was determined for each sample using the Folin-Ciocalteu method described by Singleton *et al*. (1999), with some modifications. Extracts (0.5 mL) were added to test tubes followed by 9.5 mL of distilled water, 0.5 mL of Folin-Ciocalteu reagent and the reaction was neutralized with 2 mL of 10% sodium carbonate solution. The contents of each test tube were mixed thoroughly. After standing for 1 h at room temperature, the absorbance was measured at 730 nm with a UV-visible spectrophotometer (SHIMADZU 1700, Japan). Using gallic acid as standard, the total

phenolics content was expressed as mg of gallic acid equivalents per gram dry matter.

Antioxidant capacity assays

DPPH radical scavenging activity

The radical scavenging activity of the phenolics extracts on the DPPH radical was measured according to the method described by Ragaee *et al*. (2006), with some modifications. The extract (0.4 mL) was mixed with 5 mL of 40% ethanol solution and 0.6 mL of 0.8 mmolL^{-1} DPPH solution. The mixture was vigorously shaken and left to stand for 30 min under subdued light. The absorbance was measured at 517 nm with a UV-visible spectrophotometer. The results were expressed as mg Trolox equivalents per gram dry matter.

Ferric reducing ability power (FRAP)

The FRAP of the phenolics extracts was measured according to the method of Wong *et al*. (2006), with some modifications. The FRAP reagent consisted of 10 mmolL^{-1} TPTZ in 40 mmolL^{-1} HCl, 20 mmolL^{-1} $FeCl_3.6H_2O$ and 0.1 mmolL^{-1}sodium acetate buffer, at pH 3.6 in the ratio of 1:1:10. The extracts (0.1 mL) were added to 3 mL of FRAP reagent and mixed thoroughly. After standing for 8 min at room temperature, the absorbance was measured at 593 nm with a UV-visible spectrophotometer. The results were expressed as mg Trolox equivalents per gram dry matter.

Statistical analysis

Each experiment was performed in triplicate. Differences in results obtained between treatments were analyzed utilizing analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Water hydration of paddy rice

Figure 1 shows the hydration characteristics of the paddy rice, the husk and brown rice were separated from the paddy rice. In the early stage of soaking, the water absorption rate of the paddy rice increased rapidly until after 15 h, when the rate decreased gradually. The initial moisture content of the paddy rice was 12.68%. After soaking, the paddy rice moisture content increased to 22.10, 25.78 and 28.31% on a wet basis, after soaking for 5, 10 and 15 h, respectively.

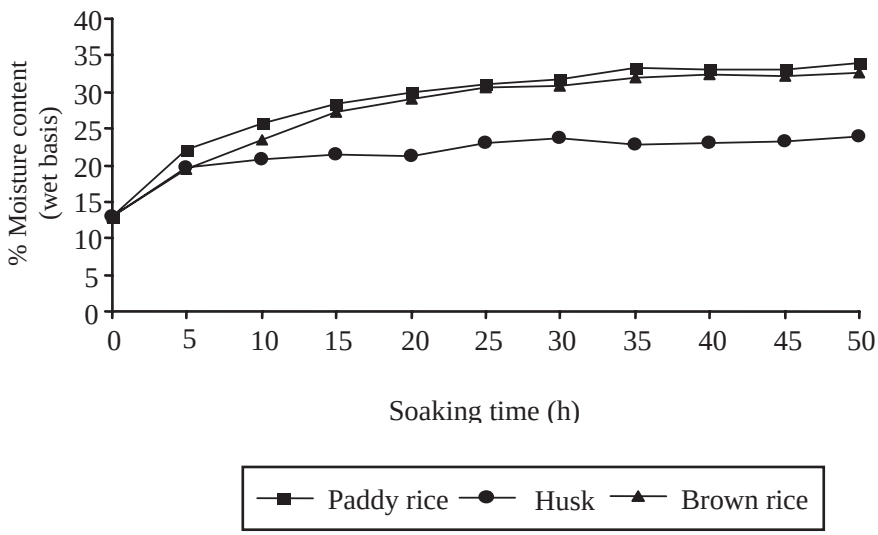

Figure 1 Moisture content of paddy rice, husk and brown rice following soaking in water for 0-50 h.

The differences between the moisture content of paddy rice and brown rice soaked for these same periods were 2.67, 2.35 and 1.12%, respectively. After soaking the paddy rice for more than 20 h, the moisture content of the paddy rice and the brown rice was nearly the same. The husk is the barrier that protects against water penetration into the paddy grain during the early stage of soaking. After water has penetrated the husk of the paddy grain, it is stored in spaces between the husk and the brown rice. Water does not penetrate immediately into the endosperm, because of the pericarp and the seed coat (Figures 4 b and c; Bello *et al*., 2004 and Abhay and Gupta, 2006). The first area which the water penetrates is the ventral site of the embryo, by diffusion through the endosperm (Hwang *et al*., 2009). When the soaking time had increased to more than 35 h, the moisture content of the paddy rice, husk and brown rice reached an equilibrium of 33.25, 22.75 and 32.32% on a wet basis, respectively. The moisture content of the rice grain is related to the germination percentage and the germination quality (Puangwerakul, 2007). Lamkin *et al*. (1983) reported that the germination percentage had a highly significant correlation (r=0.916***) with the activity of glutamic acid decarboxylase (GAD) in barley. Paddy rice could germinate at a moisture content of 18-40% or after soaking for 12-60 h. Differences in soaking time and moisture content are due to differences in the paddy rice variety (Puangwerakul, 2007). In addition, if the soaking temperature is high, then the rate of water absorption increases. However, this depends on the purpose of the soaking. The moisture content of the paddy would cause the rice to reach a state of anoxia or conditions of water stress, which induces the accumulation of GABA content and changes other constituents.

GABA content

The GABA content of the embryos of paddy rice and germinated paddy rice was determined with HPLC and the chromatogram as shown in Figure 2. The GABA content of the embryo of paddy rice could not be detected because of the detection limit. When paddy rice was soaked for 50 h, the GABA content increased to 60 mg/ 100 g embryo fresh weight (Figure 3). This result indicated that soaking contributed to increase the GABA content. Similar results were reported for soaked rice germ (Varanyanond *et al*., 2005; Choi *et al*., 2006) and brown rice (Saikusa *et al*., 1994; Ohtsubo *et al*., 2005; Komatsuzaki *et al*., 2007). In addition, this result was consistent with Howell *et al*. (2009), who reported that after soaking the rice embryo for one hour, the mapping metabolic transcript levels rapidly changed the metabolism, including an increase in hexose phosphates, tricarboxylic acid cycle (TCA) intermediates and γ-aminobutyric acid.

Later, changes in the metabolic process included those involved in carbohydrate, amino acid and cell wall metabolism. The increase in the GABA content during soaking is due to the activation of glutamate decarboxylase (GAD) that catalyzes the decarboxylation of L- glutamic acid to carbon dioxide and GABA, which causes the glutamic acid to decrease (Oh, 2003; Komatsuzaki *et al*., 2007). Moreover, soaking could lead to anoxia (Reggiani *et al*., 1988; Dewar *et al*., 1997) and suspension cells adapted to water stress (Rhodes *et al*., 1986). Tissue stress and anoxia, which reduce respiration and the NAD/NADH ratio, can restrict the production of succinate. Such stress may also contribute to the accumulation of GABA, by reducing the oxidation of succinic semialdehyde to succinate. Following the removal of the stress, GABA provides an immediate substrate for the Krebs cycle (Wallace *et al*., 1984).

It was found that the GABA content increased continuously with germination time. After 12-60 h germination of paddy rice, the GABA content increased from 80 to 220 mg /100 g embryo fresh weight (Figure 3). The increase in the GABA content during germination might have been due to the seed growth and development of

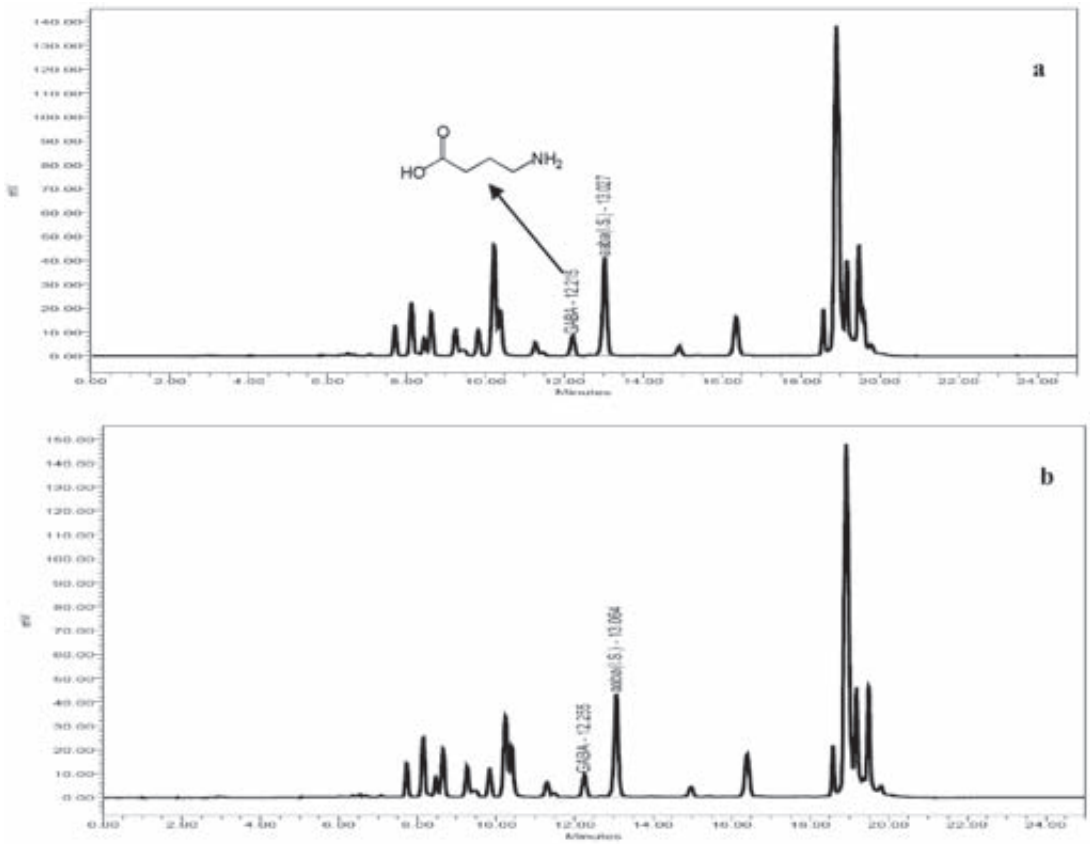

Figure 2 Chromatogram of : (a) standard GABA 25pmol; and (b) paddy rice embryo germinated 24 h.

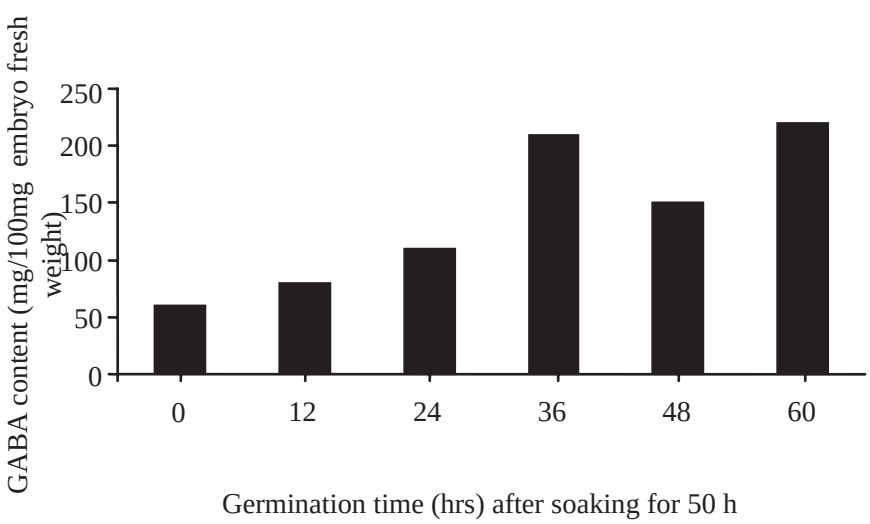

Figure 3 GABA content of paddy rice embryos after soaking and various germination times.

the plant (Shelp *et al*., 1999). Bown and Shelp (1997) suggested that GABA accumulation and efflux were part of an intercellular signal transduction pathway leading to the regulation of growth and development. Moreover Aurisano *et al*. (1995) found that germinating seed rice under anaerobic conditions for 24 h induced GABA accumulation in the shoot and root. Thus, the accumulation of GABA that occurred in embryo, as a part of the general increase in the GABA content during germination might have come from the shoot and root part (Figures 3, 4a and 4d).

Chemical composition

The chemical composition of the samples is presented in Table 1, showing that the germination process caused changes in the chemical composition. The protein, fat, amylose and starch content decreased continuously with increasing germination time, with a notable significant ($p<0.05$) difference after a germination time of 48 h. The dietary fiber content showed a

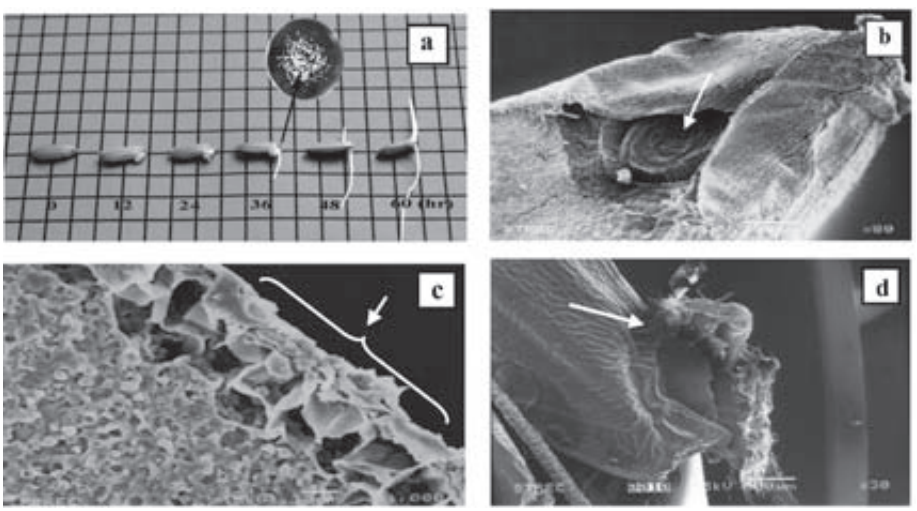

Figure 4 Paddy rice: (a) physical characteristics at various germination times: (b) paddy rice embryo, x80; (c) aleuron layer at the ventral site, x1000; and (d) germinated paddy rice embryo at 36 h, x30.

Table 1 The chemical composition of brown rice at various germination times.

Chemical composition (% wet basis)	Germination time (h)					
	0	12	24	36	48	60
Moisture	8.89±0.14^{d}	8.59±0.10^{c}	8.13±0.13^{b}	7.85±0.10^{a}	8.07±0.10^{b}	8.77±0.08^{cd}
Fat	3.08±0.11^{a}	3.06±0.06^{a}	2.97±0.18^{a}	2.92±0.17^{a}	2.86±0.16^{a}	2.81±0.11^{a}
Protein	6.29±0.06^{d}	6.21±0.09^{d}	6.17±0.11^{cd}	6.04±0.11^{bc}	5.96±0.04^{ab}	5.84±0.09^{a}
Ash	1.26±0.08^{a}	1.29±0.03^{a}	1.37±0.06^{ab}	1.46±0.09^{bc}	1.48±0.03^{bc}	1.56±0.06^{c}
Dietary fiber	2.05±0.01^{a}	2.07±0.06^{a}	2.51±0.01^{b}	2.69±0.00^{c}	2.83±0.04^{d}	2.92±0.06^{d}
Amylose	6.34±0.06^{c}	6.28±0.23^{c}	6.08±0.25^{c}	5.68±0.15^{b}	5.18±0.06^{a}	5.11±0.20^{a}
Starch	75.72±0.77^{b}	73.34±0.49^{ab}	73.97±0.22^{ab}	72.94±1.04^{ab}	72.39±2.19^{ab}	72.05±1.99^{a}

Data shown as mean±standard error

Means in rows followed by different letter superscripts are significantly different ($p<0.05$).

significant ($p<0.05$) increase after a germinating time of 36 h. Similar results have been reported for germinated paddy rice (Evelyn and Juliano, 1972; Ayernor and Ocloo, 2007) and germinated brown rice (Choi *et al*., 2006; Watchraparpaiboon *et al*., 2007). During germination, the metabolic rate in the grains increased rapidly, due to the action of enzymes leading to the utilization of protein fat and carbohydrate for energy and growth (Evelyn and Juliano, 1972). Ayernor and Ocloo (2007) reported that the decrease in the starch content of the grains during germination was also due to the action of hydrolytic enzymes, such as a and b-amylases, which hydrolyze starch into low molecular weight carbohydrates, such as maltose, glucose and dextrins.

Total phenolics content and antioxidant capacity

The total phenolics content and antioxidant capacity of germinated paddy rice are presented in Table 2. After soaking, the total phenolics content in germinated paddy rice was 0.4785 mg gallic acid/g, which was significantly ($p<0.05$) different at germination time of 24, 36, 48 and 60 h, and was a similar result to that reported by Tian *et al*. (2004). As the germination time increased, the total phenolics content increased as well. The greatest increase in the total phenolics content was 0.6432 mg gallic acid/g at a germination time of 60 h. Cereal grains contain phenolic acids and glycosides, in both soluble and insoluble form, which are bound with polysaccharides at the cell wall. Most of the phenolic compounds are in an insoluble form (Miller *et al*., 2000). However, the soluble phenolics in brown rice contain free phenolic acids and a hydroxycinnamate sucrose ester, consisting of feruloylsucrose and sinapolysucrose. However, insoluble phenolics contain mostly ferulic acid and *ρ*-coumaric acid (Sosulski *et al*., 1982; Adom and Liu, 2002).

During germination, the brown rice levels of feruloylsucrose and the sinapolysucrose were about 70% and they decreased, whereas the level of sinapinic acid increased nearly 10 times and insoluble phenolics (ferulic acid and *ρ*-coumaric acid) increased about 1-2 times. Tian *et al*. (2004) explained that the increase in the amount of free form phenolics in germinated brown rice is due to the decomposition of the cell wall during germination. The increase in the insoluble form might facilitate an increase in the hydrolyzation of insoluble phenolics during germination (Tian *et al*., 2004; Adom and Liu, 2002).

Antioxidant capacity can be measured by DPPH radical scavenging activity and ferric reducing ability power (FRAP), based on the reaction of the reagent with antioxidant compounds that are electron-donating or produce

Table 2 Total phenolics content and antioxidant capacity of germinated brown rice during germination.

Germination time (h)	Total phenolics (mg gallic acid/g dry matter)	Antioxidant capacity	
		DPPH (mg Trolox/g dry matter)	FRAP (mg Trolox/g dry matter)
0	0.4785 ±0.03^{a}	0.2712 ±0.01^{a}	0.6888 ±0.00^{a}
12	0.4848 ±0.01^{ab}	0.2714 ±0.00^{a}	0.6949 ±0.01^{a}
24	0.5572 ±0.01^{c}	0.2868 ±0.01^{ab}	0.7461 ±0.00^{c}
36	0.6245 ±0.02^{d}	0.2977 ±0.01^{b}	0.7592 ±0.00^{d}
48	0.5307 ±0.00^{bc}	0.2823 ±0.0^{ab}	0.7234 ±0.00^{b}
60	0.6432 ±0.03^{d}	0.3035 ±0.01^{b}	0.8570 ±0.00^{e}

Data shown as mean±standard error

Means in a column followed by different letter superscripts are significantly different ($p<0.05$).

hydrogen radicals. The germination time modified the antioxidant capacity as shown in Table 2. Both methods showed a trend change during germination, with the antioxidant capacity increasing continuously with germination time. The antioxidant capacity of germinated paddy rice was directly related to the total phenolics content. This result was similar to Velioglu *et al.* (1998). The capacity of DPPH radical scavenging in germinated paddy rice ranged from 0.2712 to 0.3035 mg Trolox/g. The ferric reducing ability power or FRAP of the germinated paddy rice ranged from 0.6888 to 0.8570 mg Trolox/g. With an increase in the germination time, the free radical scavenging activity of DPPH increased slightly, while FRAP tended to increase significantly ($p<0.05$) during germination from 24 to 60 h. However, the antioxidant capacity depended on the chemical structure of the substrates that reacted with the reagent. Adom and Liu (2002) reported that insoluble phenolics were the major contributors to the antioxidant capacity, which was about 71% in rice.

Rice bran is a rich source of vitamin E, with the major components being α-tocopherol, γ-tocopherol, α-tocotrienol, γ-tocotrienol and γ-oryzanol. All these components exhibited significant antioxidant capacity in the inhibition of cholesterol oxidation (Xu *et al.*, 2001). Kayahara and Tsukahara (2000) reported that during the germination of brown rice, vitamin E (tocopherol, and tocotrienol) increased nearly four times. Moreover, Butsat and Siriamornpun (2010) reported that the rice husk showed greater phenolics concentration than rice bran, brown rice and milled rice. The phenolics might diffuse into the rice grain during soaking, which could, explain the increased antioxidant capacity of germinated paddy rice.

CONCLUSIONS

The germination process of Thai waxy paddy rice significantly ($p<0.05$) affected the GABA content, chemical composition, total phenolics content and antioxidant capacity. The GABA content, dietary fiber, total phenolics content and the antioxidant capacity increased dramatically during germination, while the fat, protein, amylose and starch content decreased slightly. Germinated paddy rice could be considered as a source of GABA and dietary fiber with a high antioxidant capacity. Germinated paddy rice could be utilized in the food chain in several ways, including in germinated rice, snack foods, infant foods and alcoholic beverages.

ACKNOWLEDGEMENTS

The authors are grateful to the Phranakorn Rajabhat University for financial support and special thanks are recorded to the Ubon Ratchathani Rice Research Center for providing the paddy rice.

LITERATURE CITED

AACC. 1990. **Approved Methods of the American Association of Cereal Chemists.** 9th ed. American Association of Cereal Chemists, In., St. Paul, Minnesota.

Abhay, Kr.T. and A.K. Gupta. 2006. Water absorption characteristics of paddy, brown rice and husk during soaking. **J. Food Eng.** 75: 252-257.

Adom., K.K. and R.H. Liu. 2002. Antioxidant activity of grains. **J. Agric. Food Chem.** 50: 6182-6187.

AOAC. 1990. **Official Analytical Methods of Analysis.** 15th ed. The Association of Official Analytical Chemists. Arlington, Virginia. 1298 pp.

AOAC. 2000. **Official Analytical Methods of Analysis**.18th ed. The Association of Official Analytical Chemists. Arlington, Virginia. 2200 pp.

Aurisano, N., A. Bertani and R, Reggiani. 1995. Anaerobic accumulation of 4-aminobutyrate in rice seedling; causes and significance. **Phytochem.** 38(5): 1147-1150.

Ayernor, G.S. and F.C.K. Ocloo. 2007. Physicochemical changes and diastatic activity associated with germinating paddy rice (PSB.Re 34). **Afr. J. Food Sci.** 1: 37-41.

Bamforth, C.W. and A.H.P Barclay. 1993. Malting technology and the uses of malt, pp. 297-354. *In* A.W. MacGregor and R.S. Bhatty (eds). **Barley Chemistry and Technology**. American Association of Cereal Chemistry, St. Paul, MN.

Bello, M., P.T., Marcela and S. Constantino. 2004. Factors affecting water uptake of rice grain during soaking. **Lebensm. Wiss.u Technol.** 37: 811-816.

Bown, A.W. and B.J. Shelp. 1997. The metabolism and functions of g-aminobutyric acid. **Plant Physiol.** 115: 1-5.

Butsat, S. and S. Siriamornpun. 2010. Antioxidant capacities and phenolic compounds of the husk, bran and endosperm of Thai rice. **Food Chem.** 119: 606-613.

Champagne, E.T., D.F. Wood., B.O. Juliano and D.B. Bechtel. 2004. The rice grain and its gross composition, pp.77-107. *In* E.T. Champagene (ed.). **Rice.** American Association of Cereal Chemistry, St.Paul,MN.

Choi, I., D. Kim., J. Son., C.Yang., J. Chun and K. Kim. 2006. Physical-chemical properties of giant embroyo brown rice (Keunnunbyeo). **Agric. Chem. Biotechnol.** 49 (3): 95-100.

Dewar, J., J.R.N., Taylor and P. Berjak. 1997. Determination of improved steeping conditions for sorghum malting. **J. Cereal Sci.** 26: 129-136.

Evelyn, P.P. and B.O. Juliano. 1972. Biochemical changes in the grain during germination. **Plant Physiol.** 49: 751-756.

Hayakawa, K., M. Kimura and K. Kamata. 2002 Mechanism underlying gamma- aminobutyric acid induced antihypertensive effect in spontaneously hypertensive rats. **Eur. J. Pharm.** 438: 107-1 13.

Howell, K.A., R. Narsai, A. Carrol, A. Ivanova, M. Lohse and B. Usadel. 2009. Mapping metabolic and transcript temporal switches during germination in rice highlights specific transcription factors and the role of RNA instability in the germination process. **Plant Physiol.** 149: 961-980.

Hwang, S.S., Y.C. Cheng, C. Chang, H.S. Lur and T.T. Lin. 2009. Magnetic resonance imaging and analyses of tempering processes in rice kernels. **J. Cereal Sci.** 50: 36-42.

Ito, Y., A. Mizukuchi, M. Kise, H. Aoto, S.Yamamoto, R.Yoshihara and J. Yokoyama. 2005. Postprandial blood glucose and insulin responses to pre-germinated brown rice in healthy subjects. **J. Med. Invest.** 52: 159-164.

Jakobs, C., J. Jaeken and K.M. Gibson. 1993. Inherited disorders of GABA metabolism. **J. Inherit. Metab. Dis** 16: 704-715.

Kayahara, H. and K. Tsukahara. 2000. Flavor, health and nutritional quality of pre-germinated brown rice. Presented at **2000 International Chemical Congress of Pacific Basin Societies in Hawaii**, December 2000.

Komatsuzaki, N., K. Tsukahara, H. Toyoshima, T. Suzuki, N. Shimizu and T. Kimura. 2007. Effect of soaking and gaseous treatment on GABA content in germinated brown rice. **J. Food. Eng.** 78: 556-560.

Lamkin, W.M., S.W. Nelson, B.S. Miller and Y. Pomeranz. 1983. Glutamic acid decarboxylase activity as a measure of percent germination for barley. **Cereal Chem.** 60(2): 166-171.

Liu, H.J., B.Y. Chang, H.W. Yan, F.H. Yu and X.X. Liu. 1995. Determination of amino acids in food and feed by derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate and reversed-phase liquid chromatographic separation. **J. AOAC International** 78(3): 736-744.

Liu, L.L., H.Q. Zhai and J.M. Wan. 2005. Accumulation of g-Aminobutyric acid in

giant-embryo rice grain in relation to glutamate decarboxylase activity and its gene expression during water soaking. **J. Cereal Chem.** (82)2: 191-196.

Mariotti, M., C. Alamprese, M.A. Pagani and M. Lucisano. 2006. Effect of puffing on ultrastructure and physical characteristics of cereal grains and flours. **J. Cereal Chem.** 43: 47-56.

Miller, H.E., F. Rigelhof, L. Marquart, R.D.A. Prakash and M. Kanter. 2000. Antioxidant content of whole grain breakfast cereals, fruits and vegetable.. **J. Am. College Nutr.** 19: 312s-319s.

Miura, D., Y. Ito, A. Mizukuchi, M. Kise, H. Aoto and K.Yagasaki. 2006. Hypocholesterolemic action of pre-germinated brown rice in hepatoma-bearing rats. **J. Life Sci.** 76: 259-264.

Oh, S.H., J.R. Soh and Y.S. Cha. 2003. Germinated brown rice extract shows a nutracetical effect in the recovery of chronic alchol-related symptoms. **J. Med. Food.** 6: 115-121.

Oh, S.H. 2003. Stimulation of g- amino butyric acid synthesis activity in brown rice by a chitosan/glutamic acid germination solution and calcium/calmodulin. **Biochem. Mol. Boil.** 36(3): 319-325.

Ohtsubo, K., K. Suzuki, Y. Yasui and K. Kasumi. 2005. Bio-functional components in the processed pre-germinated brown rice by a twin-screw extruder. **J. Food Comp. Anal.** 18: 303-316.

Puangwerakul, Y. 2007. Malt Characteristic of 40 Rice Varieties Cultivated in Thailand. **Kasetsart J. (Nat. Sci.)** 41: 15-20**.**

Ragaee, S., E.S.M. Abdel-Aal and M. Noaman. 2006. Antioxidant activity and nutrient composition of selected cereals for food use. **Food Chem.** 98: 32-38.

Reggiani, R., C.A. Cantu. I. Bramibilla and A. Bertani. 1988. Accumulation and interconversion of amino acids in rice roots under anoxia. **Plant Cell Physiol.** 29: 981-987.

Rhodes, D., S. Handa. and R.A. Bressan. 1986. Metabolic changes associated with adaptation of plant cells to water stress. **Plant Physiol.** 82: 890-903.

Saikusa, T., T. Horino and Y. Mori. 1994. Accumulation of g- aminobutyric acid (GABA) in the rice germ during water soaking. **J. Biosci. Biotech. Biochem.** 58 (12): 2291-2292.

Seiki, T., R. Nagase, M. Torimitsu, M. Yanagi. Y. Ito, M. Kise, A. Mizukuchi, N. Fujimura, K. Hayamizu and T. Ariga. 2005. Insoluble fiber is a major constituent responsible for lowering the post-prandial blood glucose concentration in the pre-germinated brown rice. **Biol Pharm Bull.** 28:1539-1541.

Shelp, B.J., A.W. Bown and M.D. McLean. 1999. Metabolism and functions of gamma-aminobutyric acid. **Trends Plant Sci.** 4: 446-452.

Shoichi, I. and Y. Ishikawa. 2004. Marketing of value-added rice products in Japan: germinated brown rice and rice bread, pp 1-10. *In* **FAO International Rice Year, 2004 Symposium**. Food and Agriculture Organization of the United Nations, Italy.

Singleton, V.L., R. R. Orthofer and R.M. Lamuela-Raventors. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by mean of Folin-Ciocalteu reagent. **Methods Enz.** 299:152-178.

Sosulski, F., K. Krygier and L. Hogge. 1982. Free, esterified and insoluble-bound phenolic acids. 3. Composition of phenolic acids in cereal and potato flours. **J. Agric. Food Chem.** 30: 337-340.

Sunte, J., V. Srijesdaruk and R. Tangwongchai. 2007. Effects of soaking and germinating process on gamma-aminobutyric acid (GABA) content in germinated brown rice (Hom mali 105). **Agric. Sci. J.** 38(6) (Suppl): 103-106.

Tian., S., K. Nakamura and H. Kayahara. 2004.

Analysis of phenolic compounds in white rice, browne rice and germinated brown rice. **J. Agric. Food Chem.** 52: 4808-4813.

Varanyanond, W., P. Tungtrakul, V. Surojanametakul, L. Wattanasiritham and W. Luxiang. 2005. Effects of water soaking on gamma-aminobutyric acid (GABA) in germ if different Thai rice varieties. **Kasetsart J. (Nat. Sci.)** 39: 411-415.

Velioglu, Y. S., G. Mazza, L. Gao and B.O. Oomah. 1998. Antioxidant activity and total phenolics in selected fruits vegetables and grain products. **J. Agric. Food Chem.** 46: 4113-4117.

Wallace, W., J. Secor and L. Schrader. 1984. Rapid accumulation of –aminobutyric acid and alanine in soybean leaves in response to an abrupt transfer to lower temperature, darkness, or mechanical stress. **Plant Physiol.** 175: 170-175.

Watchraparpaiboon, W., P. Laohakunjit, O. Kerdchoechuen and S. Photchanachai. 2007. Effects of pH, temperature and soaking time on qualities of germinated brown rice **J. Agric. Sci (Suppl.)** 39(6): 169-172.

Wong, S.P., L.P. Leong and J.H. William Koh. 2006. Antioxidant activities of aqueous extracts of selected plants. **Food Chem.** 99: 775-783.

Xu, Z., N. Hua and J.S. Godber. 2001. Antioxidant activity of tocopherols, tocotrienols, and γ-oryzanol components from rice bran against cholesterol oxidation accelerated by 2,2-azobis (2-methylpropionamidine) dihydrochloride. **J. Agric. Food Chem.** 49: 2077-2081.