SNRU Journal of Science and Technology

Journal home page: snrujst.snru.ac.th

Determination of biochemical compositions and antioxidant activities of Hom Mali 105 rice

Jinda Jandaruang[1,*], Sutthidech Preecharram[2]

[1]Chemistry program, Faculty of Science and Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon, 47000 Thailand

[2]Faculty of Science and Engineering, Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon, 47000 Thailand

***Corresponding Author**: jinda.j@snru.ac.th

Received: 30 June 2019; **Revised:** 7 October 2019; **Accepted:** 9 October 2019; **Available online:** 1 January 2020

Abstract

This study was determination of protein, γ-aminobutyric acid (GABA), total phenolic compounds and antioxidant activity in germinated brown rice and germinated Hang rice (Hom Mali 105). The rice grain was divided to two groups; pre-harvest (100 days) and harvest stage (120 days). The results showed that uncooked of harvest stage germinated brown rice (UHBR) extract showed the highest protein content which was 389.00 mg/100 g dry weight. The molecular weight of all protein samples appeared on the range of 14.40 to greater than 97.00 kDa. The comparison between germinated brown rice and germinated Hang rice indicated that either pre-harvest or harvest stage germinated brown rice had GABA, total phenolic contents and antioxidant activities more than germinated Hang rice. Uncooked of pre-harvest stage germinated brown rice (UPBR) exhibited the highest of GABA, total phenolic contents and antioxidant activities which was 311.30, 131.10 mg/100 g dry weight and 82.10%, respectively. Therefore, based on the data, the pre-harvest rice has the highest GABA, total phenolic content and antioxidant activity which are suitable for the production of germinated brown rice and germinated Hang rice.

Keywords: pre-harvest rice; germinated brown rice; germinated Hang rice; protein; GABA; antioxidant

1. Introduction

Rice is the main food that Southeast Asian people prefer to consume. In which the grain contains many useful nutrients, primary substances such as carbohydrates, fats, proteins, vitamins and secondary substances such as phenolic compounds. In addition, the biochemical process happens during germination. The carbohydrates are transformed into oligosaccharides, monosaccharides and reducing sugars. The proteins are digested to peptides, amino acids and many nutrients such as γ-oryzanol, tocopherol and GABA. These above substances have antioxidant properties. Protein is a component of milled rice approximately 6 – 7% [1]. The rice protein is generally regarded as hypoallergenic [2] and its nutritional quality is estimated to be equivalent or higher than that of other cereals but lower than proteins derived from animal sources, legumes and oilseed crops [3]. Rice protein has been limited to attend, due to the relatively low protein content of rice and the low solubility of rice proteins in water. However, in the past year, as per the perceived nutritional and health properties of plant protein has increased. Method for extraction of proteins from rice bran and rice flour were studied and applied industrially [4]. Rice protein is used as an ingredient that adds nutritional value to nutritional products, including sports nutrition supplements, as an alternative

protein [5]. GABA is a non-protein amino acid which was produced during the germination of rice by mean of decarboxylation of L-glutamic acid with Glutamate decarboxylase enzyme. GABA is a neurotransmitter in the brain of mammals and tranquillizing effects. Furthermore, GABA are also used as to inhibit cancer cell proliferation [6], and other beneficial health effects. Phenolics are the main compounds in rice which act as antioxidants by hydroxyl groups. The hydroxyl groups are donating hydrogen to reactive oxygen and reactive nitrogen species which are breaks the cycle of new radicals. Phenolic compounds show the antioxidant activities dependent on the amount of substances.

There are found that unpolished rice has these substances higher than polished rice. Brown rice is a kind of unpolished rice which is a highly nutritious grain. The brown rice has GABA content higher than white rice [7]. Brown rice nutrition is very impressive and offers many health benefits, including reducing the risk of heart disease. Moreover, the brown rice extracted was high antioxidant activity, prevention of cancer [8], control of blood lipids and related diseases [9] and the prevention of diabetic complications [10]. Hang rice is a kind of unpolished rice which is the traditional product of Sakon Nakhon province, Thailand. The Hang rice process consists of soaking, steaming, drying and dehusking without polishing [11]. The germination is included in Hang rice process. The germination of rice increases the content of GABA and antioxidants substances [12]. Germinated rice has the GABA 10 folds more than white rice [13]. Moreover, the time for harvesting grain may also affect the amount of substances in the grain as well. However, there is still little research to study the effect of rice harvesting time on the amount of various substances in the grain. In this experiment, rice grain samples were harvested at two stages: the pre-harvest stage is harvested after flowering for 28 – 30 days. The rice grains are yellow color about 85% of all grain. The harvest stage, rice grain is harvested in the form of paddy rice. These rice grains are full grain which is yellow grain more than 85% of all grain.

Therefore, in this research, we studied the content of protein, GABA, total phenolic compounds and antioxidant activity in various Hom Mali 105 rice samples which is a popular rice for Thai consumers because it is fragrant, soft and tasty when cooked. The grain of germinated brown rice and germinated Hang rice are divided to 2 major groups, pre-harvest and harvest stages and both stages were divided as uncooked and cooked rice.

2. Materials and methods

Reagents and standards

Low molecular weight calibration kit purchased from GE Healthcare Life Sciences, Sweden. Standard γ-aminobutyric acid (GABA), Folin-Ciocalteu phenol reagent, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and gallic acid were purchased from Sigma-Aldrich Chemical Co. USA. All the other chemicals and solvents were analytical grade.

Rice samples

Rough rice of *Oryza sativa* L., cultivar Hom Mali 105 was selected for the study and it was harvested from Phanna Nikhom District in Sakon Nakhon province, Thailand. The rice grain was harvested in 2 period which were the pre-harvest (100 days) and harvest stage (120 days). The germinated Hang rice was prepared by soaked with water (1:1.50w w^{-1}) at room temperature (28 ± 5 °C) for 12 hrs and then drained. After that they were incubated for 18 hrs, stream at boiling water for 30 min and then sun drying. The dry grains were dehulled and packaged in plastic box, labeled and stored in a cold chamber. Germinated brown rice was prepared like germinated Hang rice, except no strep of streaming.

Cooking conditions

One hundred grams of each rice samples (pre-harvest germinated brown rice, harvest germinated brown rice, pre-harvest germinated Hang rice and harvest germinated Hang rice) was cooked for 30 min in rice cooker (Model A102T, 500 W, Extra electronic Co, Ltd.)

Rice extraction

One hundred grams of eight types on rice samples (uncooked pre-harvest germinated brown rice (UPBR), uncooked harvest germinated brown rice (UHBR), cooked pre-harvest germinated brown rice (CPBR), cooked harvest germinated brown rice (CHBR), uncooked pre-harvest germinated Hang rice (UPHR), uncooked harvest germinated Hang rice (UHHR), cooked pre-harvest germinated Hang rice (CPHR) and cooked harvest germinated Hang rice (CHHR)) were mashed and dissolved with 80% ethanol in Erlenmeyer flask. These samples were shaken at 150 rpm for 24 hrs. The supernatant was separated by centrifugation at 3,500 rpm for 10 min. The extracts were evaporated under vacuum by a rotary evaporator. The crude extracted was kept at – 40 °C. These crudes extract were used for GABA, total phenolic compounds and antioxidant activity analysis.

Determination of protein by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

The extraction of rice protein fractions were performed according to the method of Conor *et al.* [14]. The rice samples were mashed, and then dissolved with 0.45% NaOH by ratio of 1:30%w v^{-1}. These suspensions were mixed and shaken at 150 rpm for 1 hr and then centrifuged at 3,500 rpm for 10 min. The rice proteins were precipitated from the supernatants by adjusting the pH to 4.50 by 1 M HCl. The precipitated proteins were washed three times with deionized water, adjusted to pH 7.00, and then stored at 4 °C for subsequent analysis. The protein concentration was determined by the Bradford method [15]. The range of 5 – 100 µg Bovine serum albumin were as standard protein concentration. Twenty microliters of standard protein solution or protein samples solution to each tube and mix well with 1 ml of Bradford solution. Incubate at room temperature for 10 min and measure absorbance at 595 nm. Proteins concentration were calculated by the absorbance of protein sample comparing with standard curve of Bovine serum albumin ($y = 0.1571x + 0.022$, $R^2 = 0.9989$).

The molecular weight of rice proteins was determined by SDS-PAGE which was conducted according to the method of Dimino [16]. Briefly, 15% separating gel and 4% stacking gel were prepared. Each rice extracted were prepared by mixing with 2X solubilizing buffer (0.5 M Tris–HCl buffer, pH 6.80, 0.50% (w v^{-1}) bromophenol blue, 10% (v v^{-1}) glycerol, 2% (w v^{-1}) SDS, and 10% (v v^{-1}) β-mercaptoethanol) ratio of 1:1 (v v^{-1}) and then boiled for 5 min. The protein bands were stained with Coomassie brilliant blue R250 (CBB R-250). Low molecular weight calibration kit (GE Healthcare Life Sciences, Sweden) was used as the standard protein marker which 14.40, 20, 30, 45, 66 and 97 kDa.

Determination of GABA contents

GABA contents were prepared according to the method of Karladee *et al*. [17], with some modifications. Each rice extracted were added with 20 µl of 0.20 M borate buffer and 100 µl of 6% phenol. The solutions were mixed thoroughly and cooled in a cooling bath for 5 min. Next, 30 µl of 15% CaOCl was added, and the solution was shaken vigorously for 1 min, and again cooled in a cooling bath for 5 min. Finally, the solution was boiled at 90 °C for 1 min and allowed to cool. The solution was measured at a wavelength of 630 nm, with distilled water as a blank. GABA (0.50 – 4.00 mg ml^{-1}) was used as a standard compound. GABA content was quantified by comparing the optical density reading with the standard GABA content curve ($y = 0.1468x + 0.0213$, $R^2 = 0.9992$) as milligrams of GABA equivalents per 100 grams of samples.

Determination of total phenolics compounds

The determination of total phenolic contents was determined by using the Folin–Ciocalteau phenol reagent [12] and reported as gallic acid equivalents (mg/100 g dry weight), A 50 µl of each rice extracted were added with 80 µl of 10% Folin–Ciocalteau phenol reagent and 150 µl of 7% sodium carbonate. The reaction was settled for 2 hrs in the dark at room temperature. Gallic acid solution (0.10 – 0.90 mg ml^{-1}) was used to produce standard calibration curve. The absorbance of the sample and standards were measured spectrophotometrically at 765 nm. Methanol was applied as a control. Total phenolics compounds were calculated by the absorbance of sample according to standard curve ($y = 0.4927x + 0.0270$, $R^2 = 0.9983$) as milligrams of gallic acid equivalents per 100 grams of samples.

Determination of antioxidant activity by DPPH scavenging assay

The antioxidant activity was assessed in terms of radical scavenging abilities using the stable DPPH radical scavenging assay [18]. Aliquots of rice extract 50 µl were mixed with 0.1 mM DPPH methanolic solution. The resulting solution was then left to stand for 20 min at room temperature in a dark room. Methanol was used as a control. Radical scavenging activity was expressed as the inhibition percentage and was calculated using equation (1);

$$\text{DPPH scavenging effect (\%)} = [\ (A_{control} - A_{sample}) / A_{control}\ ] \times 100 \quad (1)$$

$A_{control}$ is absorbance of the control, A_{sample} is absorbance of the test sample

Statistical analysis

All experiments were performed in triplicates. The results were carried out as mean value ± standard deviation (SD). Statistical analyses were compared with the Duncan Multiple Range Test and $p < 0.05$ was applied.

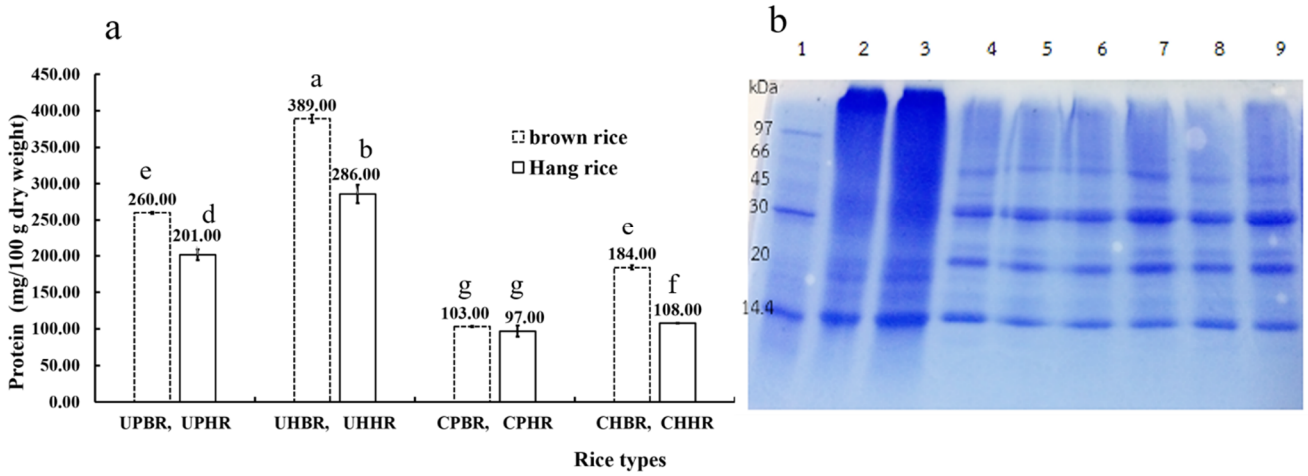

Fig. 1 Protein contents of uncooked and cooked rice extracted in germinated brown rice and germinated Hang rice which period of pre-harvest and harvest stage (a). Data expressed as means ± SD of triplicate determinations. Letter; a-g means of each sample type with different letters are significantly different ($p < 0.05$). 15% SDS-PAGE of uncooked and cooked rice extracted in germinated Hang rice and germinated brown rice which period of pre-harvest and harvest stage (b), lane 1 – 9 are standard protein marker, UPBR, UHBR, CPBR, CHBR, UPHR, UHHR, CPHR and CHHR, respectively.

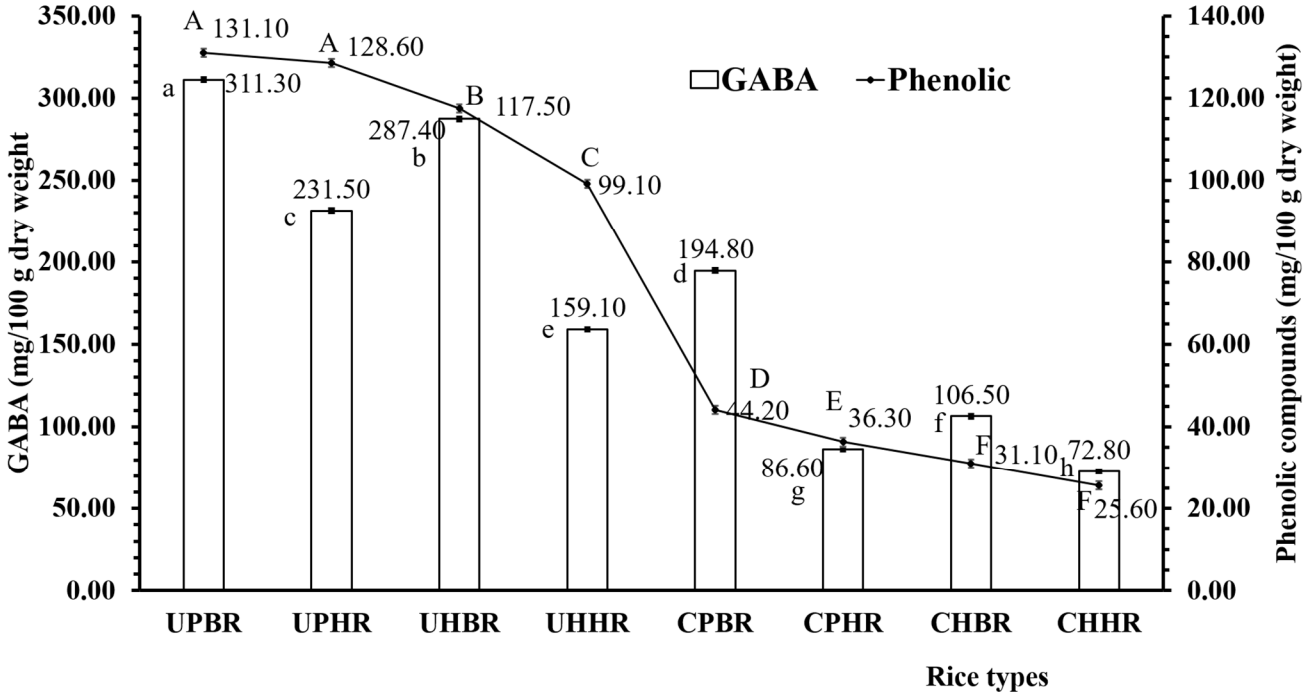

Fig. 2 GABA contents (bar □) and total phenolics (line ——) of uncooked and cooked rice extracted in germinated brown rice and germinated Hang rice which period of pre-harvest and harvest stage. Data reported are the mean ± SD of triplicate determinations. Letter; a – h means of each GABA and A – F means of each phenolics sample type with different letters are significantly different ($p < 0.05$).

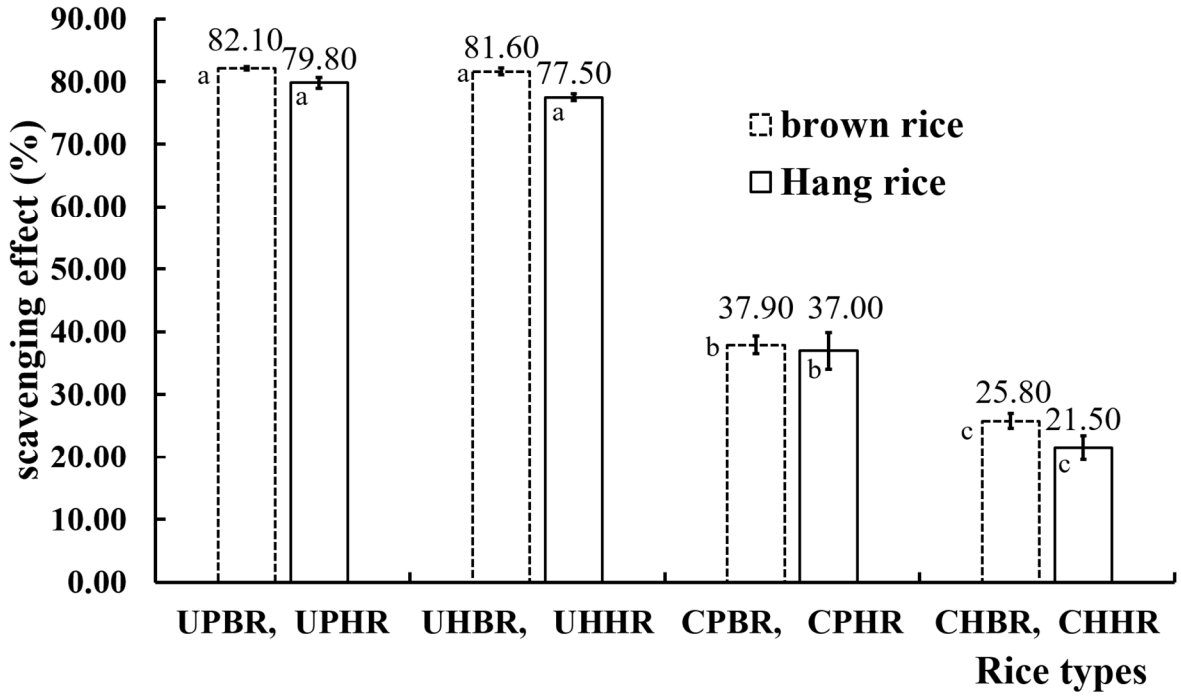

Fig. 3 Antioxidant activities of uncooked and cooked rice extracted in germinated brown rice and germinated Hang rice which period of pre-harvest and harvest stage. Data expressed as means ± SD of triplicate determinations. Letter; a – c means of each sample type with different letters are significantly different ($p < 0.05$).

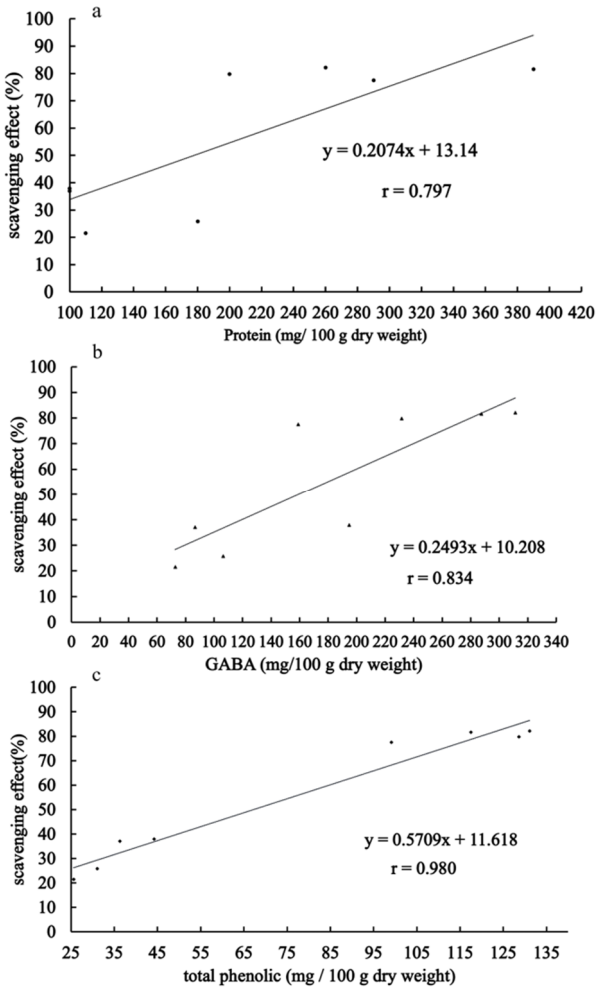

Fig. 4 Correlation plots between DPPH free radical scavenging activity and protein contents (a), GABA contents (b) and total phenolic compounds (c) of germinated brown rice and germinated Hang rice extracts.

3. Results and Discussion

The protein of rice extract

The total protein contents of germinated brown rice and germinated Hang rice were analyzed by Bradford's method. The results (Fig. 1) show that all sample have protein content between 97.00 – 389.00 mg/100 g dry weight. The uncooked rice has total protein more than cooked rice, germinated brown rice also more than germinated Hang rice too. The highest total protein content was found on UHBR with 389.00 mg/100 g dry weight. This result may be due to the heat by cooking process that cause the damage of protein [19]. The total protein content from all sample except CPBR and CPHR showed a significant difference at the 95% statistically significant confidence level.

The protein extracted were determine molecular weight by SDS-PAGE. The results indicated that all 8 types of rice samples had proteins with a molecular weight between 14.4 to greater than 97 kDa. The main protein patterns were found around at 14.40, 22.00, 30.00 and 57.00 kDa regions which shown in Fig 1(b). These protein patterns related with the molecular weight of rice bran protein. Band of protein in SDS-PAGE with 14.00, 22.00, 30.00 and 57.00 kDa may be prolamin [1], globulin, albumin and glutelin [20], respectively. These divergent may be due to the heterogeneous nature of polypeptide in rice bran and the different varieties of rice.

Quantification of GABA content on rice extract

The GABA content of germinated brown rice and germinated Hang rice were determined. All samples show GABA content in the range of 72.80 – 311.30 mg/100g dry weight (Fig. 2). The highest of GABA content was exhibited on UPBR with 311.30 mg/100g dry weight. When compare GABA content between germinated brown rice and germinated Hang rice, indicated that germinated brown rice shows higher GABA content. Likewise, uncooked rice has GABA content more than cooked rice and the grain of pre-harvest stage have GABA content more than grain of harvest stage. From this research, the highest GABA content is 311.30 mg/100g dry weight which is different from a study with Hom Mali 105 rice which has the highest GABA content of 23.48 mg/100g dry matter [17].

Germinated brown rice extract had a significantly greater of GABA than germinated Hang rice. This result may be due to the production of germinated Hang rice. The production of germinated Hang rice as same as germinated brown rice production except addition hot streamed for cook rice. This process may be the loss of GABA. Since GABA is a substance easily soluble in water, the longtime of streaming may result in dissolving with water during streaming. Prior study in Mung bean, found that microwave cooking process can reduce losing GABA [21]. Therefore, it may be possible the use microwave cooker instead of cooking for prevention of losing GABA [21].

Quantification of total phenolic content on rice extract

The total phenolic contents were determined, by following a modified Folin-Ciocalteu reagent method and the results were expressed as gallic acid equivalents (Fig. 2). Germinated brown rice and germinated Hang rice showed total phenolic contents in the range of 25.60 – 131.10 mg/100g dry weight. The UPBR contained the highest amount of total phenolic followed by UPHR and UHBR with 131.10, 128.60 and 117.50 mg/100g dry weight, respectively. When compared total phenolic content between germinated brown rice and germinated Hang rice, the phenolic content of germinated brown rice was higher than germinated Hang rice and the grain rice on pre-harvest stage was more than the grain rice of harvest stage. Total phenolic content of cooked rice was lower than that in uncooked rice. This result related with the study of Pitiwiwattanakul *et al*. [22], that total phenolic of Homdam Sukhothai 2 on heat treatment is reduced for 75 – 82%. Tian *et al*. [23] have been reported that major phenolic compounds in rice grain are mainly in a water-soluble form such as 6'-O-(E)-feruloylsucrose and 6'-O-(E)-sinapoylsucrose. However, many factors have the effect of total phenolic content such as extraction methods, varieties, growing conditions and germination time [24].

Antioxidant activity

In this study antioxidant of germinated brown rice and germinated Hang rice extract were determined by DPPH assay. The result is shown in Fig.3. The scavenging activity of all samples were exhibited the range of 21.50 – 82.10%. The maximum antioxidant was found in UPBR. The uncooked rice showed higher scavenging activity than cooked rice. The reduction in antioxidant activity are related to the decrease content of protein, GABA, total phenolic compounds on cooked rice which due to the loss of part of these compounds in the water and thermal decomposition.

The relationship between protein content, GABA content, total phenolic compounds and antioxidant activity

The correlations between protein content, GABA content, total phenolic compounds and antioxidant activities were computed. The linear correlation coefficients (r) was obtained in Fig. 4. The antioxidant activity of the rice extract was positively and significantly correlated with the concentrations of protein ($r = 0.797$), GABA ($r = 0.834$) and total phenolic compounds ($r = 0.980$) (Fig. 4). The correlation of antioxidant activity and total phenolic compounds was the highest, indicating that these compounds were the main components responsible for the antioxidant activity in the germinated rice extracted. A similar correlation for rice grains was observed by Karladee *et al*.

[17]. A correlation was also observed for other foods, such as wheat and oats [25]. The rice extract was shown antioxidant activity correlating with the reduction of oxidative stress both of in vitro and ex vivo assays, such as aid in the prevention of cancer [8], control of blood lipids and related diseases [9] and the prevention of diabetic complications [11], suggesting that rice grains with higher concentrations of phenolic compounds may have beneficial effects on health.

4. Conclusion

Germinated brown rice extracts contained protein content (389.00 mg/100 g dry weight for UHBR), GABA content (311.30 mg/100 g dry weight for UPBR), total phenolic compounds (131.10 mg/100 g dry weight for UPBR) and antioxidant activity (82.10% weight for UPBR) higher than the germinated Hang rice. Moreover, germinated brown rice on pre-harvest stage showed the highest amount of various substances. Therefore, the rice seeds in the pre-harvest stage are suitable for production as germinated brown rice and germinated Hang rice which will give the highest nutritional value to functional food products.

5. Suggestions

The information about protein content, GABA content, total phenolic compounds and antioxidant activity in germinated brown rice and germinated Hang rice were completely obtained. This information can be useful in germinated brown rice and germinated Hang rice marketing promotion. In the future, the cooking process on germinated rice with high nutritional should be study.

6. Acknowledgement

This investigation was the financial support by the research and development institute Sakon Nakhon Rajabhat University, Thailand.

7. References

[1] L. Amagliani, J. O'Regan, A.L. Kelly, J.A. O'Mahony, Composition and protein profile analysis of rice protein ingredients, J Food Compost Anal. 59 (2017) 18 – 26.
[2] R.M. Helm, A.W. Burks, Hypoallergenicity of rice protein, CEREAL FOODS WORLD. 41 (1996) 839 – 843.
[3] L. Day, Proteins from land plants – potential resources for human nutrition and food security, Trends Food Sci. Technol. 32 (2013) 25 – 42.
[4] C. Fabian, Y. Ju, A review on rice bran protein: its properties and extraction method, Crit Rev Food Sci Nutr. 51(2011) 816 – 827.
[5] M. Reche, C. Pascual, A. Fiandor, I. Polanco, M. Rivero-Urgell, R. Chifre, S. Johnston, M. Martin-Esteban, The effect of a partially hydrolysed formula based on rice protein in the treatment of infants with cow's milk protein allergy, Pediatr. Allergy Immunol. 21 (2010) 577 – 585.
[6] C.H. Oh, S.H. Oh, Effects of germinated brown rice extracts with enhanced levels of GABA on cancer cell proliferation and apoptosis, J Med Food. 7 (2004) 19 – 23.
[7] M. Walter, E. Marchesan, P.F.S. Massoni, L.P.D. Silva, G.M.S. Sartori, R.B. Ferreira, Antioxidant properties of rice grains with light brown, red and black pericarp colors and the effect of processing, Food Res Int. 50 (2013) 698 – 708.
[8] P.N. Chen, W.H. Kuo, C.L. Chiang, H.L. Chiou, Y.S. Hsieh, S.C. Chu, Black rice anthocyanins inhibit cancer cells invasion via repressions of MMPs and u-PA expression, Chem Biol Interact. 163(3) (2006) 218 – 229.
[9] W.H. Ling, Q.X. Cheng, J. Ma, T. Wang, Red and black rice decrease atheroscletoric plaque formation and increase antioxidant status in rabbits, J Nut. 131 (2001) 1421 – 1426.

[10] Y. Morimitsu, K. Kubota, T. Tashiro, E. Hashizume, T. Kamiya, T. Osawa, Inhibitory effect of anthocyanins and colored rice on diabetic cataract formation in the rat lenses, Int Congr Ser. 1245 (2002) 503 – 508.
[11] K. Phattayakorn, P. Pajanyor, S. Wongtecha, A. Prommakool, W. Saveboworn, Effect of germination on total phenolic content and antioxidant properties of 'Hang' rice, INT FOOD RES J. 23 (2016) 406 – 409.
[12] A. Moongngarm, N. Saetung, Comparison of chemical compositions and bioactive compounds of germinated rough rice and brown rice, Food Chem. 122 (2010) 782 – 788.
[13] H. Kayahara, K. Tsukahara, Flavor, health and nutritional quality of pre-germinated brown rice, 10th International Flavor Conference 2000; Paros, Greece. December 2000, 546 – 551.
[14] M.A. Conor, R.M. Saunders, G.O. Kohler, Rice bran protein concentrates obtained by wet alkaline extraction, Cereal Chem. 53 (1976) 488 – 496.
[15] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal Biochem. 72 (1976) 248 – 254.
[16] M.L.D.A. F.Palmer, Purification of bovine hemoglobin via fast performance liquid chromatography, J CHROMATOGR B. 856 (2007) 353-357.
[17] D. Karladee, S. Suriyong, r-Aminobutyric acid (GABA) content in different varieties of brown rice during germination, SCIENCEASIA. 38 (2012) 13 – 17.
[18] J. Jandaruang, J. Siritapetawee, K. Thumanu, C. Songsiriritthigul, C. Krittanai, S. Daduang, A. Dhiravisit, S. Thammasirirak, The effects of temperature and pH on secondary structure and antioxidant activity of *Crocodylus siamensis* hemoglobin, Protein J. 31 (2012) 43 – 50.
[19] E. Tornberg, Effects of heat on meat proteins – Implications on structure and quality of meat products, Meat Sci. 70 (2005) 493 – 508.
[20] A.P. Adebiyi, A.O. Adebiyi, Y. Hasegawa, T. Ogawa, K. Muramoto, Isolation and characterization of protein fractions from deoiled rice bran, Eur Food Res Technol. 228 (2009) 391 – 401.
[21] T. Kasarin, L. Pairoj, V. Warunee, C. Hansawasdi, GABA (γ-aminobutyric acid) production, antioxidant activity in some germinated dietary seeds and the effect of cooking on their GABA content, Food Sci Technol. 36 (2016) 313 – 321.
[22] V. Pitiwiwattanakul, S. Phimphilai, S. Nuglor, C. Chiyasut, Effects of germinating conditions on antioxidant properties, total polyphenol and phytate contents in quick-cooking husked Hom Dam Sukhothai 2 rice, As. J. Food Ag-Ind. 4 (2011) 297 – 305.
[23] S. Tian, K. Nakamura, H. Kayahara, Analysis of phenolic compounds in white rice, brown rice, and germinated brown rice, J Agric Food Chem. 52(2004) 4808 – 4813.
[24] H. Ti, R. Zhang, M. Zhang, Q. Li, Z. Wei, Y. Zhang, X. Tang, Y. Deng, L. Liu, Y. Ma, Dynamic changes in the free and bound phenolic compounds and antioxidant activity of brown rice at diffenrent germination stages, Food Chem. 161 (2014) 337 – 344.
[25] Y.S. Velioglu, G. Mazza, L. Gao, B.D. Oomah, Antioxidant activity and total phenolics in selected fruits, vegetables and grain products, J Agric Food Chem. 46 (1998) 4113 – 4117.