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Study on Total Phenolic Contents and their Antioxidant Activities of Thai White, Red and Black Rice Bran Extracts

N. Muntana and S. Prasong
The Center of Excellence for Innovation in Chemistry, Department of Chemistry,
Faculty of Science, Mahasarakham University, Mahasarakham 44150, Thailand

Abstract: In this study, different brans of Thai rice cultivars which were divided into 3 groups: white color (Hom mali 105, Supan, Saohai, Hom chaiya and Hom jun), red color (3256, GS 18003, 21606, 21699 and 5718) and black color (96041-20, 96051-37, 96004-49, 96023-35 and 96065-42) were investigated their Total Phenolic Content (TPC) and antioxidant activities. The rice brans were firstly extracted using methanol. The extracts were analyzed using Folin-Ciocalteau method for total phenolic content while thiocyanate method and 1, 1-diphenyl-2-picrylhydrasyl (DPPH) free radical-scavenging assay were used for antioxidant activities determination. The results indicated that the total phenolic content of white, red and black rice bran extract were in the range of 0.8931-0.9884, 1.0103-1.0494 and 1.0810-1.2239 mg gallic acid equivalent (GAE mg^{-1}), respectively. With thiocyanate method, percentage inhibition were in the range of 10.15-20.68, 30.64-38.80 and 25.52-26.28 for white, red and black rice bran extract, respectively. With DPPH radical-scavenging assay, methanolic extract of 5718 showed the highest (IC_{50}=0.0057 mg mL^{-1}) while Homchaiya showed the lowest (IC_{50}=0.2582 mg mL^{-1}) activities. All of extracts showed lower activity than BHA (IC_{50}=0.0012 mg mL^{-1}). However, the antioxidant activity of all rice bran extracts indicated high antioxidant efficiency in the following order: red>black>white color rice brans. It is a promising that Thai rice bran are potential antioxidant sources.

Key words: Antioxidant activity, free radical-scavenging, Thai rice cultivars, total phenolic compounds

INTRODUCTION

Rice bran is considered one of natural resources that contain high amount of phytonutrients including phenolic compounds (Chen and Bergman, 2005). It is universally distributed in the plant (Martinez-Valverde *et al*., 2000). The phenolic compounds are natural antioxidants (Zheng and Wang, 2001). Most of them are widely recognized as being capable of maintaining human health that associated heart and cancer (Newmark, 1996; Velioglu *et al*., 1998). In addition, nitrogen compounds, carotenoids as well as ascorbic acids are natural antioxidants which were obtained from plant (Laandrault *et al*., 2001; Iqbal *et al*., 2005).

Generally, all phytochemical compounds usually accumulate in pericarp and testa or bran of the rice kernel. These compounds are pigment-containing related to distinct colors such as red, purple and black. A number of colored rice varieties are wildly grown throughout Asia. Thai glutinous black rice varieties known as black sticky rice or kao niow dahm are commonly available throughout the world rice market. Moreover, non-glutinous Thai black rice varieties, e.g., Khao Hom Nin (KHN), has also been exported and finding higher popularity and demand higher prices in Asian rice market nowadays. Many studies have been reported that black rice contains rich of anthocyanin and other polyphenolic compounds much more abundantly than white rice (Ryu *et al*., 1998; Zhang *et al*., 2006). In addition, a significant positive correlation between in the black rice extract and their antioxidative potency was obtained (Ling *et al*., 2001; Hu *et al*., 2003). Therefore, black rice has attracted increasing study for medicinal merits including the prevention of various diseases associated with oxidative stress.

This research was aimed to investigate the antioxidant activities of some white, red and black rice bran extracts of Thai cultivars. Firstly, Total Phenolic Content (TPC) of rice bran was measured following the Folin-Ciocalteu method using gallic acid as a standard. Total Antioxidant Activity (TAA) was determined using ferric thiocyanate method and DPPH assay.

MATERIALS AND METHODS

This study was done for 12 months from February 1, 2008-March 5, 2009. All of experiment was carried out at

Corresponding Author: Srihanam Prasong, The Center of Excellence for Innovation in Chemistry, Department of Chemistry, Faculty of Science, Mahasarakham University, Mahasarakham 44150, Thailand Tel: +66-43-754246 Fax: +66-43-754246

the Department of Chemistry, Faculty of Science, Mahasarakham University, Thailand.

Materials: Thai rice cultivars used in this study were non-glutinous rice white color (Hom mali 105, Supan, Saohi, Hom chaiya and Hom jun) or red color (3256, GS 18003, 21606, 21699 and 5718) and glutinous of black rice (96041-20, 96051-37, 96004-49, 96023-35 and 96065-42). All of rice was cultured in the field of Mahasarakham University, Thailand. The rice seed were grown and then cultured in the selected area. In the culture time, the rice was supplemented with water and organic fertilizer. The environmental condition was about 30-32°C throughout the cultured periods. The age of the rice used in this study was 4 months.

Reagents and chemicals: Gallic acid, Folin-Ciocalteau reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), linoleic acid, NaOH-phosphate buffer (pH 7), distilled water, ammonium thiocyanate, butylated hydroxyaniline, sodium carbonate and ferric chloride were purchased from Fluka (Switzeland). All other reagents and solvents used were of analytical and HPLC grade.

Preparation of crude extraction: Brans of rice samples (25 g) were extracted with methanol 3 times and each for 30 min at room temperature. The extracts were pooled and filtered through a 0.45 μm of Nylon membrane filter. The extracts were then concentrated which gradually reduced pressure on a rotary evaporator. The crude extracts were used for the determination of antioxidant activity (Osawa and Namiki, 1981).

Total phenolic content: The total phenolic contents of crude rice bran extracts were determined by spectrophotometric method using Folin-Ciocalteu's phenol reagent (Osawa and Namiki, 1981). The crude extract (0.5 mL) was diluted to 5.0 mL with distilled water. Folin-Ciocalteu reagent (5.0 mL) was added and mixed thoroughly. After 3.0 min, 5 mL of 10% sodium carbonate solution was added and the mixture was allowed to stand for 1 h with intermittent shaking. The mixture solution was measured at 750 nm using a Thermo Spectronic 4001/4 (USA). The total phenolic content was analyzed against gallic acid calibration curve standard. This experiment was carried out in triplicate and averaged of values contents.

Thiocyanate assay: Erlenmeyer flask containing linoleic acid (0.13 mL) in 0.2 M NaOH-phosphate buffer (10 mL, pH 7) was firstly prepared then crude extract (1 mg) of different rice brans was added and adjusted volume to 25 mL with distilled water (Osawa and Namiki, 1981). The flasks were incubated at 40°C for a two weeks and the degree of oxidation was measured by thiocyanate method. Briefly, the incubation mixture (0.2 mL) was reacted with NH_4SCN (30%, 0.2 mL), 9.4 mL of 75% EtOH and 0.2 mL of $FeCl_2$ (2.53×10^{-2} g/10 mL of 3.5 % HCl) solution. The sample was measured using spectrophotometer at 500 nm. The control solution was prepared in a similar method without some extracts, while α-tocopherol was used as standard. The experiment was carried out in triplicate and averaged of value activities.

DPPH free radical-scavenging assay: The crude extracts and BHA (5-40 mg mL^{-1}) were added to 1.5 mL of 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) in ethanol. The mixture was shaken vigorously and left to stand for 20 min at room temperature in the dark. The absorbance was measured using spectrometer at 517 nm. The radical scavenging activity was calculated by the following equation:

$$\text{Scavenging activity (\%)} = ((A_c-A_s)/A_c)\times100$$

where, A_c and A_s are the absorbance at 517 nm of the control and extract or standard, respectively. The experiment was carried out in triplicate and averaged for their scavenging activity.

Statistical analysis: All of data were expressed as the Mean±SD and percentage of each value.

RESULTS

Total phenolic content: With Folin-Ciocalteu reagent method using gallic acid as the standard, the average quantity of total phenolic compounds found in white, red and black rice bran extracts were in the ranged from 0.8931-0.9884, 1.0103-1.0494 and 1.0810-1.2239 mg GAE mg^{-1} of extract, respectively as shown in Fig. 1. The red rice bran was remarkably the highest of total phenolic content compared to the black and white rice bran extracts. Black and white rice bran extracts showed very similar of total phenolic content and slightly lower than that of red rice. The highest value was 5718 and the next is 21606 strains for red rice. The total phenolic compounds were very similar for white rice, except Homchaiya was the lowest. For black rice, all of rice strains were similar content of total phenolic compounds. From the results, the different values of total phenolic compounds in all of rice cultivars did not dramatically different.

Fig. 1: Total phenolic content of Thai rice bran extracts measured by the Folin-Ciocalteu method expressed as mg GAE/g: white rice; 1 (Mali105), 2 (Supun), 3 (Saohai), 4 (Homjun), 5 (Homchaiya), red rice; 6 (3256), 7 (GS18003), 8 (21606), 9 (21699), 10 (5718), black rice; 11 (96041-20), 12 (96051-37), 13 (96004-49), 14 (96023-35) and 15 (96065-42)

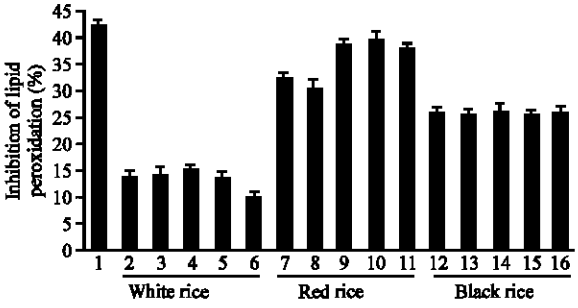

Fig. 2: Antioxidative activity of crude extracts of Thai rice bran using thiocyanate method expressed as % inhibition of lipid peroxidation: white rice; 2 (Mali105), 3 (Supun), 4 (Saohai), 5 (Homjun), 6 (Homchaiya), red rice; 7 (3256), 8 (GS18003), 9 (21606), 10 (21699), 11 (5718), black rice; 12 (96041-20), 13 (96051-37), 14 (96004-49), 15 (96023-35) and 16 (96065-42) which compared with BHA (No. 1)

Anti-oxidative assay using thiocyanate method: With thiocyanate method, the red rice samples showed higher antioxidant activities than those of black and white rice. However, all of samples were lower antioxidant activity than BHA (Fig. 2). The bran extract of Homchaiya, however, exhibited the lowest inhibition of peroxidation of 10.15%, whereas 5718 showed the highest inhibition of peroxidation of 41.80%. The result showed that white rice was significantly lower of antioxidant activity compared to red and black rice. It is slightly surprised that black rice was similar of antioxidant activity in all of strains. Most of white strains are also composed of antioxidant activity in similar percentages, except Homchaiya. Moreover, 21606, 21699 and 5718 of red rice composed very high antioxidant activity. This result was positively correlated to the total phenolic content of the rice cultivars.

Table 1: Radical-scavenging activity of Thai rice bran extracts and BHA expressed by EC_{50} (mg mL^{-1})

Color	Cultivars	EC_{50} (mg mL^{-1})
White	Mali105	0.2516
	Supun	0.1576
	Saohai	0.1644
	Homjun	0.2336
	Homchaiya	0.2582
Red	3256	0.0155
	GS18003	0.0140
	21606	0.0169
	21699	0.0084
	5718	0.0057
Black	96041-20	0.0267
	96051-37	0.0243
	96004-49	0.0359
	96023-35	0.0218
	96065-42	0.0256
Standard	BHA	0.0021

DPPH-free radical scavenging activity: The EC_{50} of the rice bran extracts divided into 3 groups which were the lowest (red rice), middle (black rice) and highest (white rice) values (Table 1). In the highest group, 5718 showed the best free-radical scavenging activity (0.0057 mg mL^{-1}) and the next is 21699. Similar activity but lower was found in other cultivars. For middle group, 96023-35 showed the best free-radical scavenging activity (0.0218 mg mL^{-1}) while 96004-49 (0.0359 mg mL^{-1}) is the worst activity. Similar activity was also found in other cultivars. In the lowest group, Supan (0.1576 mg mL^{-1}) and Saohai (0.1644 mg mL^{-1}) appeared the highest free-radical scavenging activity whereas Mali 105 (0.2516 mg mL^{-1}) and Homchaiya (0.2582 mg mL^{-1}) gave the lowest activity in this group. The free-radical scavenging activity of all rice bran extracts were higher EC_{50} values than BHA (0.0021 mg mL^{-1}).

DISCUSSION

Black and red rice are contained color pigments. These cultivars of rice have long history for people consumption, especially in Southeastern Asia (Hu *et al*., 2003). Rice bran extracts have been reported on a hypocholesterolemic effect as well as antioxidant activity (Sugano and Tsuji, 1997). In addition, consumption of black rice has resulted to artherosclerotic lesions (Ling *et al*., 2001), reduce oxidative stress and inflammatory (Xia *et al*., 2003) or cardiovascular protection (Xu *et al*., 2001). Antioxidant activity in white rice hull has been reported (Lee *et al*., 2003). However, pigmented rice, such as red and black rice, composed of high content of phenolic compounds (Oki *et al*., 2002; Clifford, 2000). They are distributed in the plant as

secondary structure metabolite (Matinez-Valverde *et al*., 2000). Various benefits of the phenolic compounds are known to use many effects in human including oxidative damage of lipid and low density lipoproteins inhibiting platelet aggregation (Daniel *et al*., 1999) and reducing coronary heart disease and cancer risk (Matinez-Valverde *et al*., 2000; Newmark, 1996). Fruits and vegetables are major dietary sources of phenolic compounds, however, pigmented rice has also been found as an excellent source of the phenolic compounds (Tian *et al*., 2004).

The results from this study indicated the contents both total phenolic content and antioxidant activity were similar with many previously reported. Moreover, significantly different either total phenolic content or antioxidant activity compounds of black and red rice were higher than that of white rice. It might be suggested that both red and black rice composed of higher color pigment which were the main compounds for antioxidant activity. On the other hand, environment conditions such as temperature, light, water or soil may concern on composition of the compounds. It can not compare or imply that some substances in the rice cultured from different places. However, it is a promising that the color rice tendency compose many benefits compounds in higher than that of de-color rice. Moreover, the component of phenolic compounds in each section of the rice seed is also interesting to study. Furthermore, comparison between those of factors for rice culture should be explored.

CONCLUSION

The results showed that Thai red rice cultivars possessed relatively strong antioxidant activity. There was also a correlation between the specific antioxidation capacities and total phenolic contents of the red rice bran samples studies. The results indicated that antioxidant activities in the red rice bran extracts were largely owing to the phenolic compounds. Black rice bran extracts composed of high total phenolic content and free-radical scavenging activity as like as red rice, but in lower. On the other hand, white rice showed the lowest both total phenolic content and antioxidant activity. However, the results still appeared in high content compared to other plants. Therefore, Thai rice bran extracts should be acted as a potential source of antioxidative phytochemicals and useful ingredient for nutraceutical or functional food products.

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REFERENCES

Chen, M.H. and C.J. Bergman, 2005. A rapid procedure for analysing rice bran tocopherol, tocotrienol and β-oryzanol contents. J. Food Comp. Anal., 18: 319-331.

Clifford, M.N., 2000. Anthocyanins: Nature, occurrence and dietary burden. J. Agric. Food Chem., 80: 1063-1072.

Daniel, O., M.S. Meier, J. Schlatter and P. Frischknecht, 1999. Selected phenolic compounds in cultivated plants: Ecologic functions, health implications and modulation by pesticides. Environ. Health Perspect., 107: 109-114.

Hu, C., J. Zawistowski, W. Ling and D.D. Kitts, 2003. Black rice (*Oryza sativa* L. indica) pigmented fraction suppresses both reactive oxygen species and nitric oxide in chemical and biological model systems. J. Agric. Food Chem, 51: 5271-5277.

Iqbal, S., M.I. Bhanger and F. Anwar, 2005. Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. Food Chem., 93: 265-272.

Laandrault, N., P. Pouchert, P. Ravel, F. Gase, G. Cros and P.L. Teissedro, 2001. Antioxidant activities and phenolic level of French wines from different varieties and vintages. J. Agric. Food Chem., 49: 3341-3343.

Lee, S.C., J.H. Kim, S.M. Jeong, D.R. Kim, J.U. Ha, K.C. Nam and D.U. Ahn, 2003. Effect of far-infrared radiation on the antioxidant activity of rice hulls. J. Agric. Food Chem., 51: 4400-4403.

Ling, W.H., Q.X. Cheng, J. Ma and T. Wang, 2001. Red and black rice decrease atherosclerotic plaque formation and increase antioxidant status in rabbits. J. Nutr., 131: 1421-1426.

Matinez-Valverde, I., M.J. Periago and G. Ros, 2000. Nutritional importance of phenolic compounds in the diet. Arch. Latinoam. Nutr., 50: 5-18.

Newmark, H.L., 1996. Plant phenolics as potential cancer prevention agents. Adv. Exp. Med. Biol., 401: 25-34.

Oki, T., M. Masuda, M. Kobayashi, Y. Nishiba, S. Furuta, I. Suda and T. Sato, 2002. Polymeric procyanidins as radical-scavenging components in red-hulled rice. J. Agric. Food Chem., 50: 7524-7529.

Osawa, T. and M. Namiki, 1981. A novel type of antioxidant isolated from leaf wax of Eucalyptus leaves. Agric. Biol. Chem., 45: 735-739.

Ryu, S.N., S.Z. Park and C.T. Ho, 1998. High performance liquid chromatographic determination of anthocyanin pigments in some varieties of black rice. J. Food Drug Anal., 6: 729-736.

Sugano, M. and E. Tsuji, 1997. Rice bran oil and cholesterol metabolism. J. Nutr., 127: 521S-524S.

Tian, S., K. Nakamura and H. Kayahara, 2004. Analysis of phenolic compounds in white rice brown rice and germinated brown rice. J. Agric. Food Chem., 52: 4808-4813.

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

Xia, M., W.H. Ling, J. Ma, D.D. Kitts and J. Zawistowski, 2003. Supplementation of diets with black rice pigment fraction attenuates atherosclerotic plaque formation in apolipoprotein E deficient mice. J. Nutr., 133: 744-751.

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

Zhang, M.W., B.J. Guo, R.F. Zhang, J.W. Chi and Z.C. Wei *et al*., 2006. Separation, purification and identification of antioxidant compositions in black rice. Agric. Sci. China, 5: 431-440.

Zheng, W. and S.Y. Wang, 2001. Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem., 49: 5165-5170.