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Short Communication

Bioactive compounds and antioxidant activities of red (Brown Red Jasmine) and black (Kam Leum Pua) native pigmented rice

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Abstract

Bioactive compounds (total phenolic, anthocyanin and proanthocyanidin contents) and their antioxidant activities (DPPH, ABTS radical scavenging and ferric reducing antioxidant power assays; FRAP) of red (Brown Red Jasmine rice: BRJ), black (Kam Leum Pua rice: KLP) and white rice (Japanese Brown rice: JBR) were investigated. These rice were extracted by 70% ethanol as solvent for 6 hours at room temperature. Results showed that BRJ red rice extract composed of the significant higher total phenolic content (1.018 mg GAE/mg extract) than those of KLP black and JBR white rice (0.755 and 0.069 mg GAE/mg extract, respectively) ($p<0.05$). The proanthocyanidin compound was only found in BRJ red rice extract (3.168 mg CE/mg extract) whereas the anthocyanidin was only occurred in extract of KLP black rice (17.784 mg/ml extract). Moreover, the antioxidant activities from these 3 assays (DPPH, ABTS, FRAP) tended to be higher activities in BRJ red rice (7.528, 84.476, 10.792 mg TE/mg extract) than those of KLP black rice (7.227, 79.347, 8.727 mg TE/mg extract) and JBR white rice (0.537, 18.199, 0.640 mg TE/mg extract) ($p<0.05$). These results suggested that the native Thai pigmented rice, especially red BRJ rice, had the high bioactive compound with high antioxidant capacity. Its extract may be applied as active ingredient in cosmetic, food and other industries.

Introduction

Free radicals and related species play an important role in our body that is produced by endogenous systems, exposure to chemicals condition or pathological states (Lobo *et al*., 2010). It has been widely accepted that an excess of generation of free radicals leading to oxidative damage which are responsible for the age-related damage at cellular and tissue levels (Fusco *et al*., 2007). Thus, a balance between oxidant and antioxidant is necessary in order to reduce the rate of formation of aging changes and disease pathogenesis (Rohrer and Siebenmorgen, 2004). Many studies have attempted in search of natural antioxidant compounds which have been safer than synthetic substances such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) (PaPas, 1999). Natural antioxidant compounds can be found in various plant sources such as berries, cherries, spinach, green tea, black tea, and rice (Lobo *et al*., 2010).

Rice (*Oryza sativa* L.) consumes as a staple food in Asia and also is one of the main economic plants in Thailand. Rice can be classified into 2 types by its color; non-pigmented rice and pigmented rice. In the past, people would like to more consume white rice than pigmented rice that lead to be the low price of these color rice. Recently, consumers more concern in their health and beauty, thus the consumption of pigmented rice tend to be increase. Pigmented rice are also known as source of antioxidant compounds including flavonoid, anthocyanin, phytic acid, proanthocyanidin, tocopherols, tocotrienols, γ-oryzanol, and phenolic compounds (Butsat and Siriamornpun, 2010; Goufo and Trindade, 2014). There are many varieties of rice that contained color pigments. The pigment of rice is located in the aleurone layer of rice grain and can be classified into black, purple and red color by the kinds of pigment compounds such as anthocyanin and proanthocyanidin (Finocchiaro *et al.*, 2010; Pereira-Caro *et al*., 2013a, 2013b). In Thailand, the pigmented rice from other country (e.g. Japanese Brown rice) come to be more popular consume, although many variety of Thai pigmented rice are present. It may caused by the advertising of advantage of Japanese Brown pigmented rice and lack of information in comparison bioactive compound and their activity of pigmented rice from Thai and other country. Thus, the objective of this study was to determined

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bioactive compounds and their activities of two Thai native pigmented rice [Red rice: Brown red jasmine rice (BRJ) and black rice: Kam Leum-Pua (KLP)] and one Japanese Brown rice (JBR).

Materials and Methods

Plant materials and Chemicals

Three pigmented rice varities (*Oryza sativa* L.); including Brown Red Jasmine rice (BRJ), Black glutinous rice (Kam Leum Pua; KLP) and Japanese Brown rice (JBR) were obtained from Northern Thailand. The grains were re-dried by hot air oven at 45°C for 48 hours and kept at room temperature until used. 2,2-Diphenyl-1-picryhydrazyl (DPPH), 2,2'-Azino-bis(3-ethylbenzothiakine-6-sulfonix acid) (ABTS), 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ), epicatechin, gallic acid, quercetin and trolox were purchased from Sigma Chemical (St.Louis, MO, USA). Dimethyl sulfoxide, ethanol, Folin-Ciocalteu reagent and ferric chloride were purchased from Merck (Damstadt, Germany). All chemicals and reagents used in the study were of analytical grade.

Extraction of bioactive compound

Twenty gram each of rice grains were extracted with 70% ethanol at ratio sample: solvent (ratio 1:10; w/v) using shaking methods by 150 rpm at room temperature for 6 hours. All extracts were filtered through filter paper (Whatman No. 1) and removed the solvent by rotary evaporator. Extracts were freeze-dried and kept at -20°C until use.

Total phenolic content (TPC)

Total phenolic content (TPC) in each of rice extracts were determined by colorimetric method (Singleton and Rossi, 1965). Briefly, 20 µl of extracts were reacted with 100 µl of 0.2 M Folin-Ciocalteu reagent for 1 min. Then, 80 µl of 7.5% (w/v) Na_2CO_3 was added into the reaction mixture. After incubation at room temperature for 30 min, the absorbances of extracts were measured at 765 nm by microplate reader. Gallic acid was used as a reference standard, and the results were expressed as milligram gallic acid equivalents (mg GAE/mg extract).

Total anthocyanin content

For total anthocyanin contents, it was measured by the pH differential method (AOAC Offical Method, 2005). Briefly, 50 µl of rice extracts were diluted with 0.025 M potassium chloride buffer (pH1.0) in a ratio of 1:4 (extract: buffer v/v) and kept in dark place for 30 min at room temperature. The absorbance was measured at 520 and 700 nm, respectively. The difference in absorbance between pH values and wavelength was calculated as follow:

$$A = (A_{520nm}\text{-}A_{700nm})_{pH1.0} - (A_{520nm} - A_{700nm})_{pH4.5}$$

The concentration of total anthocyanin contents expressed as cyaniding-3-glucoside equivalents (mg /L).

Total proanthocyanidin content

The content of proanthocyanidin in pigment rice extracts was measured by the vanillin-sulfuric acid method according to Singleton and Rossi (1965). Briefly, 20 µl of the diluted sample was reacted with 50 µl of 1% Vanillin and 50 µl of 25% H_2SO_4 in methanol. The absorbance was taken at 500 nm after incubation at room temperature for 15 min in the dark. Epicatechin was used as a reference standard and the results were expressed as milligram catechin equivalents (mg CE/mg extract).

DPPH radical scavenging activity

The DPPH radical scavenging activity was measured according to Thaipong *et al*. (2006). Briefly, 10 µl of rice extracts were mixed with 190 µl of DPPH reagent. The mixture was vortexed and then kept in dark place for 30 min at room temperature. The absorbance was measured at 515 nm. Trolox was used as a reference standard, and the results were expressed as Trolox equivalents (mg TE/mg extract).

ABTS radical scavenging activity

The ABTS radical scavenging activity was measured according to Thaipong *et al.* (2006). Briefly, the mixture of ABTS radical cation was prepared by adding 7 mM ABTS with 2.45 mM potassium persulphate and then the mixture was left to stand overnight in the dark at room temperature. The ABTS radical cation solution was diluted with 50 mM phosphate buffer in a ratio of 1:20 (v/v). For analysis, 10 µl of rice extracts were mixed with 190 µl of ABTS radical cation solution. The mixture was vortexed and then kept in dark place for 15 min at room temperature. Absorbance was measured at 734 nm. Trolox was used as a reference standard, and the results were expressed as Trolox equivalents (mg TE/ mg extract).

Ferric reducing antioxidant power (FRAP) assay

Reducing power was measured according to Benzie and Strain (1996) as followed. FRAP solution was prepared freshly by mixing 1.0 ml of 2,4,6-TPTZ solution in 40 mM hydrochloric acid with 1.0 ml of 20 mM ferric chloride and 10 ml of 0.3 M acetate

Table 1. Total phenolic, anthotcyanin and proanthocyanidin of red (Brown red jasmine) and black (Kam Leum Pua) pigmented rice and Japanese brown rice

Rice Extract	TPC (mg GAE/mg extract)	Anthocyanin content (mg/ml extract)	Proanthocyanidin content (mg CE/mg extract)
Brown red jasmine rice (BRJ)	1.018 ± 0.095[a]	ND	3.168 ± 0.078
Kam Leum Pua rice (KLP)	0.755 ± 0.009[b]	17.784 ± 0.172	ND
Japanese brown rice (JBR)	0.069 ± 0.002[c]	ND	ND

Note: ND = Not Detectable. TPC = Total Phenolic Compound.
Data expressed as mean ± S.D. (n=4).
Difference in superscribe letters (a, b, c) within the same column indicates statistically different value among sample (ANOVA, Duncan's multiple range tests, $p<0.05$).

buffer (pH3.6). For analysis, 10 µl of rice extracts or solvent was mixed with 190 µl of FRAP solution. The mixture was vortexed and then left to stand in dark place for 15 min at room temperature. The absorbance was measured at 593 nm. Trolox was used as a reference standard, and the results were expressed as Trolox equivalents (mg TE/mg extract).

Statistical analysis

All obtained data were carried out at least in triplicate and expressed as mean ± SD and statistically analyzed by using SPSS program version 18 (SPSS Inc, Chicago, USA). Comparisons among samples were determined by one-way analysis of variance (ANOVA) with Duncan's multiple range tests at the significance level $p<0.05$.

Results and Discussion

Total phenolic contents of rice extracts

Total phenolic contents (TPC) of rice extracts were shown in table 1. Results showed that the native Thai pigmented rice [Brown Red Jasmine (BRJ) and Kam Leum Pua (KLP)] had significantly higher total phenolic contents than the Japanese brown rice (JBR) ($p<0.05$). Moreover, BRJ red rice extract contained the highest TPC (1.018 mg GAE/ mg extract) which was 10 times greater than KLP black rice extract (0.755 mg GAE/ mg extract) and 14 times higher than BJP white rice extract (0.069 mg GAE/ mg extract). It was similar to previous studies that ethanol extracts of red pigmented rice had higher TPC than those of black pigmented rice as well as rice varieties from other country (Ratanachithawat *et al*., 2010; Jun *et al*., 2012) and pigmented rice tended to have higher total phenolic contents than non-pigmented rice (Chen *et al*., 2012).

Total anthocyanin and proanthocyanidin contents of rice extracts

Total anthocyanin and proanthocyanidin were summarized in table 1. The anthocyanin content was only found in black rice (KLP) extract (17.784 mg/ ml extract), while BRJ red rice extract and JBR white rice could not detect. Similar to other studies, black pigmented rice showed the higher total anthocyanin contents than red pigmented and non-pigmented rice extracts (Yodmanee *et al*., 2011; Chen *et al*., 2012).

On the other hand, the proanthocyanidin was only found in BRJ red rice extract (3.168 mg CE/ mg extract), while it could not be detected in KLP black and JBR white rice (table 1). According to previous studies, red rice is characterized by the presence of proanthocyanidin whereas black rice is characterized by the anthocyanins content (Finocchiaro *et al.,* 2010).

Antioxidant activity of rice extracts

Antioxidant activity of rice extracts was determined using three methods; DPPH$^{\bullet}$ radical scavenging assay, $ABTS^{+\bullet}$ cation decolorization assay and ferric reducing antioxidant power (FRAP) assay. These activities of extract were significant difference between red, black and brown rice ($p<0.05$, Figure 1) that two Thai native pigmented rice tended to be greater antioxidant capacity than JBR white rice.

For DPPH$^{\bullet}$ scavenging assay, BRJ red rice extract (7.528 mg TE/mg extract) had higher antioxidant activity than those of KLP black rice and JBR white rice (7.227 and 0.537 mg TE/mg extract, respectively). Similar to $ABTS^{+\bullet}$ assay, BRJ red rice extract showed higher antioxidant than those of KLP black rice and JBR white rice (84.476, 79.347 and 18.199 mg TE/mg extract, respectively). In FRAP result, BRJ red rice extract (10.792 mg TE/ mg extract) had also higher reducing antioxidant power which can induce the Fe (II)-TPTZ to its oxidized Fe (III)-TPTZ form than those of KLP black rice extract (8.727 mg TE/ mg extract) and BJP white rice extract

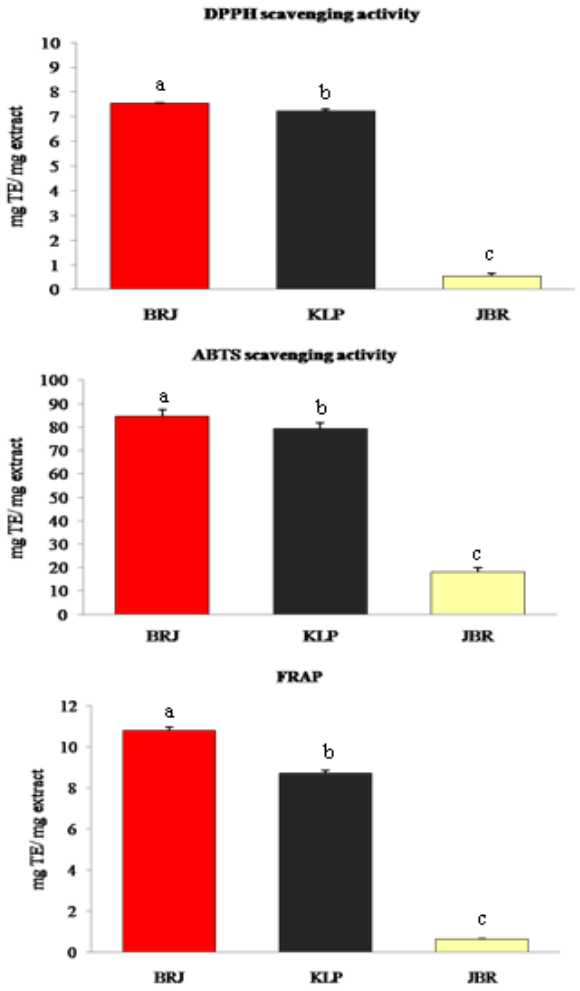

Figure 1. Antioxidant activity of red (Brown Red Jasmine;BRJ) and black (Kam Leum Pua;KLP) pigmented rice and Japanese brown rice (JBR) were determined by DPPH$^{\bullet}$ radical scavenging assay, $ABTS^{+\bullet}$ cation decolorization assay and ferric reducing antioxidant power (FRAP) assay. Difference in letters (a, b, c) above the bar graph indicates statistically different value among sample (ANOVA, Duncan's multiple range tests, $p<0.05$)

(0.640 mg TE/ mg extract) ($p<0.05$) as shown in Figure 1. The tendency of the total phenolic contents related to the results of antioxidant capacity of rice extract. It suggested that the phenolic compound may be a major antioxidant compounds in pigmented rice, like previous studies (Rattanachitthawat *et al*., 2010; Jun *et al.*, 2012; Min *et al.*, 2012)

Conclusion

Two native Thai pigmented rice (Red: Brown Red Jasmine rice and Black: Kam Leum Pua) compose of the higher phenolic compounds and greater antioxidant activities than Japanese Brown rice. The greatest amount of these compounds and activities was found in red rice (Brown Red Jasmine rice). Results suggested that the extract of these native Thai pigmented rice, especially Brown Red Jasmine rice, might be applied as antioxidant or anti-aging ingredients in cosmetic, food and other industries.

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References

AOAC Official Method. 2005. Total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines. Journal of AOAC International 88(5): 1269-1278.

Benzie I.F. and Strain J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry 239(1):70-76.

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

Chen X.Q., Nagao N., Itani T. and Irifune K. 2012. Anti-oxidative analysis, and identification and quantification of anthocyanin pigments in different coloured rice. Food Chemistry 135(4): 2783-2788.

Fusco D., Colloca G. Monaco M.R. and Cesari M. 2007. Effects of antioxidants supplementation on the aging process. Clinical Interventions of Aging 2(3): 377–387.

Finocchiaro F., Ferrari B. and Gianinetti A. 2010. A study of biodiversity of flavonoid content in the rice caryopsis evidencing simultaneous accumulation of anthocyanins and proanthocyanidins in a black-grained genotype. Journal of Cereal Science 51(1); 28-34.

Goufo P. and Trindade H. 2014. Rice antioxidants: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid. Food Science and Nutrition 2(2): 75-104.

Jun H.I., Song G.S., Yang E.I., Youn Y. and Kim Y.S. 2012. Antioxidant activities and phenolic compounds of pigmented rice bran extracts. Journal of Food Science 77(7):C759-C764.

Lobo V., Patil A., Phatak A. and Chandra N. 2010. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy Reviews 4(8): 118–126.

Min B., Gu L., McClung A.M., Bergman C.J. and Chen M-H. 2012. Free and bound total phenolic concentrations, antioxidant capacities, and profiles of

proanthocyanidins and anthocyanins in whole grain rice (*Oryza sativa* L.) of different bran colours. Food Chemistry 133(3): 715-722.

Papas AM. 1999. Diet and antioxidant status. Food and Chemical Toxicology 37:999–1007.

Pereira-Caro G., Cros G., Yokota T. and Crozier A. 2013a. Phytochemical profiles of black, red, brown, and white rice from the Camargue region of France. Journal of Agricultural and Food Chemistry 61(33):7976-7986.

Pereira-Caro G., Watanabe S., Crozier A., Fujimura T., Yokota T. and Ashihara H. 2013b. Phytochemical profile of a Japanese black-purple rice. Food Chemistry 141(3):2821-2827.

Rohrer C.A. and Siebenmorgen T.J. 2004. Nutraceutical Concentrations within the Bran of Various Rice Kernel Thickness Fractions. Biosystems Engineering 88(4): 453–460.

Rattanachittahawat S., Suwannalert P., Riengrojpitak S., Chaiyasut C. and Puntuwatana S. 2010. Phenolic content and antioxidant activities in red unpolished Thai rice prevents oxidative stress in rats. Journal of Medicinal Plants Research 4(9): 796-801.

Singleton V.L. and Rossi J.R. 1965. Colorimetry of Total Phenolics with Phosphomolybdic- Phosphotungstic Acid Reagents. American Journal of Enology and Viticulture 16: 144–158.

Thaipong K., Boonprakob U., Crosby K., Cisneros-Zevallos L. and Byrne D.H. 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis 19(6-7): 669-675.

Yodmanee S., Karrila T.T. and Pakdeechanuan P. 2011. Physical, chemical and antioxidant properties of pigmented rice grown in Southern Thailand. International Food Research Journal 18(3): 901-906.