

Full Length Research Paper

Anti-oxidative biochemical properties of extracts from some Chinese and Thai rice varieties

Chanida Saenkod², Zhonghua Liu^{1,2*}, Jianan Huang^{1,2} and Yushun Gong^{1,2}

¹Key Laboratory of Tea Science of Ministry of Education and Hunan Province and Key Laboratory of Crop Germplasm Innovation and Utilization, Hunan Agricultural University, Changsha, Hunan 410128, People's Republic of China.

²National Research Center of Engineering and Technology for Utilization of Botanical Functional Ingredients, Changsha, Hunan 410128, People's Republic of China.

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Total phenolic compounds (TPC), total flavonoid contents (TFC) and antioxidant activity of extracts from 8 varieties of Chinese and Thai rice grains were evaluated. Extraction was done using water (at 25°C), hot water (at 50°C) and 70% ethanol. Highest TPC and TFC were seen in all rice varieties after extraction with 70% ethanol. The Heimi variety of Chinese rice showed the highest TPC and TFC (634.83 and 158.47 mg/kg, respectively) followed by Jing Nian variety, also from China, Dok Kam and Niaow Deang varieties both from Thailand, respectively. Highest antioxidant activity was observed in Heimi followed by Jing Nian, Dok Kam and Niaow Deang varieties. The color of Heimi and Jing Nian is black, and that for Dok Kam and Niaow Deang is red, so TPC and TFC were correlated with the color of rice. However, no correlation was observed with the Nang Dum variety from Thailand even though it has a black color. Its TPC and TFC were found to be statistically the same as that of the white-colored Ji Nong Da, T-You 597 and Xiang Wan Nuo 1 Hao varieties from China. Antioxidant activities from each solvent system extraction (water, hot water and 70% ethanol) were also found to be directly correlated with the quantity of TPC and TFC. The TPC had positive correlations with DPPH scavenging ($R^2 = 0.9155, 0.9358$ and 0.9625), reducing power ($R^2 = 0.9799, 0.8521$ and 0.9733) and inhibition of lipid peroxidation ($R^2 = 0.7621, 0.7429$ and 0.9610). TFC also had positive correlations with DPPH scavenging ($R^2 = 0.9098, 0.9246$ and 0.9477), reducing power ($R^2 = 0.9916, 0.9745$ and 0.9844) and inhibition of lipid peroxidation ($R^2 = 0.7548, 0.7178$ and 0.8283), respectively.

Key words: Rice grain, bioactive compounds, total phenolic, flavonoids, antioxidant component.

INTRODUCTION

Antioxidants for oxidative stress protection from natural sources such as grain crops, vegetables and fruits are becoming a profitable alternative as compared to synthetic antioxidants that have adverse effects. Free radicals have been claimed to induce oxidative stress in various cell components including proteins, lipids and DNA, ultimately leading to certain diseases such as cancer, cardiovascular diseases and ageing and inflammatory diseases.

Rice (*Oryza sativa* Linn.) is the most important cereal crop in the world, either directly as human foods or indirectly as animal feeds. In China, rice is the staple food for more than 60% of the Chinese people, accounting for 35% of all rice grain production (Cao et. al., 2010). Thailand, Rice is one of the main food and source of nutrition for most of Thai population. Rice is rich in many nutrient components including carbohydrates, proteins, certain fatty acids and micronutrients (vitamins and trace

Table 1 Description of the rice varieties used in the study.

Rice Variety	Abbreviation	Description	Color	Source
Heimi	BB	Brown Heimi	Black	China
Ji Nong Da 37	BJ	Brown Ji Nong Da	White	China
T-You 597	BT	Brown T-You 597	White	China
Xiang Wan Nuo 1 Hao	BW	Brown Xiang Wan Nuo 1 Hao	White	China
Jing Nian	BY	Brown Jing Nian	Black	China
Niaow Deang	BND	Brown Niaow Deang	Red	Thailand
Nang Dum	BN	Brown Nang Dum	Black	Thailand
Dok Kam	BD	Brown Dok Kam	Red	Thailand

minerals). It is also a good source of many bioactive non-nutrient compounds, known as antioxidants, including phenolic compounds.

Several studies indicate that phytochemicals are bioactive compounds that include phenylpropanoids, tannins, lignins, γ -oryzanol, tocotrienols, tocopherols, phenolics compounds and flavonoids. Most common groups of phenolic compounds are flavonoids which are water-soluble plant pigments with many colors (Hansakul et al., 2011). Phenolic compounds and flavonoid contents are potential antioxidative phytochemicals that can act as metal ion chelators, free radical scavengers and reducing agents thus offer human health benefits, which also can be found in pigmented rice (Srisawat et al., 2010; Lum and Chong, 2012). According to previous studies, antioxidant activity and phenolic content of black rice is greater than that of white rice (Higashi-Okai et al., 2008; Muntana and Prasong, 2010). In addition, pigmented rice extracts have been reported to effectively decrease inflammation and oxidative stress as well as atherosclerotic lesions (Ling et al., 2001; Xia et al., 2003).

The main objective of this study was to compare the total phenolic, total flavonoid and antioxidant components of extract from different varieties of Chinese and Thai rice grains using water (at 25°C), hot water (at 50°C) and 70% ethanol for extraction.

MATERIALS AND METHODS

Eight rice grain samples (*O. sativa* L.) were used in this study, as described in Table 1. The four varieties of de-hulled samples were obtained from different growth site in China, and were given the terms Brown Heimi (BB), Brown Ji Nong Da (BJ), Brown T-You 597 (BT) and Brown Xiang Wan Nuo 1 Hao (BW). One variety was purchased from a supermarket in China and was described as Brown Jing Nian (BY). Other three varieties were obtained from the southern of Thailand and were given the terms Brown Niaow Deang (BND), Brown Nang Dum (BN) and Brown Dok Kam (BD).

Extraction

One hundred grams (100 g) of rice grains from each variety were soaked in solvents, namely water at 25°C, hot water at 50°C and

70% ethanol at 25°C; ratio 1:5 (w/v) in a serial manner. Extraction was done for 40 min using an ultrasonic bath. A constant temperature was set throughout the extraction. The extracts were centrifuged at 5,000 rpm for 15 min and then the supernatants were collected. Each solvent extraction was repeated twice and each of the extract solution was combined and dried under vacuum rotary evaporator. All dried extracts were kept in the freezer (-20°C) until used for analysis.

Determination of total phenolic compounds (TPC)

The total phenolic content of extracts was determined using the Folin-Ciocalteu phenol reagent following the method of Iqbal et al. (2005). A 0.2 mL of each sample (concentration 0.2 mg/mL) was added to 0.8 mL of freshly diluted 10-fold Folin-Ciocalteu phenol reagent (Sigma, St. Louis, USA). After 10 min, 2 mL of 7.5% NaCO₃ was added to the mixture. The reaction was allowed to complete for 1 h in the dark at room temperature then measured at 765 nm on a spectrophotometer (UV-Vis 2450 Shimadzu, Japan). Gallic acid (Tianjin Kermel Chemical Reagent Development Center, Wangtai, China) was used as a standard and the results were calculated as gallic acid equivalents (mg/kg) of the sample. The procedure was conducted in triplicate and the results were averaged.

Determination of total flavonoid contents (TFC)

Total flavonoid contents of each sample were determined using colorimetric method with some modifications (Vichapong et al., 2010). Briefly, 0.2 mL of extract solution was mixed with 0.3 mL of 5% NaNO₃. After 5 min, 0.3 mL of 10% AlCl₃ was added then incubated for 6 min and 2 mL of 1 mol/L NaOH was subsequently added. The absorbance was measured immediately at 510 nm. The total flavonoid content was calculated from the calibration curve of catechin standard (Hefei Hiromi Biotechnology Co., Ltd., China). The measurements were calibrated to a standard curve of prepared catechin solution, and were expressed as 1 mg catechin equivalent per 100 g rice (mg/kg).

Scavenging effect on DPPH radical

Radical DPPH scavenging activity was determined using the method of Lai et al. (2009) with some modification. A 0.1 mL of the extraction solution (1 mg/mL in dH₂O) in test tube was mixed with 3.9 mL of 95% ethanol and 1 mL of DPPH solution (1 mM in 95% ethanol). The mixture was kept at room temperature for 30 min prior to measurement of the absorbance at 517 nm (A_{517nm}). All measurements were done in triplicate. The scavenging ratio was

Table 2. Total phenolic compounds (mg/kg) of different rice varieties using different solvents.

Rice varieties	Solvent		
	Water (at 25°C)	Hot water (at 50°C)	70% ethanol (at 25°C)
BB	433.99±0.00 ^a	610.92±0.00 ^a	634.83±0.00 ^a
BJ	41.213±0.77 ^b	45.53±0.54 ^b	65.78±1.97 ^{bc}
BT	41.87±0.79 ^b	45.97±0.00 ^b	54.86±0.68 ^b
BW	42.33±0.46 ^b	43.70±0.46 ^b	72.62±3.42 ^c
BY	286.21±0.46 ^c	434.45±4.18 ^c	462.45±0.60 ^d
BND	101.99±1.72 ^d	137.51±2.05 ^d	270.49±3.99 ^e
BN	45.97±1.72 ^b	49.16±0.82 ^b	91.90±8.06 ^f
BD	135.69±0.23 ^e	170.60±1.65 ^e	382.07±0.68 ^g

Values are the mean of triplicate measurements ± standard error (Means±SE); (n = 3). Mean followed by different superscript letters in a column are significantly ($p \leq 0.05$) different from each other.

calculated using the formula:

$$\text{DPPH scavenging (\%)} = 1 - \frac{A_{517\text{nm, sample}}}{A_{517\text{nm, control}}} \times 100$$

Determination of reducing power

According to the method of Lai et al. (2009), 2.5 mL of the extract solution (1 mg/mL in dH₂O) was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6). Then, each sample was mixed with 2.5 mL of 1% K₃[Fe(CN)₆] and incubated at 50°C for 20 min. About 2.5 mL of 10% trichloroacetic acid were added and the mixture was centrifuged for 10 min. The upper layer of the solution 2.5 mL was mixed with distilled water 2.5 mL and 0.5 mL of 1% FeCl₃.6H₂O and absorbance was measured at 700 nm, indicative of the reducing power, which was recorded on the final mixture. The procedure was done in triplicate.

Inhibition of lipid peroxidation

The procedure followed the method of Badmus et al. (2011). Briefly, 0.5 ml of egg yolk homogenate (10% v/v) was added to each sample extract of 0.1 mL (10 µL/ml). The volume of the sample was made to 1 ml by adding distilled water and then 0.05 ml of FeSO₄.7H₂O was added. After 30 min, 1.5 ml of 0.8% thiobarbituric acid in 1.1% SDS was added. The mixed solution was then incubated at 95°C for 1 h. After cooling, 5 mL of butanol was added and the mixture was centrifuged at 3,000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm and the percentage of lipid peroxidation inhibition was calculated with the formula:

$$\text{Inhibition on lipid peroxidation (\%)} = 1 - \frac{A_{532\text{nm, sample}}}{A_{532\text{nm, control}}} \times 100$$

Statistical analysis

All experiments were conducted in triplicates and the results were expressed as mean ±SE. Significance of mean differences between chemical compositions and bioactive compounds within different rice varieties were statistically compared using an analysis of variance (ANOVA) at the 5% probability level ($p \leq 0.05$) with

individual pairwise comparisons made using Tukey's HSD test through an SPSS program version 17.

RESULTS AND DISCUSSION

Total phenolic compounds (TPC) and total flavonoid contents (TFC)

Several studies have reported the use of different solvent systems to extract rice such as hexane, methanol, ethanol-water (70:30 v/v) and water at room temperature (Srisawat, et al., 2010). In this study, different solvents were used namely, water (at 25°C), hot water (50°C) and 70% ethanol (at 25°C) to extract Chinese and Thai rice varieties. The total phenolic compound in different rice varieties using different solvent extraction was investigated following a modified Folin-Ciocalteu phenol reagent method. The results were expressed as gallic acid equivalents (Table 2). In extract composition, samples extracted using 70% ethanol (at 25°C), hot water (50°C) and water (at 25°C) had the TPC content range from 54.85 to 634.83, 43.69 to 610.92 and 41.21 to 433.95 mg/kg, respectively. Statistics of TPC values of all crude extracts from different rice varieties with the solvent applied suggests a significant difference at 95% confidence level. But there was no significance between BJ, BT, BW and BN from water and hot water extracted. However, the levels of TPC were slightly different from those studied by Lum and Chong (2012) where the quantity of TPC in Malaysia rice ranged from 22.59 to 329.53 mg/kg. In addition, BB rice, a black rice variety from China, contained the highest TPC from all the solvents extraction. These data indicate that black rice has higher total phenolic compound than red and white rice, which is similar to those reported by Muntana and Prasong (2010) that high phenolic compounds can be found in pigmented rice such as red and black rice.

The same effect and trend can be observed in the total flavonoid contents (TFC) in all rice varieties (Table 3) where samples extracted with 70% ethanol (at 25°C)

Table 3. The total flavonoid compounds (mg/kg) of different rice varieties using different solvents.

Rice varieties	Solvent		
	Water (at 25°C)	Hot water (at 50°C)	70%ethanol (at 25°C)
BB	79.47±1.05 ^a	143.15±0.32 ^a	158.47±3.46 ^a
BJ	15.95±0.41 ^{bc}	16.63±0.02 ^{be}	17.06±0.03 ^b
BT	13.28±0.49 ^{bf}	18.51±1.78 ^b	21.92±2.25 ^b
BW	18.74±1.03 ^c	19.38±1.00 ^b	20.01±1.34 ^b
BY	74.37±0.57 ^d	113.30±0.48 ^c	123.68±0.42 ^c
BND	26.17±0.73 ^e	35.91±0.16 ^d	66.71±0.16 ^d
BN	11.65±0.99 ^f	13.56±0.21 ^e	16.98±0.78 ^b
BD	34.15±0.58 ^g	42.45±0.28 ^f	89.38±0.72 ^e

Values are the mean of triplicate measurements ± standard error (Means±SE); (n = 3). Mean followed by different superscript letters in a column are significantly ($p \leq 0.05$) different from each other.

shows that the TFC range from 16.98 to 158.47 mg/kg, 13.56 to 143.15 mg/kg for hot water (at 50 C) and 11.65 to 79.47 mg/kg for water (at 25°C) were significantly different ($p \leq 0.05$) between each other, except BT, BW from hot water extracted, and BJ, BT, BW and BN from 70% ethanol extracted were not significance. The result showed that TFC is lower than total flavonoid content of the ethanolic extract from Thai rice (Hansakul et al., 2011). Even though all the black rice varieties had the highest TFC than red and white rice varieties, some red rice varieties accessions still had lower contents than white varieties. This association was also observed by Shen et al. (2009), who reported that the flavonoids content of black rice was higher than that of red and white rice. However, some flavonoid contents in red rice were lower than that of white rice. It is possible that other flavonoid compounds such as flavonols and flavanones, rather than anthocyanins, were higher in white rice than those in red rice.

Among all the rice extracts, the 70% ethanol crude extracts contained more TPC and TFC than water (at 25°C) and hot water (at 50°C) crude extracts in the same rice variety. Ethanol can effectively increase permeability of cell wall thus facilitate efficient extraction of polar substances which when added to water can cause further solubility of polar substances in the cell (Veronique, 2006; Anwar and Przybylski, 2012).

Antioxidant components

The scavenging effect on DPPH radical of rice extracts were evaluated using the DPPH method (Figure 1A). Absorbance at 517 nm of DPPH radical in this method was recorded at 0 to 10 min intervals from initiation of the reaction. Scavenging capacity was similar at the beginning of the reaction and was changed with the increase of the reaction time until it stabilized at 10 min. The DPPH radical scavenging capacity varied from 6.28 to 55.86% for water (at 2°C) crude extracts, 7.99 to 68.58% for hot

water (at 50°C) crude extracts and 12.81 to 70.82% for 70% ethanol (at 25°C) crude extracts with significant difference ($p \leq 0.05$). The highest scavenging capacity was found in 70% ethanol extract of BB variety (70.82%) as compared to other rice varieties and solvent system. Higher percentage of DPPH scavenging is correlated with higher antioxidant capacity (Sultana et al., 2009).

The reducing power of different extracts from all varieties of rice at 1 mg/mL concentration is depicted in Figure 1B. In this assay, the absorbance values of rice extract using water (at 25°C), hot water (at 50°C), and 70% ethanol (at 25°C) at 700 nm were, respectively, 0.13 to 2.29, 0.10 to 1.66 and 0.11 to 0.49, which were significantly different ($p \leq 0.05$) between each other. But there was no significance in BJ, BT, BW and BN from water and 70% ethanol extracted. The highest value of absorption in rice extracts was observed in 70% ethanol extract (at 25°C) of BB variety at 2.29. Reducing power in BB variety was higher than other varieties which were found to be the same with the TPC, TFC and scavenging effect on DPPH radical. In addition, the reducing power of rice bran extracts was related to the total phenolic compound contents.

Inhibitory capacity of all rice varieties extracts was determined using linoleic acid test system that can inhibit hydrogen peroxide production in the peroxidation of linoleic acid. The inhibitory activity of rice extracts against lipid peroxidation was shown to decrease absorbance at 532 nm. This is due to the diminution of the lipid oxidation products of linoleic acid, specially the conjugated dienes. In this study, Figure 1C shows that the inhibition of lipid peroxidation of crude extract using 70% ethanol (at 25°C) was the most promising among the extracts examined accounting for 67.86% in BB variety. This can be attributed to the superior antioxidant activities of the sample extracted using 70% ethanol (at 25°C) that exhibited comparatively greater antioxidant compositions than samples extracted with water (at 25°C) and hot water (at 50°C). Statistics of lipid peroxidation inhibition values of all crude extracts from different rice varieties with the

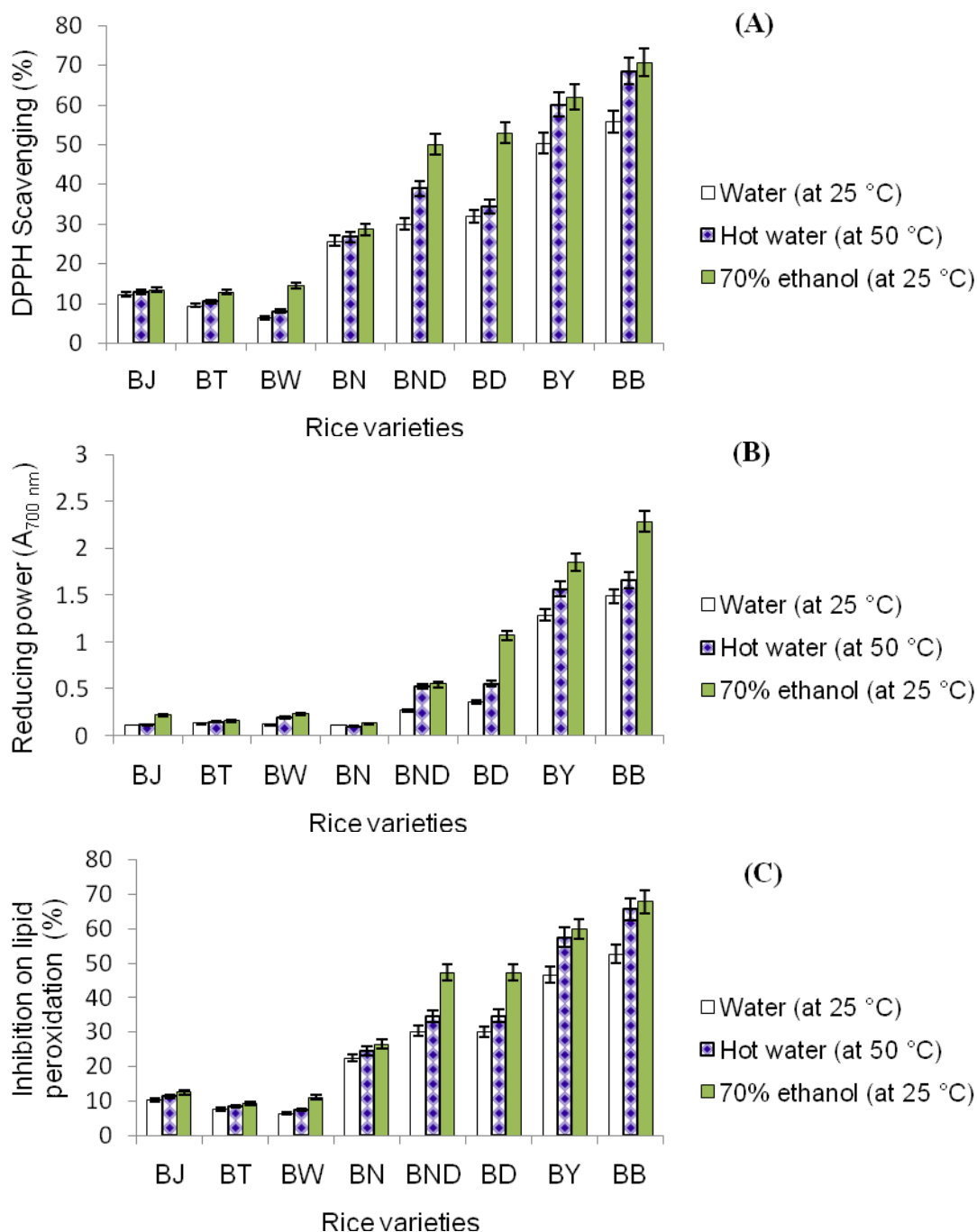


Figure 1. Antioxidant components of rice gRain extracts; (A) scavenging capacity on DPPH radical, (B) reducing power and (C) inhibition on lipid peroxidation. Values are the mean of triplicate measurements \pm standard error (Means \pm SE) in each solvent extract; Statistical significance was determined by Tukey's HSD test ($p \leq 0.05$).

solvent applied suggests a significant difference at 95% confidence level. However, there was no significance between BB and BY extracted with water.

Total phenolic compounds, total flavonoids component and antioxidant activity (DPPH scavenging, reducing power and inhibition of lipid peroxidation) were used to

determine the antioxidant activity of the extracts from different rice varieties. An integration of different methods is necessary because their actions and natural antioxidants from plant materials are complex. Furthermore, the correlation coefficient (R^2) between TPC or TFC and antioxidant activities (DPPH scavenging, reducing power

and inhibition of lipid peroxidation) in each rice variety extract was determined. The antioxidative activities (DPPH scavenging activity, reducing power, and inhibit on lipid peroxidation) as a function of total phenolic compounds and total flavonoid contents has a positive correlation coefficient (R^2) (data not show). According to Butsat and Siriamornpun (2010), the antioxidant activity of different Thai Rice varieties was positively correlated with total phenolic contents.

Dietary antioxidants protect against reactive oxygen species in the human body. An increased intake of antioxidants may therefore have a number of health effects, such as reducing the incidence of cancer and cardiovascular diseases (Diplock et al., 1998; Halliwell and Gutteridge, 1999). Due to the detection of many new bioactive compounds in food with possible antioxidant activity, and the increased interest in the relationship between antioxidants and disease risks and mechanisms, there is an urgent need to establish the antioxidant capacity in different foods, especially the rice crop which constitute the main food for populations in different countries.

Conclusions

In all (Chinese and Thai) varieties of rice used in this study, ethanol was shown to be the best solvent for extracting phenolic compounds, while the least effective was the use of water (25°C). Same observation was also found after extracting flavonoid compounds. The variety of rice that showed the highest phenolic and flavonoid compounds after extraction was Heimi (BB) from China, followed by Jing Nian (BY), also from China, Dok Kam (BD) and Niaow Deang (BND) both from Thailand, respectively. The pigment of BB and BY is black while red for BD and BND, which according to Shen et al. (2009), TPC and TFC has a correlation with the color of rice. However, the correlation was not observed with the BN variety from Thailand even though it has a black color, its TPC and TFC was found to be almost insignificantly different from the BJ, BT and BW from China that has white color. Antioxidant activity was also found to be correlated with the quantity of TPC and TFC where BB has the highest, followed by BY, BD and BND, respectively.

The findings of this study suggest that nutritional values of these rice varieties extract contains bioactive compounds, may have potential to protect against diseases. Therefore, these rice should be developed in food applications as health products, food industry and pharmaceutical to add value to rice.

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