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POTENTIAL ANTIOXIDANT PROPERTIES OF PIGMENTED RICE FROM SABAH, MALAYSIA

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ABSTRACT

In this study, the methanolic crude extracts of four Sabah rice varieties namely black, red, brown and white rice were screened for their total phenol acids content, tocopherol content, antioxidant and free radical scavenging properties. The red rice contained the highest quantity of phenolic acids (329.93 ± 19.17 mg/100 g) and tocopherols (200.33 ± 13.61 mg/100 g). To detect antioxidant activity of the pigmented rice extracts, ferric thiocyanate (FTC) assay was used and compared with the thiobarbituric acid (TBA) method. Scavenging activity was measured by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Red rice extract showed the highest activity for all the three tests, FTC (0.302), TBA (0.329) and DPPH (65.54% ± 0.57) due to its high content of phenolic acids and tocopherols.

The results indicate that the pigmented rice varieties possess protective effects against the generation of hydroperoxide and free radical scavenging activity. The antioxidant activity of the extracts differed and was dependent on the bran color of the rice. The results showed that the antioxidant activity was in the following order: red rice > black rice > brown rice > white rice. In conclusion, antioxidant activity was strongly correlated to the total phenolic acids and tocopherols content.

KEYWORDS: Antioxidant, Phenolic Acids, Pigmented Rice, Tocopherol

INTRODUCTION

Rice (Oryza sativa) is the major and staple cereal crop of over half of the world population. In China and India whose people constitute almost half the world's population, rice serves as the staple food for their daily life (Sasaki & Burr, 2000; Khan & Komatsu, 2004) and unsurprisingly, 95% of the world rice production is in Asian countries (Bhattacharjee *et al.*, 2002).

In Sabah, located in South East Asia, there are various indigenous rice cultivars with different bran color such as purple, black, red, and brown but the most common type is white rice. Pigmented rice varieties are usually named according to their bran color formed by deposition of anthocyanins in the pericarp, seed coat and aluerone (Chaundry, 2003).

Pigmented rice is a potential source of antioxidants in various types of functional food production (Yawadio *et al.*, 2007). Potential antioxidative phytochemicals such as acetylated procyanidin, anthocyanins, and other phenolic acids, which can be found in these pigmented rice, can help prevent oxidative stress, inhibits the initiation and formation of cancer, reduces plasma cholestrol levels and may prevent cardiovascular disease (Romero, 2009).

Previous studies show that red rice has gained popularity in Japan as a functional food due to its high polyphenols and anthocyanin content (Itani & Ogawa, 2004) and black rice has also been shown to possess nutritional advantages over

common rice such as higher protein content, vitamins and minerals (Suzuki *et al*., 2004) or as an organic food coloring agent (Chaudhary, 2003).

However, information regarding the phytochemicals profile of pigmented rice is scarce and rice is not purposely utilized by local people based on phytochemical content. This study was done to determine the potential antioxidative properties of four local Sabah rice varieties that can be exploited by pharmaceutical and health products industries.

MATERIALS AND METHODS

Collection of Pigmented Rice

Black, red, brown and polished white rice were bought from the market and ground into rice flour. The whole grain and the rice flour samples were kept at 4 ^{O}C.

Solvent Extraction

Methanol was used for extraction. The rice flours were macerated in the solvent for 72 hours. After removing the rice residues by filtration, each filtrate was evaporated to dryness in vacuo and weighed.

Determination of Total Phenolic Content

Total phenolic content of each rice bran extract was measured according to the method reported by Singleton *et al*. (1999) by using Folin-Ciocalteu reagent with some modification. To each of 0.1 mL of samples (triplicate), 50 µL of 2 N Folin-Ciocalteu reagent was added, mixed and allowed to stand for 3 to 5 min at room temperature. A 0.3 mL of a 20% (w/v) sodium carbonate solution was added, mixed and kept aside for 15 min. Finally, 1 mL of distilled water was added. The absorbance of all samples was measured against a reagent blank at 725 nm. Results were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/100 g dw).

Determination of Tocopherol Content

Tocopherol content was determined by colorimetric assay according to Kıvcak & Mert (2001). A 2, 2'-bipyridine (0.125 g) was dissolved in 25 mL of absolute ethanol (dark bottle) and ferric chloride hexahydrate (0.2 g) was dissolved in 100 mL of absolute ethanol. The solution was kept in the refrigerator until used. Aliquots (20, 40, 60, and 80 µL) of a 0.095 g mL^{-1} solution of tocopherol in chloroform ($CHCl_3$) were transferred to a volumetric flask and the volume was adjusted to 9.8 mL with $CHCl_3$.

Each of the solutions and 0.1 mL of 2, 2'-bipyridine reagent was pipette into a 10 mL volumetric flask and mixed. A portion of ferric chloride reagent (0.1 mL) was added to the 10 mL volumetric flask and the mixture was shaken for 10 second. The absorbance of the mixture was read at 522 nm in a 1 cm cell 50 second after adding the ferric chloride. A blank was run by using 9.8 mL of $CHCl_3$, 0.1 mL of 2, 2'-bipyridine reagent, and 0.1 mL of ferric chloride reagent. The tocopherol content in the extracts was then calculated from the standard curve.

Ferric Thiocyanate (FTC) Method

The standard method as described by Kikuzaki & Nakatani (1993) was used to measure the amount of peroxide at the beginning of lipid peroxidation. Reaction of peroxide with ferrous chloride form ferric ions and ferric ions will then unite with ammonium thiocyanate and produce ferric thiocyanate. The substance is red, and a denser color is indicative of higher absorbance. A screw-cap vial containing a mixture of 4 mg of a sample in 4 mL of 99.5% ethanol (0.02%), 4.1 mL of 2.51% linoleic acid in 99.5% ethanol, 8.0 mL of 0.02 M phosphate buffer (pH 7.0) and 3.9 mL of water were placed in

an oven at 40 ^{o}C in the dark. A 9.7 mL of 75% (v/v) ethanol and 0.1 mL 30% ammonium thiocyanate were added to 0.1 mL of the mixture above. Three minutes after addition of 0.1 mL of 2×10^{-2} M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance was measured at 500 nm. This step was repeated every 24 hours until the control reached its maximum absorbance value. The absorbance of an aliquot without the crude extract sample (control) and the reference compound DL-α-tocopherol was also measured. All the determinations were performed in three replicates.

Thiobarbituric Acid (TBA) Method

The method of Ottolenghi (1959) was used to measure the free radicals present after peroxide oxidation. One milliliter (1 mL) of 20% aq. trichloroacetic acid and 2 mL of 0.67% thiobarbituric acid was added to 2 mL of the sample solution prepared using the FTC method. The mixture was then placed in a boiling water bath for 10 min. After cooling, it was centrifuged at 3000 rpm for 30 min. The absorbance of the supernatant was then measured at 532 nm. The antioxidant activity was based on the absorbance on the final day for the FTC method. The absorbance of the sample without the crude extract samples (control) and the reference compound DL-α-tocopherol was also measured. All the determinations were performed using three replicates.

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Method

The scavenging activity of DPPH free radicals by the rice extracts was determined according to the method reported by Cuendet *et al*. (2000). A methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (0.1 mM) was prepared and stored at 10 ^{o}C in the dark. A methanol solution of the test compound was prepared (1 mg mL^{-1}). A 1.5 mL aliquot of the methanol solution was added to 1.5 mL of DPPH solution.

Absorbance measurements were recorded immediately with a UV-visible spectrophotometer at 517 nm. The decrease in absorbance was determined continuously at 3 min intervals until the absorbance stabilized. The time when the absorbance stabilized was recorded. The absorbance of the DPPH radical without the crude extract samples (control) and the reference compound DL-α-tocopherol was also measured. All the determinations were performed in three replicates. The percentage of inhibition (PI) of the DPPH radical was calculated by using the following formula:

Scavenging ability (%) = [$Absorbance_{517\,nm}$ of control – $Absorbance_{517\,nm}$ of sample/ $Absorbance_{517\,nm}$ of control] × 100%

Statistical Analysis

Data for each parameter are reported as the mean ± standard deviation of the triplicate samples used for analyses of the same extract. All statistical analyses were carried out using SPSS version 17.

Correlation analysis was used to describe the relationship between the attributes. Mean comparison between the rice varieties and control was done post analysis of variance at 5% significance level using Tukey's test.

RESULTS AND DISCUSSIONS

Total Phenolic Content (TPC)

TPC of the various rice colored varieties are presented in Table 1. There were significant differences in the TPC between all four rice varieties ($p\leq0.05$). Red rice variety contained the highest quantity of phenolic acids, 329.93 ± 19.17 mg/100 g followed by black rice variety, 290.77 ± 13.72 mg/100 g of dry sample.

Brown rice variety contained 69.63 ± 5.58 mg/100 g and the rice variety with lowest content of phenolic acids was white rice with a content of 22.59 ± 1.31 mg/100 g of rice powder.

In a previous study, Sompong *et al.* (2010) observed large variations in total phenolic acids between red rice and black rice grown at different locations in Sri Lanka. They reported that TPC for red rice 3, Bahng Gawk (BG) red rice, Niaw Dam Pleuak Dam (PD) black rice and Niaw Dam Pleuak Khao (PK) black rice was 79.18 ± 5.09, 691.37 ± 28.06, 336.69 ± 0.72 and 665.16 ± 22.05 mg/100 g respectively. Another study by Shen *et al.* (2009) found that mean phenolic contents of red rice was 470.1 mg/100 g and for black rice was 1055.7 mg/100 g. Total phenolic acids content on rice bran of different cultivars was reported by Chotimarkorn *et al.* (2008) to range from 2.2 ± 0.3 mg g^{-1} to 3.2 ± 0.2 mg g^{-1}.

The TPC of rice reported by Sompong *et al.* (2010) was dependent on the planting location even if the same variety of rice was used. Chotimarkorn *et al.* (2008) showed that the total phenolic acids content differed with cultivars. Higher total phenolic acids in pigmented rice than non-pigmented rice bran showed that the deposition of phytochemical compounds caused the color pigmentation in black and red rice.

Table 1: Total Phenolic Content, Tocopherol Content and Antioxidant Capacity of Pigmented Rice Varieties

	TPC (mg/ 100 g)	Tocopherol (mg/ 100 g)	DPPH (% remaining)
Black rice	290.77±13.72[a]	124.33±31.34[a]	37.66±3.85[a]
Red rice	329.93±19.17[b]	200.33±13.61[b]	65.54±0.57[b]
Brown rice	69.63±5.58[c]	82.33±4.93[a]	13.74±11.77[c]
White rice	22.59±1.31[d]	83.67±1.45[a]	ND
Tocopherol (control)	-	-	79.18±1.51[b]

[1] Different letters in the same column indicate significant differences ($p<0.05$)

[2] Mean % ± SD obtained from analysis of three replicates

[3] ND= not determined by this method

Tocopherol Content

Total tocopherol content in the four colored rice varieties is shown in Table 1. The red rice variety had the highest content of tocopherol (200.33 ± 13.61 mg/100 g) which was significantly different from the other three rice varieties ($p\leq0.05$).

The quantity of tocopherol found in black, brown and white rice varieties was not significantly different wherein the black rice variety contained 124.33 ± 31.34 mg/100 g of tocopherol, brown rice contained 82.33 ± 4.93 mg/100 g and white rice having 83.67 ± 1.45 mg/100 g. However the TPC of the black rice was about 50% more than that of brown and white rice. Chotimarkorn *et al.* (2008), reported that total tocopherol content in five different Thailand long-grained rice brans varied from 0.35 ± 0.03 mg g^{-1} to 0.77 ± 0.03 mg g^{-1}. Schramm *et al.* (2007) also reported differences in TPC of the rice bran of two different varieties, Cypress and Cheniere withmean tocopherol content of 202.13 ± 11.71 µg g^{-1} and 217.02 ± 13.86 µg g^{-1} respectively.

Tocopherol content of the rice varieties in this study was higher compared to that reported by Chotimarkorn *et al.* (2008) and Schramm *et al.* (2007). Black and red pigmented rice showed higher tocopherol contents compared with other

colored rice brans. This indicates that the color pigmentation of these rice varieties is caused by the deposition of various phytochemical compounds. Tocopherol which is a powerful antioxidant compound is also shown to be very sensitive to environmental conditions. Long storage or exposure of the rice samples to ambient environment may initiate the degradation of the tocopherol compounds and reduce the tocopherol content (Htwe *et al*., 2010).

Antioxidant Assay

Ferric thiocyanate (FTC) method was used to determine the amount of hydroperoxide of linoleic acid at an early stage of lipid peroxidation. The data obtained at different time intervals are presented in Figure 1.

From Figure 1, hydroperoxide formation in methanol, the negative control started at day one and reached the maximum value at day five. All the pigmented rice extracts tested showed low absorbance values which indicated a high level of antioxidant activity. Red rice extract had the highest activity with lowest absorbance of 0.302 followed by brown rice extract (0.342) and black rice extract (0.372) and the highest absorbance was for white rice extract with 0.389. None of the extracts showed absorbance values greater than the negative controls (1.514) at the end point of the experiment using this method. The antioxidant activity of all the rice extracts tested was not higher than the commercial standard antioxidant, alpha-tocopherol with the lowest absorbance of 0.211 at day six.

Although the absorbance of the four rice varieties was slightly different, there was no significant difference in the antioxidant activity between all the four rice varieties ($p \leq 0.05$). There were significant differences between the four rice extracts, the α- tocopherol and methanol ($p \leq 0.05$). However, there were significant effects of time on the antioxidant activity in all four rice varieties and α- tocopherol ($p \leq 0.05$).

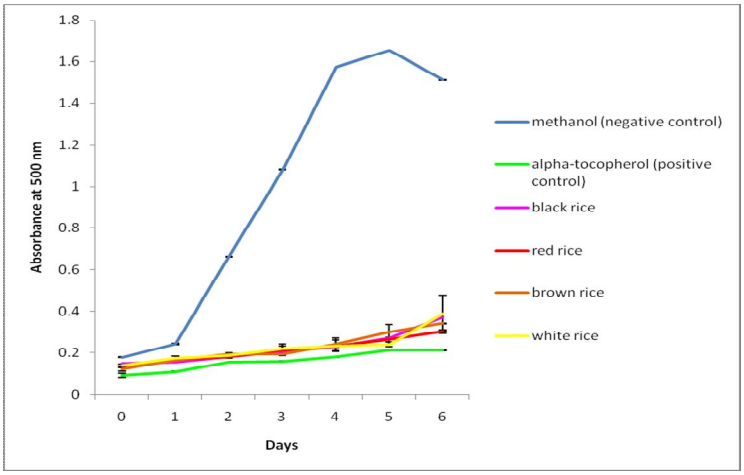

Fig. 1: Antioxidant Properties of Negative and Positive Controls, and Pigmented Rice Extracts Determined by the FTC Method

Thiobarbituric acid (TBA) method was used to measure the decomposed peroxide that forms carbonyl compounds at a later stage of lipid oxidation. The results are shown in Figure 2. Red rice had the highest antioxidant activity compared to the other three rice varieties with the lowest absorbance (0.329) but not lower than the α- tocopherol (0.160). Black rice had the second highest antioxidant properties (0.364) followed by brown rice (0.411) and white rice which showed the lowest antioxidant activity (0.420). Red rice extract exhibited the highest antioxidant activity for both methods. Among the extracts examined, white rice extract possessed the lowest antioxidant activity. However, black and brown rice showed different strengths in antioxidant activity for both the FTC and TBA methods. From Table 1, the amount of the phenolic acids and tocopherols detected in the four rice varieties were different. Variations in the amounts of the phytochemical

compounds in the four rice types tested can be one of the reasons causing differences in antioxidant activity of the extracts. Antioxidant activity using the TBA method was higher than for the FTC method because the amount of peroxide in the initial stage of lipid peroxidation was less than the amount of peroxide in the secondary stage after the decomposition of the peroxides and the secondary product, malonaldehyde was much more stable for a period of time (Aqil *et al.*, 2006).

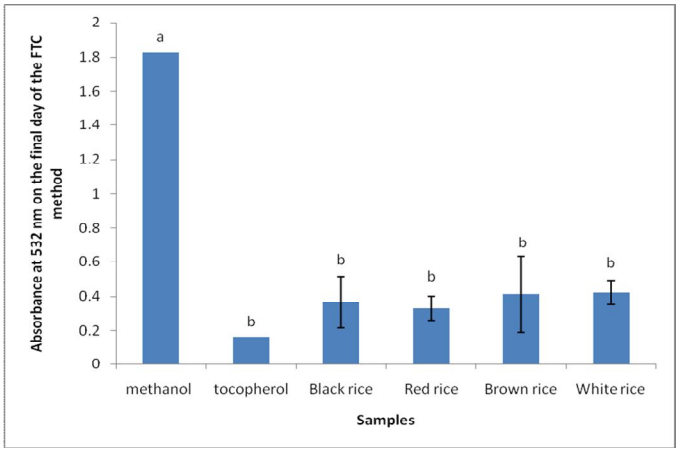

Fig. 2: Antioxidant Activities of Pigmented Rice Extracts Determined by TBA Method

Free radicals have been implicated in many disease conditions, the important ones being superoxide, hydroxyl and peroxyl radicals, and single oxygen (Aqil *et al.*, 2006). The reduction in absorbance is the result of color change from purple to yellow as the radical was scavenged by antioxidants through donation of hydrogen to form the stable DPPH-H. The percentage values of the decolorization of DPPH radicals are shown in Table 1.

In this study, red rice had the highest free radical scavenging activity, 65.54 ± 0.57% compared to other three rice varieties and was not significantly different from the α- tocopherol, 79.18 ± 1.51% ($p \leq 0.05$). Black rice had the second highest free radical scavenging activity of 37.66 ± 3.85% and brown rice only had 13.74± 11.77% free radical scavenging activity. The antioxidant activities of the red, black and brown rice were significantly different ($p \leq 0.05$). Free radical scavenging activity in white rice cannot be determined in this test and this could be due to the low content of phytochemical compounds such as phenolic acids that is able to donate hydrogen to form the stable DPPH-H in white rice. From Table 1, total phenolic acids content of white rice is the lowest compared with others.

Red rice extracts had the highest antioxidant activity in FTC, TBA and DPPH methods and also found to have the highest content of phenolic acids (329.93 ± 19.17 mg/100 g) and tocopherol (200.33 ± 13.61 mg/100 g). The phenolic acids have significant effect on DPPH radical scavenging. White rice with the lowest content of phenolic acids (22.59 ± 1.31 mg/100 g) do not posses any free radical scavenging activity in the DPPH radical scavenging test but posses good and similar antioxidant activity for FTC and TBA methods in brown rice extracts.

Tocopherol content of brown and white rice extracts were similar; 83.67 ± 1.45 mg/100 g for white rice extract and 82.33 ± 4.93 mg/100 g for brown rice extract. Antioxidant activity of all the rice extracts was lower than for α-tocopherol because it is a purified compound compared with the methanol extracts which may contain many other compounds.

There was positive correlation which indicated that the antioxidant properties were strongly correlated with the total phenolic acids (R^2=0.951; Figure 3) and tocopherol content (R^2= 0.963; Figure 4) ($p \leq 0.05$). Free radical scavenging activity of the red rice was highest with the highest phenolic acids and tocopherol contents.

Rattanachitthawat *et al.* (2010) and Goffman & Bergman (2004) also reported that the antioxidant properties are directly correlated to the TPC and TPC was higher in pigmented rice varieties. Red and black color pigmentation in plants was found to be a good source of phenolic acids (Yawadio & Morita, 2007; Gould *et al.*, 2009) and has a crucial role in antioxidant activity (Kong & Lee, 2010).

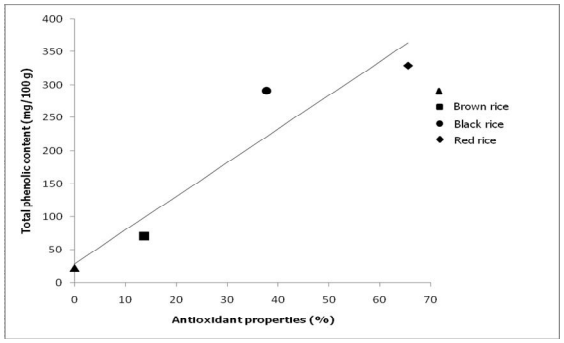

Fig. 3: Correlation between Antioxidant Properties of Pigmented Rice Extracts and Total Phenolic Acids Content (R^2 = 0.951)

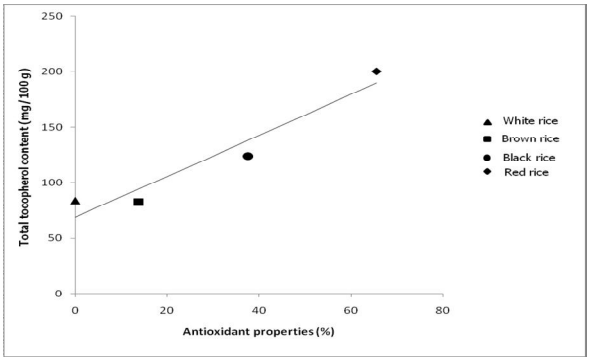

Fig. 4: Correlation between Antioxidant Properties of Pigmented Rice Extracts and Total Tocopherol Content (R^2 = 0.963)

CONCLUSIONS

In conclusion, red rice variety which contained the highest TPC and tocopherol contents had the highest antioxidant properties for all the three tests. In this study, the effect of color or pigmentation was shown to have affected the antioxidant activities with the results showing that the TPC increased with pigmentation.

These results could be used as a basis to promote the consumption or sales of pigmented rice to the local or international market due to its health benefits. Pigmented rice varieties were shown to be primary or secondary antioxidants, being free radical scavengers in prevention of lipid oxidation and a promising source of potential antioxidants. Further studies can be carried out on isolating and identifying the phytocompounds responsible for the antioxidant activity of the rice extracts, which may be further exploited in healthcare and pharmaceutical formulations.

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