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Antioxidative Activities of Bran Extracts from Pigmented Rice Cultivars

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Abstract

Introduction : Pigmented rice bran has beneficial effects in the human diet and potential to promote human health since it contains antioxidative compounds that can inhibit the formation and reduce the concen trations of reactive cell-damaging free radicals. The objectives of this study were to determine the relative antioxidative activities and identify anthocyanins in different pigmented rice brans and to discover new rice varieties with high antioxidative potential. **Methods:** Rice brans cultivar Hom-nin, Homdum-sukothai, Homnin-jakkapat, Hommae-payathongdum, Riceberry, Khawklum, Homdang-suk othai and Saohai were extracted for anthocyanins by methanol–water (70:30, v/v) containing 0.1% hydrochloric acid. The rice bran extraction samples were determined for anthocyanins by high perfor mance liquid chromatography and determined antioxidant activity by 1-1 diphenyl-2-picryl hydrazyl (DPPH). **Results:** The total anthocyanin contents varied significantly and exhibited a range of 2-3.42 mg /g. Five anthocyanin compounds were observed and three anthocyanin compounds were chara cterized by comparison of the spectroscopic and chromatographic properties with those of authentic standards. The most abundant anthocyanins were cyanidin-3-glucoside in black rice, pelargonidin-3-glucoside in blue rice, and delphinidin-3-glucoside in purple rice. The black rice bran which contained high content of cyanidin-3-glucoside had the high antioxidative activities. **Conclusion:** Pigmented rice bran cultivar Hommae-payathongdum and Hom-nin following had highest antioxidative activities. These pigmented rice cultivars, with high antioxidative potential, may provide a source of new antioxidants, for new improved varieties, for use in foods with pharmaceutical properties, thereby increasing rice consumption and contributing to the prevention of diseases caused by oxidative damage to cells.

Keywords: Pigmented rice, Anthocyanin, Antioxidant

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1. Introduction

Rice is the principle cereal food and the staple food of the world's population (Han *et al.*, 2004). Colored rice is a hulled grain having red or purple color in addition to light gray on its bran, especially black rice has long been consumed and considered to be a healthy food. It is reported that colored rices contain a lot of anthocyanin pigments (Ryu *et al.*, 2003). Anthoc yanins are belong to water-soluble plant pigments and representatives of flavonoids which are responsible for the blue, purple and red color of many plant tissues. The colors are red to purple or blue, depending on pH. They occur primarily as glycosides of their respective molecule, the position of this attachment, aglycone anthocya nidin chromophores. The differences between individual anthocyanins relate to the number of hydroxyl groups, the nature and number of sugars attached to the and the nature and number of aliphatic or aromatic acids attached to sugars in the molecule (Han et al., 2006). Reactive free radicals have been postulated to contribute to the causes of chronic inflammatory proliferative diseases (CIPD), especially arteriosclerosis and cancer, through oxidative damage of essential enzymes, cells, and tissues (Klaunig and Kame ndulis, 2004). Aerobic respiratory organisms use of oxygen to produce energy for living, but reactive oxygen species (ROSs) are also gene rated during the oxidative metabolisms. ROSs may induce cellular damage when ROSs increased and antioxidant defense systems are overwhelmed. There is therefore widespread interest in defining the possible role of the diet in preventing and reversing reactive oxygen species (ROs)-induced chronic diseases (Takahashi *et al.*, 2004).

Anthocyanins may reduce the risks of cardiovascular diseases and cancer with antii nflammatory, antioxidant and chemoprotective properties and they were found to inhibit low density lipoprotein oxidation *in vitro*. Delphinidin could inhibit cell invasion of human fibrosarcoma HT-1080 cell *in vitro*. (Chen *et al.*, 2006). The present study was aimed at characterizing anthocyanin composition in a diverse colored rice brans to identify anthocyanin-rich grains and their antioxidative potential for the development of functional foods and/or functional food colorants.

2. Materials and methods

2.1 Cereal grains

A diverse array of black, blue, purple, red, and white rice were used in the present study. These included black rice (cultivars Hom-nin, Homdum-sukothai, Homnin-jakkapat, Homma e-payathongdum), blue rice (cultivar Riceberry), purple rice (cultivar Khawklum), red rice (cultivar Homdang-sukothai), white rice (cultivar Saohai). Rice samples were obtained from Rice Research Center, Pathumthani province, Thailand.

2.2 Anthocyanin extraction

Anthocyanins in rice brans were extracted according to the method described by Abdel-Aal and Hucl (2003) with slight modifi cations. Three grams of the ground materials was extracted twice by mixing with 24 mL of methanol acidified with 1.0 N HCl (70:30, v/v) and shaking on rotatory shaker at 200 rpm for 24 hours. The apparent pH of the mixture was adjusted to 1.0 before shaking and was checked and readjusted if necessary after 15 and 30 minutes of shaking. The crude extracts were centrifuged at 10,000 *g* and 4 ºC for 20 minutes and then refrigerated for 2 days to precipitate large molecules. The extracts were recentrifuged at 10,000 *g* and 4ºC for 20 minutes.

2.3 Quantification of total anthocyanin

Total anthocyanin content (TAC) deter mination was based on a pH-differential method and expressed as delphinidin-3-glucoside equiva lents (Giusti and Wrolstad, 2005) according to the following formula:c [mg/L] = A·M·DF/ƐM·d, with A = absorption value, M = molecular weight of delphinidin-3-glucoside (465 g/mol), DF = dilution factor, ƐM = molar extinction coefficient of delp hinidin-3-glucoside at pH 1 (29,000 L/mol· cm), and d = path length of the cuvette (1 cm).

2.4 DPPH (1-1 Diphenyl-2-picryl hydrazyl) assay

The free radical scavenging activity of different fractions was measured by the DPPH scavenging method proposed by Shimada, *et al*., (1992). 2.5 x 10^{-4} M solution of DPPH in methanol was prepared and 2.0 mL of this solution was added to 2.0 mL of different rice extracts obtained in different storage conditions. The mixture was shaken vigorously and left to stand for 30 minutes in the dark, and the absorbance was then mea sured at 517 nm against a blank. The DPPH radical-scavenging activity was calculated accor ding to the following: % of DPPH scavenging activity = {1- (AbS/AbC)} x 100, where AbC was the absorbance of the control and AbS was the absorbance in the presence of the test compound. EC_{50} is the effective concentration in mg extract/ mL which inhibits the DPPH activity by 50%. Value was obtained by interpolation from linear regression analysis. Butylated hydroxytoluene (BHT) was used for comparison.

2.5 Analysis of anthocyanins

Anthocyanins in the extracts were sep arated and quantified with an 1100 series chromatograph (Agilent, Mississauga, ON, Canada) equipped with a G1311A quaternary pump, G1329A temperature controlled injector, G1316A temperature-controlled column therm ostat, G1322A degasser, G1315B UV-visible detector. A 150 × 2.00 mm, 5 µm particle size Phenomenex Luna C18 (2) rapid resolution column was employed for separation. Separation of anthocyanins was conducted at temperatures, 60 ºC, to improve separation efficiency, partic ularly for those grains exhibiting complex anthoc yanin composition. The column was eluted with a gradient mobile phase consisting of (A) 6% formic acid and (B) absolute methanol at 1 mL/ minutes. The gradient was programmed as follows: 0-7 minutes, 82-80% A; 7-10 minutes, 80-75% A; 10-25 minutes, 75-40% A; 25-26 minutes, 40-82% A; and 26-28 minutes, hold at 82% A. The separated anthocyanins were detected and measured at 520 nm. Five selected pure anthocyanin compounds including delphin idin 3-glucoside, cyanidin 3-glucoside, pelargon idin 3-glucoside, peonidin, malvidin were purchased from Polyphenols Laboratories (Sandens, Norway) (Zhang *et al*., 2004).

2.6 Statistical analysis

The data were subjected to analysis of variance to determine differences between samples. The data were reported as mean of triplicates standard deviation (S.D.).

3. Results

UV-vis data also provide some means of confirming some of the aglycones. Table 1 shows UV-vis maximum absorption wavelength (λ_{max}) for delphinidin-3-glucoside at 524-529 nm, for cyanidin-3-glucoside at 514-517 nm, for pelargonidin-3-glucoside observed at 500-504 nm, for peonidin at 522-526 nm and for malvidin at 527-532 nm.

Table 1 Maximum absorption wavelength of anthocyanins in pH 1 buffer

Anthocyanin	λ_{max}
Delphinidin-3-glucoside	527
Cyanidin-3-glucoside	516
Pelargonidin-3-glucoside	502
Peonidin	524
Malvidin	529

The TAC varied significantly between black, blue, purple, red, and white rice brans (Table 2). Significant differences in the concentration of total anthocyanins were found between black, blue purple and red rice bran. Black rice brans had a wide range of total anthocyanins depending upon cultivar (Ryu *et al.*, 1998). In the present study, black rice brans, with an approximate of 2,400 µg/g, was found to possess the highest TAC among all of the studied colored rice bran, which is 24 times higher than that of red rice bran (102 µg/g) (Table 1). On the other hand, white rice bran had a very small concentration of TAC (12 µg/g), which may belong to one or more other groups of pigments because no anthocyanin peaks were detected by HPLC analyses in the white rice bran extract. The results on free radical quenching capability of rice bran antioxidants obtained by solvent extractions are presented in Table 1. Total antioxidant activity of the rice bran extracts increased with the increasing concen tration of the extracts and a significant was deter mined for of the extract (Table 1). However, the total antioxidant activities of all the rice bran extracts were less than that of the positive control synthetic antioxidant, BHT (EC_{50} of 27.30 mg/L).

Figure 1 shows HPLC-UV-vis chroma tograms of anthocyanin standard mixture sep arated on a C18 column. The peaks show the major anthocyanins that are identified. For a given saccharide combined with the various possible aglycones, the relative HPLC elution of the compounds follows the order delphinidin-3-glucoside, cyanidin-3-glucoside, pelargonidin-3-glucoside, peonidin and malvidin.

Figure 1 HPLC profiles of acidified methanol (1% HCl) anthocyanin on a C18 column. Detection was at 520 nm: (a) delphinidin-3-glucoside; (b) cyanidin-3-glucoside; (c) pelargonidin-3-glucoside; (d) peonidin; (e) malvidin.

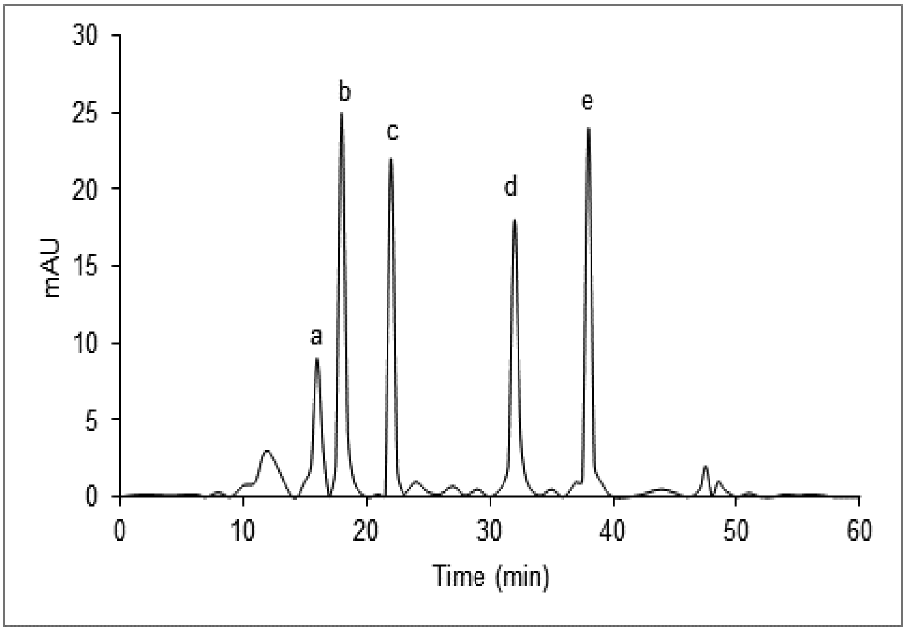

Table 2 Total anthocyanin contents (TAC) determined by pH-differential method and antioxidant capacity of pigmented rice brans

Rice cultivar	Pigmented rice	TAC (μg/g)	DPPH (EC_{50})*(mg/L)
Hom-nin	Black rice	1,937±78.6[a]	72.62±4.26[a]
Homdum-sukothai	Black rice	2,025±32.7[b]	92.17±6.15[b]
Homnin-jakkapat	Black rice	3,594±64.1[c]	106.44±9.34[b]
Hommae-payathongdum	Black rice	2,042±49.6[b]	61.32±8.72[a]
Riceberry	Blue rice	1,719±44.2[d]	152.46±16.46[c]
Khawklum	Purple rice	1,261±51.4[e]	94.40±7.19[b]
Homdang-sukothai	Red rice	102±7.8[f]	177.94±12.32[c]
Saohai	White rice	12±3.6[g]	673.69±48.64[d]

Values are expressed as mean ± S.D. (n = 3). Values within each column with the same superscript are not significantly different at $p < 0.05$.

*EC_{50} value, the effective concentration at which the antioxidant activity was 50%; the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was scavenged by 50%.

Anthocyanins were extracted from pigmented rice brans. The anthocyanin was obtained from 70:30 (v/v) methanol:water contai ning 0.1% HCl fraction. TACs in the pigmented rice brans and non-pigmented rice bran were 102-3,594 μg/g and 12 μg/g, respectively (Table 2). The anthocyanin extract HPLC chromatogram has three peaks which peak were delphinidin-3-glucoside, cyanidin-3-glucosde and the other is pelargonidin-3-glucoside. Cyanidin-3-glucoside was found in all pigmented rice brans in this study. It was the major anthocyanin in black and red rice bran. In addition, delphinidin-3-glucoside and pelargonidin-3-glucoside was also found in black rice brans. Delphinidin-3-glucoside was the major anthocyanin found in purple rice bran while pelargonidin-3-glucoside was the major anthoc yanin in blue rice bran but it was also found in small amount in red rice bran (Table 3).

Table 3 Average concentration of anthocyanins in black, blue, purple and red rice brans

Rice cultivar	Anthocyanin (μg/g)				
	Dp-3-Glu	**Cy-3-Glu**	**Pp-3-Glu**	**Pn**	**Mv**
Hom-nin	43±2.7[a]	1,170±14.6[a]	220±6.2[a]	nd	nd
Homdum-sukothai	36±6.4[a]	1,490±23.3[b]	46±7.3[b]	nd	nd
Homnin-jakkapat	170±4.4[b]	2,710±12.8[c]	239±8.6[c]	nd	nd
Hommae-payathongdum	75±8.9[c]	1,760±15.3[d]	227±6.9[c]	nd	nd
Riceberry	nd	236±6.3[e]	1,242±14.7[d]	nd	nd
Khawklum	862±12.7[d]	124±8.2[f]	nd	nd	nd
Homdang-sukothai	nd	47±5.8[g]	16±1.4[e]	nd	nd
Saohai	nd	nd	nd	nd	nd

Values are expressed as mean ± SD (n = 3). Values within each column with the same superscript are not significantly different at $p < 0.05$. Dp, delphinidin; Cy, cyanidin; Pg, pelargonidin; Glu, glucoside; Pn, peonidin; Mv, malvidin. nd, not detected.

4. Discussion

The TAC results indicate that colored rice bran such as black, blue and purple rice bran may hold promise for the development of functional foods and/or natural colorants. The availability and agronomic performance of these rice brans will determine their potential market. Blue rice bran had an average TAC of 1,719 μg/g (Table 2), which is higher than that red rice bran (102 μg/g). Additionally, anthocyanin concentrations were significantly influenced by growing conditions and environment in pigmented rice due to the pigment location in the outer pericarp or fruit coat (Abdel-Aal and Hucl, 2003). Thus, anthocyanins in pigmented rices are more prone to environmental effects. Purple rice bran used in the present study contained lower TAC compared to blue rice bran (Table 2). Red and white rice bran exhibited small concentrations of TAC. These data are in agreement with previous results (Abdel-Aal and Hucl, 2003). The HPLC analysis of white rice bran extracts showed an absence of anthocyanin compounds, which indicates that the small amount of TAC may be contributed by one or more other groups of pigment. Fractionation of rice kernels into bran by abrasive or roller milling was able to concentrate anthocyanin pigments in the bran fractions. In black, brown, and red the bran fraction contained 3-4 times higher anthocyanin content than the whole grains. The addition of time of abrasion might increase the pigment recovery in the combined bran fractions obtained from pigmented rices (Awika *et al*., 2004).

In general, the colored grains studied showed substantial differences in their TAC, and some of them, such as black rice, purple corn, and blue wheat, had remarkable levels of anthocyanins. When the relationship between the total anthocyanin content determined by colorimetry (Table 2) versus that determined by HPLC (Table 3) was examined, a significant positive correlation was obtained with a correlation coefficient (*r*) of 0.987 and a slope of 1.1889. This shows that the colorimetric method overestimated the HPLC values by 19%. The difference may be due to the contribution of other pigments present in the grains that have an absorbance at 535 nm. This overestimation was consistent from one grain to another. Anthocyanins have been recognized as health-enhancing substances and have been found in many types of grains. The present study showed a diversity of anthocyanins in a selection of black, blue, purple, and red rice brans. It also shows substantial differences in anthocyanin content and composition among the rice brans studied. Such diversity in anthocyanin composition would help in the selection process for the development of anthocyanin-rich grain products. In addition, the anthocyanin pigments in rice brans can be concentrated by dry milling and fractionation processes to produce fractions that are high in anthocyanin contents, even much higher than those found in fruits and vegetables. The data suggest that some of the colored rice brans such as black rice brans, may hold promise for the development of grain-based functional foods or natural colorants on the basis of their anthocyanin content and composition.

The colored grains investigated exhibited diverse anthocyanin compositions. Cyanidin-3-glucoside was the most abundant anthocyanin in black rice and red rice, accounting for 81-95% and 77% of the total anthocyanins, respectively. Pelargonidin-3-glucoside came second in black and red rice, whereas delphinidin-3-glucoside was the third major anthocyanin in black rice brans (Table 3). Ryu et al. (1998) found two main anthocyanins in 10 black rice varieties in which cyanidin-3-glucoside is the most common (0.0-470 mg/100 g), whereas peonidin-3-glucoside (0.0-40 mg/100 g) is the second. The anthocyanin composition of blue rice bran differed from that of black rice brans. This study identified the main anthocyanin in blue rice bran as pelargonidin-3-glucoside, being 84% of the total anthocyanins. Cyanidin-3-glucoside was the second dominant anthocyanin at 16% of the total anthocyanins. In purple rice bran, 2 anthocyanin compounds were observed with delphinidin-3-glucoside and cyanidin-3-glucoside. Delphinidin-3-glucoside was the predominant anthocyanin in purple rice bran. Anthocyanin was not detected in white rice bran. This indicates that no other pigment contribute to the color of the grain in white rice (Table 3).

The main anthocyanin pigments of black rice have been reported to be cyanidin-3-glu coside, peonidin-3-glucoside and petunidin-3-glucoside (Pt-3-Glu) (Yao *et al*., 2010). In this study, it was found that black rice (Homnin-jakkapat) contained higher TAC than the others, especially cyanidin-3-glucoside which had strong antioxidant activities *in vitro* system. From the results, it could be concluded that Homnin-jakkapat has high amount of anthocyanin contents on its bran and anthocyanins can be isolated from the Homnin-jakkapat by chromatography successively. In red rice, it is not so clear: Abdel-Aal *et al.* (2006) reported that cyanidin-3-glucoside was the main anthocyanin, whereas Kim *et al*. (2008) concluded that red rice did not contain anthocyanin pigments. However, in this study, Homdang-sukothai rice bran was found that it contained pelargonidin-3-glucoside and cyanidin-3-glucoside that corresponded to the previous report of Abdel-Aal *et al*. (2006). Anthocyanins are the most prominent pigments in rice bran and they are strong antioxidants. Their double bond conjugate systems allow electron delocalization, resulting in very stable structures and a powerful antioxidant activity. Furthermore, the extent and position of hydroxylation and methoxylation in the B ring modulates their stability and reactivity (Pereira *et al*., 1997). Differences in antioxidant activities between various anthocyanins have been noted in several studies. Although Homnin-jakkapat contains higher TAC than the others, however, Hommae-payathongdum has highest antioxidant capacity. The increase in anthocyanin content in rice bran was not always associated with a similar proportional increase in antioxidant capacity. It has been reported that pH differences have a major influence on scavenging capacity of anthocyanins and that the presence of acid in the solvent has a negative influence on the antioxidant capacity of samples.

ROSs plays a crucial role in a wide range of common diseases and age-related degenerative conditions including cardiovascular disease, inflammatory conditions, and neurodegenerative diseases such as Alzheimer's disease, mutations and cancer (Han *et al*., 2004). So antioxidant capacity is widely used as a parameter to characterize food or medicinal plants and their bioactive components. In this study, the antioxidant activity of the anthocyanin extract was evaluated and it showed very strong antioxidant activity. Thus, these results suggest that anthocy anin extract from black rice can be used as anti oxidant material, food additives. This suggests that use of anthocyanin extract from black rice may offer an attractive new antioxidant agent against ROS. In Table 3, the anthocyanins for which structures are noted as confirmed on the basis of congruence of properties with authentic standards are completely named because glucose has been reported as the most common hexose in grain anthocyanins (Moreno *et al*., 2005).

It is known that the anthocyanin bio synthesis pathway is controlled in response to different developmental and environmental cues. The colour of rice results from the accumulation of the corresponding pigments, the orange to red pelargonidin, the red to magenta cyanidin and the violet to blue delphinidin. Each variety of pigment rice has a unique set of anthocyanins (Mazza and Miniati, 1993). In the present work, the TAC was calculated value and identified five anthocyanins and in contrast to pigment rice brans considering the contribution of individual anthocyanins to the TAC on the basis of their concentration and antioxidant capacity.

5. Conclusion

This study suggests that medicinal rice plants can be promising sources of potential black rice bran antioxidants and anticancer activity. The present results will form the basis for selection of black rice species for further investigation in the potential drug discovery of new natural bioactive compounds. Homnin-jakkapat and Hommae-payathongdum are good choices for the plant scientists to develop new rice cultivars with high bioactive compounds with high nutritive value.

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