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Anthocyanins, phenolic compounds and antioxidant activities in colored corn cob and colored rice bran

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Abstract

Colored rice and corn have been widely cultivated in Thailand. Some varieties of both plants contain high amounts of bioactive compounds which may contribute to antioxidant activity. HPLC analysis showed that red (Hom Mali Deang; HMD) and black (Hom Nin; HN, Khao Niaw Dum; KND, Khao Niaw Dum Mong; KNDM and Maled Pai; MP) rice brans, and two corn varieties, KND and KGW, with red (R-KND and R-KGW, respectively) and black (B-KND and B-KGW, respectively) color cobs, contained two major anthocyanins, cyanidin-3-glucoside and peonidin-3-glucoside; however, pelargonidin-3-glucoside was detected in purple corn cob. Syrigic acid was the dominant phenolic compound in rice bran and corn cob, whereas, ferulic acid, gallic acid, vanillic acid and 4-hydroxybenzoic acid were found in smaller amounts. Among corn varieties, B-KND had highest anthocyanins and phenolic content; among rice varieties the highest anthocyanins content was found in MP, and the highest phenolic content in HMD. The highest antioxidant activity using $ABTS^{\bullet+}$ and FRAP assays were observed in B-KND; HMD showed highest antioxidant activity with the $DPPH^{\bullet}$ assay. The results suggest that corn cob of B-KND and rice bran from MP, HMD could be a potential natural source of functional antioxidant food ingredients.

Introduction

Presently, there are several natural sources of antioxidants including fruits, vegetables and cereals (Chu *et al.*, 2000; Harnly *et al.*, 2006; Menga *et al.*, 2010). The consumption of foods containing anthocyanins and phenolics is now widespread due to the antioxidant activities of these food components (Bagchi *et al.*, 2004; Li *et al.*, 2012), that are associated with potential health benefit effects such as anti-cancer (Nikkhah *et al.*, 2008), cardiovascular diseases (Wallace, 2011), detoxification activity (Ajiboye *et al.*, 2011), anti-proliferation (Lo *et al.*, 2007), anti-angiogenic activity (Bagchi *et al.*, 2004) and anti-inflammatory (Bowen-Forbes *et al.*, 2010).

Anthocyanins are water soluble compounds and belong to a group of phenolics which provide the orange, pink, red, violet and blue colors in some plants; six anthocyanins which commonly occur in nature are cyanidin, peonidin, pelargonidin, malvidin, delphinidin and petunidin which are different in the number and position of methoxyl and hydroxyl groups (Castañeda-Ovando *et al.*, 2009). The common sources of anthocyanins are fruits and vegetables; for example, blackberry (245 mg/100g), chokeberry (1480 mg/100g), black currant (476 mg/100g), black raspberry (687 mg/100g), Concord grape (120.1 mg/100g), elderberry (1375 mg/100g), red cabbage (322 mg/100g), red radish (100.1 mg/100g) (Wu et al., 2006). Additionally, some pigmented cereals have been considered as a source of anthocyanins and phenolics with potential application as food colorant and health food ingredients (Escribano-Bailón *et al.*, 2004; Dykes and Rooney, 2007). Anthocyanins have been reported to be concentrated in the bran fraction of cereals, such as black sorghum bran (980 mg/100g) (Awika *et al.*, 2005), purple wheat bran (115.5 mg/100g) (Li *et al.*, 2007a) and black barley bran (15.87 mg/100g) (Siebenhandl *et al.*, 2007), suggesting that the bran fraction of cereals could be a source of anthocyanins. Recently, purple corns have been reported to be a good source of anthocyanins (Jing *et al.*, 2007; Yang and Zhai, 2010b), and are found in relatively high quantities in corn husk (Li *et al.*, 2008), corn cobs (Jing *et al.*, 2007; Yang and Zhai, 2010a) and corn silk (Sarepoua

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et al., 2015). In corn, the anthocyanins and phenolic compounds in whole cob section account for 54.6% and 59.3%, respectively of the corn (Cevallos-Casals and Cisneros-Zevallos, 2003), while, in rice 33-45% of the phenolic compounds is in rice bran (Butsat and Siriamornpun, 2010). The previous studies have been reported about the application of phytochemical compounds in purple corn cob and colored rice. Cevallos-Casals and Cisneros-Zevallos (2004) reported that anthocyanins from purple corn under acidic solution showed similar shade of color to synthetic red dye. In general, the application of anthocyanins as food colorant are usually used in acidic food system such as fruit beverages and jams because they provide an attractive red color (Jing and Giusti, 2005). However, Jing and Giusti (2005) suggested that anthocyanin extracts from purple corn cob showed effective colorant in neutral pH food such as milk. Besides, Jing (2006) reveled that phytochemical compounds in purple corn cob are associated with potential beneficial effects on health that can be used as value-added ingredients in dietary supplement. Likewise in colored rice, Wang *et al.* (2007) reported that the supplementation of black rice pigment fraction showed positive effect in patients with coronary heart disease.

There are many varieties of colored rice which have been introduced in Thailand, that provide a range of desirable colors and contain high levels of both anthocyanin and phenolic compounds (Sompong *et al*., 2011). Purple corn is originated in Latin America, especially in Peru and Bolivia. The special varieties of waxy purple corn of an open-pollinated and a new hybrid variety were developed at Khon Kaen university, Thailand, with improved eating quality and high anthocyanin contents (Lertrat and Thongnarin, 2008; Harakotr *et al*., 2014). However, some desirable varieties of colored rice bran and corn cob in Thailand have not been characterized in terms of their anthocyanin and phenolic contents as well as antioxidant activities, in order that they can be considered as the important natural food colorants or functional food ingredients.

The objectives of this study were to identify and compare the main anthocyanins, phenolic compounds and antioxidant activities present in varieties of colored corn cob and rice bran, as well as to provide database information for phytochemical-rich plants in Thailand.

Materials and Methods

Chemicals and reagents

2,2'-azino-bis(3-ethylbenzothiazoline-6-solfonic acid diammonium salt (ABTS), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), vanillic acid, caffeic acid, and ferulic acid were purchased from Fluka. 2,2-diphenyl-1-picrylhydrazyl (DPPH), 4-hydroxybenzoic acid, gallic acid, syringic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), peonidin-3-glucoside (Pn-3-G), and pelargonidin-3-glucoside (Pg-3-G) were purchased from Sigma-aldrich. Cyanidin-3-glucoside (Cy-3-G) was purchased from Chromadex®. Folin-ciocalteu reagent was purchased from Merck.

Materials

Rice bran of five colored rice varieties which included one red rice (Hom Mali Deang, HMD) and four black rice (Hom Nin, HN; Khao Niaw Dum, KND; Khao Niaw Dum Mong, KNDM and Maled Pai, MP) was milled using a laboratory rice miller (Thongtavee rice mill Co., Ltd., Thailand). Dried corn cobs from four corn varieties of purple waxy corn cob which included red and black color of an open-pollinated, KND (R-KND and B-KND, respectively) and F1-hybrid, KGW (R-KGW and B-KGW, respectively) were crushed into coarse particles and ground into a fine powder using a hammer mill (Perten lab mill 3100, Sweden). All materials were obtained from the Plant Breeding Research Center for Sustainable Agriculture, Department of Agronomy, Faculty of Agriculture, Khon Kaen University, Thailand.

Sample extraction

The extraction procedure for anthocyanins and phenolics was based on a method of Awika *et al*. (2005) with some modifications; methanol was selected as an extraction solvent according to Ramos-Escudero *et al.* (2012) and Lai *et al.* (2009). All samples (1.0 g) were extracted with 10 ml of methanol then shaken for 2 h and then centrifuged at 3,000 rpm for 10 min. The mixture was filtered (Whatman No.1 filter paper) and the residues were re-extracted two times with 5 ml of methanol using the same procedure. The three aliquots were combined and stored at -18°C in the dark until analyzed.

Determination of total phenolic content (TPC)

TPC of the extracts was determined using the method of Dewanto *et al*. (2002). The reaction mixture containing 125 µl of extract (with appropriate dilution) and 250 µl Folin-Ciocalteu reagent followed by the addition of 3 ml distilled water, was mixed well and then allowed to stand for 6 min, then added to 2.5 ml of 7% sodium carbonate solution. The reaction mixture was allowed to stand for 90 min at room

temperature before measuring absorbance at 760 nm (Shimadzu UV-1800 spectrophotometer, Japan). Gallic acid was used as a calibration standard and results were expressed as mg gallic acid equivalent per 100 g sample.

Determination of total anthocyanin content (TAC)

TAC was determined using the pH-differential method described by Lee *et al.* (2005). Two aliquots of 50 µl of extract were added with 3 ml of 0.025 M of potassium chloride buffer at pH 1.0 and 0.4 M of sodium acetate buffer at pH 4.5, then allowed to stand for 20 min before measuring absorbance at 520 and 700 nm. Total anthocyanins content was calculated using the following equation and expressed as cyanidin-3-glucoside equivalent per 100g sample.

$$\text{Total anthocyanins (mg/100g)} = (\Delta A/\epsilon L) \times MW \times D \times (V/G) \times 100$$

Where ΔA is absorbance ((A_{520}nm – A_{700}nm) pH 1.0 – (A_{520}nm – A_{700}nm) pH 4.5), ϵ is molar extinction coefficient of Cy-3-G (29,600 $M^{-1}\,cm^{-1}$), L is cell path length of cuvette (1 cm), MW is molecular weight of anthocyanins (449.2 g/mol), D is a dilution factor, V is a final volume (ml) and G is weight of sample (g).

Determination of $ABTS^{\bullet+}$ scavenging assay

The procedure described by Stratil *et al.* (2006) was used, with some modifications. Radical cation of $ABTS^{\bullet+}$ was generated by reacting ABTS (7 mM) with $K_2O_8S_2$ (4.95 mM) with the ratio 1:1 (v/v) for 12 h at room temperature in the dark. $ABTS^{\bullet+}$ working solution was diluted with phosphate buffer saline (PBS, pH 7.4) to absorbance 1.0 AU at 734 nm. Aliquots of 40 µl sample extracts (with appropriate dilution) were mixed with 4.0 ml working solution, then allowed to stand for 10 min before measurement. Antioxidant activity was expressed as mg Trolox equivalent per 100 g sample.

Determination of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•) scavenging assay

DPPH free radical scavenging activity was determined according to the method described by Leong and Shui (2002), with some modifications. Freshly prepared solution of 0.1 mM solution of $DPPH^{\bullet}$ in methanol was prepared with absorbance 1.0 AU at 517 nm. An aliquot of 100 µl of each sample (with appropriate dilution) was mixed with 4.0 ml of $DPPH^{\bullet}$ solution, then allowed to stand at room temperature for 30 min before measurement. Antioxidant activity was expressed as mg Trolox equivalent per 100g sample.

Determination of Ferric Reducing Ability of Power (FRAP) assay

FRAP assay was determined using the method described by Benzie and Strain (1996), with some modifications. FRAP reagent was prepared by mixing 1 volume of an aqueous 10 mM solution of TPTZ reagent in 40 mM HCl with 1 volume of 20 mM $FeCl_36H_2O$ and 10 volume of acetate buffer of pH 3.6 (3.1 g sodium acetate and 16 ml acetic acid per liter). An aliquot of 150 µl sample extract (with appropriate dilution) was added to 2.90 ml of FRAP reagent, then allowed to stand at room temperature for 30 min before measurement at 593 nm. Fresh working solutions of ferrous sulfate ($FeSO_4$) were used as standard of calibration curve and expressed as mg $FeSO_4$ equivalent per 100g sample.

HPLC-DAD analysis of phenolic compounds

HPLC analyses were performed using a 1100 Series Agilent Technology (Agilent,Palo Alto, CA, USA), equipped with photodiode array detector. The extracts were passed through a 0.45 µm filter before injection into the HPLC system. 30 µl filtered extract was loaded onto the 3 µm particle diameter, 150×3.9 mm i.d. Atlantis TM d C18 column coupled to a 0.2 µm, Phenomenex guard column. The mobile phase was a gradient solvent containing methanol (A) and 3% acetic acid in water (B); elution conditions were as follows: 0% A at 0 min, increasing to 20% A within 5 min, to 30% A within the next 5 min, holding at 30% A for 5 min and increasing to 35% A within the next 5 min, to 40% A within the next 10 min and decreasing to 30% A within the next 10 min, before equilibration at 0% A. The solvent flow rate was set at 0.5 ml/min and the column temperature at 25°C. Phenolic compounds were detected at 280 nm.

HPLC-DAD analysis of anthocyanins

HPLC analyses were performed using a 1100 Series Agilent Technology which was equipped with diode array detector. 50 µl extract was loaded into a 5 µm particle diameter, 250×4.0 mm i.d. Hypersil ODS C18, column coupled to an 5 µm, ODS guard column, operating at 25°C at a flow rate of 1.0 ml/min and the chromatogram was recorded at 520 nm. Mobile phases consisted of acetonitrile (A) and 4% phosphoric acid (B). The solvent gradient was as follows: 6% A at 0 min, increasing to 25% A within 10 min, holding at 25% A for 5 min and decreasing to 6% A within the next 10 min, and equilibrated before the next injection.

Statistical analysis

All measurements were done in triplicate and the data reported as means + SD. The statistical analysis was determined by one-way ANOVA using IBM® SPSS Statistics (version 19.0). Duncan's multiple range test was used to determine significant differences between means at $p<0.05$. Correlation coefficient was performed between TPC, TAC and antioxidant activities by Pearson's correlation analysis. All measurements were done in duplicate.

Results and Discussion

Total phenolic and anthocyanin content

TPC and TAC (expressed as gallic acid and Cy-3-G equivalent per 100g of sample, respectively) are shown in Table 1. The highest TPC was observed in B-KND (2,246.80+22.52 mg/100g) compared to all the corn cob extracts; the red corn cob extract from the same KND variety had a significantly lower TPC (833.42+131.72 mg/100g). TPC in KND and KGW corn cob was similar to that in corn cob studied by Jing et al. (2007) (950-3,516 mg/100g) but lower than that reported by Cevallos-Casals and Cisneros-Zevallos (2003) (4,395+84 mg/100g). Among rice varieties, red Thai rice, HMD contained 1,612.13+60.05 mg/100g TPC, black rice bran extracts contained TPC in the range of 824.92-1,317.23 mg/100g) which is lower than TPC in pigmented rice bran reported by Laokuldilok *et al.* (2011) (1,526-3,289 mg/100g) and Zhang *et al.* (2010) (2,086-7,043 mg/100g). Our results indicate that TPC in red rice bran was significantly higher compared to black rice bran; this is in an agreement with results reported by Sompong *et al.* (2011) and Chen *et al*. (2012). However, Yao *et al.* (2010) reported that TPC in black rice was higher than those of red and purple, suggesting that rice cultivar mainly impact phenolic content rather than their color (Chen *et al*., 2012).

The extract from B-KND variety was significantly higher in TAC (1,426.24+76.99 mg/100g) compared to those of corn cobs (202.40-310.53 mg/100g). TAC in corn cobs was similar to that in corn cob studied by Jing *et al*. (2007) (290-1,333 mg/100g), but lower than that reported by Li *et al.* (2008) (2,404 mg/100 g) and Cevallos-Casals and Cisneros-Zevallos (2003) (3,752 mg/100g). The TAC and TPC of corn cob are affected by many factors such as cultivars as well as growing conditions (Jing *et al.*, 2007). Black rice bran of MP showed highest TAC (668.56+190.14 mg/100g) and the lowest was found in red rice bran HMD (28.42+11.31 mg/100g); all black rice bran extracts were significantly higher than the red rice bran extract. These results suggest that higher TAC might be related to black color of both black color corn cob and black color rice bran. Similar results have been reported amongst cereals such as rice (Laokuldilok *et al*., 2010; Sompong *et al*., 2011), wheat (Knievel *et al.*, 2009), corn seed (Zhao *et al*., 2009) and sorghum (Dykes *et al.*, 2006). Our results suggest that B-KND variety can be considered as an inexpensive and excellent source of phenolics as well as anthocyanins.

Table 1. Total phenolic and anthocyanins content of different purple corn cob and colored rice bran varieties

Sample varieties	TPC (mg gallic eq./100g)	TAC (mg Cy-3-G eq./100g)
Purple corn cob		
R-KGW	644.05 ± 75.82 ^{f}	202.40 ± 29.60 ^{c}
R-KND	833.42 ± 131.72 ^{e}	310.53 ± 34.57 ^{c}
B-KGW	780.16 ± 17.41 ^{e}	254.25 ± 64.15 ^{c}
B-KND	2,246.80 ± 22.52 ^{a}	1,426.24 ± 76.99 ^{a}
Rice bran		
HMD	1,612.13 ± 60.05 ^{b}	28.42 ± 11.31 ^{d}
KNDM	1,254.41 ± 31.44 ^{c}	314.72 ± 50.69 ^{c}
MP	1,317.23 ± 48.35 ^{c}	668.56 ± 190.14 ^{b}
KND	824.92 ± 25.97 ^{e}	252.18 ± 79.14 ^{c}
HN	992.58 ± 26.73 ^{d}	252.11 ± 44.56 ^{c}

Values within each column with the same letter are not significantly different ($p<0.05$).

Identification of individual phenolic and anthocyanin compounds

The chromatographic profiles of the major anthocyanins in corn cob and rice bran are shown in Figure 1. The results indicate the presence of three major anthocyanins in colored corn cobs, Cy-3-G (peak 1), Pg-3-G (peak 2) and Pn-3-G (peak 3) with the concentrations of 684.35-852.59, 12.9-48.94 and 53.87-217.39 mg/100g, respectively (Table 2); these anthocyanins in colored corn cob have been identified in colored corn cob by other researchers (Jing and Giusti, 2007; Yang and Zhai, 2010a). In addition, acylated anthocyanin containing malonic acid, including cyanidin-3-(6″-malonylglucoside), pelargonidin-3-(6″-malonylglucoside) and peonidin-3-(6″-malonylglucoside) have also been found in purple corn (Jing and Giusti, 2007; Yang and Zhai, 2010a). Yang and Zhai (2010b) reported that Cy-3-G was present with highest amount in purple corn cob, followed by Pn-3-G, Pg-3-G, respectively, these reports are consistent with our results.

Amongst rice cultivars, Cy-3-G was found to be the major anthocyanin in colored rice bran, followed by Pn-3-G; both compounds have been found in rice in previous studies (Frank *et al*., 2012; Hou *et al*., 2013). Black rice brans contain Cy-3-G and Pn-3-G in the range of 694.29-898.62 and 12.41-105.66

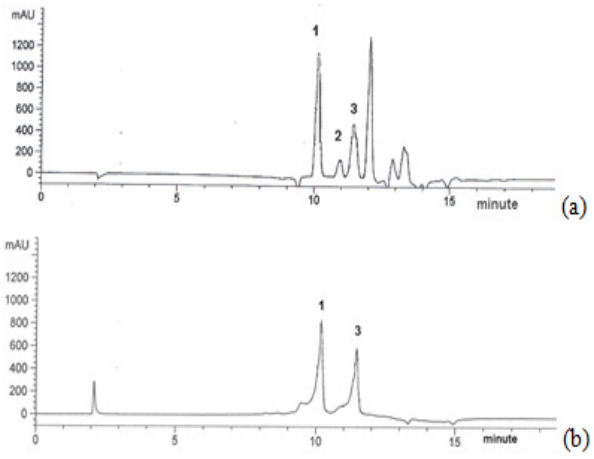

Figure 1. HPLC chromatograms of anthocyanins from (a) purple corn cob and (b) colored rice bran

mg/100g, respectively, however, both compounds were lower than those in black rice bran reported by Zhang *et al.* (2010) (736.6-2,557.0 and 100.7-534.2 mg/100g, respectively) and Laokuldilok *et al.* (2011) (662.2-2,316.7 and 141.7-472.3 mg/100g, respectively). Red rice bran, HMD contained Cy-3-G and Pn-3-G in the amount of 28.02 and 2.12 mg/100g, respectively, which are lower than those in black rice brans. However, Cy-3-G and Pn-3-G in HMD were lower than in red rice bran reported by Laokuldilok *et al.* (2011) (179.0 and 9.1 mg/100g, respectively). In addition, Pg-3-G was found only in KNDM and MP, with the concentration of 0.93 and 6.44 mg/100g, respectively. In a previous study by Pereira-Caro *et al*. (2013), small amounts of Pg-3-G was detected in Japanese black–purple rice seed; however, some colored rice have been reported to contain small amounts of delphindin and petunidin (Kim *et al.*, 2008; Yao *et al*., 2010). A number of studies have reported that anthocyanins may act as the protective effect on biological oxidative damage (Tsuda *et al*., 1994). Cy-3-G was a predominate anthocyanin detected in colored rice bran and corn cob from our materials. The study of Tsuda *et al.* (2000) found that Cy-3-G could act as an effective antioxidant in rat under oxidative stress and could elevate antioxidant enzymes (Shih *et al.*, 2007). Beside this, some reports indicated that Cy-3-G from purple corn has a potential for a prevention of obesity, diabetes (Tsuda *et al.*, 2003) as well as reduce the risks of cancer (Seeram *et al*., 2003). Although, Pn-3-G has been found in a second highest in all samples, however a previously study reported that Pn-3-G could prevent of lung cancer metastasis (Ho *et al.*, 2010). Moreover, Pg-3-G has been detected with the smallest amount in purple corn cob and some rice varieties, KNDM and MP, but this compound showed positive effect on prevention of atheromatosis (Hidalgo *et a*l., 2012).

Table 2. The individual major anthocyanin concentration in purple corn cob and colored rice bran

Sample varieties	Anthocyanin (mg/100g sample)		
	Cy-3-G	Pg-3-G	Pn-3-G
Purple corn cob			
R-KGW	752.29	17.79	76.40
R-KND	769.33	13.34	80.14
B-KGW	684.35	12.90	53.87
B-KND	852.59	48.94	217.39
Rice bran			
HMD	28.02	ND	2.12
KNDM	694.29	0.93	27.49
MP	963.20	6.44	47.05
KND	832.65	ND	12.41
HN	898.62	ND	105.66

ND: not detected

The most abundant phenolics found in colored rice bran was syringic acid (6.00-199.85 mg/100g), and with smaller concentrations of ferulic acid (7.14-7.47 mg/100g), vanillic acid (1.53-9.54 mg/100g) and 4-hydroxybenzoic acid (2.13-6.28 mg/100g). These results indicated that those materials were a good source of syringic acid, this compound could be an alternative treatment of cerebral ischemia on rat brain (Güven *et al*., 2015) and anti-hypertensive effect in rat (Kumar *et al*., 2012). Gallic acid was only detected in KNDM (2.58 mg/100g). Previous studies have been reported that gallic acid showed strong antioxidant activities using ORAC and TEAC assay (Yeh and Yen, 2003) as well as showed positive effect on the prevention of oxidative stress (Parihar *et al.*, 2014) and anti-inflammatory agent (Yang *et al.*, 2015). Black color rice bran and corn cob showed higher contents of all compounds as compared to red rice. Caffeic acid was not detected in all samples. However, ferulic acid was not found in three varieties of rice, HMD, KNDM and MP. Several studies have reported that ferulic acid was the predominant phenolic compound in corn (Del Pozo-Insfran *et al*., 2006; Cuevas Montilla *et al*., 2011) and rice (Sompong *et al*., 2011; Jun *et al*., 2012). Others phenolic have been found in rice with a small amounts including vanillic acid and p-coumaric acid (Butsat *et al*., 2010; Jun *et a*l., 2012), sinapic acid, caffeic acid, protocatechuic acid, hydroxybenzoic acid and syringic acid (Tain *et al*., 2005). Vanillic acid, p-coumaric acid and protocatechuic acid have been also detected in corn (Hu and Xu, 2011). However, phenolic composition in our methanol extracts was different from previous reports that could be due to the different extract condition. Some studies

Table 3. The individual phenolic concentration in purple corn cob and colored rice bran

Sample varieties	Phenolic (mg/100g sample)					
	caffeic acid	gallic acid	vanillic acid	4-hydroxybenzoic acid	ferulic acid	syringic acid
Purple corn cob						
R-KGW	ND	ND	5.87	2.71	9.34	43.33
R-KND	ND	ND	2.40	0.73	7.34	31.30
B-KGW	ND	ND	7.05	3.12	10.97	49.53
B-KND	ND	ND	1.42	2.17	10.73	102.78
Rice bran						
HMD	ND	ND	1.53	2.13	ND	6.00
KNDM	ND	2.58	3.35	3.72	ND	87.68
MP	ND	ND	4.00	6.28	ND	199.85
KND	ND	ND	8.83	ND	7.47	129.96
HN	ND	ND	9.54	ND	7.14	169.41

ND: not detected

reported that alkaline extraction of cereals could liberate soluble-conjugated and also bound form phenolic compounds whereas free form phenolics were the predominated found in alcohol extract (Li *et al*., 2007b; Park *et al.*, 2014). Van Hung *et al.* (2011) reported that ferulic acid was the major phenolic found in alkaline extract of sprouted wheats, whereas dominate phenolic acid found in alcohol extract was syrigic acid. However, there are still some phenolic compounds that remained unidentified in this study and follow up work is needed.

Antioxidant activities

Antioxidant activities were determined by $ABTS^{\bullet+}$, $DPPH^{\bullet}$ and FRAP assay, and reported as mg Trolox equivalent for both $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ assay, and mg $FeSO_4$ equivalent per 100 g sample, respectively; these assays have been widely used as measures of antioxidant capacities in plant (Dudonne *et al*., 2009). Among corn cob, the highest antioxidant activities using $ABTS^{\bullet+}$, $DPPH^{\bullet}$ and FRAP assay were observed for B-KND (3,033.64+160.56, 2,592.18+242.96 and 10,976.35+892.71 mg/100g, respectively), followed by R-KND variety (Figure 2). There were no significant differences in antioxidant activities between corn cob of B-KGW and R-KGW variety. Harakotr *et al*. (2014) compared antioxidant activities in different colored waxy corn seed cultivated in Thailand, and found that purplish black color of waxy corn seed showed higher antioxidant activities compared to dark pink and light purple color; however, little information is available with regards to antioxidant activity in different colors of corn cob. The highest antioxidant activity among rice brans was observed in HMD. The antioxidant activities of corn cob using $ABTS^{\bullet+}$ showed similar trend to $DPPH^{\bullet}$ and FRAP assay; similar results were obtained for rice brans. These results indicate that the extracts from corn cob and rice bran showed highly significant correlations between their radical scavenging activities ($ABTS^{\bullet+}$ and $DPPH^{\bullet}$ assay) and ferric reducing capacities (FRAP assay) (all $r>0.846$, $p<0.01$, data not shown).

Among corn varieties, antioxidant activities were significantly correlated with TPC and TAC; this is in agreement with Yang and Zhai (2010a) who reported that anthocyanin contents in purple corn cob contributes to antioxidant activities since these compounds can donate a hydrogen atom from their hydroxyl groups to reactive free radicles (Kay *et al*., 2004) and are able to reduce ferric ions (Yang and Zhai, 2010a). TPC in rice brans was highly correlated to all antioxidant activity assay used ($r>0.931$, $p<0.01$), but no relationship was observed between TAC and antioxidant activities (data not shown), that may be due to some rice brans such as HMD contained lower amounts of anthocyanins than phenolics (Pitija *et al.*, 2013). Our results were consistent with those of Sompong *et al*. (2011), who studied antioxidant activities in some colored rice varieties, and reported that TPC had highly positive correlation with antioxidant activities using FRAP and TEAC (Trolox equivalent antioxidant capacity).

A previous report indicated that anthocyanins, particular Cy-3-G, are the major contributor to antioxidant activity in black rice (Zhang *et al.*, 2006); this was not observed in our study. In general, rice barn contains γ-oryzanol and phenolic acid which are the major antioxidants for red rice bran (Laokuldilok *et al*., 2011). Butsat and Siriamornpun (2010) reported that γ-oryzanol in rice bran is the main antioxidant.

Our study has suggested that cob of KND variety of waxy corn which has been developed and cultivated in Thailand as well as rice bran obtained from MP and HMD contains several compounds correlated to antioxidant activities and could be a low cost and valuable source of natural antioxidants

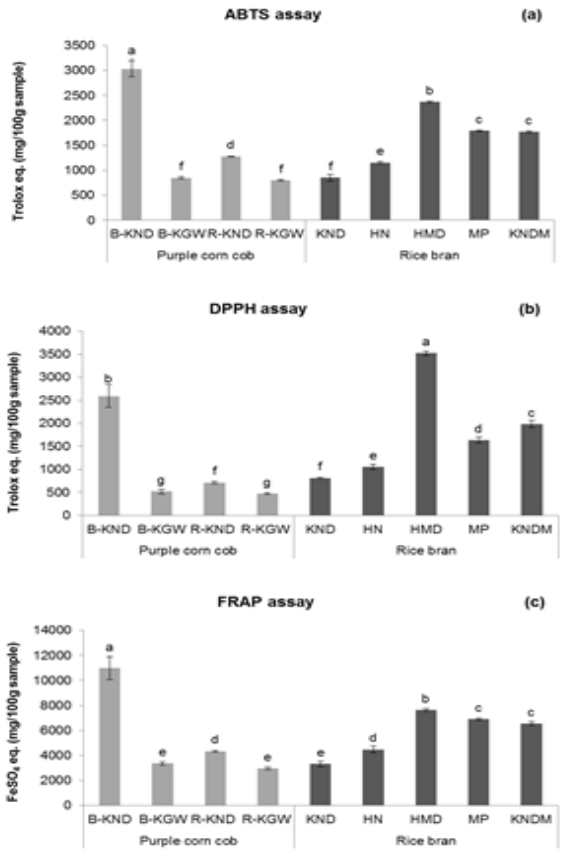

Figure 2. Antioxidant activities of purple corn cob and colored rice bran. (a) ABTS assay, (b) DPPH assay and (c) FRAP assay

Values are mean + SD. Values within the same assay with the same letter are not significantly different ($p<0.05$).

and has the potential to be used as food colorant or functional food ingredient.

Conclusions

The results of this study show that purple corn cob and colored rice bran which were developed in Thailand, were found to have high amount of phenolics, anthocyanins and antioxidant activities. Purple corn cob of an open-pollinated, B-KND has the highest antioxidant activities which could be attributed to the highest of TPC and TAC. Among colored rice brans, black color bran of MP shows the highest content of TAC whereas the highest of TPC and antioxidant activities was observed in red rice bran, HMD. Cy-3-G and Pn-3-G were the major anthocyanins identified in purple corn cob and colored rice bran. In addition, Pg-3-G was also detected in purple corn cob with the lowest amount, and only found in KNDM and MP of rice varieties. Syrigic acid was identified as the predominate phenolic in all extracts. Our results could be suggested that B-KND of purple corn cob, MP and HMD of rice bran might be used as economic sources of natural antioxidant of food ingredients.

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