International Food Research Journal 18(3): 901-906 (2011)

Physical, chemical and antioxidant properties of pigmented rice grown in Southern Thailand

Yodmanee, S., *Karrila, T.T. and Pakdeechanuan, P.

Department of Food Science and Nutrition, Faculty of Science and Technology, Prince of Songkla University, Pattani Campus, Pattani, 94000, Thailand

Abstract: Eight varieties of pigmented rice grown in southern Thailand, as a dehusked grain, were studied for their chemical compositions, antioxidant properties and color parameters. Moisture, protein, lipid, crude fiber and ash contents of all varieties were in the ranges of 5.96-8.19, 6.63-8.46, 1.44-3.47, 0.16-0.35 and 1.35-2.15 g/100 g (db), respectively. The iron and polyphenol contents in these rice samples were in the range of 0.91-1.66 mg/100 g and 58-329 mg GAE/100 g sample, respectively. The antioxidant capacity of dehusked rice grain extract was positively correlated ($p< 0.01$) with polyphenol content (r =0.923). Rice grain color parameters (L^*, a^* and b^*) had negative correlations ($p<0.01$) with iron content (r = -0.646, -0.654 and -0.791), polyphenol (r = -0.893, -0.851 and -0.928) and antioxidant capacity (r = -0.794, -0.629 and -0.770). The results showed that dark purple grain has higher iron content, polyphenol content and antioxidant capacities than red brown grain. This study can guide in the selection and production of rice varieties with enhanced nutritional qualities, suggesting the use of color parameters as a practical indicator of some key nutritional characteristics.

Keywords: Pigmented rice, polyphenol, iron content, antioxidant activity

Introduction

Pigmented rice or colored rice is distinguished by the rice grain having red brown or dark purple color in its covering layers. Pigments, which are located in the aleurone layer of rice grain, have been reported as a mixture of anthocyanin compounds, which belong to the family of flavonoids (Yawadio *et al*., 2007). The phenolic compounds have been found as a major active component for antioxidation (Iqbal *et al*., 2005; Zhang *et al*., 2006; Yawadio *et al*., 2007; Tabart *et al*., 2009). For pigmented rice, the main substance of phenolic compounds has been reported as anthocyanins (Iqbal *et al*., 2005; Zhang *et al*., 2006; Yawadio *et al*., 2007). Anthocyanins in pigmented rice have been identified. They are cyanidin-3-glucoside and peonidin-3-glucoside (Hu *et al*., 2003); malvidin, pelargonidin-3, 5-diglucoside, cyanidin-3-glucoside and cyanidin-3, 5-diglucoside (Zhang *et al*., 2006); cyanidin-3-glucoside, pelargonidin-3-glucoside (Yawadio *et al*., 2007). The content of antioxidative substances, i.e polyphenol in rice grain, is affected by genotype and environment (Goffman and Bergman, 2004). Health benefits of pigmented rice extracts have also been reported. The isolated compounds from pigmented rice (anthocyanins; cyanidin-3-glucoside, pelargonidin-3-glucoside) showed aldose reductase inhibitory activities and hence they would have benefits in diabetic prevention (Yawadio *et al*., 2007). It also has been reported that a diet containing black rice extracts which had anthocyanin (31.3 g/100 g) decreased cholesterol, LDL cholesterol and concentration of triacylglycerol in plasma of rats (Zawistowski *et al*., 2009). Antioxidative activity of pigmented rice has been reported by Zhang *et al*. (2006); Nam *et al*. (2006); Chung and Shin (2007) and Hiemori *et al*. (2009). Brown rice grain (or de-hulled grain) is also an important source of vitamins and minerals Meng *et al*. (2005) have reported that black rice contains iron, zinc, calcium, copper and manganese higher than those in red rice.

Rice production and export are significant to Thailand. More than 5,000 rice varieties are known within the country. The rice most suitably grown in each part of the country varies due to environmental differences. Southern Thailand is set between the Indian and Pacific oceans. Rice grown in this area is different from that grown in other parts of Thailand, while the production quantity does not match that of the rest of Thailand. However there is no information of rice characteristics grown in this area, relating varieties to their best matching applications. There are many pigmented varieties of rice grown in southern, Thailand which have not been investigated but might prove beneficial. The aim of this work was to search for such beneficial varieties which could have high potential in providing micronutrients, particularly iron, and antioxidant substances.

*Corresponding author.
Email: *taewee.tongdang@gmail.com*
Tel: +66 (0)73312160; Fax: +66 (0)73335130

Materials and Methods

Rice samples and chemicals

Colored rice samples used in this study were grown in Southern Thailand, and provided by Pattani Rice Research center, Pattani, Thailand. There were 8 rice varieties in total, with 3 non waxy varieties; Homkradunga (HK), Kamyan (KN), Sangyod (SY), and 5 waxy varieties; Red waxy rice-96060 (RWR-96060), Kramrad (KR), Black waxy rice-96044 (BWR-96044), Black waxy rice-96025 (BWR-96025) and Chormaiphai (CMP). All paddy rice samples were kept at 4°C. This experiment was performed when these samples had been stored for 3 months. The amylose contents of these rice samples were 28.19, 25.30, 18.30, 8.57, 7.31, 8.74, 7.25 and 8.00 g/100 g, respectively. Paddy rice grain was dehusked using a rubber roller. Broken kernels were separated from the whole dehusked grain obtained. The dehusked grains were ground using Cyclotec milling (Cyclotec™ 1093, Foss, Sweden) and sieved through a 100 micrometer sieve**.** Pigmented rice flour samples were then vacuum packed in plastic bags and stored at 4°C. The chemicals used in this study were azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, gallic acid, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), Folin–Ciocalteu reagent and 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) from Fluka Chemie AG (Buchs, Switzerland).

Color of dehusked rice grain

Color of dehusked grain was determined by Hunter-Lab (Ultrascan XE, Hunter-Lab, USA). Prior to color measurements, the instrument was calibrated with light tap and white calibration tile. The colorimeter was set to an illuminant condition D_{65} and a 10° standard observer. Each sample was put in a cuvette and replaced in to the specular port site, the color parameters (a^*, b^* and L^*) were then read (Lamberts *et al*., 2007). Fifteen replicates for each sample were determined.

Proximate analysis

Moisture content, protein, lipid, fiber and ash were determined with standard procedures (AOAC, 2000). Protein was estimated from total nitrogen using a conversion factor of 5.85.

Iron (Fe) content

The iron contents in rice were determined using atomic absorption spectrophotometer-AAS (100 Analyzer, Perkin Elmer, Germany). The iron detection limit of AAS used in this study was 0.039 mg/kg. Dehusked rice flour samples (2–4 g) were wet-acid digested with nitric acid and solubilized with 10 mL of deionized water (Lestienne *et al*., 2005). The absorbance of solubilized samples was measured. The absorbance of iron solutions (0.5, 1, 2 and 4 g/mg) were measured in the same manner as the samples, and standard curve was created. The regression equation obtained was y = 0.0358x-0.0028 (R^2 = 0.9993).

Total polyphenols

Whole meal flour (150 g) of each accession were extracted with 150 mL of a mixture of $CH_3OH/H_2O/HCOOH$ (50:48.5:1.5) for 24 h at room temperature. The mixture was centrifuged at 2500 g for 15 min and the supernatant was collected and stored at 4°C (Gómez-Alonso *et al*., 2007). Total polyphenols were assayed using the Folin–Ciocalteau method (Aguilar-Garcia *et al*., 2007). Briefly, Folin-Ciocalteau reagent was diluted with water 1:9 (v/v). To 2.5 mL of this reagent, 60 µL of the sample extract was added and after 2 min incubation at room temperature, 2 mL sodium carbonate solution (75 g/L) was added. The mixture was incubated for 15 min at 50°C and cooled quickly in an ice-water bath. The absorbance at 760 nm was read within 15 min by spectrophotometer (Libra S22, Biochrom, England). The standard curve was made using gallic acid. The obtained regression equation was y = 2.4646x-0.0228, (R^2 = 0.9967).

Anthocyanin content

The analysis method for anthocyanin content was modified from the method used by Hosseinian *et al* (2008). The pigmented rice extract (20 µL) was added into 2 mL of potassium chloride buffer (0.03 mol/L, pH 1.0) and 2 mL of sodium acetate buffer (0.4 mol/L, pH 4.5). Each of them was left for 15 min before taking an absorption measurement using spectrophotometer (Libra S22, Biochrom, England) at 550 nm and 700 nm. Distilled water was used as a blank. The anthocyanin concentration (mg/L) of sample was calculated according to the following formula and expressed as Cyanidin-3-glucoside equivalents:

$$\frac{A \times MW \times DF \times 1000}{\varepsilon \times 1}$$

A was $(A_{\lambda700-\lambda550})$ pH 1.0 - $(A_{\lambda700-\lambda550})$ pH 4.5

MW was the molecular weight of Cyanindin-3-glucoside (449.2 g/mol)

DF was the dilution factor (20 µL sample is diluted to 2 mL, DF = 1000)

ε was the extinction coefficient ($L \times cm^{-1} \times mol^{-1}$) = 26,900 for Cyanindin-3-glucoside, where L (path length in cm) = 1

Radical ABTS$^{\cdot+}$ scavenging activity

Total antioxidant capacity of rice extracts was determined using a spectrophotometer (Libra S22, Biochrom, England) and the method described by Choi *et al*. (2007). The ABTS$^{\cdot+}$ solution was prepared by adding 7 mM ABTS$^{\cdot+}$ into 2.45 mM potassium persulfate; and the mixed solution was kept in a dark place for over night. The absorbance of the ABTS$^{\cdot+}$ solution at 414 nm was adjusted to be in the range of 1.4-1.5. The extracts 0.6 mL of which had concentration in the range of 0, 0.005, 0.01, 0.015 and 0.02 mg/mL were added into 6 mL ABTS$^{\cdot+}$ solution and mixed thoroughly. The reaction mixture was kept at room temperature for 1 hr and the absorbance was immediately recorded at 414 nm. The standard solution for ABTS$^{\cdot+}$ scavenging activity was Trolox which had the concentration in the range of 0-0.02 mg/mL. The regression equation obtained was y = 4438.1x-12.331 (R^2 = 0.905). The percentage of inhibition was expressed using the following equation:

$$Inhibition(\%) = \frac{(Abs_{t=0} - Abs_{t=30})}{Abs_{t=0}} \times 100$$

Where $Abs_{t=0}$ = Absorbance of sample at 0 min
$Abs_{t=30}$ = Absorbance of sample at 30 min

Results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC, mmol/L).

Radical DPPH$^{\cdot}$ scavenging activity

Five different concentrations of rice extract (0, 0.1, 0.2, 0.4 and 0.8 mg/mL) were added to 3 mL of 200 µM DPPH$^{\cdot}$ solution in methanol. After incubation at room temperature for 30 min, the absorbance at 517 nm was measured using spectrophotometer (Libra S22, Biochrom, England). The standard solution for DPPH$^{\cdot}$ scavenging activity was Trolox which was used in the range of 0-0.02 mg/mL. The obtained regression equation was y = 4239.5x-10.818 (R^2= 0.930). The percentage of inhibition was expressed using the following equation:

$$Inhibition(\%) = \frac{(Abs_{t=0} - Abs_{t=30})}{Abs_{t=0}} \times 100$$

Where $Abs_{t=0}$ = Absorbance of sample at 0 min
$Abs_{t=30}$ = Absorbance of sample at 30 min

The inhibition percentage was plotted against quantity of rice extract solution to obtain a regression line (Zigoneanu *et al*., 2007).

Statistical analysis

All the analyses were carried out at least in triplicate and expressed as mean and standard deviation. Data were statistically analyzed by analysis of variance (ANOVA) and significant differences were identified by Duncan's Multiple Range test (p<0.05). The Pearson correlation analysis was performed. The parameter pairs with a correlation coefficient (r) = 0.800 or higher were selected to further fit a regression line, and the coefficient of determination (R^2) was obtained.

Results and Discussion

Dehusked grain color

Table 1 shows the color parameters (L^*, a^* and b^*) of the 8 varieties of de-hulled grains. L^* values, which expresses the brightness, were in the range of 40.82-51.78. The values of a^* and b^* were in the range of 3.84-12.15 and 3.30-15.71 respectively. It can be noticed that a^* and b^* values in waxy rice varied more than those in non-waxy rice. From this result, rice samples could be grouped, according to their color, into 2 groups. The first group was rice grains which had lower L^*, a^* and b^* values (BWR-96044, BWR-96025 and CMP) and showed dark purple color, while the second group had the higher values (SY, KR, RWR-96060, HK and KN) and showed red brown color. The differences in grain color could depend on the form of anthocyanins and rice genotypes (Yawadio *et al*., 2007; Escribano-Bailón *et al*., 2004).

Table 1. Color parameters of dehusked pigmented rice grains

Rice varieties		L*	a*	b*
Non-waxy rice	HK	49.77±1.59^{d}	7.50±0.56^{c}	11.66±0.70^{de}
	KN	49.63±0.87^{d}	9.68±0.38^{d}	13.07±0.57^{f}
	SY	47.41±0.65^{c}	10.67±0.63^{e}	12.08±0.54^{e}
Waxy rice	BWR-96025	41.20±0.96^{a}	4.18±0.58^{a}	3.84±0.80^{b}
	RWR-96060	51.78±0.66^{e}	12.15±0.49^{f}	15.71±0.48^{g}
	CMP	44.04±1.68^{b}	4.80±0.48^{b}	5.92±1.17^{c}
	KR	51.44±1.27^{e}	11.00±0.97^{e}	11.44±1.04^{d}
	BWR-96044	40.82±1.04^{a}	3.84±0.74^{a}	3.30±0.84^{a}

Mean value + standard deviation of fifteen replicates
Mean values with different letters in the same column are significantly different (p<0.05).

Proximate compositions

The proximate compositions; moisture content, protein, lipid and crude fiber of 8 rice varieties are presented in Table 2. The moisture contents of all varieties were in the range of 5.96-8.19 g/100 g. Protein contents for all varieties were in the range of 6.63-8.46 g/100 g. It can be noticed that 5 in 8 varieties had protein >8 g/100 g (db). The lipid content of KN variety was the highest one (2.17 g/100 g) among these rice samples. The rest had lipid contents in the

Table 2. Chemical compositions of dehusked pigmented rice grains

Rice varieties	Moisture (g/100g)	Protein (g/100g db)	Lipid (g/100g db)	Fiber (g/100g db)	Ash (g/100g db.)	Iron content (mg/100 g db.)	Polyphenol (mg GAE/100g db.)	Anthocyanin (mg Cy-3-glc/100g db.)
HK	6.76±0.26^{b}	6.96±0.10^{b}	1.47±0.09^{ab}	0.28±0.00^{b}	1.44±0.10^{ab}	1.16±0.03^{b}	80.44±6.61^{b}	10.68±2.31^{a}
KN	8.65±0.14^{f}	6.63±0.11^{a}	2.17±0.04^{c}	0.35±0.05^{c}	1.64±0.13^{ab}	1.26±0.03^{cd}	58.89±6.89^{a}	9.79±1.54^{a}
SY	7.18±0.22^{c}	8.06±0.03^{d}	1.65±0.56^{ab}	0.26±0.01^{b}	2.15±0.05^{c}	1.21±0.04^{bc}	82.01±7.90^{b}	15.14±0.19^{b}
RWR-96060	8.19±0.30^{e}	8.18±0.12^{d}	1.58±0.04^{ab}	0.35±0.05^{c}	1.78±0.35^{b}	0.91±0.04^{a}	84.43±3.61^{b}	16.69±1.73^{b}
BWR-96025	7.60±0.21^{d}	8.44±0.03^{e}	1.93±0.39^{bc}	0.26±0.01^{b}	1.52±0.12^{ab}	1.48±0.04^{e}	280.15±9.83^{d}	129.36±2.50^{d}
CMP	6.08±0.23^{a}	8.46±0.17^{e}	1.50±0.04^{ab}	0.16±0.05^{a}	1.58±0.20^{ab}	1.31±0.03^{d}	208.42±0.00^{c}	114.77±1.68^{c}
KR	6.83±0.26^{bc}	7.69±0.09^{c}	1.44±0.11^{a}	0.28±0.01^{b}	1.38±0.10^{a}	1.46±0.03^{e}	80.17±4.49^{b}	11.13±1.54^{a}
BWR-96044	5.96±0.06^{a}	8.23±0.17^{d}	1.67±0.09^{ab}	0.29±0.00^{b}	1.35±0.07^{a}	1.66±0.03^{f}	329.24±6.72^{e}	245.36±3.53^{e}

Mean value + standard deviation of triplicates
Mean values with different letters in the same column are significantly different (p<0.05).

range of 1.44-1.93 g/100 g (db.). For crude fiber and ash all of these varieties were in the ranges of 0.16-0.35 and 1.35-2.15 g/100 g, respectively. There were no significant differences in crude fiber content among HK, SY, BWR-96025, KR and BWR-96044 varieties. Deepa *et al*. (2008) reported that protein, lipid, fiber and ash contents of brown rice samples were in the range of 7.95-9.52, 2.06-2.60, 4.96-8.08 and 1.27-1.54 g/100 g, respectively.

Iron content

It was found that the pigmented rice in this study had iron content in the range of 0.91-1.66 mg/100 g sample (Table 2). All waxy rice varieties, except RWR-96060, showed higher iron contents than the non-waxy varieties. They were in the order of BWR-96044>BWR96025>KR>CMP>KN>SY>HK >RWR-96060. It seems that purple color grain have higher iron content that red brown color grains. This is similar to what have been found by Meng *et al*. (2005).The differences in iron content of rice may be affected by their growing environments and genetic differences (Meng *et al*., 2005).

Total polyphenol anthocyanins and antioxidant activities

Polyphenol and anthocyanins contents are given in table 2. Dark purple color group had higher polyphenol and anthocyanin content than the red brown color group. Similar results had been reported by Goffman and Bergman (2004) and Shen *et al*. (2009). Polyphenols are the most effective antioxidative constituents in plant products consumed (Escribano-Bailón *et al*., 2004).

In this study the antioxidant activity of crude extracts from dehusked rice grain was determined by DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ assays. The results are given in Figure 1. All the varieties exhibited appreciable scavenging activity against both of the radicals and the same order of scavenging was observed by both assays. Increasing extract concentration resulted in an increase in scavenging activity. Dark purple group (BWR-96044, BWR-96025 and CMP) had higher scavenging activity (both DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ assays) than those of red brown group (RWR-96060, SY, KR, HK and KN). This was also related to polyphenol contents.

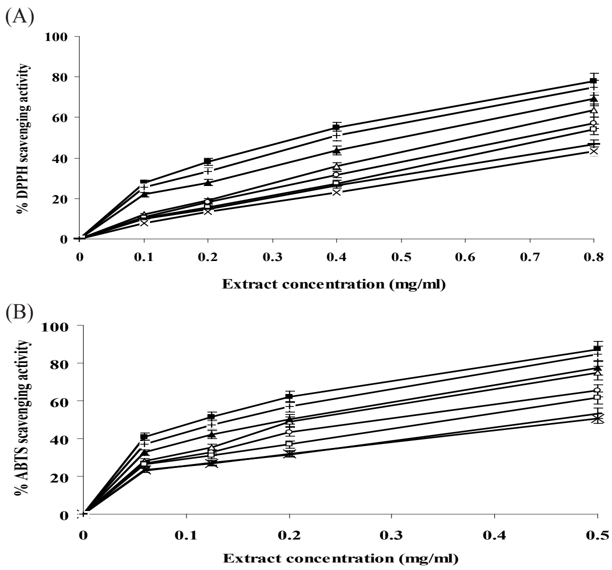

Figure 1. Antioxidant activities of the extracts from dehusked pigmented rice grain, determined by DPPH$^{\cdot}$ assay (A) and ABTS$^{\cdot+}$ assay (B): BWR-96044 (■), RWR-96060 (Δ), SY (¤), KN (×), CMP (▲), KR (□), BWR-96025 (+) and HK (-).
Error bars are standard deviations of triplicates.

Relationship between grain color, iron content, total polyphenol and antioxidant activities (ABTS$^{\cdot+}$)

The correlations between grain color, iron content, total polyphenol and antioxidant activities (ABTS$^{\cdot+}$) were computed. The linear correlation coefficients (r) and coefficient of determination (R^2) obtained are shown in Figure 2 and the discussion is as following.

The relationship between iron content and grain color was found to be strongly negative with L^*, a^* and b^* values and yielded linear correlation coefficients r = -0.646, -0.654 and -0.791. This means darker color

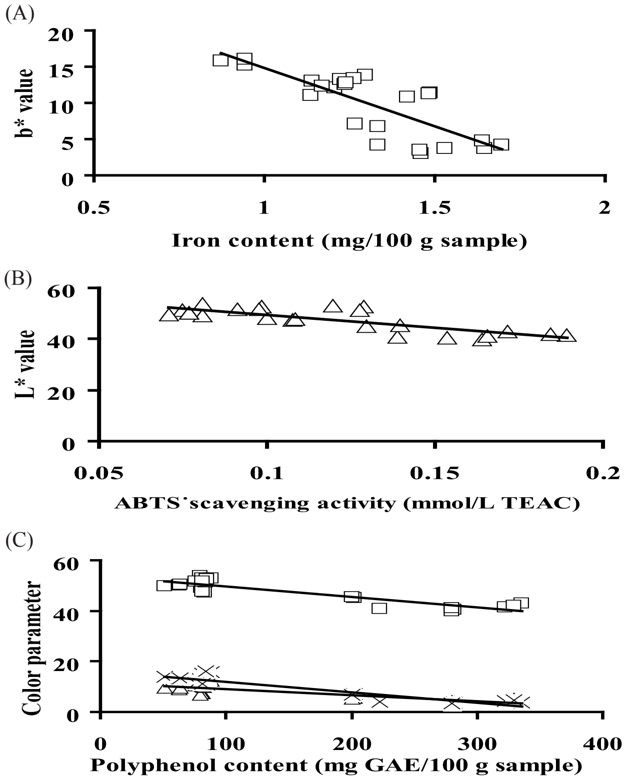

Figure 2. (A) Relationship between b* value and iron content, *r* = -0.791, $y = -15.954x + 30.688$, R^2 = 0.609

(B) Relationship between L* value and $ABTS^{\cdot +}$ scavenging activity, *r* = -0.794, $y = -0.006x + 0.413$, R^2 = 0.616

(C) Relationships between color parameters (L*(□), a*(Δ) and b*(×) and polyphenol content, *r* = -0.893, -0.851 and -0.928; $y = -0.401x + 53.448$, $y = -0.244x + 11.589$ and $y = -0.406x + 15.959$; R^2 = 0.807, 0.728 and 0.858, respectively.

is associated with higher iron content. The highest correlation suggested a linear equation between b* and iron content giving $y = -15.954x + 30.688$; (R^2 = 0.609). Iron content tends to be higher in aromatic and colored (red and black) rice varieties than in colorless varieties. Meng *et al*. (2005) found that grain color related to iron content. Iron is an important mineral as it is the only mineral in hemoglobin. Iron deficiency causes anemia. In some countries (particularly in South-East Asia), rice is consumed as staple food. Hence, it is possible to reduce iron deficiency anemia by consuming pigmented rice. However, the variety of pigmented rice should also be considered and improved, based on its iron content.

Correlations between grain color (L*, a* and b*) and total polyphenol in pigmented rice were negative (*r* = -0.893, -0.851 and -0.928). The corresponding linear regression equations were $y = -0.401x + 53.448$, $y = -0.244x + 11.589$ and $y = -0.406x + 15.959$ (R^2 = 0.807, 0.728 and 0.858, respectively). The relationship between grain color (L*) and antioxidant activity was highly negative correlated (*r* = -0.794) and the corresponding linear regression equation was $y = -0.006x + 0.413$ (R^2 = 0.616). It seemed to imply that darker grain (red or purple) contained more polyphenol and showed higher antioxidant capacity. Shen *et al*. (2009) also found that color parameters (L*, a* and b*) had a relationship with polyphenol and antioxidant activities. The color parameters L*, a* and b* were negatively correlated, in our current study, with antioxidant capacity and iron content. Thus, breeding lines high in antioxidant could be indirectly persued by selecting grain with low L*, a* and b* values.

Conclusions

According to dehusked grain color 8 varieties of pigmented rice in this study could be grouped into two groups, dark purple group (BWR-96044, BWR-96025 and CMP) and red group (HK, KN, SY, KR and RWR-96060). Dark purple group had iron content, polyphenol contents and antioxidant capacities higher than those of red group. Negative correlations between grain color parameters and iron content as well as polyphenol content and antioxidant activity were found in this study. These relationships could also serve as indicators to indirectly select rice varieties for a specific purpose of industrial products. The relation between rice color and iron, and antioxidant content can be useful information or a simple tool to help the rice breeders in screening rice for higher iron and antioxidant varieties. These data also provide opportunities to further increase the nutritional benefits of some food products from rice.

Acknowledgements

The authors would like to thank Prince of Songkla University, Pattani campus and Graduate School, Prince of Songkla University, Thailand for the financial support. Pattani Rice Research Center, Thailand is also acknowledged for the rice samples.

References

Aguilar-Garcia, C., Gavino, G., Baragaño-Mosqueda, M., Hevia, P. and Gavino, V.C. 2007. Correlation of tocopherol, tocotrienol, γ-oryzanol and total polyphenol content in rice bran with different antioxidant capacity assays. Food Chemistry 102 (4): 1228-1232.

AOAC. 2000. Official Method of Analysis. 16th ed. Verginia: The Associate Analysis Chemists.

Choi, Y., Jeong, H.S. and Lee, J. 2007. Antioxidant activity of methanolic extracts from some grains consumed in Korea. Food Chemistry 103 (1): 130-138.

Chung, H.S. and Shin, J.C. 2007. Characterization of antioxidant alkaloids and phenolic acids from anthocyanin-pigmented rice (*Oryza sativa* cv. *Heugjinjubyeo*). Food Chemistry 104 (4): 1670-1677.

Deepa, G., Singh, V. and Naidu, K.A. 2008. Nutrient composition and physicochemical properties of Indian medicinal rice-Njavara. Food Chemistry 106 (1): 165-171.

Escribano-BailÓn, M.T., Santos-Buelga, C. and Rivas-Gonzalo, C. 2004. Anthocyanins in cereals. Journal of Chromatography A 1054 (1-2): 129-141.

Goffman, F.D. and Bergman, C.J. 2004. Rice kernel phenolic content and its relationship with antiradical efficiency. Journal of the Science of Food and Agriculture 84 (10): 1235–1240.

Gómez-Alonso, S., García-Romere, E. and Hermosín-Gutiérrez, I. 2007. HPLC analysis of diverse grape and wine phenolics using direct injection and multidetection by DAD Fluorescence. Journal of Food Composition and Analysis 20 (7): 618-626.

Hamalatha, S., Platel, K. and Srinivasan, K. 2007. Zinc and iron contents and their bioaccessibility in cereals and pulses consumed in India. Food Chemistry 102 (4): 1328-1336.

Hiemori, M., Koh, E. and Mitchell, A.E. 2009. Influence of Cooking on Anthocyanins in Black Rice (*Oryza sativa* L. *japonica* var. SBR). Journal of Agricultural and Food Chemistry (57): 1908-1914.

Hosseinian, F.S., Li, W. and Beta, T. 2008. Measurement of anthocyanins and other phytochemicals in purple wheat. Food Chemistry 109 (4): 916-924.

Hu, C., Zawistowski, J., Ling, W. and Kitts, D.D. 2003. Black Rice (*Oryza sativa* L. *indica*) Pigmented Fraction Suppresses both Reactive Oxygen Species and Nitric Oxide in Chemical and Biological Model Systems. Journal of Agriculture and Food Chemistry (51): 5271-5277.

Iqbal, S., Bhanger, M.I. and Anwar, F. 2005. Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. Food Chemistry 93 (2): 265-272.

Lamberts, L., Bie, E.D., Vandeputte, G.E., Veraverbeke, W.S., Derycke, V., Man, W.D. and Delcour, J.A. 2007. Effect of milling on color and nutritional properties of rice. Food Chemistry 100 (4): 1496-1503.

Lestienne, I., Icard-Vernière, C., Mouquet, C., Picq, C. and Trèche, S. 2005. Effect of soaking whole cereal and legume seeds on iron, zinc and phytate contents. Food Chemistry 89 (3): 421-425.

Meng, F., Wei, Y. and Yang, X. 2005. Iron content and bioavailability in rice. Journal of Trace Elements in Medicine and Biology 18 (4): 333–338.

Nam, S.H., Choi, S.P., Kang, M.Y., Koh, H.J., Kozukue, N. and Friedman, M. 2006. Antioxidative activities of bran extracts from twenty one pigmented rice cultivars. Food Chemistry 94 (4): 613-620.

Shen, Y., Jin, L., Xiao, P., Lu, Y. and Bao, J. 2009. Total phenolics, flavonoids, antioxidant capacity in rice grain and their relations to grain color, size and weight. Journal of Cereal Science 49 (1): 106-111.

Tabart, J., Kevers, C., Pincemail, J., Defraigne, J. and Dommes, J. 2009. Comparative antioxidant capacities of phenolic compounds measured by various tests. Food Chemistry 113 (4): 1226-1233.

Yawadio, R., Tanimori, S. and Morita, N. 2007. Identification of phenolic compounds isolated from pigmented rices and their aldose reductase inhibitory activities. Food Chemistry 101 (4): 1616-1625.

Zawistowski, J., Kopec, A. and Kitts, D.D. 2009. Effects of a black rice extract (*Oryza sativa* L. *indica*) on cholesterol levels and plasma lipid parameters in Wistar Kyoto rats. Journal of Functional Foods 1 (1): 50-56.

Zhang, M.W., Guo, B.J., Zhang, R.F., Chi, J.W., Wei, Z.C., Xu, Z.H., Zhang, Y. and Tang, X.J. 2006. Separation, purification and identification of antioxidant compositions in black rice. Agricultural Science in China 5 (6): 431-440.

Zigoneanu, I.G., Williams, L., Xu, Z. and Sabliov, C.M. 2007. Determination of antioxidant components in rice bran oil extracted by microwave-assisted method. Bioresource Technology 99 (11): 4910-4918.