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Original Article

Chemometric classification of pigmented rice varieties based on antioxidative properties in relation to color

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Abstract

The pigmented Thai rice varieties including red and black color and non-pigmented rice (white) collected from different growth sites in the north of Thailand and were determined for color and antioxidant properties. Anthocyanins were the major compound in group of black rice (21.15-441.96 mg/100 g rice). Total phenolic, flavonoid, and α-tocopherol contents were highest in the black rice followed by red rice and antioxidant capacities were predominant in pigmented varieties. Black rice grown in mountainous area presented the highest antioxidant activity compared to the other growing locations. The color parameters, especially *L** value presented the negative correlations with antioxidant parameters, while the antioxidant contents, excepted γ-oryzanol content had significant correlation with antioxidant capacities. Pigmented rice varieties could be clearly classified into 4 groups using PCA and HCA, which provided a good indicator to classify pigmented rice varieties based on color and antioxidative properties.

Keywords: pigmented rice, color, antioxidant capacities, hierarchical cluster analysis, principal component analysis

1. Introduction

Rice (*Oryza sativa* L.) is a major cereal crop in the world, which was consumed as a staple food for over half of the world's population (Bhattacharjee *et al.*, 2002). Milled rice is mostly consumed, due to the texture of cooked milled rice is softer compared to that of cooked brown rice. However, brown rice or whole-grain rice made up from the endosperm, germ and bran of the grain had long been considered an excellent source of energy, nutrients, and phytochemicals (de Mira *et al.*, 2009). The bran layers covering the rice grain contain high amount of nutrients and phytochemicals, such as tocopherols, tocotrienols, γ-oryzanol, vitamin B complex and phenolic compounds (Sookwong *et al.*, 2007, Yu *et al.*, 2007), which is believed to exhibit the important roles for protection against various degenerative diseases (Goffman and Bergman. 2004).

In addition to traditional white rice or common rice, other specialty rice types, pigmented rice have been developed to possess nutritional, textural or other properties that often gain higher prices in the market place. Pigmented rice is reported as a potent source of phenolic compounds and much greater amount in comparison with that of white rice (Vichapong *et al.*, 2010) and it has a number of nutritional advantages over common rice, such as protein, vitamin, and minerals, depended on varieties and production location. Furthermore, positive health effects of the pigments presented in the bran layer of rice grain have been reported, due to the presence of phenolics, flavonoids, tocopherols, tocotrienols and γ-oryzanol (Tananuwong and Tewaruth. 2010).

Several studies reported the relation of pericarp or pigment substances on rice grain on the potential health benefits. Pigmented rice has higher DPPH radical-scavenging activity than white rice due to the polymeric procyanidins

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which were the major components responsible for that activity (Oki *et al.*, 2002). The fed of rice with red or purple bran color have significantly decreased the liver reactive oxygen species, aortic malondialdehyde and the area of atherosclerotic plaque in rabbits, which lower than in those fed rice with white bran (Ling *et al.*, 2001). In addition, Toyokuni *et al.* (2002) reported that a protective effect against renal lipid peroxidation in rats fed with dark colored bran rice was higher than those of rats fed rice with light colored bran rice.

Nowadays, pigmented rice has been receiving more attention in commerce, consequently it is of interest to study their properties in more details. There have been a number of studies on antioxidant in pigmented rice varieties, however, the relation and classification of pigmented rice according to their color and antioxidant properties has not been performed. This study aimed to determine color parameters and anti-oxidant properties, both contents and capacities, of the rice samples, as well as the relationship of these parameters. In addition, classification of the rice samples based on color and antioxidant parameters using chemometric tools was also performed.

2. Materials and Methods

2.1 Plant materials

Twenty paddy rice samples used in this study included three colors (white, red, and black) as shown in Table 1. All rice samples were harvested during June to October 2011 and collected from different growing locations in the north and lower north of Thailand.

The paddy rice samples were dried at 50°C by hot air oven to reduce moisture content to approximately 12% (wb). The rice samples were de-husked to obtain the unpolished rice by a local milling system. The unpolished samples were ground until obtain a fine powder and kept at 4°C.

2.1 Reagents and chemicals

The Folin–Ciocalteu phenol reagent, gallic acid, catechin, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,4,6-tripyridyl-s-triazine (TPTZ), and α-tocopherol were

Table 1. Varieties, color and growing locations of pigmented and non-pigmented rice samples.

Samples * (color/ rice varieties)	Abbreviation	Growing locations
White		
Phitsanulok 2	WPL2	Phitsanulok
Hom Mali 105	WHM105	Phitsanulok
San Pa Tong	WSP	Phrae
Red		
Hom Man Puu	RMP	Phayao
Sung Yod	RSY	Phayao
Hom Daeng Sukhothai 1	RST1	Sukhothai
Hom Daeng Sukhothai 4	RST4	Sukhothai
Black		
Hom Nil (1)	BHN(1)	Phayao
Hom Nil (2)	BHN(2)	Phichit
Rice Berry	BRB	Phichit
Hom Dam Sukhothai 2	BST2	Sukhothai
Khao Jao Dam	BJD	Phetchaboon
Niaw Dam Pleuk Khow (1)	BPK(1)	Phetchaboon
Niaw Dam Pleuk Khow (2)	BPK(2)	Phetchaboon
Niaw Dam Pleuk Dam (1)	BPD(1)	Phetchaboon
Niaw Dam Pleuk Dam (2)	BPD(2)	Phichit
Kam Phayao	BPY	Phayao
Kam Doi Saket	BSK	Phayao
Kam Doi Muser	BMS	Phayao
Kam Leum Pua	BLP	Phayao

* Number in brackets presented the explanation of rice varieties which were collected from the different growing locations.

purchased from Sigma–Aldrich (Seelze, Germany). Oryzanol was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The solvents for HPLC analysis were HPLC-grade and all other chemicals were of analytical grade.

2.2 Color measurement

Color values of different unpolished rice samples were measured using Hunter Lab (MiniScan XE Plus), where L^* indicates degree of whiteness or darkness (0 = black and 100 = white), a^* indicates degree of redness (+) and greenness (–), and b^* indicates degree of yellowness (+) and blueness (–). Hue angle (H°) was used to express the dimension of color and calculated as $H° = \tan^{-1}(b^*/a^*)$. Chroma (C) value was determined for the intensity or saturation of color and calculated as $C = (a^{*2} + b^{*2})^{1/2}$.

2.3 Total anthocyanins content

The total anthocyanins content (TAC) was determined using pH differential method according to a modified method of Giusti and Wrolstad (2005). Rice flour samples were extracted three times with acidified methanol (methanol and 1 M HCl, 85:15, v/v) with a solvent to sample in ratio of 10:1. One milliliter of rice extract was diluted to 10 mL using potassium chloride buffer, pH 1.0 and sodium acetate buffer, pH 4.5 to prepare two pH sample solutions. The absorbance of each dilution was measured at 520 and 700 nm with a UV-vis spectrophotometer (DR/4000, HACH, USA). The TAC was calculated in terms of cyanidin-3-glucoside using the following equation:

$$\text{Monomeric anthocyanin pigment (mg/L)} = [A_{diff} \times MW \times DF \times 1000] / \varepsilon \quad (1)$$

where MW represents molecular weight of cyanidin-3-glucoside (449.2), DF is the dilution factor (10), ε is molar absorptivity of cyanidin-3-glucoside (26,900 L/mol cm) and A_{diff} was calculated from the following equation:

$$A_{diff} = (A_{520}-A_{700})_{pH1.0} - (A_{520}-A_{700})_{pH4.5} \quad (2)$$

2.4 Sample extraction

Crude extract from rice samples was extracted according to the modified procedure reported by Sompong *et al.* (2011). One gram of rice flour samples was mixed with 20 mL of 85% aqueous methanol and shaken at 150 rpm using a shaker (GFL 3017, Burgwedel, Germany) at room temperature for 6 hrs. The mixtures were centrifuged (MPW-350R, Warsaw, Poland) at 2,500 xg for 10 min. The supernatants were collected and the residues were re-extracted twice under the same conditions. The supernatants of each cycle were combined and the solvent was removed using a rotary evaporator (Buchi R-200, Flawil, Switzerland) at 40°C. The final volume of crude extract was adjusted to 25 mL with methanol and then used for determination of total phenolic and flavonoid contents as well as antioxidant capacities.

2.5 Determination of total phenolic content

The total phenolic content (TPC) was determined by the Folin–Ciocalteu assay as described by Singleton *et al.* (1999) with a slight modification. The crude extracts (100 µL) were mixed with 500 µL of the freshly diluted 10-fold folin-ciocalteu reagent. One milliliter of sodium carbonate (15% w/v) was added to the mixtures after the reaction time for 2 min. The final volume was made up to 5.0 mL with distilled water and incubated at room temperature for 2 hrs. The absorbance at 750 nm was measured against a blank using a spectrophotometer. Gallic acid was used as standard and the TPC was expressed as mg gallic acid equivalent (GAE) per 100 g flour.

2.6 Determination of total flavonoid content

The total flavonoid content (TFC) was determined following a modified method of Zhishen *et al.* (1999). The crude extracts (500 µL) were diluted with 2.0 mL of distilled water and then 150 µL of 5% $NaNO_2$ solution were added to the mixtures. After 5 min, 150 µL of 10% $AlCl_3$ were added and 1.0 mL of 1 M NaOH was added 6 min later. Total volume was made up to 5.0 mL with distilled water. The mixture was measured for the absorbance at 510 nm using a spectrophotometer. The TFC was expressed as mg catechin equivalent (CE) per 100 g flour.

2.7 Determination of DPPH radical-scavenging ability

DPPH radical-scavenging ability described by Pellati *et al.* (2004) was measured with slight modification. One milliliter of crude extracts was mixed with 2 mL of a 0.1 mM methanolic solution of DPPH. The mixtures were stored at room temperature in the dark for 30 min and the absorbance was measured at 517 nm against a blank using a spectrophotometer. The percentage of radical-scavenging ability was calculated by using the formula:

$$\text{Scavenging ability (\%)} = [1 - (A_{sample}/A_{control})] \times 100 \quad (3)$$

The DPPH scavenging activity of crude extracts was expressed as 50% inhibitory concentration, IC_{50} (mg/mL) and obtained by interpolation from linear regression analysis.

2.8 Determination of ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) assay based on the reduction of the Fe(III)-TPTZ complex to the ferrous form was determined according to the method of Benzie and Strain (1999). The FRAP reagent was daily prepared and included 0.3 M acetate buffer (pH 3.6), 10 mM

TPTZ in 40 mM HCl, and 20 mM. $FeCl_3$ at a ratio of 10:1:1 (v/v/v). Two hundred microliters of crude extracts were mixed with 1.3 mL of FRAP reagent. The absorbance at 595 nm was measured after incubation at 37°C for 30 min. The FRAP value was expressed as mmol of Fe(II) equivalents per 100 g flour.

2.9 Determination of trolox equivalent antioxidant capacity (TEAC) assay

The ABTS radical cation scavenging assay was analyzed as described by Pellegrini *et al.* (2003). A stable stock solution of ABTS radical cation was produced by the reacting of 7 mM aqueous solution of ABTS with potassium persulfate in the dark at room temperature for 12-16 hrs before use (absorbance of 0.70±0.02 AU at 734 nm). The crude extracts (120 µL) were reacted with 1.5 mL of a diluted ABTS radical cation solution. The absorbance of the mixtures was measured at 734 nm after reaction time for 1 min. The TEAC result was expressed as mmol of trolox per 100 g flour.

2.10 Extraction and determination of α-tocopherol and γ-oryzanol

Rice flour samples (0.25 g) were extracted with 0.8 ml of 70% ethanol by shaking for 30 min at room temperature and then centrifuged at 13,000 xg at 4°C for 10 min. The supernatants were collected and the residues were re-extracted three times with the same conditions. The combined supernatants were used for determination of the α-tocopherol and γ-oryzanol contents.

The α-tocopherol and γ-oryzanol contents were determined by a high performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan) equipped with a LC-10ADVP pump, SPD-10AVP photo diode array detector and RF-10AXL fluorescence detector according to the method described by Pascual *et al.* (2013). The chromatograms were acquired on the Shimadzu Class-VP software. Analytical separation was carried out at 30°C using an Inertsil ODS-3 (4.6x250 mm, 5 µm) column (GL Sciences Inc., Tokyo, Japan). The elution was performed at a flow rate of 1 mL/min. The gradient elution system was initially at 45% acetonitrile, 45% methanol, and 10% isopropanol for 6 min, followed by a linear gradient to 25% acetonitrile, 70% methanol, and 5% isopropanol in 10 min, and this condition was held for 12 min. The eluent composition was reset to the initial condition and held for 7 min to stabilize the baseline. The α-tocopherol was monitored using the fluorescence detector at an excitation wavelength of 298 nm and an emission wavelength of 328 nm, while the γ-oryzanol was monitored with PDA detection at 325 nm.

2.11 Statistical analysis

The experimental data were expressed as mean ± SD (n=3) and all statistical analyses were carried out using the SPSS software package. Analysis of variance (ANOVA) and Duncan's new multiple range test were performed to determine the significant differences between samples. Pearson correlation was used to evaluate the strength of correlations among the evaluated parameters. Multivariate analysis including hierarchical cluster analysis and principal component analysis were performed to differentiate rice samples based on their color and antioxidative properties.

3. Results and Discussion

3.1 Color properties

The color parameters of unpolished rice samples included *L*, a*, b**, hue angle, and chroma values are shown in Table 2. The whiteness (*L**) was more dominant in the white rice group followed by the red rice group, while the black rice group gave the lowest of *L** values except BSK and BLP varieties. The positive *a** values or redness were the highest for the group of red rice varieties due to the reddish colored external layers (Saikia *et al.*, 2012). The yellowness (*b**) presented the same trend with the whiteness. The hue angle was higher for the group of white rice than that of the group of red and black rice varieties, which indicated that the red and black rice varieties were more in red color than that of the white rice varieties. Furthermore, the chroma was high in both of the white and red rice varieties, while one of black rice varieties BLP also presented high value of chroma, due to white and yellow color that appeared on rice grain as different from the other of black rice varieties.

3.2 Antioxidant contents

The TAC of crude extracts was prominent in the black rice varieties, followed by the red rice varieties (Table 3). The highest TAC was found in BPK(1) (441.96 mg/100 g), followed by four black rice varieties, BJD, BPK(2), BPD(1), and BLP, which contained high level of anthocyanin (>200 mg/100 g). Abdel-Aal *et al.* (2006) reported that cyanidin-3-glucoside and peonidin-3-glucoside were identified as two major anthocyanins in pigmented rice, especially black rice. In addition, these two anthocyanins were found in black Japonica rice bran with a value of 90-95% of total anthocyanin content (Laokuldilok *et al.*, 2011).

The TPC of crude rice extracts was not clearly differentiated among the red and black rice varieties with a range of 221.56-368.16 and 138.49-634.13 mg GAE/100 g, respectively; whereas the white rice varieties showed the lowest of TPC content (Table 3). The highest TPC was found in BPK(1) and the high levels of TPC was also found in other black rice samples, BJD, BPK(2), and BPD(1). Shao *et al.* (2014) studied phenolic acids, anthocyanins, and antioxidant capacity in white, red, and black rice grains. The results showed that protocatechuic acid, vanillic acid, syringic acid, *p*-coumaric acid, ferulic acid, and isoferulic acid were detected as the major phenolic compounds in rice samples, but protocatechuic acid and vanillic acid were not found in white rice

Table 2. Color properties of pigmented and non-pigmented rice varieties.

Samples (Color/ rice varieties)	Color properties				
	*L**	*a**	*b**	Hue angle (°)	Chroma
White					
WPL2	34.98 ± 1.40^{c}	1.49 ± 0.19^{g}	9.09 ± 0.33^{ab}	80.64 ± 0.61^{b}	9.21 ± 0.34^{de}
WHM105	39.87 ± 1.17^{b}	0.66 ± 0.09^{gh}	9.73 ± 0.41^{a}	86.09 ± 0.20^{ab}	9.75 ± 0.41^{cd}
WSP	45.60 ± 1.16^{a}	0.34 ± 0.17^{h}	9.80 ± 0.17^{a}	87.99 ± 0.19^{a}	9.80 ± 0.17^{bc}
Red					
RMP	22.77 ± 0.71^{ef}	6.74 ± 0.18^{bc}	7.63 ± 0.10^{c}	48.56 ± 0.78^{cdef}	10.18 ± 0.16^{bc}
RSY	23.93 ± 0.26^{e}	8.61 ± 0.30^{a}	9.13 ± 0.35^{ab}	46.66 ± 0.45^{cdef}	12.55 ± 0.42^{a}
RST1	21.18 ± 0.51^{fg}	7.46 ± 0.37^{b}	6.59 ± 0.13^{d}	41.48 ± 1.01^{fgh}	9.95 ± 0.28^{bc}
RST4	19.70 ± 0.25^{g}	6.47 ± 0.23^{c}	6.17 ± 0.45^{d}	43.59 ± 2.49^{efg}	8.95 ± 0.29^{e}
Black					
BHN(1)	16.36 ± 0.39^{hijk}	3.19 ± 0.23^{de}	3.16 ± 0.63^{ef}	44.66 ± 2.47^{defg}	4.49 ± 0.26^{g}
BHN(2)	15.89 ± 0.91^{hijk}	3.28 ± 1.40^{de}	2.37 ± 0.70^{fg}	35.59 ± 5.34^{hi}	4.05 ± 0.41^{gh}
BRB	14.20 ± 0.41^{jk}	1.36 ± 0.36^{g}	1.52 ± 0.23^{g}	48.33 ± 2.81^{cdef}	2.04 ± 0.28^{l}
BST2	15.34 ± 1.47^{hijk}	2.60 ± 0.60^{e}	1.73 ± 0.04^{g}	33.99 ± 4.44^{i}	3.13 ± 0.38^{ij}
BJD	14.49 ± 0.83^{ijk}	1.28 ± 0.43^{g}	1.54 ± 0.48^{g}	50.14 ± 8.56^{cde}	2.01 ± 0.18^{l}
BPK(1)	14.88 ± 0.59^{hijk}	1.64 ± 0.53^{fg}	2.07 ± 0.46^{g}	51.66 ± 3.50^{cd}	2.64 ± 0.27^{jk}
BPK(2)	16.41 ± 0.52^{hij}	3.13 ± 0.32^{de}	3.04 ± 0.40^{ef}	44.10 ± 5.55^{efg}	4.38 ± 0.26^{gh}
BPD(1)	16.27 ± 2.05^{hijk}	3.05 ± 1.30^{de}	3.00 ± 1.88^{ef}	45.24 ± 2.82^{defg}	4.33 ± 0.59^{gh}
BPD(2)	14.13 ± 0.92^{k}	1.64 ± 0.59^{fg}	1.68 ± 0.43^{g}	45.73 ± 4.35^{defg}	2.37 ± 0.17^{kl}
BPY	16.74 ± 0.96^{hi}	3.41 ± 0.54^{de}	1.73 ± 0.48^{g}	26.99 ± 2.61^{j}	3.83 ± 0.56^{h}
BSK	23.72 ± 2.74^{e}	2.52 ± 0.51^{ef}	2.01 ± 0.26^{g}	38.65 ± 2.88^{ghi}	3.24 ± 0.18^{i}
BMS	16.90 ± 0.66^{h}	3.94 ± 1.00^{d}	3.87 ± 0.40^{e}	44.59 ± 2.41^{defg}	5.53 ± 0.63^{f}
BLP	27.03 ± 3.69^{d}	6.22 ± 0.79^{c}	8.30 ± 0.56^{bc}	53.26 ± 1.68^{d}	10.38 ± 0.84^{b}

Mean values in a column superscripted by the same letter are not significantly different at $p<0.05$.

and red rice varieties.

The TFC of the rice samples followed a similar trend to that of TPC, with a range of 57.35-279.32, 100.59-165.65, and 15.43-16.67 mg CE/100 g for the group of black, red, and white rice, respectively (Table 3). The BPK(1) also contained the greatest TFC, followed by three black rice samples, including BJD, BPK(2), and BPD(1). Anthocyanin is well known as the predominant flavonoid in pigmented rice. in addition, kaempferol and quercetin were identified as the predominant flavonols, while apigenin was the major component of flavones in pigmented rice (Kim *et al.*, 2010).

The α-tocopherol content of crude extracts determined by HPLC was in the range of 0.58-0.71, 0.37-0.73, and 0.62-2.18 mg/100 g for the group of white, red, and black rice samples, respectively (Table 3). The highest level of α-tocopherol was found in four black rice varieties (BJD, BPK (1), BPD(1), and BLP), while the low content of α-tocopherol was presented in all groups of rice samples including WPL2, RSY, RST1, RST4, and BPD(2). Therefore, the variation of α-tocopherol content was not affected by color on the rice grain, but its effect came from rice varieties. Kong and Lee (2010) studied antioxidants in milling fractions of black rice varieties in Korea and reported that tocopherols and tocotrienols were mainly located in rice bran fraction, which were approximately 5-folds higher than those of whole grain fraction.

The γ-oryzanol content of crude extracts was significant differences detected amongst rice varieties, but the trend of these contents was not same as that found for the other antioxidant contents. The highest γ-oryzanol content was presented in BSK (58.39 mg/100 g), which did not show a prominent content of TAC, TPC, TFC, and α-tocopherol content. However, the other black rice varieties also contained high levels of γ-oryzanol in the range of 38.21-49.56 mg/100 g (Table 3).

The γ-oryzanol content of rice samples in this study was higher than those of brown rice samples from Brazil that investigated by Pascual *et al.* (2013), which was in the range of 15.10-22.81 mg/100 g. In addition, Kong and Lee (2010) reported that γ-oryzanol content in whole grain of black rice varieties in Korea ranged from 60-75 mg/100 g (db), which was higher than those found in this study. The obtained results could have been due to the different kinds of rice varieties and extraction solvents.

Table 3. Antioxidant content and capacity of pigmented and non-pigmented rice varieties.

Samples (color/ rice varieties)	TAC (mg/100 g)	TPC (mg GAE/ 100 g)	TFC (mg CE/ 100 g)	α-tocopherol (mg/100 g)	γ-oryzanol (mg/100 g)	DPPH (IC50; g/mL)	FRAP (mmol Fe(II)/ 100 g)	TEAC (mmol TE/ 100 g)
White								
WPL2	nd	31.22 ± 1.56^{r}	16.41 ± 1.12^{m}	0.58 ± 0.04^{fgh}	28.64 ± 1.67^{ij}	43.30 ± 1.22^{a}	0.11 ± 0.02^{l}	0.33 ± 0.01^{o}
WHM105	nd	37.47 ± 4.13^{r}	15.43 ± 2.55^{m}	0.71 ± 0.03^{efg}	32.98 ± 0.64^{ghi}	42.92 ± 0.70^{a}	0.10 ± 0.02^{l}	0.36 ± 0.01^{o}
WSP	nd	46.87 ± 5.63^{q}	16.67 ± 2.36^{m}	0.67 ± 0.03^{fg}	36.94 ± 1.57^{fg}	34.80 ± 0.69^{b}	0.07 ± 0.01^{l}	0.37 ± 0.01^{o}
Red								
RMP	9.46 ± 0.96^{h}	368.16 ± 0.90^{f}	165.65 ± 3.77^{c}	0.73 ± 0.02^{ef}	25.99 ± 0.41^{jk}	1.87 ± 0.03^{mn}	1.84 ± 0.18^{cd}	1.83 ± 0.09^{f}
RSY	15.59 ± 3.48^{gh}	221.56 ± 3.12^{m}	100.59 ± 1.47^{h}	0.40 ± 0.01^{h}	23.10 ± 0.57^{ik}	3.94 ± 0.01^{h}	1.05 ± 0.02^{h}	1.48 ± 0.03^{i}
RST1	13.36 ± 1.67^{gh}	306.07 ± 3.12^{i}	143.30 ± 3.89^{f}	0.37 ± 0.02^{h}	31.23 ± 0.91^{hi}	2.67 ± 0.05^{jk}	1.38 ± 0.17^{f}	1.52 ± 0.01^{hi}
RST4	10.02 ± 1.67^{h}	325.62 ± 0.90^{h}	147.36 ± 1.12^{f}	0.46 ± 0.03^{gh}	34.09 ± 0.89^{gh}	2.54 ± 0.08^{ki}	1.49 ± 0.02^{e}	1.55 ± 0.01^{gh}
Black								
BHN(1)	21.15 ± 2.55^{gh}	138.49 ± 0.90^{p}	60.76 ± 1.85^{k}	0.81 ± 0.07^{ef}	38.50 ± 0.24^{efg}	10.10 ± 0.07^{c}	0.40 ± 0.02^{k}	0.84 ± 0.03^{n}
BHN(2)	27.27 ± 1.93^{gh}	198.20 ± 1.56^{n}	79.32 ± 0.73^{k}	1.09 ± 0.06^{d}	42.81 ± 2.89^{cde}	6.26 ± 0.05^{e}	0.52 ± 0.00^{k}	1.00 ± 0.04^{m}
BRB	33.95 ± 1.93^{g}	148.44 ± 7.16^{o}	57.35 ± 1.95^{l}	0.69 ± 0.05^{efg}	49.30 ± 1.53^{b}	9.06 ± 0.16^{d}	0.40 ± 0.07^{k}	0.81 ± 0.03^{n}
BST2	61.79 ± 3.34^{f}	241.35 ± 3.93^{k}	93.26 ± 1.47^{i}	1.33 ± 0.22^{c}	45.61 ± 3.49^{bcd}	5.39 ± 0.06^{fg}	0.71 ± 0.04^{ij}	1.18 ± 0.01^{l}
BJD	85.19 ± 23.40^{b}	487.01 ± 1.56^{d}	188.29 ± 3.31^{d}	2.04 ± 0.27^{a}	38.21 ± 2.45^{efg}	2.01 ± 0.02^{imn}	1.74 ± 0.09^{d}	2.17 ± 0.07^{d}
BPK(1)	441.96 ± 19.92^{a}	634.13 ± 8.60^{a}	279.32 ± 5.98^{a}	2.15 ± 0.32^{a}	49.56 ± 4.21^{b}	1.56 ± 0.01^{n}	2.30 ± 0.07^{a}	2.58 ± 0.04^{b}
BPK(2)	265.51 ± 27.59^{c}	521.35 ± 9.49^{c}	236.77 ± 2.36^{b}	1.66 ± 0.13^{b}	38.27 ± 2.40^{efg}	1.94 ± 0.00^{imn}	1.86 ± 0.04^{c}	2.40 ± 0.01^{c}
BPD(1)	380.73 ± 20.86^{b}	541.23 ± 3.25^{b}	227.76 ± 2.55^{c}	2.06 ± 0.21^{a}	46.32 ± 2.39^{bcd}	1.79 ± 0.02^{mn}	2.05 ± 0.02^{b}	2.66 ± 0.03^{a}
BPD(2)	103.53 ± 7.28^{e}	338.89 ± 0.00^{g}	128.12 ± 2.36^{g}	0.62 ± 0.09^{fgh}	48.48 ± 4.86^{b}	3.31 ± 0.06^{i}	1.04 ± 0.04^{h}	1.61 ± 0.04^{g}
BPY	81.27 ± 7.71^{f}	229.50 ± 2.70^{l}	88.90 ± 1.47^{j}	0.77 ± 0.10^{ef}	48.88 ± 7.56^{b}	5.84 ± 0.06^{ef}	0.65 ± 0.01^{j}	1.27 ± 0.02^{k}
BSK	118.56 ± 3.34^{e}	268.62 ± 9.75^{j}	99.46 ± 0.42^{h}	0.93 ± 0.03^{de}	58.39 ± 2.85^{a}	5.05 ± 0.00^{g}	0.79 ± 0.01^{i}	1.42 ± 0.03^{j}
BMS	158.64 ± 11.69^{d}	343.13 ± 3.25^{g}	146.52 ± 1.12^{f}	1.74 ± 0.06^{b}	47.32 ± 0.50^{bc}	3.22 ± 0.01^{ij}	1.24 ± 0.05^{g}	1.86 ± 0.03^{f}
BLP	261.06 ± 10.74^{c}	407.24 ± 3.12^{e}	166.68 ± 4.09^{e}	2.18 ± 0.18^{a}	41.13 ± 4.98^{def}	2.42 ± 0.02^{kim}	1.50 ± 0.04^{e}	2.03 ± 0.07^{e}

nd: not detected.
Mean values in a column superscripted by the same letter are not significantly different at $p < 0.05$.

3.3 Antioxidant capacities

The DPPH radical-scavenging ability is frequently used to evaluate the hydrogen donating of the antioxidants and the results are expressed as IC_{50} values (Table 3), indicating the concentration of antioxidant that caused the decrease of DPPH radicals to half of its initial concentration. Therefore, the lower of IC_{50} value provides higher antioxidant efficiency. The IC_{50} values for the black rice varieties varied from 1.56 to 10.10 mg/mL, while the red and white rice varieties were in the range of 1.87-3.94 and 34.80-43.30 mg/mL, respectively. The lowest IC_{50} value was found in BPK(1), corresponded to the highest content of TAC, TPC, TFC, and α-tocopherol content that observed in this sample. These results also corresponded with the previous study by Sompong *et al.* (2011) that determined the DPPH scavenging ability in red and black rice varieties from Thailand, China, and Sri Lanka and reported that no significant difference between red and black color of rice grain.

The FRAP values of crude extracts from different rice samples are given in Table 3. The red and black rice varieties were not clearly differentiated in terms of FRAP values, whereas the lowest of FRAP value was observed from the white rice varieties. The BPK(1) also presented the greatest FRAP value (2.30 mmol Fe(II)/100 g), followed by RMP, BJD, BPK(2), and BPD(1). No significant effect of rice grain color on ferric reducing ability was also reported by Sompong *et al.* (2011).

The TEAC values of the different crude rice extracts were performed using ABTS radical scavenging activity (Table 3). The result of TEAC showed a similar trend to that of FRAP values. The TEAC values of four black rice varieties (BJD, BPK(1), BPK(2), and BPD(1)) ranged from 2.17 to 2.66 mmol TE/100 g, which were higher than those of other black rice varieties as well as white and red rice varieties. Sompong, *et al.* (2011) reported that the variation of TEAC values was found within red and black rice varieties, which ranged between 2.1-12.6 and 5.0-12.1 mmol TE/100 g for red and black rice varieties, respectively.

Among the antioxidant contents and capacities determined in this study, the observed results were greatly correlated in terms of TAC, TPC, TFC, α-tocopherol content, DPPH activity, FRAP, and TEAC. The highest antioxidant contents and capacities were found in four black rice varieties, including BJD, BPK(1), BPK(2), and BPD(1). These samples were obtained from Khao Kho, Phetchabun, Thailand, which located about 500-1,400 meters above the sea level and the weather is relatively cool throughout the year, which the average temperature of 22.2-33.5°C. Iqbal and Bhanger (2006) reported that agroclimatic locations and

seasons have profound effects on the antioxidant activity of M. oleifera leaves in Pakistan, which the sample from cold areas contained relatively higher antioxidant activity than those from temperate regions.

3.4 Correlation analysis of color and antioxidant parameters

Among the antioxidant parameters, the positive correlations were detected for all parameters, except DPPH activity that presented the negative correlation with other parameters (Table 4). The highest significant correlations were presented between the TPC and TFC ($r = 0.989$), while the TPC and TFC presented highly significant correlations with FRAP and TEAC ($r > 0.900$). However, γ-oryzanol content exhibited a lower correlation coefficient with other parameters, which might be due to the fact that γ-oryzanol was located in bran layer of rice grain and not depended on the color on rice grain. Aguilar-Garcia *et al.* (2007) reported that γ-oryzanol content of rice bran powder (155.0-272.0 mg/100 g) was higher than that of brown rice powder (20.1-38.8 mg/100 g). It was due to brown rice is made up from a starchy endosperm and embryo coated with the bran layer, as a result the micronutrients are located in the bran rather than in the endosperm. Furthermore, the FRAP and TEAC exhibited the highly significant correlation in term of antioxidant capacity parameters ($r = 0.962$).

The accession of relationship among the color parameters and antioxidant properties was shown in Table 5. The L^* value presented the negative correlations with all antioxidant parameters, excepted only for DPPH radical scavenging ability that means the white rice varieties with a high L^* values presented a lower for antioxidant contents and capacities, corresponded with the data in Table 3.

3.5 Principal component analysis

The score plot from the principal component analysis (PCA) of different rice varieties was presented in Figure 1a. Eigenvalues of two principal components (PC) could explain 95.81% of total variance. The PC1 was the most important one, accounting for 80.57% of total variance, which mainly attributed to the pigmented rice varieties. However, the variation was not clearly distinguished between black and red rice varieties. The PC2 accounted for additional 15.24% of total variance, which represented the three varieties of white rice.

The score plot of different color and antioxidant parameters was presented in Figure 1b. The 83.69% of total variance could be explained by two PCs (47.34% for PC1 and 36.35% for PC2). The PC1 represented the hue value, TAC, TPC, TFC, α-tocopherol content, FRAP, TEAC, and DPPH activity, while other color parameters and γ-oryzanol content were observed on PC2. The obtained results indicated that the hue value and γ-oryzanol content exhibited a low relationship with the other color and antioxidant parameters, corresponded to the lower correlation coefficient of the γ-oryzanol content (Table 4). This result could indirectly indicate that γ-oryzanol, a compound that is abundant in rice bran fraction, show low association to pigments in rice grains.

3.6 Hierarchical cluster analysis

The dendrogram of rice samples using hierarchical cluster analysis (HCA) ranged from zero (greater similarity) to 25 (lower similarity) was presented in Figure 2. Four clusters of rice samples were well defined at degree of similarities equal to 8. The cluster 1 was classified for four red and one of black rice varieties. It is interesting that BLP was grouped into the same cluster with the red rice varieties, which corresponded to its color properties that closed to the color properties of red rice varieties. The cluster 2 consisted of eight black rice varieties, while the other flour black rice varieties, BPK(1), BPD(1), BJD, and BPK(2), that showed the highest

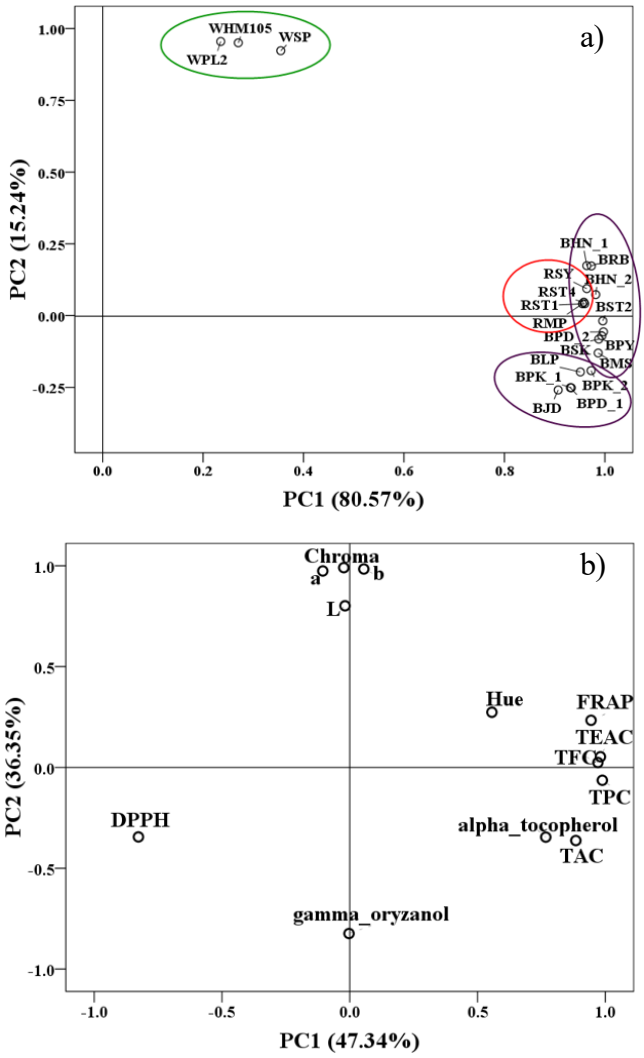

Figure 1. Principal component analysis (PCA) scores plot for (a) pigmented and non-pigmented rice varieties and (b) tested parameters on PC1 and PC2 based on color and antioxidant parameters.

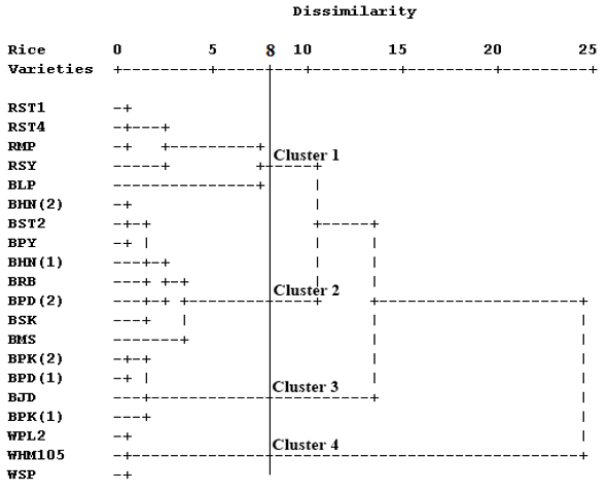

Figure 2. Dendrogram of hierarchical cluster analysis (HCA) of pigmented and non-pigmented rice varieties based on color and antioxidant parameters.

levels of antioxidant properties were grouped to the cluster 3. Furthermore, the cluster 4 included three white rice varieties, which exhibited the lowest values in terms of antioxidant contents and capacities. The result of HCA supported the result of PCA (Figure 1a) and it should be noted that the result of HCA was clearly classified between red, black, and white rice varieties.

4. Conclusions

Among the rice varieties, antioxidant contents and capacities of pigmented rice varieties were much higher than those of white rice varieties and the relationship between color and antioxidant parameters was observed by correlation analysis. The white rice varieties were negatively correlated with antioxidant contents and capacities, while γ-oryzanol content exhibited a lower correlation coefficient with other antioxidant properties. The obtained PCA result was good for classification between pigmented rice and white rice varieties and it was more clearly differentiated using HCA. Therefore, these two multivariate techniques are effective method to classify the rice varieties in this study. The result of this study was a useful tool in further making recommendations and selecting the pigmented rice with high antioxidant content, as well as the providing opportunities for rice breeder to use as indexes to indirectly select rice breeding lines and to promote the production of rice with enhance levels of phytochemicals. This research demonstrates clearly that the pigmented rice could be incorporated in to food, pharmaceutical, and medical products to increase their nutritional value and efficacy.

Table 4. Correlation coefficients between antioxidant parameters of pigmented and non-pigmented rice varieties.

Parameters	TAC	TPC	TFC	α-tocopherol	γ-oryzanol	DPPH	FRAP	TEAC
TAC								
TPC	0.874[a]							
TFC	0.816[a]	0.989[a]						
α-tocopherol	0.894[a]	0.726[a]	0.687[a]					
γ-oryzanol	0.320	0.259	0.178	0.403				
DPPH	-0.541[b]	-0.738[a]	-0.724[a]	-0.368	-0.318			
FRAP	0.703[a]	0.963[a]	0.974[a]	0.602[a]	0.037	-0.712[a]		
TEAC	0.845[a]	0.984[a]	0.974[a]	0.704[a]	0.221	-0.771[a]	0.962[a]	

[a] Significant at 0.01 probability level.
[b] Significant at 0.05 probability level.

Table 5. Correlation coefficients between color and antioxidant parameters of pigmented and non-pigmented rice varieties.

Parameters	TAC	TPC	TFC	α-tocopherol	γ-oryzanol	DPPH	FRAP	TEAC
*L**	-0.229	-0.595[a]	-0.579[a]	-0.332	-0.434	0.835[a]	-0.506[b]	-0.591[a]
*a**	-0.442	0.192	0.251	-0.196	-0.481[b]	-0.495[b]	-0.376	-0.294
*b**	-0.283	-0.433	-0.377	0.365	-0.765[a]	-0.592[a]	-0.242	-0.387
Hue angle	0.423	-0.452[b]	-0.435	-0.152	-0.410	0.873[a]	-0.393	-0.490[b]
Chroma	-0.363	-0.337	-0.271	-0.400	-0.801[a]	0.367	-0.117	-0.265

[a] Significant at 0.01 probability level.
[b] Significant at 0.05 probability level.

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