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Antioxidant Activities of Some Red, Black and White Rice Cultivar from West Sumatra, Indonesia

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Abstract: Food with antioxidant is very important to reduce the radical activity can caused oxidative stress. Antioxidant react as scavenger to reduce the radical actions, polyphenols were the common antioxidant in some plants. Colored rice which have some pigment have contribution as antioxidant activity. Red and black pigment on the rice seeds which give color to colored rice were polyphenols. We identified the 1,1-Diphenyl-2-picrylhydrazyl (DPPH), radical scavenging activity, total polyphenol, anthocyanin and protein content of eighteen colored rice from West Sumatra Indonesia, nine red rice cultivar, six black rice cultivar and two white rice cultivar as control. We found that some of the colored rice were polished at various level. The aim of this study was to identifiy the antioxidant activity of colored rice include polished and non polished rice. The result showed that the non polished colored rice have higher antioxidant activity than white rice. A variety of red rice cultivar from Solok Selatan was the best cultivar among other with antioxidant activity 54.2% at concentration 0.25 mg/ml, total polyphenol content 31.3 mg/ml and protein content 7.9%.

Key words: Rice, coloured rice, antioxidant activity, polished rice

INTRODUCTION

Rice (*Oryza sativa*) attracted more attention because of many country used as staple food. Rice divided into four groups based on the presence of pigment: black (anthocyanin), red (tannin), green (chlorophyll) and white rice (without pigment) (Itani, 2000). Red rice had red color on the surface, in West Sumatra usually cook as complementary with fried banana. Black rice, have very deep violet color because the present of anthocyanins. In West Sumatra black rice was fermented to produce fermented food.

Food become a very important sources of antioxidants due to the presence of hydroxyl substituents and their structure, which enables them to scavenge free radicals (Villano *et al*., 2007). Antioxidants are compounds that inhibit the oxidation of other molecules by inhibiting the initiation of oxidizing chain reactions. Natural antioxidants can be polyphenol compounds (tocopherols, flavonoids and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids and amines) (Velioglu *et al*., 1998). Colored rice are reported to be potent sources of antioxidants and their consumption as one of the sources is encouraged (Yawadio *et al*., 2007).

West Sumatra has many cultivar of black, red and white rice. Nine red rice cultivars, six red rice cultivars and 2 white rice cultivars were evaluate for the DPPH Radical Scavenging Activity (DPPHRSA), total polyphenols, anthocyanins and protein content. The main objective was to discover varieties from West Sumatra with high antioxidative potential.

MATERIALS AND METHODS

Plant materials: Nine red (include three polished red rice cultivars), six black and two white rice cultivars as control were used in this study (Fig. 1). These materials were derived from different planting area in West Sumatra region: Red rice: Batu Sangkar (S) (1), Padang Panjang (2), Kubang Putih (3), Lembah Gumanti (S) (4), Solok (5), Ombilin Merah Talang (6), Painan (7), Lembah Gumanti 2 (S) (8), Sariak Alahan Tigo (9), Pasaman (10). Black rice: Painan (11), Batusangkar (12), Palembayan (13), Lembah Gumanti (14), Solok Selatan (15) and Sariak Alahan Tigo (16). White rice: Kuriak Batusangkar (17) and Seratus Hari Kamang (18). (S) mean polished rice.

Chemicals: 1,1-Diphenyl-2-picrylhydrazyl (DPPH), ethanol, methanol, gallic acid, Na_2CO_3, KCl, HCl, sodium acetate, CH_3CO_2Na, distilled water, sulphuric acid, ammonium sulphate, brucine reagent, sodium hydroxide.

Color measurements: The Hunter L*, a*, b* values of reflected color of hulled rice grains in a Petri dish were measured using a colorimeter (CR-200, Minolta Co. Ltd., Osaka, Japan) with stirring 10 times.

DPPH radical scavenging activity: DPPH radical scavenging activity was determined using the method originally developed by Blois (1958). A portion (0.1 ml) of the extract solution (1.0 mg/ml methanol) in a test tube was well mixed with 3.9 ml of methanol and 1.0 ml of a

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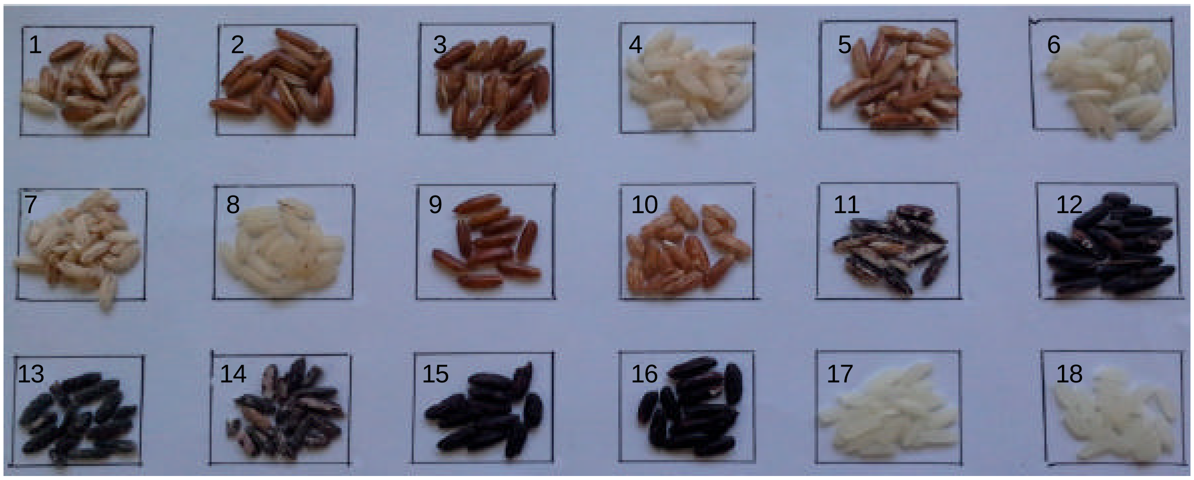

Fig. 1: Colored rice from West Sumatra

DPPH solution (1.0 mM in methanol). The mixture was kept at ambient temperature for 30 min prior to measurement of the absorbance at 517 nm (A517 nm). All measurements were done in triplicate:

$$\text{DPPH-RSA (\%)} = \frac{\text{(control absorbance-extract absorbance)}}{\text{control absorbance}} \times 100$$

Total polyphenol: The total phenolic content of the extract was measured with the Folin-Ciocalteau method (Taga *et al*., 1984), using gallic acid as a standard. A 0.1 ml of the extract solution was sampled into 2 ml of 2% Na_2CO_3 and mixed for 3 min. After adding 0.1 ml of 50% Folin-Ciocalteau reagent, the final mixture was left for 30 min before reading the absorbance at 750 nm. All measurements were conducted in triplicate and the data were expressed as g gallic acid equivalent (GAE) per kg of the extract, based on the calibration curve of gallic acid.

Anthocyanin content (Lee, 2005): 1.86 g KCl into a beaker and add distilled water to ca 980 mL. Measure the pH and adjust pH to 1.0 (±0.05) with HCl (ca 6.3 mL). Transfer to a 1 L volumetric flask and dilute to volume with distilled water. (b) pH 4.5 buffer (sodium acetate, 0.4M). Weigh 54.43 g $CH_3CO_2Na•3H_2O$ in a beaker and add distilled water to ca 960 mL. Measure the pH and adjust pH to 4.5 (±0.05) with HCl (ca 20 mL). Transfer to a 1 L volumetric flask and dilute to volume with distilled water.

Calculate anthocyanin pigment concentration, expressed as cyanidin-3-glucoside equivalents, as follows:

Anthocyanin pigment (cyanidin-3-glucoside equivalents, mg/L) = where, A = (A520-700 nm) pH 1.0-(A520-700 nm) pH 4.5:

$$\text{(mg/L)} = \frac{(A \times MW \times DF \times 1000)}{(\varepsilon \times d)}$$

where, MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor established in D; l = path length in cm; = 26 900 molar extinction coefficient, in L and mol-1 and cm-1, for cyd-3-glu and 103 = factor for conversion from g to mg.

Protein content: The crude protein was determined by the Kjeldahl method with slight modification. 0.5 g of the powdery form of each colored rice variety was digested with 5 ml of concentrated sulphuric acid in the presence of Kjeldahl catalyst. The nitrogen from the protein in the sample was converted to ammonium sulphate that reacted with 2.5 ml of 2.5% Brucine reagent, 5 ml of 98% sulphuric acid to give a coloured derivative and the absorbance read at 470 nm. The percentage nitrogen was calculated and multiplied by 6.25 to obtain the value of the crude protein (A.O.A.C., 1990).

RESULTS and DISCUSSION

Table 1 shows the grain reflected color of eighteen colored rice cultivar from West Sumatra, include three red rice cultivar with polished with different level. In some region of West Sumatra, people used black rice for consume, but they polished it first before consume. Because of this reason the L* value had significant different among the red rice cultivar.

The high level of polished rice give high value of L*. The L* value for red rice range from 26.42-65.98. The red rice from Sariak Alahan Tigo have the darkest color among other. While the red rice from Ombilin Merah Talang have the same L* value with white rice, because of the level of polished rice almost 90%. Which means the red rice from Ombilin Merah Talang have no pigment on the surface. The L* value of black rice range from 19.08-39.34. Solok Selatan have the highest L* value among other and become the darkest color among other. Some of the black rice cultivar also polished at different level. Solok Selatan is completely non polished rice which have a very deep purple color.

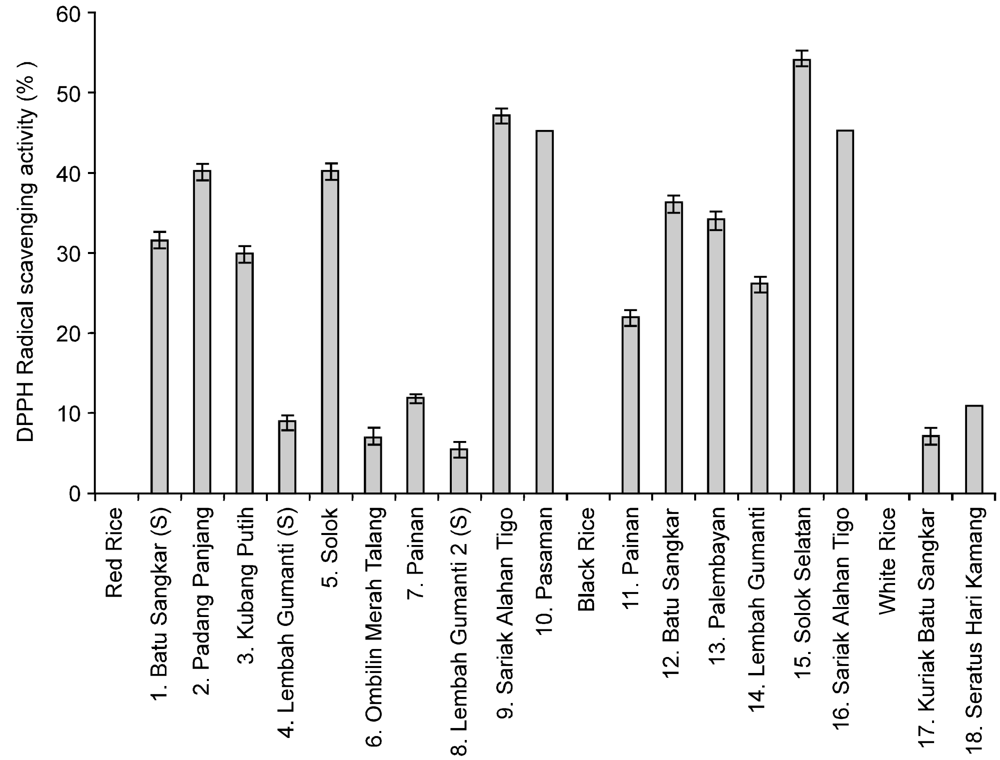

Fig. 2: DPPH-RSA of colored rice

Table 1: Hunters reflected color values

	Hunter reflected color		
Name of varieties	L*	a*	b*
Red rice			
Batu sangkar	44.57±0.4	9.34±0.2	11.47±0.2
Padang panjang	32.50±0.1	10.12±0.1	9.34±0.2
Kubang putih	29.52±0.9	9.91±0.2	9.06±0.3
Lembah gumanti	63.85±0.3	6.20±0.2	12.47±0.1
Solok	43.88±1.5	9.53±0.3	10.66±0.1
Ombilin merah talang	65.98±0.1	3.57±0.1	13.58±0.1
Painan	57.84±0.5	8.15±0	12.89±0.2
Lembah gumanti 2	57.19±0.4	6.25±0.1	14.31±0.2
Sariak alahan tigo	26.42±0.5	9.60±0.5	8.77±1.3
Pasaman	38.37±0.7	9.28±0.2	9.08±1.3
Black rice			
Painan	39.34±1.6	3.24±0.0	3.57±0.2
Batu sangkar	22.68±0.2	1.88±0.2	1.47±0.1
Palembayan	31.25±0.6	2.01±0.3	1.67±0.5
Lembah gumanti	33.39±0.1	2.09±0.1	2.06±0.1
Solok selatan	19.08±0.8	1.99±0.1	-0.17±0.1
Sariak alahan tigo	19.48±1.2	1.89±1.2	0.66±0.2
White rice			
Kuriak batu sangkar	68.32±1.4	0.68±0.3	12.77±0.3
Seratus hari kamang	68.79±1.1	0.51±0.3	12.49±0.2

Mean of triplicate determination±standard deviation

The a* value of red rice range from 3.57-10.12, black rice range from 1.88-3.24 and white rice range from 0.51-0.68. The b* value of red rice, black rice and white rice range from 8.77-13.58, -0.17-3.57, 12.49-12.77.

From the grain color data, we can determined the antioxidant activity and the total polyphenol of colored rice. The L*, a* and b* value can give information about the pigment presence in rice color and the polished and non polished rice.

DPPH radical scavenging activity (DPPHRSA) of colored rice: DPPHRSA was a measurement to estimate the antioxidant activity of colored rice. We incubate the DPPH solution and rice samples about 30 min. The rice samples was prepared in methanolic extract. Methanol extract had superior antioxidant activities to the ethanol extract, which exhibited a comparatively greater amount of the antioxidant compositions, caused by the possibility of more polar phenolic compounds in the methanol extract than in the ethanol (Lai *et al*., 2009).

Figure 2 showed that rice pigmented with red and black had higher DPPHRSA at concentration 0.25 mg/ml than the polished rice. The following colored rice cultivars had the highest DPPHRSA in the test: Black rice cultivar from Solok Selatan (15) and Red rice cultivar from Sariak Alahan Tigo (9) followed by Pasaman (10). These red and black rice cultivar is a nonpolished rice. The darkest color of rice showed the highest antioxidant activity. The red and purple color determined the antioxidant properties in colored rice. The red rice from Lembah Gumanti, Ombilin Talang, Painan and Lembah Gumanti 2 have almost the same antioxidant activity with white rice cultivar.

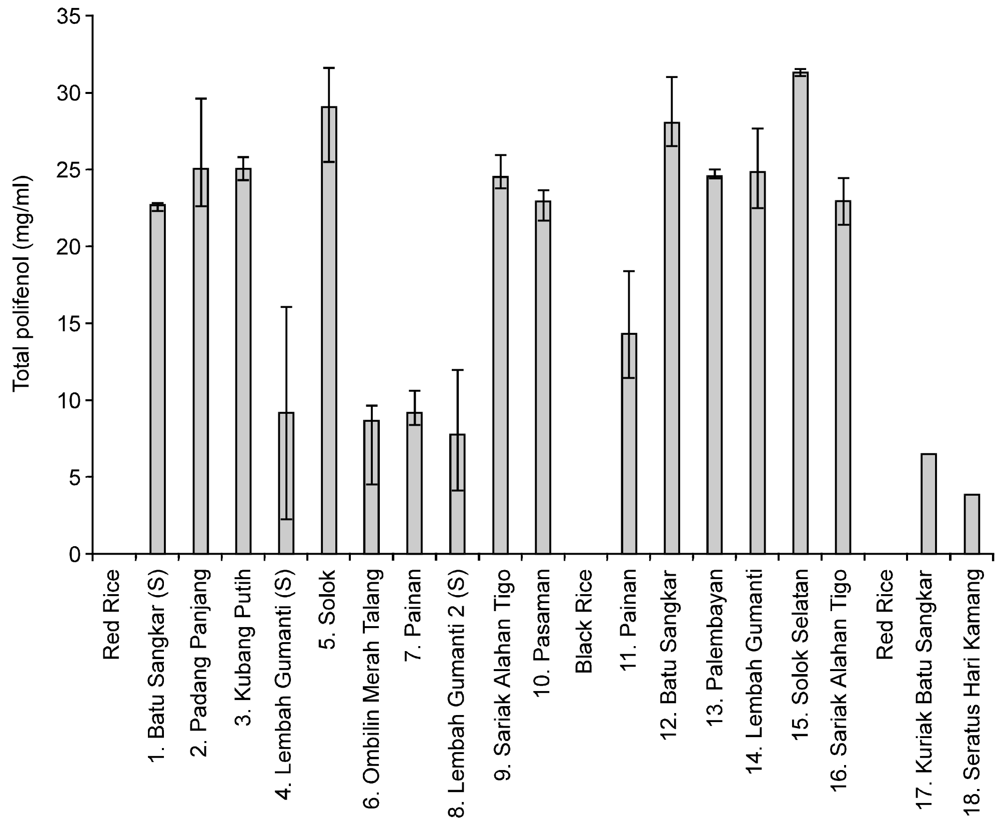

Fig. 3: Total polyphenol of colored rice

The reflected color have correlation with the antioxidant activity of coloured rice. A red rice cultivar from Sariak Alahan Tigo have the lowest L* value and have the highest antioxidant activity. A Cultivar from Solok Selatan also have the lowest L* value and have the highest antioxidant activity. Which mean we can estimate the antioxidant activity by the grain color because of the pigment contain in rice cultivar.

According to Hyun *et al*. (2006) the pigmented rice with high antioxidative potential may provide a source of new antioxidants as well as genes. Rice consumption can contributing to the prevention of chronic disease caused by oxidative damage cells. The information about the antioxidant activity will give an understanding for change the habitual to consume no polished rice cultivar.

DPPH (1,1-diphenyl-2-picrylhydrazyl) causes a deep violet color with λ_{max} around 520 nm. When a solution of DPPH is mixed with a substrate acting as hydrogen atom donor, a stable nonradical form of DPPH is obtained with simultaneous change of the violet color to pale yellow (Molineux, 2004).

Total polyphenol: Polyphenols are one of the most effective antioxidant compounds in plant foods including fruit, vegetables and grains (Velioglu *et al*., 1998).

Figure 3 shows the result of total polyphenols from each region of West Sumatra. The result from the different cultivars can be divided into high, medium and low polyphenol contents. The following cultivars exhibited high polyphenol; Red Rice: Solok, Padang Panjang, Kubang Putih, Sariak Alahan Tigo, Batu Sangkar and Pasaman; Black Rice: Solok Selatan, Batu Sangkar, Sariak Alahan Tigo, Palembayan and Lembah Gumanti. In contrast, the red rice cultivar from Lembah Gumanti, Painan, Ombilin Merah Talang and one black rice cultivar fromPainan showed medium polyphenols. The activity of white rice was the weakest.

The relatively high total antioxidant activity in red rice compared to black rice, green rice and white rice showed a significant correlation with total polyphenol as potential antioxidant (Rao *et al*., 2001). The same result also explained with the positive correlation between antioxidant activity and the total polyphenol content in rice. Solok Selatan have the highest antioxidant activity and followed by the highest total polyphenol.

The total polyphenol of red rice range from 7.7-24.5 mg/ml. Red rice cultivar from Sariak Alahan Tigo have the highest total polyphenol and Lembah Gumanti 2 was the lowest. Red cultivar from Lembah Gumanti 2 was a polished rice which have less pigment than others.

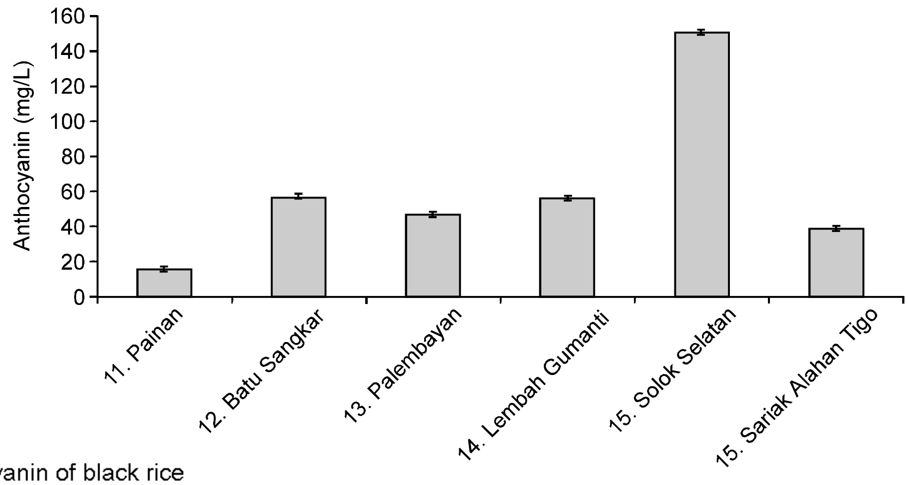

Fig. 4: Anthocyanin of black rice

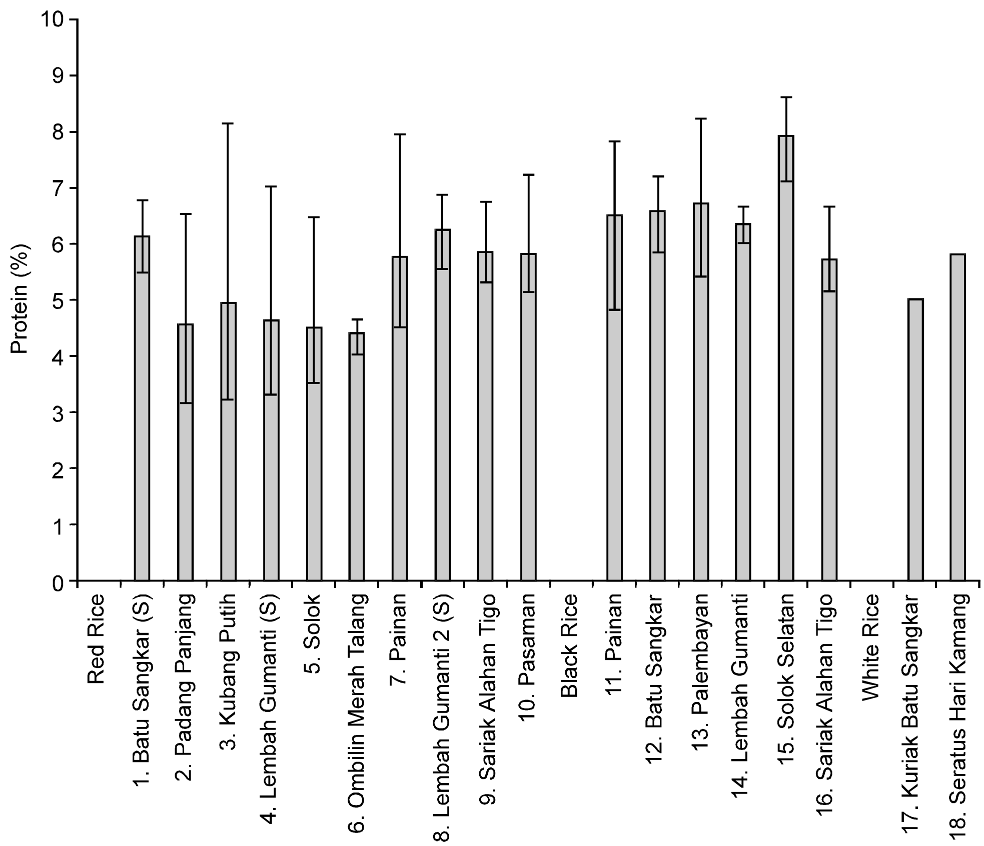

Fig. 5: Protein content of colored rice

Anthocyanin content: The anthocyanin content was measured for black rice. The data showed in Fig. 4. Anthocyanin was a polyphenol pigment in black rice cultivar. Anthocyanins are responsible for the red, purple and blue color present in fruits, vegetables and grains. There are six common anthocyanidins (pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin), whose structures can vary by glycosidic substitution at the 3 and 5 positions (Lee, 2005).

Among the black rice cultivars as seen on Fig. 4. Solok Selatan cultivars had the highest anthocyanin among others. A significant correlation was also noted between grain color, DPPHRSA, total polyphenol and anthocyanin content. Solok Selatan Cultivar had the darkness color, the highest total polyphenols, anthocyanin and antioxidant activity. The antioxidant compounds include anthocyanins (glycosides)-cyanidin-3-O-β-D-glycoside and peonidin-3-O-β-glycosides (Hu *et al.*, 2003). The pH differential method has been used extensively by food technologists and horticulturists to assess the quality of fresh and processed fruits and vegetables (Lee, 2005). By polished the rice will decrease the anthocyanin in black rice which influence the antioxidant activity and total polyphenol. Red rice cultivar from Painan have the lowest anthocyanin content.

Anthocyanins are glycosides of polyhydroxy 2-phenylbenzopyrylium cations belonging to a larger group of flavonoids, a subgroup of polyphenol (Wu *et al*., 2002). Purple seeds contain anthocyanins and red seeds contain proanthocyanidins (Furukawa *et al*., 2005). Anthocyanin-pigmented rice, which possesses potent antioxidant components, can contribute, not only to the reduction of oxidative stress diseases but also to improvements in the color and taste of foods made with rice (Suk and Chul, 2007).

Protein content: Figure 5 showed that black rice, red rice and white rice contain high levels of protein. Solok Selatan was a cultivar with high protein and antioxidant activity become the best cultivars among others. Beside amylose, protein was the important component to human health. Lack of protein will decrease the nutrition and will influence the metabolism. The protein content of colored rice have no correlation with the grain color, the antioxidant activity nor the total polyphenol, but the higher protein content will made the rice quality more better. The Data on Fig. 5 showed red rice, black rice and white rice range from 4.4-7.9%. The red rice cultivar from Lembah Gumanti 2 have the highest protein contain among other red rice cultivar. Solok Selatan was the highest protein content among the red rice cultivar and Seratus Hari Kamang was the highest protein contain among white rice cultivar.

Conclusion: The result suggest that red and black rice have antioxidant potencies of natural product present in rice. Lack of antioxidant compound in polished and white rice. A variety of red rice cultivar from Solok Selatan was the best cultivar among other with antioxidant activity 54.2% at concentration 0.25 mg/ml, total polyphenol content 31.3 mg/ml, protein content 7.9% and amylose content 4.8%.

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