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Phytochemical Compound and Nutritional Value in Black Rice from Java Island, Indonesia

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ABSTRACT

A study of anthocyanin in whole brain of pigmented rice has been concern to deeply determine as healthy nutrition since the anthocyanin function of rice bran has proved clearly. This study purposed to characterize the nutrition chemical composition, amino acids residues & phytochemical properties, total anthocyanin and its function in pigmented rice. Five rice varieties from Java Island were determined using proximate analysis, amino acids content, qualitative phytochemical analysis and IC50 anti-oxidative testing using DPPH analysis. The result showed the lipid content in all black rice higher than white and red rice. In line with IC50 testing result in black rice was low and closed with ascorbic acid value. Phytochemical profile determined the Toraja black rice from west Java (BRWJ) have highest content leucoanthocyanidin, phenol, flavonoids, quinone, antraquinone and glucoside and also total anthocyanin. The Toraja black rice from West Java conducted that has a higher content of nutrition values and phytochemical compounds compare with others. The total anthocyanins in all black and red rice indicated have anti-oxidative activity.

Keywords: anthocyanin, anti-oxidative, nutritional food, phytochemical, pigmented rice.

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INTRODUCTION

The beneficial effects of whole grains of pigmented rice consumption might have a lot of biological function. Anthocyanins of black rice predicted has a function as bioactive compounds as an antioxidant activity, anti-obesity, anti-inflammatory, and other functions that have promoting health benefit to control metabolic mechanism [1]. Rice (*Oryza sativa L.*) is one of the important plants that play a role in food sources for most of the world's population. Rice is one of the ordinary main foods of Indonesian people. A lot of native paddy variants are not only white rice but also wild-pigmented paddy flourish in several islands in Indonesia. Based on the pigments contained in rice, pigmented-rice is classified into black, brown, red, and white that belongs to genus Oryza [2].

Oryza sativa is divided into two subspecies japonica and indica. The have spesific characters that *O. sativa L.* indica has long and non-sticky rice characters, and *O. sativa L. japonica* has short and sticky rice characters [3, 4]. Population of Southern Asia, Northern China, Japan and America Latino consumed rice of *Oryza sativa L.* japonica. Population in Eastern and Southern Asia countries were consuming rice of *Oryza sativa L.* indica [5, 6]. Recent studies reported that black rice in Indonesia has diverse strains of black colors with high content of anthocyanins [7]. Moreover [8] state that black rice in West Java is pitch black in stems, leaves and grains of rice. The black rice is other region such as Java, East Nusa Tenggara (NTT), Borneo and Sumatra islands that they have a black color only on rice grains. The difference color morphology indicates the influence of environmental factors and the formation of rice anthocyanin production genes.

Environmental factors such as UV-B regulate the activation of anthocyanin biosynthetic gene transcription factors [9, 10]. The anthocyanin content in different black rice is caused by environmental factors such as exposure to biotic and abiotic stress, UV rays (UV-B and UV-C) and soil environmental conditions [11, 12]. The black rice contains phytochemical compounds such as flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, oryzanols, and phenol [11, 13]. Anthocyanins and proanthocyanidins are a type of flavonoids and include bioactive compounds found in black, brown and red rice.

Anthocyanin is most commonly found in black rice, which gives a purple to black color to the pericarp layer (the layer near the seed coat) of black rice [9]. In plants, anthocyanin is acted to attract insect pollinators, seed dispersal and as a photo protectant by capturing free radicals during photosynthesis [14]. Most anthocyanins are formed from three basic types of anthocyanidins, namely cyanidin, delphinidin and pelargonidin that the there are different structure at the B-group hydroxyl ring. The higher amount of hydroxyl in B-ring will take an impact on the blue color, which expressed the color of anthocyanin [9]. In studies, we explore five varieties of black, red, and white rice from West, Central and East Java prefecture. We focus to identify the content of chemical properties, amino acid residues composition, and total anthocyanin & its characteristics in five-varieties of pigmented rice.

MATERIAL AND METHODS

Sample Collection

Five varieties of rice were collected from three provinces in Java Island, Indonesia. They were Mentik Wangi white rice (WREJ) from East Java, Mentik red rice (RREJ) from East Java, NTT local (N790) black rice (BREJ) from East Java, Melik black rice (BRCJ) from Central Java, and Toraja local black rice (BRWJ) from West Java. The rice samples were stored at room temperature until used for further analysis.

Proximate Analysis of Pigmented Rice
The proximate composition of all rice samples were determined by an Indonesian National Standard (SNI 01-2891-1992) method used for carbohydrate, ash, protein, lipid, and water content. The analysis was done in Central Laboratory of Life Sciences, Brawijaya University, Malang, Indonesia.

Determination of Amino Acids in Pigmented Rice
The determination of amino acids of the all rice was used The Ultra Performance Liquid Chromatography (UPLC). The UPLC was used to identify the 17 amino acids in the all samples, namely L-histidine (His), L-threonine (Thr), L-valine (Val), L-methionine (Met), L-lysine (Lys), L-isoleucine (Iso), L-leucine (Leu), L-phenylalanine (Phe), L-aspartic acid (Asp), L-serine (Ser), L-glutamic acid (Glu), glycine (Gly), L-arginine (Arg), L-alanine (Ala), L-proline (Pro), L-cystine (Cys), and L-thyrosine (Tyr). The UPLC analysis performed was based on the Waters Acquity UPLC H Class and H Class Bio amino Acid Analysis System Guide (Waters, 2012). One microliter of each sample was injected onto the AccQ.Tag Ultra C18 1.7 µm column (2.1 x 100 mm). The column was maintained at 49°C. The following mobile phases (A–D) were used for the separation, namely, A: Eluent A concentrate Amino Acid Analysis AccQ.Tag Ultra; B: Eluent B Amino Acid Analysis AccQ.Tag Ultra 10% in water; C: Aquabidest; D: Eluent B Amino Acid Analysis AccQ.Tag Ultra. The flow rate was fixed at 0.5 mL/min. Amino acids were detected using photometric diode array (PDA) detector at the wavelength of 260 nm.

The L-tryptophan (Trp) in rice samples was analyzed using High Performance Liquid Chromatography (HPLC) based on the AOAC official method (Szkudzińska et al., 2017). We used the Lichrospher RP-18 (250 mm x 4.0 mm, 5µm) column (Merck, Germany) for separation with the injection volume of 15 µL. The column had an ambient temperature and isocratic pump system with the flow rate of 1.5 mL/min. The mobile phase consisted of A = Sodium Acetate 0.0085 M pH 4 and B = Methanol (A: B = 95: 5). The L-tryptophan was detected at 280 nm of wavelength using the PDA detector.

Pigmented Rice Extraction
The powdered rice samples were extracted by maceration using organic solvents, namely aquadest, ethanol, and methanol. The maceration was carried out overnight at room temperature [15]. The homogenates were filtered with Whatman filter papers (0.45 µm). The filtrates were used for the qualitative phytochemical analysis.

Qualitative Phytochemical Analysis of Pigmented Rice
The rice extracts were assessed for the existence of phytochemical compounds. The determination of anthraquinones, flavonoid, glycoside, tannin, phenolic, alkaloid, protein, and leucoanthocyanidin were based on the standard methods [16–18]. Different wavelengths were used in each test to measure the absorbance of the samples using a spectrophotometer, namely 210 nm (glycoside test), 280 nm (phenolic and protein tests), 430 nm (flavonoid test), 470 nm (alkaloid test), 515 nm (anthraquinone test), 535 nm (leucoanthocyanidin test), and 700 nm (tannin test).

Determination of Total Anthocyanin Content
The rice sample (25 g) was mixed with 250 ml of 0.1% HCl in 85% methanol and homogenized at room temperature overnight. The homogenate was filtered using Whatman filter paper (0.45 µm) and evaporated with rotary evaporator at 700 rpm, 70°C for 4 hours. Total anthocyanin content of the extract was determined with the pH-differential method and expressed in mg/L [19, 20].

Total Anthocyanin Characterization of Pigmented Rice based on pH, IR Spectrum and TLC Analysis
The color intensity measurement of total anthocyanin extract was performed on seven pH conditions (1, 2, 3, 4.5, 7, 8, and 11). The absorbance of each solution was measured with a UV-1700 UV-VIS spectrophotometer (Shimadzu, Japan) at the wavelength range of 200-800 nm [21]. Thin layer chromatography (TLC) was used for the qualitative analysis of anthocyanins in the extracts. The 0.2 mg/ml of anthocyanin extract was dripped on the F254 silica plate with the mobile phase of n-butanol: acetic acid: water (3: 1: 1). UV visualized the anthocyanin profile of pigmented rice at the wavelength of 365 nm [22]. The Fourier transform infrared spectrometry (FTIR) was carried out according to the standard protocol in the Analysis and Measurement Units of Chemistry Department, Faculty of Mathematics and Natural Sciences, Brawijaya University, Indonesia. FTIR was used to analyze the functional groups of total anthocyanins in the rice samples [23].

Antioxidant activity of Pigmented Rice using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assays
Total anthocyanins of pigmented rice extract (0, 2, 4, 6, 8, 10 µg/mL) 200µL were added into 50µg/mL of 3.8mL DPPH solution. The reaction was incubated at 37°C for 30 minutes and measured on 517nm. Ascorbic acid was used as positive control. The antioxidant activity was calculated by percentage of DPPH scavenging [24].

RESULT
Chemical nutrient properties and amino acid residues of the compounds in pigmented rice
To determine the chemical nutrient composition, all rice samples was analyzed using proximate analysis and amino acid residues contents by UPLC (Figure 1). Among pigmented rice, the carbohydrate, ash, protein, fat and water contents are no different (Figure 1a). The composition of essential and non-essential amino acids is higher in all black rice compared to red and white rice (Figure 1b).

Phytochemical properties of pigmented rice
Biochemical analysis in this research includes extraction using several solvents, such as water, n-Hexane, ethanol and methanol. The use of various solvents for the extraction of pigmented rice aims to find out the right solvent to isolate the bioactive compounds of black rice. Pigmented rice extract with organic solvents, include distilled water, ethanol, methanol and n-Hexane. The screening of phytochemical compounds in pigmented rice using various solvents showed that BRWJ contained leucoanthocyanidin, phenol, tannin, flavonoid, Quinone, antraquinone, and glycoside compounds higher than the others (Figure 2). Pigmented rice water extracts showed the presence of

A Component	Concentration (%) White Rice East Java (WREJ)	Red Rice East Java (RREJ)	Black Rice East Java (BREJ)	Black Rice Central Java (BRCJ)	Black Rice West Java (BRWJ)
Carbohydrate	74.62	74.99	73.24	73.45	72.88
Ash	1.26	1.36	1.56	1.16	1.43
Protein	10.12	7.86	9.93	9.53	10.15
Fat	2.92	2.48	2.73	2.85	2.75
Water	11.08	13.32	12.54	13.01	12.69

B Amino acids	Concentration (ppm) White Rice East Java (WREJ)	Red Rice East Java (RREJ)	Black Rice East Java (BREJ)	Black Rice Central Java (BRCJ)	Black Rice West Java (BRWJ)
Essentials					
L-Histidine	1601.93	1639.63	1883.42	2020.28	2326.75
L-Threonine	2440.98	2810.27	3033.58	2814.3	3439.24
L-Valine	3709.92	4286.54	4555.81	3861.18	5038.88
L-Methionine	1568.58	1646.34	1864.05	1924.42	2160.6
L-Lysine	3180.17	3873.19	4227.14	2950.38	4257.05
L-Isoleucine	2569.32	3179.54	3209.94	2626.28	3581.15
L-Leucine	5396.42	6569.97	6599.23	5572.26	7320.57
L-Tryptophan	754.34	903.2	1016.46	658.45	1000.69
L-Phenylalanine	3463.56	3741.83	4128.3	4196.98	4938.84
Non-essentials					
L-Aspartic acid	6266.62	7364.9	7327.9	5432.35	7852.92
L-Serine	3662.38	3999.47	4249.62	3942.5	4683.39
L-Glutamic acid	13222.84	16347.92	15988.43	12008.68	17167.57
Glycine	3107.44	3526.4	3689.79	3647.65	4293.47
L-Arginine	4709.1	5041.19	5632.01	5341.29	6580.92
L-Alanine	4297.52	4922.48	4944.03	4216.84	5254.14
L-Proline	2927	3416.13	3478.28	3046.39	3926.81
L-Cysteine	325.48	382.09	390.73	416.91	480.32
L-Tyrosine	2110.75	2367.88	2567.56	2935.56	3054.78

Figure 1. Chemical properties and amino acid residues of the compounds in pigmented rice. A. Proximate analysis of pigmented rice, and B. Rice amino acid residues composition analyzed by UPLC.

various phytochemical compounds, white rice showed the lowest phytochemical compounds. Likewise, methanol and ethanol extracts of black rice showed the presence of phytochemical compounds in black rice, but the ethanol extract of BRCJ was not detected by antraquinone. WREJ white rice methanol extract was also not detected the presence of leucoanthocyanidin, protein, quinone, antra-quinone, and glycosides.

Solvent	Sample	Compounds: Leucoanthocyanidins	Phenol	proteins	Tannin	Flavonoids	Quinones	Antraquinone	Glycoside
Water extract	WREJ	+	+	+	+	+	+	+	+
	RREJ	+	++	+	+	+	+	++	++
	BREJ	+++	+++	+++	+++	+++	+++	+++	+++
	BRCJ	++	++	++	++	++	++	++	++
	BRWJ	++++	++++	++++	++++	++++	++++	++++	++++
Methanolic extract	WREJ	-	+	-	+	+	-	-	-
	RREJ	+	++	+	++	++	+	+	+
	BREJ	++	+++	++	+++	+++	++	++	++
	BRCJ	+++	++++	+++	++++	++++	+++	+++	+++
	BRWJ	++++	++++	++++	++++	++++	++++	++++	++++
Ethanolic extract	WREJ	-	+	++	+	-	+	+	-
	RREJ	+	+++	+++	++	+	++	+	-
	BREJ	+++	+	+	+++	++	+++	++	+++
	BRCJ	++	+	+	++++	+++	+++	-	++
	BRWJ	++++	++	++++	++++	++++	++++	+++	++++

Figure 2. The phytochemical screening in pigmented rice, - not detected, + detected phytochemical in low intensity of color, ++ detected phytochemical compound with medium color intensity, +++ detected phytochemical compound with high color intensity, ++++ detected phytochemical compound with very high color intensity.

The total anthocyanin and its characteristics in pigmented rice

Table 1. The wavenumber values at the FTIR absorption peaks for total anthocyanin of pigmented rice samples and their probable functional groups.

Wave Number (cm^{-1})					**Probable functional group**
WREJ	**RREJ**	**BREJ**	**BRCJ**	**BRWJ**	
3388.7		3355.91	3380.98	3369.41	N-H Stretching; Aliphatic primary amines
	3299.98				N-H Stretching; Aliphatic primary amines
	3010.67	3010.67	3008.75	3010.67	O-H stretching alcohol, C-H stretching alkene, alkane
2923.88	2925.81	2925.81	2925.81	2925.81	N-H stretching; Amine salt, C-H alkane
2852.52	2854.45	2856.38	2854.45	2856.38	C-H stretching; Alkane
	1741.6	1741.6	1741.6	1741.6	C-H bending; Aromatic compound
1704.96	1712.67	1708.81	1712.67	1708.81	C-H bending; Aromatic compound
1645.17	1647.1	1645.17	1645.17	1643.24	C=C stretching; Alkene
	1620.09	1627.81	1631.67		C=C stretching; Alkene, N-H Bending; amine, C=C stretching; Cyclic alkene
1548.73		1544.88		1546.8	N-O stretching; Nitro compound
			1537.16		N-O stretching; Nitro compound
1515.94	1517.87	1515.94	1515.94	1517.87	N-O stretching; Nitro compound
1463.87		1461.94		1460.01	C-H bending; Alkane, Methylene group
	1454.23		1456.16		C-H bending; Alkane, Methyl group
			1438.8		C-H bending; Alkane, Methyl group; O-H bending; Carboxylic acid
1415.65	1398.3				O-H bending; Alcohol
		1367.44	1369.37	1334.65	O-H bending; Alcohol; O-H bending; Phenol
	1280.65	1267.14	1276.79	1280.65	C-N stretching; Aromatic amine; C-O stretching; Aromatic ester
	1242.07			1242.07	C-N stretching; Amine
		1201.57	1201.57	1201.57	C-O stretching; tertiary alcohol; C-O stretching; Ester
1153.35	1161.07	1166.85	1172.64	1172.64	C-O stretching; tertiary alcohol; C-O stretching; Ester
	1107.06				C-O stretching; Secondary alcohol
1080.06	1074.28	1076.21	1076.21	1074.28	C-O stretching; Primary alcohol
1027.99		1029.92	1035.7	1031.85	
933.48					C=C bending; Alkene (monosubtituted)
	819.69	823.55		825.48	C=C bending; Alkene (trisubtituted); C-H bending 1,4-disubtituted
	777.26				C-H bending; 1,3-disubtituted
694.33	705.9	721.33	721.33	719.4	C=C bending; Alkene (disubtituted)
		626.82		636.47	C-H bending; Alkyne
576.68	574.75	580.53	576.68	580.53	Not determined
		513.03	518.82	522.67	Not determined

The highest total content of anthocyanin is at BRWJ, followed by BREJ. BRCJ black rice has a total anthocyanin content that is not significantly different from RREJ red rice (Figure 3a). Based on spectrum, the total anthocyanins of pigmented rice are absorbed maximum at UV wavelengths (200-300 nm) and visible light (400-600 nm) (Figure 3B-F). However, WREJ does not show the maximum absorption of visible light (Figure 3B). RREJ pH 1 shows the absorption The RREJ red rice shows a small total anthocyanin. Black rice BREJ pH 1 and pH 5 show the maximum absorption of visible light 450-550 nm, at pH 9 and pH11 the maximum absorption of BREJ at wavelengths 350-420 nm. BRCJ has a maximum absorption at

450-575 nm at pH 1 and pH 3, whereas in the UV region it is absorbed maximum at 330-420 nm at pH 7 and pH 9.

The FTIR profile shows the same pattern between red rice and the black rice (Figure 2G-K). But some characters in white rice is not found, such as the wavelength of 3200 - 2800 cm-1 (Figure 2G). Specific functional groups only in red and black rice indicate the presence of functional groups that contain benzene structure. The structure of benzene is found in phenolic compounds, flavonoids and anthocyanins. The functional group which is only owned by red rice and the three types of black rice is 14338.8 (CH bending; Alkane, Methyl group; OH bending; Carboxylic acid) 1334-1367.44 cm-1 (OH bending/phenolic group), 1267.14-1280.65 cm-1 (CN stretching / aromatic amine/aromatic ester), 1242.07 cm-1 (CN stretching/amine), 1201.57 cm-1 (CO- stretching/alcohol/ester), 1107.06 cm-1 (CO stretching/secondary alcohol), 819.69 - 825.48 cm-1 (C = C bending/alkene tri-substitute; CH bending/1,4-disubtituded), 777.26 cm-1 (CH bending/1.3 di-substitute) (Figure 3H-K and Table 1).

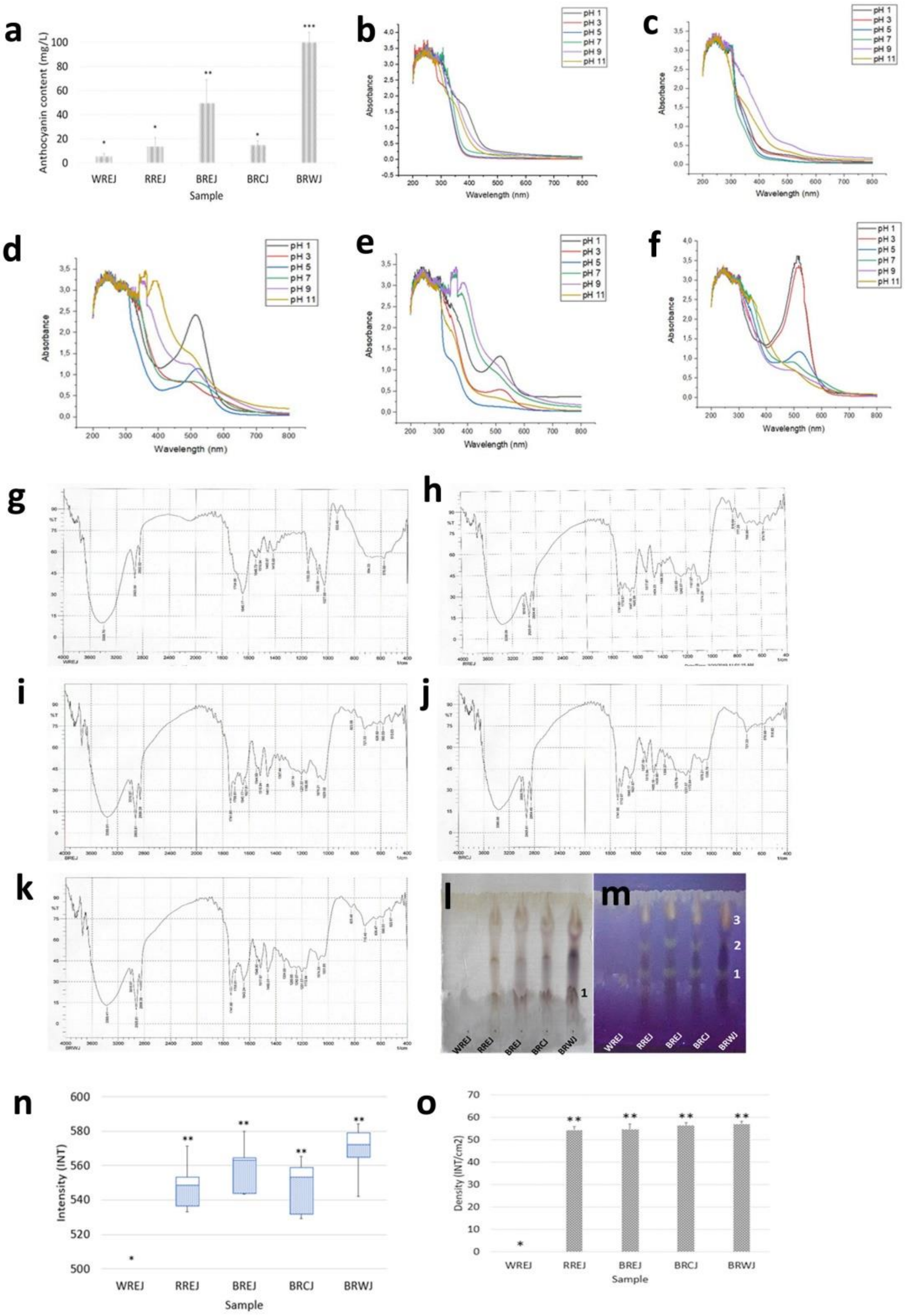
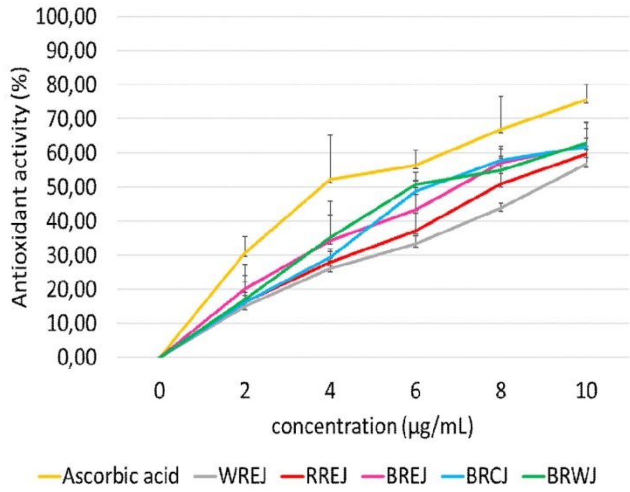


Figure 3. The total anthocyanin and its characteristics in pigmented rice. Total content of pigmented rice anthocyanin (a); Anthocyanin characterization based on pH differences in WREJ (b), RREJ (c), BREJ (d), BRCJ (e), BRWJ (f); Characterization of functional groups based on IR spectrum analysis on WREJ (g), RREJ (h), BREJ (i), BRCJ (j), BRWJ (k); Total anthocyanin profile of pigmented rice analyzed by thin layer chromatography (TLC) on direct visualization (l) and determined by UV (m), total anthocyanin color density based on TLC data (n) and its intensity (o).

Total TLC analysis of anthocyanin pigmented rice showed that there were three spots on red rice and three types of black rice, whereas white rice was not detected (Figures 3L and M). The three spots are thought to be anthocyanin compounds in red and black rice. Based on TLC spot intensity in pigmented rice, BRWJ significantly has the highest intensity. BREJ has a higher intensity than BRCJ black rice and RREJ red rice (Figure 3N). Based on the TLC spot density of the four-pigmented rice types (red and black rice) the fourth density was higher than white rice, but the density between the four-pigmented rice was not significantly different (Figure 3O).



Sample	IC50 (µg/mL)
Ascorbic acid	5.43
WREJ	8.85
RREJ	8.07
BREJ	7.25
BRCJ	7.21
BRWJ	7.08

Figure 4. Total antioxidant activity of pigmented rice anthocyanins against the scavenging of DPPH as free radicals

DPPH is a free radical compound that can be used to analyze compound activity in extracts. The principle of DPPH analysis is by measuring the decrease in DPPH solution absorption at the optimum wavelength in the reaction solution. To test the percentage of antioxidant and IC50 activity of total rice anthocyanin extracts, were analyzed using DPPH and ascorbic acid as a control (Figure 4.). The antioxidant activity in the sample is equivalent to the amount of DPPH that is reduced in the reaction solution. In Figure 4, the antioxidant activities have no significant different among BRWJ, BREJ, RREJ and BRCJ compared to white rice (WREJ).

DISCUSSION

The rice has macronutrient contents involved carbohydrates, proteins, and fat. However, the black rice from West Java (BRWJ) has the lowest carbohydrates and the highest protein composition. The lowest fat content found in red rice from East Java (RREJ). The chemical nutrient property of black rice from central Java (BRCJ) is similar with white rice. Living organism needed not only the macronutrients but also micronutrients in their dietary nutrients. The compositions of amino acid residues in black rice are more abundant than red and white rice. The highest amino acid residues contents were found in black rice from West Java and then East Java.

The L-glutamic acid residue was dominated in all rice. In the body the glutamic acid is intermediate in Krebs cycles and has a function for energy metabolism and glutamate or glutamine biosynthesis. Glutamate is neurotransmitter to prevent the neurological disorder [25]. The food-rich amino acids are an essential nutrient for lymphocyte proliferation and cytokine production to mediate the immunity response. The expression of genes susceptibility immune system is predominantly dependent on glutamine presence [26]. Recently study reported the content of arginine and glutamate acid in foods is good for the regulation of the body's metabolism [27]. Glutamine, a derivative of glutamate acid, increases the expression of proliferation genes in the small intestine and decreases oxidants in the body, besides that arginine can increase lipolysis activity and decrease the expression of fat-forming genes in adipose tissue. Methionine, histidine, serine and glycine play a role in histone modification such as acetylation, methylation and phosphorylation. Modifications of histones in cells play a role in the body's physiological functions and regulation of gene expression [26–28]. Based on nutrient content, both proximate testing and amino acid analysis, BRWJ black rice has higher nutrition than other pigmented rice. The amino acids L-Tryptophan, L-phenylalanine and L-Tyrosine are highest in black rice, especially BRWJ. These three amino acids act as the main precursors for the formation of phenolic compounds, flavonoids and anthocyanins. The high amino acid content in black rice indicates that black rice is good for health.

Pigmented rice extract with solvent distilled water, ethanol and methanol shows the content of secondary metabolites such as leucoanthocyanidin, flavonoids, phenolic, tannins, Quinone, antraquinones, and glycosides. Whereas pigmented rice extract with n-Hexane solvent showed no tested compounds. Leucoanthocyanidin compounds are mostly obtained from ethanol and methanol extracts. The anthocyanin as a bioactive compound of black rice was extracted with methanol solvent. Huang and Lai, (2016) reported that anthocyanin was most obtained from methanol extracts with pH <2 [15]. The N-Hexane solvent took an extract of phytosterols or oils in plants [29] . And the next steps, it carried out the bioactive compounds extraction of black rice with methanol at pH > 2 and total anthocyanin characterized by UV-Vis spectrophotometry. In ethanol extracts there were no leucoanthocyanidin, flavonoid, and glycoside compounds detected. Extracts of water, methanol and ethanol of the three types of black rice showed the presence of leucoanthocyanin, phenol, tannin, flavonoid, quinone and glycoside compounds which were higher than WREJ white rice and RREJ red rice. These groups of compounds play an important role in the inhibition of metabolic diseases. Leucoantocyanidin is a precursor compound in the formation of anthocyanin. Phenolic compounds, tannins, flavonoids, and glycosides may have function as antioxidants.

In this study, Black rice BRWJ shows maximum absorption at the UV region (220-330 nm) at all pH and are absorbed at wavelengths of 400-590 nm at pH 1, pH 3 and pH 5. The maximum absorption of visible light indicates the presence of anthocyanin compounds. All compounds in plants can capture the UV waves. The black rice in acidic conditions showed the spectra profile in the area of visible light with a wavelength of 400-590 nm. According to the TLC, there are spots suspected as anthocyanin compounds that are only detected in red rice and all three types of black rice.

These data have shown similarity activities in pigmented rice bran. The antioxidant activities of the back and red rice bran in free fractions were much higher than that in bound fractions [30]. *In vivo* and *in silico* studies revealed that anthocyanins in pigmented rice has some biological function such as antioxidant [15], anti-inflammatory [31] and anti-apoptosis [1]. As future study, we perform genetic basis of pigmentation and nutritional values of pigmented rice of Java Island, which it may has anti-diabetic, anti-adipogenesis, anti-obesity, anti-hyperlipidemic, and anti-cancer activity.

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