PAPER

EFFECT OF DEGREE OF RICE MILLING ON ANTIOXIDANT COMPONENTS AND CAPACITIES

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ABSTRACT

De-husking and milling-induced changes in the content of antioxidant compounds and the antioxidant capacities of rice fractions were investigated in this study. Six fractions - rice husk, brown rice, and milled rice (MR) after four different degrees of milling (MR-3.5, MR-5.3, MR-7.1, and MR-9.9) - were extracted with 70% aqueous ethanol or water. Total phenolic and flavonoid contents decreased significantly ($p < 0.05$) as the degree of milling increased. Rice husk and brown rice fractions showed higher 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activities than those of the MR, as well as higher levels of antioxidant components (total phenolics and total flavonoids). Phytochemicals such as phenolic compounds and vitamin E are mainly concentrated in the outer layers of the grains rather than in the endosperm. These findings suggest that consuming rice milled to a lesser degree may have certain health benefits.

Keywords: rice, degree of milling, antioxidant, phenolics, vitamin E

1. INTRODUCTION

Rice (*Oryza sativa*) is one of the world's most important cereal crops and a main staple food in Korea and many other Asian countries. As much as 75% of the daily caloric intake of the population of some Asian countries is derived from rice (HUANG *et al*., 2015). The rice grain has a hard husk that protects the kernel within. Brown rice is obtained by removing the husk but leaving the bran, germ, and endosperm. Milled rice is produced by removing the bran layers of the rough rice kernel by milling (BUTSAT and SIRIAMORNPUN, 2010). Although brown rice and under-milled rice are considered excellent sources of calories and nutrients such as vitamins and minerals (ZHANG *et al*., 2014), white rice is preferred by consumers because of its good eating quality. However, because of growing health consciousness, some consumers in Asia have recently started consuming rice milled to a lesser degree or even brown rice.

Plant-derived phytochemicals, which are potential sources of natural antioxidants, may combat oxidative stress in the human body by maintaining the balance between oxidants and antioxidants (TEMPLE, 2000). Many naturally occurring phytochemicals in plant-derived products contain a complex mixture of phenolic compounds that can exert several biological effects including antioxidant activity (PANDEY and RIZVI, 2009). It has been reported that phenolic compounds may function as free radical scavengers and quenchers of singlet oxygen. Their antioxidant activities have been attributed to their redox properties (PATEL *et al*., 2011). Antioxidant phytochemicals that quench free radicals may play a significant role in human health. A wide variety of biologically active phytochemicals are concentrated mainly in the pericarp and aleurone layers in cereal grains such as rice, wheat, and oats (BUTSAT and SIRIAMORNPUN, 2010; HA *et al*., 2006). Several studies have demonstrated that the aleurone layer has high nutritive value and has beneficial health effects, such as decreasing the incidence of atherosclerotic disease (DANIEL *et al*., 1999; MORTON *et al*., 2000), lowering blood cholesterol (OKARTER and LIU, 2010), and preventing cardiovascular disease (MARTINEZ-VALVERDE *et al*., 2000). It has also been reported to have an anticancer effect (NEWMARK, 1996). Some of these protective effects may be attributed to polyphenols, which comprise several classes of flavonoids and vitamin E as well as other phenolic constituents and are present in the aleurone layer. However, dehusking and milling decreases the content of antioxidant compounds in the grain. Thus, the degree of milling is an important factor in the nutritional value of milled rice.

Previous studies have reported the effect of milling on the physicochemical properties of rice and the cooking and textural properties of milled rice (SINGH *et al*., 2005; LIU *et al*., 2015). Several studies have also reported the content of antioxidant compounds and the antioxidant activity of white or black rice and the contents of various beneficial components (several phenolic compounds, tocopherols, tocotrienols, and γ-oryzanol) of rice bran or husks (ADOM and LIU, 2002; ZHANG *et al*., 2010; BUTSAT *et al*., 2009). However, limited information is available on the correlation between the antioxidant compound content and activity of rice husks, brown rice, and rice milled to different degrees. Therefore, the aim of this study was to determine the antioxidant compound content and antioxidant activity of milled rice fractions and to evaluate husks as a source of natural antioxidants.

2. MATERIALS AND METHODS

2.1. Materials

1,1-Diphenyl-2-picrylhydrazyl radical (DPPH), the diammonium salt of 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Folin-Ciocalteu's (FC) phenol reagent, tocopherol (α-, β-, γ-, and δ-T), tocotrienol (α-, β-, γ-, and δ-T3), ascorbic acid, gallic acid, (+)-catechin, butylated hydroxytoluene (BHT), potassium hydroxide, and sodium chloride were purchased from Sigma-Aldrich (St. Louis, USA). All other chemicals and solvents used were of analytical or HPLC-grade.

2.2. Dehusking and milling of rice

The paddy rice sample (*O. sativa* L. Chucheongbyeo, short-grain rice) used in this study was obtained from the Korea Food Research Institute. The paddy sample was harvested in 2014. The rice variety used in the study is commercially available in Korea. Rice was threshed to separate the rice husk (RH) and brown rice (BR) using an automatic rice husker (Kett TR200, Kokyo, Japan). The brown rice samples were polished in a McGrill no. 2 mill (Ricepal32, Yamamoto Co., Ltd., Tendo, Japan) to obtain rice grains milled to different degrees (3.5% [MR-3.5], 5.3% [MR-5.3], 7.1% [MR-7.1], and 9.9% [MR-9.9]). The degree of milling was determined using the following relationship: [1 − (1000 − kernel weight of milled rice)/(1000 − kernel weight of brown rice)] × 100. All fractions, except the bran, were stored in double-sealed polythene bags at -25°C prior to analysis.

2.3. Sample preparation

All fractions were ground using a blender. Samples of approximately 200 g were extracted using 1 L of 70% aqueous ethanol or distilled water in a shaker (JSSI-100T, JS Research Inc., Gongju, Korea) at 25±3°C for 24 h. The extracts were filtered using Whatman no. 2 filter paper, and the residues were discarded. The filtrate was concentrated at 40°C using a vacuum rotary evaporator (R-205, Büchi, Flawil, Switzerland), lyophilized, and then stored at -20°C until further analysis.

2.4. Determination of total phenolic and flavonoid contents

The concentrations of total phenolic and flavonoid compounds in the extracts were measured spectrophotometrically. Total phenolic content (TPC) was determined using the FC-method (DEWANTO *et al*., 2002) with some modifications. Standard solution or sample extracts were mixed with 2 mL of 2% sodium carbonate solution and 100 μL of 1 N Folin-Ciocalteu reagent. After incubating for 30 min at 25±3°C, the absorbance at 750 nm was measured using a spectrophotometer. The results are expressed as mg of gallic acid equivalents per 100 g of sample (the equation of the standard curve was $y = 0.0024x - 0.0897$, $R^2 = 0.998$).

Total flavonoid content (TFC) in the samples was determined by a colourimetric method (TIAN *et al*., 2011). Briefly, either standard solution or sample extracts were mixed with 1.25 mL of distilled water and 75 μL of 5% $NaNO_2$. After incubating for 30 min at 25±3°C, 150 μL of 10% $AlCl_3 \cdot 6H_2O$ was added, and the mixture was allowed to stand for 5 min before the addition of 0.5 mL of 1 M NaOH. The absorbance at 510 nm was then measured. The results are expressed as (+)-catechin equivalents per 100 g of sample (the equation of the standard curve was $y = 0.0024x + 0.0275$, $R^2 = 0.998$).

2.5. Determination of the vitamin E composition

Total vitamin E content and the content of each of its isomers in the samples were determined using HPLC with a fluorescence detector (WIE *et al*., 2009). A 10 mL aliquot of ethanol containing pyrogallol (6%, w/v) was added to 1 g of sample in a saponification vessel. After sonication for 5 min, 5 mL of 60% potassium hydroxide was added, and the vessel was flushed with nitrogen gas for 1 min. An air condenser was attached to the vessel, and the contents were digested at 70°C for 50 min in a shaking water bath. The contents were cooled in an ice bath, 20 mL of 2% sodium chloride was added, and the mixture was extracted three times with 20 mL of hexane/ethyl acetate (85:15, v/v) containing 0.001% BHT. The extracts were pooled and diluted to 50 mL and filtered through a 0.45 μm nylon membrane filter. An aliquot of the filtered extract was analysed using a normal-phase HPLC system (PU-1580; Jasco, Tokyo, Japan). Analysis of tocopherol and tocotrienol isomers was performed on a LiChrospher® Diol column (250 × 4 mm, 5 μm; Merck, Darmstadt, Germany) using a mobile phase of hexane/isopropanol (98.7:1.3, v/v) at a flow rate of 1.0 mL/min. Peaks were detected by fluorescence (FP-1520 l Jasco) using an excitation wavelength of 290 nm and an emission wavelength of 330 nm. Tocopherol and tocotrienol peaks were identified by comparing their retention times to those of standards (the standard curve equations and R^2 values were α-T, $y = 0.000001x + 0.021928$, $R^2 = 0.998$; α-T3, $y = 0.000001x + 0.018294$, $R^2 = 0.997$; β-T, $y = 0.000001x + 0.005295$, $R^2 = 0.997$; γ-T, $y = 0.000001x + 0.024276$, $R^2 = 0.998$; γ-T3, $y = 0.000001x – 0.000236$, $R^2 = 0.998$; δ-T, $y = 0.0000005x + 0.0036275$, $R^2 = 0.998$; δ-T3, $y = 0.0000003 + 0.0110694$, $R^2 = 0.999$).
All analyses, except for that of vitamin E, were conducted in triplicate.

2.6. Determination of DPPH radical scavenging activity

DPPH radical scavenging activity was assayed by the method described by Choi and Lee (2009) with some modifications. Briefly, 0.1 mL of 0.2 mM DPPH radical solution (1 mL) was added to 20 μL of sample extract or a standard solution of ascorbic acid, and the mixture was allowed to stand for 30 min. The absorbance of the mixture at 520 nm was measured against a blank of distilled water and an ascorbic acid standard calibration curve was constructed. The DPPH radical scavenging activity was expressed as ascorbic acid equivalent antioxidant activity (AEAC) and defined as mg of ascorbic acid equivalents per 100 g of sample (the equation of the standard curve was $y = -0.0268x + 1.4586$, $R^2 = 0.991$).

2.7. Determination of ABTS radical scavenging activity

Total antioxidant capacity of each sample extract was determined with an improved cationic ABTS radical method using a spectrophotometer (GONG *et al*., 2013). The ABTS radical was generated by adding 7 mM ABTS to a 2.45 mM potassium persulfate solution and letting the mixture stand overnight in the dark at 25±3 °C. The solution of positively charged ABTS radicals was diluted with distilled water to obtain an absorbance of 1.0 at 734 nm. Diluted ATBS radical solution (1 mL) was then added to 50 of μL sample extract or ascorbic acid standard solution. After 90 min, the absorbance of the mixture was measured at 734 nm. The antioxidant activity of the extracts was expressed as mg of AEAC in 100 g of sample (the equation of the standard curve was $y = -0.0055x + 1.1115$, $R^2 = 0.995$). All analyses were performed in triplicate.

2.8. Statistical analysis

Data from the analyses of each of the samples in triplicate were reported as the mean±SD. Different samples were compared using one-way analysis of variance using SAS, version 8.1 (SAS Institute, Cary, USA). A value of $p < 0.05$ was considered to be statistically significant.

3. RESULTS AND DISCUSSIONS

Antioxidants can be classified into two groups according to their solubility; hydrophilic antioxidants, such as phenolic and flavonoid compounds, and lipophilic antioxidants (fat-soluble), such as vitamin E (DHIBI *et al*., 2012). These compounds exhibit antioxidant properties in many in vitro model systems and have the potential to reduce the risk of chronic diseases associated with oxidative stress (LIU, 2003).

3.1. Total phenolic content

Phenols are one of the most effective antioxidative components in plant-derived foods, including fruits, vegetables, and grains (CHOI and LEE, 2009). These compounds are effective antioxidants because of their ability to donate a hydrogen atom or an electron to form stable radical intermediates. Therefore, it is important to quantify the TPCs to determine their contribution to the antioxidant activities of the test samples. The results of TPC analysis are expressed as mg of gallic acid equivalent/100 g of sample (Table 1).

Table 1. Total phenolic and flavonoid contents of milled fractions obtained from paddy rice. Means with different letters in a column are significantly different ($p < 0.05$).

Solvent	Rice fraction	Total phenolic content [1)]	Total flavonoid content [2)]
70% ethanol	RH	166.02±4.48^{a} [3)]	18.42±0.84^{a}
	BR	45.94±0.51^{b}	9.53±0.91^{b}
	MR-3.5	20.23±0.13^{c}	5.84±0.34^{c}
	MR-5.3	16.94±0.14^{c}	4.84±0.20^{d}
	MR-7.1	10.61±0.11^{d}	2.77±0.33^{e}
	MR-9.9	7.64±0.08^{d}	1.57±0.05^{f}
Water	RH	32.39±1.95^{a}	5.71±0.23^{a}
	BR	26.15±0.26^{b}	5.44±0.54^{a}
	MR-3.5	17.61±1.06^{c}	4.77±0.42^{b}
	MR-5.3	10.42±0.21^{d}	2.67±0.29^{c}
	MR-7.1	10.38±0.07^{d}	2.45±0.10^{cd}
	MR-9.9	6.69±0.15^{e}	1.89±0.04^{d}

[1)] Means±standard deviation (SD) for triplicate determinations expressed as mg of gallic acid equivalents/100 g of sample.

[2)] Means±SD for triplicate determinations expressed as mg of catechin equivalents/100 g of sample.

TPCs in the 70% ethanol and water extracts were 7.64-166.02 and 6.69-32.39 mg/100 g of sample, respectively. The determination of TPC was affected by the extraction solvent; 70% ethanol was more effective than water. Solvent extraction is frequently used to isolate antioxidants, and both the yield and antioxidant activity of the extracts are strongly dependent on the solvent; this is because compounds with different polarities often have different antioxidant potentials (MARINOVA and YANISHLIEVA, 1997). Many studies have indicated that using 70% ethanol in water as the extraction solvent affords significantly higher quantities of phenolic compounds than other solvents, such as methanol and water (AJILA *et al*., 2011). The ethanol extracts show the highest antioxidant activities, which is consistent with our results. Ethanol-water extraction systems were used in the present study since they are the most widely employed solvents for reasons of chemical hygiene and ease of availability. More importantly, these solvents are compatible with the production of food-grade materials (SOONG and BARLOW, 2004). TPCs in the rice fractions for all samples were found to be in the following order: RH > BR > MR-3.5 > MR-5.3 > MR-7.1 > MR-9.9. The ethanol extract of the husk fraction exhibited the highest TPC (166.02±4.48 mg/100 g of sample), and the TPC decreased significantly ($p < 0.01$) as the degree of milling increased. Phenolic compounds are located predominantly in the bran layer, which is progressively removed during the milling process (BUTSAT *et al*., 2009). Rice husk and rice bran are also rich in TPC (VIJAYALAXMI *et al*., 2015). The outer layers of the cereal grains, such as husk, pericarp, testa and aleurone cells, contain the highest concentrations of TPC, whereas its concentration is considerably lower in the endosperm (KAHKONEN *et al*., 1999). The effect of dehusking and milling on TPC could be due to the variable distribution of phenolic compounds in the husk and bran.

3.2. Total flavonoid content

Flavonoids are a group of phenolic compounds that contain two aromatic rings linked by three carbons that are usually part of an oxygenated heterocycle. These compounds have potent antioxidant and anticancer activities (SHEN *et al*., 2009). The TFC, expressed as mg of catechin equivalents per 100 g of sample, was lower than the TPC in all the samples tested (Table 1). The TFCs in the milled fractions were 1.57-18.42 and 1.89-5.71 mg/100 g of sample in the 70% ethanol and water extracts, respectively. RH had the highest TFC among the six rice fractions, followed by BR, MR-3.5, MR-5.3, MR-7.1, and MR-9.9 in that order. The order of abundance of TFC was similar to that of TPC in all samples. This result indicates that the concentration of flavonoids increases from the endosperm to the aleurone layer.

3.3. Total content of vitamin E and its isomers

Vitamin E is another antioxidant present in grains that protects polyunsaturated fatty acids in cell membranes from oxidative damage (SLAVIN *et al*., 1999). The antioxidant activity of the tocopherols and tocotrienols (collectively known as chromanols) is mainly due to their ability to donate their phenolic hydrogens to lipid free radicals. Vitamin E is synthesized only by plants. Therefore, it is a vital nutrient for humans and animals that can be obtained only from dietary sources (KAHKONEN *et al*., 1999). The individual concentrations of eight vitamin E isomers (α, β, γ, and δ-T and α, β, γ, and δ-T3) and their total contents in different parts of the rice grain are presented in Table 2. Four tocopherol isomers, α, β, γ, and δ-tocopherol, and three tocotrienol isomers, α, γ, and δ-tocotrienol, were identified. β-Tocotrienol was not detected.

Table 2. Composition of eight vitamin E isomers in the milled fractions obtained from paddy rice. Means with different letters in the columns for each fraction are significantly different ($p < 0.05$).

Rice fractions	Tocopherol (T)				Tocotrienol (T3)				Total vitamin E
	α-T	β-T	γ-T	δ-T	α-T3	β-T3	γ-T3	δ-T3	
RH	1.15±0.05^{b}	0.04±0.00^{bc}	0.18±0.00^{c}	0.51±0.00^{c}	0.35±0.01^{b}	ND	0.51±0.00^{c}	0.02±0.00^{d}	2.27±0.08^{c}
BR	1.25±0.09^{b}	0.04±0.01^{b}	0.21±0.01^{b}	1.19±0.02^{bc}	0.76±0.05^{a}	ND	1.19±0.02^{a}	0.05±0.00^{a}	3.53±0.15^{b}
MR-3.5	1.61±0.03^{a}	0.06±0.00^{a}	0.28±0.01^{a}	1.23±0.05^{a}	0.70±0.04^{a}	ND	1.23±0.05^{a}	0.05±0.00^{a}	3.98±0.11^{a}
MR-5.3	0.88±0.04^{c}	0.03±0.00^{c}	0.16±0.02^{c}	0.66±0.03^{bc}	0.32±0.01^{b}	ND	0.66±0.03^{b}	0.04±0.00^{b}	2.12±0.11^{cd}
MR-7.1	0.72±0.14^{c}	0.02±0.00^{d}	0.13±0.02^{d}	0.56±0.06^{bc}	0.26±0.04^{b}	ND	0.56±0.06^{bc}	0.03±0.00^{bc}	1.74±0.26^{d}
MR-9.9	0.30±0.06^{d}	0.01±0.00^{e}	0.06±0.00^{e}	0.47±0.10^{b}	0.15±0.03^{c}	ND	0.47±0.10^{c}	0.03±0.00^{c}	1.06±0.20^{e}

Means±standard deviation (SD) of duplicates (mg/100 g of sample).
ND, not detected.

The vitamin E level was 0.86-4.09 mg/100 g of sample in all the extracts of the six fractions. MR-3.5 was the best source of vitamin E (3.98±0.11 mg/100 g), followed by BR (3.53±0.15 mg/100 g), RB (2.27±0.08 mg/100 g), MR-5.3(2.12±0.11 mg/100 g), MR-7.1(1.74±0.26 mg/100 g), and finally MR-9.9 (1.06±0.20 mg/100 g). Tocopherols are known to be the principal antioxidants present in rice bran. The average content of total vitamin E in BR was higher than that in RH (HUANG and NG, 2011). The levels of vitamin E detected in the BR and RH fractions in our study were similar to previously reported values of 1.04-3.25 mg/100 g of sample (KAHKONEN *et al*., 1999), 3.62–3.82 mg/100 g of sample and 1.81-4.08 mg/100 g of BR, and 0.41–5.50 μg/100 g of RH (SOONG and BARLOW, 2004; OKARTER and LIU, 2010). The major forms of vitamin E were α-T and γ-T3, whereas β-T and δ-T3 were present in trace amounts and β-T3 was not detected. These results were in agreement with the finding that the major isomers of vitamin E in rice samples were γ-T3 and α-T, whereas the δ-T and β-T3 contents were the lowest (HA *et al*., 2006). Similar to the present study, previous studies have shown that vitamin E was more concentrated in regions close to the bran and husk layers than in the endosperm (SOONG and BARLOW, 2004). The removal of the husk, the aleurone layer, and the germ during milling reduces the vitamin E content of the final milled products (OKARTER and LIU, 2010). Lipid fractions are distributed mainly in the outer layers of rice grains (ADOM and LIU, 2002). These findings are in good agreement with our data in which the vitamin E content decreased as the degree of milling increased. Therefore, our results indicate that antioxidant components comprising total phenolics, total flavonoids, and vitamin E are primarily concentrated in the outer layers rather than in the endosperm.

3.4. DPPH radical scavenging activity

The DPPH and ABTS radical scavenging activities are related to the nature of the phenolic compounds as that affects their electron transfer/hydrogen donating ability (WETTASINGHE and SHAHIDI, 2000; CHOI *et al*., 2007). The increase in free radicals can accelerate the oxidation of foods and decrease their quality. The radical scavenging activity is very important due to the deleterious role of free radicals in foods and in biological systems. Therefore, this study investigated the free radical scavenging activity of the 70% ethanol and water extracts of different rice fractions using DPPH and ABTS

assays. Both these radicals are commonly used for in vitro assessment of antioxidant activity.

The DPPH radical is stable and is widely used to evaluate the free radical scavenging activities of many plant extracts. The capacity of the six rice fractions to act as hydrogen donors in the transformation of the DPPH radical to its reduced form was examined. AEAC values are often used to rank the antioxidant activity of unknown mixtures (KONG and LEE, 2010). The AEAC of a sample determines its antioxidant activity relative to that of ascorbic acid. The DPPH radical scavenging activities of the milled rice fractions, expressed as mg of AEAC per 100 g, are shown in Fig. 1. The colour of the DPPH reagent changes from purple to yellow as a result of the antioxidant activity of the test sample. Similar to the antioxidant content, the free radical scavenging activity was affected by the extraction solvent; 70% ethanol was more effective than water. The DPPH radical scavenging activities of the extracts of the six samples were in the order RH > BR > MR-3.5 > MR-5.3 > MR-7.1 > MR-9.9 for both solvents. The results show that the antioxidant capacity of all extracts decreased during the milling process. The high TPC and TFC in the husks may account for the strong DPPH radical scavenging activity. This finding is consistent with the finding of a previous study that found that the antioxidant activity was dependent on the composition of the milled fractions (SHEN *et al*., 2009; BUTSAT and SIRIAMORNPUN, 2010). The DPPH radical scavenging activity correlated directly with the TPC and TFC in the extracts of the six samples with correlation coefficients (R2) of 0.880 and 0.942, respectively. However, there was no correlation between DPPH radical scavenging activity and total vitamin E content (R2 = 0.274). Thus, there is a strong association between the TPC and TFC of different milled fractions and their respective DPPH radical scavenging activities.

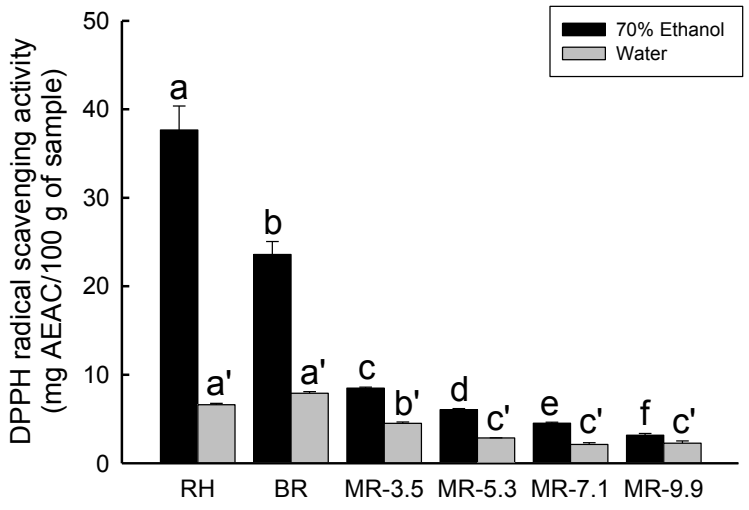

Figure 1. DPPH radical scavenging activity of milled fractions obtained from paddy rice. Data are expressed as the mean±SD of the results for the six rice fractions. [a-f, a'-c'] Means with different letters are significantly different ($p < 0.05$) by one-way analysis of variance.

3.5. ABTS radical scavenging activity

Total antioxidant content (TAC) was measured using the ABTS assay. The ABTS radical scavenging test is widely used to determine the antioxidant activity of both hydrophilic and lipophilic compounds and to measure the relative radical scavenging activity of hydrogen-donating and chain-breaking antioxidants in many plant extracts (APAK *et al*., 2007). The cationic ABTS radical assay can be used over a wide pH range, is inexpensive, and is more rapid than the DPPH radical assay (SLAVIN *et al*., 1999). Fig. 2 shows the antioxidant activities of the extracts expressed as mg of AEAC per 100 g of sample. The extraction solvent influenced the antioxidant activity of the extracts of all the fractions. Of the two methods used in this study, extraction with 70% ethanol afforded higher antioxidant activities. TAC varied among the different fractions obtained from different degrees of milling. The TAC of 70% ethanol and water extracts ranged from 5.20-48.74 and 1.88-4.13 mg of AEAC per 100 g sample, respectively. The TAC of the samples tested in decreasing order was RH > BR > MR-3.5 > MR-5.3 > MR-7.1 > MR-9.9. Thus, the TAC in rice decreased significantly ($p < 0.01$) as the degree of milling increased. Many bioactive compounds including phenolic antioxidants, tocopherol, and tocotrienol were present in higher quantities in the removed husk and bran than in the remaining endosperm (IQBAL *et al*., 2005; HA *et al*., 2006). The highest TAC was detected in RH, which was consistent with the results of the analyses of TPC, TFC, vitamin E and DPPH radical scavenging ability. The lowest TAC was observed for MR-9.9, which exhibited markedly lower TPC, TFC, and vitamin E levels, and lower DPPH radical scavenging capacity than those of the other samples. There was a positive correlation (R2 = 0.996) between ABTS and DPPH radical cation scavenging activities. The decrease in TAC might have been the result of decreased levels of antioxidant compounds. TAC was positively correlated with TPC and TFC. The correlation coefficients (R2) between the ABTS assay and TPC and between the ABTS assay and TFC were 0.800 and 0.873, respectively. There was no correlation between the vitamin E and TAC. These results confirm that the phenolic and flavonoid compounds may be the major contributors to the antioxidant activity of the grains, which is consistent with the findings of earlier studies (BUTSAT and SIRIAMORNPUN, 2010; ADOM and LIU, 2002).

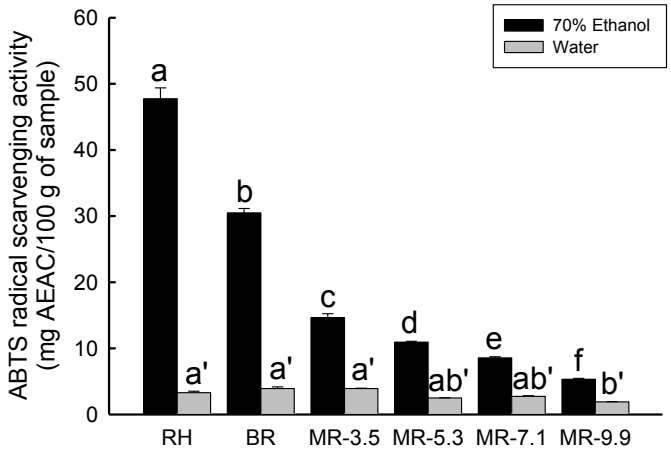

Figure 2. ABTS radical scavenging activity of milled fractions obtained from paddy rice. Data are expressed as the mean±SD of the results for the six rice fractions. [a-f, a'-b'] Means with different letters are significantly different ($p < 0.05$) by one-way analysis of variance.

4. CONCLUSIONS

This study shows that the antioxidant strength of rice depends on the degree of milling. BR contains more components that have health benefits than MR, such as polyphenolics, flavonoids, and isomers of vitamin E. These results demonstrate that brown rice is a good dietary source of antioxidants and has health benefits. Thus, it is important to modulate the milling process to preserve bioactive compounds.

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