

Red Pericarp Advanced Breeding Lines Derived from *Oryza Rufipogon* × *Oryza Sativa*: Physicochemical Properties, Total Antioxidant Activity, Phenolic Compounds and Vitamin E Content

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Abstract: Two new red pericarp transgressive variants (advanced breeding lines from BC₂F₇ generation) with high yield, derived from a cross between the wild relative, *O. rufipogon* Griff. and *O. sativa* subsp. *indica* cv. MR219, were analysed to determine their proximate composition, total antioxidant activity, phenolic acid composition and tocopherol content in comparison with two commonly consumed rice varieties, MR219 (brown coloured) and Thailand rice (red coloured). The red pericarp variants were not significantly different in grain quality related traits. For fat content, neither variant showed any significant difference to the recurrent parent MR219, however for amylose content they possessed lower levels compared to MR219 but for both traits results were comparable to Thailand red rice. Variants G33 and G37 produced significantly ($p < 0.05$) higher total phenolic content (0.49 and 0.51 mgGAE/g, respectively) than the white control sample, MR219 (0.32 mgGAE/g) but lower than Thailand red rice (1.59 mgGAE/g) ($p < 0.05$). Ferric-Reducing Ability Power (FRAP) was significantly ($p < 0.01$) higher in both variants compared to MR219 but lower than in Thailand red rice. For DPPH radical scavenging, both variants were not significantly different from both controls. Caffeic and ferulic acid detected in all samples were in higher amounts compared to the other compounds and hydroxycinnamic acids were considered as the main phenolic acids. Across all samples, the content of total E vitamin was higher in G37 and γ -tocotrienol, which was the most abundant tocol. In conclusion both red pericarp variants can be used in cultivar development program for red rice with high nutritional value.

Keywords: Antioxidant properties, proximate composition, tocopherol content, transgressive variants

INTRODUCTION

Rice (*Oryza sativa* L.) is an important cereal crop and main source of food for more than half of the world's human population (Khush, 2005). Even though white rice is generally consumed, there are many specific cultivars of rice that have colour pigments, such as black and red. China, Korea, Japan and many other countries in Southeast Asia have traditionally consumed pigmented rice (*Oryza sativa* L.). More recently, whole grain pigmented rice has been classified a functional food because of the appreciable presence of phenolic compounds, especially anthocyanins in the pericarp (Abdel-Aal *et al.*, 2006; Ryu *et al.*, 1998; Yawadio *et al.*, 2007). Changing one's diet by increasing the intake of food that is 'relatively high' in selected natural antioxidants, such as plant polyphenols, vitamin C or flavonoids, has been reported to reduce the incidence of

chronic and degenerative diseases (Laandrault *et al.*, 2001; Shahidi, 2000; Wilson, 1999). Whole grain cereals are a great source of antioxidant compounds which have the potential to reduce oxidative stress (Slavin, 1994). The nutritional properties of pigmented rice also include the capacity to prevent atherosclerosis in the mouse model and human study (Lu *et al.*, 2008; Xia *et al.*, 2006). Pigmented rice was reported to have a greater antioxidative capacity than white rice (Choi *et al.*, 2007). Since rice is consumed mainly as a whole grain, the texture is a matter of primary concern. Texture is a key attribute of food acceptance by consumers and as such, an important step in quality assessment. The most important quality criteria on the basis of chemical characteristics are moisture, crude protein, crude fat and amylose content.

Given the importance of promoting whole grain consumption for its healthful bioactive content, the objectives of this study were to evaluate the proximate

composition and antioxidant properties of two red pericarp advanced breeding lines developed by the National University of Malaysia (UKM) and the Malaysian Agricultural Research and Development Institute (MARDI).

MATERIALS AND METHODS

Rice samples: The new red pericarp rice variants are the subset of transgressive variants (advanced breeding lines from BC₂F₅ and BC₂F₆ generation) derived from a cross between the wild rice *O. rufipogon* Griff. and *O. sativa* L. subsp. *indica* cv. MR219. The red pericarp variants were generated from a backcross breeding programme to increase the yield of cultivated rice by using wild rice (Sabu *et al.*, 2006). Lines with high yield were detected in the segregating backcross lines. The variants G33 (R14-3-66-4-B-B) and G37 (R19-2-93-3-B-B) used in the present study (Fig. 1) were selected on the basis of field performance and pericarp colour in BC₂F₅ and BC₂F₆ generation (Bhuiyan *et al.*, 2011). Based on the field experiments conducted over two seasons (offseason and main season) at a single location under the research field of Malaysian Agricultural Research and Development Institute (MARDI), Seberang Perai, Pulau Pinang (Latitude 05°25'N and Longitude 100°15'E), variant G37 produced similar yield per plot (4.5 t/ha) to control MR219 (4.5 t/ha) while variant G33 produced a significantly higher yield (5.2 t/ha) than MR219 (Bhuiyan *et al.*, 2011). Variant G33 also showed a lower glycemic index (GI = 51) than white rice (GI = 86) (Karupaiah *et al.*, 2011). A precise selection for regaining the grain type of MR219 was carried in each generation and the variants selected for grain quality evaluation had a physical appearance of grain type that was close to that of the recurrent parent MR219 (Fig. 1).

Preparation of samples: Samples of rough rice were hulled with a dehusker machine (Motion Smith Co., Singapore), and passed through a 500 µm sieve screen to obtain rice powder at UPM-BERNAS Research Laboratory located at the Faculty of Food Science and Technology, Universiti Putra Malaysia, in 2008. Moisture was determined by drying at 130°C to constant mass according to the standard procedures described in ISO711 (1985) method. A Malaysian high yielding rice variety, MR219 and a commonly available brand of Thailand red rice, purchased from a local supermarket were used as controls.

Proximate analysis: The length, width and thickness of brown rice (50 grains per sample) were measured with the help of a digital micrometer (Steinmeyer, Germany). Size and shape were determined according to the scale of RTWG (1997). A colorimeter (HunterLab, UltraScan Pro D65, USA) was used for all color determinants (Bao *et al.*, 2005). Color measurements were expressed as



Fig. 1: Grain type of the parents and the transgressive variants used in this study

tristimulus parameters, L^* , a^* and b^* [L^* indicates lightness (100 = white and 0 = black), a^* indicates redness-greenness and b^* indicates yellowness-blueness] (Bao *et al.*, 2005). Fat content of rice flour was determined according to AOAC approved standard method (AOAC, 2005). Protein was determined according to the Kjeldahl standard method (MS1194, 1991) using the factor 5.95×N for conversion.

Amylose Content (AC): Amylose content was determined based on ISO-cited methodology (ISO, 2005a, b). Rice flour was defatted by refluxing with methanol for 6 h in a Soxtec Extraction Unit (Soxtec™ 2050, FOSS Analytical, Denmark). After defatting, samples were left for two days in the same room to allow evaporation of residual methanol. About 0.1 g of defatted rice sample were weighed and transferred in to 100 mL volumetric flask. A total of 9 mL of sodium hydroxide solution (1 mol/L) and 1 mL ether were added. The mixture was heated in a boiling water bath for 10 min. Samples were allowed to stand at room temperature overnight. Distilled water was added to the samples and mixed vigorously. The absorbance was measured using Flow Injection Analyser (FIA) (FOSS Co., Sweden).

Extraction procedure to determine the antioxidant properties: Extraction of total antioxidants was adapted from the method of Zuo *et al.* (2002). Approximately 2 g of finely ground samples were mixed with 10 mL of 80% aqueous methanol (1:10, w/v) at room temperature. The suspended samples in methanol were shaken for 3 h in an incubator shaker (Innova4080, New Brunswick Scientific, Herisau, Switzerland) before centrifuging (Hermle Labortechnik Z323K, Germany) at 2500 rpm for 20 min to obtain the supernatant. The remaining residue was re-extracted with 8 mL of 80% methanol containing 150 µL HCl at room temperature, shaken for 3 h and then centrifuged. Supernatants from both extractions were combined and finally evaporated to dryness at 45°C using a rotary evaporator (Heidolph Laborota 4000 efficient, Germany). Dry residues were re-suspended in 10 mL methanol before storage at -20°C pending analysis.

Determination of Total Phenolic Content (TPC): The Total Phenolic Content (TPC) of rice samples was determined using the Folin-Ciocalteu method (Liu and Yao, 2007). Briefly, 100 μ L of sample extract was shaken for 1 min with 1 mL of diluted Folin-Ciocalteu reagent (1:10 with methanol). Then, 800 μ L of 10% Na_2CO_3 was added, and made up to 5 mL volume with distilled water. Absorbance was measured at 760 nm after 2 h of reaction using a UV-vis spectrophotometer (Sunnyvale, CA, USA) equipped with a microplate reader (VERSAmax, Tunable). TPC was estimated using a standard curve prepared with gallic acid. Results were expressed as mg gallic acid equivalents per g of grain.

Determination of 2,2'-diphenyl-1-picrylhydrazyl (DPPH[•]) radical scavenging ability: The DPPH[•] assay is simple and rapid and needs only a UV-vis spectrophotometer to perform, which probably explains its widespread use in antioxidant screening (Noruma *et al.*, 1997). The free-radical scavenging capacity of sample extract was evaluated using a published method (Liyana-Pathirana and Shahidi, 2007). A sample volume of 100 μ L was added to 1.9 mL of freshly prepared 0.1 mM DPPH[•] solution and the reaction was allowed to proceed without light at room temperature for 30 min. The absorbance was read at 517 nm relative to the control containing only 2 mL of 0.1 mM DPPH[•]. The percentage of scavenging activity was calculated from the formula:

$$[1 - (A_{517} \text{ of sample} / A_{517} \text{ of control})] \times 100$$

where, A_{control} = absorbance of DPPH[•] + methanol;
 A_{sample} = absorbance of DPPH[•] + rice extract.

Determination of Ferric-Reducing Antioxidant Power (FRAP): The FRAP assay is a method of measuring the reducing ability of reductants (antioxidants) from Fe^{3+} to Fe^{2+} . The formation of blue coloured Fe^{2+} -TPTZ complex (Fe^{2+} tripyridyltriazine) increases the absorbance at 593 nm (Kubola and Sirithon, 2008). The stock solutions were 300 mM acetate buffer pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. These stock solutions were used to make the fresh FRAP working solution in a ratio of 10:1:1 which was warmed at 37°C before use. Sample extracts (300 μ L) were allowed to react with 1.7 mL of the FRAP solution. The absorbance at 593 nm of the samples were measured after 60 min. Ferric-reducing ability was determined from a standard curve prepared with known concentrations of FeSO_4 and expressed as per mol FeSO_4 /g fresh weight.

Extraction of phenolic compounds and HPLC analysis: For UPLC analysis, each residue was dissolved in 5 mL 80% methanol, mixed well and centrifuged for 30 min. The aqueous layer was removed and centrifuged for 30 min. The extracts were separated by using UPLC (Waters, USA) equipped with the C18 column (1.7 μ m, 21 \times 50 mm) and Waters PDA detector. The mobile phase

consisted of purified water with 0.002% trichloroacetic acid (solvent A) and acetonitrile (30%) with methanol (70%) (solvent B) at a flow rate of 0.350 mL/min. Gradient elution was performed as follows: 0 min, 100% solvent A; 3 min, 100% solvent A; 15 min, 65% solvent A and 35% solvent B; 18 min, 100% solvent B; and 20 min, 100% solvent A. Column temperature was set at room temperature. The detector was set at 280 nm. Pure standards of caffeic acid, p-coumaric acid, ferulic acid, vanillic, syringic and sinapic were used to calibrate the standard curves.

Extraction of tocopherols and HPLC analysis: The extraction and determination of tocopherols and tocotrienols were performed, according to the method of Adam *et al.* (2007). Approximately 3 g of finely ground brown rice samples were extracted into 30 mL of chloroform and methanol (2/1), (v/v) and shaken vigorously at room temperature. The residual was further extracted four times, and the supernatants were combined. The solvent was filtered through a Whatman filter paper No. 4. Subsequently, the combined filtrate was evaporated at 70°C. The dry residue was redissolved in 1 mL of hexane and filtered prior to being injected to HPLC system. A sample volume of 20 μ L was injected for the chromatographic analysis. The yields of extract (%) were expressed on a dry basis, i.e., mass of extract per mass of dry matter. To determine tocopherol and tocotrienol isomers in brown rice, the oil obtained from the rice sample was diluted with 1 mL n-hexane. An aliquot of the diluted sample was subjected to HPLC analysis to determine the tocopherols. The LC system consisted of an HPLC system (Hewlett Packard HP 1100, FLD) comprising a YMC column (150 \times 6.0 mm, I.D.), using a mobile phase of IPA/Hexane (0.5%) at a flow rate of 1.0 mL/min. Peaks were detected by fluorescence using excitation wavelength of 295 nm and emission wavelength of 330 nm. The contents of tocopherols were calculated from the peak areas using standard curves of tocopherols (α -T, γ -T) and tocotrienols (α -T3, γ -T3, δ -T3).

Statistical analysis: All analysis was performed using duplicate samples and analytical results were expressed on a dry matter basis as mean \pm standard deviation. The significance of differences among treatment means was determined by analysis of variance using SAS version 9.1 (SAS Institute, Cary, NC, USA) with a significant level of 0.05. Multiple mean comparisons within the sample set were carried out at the 5% significance level using the Duncan's multiple range test. Correlations from regression analysis among the parameters were also determined.

RESULTS

Proximate composition: Based on the length and length/ breadth ratio, all samples could be classified as

Table 1: Mean value of physicochemical properties of the evaluated red pericarp transgressive variants

Variants	MOI	FAT	PRO	AC	Amylose classification
G33	12.5±0.1 ^a	2.35±0.0 ^a	6.80±0.0 ^b	18.98±0.1 ^c	Low amylose
G37	12.2±0.1 ^b	2.55±0.2 ^a	6.50±0.1 ^b	19.70±0.0 ^b	Low amylose
MR219	12.2±0.1 ^b	2.38±0.1 ^a	8.35±0.4 ^a	21.18±0.1 ^a	Intermediate amylose
Thailand rice	11.8±0.1 ^c	2.05±0.0 ^b	8.18±0.0 ^a	8.52±0.0 ^d	Very low amylose

Data expressed as mean±standard deviation; Mean values within a column superscripted by the same letter are not significantly different at p<0.05; MOI: Moisture content; FAT: Fat content; PRO: Protein content; AC: Amylose content

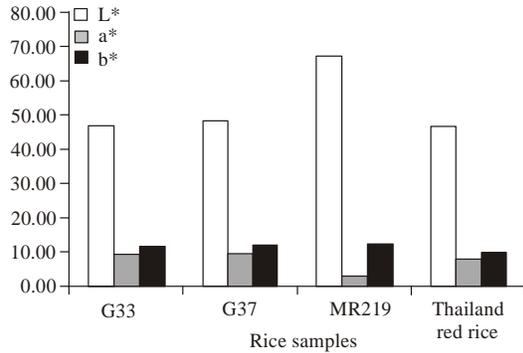


Fig. 2: Mean color parameters of white and red rice

Table 2: Total phenolic content, % inhibition DPPH' radical and FRAP value of red and white rice samples

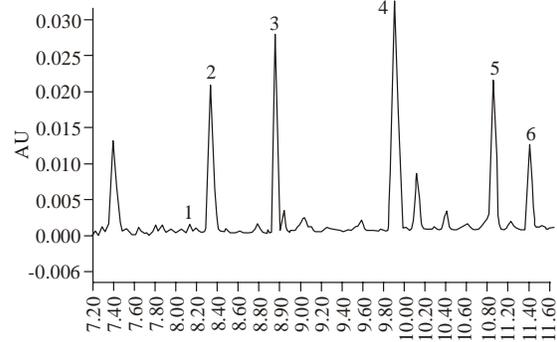
Variants	TPC (mg GAE/g)	% inhibition DPPH' radical	FRAP (µmolFeSO ₄ /g)
G33	0.49±0.02 ^b	86.6±0.41 ^{ns}	107.72±1.57 ^a
G37	0.51±0.01 ^b	86.4±0.43	90.94±0.95 ^b
MR219	0.32±0.01 ^c	85.9±0.81	69.71±0.64 ^c
Thailand rice	1.58±0.01 ^a	85.6±0.69	111.50±3.18 ^a

Data expressed as mean±standard deviation; Mean values within a column superscripted by the same letter are not significantly different at p<0.05; ns: Not significant

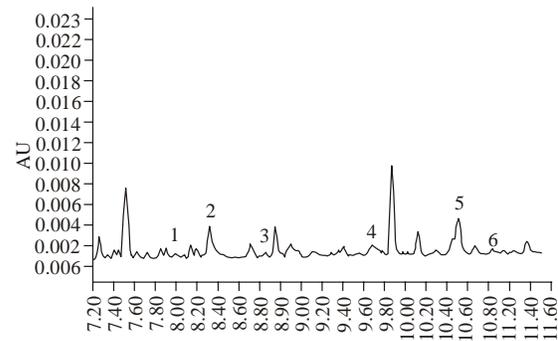
long and slender. Table 1 shows the results of ANOVA for proximate composition of samples. Both red pericarp variants showed similar moisture content (11.8 to 12.5%, p<0.01). Significant differences for protein content (p<0.01) for the tested rice samples were found, with values for both variants (6.8 and 6.5% for G33 and G37, respectively) lower than the controls (8.35 and 8.18% for MR219 and Thailand rice, respectively) (Table 1). Variants G37 and G33 produced 19.7 and 18.9% amylose content, respectively, which were lower than MR219 (21.8%) but higher than Thailand rice (8.52%).

Dehusked grain color: Figure 2 shows the color parameters (*L**, *a** and *b**) of all samples of de-hulled grains. *L** values, which express brightness, were in the range of 46.6-67.2. The values of *a** and *b** were in the range of 3.1-9.4 and 9.7-12.2, respectively. It was observed that *L** and *b** values in red rice were smaller than those of the white rice but parameter *a** values were higher in red pericarp samples than MR219 (Fig. 2).

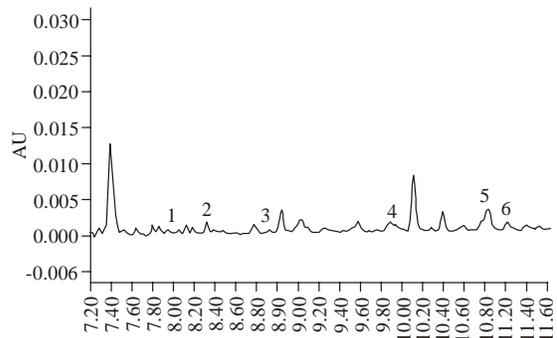
Antioxidant properties: The DPPH' scavenging activity of the samples is presented in Table 2. All samples analyzed showed appreciable scavenging activity



(a)



(b)



(c)

Fig. 3: Typical chromatograms of A) standard phenolic acids, B) MR219 and C) G37. Peaks: (1) vanillic acid, (2) caffeic acid, (3) syringic acid, (4) *p*-coumaric acid, (5) ferulic acid and (6) sinapic acid.

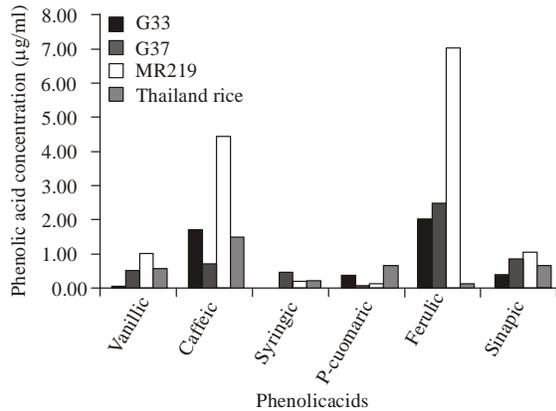


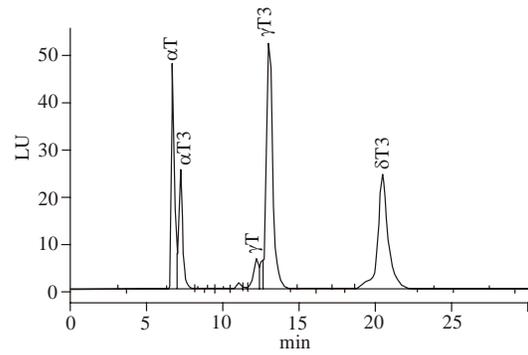
Fig. 4: Distribution of phenolic acids for all rice samples

opposing the radicals, though, there was no significant difference between samples ($p > 0.05$). The DPPH[•] scavenging varied from 85.6% (Thailand rice) to 86.6% (G33).

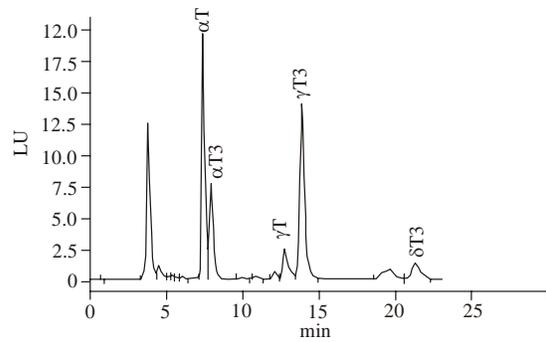
Total Phenolic Content (TPC): Significant differences ($p < 0.05$) were observed for TPC between samples. Compared with white rice, red rice contained higher TPC ($p < 0.01$) and the total phenolic content was in the order of Thailand rice > G37 > G33 > MR219. TPC content varied between 0.32 ± 0.01 to 1.58 ± 0.01 mg GAE/g with higher value from Thailand rice and lower from MR219 (Table 2).

Phenolic acids composition: Both red and white rice samples contained free phenolic acids including vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid and sinapic acid except the red sample, G33 in which syringic acid could not be detected. Typical HPLC chromatograms of standard phenolic acids, G37 and MR219 are shown in Fig. 3. The most abundant phenolic acids found in all samples were caffeic and ferulic acids, with concentration from 0.71-4.40 and 0.14-6.97 µg/g, respectively (Fig. 4). In addition, sinapic, vanillic, syringic and *p*-coumaric acids were minor constituents of brown rice.

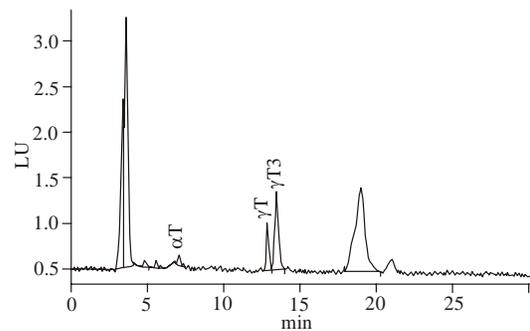
Ferric-Reducing Antioxidant Power (FRAP): Ferric-reducing antioxidant power assay was used in this study since it is quick and simple to perform, and the reaction is reproducible and linearly associated with the molar concentration of the antioxidants. The antioxidant capacities of the samples are given in Table 2. FRAP values were significantly different between samples with red rice having higher FRAP values ($p < 0.01$). The Thailand variety showed the highest value ($p < 0.01$). FRAP values ranged from 69.71 to 111.50 µmol FeSO₄/g. Within red rice types, variations were also found ($p < 0.01$).



(a)



(b)



(c)

Fig. 5: Typical chromatograms of: (A) standard mixture (B) G37 and (C) Thailand rice. Chromatographic conditions with mobile phase composed of IPA/Hexane (0.5%) at a flow rate of 1.0 mL/min. αT: α-tocopherol, γT: γ-tocopherol, αT3: α-tocotrienol, γT3: γ-tocotrienol, δT3: δ-tocotrienol.

The contents of tocopherols and tocotrienols: Efficiency of extracts is an essential factor in the contrast of antioxidant compounds. Because of the different solubilities of active compounds, antioxidants in grains are difficult to extract (Miller *et al.*, 2000). Methanol-chloroform was selected as an extraction solvent in this study. The yield of methanol-chloroform extracts obtained

Table 3: The contents of tocopherols (T) and tocotrienols (T3) (in whole grains) of different variants (mg/100g d.m.) and extraction yields

	G33	G37	MR219	Thailand rice
Tocopherols				
α -tocopherol	0.10±0.0 ^b	0.88±0.02 ^a	0.06±0.0 ^c	0.00±0.00 ^d
γ -tocopherol	0.21±0.0 ^b	0.15±0.0 ^c	0.34±0.0 ^a	0.02±0.00 ^d
Total	0.31±0.0 ^c	1.03±0.03 ^a	0.40±0.0 ^b	0.02±0.00 ^d
Tocotrienols				
α -tocotrienol	0.03±0.01 ^c	0.53±0.0 ^a	0.06±0.0 ^b	0.00±0.00 ^d
γ -tocotrienol	0.76±0.01 ^b	0.93±0.02 ^a	0.62±0.0 ^c	0.04±0.00 ^d
δ -tocotrienol	0.07±0.0 ^c	0.14±0.0 ^b	0.08±0.0 ^b	0.00±0.00 ^d
Total	0.86±0.02 ^b	1.60±0.03 ^a	0.76±0.0 ^c	0.04±0.00 ^d
Total vitamin E	1.17±0.02 ^b	2.63±0.05 ^a	1.16±0.0 ^b	0.06±0.00 ^c
Extraction yield (%)	2.6	3.0	3.4	2.1

Data expressed as mean±standard deviation, Mean values within a row superscripted by the same letter are not significantly different at $p < 0.05$

from grains is presented in Table 3 and the grains gave a yield of 2.1-3.4%. Typical HPLC chromatograms of standard tocopherol and tocotrienol extracts obtained from whole rice grain are shown in Fig. 5. All 5 tocopherol compounds were well separated in a total run time of 30 min, with good peak resolution, sharpness and symmetry.

Concentration of tocopherol (α -T and γ -T) and tocotrienol (α -T3, γ -T3 and δ -T3) homologs in brown rice powder of different colors are listed in Table 3. The concentration of total vitamin E (the sum of tocopherols and tocotrienols) in the whole rice grain samples ranged from 0.06 to 2.63 mg/100 g rice (dry basis) ($p < 0.01$). Variant G37 (red pericarp) contained the highest concentration of total vitamin E, followed by the G33 (red pericarp) and MR219 (light brown); while Thailand rice (red pericarp) had the lowest concentration. The levels of γ -tocotrienol, the most abundant tocol found in the samples, ranged between 0.04 and 0.93 mg/100 g and averaged at 0.6 mg/100 g ($p < 0.01$), which corresponds to 46.9% of total tocols content. α -tocopherol, α -tocotrienol, γ -tocopherol and δ -tocotrienol were present at lower concentrations representing 20.9, 12.3, 14.1, and 5.8% of total tocols, respectively. No other vitamin E homologues were detected.

DISCUSSION

Despite being a widely untapped reservoir of genes for resistance to various biotic or abiotic stresses, wild species also include genes that can increase productivity and yield in the background of adapted cultivars. *O. rufipogon* (IRGC105491) was used in different studies to introgress novel alleles from this accession into the genetic background of several adapted cultivars (Xiao *et al.*, 1998; McCouch *et al.*, 2007; Thomson *et al.*, 2003; Cheema *et al.*, 2008; Septiningsih *et al.*, 2003; Xie *et al.*, 2006). In this study, a limited backcross programme was used to introgress alleles linked with yield and grain quality components from *O. rufipogon* (acc. IRGC105491) to cultivated rice, *O. sativa* cv. MR219. The progenies were derived under selection pressure in BC₂F₅ and BC₂F₆ generations to preserve the commercially relevant agronomic attributes of MR219.

For ease of breeding, indirect selection approaches are often used in the breeding program. One of these

indirect selection methods is to use the color parameters that can be easily measured in an automatic color difference meter. Colour is used as one criterion of quality in all rice development and is often referred to as “general appearance” however, the assessment, is performed on whole grain rice. Yodmanee *et al.* (2011) reported the values of a^* , b^* and L^* for non-waxy coloured rice samples were in the range of 7.5-10.67, 11.66-12.08 and 47.41-49.77, respectively similar to this study. The differences in grain colour may depend on rice genotypes and the form of anthocyanins present (Escribano-Bailón *et al.*, 2004; Yawadio *et al.*, 2007).

Moisture content has a considerable influence on the vigor and life of the seed and thus should be lower than 14% and preferably lower than 12% for longer storage times (www.knowledgebank.irri.org). Air-oven or vacuum-oven, methods are basic procedures for measuring moisture in rice (Owens, 2001). In our study, moisture content was in the range of 11-13% for all variants. Sompong *et al.* (2011) reported the moisture content for ten red rice varieties varying between 9.3 and 13.1%, which is a larger range than our study finding. The results however suggest that the moisture content found in the present study is within limits as in all variants it was below 13%.

The Kjeldahl method indirectly measures the total protein content of foods by direct nitrogen measurement and following multiplication by a conversion factor (Moore *et al.*, 2010). The range of protein levels for red rice variants was lower than that for the commercial standards. Recently, Yodmanee *et al.* (2011) and Sompong *et al.* (2011) reported protein contents for red rice samples in the range of 6.63-8.46 g/100 g and 7.16-10.36%, respectively.

Rice bran with germ has a slightly higher fat content than other cereal bran (Orthofer, 1996). Fat content in G37 was higher than in Thailand rice, suggesting that some red rice types could be a genetic resource for increased levels of lipid. This finding supports the data previously reported in a study where the fat values of ten coloured rice varieties were 1.15-3.19% (Sompong *et al.*, 2011). Rice contains only a trace of fat and no cholesterol which makes it a healthier choice of food (Rice Data Center, 2003).

The amylose content of the rice starch is an important factor in eating quality. It is associated directly with volume expansion and water absorption during cooking and with the hardness, whiteness and dullness of cooked rice (Juliano, 1985). The amylose content in samples from this study ranged from 18.9 to 19.7% which classifies the samples as low amylose rice relative to MR219 (21.2%) which is classified as intermediate (Juliano, 1979). In a study by Sompong *et al.* (2011) the amylose content of Thailand red rice samples ranged from 7.47 to 41.95%. Shi and Zhu (1996) suggested that maternal effects, direct seed effects and cytoplasmic effects were the main factors in controlling amylose content, alkali spreading score and gel consistency, respectively.

Phenolic compounds are reported to exert an anti-oxidative stress effect (Lima *et al.*, 2006; Montilla *et al.*, 2006). Compared with white rice, red rice contains more phenolic compounds. The total phenolic content (TPC) values of red rice samples were higher than those of white rice. Goffman and Bergman (2004) have found that TPC was highly positively correlated with antiradical efficiency which suggests that phenolics are the main compounds responsible for the free radical scavenging activity in rice bran methanolic extracts. Based on Goffman and Bergman (2004), phenolic concentrations in rice appears to be highly correlated to bran colour, with cultivars with red and purple bran exhibiting up to 20 times greater concentrations as compared with those with white or light brown bran. These results are within the range cited by Sompong *et al.* (2011), who observed for red rice varieties, mean phenolic contents of 79.2 and 691.4 mg/100 g (expressed as ferulic acid on DM basis in methanolic extracts).

The stable DPPH[•] radical is frequently used to investigate free radical-scavenging activities of hydrogen donating antioxidants in many plant materials. No significant difference between both colours and within red colours was observed. Oki *et al.* (2002) found that the polymeric procyanidins are the major components responsible for the DPPH[•] radical scavenging activity. A negative correlation ($r = -0.43$) between TPC and DPPH[•] radical-scavenging ability was found. A similar correlation was also reported by Sompong *et al.* (2011).

The measurement of the total antioxidant capacity of cereals is of interest, because phenolic compounds are among the most effective antioxidants. The FRAP assay directly measures antioxidants with a reduction potential below that of the Fe³⁺/Fe²⁺ couple (Halvorsen *et al.*, 2002; Halvorsen *et al.*, 2006). The literature reports (Siddhuraju *et al.*, 2002; Yildirim *et al.*, 2001) that the reducing power of bioactive compounds is correlated with their antioxidant activity. Our results showed that the FRAP values were higher in red pericarp variants relative to white control, MR219 same as TPC results but it was lower than Thailand rice; this indicates that colour has

significant bearing on ferric-reducing ability. There was a positive but not significant correlation between TPC and FRAP ($r = 0.66$). Many studies have shown a good positive linear correlation between antioxidant capacity and total phenolic content of rice samples (Chi *et al.*, 2007; Jin *et al.*, 2009; Yafang *et al.*, 2011).

The term "phenolic acids" is generally used to designate phenols that only hold one carboxylic acid functionality (Robbins, 2003). When it comes to plant metabolites, they are a special group of organic acids (Stalikas, 2007). The basic chemical structures of natural phenolic acids are classified by two frameworks: hydroxycinnamic and hydroxybenzoic structures. The highest concentration of caffeic acid was found in MR219 was 4.40 µg/mL. Besides ferulic acid and caffeic acid, sinapic acid was also found in significant quantities (0.36 to 1.03 µg/mL). Considering that these three phenolic acids are hydroxycinnamic acids, it can be concluded that hydroxycinnamic acids were the main phenolic acids. In contrast to hydroxycinnamic acids, the hydroxybenzoic acids (vanillic and syringic acids) were not in such high levels (Fig. 4). Generally, hydroxycinnamic acids are considered to be more effective than their hydroxybenzoic counterparts probably because of the presence of CH = CH-COOH group. Syringic acid, although not dominant in G33, was found in all samples, ranging from 0.17 to 0.45 µg/mL. These findings are consistent with previous data for rice where the main phenolic acids were reported to be primarily ferulic (FA) and Protocatechuic Acid (PCAs) (Hudson *et al.*, 2000; Zhou *et al.*, 2004; Sompong *et al.*, 2011).

Vitamin E is another antioxidant in grains that supports polyunsaturated fatty acids in cell membranes by preventing their oxidative damage (Slavin *et al.*, 1999). For isolation of plant antioxidant compounds, solvent extraction is the most commonly used procedure. In a study by Badrinathan *et al.* (2011), using six different solvents, cold extraction with chloroform and crude methanol: chloroform extracts showed the highest yield. Table 3 illustrates the methanol: chloroform extraction yield of the rice samples that was, in decreasing order of yield: MR219 (3.04%) >G37 (3.0%) >G33 (2.6%) > Thailand red rice (2.1%). Variations in the yield of extracts from the plant materials tested might refer to the availability of different extractable components, resulting from the varied chemical composition of plants, nature of the soil and agro-climatic conditions (Hsu *et al.*, 2006). Among other parameters, capability of the extracting solvent to dissolve endogenous compounds might be a contributing factor (Siddhuraju and Becker, 2003; Sultana *et al.*, 2007). A previous study (Choi *et al.*, 2007) showed that the yield of methanolic extracts obtained from nine grains was in the range of 2.3-6.8%. The chromatographic

separation of tocopherols in a solution of purified standard is reported in Fig. 5. The antioxidant activities of tocopherols and tocotrienols are attributed to their capabilities to give their phenolic hydrogen to lipid free radicals and delay the autocatalytic lipid peroxidation processes (Seppanen *et al.*, 2010). Of the vitamin E components, γ -tocotrienol contributed to 46.9% of total tocopherols, followed by α -tocopherol, α -tocotrienol, γ -tocopherol and δ -tocotrienol in minor quantities and the tocopherols and tocotrienols were 35 and 65% of total E vitamin content, respectively. Likewise, γ -tocotrienol (46% of total tocopherols), followed by α -tocopherol (28%) have been reported as major components in 22 *indica* rice cultivars (Heinemann *et al.*, 2008) whilst γ -tocotrienol composed 74% of total tocopherols followed by α -tocopherol (11%) of cultivars from Brazil (Pascual *et al.*, 2011) and these observations were consistent with other studies (Chen and Bergman, 2005; Auilar-Garcia *et al.*, 2008). In our study, the concentration of total tocotrienols was more than 1.8-fold higher than that of total tocopherols in all rice samples. Based on Bergman and Xu (2003) and Heinemann *et al.* (2008), the lack of association between the levels of α - and γ -homologues indicates that the biosynthesis of these tocopherols happens through different metabolic pathways, which may describe why cultivars have specific homologues as major components. In this study, the concentrations of tocopherol and tocotrienol homologues varied among rice variants and were different in rice variants within the same color classification. However, γ -tocopherol (γ -T) was higher than α -T in some samples including MR219 and G33. Khatoun and Gopalakrishna (2004) reported much lower levels of α -tocopherol contents than those found in the present study. These differences in tocopherols concentration may be due to the differences of the rice variants, different methods of extraction used in the studies and possibly due to varying growth conditions.

CONCLUSION

In summary, amongst the rice samples, red pericarp advanced breeding lines extracts were the most effective in antioxidative reactions, unlike the MR219, which showed weak antioxidant abilities. Even though both red pericarp advanced breeding lines showed only half of the activity of Thailand rice in the TPC and FRAP assays, their tocopherol content was comparable to the mean values of Thailand rice. However, due to the smaller number of investigated rice variants in this study, the effect of colour or pigmentation was not as pronounced as that found in other studies. We demonstrated that whole-kernel red rice is a good source of phenolics and tocopherols. Consumption of red rice, which has been shown to be a

rich source of these compounds, could then be recommended in ordinary diet to enhance the daily intake of vitamins and thus are interesting for food products.

ACKNOWLEDGMENT

The authors are grateful to the Ministry of Science, Technology and Innovation (MOSTI) for funding this study under Project No.UKM-ABI-NBD0001-2007. Cooperation was also provided by Ms. Ani Fadlinha Mustaffa, Ms. Maizura Murad, Dr. Anna Permatasari Kamarudin, and UPM-BERNAS Research Laboratory staff are gratefully acknowledged. We thank Dr. Ravigadevi Sambanthamurthi and Dr. Abdul Gapor Mat Top from the Malaysian Palm Oil Board, for facilitating the phenolic component and vitamin E analysis and Ms. Jabariah and Ms. Mahani for their technical assistance.

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