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Antioxidant capacities and phenolic compounds of the husk, bran and endosperm of Thai rice

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ABSTRACT

Four fractions, namely, rice bran, rice husk, brown rice and milled rice of a Thai rice variety (Khao Dawk Mali 105), collected from three different growth sites, were analysed to determine phenolic acid composition, γ-oryzanol and tocopherols content and their antioxidant capacity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH·) radical scavenging and ferric reducing ability power (FRAP) assays. The bran and husk fractions showed higher values of antioxidant activity based on the DPPH· and FRAP assays, compared to the other fractions. In addition, the bran fraction had the highest γ-oryzanol and tocopherols content. On the other hand, the husk fraction showed a greater phenolic acids concentration than the other fractions. The three major phenolic acids found in all fractions, despite different growth sites, were ferulic, vanillic and *p*-coumaric acids. Ferulic acid was most evident in the bran, whereas vanillic and *p*-coumaric acids were mostly found in the husk. Significant differences ($p < 0.05$) in the antioxidant capacities of each fraction depended on the growth sites and antioxidant components. We propose that variation in phenolic acid content could be affected by the growth sites. This study demonstrates that rice bran and husk can be considered as valuable sources of bioactive components with high antioxidant properties.

1. Introduction

Rice is a rich source of many bioactive compounds including phenolic antioxidants that have the potential to reduce the risk of disease, such as inhibiting platelet aggregation (Daniel, Meier, Schlatter, & Frischknecht, 1999), reducing the risk of coronary heart disease and cancer (Martinez-Valverde, Periago, & Ros, 2000; Newmark, 1996), and preventing oxidative damage of lipid and low-density lipoproteins (Morton, Abu-Amsha, Puddey, & Croft, 2000). Cereal grains, especially rice, contain special phenolic acids (such as ferulic acid, *p*-coumaric and diferulate) that are not present in significant quantities in fruit and vegetables (Adom & Liu, 2002). Most of these compounds are found in different parts of plants (Kähkönen et al., 1999), particularly in distinct fractions from milling the grains (Onyeneho & Hettiarachchy, 1992). Rice bran is a rich source of steryl ferulate esters, commonly referred to as oryzanols (Norton, 1995; Seetharamaiah & Prabhakar, 1986). In addition, rice bran is a potential source of tocopherols, tocotrienols and phenolic compound (Nicolosi, Rogers, Ausman, & Orthoefer, 1994). These bioavailable materials have shown potential for antioxidant activity (Nam, Choi, Kang, Kozukue, & Friedman, 2005; Xu, Hua, & Godber, 2001). In general, the rice grain has a hard husk protecting the kernel inside. After the husk is removed, the remaining product is called brown rice, including bran, germ and endosperm. The commercial rice-milling process leads to products with low-value fractions, such as husk and bran. Because rice husks are inedible, they are used in various non-food applications as low-value waste materials. However, rice husks offer the valuable nutritional advantage that they contain an antioxidant defence system to protect the rice seed from oxidative stress (Ramarathnam, Osawa, Namiki, & Kawakishi, 1988). In addition, Jeon et al. (2006) have reported that phenolic compounds, from methanolic extracts of rice husk, exhibit high antioxidant activity against scavengers of singlet oxygen and inhibit high hydrogen peroxide-induced damage to cellular deoxy nucleic acid (DNA) in human lymphocytes. Recently, Lee et al. (2003) demonstrated that the antioxidant activity of rice husks was increased by far-infrared radiation, when using them for cooking turkey breast. However, there is limited published information available about phenolic acid composition and antioxidant activities of the morphological fractions of the rice grain.

Therefore, the objective of this study was to investigate the significance of rice as an antioxidant source. The focus was on four fractions from rice grain grown at different sites. In addition, phenolic acids, γ-oryzanol and tocopherols contents were determined

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by reversed-phase high-performance liquid chromatographic (HPLC). The variety chosen for these studies was Khao Dawk Mali 105 (KDML 105), one of the best-known varieties in Thailand, in terms of both production and export. It is fragrant and of good-cooking quality (Mahatheeranont, Keawsa-ard, & Dumri, 2001). The growth district chosen was Tung Kula Rong-Hai, the biggest production region of Thailand for the popular variety KDML 105.

2. Materials and methods

2.1. Rice samples

Paddy-rice samples (KDML 105 variety) were obtained from three different growth sites in the Tung Kula Rong-Hai region (Roi-Et province), in northeastern Thailand as described in Table 1. These grains were milled to separate husk from brown rice and germ was manually removed from brown rice but not included in the analysis. Then the brown rice was polished to obtain milled rice and bran. All fractions, exception the bran, were ground and passed through a 500 μm sieve screen. Moisture was determined by drying at 110 °C to constant mass. This and all other analysis were performed using triplicate samples and analytical results were expressed on a dry matter basis. The samples were stored at −20 °C prior to analysis.

2.2. Chemicals and reagents

The compounds 2,2-diphenyl-1-picrylhydrazyl (DPPH·), 6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tripiridyl-s-triazine (TBTZ), 2,6-di-tert-butyl-4-methyphenol (BHT), Folin–Ciocalteu's reagent, standards of gallic, ferulic, *p*-hydroxybenzoic, protocatechuic, *p*-coumaric, caffeic, syringic, sinapic, chlorogenic and vanillic acids were purchased from Sigma–Aldrich Fine Chemicals (St. Louis, MO). Oryzanol (Food Grade, 99.9% purity) was obtained from Tsuno Rice Fine Chemicals Co., Ltd. (Wakayama, Japan). The phosphoric acid, methanol and acetonitrile used in the HPLC analysis were purchased from Merck (Darmstadt, Germany). All other solvents were purchased from Fisher Scientific were of the highest available purity.

2.3. Extraction of crude phenolics of rice fractions

The crude phenolic compounds present in rice fractions were extracted with 80% aqueous methanol (1:10, w/v) at 25 °C with shaking in an incubator at 150 rpm for 16 h. The mixtures were centrifuged at 2500 rpm for 20 min and the supernatants were collected. The residues were re-extracted under the same conditions, and supernatants from both extractions were combined. The solvent was removed under vacuum at 40 °C and the resulting concentrated slurries were lyophilised and stored at −20 °C pending analysis.

2.4. Determination of total phenolic content

The total phenolic content (TPC) of each fraction was determined using the Folin–Ciocalteu method with some modifications (Liu & Yao, 2007). Briefly, 200 μl of extract solution was shaken for 1 min with 1 ml of diluted (1:10 with water) Folin–Ciocalteu reagent. After the mixture was shaken, 800 μl of 10% Na_2CO_3 was added, and final volume was made up to 5 ml with distilled water. After 2 h of reaction, the absorbance at 760 nm was determined and used to estimate the phenolics-acid content using a standard curve prepared using gallic acid.

2.5. Free-radical scavenging activity on DPPH·

The free-radical scavenging capacity of each extract was evaluated according to the procedure of Liyana-Pathirana and Shahidi (2007) with some modifications. Briefly, 100 μl of extract was added to the freshly prepared 0.1 mM DPPH· solution (1.9 ml), and the mixture was kept at room temperature in a dark room for 30 min. The absorbance was read at 517 nm relative to the control (as 100%) and the percentage of scavenging effect was expressed as $[1 - (A_{517}$ of sample$/A_{517}$ of control$)] \times 100$.

2.6. Ferric reducing ability of plasma (FRAP) assay

The FRAP assay is a method of measuring the ability of reductants (antioxidants) to reduce Fe^{3+}–Fe^{2+}. The formation of blue-coloured Fe^{2+}-TPTZ complex (Fe^{2+} tripyridyltriazine) increases the absorbance at 593 nm. The method of Kubola and Sirithon (2008) was used with some modifications. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3{\cdot}6H_2O$ solution. The fresh working solution was warmed at 37 °C before using. Rice extracts (300 μl) were allowed to react with 1.7 ml of the FRAP solution. The absorbance at 593 nm of the mixture was measured after 60 min of reaction. The results were calculated by standard curves prepared with known concentrations of $FeSO_4$, and were expressed as μmol $FeSO_4$/g fresh weight.

2.7. Determination of phenolics-acid composition

For HPLC analysis, each residue was dissolved in 5 ml methanol (HPLC) and then was passed through a 0.45 μm filter. A 20 μl aliquot of sample solution was fractionated using a Shimadzu HPLC system equipped with a diode array detector on a 250 mm × 4.6 mm i.d., 5 μm, Inertsil C18 analytical column. The mobile phase consisted of purified water with phosphoric acid pH 2.58 (solvent A) and acetonitrile (solvent B) at a flow rate of 0.8 ml/min. Gradient elution was performed as follows: from 0 to 5 min, linear gradient from 5% to 9% solvent B; from 5 to 15 min, 9% solvent B; from 15–22 min, linear gradient from 9% to 11% solvent B; from 22 to 38 min, linear gradient from 11% to 18% solvent B, 38–43 min, from 18% to 23% solvent B, 43–44 min, from 23% to

Table 1
Description of the sites where the rice samples were obtained in this study.

Sites	Source	Soil properties[a]
1	Suwanaphum district	This group of soils consists of poorly drained, coarse-textured soils that are salt affected and occupy a low-lying terrain of the north–east plateau and coastal plain. Most of the areas are paddy rice but the yield is relatively variable due to degree of salinity
2	Pone-Sai district	Deep grey clay soils from old alluvium, flat, grayish brown clay loam with mottling top soils, grey brown clay or silty clay and yellow or red mottling of laterite, slow permeability, poorly drained, moderate to high fertility, moderate phosphorous and potassium availability, neutral to low acid, water logging in rain season
3	Kaset-Wisai district	Very deep grey loamy soils from alluvial deposit, poorly drained to poorly drained, medium textured (silt loam grading to silty clay loam) soils that developed mostly in the areas of alluvial plain or flood plain

[a] Cited from Roi-Et Land Development Station (2002).

90% solvent B, 44–45 min, linear gradient from 90% to 80% solvent B, 45–55 min, isocratic at 80% solvent B, 55–60 min, linear gradient from 80% to 5% solvent B and re-equilibration period of 5 min with 5% solvent B were used between individual runs. Column temperature was set at 38 °C. Hydroxybenzoic acid compounds were detected at a wavelength of 280 nm and hydroxycinnamic acid compounds at 325 nm. Phenolic compounds in the samples were identified by comparing their relative retention times and UV spectra with authentic compounds and were detected using an external standard method.

2.8. Extraction and determination of γ-oryzanol and tocopherols contents

One-step equilibrium direct-solvent extraction was conducted by the method of Chen and Bergman (2005a) with some modifications. Each sample (1 g) was extracted with acetone ratio of 1:10 w/v, vortexed at maximum speed for 1 min then centrifuged at 2500 rpm for 20 min, after which the solvent was removed. The residual was further extracted twice, and the supernatants were combined before evaporating them to dryness under nitrogen gas. The determinations were made in triplicate.

The contents of γ-oryzanol and tocopherols were determined using HPLC. The crude extracts were dissolved in the mobile phase and filtered through a 0.45 μm pore size syringe-driven filter. The RP-HPLC system (Shimadzu) consisted of an auto sampler and column oven equipped with Inertsil ODS (4.6 mm × 250 mm, 5 μm) with mobile phase of acetonitrile/methanol (25:75, v/v), flow rate 1.5 ml/min and photodiode-array detector at 292 nm for the analysis of tocopherols and at 325 nm for the analysis of γ-oryzanol. Calibration curves were constructed with the external standards.

2.9. Statistical analysis

Each antioxidant activity assay was carried out three times from the same extract in order to determine their reproducibility. Analysis of the variance was used to determine any difference in antioxidant activities resulting from these methods. Duncan's new multiple-range test was used to determine significant differences. Statistical significance was declared at $p < 0.05$.

3. Results and discussion

3.1. Total phenolic content (TPC)

The TPC of four fractions of KDML 105 rice grown at different locations are shown in Table 2. There were significant differences detected amongst these fractions. The TPC of bran fraction had the highest value (2.5–2.7 mg GAE/g) whilst milled rice had the lowest TPC (0.5–0.7 mg GAE/g). All fractions of rice from site 2 showed the greatest TPC compared to other sites. The TPC of husk and brown rice fractions from different sites were significantly different ($p < 0.05$). Rice fractions from site 1 showed the lowest TPC results. However, there were no significant differences between the fractions of rice samples from site 2 and site 3 observed. This might be due to similarities in growth conditions.

3.2. DPPH· radical scavenging activity

The free-radical scavenging activity of the extracts of rice fractions were evaluated using the DPPH· method (Table 2). The ability to scavenge DPPH· radical by rice fractions was in the order of bran > husk > brown rice > milled rice for all samples. The DPPH radical scavenging varied from 42.0% to 89.5% of husk, 37.5% to 68.0% of brown rice and 25.2% to 39% of milled fractions. The corresponding values for the bran fraction were 85.9% to 86.7%. However, the samples, especially those of husk fractions, from site 2 demonstrated the highest scavenging capacity (89.5%). The varied radical scavenging activity of the extracts depended on the amount of total phenolic in each fraction. This finding supports the data previously reported in a study where the antioxidant activity was dependent on the actual composition of milling fractions (Liyana-Pathirana & Shahidi, 2007). In addition, all rice fractions (except for milled rice) showed the ability to scavenge the DPPH· radical at a rate higher than BHT (0.2 mg/ml) but lower than ascorbic acid with the concentration at 0.2 mg/ml and Trolox with the concentration 0.1 mM (Fig. 1).

3.3. Ferric reducing ability power (FRAP) of rice fractions

The ferric reducing ability power of all extracts of rice fractions expressed in FRAP value (μmol $FeSO_4$/g) are shown in Table 2. The different fractions from three sites showed significant ($p < 0.05$) differences in FRAP value, indicating that the location sites significantly influenced the ferric reducing power evaluation. Regardless of the location sites, the FRAP value of different fraction extracts indicated that the bran fraction had the greatest reducing power (27.5–32.2 μmol $FeSO_4$/g) followed by husk (12.4–28.2 μmol

Table 2
Total phenolic content, % inhibition DPPH· radical and FRAP value of four fractions from three sites.

Sites	Rice fractions	TPC (mg GAE/g)	% inhibition DPPH radical	FRAP (μmol $FeSO_4$/g)
1	Bran	2.5 ± 0.3^{ax}	85.9 ± 0.2^{ax}	27.5 ± 2.5^{ax}
	Husk	1.2 ± 0.1^{by}	42.0 ± 0.2^{bz}	12.4 ± 0.8^{bz}
	Brown rice	0.6 ± 0.1^{cy}	37.5 ± 0.5^{cy}	6.3 ± 0.2^{cy}
	Milled rice	0.5 ± 0.1^{cx}	25.2 ± 1.0^{dz}	4.6 ± 0.3^{dz}
2	Bran	2.7 ± 0.3^{ax}	86.7 ± 0.7^{bx}	32.2 ± 4.5^{ax}
	Husk	2.2 ± 0.3^{bx}	89.5 ± 1.4^{ax}	28.2 ± 1.8^{ax}
	Brown rice	1.3 ± 0.2^{cx}	68.0 ± 1.3^{cx}	12.5 ± 1.1^{bx}
	Milled rice	0.7 ± 0.2^{dx}	39.0 ± 0.5^{dx}	6.5 ± 0.5^{cx}
3	Bran	2.5 ± 0.2^{ax}	86.5 ± 0.3^{ax}	28.9 ± 4.1^{ax}
	Husk	1.9 ± 0.2^{bx}	77.8 ± 0.8^{by}	22.8 ± 2.3^{by}
	Brown rice	1.2 ± 0.1^{cx}	67.8 ± 1.2^{cx}	12.4 ± 1.5^{cx}
	Milled rice	0.5 ± 0.1^{dx}	30.3 ± 0.3^{dy}	5.3 ± 0.2^{dy}

The data are presented as mean ± SD for three replicates. ND, not detected.
$^{(a-d)}$Significant differences between means for a given within the fractions.
$^{(x-z)}$Significant differences between means for a given within the sites.

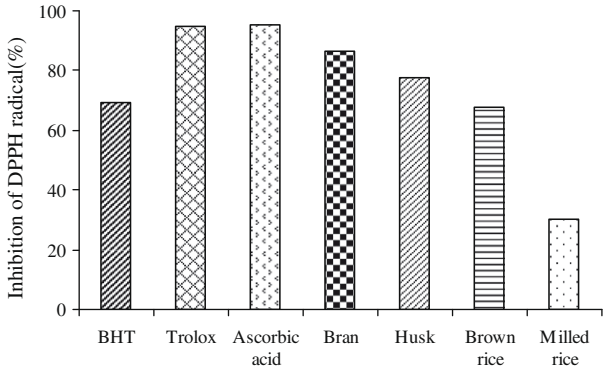

Fig. 1. Free radical scavenging activity of four fractions of KDML 105 rice analysed by DPPH· method. (results are means ± SD of three sites each fraction) compared amongst BHT (0.2 mg/ml), Trolox (0.1 mM) and ascorbic acid (0.2 mg/ml).

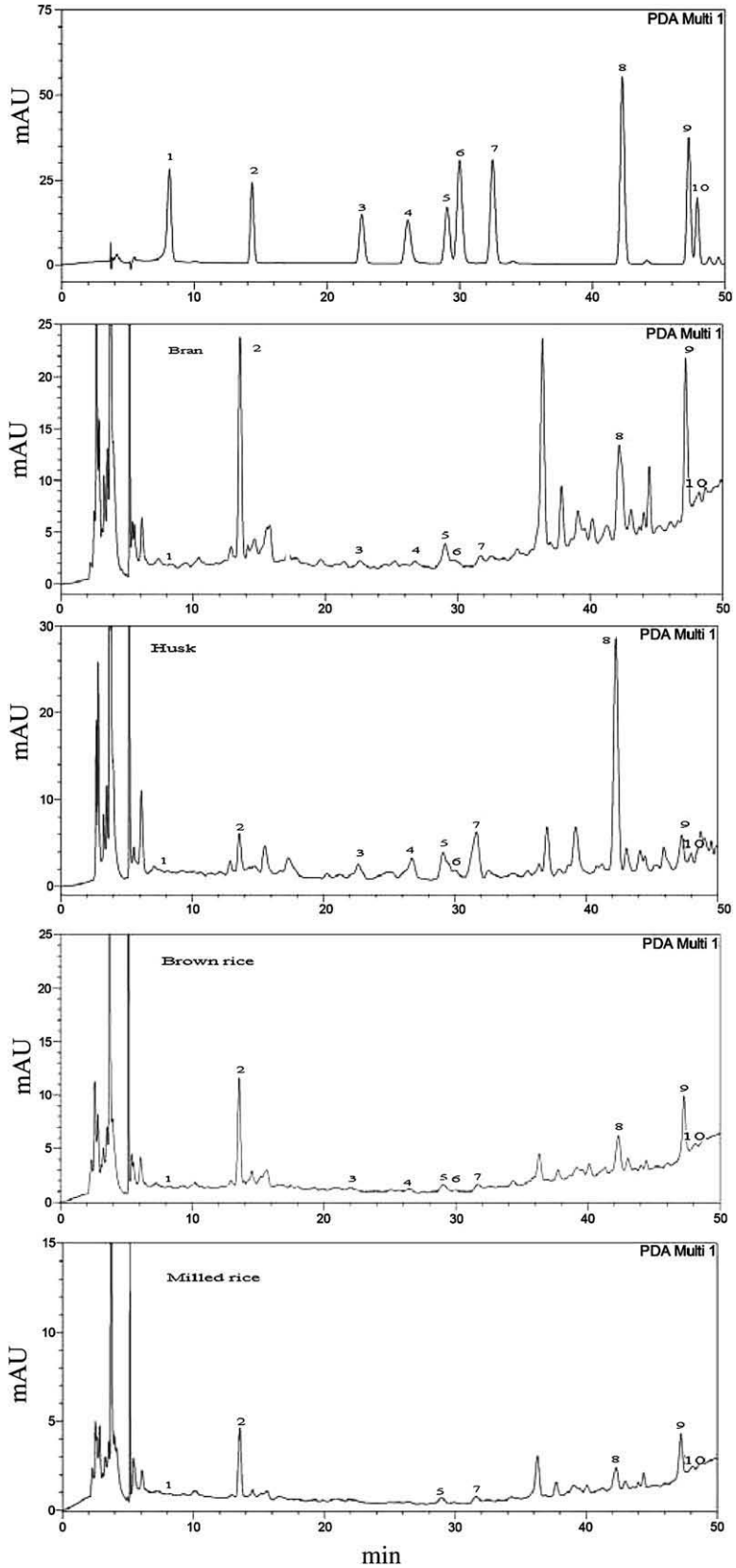

Fig. 2. Typical chromatograms of standard phenolic acids and four fractions of KDML 105 rice. Peaks: (1) gallic acid, (2) protocatechuic acid, (3) p-hydroxybenzoic acid, (4) chlorogenic acid, (5) vanillic acid, (6) caffeic acid, (7) syringic acid, (8) *p*-coumaric acid, (9) ferulic acid and (10) sinapic acid.

$FeSO_4$/g), brown rice (6.4–12.5 μmol $FeSO_4$/g), and then milled rice (4.6–6.5 μmol $FeSO_4$/g). In addition, all fractions of rice sample obtained from site 1 possessed lower FRAP values compared to other sites, except for the bran fractions. The bran fractions gave high

FRAP values that may be due to their higher TPC. The results showed that the FRAP values were dependent on the variations in TPC. However, the husk from site 2 showed that the FRAP values were consistent with antioxidant capacity detected by the DPPH· assay.

3.4. The phenolic acids composition in crude extract of rice fractions

Typical HPLC chromatograms of standard phenolic acids and four fractions of KDML 105 rice are shown in Fig. 2. The distribution of phenolic acids in all rice fractions from the three sites is presented in Fig. 3. The most abundant phenolic acids found in all fractions were ferulic, *p*-coumaric and vanillic acids. By comparing the phenolic acids to all fractions from all three sites, the bran fractions contained the highest level of ferulic acid, with concentrations from 20.70 to 33.45 μg/g. Vanillic acid and *p*-coumaric acid were the most dominant phenolic acids in husk fractions, with concentration from 14.4–26.7 μg/g and 4.84–28.90 μg/g, respectively. In addition, ferulic, *p*-hydroxybenzoic, protocatechuic and chlorogenic acids were minor constituents in the husk. The distribution of phenolic acids was similar for both brown rice and milled rice; however, some phenolic acids (*p*-hydroxybenzoic, chlorogenic, caffeic and syringic acids) were not detected in either of these fractions. In general, hydroxyl cinnamic acids such as ferulic acid, *p*-coumaric acid, were bound to phytosterols which have the structure, oryzanol (Miller & Engel, 2006). Previous research found that the concentration of individual phenolic acids was higher in brown rice than in milled rice (Zhou, Robards, Helliwell, & Blanchard, 2004). However, there has been no report of phenolic acid in husk fraction investigated, particularly, KDML 105 variety.

The total phenolic acids content in all fractions from different sites typically contributed 33–45% of the total phenolic acids in bran, 42–50% in husk, 8–13% in brown rice and 2–5% in milled rice, that was dependent on the growth sites and on the particular phenolic acid investigated. The two main phenolic acids in the husk were vanillic and *p*-coumaric acids. The contents of both these phenolics were 32% and 25% for site 1, 32% and 28% for site 2, and 17% and 34% for site 3. For bran fraction, ferulic acid was found to be the major phenolic acid, accounted for 43% for site 1, 50% for site 2 and 56% for site 3. These results indicate that the

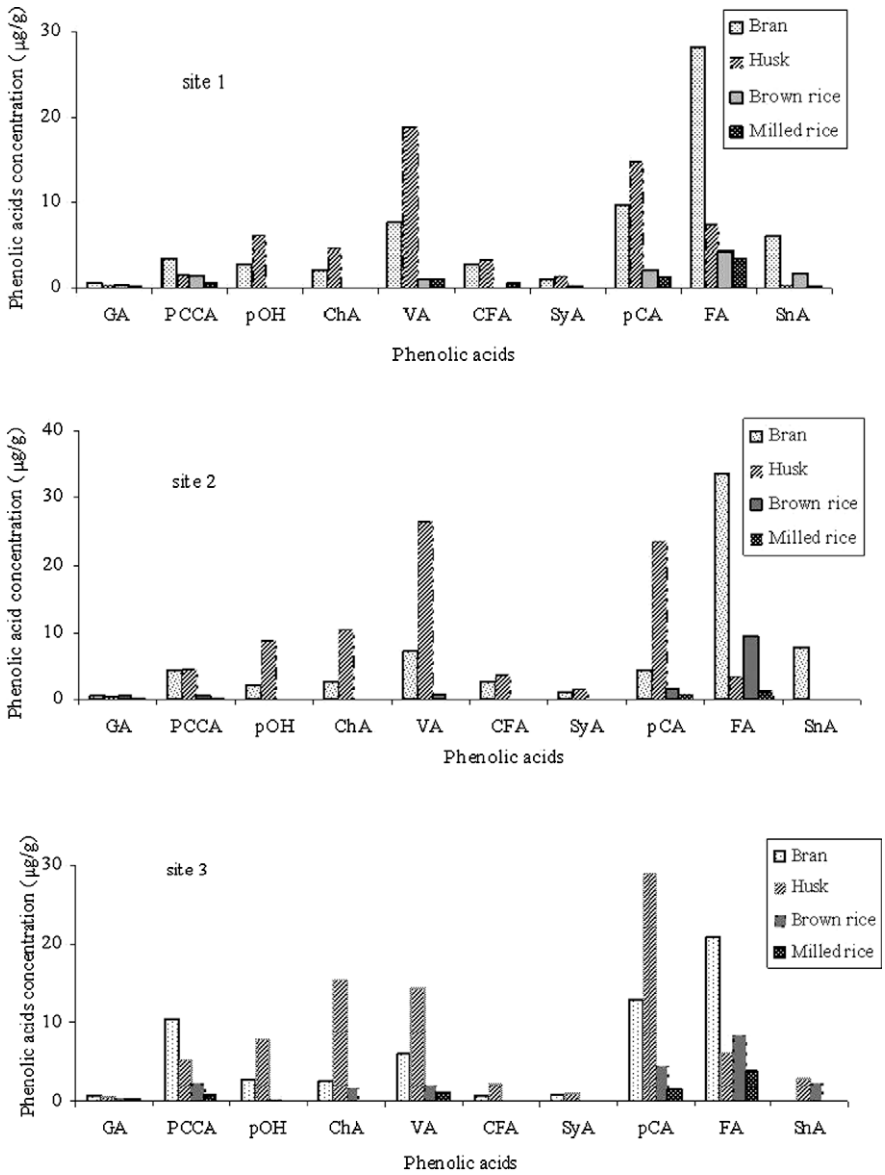

Fig. 3. Distribution of phenolic acids in four fractions of KDML 105 rice from three sites.

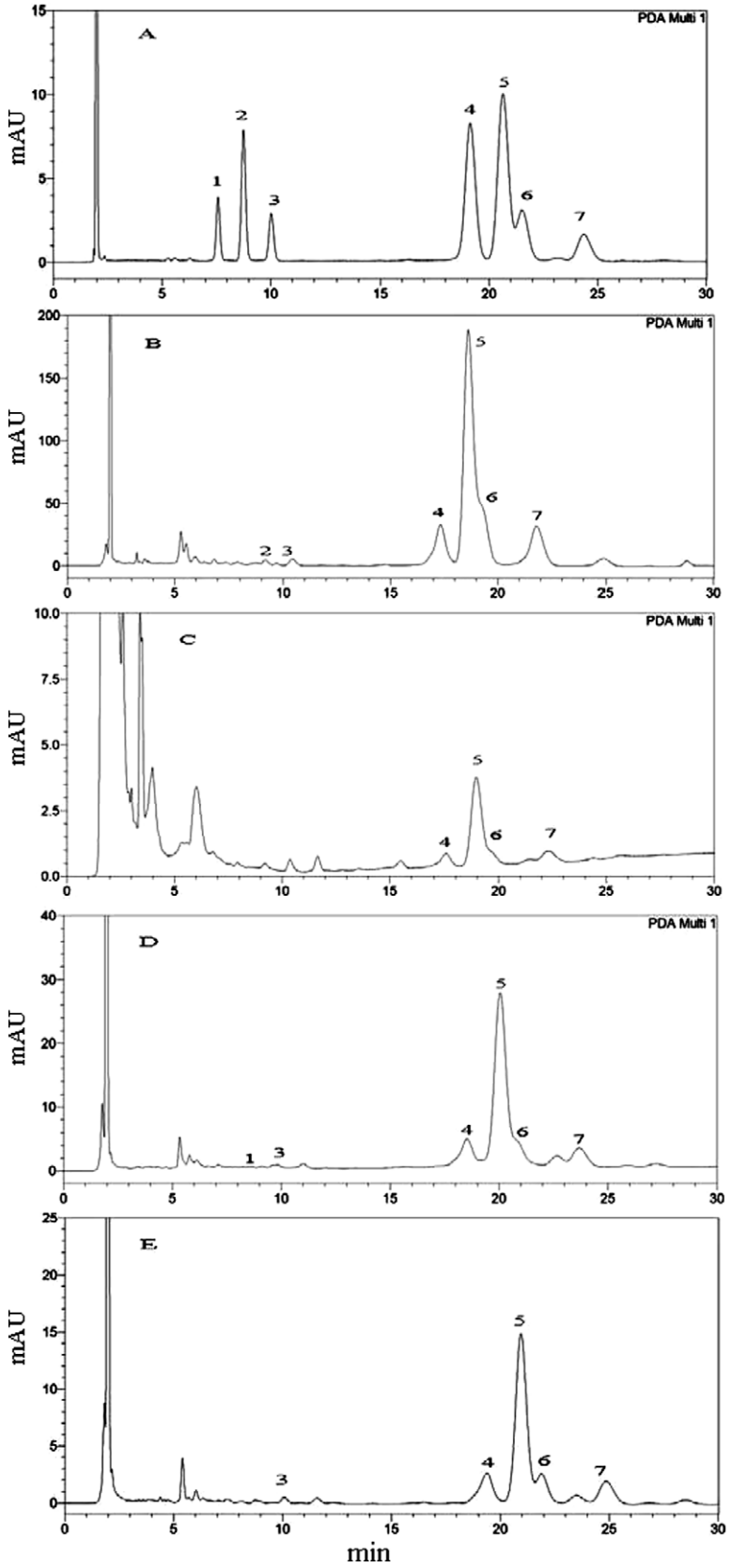

Fig. 4. Typical chromatograms of: (A) standard phenolic acids and four rice faction (B) bran, (C) husk, (D) brown rice and (E) milled rice. Peaks: (1) δ-tocopherol, (2) γ-tocopherol, (3) α-tocopherol and (4–7) γ-oryzanol.

concentrations of phenolic acids increase from endosperm to the aleurone layer. This conclusion sheds light on the concept that *p*-coumaric acid is primarily cross-linked with the lignin of the husk cell walls (Salomonsson, Theander, & Aman, 1980). On the

Table 3
The γ-oryzanol and tocopherols contents of four fractions of KDML 105 rice from three sites.

Sites	Rice fractions	γ-oryzanol (mg/g)	α-tocopherol (μg/g)	γ-tocopherol (μg/g)	δ-tocopherol (μg/g)
1	Bran	3.43 ± 0.03^{ay}	69.2 ± 1.30^{ay}	1.47 ± 0.03^{ax}	ND
	Husk	0.06 ± 0.01^{dy}	ND	ND	ND
	Brown rice	0.43 ± 0.01^{by}	11.6 ± 0.08^{by}	Trace	6.96 ± 0.90^{ax}
	Milled rice	0.20 ± 0.01^{cz}	5.43 ± 0.10^{cy}	ND	ND
2	Bran	5.38 ± 0.09^{ax}	82.8 ± 2.94^{ax}	4.74 ± 0.10^{ay}	ND
	Husk	0.16 ± 0.01^{dy}	ND	ND	ND
	Brown rice	0.95 ± 0.03^{bx}	24.6 ± 3.13^{bx}	Trace	3.01 ± 0.18^{az}
	Milled rice	0.33 ± 0.01^{cx}	9.98 ± 0.23^{cx}	ND	ND
3	Bran	5.18 ± 0.11^{ax}	70.7 ± 1.70^{ay}	3.46 ± 0.08^{az}	ND
	Husk	0.07 ± 0.01^{dy}	ND	ND	ND
	Brown rice	0.41 ± 0.01^{bx}	11.8 ± 0.53^{by}	Trace	4.89 ± 0.15^{ay}
	Milled rice	0.27 ± 0.01^{cy}	10.9 ± 0.23^{bx}	ND	ND

The data are presented as mean ± SD for three replicates. ND, not detected.
$^{(a-d)}$Significant differences between means for a given within the fractions.
$^{(x-z)}$Significant differences between means for a given within the sites.

other hand, our finding that ferulic acid is the major component found in bran makes sense if it is linked to arabinoxylan in cell walls of the aleurone layers (McKeehen, Busch, & Fulcher, 1999). In addition, our results indicate that the husk fraction provides the highest antioxidant activity. This means that the husk fraction can no longer be regarded as a worthless part of the rice grain. We now see the husk as a valuable source of phenolic acids and as major source of antioxidant activity.

3.5. The contents of γ-oryzanol and tocopherols

Typical HPLC chromatograms of standard tocopherols, γ-oryzanol and extracts obtained from crude rice fractions are shown in Fig. 4. The quantity of γ-oryzanol varied amongst all rice fractions irrespective of all growth sites, as shown in Table 3. For all extracts of the four fractions, the amount of γ-oryzanol ranged from 0.06 to 5.38 mg/g. The bran fraction was the best source of γ-oryzanol (3.43–5.38 mg/g), followed by brown rice (0.41–0.95 mg/g), milled rice (0.20–0.33 mg/g) and finally husk fractions (0.06–0.16 mg/g). All fractions obtained from site 2 gave the most γ-oryzanol content ($p < 0.05$). These results imply that growing conditions might affect the γ-oryzanol content of rice fractions. The level of γ-oryzanol detected in bran fractions was higher compared to previous studies, namely 2.4–3.1 mg/g (reported by Shin & Godber, 1996), 0.5–0.8 mg/g (reported by Iqbal, Bhanger, & Anwar, 2005), and 2.65 mg/g (reported by Proctor & Bowen, 1996). However, different extracting solvents were used in these other studies. The higher extractability of γ-oryzanol in the present study might be due to the use of acetone as the extracting solvent, where γ-oryzanol is present in both non-polar (Xu & Godber, 1999) and polar (Qureshi, Mo, Packer, & Peterson, 2000) components thus lipid fractions were extracted. Brown rice and milled rice fractions in our study showed higher γ-oryzanol contents than brown rice (0.5–0.7 mg/g) and milled rice (0.07–0.12 mg/g) as reported by Khatoon and Gopalakrishan (2004), and also higher levels than those for rice bran (1.55–2.72 mg/g) and for brown rice (0.20–0.39 mg/g) for three varieties of Venezuelan rice (Aguilar-Garcia, Gavino, Baragano-Mosqueda, Hevia, & Gavino, 2007).

The content of tocopherols (including α-tocopherol, γ-tocopherol and δ-tocopherol) varied widely between fractions. Importantly, γ-tocopherol was only found in the bran fraction and δ-tocopherol was only detected in brown rice (Table 3). All fractions of rice grown at site 2 gave the highest content of α-tocopherol, when compared to the rice from other sites. A previous study has reported a total tocopherol content of 31.3–48.7 μg/g for brown rice from four varieties (Gopala Krishna, Prabhakar, & Sen, 1984). Khatoon and Gopalakrishan (2004) reported the α-tocopherol contents of Basmati brown rice (12.1 μg/g) and Jaya brown rice (9.9 μg/g). Recently, Aguilar-Garcia et al. (2007) reported the levels of tocopherol in rice bran (<0.5–35.0 μg/g) and brown rice (<0.5–8.5 μg/g) for three varieties of Venezuelan rice. These differences in γ-oryzanol and tocopherols concentration may be due to the different methods of extraction used in the studies, and may also be due to differences between rice varieties and growth conditions. According to the results obtained from this study, it could be claimed that acetone is the best extracting solvent for γ-oryzanol and tocopherols with respects to contents because acetone extracts both polar and non-polar components. These findings also show to the extent that growth sites alter levels of γ-oryzanol and tocopherols in the grain fractions. Previous studies reported effects of genotype and environment as well as kernel thickness on the γ-oryzanol content in rice bran (Bergman & Xu, 2003; Chen & Bergman, 2005a; Chen & Bergman, 2005b; Lloyd, Siebenmorgen, & Beers, 2000). Other studies have reported that brown rice grown at different sites or in different seasons influence γ-oryzanol content (Miller & Engel, 2006). However, the actual environmental growing conditions for the samples in this study were not analysis. It is well known that tocopherols and γ-oryzanol are the main antioxidants present in rice bran. Antioxidant activity of γ-oryzanol is almost 10 times higher than that of tocopherols (Abdel-Aal & Hucl, 1999). In our study, bran fractions had the highest γ-oryzanol and α-tocopherol contents, with high antioxidant capacities compared to those of brown rice and milled rice fractions. Despite having a low amount of γ-oryzanol and absence of tocopherols in husk fractions, crude extract of husk fractions showed substantially high antioxidant capacities, especially husk fraction of rice from site 2 and site 3. Therefore, it was presumed that the major antioxidant components found in husk fraction may be phenolic acids.

4. Conclusions

The concentrations of bioactive constituents, including phenoilc acids, γ-oryzanols and tocopherols, were found in greater amounts in the external layers; thus the bran and husk fractions demonstrated a higher antioxidant activity than those of other milling fractions. The antioxidant activity may also vary depending on the actual composition of these fractions. All fractions of rice obtained from three growth locations differed significantly in their radical scavenging activities against the DPPH$^{\cdot}$ radical and ferric reducing power. Results depended on the site origin and the antioxidant components (γ-oryzanol, α-tocopherol and phenolic acids). These results indicate that antioxidant activity could be affected by growth sites, as well as by grain morphology, because

antioxidant properties respond to the environmental changes differently. The level of phenolics in rice has been correlated with UV-B tolerance (Caasilit, Whitecross, Nayudu, & Tanner, 1997). These variations in phenolic content could be affected by growth conditions. Antioxidant activity of bran and husk fraction extracts proved to be higher when compared with those of amongst fractions. However, the husk of rice grain grown at site 2 showed higher antioxidant activity than brown rice and milled rice. The distribution of phenolic acids varied amongst the rice fractions; the husk contained the highest total levels of phenolic acids. The study indicated that rice husk, as a valuable source of phenolic acids, is an effective source of natural antioxidants. Our findings now provide a valuable basis for developing rice-milling fractions (previously thought to be "worthless") as valuable food additives to enhance human nutrition via their phenolic acid composition and antioxidant activity.

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