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Antioxidant components and properties of five long-grained rice bran extracts from commercial available cultivars in Thailand

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ABSTRACT

Five varieties of long-grained rice bran, which are the most commonly cultivated varieties in Thailand, are abundant in antioxidant components. The antioxidative activity of rice bran extracts was investigated using various established *in vitro* systems, including 2,2′-diphenyl-1-picrylhdrazyl free radical-scavenging (DPPH·), total reducing power, ferrous ion-chelating activity and lipid peroxidation inhibition. The total phenolic and flavonoid contents, and gamma-oryzanol, tocopherol and tocotrienol isomer contents of rice bran extract were also determined by colorimetric assay and high performance liquid chromatography. The methanolic rice bran extracts produced strong results with DPPH free radical-scavenging (EC_{50} 0.38–0.74 mg/ml), reducing power (EC_{50} 0.10–0.53 mg/ml), ferrous ion-chelating activity (EC_{50} 0.11–0.55 mg/ml) and inhibition of lipid peroxidation (EC_{50} 0.14–0.57 mg/ml). Total phenolic and flavonoid contents, and gamma-oryzanol, tocopherol and tocotrienol contents of rice bran extract were in the range 2.2–3.2, 0.03–0.10, 0.56–1.08, 0.35–0.77 and 0.22–0.46 mg/g rice bran, respectively. These results indicated that the methanolic components of the long-grained rice bran extracts might potentially be natural antioxidants.

1. Introduction

Rice is the main staple food in Thailand and many countries in Asia. It is also the main export product of Thailand. During rice processing, rice bran is produced as a by-product of rice milling. Rice bran powder has a high nutritive value (Saunders, 1990), offering benefits such as the lowering of blood cholesterol (Kahlon, Chow, & Sayre, 1994), decreasing the incidence of atherosclerosis disease (Saunders, 1985) and having a laxative effect (Saunders, 1990). The beneficial components of rice bran comprise sterols, higher alcohols, gamma-oryzanol, tocopherols, tocotrienols and phenolic compounds (Aguilar-Garcia, Gavino, Baragaño-Mosqueda, Hevia, & Gavino, 2007; Nicolosi, Rogers, Ausman, & Orthoefer, 1994). Gamma-oryzanol, which is ubiquitous as a component of primary plant cell walls, offers some benefits (Tanaka, 1971), such as lowering of blood cholesterol (Guardiola, Codony, Addis, Rafecans, & Boatella, 1996) and antioxidant properties (Xu, Hua, & Godber, 2001). In addition to tocopherols, tocotrienols and several phenolic compounds have potentially beneficial effects (Liu, 2003), such as antioxidative activity (Pietta, 2000) and antibacterial properties (Kim & Kim, 2006).

The addition of an antioxidant is required to retard lipid peroxidation, and preserve the flavour, colour and vitamins of food during storage. Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are commonly used in food to retard lipid peroxidation during storage (Chu & Hus, 1999). However, many researchers have reported adverse effects of synthetic antioxidants, such as toxicity and carcinogenicity (Ito et al., 1986; Whysner, Wang, Zang, Iatropoulos, & Williams, 1994; Williams, Iatropoulos, & Whysner, 1999). Safety concerns over synthetic antioxidants have led to an increasing interest in identifying naturally occurring antioxidants from edible materials, edible by-products and residual sources (Moure et al., 2001). The replacement of synthetic antioxidants by antioxidants from natural sources has many benefits due to their functionalities, e.g. health benefits and solubility.

Nevertheless, no basic information regarding the antioxidant components and properties of rice bran extract from commercially available cultivars in Thailand has been reported. The object of this study was to investigate the antioxidant components and properties of rice bran from five varieties of long grain rice, which are the most commonly cultivated varieties in Thailand, to provide information on functional food for commercial practices.

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2. Materials and methods

2.1. Materials

Standards of tocopherols (α-T, β-T, γ-T and δ-T), tocotrienols (α-T3, β-T3, γ-T3 and δ-T3), 2,2′-diphenyl-1-picrylhydrazyl (DPPH·), Folin–Ciocalteu reagent, catechin, butylated hydroxytoluene (BHT) and gallic acid were purchased from Sigam–Aldrich Chemical Co., (St. Louis, Mo, USA). Disodium ethylenediamine tetracetate (Na_2EDTA) was purchased from Fluka Chemical (Buchs, Switerland). Trichloroacetic acid was purchased from BDH (Poole, UK). Gamma-oryzanol (Oryza Oil & Fat Chemical, Co., Ltd., Aichi Pref, Japan) was kindly given by Prof. Dr. Hideki Ushio, Department of Food Science and Technology, Tokyo University of Marine Science and Technology, Tokyo, Japan. HPLC grades of methanol, acetonitrile and dichloromethane were purchased from BDH (Poole, UK). The analytical grade methanol and other organic solvents were purchased from Merck (Darmstadt, Germany). Other chemicals used in this study were of analytical grade and used without further purification.

2.2. Methods

2.2.1. Preparation of rice bran extract

Rice bran powders of five long grain rice cultivars, namely *Oryza Sativa* L. CV. Khao Dawk mali 105; RB-1, *O. Sativa* L. CV. Khoa Pathum Thani 60; RB-2, *O. Sativa* L. CV. Khao Suphan buri 90; RB-3, *O. Sativa* L. CV. Khao Chinat 1; RB-4 and *O. Sativa* L. CV. Khao Gokho 13; RB-5, were obtained by milling rice grain in a local grinding mill, followed by sieving to separate grain from rice bran. Rice bran was ground, then passed through 177-297 μm sieves and heated at 100 °C for 15 min to inactivate endogenous lipase (Juliano, 1985). Rice bran powder (10.0 g) was extracted with methanol (150 ml) for 12 h in an electrical shaker at room temperature. The extract was filtered through Whatman No.1 filter paper and the solvent was removed. The residual rice bran powder was further extracted twice, with methanol and the extracts were combined before evaporating under vacuum using a rotary evaporator N–N Series, Eyela (Tokyo, Japan) and then a flow of nitrogen gas to dryness. The residual crude methanolic rice bran extract was weighed and stored at −20 °C under a nitrogen gas stream.

2.2.2. Determination of total phenolic content

Total phenolic content of rice bran extract was measured according to the method reported by Singleton, Orthofer, and Lamuela-Raventos (1999) by using Folin–Ciocalteu reagent with some modification. Rice bran extract was dissolved methanol. An aliquot of rice bran extract (250 μl) at appropriate dilution of sample was mixed with 500 μl of the freshly prepared Folin–Ciocalteu reagent and a further 6.0 ml of distilled water. The mixture was shaken vigorously and 2.0 ml of sodium carbonate (15% w/v) were added and the mixture was again shaken vigorously for 2 min. The final volume was made up to 10.0 ml with distilled water. After the mixture was left to stand for 2 h at room temperature, the absorbance at 750 nm was measured by using a UV–vis spectrophotometer (model Lambda EZ201 UV/vis spectrophotometer (Perkin Elmer, USA). The results of total phenolic content were expressed as μg gallic acid equivalents per g of rice bran.

2.2.3. Determination of total flavonoid content

Total flavonoid content of rice bran extract was determined according to the method reported by Jia, Tang, and Wu (1998). Briefly, rice bran extract was dissolved in methanol and an appropriate dilution of rice bran extract (250 μl) was diluted with distilled water 1.25 ml and 75 μl of 5% $NaNO_2$ solution were added. The mixture was allowed to stand at room temperature for 6 min before 150 μl of 10% $AlCl_3$ were added. This mixture was allowed to stand for a further 5 min before 0.5 ml of 1 M NaOH was added. The solution was shaken vigorously before absorbance at 510 nm was measured with a UV–vis spectrophotometer (model Lambda EZ201 UV/vis spectrophotometer (Perkin Elmer, USA). The results were expressed as μg catechin equivalents per g of rice bran.

2.2.4. Determination of gamma-oryzanol content in rice bran extract

Gamma-oryzanol content in rice bran extract was measured by using reverse phase high performance liquid chromatography (RP-HPLC) according to the method reported by Rogers, Rice, and Romancyzk (1993) with some modification. Rice bran extract 100.0 mg was dissolved in 1.0 ml of methanol before filtering through a syringe filter with PTFE (0.2 μm; Ascordic syringe filter). The RP-HPLC consisted of an Agilent 1100 series (Palo Alto, Ca, USA), including auto sample and column oven equipped with Hypersil ODS (4.0 × 250 mm, 5 μm, Agilent Technologies, Palo Alto, CA, USA), and a variable wavelength UV–vis detector (model G1379A) at 330 nm by using a mixture of methanol:acetonitrile:dichloromethane:acetic acid (50:44:3:3 v/v/v/v) as a mobile phase at a flow rate of 1.0 ml/min. The content of gamma-oryzanol was calculated from the peak area of standard gamma-oryzanol.

2.2.5. Determination of total tocopherol, total tocotrienol, tocopherol and tocotrienol isomer contents

Tocopherol isomer content of rice bran extract was determined by RP-HPLC according to the method reported by Xu et al. (2001). Rice bran extract (100.0 mg) was dissolved in methanol. Tocopherol isomers were separated on a RP-HPLC Agilent 1100 series equipped with a Mightysil RP-18 GP column (4.6x250 mm, 3 μm, Kanto Chemical Co., Inc., Tokyo, Japan) and a FLD G1321A fluorescence detector operating with excitation and emission wavelengths of 290 and 330 nm, respectively. The mobile phase was methanol:acetonitrile:dichloromethane (50:44:6, v/v/v) with a flow rate of 1 ml/min. Peak areas of each tocopherol isomer were used for calculating total tocopherol and tocopherol isomer contents.

2.2.6. Determination of 2,2′-diphenyl-1-picrylhydrazyl radical-scavenging activity

DPPH radical-scavenging activity of rice bran extract was determined according to the method reported by Brand-Williams, Cuvelier, and Berset (1995) with some modification. An aliquot of 0.5 ml of sample solution in methanol was mixed with 2.5 ml of a 0.5 mM methanolic solution of DPPH·. The mixture was shaken vigorously and incubated for 30 min in the dark at room temperature. The absorbance was measured at 517 nm against a blank, using a UV–vis spectrophotometer (model Lambda EZ201 UV/vis spectrophotometer, Perkin Elmer, USA). DPPH free radical-scavenging ability was calculated by using the formula: scavenging ability (%) = [$Absorbance_{517\,nm\ of\ control}$ − $Absorbance_{517\,nm\ of\ sample}$/$Absorbance_{517\,nm\ of\ control}$] × 100. The scavenging activity of rice bran extract was expressed as 50% effective concentration, EC_{50} (mg/ml) and was obtained by interpolation from linear regression analysis. BHT was used for comparison.

2.2.7. Determination of reducing power

The reducing power of rice bran extract was measured according to the method reported by Yen and Duh (1993) with some modification. Each rice bran extract in methanol (2.5 ml) was mixed with 2.0 M sodium phosphate buffer at pH 6.6 (2.5 ml). The dilute sample was then mixed with 5.0 ml of 1% potassium fericyanide and the mixture was incubated at 50 °C for 20 min. Trichloroacetic acid (10%, 5.0 ml) was added to mixture, which was then centrifuged at 6000*g* for 10 min. The upper solution (5.0 ml)

was mixed with distilled water (5.0 ml) and 1.0 ml of ferric chloride (1.0%). The absorbance was measured at 700 nm by using UV–vis spectrophotometer (model Lambda EZ201 UV/vis spectrophotometer, Perkin Elmer, USA) and BHT was used for comparison.

2.2.8. Determination of ferrous ion-chelating activity

Ferrous ion-chelating ability of rice bran extract was measured according to the method reported by Shimada, Fujikawa, Yahara, and Nagamura (1992). The reaction mixture (2.0 ml) contained hexamine (30 mM), potassium chloride (30 mM) and ferrous sulphate (9 mM) was added each rice bran extract in methanol (2.0 ml) and 200 μl of 1 mM tetramethyl murexide (TMM). The mixture was shaken vigorously and left to stand for 3 min at room temperature. Absorbance of the mixture was determined at 485 nm against a blank. Ferrous ion-chelating ability was expressed as 50% effective concentration EC_{50} (mg/ml) and was obtained by interpolation from linear regression analysis. Disodium ethylenediaminetetracetate (Na_2EDTA) was used for comparison.

2.2.9. Determination of lipid peroxidation inhibition

Inhibition of lipid peroxidation of rice bran extract was measured according to the method reported by Lingnert, Vallentin, and Eriksson (1979). Each rice bran extract in methanol (200 μl) was mixed with 4.0 ml of linoleic acid (10 mM) in sodium phosphate buffer (0.2 M) and left to stand in darkness at 37 °C for 15 h to accelerate lipid peroxidation. After the end of incubation, 6.0 ml of 60% methanol were added and the absorbance measured at 234 nm against a blank, using a UV–vis spectrophotometer (model Lambda EZ201 UV/vis spectrophotometer, Perkin Elmer, USA). BHT was used for comparison. The inhibition of lipid peroxidation was calculated by using the formula: % of inhibition of lipid peroxidation = [Absorbance $_{234\ of\ control}$ − Absorbance $_{234\ of\ sample}$/ Absorbance $_{234\ of\ control}$] × 100 and reported as EC_{50} (mg/ml) value.

2.3. Statistical analysis

The experimental data were subjected to a one-way analysis of variance for a completely random design to determine the least significant difference at the level of 0.05. The data values were expressed as mean ± SD ($n = 3$).

3. Results and discussion

The total phenolic content of rice bran extracts, as determined by the Folin–Ciocalteu reagent, ranged from 2.2 ± 0.3 to 3.2 ± 0.2 mg gallic acid eq/g rice bran (Table 1). The phenolic compound contents, ranked the samples in descending order, as follows: RB-2, RB-1, RB-3, RB-5 and RB-4, the values of which were 3.2 ± 0.2, 2.9 ± 0.1, 2.8 ± 0.1, 2.7 ± 0.2, and 2.2 ± 0.3 mg gallic acid equivalents/ g rice bran, respectively. RB-2 had the highest content of phenolic compounds, while their lowest content was found in RB-4 ($p \leqslant 0.05$). The total phenolic content was not significantly different between varieties RB-1, RB-3 and RB-5 ($p > 0.05$). This supports the findings of other studies, where phenolic compounds exhibiting effective antioxidant properties were found in some extracts of commercially available varieties, such as rice bran from Pakistan (Iqbal, Bhanger, & Anwar, 2005), long-life and short-life seed hull extracts (Ramarathnam, Osawa, Namiki, & Tashiro, 1986) and defatted rice bran (Devi & Arumughan, 2007; Devi, Jayalekshmy, & Arumughan, 2007).

The total flavonoid content ranged from 0.03 ± 0.002 to 0.10 ± 0.002 mg catechin equivalents/g rice bran. The highest flavonoid content was found in RB-4, which had 0.10 ± 0.002 mg catechin equivalents/g rice bran; on the other hand, RB-3 had the lowest (0.03 ± 0.002 mg catechin equivalents/g rice bran) ($p \leqslant 0.05$). Flavonoid content extracted from wheat bran was higher than that of rice bran (Iqbal, Bhanger, & Anwar, 2007). This could be due to the different kinds of bran.

The gamma-oryzanol, tocopherol, and tocotrienol profiles of methanolic rice bran extracts are given in Table 2. The highest contents of gamma-oryzanols, tocopherols and tocotrienols were found in RB-2, the values of which were, 1.08 ± 0.07, 0.77 ± 0.03 and 0.46 ± 0.02 mg/g rice bran, respectively. The gamma-oryzanols contained in methanolic rice bran extracts were from 0.56 ± 0.03 to 1.08 ± 0.07 mg/g of rice bran. According to Xu and Godber (1999), gamma-oryzanols are a mixture of phytosteryl and campesteryl ferulates. They have also been shown to have antioxidant proper-

Table 1
Total phenolic and flavonoid compounds from methanolic rice bran extracts from *Oryza Sativa* L. CV. Khao Dawk mali 105 (RB-1), *Oryza Sativa* L. CV. Khoa Pathum Thani 60 (RB-2), *Oryza Sativa* L. CV. Khao Suphan buri 90 (RB-3), *Oryza Sativa* L. CV. Khao Chinat 1 (RB-4), *Oryza Sativa* L. CV. Khao Gokho 13 (RB-5)

Rice bran extract	Total phenolic content (mg gallic acid eq/g rice bran)	Total flavonoid content (mg catechin eq/g rice bran)
RB-1	2.9 ± 0.1b	0.08 ± 0.002c
RB-2	3.2 ± 0.2c	0.06 ± 0.001b
RB-3	2.8 ± 0.1b	0.03 ± 0.002a
RB-4	2.2 ± 0.3a	0.10 ± 0.002d
RB-5	2.7 ± 0.2b	0.09 ± 0.002c

Results represent means ± standard deviation ($n = 3$). In each column, different letters mean significant differences ($p \leqslant 0.05$).

Table 2
Tocopherol, tocotrienol and gamma-oryzanol contents from methanolic rice bran extracts from *Oryza Sativa* L. CV. Khao Dawk mali 105 (RB-1), *Oryza Sativa* L. CV. Khoa Pathum Thani 60 (RB-2), *Oryza Sativa* L. CV. Khao Suphan buri 90 (RB-3), *Oryza Sativa* L. CV. Khao Chinat 1 (RB-4), *Oryza Sativa* L. CV. Khao Gokho 13 (RB-5)

Rice branextract	Tocopherols (mg/g rice bran)				
	α	β	γ	δ	Total
RB-1	0.33 ± 0.02	0.21 ± 0.01	0.10	± 0.01 0.03 ± 0.001	0.67 ± 0.05c
RB-2	0.38 ± 0.02	0.25 ± 0.03	0.13	± 0.02 0.01 ± 0.002	0.77 ± 0.03d
RB-3	0.12 ± 0.02	0.08 ± 0.01	0.12	± 0.02 0.03 ± 0.001	0.35 ± 0.03a
RB-4	0.23 ± 0.02	0.18 ± 0.01	0.06	± 0.01 0.01 ± 0.001	0.48 ± 0.06b
RB-5	0.28 ± 0.02	0.20 ± 0.02	0.08	± 0.01 0.04 ± 0.003	0.60 ± 0.06c
Rice branextract	γ-Oryzanol contents(mg/g rice bran)	Tocotrienols (mg/g rice bran)			
		α	β	γ	Total
RB-1	0.99 ± 0.05c	0.11 ± 0.01	0.20 ± 0.01	–	0.31 ± 0.02b
RB-2	1.08 ± 0.07c	0.20 ± 0.02	0.26 ± 0.02	–	0.46 ± 0.02c
RB-3	0.56 ± 0.03a	0.21 ± 0.02	0.20 ± 0.01	–	0.41 ± 0.02c
RB-4	0.79 ± 0.09b	0.17 ± 0.02	0.16 ± 0.02	–	0.33 ± 0.02b
RB-5	0.61 ± 0.10a	0.10 ± 0.01	0.12 ± 0.01	–	0.22 ± 0.02a

Results represent means ± standard deviation ($n = 3$). In each column, different letters mean significant differences ($p \leqslant 0.05$).

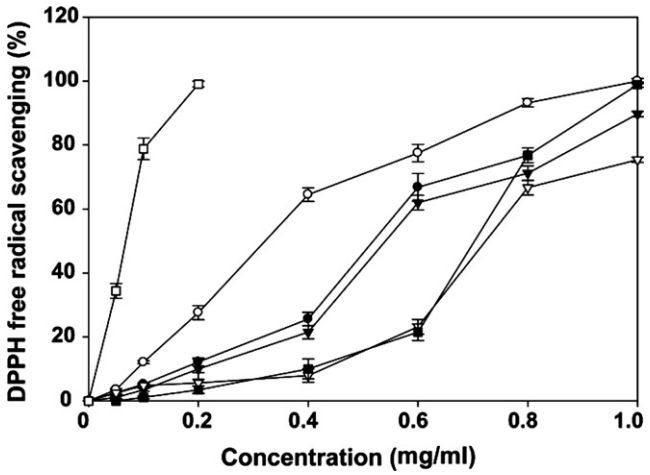

Fig. 1. DPPH radical-scavenging activity (%) of different amounts of methanolic rice bran extracts from *Oryza Sativa* L. CV. Khao Dawk mali 105 (●; RB-1), *Oryza Sativa* L. CV. Khoa Pathum Thani 60 (○; RB-2), *Oryza Sativa* L. CV. Khao Suphan buri 90 (▼; RB-3), *Oryza Sativa* L. CV. Khao Chinat 1 (∇; RB-4), *Oryza Sativa* L. CV. Khao Gokho 13 (■; RB-5) and BHT (□) by 1,1,-diphenyl-2-picrylhydrazyl (DPPH) radicals. Results represent means ± standard deviation ($n = 3$).

ties in many types of *in vitro* model systems (Nyström, Mäkinen, Lampi, & Piironen, 2005; Xu et al., 2001). The main tocopherol was α-tocopherol, ranging from 0.12 ± 0.02 to 0.38 ± 0.02 mg/g of rice bran, whereas δ-tocopherol was present in small amounts (0.01 ± 0.002–0.04 ± 0.003 mg/g of rice bran). Different forms of tocopherol exhibit different antioxidative effects, which were ranked in the descending order δ > γ > β > α, with increasing temperature, by Madhavi, Singhal, and Kulkarni (1996). The strong antioxidative effect of tocopherol is achieved by transference of a hydrogen atom to a lipid peroxy radical to form lipid hydroperoxide and tocopheryl radicals (Kamal-Eldin & Appelqvist, 1996). The majority of tocotrienols found in methanolic rice bran extracts were α- and β-forms, ranging from 0.10 ± 0.01 to 0.21 ± 0.02 and 0.12 ± 0.01 to 0.26 ± 0.02 mg/g of rice bran, respectively. This showed that rice bran extracts contained high phenolic, flavonoid, gamma-oryzanol, tocopherol and tocotrienol contents, as would be required in a source of natural effective antioxidants.

Various mechanisms, such as free radical-scavenging, reducing capacity, metal ion-chelation and inhibition of lipid peroxidation (Hollman & Katan, 1999; Pietta, 2000; Rice-Evans, Miller, & Paganga, 1997), have been studied to explain how rice bran extracts could be used as effective antioxidants. The DPPH free radical method has been used extensively to evaluate reducing substances, based on the reduction of methanolic DPPH· solution in the presence of a proton-donating substance, resulting in the formation of diamagnetic molecules (Soares, Dins, Cunha, & Ameida, 1997). The scavenging effects of all extracts on DPPH radicals increased with increasing of concentration (Fig. 1) and DPPH radical-scavenging at EC_{50} values of rice bran extracts is shown in Table 3. With regard to EC_{50} values of DPPH·-scavenging activity, the highest DPPH·-scavenging activity of rice bran extracts was found in RB-2 (0.38 ± 0.02 mg/ml) and the lowest activity was in RB-4 (0.74 ± 0.05 mg/ml) ($p \leqslant 0.05$). The effective scavenging activities, ranked in descending order, were as follows: RB-2, RB-1, RB-3, RB-5 and RB-4. According to other research, DPPH free radical-scavenging of Pakistani rice bran extracts (Iqbal et al., 2005), rice hulls (Lee et al., 2003) and unsaponifiable matter from rice bran (Lee et al., 2005) produced similar results. However, DPPH free radical-scavenging of all the rice bran cultivars was less than that of BHT, a synthetic antioxidant, at the same concentration. As rice bran extracts have a high ability to donate hydrogen atoms, the results of DPPH free radical-scavenging might be due to hydrogen donation ability (Shimada et al., 1992).

Reducing power assay has also been used to evaluate the ability of natural antioxidants to donate electrons (Dorman, Peltoketo, Hiltunen, & Tikkanen, 2003). The reducing power of rice bran extracts and BHT is shown in Fig. 2. The reducing power of rice bran extracts increased with increasing concentration and a significant change in reducing power ($p \leqslant 0.05$) was observed between 0.05 to 1.0 mg/ml concentration of rice bran extracts. At 0.10 mg/ml of methanolic rice bran extracts, the absorbance values of RB-1, RB-2, RB-3, RB-4 and RB-5 at 700 nm were, 0.46 ± 0.03, 0.55 ± 0.03, 0.33 ± 0.02, 0.12 ± 0.04 and 0.14 ± 0.03, respectively. However, the reducing powers of all the rice bran cultivars were less than that of BHT (2.25 ± 0.05) at the same concentration. The highest reducing power of rice bran extracts was observed in RB-2 (EC_{50} = 0.10 ± 0.01 mg/ml) (Table 3). The reducing power of rice bran extracts was related to the total content of phenolic

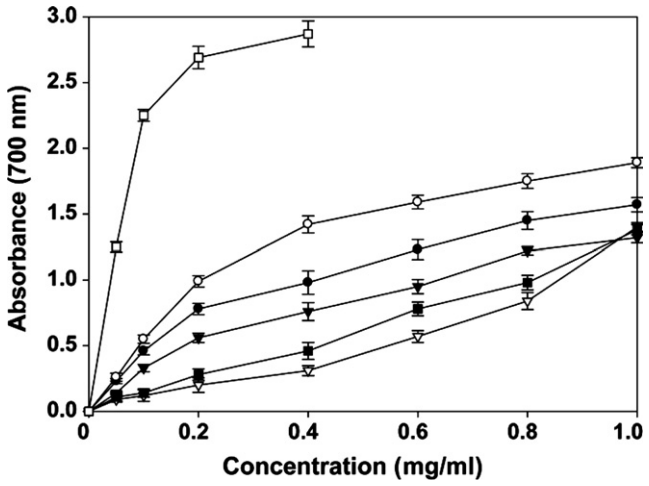

Fig. 2. Reducing power of different amounts of methanolic rice bran extracts from *Oryza Sativa* L. CV. Khao Dawk mali 105 (●; RB-1), *Oryza Sativa* L. CV. Khoa Pathum Thani 60 (○; RB-2), *Oryza Sativa* L. CV. Khao Suphan buri 90 (▼; RB-3), *Oryza Sativa* L. CV. Khao Chinat 1 (∇; RB-4), *Oryza Sativa* L. CV. Khao Gokho 13 (■; RB-5) and BHT (□). Results represent means ± standard deviation ($n = 3$).

Table 3
EC_{50} value (mg/ml) of reducing power, DPPH free radical-scavenging activity and inhibition on lipid peroxidation of methanolic rice bran extract from *Oryza Sativa* L. CV. Khao Dawk mali 105 (RB-1), *Oryza Sativa* L. CV. Khoa Pathum Thani 60 (RB-2), *Oryza Sativa* L. CV. Khao Suphan buri 90 (RB-3), *Oryza Sativa* L. CV. Khao Chinat 1 (RB-4), *Oryza Sativa* L. CV. Khao Gokho 13 (RB-5)

Rice bran extract (mg/ml)	RB-1	RB-2	RB-3	RB-4	RB-5
Reducing power	0.12 ± 0.01a	0.10 ± 0.01a	0.18 + 0.01b	0.53 + 0.02d	0.40 ± 0.02c
DPPH free radical-scavenging	0.52 ± 0.02b	0.38 ± 0.02a	0.58 + 0.03b	0.74 + 0.05d	0.64 ± 0.03b
Ferrous ion-chelating activity	0.14 + 0.02a	0.11 + 0.01a	0.31 + 0.02b	0.55 + 0.03d	0.45 + 0.04c
Inhibition on lipid peroxidation	0.28 ± 0.02b	0.14 + 0.01a	0.35 ± 0.02d	0.57 ± 0.04c	0.35 ± 0.02c

Results represent means ± standard deviation ($n = 3$). In each line, different letters mean significant differences ($p \leqslant 0.05$).

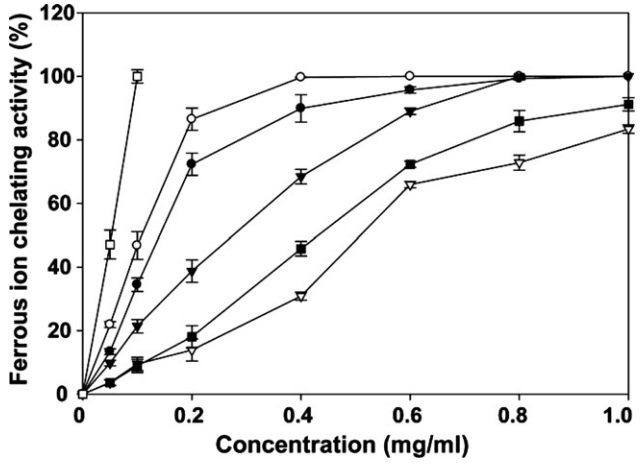

Fig. 3. Ferrous ion-chelating activity (%) of different amount of methanolic rice bran extracts from *Oryza Sativa* L. CV. Khao Dawk mali 105 (●; RB-1), *Oryza Sativa* L. CV. Khoa Pathum Thani 60 (○; RB-2), *Oryza Sativa* L. CV. Khao Suphan buri 90 (▼; RB-3), *Oryza Sativa* L. CV. Khao Chinat 1 (▽; RB-4), *Oryza Sativa* L. CV. Khao Gokho 13 (■; RB-5) and Na_2EDTA (□). Results represents mean ± standard deviation ($n = 3$).

compounds. The highest values of EC_{50} for reducing power in rice bran extracts were observed in RB-4, which had the lowest content of phenolic compounds. These results agreed with other studies (Iqbal et al., 2005; Nam et al., 2006). The results demonstrate that some compounds of rice bran extracts were electron donors to free radicals to terminate or stabilize radical chain reaction.

The results of the ferrous ion-chelation of rice bran extract and Na_2EDTA are shown in Fig. 3. Ferrous ions were shown to stimulate lipid peroxidation by Ferton reaction and are an effective prooxidant in food systems (Yamaguchi, Tatsumi, Koto, & Yoshimitsu, 1988). The ferrous ion-chelating activities of methanolic rice bran extracts were dose-dependent. At 0.10 mg/ml of methanolic rice bran extracts, the ferrous ion-chelating activities of RB-1, RB-2, RB-3, RB-4 and RB-5 were 34.5 ± 2.1, 46.8 ± 4.3, 21.4 ± 2.1, 9.5 ± 2.2 and 8.9 ± 1.9%, respectively. With regard to EC_{50} values of ferrous chelating activity, the activities of RB-2 (0.11 ± 0.01 mg/ml) and RB-1(0.14 ± 0.02 mg/ml) were better than those of the others (Table 3). Iqbal et al. (2005) reported that 80% of methanol extracts obtained from Pakistani rice bran showed a chelating effect. These results indicate that the ferrous ion-chelating activity was greatest for the RB-2 and RB-1, which is similar to the pattern for reducing power and DPPH free radical-scavenging.

The antioxidative effect of methanolic rice bran extracts through linoleic acid peroxidation, by determination of the conjugated diene during accelerated lipid peroxidation at 37 °C for 15 h, is shown in Fig. 4. At 0.10 mg/ml concentration of methanolic extracts, RB-2 exhibited strong inhibition of lipid peroxidation (46.7 ± 3.2%). The other extracts obtained from RB-1, RB-3 and RB-5 exhibited moderate inhibitions, which were, 17.8 ± 2.1, 13.3 ± 1.5 and 17.4 ± 0.9%, respectively, and the lowest inhibition was observed in RB-4 (8.8 ± 0.3%). The antioxidative activities of methanolic rice bran extracts in all cultivars were found to be lower than that of BHT (EC_{50} = 0.05 mg/ml).

The significant differences in antioxidative activities for rice bran extracts obtained from different cultivars indicated that the kind of cultivar and the quantities of phenolics, flavonoids, tocopherols, tocotrienols and gamma-oryzanol compounds they contained had significant effects on their antioxidant properties. It is concluded that long-grained rice bran extracts from Thai cultivars contain natural antioxidants, which could have implications for commercial practice in the food industries.

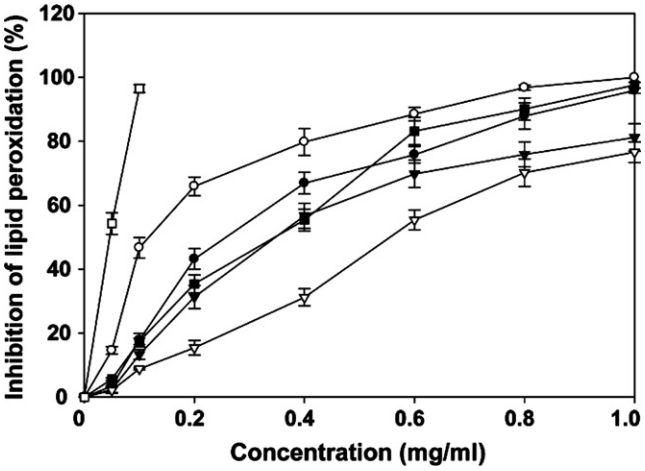

Fig. 4. Inhibition of lipid peroxidation (%) of of different amount of methanolic rice bran extracts from *Oryza Sativa* L. CV. Khao Dawk mali 105 (●; RB-1), *Oryza Sativa* L. CV. Khoa Pathum Thani 60 (○; RB-2), *Oryza Sativa* L. CV. Khao Suphan buri 90 (▼; RB-3), *Oryza Sativa* L. CV. Khao Chinat 1 (▽; RB-4), *Oryza Sativa* L. CV. Khao Gokho 13 (■; RB-5) and BHT (□). Results represent means ± standard deviation ($n = 3$).

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