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Antioxidant activity of methanolic extracts from some grains consumed in Korea

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Abstract

The objectives of this study were to determine antioxidant activity of the methanolic extracts from some grains and to investigate relationships between antioxidant activities and antioxidant contents in the extracts. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities, 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation scavenging activities, inhibitory effect on lipid peroxidation, chelating activity and reducing power have been used to investigate the relative antioxidant activities of the extracts from grains. The concentrations of total polyphenolics and carotenoids in the extracts were measured by spectrophotometric methods and vitamin E analysis was carried out by HPLC. The methanolic extracts prepared from red sorghum and black rice showed significantly higher antioxidant activities and contained higher polyphenolic contents than other grains such as white rice, brown rice, mungbean, foxtail millet, prosomillet, barley, and adlay. Polyphenolic compounds were the major naturally occurring antioxidants in grains. The correlation coefficient between total polyphenolic content and ABTS radical cation scavenging activity in the extracts was >0.99. However, no relationship was found between antioxidant activities and carotenoids and vitamin E derivatives.

Keywords: Grains; Antioxidant activity; Polyphenolics; Antioxidant components

1. Introduction

Free radicals produced by radiation, chemical reactions and several redox reactions of various compounds may contribute to protein oxidation, DNA damage, lipid peroxidation in living tissues and cells (Halliwell, 1996; Morrissey & O'Brien, 1998). This oxidative stress may be related to many disorders such as cancer, atherosclerosis, diabetes and liver cirrhosis (Halliwell & Gutteridge, 1984; Muramatsu et al., 1995; Steinberg, Parthasarathy, Carew, Khoo, & Witztum, 1989). Recent epidemiological studies have suggested that increased consumption of whole grains, fruits and vegetables is associated with reduced risks of chronic diseases (Hu, 2002). This association may be attributed to the natural antioxidants from plant foods such as vitamin C, tocopherol, carotenoids, polyphenolics and flavonoids which prevent free radical damage (Diplock et al., 1998).

Grains and their products are one of the most commonly consumed food items and a staple in Korean diet. Moreover, based on Dietary Guidelines for Americans (2005), the new Food Guide Pyramid recommends the consumption of three or more servings of whole grain per day to promote health and reduce the risk of chronic diseases (US Department of Agriculture, 2005). Grains are important sources of energy, protein, dietary fibre, minerals, vitamins and phytochemicals such as phenolic acid, phytic acids, lignans and phytoestrogens (Slavin, Martini, Jacobs, & Marquart, 1999). Phenolic acids, particularly ferulic acid, *p*-coumaric acid and vanillic acids, are predominant in bran layer of grains and are mainly present as a covalently bound form with insoluble polymers. Therefore, the antioxidant activity of grains could be further enhanced

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by gastrointestinal pH and microbial dependent enzymatic hydrolysis during colonic digestive processes (Baublis, Lu, Clydesdale, & Decker, 2000). Generally grains contain low to moderate levels of tocopherol due, but to the large amount consumed in Korean diet, they provide a significant and consistent source of tocopherols. Tocopherols are regarded as intracellular antioxidants due to their activity of inhibiting the peroxidation of polyunsaturated fatty acids in biological membranes (Qureshi, Mo, Packer, & Peterson, 2000). Also, phytic acid occurring in grains acts as an antioxidant by the formation of chelates with prooxidant transition metals. Although phytic acid is generally regarded as an antinutrient due to its mineral binding activity, it is known to reduce the risk for colon and breast cancer in animals (Graf & Eaton, 1990). Recent studies have reported the antioxidant activities of black rice (Hu, Zawistowski, Ling, & Kitts, 2003), buckwheat (Holasova et al., 2002), oat (Handelman et al., 1999), sorghum (Awika, Rooney, Wu, Prior, & Cisneros-Zevallos, 2003) and cereal products including oat bran, oat meal, wheat meal, and ready-to-eat cold cereal (Yu, Davy, Wilson, & Melby, 2002).

The objectives of this study were to determine antioxidant activity of methanolic extracts from grains, white rice, black rice, red sorghum, brown rice, mungbean, foxtail millet, prosomillet, barley, and adlay, frequently consumed in Korea and to explore relationship between antioxidant activity and antioxidant content in the samples.

2. Materials and methods

2.1. Chemicals

Gallic acid, ascorbic acid, Folin–Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), diammonium salt of 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), potassium ferricyanide, ferric chloride, ferrous chloride, ferrozine (3-(2-pyridyl)-5,6-bis-(4-phenylsulphonic acid)-1,2,4-triazine), linoleic acid, ammonium thiocyanate, butylated hydroxytoluene (BHT), and potassium persulphate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Trichloroacetic acid was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan) and tocopherol and tocotrienol standards were obtained from Merck (Darmstadt, Germany). All other reagents and solvents used were of analytical and HPLC grade.

2.2. Sample preparation

Grain samples of white rice (Chucheongbyeo), black rice (Hukjinjubyeo), red sorghum (P931), brown rice (Chucheongbyeo), mungbean (Bangasa), foxtail millet (Morejo), prosomillet (Gagijang), barley (Ollbori), adlay (Johyeong adlay) were purchased from a local market in Korea during the period of July to October in the year of 2003. All samples were harvested in 2003.

Approximately 10 g of finely ground samples were extracted into 200 ml of methanol in a shaker (Eyela Model MMS-300, Tokyo Rikakikai Co., Ltd., Japan), at room temperature for 24 h. Subsequently, the extracts were centrifuged at 8000 rpm (6720*g*) for 15 min and the supernatants were filtered through a Whatman No. 2 filter paper. The combined filtrate was evaporated at 40 °C. The dried extract was redissolved in methanol to a concentration of 4 mg/ml and stored at −20 °C until analysis.

2.3. Determination of antioxidant compounds

The concentration of total polyphenolics and carotenoids in the methanolic extracts was measured spectrophotometrically while vitamin E analysis was carried out by HPLC. Total phenolic content in the extracts was determined using the Folin–Ciocalteu method (Dewanto, Wu, Adom, & Liu, 2002) with some modifications; results were expressed as mg gallic acid equivalents per 100 g of grain.

Total carotenoid contents in the extracts were determined by a spectrophotometric assay described by Lee and Castle (2001) with some modifications. Approximately, 5 ml of extract were mixed with equal volume of distilled water and 15 ml of hexane/acetone/methanol (50/25/25, v/v) solution. The mixture was then homogenized with a Polytron® and centrifuged at 3000 rpm (940*g*) for 10 min. The absorbance of the top layer of hexane was measured at 450 nm using a spectrophotometer. Total carotenoid contents of the samples were calculated as μg β-carotene per 100 g of sample using an extinction coefficient of $E^{1\%}_{1\ cm} = 2505$ (De Ritter & Purcell, 1981).

Vitamin E content in the extracts was determined according to the procedure described by Lee, Suknark, Kluvitse, Phillips, and Eitenmiller (1999) with some modifications. In brief, an aliquot of each methanolic extract was evaporated under nitrogen gas. The residues were redissolved in *n*-hexane, filtered and analyzed by normal phase HPLC. Analysis of tocopherols and tocotrienols was performed on LiChrosphere® Diol 100 column (250 × 4 mm, i.d., 5 μm) using a mobile phase of hexane/isopropanol (98.7:1.3, v/v) at a flow rate of 1.0 ml/min. Peaks were detected by fluorescence using excitation wavelength of 290 nm and emission wavelength of 330 nm. All analyses except vitamin E were conducted in triplicate.

2.4. Determination of antioxidant activity

The DPPH radical scavenging activity of grains was estimated according to the method explained by Cheung, Cheung, and Ooi (2003) with some modifications. Aliquots of 0.8 ml of 0.2 mM DPPH methanol were mixed with 0.2 ml of the extracts. The mixtures were vigorously shaken and left to stand for 10 min under subdued light. The absorbance at 520 nm was measured against water as a blank.

The scavenging activity of grains on ABTS radical cation was estimated according to the method of Re et al.

(1999) with some modifications. ABTS radical cation was generated by adding 7 mM ABTS to 2.45 mM potassium persulphate solution and the mixture was left to stand for overnight in the dark at room temperature. The ABTS radical cation solution was diluted with distilled water to obtain an absorbance of 1.4–1.5 at 414 nm (molar extinction coefficient $\varepsilon = 3.6 \times 10^4\ mol^{-1}\ 1\ cm^{-1}$, Forni, Mora-Arellano, Packer, & Willson, 1986). Diluted ABTS radical cation solution (1 ml) was added to 50 μl of extracts or ascorbic acid standard solution. The absorbance was measured at 414 nm after 1 h. The ABTS radical cation scavenging activity was expressed as ascorbic acid equivalent antioxidant activity (AEAC) and defined as the mg ascorbic acid equivalents per 100 g of sample (Leong & Shui, 2002).

The reducing power of grain extracts was determined according to the method of Oyaizu (1986) with some modifications. Methanolic grain extracts (250 μl), 200 mM sodium phosphate buffer (250 μl, pH 6.6) and 1% potassium ferricyanide (250 μl) were mixed and incubated in a water bath at 50 °C. After 20 min, 250 μl of 10% trichloroacetic acid (w/v) were added to the mixture and centrifuged at 1000 rpm (240*g*) for 10 min. The supernatant (500 μl) was then mixed with equal volume of distilled water and ferric chloride solution (0.1%, w/v). The intensity of blue green colour was measured at 700 nm using a spectrophotometer.

The chelating activity of the extracts was determined according to the method explained by Dinis, Madeira, and Almeida (1994). The extract (1 ml) was reacted with 100 μl of ferrous chloride (2 mM) and ferrozine (5 mM) for 10 min, and the absorbance of the mixture was measured at 562 nm.

The efficacy of inhibiting lipid peroxidation of the extracts was determined according to the method described by Zin, Abdul-Hamid, and Osman (2002). In brief, screw-cap tube containing a mixture of sample extract (4 ml), 2.51% linoleic acid in 99.5% ethanol (4.1 ml), 0.05 M phosphate buffer (pH 7, 0.8 ml), and distilled water (3.9 ml) was kept at 40 °C in the dark. Subsequently, the degree of oxidation was measured using ferric thiocyanate method (Kikuzak & Nakatani, 1993). Every 24 h, 0.1 ml of this reaction mixture was drawn and mixed with 75% ethanol, 30% ammonium thiocyanate and 0.02 M ferrous chloride in 3.5% hydrochloric acid. After 3 min, the intensity of red colour was measured at 500 nm. Absorbance was measured until the control, where there was no addition of sample extract, reached maximum absorbance. BHT (100 ppm) was used as a positive control.

2.5. Statistical analysis

The results were reported as mean ± standard deviation (SD). The significance of differences among treatment means was determined by analysis of variance (one-way ANOVA) using SAS version 8.1 (SAS Institute, Cary, NC, USA) with a significant level of 0.05. Correlations from regression analysis between the parameters were also determined.

3. Results and discussion

3.1. Yields of methanolic extracts

Efficiency of extracts is an important factor for the comparison of antioxidant activity. However, antioxidants in grains are difficult to be extracted due to different solubility of active compounds (Miller, Rigelhof, Marquart, Prakash, & Kanter, 2000). Previous studies reported that relatively higher antioxidant activities were observed from methanolic extracts in grains compared to the other solvents including *n*-hexane, diethyl ether, ethyl acetate, acetone and water (Oki et al., 2002; Sosulski, Krygier, & Hoogge, 1982; Zieliński & Kozłowska, 2000). Therefore, methanol was selected as an extraction solvent in this study. The yield of methanolic extracts obtained from nine grains is presented in Table 1 and the grains gave a yield of 2.3–6.8%.

3.2. Antioxidant compounds of the methanolic extracts from grains

It has been found that polyphenolic compounds are one of the most effective antioxidative constituents in plant foods including fruit, vegetables, and grains (Velioglu, Mazza, Gao, & Oomah, 1998), hence it is important to quantify polyphenolic contents and to assess their contribution to antioxidant activity. The polyphenolic contents in the methanolic extracts of nine grains were expressed as mg gallic acid equivalents per 100 g of sample (Table 1). The order of polyphenolic contents was as follows: red sorghum (733 mg/100 g), black rice (313 mg/100 g), brown rice (54 mg/100 g), barley (50 mg/100 g), foxtail millet (47 mg/100 g), mungbean (45 mg/100 g), adlay (43 mg/100 g), prosomillet (29 mg/100 g), and white rice (18 mg/100 g). The red sorghum and black rice were remarkably high in polyphenolic content compared to the other grains,

Table 1
Polyphenolics and total carotenoids contents of the methanolic extracts obtained from the grains and extraction yields

Source	Polyphenolics[a]	Total carotenoids[b]	Yield (%)
White rice	18 ± 0.6	1 ± 0.5	2.3
Black rice	313 ± 31.5	77 ± 6.3	5.1
Sorghum	733 ± 48.4	22 ± 1.1	5.3
Brown rice	54 ± 2.2	14 ± 2.0	3.8
Mungbean	45 ± 1.9	102 ± 3.7	6.1
Foxtail millet	47 ± 1.4	80 ± 5.8	6.8
Prosomillet	29 ± 1.2	74 ± 7.4	4.5
Barley	50 ± 2.9	15 ± 0.9	2.4
Adlay	43 ± 2.0	10 ± 3.1	5.1

[a] Mean of triplicate determinations ± SD expressed as mg gallic acid equivalents per 100 g of grain (wet weight basis).
[b] Mean of triplicate determinations ± SD expressed as μg β-carotene per 100 g of grain (wet weight basis).

apparently due to their intensive purple colour. The brown rice contained about 3 times higher polyphenolics as compare to white rice in the methanolic extracts. This suggests that the milling process might have removed a significant amount of polyphenolics (Tian, Nakamura, & Kayahara, 2004). The polyphenolic contents in the methanolic extracts of the grains in this study were relatively lower than the study by Adom and Liu (2002). This is possibly due to the fact that extraction with methanol does not release bound phenolics from the grain cell walls (Baublis et al., 2000). However, recently, the antioxidant activity of water, methanol, ethyl acetate, dichloromethane, and hexane extracts from plant-derived foods has been reported in various in vitro models. Among all of the extracts, methanol extract was reported to possess higher antioxidant activity than that of other extracts in various model systems (Chyau, Tsai, Ko, & Mau, 2002; Jung et al., 2004; Murthy, Jayaprakasha, & Singh, 2002). Differences in the polarity of the extracting solvents could result in a wide variation in the polyphenolic contents of the extract. Because methanol is a relatively polar organic solvent compared to other extracting solvents including ethanol, ethyl acetate, acetone, and hexane, most polyphenolics evaluated in this study are likely polar compounds. Although the methanolic extracts prepared from red sorghum and black rice showed significantly higher antioxidant activities and contained higher polyphenolic contents than other grains, the limitation of our results is that we evaluated only the antioxidant activities and antioxidant compounds of the free and soluble-esterified polyphenolics from the methanolic extracts in vitro (Krygier, Sosulski, & Hogge, 1982). In vitro trials do not directly reflect the results of in vivo studies, however, in vitro experiments could be much valuable, simpler and controllable screening tools for antioxidant studies.

Carotenoids may act as singlet oxygen quencher and can transfer one electron to the radicals, giving rise to a stable carotenoid radical cation regenerating the original molecule (Mortensen & Skkibsted, 1997). Total carotenoid content in grains, expressed as μg β-carotene equivalents per 100 g of sample, was relatively lower in all the tested samples than the polyphenolic and α-tocopherol (α-T) contents (Table 1). The order of total carotenoids was as follow: mungbean (102 μg/100 g), foxtail millet (80 μg/100 g), black rice (77 μg/100 g), prosomillet (74 μg/100 g), red sorghum (22 μg/100 g), barley (15 μg/100 g), brown rice (14 μg/100 g), adlay (10 μg/100 g), white rice (1 μg/100 g).

Vitamin E is another antioxidant present in grains that protects polyunsaturated fatty acids in cell membranes against oxidative damage (Slavin et al., 1999). The order of α-tocopherol content was as follow: black rice (1.24 mg/100 g), brown rice (0.42 mg/100 g), barley (0.23 mg/100 g), red sorghum (0.14 mg/100 g), foxtail millet (0.06 mg/100 g), mungbean (0.05 mg/100 g), white rice (0.07 mg/100 g), adlay (0.02 mg/100 g) and prosomillet (0.00 mg/100 g). The recent Institute of Medicine report on vitamin E (IOM (Institute of Medicine), 2000) has a recommended dietary allowance (RDA) of 15 mg/day of α-T. This RDA is based only on the α-T form of vitamin E. However, γ-tocopherol (γ-T) was higher than α-T in some samples including sorghum, mungbean, foxtail millet, prosomillet, barley, and adlay. β-Tocopherol was not detected in any of the samples. Tocotrienols (T3), especially α-T3 and γ-T3, were the predominant vitamin E forms in white rice, black rice, and brown rice. β-T3 and δ-T3 were measurable in some grain samples including sorghum, barley, and adlay (Table 2).

3.3. Antioxidant activities of grains

The stable DPPH radical, which has a maximum absorption at 515 nm, is widely used to evaluate the free radical scavenging activity of hydrogen donating antioxidants in many plant extracts (Brand-Williams, Cuvelier, & Berset, 1995; Wettasinghe & Shahidi, 2000). The DPPH radical scavenging activity of the methanolic extracts (4 mg/ml) is presented in Fig. 1. High pigmented red sorghum (92%) and black rice (87%) showed relatively higher radical scavenging activity than non-pigmented samples (7–67%). These results are consistent with those of Miller

Table 2
Tocopherol and tocotrienol contents in the methanolic extracts obtained from the grains

Sample[a]	α-T[b]	β-T	γ-T	δ-T	α-T3	β-T3	γ-T3	δ-T3	Total
White rice	0.07	–[c]	0.01	–	0.08	–	0.58	–	0.74
Black rice	1.24	–	0.25	3.86	1.38	–	2.64	–	9.37
Sorghum	0.14	–	1.33	0.02	0.21	–	0.08	0.01	1.79
Brown rice	0.42	–	0.12	0.00	0.56	–	1.54	–	2.64
Mungbean	0.05	–	9.78	0.70	0.04	–	0.32	0.03	10.92
Foxtail millet	0.06	–	2.62	0.04	0.05	–	0.57	–	3.34
Prosomillet	0.00	–	1.33	0.83	0.00	–	0.06	–	2.22
Barley	0.23	–	0.32	0.02	1.07	0.24	–	0.04	1.92
Adlay	0.02	–	1.67	0.16	0.09	–	1.31	0.17	3.42

[a] Mean of duplicate determinations expressed as mg per 100 g of grain (wet weight basis).
[b] Corresponding tocopherols and tocotrienols.
[c] Not detected.

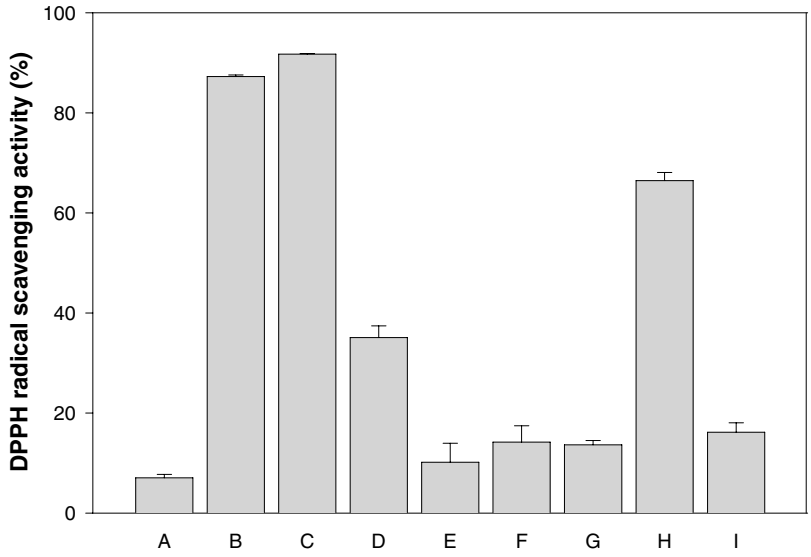

Fig. 1. Scavenging activity of methanolic extracts (4 mg/ml) from grains on DPPH radical. A, white rice; B, black rice; C, sorghum; D, brown rice; E, mungbean; F, foxtail millet; G, prosomillet; H, barley; I, adlay.

et al. (2000) who reported relatively higher levels of antioxidant activity in highly pigmented berries, raisins, dates, and prunes. No correlation between contents of carotenoid and vitamin E and DPPH radical scavenging activity was observed. Anthocyanins are the major extractable polyphenols from red sorghum and black rice. The colour interference of anthocyanin with DPPH radical resulted in a relatively lower radical scavenging activity due to the fact that anthocyanins absorbed maximally at 475–485 nm (Arnao, 2000). Therefore, lack of a strong correlation ($R^2 = 0.6200$, $p > 0.05$) between polyphenolic content and DPPH radical scavenging activity due to colour interference may lead to underestimation of antioxidant activity of samples with high anthocyanin content. Velioglu et al. (1998) reported a similar colour interference of the DPPH radical with the anthocyanin-rich plant materials, such as blueberries, cherries, red onion and purple potatoes.

The ABTS method is widely employed for measuring the relative radical scavenging activity of hydrogen donating and chain breaking antioxidants in many plant extracts (Brand-Williams et al., 1995; Netzel et al., 2003). The ABTS radical cation scavenging activity of methanolic extracts (4 mg/ml), expressed as mg ascorbic acid equivalents per 100 g of sample (mg AEAC), is presented in Fig. 2. High pigmented red sorghum (1219 mg AEAC) and black rice (572 mg AEAC) showed much higher radi-

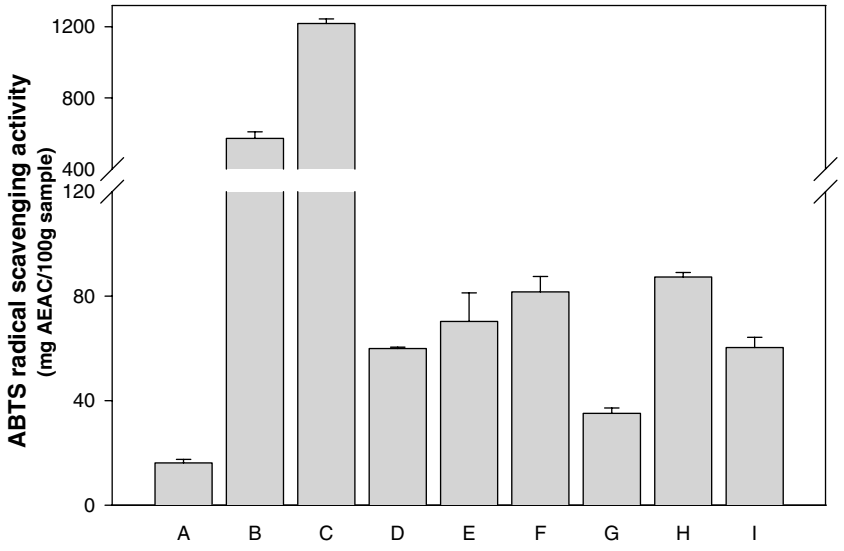

Fig. 2. Scavenging effect of methanolic extracts (4 mg/ml) from grains on ABTS radical cation. A, white rice; B, black rice; C, sorghum; D, brown rice; E, mungbean; F, foxtail millet; G, prosomillet; H, barley; I, adlay.

cal scavenging activity than other samples (16–87 mg AEAC). There was a positive and significant correlation ($R^2 = 0.9973$, $p < 0.01$) between polyphenolic content and ABTS radical cation scavenging activity. The results confirm that the polyphenolic compounds may be responsible for the major portion of the antioxidant activity of grains. Many previous studies have reported significant correlation between polyphenolics and antioxidant activities in fruits, barley, and mushrooms (Choi, Ku, Chang, & Lee, 2005; Goupy, Hugues, Boivin, & Amiot, 1999; Leong & Shui, 2002). On the other hand, no correlation between antioxidant compounds (carotenoids and α-vitamin E) and ABTS radical cation scavenging activity was observed (Table 3).

The reducing power of the methanolic extracts (4 mg/ml) is presented in Fig. 3. In this method, ferric–ferricyanide complex is reduced to the ferrous form depending on the presence of antioxidants (Amarowicz, Pegg, Rahimi-Moghaddam, Barl, & Weil, 2004). Highly pigmented red sorghum ($A_{700} = 3.18$) and black rice ($A_{700} = 2.46$) had relatively higher reducing power than other samples ($A_{700} = 0.22$–0.54), showing a significant correlation ($R^2 = 9075$, $p < 0.05$) with polyphenolic content. This indicates that polyphenolics in methanolic extracts of grains may play a role as electron and hydrogen donors.

Although iron is essential for oxygen transport, respiration, and activity of enzyme, it is a reactive metal that catalyzes oxidative damage in living tissues and cells (Miller, 1996). The chelating effects (%) of the methanolic extracts (4 mg/ml) on ferrous ion are presented in Fig. 4. Mungbean (92%), red sorghum (80%), and foxtail millet (79%) showed relatively higher chelating effects than other samples (26–67%). No correlation was observed with any other antioxidant compounds such as polyphenolics, carotenoids, and α-tocopherol (Table 3). This indicates that factors other than antioxidant compounds determined in this study may play a major role in the chelating activity of grains such as mungbean, red sorghum, and foxtail millet (Graf & Eaton, 1990).

The autoxidation of linoleic acid without extracts and BHT was accompanied by a rapid increase of peroxide value (Fig. 5). Significant difference ($p < 0.01$) in peroxide value was found between the negative control and the methanolic extracts (4 mg/ml) or BHT (100 ppm). However, there was no significant difference ($p > 0.05$) among the samples due to their excellent antioxidant activities against lipid peroxidation except white rice and adlay. Similar to the chelating effects, no correlation was observed with any other antioxidant compounds such as polyphenolics, carotenoids, and α-tocopherol (Table 3).

In conclusion, highly pigmented red sorghum and black rice showed significantly higher antioxidant activities and contained higher polyphenolic contents than other samples in the methanolic extracts. Although carotenoids and vitamin E contents were relatively lower than polyphenolics

Table 3
Correlation analysis between antioxidant contents and antioxidant activities

	Antioxidant contents		
	Polyphenolics	Carotenoids	α-Tocopherol
DPPH radical scavenging activity	0.6200[a]	0.0094	0.3973
ABTS radical cation scavenging activity	0.9973**[b]	0.0011	0.0940
Reducing power	0.9075*	0.0012	0.2739
Chelating effect	0.1846	0.4507	0.0166
Inhibition of lipid peroxidation	0.0789	0.3275	0.1782

[a] Correlation coefficient R^2.
[b] Significantly different: $^*p < 0.05$; $^{**}p < 0.01$.

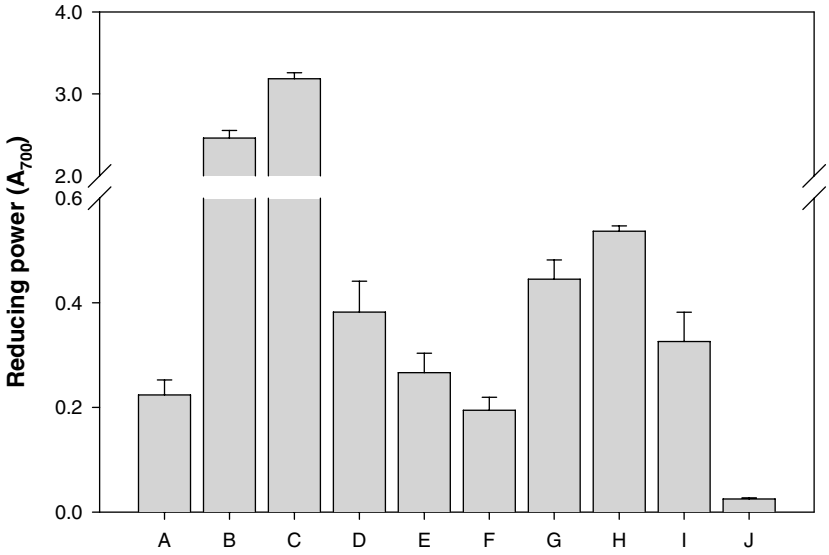

Fig. 3. Reducing power of methanolic extracts (4 mg/ml) from grains. A, white rice; B, black rice; C, sorghum; D, brown rice; E, mungbean; F, foxtail millet; G, prosomillet; H, barley; I, adlay; J, negative control.

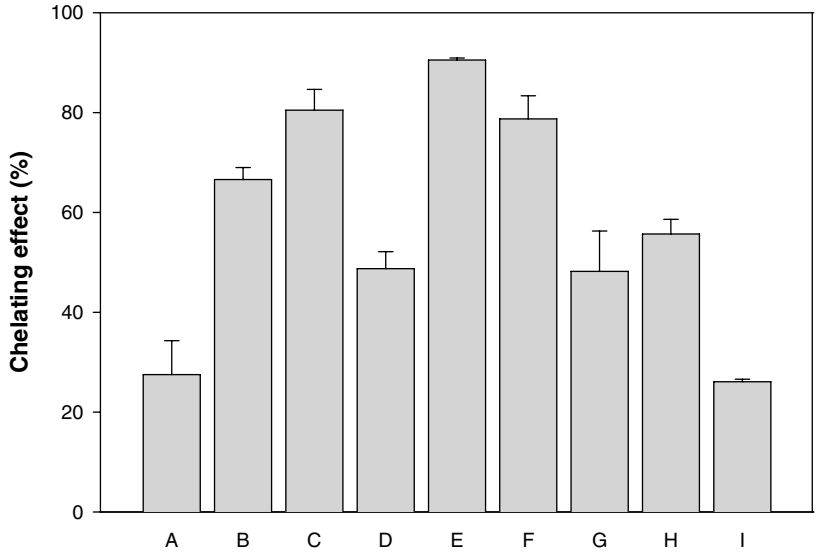

Fig. 4. Chelating effect of methanolic extracts (4 mg/ml) from grains on ferrous ion. A, white rice; B, black rice; C, sorghum; D, brown rice; E, mungbean; F, foxtail millet; G, prosomillet; H, barley; I, adlay.

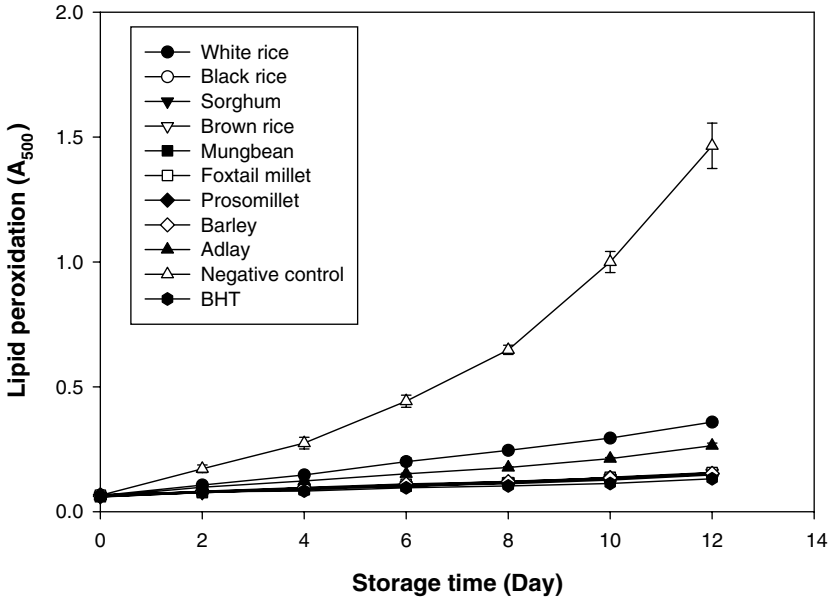

Fig. 5. Inhibition of lipid peroxidation of methanolic extracts (4 mg/ml) from grains.

among samples, grains may contribute to the significant supply of antioxidant to prevent oxidative stress due to the fact that grains are used as a staple food and consumed large amount in our diets. Our results could have a direct impact on grain consumption by increasing consumer awareness of the health benefits of grains.

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