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Antioxidative activities of bran extracts from twenty one pigmented rice cultivars

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Abstract

Ethanol–water (70:30, v/v) extracts from the bran of rice seeds from twenty one pigmented and one nonpigmented rice cultivars were evaluated for antioxidative activities using the following tests: inhibition of peroxidation of linoleic acid; inhibition of peroxidation of rabbit lipid erythrocyte membranes; reduction of potassium ferricyanide, and scavenging of superoxide anions and hydroxyl radicals. With some exceptions, extracts from the pigmented rice seeds had higher antioxidative activity than did the nonpigmented variety. The following pigmented cultivars had the highest antioxidative activities in all tests: Jumlalocal-1, Parnkhari 203, DZ78, LK1-3-6-12-1-1, and Elwee. A significant correlation was also noted between reducing power, inhibition of erythrocyte ghost membrane peroxidation, and superoxide anion and hydroxyl radical scavenging. The results suggest that: (a) ferricyanide reducing power might be a useful and simple index for large-scale evaluation of antioxidative potencies of natural products present in rice; (b) pigmented rice varieties with high antioxidative activities provide a source of antioxidants and a genetic resource to develop new health-promoting rice cultivars.

Keywords: Pigmented rice; Antioxidative activity; Radical scavenging activity; Anthocyanin pigments; Health-promoting rice

1. Introduction

Reactive free radicals have been postulated to contribute to the causes of chronic inflammatory proliferative diseases (CIPD), especially arteriosclerosis and cancer, through oxidative damage of essential enzymes, cells, and tissues (Ames, 1983; Ishihara & Hirano, 2002; Klaunig & Kamendulis, 2004). There is therefore widespread interest in defining the possible role of the diet in preventing and reversing reactive oxygen species (ROS)-induced chronic diseases (Sander, Chang, Hamm, Elsner, & Thiele, 2004; Shahidi & Naczk, 2004; Takahashi, Ogra, & Suzuki, 2004).

Rice (*Oryza sativa* L.) is a basic food for a large part of the world's population (Friedman, 1996; Juliano, 1985; Khush & Toeenniesson, 1991). Rice bran was found to be a palatable ingredient in dog food (Spears, Grieshop,

Abbreviations: BHT, butylated hydroxytoluene; CIPD, chronic inflammatory proliferative diseases; DETAPAC, diethylenetriaminepentaaacetic acid; DMPO, 5,5-dimehtyl-1-pyrroline-*N*-oxide; ESR, electron spin resonance; HPX, hypoxanthine; MTT, 3-[4,5-dimethylthiazolyl-2-yl]2,5-diphenyltetrazolium bromide; NAD(P)H, reduced nicotine dinucelotide; XOD, xanthine oxidase.

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& Fahey, 2004). In addition to good quality protein, high fiber content, and vitamin content, pigmented rice varieties have the potential to promote human health because they contain antioxidative compounds that have the ability to inhibit the formation or to reduce the concentrations of reactive cell-damaging free radicals (Acquaviva et al., 2003; Adom & Liu, 2002; Choi, Nam, & Choi, 1996; Hu, Zawistowski, Ling, & Kitts, 2003; Hyun & Chung, 2004; Ichikawa et al., 2001; Lee et al., 2003; Oki et al., 2002; Parrado et al., 2003; Toyokuni et al., 2002). These compounds include anthocyanins (glycosides) – cyanidin-3-*O*-β-D-glucoside and peonidin-3-*O*-β-glucoside (Hu et al., 2003; Ichikawa et al., 2001; Ryu, Park, & Ho, 1998); anthocyanidins (aglycones) – cyanidin and malvidin (Hyun & Chung, 2004); polymeric procyanidins (Oki et al., 2002); the phenolic compounds anisole, 4-hydroxycinnamic acid (*p*-coumaric), 4, 7-dihydroxyvanillic acid, protocatechuic acid methyl ester, syringaldehyde, and vanillin (Asamarai, Addis, Epley, & Krick, 1996; Goffman & Bergman, 2004; Lee et al., 2003; Miyazawa, Oshima, Koshio, Itsuzaki, & Anzai, 2003); the phenolic compounds ferulic and sinapinic acids and the sucrose esters 6′-*O*-(E)-feruoylsucrose and 6′-*O*-(E)-sinapoylsucrose (Tian, Nakamura, & Kayahara, 2004); the ferulic acid sterol ester γ-oryzanol (Parrado et al., 2003); and the alkaloid 4-carbomethoxy-6-hydroxy-2-quinolone (Chung & Woo, 2001).

These observations suggest that pigmented non-polished rice varieties may have beneficial effects in the human diet. Such rice cultivars may also provide genes for plant breeders interested in developing, through plant breeding and plant molecular biology techniques, new rice varieties with high antioxidant activities to be used as health-promoting foods. To contribute to these goals, we determined the relative antioxidative activities in several tests of aqueous-ethanol extracts of rice brans from 21 pigmented and one nonpigmented rice cultivar used as an internal control. The main objective was to discover new varieties with high antioxidative potential.

2. Materials and methods

2.1. Samples and chemicals

Twenty-one pigmented rice cultivars and one nonpigmented cultivar (Chuchung) – used as an internal control – were grown and harvested at the experimental rice field of the College of Agriculture and Life Science, Seoul National University (Suwon, Korea). The rice seeds were cleaned and stored at 4 °C prior to use. New Zealand white rabbits were obtained from Samyook Experimental Animals Co. (Osan, Korea). Xanthine oxidase (EC1.1.3.22) and other biochemicals were purchased from Sigma (Louis, MO).

2.2. Extractions of rice bran

The rice seeds were dehulled, degermed, polished in a laboratory mill, and then passed through a 60-mesh sieve, resulting in a uniform fraction of rice bran. The pigments in the hull were extracted by shaking overnight at room temperature with 10 times the sample weight of 70:30 ethanol–water (v/v). The solvent was then removed from the extract by rotary evaporation at room temperature.

2.3. Reducing power of rice brans by ferricyanide test

The reducing power of each extract was determined by the method of Oyaizu (1986) with some modifications as follows: An aliquot (20 μl) of the extracts was diluted to a desired final concentration (0.16–3.3 mg/ml of rice bran) with 0.7 ml of 50 mM phosphate buffer (pH 6.6). The dilute sample was then mixed with 0.5 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$] and the mixture was incubated at 50 °C for 20 min. Trichloroacetic acid (10%; 0.5 ml) was added to the mixture, which was then centrifuged at 1000*g* for 10 min. Aliquots (0.5 ml) from the upper layer of the solution were mixed with the same 0.5 ml volume of deionized water and 0.1% $FeCl_3$ solution (0.1 ml). The absorbance (λ_{max} = 700 nm) was then measured using a UV/VIS spectrophotometer (V-550, JASCO, Tokyo, Japan).

2.4. Inhibition of linoleic acid peroxidation

The in vitro antioxidant activity of the rice extracts was determined by the thiocyanate method (Mitsuda, Yasumoto, & Iwami, 1966). Briefly, an aliquot of the sample in distilled water was mixed with 5 volumes of 0.02 M linoleic acid emulsion and 4 volumes of 0.2 M phosphate buffer (pH 7.0) in a test tube. The mixture was then placed in the dark at 4 °C for 8 days to accelerate lipid oxidation. After addition of the ferric chloride and thiocyanate solutions, the peroxidation value was measured by the absorbance of the resulting chromophore (λ_{max} = 500 nm).

2.5. Inhibition of peroxidation of rabbit erythrocyte membranes

Preparation of erythrocyte membrane ghosts and subsequent determination of the antioxidant activity of each extract, based on the chemically induced lipid peroxidation were done as described previously (Tsuda, Shiga, Ohshima, Kawakishi, & Osawa, 1996), with some modification as follows: rabbit blood (100 ml), collected by cardiac puncture, was subjected to an isotonic buffer (10 mM phosphate/152 mM KCl, pH 7.4), followed by

centrifugation at 1500*g* for 20 min to a pellet of erythrocyte membrane ghosts. The reaction mixture, consisting of 0.9 ml of rabbit erythrocyte membrane ghost containing 1.8 mg protein, 50 μl of 24 mM *tert*-butylhydroperoxide, and 3 mg of the test extracts in the same phosphate buffer solution (50 μl) was incubated for 30 min at 37 °C with constant shaking. Trichloroacetic acid (0.67%; 0.25 ml) was then added to stop the reaction. The chromophore (λ_{max} = 535 nm) resulting after addition of the thiobarbituric acid solution was then measured.

2.6. Superoxide anion-scavenging test

Scavenging activity of the rice bran extracts of superoxide anions (O_2^-) generated from the xanthine/xanthine oxidase (HPX/XOD) reaction was measured by an electron spin resonance (ESR) technique using 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) as a spin trap (Mitsuta, Mizuta, Kohno, & Hiramatsu, 1990). The aqueous extracts, in 50 μl of 0.1 M phosphate buffer solution (pH 7.4), were mixed with 50 μl of 2 mM hypoxanthine, 35 ml of 5.5 mM diethylenetriaminepentaacetic acid (DETAPAC), 15 μl of 9.2 mM DMPO and 0.4 U of xanthine oxidase. ESR spectra were then recorded with an ESR spectrometer (TE-200, JEOL, Tokyo, Japan) using the following instrumental conditions: modulation amplitude, 0.1 mT; recording range, 4 mT; recording time, 1 min; time constant, 0.1 s; microwave power, 1.8 mW; and microwave frequency, 9.40432. Scavenging ability was defined as the % reduction of the peak heights (representing the DMPO-O_2 spin trap adduct) as compared to the peak heights measured in the absence of the rice extracts.

2.7. Inhibition of xanthine oxidase activity

Inhibition of xanthine oxidase activity by the rice bran extracts was determined as described previously (Noro et al., 1987).

2.8. Hydroxyl radical scavenging activity

Hydroxyl radical (·OH) scavenging activity was determined by quantification of DMPO-OH spin trap adducts resulting from the reaction of DMPO with the radicals generated by the Fenton reaction (Rosen & Rauckman, 1981). ESR spectra were recorded after each extract, in 50 μl of 0.1 M phosphate buffer solution (pH 7.4), was mixed with 50 μl of 0.3 mM DMPO, 50 μl of 10 mM $FeSO_4$, and 50 μl of 10 mM H_2O_2. Conditions used in the ESR experiments were the same as those described above. The percentage of scavenging by bran extracts equals the ratio of the peak heights of spectra observed with DMPO-OH spin trap adducts to that of the heights measured without extracts.

2.9. Ferrous ion binding

Ferrous ion (Fe^{2+}) binding by rice bran extracts was determined as described previously (Carter, 1971).

2.10. Statistical analysis

Tests were run in triplicate and the values expressed as means ± SD. Differences in treatment means and correlations of values from the different antioxidative methods were analyzed using SPSS PC+ software (version 10.0), significant at $P < 0.05$.

3. Results and discussion

3.1. Inhibition of lipid peroxidation

Table 1 shows the results of antioxidative activities of pigmented rice bran extracts obtained with the linoleic acid peroxidation system. The results for the different cultivars can be divided into those with high, low and insignificant activities. The following cultivars exhibited high activity: RSG No. 336, LK1B-2-1-1, LK1B-4-12-1-1, LK1A-2-12-1-1 and LK2-7-12-1-1. Extracts from RSG No. 336, LK1B-2-1-1, and LK1B-4-12-1-1 suppressed lipid peroxidation more effectively than did butylated hydroxytoluene (BHT), an antioxidant widely used as a food additive. In contrast, the extracts from SC-5, HP 833-1-3-1-1-1, HP 833-1-1-1-B-1-1-1, LK1D-2-12-1 and LK1-3-6-12-1-1 showed weak activity. The activity of the LK1-3-6-12-1-1 cultivar was the weakest, similar to that of the white Chuchung rice. The extracts from eleven cultivars (DK-1, SC-45, B-89-11-2, Muthmanikan, Jamlalocal-1 Parnkhari 203, DV85, DZ78, Elwee, Kele and wx124-163-45-7-1-1-1) exhibited pro-oxidant activity, suggesting the presence of free radicals promoting chain reactions during the linoleic acid peroxidation.

Although the linoleic acid peroxidation method is convenient to use, the in vitro assay may not reflect in vivo lipid peroxidation, where various cellular factors could affect the results. We therefore also carried out an ex vivo assay using rabbit erythrocyte membrane ghosts. Table 2 shows marked discrepancy in the activity profiles between the two test systems. Thus, cultivars such as DZ78, Elwee, Parnkhari 203, Jumlalocal-1, DV85, Muthmanikan, B-89-11-2, SC-45, and DK1, with pro-oxidative activity in the linoleic acid assay, behaved as antioxidants in the membrane assay. On the other hand, cultivars RSG No. 336, LK1B-2-1-1, LK1B-4-12-1-1 and LK1A-2-12-1-1, exhibiting high activities in

Table 1
Antioxidant activity of pigmented rice bran extracts measured by inhibition of peroxidation of linoleic acid and of erythrocyte ghost membranes[a]

Rice cultivar	Linoleic acid peroxidation		Rabbit erythrocyte ghost membrane peroxidation	
	$A_{535\ nm}$	Inhibition (%)	$A_{535\ nm}$	Inhibition (%)
Negative control[b]	0.200 ± 0.087	0	2.33 ± 0.076	0
Positive control[c]	0.098 ± 0.055^{h}	51.00	Not determined	Not determined
Chuchung white rice	0.183 ± 0.008^{cdef}	8.42	2.01 ± 0.077^{b}	13.43
B-89-11-2	0.216 ± 0.039^{bcde}	−8.27	1.32 ± 0.095^{hi}	43.33
DK 1	0.203 ± 0.018^{bcde}	−1.50	1.78 ± 0.076^{d}	23.6
DV85	0.240 ± 0.036^{abcd}	−20.34	0.902 ± 0.042^{l}	61.21
DZ78	0.223 ± 0.002^{abcde}	−11.92	0.594 ± 0.096^{o}	74.47
Elwee	0.286 ± 0.080^{a}	−43.4	0.667 ± 0.100^{n}	71.32
HP 833-1-3-1-1-1	0.173 ± 0.031^{defg}	13.2	2.21 ± 0.075^{a}	5.09
HP 883-1-1-1-B-1-1-1	0.168 ± 0.029^{efg}	15.8	1.84 ± 0.036^{d}	21.10
Jumlalocal-1	0.256 ± 0.057^{ab}	−28.4	0.893 ± 0.111^{l}	61.60
Kele	0.203 ± 0.014^{bcde}	−1.90	1.33 ± 0.068^{h}	42.69
LK1A-2-12-1-1	0.130 ± 0.027^{fgh}	34.81	1.27 ± 0.130^{ij}	45.4
LK1B-2-1-1	0.090 ± 0.023^{h}	54.91	2.17 ± 0.164^{a}	16.34
LK1B-4-12-1-1	0.092 ± 0.025^{h}	54.0	1.50 ± 0.100^{f}	35.54
LK1D-2-12-1	0.174 ± 0.006^{cdefg}	12.9	1.421 ± 0.124^{g}	38.91
LK2-7-12-1-1	0.114 ± 0.029^{gh}	42.94	1.61 ± 0.092^{e}	31.00
LK1-3-6-12-1-1	0.187 ± 0.046^{bcdef}	6.11	1.24 ± 0.078^{j}	46.72
Muthmanikan	0.240 ± 0.039^{abcd}	−20.14	1.14 ± 0.068^{k}	51.2
Panrkhari 203	0.241 ± 0.044^{abcd}	−20.7	0.739 ± 0.075^{m}	68.22
RGS No. 336	0.086 ± 0.024^{h}	57.1	2.06 ± 0.158^{b}	11.3
SC-45	0.244 ± 0.044^{abc}	−22.2	1.62 ± 0.117^{e}	30.40
SC-5	0.171 ± 0.012^{defg}	14.23	1.93 ± 0.009^{c}	16.94
wx124-163-45-7-1-1-1	0.200 ± 0.027^{bcde}	−0.35	1.330 ± 0.122^{hi}	42.92

[a] Values expressed as means ± SD ($n = 3$). Values within each column with the same superscript are not significantly different at $P < 0.05$.
[b] Control treatment without bran extract.
[c] Treatment with the synthetic antioxidant butylated hydroxytoluene, BHT.

Table 2
Ferricyanide reducing power of pigmented rice bran extracts at the indicated concentrations[a]

Rice cultivar	Reducing power ($A_{700\ nm}$)				
	0.083 mg/ml	0.16 mg/ml	0.83 mg/ml	1.6 mg/ ml	3.3 mg/ml
Chuchung white rice	0.233 ± 0.031^{m}	0.276 ± 0.027^{n}	0.669 ± 0.058^{k}	1.12 ± 0.077^{m}	1.88 ± 0.073^{l}
B-89-11-2	0.510 ± 0.063^{cdef}	0.816 ± 0.039^{cd}	2.45 ± 0.042^{cde}	2.63 ± 0.028^{gh}	2.73 ± 0.055^{ij}
DK 1	0.282 ± 0.054^{bm}	0.426 ± 0.051^{jkl}	1.40 ± 0.080^{i}	2.23 ± 0.126^{j}	2.62 ± 0.061^{jk}
DV85	0.516 ± 0.107^{cdef}	0.839 ± 0.113^{cd}	2.51 ± 0.025^{abcd}	2.95 ± 0.044^{abcd}	3.01 ± 0.066^{bcdefgh}
DZ78	0.691 ± 0.094^{b}	1.15 ± 0.120^{b}	2.76 ± 0.036^{ab}	3.01 ± 0.089^{ab}	3.26 ± 0.091^{a}
Elwee	0.724 ± 0.079^{b}	1.22 ± 0.104^{b}	2.74 ± 0.046^{abc}	3.09 ± 0.042^{a}	3.20 ± 0.097^{abc}
HP 883-1-1-1-B-1-1-1	0.259 ± 0.072^{lm}	0.364 ± 0.096^{lmn}	1.07 ± 0.064^{j}	1.79 ± 0.085^{k}	2.78 ± 0.097^{ghij}
HP833-1-3-1	0.294 ± 0.028^{klm}	0.398 ± 0.045^{klm}	1.11 ± 0.025^{j}	1.87 ± 0.010^{k}	2.92 ± 0.090^{defghi}
Jumlalocal-1	0.573 ± 0.057^{cj}	0.930 ± 0.022^{c}	2.73 ± 0.089^{abc}	3.00 ± 0.075^{ab}	3.02 ± 0.101^{bcdefg}
Kele	0.368 ± 0.074^{ghijkl}	0.557 ± 0.086^{ghi}	1.73 ± 0.014^{h}	2.71 ± 0.103^{fg}	2.89 ± 0.097^{efghi}
LK1-3-6-12-1-1	1.07± 0.042^{a}	1.69 ± 0.044^{a}	2.8 ± 0.015^{a}	2.90 ± 0.082^{bcde}	3.23 ± 0.128^{ab}
LK1A-2-12-1-1	0.542 ± 0.006^{cde}	0.845 ± 0.031^{cd}	2.57 ± 0.073^{abcd}	2.41 ± 0.014^{i}	2.72 ± 0.085^{ij}
LK1B-2-1-1	0.340 ± 0.034^{hijklm}	0.521 ± 0.014^{hij}	1.62 ± 0.074^{hi}	2.48 ± 0.061^{hi}	2.90 ± 0.127^{efghi}
LK1B-4-12-1-1	0.425 ± 0.042^{efghij}	0.606 ± 0.043^{fghi}	1.80 ± 0.100^{gh}	2.76 ± 0.048^{efg}	3.19 ± 0.111^{abc}
LK1D-2-12-1	0.479 ± 0.019^{cdefg}	0.747 ± 0.037^{de}	2.22 ± 0.011^{ef}	2.91 ± 0.100^{bcde}	2.98 ± 0.070^{cdefgh}
LK 2-7-12-1-1	0.450 ± 0.029^{defgh}	0.673 ± 0.011^{efg}	2.09 ± 0.027^{f}	2.50 ± 0.077^{hi}	2.88 ± 0.052^{efghi}
Muthumanikan	0.520 ± 0.054^{cdef}	0.841 ± 0.060^{cd}	2.67 ± 0.034^{abcd}	2.80 ± 0.106^{def}	2.78 ± 0.026^{ghij}
Parnkhari 203	0.541 ± 0.104^{cde}	0.901 ± 0.103^{c}	2.62 ± 0.092^{abcd}	2.93 ± 0.095^{abcd}	3.07 ± 0.098^{abcdef}
RGS No. 336	0.318 ± 0.091^{jklm}	0.517 ± 0.030^{hij}	1.69 ± 0.028^{h}	2.52 ± 0.033^{hi}	2.99 ± 0.080^{cdefgh}
SC-45	0.561 ± 0.059^{cd}	0.927 ± 0.080^{c}	2.43 ± 0.025^{de}	2.45 ± 0.019^{i}	2.77 ± 0.057^{hij}
SC-5	0.235 ± 0.055^{m}	0.316 ± 0.055^{lmn}	0.921 ± 0.085^{jk}	1.54 ± 0.093^{l}	2.47 ± 0.020^{k}
wx124-163-45-7-1-1-1	0.408 ± 0.033^{fghij}	0.622 ± 0.059^{fgh}	1.80 ± 0.069^{gh}	2.82 ± 0.059^{cdef}	3.12 ± 0.094^{abcde}

[a] Each value expressed as mean ± SD ($n = 3$). Values within each column with the same superscript letters are not significantly different at $P < 0.05$.

the linoleic acid assay, showed weak or no activity in the ex vivo assay.

Rice extracts showing strong antioxidative activity in the erythrocyte membrane assay behaved mostly as pro-oxidants in the linoleic acid assay. Such pro-oxidant activity by naturally occurring antioxidants is a well-known phenomenon (Zhang & Omaye, 2001). These results suggest that the determination of antioxidative activity using rabbit erythrocyte membrane ghosts, which mimics complex oxidation-reduction events occurring in living cells, is probably more relevant to human health than is determination by the linoleic acid peroxidation assay.

3.2. Reducing activities in the fericyanide test

Table 2 shows that, with the exception of SC-5 and HP833-1-1-1-B-1-1-1 cultivars, the electron-donating (reducing) powers of the pigmented rices were generally stronger than those of Chuchung white rice. The data also show that reducing power increased in a dose-dependent manner, reaching a maximum at ~1.6 mg/ml. To interpret these results, we classified the rice cultivars into five groups, based on their $A_{700\ nm}$ values, with high, moderate, low, insignificant, and negative activity, respectively. The high-activity group, with $A_{700\ nm} > 2.8$, includes Parnkhari 203, DV85, DZ78, Elwee, LK1-3-6-12-1-1, Jumlalocal-1 and LK1D-2-12-1 cultivars. Moderate activity ($A_{700\ nm}$ between 2.8 and 2.5) was observed with B-89-11-2, Muthmanikan, Kele, wx124-163-45-7-1-1-1, and LK1B-4-12-1-1 cultivars. Low reducing activity ($A_{700\ nm}$ between 2.5 and 1.7) was observed with DK 1, SC-45, HP 883-1-1-1-B-1-1-1, LK1A-2-12-1-1, LK 2-7-12-1-1, LK1B-2-1-1, RSG No. 336 and HP 883-1-3-1-1-1 cultivars. Cultivar SC-5 had no reducing power; the levels are nearly the same as that observed with the nonpigmented Chuchung rice.

The observed significant correlations between reducing powers and the antioxidative properties from the cell membrane assays, over a wide range of concentrations tested ($r = 0.626$, 0.755 and 0.738 at concentrations of 0.083, 0.83, and 1.6 mg/ml, respectively, $P < 0.05$) suggest that the pigmented rice extracts possess components acting as electron donors which can terminate lipid peroxidation chain reactions, possibly through conversion of lipid peroxy radicals to more stable products (Tsuchihashi, Kigoshi, Iwatsuki, & Niki, 1995).

3.3. Scavenging of superoxide anions

Superoxide anions can be generated by a one-electron reduction of a triplet oxygen species or by catalytic action of NAD(P)H oxidase or xanthine oxidase in living cells (Deliconstantinos, Villiotou, & Stavrides, 1996; Heyneman, 1983). Although not harmful by themselves, superoxide anions can be converted to hydrogen peroxide via enzymatic or nonenzymatic pathways, leading to generation of cell-damaging hydroxyl radicals (Wyllie & Liehr, 1997). Table 3 shows that scavenging abilities increased with concentration of the rice extracts. Activity was strongest with the LK1-3-6-12-1-1 cultivar at both

Table 3
Scavenging of superoxide anions (O_2^-) and inhibition of xanthine oxidase (XOD) by pigmented rice bran extracts at the indicated concentrations[a]

Rice cultivar	(O_2^-) scavenging (%)		(XOD) inhibition (%)	
	0.5 mg/ml	5 mg/ml	0.5 mg/ml	5 mg/ml
Chuchung white rice	16.7 ± 7.17^{j}	43.8 ± 0.85^{j}	-14.4 ± 4.70	-1.74 ± 15.8
B-89-11-2	86.1 ± 3.42^{cd}	94.4 ± 0.97^{b}	-34.4 ± 13.27	61.01 ± 18.1
DK 1	66.7 ± 1.33^{h}	84.7 ± 1.01^{ef}	-34.2 ± 5.24	23.2 ± 8.88
DV85	77.0 ± 0.74^{fg}	94.4 ± 1.23^{b}	-25.8 ± 7.50	67.3 ± 15.7
DZ78	87.5 ± 1.11^{bc}	94.4 ± 0.48^{b}	-3.48 ± 14.61	68.0 ± 5.31
Elwee	90.3 ± 1.70^{ab}	94.4 ± 1.55^{b}	-12.0 ± 5.67	64.2 ± 9.23
HP 883-1-1-1-B-1-1-1	45.8 ± 1.51^{i}	66.7 ± 1.08^{h}	-16.8 ± 9.30	-3.19 ± 3.38
HP883-1-3-1-1-1	73.8 ± 1.17^{g}	59.0 ± 0.76^{i}	-27.8 ± 7.73	19.6 ± 5.01
Jumlalocal-1	82.6 ± 0.71^{de}	93.8 ± 0.34^{b}	-18.4 ± 4.11	72.3 ± 4.04
Kele	79.9 ± 1.68^{ef}	90.3 ± 1.20^{c}	-30.2 ± 5.59	44.2 ± 2.05
LK1-3-6-12-1-1	93.1 ± 1.24^{a}	96.5 ± 1.11^{a}	32.3 ± 0.50	100 ± 9.16
LK1A-2-12-1-1	66.7 ± 1.90^{h}	86.1 ± 1.96^{de}	-16.5 ± 2.30	80.0 ± 9.20
LK1B-2-1-1	47.9 ± 0.99^{i}	75.0 ± 0.31^{g}	-10.0 ± 3.04	52.0 ± 9.45
LK1B-4-12-1-1	64.6 ± 1.41^{h}	86.8 ± 0.70^{d}	6.23 ± 2.66	96.4 ± 13.7
LK1D-2-12-1	66.0 ± 0.82^{h}	84.0 ± 0.65^{f}	-20.6 ± 3.26	72.5 ± 4.46
LK2-7-12-1-1	74.3 ± 1.01^{g}	90.3 ± 0.49^{c}	-11.2 ± 5.66	77.5 ± 17.0
Muthumamikan	81.9 ± 4.03^{e}	94.4 ± 1.86^{b}	-24.9 ± 5.44	65.2 ± 10.6
Parnkhari 203	88.9 ± 0.99^{bc}	93.1 ± 1.14^{b}	-19.0 ± 4.53	55.2 ± 9.79
RGS No. 336	66.0 ± 0.28^{h}	0.75 ± 0.40^{k}	-7.54 ± 1.53	70.0 ± 9.83
SC-45	80.6 ± 1.37^{ef}	94.5 ± 0.82^{b}	-14.8 ± 5.27	65.1 ± 12.0
SC-5	20.1 ± 2.98^{j}	58.33 ± 2.00^{i}	-39.3 ± 4.02	-21.9 ± 8.80
wx124-163-45-7-1-1-1	65.1 ± 0.31^{h}	$85.4 \pm 1.00\ d^{ef}$	-21.9 ± 4.04	41.2 ± 2.66

[a] Values expressed as means ± SD ($n = 3$). Values within each column with the same superscripts are not significantly different at $P < 0.05$.

concentrations tested (93.0 and 96.5% inhibition, respectively).

These pigmented rice cultivars can be classified into four groups with regard to their scavenging activities: high activity, >80% inhibition; moderate activity, 50–79% inhibition; low activity, 30–49% inhibition; insignificant activity, <30% inhibition. At a concentration of 0.5-mg/ml, B-89-11-2, DZ78, Elwee, Jumlalocal-1, LK1-3-6-12-1-1, Muthumanikan, Parnkhari 203, and SC-45 cultivars had high activity. Moderate activity was shown by cultivars DK1, DV85, HP833-1-3-1-1-1, Kele, LK1A-2-12-1-1, LK1B-4-12-1-1, LK1D-2-12-1, LK2-7-12-1-1, RSG No. 336 and wx124-163-45-7-1-1-1. Activities of HP833-1-1-1-B-1-1-1, LK1B-2-1-1 and SC-5 were low, and that of SC-5 insignificant. A significant correlation was observed between the activity and the concentrations used ($r = 0.578$, $P < 0.05$), suggesting the involvement of active constituents in the extracts that can scavenge superoxide anions.

Results (Table 3) of the xanthine oxidase (XOD) inhibition assay (Muraoka & Miura, 2004) suggest that the superoxide scavenging by LK1-3-6-12-1-1, LK1A-2-12-1-1 and LK1B-4-12-1-1 cultivars might be caused by a blockage of enzyme action of XOD, not by direct quenching of the radicals at concentrations of 5 mg/ml. In contrast, HP883-1-3-1-1-1, and SC-5 cultivars seem to exert their action via direct quenching, because we could not obtain any evidence of inhibition of XOD by the extracts at this dose.

3.4. Scavenging of hydroxyl radicals

In cells, hydroxyl radicals are generated from hydrogen peroxide via the so-called Fenton reaction (Huang et al., 2002). We therefore also examined whether pigmented rice extracts can scavenge hydroxyl radicals generated by this reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{-}OH + {}^{\cdot}OH$) in vitro. Table 4 shows that hydroxyl-radical scavenging activity was less than superoxide-anion scavenging activity. At a dose of 0.5 mg/ml, five out of twenty one pigmented rice extracts (DZ78, Jumlalocal-1, LK1-3-6-12-1-1, Parnkhari 203, and SC-45) showed moderate scavenging ability (50–69% inhibition); low activity (30–49% inhibition) was shown by extracts from eleven cultivars (B-89-11-2, DK1, DV85, Elwee, Kele, LK1A-2-12-1-1, LK1B-4-12-1-1, LK1D-2-12-1, LK2-7-12-1-1, Muthumanikan, and wx124-163-45-7-1-1-1); and insignificant activity (<30% inhibition) by five cultivars (HP883-1-1-1-B-1-1-1, HP883-1-3-1-1-1, LK1B-2-1-1, RSG No. 336, and SC-5).

The data also show that the activity was greatest with the cultivars SC-45, Jumlalocal-1, Parnhhari 203, and LK1-3-6-12-1-1 at both doses (0.5 and 5 mg/ml) tested. Extracts from three cultivars (HP883-1-1-1-B-1-1-1, HP883-1-3-1-1-1, and SC-5) displayed the same (or lower) scavenging activities as the nonpigmented rice.

To examine whether the scavenging effects were caused by depletion of free Fe^{2+}, we also determined the Fe^{2+} binding capacity of the rice extracts (Table

Table 4
Scavenging of hydroxyl radicals (‘OH) and chelation of ferrous ion (Fe^{2+}) by pigmented rice bran extracts at the indicated concentrations[a]

Rice cultivar	(‘OH) scavenging (%)		(Fe^{2+}) chelation (%)	
	0.5 mg/ml	5 mg/ml	0.5 mg/ ml	5 mg/ml
Chuchung white rice	21.8 ± 3.00^{j}	67.3 ± 3.49^{lm}	1.41 ± 1.92	-0.91 ± 3.27
B-89-11-2	32.7 ± 2.94^{g}	84.8 ± 0.30^{bc}	15.9 ± 0.63	28.0 ± 6.99
DK 1	40.2 ± 1.45^{e}	82.0 ± 1.05^{def}	18.2 ± 5.87	32.5 ± 6.26
DV85	40.8 ± 0.86^{e}	81.0 ± 1.14^{ef}	9.27 ± 0.55	27.1 ± 6.39
DZ78	53.5 ± 1.00^{b}	83.4 ± 0.35^{cde}	6.86 ± 1.81	28.5 ± 11.97
Elwee	33.2 ± 0.79^{g}	78.3 ± 0.56^{gh}	-3.61 ± 3.08	30.8 ± 9.03
HP 883-1-1-1-B-1-1-1	18.5 ± 0.81^{k}	51.3 ± 1.49^{o}	2.65 ± 4.77	15.8 ± 3.47
HP883-1-3-1-1-1	17.0 ± 1.09^{kl}	67.0 ± 0.95^{lmn}	-0.48 ± 5.12	14.7 ± 0.55
Jumlalocal-1	63.7 ± 0.85^{a}	88.2 ± 0.33^{a}	10.7 ± 3.79	44.0 ± 9.22
Kele	32.2 ± 1.04^{g}	72.3 ± 1.22^{j}	5.42 ± 3.82	14.2 ± 1.27
LK1-3-6-12-1-1	63.9 ± 1.24^{a}	86.9 ± 0.18^{ab}	-2.17 ± 5.42	29.1 ± 1.37
LK1A-2-12-1-1	49.9 ± 0.38^{c}	81.9 ± 0.35^{def}	2.65 ± 2.71	25.8 ± 0.91
LK1B-2-1-1	26.0 ± 0.80^{i}	$71.3 \pm 1.^{26jk}$	9.51 ± 6.07	21.4 ± 5.41
LK1B-4-12-1-1	39.4 ± 0.95^{e}	77.9 ± 0.83^{gh}	4.45 ± 9.39	16.9 ± 4.35
LK1D-2-12-1	36.4 ± 0.46^{f}	69.2 ± 0.66^{kl}	3.01 ± 1.99	21.1 ± 2.35
LK2-7-12-1-1	47.5 ± 0.83^{d}	82.5 ± 0.52^{cdef}	4.21 ± 0.91	23.1 ± 2.37
Muthumamikan	39.3 ± 3.40^{e}	84.0 ± 2.51^{cd}	14.8 ± 0.96	32.7 ± 7.99
Parnkhari 203	63.5 ± 0.98^{a}	87.8 ± 1.00^{a}	6.38 ± 1.37	42.5 ± 9.97
RGS No. 336	28.7 ± 0.16^{h}	77.9 ± 0.32^{gh}	7.94 ± 0.96	20.8 ± 6.46
SC-45	63.6 ± 0.98^{a}	88.9 ± 1.46^{a}	20.3 ± 6.97	36.6 ± 7.78
SC-5	22.7 ± 3.44^{j}	66.2 ± 2.07^{mn}	15.5 ± 4.74	31.7 ± 9.18
wx124-163-45-7-1-1-1	39.6 ± 0.24^{e}	74.9 ± 0.95^{hi}	6.98 ± 2.05	15.6 ± 4.17

[a] Values expressed as means ± SD ($n = 3$). Values within each column with the same superscript are not significantly different at $P < 0.05$.

4). The results suggest that Fe^{2+} binding by the rice extracts did not seem to affect the hydroxyl radical scavenging ability. Because of a significant correlation between binding capacity and radical scavenging ability, especially at high (5 mg/ml) dose ($r = 0.683$, $P < 0.05$), direct quenching of free radicals may not be an exclusive pathway for hydroxyl radical scavenging by rice extracts.

4. Conclusions and significance for agriculture and human health

The bran of pigmented rice varieties has, with some exceptions, greater antioxidant and free-radical scavenging activities than bran of nonpigmented cooking rice. The results also show that several pigmented rice extracts acted as pro-oxidants in the linoleic peroxidation assay, possibly by mechanisms described for the pro-oxidant activities of γ-tocopherol and ascorbic acid (Buettner, 1986; Yamauchi, Miyake, Kato, & Ueno, 1993). Pigmented rice extracts scavenged superoxide anions more effectively than hydroxyl radicals.

Comparative results from the in vitro linoleic acid and the ex vivo rabbit erythrocyte membrane ghost peroxidation assays suggest that the latter might be a more reliable method for predicting redox reactions occurring in vivo. Significant correlations were observed between the reducing powers of the rice extracts and inhibition of lipid peroxidations of erythrocyte membranes over a wide range of concentrations. The ferricyanide test of reducing power, the simplest of the four assays evaluated in this study, might therefore be a useful index for large-scale screening of antioxidant potencies.

A major aim of this study is to facilitate development of novel rice cultivars with high antioxidative capacity. The following five cultivars had very high antioxidative activities in all four indices of antioxidative activity tested (inhibitory activity to lipid peroxidation, reducing power, superoxide radical-scavenging activity, and hydroxyl radical-scavenging activity): Jumlalocal-1, Parnkhari 203, DZ78, LK1-3-6-12-1-1 and Elwee. These pigmented rice cultivars, with high antioxidative potential, may provide a source of new antioxidants as well as genes, for new improved varieties, for use in foods with medicinal properties, thereby increasing rice consumption and contributing to the prevention of chronic diseases caused by oxidative damage to cells.

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References

Acquaviva, R., Russo, A., Galvano, F., Galvano, G., Barcellona, M. L., Li Volti, G., et al. (2003). Cyanidin and cyanidin3-*O*-β-D-glucoside as DNA cleavage protectors and antioxidants. *Cell Biology and Toxicology, 19*(4), 243–252.

Adom, K. K., & Liu, R. H. (2002). Antioxidant activity of grains. *Journal of Agricultural and Food Chemistry, 50*, 6170–6182.

Ames, B. N. (1983). Dietary carcinogens and anticarcinogens. Oxygen radicals and degenerative diseases. *Science, 221*(4617), 1256–1264.

Asamarai, A. M., Addis, P. B., Epley, R. J., & Krick, T. P. (1996). Wild rice hull antioxidants. *Journal of Agricultural and Food Chemistry, 44*, 126–130.

Buettner, G. R. (1986). Ascorbate autoxidation in the presence of iron and copper chelates. *Free Radical Research Communications, 1*(6), 349–353.

Carter, P. (1971). Spectrophotometric determination of serum iron at the submicrogram level with a new reagent (ferrozine). *Analytical Biochemistry, 40*(2), 450–458.

Choi, S. W., Nam, S. H., & Choi, H. C. (1996). Antioxidative activity of ethanolic extracts of rice brans. *Foods and Biotechnology, 5*(4), 305–309.

Chung, H. S., & Woo, W. S. (2001). A quinolone alkaloid with antioxidant activity from the aleurone layer of anthocyanin-pigmented rice. *Journal of Natural Products, 64*(12), 1579–1580.

Deliconstantinos, G., Villiotou, V., & Stavrides, J. C. (1996). Alterations of nitric oxide synthase and xanthine oxidase activities of human keratinocytes by ultraviolet B radiation. Potential role for peroxynitrite in skin inflammation. *Biochemical Pharmacology, 51*(12), 1727–1738.

Friedman, M. (1996). The nutritional value of proteins from different food sources. *Journal of Agricultural and Food Chemistry, 43*, 3–29.

Goffman, F. D., & Bergman, C. J. (2004). Rice kernel phenolic content and its relationship with antiradical efficiency. *Journal of the Science of Food and Agriculture, 84*, 1235–1240.

Heyneman, R. A. (1983). Subcellular localization and properties of the NAD(P)H oxidase from equine polymorphonuclear leukocytes. *Enzyme, 29*(3), 198–207.

Hu, C., Zawistowski, J., Ling, W., & Kitts, D. D. (2003). Black rice (*Oryza sativa* L. *indica*) pigmented fraction suppresses both reactive oxygen species and nitric oxide in chemical and biological model systems. *Journal of Agricultural and Food Chemistry, 51*(18), 5271–5277.

Huang, X., Dai, J., Fournier, J., Ali, A. M., Zhang, Q., & Frenkel, K. (2002). Ferrous ion autoxidation and its chelation in iron-loaded human liver HepG2 cells. *Free Radical Biology and Medicine, 32*(1), 84–92.

Hyun, J. W., & Chung, H. S. (2004). Cyanidin and malvidin from *Oryza sativa* cv. Heungjinjubyeo mediate cytotoxicity against human monocytic leukemia cells by arrest of G(2)/M phase and induction of apoptosis. *Journal of Agricultural and Food Chemistry, 52*, 2213–2217.

Ichikawa, H., Ichiyanagi, T., Xu, B., Yoshii, Y., Nakajima, M., & Konishi, T. (2001). Antioxidant activity of anthocyanin extract from purple black rice. *Journal of Medicinal Food, 4*(4), 211–218.

Ishihara, K., & Hirano, T. (2002). IL-6 in autoimmune disease and chronic inflammatory proliferative disease. *Cytokine and Growth Factor Reviews, 13*(4–5), 357–368.

Juliano, B. O. (Ed.). (1985). *Rice: Chemistry and technology*. Minneapolis, MN: American Association of Cereal Chemists.

Khush, G. S., & Toeenniesson, G. (Eds.). (1991). *Rice biotechnology*. Wallingford, UK: CAB International.

Klaunig, J. E., & Kamendulis, L. M. (2004). The role of oxidative stress in carcinogenesis. *Annual Reviews of Pharmacology and Toxicology, 44*, 239–267.

Lee, S. C., Kim, J. H., Jeong, S. M., Kim, D. R., Ha, J. U., Nam, K. C., et al. (2003). Effect of far-infrared radiation on the antioxidant activity of rice hulls. *Journal of Agricultural and Food Chemistry, 51*, 4400–4403.

Mitsuda, H., Yasumoto, K., & Iwami, K. (1966). Antioxidative action of indole compounds during the autoxidation of linoleic acid. *Eiyo To Shokuryo, 19*, 210–214.

Mitsuta, K., Mizuta, Y., Kohno, M., & Hiramatsu, M. (1990). The application of ESR spin-trapping technique to the evaluation of SOD-like activity of biological substances. *Bulletin of the Chemical Society of Japan, 63*, 187–190.

Miyazawa, M., Oshima, T., Koshio, K., Itsuzaki, Y., & Anzai, J. (2003). Tyrosinase inhibitor from black rice bran. *Journal of Agricultural and Food Chemistry, 51*, 6953–6956.

Muraoka, S., & Miura, T. (2004). Inhibition of xanthine oxidase by phytic acid and its antioxidative action. *Life Sciences, 74*(13), 1691–1700.

Noro, T., Noro, K., Miyase, T., Kuroyanagi, M., Umehara, K., Ueno, A., et al. (1987). Inhibition of xanthine oxidase by anthraquinones. *Chemical and Pharmaceutical Bulletin, 35*(10), 4314–4316.

Oki, T., Matsuda, M., Kobayashi, M., Nishiba, Y., Furuta, S., Suda, I., et al. (2002). Polymeric procyanidins as radical-scavenging components in red-hulled rice. *Journal of Agricultural and Food Chemistry, 50*, 7524–7529.

Oyaizu, M. (1986). Studies on products of the browning reaction. Antioxidative activities of browning reaction products prepared from glucosamine. *Eiyogaku Zasshi, 44*(6), 307–315.

Parrado, J., Miramontes, E., Jover, M., Marquez, J. C., Angeles Mejias, M., Collantes De Terran, L., et al. (2003). Prevention of brain protein and lipid oxidation elicited by water-soluble oryzanol enzymatic extract derived from rice bran. *European Journal of Nutrition, 42*, 307–314.

Rosen, G. M., & Rauckman, E. J. (1981). Spin trapping of free radicals during hepatic microsomal lipid peroxidation. *Proceedings of the National Academy of Sciences of the United States of America, 78*(12), 7346–7349.

Ryu, S. N., Park, S. Z., & Ho, C.-T. (1998). High performance liquid chromatographic determination of anthocyanin pigments in some varieties of black rice. *Journal of Food and Drug Analysis, 6*, 729–736.

Sander, C. S., Chang, H., Hamm, F., Elsner, P., & Thiele, J. J. (2004). Role of oxidative stress and the antioxidant network in cutaneous carcinogenesis. *International Journal of Dermatology, 43*(5), 326–335.

Shahidi, F., & Naczk, M. (2004). *Phenolics in food and nutraceuticals*. Boca Raton, FL: CRC Press.

Spears, J. K., Grieshop, C. M., & Fahey, G. C. J. (2004). Evaluation of stabilized rice bran as an ingredient in dry extruded dog food. *Journal of Animal Science, 82*, 1122–1135.

Takahashi, Y., Ogra, Y., & Suzuki, K. T. (2004). Synchronized generation of reactive oxygen species with the cell cycle. *Life Sciences, 75*(3), 301–311.

Tian, S., Nakamura, K., & Kayahara, H. (2004). Analysis of phenolic compounds in white rice, brown rice, and germinated brown rice. *Journal of Agricultural and Food Chemistry, 52*, 4808–4813.

Toyokuni, S., Itani, T., Morimitsu, Y., Okada, K., Ozeki, M., Kondo, S., et al. (2002). Protective effect of colored rice over white rice on Fenton reaction-based renal lipid peroxidation in rats. *Free Radical Research, 36*(5), 583–592.

Tsuchihashi, H., Kigoshi, M., Iwatsuki, M., & Niki, E. (1995). Action of β-carotene as an antioxidant against lipid peroxidation. *Archives of Biochemistry and Biophysics, 323*, 137–147.

Tsuda, T., Shiga, K., Ohshima, K., Kawakishi, S., & Osawa, T. (1996). Inhibition of lipid peroxidation and the active oxygen radical scavenging effect of anthocyanin pigments isolated from *Phaseolus vulgaris* L. *Biochemical Pharmacology, 52*, 1033–1039.

Wyllie, S., & Liehr, J. G. (1997). Release of iron from ferritin storage by redox cycling of stilbene and steroid estrogen metabolites: a mechanism of induction of free radical damage by estrogen. *Archives of Biochemistry and Biophysics, 346*(2), 180–186.

Yamauchi, R., Miyake, N., Kato, K., & Ueno, Y. (1993). Reaction of α-tocopherol with alkyl and alkylperoxyl radicals of methyl linoleate. *Lipids, 28*(3), 201–206.

Zhang, P., & Omaye, S. T. (2001). DNA strand breakage and oxygen tension: effects of β-carotene, α-tocopherol and ascorbic acid. *Food and Chemical Toxicology, 39*(3), 239–246.