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Antioxidant Properties of Different Polysaccharides Extracted with Water and Sodium Hydroxide from Rice Bran

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Abstract The antioxidant properties of water extracted polysaccharides (PW), 1%(w/v) NaOH extracted polysaccharides (PN1), and 5%(w/v) NaOH extracted polysaccharides (PN5) were assessed in this paper. PW showed good capability of scavenging H_2O_2, anti-lipid peroxidation, reduction power, and scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH). The maximum values were all observed at the dose of 1 mg/mL sample. As far as the antioxidant activities of PN1 were concerned, the capability of scavenging superoxide radical, chelating metal, and total antioxidation showed higher than those of PN5. When polysaccharide concentration was 1 mg/mL, the 3 index described above were attached 71.8%, 51.6%, and 446.3 μM Trolox equivalent, respectively. With respect to PN5, higher capabilities of scavenging hydroxyl free radicals were obtained while the peak of 84.8% was observed at the concentration of 1.0 mg/mL. Moreover, the concentration-dependent influences were characterized in all cases.

Keywords: rice bran, polysaccharide, antioxidant activity, radical scavenging activity, reducing power, chelating ability

Introduction

Oxidative stress, induced by oxygen radicals, is a common reaction and an essential biological process in many organisms. However, a vast amount of evidence implicated that the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as cataract, cancer, rheumatoid arthritis, Alzheimer's diseases, and atherosclerosis as well as in degenerative process of aging (1). Therefore, it is essential to develop and utilize effective antioxidants which can scavenge free radicals in the human bodies (2). In order to reduce damage to human body, many antioxidants have been synthesized industrially. However, most of them such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are suspected to being responsible for liver damage and carcinogenesis (3). In recent years, research on antioxidants, especially exploration of potent natural compounds with low cytotoxicity from plants, have become an important branch of biomedicine.

Rice is consumed in its polished form as a stable food in many countries. Rice bran is the outer layer of brown rice, obtained as a by-product of the rice milling industry. However, most of them are discarded, except used in feeds (4). Due to the abundance of some functional compounds, rice bran has drawn the attention of chemists and pharmacologists in recent years (5). Among the functional compounds, many biological active polysaccharides extracted from rice bran appeared to elicit excellent physiological properties in maintaining health, preventing diseases such as anti-tumor, enhancing the immune function, and increasing the peripheral blood lymphocytes (5). It has been reported that polysaccharides from different resource have strong antioxidant properties and can be explored as novel potential antioxidants (6,7). However, as far as our literature survey could ascertain, no information was available on the *in vitro* antioxidative activities of polysaccharides from rice bran. Therefore, the aim of this study was to investigate the *in vitro* antioxidant capacities of polysaccharides from rice bran. Eight *in vitro* test models were employed here. The obtained information will be helpful in protecting the human body from free radicals and retarding the progress of many chronic diseases.

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Materials and Methods

Materials Rice bran was supplied by Anhui Tianchuang Biotechnology Co., Ltd. (Suzhou, Anhui, China). It was squeezed and ground, passed through 1-mm sieve, and then stored at 4°C until use. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and Trolox were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2,2-Azino-bis(3-ethylbenzthiazoline-6-sulphonate) (ABTS) was obtained from Shanghai Biotechnology Co., Ltd. (Shanghai, China). All other chemicals were analytical grade and purchased from Shanghai Boer Chemical Reagent Co., Ltd. (Shanghai, China).

Preparation of polysaccharides The powdered material was pre-extracted for 48 hr in a Soxhlet system with acetone and subsequently for another 48 hr with MeOH to remove impurities. The extract was discarded. The residue was dried at 40°C for 48 hr. For preparation of polysaccharides extracted with water from rice bran (PW), the above dried powder (100 g) was extracted with distilled water at 50-60°C for 1 hr. After centrifugation for 15 min at 12,000×g, supernatants were collected and the pellet was re-extracted 4 times to recover the residual water soluble polysaccharides. The combined supernatants constituted the polysaccharide fraction of PW. For preparation of polysaccharides extracted with 1% NaOH from rice bran (PN1), the residue after PW extraction was dried at 60°C and extracted 3

times with 2,000 mL NaOH at concentration of 1% and 0.5 g polyvinylpyrrolidone for 2 hr at room temperature. The combined supernatants were the polysaccharide fractions of PN1. For preparation of polysaccharides extracted with 5.0% NaOH from rice bran (PN5), the residue after PN1 extraction was washed with distilled water for 3 times and dried at 60°C for 48 hr. After that, the extraction procedure was the same as that for PN1 extraction, except NaOH concentration at 5.0%. With respect to removing impurities such as proteins, purification methods were further employed. For example, the combined extracts of PW were concentrated to a certain volume and centrifuged at a speed of 12,000×g for 30 min. The supernatant was added ethanol to a final concentration of 80%(v/v) and centrifuged, and the precipitate was dissolved in appropriate distilled water. This process was repeated 3 times. The precipitate was then washed with Sevag reagent (isoamyl alcohol:$CHCl_3$ in 1:4 ratio) (8) and dried in a vacuum, to give the crude polysaccharide in rice bran for all experiments. For PN1 and PN5, the removing methods were the same as those of PW. The content of polysaccharides was determined by phenol-sulphate acid method (8).

Monosaccharide analysis and molecular weight detection Monosaccharide composition and molecular weight of polysaccharide from rice bran were investigated according to our previous reports (9).

Hydroxyl radical scavenging activity Hydroxyl radical scavenging activity was measured according to Smirnoff and Cumbes (10) with some modification. The reaction mixture containing 0.2 mL different samples at the concentration of 0.01, 0.1, 0.3, 0.5, and 1.0 mg/mL, was incubated with 0.15 mM ferric ethylenediamine tetraacetic acid (2.0 mL), 2.0 mM salicylic acid (0.8 mL), 6.0 mM H_2O_2 (2.0 mL), and 0.8 mL distilled water for 60 min at 37°C, and hydroxyl radical was detected by monitoring absorbance at 510 nm. In the control, sample was substituted with distilled water, and sodium phosphate buffer replaced H_2O_2. The capability of scavenging hydroxyl radical was calculated using the following equation:

$$\text{Scavenging effect (\%)} = [1-(A_{\text{sample 510 nm}}-A_{\text{sample blank 510 nm}})/A_{\text{control 510 nm}}]\times 100$$

where $A_{control}$, A_{sample} and $A_{sample\ blank}$ represents the absorbance of the control group, the test group and the single samples, respectively.

Superoxide anion scavenging activity The assay was based on the capacity of the sample to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) in nicotinamide adenine dinucleodide-reduced (NADH)-NBT-phenazine methosulfate (PMS) according to Wang *et al.* (11) with minor modification. One mL polysaccharide sample at different concentration (0.01-1.0 mg/mL) was added to 2.0 mL Tris-HCl buffer (16 mM, pH 8.0) containing 76 μM NBT and 394 μM NADH. Reactions were started by addition of 0.4 mL of PMS (56 μM in 16 mM Tris-HCl buffer, pH 8.0) and incubated at room temperature for 5 min, and the absorbance was measured at 560 nm against blank. The control was prepared with Tris-HCl substituting for polysaccharides. The capacity of scavenging to superoxide radical was calculated using the following equation:

$$\text{Scavenging effect (\%)} = [1-(A_{\text{sample 560 nm}}-A_{\text{sample blank 560 nm}})/A_{\text{control 560 nm}}]\times 100$$

where $A_{control}$, A_{sample}, and $A_{sample\ blank}$ represents the absorbance of the control group, the test group, and the single samples, respectively.

Scavenging activity of DPPH radical The scavenging activity of DPPH radical was measured according to the method of Xie *et al.* (12) with minor modification. Polysaccharide sample (0.01 to 1.0 mg/mL) of 0.5 mL was added to 3.0 mL of a 0.004% ethanol solution of DPPH. Absorbance at 517 nm was measured after 30 min, and the % scavenging activity was calculated by the following equation:

$$\text{Scavenging effect (\%)} = [1-(A_{\text{sample 517 nm}}-A_{\text{sample blank 517 nm}})/A_{\text{control 517 nm}}]\times 100$$

where $A_{control}$, A_{sample}, and $A_{sample\ blank}$ represents the absorbance of the control group, the test group, and the single samples, respectively.

H_2O_2 scavenging activity The ability of samples to quench H_2O_2 was determined spectrophotometrically (13). Polysaccharide sample (0.01 to 1.0 mg/mL) with 0.5 mL was added to 1.0 mL of phosphate buffered saline (PBS) buffer (0.1 M, pH 7.4), and then mixed with 3.0 mL solution of H_2O_2 (40 nM). Absorbance of H_2O_2 at 230 nm was determined 1 min later in a spectrophotometer. For each concentration, a separated blank sample was used for background subtraction. The inhibition of H_2O_2 production was calculated as follows:

$$\text{Inhibition rate (\%)} = [1-(A_{\text{sample 230 nm}}-A_{\text{sample blank 230 nm}})/A_{\text{control 230 nm}}]\times 100$$

where $A_{control}$, A_{sample}, and $A_{sample\ blank}$ represents the absorbance of the control group, the test group, and the single samples, respectively.

Chelating effect on ferrous ion The chelating effect of different polysaccharide on ferrous ion was assayed according to Yuan *et al.* (14) with minor modification. One mL of samples at different concentrations (0.01-1.0 mg/mL) were mixed with $FeCl_2$ (0.1 mL, 2 mM) and ferrozine (0.2 mL, 5 mM), shaken well, stood for 10 min at room temperature, and then the absorbance of the mixture was determined at 562 nm. A lower absorbance indicated stronger chelating ability.

Anti-lipid peroxidation assay The effect of polysacchrides on $FeCl_2$-ascorbic acid induced lipid peroxidation in rat liver was determined by the method of Yoshiyuki *et al.* (15) with some modifications. In brief, a reaction mixture consisted of 0.1 mL of liver homogenate, 0.1 mL of Tris-HCl buffer (pH 7.2) containing 0.1 mM ascorbic acid, 4 mM $FeCl_2$, and 0.05 mL of various concentrations of polysaccharides (0.01-1.0 mg/mL) and incubated at 37°C for 1 hr, followed by centrifugation (4,000×g, 10 min). After 0.9 mL of distilled water and 2 mL of 0.6% thiobarbituric acid (TBA) were added, the mixture was heated in a boiling water bath at 100°C for 15 min. After

cooling, 5 mL of *n*-butanol was added and the mixture was then shaken vigorously. The *n*-butanol layer was separated by centrifugation (4,000×g, 10 min), followed by subjecting to the measurement of TBA reactive substances (TBARS) production at 532 nm.

Reducing power assay The reducing power of polysaccharides was determined referring to the reference (14). One mL of samples in different concentrations (0.01-1.0 mg/mL) was mixed with 0.2 mL phosphate buffer (0.2 M, pH 6.6) and 0.5 mL potassium ferricyanide (1.0%, w/v). The mixture was incubated at 50°C for 20 min. One mL of trichloroacetic acid (TCA, 10%, w/v) was added to the mixture which was then centrifuged for 10 min at 3,000×g. The supernatant (1.5 mL) was mixed with 0.2 mL $FeCl_3$ (1.0%, w/v) solution and 3.0 mL distilled water. The absorbance was measured at 700 nm.

Total antioxidant activity of different polysacchaides This assay assesses the total radical scavenging capacity, based on the ability of a compound to scavenge the stable ABTS radicals (16). The stock solutions included 7 mM ABTS solution (A) and 140 mM potassium persulphate ($K_2S_2O_8$) solution (B). For ABTS radicals production, the working solution was prepared by mixing 5 mL of A and 88 μL of B, and allowing them to react for 12 hr at room temperature in dark. The solution was then diluted by mixing 1 mL ABTS solution with 50 mL methanol in order to obtain an absorbance in the inspection range of UV-1600 spectrophotometer (Ruili, Beijing, China) at 734 nm. Fresh ABTS solution was prepared for each assay. Sample (200 μL) was mixed with 3 mL of ABTS solution and the mixture was left at room temperature for 1 hr in dark. The absorbance was then measured at 734 nm using the spectrophotometer. A standard curve of Trolox ranging from 100 to 600 μM was prepared. The activity was expressed as μmol Trolox equivalents (TE)/g polysaccharides.

Data analysis Data were analyzed using SPSS package for Windows. All values were expressed as mean±standard deviation (SD) from 3 independent replicates. Results were evaluated by one-way analysis of variance (ANOVA), followed by Student's test for statistical analysis. Difference was considered significant when *p*-value was less than 0.05.

Results and Discussion

Polysaccharide composition and molecular weight The monosaccharide composition was determined by gas chromatography-mass spectrometry (GC-MS) and GC. Results showed that PW compromised Glu, Man, Gal, Rib, and Ara in a molar ratio of 8.68:1.32:4.87:1.41:1.00. PN1 is composed of Glu, Man, Gal, Rib, and Ara in a molar ratio of 11.04:2.28:0.89:0.48:1.00. PN5 consists of Glu, Man, Gal, Rib, and Ara in a molar ratio of 7.23:1.70:5.31:1.87:1.00. The average molecular weight of different polysaccharide fractions was estimated by high performance liquid chromatography (HPLC) system. Results showed that the 3 fractions were not homogenous compounds and each fraction contained some polysaccharides with different molecular weights. The molecular weight distribution of PW, PN1, and PN5 ranged from 5.3×10^3 to 6.3×10^6 Da, 7.2×10^3 to 5.1×10^6 Da, and 6.6×10^3 to 4.7×10^6 Da, respectively.

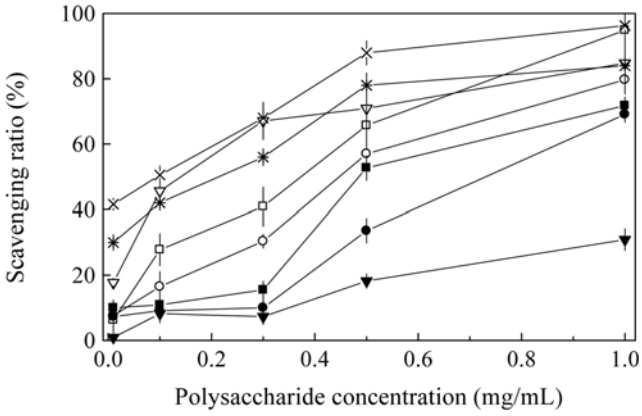

Fig. 1. Scavenging effect on hydroxyl radical and superoxide radical by different polysaccharide fractions from rice bran. Each value is the mean±SD of triplicate measurements. PW, polysaccharide fraction extracted with distilled water; PN1, polysaccharide fraction extracted with 1% NaOH from rice bran after PW extraction; PN5, polysaccharide fraction extracted with 5% NaOH from rice bran after PN1 extraction. -○- Hydroxyl radical scavenged by PW; -□- Hydroxyl radical scavenged by PN1; -▽- Hydroxyl radical scavenged by PN5; -●- Superoxide radical scavenged by PW; -■- Superoxide radical scavenged by PN1; -▼- Superoxide radical scavenged by PN5; -x- Hydroxyl radical scavenged by Ve; -✱- Superoxide radical scavenged by BHT.

Hydroxyl radical scavenging activity of different polysaccharide fractions It is well known that hydroxyl radical is a powerful oxidant that can react with all biological molecules such as proteins, lipids, and carbohydrates (14). The oxidative stress can mediate a wide variety of degenerative processes and diseases (12). The Fenton reaction, i.e., $Fe^{2+}+H_2O_2\rightarrow\cdot OH+OH^-+Fe^{3+}$, is a standard method employed in investigation of the capabilities of hydroxyl radical scavengers. This method was used in this study because the requirements for such a reaction are often fulfilled *in vivo*. As shown in Fig. 1, all the samples exhibited obvious scavenging activity on hydroxyl radical in a dose-dependent manner. Among the 3 polysaccharide fractions, the highest value (94.9%) was observed in PN1 group at the concentration of 1 mg/mL, which was as effective as that of ascorbic acid. Previous studies reported 2 types of antioxidant mechanism such as suppression against hydroxyl radical generation and cleaning the hydroxyl radical generated (17). The former mechanism was related to the transition metal ions. In the absence of transition metal ions, H_2O_2 was fairy stable. However, hydroxyl radicals acted in superoxidation by H_2O_2 with metal ions. The molecules that could chelate iron and render them inactive in Fenton reaction might have scavenging ability on hydroxyl radical (18).

Superoxide radical scavenging activity of different polysaccharide fractions The superoxide radical was generated in PMS/NADH system and assayed by the reduction of NBT. The scavenging ability of the 3

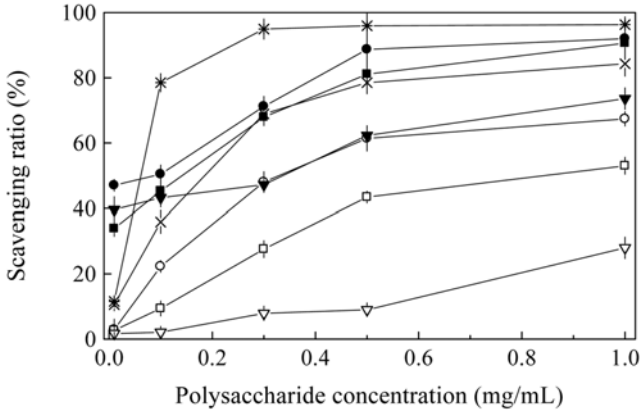

Fig. 2. Scavenging effect on DPPH radical and H_2O_2 by different polysaccharide fractions from rice bran. Each value is the mean±SD of triplicate measurements. PW, polysaccharide fraction extracted with distilled water; PN1, polysaccharide fraction extracted with 1% NaOH from rice bran after PW extraction; PN5, polysaccharide fraction extracted with 5% NaOH from rice bran after PN1 extraction. -○- DPPH radical scavenged by PW; -□- DPPH radical scavenged by PN1; -▽-, DPPH radical scavenged by PN5; -●- H_2O_2 scavenged by PW; -■- H_2O_2 scavenged by PN1; -▼- H_2O_2 scavenged by PN5; -x- DPPH radical scavenged by Ve; -✱- H_2O_2 scavenged by BHT.

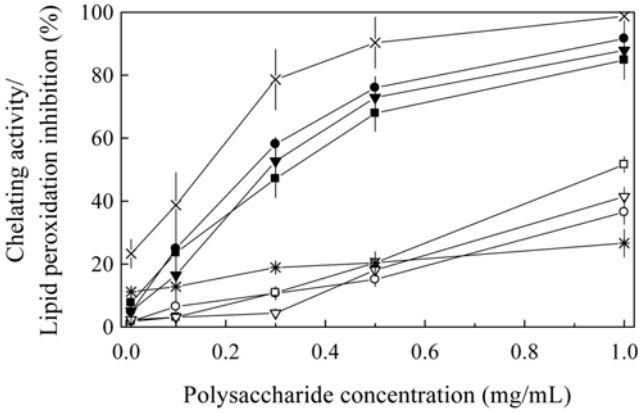

Fig. 3. Chelating effect on ferrous ions and inhibitory activities on lipid peroxidation of different polysaccharide fractions from rice bran. Each value is the mean±SD of triplicate measurements. PW, polysaccharide fraction extracted with distilled water; PN1, polysaccharide fraction extracted with 1% NaOH from rice bran after PW extraction; PN5, polysaccharide fraction extracted with 5% NaOH from rice bran after PN1 extraction. -○- Chelating activity for PW; -□- Chelating activity for PN1; -▽- Chelating activity for PN5; -●- Lipid peroxidation inhibited by PW; -■- Lipid peroxidation inhibited by PN1; -▼- Lipid peroxidation inhibited by PN5; -x- Chelating activity for EDTA; -✱- Lipid peroxidation inhibited by Ve.

polysaccharide types on superoxide radical were all tested and significantly exhibited in a concentration-dependent manner (Fig. 1). For each polysaccharide fractions, the peaks were all observed at the dose of 1 mg/mL. As far as the scavenging ability is concerned, the capability of PN1 at the concentration over 0.3 mg/mL was much higher than those of PW and PN5, while PN5 had the weakest effects. Among all samples, as much as 71.8% of superoxide radical was scavenged by PN1 at a concentration of 1.0 mg/mL. Although superoxide radical was a weak oxidant in most organisms, it could produce H_2O_2 and hydroxyl radical through dismutation and other types of reaction and is the source of free radicals formed *in vivo*. Moreover, superoxide radical and its derivatives are cell-damaging through causing damage to DNA and membrane of cell (18). These results clearly indicated that the antioxidant activities of all samples were related to the abilities of scavenging superoxide.

DPPH radical scavenging activity of different polysaccharide fractions Most free radicals are of high activities and exist only in short time. DPPH is one of the small amounts of free radicals which can keep stable even at room temperature (19). DPPH assay, a representative of single electron transfer reaction, is often used to evaluate the ability of antioxidant to scavenge free radicals, which is known to give reliable information concerning the antioxidant ability of the tested compounds (20). DPPH radical shows strong absorbance at 517 nm and it reduces gradually while the free radicals are scavenged. The method is based on the reduction of absorbance of methanolic DPPH solution at 517 nm in the presence of a proton-donating substance, due to the formation of the diamagnetic molecule by accepting an electron or hydrogen radical. It is noticeable by eye that there is a discoloration from modena to yellow induced by antioxidants. Figure 2 illustrates the scavenging ability of different polysaccharide fractions from rice bran on DPPH radical. The higher concentration the higher level of scavenging ability was found for polysaccharides in the samples used in the test. Similar results have been reported in polysaccharides from *Ligusticum chuanxiong* Hort. (14). At 0.3 mg/mL, the polysaccharides showed inhibition of 7.9-48.1%. At 1.0 mg/mL, inhibiting abilities increased to 28.0-67.4%. In all cases, the DPPH radical scavenging ability decreased in the order of PW>PN1>PN5.

H_2O_2 scavenging activity of different polysaccharide fractions Although H_2O_2 itself is not very reactive, it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. Levels of H_2O_2 at or below 20-50 mg seem to have limited cytotoxicity to many cell types. In most organisms, H_2O_2 can be formed from superoxide anion-radical by superoxide dismutase. In the absence of transition metal ions, H_2O_2 is fairly stable. However, hydroxyl radicals can be formed by the reaction of superoxide with H_2O_2 in the presence of metal ions, usually ferrous or copper (18). H_2O_2 can cross membranes and may slowly oxidize a number of compounds (21).

The scavenging activities of different polysaccharide fractions against H_2O_2 were determined and shown in Fig. 2. The scavenging activity of PW and PN1 against H_2O_2 was significantly higher than that of PN5 ($p<0.05$). In the range of 0.3-1.0 mg/mL, the scavenging rate of PW and PN1 ranged from about 66.0 to 92.0%, in the meanwhile that of PN5 from 47.2 to 13.6%. In this concentration range, the order of scavenging ability with PW>PN1> PN5 was also observed.

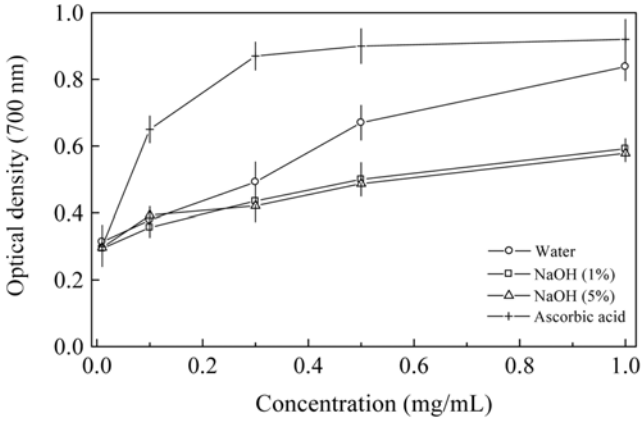

Fig. 4. Reducing power of different polysaccharide fractions from rice bran. Each value is the mean±SD of triplicate measurements.

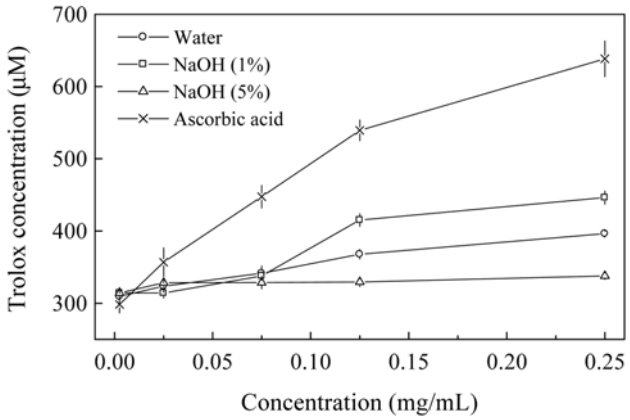

Fig. 5. Scavenging effect on ABTS radical by different polysaccharide fractions from rice bran. Each value is the mean±SD of triplicate measurements.

Metal chelating activity of different polysaccharide fractions Previous studies suggested that transition metal ions are involved in many oxidation reactions *in vivo*. The scavenging of hydroxyl radicals by antioxidant was effective mainly via chelating of metal ions. Since compound interfering with the catalytic activity of metal ions could affect the peroxidative process, the measurement of chelating ability is important for evaluating free radical scavenging activity of the compound (22). In this study, metal chelation potential as a measure of polysaccharide activity was assayed by Fenton reaction system in which Ferrous ions were formed. Ferrous ions can catalyze Haber-Weiss reaction and induce superoxide anion to form more hazardous hydroxyl radicals which quickly react with the adjacent biomolecules and induce severe damage (12). As shown in Fig. 3, the metal chelating activity of polysaccharides increased with increasing concentrations used in the test. At low concentration below 0.3 mg/mL, the samples exhibited slight chelating effects which ranged from 1.8 to 10.7% for PW, 1.8 to 10.9% for PN1, and 2.3 to 4.4% for PN5. Once the dose was higher than 0.3 mg/mL, the activity was increased rapidly where 51.6% for PN1, 36.5% for PW, and 41.4% for PN5 were achieved at the concentration of 1.0 mg/mL. It has been recognized that the metal chelating ability might be involved in antioxidant activity and affected other functions that contribute to the antioxidant activity (23). Therefore, at least partly, the chelating effect of polysaccharides from rice bran on ferrous ions might affect the other activities of scavenging free radicals to protect organism against oxidative damage. Since ferrous ions are the most effective pro-oxidants in the food system (24), the high ferrous-ion chelating abilities of polysaccharides from rice bran would be somewhat beneficial.

Lipid peroxidation inhibition of different polysaccharide fractions Lipid peroxidation is a typical free radical oxidation and proceeds via a cyclic chain reaction (14). The data obtained from Fig. 3 revealed that the polysaccharides from rice bran demonstrated great capacity for inhibiting effects on lipid peroxidation. In all cases, the phenomenon of concentration-dependence was obvious. The inhibiting effects rose from 4.8 to 91.6% for PW, 7.7 to 84.8% for PN1, and 5.1 to 87.9% for PN5 with the concentration increasing from 0.01 to 1.0 mg/mL. Similar results were also found in polysaccharides from green tea (6).

Reducing power of different polysaccharide fractions Samples with higher reducing power have better abilities to donate electron. Reducing power assay is often used to evaluate the ability of natural antioxidant to donate electron or hydrogen (25). It has been widely accepted that higher absorbance value at 700 nm means stronger reducing power of samples. The reducing power of different polysaccharides from rice bran determined at 700 nm is depicted in Fig. 4. In this assay, the reducing power of the tested polysaccharides steadily improved with increasing sample concentration. The notable difference of reducing power was not observed between PN1 and PN5 at the same concentration ($p<0.05$). At 1.0 mg/mL, the reducing power of PW was 0.84 which increased 45% than those of PN1 and PN5. The main reason could be their different molecular structure. Mau *et al.* (26) reported that the reducing power of ascorbic acid, α-tocopherol, and BHA were 0.80, 0.89, and 0.92, respectively, at 1.0 mg/mL. Earlier authors have revealed that there was a direct correlation between antioxidant activity and reducing power (27). Free radicals form stable substances by accepting donated electron, the free radical chain reactions are thus interrupted (28). In this study, the data on the reducing capacity of polysaccharides suggested that reductone-associated and hydroxide groups of polysaccharides can act as electron donors and can react with free radicals to convert them to more stable products and thereby terminate radical chain reactions.

Total antioxidant activity of different polysaccharide fractions The total antioxidant activity was determined by the Trolox equivalent antioxidant capacity (TEAC) method (16). The antioxidant activities are expressed as equivalents of Trolox which is a water soluble vitamine E analogue. The TEAC value is defined as the molar concentration of Trolox solution having the antioxidant capacity equivalent to the sample solution being tested (29). TEAC values of polysaccharides from rice bran were calculated using a

Table 1 EC_{50} values of polysaccharides from rice bran in antioxidant properties

	EC_{50} (mg/mL)[1)]		
	PW[2)]	PN1	PN5
Scavenging effect on hydroxyl radical	0.54	0.49	0.26
Scavenging effect on superoxide radical	0.75	0.64	>1
Scavenging effect on DPPH radical	0.54	0.82	>1
Chelating effect on ferrous ion	>1	>1	>1
Reducing power	0.37	0.61	0.64
Inhibitory activity on lipid peroxidation	0.37	0.43	0.38
Scavenging effect on H_2O_2	0.05	0.14	0.26
Scavenging effect on ABTS radical	0.1	0.07	>0.25

[1)]Represent the effective concentration at which hydroxyl radical, superoxide radical, DPPH radical, H_2O_2, or ABTS radical were scavenged by 50%; ferrous ions were chelated by 50%; the absorbance was 0.5 for reducing power; and Fe^{2+} induced lipid peroxidation of mouse liver homogenates were inhibited by 50%. EC_{50} values were calculated by interpolation from linear regression analysis.

[2)]PW, polysaccharide fraction extracted with distilled water; PN1, polysaccharide fraction extracted with 1% NaOH from rice bran after PW extraction; PN5, polysaccharide fraction extracted with 5% NaOH from rice bran after PN1 extraction.

Trolox standard curve. The standard curve was characterized as Y=–0.0019X+1.2039 where the correlation index was 0.998 (Fig. 5). Results suggested that all minor component polysaccharides possessed higher TEAC values. In the cases of PW and PN1, the total antioxidant ability significantly enhanced with increasing sample concentration (Fig. 5). Compared with the profile of PW and PN1, the activities of PN5 were steady in the tested concentration. PN1 showed a higher ability to scavenge ABTS radical than those of PW and PN5. When the concentration of polysaccharides was over 0.075 mg/mL, the total antioxidant activity of the samples decreased in the order of PN1>PW>PN5.

EC_{50} values in antioxidant properties The antioxidant properties were summarized in Table 1 and the results were normalized and expressed as EC_{50} values (mg/mL) for comparison. Effectiveness in antioxidant properties inversely correlated with EC_{50} value which defined as the antioxidant needed to react 50% of substrates in the tested solution. For the polysaccharides extracted from rice bran with water, EC_{50} value followed the ascending sequence: H_2O_2 < ABTS radical < reducing power/lipid peroxidation<DPPH radical/hydroxyl radical < superoxide anion < ferrous ions chelating. However, for that of polysaccharides extracted with NaOH (1.0%, w/v), the sequence was ABTS radical < H_2O_2 < lipid peroxidation < hydroxyl radical < reducing power < superoxide anion < DPPH radical < ferrous ions chelating. The EC_{50} value of polysaccharides extracted from rice bran with NaOH (5.0%, w/v) of all tested antioxidant activities, except hydroxyl radical, were higher than those of PW and PN1. In this study, the lowest EC_{50} value on antioxidation of rice bran polysaccharides was determined as 0.64 mg/mL for superoxide anion radical, 0.26 mg/mL for hydroxyl radical, 0.05 mg/mL for H_2O_2, 0.37 mg/mL for lipid peroxidation and reducing power, 0.54 mg/mL for DPPH radical, 0.07 mg/mL for ABTS radical, and over 1.0 mg/mL for ferrous ions chelating. Tseng *et al.* (7) have reported the antioxidant properties of polysaccharides from a famous traditional Chinese medicine of *Ganoderma tsugae* and indicated that the lowest EC_{50} value of reducing power, DPPH radical, hydroxyl radical, and ferrous ions chelating was 6.67, 2.84, 17.95, and 2.08 mg/mL, respectively. Yuan *et al.* (14) isolated a good antioxidant polysaccharide from *L. chuanxiong* Hort. and found that the lowest EC_{50} value was 0.87 mg/mL for DPPH radical, 6.36 mg/mL for hydroxyl radical, 0.82 mg/mL for superoxide anion radical, 3.19 mg/mL for ferrous ions chelating, and 1.68 mg/mL for lipid peroxidation. By comparison with EC_{50} value, the strong antioxidant ability of polysaccharides from rice bran was easily known.

In summary, several *in vitro* assays were applied to evaluate the antioxidant potential of polysaccharides from rice bran in this paper. Among the 3 polysaccharide fractions, polysaccharide extracted with water and NaOH (1%, w/v) have more potential for antioxidation than that of polysaccharide extracted with NaOH (5%, w/v). Due to the high antioxidant activity and abundance in raw materials, polysaccharides extracted from rice bran can be developed as a new dietary supplement and functional food to replace some rare medicinal plants. Moreover, the bioactivities of polysaccharides are related to many factors including chemical components, molecular mass, structure, conformation, and even the extraction and isolation methods, further research on antioxidant mechanisms of polysaccharides are in progress.

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