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Chemical Analysis and Antioxidant Activity of Polysaccharide Extracted from Rice Bran

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Abstract: Three rice bran polysaccharide (RBP1, RBP2 and RBP3) fractions were successfully purified from the crude polysaccharide of rice bran by DEAE-Cellulose and Sephadex G-200 column chromatography. The average molecular weights (Mws) of these fractions were 51.5, 26.1 and 6.95 kDa, respectively. Monosaccharide components analysis indicated that RBP1 and RBP2 were composed of arabinose, xylose, mannose, glucose and galactose in a molar ratio of 1.00:2.82:57.11:140.82:7.76 and 1.00:1.62:1.18:77.5:7.79. RBP3 was composed of arabinose, mannose, glucose and galactose in a molar ratio of 1.00:1.03:8.84:2.00. On the basis of antioxidant test in *vitro*, RBP2 exhibited the highest antioxidant ability among these fractions.

Key words: Antioxidant activity · Composition · Free radicals · Polysaccharide · Rice bran

INTRODUCTION

Oxidative stress, induced by oxygen radicals, is a common reaction and an essential biological process to many organisms for the production of energy. However, a vast amounts 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, 2]. Therefore, it is essential to develop and utilize effective antioxidants so that they can scavenge free radicals in the human bodies [3]. In order to reduce damage to human body, many antioxidants have been synthesized industrially. However, most of them such as butylated hydroxyanisole and butylated hydroxytoluene are suspected to being responsible for liver damage and carcinogenesis [4]. In recent years, research on antioxidants, especially exploration of potent natural compounds with low cytotoxicity from plants, have become an important branch of biomedicine. The importance of rice to the world population's dietary requirement is evident from its presence in the diet of a quarter of the world's people [5]. Rice processing or milling produces several streams of material, including husks, milled rice and bran. 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 [6]. Due to the abundance of some functional compounds, rice bran has drawn the attention of chemists and pharmacologists in recent years [7, 8]. Among the functional compounds, many biological active polysaccharides extracted from rice bran appeared to elicit excellent physiological properties in maintaining health and preventing diseases [9], enhancing the immune function [10] and increasing the peripheral blood lymphocytes [11]. It has been reported that polysaccharides from different resource have strong antioxidant properties and can be explored as novel potential antioxidants [12, 13]. Rice bran saccharide (RBS), a polysaccharide component contained in rice bran, exhibits anti-tumor capabilities. In a study on tumor prevention and suppression of tumor growth in rats, RBS was found to suppress carcinogenesis and to prolong survival rate [14]. We have not seen any published literature regarding the fractionation of the bran layer to examine RBS concentration. If RBS can be extracted successfully, rice bran could be a source for a high-value

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pharmaceutical product [15, 16]. 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 thein vitro antioxidant capacities of different fractions of polysaccharides extracted with hot-water from rice bran. The obtained information will be helpful not only in protecting the human body from free radicals and retarding the progress of many chronic diseases, but also in supplying a new way to improve the value of rice bran.

MATERIALS AND METHODS

Materials: Rice bran (RB) was obtained from a local market. DEAE-Cellulose and Vitamin C were purchased from Sigma Chemical Co. Sephadex G-200 and Dextrans of different molecular weights were purchased from Pharmacia Co. The standard monosaccharides (glucose, mannose, rhamnose, galactose, xylose and arabinose) were purchased from Merk Chemical Co. DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical was purchased from Sigma-Aldrich. Trifluoroacetic acid (TFA), pyridine, methanol and acetic acid, ethanol, acetic anhydride and all other chemicals and reagents were of grade AR.

Extraction of Polysaccharides: The powdered of rice bran was pre-extracted for 48 h in a Soxhlet system with acetone and subsequently for another 48 h with MeOH. The extract was discarded. The residue was dried at 40°C for 48 h and extracted with distilled water at 50-60°C for 1.0 h. After centrifugation for 15 min at 12,000g, supernatants were removed and the pellet was re-extracted four times to recover the residual water-soluble polysaccharides. The combined supernatants were concentrated to a certain volume and centrifuged at a speed of 12,000g for 30 min, which constituted the water-soluble polysaccharide fraction (RBP). For preparation of rice bran polysaccharide (RBP), ethanol was added to RBP1 at a final concentration of 40% (v/v) and centrifuged and the precipitate was dissolved in appropriate distilled water. In this procedure, the obtained supernatant of 40% ethanol solution was used to prepare RBP2. Ethanol was added to PS1 at a final concentration of 60% (v/v) and the precipitate was dissolved in appropriate distilled water (RBP2) after centrifugation. For preparation of RBP3, ethanol was also added to the ethanol solution obtained in the process of RBP2 preparation at a final concentration of 80% (v/v). Sevag method was also performed to remove proteins in samples of RBP1, RBP2 and RBP3.The content of polysaccharides was determined by Phenol-Sulphate acid method [17]. To calculate the efficiency of polysaccharide extraction and polysaccharide fraction content, the polysaccharide preparation procedures were repeated for three times.

Monosaccharide Analysis: GC-MS was used for identification and quantification of the monosaccharides. First, the polysaccharide (10.0 mg) was hydrolyzed with 2.0 M TFA at 110°C for 4h in a sealed glass tube [18]. Then the hydrolysate was evaporated to dryness and dissolved in 0.5 mL of pyridine, after 10.0 mg hydroxylamine hydrochloride and 2.0 mg myo-inositol (as internal reference) were added to the solution, it was allowed to react at 90°C for 30 min. The tube was cooled to room temperature and then 0.5 mL of acetic anhydride was added and mixed thoroughly by vortexing. The tube was sealed and incubated in a water bath shaker set at 90°C for 30 min. After cooled, approximately 1.0µl of clear supernatant was loaded onto an Rtx-5SilMS column (30 m × 0.32 mm × 0.25µm) of the GC-MS. Alditol acetates of authentic standards (glucose, mannose, rhamnose, galactose, xylose and arabinose) with myo-inositol as the internal standards were prepared and subjected to GC-MS analysis separately in the same way. The operation was performed in the following conditions—injection temperature: 240°C; detector temperature: 240°C; column temperature programmed: 160°C holding for 2 min,then increasing to 240°C at 5°C/min and finally holding for 5 min at 240°C. Nitrogen was used as the carrier gas and maintained at 1.0 mL/min.

Molecular Weight Determination: The molecular weight of the rice bran (RB) polysaccharide was determined due to the method previously described by [19] by gel permeation chromatography (GPC) on a Sephadex G-150 column (2.6 cm x 70 cm). Standard dextrans (40, 500 and 2000 kDa, Fluka Chemical Co. Buchs, Switzerland) and glucose were used and the elution volumes were plotted against the logarithm of their respective molecular weights. The elution volume of the purified polysaccharide was plotted in the same graph and the molecular weight was determined.

Infrared Spectra of Polysaccharides: The structural characteristics of the rice bran polysaccharides RBP fractions were determined by Fourier transform IR spectrophotometer (Perkin Elmer Corp. USA). The purified

polysaccharides were ground with KBr powder and then pressed into pellets for transform IR spectral measurement in the frequency range of 500-4000 cm^{-1} [20].

Preparation of Rat Liver Homogenates: Liver homogenates were prepared from male Wistar albino rats (180-200 g) fed on a standard laboratory diet and receiving water ad libitum. The animals were fasted overnight but allowed free access to drinking water, killed the next day, dissected and abdominal cavity was perfused with saline. The liver from each animal was collected; a weigh amount of liver was processed to obtain 20% homogenate in ice cold phosphate buffer, pH 7.4 and centrifuged for 15 min to remove the cell debris. The supernatant was used for the in vitro studies [21].

Scavenging Activity of DPPH Radical: The scavenging activity of 1, 1-dihpenyl-2-picrylhydrazyl (DPPH) radical was measured according to the method of Braca *et al.* [24] with minor modification. Polysaccharide sample (0.01-1.0 mg/ml) with 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 against blank.

Hydroxyl Radical Scavenging Activity: Hydroxyl radical scavenging activity was measured according to the method of Smirnoff and Cumbes [22]. The reaction mixture containing 0.2 ml different samples (0.01-1.0 mg/ml), was incubated with 0.15 mM EDTA-Fe (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.

Superoxide Anion-Scavenging Activity: The assay was based on the capacity of the sample to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) in NADH-NBT- phenazine methosulphate (PMS) according to Wang *et al.* [23]. One milliliter 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 76µM NBT and 394µM NADH. Reactions were started by addition of 0.4ml of PMS (56µMin16mM Tris-HCl buffer, pH 8.0) and incubated at room temperature for 5.0 min and the absorbance was measured at 560 nm against blank. In the control, sample was substituted with Tris-HCl buffer.

Chelating Effect on Ferrous Ion: The chelating effect of different polysaccharides on ferrous ion was assayed according to [25]. One milliliter of samples in 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), shook well, stayed for 10 min at room temperature and then the absorbance of the mixture was determined at 562 nm against the reference blank. A lower absorbance indicated stronger chelating activity.

Reducing Power Assay: The reducing power of polysaccharides was determined referring to the reference of Yuan *et al.* [25] with some modifications. One milliliter 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 milliliter of trichloroacetic acid (TCA) (10%, w/v) was added to the mixture which was then centrifuged for 10 min at 3000g. 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.

Anti-Lipid Peroxidation Assay: The effect of polysaccharides on $FeCl_2$-ascorbic acid induced lipid peroxidation in rat liver was determined by the method of Yoshiyuki *et al.* [26]. 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 h, followed by centrifugation (4000g, 10 min). Then 0.9 ml of distilled water and 2 ml of 0.6% thiobarbitric acid (TBA) were added, the mixture was heated for 30 min 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 (4000g, 10 min), followed by subjecting to the measurement of (TBA) reactive substances (TBARS) production at 532 nm.

Total Antioxidant Activity: This assay assesses the total radical scavenging capacity, based on the ability of a compound to scavenge the stable ABTS radicals [27]. The stock solutions included 7.0 mM (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) 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 5.0 ml of A and 88µl of B and allowing them to react for 12 h 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 at 734 nm. Fresh ABTS solution was prepared for each assay. Sample (200 µl) was mixed with 3.0 ml of ABTS solution and the mixture was left at room temperature for 1.0 h in the 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.

Statistical Analysis: All the data were expressed as means ± standard deviations (SD) from three independent replicates. Results were evaluated by oneway ANOVA, followed by Student's test for statistical analysis. Difference was considered significant when *P*-value was <0.05. SPSS Version 10 (SPSS, Chicago, IL) was used as described by Dytham [28].

RESULTS AND DISCUSSION

Isolation and Purification of Polysaccharide: The polysaccharide, named RBP, was obtained from rice bran by the method of water-extraction and ethanol precipitation. The total yield rate of crude polysaccharides was 15.3% by this isolation procedure. RBP was fractionated by ionexchange chromatography on a DEAE-Cellulose column eluted with deionized water and NaCl and was separated into RBP1 (eluted with water), RBP2 (eluted with 0.05 M NaCl) and RBP3 (eluted with 0.1 M NaCl) (Fig. 1), as detected by the phenol-sulfuric acid assay. The recovery rate of the eluted polysaccharides was 85.7%. The polysaccharide was further separated and sequentially purified through Sephadex G-200 column. Fractions (3.0 ml) were collected. Three major polysaccharide peaks, RBP1 (Fig. 2), RBP2 (Fig. 3) and RBP3 (Fig. 4), were collected and then freeze-dried. High performance gel permeation chromatography was often employed to determine the molecular weight of polysaccharide. The molecular weights (Mws) of RBP1, RBP2 and RBP3 were estimated to be 51.5, 26.1 and 6.95 kDa, respectively [16].

Monosaccharide compositions of RBP1, RBP2 and RBP3 were determined by the trifluoroacetic acid hydrolysis and GC-MS analysis method. The results indicated that mannose and glucose were the major monosaccharide constructing the backbones of RBP1.

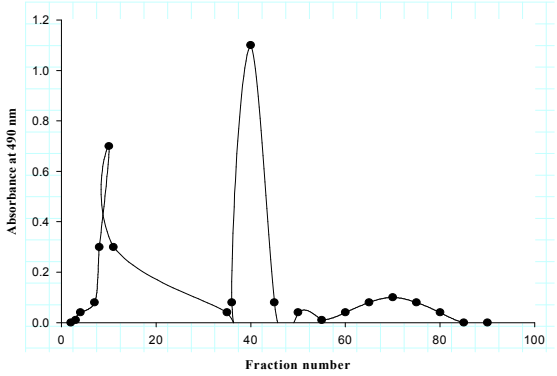

Fig. 1: Chromatography of eluted crude polysaccharide (RBP) on DEAE-Cellulose column (26 mm×300 mm). RBP1 eluted with distilled water; RBP2 eluted with 0.05 M NaCl; RBP3 eluted with 0.1 M NaCl

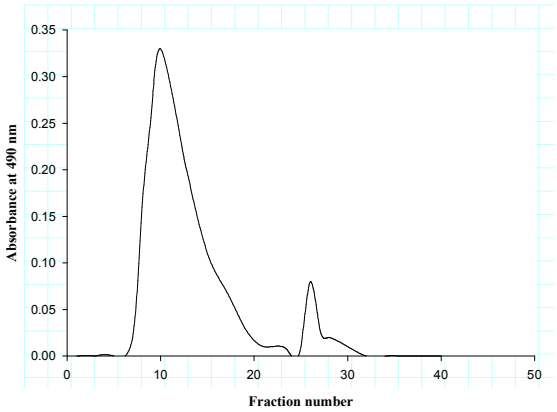

Fig. 2: Fraction (RBP1) on a chromatography column of Sephadex G-200 (eluted with distilled water)

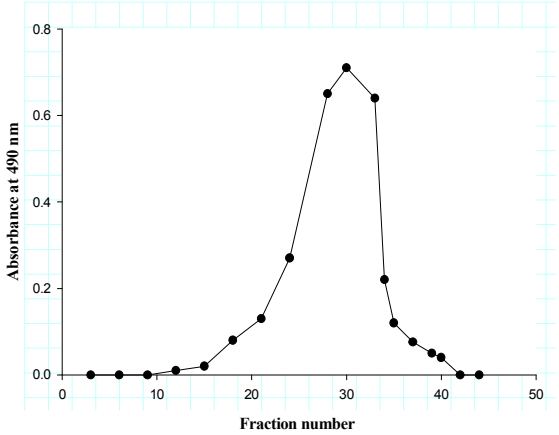

Fig. 3: Sephadex G-200 column chromatogram of RBP2 from distilled water elute, one fraction (RBP2) was obtained.

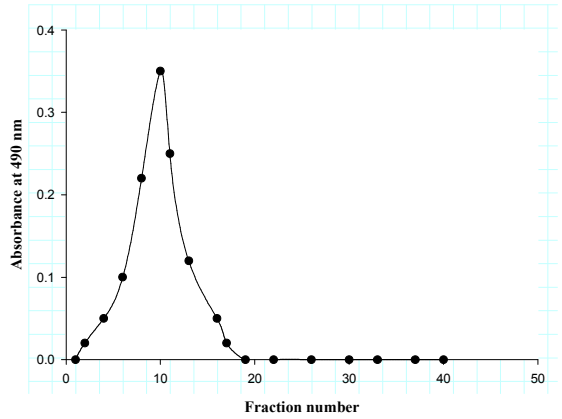

Fig. 4: Sephadex G-200 column chromatogram of RBP3 from distilled water elute, one fraction (RBP3) was obtained.

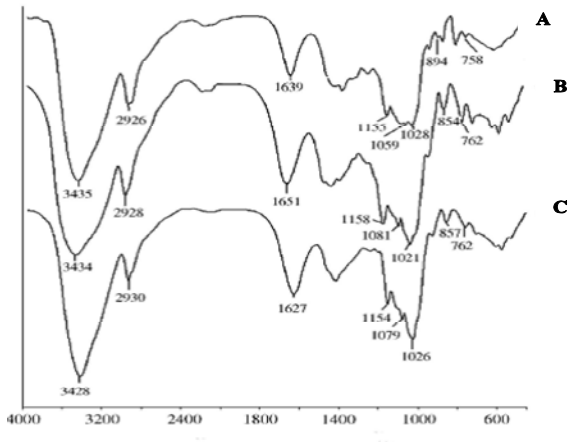

Fig. 5: FT-IR spectra of the polysaccharides of RBP1, RBP2 and RBP3. A for RBP1, B for RBP2 and C for RBP3.

The molar ratio of monosaccharide compositions in RBP1 was described as follows: arabinose: xylose: mannose: glucose: galactose = 1.00:2.82:57.11:140.82:7.76. Glucose was found as the major monosaccharide in RBP2 and the molar ratio of glucose, arabinose, xylose, mannose and galactose was 77.5:1.00:1.62:1.18:7.79. RBP3 was only composed of four monosaccharides: glucose, mannose, arabinose and galactose in a molar ratio of 8.84:1.00:1.03:2.00 [29].

Infrared Spectra of RBP1, RBP2 and RBP3: The FT-IR spectra of the three fractions were presented in Fig. 5. All samples exhibited a broad stretching intense characteristic peak at around 3429 cm^{-1} for the hydroxyl group and a weak C-H band at around 2929 cm^{-1}. The band at 1627-1651 cm^{-1} was due to the bound water [30]. Each particular polysaccharide has a specific band in the 1200-1000 cm^{-1} region; this region was dominated by ring vibrations overlapped with stretching vibrations of (C-OH) side groups and the (C-O-C) glycosidic band vibration [31]. A characteristic peak at around 894 cm^{-1} was found in RBP1, indicating the β-configuration of the sugar units [32]. The positive specific rotation and the characteristic absorption at 854 and 857 cm^{-1} was found in the IR spectrum of RBP2 and RBP3, respectively, indicated α-configuration of the sugar units.

Antioxidant Activities Analysis:

Effect of Scavenging DPPH Radicals: DPPH radical is a widely used method to evaluate the free radical scavenging ability of natural compounds [33]. DPPH is a stable free radical that shows maximum absorption at 517 nm in methanol. In the test, the antioxidants were able to reduce the stable DPPH radical and the absorbance at 517 nm. The effect of antioxidants on DPPH radical scavenging was conceived to be due to their proton-donating ability. Therefore, the antioxidant activity of a substance can be expressed as its ability in scavenging the DPPH free radical. In this experiment, the scavenging ability of purified polysaccharides (RBP1, RBP2 and RBP3) on DPPH free radical was shown in Fig. 6. The results indicated that RBP2 and RBP3 showed in all concentrations dose-dependent DPPH radical scavenging activities. Furthermore, the scavenging activities of RBP2 increased very significantly with increasing concentrations and stronger than RBP1 and RBP3 at every concentration point. Especially in the higher doses (2.0 mg/ml), RBP2 exhibited very high radical

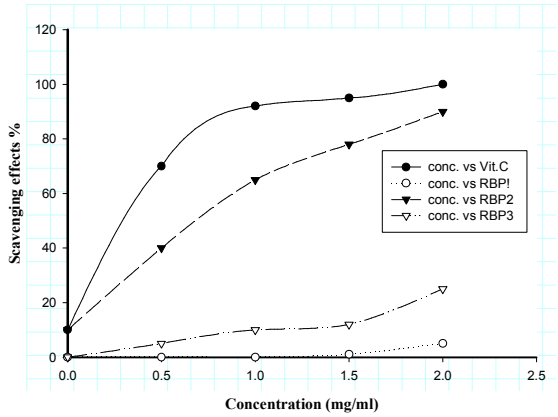

Fig. 6: The scavenging effect of different polysaccharides fractions on DPPH radical.

scavenging activity (89.3%), which was close to that of Vitamin C (98.4%). However, in the lower doses (0.01-1.0 mg/ml), the radical scavenging activity of RBP2 was far lower than that of Vitamin C. Therefore, it was obvious that the polysaccharide of RBP2 has strong antioxidant activity in the higher doses, followed by RBP3. RBP1 has no significant effects on DPPH radical scavenging.

Scavenging Activity of Superoxide Anion: The scavenging ability of the three polysaccharide types on superoxide radical were all tested and significantly exhibited in a concentration-dependent manner (Fig. 7). For each polysaccharide types, the peaks were all observed at the polysaccharide concentration of 1.0 mg/ml. The highest scavenging ability of 82.3% was obtained by RBP1 at the concentration of 1.0 mg/ml. Among all samples, the scavenging ability on superoxide radical decreased in the following order: RBP1 > RBP2 > RBP3. In addition, RBP1 has a high-level of radical-scavenging effect and the percentage inhibition is close to that of Butylated hydroxytoluene (BHT) at the dose of 1.0 mg/ml. Although, superoxide radical was a weak oxidant in most organisms, it could produce hydrogen peroxide 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 [34]. These results clearly indicated that the antioxidant activities of all samples were related to the abilities of scavenging superoxide radical and it is important to scavenge superoxide radical.

Scavenging Effects of Polysaccharide on Hydroxyl Radicals: Hydroxyl radicals were the most harmful ROS and were mainly responsible for the oxidative injury of biomolecules generated by reaction of iron-EDTA complex with H_2O_2 in the presence of ascorbic acid, attack deoxyribose to form products that, upon heating with 2-thiobarbituric acid under acid conditions, yield a pink tint [35]. Added hydroxyl radical scavengers compete with deoxyribose for the resulted hydroxyl radicals and diminish tint formation [36, 37]. The method was used to evaluate the hydroxyl radicals scavenging ability of natural compounds. The results of hydroxyl radical scavenging activities of the three polysaccharide fractions and Vitamin C were given in Fig. 8. As is illustrated in the figure, all the samples exhibited obvious scavenging activity on hydroxyl radical in

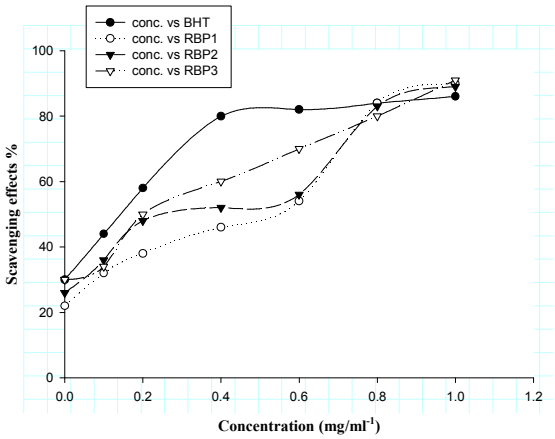

Fig. 7: Scavenging effect on superoxide radical of different polysaccharide fractions from rice bran.

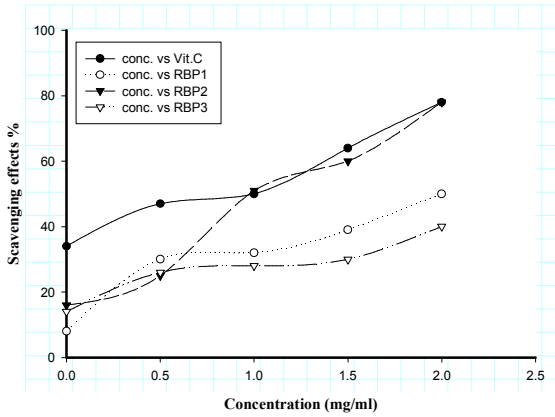

Fig. 8: The scavenging effect of different polysaccharides fractions on hydroxyl radical.

a concentration-dependent manner. These three polysaccharide fractions were found to have the ability to scavenge hydroxyl radicals at concentrations between 0.5 and 2.0 mg/ml. The ability of RBP2 was the strongest, followed by RBP1. RBP3 showed the weakest ability. At 2.0 mg/ml, RBP2 showed an excellent hydroxyl radical scavenging activity (78.9%) among all the fraction samples, also higher than Vitamin C (77.6%). The hydroxyl radical scavenging ability decreased in the order of RBP2>Vitamin C>RBP1>RBP3. Therefore, these results clearly showed that RBP2 has potential antioxidant ability of scavenging hydroxyl radical

Chelating Ability of Ferrous Ion:

Metal-chelating Activity: Chelating effect of all fraction of rice bran dietary fiber increased with the increase in concentration Fig. 9. Among all samples, although the

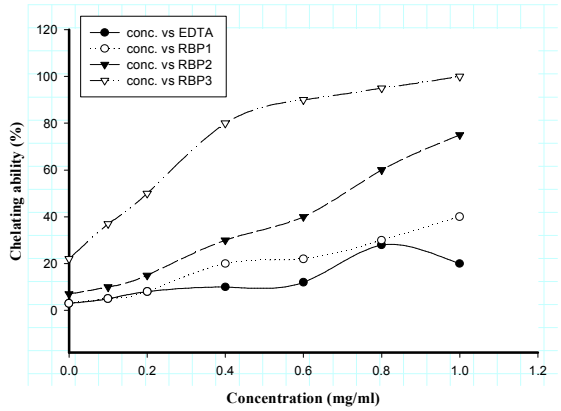

Fig. 9: Scavenging effect on chelating effect of different polysaccharide fractions from rice bran.

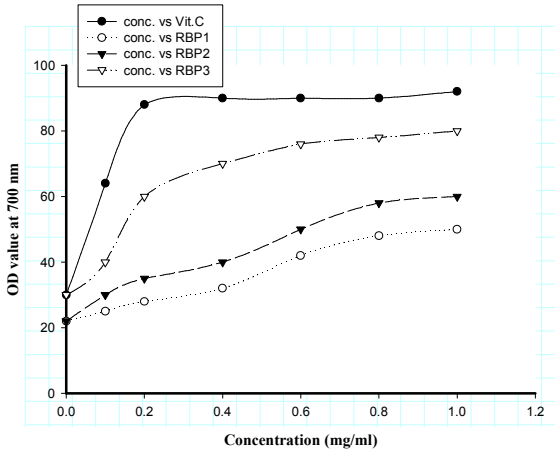

Fig. 10: Reducing power of different polysaccharide fractions from rice bran.

three polysaccharide types have similar concentration-dependent profiles, the metal chelating ability of RBP1 was much weaker than that of RBP3 while that of RBP2 was between RBP1 and RBP3. Compared with EDTA, the chelating ability of the samples on ferrous ion was weaker. At the concentration of 1.0 mg/ml, the chelating ratio reached 71% which was about 3.7-fold of RBP1 and 2-fold of RBP2. In the tested concentration range (0.01-1.0 mg/mL), the scavenging ratio only increased from 4.7% to 71% while that of RBP1 only increased from 2.9% to 20.8%. 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 [38]. 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 prooxidants in the food system [39], the high ferrous ion chelating abilities of polysaccharides from rice bran would be somewhat beneficial as functional food.

Reducing Power Assay: The antioxidant activity has been reported to have a direct, positive correlation with the reducing power. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [40]. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing power of all samples was shown in Fig. 10. Among the samples, the scavenging ability was found to be decreased in the order of RBP1>RBP2>RBP3. Earlier authors have revealed that there was a direct correlation between antioxidant activity and reducing power [41]. Free radicals form stable substances by accepting donated electron, the free radical chain reactions are thus interrupted [42]. The abtained data on the reducing capacity of polysaccharides showed 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

Anti-lipid Peroxidation Activities of Different Polysaccharide Fractions: The data obtained from Fig.11 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 29.2% to 49.3% for RBP1, 10.5% to 27.1% for RBP2 and 9.4% to 22.7% for RBP3 with the concentration increasing from 0.01 to 1.0 mg/ml. Similar results were also found in other plant species [43]. In the tested concentration range, the inhibiting effect of RBP1 was much higher than that of ascorbic acid.

Total Antioxidant Activity of Different Polysaccharide Fractions: ABTS assay is often used in evaluating total antioxidant power of single compounds and complex mixtures of various plants [44]. In this assay, which employs a specific absorbance (734 nm) at a wavelength remote from the visible region and requires a short reaction time, can be used in both organic and aqueous solvent systems and can also be an index reflecting the antioxidant activity of the test samples [45, 46]. Therefore,

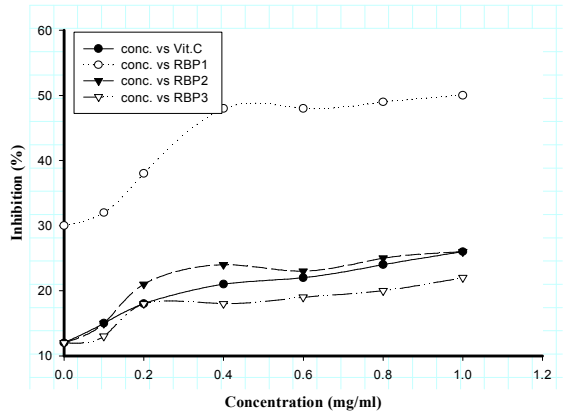

Fig. 11: Inhibition on lipid peroxidation of different polysaccharide fractions from rice bran.

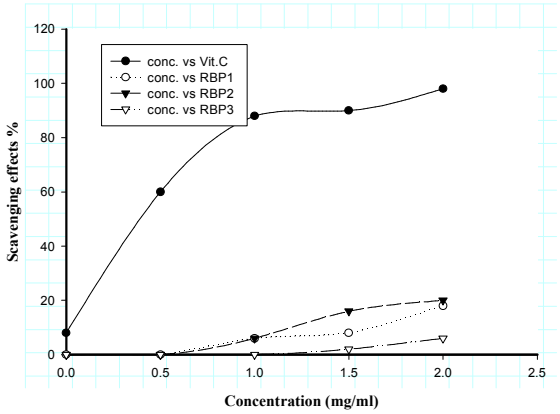

Fig. 12: The scavenging effect of different polysaccharides fractions on ABTS radical.

the antioxidant activity of scavenging ABTS free radical was measured in terms of this method. In the experiment, the scavenging ability of purified polysaccharides (RBP1, RBP2 and RBP3) on ABTS free radical was shown in Fig. 12. As seen from the figure, their activities of all the samples increased in a concentration-dependent manner. The comparison standard Vitamin C showed valuable high radical scavenging activity (85.8-90.1%) in the higher doses (from1.0 to 2.0 mg/ml). RBP1 and RBP3 exhibited very low radical scavenging activity at every. In this experiment, three polysaccharides showed different degree antioxidant effects. The antioxidant abilities of these polysaccharide fractions were supposed to relate to the configuration of the sugar units and monosaccharide compositions. In the infrared spectra analysis, RBP1 exhibited the characteristic absorption of β-configuration. On the contrary, RBP2 exhibited the characteristic absorption of α-configuration. Although RBP3 owned α-configuration of the sugar units, the monosaccharide compositions were different between RBP3 and RBP2. Xylose was found in RBP2, but not in RBP3. Glucose was the major monosaccharide in RBP1 and RBP2, but the amounts of mannose were smaller in RBP2 than in RBP1.

CONCLUSION

According to the results above, it was concluded that the water-extracted crude polysaccharides (RBP) from rice bran extract were purified by DEAE-Cellulose and Sephadex G-200 column chromatography. Three major polysaccharide fractions (RBP1, RBP2 and RBP3) were gained and the purification polysaccharides prepared were conformed of high purity. Antioxidant test indicated that the three polysaccharide fractions from rice bran showed different antioxidant activities with different evaluation system. Moreover, 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.

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