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Characterization and Free Radical Scavenging Activity of Rapeseed Meal Polysaccharides WPS-1 and APS-2

JIANFEI ZHU AND MOUCHENG WU*

Laboratory of Functional Food and Nutrition, College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, People's Republic of China

Two major polysaccharide fractions, WPS-1 and APS-2, were isolated from water-soluble and alkali-soluble extracts of Huaza No. 4 rapeseed meal with a stepwise procedure of D3520 macroporous adsorption resin column chromatography, ethanol precipitation, and DE-52 cellulose column chromatography. Physicochemical properties of the polysaccharides were determined by chemical methods, high -performance liquid chromatography (HPLC), gel permeation chromatography (GPC), and Fourier transform infrared spectrometry (FT-IR). The chemiluminescence (CL) method was used to investigate the free radical scavenging activity of the polysaccharide fractions. The polysaccharides were primarily polymers of arabinose, galactose, and glucose, associated with protein portions consisting of 13 different amino acids. The average molecular masses of WPS-1 and APS-2 were 7.20×10^5 and 1.61×10^5 Da, respectively. Compared with APS-2, WPS-1 was more effective at scavenging superoxide radical ($O_2^{\bullet -}$) and hydroxyl radical ($HO^{\bullet}$), but less effective at scavenging hydrogen peroxide (H_2O_2). In decreasing order, the free radical scavenging activity of WPS-1 and APS-2 toward reactive oxygen species (ROS) was $H_2O_2 > HO^{\bullet} > O_2^{\bullet -}$.

KEYWORDS: Rapeseed meal; polysaccharides; physicochemical properties; free radical scavenging activity; chemiluminescence

INTRODUCTION

Since the 1980s, China has been steadily increasing rapeseed production and become the largest rapeseed-producing country in the world. Most rapeseed grown in China is for edible oil production. At the present, China produces 8.5 million tons of edible oil each year with 3.5 million tons being rapeseed oil. Rapeseed currently ranks as the third source of vegetable oil (after soy and palm) and the third largest source of oil meal (after soy and cotton) (*1*). Rapeseed meal, originating from the process of rapeseed oil extraction, is an important agricultural byproduct, owing to its enriched content of bioactive compounds such as proteins, phenolic compounds, phytic acid, and polysaccharides. The development and utilization of these bioactive compounds have received considerable attention in recent years to increase the profitability of rapeseed as an agricultural commodity (*2*).

Rapeseed meal is currently used as animal feed because it is a valuable source of protein with a well-balanced amino acid composition, but it has the potential to be exploited as a functional additive for further applications (*3*). The phenolic compounds in rapeseed meal can offer natural options as potential antioxidants in lipid-containing systems. Thiyam et al. (*4*) demonstrated that phenolic compounds in rapeseed could be effectively applied to increase the oxidative stability of rapeseed oil. Phytic acid, a common plant constituent, also possesses strong antioxidant properties due to its relatively high binding affinity for iron, making it also a promising food preservative. Stodolak et al. (*5*) found that phytic acid effectively inhibited lipid peroxidation and could increase the stability of both raw and cooked meat, including beef and pork. Previously, rapeseed meal was regarded as a source of pectic substances, using cyclohexanediaminetetraacetic acid (CDTA) (*6*), ethylenediaminetetraacetic acid (EDTA) (*7*), and ammonium oxalate (*8*) as chelating agents for extraction. However, research on hemicellulosic polysaccharides in rapeseed has received little attention until now.

There are many types of free radicals, but those of most concern in biological systems are derived from oxygen and are collectively known as reactive oxygen species (ROS). A role of ROS is becoming increasingly recognized in the pathogenesis of many human diseases, including cancer, aging, and atherosclerosis (*9*, *10*). However, the chain reaction of damage caused by free radicals in the human body can be interrupted by antioxidants. Naturally occurring polysaccharides in many foodstuffs can play an important role as dietary free radical scavengers that aid in the prevention of oxidative damage. To our knowledge, the free radical scavenging activity of polysaccharides from rapeseed meal has not yet been investigated.

* Corresponding anthor (telephone +86 27 87283263; fax +86 27 87396538; e-mail wumch98@mail.hzau.edu.cn).

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In the present study, the preparation of polysaccharides from rapeseed meal was investigated and their structural features were demonstrated. The in vitro free radical scavenging activity of the obtained polysaccharides was also investigated using the chemiluminescence (CL) method.

MATERIALS AND METHODS

Materials. Defatted Huaza No. 4 rapeseed meal was a generous gift of Hubei WaeA Oils & Fats Technology Co. (Hubei, China).

Chemicals and Reagents. D3520 macroporous adsorption resin was purchased from Nankai University Chemical Co. (Tianjin, China); dialysis bags (molecular weight cutoff of 8000 Da) were bought from Beijing Biodee Biotechnology Co. (Beijing, China); DE-52 cellulose was purchased from Whatman Co. (Maidstone, Kent, U.K.); Amino acid standards were obtained from Waters Co. (Milford, MA); mannose (Man), rhamnose (Rha), glucose (Glc), galactose (Gal), arabinose (Ara), fucose (Fuc), glucuronic acid (GlcA), galacturonic acid (GalA), Folin−Ciocalteu reagent, tannic acid, bovine serum albumin (BSA), and luminol were provided by Sigma Chemical Co. (St. Louis, MO); standard dextrans T-2000, T-500, T-70, T-40, and T-10 were bought from Pharmacia Co. (Uppsala, Sweden). Other chemicals were of analytical grade. Double-distilled water prepared in our laboratory was used for all reagents and solutions.

Preparation of WPS-1 and APS-2. Defatted Huaza No. 4 rapeseed meal was extracted twice with 80% (v/v) EtOH (1 g/20 mL) at 80 °C for 2 h to remove most of the phenolic compounds and oligosaccharides. The pretreated rapeseed meal was then air-dried, ground, and extracted three times with water (1 g/28 mL) at 94 °C for 2.9 h. Insoluble material was collected by filtration on filter paper using a Buchner funnel, to produce a filtrate containing water-soluble polysaccharides (solution 1). The insoluble residue on the filter paper was then resuspended and further treated (1 g/20 mL) with 5% NaOH and heated at 60 °C for 2 h. Alkali-insoluble material was removed by filtration with the Buchner funnel, and the filtrate was acidified to pH 5 with 4 mol/L HCl to yield an alkali-soluble polysaccharide fraction (solution 2). Solutions 1 and 2 were each applied to individual D3520 macroporous adsorption resin columns (copoly styrene−divinylbenzene; nonpolar; 0.3−1.25 mm particle diameter; 8.5−9.0 nm average pore diameter) to adsorb free proteins and phenolic compounds. EtOH to 40% concentration was added to the eluate of solution 1, and precipitation was allowed to take place for 5 h. The mixture was then centrifuged (5000 *g*, 15 min), the pellet was discarded, and the supernatant was brought up to a final EtOH concentration of 80%. After another 5 h precipitation period, the mixture was centrifuged again as above. The pellet was dissolved in water and extensively dialyzed against water for 48 h to remove low molecular weight materials. Dialyzed polysaccharides were recovered by lyophilization, to give a water-soluble polysaccharide (WPS) extract. A similar procedure was carried out for the eluate for solution 2, except that the final concentration of EtOH was 60%, not 80%, to give an alkali-soluble polysaccharide (APS) extract.

Twenty milligrams each of WPS and APS was each dissolved in water and applied to individual DE-52 cellulose columns (16 × 250 mm, i.d.). The WPS column was first eluted with water (100 mL), 0.1 mol/L NaCl (200 mL), and then 0.2 mol/L NaCl (200 mL) at 5 mL/10 min/tube, whereas the APS column was first eluted with water (150 mL), 0.2 mol/L NaCl (200 mL), and finally 0.5 mol/L NaCl (200 mL). Eluates from the WPS column were combined into two fractions, referred to as WPS-1 and WPS-2. Four eluate fractions, designated APS-1, APS-2, APS-3, and APS-4, were obtained from the APS column. Each eluate fraction was further dialyzed and lyophilized as described above. This procedure for isolation and purification of rapeseed meal polysaccharides is illustrated schematically in **Figure 1**. WPS-1 and APS-2, as the main fractions, were used for further analysis.

Component Analysis. The carbohydrate content was determined by the phenol− H_2SO_4 method, using Glc as a standard (*11*). The protein content was measured according to the Bradford method, using BSA as a standard (*12*). The phenolic content was measured by the Folin−Ciocalteu method, using tannic acid as a standard (*13*).

Monosaccharide Composition Analysis. Monosaccharides were analyzed by HPLC using precolumn derivatization with 1-phenyl-3-methyl-5-pyrazolone (PMP) (*14*), which was recrystallized twice from methanol before use. Ten milligrams each of WPS-1 and APS-2 was hydrolyzed to monosaccharides by the addition of 2 mL of trifluoroacetic acid. Monosaccharides were labeled with PMP by adding 20 μL of PMP solution (0.5 mol/L in methanol) and 20 μL of 0.3 mol/L NaOH, vortexing, and incubating at 70 °C for 2 h. The mixture was neutralized by adding 20 μL of 0.3 mol/L HCl, and then 500 μL of butyl ether was added and mixed thoroughly by vortexing for at least 5 s. Phase separation was enhanced by brief centrifugation. The organic phase (upper layer) was carefully removed and discarded. This phase partition extraction process was repeated two more times. The resulting aqueous phase was diluted with 150 μL of water for HPLC analysis. A set of monosaccharide standards, 2 mmol/L of Man, Rha, Glc, Gal, Ara, Fuc, GlcA, and GalA, was treated identically as the hydrolyzed samples. The PMP-labeled monosaccharides were analyzed by HPLC (HPLC-335 Varian). An ODS column (250 × 4.6 mm, i.d.; Kromasil) was used for the separation of PMP-labeled carbohydrates at a flow rate of 1 mL/min and UV detection at 245 nm. PMP-monosaccharides were separated in a gradient system consisting of buffer A (50 mmol/L phosphate, pH 6.9 + 15% acetonitrile) and buffer B (50 mmol/L phosphate, pH 6.9 + 40% acetonitrile) run in the following gradient program: 0−10% B in 9 min, to 55% B in 25 min.

Analysis of Amino Acids. Ten milligrams each of WPS-1 and APS-2 was dissolved in 6 mol/L HCl and hydrolyzed at 110 °C for 18 h. The amino acid constituents were analyzed by the HPLC AccQ method (*10*). Separations were carried out on an AccQ Tag C_{18} column (150 × 3.9 mm, i.d.; Waters) with a flow rate of 1.0 mL/min at 40 °C, using gradient elution with mobile phase A (40 mmol/L KH_2PO_4) and mobile phase B [acetonitrile/water = 60/40 (v/v)]. Preliminary calibration of the column was conducted using 24 standard amino acids.

Homogeneity and Molecular Weight. The homogeneity and molecular weight of WPS-1 and APS-2 were determined by GPC in an Agilent 1100 GPC apparatus using a PL aquagel-OH Mixed 8 μm column (300 × 7.5 mm, i.d.; Agilent), eluted with 0.05 mol/L Na_2SO_4, at a flow rate of 0.5 mL/min and at a column temperature of 25 °C. The eluate was monitored with a refractive index detector. The standard dextrans T-2000, T-500, T-70, T-40, and T-10 were used as molecular weight markers. All data provided by the GPC system were collected and analyzed using the Workstation software package.

FT-IR Analysis. WPS-1 and APS-2 were analyzed with FT-IR (Thermo Nicolet Nexus) for detecting functional groups. The FT-IR spectra (KBr pellets) were recorded in a range of 400−4000 cm^{-1}.

Determination of $O_2^{\bullet-}$ Scavenging Activity. The scavenging ability of WPS-1 and APS-2 for $O_2^{\bullet-}$ was determined by the pyrogallol−luminol system on a BPCL Ultraweak luminescence analyzer (Institute of Biophysics, Academia Sinica, Beijing, China) (*15*). Ten microliter samples at different dilutions (using sample buffer solution as control) and 50 μL of pyrogallol (6.25×10^{-4} mol/L) were mixed in the sample cell. The luminescence reaction was initiated soon after 940 μL of a mixture containing luminol (0.1 mmol/L) and carbonate buffer (0.05 mol/L, pH 10.2) was added. The CL intensity integral was recorded every 2 s, and the total CL intensity integral over 300 s was determined. Test conditions were as follows: Hi-V, 800; Kv, −1; spectral range of CL, 180−800 nm; and temperature, 30 °C.

Determination of $HO^{\bullet}$ Scavenging Activity. The scavenging ability of WPS-1 and APS-2 for $HO^{\bullet}$ was measured in a $CuSO_4$−phenanthroline−ascorbate−H_2O_2 CL system (*16*). A 50 μL sample was added to the sample cell (using sample buffer solution as control), and then 50 μL of 50 mmol/L $CuSO_4$, 50 μL of 50 mmol/L 1,10-phenanthroline, and 780 μL of 50 mmol/L sodium tetraborate (pH 9.25) were added sequentially and adequately mixed. Next, 20 μL of 1 mmol/L ascorbic acid and 50 μL of 0.15% H_2O_2 were added to initiate the reaction. The CL curves were obtained at 6 s intervals over a period of 400 s.

Determination of H_2O_2 Scavenging Activity. The scavenging ability of WPS-1 and APS-2 for H_2O_2 was determined by a CL method in the luminol−H_2O_2 system (*17*). A 50 μL sample was added to the sample cell (using sample buffer solution as control). The luminescence reaction was initiated after 50 μL of 0.15% H_2O_2 and 900 μL of a

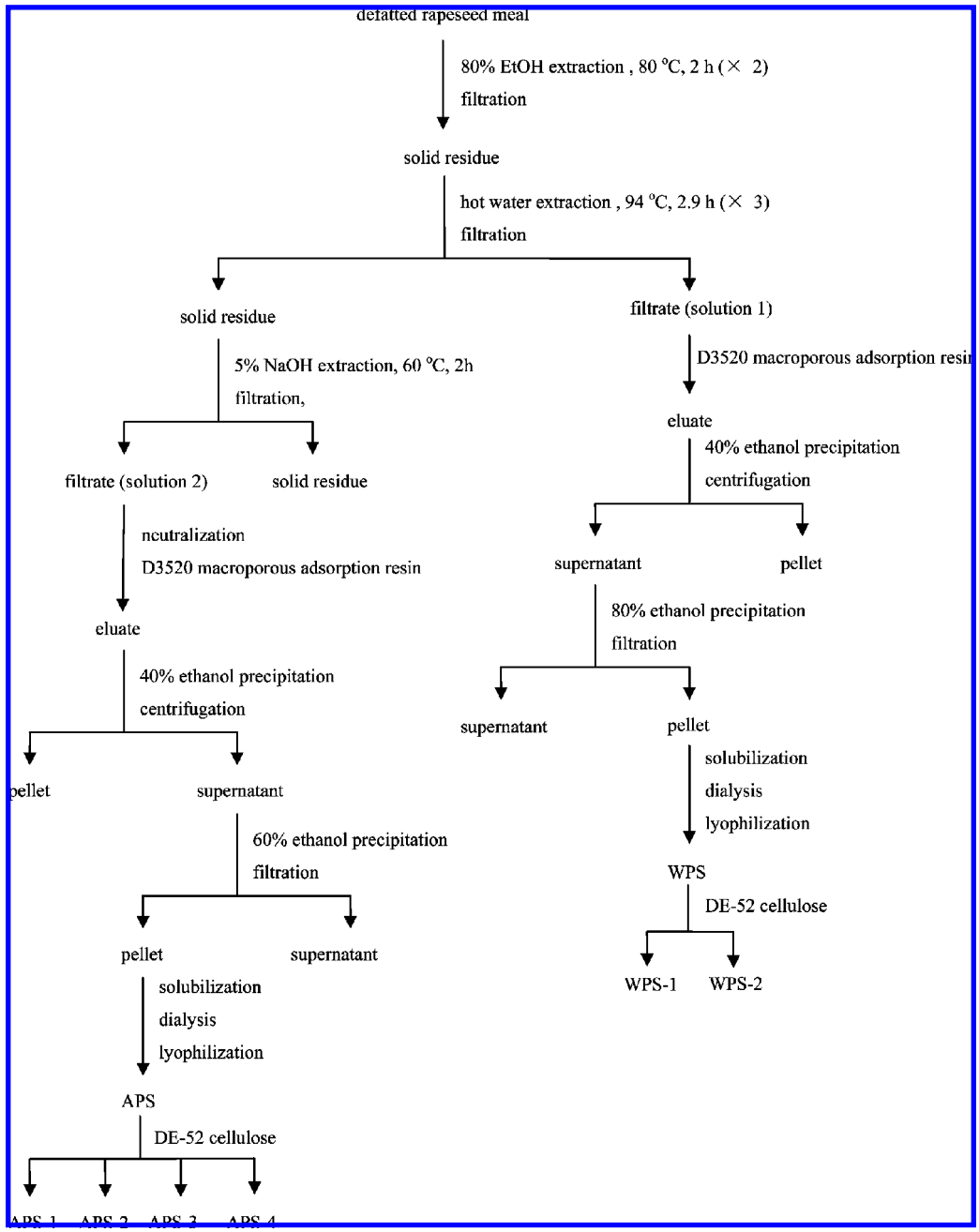

Figure 1. Isolation and purification of rapeseed meal polysaccharides.

mixture containing luminol (0.1 mmol/L) and carbonate buffer (0.05 mol/L, pH 10.2) were added. CL intensity was recorded for 180 s at 2 s intervals.

Data Statistics. Each experiment was performed in triplicate. The integrated area of the curve was used to express the intensity of the relative luminescence. The scavenging activity was calculated according to the equation

$$\text{scavenging activity (\%)} = \frac{CL_{control} - CL_{sample}}{CL_{control}} \times 100\%$$

where $CL_{control}$ is the relative luminscence intensity of the blank control and CL_{sample} is the relative luminescence intensity of the experimental sample.

Statistical analysis was performed using Origin 7.5 software. Analytical data were expressed as means ± SD.

RESULTS

Isolation and Purification of Rapeseed Polysaccharides. The yields of WPS and APS were 0.27 and 2.41%, respectively. DE-52 cellulose anion exchange chromatography allowed the following polysaccharide fractions to be separated from WPS: WPS-1 (eluted with 0.1 mol/L NaCl) and WPS-2 (eluted with 0.2 mol/L NaCl) (**Figure 2A**). From APS, the following fractions were obtained: APS-1 (eluted with water), APS-2 (eluted with 0.2 mol/L NaCl), APS-3, and APS-4 (eluted with 0.5 mol/L NaCl) (**Figure 2B**). The yields of WPS-1 and APS-2 were 74.4 and 69.8%, respectively. The yields of WPS-2, APS-1, APS-3, and APS-4 were too low to be calculated.

Chemical Composition. No reaction occurred with Folin−Ciocalteu reagent, indicating no phenolic compounds in WPS-1 and APS-2. The carbohydrate content, protein content, and monosaccharide composition of WPS-1 and APS-2 are shown in **Table 1**. WPS-1 consisted primarily of Ara (66.6 mol %) and Gal (18.3 mol %) accompanied by some Glc (12.6 mol %) and GlcA (2.51 mol %). APS-2 consisted primarily of Ara (52.8 mol %) and Gal (21.7 mol %) accompanied by some Glc (10.6 mol %), Man (4.98 mol %), GalA (4.82 mol %), GlcA (3.64

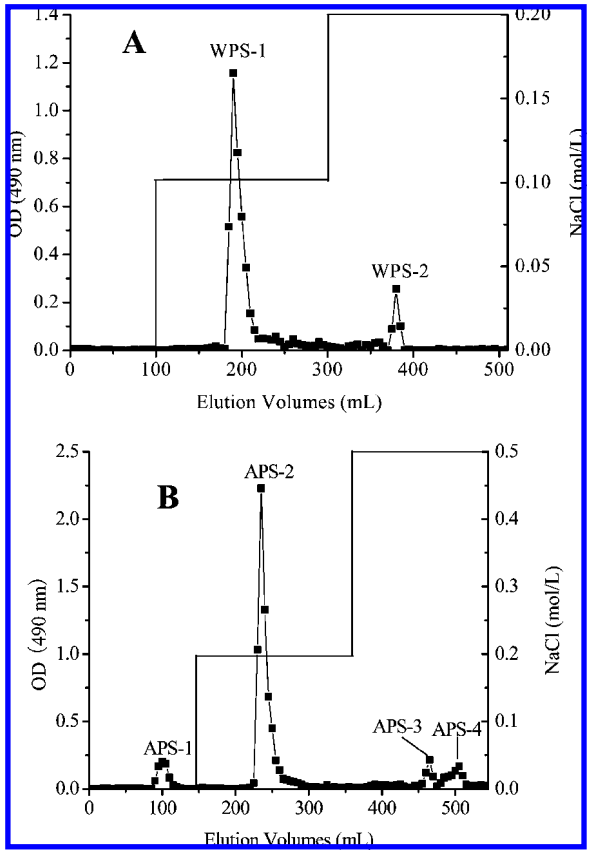

Figure 2. Elution curves of WPS (**A**) and APS (**B**) from DE-52 cellulose columns. Polysaccharide-containing fractions were detected by the phenol−H_2SO_4 method and measuring absorbance at 490 nm.

Table 1. Compositions of WPS-1 and APS-2

sample	WPS-1	APS-2
protein (wt %)	3.42	8.37
neutral sugar (wt %)	83.2	66.8
uronic acid (wt %)	6.09	9.28
monosaccharide compositions (mol %)		
Ara	66.6	52.8
Gal	18.3	21.7
Glc	12.6	10.6
Man	nd[a]	4.98
Rha	nd	1.42
GlcA	2.51	3.64
GalA	nd	4.82

[a] nd, not detected.

mol %), and Rha (1.42 mol %). Amino acid analysis indicated that the protein portion of both WPS-1 and APS-2 consisted of 13 amino acids (**Table 2**). Total amino acid contents of WPS-1 and APS-2 were 3.61 and 8.01%, respectively, which agreed well with the results obtained using the Bradford method (**Table 1**). The results showed that WPS-1 and APS-2 were both proteoglycans consisting of both polysaccharides and proteins.

Molecular Mass Determination. WPS-1 and APS-2 both appeared as single peaks on the GPC (**Figure 3**), indicating that both were homogeneous polysaccharides. The estimated equivalent dextran molecular mass of WPS-1 was 7.20×10^5 Da and that of APS-2 was 1.61×10^5 Da, with reference to standard dextrans.

FT-IR Analysis. The IR spectra of WPS-1 and APS-2 are shown in **Figure 4**. The fractions exhibited a broad O−H stretch vibration band around 3400 cm^{-1} and a weak C−H stretch

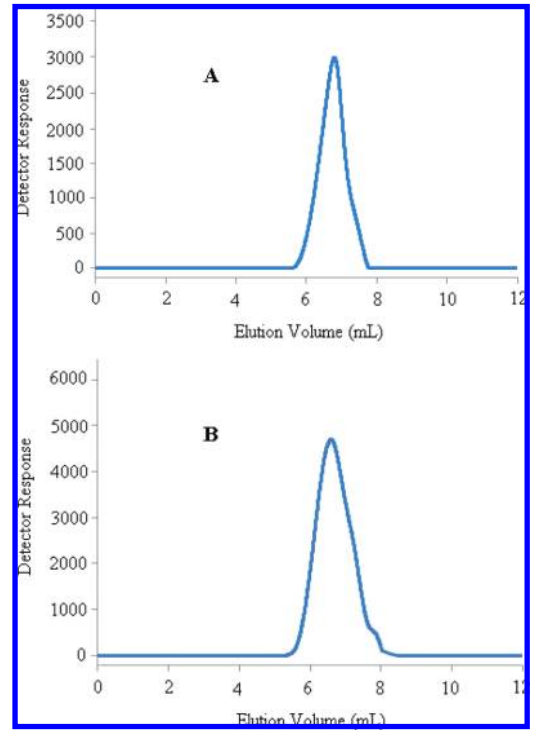

Figure 3. GPC of WPS-1 (**A**) and APS-2 (**B**).

Table 2. Amino Acid Contents of WPS-1 and APS-2

	content (mg/100 mg)	
amino acid	WPS-1	APS-2
aspartic acid	0.28	0.53
glutamic acid	0.42	1.16
serine	0.09	0.19
glycine	0.31	0.24
arginine	0.19	0.90
threonine	0.46	0.63
alanine	0.17	0.24
tyrosine	0.10	2.01
valine	0.42	0.55
cystine	0.21	0.32
isoleucine	0.26	0.41
leucine	0.15	0.26
phenylalanine	0.55	0.57
total	3.61	8.01

vibration band around 2935 cm^{-1}. The peak around 1640 cm^{-1} was a characteristic absorption band of proteins (*18*). The intense band around 1090 cm^{-1} was attributed to the O−H deformation vibration (*19*).

$O_2^{\bullet-}$ Scavenging Activity. Pyrogallol is known to autoxidize in alkaline conditions to generate $O_2^{\bullet-}$, and the decay from the excited state back to the ground state is accompanied by the emission of light (luminescence). Light emission in the pyrogallol−luminol system increased for the first 10 s after the luminescence measurement was initiated, followed by a rapid decline. The scavenging effect for $O_2^{\bullet-}$ was observed in the concentration range of 20−2000 μg/mL when WPS-1 and APS-2 were added. The addition of WPS-1 and APS-2 resulted in an increase in the peak of CL, especially at the high concentrations. However, the integral area of the curve was decreased. This phenomenon indicates further that WPS-1 and APS-2 both had an accelerant−oxidant effect in the initial period, but they had an antioxidant effect with prolonged reaction times. Both of these reactions showed a dose-dependent relationship (**Figure 5A**,**B**). The maximum inhibition rates of

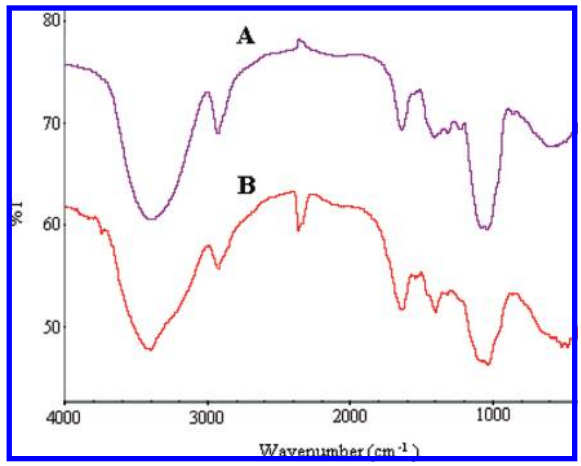

Figure 4. FT-IR spectra of WPS-1 (**A**) and APS-2 (**B**).

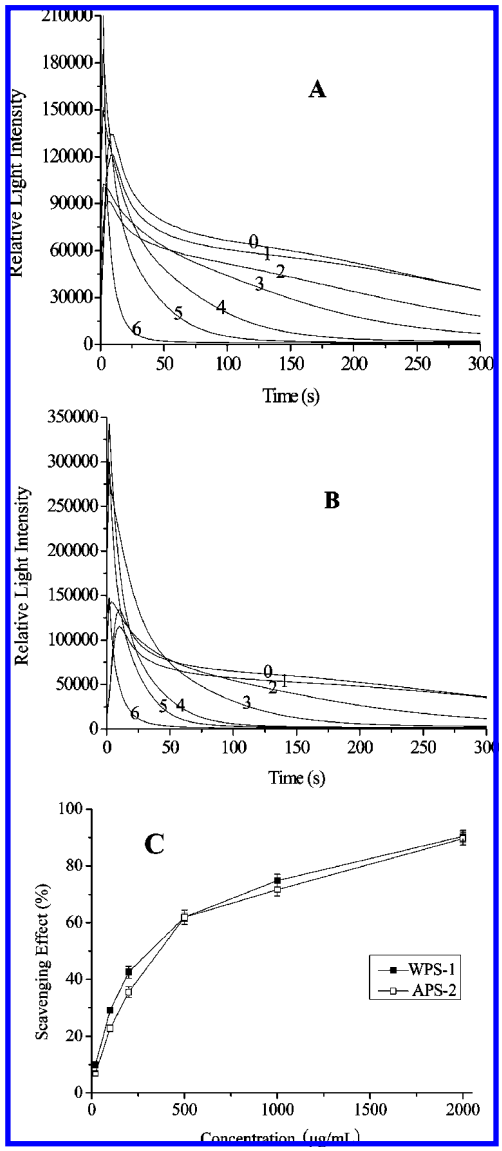

Figure 5. Effect of WPS-1 (**A**) and APS-2 (**B**) on luminescence of the pyrogallic acid−luminol system: 0, 0 μg/mL; 1, 20 μg/mL; 2, 100 μg/mL; 3, 200 μg/mL; 4, 500 μg/mL; 5, 1000 μg/mL; 6, 2000 μg/mL. (**C**) Concentration-dependent inhibitory effect of WPS-1 and APS-2 on $O_2^{\bullet-}$ ($\bar{X} \pm$ SD, $n = 3$).

WPS-1 and APS-2 were 90.4 and 89.6% at the 2000 μg/mL concentration, respectively. Their half-maximal inhibitory concentration (IC_{50}) values were 400 ± 43.8 μg/mL for WPS-1 and 450 ± 64.3 μg/mL for APS-2. WPS-1 was slightly more effective than APS-2 at scavenging $O_2^{\bullet-}$ (**Figure 5C**).

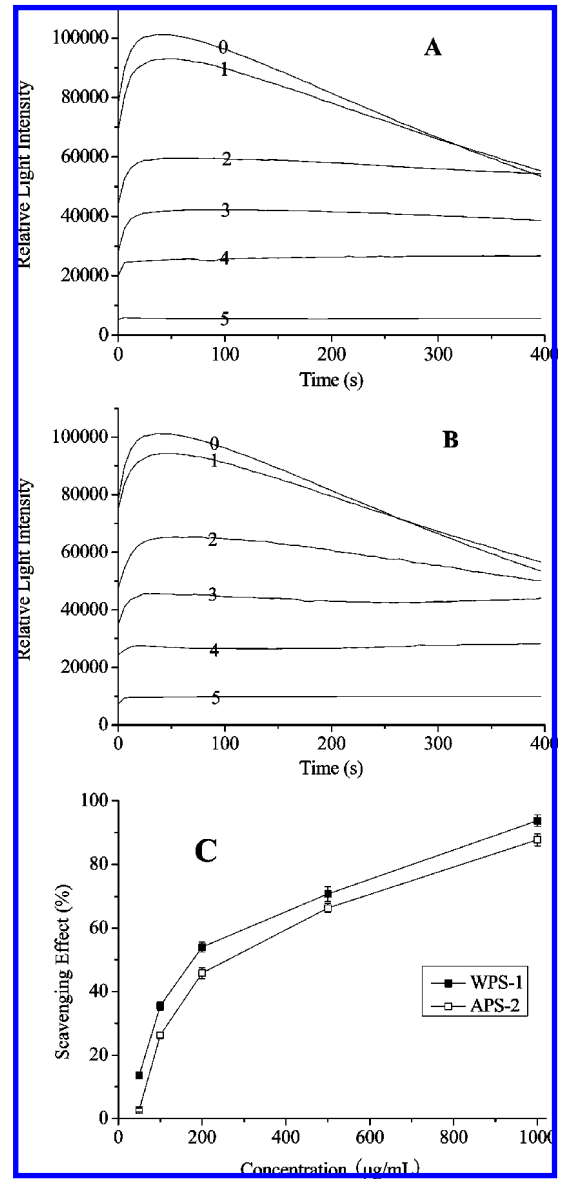

Figure 6. Effect of WPS-1 (**A**) and APS-2 (**B**) on luminescence of the $CuSO_4$−phenanthroline−ascorbate−H_2O_2 system: 0, 0 μg/mL; 1, 50 μg/mL; 2, 100 μg/mL; 3, 200 μg/mL; 4, 500 μg/mL; 5, 1000 μg/mL. (**C**) Concentration-dependent inhibitory effect of WPS-1 and APS-2 on HO$^{\bullet}$ ($\bar{X} \pm$ SD, $n = 3$).

HO$^{\bullet}$ Scavenging Activity. In the $CuSO_4$−phenanthroline−ascorbate−H_2O_2 system, Cu(II) mediates the oxidation of ascorbate, then Cu(I) oxidizes H_2O_2 to generate HO$^{\bullet}$ primarily by the Fenton reaction. Phenanthroline is excited through its oxidation by HO$^{\bullet}$, and its decay from the excited state back to the ground state is accompanied by CL. The kinetics of the reaction showed maximal CL in the initial 50 s after the reactants were mixed, followed by a decline. The effect of WPS-1 and APS-2 were tested in the concentration range of 50−1000 μg/mL. With the addition of samples, the peak CL decreased in a dose-dependent manner (**Figure 6A**,**B**). WPS-1 and APS-2 could both scavenge HO$^{\bullet}$, and the maximum inhibitions of CL by WPS-1 and APS-2 were 93.8 and 87.7%, respectively. The IC_{50} value of WPS-1 was 240 ± 17.5 μg/mL, whereas that of APS-2 was 293 ± 24.4 μg/mL. WPS-1 was more effective than APS-2 at scavenging HO$^{\bullet}$ (**Figure 6C**).

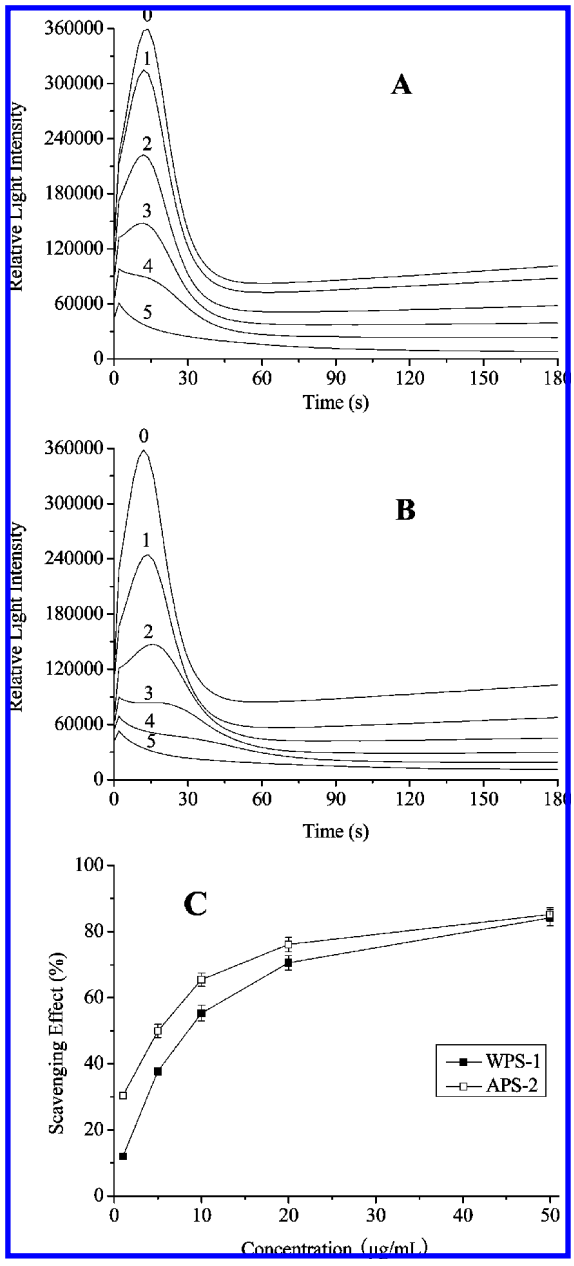

Figure 7. Effect of WPS-1 (**A**) and APS-2 (**B**) on luminescence of the H_2O_2−luminol system: 0, 0 μg/mL; 1, 1 μg/mL; 2, 5 μg/mL; 3, 10 μg/mL; 4, 20 μg/mL; 5, 50 μg/mL. (**C**) Concentration-dependent inhibitory effect of WPS-1 and APS-2 on H_2O_2 ($\bar{X} \pm$ SD, $n = 3$).

H_2O_2 Scavenging Activity. In the presence of oxygen and in an alkaline condition, H_2O_2 can oxidize luminol to produce luminescence. The light emission intensity in the H_2O_2−luminol system increased to the maximum in the first 20 s, followed by a rapid decline. The inhibition of H_2O_2-induced CL in the presence of WPS-1 and APS-2 at different concentrations is shown in **Figure 7**. WPS-1 and APS-2 could effectively inhibit CL formation in a concentration-dependent manner (**Figure 7A**,**B**), indicating that they possess H_2O_2 scavenging ability. The maximum inhibition by WPS-1 was 84.1% and that by APS-2 was 85.2%. The IC_{50} value of WPS-1 was 10.0 ± 0.83 μg/mL, whereas that of APS-2 was 6.05 ± 0.52 μg/mL. It was concluded that APS-2 was more effective than WPS-1 at scavenging H_2O_2 (**Figure 7C**).

DISCUSSION

Excessive ROS production may cause oxidative stress and tissue damage in biological systems. Oxidative stress has been implicated in the pathogenesis of neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. Although each disease has distinctive morphological and biochemical characteristics, the pathology of each is consistent with oxidative damage. Although excessive generation of ROS cannot account for the entire disease process, the specific nature of these diseases suggests that ROS play a role in disease pathogenesis. Because of current interest in ROS, researchers are paying more attention to complex antioxidant materials that widely exist in natural products, and which can act to counteract or scavenge ROS for health protection (*20*).

CL is a powerful analytical technique that has excellent sensitivity, a wide linear dynamic range, and requires relatively simple and inexpensive instrumentation. Moreover, CL is an effective method for research into ROS, because a promising combination exists between the instantaneousness of CL emission and the short lifetime of free radicals. CL measurement has therefore been labeled as an alternative diagnostic and biochemical research tool. In clinical chemistry, CL systems have been widely used to replace radioisotopes in a number of standard laboratory tests. Different classes of CL labels including luminol and lucigenin have been applied to biological assays (*21*).

Since 1970s, rapeseed polysaccharides have been studied. Siddiqui and Wood (*7*) used 2% EDTA followed by 0.5% hot ammonium oxalate to isolate the polysaccharides from rapeseed meal. The ammonium oxalate fraction was found to consist mainly of pectin, heavily branched with mostly Ara and Xyl units. Aspinall et al. (*22*) used 10% NaOH (containing 4% boric acid) as extracting agent to extract rapeseed hulls and isolated a polysaccharide mainly composed of xyloglucan. Aspinall and Jiang (*8*) also isolated a polysaccharide composed of 76% uronic acid from rapeseed hulls using hot ammonium oxalate as the extracting agent. Different results have been reported because rapeseed polysaccharides can have varied monosaccharide compositions, depending on the variety of rapeseed, the extracting agent, and the preparation procedures.

In this study, solutions 1 and 2 were applied to a column of D3520 macroporous adsorption resin, which had excellent adsorption capacity for free proteins and phenolic compounds but nearly no adsorption for polysaccharides. It was assumed that hydrogen bonding, hydrophobic interaction, and $\pi-\pi$ interaction were involved in the adsorption process. The eluates were subjected to precipitation with 40% EtOH. Fractions with high amounts of impurities and low carbohydrate content were discarded following precipitation. WPS and APS were precipitated with 80 and 60% EtOH from solutions 1 and 2, respectively. Solution 2 contained NaCl, which was likely to decrease the concentration of EtOH necessary for precipitation. The amounts of precipitate did not increase with further increases in EtOH concentration.

Early studies on rapeseed polysaccharides were mainly concerned with content and composition. It was not until the 1990s that their structural characteristics began to be investigated. Monosaccharide composition analysis indicated that the molar percentages of Ara in WPS-1 and APS-2 were 66.7 and 52.8 mol %, respectively. Thus, Ara was the major monosaccharide in WPS-1 and APS-2 and suggested the presence of arabinan. From the ^{13}C NMR and ^{1}H NMR spectra of WPS-1 and APS-2 (spectra not shown), an arabinan

fragment agreed well with the results reported by Eriksson et al. (*23*). Therefore, it was assumed that the arabinose moieties of WPS-1 and APS-2 were also mainly (1→5) and (1→2) linked.

The free radical scavenging activity of the two polysaccharide fractions extracted with water (WPS-1) and dilute alkali (APS-2) were compared in this work. WPS-1 and APS-2 both had scavenging activity for $O_2^{\bullet -}$, $HO^{\bullet}$, and H_2O_2; WPS-1 was more effective than APS-2 at scavenging $O_2^{\bullet -}$ and $HO^{\bullet}$, but less effective at scavenging H_2O_2. Referring to IC_{50} values, the free radical scavenging activities of both WPS-1 and APS-2 toward ROS were, in decreasing order, $H_2O_2 > HO^{\bullet} > O_2^{\bullet -}$.

The bioactivity of polysaccharides could be affected by many factors including chemical components, molecular mass, structure, conformation, and even the extraction and isolation methods (*24*). WPS-1 and APS-2 had similar chemical compositions. However, the carbohydrate content of WPS-1 was greater than that of APS-2; the protein content of WPS-1 was less than that of APS-2; and the molecular mass of WPS-1 (7.20×10^5 Da) was larger than that of APS-2 (1.61×10^5 Da).

Liu et al. (*25*) reported that the $O_2^{\bullet -}$ scavenging activity of polysaccharide extracts appears to depend on the amount of protein present as proteoglycans. Tsiapali et al. (*26*) described the free radical scavenging activity of a variety of polysaccharides and demonstrated that their free radical scavenging activity was due, in part, to their monosaccharide constituents. It seemed that the activity of the rapeseed polysaccharides was not a function of a single factor but rather a combination of factors. It was suggested that the antioxidant activity of the rapeseed polysaccharides lies in their ability to act as free radical scavengers, as had been suggested for other sources of polysaccharides. However, further investigation of in vivo antioxidant activity and the mechanisms of WPS-1 and APS-2 free radical scavenging is warranted. In addition, it should be noted that the free radical scavenging models are artificial (in vitro) and the results do not necessarily apply to in vivo models.

In conclusion, polysaccharides may represent one form of bioactive component in rapeseed. To better understand the bioactivity of rapeseed polysaccharides, further in vivo investigation of their antioxidant activity and mechanisms will be carried out in our future work. The exact correlation between the chemical characteristics and the antioxidant activity of rapeseed polysaccharides requires further investigation.

ABBREVIATIONS USED

HPLC, high-performance liquid chromatographic; GPC, gel permeation chromatography; FT-IR, Fourier transform infrared spectrometry; CL, chemiluminescence; $O_2^{\bullet -}$, superoxide radical; $HO^{\bullet}$, hydroxyl radical; H_2O_2, hydrogen peroxide; ROS, reactive oxygen species; CDTA, cyclohexanediaminetetraacetic acid; EDTA, ethylenediaminetetraacetic acid; Man, mannose; Rha, rhamnose; Glc, glucose; Gal, galactose; Ara, arabinose; Fuc, fucose; GlcA, glucuronic acid; GalA, galacturonic acid; BSA, bovine serum albumin; WPS, water-soluble polysaccharide; APS, alkali-soluble polysaccharide; PMP, 1-phenyl-3-methyl-5-pyrazolone; IC_{50}, half-maximal inhibitory concentration.

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