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Degradation of sulfated polysaccharides from *Enteromorpha prolifera* and their antioxidant activities

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ABSTRACT

The effects of degradation on molecular weights (Mws) of polysaccharides from *Enteromorpha prolifera* were investigated. Microwave-assistance could highly accelerate reaction rate. Six representative sulfated polysaccharides (Mw 446.5, 247.0, 76.1, 19.0, 5.0 and 3.1 KDa) were prepared by a microwave-assistance acid hydrolysis method. Chemical analysis and FT-IR spectrum showed only glycosidic linkages were cleft without breaking significant structural units. Antioxidant activities of representative polysaccharides revealed that all samples showed great inhibitory effects on superoxide radical at a low concentration compared to Vitamin C and samples with high Mws exhibited higher inhibitory effects. On the contrary, samples with low Mws possessed stronger inhibitory effects on hydroxyl radical, IC_{50} of Mw 3.1 KDa was 0.39 mg/mL. The chelating effect of Mw 3.1 KDa was 77.3% at 5 mg/mL, which was twice more than initial polysaccharide. The study indicated Mw was the most significant factor to influence antioxidant activities of polysaccharides from *E. prolifera*.

1. Introduction

Recent years green algae belonging to *Ulvae* (including *Ulva* and *Enteromprpha* species) had been frequently involved in algal proliferation in China's Qingdao coastal areas and amounts of them were identified *Enteromorpha prolifera* (Muell.) J. Agardh. High quantity of algae wrack accumulating along shorelines and on the beaches produced smelly odors and arose technological issues concerning, for example, the collection and storage of this algal material. Therefore, it is urgent to find how to achieve better resource use to protect environment and reduce processing cost. It has been reported many bioactive materials in *Enteromorpha* species are considered to be nutritious and low-calorie food (Lahaye & Jegou, 1993). In Asia, *Enteromprpha* species has been used as pharmaceutical product and healthcare food for millennia.

Polysaccharides from *Enteromorpha* species are a group of sulfated heteropolysaccharides and their unique chemical and physicochemical properties make this family of polysaccharides attractive candidates for novel functional and biologically active polymers (Lahaye & Robic, 2007). Many researches proved sulfate polysaccharides from green algae possessed potential antioxidant activities and various classes of them had been shown as potent antioxidants. Costa reported that the sulfate polysaccharides from *Codium isthmocladum* (Chlorophyta) showed antioxidant activity (Costa et al., 2010). Zhang illustrated that all sulfated polysaccharides from three green algae *Ulva pertusa*, *Enteromorpha lina* and *Bryopsis plumose* possessed antioxidant activities in certain assays (Zhang et al., 2010). The studies on antioxidant activities of bioactive compounds from *E. prolifera* mainly focused on polyphenols and flavonoids in it (Ahn, Park, & Je, 2012; Cho, Lee, Kang, Won, & You, 2011) while few works were reported on polysaccharides.

Furthermore, some reports indicated that molecular weights (Mws) of polysaccharide from marine algae had great influence on their antioxidant activities. Hou illustrated fucoidans with low Mws had better hydroxyl radical scavenging activities, reducing powers and superoxide radical scavenging activities (Hou, Wang, Jin, Zhang, & Zhang, 2011). Qi reported that sulfated polysaccharides with low Mws from *U. pertusa* Kjellm had stronger antioxidant activities (Qi et al., 2005). In order to obtain polysaccharides with low Mws, several chemical and physical methods were used including enzymatic methods, acid hydrolysis and oxidative degradation. Aarstad used enzyme engineering to produce different alginate sequences (Aarstad, Tondervik, Sletta, & Skjak-Braek, 2012). Enzymes were highly specific for cleaving glycosidic bonds in the polysaccharide chain but they were still not available for commercial preparation and utilization. Anastyuk obtained oligosaccharide fragments with low Mws from *Costaria costata* by

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mild acid hydrolysis (Anastyuk, Imbs, Shevchenko, Dmitrenok, & Zvyagintseva, 2012). Zhao prepared porphyran with different Mws from *Porhyra haitanensis* by ascorbate and H_2O_2 in combination (Zhao et al., 2006). Among acid hydrolysis and H_2O_2 degradation method, higher concentration or longer reaction time would be unavoidable to obtain products with low Mws, which would violently change structures of sugar units and break necessary bioactivity groups (Qin, Du, & Xiao, 2002).

As an unconventional energy source, microwave irradiation has received a great attention mainly due to considerable saving processing time, improving reaction efficiency and increasing yield of product. Microwave-assistance technology has been successfully applied in biologically active compounds extraction and synthesis, while there are few studies on degradation of polysaccharides from marine algae with microwave-assistance. Yu acquired two sulfated polysaccharides with Mw 151.6 and 28.2 KDa from *U. pertusa* by using a microwave degradation oven (Yu et al., 2003). Sun prepared polysaccharides with different Mws (from 2918 to 256.2, 60.66 and 6.55 kDa) from *Porphyridium cruentum* with microwave irradiation (Sun, Wang, Shi, & Ma, 2009). However, there are no reports about degradation of sulfated polysaccharides from *E. prolifera* with microwave-assistance.

In this study, the effects of degradation with microwave-assistance on Mws of polysaccharides from *E. prolifera* were investigated. A dedicated multimode microwave reactor was employed to precisely control reaction temperature and time. Six representative sulfated polysaccharides with different Mws were prepared and their monosaccharide compositions, sulfated contents and uronic acids were characterized. Finally the relationship between Mws and antioxidant activities of polysaccharides were investigated.

2. Materials and methods

2.1. Materials and equipments

E. prolifera was collected on the Number One Bathing Beach of Qingdao, China 2011. The algae was washed with tap water, air dried, ground into powder and kept in plastic bags at room temperature before being used.

Nitro blue terazolium (NBT), phenazine methosulfates (PMS), nicotinamide adenine dinucleotide-reduced (NADH), hydrogen peroxide (H_2O_2), ferrozine, trichloroacetic acid (TCA) and standard sugars (glucuronic acid, ribose, fucose, mannose, galactose, glucose, rhamnose and xylose) were purchased from Sigma Chemicals Co. All other reagents were of analytical grade. Dialysis membranes were produced by Spectrum Co., Ltd. and molecular weight was cut off at 1000, 3600 and 14,000 Da. Microwave synthesis/extraction reaction station (Type: MAS-II) was purchased from Shanghai SINEO Microwave Chemistry Technology Co., Ltd.

2.2. Preparation of initial polysaccharide from E. prolifera

Dry algae powder (100 g) was extracted with distilled water (7500 mL) at 90 °C for 4 h under continuous stirring. After filtered through gauze, the hot liquid supernatant was centrifuged and filtered by siliceous earth. The residue was washed with additional water and the liquid was collected. The residue was extracted once more under similar condition. The combined solution was concentrated and dialyzed against tap water and distilled water for 48 h respectively, and then the solution was concentrated to about 1000 mL under reduced pressure. The polysaccharide was precipitated by the addition of 4000 mL ethanol then lyophilized to yield white powdered products and referred as IEP (mean yield, 20.3%).

2.3. Preparation of polysaccharides with different Mws

Reaction solution (1%, w/v) was prepared by dissolving appropriate IEP (0.5 g) in distilled water (50 mL). Then hydrogen chloride (HCl) of different volumes was added to the solution, temperatures and times were changed. Microwave irradiation was performed in a laboratory microwave reaction station with a magnetic stirrer and infrared thermometer. The details of the reaction conditions are given in Section 3. At the end of interval time, 0.5 mL reaction mixture was taken out for gel permeation chromatography analysis.

2.4. Characterization

The Mw was measured by an Agilent 1260 gel permeation chromatography (Agilent Technologies, USA) equipped with a refractive index detector. Chromatography was performed on TSK G4000-PW_{xl} column, using 0.05 M $NaNO_3$ aqueous solution as mobile phase at a flow rate of 0.5 mL/min with column temperature at 30 °C. The standards used to calibrate the column were dextrans Mw 1000, 5000, 12,000, 25,000, 50,000, 80,000, 270,000 and 670,000 Da (Sigma, USA).

The molar ratio of monosaccharide composition was analyzed by 1-phenyl-3-methyl-5-pyrazolone (PMP) precolumn derivation HPLC (Zhang, Zhang, Wang, Shi, & Zhang, 2009). Briefly speaking, a solution of sample (10 mg/mL) was hydrolyzed in 2 M trifluoroacetic acid in a 10 mL ampoule. The ampoule was sealed in a nitrogen atmosphere and hydrolyzed for 4 h at 110 °C. Then the hydrolyzed mixture was neutralized to pH7 with sodium hydroxide. Later the mixture was converted into its 1-phenyl-3-methyl-5-pyrazolone derivatives and separated by HPLC.

The total sugar content was analyzed with the phenol–sulfuric acid method using rhamnose as the standard (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956). The protein content was measured according to Bradford's method, using bovine serum albumin (BSA) as the standard (Bradford, 1976). Uronic acid was estimated with a modified carbazole method using glucuronic acid as the standard (Bitter & Muir, 1962). The sulfated content was determined by ion chromatography on Shodex ICSI-52 4E column (4.0 mm × 250 mm) eluted with 3.6 mM Na_2CO_3 at a flow rate of 0.8 mL/min at 45 °C. Fourier transform infrared (FT-IR) spectra of polysaccharides were measured by a Thermo Scientific Nicolet iS10 FT-IR spectrometer in KBr disks.

2.5. Inhibitory effect on superoxide radical assay

Superoxide radical was generated in the PMS–NADH system containing 3 mL Tris–HCl buffer (16 mM, pH 8.0), 338 μM NADH, 72 μM NBT, 30 μM PMS and varying concentration of polysaccharide sample (0.01–0.14 mg/mL). The mixture prepared earlier was incubated at room temperature for 5 min and the absorbance was read at 560 nm against the blank (Nishikimi, Appaji Rao, & Yagi, 1972). In the control, sample was substituted with Tris–HCl buffer. The inhibitory effect on superoxide radical was calculated using the following equation:

$$\text{inhibitory effect}\ (\%) = \left(1 - \frac{A_{\text{sample}}\ 560\,\text{nm}}{A_{\text{control}}\ 560\,\text{nm}} \times 100\right)$$

2.6. Inhibitory effect on hydroxyl radical assay

Inhibitory effect on hydroxyl radical was determined by a modified method of Smirnoff and Cumbes (1989). The reaction system contained 1 mL sodium phosphate buffer (15 mM, pH 7.4), 1 mL 360 μg/mL safranin T, 0.5 mL 2 mM EDTA–$FeSO_4$, 1 mL 3% H_2O_2 and

1 mL polysaccharide solution (0.14–2.22 mg/mL). After incubation at 37 °C for 30 min, hydroxyl radical was detected by monitoring the absorbance at 520 nm against a blank. In the control, polysaccharide sample was substituted with distill water and the H_2O_2 was substituted with sodium phosphate buffer. The inhibitory effect on hydroxyl radical was calculated using following equation:

$$\text{inhibitory effect } (\%) = \left(1 - \frac{A_{\text{sample}}\ 520\,\text{nm}}{A_{\text{control}}\ 520\,\text{nm}} \times 100\right)$$

2.7. Metal chelating effect assay

The ferrous chelating effect was investigated with slight modified method of Decker and Welch (1990). 0.5 mL solution containing polysaccharide sample (0.1–5.0 mg/mL) was mixed with $FeCl_2$ (0.1 mL, 2 mM) and ferrozine (0.4 mL, 5 mM), shook well, stayed still for 10 min at room temperature and the absorbance of mixture was determined at 562 nm. The ferrous chelating effect was given by the following equation:

$$\text{chelating effect } (\%) = \left(1 - \frac{A_{\text{sample}}\ 562\,\text{nm}}{A_{\text{control}}\ 562\,\text{nm}} \times 100\right)$$

2.8. Reducing power assay

The reducing power was determined as described by Yen and Chen (1995). Briefly, 1 mL polysaccharide sample solution (0.25–2.5 mg/mL) in phosphate buffer (0.2 M, pH 6.6) was mixed with 1 mL of potassium ferricyanide (1%, w/v) and incubated at 50 °C for 20 min. Afterwards 2.0 mL of TCA (10%, w/v) was added to the mixture to terminate the reaction. Then the solution was mixed with 1.25 mL ferric chloride (0.1%, w/v) and the absorbance was measured at 700 nm.

2.9. Statistical analysis

All data were shown as mean ± standard deviation (S.D.). Statistical differences between the experimental groups were determined by one way ANOVA, and differences were considered to be statistically significant if $P < 0.05$.

3. Results and discussion

3.1. Influence of degradation on the Mws of polysaccharides

The effects of reaction temperature, concentration of HCl, with microwave irradiation or conventional heating were studied. Samples were taken at interval during the reaction.

Fig. 1A illustrates the influence of different reaction temperatures on the Mws of degraded IEP. 60 °C, 70 °C, 80 °C, and 90 °C were cautiously selected for testing under reaction system of 1 M HCl with microwave irradiation 600 W over 60 min. The degradation rate sustained low level at comparatively low temperature. The Mws slowly changed at 60 °C and 70 °C at the beginning 30 min and decreased to 15 KDa and 5.18 KDa respectively after 60 min. On the contrary, the Mw violently decreased to below 20 KDa at the previous 5 min when temperature reached 80 °C or 90 °C, which implied high temperature was favorable to polysaccharides degradation.

The initial reaction condition was fixed on 70 °C with microwave irradiation 600 W in Fig. 1B. HCl concentration ranged from 0 M to 1 M. Subjected to 0.1 M HCl, the Mw of product decreased to approximately 100 KDa after 60 min, while the Mw sharply decreased to 5.17 KDa when the concentration of HCl reached to 1 M. The degradation reaction still occurred without HCl existence but the degradation efficiency was remarkably increased as the acidity was enhanced.

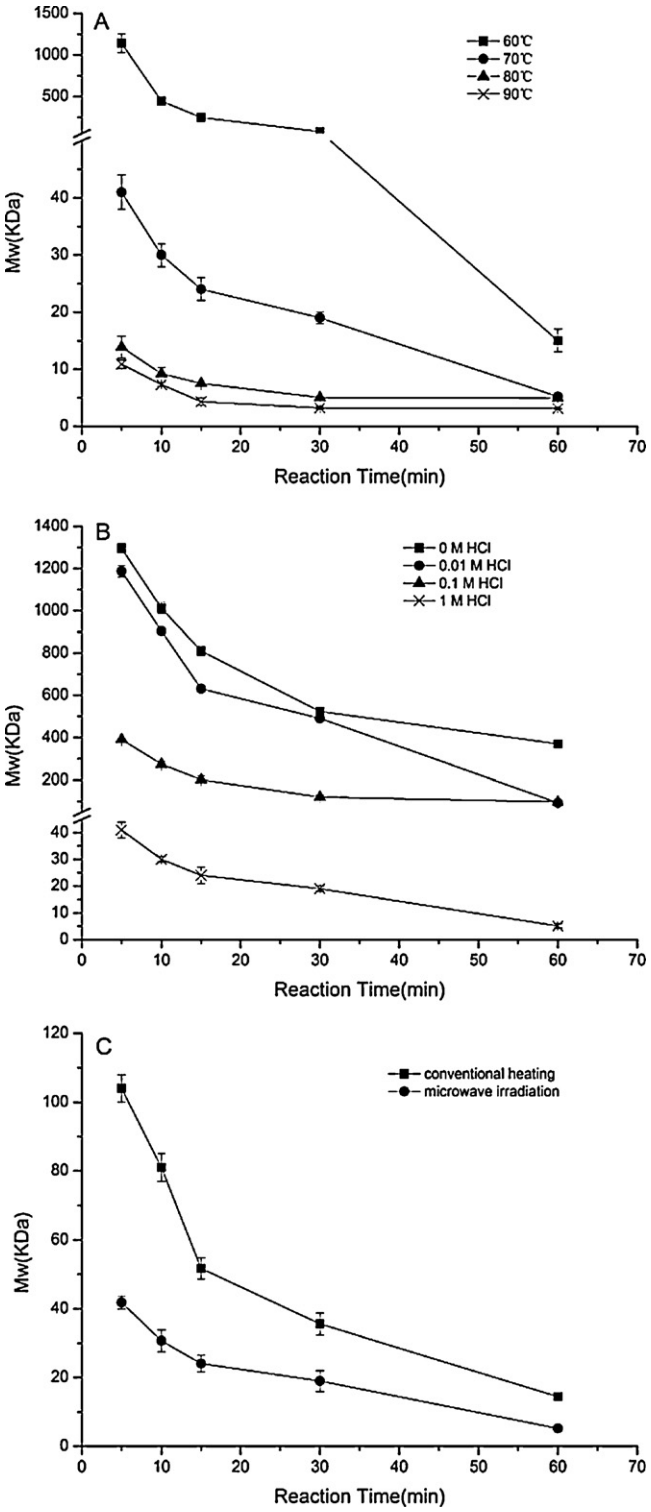

Fig. 1. Effects of reaction temperature (A), concentration of HCl (B) microwave irradiation or conventional heating (C) on the Mws of polysaccharides.

The reaction was carried out using 1 M HCl at 70 °C. As shown in Fig. 1C, the Mw decreased to 41.7 KDa while it was 105 KDa without microwave irradiation in 5 min. The Mw of polysaccharides decreased to 19.0 KDa in 30 min with microwave irradiation while it cost 60 min to reach the same Mw region by conventional heating. During the whole degradation reaction, the acquired Mws

Table 1
Properties of the seven samples.

Sample	Mw/KDa	Total sugar%	Uronic acid/%	Sulfate/%	Protein/%	Yield/%	Molar ratios of the neutral sugar components (rhamnose as 1)					
							Rhamnose	Mannose	Glucose	Galactose	Xylose	Fucose
IEP	–	52.83	26.46	21.98	1.04	–	1	0.054	0.97	0.074	0.18	0.0089
DEP1	446.5	43.03	26.76	24.52	1.12	65.45	1	0.047	0.89	0.074	0.32	0.0145
DEP2	247.0	52.74	27.43	23.17	1.01	64.32	1	0.044	0.91	0.074	0.33	0.0171
DEP3	76.1	49.95	28.9	24.67	0.91	56.13	1	0.045	0.91	0.077	0.31	0.0143
DEP4	19.0	51.08	28.27	21.4	0.58	60.74	1	0.051	0.97	0.083	0.30	0.0134
DEP5	5.0	45.4	30.45	22.65	0.35	40.21	1	0.056	0.97	0.084	0.29	0.0128
DEP6	3.1	46.76	29.76	24.4	0.26	23.78	1	0.054	1.03	0.091	0.28	0.0125

with microwave-assistance were much lower than those of conventional heating, which showed the utilization of microwave highly accelerated reaction rate.

The study showed polysaccharides from *E. prolifera* with different Mws could be prepared by changing reaction condition. The microwave-assistance acid hydrolysis method was feasible. Then a series of chemical analysis of products were accomplished to investigate whether the degradation process might violently damage the structures of sugar units and necessary bioactivity groups.

3.2. Chemical analysis

According to above method, six representative polysaccharides with different Mws were prepared. The chemical compositions of initial polysaccharide (IEP) and degraded polysaccharides from *E. prolifera* with different Mws (DEP1–6) are given in Table 1. Essentially monosaccharide compositions of samples and sulfate contents were closely similar. Rhamnose and glucose were the major proportions (almost 1:1) with small amounts of xylose, mannose and galactose and trace amounts of fucose. The yields of products declined severely as the Mws of samples decreased to below 10 KDa.

The characteristic absorptions of IEP, DEP2, DEP4 and DEP6 were selected to be identified in the FT-IR spectra in Fig. 2. Typical absorption at 842, 1248 and 1642 cm^{-1} were clearly observed for all samples, which were attributed to the bending vibration of C—O—S of sulfate in axial position, the stretching vibration of S=O and C=O respectively. Featured absorption at 788 cm^{-1} might correspond to the bending vibration of C—O—S of sulfate in equatorial position. The chemical analysis implied that during the whole degradation reaction only glycosidic linkages were cleft specially. Those important structural units of initial polysaccharide were not broken, which would not influence the polysaccharide activities significantly (see Fig. 2).

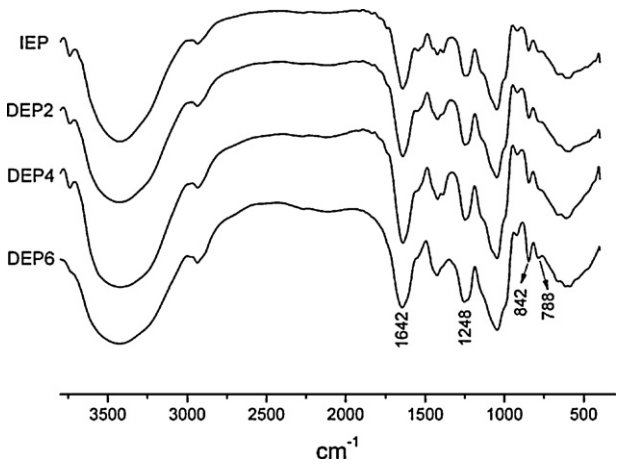

Fig. 2. FT-IR spectra of selected polysaccharides with different Mws.

3.3. Antioxidant activity

3.3.1. Inhibitory effects on superoxide radical

Numerous biological and photochemical reactions could create superoxide anion radical, which indirectly initiates lipid peroxidation as a result of producing other ROS such as singlet oxygen and hydroxyl radical. Superoxide radical was determined by PMS–NADH superoxide generating system and the results are shown in Fig. 3.

All tested samples had inhibitory effects on superoxide radical in a concentration-dependent manner (0.01–0.15 mg/mL). IC_{50} (the concentration inhibiting 50% radical generation) of DEP1–4 were 0.016, 0.0165, 0.0167 and 0.028 mg/mL respectively. Although the IC_{50} of DEP5 and DEP6 could not be read, their inhibitory effects could reach 46.6% and 42.9% when the concentration was as high as 0.14 mg/mL. All the tested samples showed great inhibitory effects at a low concentration compared to Vitamin C, which was reported that the IC_{50} of Vitamin C for superoxide radical was higher than 1.75 mg/mL (Xing et al., 2005). The results suggested that the samples with higher Mws exhibited stronger inhibitory effects compared with lower ones. Qi et al. reported that sulfated polysaccharides with different Mws from *U. pertusa* Kjellm (Chlorphyta) had similar IC_{50} value (0.0221 mg/mL) (Qi et al., 2005) with our results in the inhibitory effects on superoxide radical.

3.3.2. Inhibitory effects on hydroxyl radical

The $EDTANa_2$–Fe(II)–H_2O_2 system was used to generate hydroxyl radical which would discolor safranin T. Added hydroxyl radical inhibitor could inhibit the bleaching. The above mentioned model was used to measure inhibitory effects of all samples on hydroxyl radical. The results indicated that inhibitory effects of the samples (0.14–2.22 mg/mL) were concentration-dependent and

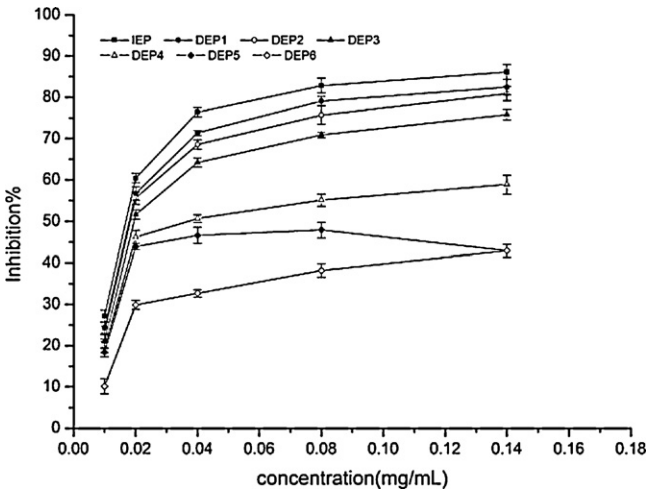

Fig. 3. Inhibitory effects of samples on superoxide radical. Values are means ± S.D. ($n = 3$).

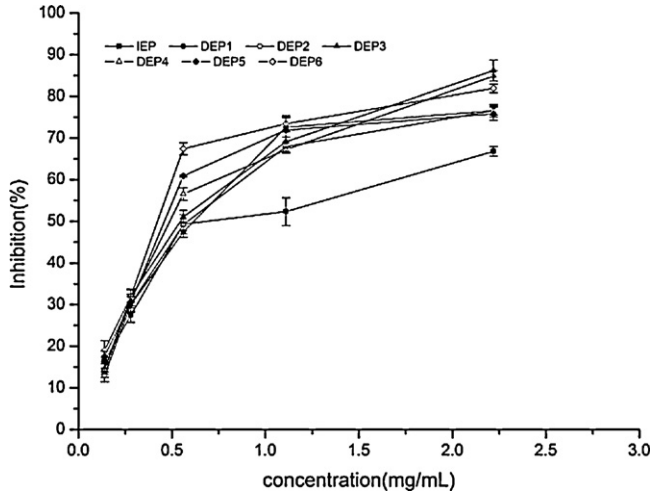

Fig. 4. Inhibitory effects of samples on hydroxyl radical. Values are means ± S.D. ($n = 3$).

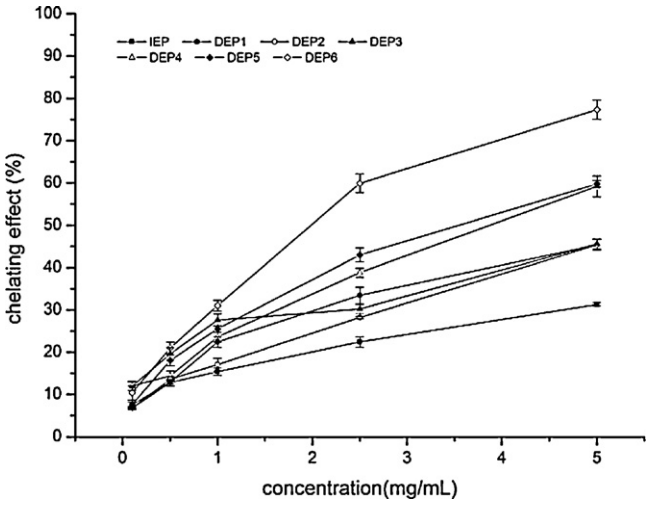

Fig. 5. Metal chelating effects of samples. Values are means ± S.D. ($n = 3$).

the samples possessed higher effects at higher concentration in Fig. 4. The IC_{50} of IEP and DEP1–6 was 0.58, 0.61, 0.56, 0.54, 0.48, 0.42 and 0.39 mg/mL respectively. It was reported that inhibitory effect on hydroxyl radical of Vitamin C was between 50% and 60% at 1.63 mg/mL (Xing et al., 2005), which was higher than all of samples. The results also showed the inhibitory effects on hydroxyl radical were keeping on a high level and enhancing with the decreased Mws.

Comparing with other reactive oxygen species, hydroxyl radical (•OH) has the highest activity which could induce severe damage to organism. Previous studies had reported two types of antioxidant mechanism: suppression against hydroxyl radical generation and cleaning the hydroxyl radical generated (Halliwell, Gutteridge, & Aruoma, 1987; Shon, Kim, & Sung, 2003). The former mechanism is related to the transition of metal ions that catalyze the generation of hydroxyl radicals so the chelating ability may affect the hydroxyl radicals. Earlier researchers suggested both of the two mechanisms might be responsible for the hydroxyl radical inhibitory effects of sulfated polysaccharide (Xing et al., 2004; Yu et al., 2003). Chemical analysis and FT-IR spectra showed all the samples contained sulfate, glucuronic acid and hydroxyl groups which had strong chelating ability. Considering that sulfate contents and glucuronic acid contents were almost equal, we suggested that the samples with lower Mws contained more polysaccharide fractions which might enhance the hydroxyl radical inhibitory effect under same concentration. However, the antioxidants of the tested samples were not determined by single factor but a combination of several factors, varying from spatial structure to monosaccharide composition. The mechanism of *E. prolifera* polysaccharides with different Mws on the hydroxyl radical inhibitory effects need to be further investigated.

3.3.3. Metal chelating effects on ferrous ions

Ferrozine can quantitatively form complexes with Fe^{2+}, which has absorbance at 562 nm. In the presence of other chelating agent, the complex formation is disrupted with the result that the red color of the complexes decreases. The chelating effect is estimated by measurement of color reduction. The chelating effects of ferrous of different samples were concentration-dependent (0.1–5 mg/mL) as shown in Fig. 5. The chelating effect of IEP and DEP1–6 was 31.3%, 45.4%, 45.4%, 45.6%, 59.2%, 59.8% and 77.3% at high concentration 5 mg/mL. The chelating effect of DEP6 (Mw 3.1 KDa) was twice more than that of IEP. The samples with lower Mws showed stronger chelating effects.

Ferrum is known as most important lipid oxidation prooxidant due to its high reactivity. Fe^{2+} could accelerates lipid oxidation by breaking down hydrogen and lipid peroxidase to reactive free radicals vie the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH$) (Qiao et al., 2009). Therefore, metal chelating effects of tested sample to Fe^{2+} would influence its antioxidant activity. Qi reported the metal chelating effects of polysaccharide derivatives with higher sulfate contents were more pronounced than lower ones at high concentration (Qi et al., 2005). Wang suggested the ratio of sulfated/fucose content of sulfated polysaccharide fractions from *Laminaria japonica* was obviously related with the metal chelating effect (Wang, Zhang, Zhang, & Li, 2008). Our results replenished that the Mws of polysaccharides from *E. prolifera* significantly influenced metal chelating effects and the metal chelating effects were consistent with the conclusion of the inhibitory effects on hydroxyl radical, which implied the metal chelating effect might play a significant role in prompting the antioxidant activity.

3.3.4. Reducing powers

In the reaction system, antioxidant substances in samples cause reduction of Fe^{3+}/ferricyanide complex to the Fe^{2+} form. Therefore, Fe^{2+} can be monitored by measuring the formation of Prussian blue at 700 nm (Xing et al., 2008). Higher absorbance value means stronger reducing power of sample. Fig. 6 depicts the reducing

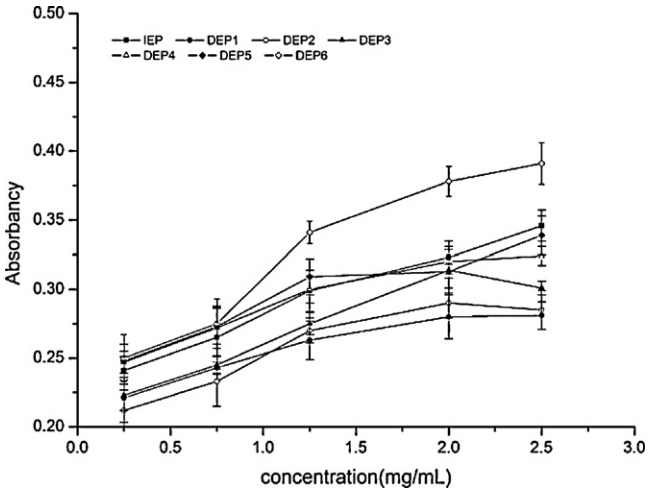

Fig. 6. Reducing powers of samples. Values are means ± S.D. ($n = 3$).

powers of all samples and all of them exhibited concentration-dependent. The reducing power of IEP and DEP1–6 was 0.346, 0.281, 0.285, 0.301, 0.324, 0.339 and 0.391 at 2.5 mg/mL respectively, which implied samples of lower Mws possessed stronger reducing powers. The reducing powers of tested samples were much weaker than those of ascorbic acid, α-tocopherol and BHA (Mau, Chang, Huang, & Chen, 2004) but they were higher than the sulfated polysaccharide fractions extracted from *L. japonica* (all below 0.15 at 2.5 mg/mL) (Wang et al., 2008).

The reducing power was generally associated with the presence of reductones, which had been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. Reductones were also reported to react with certain precursors of peroxide, thus preventing peroxide formation (Song, Zhang, Zhang, & Wang, 2010). The results suggested that all the samples might supply reducing agents or groups to enhance their antioxidant activities.

4. Conclusion

This study established an efficient degradation method of *E. prolifera* polysaccharide with microwave-assistance acid hydrolysis method. Microwave-assistance highly accelerated the reaction rate. Polysaccharides with different Mws could be obtained by strictly controlling reaction condition. Representative polysaccharides with different Mws (Mw 446.5, 247.0, 76.1, 19.0, 5.0 and 3.1 KDa) were prepared and the relationship between Mws and antioxidant activities of polysaccharides was firstly investigated. Compared with conventional heating, the reaction time to prepare products with same Mws was dramatically reduced two third to one half with microwave assistance and the Mw of *E. prolifera* polysaccharides could be decreased to 3.1 KDa.

Initial *E. prolifera* polysaccharide and the polysaccharides with different Mws all possessed antioxidant activities. All the tested samples showed great inhibitory effects at a low concentration compared to Vitamin C and samples with higher Mws exhibited stronger inhibitory effects. On the contrary, the lower Mws samples exhibited stronger inhibitory effects on hydroxyl radical, reducing powers and chelating effects. IC_{50} of 3.1 KDa on hydroxyl radical was 0.39 mg/mL and the chelating effect was 77.3% at the concentration 5 mg/mL. The study indicated the Mw was a significant factor when sulfate content and monosaccharide composition of the samples were similar. However, the mechanism of antioxidant activities in vivo and the safety of sulfated polysaccharides from *E. prolifera* for human consumption should be further researched.

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