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Degradation of *Laminaria japonica* fucoidan by hydrogen peroxide and antioxidant activities of the degradation products of different molecular weights

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ABSTRACT

Conditions of fucoidan degradation by hydrogen peroxide and the antioxidant activities of fucoidans with different molecular weights (Mws) were studied. Fucoidan degradation was monitored by gel permeation chromatography (GPC). Results showed that higher reaction temperature, hydrogen peroxide concentration and reaction time were the main factors that determined the decrease in Mw. There were no significant chemical changes in the backbones of the fucoidans treated with hydrogen peroxide, and the sulphate group contents barely change as compared to the raw polysaccharide. The antioxidant activities of seven fucoidans with different Mws (1.0 kDa, 3.8 kDa, 8.3 kDa, 13.2 kDa, 35.5 kDa, 64.3 kDa, 144.5 kDa) were assessed. The results showed that the relationship between Mws of fucoidans and their antioxidant activities is not simply linear. The samples with Mws of 3.8 kDa, 1.0 kDa and >8.3 kDa have better hydroxyl radical scavenging activity, reducing power and superoxide anion scavenging activity, respectively.

1. Introduction

Fucoidan is a heteropolysaccharide containing substantial amounts of L-fucose and sulphate groups. This molecule is extracted mainly from brown algae. Fucoidan has a variety of biological activities, including those of an anticoagulant, antivirulent, anti-inflammatory agent and antioxidant.

In recent years, the antioxidant activities of fucoidans have been widely studied. The increasing popularity of computers and mobile phones and significant environmental pollution have led to increasing numbers of free radicals in the human body. The presence of free radicals accelerates aging and causes cancer and cardiovascular diseases (Ames, 1983; Stadtman, 1992) by attacking vital biological macromolecules, such as the lipid protein and DNA (Kellogg & Fridovich, 1975; Lai & Piette, 1977; Wiseman & Halliwell, 1996). Antioxidants are able to prevent the formation of free radicals and slow down the progress of chronic diseases. Thus, the use of antioxidants in the food and pharmaceutical industries has become an area of intense interest (Kinsella, Frankel, German, & Kanner, 1993). Letutour found that the extracts of two species of algae, *Laminaria digitata* and *Himanthalia elongata*, could preserve sunflower oil and synergistically enhance the antioxidant activity of vitamin E (Letutour, 1990). Ruperez, Ahrazem, and Leal (2002) demonstrated that sulphated polysaccharides from edible seaweeds could potentially be used as natural antioxidants by the food industry (Ruperez et al., 2002). Wang reported that over-sulphated, acetylated and benzoylated fucoidans exhibited stronger antioxidant activities in vitro (Wang, Liu, et al., 2009).

The antioxidant activity of sulphated polysaccharides depends on several structural parameters, such as the number of sulphate groups, the Mw and the molar ratio of sulphate/fucose and sulphate/total sugar. The Mw plays an important role in antioxidant activity (Zhang et al., 2010). It may be difficult for the crude fucoidan with a higher Mw to permeate the basement membrane and exert its effects in vivo. A low-molecular-weight, sulphated polysaccharide prepared from *Laminaria japonica* had effective scavenging activities for superoxide radical, hydroxyl radical and hypochlorous acid in vitro (Zhao et al., 2004). Another low-molecular-weight, sulphated polysaccharide had a stronger effect against low-density lipoprotein oxidation than the crude fucoidan (Xue et al., 2001). These results indicate that a lower Mw may favour better antioxidant activity.

For the preparation of low-molecular-weight fucoidans, acid, radical and enzymatic methods have been widely used. In the acid method, higher temperature or acidity can lead to lower Mw products as well as a lower sulphated group content, and the sulphated group was necessary for many bioactivities of the polysaccharides (Zhang et al., 2003). Enzymes isolated from bacteria and the digestive glands of marine invertebrates (Holtkamp, Kelly, Ulber, & Lang, 2009) have also been studied. These enzymes are highly specific for cleaving glycosidic bonds in the polysaccharide chain;

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however, the commercially preparation and utilization of these enzymes is still infeasible.

The radical method usually utilises hydrogen peroxide as the catalyst. Miller found that polysaccharides, such as xylan, galacturonan, arabinogalactan and cellulose, could be hydrolysed in the presence of 0.1–10 mM hydrogen peroxide hydrogen (Miller, 1986). Hydrogen peroxide is able to generate reactive oxygen species, such as HOO^-, $HO^\bullet$, and $^\bullet O_2^-$. These radicals degrade the polysaccharides by attacking and breaking the glycosidic linkages. The radical method is mild, and the structures of the sugar units are not significantly changed. This method is a viable alternative for the preparation of low-molecular-weight fucoidans; however, there are few reports that address peroxide degradation and the effect of radical hydrolysis on the antioxidant activities of the sulphated polysaccharides. There is one report published in the *Periodical of Ocean University of China* in Chinese (Dong et al., 2006), but this study introduced metal ions into the hydrolysis process. Although these ions are able to accelerate the reaction rates, they are environmentally unfriendly and must be removed by a cheating resin. For the antioxidant activities, only three kinds of fucoidans with different Mws (raw fucoidan, Mw < 5 kDa and Mw 5–15 kDa) were chosen.

This study examines the depolymerisation of fucoidan using hydrogen peroxide, and factors affecting the Mws of the products are investigated. Seven fucoidans with different Mws, ranging from 1.0 kDa to 144.5 kDa, were then chosen to study the relationship between the Mws and the antioxidant activities of the fucoidans.

2. Materials and methods

2.1. Materials

L. japonica cultured in Shazikou, Qingdao, China, was collected in August 2009. Fucoidan was extracted as described by Wang, Zhang, Zhang, Zhang, and Li (2009). Nitro blue tetrazolium (NBT), phenazine methosulphate (PMS), nicotinamide adenine dinucleotide-reduced (NADH) and standard sugars (glucuronic acid, ribose, fucose, mannose, galactose, glucose, rhamnose and xylose) were purchased from Sigma Chemicals Co. All other commercial reagents were of analytical grade and were used without further purification.

2.2. Degradation of fucoidan

A fucoidan to liquor ratio of 1:30 was used. Fucoidan powder was introduced into a reactor containing liquor at the desired pH and hydrogen peroxide concentration. The pH was adjusted using 0.1 M HCl and NaOH. The reactor was kept in a thermostatic water bath. Every hour, 1 mL of the reaction liquid was removed. After adding sodium bisulphite, the product was analysed using high performance gel permeation chromatography (HPGPC).

For the samples used in activity analysis, after reaction, sodium bisulphite addition and neutralisation, the reaction mixtures were dialysed against tap water for 48 h and distilled water for 24 h using dialysis membranes with a 1 kDa Mw cut off. The liquid was concentrated with a rotary evaporator under diminished pressure at 50 °C and then centrifuged. The supernatants were lyophilised.

2.3. Analytical methods

The Mws were determined by HPGPC with a TSKgel G3000PWxl column. The mobile phase was 0.2 M Na_2SO_4 aqueous solution, and the flow rate was 0.5 mL/min. The column temperature was maintained at 40 °C, and samples were detected by a refractive index detector (Shimadzu RID-10A). Dextran standards were used to calibrate the column. All data were recorded and processed using the Shimadzu LC-Solution software.

Neutral sugars were analysed by 1-phenyl-3-methyl-5-pyrazolone (PMP) precolumn derivation HPLC. The details have been described previously (Zhang, Zhang, Wang, Shi, & Zhang, 2009). The following neutral sugars were analysed: fucose, mannose, galactose, glucose, rhamnose and xylose.

The total sugar and fucose contents were analysed with the phenol-sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and cysteine hydrochloride method, respectively (Gibbons, 1955), using fucose as the standard. Uronic acid was estimated with a modified carbazole method (Bitter & Muir, 1962) using glucuronic acid as the standard. Sulphate group content was analysed using the barium chloride-gelatin method (Kawai, Seno, & Anno, 1969). Infrared spectra were recorded for polysaccharide powder in a KBr pellet on a Nicolet-360 FTIR spectrometer.

2.4. Antioxidant activity assays

Superoxide anion scavenging activity was assessed by the modified method of Nishikim (Nishikim, Appaji, & Yagi, 1972). The 4.5 mL reaction system contained 16 mM Tris–HCl buffer, pH 8.0, 338 μM NADH, 72 μM NBT, 30 μM PMS, and varying concentrations of samples that ranged from 5 to 100 μg/mL. The mixture was incubated at room temperature for 5 min, and the absorbance was measured at 560 nm against a blank. The control was prepared as above without any sample. The superoxide radical scavenging ability was calculated from the following equation:

$$\text{Scavenging activity}\ (\%) = \left(1 - \frac{A_{\text{sample 560 nm}}}{A_{\text{control 560 nm}}}\right) \times 100\% \quad (1)$$

Hydroxyl radical scavenging activity was determined by a modified method of Smirnoff and Cumbes (Smirnoff & Cumbes, 1989). The reaction system contained 1 mL of sodium phosphate buffer (15 mM, pH 7.4), 1 mL of 360 μg/mL safranin T, 0.5 mL of 2 mM EDTA-$FeSO_4$, 1 mL of 3% H_2O_2, and 1 mL of 4–20 mg/mL sample. 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, sample was substituted with distilled water, and the H_2O_2 was substituted with sodium phosphate buffer. The hydroxyl radical scavenging ability was calculated using the following equation:

$$\text{Scavenging activity}\ (\%) = \frac{A_{\text{sample 520 nm}}}{A_{\text{control 520 nm}}} \times 100\% \quad (2)$$

Reducing power was measured according to Yen's method (Yen & Chen, 1995). The buffer was 0.2 M sodium phosphate solution, pH 6.6. The samples at different concentrations (2–10 mg/mL) were dissolved in 1.25 mL of distilled water and mixed with potassium ferricyanide (1.25 mL, 1% w/v) in buffer. The mixture was incubated at 50 °C for 20 min. Then, 2.5 mL of trichloroacetic acid (10% w/v) was added to terminate the reaction. Afterwards, 1.5 mL of aqueous ferric chloride solution (0.1% w/v) was added. The absorbance was measured at 700 nm after deposition for 30 min.

2.5. Statistical analysis

All the data are shown in means ± S.D. ($n = 3$) within significance $p < 0.05$ after passing Duncan's multiple-range test. The results were processed by computer programmes: Excel and Statistica software (2003).

3. Results

3.1. Influence of reaction conditions on the Mws of products

The effects of pH, H_2O_2 concentration, reaction temperature and time were studied. Samples were taken at intervals during the hydrolysis.

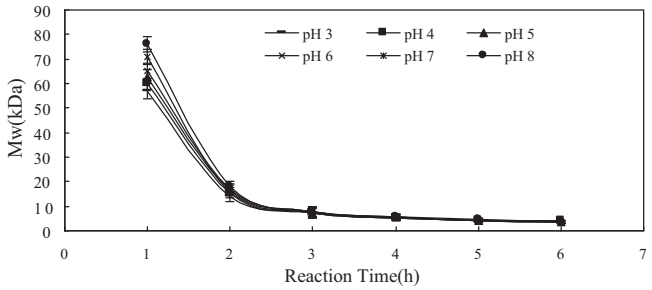


Fig. 1. Effect of pH on the Mws of degraded fucoidans (0.2 M H_2O_2, temperature 70 °C).

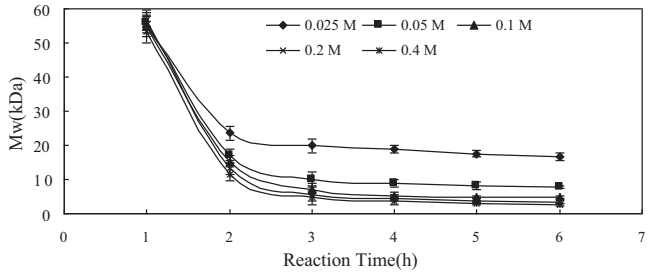


Fig. 2. Effect of H_2O_2 concentration on the Mws of degraded fucoidans (pH 3, temperature 70 °C).

Fig. 1 shows the Mw changes of fucoidans treated at different pH and time conditions in 0.2 M H_2O_2 and 70 °C. The pH ranged from 3 to 8, and the reaction time varied from 1 to 6 h. In the first 2 h, higher acidity generated lower Mw polysaccharide products, and the Mw was reduced sharply. After the second hour, the effect of pH was trivial, and the Mw changed only slightly.

Fig. 2 shows the change in Mw as the concentration of H_2O_2 was varied from 0.025 M to 0.4 M and the reaction time was varied from 1 h to 6 h. The reaction temperature was fixed at 70 °C, and pH 3 conditions were used. In the first hour, the Mws of the products obtained at different H_2O_2 concentrations were similar. However, in the first hour, curves for 0.025 M, 0.05 M and 0.1 M H_2O_2 are more distant than those for 0.1 M, 0.2 M and 0.4 M H_2O_2. In the sixth hour, the Mws are approximately 16.7 kDa, 7.7 kDa, 4.8 kDa, 3.4 kDa and 2.6 kDa, corresponding to original H_2O_2 concentrations of 0.025 M, 0.05 M, 0.1 M, 0.2 M and 0.4 M, respectively. The trend of Mw reduction, based on reaction time, is similar to that observed in Fig. 1.

To evaluate the influence of reaction temperature and time on the Mws of degraded fucoidans, temperatures of 30 °C, 50 °C, 70 °C, 90 °C were selected for testing under conditions of 0.2 M H_2O_2 at pH 3. Fig. 3 shows the changes observed over 6 h. At 30 °C, the reduction

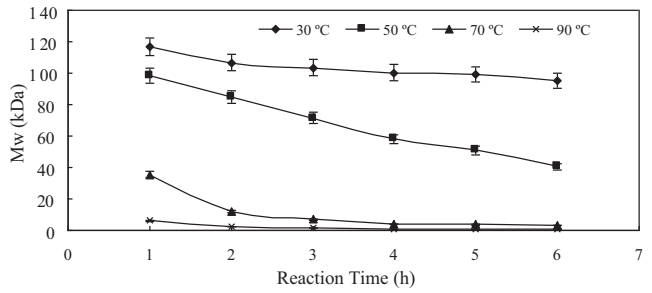


Fig. 3. Effect of reaction temperature on the Mws of degraded fucoidans (pH 3, 0.2 M H_2O_2).

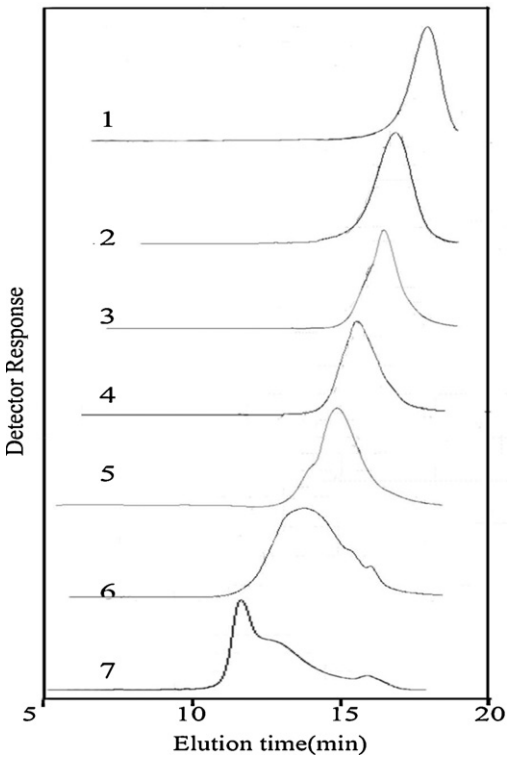


Fig. 4. HPLC profiles of the seven samples.

in Mw occurs slowly. The average Mw was above 100 kDa in the sixth hour, and the HPGPC profiles showed more than one crest and were similar to that of the raw fucoidan (diagram not shown). After 72 h at 30 °C, the Mw reduced to approximately 10 kDa. When the temperature was 50 °C, there were two crests in the first 3 h in the chromatogram and one peak in the fourth hour. The Mw was approximately 40.7 kDa in the 6th hour, 5.7 kDa in the 24th hour, and 3.0 kDa in the 48th hour. Unlike the reactions at 30 °C and 50 °C, the reduction in the Mw happened primarily in the first 2 h at 70 °C and 90 °C. The Mw was less than 1 kDa after 4 h at 90 °C.

3.2. Chemical analysis

Different Mws of the polysaccharides were prepared according to the above studies (Table 1). Samples 1–6 were the degraded fucoidans, and sample 7 was crude fucoidan. The sample Mw increased from 1 to 7.

The characteristics of the seven samples were described in Table 1. And Fig. 4 was HPLC profiles of the samples. Polydispersity index PD (Mw/Mn) was increased with the Mw. The total sugar and fucose contents of samples 1 and 2 are clearly less than those of other samples. Samples 1 and 2 contain approximately 42% and 49% total sugar, respectively, while samples 3–7 are more than 60% total sugar. The glucuronic acid contents of samples 1, 2 and 3 are lower than those of the other samples. All of the samples have similar sulphate group content, ranging from 30% to 35%. The product yields decreased with the reduction in Mw.

Neutral monosaccharide compositions of the seven samples are also shown in Table 1. There was no difference in the types of analysed monosaccharides. If the fucose content is set as 1.00, the molar ratios of other neutral sugars are reduced to a different degree.

FTIR profiles (data not shown) exhibit no significant changes among samples 1–7. All samples had the specific absorptions for fucoidans. No new bands emerged.

Table 1
Properties of the seven samples.

Sample	Mw/kDa	Mw interval/kDa	PD	Total sugar/%	Fucose/%	Glucuronic acid/%	Sulphate/%	Yield/%	Molar ratios of the neutral sugar components (fucose as 1.00)					
									Fucose	Galactose	Mannose	Glucose	Xylose	Rhamnose
1	1.0	0.8–4.4	1.17	42.21 ± 0.22	30.51 ± 0.38	4.18 ± 0.19	30.80 ± 0.12	41.91	1	0.10	0.08	0.05	0.03	0.01
2	3.8	1.2–8.6	1.42	49.22 ± 0.18	32.89 ± 1.43	4.65 ± 0.29	32.10 ± 0.02	64.09	1	0.13	0.06	0.03	0.02	0.01
3	8.3	3.5–18.2	2.82	60.32 ± 0.13	40.48 ± 2.75	5.26 ± 0.34	34.71 ± 0.31	68.28	1	0.15	0.10	0.03	0.03	0.02
4	13.1	4.8–24.1	3.07	66.90 ± 1.03	44.91 ± 1.88	6.24 ± 0.10	30.29 ± 0.66	73.36	1	0.15	0.09	0.04	0.03	0.02
5	35.5	10.5–65.2	3.66	66.49 ± 0.64	44.62 ± 1.47	6.15 ± 0.71	30.15 ± 0.33	78.31	1	0.16	0.10	0.07	0.04	0.02
6	64.3	20.4–112.6	4.01	63.54 ± 0.09	44.29 ± 1.10	6.56 ± 0.11	33.05 ± 1.28	83.85	1	0.16	0.11	0.06	0.04	0.02
7	144.5	43.1–214.7	4.58	67.551 ± 0.36	45.37 ± 0.28	6.74 ± 0.25	30.61 ± 0.35	100.00	1	0.18	0.12	0.06	0.04	0.02

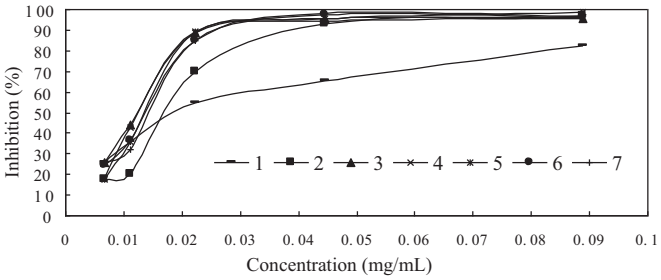


Fig. 5. Inhibitory effects of the seven samples on superoxide anions.

3.3. Antioxidant activities

Superoxide anions were generated in a PMS–NADH system, and the radicals cause the NBT to turn blue. The absorbance values indicate the superoxide radical content. The scavenging activity of the samples was calculated from Eq. (1) as described in Section 2.4. The results are shown in Fig. 5. All seven samples at the analysed concentration had inhibitory effects on superoxide anion. The weakest sample was sample 1, followed by sample 2. The other samples exhibited similar inhibitory effects.

The EDTANa_2–Fe(II)–H_2O_2 system was used to generate hydroxyl radical, and the radicals depigment safranin T. The ability of samples 1–7 to inhibit hydroxyl radical was determined by colourimetry. The scavenging activity of the samples was calculated from Eq. (2) as shown in Section 2.4. By measuring the scavenging activity of seven samples for the hydroxyl radical, we found the relationship between the activity and Mw was so complicated that seven samples was not sufficient. Thus, another five samples with Mw of 1.8 kDa, 6.1 kDa, 7.5 kDa, 20.3 kDa and 80.3 kDa were investigated. Fig. 6 shows the results for these samples. There were two peaks in the diagram; one peak was in the Mw range of 1.5–4.0 kDa, and the other peak was approximately 80 kDa. In these Mw regions, the samples possessed higher inhibiting effects. The degrees of inhibition for sample with Mw in the range of 10.0–20.5 kDa were less than 20%.

The reducing power was reflected in the colour change from yellow to green as the antioxidants reduced the Fe^{3+}-ferricyanide complex to the ferrous form. Fig. 7 shows the relationship between the absorbance and sample Mw. Higher absorbance meant stronger reducing power. For samples 1–4, reducing power increased sharply with the reduction in Mw. For samples 4–7, when the Mw is more than 13.2 kDa, the reducing power increased slowly with increasing Mw. The three aspects of antioxidant activity that were assessed in these experiments are all concentration dependent.

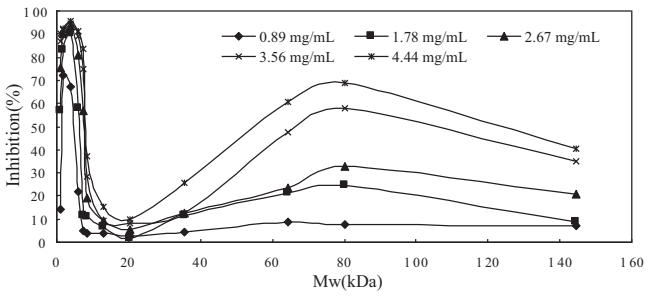


Fig. 6. Inhibitory effects of the seven samples on hydroxyl radicals.

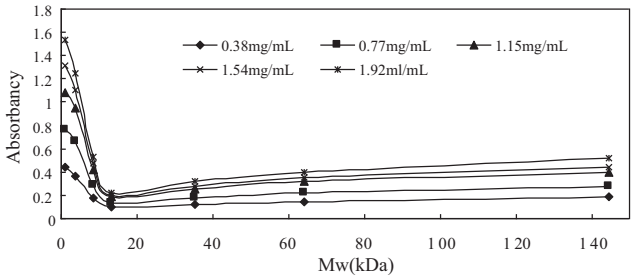
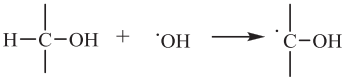


Fig. 7. Reducing power of the seven samples.

4. Discussion

4.1. Degradation by hydrogen peroxide

In the present study, we used hydrogen peroxide as a catalyst to degrade the fucoidan from *L. japonica*. Hydrogen peroxide can generate active radicals, such as HOO^-, $HO^\bullet$ and $O_2^\bullet$, which are generally regarded as the primary effective species. These free radicals possess an unpaired electron, and they can abstract a C-bonded H atom:



A previous study suggested that attacking an H atom bonded with C1, C4, or C5 on the (1→4) linked polysaccharide chain would lead to the scission of the glycosidic bond, and H-abstraction from C2, C3, or C6 produced a relatively stable glycosulose residue (Schuchmann & Sonntag, 1978). Fucoidans from *L. japonica* are primarily (1→3)-linked alpha-L-fucopyranose residues with a few (1→4)-alpha-L-fucopyranose linkages (Wang, Zhang, Zhang, Zhang, & Niu, 2010), and the attack is non-selective. Thus, if the radicals abstract the H atom from C1, C4, or C5, the polysaccharides will be degraded.

The reaction time, temperature, pH, and H_2O_2 concentration were generally considered to be the potential factors influencing the polysaccharide degradation.

The pH of the reaction mixture affect the generation efficiency of free radical, and accordingly alter the degradation rate. Fig. 1 shows that decreasing pH (pH 3–7) results in lower Mw products in the first 2 h. From the third hour onward, the H_2O_2 content is less than before, and the number of free radicals is smaller; thus, the pH has little effect. The report by Rota et al. suggested that lower pH was accompanied by a much faster depolymerisation (Rota et al., 2005). They found that there was a considerable amount of hydroxyl radical species at the earliest time points. Carbon-centred free radicals from hydrogen abstraction on the polysaccharide fragments appeared at later time points under neutral pH but disappeared at lower pH conditions.

The concentration of H_2O_2 is also critical to the reaction. The production of free radical depends on the amount of H_2O_2 in the reaction mixture. At the first hour, all H_2O_2 concentrations generated similar products (Fig. 2). The differences emerged during hours 2–6 and became smaller with the increase in H_2O_2 concentration. The H_2O_2 content in the first hour is much higher than that at later time points, and the free radicals generated by 0.025 M H_2O_2 is sufficient for the reaction. As time passed, the remainder of H_2O_2 from the higher initial concentration is more than that remaining from the lower initial concentration. Therefore, the differences were observed to begin at the second hour. The difference in the values between each concentration is then almost constant. This result may be because the H_2O_2 concentration in every reaction was stable at a small value and played a negligible role in degradation. To generate lower Mw products, continuous addition of H_2O_2 during the reaction, as described in other reports, is an alternative method (Petit et al., 2006); however, the H_2O_2 concentration at one certain reaction time is also difficult to be determined.

Reaction temperature is another significant parameter examined in this study. Generally, an increase in temperature leads to an increase in the reaction rate. A higher temperature implies higher average kinetic energy of the molecules and more collisions per unit time (Yue et al., 2008). Fig. 3 shows that higher temperatures produced lower Mw polysaccharides; however, high temperature would destroy the sugar unit and decompose the H_2O_2. This result is evidenced by sample 1 as shown in Table 1. Sample 1 was prepared at a temperature of 90 °C, and its total content is considerably lower than those of the other samples. Usually, extension of the reaction time has a positive impact on the reaction degree. This result was seen in reactions at 30 °C and 50 °C (Fig. 3). However, when the temperature was 70 °C or 90 °C, the reduction in the product Mw primarily occurred in the first 2 h (Figs. 1–3). The main reason for this result is that high temperature led to the decomposition of H_2O_2, which could be observed in the HPLC spectrum (data not shown). Therefore, low-molecular-weight fucoidans could be prepared at lower temperatures, such as 50 °C, by prolonging the reaction time. However, an extremely low reaction temperature is not advisable. The products after 72 h reaction at 30 °C have Mw higher than 10 kDa. Moreover, the decrease in glycosidic linkages may also cause a decrease in the reaction rate.

Fucoidans with different Mw could be obtained by hydrolysis using hydrogen peroxide degradation under varying reaction conditions, such as the samples shown in Table 1. The lower Mw samples have a lower yield because they were produced under more severe conditions, which may destroy the sugar unit. Regardless of the reaction environment, there was no significant change in the content of sulphated groups (Table 1). Sulphated groups are necessary for the bioactivities of fucoidan. Based on the FTIR spectra (data not shown), no new chemical groups were present. Unlike previous reports, oxo groups were introduced into the products of the free radical degradation (Miller & Fry, 2001; Qin, Du, & Xiao, 2002; Zhao et al., 2006).

4.2. Antioxidant activities of fucoidans with different Mw

In this work, we studied the antioxidant activities of fucoidans on the basis of superoxide anion radical scavenging ability, hydroxyl radical scavenging ability and reducing power.

Superoxide anion radical ($^\bullet O_2^-$) is generated by numerous biological and photochemical reactions and is highly toxic (Banerjee, Dasgupta, & De, 2005). It can generate stronger active oxidants, such as singlet oxygen and hydroxyl radicals, which cause peroxidation of lipids (Koryckadahl & Richardson, 1978). The ability to scavenge superoxides is ascribed to potential antioxidant activity of fucoidans. Sulphate content is a factor in fucoidan superoxide anion scavenging ability (de Souza et al., 2007; Zhang et al., 2003). Our samples have similar sulphate content, so the effect of the sulphate content could be avoided. The results shown in Fig. 5 suggest that the lower Mw samples (samples 1 and 2) have weaker scavenging activity while the other samples exhibit similar activity. In studies performed by Qi et al. (2005) with ulvans, a sulphated polysaccharide, the samples with different Mws (151.7, 64.5, 58.0 and 28.2 kDa) had similar IC_{50} values in the scavenging of superoxide radicals. Zhou, Wang, Ma, and He (2008) also demonstrated that the decrease in Mw did not strengthen superoxide anion radical scavenging activity of sulphated polysaccharides from *Porphyra yezoensis*. In addition to a lower Mw, the total sugar content of samples 1 and 2 are also much lower than those of the other samples. It companies with less hydroxyl group (–OH). This difference may cause the decline in the observed activity.

Hydroxyl radicals (HO•) have the highest activity among reactive oxygen species and induce severe damage to biomolecules. The results shown in Fig. 6 suggest that the relationship between the Mw and hydroxyl radical scavenging activity is complicated. There is more than one peak and trough in the diagram. There are at least two mechanisms for the antioxidant activity in vitro: hydroxyl trapping and metal chelation. For the former, a previous study demonstrated that a decrease in Mw enhanced the scavenging power for the hydroxyl radical (Xing et al., 2005). The latter mechanism is related to the transition of metal ions that catalyse the generation of hydroxyl radicals, so the chelating ability may affect the hydroxyl radical scavenging activity. The chelating effect may be determined by the sulphate and hydroxyl groups, of which the sulphate group has the stronger chelating ability (Zhou et al., 2008). However, the aforementioned reasons cannot explain the complex phenomena shown in Fig. 6. We speculate that the observed behaviour may be related to the spatial structure of the polysaccharides, which may affect the exposure of the active group.

Reducing power is also essential to assess the antioxidant activity (Duh, Du, & Yen, 1999). Previous studies have shown that the lower Mw products possessed the more reducing power (Xing et al., 2005; Zhao et al., 2006; Zhou et al., 2008). However, this effect was only observed for the fucoidans with Mw below 13.1 kDa in our study; the reducing power of other samples increased slowly with increasing Mw(Fig. 7). The reducing power depends on the amount of reductones, which could break the free radical chain by donating a hydrogen atom. Reductones also may react with certain precursors of peroxide and prevent peroxide formation (Singh & Rajini, 2004). A polysaccharide chain has one reducing terminal, so a lower Mw means more reductones are present. However, the trend exhibited by samples 4–7 could not be explained by this mechanism; instead, the trend may be influenced by the conformation of the fucoidan, which is related to steric hindrance.

5. Conclusion

We have demonstrated here that hydrogen peroxide degradation is an effective method to prepare low Mw fucoidans. The reaction is mainly influenced by the concentration of hydrogen peroxide, reaction time and temperature, and the contents of the sulphate group are not obviously reduced, which is important for the biological activity of fucoidan. For further study of the relationship between antioxidant activity and Mw, seven samples were selected, and the results indicate that the hydroxyl radical scavenging activity and reducing power of the fucoidans were significantly affected by the Mw. However, the available mechanisms could not explain the complicated results, and they require further study.

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