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Molecular Weight Controllable Degradation of *Laminaria japonica* Polysaccharides and Its Antioxidant Properties

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Abstract In this study, molecular weight controllable degradation of algal *Laminaria japonica* polysaccharides (LPS) was investigated by ultrasound combined with hydrogen peroxide. Three main factors, *i.e*., ultrasonic power (A), ultrasonic time (B), and H_2O_2 concentration (C) were chosen for optimizing parameters by employing three-factors, three-levels BBD. The influence of degradation on structure change and antioxidant activities was also investigated. A second-order polynomial equation including molecular weight (Y) of *Laminaria japonica* polysaccharides and each variable parameter, *i.e*., ultrasonic power (A), ultrasonic time (B), and H_2O_2 concentration (C), was established: Y=20718.67−4273.13A−4000.38B−1438.75C+2333.25AB+1511.00AC+873.00BC+2838.29A^2 + 2490.79B^2+873.04C^2. The equation regression coefficient value (R^2 = 0.969) indicated that this equation was valid. The value of the adjusted determination coefficient (adjusted R^2 = 0.914) also confirmed that the model was highly significant. The results of selected experimental degradation conditions matched with the predicted value. FT-IR spectra revealed that the structures of LPS before and after degradation were not significantly changed. Antioxidant activities of LPS revealed that low Mws possessed stronger inhibitory than the original polysaccharides. The scavenging effects on superoxide radicals was the highest when IC50 of crude LPS was 4.92 mg mL^{-1} and IC50 of Mw 18.576 KDa was 1.02 mg mL^{-1}, which was fourfold higher than initial polysaccharide.

Key words *Laminaria japonica*; polysaccharides; degradation; ultrasound

1 Introduction

Many researches proved that polysaccharides from green algae possess potential antioxidant activities and various classes of them have been shown as potent antioxidants. The antioxidant activity of algae polysaccharides depends on several structural parameters, such as molecular weight (Mw), number of sulphate groups and monosaccharide composition. Previous studies have found that kelp polysaccharides with different molecular weights have different biomedical effects. The Mw plays an important role in antioxidant activity (Zhang *et al*., 2010). Hou *et al*. (2012) illustrated that fucoidans with low Mws have better hydroxyl radical scavenging activities, reducing powers and superoxide radical scavenging activities than fucoidans with high Mws. Qi *et al*. (2005) reported that sulfated polysaccharide with low Mws from U. *pertusa* Kjellm has stronger antioxidant activities. Zhao *et al*. (2004) reported that low molecular weight of kelp sulfated polysaccharide has the effect of protecting the liver.

In order to obtain low Mw polysaccharides, acid, radical and enzymatic methods have been widely used. Zhao *et al*. (2008) compared acid hydrolysis and radical process degradation of the crude sulfated polysaccharide extracted from *Laminaria japonica*. The low molecular weight of 5–15 kDa exhibits a very strong antioxidant activity on superoxide and hydroxyl radicals, with higher activity than that of large molecular weight fractions. Anastyuk obtained oligosaccharide fragments with low Mws from *Costaria costata* by mild acid hydrolysis (Anastyuk *et al*., 2012). Zhao prepared porphyran with different Mws from *Porhyra haitanensis* by ascorbate and H_2O_2 in combination (Zhao *et al*., 2006). Yu *et al*. (2003) acquired two sulfated polysaccharides with Mw 151.6 and 28.2 kDa from *U. pertusa* by using a microwave degradation oven. Sun *et al*. (2009a) prepared polysaccharides with different Mws (from 2918 to 256.2, 60.66 and 6.55 kDa) from *Porphyridium cruentum* with microwave irradiation. Li *et al*. (2013) using microwave degradation

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methods obtained six representative sulfated polysaccharides (Mw 446.5, 247.0, 76.1, 19.0, 5.0 and 3.1 kDa) from *Enteromorpha prolifera*. The study found that samples with low Mw 3.1 kDa possessed stronger inhibitory effects on hydroxyl radical. Sun *et al*. (2009b) reported that hermetical microwave was used to degrade *Porphyridium cruentum* polysaccharides from 2918 to 256.2, 60.66 and 6.55 kDa. High-molecular-weight polysaccharides have no obvious antioxidant activity, but low-molecular-weight fragments after degradation exert an inhibitory effect on oxidative damage. The 6.55-kDa fragment has stronger antioxidant activity than the 60.66 and 256-kDa fragments. But in all the above reports, Mw cannot be controlled.

Ultrasonic irradiation has been recently regarded as a new technique for degradation of polymer compounds, mainly due to the fact that the reduction in the molecular weight is simply by splitting the most susceptible chemical bond without causing any changes in the chemical nature of the polymer (Zhou *et al*., 2012).

The purpose of this study was to obtain molecular weight controllable degradation of algal *Laminaria japonica* polysaccharides by ultrasound combined with hydrogen peroxide and evaluate their antioxidant activities.

2 Materials and Methods

2.1 Material and Equipment

L. japonica was purchased from Beijing market, cleaned with water, and air dried. Hydrogen peroxide (H_2O_2) was purchased from Sigma Chemicals Co. All other reagents were of analytical grade. Ultrasonic equipment (CTXNW-2B, Beijing Hong Xiang Long Co., Ltd.) with a horn-type transducer (15 mm diameter) was employed in this study (Zhao *et al*., 2011).

2.2 Degradation of Sulfated Polysaccharide (LPS)

Algae powder was extracted with distilled water at 90 ℃ for 2 h. After being filtered and centrifuged, the supernatant was concentrated and then mixed with four volumes of cold 95% ethanol (4℃) for isolating polysaccharides.

The degradation of LPS was carried out according to a modified protocol of a previously described method (Ouyang *et al*., 2010). Briefly, LPS was dissolved in distilled wate. After stirring 1 h, a certain amount of H_2O_2 solution was quickly added to the reaction system. Set preconcerted ultrasonic power, the degradation reaction was allowed to proceed for a certain time. Finally, the pH of the solution was adjusted to 7.0 by 2 mol L^{-1} NaOH solution after the reaction. The degraded polysaccharide solution was then concentrated and precipitated by four times volume of ethanol. The solution was left standing overnight, and then the degraded LPS was filtered and vacuum-dried.

2.3 Design of Experiments

Box-Behnken design (BBD) with three independent variables was used for establishing second-order polynomial equation between molecular weight and each variable parameters including ultrasonic power (A), ultrasonic time (B), and H_2O_2 concentration (C), as shown in Table 1.

Table 1 Box-Behnken design matrix and the response values

No.	Levels			Molecular Weight (Da)
	A: Ultrasonic power (W)	B: Ultrasonic time (min)	C: H_2O_2 concentration (%)	
1	300	30	3	36103.00
2	700	30	3	24337.00
3	300	90	3	23092.00
4	700	90	3	20659.00
5	300	60	1	33181.00
6	700	60	1	20166.00
7	300	60	5	25672.00
8	700	60	5	18701.00
9	500	30	1	29418.00
10	500	90	1	20015.00
11	500	30	5	26404.00
12	500	90	5	20493.00
13	500	60	3	21956.00
14	500	60	3	20168.00
15	500	60	3	20032.00

2.4 Characteristics of LPS

The viscosity of LPS solution was measured by rotational viscometer. FT-IR of polysaccharides was carried out by the potassium bromide (KBr) pellet method on FT-IR Spectrometer (FT/IR-660 Plus, JASCO) in the range of 400–4000 cm^{-1}. The average Mw of the LPS was determined by gel permeation chromatography (GPC) method. Flow rate of the mobile phase (0.9% NaCl) was 0.5 mL min^{-1} at a column temperature of 25℃. The hydroxyl radical, superoxide radicals and DPPH scavenging activity of samples were measured according to the method of Yao *et al*. (2012).

3 Results and Discussion

3.1 Effect of Single Factor on the Degradation of LPS

The viscosity of LPS was determined to reflect the degradation level of LPS. The effect of ultrasonic power, ultrasonic time and H_2O_2 concentration on the degradation of LPS is shown in Fig.1. The ultrasonic power was set at 100, 300, 500, 700 and 900 W while ultrasonic time was 40 min and H_2O_2 concentration was 5%. It could be found that the viscosity was reduced as ultrasonic power ascended from 100 to 500 W, and then decreased slowly when the ultrasonic power exceeded 500 W (Fig.1A). This indicated that 500 W was sufficient for degrading LPS. In Fig.1B, the H_2O_2 concentration was fixed at 5%, the ultrasonic power was fixed at 700 W. The viscosity of LPS declined rapidly when ultrasonic time increased from 20 to 60 min. In Fig.1C, it could be found that the degradation of LPS decreased evidently with increasing H_2O_2 concentration.

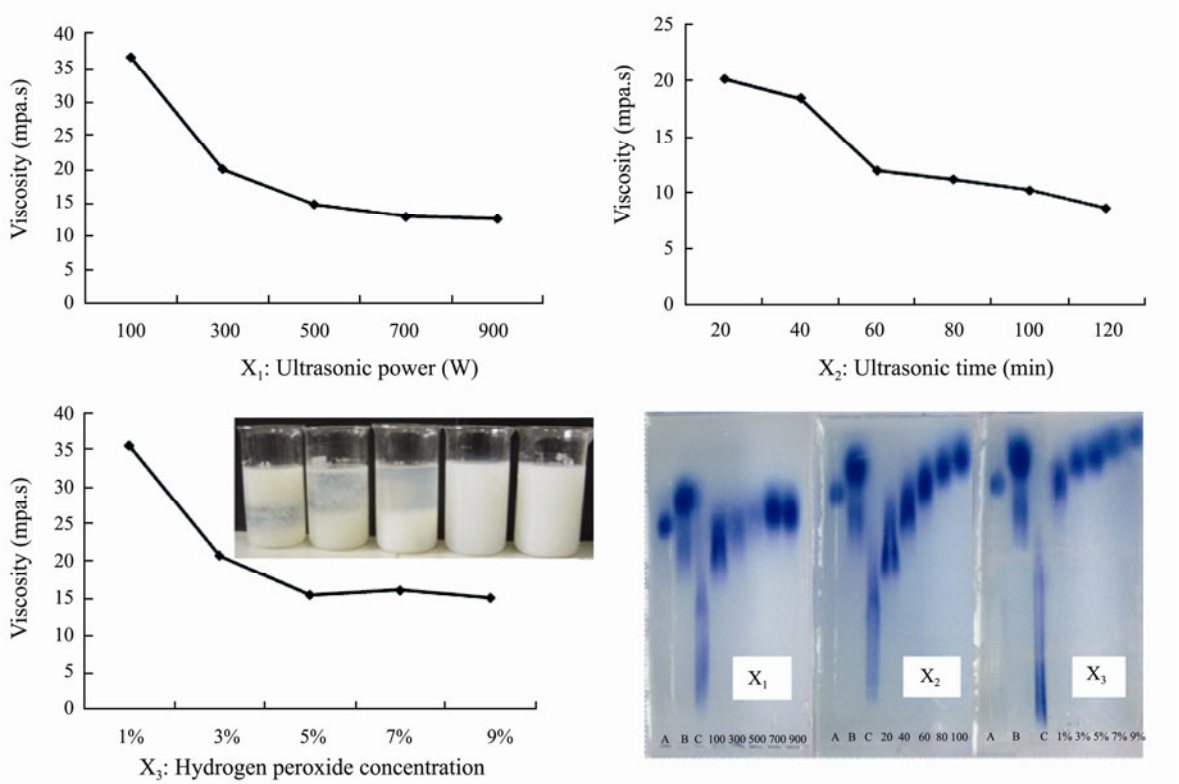

Fig.1 Effect of ultrasound power (A), ultrasound time (B) and H_2O_2 concentration (C) on the molecular weight of LPs (in electrophoresis pattern. x, Heparin; y, LPS of Mw 20kDa; z, crude LPS).

Agarose gel electrophoresis showed the variation in mobilities among LPS before and after degradation (Fig.1). The electrophoretic migration is determined by the structure and molecular weight of the polysaccharides. From Fig.1, it can be seen clearly that with increasing the individual factor, molecular weight decreased significantly.

3.2 Statistical Analysis and the Model Fitting

The values of responses at different experimental combinations are given in Table 1. By employing multiple regression analysis, the predicted response Y (Mw) can be obtained from the following equation:

$$Y=20718.67-4273.13A-4000.38B-1438.75C+2333.25AB+1511.00AC+873.00BC+2838.29A^2+2490.79B^2+873.04C^2. \quad (3)$$

The analysis of variance (ANOVA) indicated that the linear coefficients (A, B, C), quadratic coefficients (A^2, B^2) and interaction coefficient (AB) were significant, with small P-values ($P<0.05$) (Table 2). The equation regression coefficient value ($R^2 = 0.969$) indicated that this equation was valid. The value of the adjusted determination coefficient (adjusted $R^2=0.914$) also confirmed that the model was highly significant. At the same time, a very low value 6.42% of coefficient of the variation (CV) clearly indicated a very high degree of precision and a good deal of reliability of the experimental values.

The response surfaces and the contour plots are presented in Fig.2. The 3-D plot and the contour plot, which gave the ultrasonic power (A) (0 level), showed that molecular weight decreased evidently with increasing of ultrasonic power. The same trend s occurred between the other two interaction coefficients (AC, BC). Among the three parameters studied, the ultrasonic power was the most significant one to affect the molecular weight, followed by ultrasonic time and H_2O_2 concentration according to the regression coefficients significance of the quadratic polynomial model (Table 2) and gradient of slope in the 3-D response surface plot (Fig.2).

3.3 Verification of the Models

The suitability of the model equation for predicting the optimum response values was tested by using the selected optimal conditions. The predicted molecular weight and experimental molecular weight of LPS are given in Table

3. When ultrasonic power 802.36 W, ultrasonic time 71.28 min and H_2O_2 concentration 2.5% were selected, the model predicted a response of 18668.4. Considering the operating convenience, the ultrasonic power was modified as 800 W, and experimental molecular weight of LPS was 18576 Da. Another two verification predicted molecular weights were 37819 and 25753.4. And the experimental molecular weights of LPS were 37528 and 25790 Da, respectively. The obtained results fitted one basic quadratic equation.

Table 2 Regression coefficient estimation and significance test

Source	Sum of squares	d*f*	Mean *square*	*F* value	*P*-value prob > *F*
Model [a]	3.74×10^8	9	41605433	17.46774	0.0029*
A	1.46×10^8	1	1.46E+08	61.32929	0.0005*
B	1.28×10^8	1	1.28E+08	53.74996	0.0007*
C	16560013	1	16560013	6.952603	0.0462*
AB	21776222	1	21776222	9.142591	0.0293*
AC	9132484	1	9132484	3.834208	0.1075
BC	3048516	1	3048516	1.279898	0.3092
A^2	29744860	1	29744860	12.48817	0.0167*
B^2	22907236	1	22907236	9.617439	0.0268*
C^2	2814283	1	2814283	1.181557	0.3266
Residual	11909218	5	2381844		
Lack of fit	9603480	3	3201160	2.776689	0.2759
Pure error	2305739	2	1152869		
Cor total	3.86×10^8	14			

Notes: [a] $R^2 = 0.969$, R^2Adj = 0.914, C.V. = 6.42%. *Significant coefficient ($P < 0.05$).

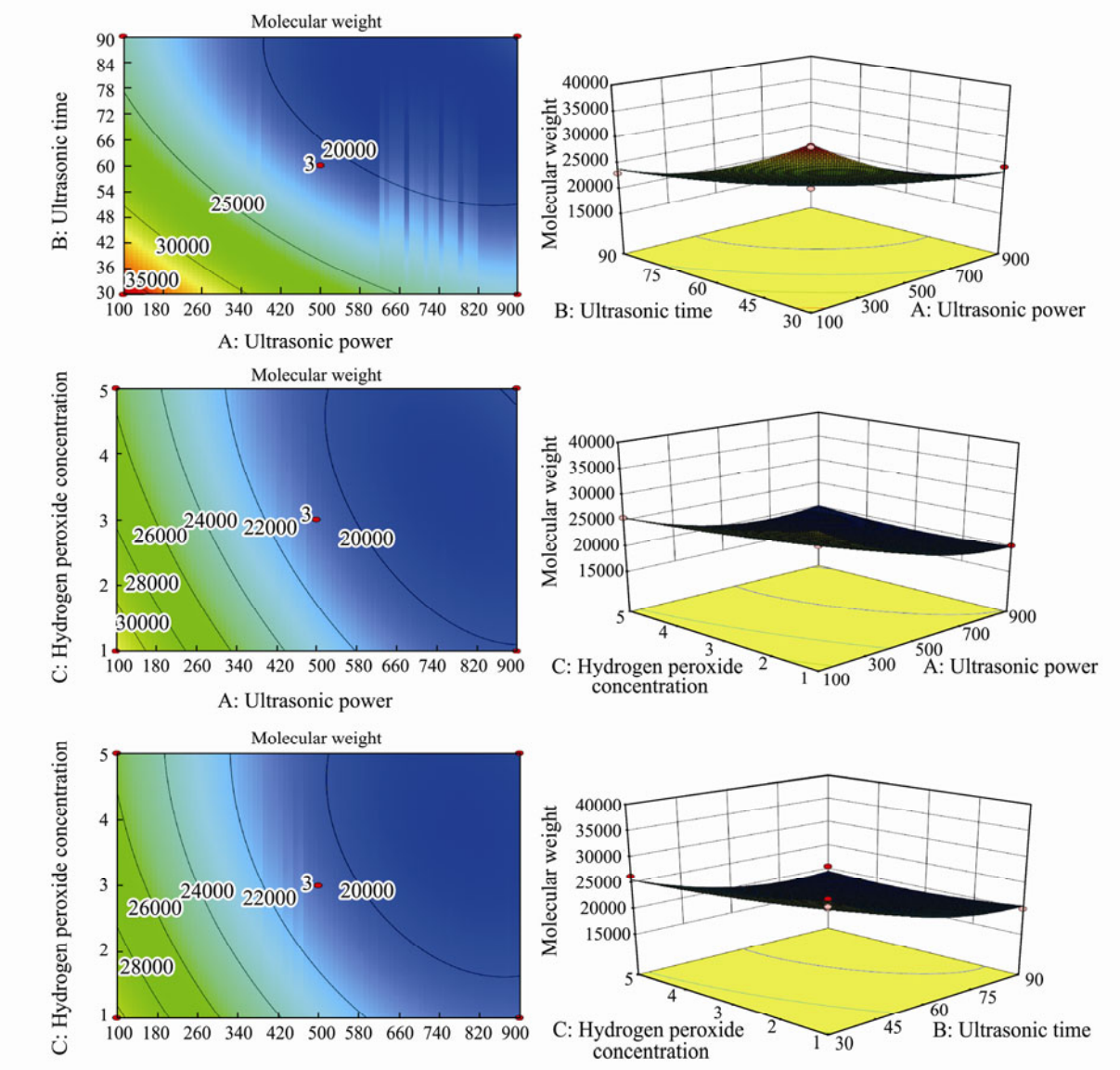

Fig.2 Response surface plots and Contour plots showing the effect of ultrasonic power (A), ultrasonic time (B) and H_2O_2 concentration (C) on the molecular weight of LPS.

Table 3 Verification of the equation

No.	Ultrasonic power (W) (modify)	Ultrasonic time (min)	H_2O_2 (%)	Molecular Weight (Da)	
				Predicted	Validation value
LPS-1	802.36 (800)	71.28	2.50	18668.4	18576
LPS-2	198.98 (200)	30.40	1.05	37819	37528
LPS-3	100.00	60.00	5.00	25753.4	25790

3.4 Characteristics and Antioxidant Activity of LPS

The monosaccharide composition of LPS was Fucose: Xylose: Mannose: Glucose: Galactose = 4:0.87:1.5:1:1.65. The content of uronic acid was 21.8% and the sulfate content was 18.9%. The average molecular weight of LPS apparently decreased from approximately 2000 kDa to 18.576 kDa after degradation. The reason may due to the fact that in the process of ethanol precipitation, the polysaccharide of smaller molecular weight was not present and washed off in ethanol. The FT-IR spectra of LPS before and after degradation are shown in Fig.3. In the spectra, these samples all had a typical large polysaccharide absorption band about 3400 cm^{-1} which was caused by a large amount of −OH stretching. The relatively strong absorption peak at around 1250 cm^{-1} reflected S=O stretching. There was no marked difference between LPS, which indicated that ultrasonic had no influence on the structure of LPS.

The scavenging effects of LPS before and after degradation on the three different radicals are shown in Table 4. The 18.576 kDa fragment had stronger radical-scavenging activity than those of the 37.528 and 25.79 kDa fragments. The IC50 values of all the degraded polysaccharides were lower than that of crude LPS, which means that the scavenging radicals' activity of degraded polysaccharides is higher than that of original crude polysaccharide.

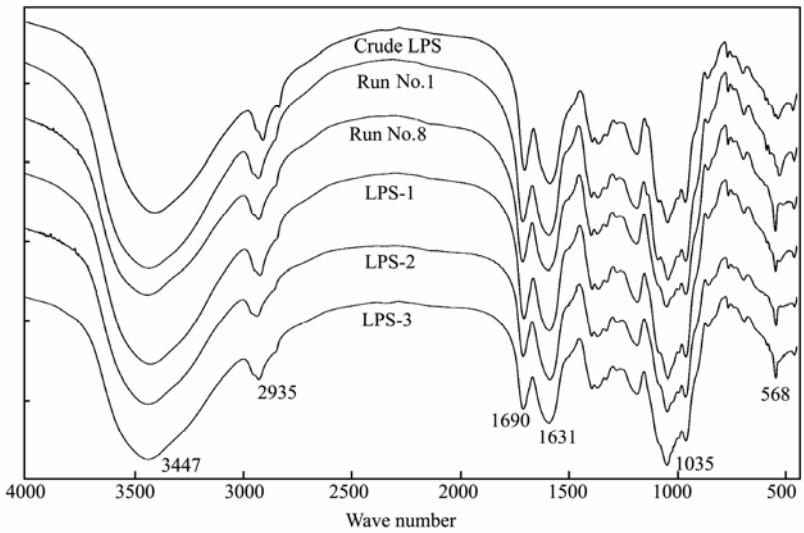

Fig.3 IR spectrum of the LPS.

Table 4 Antioxidant activities of LPs before and after degradation

Samples	IC50 of inhibitory effects (mg mL^{-1})		
	Hydroxyl radical	Superoxide radical	DPPH radical
Crude LPS	8.51	4.92	12.29
LPS-1	2.76	1.02	4.73
LPS-2	5.11	2.53	7.37
LPS-3	3.29	1.81	6.30

4 Conclusions

Molecular weight controllable degradation of *Laminaria japonica* polysaccharides was successfully made by ultrasound combined with H_2O_2. This study provides a method for preparing polysaccharides of predicted molecular weight. The study also indicates that the radical scavenging activity of *Laminaria japonica* polysaccharide after degradation is higher than that of the original polysaccharides.

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