International Journal of Research in Engineering and Science (IJRES)
ISSN (Online): 2320-9364, ISSN (Print): 2320-9356
www.ijres.org Volume 13 Issue 3 ǁ March 2025 ǁ PP. 88-93

Studies on the technology of hydrochloric acid degradation of fucoidan by orthogonal optimization and its antioxidant activity

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Abstract: *Hydrochloric acid was used to degrade fucoidan and the antioxidant activities of degraded fucoidan were studied. The optimum degradation conditions by orthogonal experimental analysis were predicted as follows: concentration of hydrochloric acid was 0.6 mol/L, temperature was 60 , time was 6h, and the sugar content was up to (56.86±0.15)%. Moreover, it was demonstrated that fucoidan has appreciable antioxidant potential on determination of reducing power, lipid peroxidation, hydroxyl radical and superoxide anion radical scavenging. The results showed that fucoidan had certain antioxidant performance.*
Key words: *fucoidan; orthogonal; antioxidant activity*

Date of Submission: 09-03-2025 Date of acceptance: 23-03-2025

I. Introduction

Fucoidan is a kind of water-soluble heteropolysaccharide with a variety of monosaccharides and sulfate groups, and its molecular weight rangs from several thousands to tens of thousands. Because of the large molecular weight, complex structure and hard absorption, the study on its physical and chemical properties as well as biological function is partly limited. With the study on the disease-resistant mechanism of polysaccharide, its advanced structure of polysaccharides, connection position of side chains, branch density and the connection forms of glycosidic bond between monosaccharides are all related to the pharmacological effects. The polymerization degree of the polysaccharide, molecular weight and the sulfate content also affect the physiological activity [1]. Hderge [2] got oligosaccharides with molecular weight less than 3000Da from the fucoidan of carrageenan using diluted sulfuric acid degradation method. So, using the appropriate method to degrade fucoidan to polysaccharides in low molecular weight is an urgent problem to be solved. Meanwhile pharmacological activity of fucoidan is related to its antioxidant activity, therefore vitro antioxidant activity of fucoidan is of great significance.

Currently, there are many methods used for degrading polysaccharide, some of them can hydrolyze polysaccharide completely so that it facilitates the study of sugar chain or analysis of polysaccharides, and some cut the specific glycosidic linkages and decrease the molecular weight. Different reagents have different effects on the glycosidic bond with different nature. Hydrochloric acid degradation of fucoidan using Orthogonal was reported barely, so viscosity characteristics of the experiments was selected as evaluation index to optimize degradation conditions. Then the vitro antioxidant experiments of degradation fucoidan were carried out.

1 Materials and methods

1.1 Materials and instruments

Crude fucoidan (laboratory preparation); hydrochloric acid; hydrogen peroxide; manganese dioxide; sodium hydroxide; 3,5 - dinitrosalicylic acid; glycerol of analytical grade; TD5A- WS-type large-capacity low-speed desktop centrifuge; UV-1600 UV-visible spectrophotometer; FA2004N electronic balance; HY31-01-type electric heated water bath; GZX-GF-19-2BS type electric blast oven; DZF-6030A type vacuum dryer; BCD-205TBDZ refrigerators; Bush funnel Ubbelohde viscometer and other instruments.

1.2 Determination of reducing sugars

Reducing sugars was determined by the DNS method [3, 4]. Standard curve was determinated by glucose, and the linear regression equation was: $Y = 15.188X + 0.0429$, $R^2 = 0.9976$. Reducing sugar content in the sample was calculated as following: Reducing sugar content = (C * 50mL) / [concentration of the sample solution (mg / mL) * 2mL] * 100%. (C was for the concentration of the sample solution sugar that was calculated by the standard curve)

1.3 Determination of the intrinsic viscosity

Using a capillary viscometer was the most convenient to determinate the [η] of the sample. Because the biggest advantage of ubbelohde viscometer is that it does not affect the volume of the solution measured, it can be taken in a gradual dilution viscometer method to obtain solutions of different concentrations. The ubbelohde viscometer was used in the following experiments. Its structure is shown in Figure1, The diameter and length of capillary H and the size of the ball E were decided by the viscosity of the solvent, which made the

solvent outflow time more than 100s, but the capillary diameter was not less than 0.5mm, otherwise it was easy to plug. The volume of ball F should be 8-10 times the volume of the tube from the "a" of tube B to the low-end of tube F, so the initial concentration can be diluted to about 1/5.

Experimental procedure was as follows:
1. Preparation of the test sample solution: Put 1g of sample into a small beaker, and dissolve it with 30mL deionized water. After dissolution, move it to the 50 ml of volumetric flask. Solution and solvent were filtered respectively with glass sand core funnel of NO.3 before starting experiments.
2. Preparation of the viscometer: Wash and dry viscometer, and place perpendicular to a thermostat to make a weight basis. Water should be over the ball of G of viscometer. The solvent and the solution were placed in a thermostat and kept at constant temperature.
3. The outflow time (t_0) of the solvent was measured: In the dry cleaner's viscometer, shift 10mL of deionized water with constant temperature using a pipette. Using the rubber tube to clamp the pipe of C, so as not to open to the atmosphere. Inhale the rubber tube of the ball B with the aurilave. Open the clip of tube C to make the tubes of B and C tube exposed to the atmosphere when the solution was up to G ball halfway through. The surface of the liquid in the ball G had decreased by gravity, and the time (t_0) was recorded when the liquid level was passing the scale opening and stopped when the liquid level was just passing the scale b, and the t_0 was the time that the solvent flowed from "a" to "b". Repeat three times, and each time was not more than 0.2s in discrepancy. Finally the average value was obtained.
4. The effluent time (t) of the solution was measured: Remove viscometer, and toss away the solvent. The pipette has taken 10mL of solution injecting into the viscometer. Same as above method, installe viscometer, measure the efflux time (t) of the solution, and add deionized water (2, 3, 5, 10 mL). We must wash ball of E viscometer by the diluted solution after each dilution, and make concentration equal everywhere inside the viscometer. all were measured by the same method.
5. Calculation method: $\eta_{sp} = (\eta - \eta_0)/\eta_0 = \eta_r - 1 = t/t_0 - 1$
6. Calculate the values of η_{sp} η_r η_{sp}/c $\ln\eta_{sp}/c$, then plot to c by using η_{sp}/c and $\ln\eta_{sp}/c$. Two straight lines were got, which helped get the [η] when the c was 0.

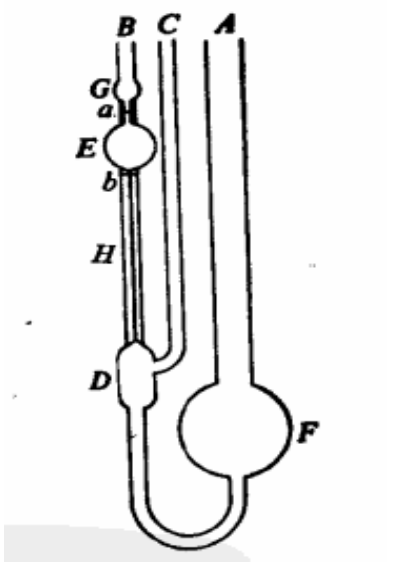

Fig. 1 Ubbelohde viscometer structural

1.4 Experimental design [5]
Effects of degradation conditions such as degradation time, degradation temperature and concentration of hydrochloric acid, on intrinsic viscosity of fucoidan, have been investigated by the single factor method. By the single-factor experimental results, three major influence factors (degradation time, degradation temperature and concentration of hydrochloric acid) were confirmed. On the basis of the single-factor experimental results, three major factors and the ranges of each factor were confirmed. Then, a three-variable and three-level orthogonal design was employed in this optimization study based on the results of the preliminary single-factor test, as shown in Table 1. Three independent variables (A, temperature; B, time; C, concentration of hydrochloric acid) at three levels were performed.

Table 1 Factors and levels for orthogonal

Factor	Level		
	1	2	3
A Temperature	50	60	70
B Time h	4	6	8
C HCl mol/L	0.2	0.4	0.6

1.5 Determination of reducing power

Concentrations of sample solution (1, 2, 3, 4, 5 and 6 mg/mL) prepared were to be tested. Take the sample solution in 2.5mL, then add 2.5 mL of phosphate buffer solution (0.2 mol/L, pH6.6) and 2.5mL of potassium ferricyanide (1%) and mix uniformly, and the mixture was heated at 50 for 20min, then cooled rapidly. Add 2.5mL of trichloroacetic acid (10% (W/V)) to the mixture. 5ml of the upper solution was from the mixture at 5000r/min centrifugal for 10min to mix 1mL of ferric chloride solution (0.1%) and 5mL of distilled water, and the mixture was measured for absorbance at 700nm. The greater the absorbance value, the stronger the reducing power[6].

1.6 Determination of lipid peroxidation

Procedure[7]:

1 A fresh egg yolk and 0.05mol / L phosphate buffer (pH = 8.0) 1:1 were mixed, then placed in a refrigerator preservation for 9 days. Diluted in a ratio of 1:20, stir them well before used.

2 The sample solution 0.2mL, 0.2mL 0.1mol / FeSO4 and 1.0mL 0.05mol / L phosphate buffer solution of L (pH = 8.0) were mixed well, added to the 4.0mL with buffer.

3 Firstly, 0.5mLl 20% trichloroacetic acid was added in control tube.

4 The control tube and the sample solution tube were vibrated in water at 37 for 2h, the tube was added 20% 0.5mL trichloroacetic acid after removed from water, let stand for 10 min after the shake.

5 Took the supernatant liquid 2.0mL(3500r/min, 10min), were added 1.0mL 0.8% thiobarbituric acid solution, the tube with gasser in 100 water bath for 15 min, and removed to cool.

6 Phosphate buffer solution was used to make a blank zero, determinate the value at A_{532} by UV spectrophotometer. With Vc as positive control.

Inhibition rate = $(A_{\text{control 532}}-A_{\text{sample 532}}) / A_{\text{control 532}} * 100\%$,

where $A_{\text{sample 532}}$ represents the absorbance of sample group and $A_{\text{control 532}}$ represents the absorbance of blank control group. All the absorbencies were read under 532 nm.

1.7 Inhibition effect of hydroxyl radical

In this study, determinate inhibition rate of fucoidan on H_2O_2/Fe^{2+} hydroxyl radical by phenanthroline-Fe^{2+} oxidation assay[8].

Specific operations: Took 2.5mL phenanthroline (5mmol/L), added 2.0mL phosphate buffer solution(pH7.4, 0.05mol/L) and mixed well, add 1.0mL $FeSO_4$(7.5mmol/L) and 1.0mL H_2O_2 (0.1%), and finally up to 10.0mL with deionized water, the solution was incubated at 37 for 1h, the absorbance (injure) was measured at 510nm. The tubes were tested according to the above operation. The 1.0mL sample solution was added 1.0mL H_2O_2 (0.1%), measured absorbance (A Sample) value at 510nm. No injured tube without H_2O_2 and polysaccharide solution was measured for absorbance (uninjured) at 510nm. With Vc as positive control.

Hydroxyl radical scavenging rate = $(A_{\text{sample}}-A_{\text{injuries}}) / (A_{\text{uninjured}} - A_{\text{sample}}) * 100\%$

1.8 Superoxide anion radical scavenging activity

Tirs-HC1 buffer solution (pH 8.20) 5mLwas added 1mL sample solution at 25 . 20 min later, added 1mL pyrogallol (3mmol/L) immediately for 4 min, adding 1mL 10mol/mL HCl solution (which can be modified to drop 2 drops) to terminate the reaction, measured the absorbance values at 320nm after 10 min. Blank of deionized water instead of the sample tubes. With Vc as positive control[9].

Inhibition rate = $(A_{\text{blank}}-A_{\text{sample}}) / A_{\text{blank}} * 100\%$

II. Results and discussion

2.1 Orthogonal experimental results

The polysaccharide molecular size was deduced by the intrinsic viscosity, and then the effect of hydrochloric

acid degradation was based on the following: the viscosity of the polysaccharide solution, which was a reflection of the size inside the liquid flow friction, generally increased with increasing concentration. The greater the molecular weight, the higher the viscosity at the same concentration. Therefore, the molecular weight of the polysaccharide can be determined by measuring the viscosity of the polysaccharide solution. The polysaccharide solution viscosity size was related to its morphology in the solvent. Therefore, the molecular weight of the polysaccharide viscosity measurement method was a relative method. The relationship between intrinsic viscosity polysaccharide solution [η] and its molecular weight (M) were expressed in the experience equation Mark-Houwink[10]:

$[\eta] = kM\alpha$,

where k and α were constants related to the nature of the solute. Fan Hui Hung et al[11] measured for [η] from copies levels by viscosity method, average molecular weight($\overline{M_W}$) was measured by laser small angle light scattering instrument (LALLS), then calculated constants k and α, and determined the sample's Mark- Houwink formula as follows: $[\eta] = 9.6924 * 10^{-3}Mw^{0.7358}$. Because of the difference of the sample nature, it cannot be accurately calculated by the above equation, while it was reasonable to determine the size of the molecular by comparing the [η], since the k and α were constants in the same sample.

Orthogonal test results of hydrochloric acid degradation were shown in Table 2:

Table 2: Three factors and three levels orthogonal of hydrochloric acid degradation

Factor / Test number	A (1)	B (2)	C (3)	Experimental index characteristic viscosity
1	50℃ 1	4 1	0.2 1	0.0303
2	50℃ 1	6 2	0.4 2	0.0922
3	50℃ 1	8 3	0.6 3	0.0717
4	60℃ 2	4 1	0.4 2	0.0274
5	60℃ 2	6 2	0.6 3	0.0032
6	60℃ 2	8 3	0.2 1	0.0536
7	70℃ 3	4 1	0.6 3	0.0280
8	70℃ 3	6 2	0.2 1	0.0681
9	70℃ 3	8 3	0.4 2	0.0636
K_1	0.1492	0.0805	0.1520	sum=1.0793
K_2	0.0842	0.0587	0.1232	
K_3	0.1397	0.1889	0.1029	
k_1	0.0497	0.0268	0.0507	
k_2	0.0281	0.0196	0.0411	
k_3	0.4657	0.0630	0.0343	
R	0.4376	0.0434	0.0164	

The results from the orthogonal showed that $R_A > R_B > R_C$. The effect of temperature on the degradation was the largest, followed by the degradation time, the concentration of hydrochloric acid degradation effect was least. Analysis of K values from the table, it ought to select combinations of $A_2B_2C_3$. From the experimental results, the best combination of experimental parameters also was $A_2B_2C_3$, which showed that the results of the orthogonal experiment were consistent with the orthogonal experimental analysis. The optimum degradation conditions by orthogonal experimental analysis as follows: temperature was 60 , degradation time was 6h and concentration of hydrochloric acid was 0.6 mol/L.

Fucoidan degradation experiments of hydrochloric acid verified in Table 3 that Changes of sugar before and after degradation were shown in Table 3:

Table 3 Changes of sugar before and after degradation

Degradation method	sugar before degradation	sugar after degradation
HCl	(18.69±0.13)%	(56.86±0.15)%

Because the glycosidic bond was broken, and reducing the group was exposed in the degradation process, the reducing sugar content can be determined as a basis for judging degradation. From Table 3, the reducing sugar content of degradation was up to (56.86 ± 0.15) %.

2.2 Determination of reducing power results

Whether a substance has the oxidation resistance or not is related to itself is a good electronic supplier, namely whether it scavenge free radical is given by their electrons. Therefore, it can measure the reducing power to judge the strength of material antioxidant activity. The stronger the reducing power, the stronger the antioxidant activity. In this study, the sample is oxidized by ferricyanide to characterize their reduction ability.

The greater the absorbance, the stronger the reduction ability. The relationship between amount of sample and its absorbance values was shown in Figure 2:

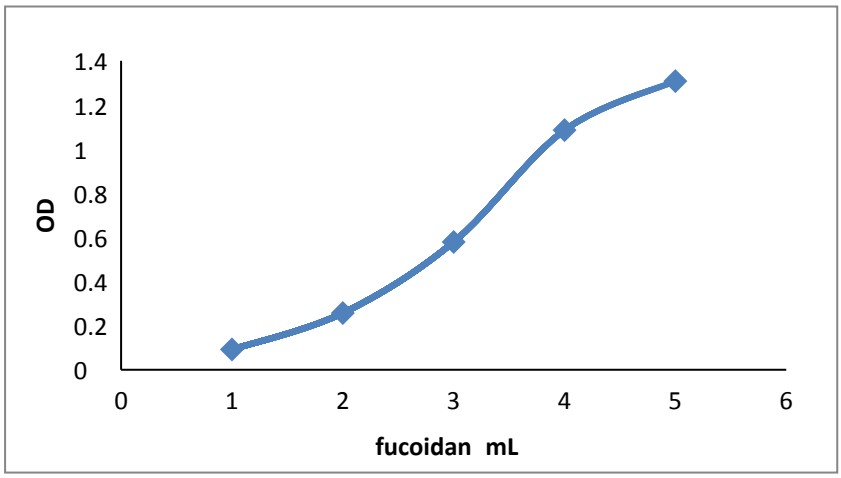

Fig.2. Measured results of reducing power

The Figure 2 showed that with the increasing amount of fucoidan, the greater the absorbance values, the stronger the reducing power. It indicated that there is a certain dose-effect relationship with the addition of the reducing power of fucoidan. Within a certain range, the higher the reactant concentration which means the more of its active ingredients, the stronger reducing power. With Vc as positive control, at the same concentration and the dose, the absorbance value of the samples of reducing power was 1.126, while the absorbance value of Vc was 2.672. It described that there were a certain degree of reducing power of samples, but not as strong as Vc.

2.3 Determination results of the antioxidant capacity

From the anti-oxidation experimental, inhibition rate of fucoidan on lipid peroxidation was 42.57%, while inhibition rate of Vc on lipid peroxidation was 51.42%. It indicated that inhibition of fucoidan on lipid peroxidation had a certain effect. However, because the concentration of the sample solution was 10mg/mL, and concentration of Vc was 2mg/mL, fucoidan on lipid peroxidation is much lower than Vc, which may related to the purity and solubility of the sample, namely the amounts of active ingredients.

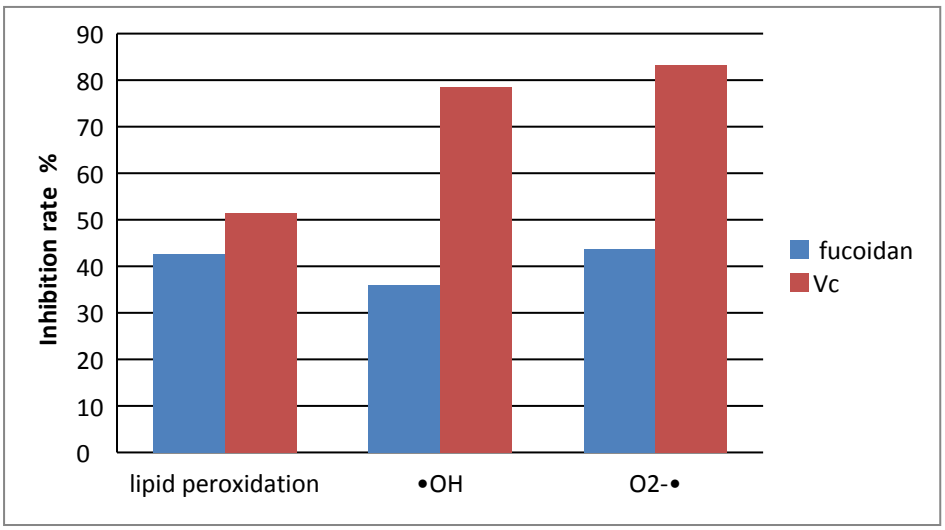

Fig.3. Comparison of the antioxidant capacity of fucoidan and Vc

Figure 3 showed that at the same concentration (2mg/mL) conditions, the inhibition rate of sample on ·OH was 36.04%, the Vc was 78.62%, which indicated that fucoidan on ·OH had certain inhibition effect, but its inhibitory effect was lower than Vc. Experiment of three phenol autoxidation method detection polysaccharide on superoxide anion free radical scavenging effect showed that the inhibition rate of sample solution on the O^{2-}• was 43.69%, the inhibition rate of Vc solution on O^{2-}• was 83.28%. Both of them were measured under the same

concentration condition (2mg/mL), which described the inhibition rate of fucoidan on O^{2-}• was closer to Vc playing the role on O^{2-}•.

III. Conclusions

In this study, the optimum degradation conditions by orthogonal experimental analysis were predicted as follows: concentration of hydrochloric acid was 0.6mol/L, degradation temperature was 60 and the degradation time was 6h. In vitro antioxidant fucoidan study, there was a certain relationship between reducing power and concentrations of fucoidan. It had certain inhibition effect on lipid peroxidation, ·OH and O^{2-}·, while the inhibitory effect was lower than Vc, which may related to the concentration, purity and solubility of the sample, namely the amounts of active ingredients.

References

[1]. Wang C. Y., Guan H S. Advances of researches on antiviral actiwities of polysacchridel I. Amtiviral activities of polusecchride.Adv Bioeng, 2000,20 (1): 17.
[2]. Shi Xiran, Xuzu Hong, Li Zhien. Degraded carrageenan polysaccharide antitumor activity [J] Ocean and Lake Algae, 2000, 31 (6): 653-656.
[3]. Wang Xu. Seaweed processing liquid waste utilization [D] Shanghai University Thesis, 2010: 18.
[4]. Zhao Kai, Xu Pengju, Gu Yeguang. 3,5 - dinitrosalicylic acid colorimetric determination of reducing sugar content of research [J]. Food Science, 2008, 29 (08): 534-536.
[5]. Xu Zhongan, Wang Tianbao, Li Changying etc. Orthogonal experimental design method Introduction [J].Information Development & Economy, 2002, 12 (5): 148-150.
[6]. Joan-HwaYang, Jeng-Leun, Pei-Tzu Ko, Ling-Chuan Huang. Oybean broth. Food Chemistry 2002, 71: 249-254.
[7]. Zhang Erxian ect. Fe^{2+} induced lipoprotein PUFA peroxidation system and the evaluation of several natural products antioxidant effect [J]. Journal of Biochemistry in Biological Physics. 1996, 28: 218-222.
[8]. Wen Liangjuan, Mao Huijun, Zhang yuanchun etc. Passion fruit peel composition analysis and the research of antioxidant activity [J]. Food Science, 2008, 29 (11): 54-58.
[9]. Liu Huiyan, Zhao Mouming. Extraction of low molecular weight Gracilaria polysaccharides and antioxidant activity [J]. Food Science and Technology, 2005, 26 (3): 67-69.
[10]. F. Danusso, G. Moraglio G. Gianott, J. Polymer Sci., 5, 475 (1961).
[11]. Fan Huihong, Guan Huashi, Zhou Ruzhen. Determination of molecular weight and its distribution of Seaweed polysaccharides [J]. Journal of Ocean University of Qingdao, 1992, 12: 43-50.