

Research Article

Extraction and Separation of Fucoidan from *Laminaria japonica* with Chitosan as Extractant

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Herein the extraction method of fucoidan from *Laminaria japonica* is reported. Firstly, chitosan, chitosan-N-2-hydroxypropyl trimethyl ammonium chloride (HACC), and hexadecyltrimethylammonium bromide (CPAB) were used to extract the fucoidan. The results showed that chitosan was the optimal extractant compared with the other two extractants. After extraction, different aqueous solutions, including NaCl, KCl, and HCl (pH2), were used to separate fucoidan from chitosan-fucoidan complex. The results showed that the separation ability of NaCl was slightly higher than that of KCl. Moreover, the price of NaCl is lower than that of KCl. Given the quality-price rate, NaCl solution was chosen as the separation solution. Thirdly, the concentration and ratio of NaCl solution : sediment influence the separation of fucoidan from chitosan-fucoidan complex. The results showed that the optimal separation conditions include 4 mol/L NaCl solution with the ratio of NaCl solution to sediment at 30 : 1. Fucoidan content was found to be affected by different separation time. Fucoidan content increased with the increase of separation time, and the optimal separation time was 6 h. Compared with traditional alkali extraction method, this method not only reduces the usage of alkali and acid and alleviate environment pollution, but also has the comparable extraction yield of fucoidan. It is a potential method for extraction of fucoidan.

1. Introduction

The brown seaweed *Laminaria japonica* Aresch. (Laminariales) is one of the most important economic seaweeds cultured in China, and it is also widely distributed in Japan and Korea. The utilization of *L. japonica* medicine has been documented in traditional Chinese medicine for over than one thousand years. In our laboratory, fucoidan was extracted from *L. japonica*. Fucoidan is a kind of sulfated fucose containing polysaccharides. Studies have shown that fucoidan has a wide spectrum of activities in biological systems including anticoagulant and antithrombotic activities, affects the inflammatory and immune systems, has antiproliferative and antiadhesive effect on cells, and protects cells from viral infection [1–8]. However, there are few reports concerning the optimization of extraction method of fucoidan from *L. japonica*. Currently, alkali extraction is the main extraction

method of fucoidan in industry. A huge amount of alkali and acid were used, which severely polluted the environment. Therefore, it is important to find a better method to extract the sulfated polysaccharide from the soaked water of seaweed. In this paper, chitosan was used to extract fucoidan. Chitosan is usually obtained from waste materials, mainly shells of crabs, shrimp, and prawns from the seafood processing industry. It is an effective adsorbent due to its nontoxicity, biocompatibility, biodegradability, and its cationic nature, which enables chitosan to form a complex with anionic fucoidan. In this paper, we studied and compared the effect of different extractants, including chitosan, chitosan-N-2-hydroxypropyl trimethyl ammonium chloride (HACC), and hexadecyltrimethylammonium bromide (CPAB) on the extraction of fucoidan. Some reaction conditions and result were studied and determined as follow: the yield and content of fucoidan; the optimal dosage and flocculate time

of extractant; and the separation of fucoïdan from chitosan-fucoïdan, HACC-fucoïdan, and CPAB-fucoïdan complexes. So far, no study has been reported on the extraction and separation of fucoïdan from chitosan-fucoïdan, HACC-fucoïdan, and CPAB-fucoïdan complexes.

2. Materials and Methods

2.1. Chemicals. *Laminaria japonica*, cultured in Shazikou, Qingdao, China, was collected in March, 2006, and the fresh seaweed was soon washed, dried by sun, and kept in plastic bags at room temperature for use. Chitosan from crab shells (Qingdao Yunzhou Biochem. Corp., China), which had a degree of deacetylation of 0.85 and average molecular weights of 560 KD, was used. Chitosan-N-2-hydroxypropyl trimethyl ammonium chloride (HACC) was prepared according to Xing's methods [9]. Hexadecyltrimethylammonium bromide (CPAB) was purchased from Sigma Chemical Co. HCl, NaCl, KCl, and other reagents were of analytical reagent grade and were used without further purification. All solutions were prepared with distilled water.

2.2. Extraction of Fucoïdan. 300 g dry algae were cut and soaked in 7000 mL water at rt for 24 h. After being soaked, the solution was separated by successive filtration through gauze and siliceous earth, and the clear solution was obtained. Then, 1% solution of chitosan, HACC, and CPAB was added slowly to each extract solution of 300 mL, respectively, with stirring, until no further formation of complex occurred. The mixture was placed for 2–8 h, and then the precipitates were centrifuged off, freeze-dried, and weighted. Future, 0.1 g of each sample was respectively suspended in different solution, such as different concentration and different volume NaCl or KCl or HCl (pH 2) solution, and each suspension solution was stirred for 10 h. The precipitate was centrifuged off, and the supernatant was fixed at 100 mL. In order to determine the separation effect of fucoïdan from chitosan-fucoïdan, HACC-fucoïdan, and CPAB-fucoïdan complexes by different separation solvents, the fucoïdan content in the supernatant was determined.

2.3. Analytical Methods

2.3.1. The Preparation of the Regression Equation for Fucoïdan. 0.01 g standard fucoïdan was dissolved into 100 mL double-distilled water. Then, 0, 0.15, 0.30, 0.45, 0.60, and 0.75 mL of the solution were transferred into six test tubes, respectively. Every tube was added with double-distilled water, and the whole volume was up to 1.0 mL. Also, 4.5 mL of 87% H₂SO₄ aqueous solution in per test tube was added to the above mixture in ice water and shaken. One minute later, six test tubes were rapidly placed into boiling water and heated for 10 min. After they were cooled at room temperature, 0.1 mL of 3% heated cysteine chloride aqueous solution was added to the mixture, and the mixture was placed for 90 min. Their absorbance at 427 nm and 396 nm was determined, respectively (according to the appendix VA of Chinese Pharmacopeia (2000)). Then, the regression equation was made between the absorbance difference.

TABLE 1: Yields and fucoïdan content of extraction of different extractants.

Extractant	Yield (%) [△]	Fucoïdan content (%) [*]
Chitosan	1.68 ± 0.02	5.51 ± 0.03
HACC	1.59 ± 0.05	5.89 ± 0.02
CPAB	0.32 ± 0.03	2.05 ± 0.03

[△]Fucoïdan to dry algae. Usually the yield of fucoïdan is 1-2% with the method of alkali extraction. Yield of fucoïdan means extraction amount of fucoïdan from the dry algae.

^{*}12 mL extractant was used to extract fucoïdan, and the extract production was suspended in 50 mL 4 mol/L NaCl. Fucoïdan content means the % of fucoïdan released to the aqueous solvent after breaking the complexes.

2.3.2. The Determination of Fucoïdan in Separation Solution from Separation of Chitosan-Fucoïdan, HACC-Fucoïdan, and CPAB-Fucoïdan Complexes. 1 g of dried sediments (chitosan-fucoïdan, HACC-fucoïdan, and CPAB-fucoïdan complexes, resp.) was milled to powder and dipped in 50 mL 4 mol/L NaCl for 6 h. Then, the mixture was agitated for 2 h at room temperature and filtered. The filtrate was diluted to 100 mL, and 0.2 mL of this solution was used to determine the concentration of fucoïdan (C, mg mL⁻¹). The determined method was as in Section 2.3.1. Then, the total separation effect of fucoïdan from chitosan-fucoïdan, HACC-fucoïdan and CPAB-fucoïdan complexes in the sediment (1g) was calculated as follows:

$$\text{the yield of fucoïdan in 1 g sediment} = C \times 100. \quad (1)$$

2.3.3. Statistical Analysis. All determinations were carried out in triplicate. All data were expressed as means ± SD. The data were analyzed by an analysis of variance ($P < 0.05$), and the means were separated by Duncan's multiple range tests. The results were processed using Excel and STATISTICA software (statsoft Inc., 1999).

3. Results

3.1. Effect of Different Extractant on Yield and Content of Fucoïdan. The fucoïdan was extracted by three different extractants, including chitosan, HACC, and CPAB. Yields and fucoïdan content of the extraction are shown in Table 1. Table 1 shows that flocculate activities of chitosan and HACC are more pronounced than that of CPAB. Although fucoïdan content of extraction with HACC is higher than that with chitosan, the yield of extraction with chitosan is higher than that with HACC. Moreover, HACC needs a further synthesis. Therefore, given the quality-price rate, chitosan was chosen as the optimal extractant.

3.2. Effect of Different Ratios of Chitosan: Soaked Water of Seaweed on Yield of Fucoïdan. Table 2 shows that the yield of extraction was influenced by different ratio of chitosan: soaked water of seaweed. The flocculate activity of chitosan was not related ratio of chitosan: soaked water of seaweed shown in Table 2. Chitosan has a different extraction activity with different ratios of chitosan: soaked water of seaweed. In a word, the yield of extraction increased at first and then

TABLE 2: Yields of extraction of different ratios of chitosan : soaked water of seaweed*.

Ratio	1:75	1:50	1:25	1:18.75	1:15
Yield of fucoïdan (%)	0.63 ± 0.03	1.31 ± 0.05	1.68 ± 0.02	1.59 ± 0.03	0.98 ± 0.02

* Concentration of chitosan is 1%.

TABLE 3: Fucoïdan content of extract production in NaCl, KCl, and HCl (pH2) solution of 50 mL.

Different solvent	NaCl	KCl	HCl (pH2)
Fucoïdan content (%)	5.51 ± 0.03	5.47 ± 0.02	1.03 ± 0.05

TABLE 4: Fucoïdan content of separation production in the different concentration of NaCl solution (50 mL).

Concentration (mol/L)	1	2	3	4
Fucoïdan content (%)	1.41 ± 0.03	1.63 ± 0.05	4.7 ± 0.02	5.51 ± 0.03

decreased with increasing ratio of chitosan : soaked water of seaweed. At 1:25, the yield of extraction was the highest, about 1.68%. So, 1:25 is the optimal ratios of chitosan: soaked water of seaweed. This phenomenon is consistent with Napper's [10] research result. Napper's result showed that the amount of flocculant is generally bigger and better, but if the amount is too large, the surface of the colloidal particles will form steric layer due to the adsorption of excess flocculant and the occurrence of steric stabilization phenomena; therefore, this result will prevent the formation of bridging structure, and be less prone to flocculation, affecting processing results. Therefore, in this paper, higher chitosan dosage is not the best.

3.3. Effect of Different Separation Solvents on Fucoïdan Content. In this paper, different solvents were used to separate fucoïdan from chitosan-fucoïdan complex. Table 3 shows that the fucoïdan content changed abruptly when an inorganic salt was added to the separation solution. The results suggest that the metal ion can promote separation of chitosan-fucoïdan complex. As shown in Table 3, their orders of separation on fucoïdan were NaCl > KCl > HCl (pH2). NaCl solution had an optimal separation effect.

3.4. Effect of the Different Concentration of NaCl Solution on Fucoïdan Content. Table 4 shows that the different concentration of NaCl solution affects the separation of fucoïdan from chitosan-fucoïdan complex. Fucoïdan content increased with increasing NaCl solution concentration. From 2 mol/L to 3 mol/L, the fucoïdan content increased abruptly from 1.63% to 4.7%; then, with the increasing concentration, fucoïdan content slowly changed. 4 mol/L was the optimal extract concentration, which might be the effect of ionic strength. Ionic strength was stronger, and the separation effect of chitosan-fucoïdan was better.

3.5. Effect of Different Ratios of NaCl Solution: Sediment on Fucoïdan Content. Different ratios of NaCl solution: sediment also influence the separation of chitosan-fucoïdan complex. In Table 5, fucoïdan content increased from 10:1 to 40:1; however, fucoïdan content slightly decreased from 40:1 to 50:1. Moreover, fucoïdan content slightly changed from 30:1 to 50:1. Therefore, for facilitating posttreatment, 30:1 was the optimal ratio.

3.6. Effect of the Different Separation Time on Fucoïdan Content. Table 6 shows the effect of the different separation time on fucoïdan content. In Table 6, the fucoïdan content increased with increasing separation time. From 4 h to 6 h, the fucoïdan content abruptly changed; then, fucoïdan content slightly changed with increasing time. Therefore, the optimal separation time is 6 h.

4. Discussion

Fucoïdan contains substantial percentages of L-fucose and sulphate ester groups. Due to these functional groups, fucoïdan has a wide spectrum of biological activities such as anticoagulant and antithrombotic activities [11], anti-inflammatory activity [12], and antitumor activity [13]. In order to obtain fucoïdan, different techniques were used to extract fucoïdan, which include the consumption of calcium-containing solvents, acid media, or plain water [14–16]. During the process of conventional production, NaOH was added to the soaked water of seaweed. With pH up to about 12 of the soaked water of seaweed, the polysaccharide mixture was separated [17]. This process needs abundant NaOH and produces a lot of wastewater containing NaOH. These methods not only pollute environment but also waste resources. Therefore, many researchers are seeking better methods to separate polysaccharide mixture from the soaked water. In this paper, in order to reduce the usage of NaOH, new methods to extract fucoïdan and to separate it from the complex of chitosan-fucoïdan were introduced. Effect of different extractants, different solvents, concentrations and volume of solution and the effect of the different separation time on yield and content of fucoïdan were researched. The results indicate that chitosan is the optimal extractant, NaCl solution has better separation effect, 4 mol/L and 30 mL are the optimal concentration and volume of NaCl solution, and the optimal separation time is 6 h.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

TABLE 5: Fucoidan content of separation production in different ratios of NaCl solution : sediment (4 mol/L).

Ratio	10 : 1	20 : 1	30 : 1	40 : 1	50 : 1
Fucoidan content (%)	1.91 ± 0.05	3.29 ± 0.03	5.41 ± 0.02	5.79 ± 0.03	5.51 ± 0.03

TABLE 6: Fucoidan content of extraction at a different separation time*.

Time (h)	2	4	6	8	10
Fucoidan content (%)	4.65 ± 0.01	4.75 ± 0.03	5.3 ± 0.02	5.35 ± 0.02	5.41 ± 0.03

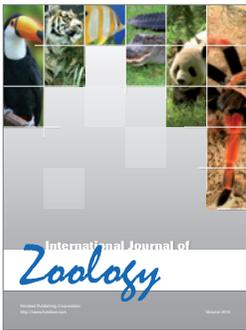
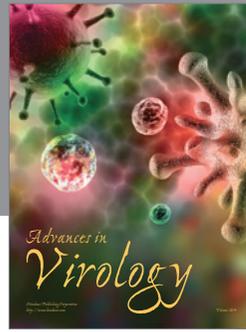
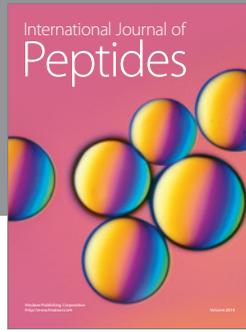
*30 mL and 4 mol/L NaCl solution were used to extract the fucoidan.

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