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A new extraction method of fucoidan from the soaked water of brown seaweed (*Laminaria japonica*)

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ABSTRACT

In this paper, a new extraction method of fucoidan from the soaked water of seaweed (*Laminaria japonica*) was introduced. Studies were carried out to compare, the effect of chitosan (CTS) and NaOH on the extraction of polysaccharide mixture from the soaked water of seaweed which are used for the production of alginate. And the conditions for the extraction of fucoidan were optimized. The results showed that chitosan was better than NaOH which were used to remove polysaccharide mixture in the algae industry. And the optimal conditions for extracting fucoidan were as followed the added volume of 1% CTS of 10 ml/150 ml soaked water, flocculation time of 8.0 h and pH of the soaked water of 6.0.

Keywords: Fucoidan; Chitosan; NaOH; Extraction; Soaked water of seaweed

1. Introduction

Brown seaweed (*Laminaria japonica*) is known to produce different polysaccharides such as alginate, laminaran and fucoidan [1]. The latter polysaccharide is a high-molecular-weight sulfated polysaccharide. Fucoidan, first isolated by Kylin almost a century ago, contains substantial percentages of L-fucose and sulphate ester groups [2]. Due to these functional groups, fucoidan has a wide spectrum of biological activities such as anticoagulant and antithrombotic activities [3–7], anti-inflammatory activity [8,9], antitumor activity [10], contraceptive activity [11,12] and antiviral activity [13,14]. Recently, the supplement of fucoidan to rats with chronic renal failure demonstrated the renoprotective effects of fucoidan [15]. Furthermore, it was also found that fucoidan prevented concanavalin A-induced liver injury by mediating the endogenous IL-10 production and the inhibition of proinflammatory cytokine in mice [16]. Therefore, it represents a source of marine compounds with potential applications in medicine. In order to obtain the potential medicine, different techniques were used to extract fucoidan. They included the action of calcium-containing solvents, acid media or plain water [17–19].

On the other hand, with the development of algae industry, the extraction of iodine and mannitol from the soaked water of seaweed has been one of advantageous projects of traditional algae industry. Nevertheless, all the kinds of polysaccharides in the soaked water of seaweed have brought troubles for the extraction of iodine and mannitol, which directly influence the production cost. During the process of traditional 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 were removed [20]. However, this process needs abundant NaOH, and produces

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vast wastewater containing NaOH. Consequently, the polysaccharide mixture were handled as waste, which not only pollute environment but also waste resource. Therefore, it is of importance to finding a better method to remove polysaccharide mixture from the soaked water of seaweed and extracting fucoidan from the polysaccharide mixture.

Chitosan, poly [β-(1→4)-2-amino-2-deoxy-D-glucopyranose], is a cationic biopolymer which is mainly produced by the alkaline deacetylation of chitin [21]. It is received much attention due to its biocompatibility, biodegradability and non-toxicity. It has been broadly applied in wastewater treatment to reuse metal ion, protein etc. [22–25]. However, it is not used to extracting fucoidan. In this paper, we firstly used chitosan to remove polysaccharide mixture and extracted fucoidan from the soaked water of seaweed in order to avoid a lot of NaOH usage and the waste of fucoidan.

2. Experimental

2.1. Materials

Chitosan was provided by Qingdao Baicheng Corp. (China). Its degree of deacetylation was 97%, and the viscosity average-molecular weight was 7.6×10^6. The standard fucoidan (99%) was purchased from Sigma Chemica Co. And the dried seaweed was purchased from market in Qingdao. The other reagents were of analytical grade and used without further purification.

2.2. The effects of chitosan and NaOH on the extraction of polysaccharide mixture

425 g seaweed was soaked by 6800 ml tap water for 24 h. Then the soaked water was filtered. And it was used in all the latter experiments.

Two replicates of 150 ml the above filtered water were prepared. One was added with 4 ml 1%chitosan in 1% acetic acid aqueous solution (CTS). And the pH of the other was adjusted to 12 with NaOH. Then they were agitated for 2 min and placed for 12 h. After 12 h, the two replicates were centrifuged for 5 min (1500 rpm). The obtained sediments were dried at 50°C and weighed.

2.3. The new extraction method of fucoidan from the soaked water of seaweed

2.3.1. Optimization of conditions for the extraction of fucoidan from the soaked water of seaweed

As the effect of chitosan was better than NaOH from the results of 2.2, optimization studies were carried out by chitosan. The conditions for optimizing extraction of fucoidan from the soaked water were the added volume of CTS (X_1), the flocculation time (X_2) and pH of the soaked water (X_3). The experimental design involved three factors (X_1, X_2 and X_3) each at three equidistant levels (1, 2 and 3), and the response variable was the percent of fucoidan in the sediments (Y). The factors, their levels and codes for level were as followed as Table 1.

The experiment was carried out by $L_9(3^4)$ as Table 2.

2.3.2. The whole process of extracting fucoidan from the soaked water of seaweed

150 ml of the soaked water was flocculated according to the results of 2.3.1. Then, it was centrifuged for 5 min (1500 rpm). The obtained sediment was dried at 50°C. 0.2176 g dried sediment was milled to powder and dipped in 30 ml double-distilled water with pH 4.00 for 12 h. Then, the mixture was agitated for 2 h at room temperature and filtered. The filtrate was added with ethanol until the concentration of ethanol in the mixture was up to 75%. After 15 min, the mixture was filtered. And the precipitate was freeze-dried. The dried precipitate was weighed and determined by Infrared spectra.

Table 1
Factors and levels

Factors	Codes	Levels		
		1	2	3
The added volume of CTS (ml)	X_1	2.0	6.0	10.0
The flocculation time (h)	X_2	2.0	4.0	8.0
pH of the soaked water	X_3	2.0	6.0	8.0

Table 2
The conditions of extracting fucoidan

Codes	X_1 (ml)	X_2 (h)	X_3
Numbers of experiment			
1	2.0	2.0	2.0
2	2.0	4.0	6.0
3	2.0	8.0	8.0
4	6.0	2.0	6.0
5	6.0	4.0	8.0
6	6.0	8.0	2.0
7	10.0	2.0	8.0
8	10.0	4.0	2.0
9	10.0	8.0	4.0

2.4. The determination of fucoidan in the obtained sediments of 2.3.1

2.4.1. The preparation of the regression equation for fucoidan

0.01 g standard fucoidan 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. 4.5 ml of 87% H_2SO_4 aqueous solution per test tube was included the above mixture in ice water and shaken. One minute later, they were rapidly placed into boiling water and heated for 10 min. After they were cooled to room temperature, 0.1 ml of 3% cysteine chloride aqueous solution was added and the mixture was placed for 90 min. The absorbance of them at 427 and 396 nm was determined, respectively. Then, the regression equation was made between the absorbance difference (396–427 nm) (Y) and the volume (X) of the fucoidan solution.

2.4.2. The determination of fucoidan in the obtained sediments of 2.3.1

1 g of dried sediments in 2.3.1 were milled to powder and dipped in 30 ml double-distilled water with pH 4.00 for 12 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 fucoidan (C, mg ml^{-1}). The determined method was as in Sect. 2.4.1. Then, the total fucoidan in the sediment (1 g) was calculated as followed

$$\text{The yield of fucoidan in 1g sediment} = C \times 100$$

2.5. Statistical analysis

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

3. Results and discussion

3.1. The comparison of chitosan and NaOH in terms of removement of polysaccharide mixture

In this experiment, the obtained sediment treated with CTS was 0.213 g which is higher than that treated with NaOH (0.105 g). Furthermore, the soaked water treated with CTS was clearer than that treated with NaOH, probably because chitosan is macromolecule which supplied more positions to combine with the polysaccharides than NaOH. In conclusion, chitosan was better than NaOH which was used to remove glycines in algae industry. Therefore, we used chitosan to handle soaked water of seaweed, and determine the optimal experimental conditions for the extraction of fucoidan and the removement of polysaccharide mixture.

3.2. The optimization of conditions for the extraction of fucoidan

The IR spectra of the obtained fucoidan was shown in Fig. 1. It was similar to the spectra published earlier [26]. The signal at 1260 cm^{-1} was attributed to the asymmetric stretching of S=O, and the signal at about 850 cm^{-1} was the stretching of C–O–S. The adsorption band at about 1100 cm^{-1} indicated the stretching of C–O and deformation vibration of O–H.

In Table 3, according to the scores of R (82.78, 45.01 and 45.37), we concluded that all the three factors influenced the yield of fucoidan. However, the added volume of CTS (X_1) had significant effect on the extraction of fucoidan. The effect of the other factors (X_2 and X_3) was junior to that of the added volume of CTS. For one factor, the optimum yield of fucoidan was obtained when the maximum of K happened. Therefore, the optimized conditions for the extraction of fucoidan from the soaked water of seaweed were the added volume of CTS of 10 ml/150 ml soaked water, flocculation time of 8.0 h and pH of the soaked water of 6.0.

3.3. The determination of fucoidan in crude fucoidan of 2.3.2

There are different methods to extract fucoidan from different algae. First extraction attempts were carried out by use of plain water, often acidified, or other solvents [6]. The first attempts to carry out systematic approaches to extraction was proved effective by Mian and Percival [27]. They developed a sequential extraction that started

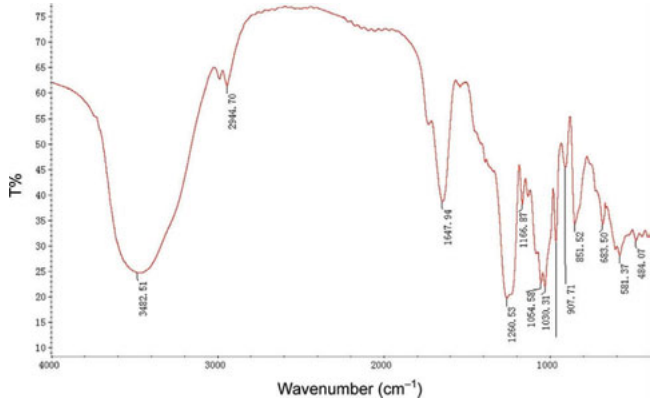

Fig. 1. IR spectra of fucoidan.

Table 3
The yield of fucoidan in 0.1 g sediment of all groups

Numbers of experiment	X_1[1] (ml)	X_2[2] (h)	X_3[3]	Y[4] (mg)
1	2.0	2.0	2.0	—
2	2.0	4.0	6.0	149.85 ± 0.03
3	2.0	8.0	8.0	170.40 ± 0.04
4	6.0	2.0	6.0	143.30 ± 0.01
5	6.0	4.0	8.0	136.12 ± 0.03
6	6.0	8.0	2.0	165.28 ± 0.03
7	10.0	2.0	8.0	—
8	10.0	4.0	2.0	165.28 ± 0.04
9	10.0	8.0	4.0	196.92 ± 0.03
K_1[5]	320.25	—	301.4	
K_2[6]	279.42	458.43	346.7	
K_3[7]	362.2	503.44	313.7	
R[8]	82.78	45.01	45.37	

The symbol "—" indicated that the determined yield of fucoidan was inaccurate because the obtained sediment was less than 0.1 g.

[1]The added volume of CTS.
[2]Flocculation time.
[3]pH of the soaked water.
[4]The yield of fucoidan in 0.1 g sediment.
[5]The sum of Y_1 when the code was 1.
[6]The sum of Y_2 when the code was 2.
[7]the sum of Y_3 when the code was 3.
[8]$\max\{K_1, K_2, K_3\} - \min\{K_1, K_2, K_3\}$

by a formaldehyde treatment, followed by an 80% ethanol extraction, in order to remove mannitol, salts, and other low-molecular weight products. A further extraction with 2% calcium chloride (at room temperature and 70°C) was used to extract fucoidans and laminaran (fixing the alginate as its calcium salt). Fucoidans were further extracted with aqueous hydrochloric acid (pH 2). At this point, the residue was extracted with sodium carbonate in order to render the alginate soluble. Two final additional solvents extracted further fucoidan fractions. This complicated sequential procedure was rarely-followed completely afterwards, but became the basis of further work [8,19]. Other authors used simpler extraction procedures, but applied elaborate purification steps [4,17].

In algae industry, iodine and mannitol were extracted from the soaked water of seaweed. However, all kinds of glycines in the soaked water bring troubles for the production of iodine and mannitol. For example, during the extraction of iodine by the method of ion-exchange chromatography polysaccharide mixture can block up ion-exchange column. Therefore, many researchers were looking for a good method to remove polysaccharide mixture from the soaked water in order to protect equipments such as ion-extrange column and save production cost.

In this paper, chitosan was firstly used to remove polysaccharide mixture and extract fucoidan from the soaked water of seaweed. On one hand, After treated with chitosan, the soaked water was clearer than that treated with NaOH, which was beneficial of prolonging the service life of equipments for the production of iodine and mannitol. On the other hand, it was a novel method to extract fucoidan. The utilization of chitosan instead of NaOH simplied the procedure of extracting fucoidan, as this method reduced the content of NaCl in the sediment. Generally, it was a new extraction method of fucoidan, which was useful for the production of iodine, mannitol and fucoidan.

4. Conclusions

In this paper, a new extraction method of fucoidan from the soaked water of seaweed was introduced. Studies were carried out to compare the effect of chitosan and NaOH in terms of removement of polysaccharide mixture and optimize the conditions for the extraction of fucoidan. The results showed that chitosan was better than NaOH which were used to remove glycines. And the added volume of 1% CTS of 10 ml/150 ml soaked water, flocculation time of 8.0 h and pH of the soaked water of 6.0 were the optimal conditions. This study would not only save production cost of iodine and mannitol, but also would utilize resources fully.

Acknowledgements

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References

[1] E. Percival and R.H. McDowell, *Chemistry and Enzymology of Marine Algae Polysaccharides.* Academic Press, New York (1967), pp. 157–175.
[2] M.S. Patankar, S. Oehninger, T. Barnett, R.L. Williams and G. F. Clark, Structure and anticoagulant activity of sulfated fucans, J. Biol. Chem., 268 (1993) 21770–21776.
[3] F.C. Church, J.B. Meade, R.E. Treanor and H.C. Whinna, Antithrombin activity of fucoidan, J. Biol. Chem., 264 (1989) 3618–3623.
[4] T. Nishino, H. Kiyohara, H. Yamada and T. Nagumo, Schkuhripinnatolides, unusual sesquiterpene lactones from Schkuhria pinnata, Phytochemistry, 30 (1991) 535–539.
[5] A. Nardella, F. Chaubet, C. Boisson-Vidal, C. Blondin, P. Durand and J. Jozefonvicz, Anticoagulant low molecular weight fucans produced by radical process and ion exchange chromatography of high molecular weight fucans extracted from the brown seaweed *Ascophyllum nodosum*, Carbohydr. Res., 289 (1996) 201–208.
[6] E. Percival, Glucuronoxylofucan, a cell-wall component of *ascophyllum nodosum,* Carbohydr. Res., 7 (1968) 272–283.

[7] L. Chevolot, B. Mulloy, J. Ratiskol, A. Foucault and S. Colliec-Jouault, A disaccharide repeat unit is the major structure in fucoidans from two species of brown algae, Carbohydr. Res., 330(2001) 529–535.
[8] A.O. Chizhov, A. Dell, H.R. Morris, S.M. Haslam and R.A. McDowell, A study of fucoidan from the brown seaweed *Chorda filum*, Carbohydr. Res., 320(1–2) (1999) 108–119.
[9] O. Berteau and B. Mulloy, Sulfated fucans, fresh perspectives: structures, functions, and biological properties of sulfated fucans and an overview of enzymes active toward this class of polysaccharide, Glycobiology, 13(6) (2003) 29R–40R.
[10] C. Zhuang, H. Itoh, T. Mizuno and H. Ito, Antitumor active fucoidan from the brown seaweed, Umitoranoo (Sargassum thunbergii), Biosci. Biotechnol. Biochem., 59 (1995) 563–567.
[11] M.C. Mahony, S. Ochninger, G.F. Clark, A.A. Acosta and G.D. Hodgen Fucoidin inhibits the zona pellucida-induced acrosome reaction in human spermatozoa, Contraception, 44 (1991) 657–665.
[12] M.C. Mahony, S. Ochninger, S. Oehninger, A.A. Acosta and G.D. Hodgen, Fucoidin binding activity and its localization on human spermatozoa, Contraception, 48 (1993) 277–289.
[13] S. Preeprame, K. Hayashi, J.B. Lee, U. Sankawa and T. Hayashi, A novel antivirally active fucan sulfate derived from an edible brown alga, Sargassum horneri, Chem. Pharm. Bull. (Tokyo), 49 (2001) 484–485.
[14] W. Zhu, E.O. Vincent, K.C. Paul and O.A.J. Put, Isolation and characterization of a sulfated polysaccharide from the brown alga Sargassum patens and determination of its anti-herpes activity, Biochem. Cell Biol., 81 (2003) 25–33.
[15] Q. Zhang, Z. Li, Z.H. Xu, X.Z. Niu and H. Zhang, Effects of fucoidan on chronic renal failure in rats, Planta Med., 69(6) (2003) 537–541.
[16] S. Ako, Y. Masashi, Y. Shiro, O. Mitsuyoshi, H. Masakazu, and N. Kimihide, Fucoidan prevents concanavalin A-induced liver injury through induction of endogenous IL-10 in mice, Hepatol. Res., 35 (2006) 190–198.
[17] M.E.R. Duarte, M.A. Cardoso, M.D. Noseda and A.S. Cerezo, Structural studies on fucoidans from the brown seaweed Sargassum stenophyllum, Carbohydr. Res., 333 (2001) 281–293.
[18] T.N. Zvyagintseva, N.M. Shevchenko, I.B. Popivnich, V.V. Isakov, A.S. Scobun, E.V. Sundukova and L.A. Elyakova, A new procedure for the separation of water-soluble polysaccharides from brown seaweeds, Carbohydr. Res., 322 (1999) 32–39.
[19] M.F. Marais and J.P. Joseleau, A fucoidan fraction from *Ascophyllum nodosum*, Carbohydr. Res., 336 (2001) 155–159.
[20] D.M. Xue, J.J. Ma, Z.Q. Pei and X.L. Wang, A method of cleaning the soaked water of seaweed, China Patent 90100214.3.
[21] P. Lertsutthiwong, N.C. How, S. Chandrkrachang and W.F. Stevens, Effect of chemical treatment on the characteristics of shrimp chitosan, J. Met. Mater. Miner., 12(1) (2002) 11–18.
[22] S. Wibowo, V. Savant, G. Cherian, T.F. Savage and J.A. Torres, Evaluation as a feed ingredient of a surimi wash water protein recovered using achitosan-alginate complex, J. Aquat. Food Prod. Technol., 14(1) (2005) 55–72.
[23] V.D. Savant and J.A. Torres, Chitosan based coagulating agents for treatment of cheddar cheese whey, Biotechnol. Progr., 16 (2000) 1091–1097.
[24] I. Uzun and F. Guzel, Adsorption of some heavy metal ions from aqueous solution by activated carbon and comparison of percent adsorption results of activated carbon with those of some other adsorbents, Turk. J. Chem., 24 (2000) 291–297.
[25] Xiaolin Chen, Cuiping Li, Xia Ji, Zhimei Zhong and Pengcheng Li, Recovery of protein from discharged wastewater during the production of chitin, Bioresour. Technol., 99 (2008) 570–574.
[26] J. Wang, L. Liu, Q.B. Zhang, Z.S. Zhang, H.M. Qi and P.C. Li, Synthesized oversulfated, acetylated and benzoylated derivatives of fucoidan extracted from *Laminaria Japonica* and their potential antioxidant activity in Vitro, Food Chem., 114 (2009) 1285–1290.
[27] A.J. Mian and E. Percival, Carbohydrates of the brown seaweeds *himanthalia lorea*, *bifurcaria bifurcata*, and *Padina pavonia*: Part I. extraction and fractionation, Carbohydr. Res., 26 (1973) 133–146.