Effect of Chopping on the Quality of Fucoidan Extracted from Sargassum filipendula

Ellya Sinurat and Diini Fithriani

1Research Center Product Processing and Biotechnology of Marine and Fisheries, Indonesia

Email: ellya_sinurat@yahoo.com

Abstract. The objective of this study was to investigate the effect of brown seaweed chopping on quality of fucoidan extracted. The brown seaweed used was Sargassum filipendula, which was obtained from Binuangun, Banten and used as the raw material for fucoidan extraction. There were two treatments, in which a batch of Sargassum sp. was chopped directly at the sampling location, whereas the other batch was not chopped. Then, Sargassum sp. was macerated with ethanol for 24 hours and dried. Fucoidan was extracted by using 0.01N HCl and filtrated by using plankonet. Then, CaCl₂ was added into the filtrate to separate the alginate from fucoidan. The fucoidan obtained was added by ethanol and dried in freeze dryer. Each treatment was performed by 3 replications. The yield, carbohydrates and sulfate ester content of fucoidan was determined. The result showed that the chopped Sargassum sp. has higher yield and total carbohydrates compared to the unchopped Sargassum filipendula, but there was no significant difference of sulfate contents.

1. Introduction

Brown seaweeds constitute an important and in traditional medicine since prehistoric times. Sargassum sp. is the most common type of brown seaweed found in Indonesia. There were 12 genes of Sargassum sp. which have been studied, i.e. Sargassum duplicatum, S. hystrix, S. echinocarpum, S. binderi Sonder, S. gracilinum, S. crassifolium, S. obtusifolium, S. microphyllum, S. polycystum, S. vulgare, S. polyceratium, S. Aquofilum [1]. Seaweed is hydrocolloid polysaccharide which consist of components such as alginate, agar and carageenan which have used in many application and Industries. Moreover, Seaweeds are also source of potentially bioactive compounds. The bioactive compounds found in brown seaweed polysaccharides are fucoidan and laminarin [2].

Fucoidans is consisted of a group of fucose-rich, sulfated polysaccharides, usually built of a backbone of α-linked L-fucose residues having various substitutions. Fucoidan polysaccharides are mainly found in brown seaweeds, but fucoidans differ in structure among brown seaweed species. Fucoidans cover several different structural entities that designate a family of fucose-containing sulfated polysaccharides [3]. The structural characteristics of fucoidan are likely to depend on the extraction technique [4], seaweed species, harvesting season [5], geographic location [6], and algal maturity [7]. These factors contribute to the fact that fucoidan structure is complex [8]. The variation of sulphate and fucose content has been studied in more depth. These are seen to vary in line with each other and follow in inverse trend to the total fucoidan content. Fucoidan content has been shown to vary over the year, with the highest content in the autumn [9]. The extraction of fucoidan from macroalgae has been...
performed by several authors in the published literature [10]. In general this consists of four main steps, i.e. an initial purification to remove pigments and lipids, often using an alcohol; an extraction step, often repeated several times to ensure full extraction of fucoidan and most commonly using calcium chloride, dilute hydrochloric acid (HCl) or water; and further purification of the extract to remove alginate and other impurities before fucoidan is finally precipitated using ethanol [11].

2. Materials and Methods

2.1. Materials

The raw materials used in this study were dried brown seaweed with moisture content of 27.92 ± 0.35%. The brown seaweed (Sargassum filipendula) was collected from Binuangeun, Indonesia. The reagents used were mostly in analytical and technical grades.

2.2. Extraction, prepurification, and hydrolysis of fucoidan

The first treatment, 1 kg of fresh brown seaweed (S. filipendula) was cleaned, chopped and dried. Then, S. filipendula was macerated with 2 L of ethanol at room temperature for 24 hours to remove the lipophilic pigments (chlorophyll, fucoxanthin, and carotene) and fats. Then, the extraction results was treated with 2 L of 0.1 N HCl (1:20) (w/v) at pH 5 and stirred for 4 hours at 85°C temperature. The filtrate was collected and centrifuged at 5,000 rpm for 15 minutes. The filtrate was subsequently neutralized with 0.5 M NaOH, and added with 2% CaCl₂ solution, incubated for 30 minutes and then centrifuged. The filtrate was collected and added to ethanol (1:2) and incubated overnight. The precipitate formed in the ethanol solution was separated by centrifugation and freeze-dried to obtain crude fucoidan.

The second treatment, 1 kg of fresh brown seaweed was cleaned and then dried without chopping procedure. Then, S. filipendula was macerated with 4 L of ethanol at room temperature for 24 hours. The extraction method used was same with the extraction method of first treatment (Modified method of Sinurat et al, 2015). The each treatment of extraction was conducted in triplicates.

2.3. Characterization of fucoidan

2.3.1 Yields

The determination of yield fucoidan obtained from fresh brown seaweed S. Filipendula.

2.3.2 Determination of total carbohydrate

The determination of total carbohydrate content was performed by phenol - sulfuric acid method (Dubois, et al, 1956). The prepurified and hydrolyzed fucoidan (5 mg) was dissolved in 1 mL of aquaest and then 2.5 mL of concentrated H₂SO₄ was added prior to incubation in an ice tub for 30 minutes. Subsequently, 0.5 ml of 5% phenol was added into the sample solution and was also incubated in the ice tub for 30 minutes. Fucose, mannose, and xylose were used as standards at various concentrations (200, 400, 600, 800, and 1000 ppm). Sample, standard, and blank solutions were measured in triplicates at 480 nm and 490 nm with a UV-VIS spectrophotometer.

2.3.3 Determination of sulfate content

The sulfate ester content in fucoidan was performed by BaCl₂ – Gelatin method. In 2 mL of 3.5 N HCl, 6 mg of prepurified and hydrolyzed fucoidan were hydrolyzed for 17-18 hours at 105-110°C by Dodgson & Price, 1962. Then, 10 μL of hydrolyzed fucoidan was placed into the 96-well plate, added with 190 μL of 3% TCA and 50 μL of 0.5% BaCl₂ – gelatin. The plate was left for 20 minutes until the barium sulfate ready to be scanned. Na₂SO₄ was used as a standard with various concentrations (100 – 500 ppm). Both sample and standard solutions were scanned against blank solution in triplicates with Microplate Reader Spectrophotometer at λ = 360 nm.
3. Results and Discussions

3.1. Isolation and chemical characteristics of crude and prepurified fucoidan

3.1.1 Yield

The yield obtained from the extraction of fucoidan from the chopped brown seaweed was significantly higher than the yield crude fucoidan obtained from unchopped brown seaweed. The fucoidan yield of the chopped and unchopped brown seaweed was 8.3% and 5.8%, respectively. The important factors that affect extraction process includes: material size, temperature extraction, and solvent. The characteristic of fucoidan showed in the Table 1.

Table 1. Yield and chemical characteristics of extracted fucoids

| Fucoidan   | Yield (%) | Total Carbohydrate (%) | Sulfate Content (%) |
|------------|-----------|------------------------|---------------------|
| Chopped    | 8.3±0.5   | 72.40±1.05             | 12.99±0.8           |
| Unchopped  | 5.8±0.43  | 63.45±1.4              | 12.63±0.7           |

3.1.2 Total carbohydrate

The total carbohydrate obtained from chopped and unchopped brown seaweed was 72.40% and 63.45%, respectively. Carbohydrates are the major constituent compounds of fucoidan, and fucose is the most dominant monomer constituent. The yield is related to total carbohydrates because the main constituents of fucoidan are carbohydrates or polysaccharides. In this study, the total carbohydrate of crude fucoidan obtained from chopped brown seaweed was higher than unchopped brown seaweed. Carbohydrates is dissolved in water and more easily extracted if the pores sizes are larger.

3.1.3 Sulfate content

The difference of the sulfate content of fucoidan obtained from chopped and unchopped brown seaweed was not significant. The sulfate content of chopped and unchopped fucoidan was 12.99% and 12.63%, respectively. This was due to the sulfate contents of fucoidan was not influenced by the size reduction of brown seaweed used.

The material size reduction was aimed to expand the material surface area, thus speeding up the penetration of solvent into the material to be extracted. The size reduction was also aimed to reduce the amount of solvent needed for extraction. The smaller particle size will increase the surface area and accelerate the solubility of a substance [12, 13]. The results obtained are in accordance with the theory that the more fine the particles used would form more pores in brown seaweed, which increased the yield obtained. Based on the result obtained, the size reduction was speculated affected the overall quality of crude fucoidan extracts. However, the mechanism involved requires further study.

4. Conclusions

The conclusions of this study were that the chopping treatment on brown seaweed influenced the quality of crude fucoidan on yield and total carbohydrate. The size reduction of brown seaweed also reduced the amount of extraction solvent and resulted higher extraction quality. The size reduction was speculated to affect the overall quality of crude fucoidan extract.

References

1. Marcel Tutor Ale and Anne S. Meyer. 2013. Fucoids from brown seaweeds: an update on structures, extraction techniques and use of enzymes as tools for structural elucidation. Royal Society of Chemistry, 3, p:8131-8141.
2. Marcel Tutor Ale, Mikkelsen D.J, and A. S. Meyer. 2011. Important Determinants for Fucoidan Bioactivity: A Critical Review of Structure-Function Relations and Extraction Methods for Fucose-Containing Sulfated Polysaccharides from Brown Seaweeds. *Mar. Drugs*, 9, p:2106–2130

3. Bilan, M. I., Grachev, A. A., Shashkov, A. S., Kelly, M., Sanderson, C. J., Nifantiev, N. E., & Usov, A. I. 2010. Further studies on the composition and structure of a fucoidan preparation from the brown alga Saccharina latissima. *Carbohydrate Research*, 345(14), 2038–2047. https://doi.org/10.1016/j.carres.2010.07.009

4. Honya, M., Mori, H., Anzai, M., Araki, Y., & Nisizawa, K. 1999. Monthly changes in the content of fucans, their constituent sugars and sulphate in cultured Laminaria japonica. *Hydrobiologia*, 398(0), 411–416. https://doi.org/10.1002/(A:1017007623005

5. Rioux, L.-E., Turgeon, S. L., & Beaulieu, M. 2007. Rheological characterisation of polysaccharides extracted from brown seaweeds. *Journal of the Science of Food and Agriculture*, 87(9), 1630–1638. https://doi.org/10.1002/jsfa.2829

6. Zvyagintseva, N. T., M Shevchenko, N., B Popivnich, I., V Isakov, V., S Scobun, A., V Sundukova, E., & A Elyakova, L. 1999. A new procedure for separation of water-soluble polysaccharides from brown seaweeds. *Carbohydrate Research*, 322.

7. Shevchenko, N. M., Anastuyk, S. D., Menshova, R. V., Vishchuk, O. S., Isakov, V. I., Zadorozhny, P. A., Zvyagintseva, T. N. 2015). Further studies on structure of fucoidan from brown alga Saccharina gurjanovae. *Carbohydrate Polymers*, 121, 207–216. https://doi.org/10.1016/j.carbpol.2014.12.042

8. Fletcher ,H.R Biller P, Ross, Adams J. 2017. The seasonal variation of fucoidan within three species of brown macroalgae. Algal Research, Vol 22, Pages 79-86

9. Anastuyk S.D , N.M. Shevchenko, Ermakova S.P., Vishchuk O.S., , Nazarenko E.L., Dmitrenok P.S., Zvyagintseva T.N., 2012. Anticancer activity in vitro of a fucoidan from the brown alga *Fucus evanescens* and its low-molecular fragments, structurally characterized by tandem mass-spectrometry, Carbohydr. Polym., 87 pp. 186-194

10. Skritssova, A., Shevchenko N., Zvyagintseva T., Imbs. 2010. Monthly changes in the content and monosaccharide composition of fucoidan from *Undaria pinnatifida* (*Laminariales, Phaeophyta*). J. Appl. Phycol., 22, pp. 79-86

11. Hernani, T. Marwati, C. Winarti,. 2007. Pemilihan Pelarut Pada Pemurnian Ekstrak Lengkuas (Alpinia galanga) Secara Ekstraksi, Jurnal Pascapanen Vol. 4 No. 1, Hal : 1 – 8.

12. Rondang Tambun , Harry P. Limbong , Christika Pinem, Ester Manurung,. 2016., Pengaruh Ukuran Partikel, Waktu Dan Suhu Pada Ekstraksi Fenol Dari Lengkuas Merah. Jurnal Teknik Kimia USU, Vol. 5, No. 4 , p:53-56.

13. Ellya Sinuratdan Rinta Kusumawati,. 2017. Optimization of Crude Fucoidan Extraction Methods from Brown Seaweed Sargassum binderi Sonder. PB Kelautan dan Perikanan Vol. 12 No. 2 p: 125-134