EXTRACTION OF FUKOIDAN CHARACTERIZATION FROM CHOCOLATE ALGAE *Sargassum sp.*

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Abstract

Fucoidan were recovered from *Sargassum polycystum* by single-step extraction with ultrasonic wave pre-treatment. Extraction were optimized using central composite design of respon surface method. Ultrasonic wave pretreatment conditions were 80% amplitude, for 15 minutes. Alga solution in 0.03 M HCl ratio 1:20 (b/v) at 70-90^{o}C for 3-5 h were evaluated during this process to establish a condition to maximize the extraction. The aim of this research was getting the optimum conditions extraction yield fucoidan *Sargassum* sp..Result this research all extraction factors had significant effects on fucoidan yield ($p<0,05$). The optimum conditions were extracting temperature 81^{o}C, extracting time 4,04 h. Under this condition, the experimental yield of crude fucoidan 7,15±5,58%. Based on the characterization result of fucoidan by fourier transform-infrared spectroscopy, we found that fucoidan *Sargassum* sp. was composed sulfate group.

Keywords: Sargassum sp., fucose, extraction method

Introduction

Fucoidan was a polysaccharide that was found in the cell wall matrix of brown algae, composed of L-fucosa and sulfate, in small amounts of galactose, mannose, xylose, glucose and rhamnosa (Shiroma *et al.,* 2008). Fucoidan has diverse biological activities that were useful as natural herbs for the body to treat various diseases, especially tumors and cancer (Ermakova *et al.,* 2011). Fucoidan can be extracted from brown algae *Sargassum* sp (Chotigeat *et al.,* 2004).

The production potential of *Sargassum* sp. was so large, this brown algae was abundant in almost all coastal waters in Indonesia (Kadi, 2004). Utilization of *Sargassum* sp. still very limited, this brown algae is classified as marine trash and the price is relatively very cheap. Price of *Sargassum* sp. Rp 1.500,- / kg at farmer level and export prices of Rp 3.000,- / kg (0.3 $), while the price of imported fucoidan was very expensive ranging between Rp 990.000,- / kg to Rp 4.050.000,- / kg according to its level of purity (PT. Marinal Indoprima, 2011).

In general, fucoidan extraction method was extracted with hot water, acid solution, alkaline solution, and $CaCl_2$ was added to precipitate alginate (Rioux *et al.,* 2007; Cumashi *et al.,* 2007). Ermakova *et al.* (2011) carried out fucoidan extraction. *Sargassum* sp hornery at 60^{o}C with 0.1 M HCl for 4 h 2 stages of extraction, precipitation with 4 volumes of ethanol 96%. Li *et al.,* (2006) extracted fucoidan Hezikia fusiforme 3 stages of extraction temperature of 70^{o}C for 6 h, precipitation with $CaCl_2$ and ethanol. Ale *et al.,* (2011a) extracted *Sargassum henslowianum* at 90^{o}C for 5 h. This fucoidan extraction took a long time and the yield was relatively low.

Ultrasonic wave extraction technique has been applied by Kwong *et al.* (2011) for fucoidan extraction from brown algae *Laminaria japonica*. Krishnaiah *et al.* (2007) extracted polysaccharides from *Eucheuma denticulatum* with ultrasonic waves. This ultrasonic wave extraction technique requires Krishnaiah *et al.* (2007) to extract polysaccharides from *E. denticulatum* with ultrasonic waves. the yield was still low. Therefore an appropriate extraction method was needed so that a good fucoidan was obtained which was a combination technique of ultrasonic initial degradation extraction followed by waterbath heating extraction.

Optimization of the extraction of polysaccharides from brown algae was carried out Ale *et al.* (2011a) in Denmark optimization of fucoidan extraction from *S. henslowianum*

by surface response method, brown algae originated from Vietnam. Qiao *et al.*, (2009) performed extraction optimization by the surface response method, purification and characterization of polysaccharides from chocolate Hyriopsis cumiialga originating from China. While the optimization of fucoidan extraction from *Sargassum* sp. with the initial degradation of the ultrasonic wave to be carried out in this study, no one has examined it. In this study optimization of the fucoidan extraction of *Sargassum* sp. was performed with the response surface method and characterized by FTIR (Fourier Transform Infra Red).

Materials And Methods

The main ingredient used in this study was *Sargassum* sp. which was obtained from the coastal waters of Poteran Sumenep island, Madura. Chloroform, methanol, aquades, hydrochloric acid (HCl) 37%, ethanol 99.8%, NaOH, CaCl2, trifluoroacetic acid (TFA) 99%, H2SO4, D-glucose and D-xylose, L-rhamnose (Sigma-Aldrich) and L-fucose (Santa Cruz). All chemicals have a degree of purity pro analys (pa) obtained from Gamma Scientific Biolab and Makmur Sejati Chemical.

Experimental Design

The experimental design in the response surface method used in this study was a two-level factorial design (2k) plus five central points for first-order experiments and central composite designs for second-order experiments. First-order experiments using a two-level factorial design plus five central points (5-9) to obtain 9 treatments. The optimization of the extraction process uses the central composite design method of RSM with 2 variables namely extraction temperature (x1) and extraction time (x2). The optimization stage of the extraction process has 13 treatments randomly ordered with 5 central point replications (treatments 9-13). Two variables examined in this study were temperature and extraction time. Temperature variations were 70^{o}C, 80^{o}C, 90^{o}C and extraction time were 1, 3 and 5 h.

Based on experimental data, a regression analysis and the suitability of the second-order polynomial equation model are performed:

$$y = \beta_0 + \sum_{i=1}^{2} \beta_i x_i + \sum_{i=1}^{2} \beta_{ii} x_i^2 + \sum \sum_{i<j} \beta_{ij} x_i x_j$$

..... (1)

Where Y was the response variable, β0 was the intercept coefficient; βi, βii, βij were the linear coefficient, quadratic, interaction, and xi, xj, codes of two independent variables extraction temperature and extraction time ($i \neq j$).

One stage fucoidan extraction

A sample of 10 kg of brown algae *Sargassum* sp. was taken from Poteran island, Sumenep district Madura. *Sargassum* sp. Was washed with fresh water to remove dirt, sand and mud then dried in the sun to dry to 17% water content. Sample *Sargassum* sp. dry mashed by dimilling using OBH Nordia mill 100 watts then sieved with 500 μm sieve and pretreatment with MeOH-$CHCl_3$-H_2O (4:2:1) at room temperature to remove color, phenol components and fats.

A number of grams of *Sargassum* sp. and hydrochloric acid solution (0.03 M HCl) at a ratio of 1:20 (w/v) degraded with ultrasonic waves (amplitude 80%, 15 minutes). Then proceed with extraction by heating waterbath temperature 70-90^{o}C and extraction time 3-5 h, after extracting the filtrate filtered with a nylon filter. Supernatant (liquid filtrate) added 1 M$CaCl_2$ (w/v) left overnight at 4^{o}C to precipitate alginate, then alginate deposits separated by filtered way. Supernatant added 3 volumes of 96% ethanol stored for 8 h at 4^{o}C to precipitate fucoidan, deposited fucoidan taken by centrifugation (speed of 10,600 g for 15

minutes at 4°C), pellets (fucoidan crude extract) were collected and transferred to petri dishes. The pellets obtained were dried in an oven overnight at 45°C (Modified by Ale *et al.*, 2011a). Then the yield was calculated as the ratio between the fucoidan weight of the extract and the initial sample weight (cip *Sargassum* sp.) Used multiplied by 100%.

$$\text{Yield} = \frac{\text{The weight of fucoidan extracted}}{\text{Initial sample weight (chip) before extracting}} x100\% \quad \text{..... (2)}$$

Fucoidan Characterization

Identification of the fucoidan functional group *Sargassum* sp. With FT-IR using the pellet potassium bromide method (Rodriguez *et al.*, 2011). Function group analysis with Infra Red Analysis Spectroscopy (FT-IR) type a Perkin-Elmer 16 PC spectrometer (Boston-USA) uses 16 scans and a frequency range of 400-4000 cm^{-1}. Fucoidan was mashed spectroscopically with potassium bromide powder then pressed into 1 mm pellets. Transition vibrational frequencies for each spectrum are corrected at baseline and absorbance was normalized between 0 and 1.

Analysis of the composition of fucoidan monosaccharides was carried out using High Performance Liquid Chromatography (HPLC), 10-15 mg of crude fucoidan extract hydrolyzed with 2 M trifluoroacetic acid (0.5 ml) at 121oC for 2 h, in a glass tube covered with N2 gas. Then the glass tube was cooled with ice-water bath, centrifuged at a speed of 5000 rpm for 5 minutes, the liquid fraction was neutralized with 2 M NaOH to pH 7. Then the sample was injected into the HPLC system. HPLC KNAUER manager system 5000, pump 1000, column oven 4050, refraction index detector S2300, Aminex HPX 87H column, temperature 65oC, mobile phase H_2SO_4 0.005 M with flow rate 0.6 ml/min, injection volume 20 µl (Rodriguez *et al.*, 2011).

Data analysis and statistics

Data analysis and statistics used Software Design Expertvers 7. Parameter measured yield of response yield. Analysis of the accuracy of the results of validation experiments was done by comparing the results of the validation with the predicted value of the Design Expert program data version at a level of confidence of 0.05 (5%).

Results And Discussions

Surface Analysis of First Order Response

The study of the effect of temperature and extraction time on the response of fucoidan *Sargassum* sp. First-order experiments, the extraction process was carried out at 70-90°C and 3-5 h extraction time. First-order experimental research results obtained response fucoidan yield ranged from 5.18% to 7.06%. The effects of temperature and extraction time on fucoidan yield are presented in Table 1.

The results showed that fucoidan yield increased at extraction temperature of 60-80°C and time of 3-4 h, decreased at extraction temperature of 90°C and time of 3-5 h. The results of the analysis of the fucoidan yield response showed that the linear regression model had a calculated F value of 0.77 smaller than the table's F value ($\alpha = 0.05$) which was 5.14. This indicates that the linear regression model was not significant to the response at the level of trust (α=0.05). The deviation parameter test of the model (curvature) has a Fcount value of 7.51 greater than the F table value (α=0.05) of 6.94, which means that the curvature was significant at the 0.05 level of confidence. Montgomery (2005) states that the curvature test has a calculated F value greater than F table 0.05, which means that the curvature was significant at the 0.05 confidence level. This shows that the optimal response quadratic polynomial model was around 80°C and 4 h.

Second-order experiments

Effect of Temperature and Extraction Time on Fucoidan Yield

Research on the effect of different temperature and extraction time on the fucoidan yield of

Sargassum sp., Extraction process was carried out at 70-90°C and 3-5 h extraction time. The results showed that the yield of *Sargassum* sp. Fucoidan ranged from 4.27% to 7.10%. The effect of temperature and extraction time on the yield of fucoidan was presented in Figure 1B.

The treatment temperature and extraction time had a very significant effect on the increase in yield. The results of Figure 1B showed that the yield of fucoidan tends to increase with the higher extraction temperature of 70-90°C and extraction time of 3-5 h, after reaching the optimal extraction point (80°C, 4 h) yields began to decrease. The lowest fucoidan yield of 4.27% occurred at an extraction temperature of 65.68°C and within 4 h, the highest fucoidan yield of 7.10% occurred at 80°C and extraction time 4 h, while at extraction temperatures of 90°C and time 5 h yields fucoidan decreased by 6.36%.

This condition was thought to be the cell wall of brown algae *Sargassum* sp. Increasingly porous with the higher extraction temperature (70-80°C) and extraction time (3-4 h) degraded brown algae increases fucoidan out of the intersellular tissue more and dissolves in HCl solvent 0,03 M, while at extraction temperature of 90°C and within 5 h in a 0.03 M HCl solution, fucoidan began to degrade so that the solubility was relatively decreased as a result, a low yield was obtained. Ale *et al.* (2011a) stated that the *Sargassum henslowianum* cell wall matrix in a weak acid solution (0.2 M HCl) tends to be porous and contracted due to increasing temperature and extraction time, yield increases at extraction temperature (30-60°C) and time (1-3 h), at a temperature of 90°C and a extraction time of 5 h the fucoidan degraded so that a low yield was obtained.

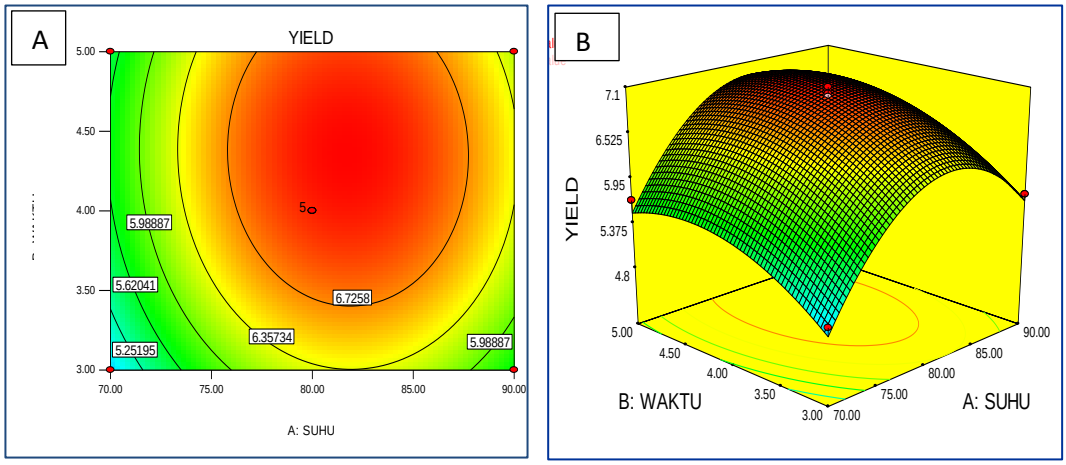

Figure 1. (A) Contour Plot (B) Graph of the effect of temperature and extraction time on the fucoidan yield of *Sargassum* sp.

Model prediction and statistical analysis

The prediction of the quadratic polynomial equation model of the fucoidan yield response from the results of the regression analysis of the Design Expert vers 7 program was expressed in the form of a second-order polynomial equation, namely:

$$y = 7{,}01+0{,}37x_1+0{,}29x_2-1{,}03x_1^2-0{,}40x_2^2-0{,}022x_1x_2, \quad R^2=0{,}984$$

Lack of Fit test found that the p value (0.0567) shows that the model inaccuracy was not significant to pure error, the model equation was right. Liu *et al.* (2010) stated that the Lack of Fit test p value greater than 0.05 indicates that the quadratic model was insignificant to the model inaccuracy. The model has a determination coefficient of 0.9840 and the value of Adj R-Squared (0.9726) shows that the model was very good and from the value of Adj

R-Squared it was shown that 97% of the total variation of fucoidan yield was determined by the independent variable and about 3% of the total variation which cannot be explained by the model.

Response surface and contour plot

The response surface 3D curves and the 2D contour plot are representative of the fucoidan *Sargassum* sp. Response regression equation (Figure 1). The response surface curve and the contour plot show the relationship between the independent variable and the response and interaction type of the two variables tested.

The effect of temperature interaction and extraction time was not significant to the fucoidan yield response, this can be seen from the two-dimensional contour plot of the circular rendering response (Figure 1A) which showed that the effect of temperature and extraction time interaction on fucoidan yield was very small. Claver *et al.* (2010) stated the shape of circular or elliptical contour plots indicates the quality of interaction between independent variables was significant or not. Circular contour plot showed weak free variable interaction, elliptical contour plot indicates the quality of free variable interaction was very significant to the response.

Optimization of Extraction and Validation

Optimization of Extraction

Optimization of fucoidan extraction of *Sargassum* sp. The treatment temperature and extraction time were statistically analyzed using Design-Expert software version 7. The computerized system calculation result of the Expert Design program obtained the optimum point solution for fucoidan extraction of *Sargassum* sp. with initial degradation of ultrasonic waves (80% amplitude, 15 minutes) suggested by the program was extraction temperature 81.00ºC and extraction time 4.04 h. The graph of the treatment temperature and extraction time at the optimum point that gave the optimal results of the fucoidant yield of *Sargassum* sp. was determined on the response surface curve in Figure 2.

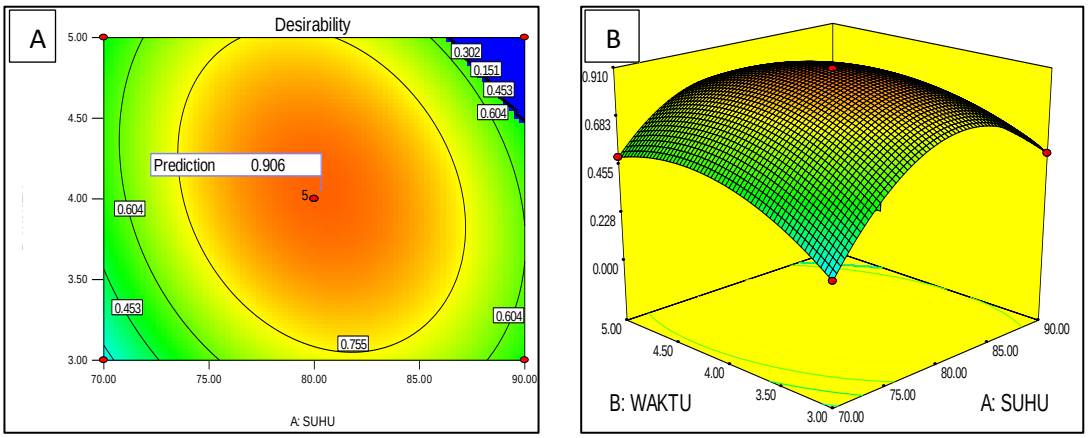

Figure 2. (A) Contour plot, (B) Graph of optimal temperature and extraction time response to *Sargassum* sp.

The maximum predictions of the fucoidan yield response program at the optimal point of extraction temperature were 81.00ºC and the extraction time was 4.04 h with initial ultrasonic wave degradation (amplitude 8%, 15 minutes) which was 7.04%. The optimum point solution for temperature and extraction time of the computerized resulted of the Design Expert program was presented in Table 1.

Table 1. The optimum point solution chosen by the Design Expert program version 7

Response	Optimal point of extraction Temperature (81,00°C) and Time (4,04 h)
Yield (%)	7,0458
Desirability	0,906

Ale *et al.* (2011a) reported optimization of *Sargassum henslowianum* fucoidan extraction which was extracted by heating waterbath with 0.03 M HCl solvent, the optimum point occurred at 90°C and extraction time 4 h. The results of the study Qiao *et al.* (2009) found that the optimal point of extraction of *Hyriopsis cumii* polysaccharides occurred at a temperature of 80°C and extraction time of 4.5 h. These showed that the optimal point of *Sargassum* sp. Fucoidan extraction which was degraded early by ultrasonic (amplitude 8%, 15 minutes) occurred at a temperature (81°C) lower than the optimal point of fucoidan extraction of *Sargassum henslowianum* by Ale *et al.* (2011a) namely at a temperature of 90°C, while the optimal time for extraction of fucoidan *Sargassum* sp. (4 h) was faster than the results of research Qiao *et al.,* (2009) with an extraction time of 4.46 h. This showed that the initial degradation with ultrasonic and continued extraction by heating waterbath was more effective compared to manually extracting waterbath heating. This condition was caused by ultrasonic wave vibrations on initial degradation breaking down the *Sargassum* sp. cell wall and increasing the penetration of solvents into cells so that fucoidan exists in cells can exit easily. Cameron and Jane (2006) in Sari *et al.* (2012) stated the mechanical effect of ultrasonic increases the penetration of fluid into the cell membrane, supported the release of cell components, and increases mass transfer. Ultrasonic cavitation produces a breaking force that will break down the cell wall mechanically and increase the transfer of material so that the components present in the cell can come out easily (Sari *et al.,* 2012).

Optimal Point Validation

The results of the optimal point validation prove the optimum point solution of the independent variables from the Design Expert program gives the results of the truly optimal response according to the solution of the program. Validation was done by comparing the response value of the actual experimental results with the response value of the calculation results of the Expert Design program. The suitability of the optimum point of fucoidan extraction was carried out by five experiments validation of optimal point extraction based on variables: initial degradation with ultrasonic waves (8% amplitude, 15 minutes), extraction temperature 81.00°C and extraction time of 4.04 h. The results of the calculation of the optimal validation point of fucoidan extraction obtained the average value of fucoidan yield of 7.15 ± 0.07%. The results of the validation experiments and the predicted value of the optimal point of fucoidan extraction of *Sargassum* sp. presented in Table 2.

Table 2. Validation and prediction experiments

Variable of Extraction		Fucoidan Yield (%)	
Temperature (°C)	Time (h)	Actual	Prediction
81.00	4.04	7.15	**7.05**
81.00	4.04	7.04	**7.05**
81.00	4.04	7.23	**7.05**
81.00	4.04	7.20	**7.05**
81.00	4.04	7.12	**7.05**

The difference in the value of the response yield of the validation results and the maximum prediction value of the Design Expert program was 1.45%. The difference in the value of the response of the validation results with the predicted value of the Design Expert program was less than 5%, this indicates that the value of the optimal free point variable was sufficient to produce a response the optimal. Sun *et al.* (2011) stated the results of validation experiments and the predicted value of the program has an error rate of less than 5% proving that the value of the optimum point variable has a high suitability. Validation of the optimal point of fucoidan extraction *S. henslowianum* from Ale *et al.* (2011a) has a level of validity of 0.891. Qiao *et al.* (2009) reported the results of the experimental validation of the optimal point of *H. cumingiiter* polysaccharide extraction having a level of suitability of 0.9823, a confidence level of 0.05 between the experimental results and the predicted values, the experimental results were good according to the predicted values of the program.

Fucoidan characterization

Characterization of functional groups

The characteristic of fucoidan *Sargassum* sp. The results of validation of the optimal extraction point were analyzed by functional groups with FTIR at 4000-400 cm^{-1} wavelength scans, and compared with commercial fucoidan Fucus vesiculosus presented in Figure 2.

The absorbance band of fucoidan *Sargassum* sp. strong and wide appear at wave numbers 3409.10 cm^{-1} and 3533.35 cm-1, standard fucoidan *Fucus vesiculosus* at wave number 3425.34 cm^{-1} it was assumed that vibrations from the OH function groups stretching from carbohydrates. According to Coates (2000) absorption band of groups OH function was in the range of wave numbers 3600-3200 cm^{-1}. Ji *et al.* (2011) stated the absorption band of OH groups from fucoidan *S. pallidum* found in wave number 3447.6 cm^{-1}. Qiao *et al.* (2009) reported stretching OH polysaccharide groups from brown algae *H. cumingiiter* can be found at wave number 3447.6 cm^{-1}. Qiao *et al.* (2009) reported stretching OH polysaccharide groups from brown algae *H. cumingiiter* can be found at wave number 3400,6 cm cm^{-1}. peak band at wave number 2935.46 cm^{-1} fucoidan *Sargassum* sp., wave numbers 2989.46 cm^{-1} and 2939.31 cm^{-1} standard fucoidan *F. vesiculosus* shows the CH stretching vibrations of carbohydrates. Coates (2000) states the absorption band in the range of wave numbers 3000-2500 cm^{-1} showed the vibrations of C-H. Ji *et al.* (2011) reported that the vibration of the C-H fucoidan *S. pallidum* was obtained at wave number 2940.9 cm^{-1}.

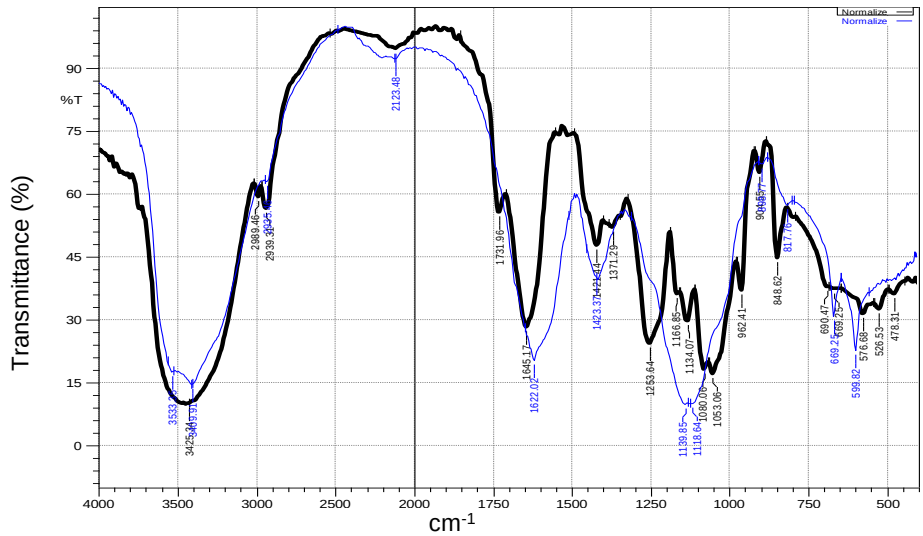

Figure 3. FT-IR spectrum of fucoidan crude *Sargassum* sp. (A), Commercial CT-IR spectrum of commercial fucoidan *F. vesiculosus* (B).

Band peak wave number 1622.02 cm^{-1} fucoidan *Sargassum* sp. and a peak of 1645.17 cm^{-1} standard fucoidan *F. vesiculosus* showed a C=C vibration indication of absorbance of uronic acid. Ale *et al.* (2011c) reported the absorption band at 1610 cm^{-1} fucoidan *S. henslowianum* and 1620 cm^{-1} standard fucoidan *F. vesiculosus* (sigma) signifying the absorbance of uronic acid. Band peak wave number 1417.58 cm^{-1} fucoidan *Sargassum* sp. and 1421.44 cm^{-1} fucoidan Fucus vesiculosus showed stretching of the C-H group from phosphat and indications of sulfate groups bound to C2 and C4 from the phosphat as well as a variety of shadow vibrations from polysaccharides consisting of glucose, mannose, xylose and rhamnosa. Fukosa has the absorption of C-H groups at wave numbers 1452.30 and 1414.69 cm^{-1}. The peak at 1420.3 ~ 1384.4 cm^{-1} showed a variety of C-H vibrations from polysaccharides consisting of D-glucose, D-mannose, D-Xylose and galacturonic acid (Ji *et al.*, 2011). Ale *et al.* (2011c) stated the absorption band in the range of 1470-1400 cm^{-1} indicates the vibration scissoring of CH_2 (galactose and mannose).

Absorption band at wave numbers 1139.85 cm^{-1} and 1118.64 cm^{-1} fucoidan *Sargassum* sp., Fucoidan *F. vesiculosus* at 1134.07; 1080.06 and 1053.06 cm^{-1} showed the CH vibrations of the phosphat and indications of sulfate, phosphat have a strong absorbance at 1200-1050 cm^{-1} wave numbers. Strong peaks between 910.34-1126.35 cm^{-1} were characteristic of the fucose and indication of stretching S = O was bound to the axial position C-4 (Kim *et al.*, 2010; Ji *et al.*, 2011). The absorption band at 898.77 cm^{-1} fucoidan *Sargassum* sp. and 904.55 cm-1 fucoidan *F. vesiculosus* showed C-H bend vibrations from polysaccharides thought to consist of galactose, rhamnose, mannose, glucose.

Zhang (1994) in Yang and Zhang (2009) reported glucose has absorption bands in the range of 905-876 cm^{-1}, galactose in the range 914-866 cm^{-1}, mannose in the range of 898-888 cm^{-1} and arabinose 855-840 cm^{-1} The absorption band at 850 cm^{-1} and 820 cm^{-1} was the C-O-S sulfate group band, the sulfate was bound to the C-2 and C-3 equatorial position of the L-fucose, and the C-4 axial position (Park *et al.*, 2012).

Ale *et al.* (2011c) reported that *S. henslowianum* and *F. vesiculosus* sulfate sulphate groups were present in wave numbers 817 cm^{-1} and 822 cm^{-1} sulfates bound to the C-2 and C-3 positions of L-phosphate, 840-absorption bands 850 cm^{-1} sulfate was bound to the axial position of the C-4 axial (Kim *et al.*, 2010). The standard fukoid sulfate group *F. vesiculosus* was found at 848.62 cm^{-1} bound to the C-4 axial position glucose. The fucoidan sulfate group *Sargassum* sp. found at 817.76 cm^{-1} bound to the L-fucosal equatorial position C-2 and C-3. The absorbance band of fucoidan *Sargassum* sp. at 817.76 cm^{-1} did not appear sharp, presumably because there were many impurities and piles with other monosaccharide components.

The peak band at 669.25 cm^{-1} fucoidan *Sargassum* sp. and fucoidan *F. vesiculosus* at wave number 690.47; 669.26 showed the CH2-S sulfate vibrations bound to the glucose and indicated xylose. Coates (2000) stated that the absorption band in the range of 710-685 cm^{-1} shows the vibration of CH2-S. Zhang (1994) in Yang and Zhang (2009) reported that xylosa has an absorption band in the range of 760-740 cm^{-1}. The peak band was at 599, 82 cm^{-1} fucoidan *Sargassum* sp. and fucoidan *F. vesiculosus* at 576.68 cm^{-1} showed CH3-S vibrations thought to be sulfate bound to the C-2 and C-3 position glucose. Coates (2000) states that the absorption band in the range of 660-630 cm^{-1} showed CH3-S. vibrations. The absorption band of 582-586 cm^{-1} asymmetry of the deformation group O = S = O confirmed that there was a significant amount of sulfate (Kim et al., 2010).

Monosaccharide Components

The results of the HPLC analysis of the *Sargassum* sp. Monosaccharide component. composed of fucosa, xylose, rhamnosa and glucose. The level of each component of dry perosite monosaccharide was fucoidan *Sargassum* sp. as follows: glucose 48.64 mg/g, xylose 35.29 mg/g, rhamnosa 21.93 mg/g, glucose 5.72 mg / g. These results indicated that

the levels of the fucoidan *Sargassum* sp. relatively higher than the fucoidan *S. henslowianum* research results Ale *et al.* (2011a) who reported that the monosaccharide component of the fucoidan constituent of *S. henslowianum* which was extracted at the optimal extraction point (90 ^{o}C, 4 h) had a monosaccharide component consisting of fucoidous 31.4 mg/g fucoidan. rhamnosa 2.0 mg/g, arabinose 0.1 mg/g, galactose 0.1 mg/g, glucose 0.8 mg/g, xylose 0.1 mg/g and mannose 0.3 mg/g.

Table 3. Components of the fucoidan monosaccharide *Sargassum* sp. after hydrolyzing with 2 M TFA

Peak	Monosaccharide Components	Retency time (minutes)	Level (mg/g)
1	Glukosa	8,32	5,72
2	Xylose	8,90	35,29
3	Rhamnosa	9,33	21,93
4	Fukosa	10,27	48,64

Conclusions

Sargassum sp. Fucoidan extraction at a temperature of 70-90^{o}C and extraction time of 3-5 h obtained the optimal point of extraction temperature of 81.00^{o}C and extraction time of 4.04 h. The results of the validation of the optimal extraction point have a yield value of 7.15 ± 5.58%. The difference in the value of the response of the validation results and the maximum prediction value of the Design Experts program was 1.45%. Based on FTIR and HPLC characterization, *Sargassum* sp. composed of glucose, rhamnosa, xylose, glucose and sulfate.

References

Ale, M.T., Mikkelsen J.D. and Meyer A.S., 2011a.Designed optimization of a single-step extraction of fucose-containing sulfated polysaccharides from *Sargassum sp. J. Appl. Phycol*.doi:10.1007/s10811-011-9690-3.

Ale, M. T., Mikkelsen J. D. and Meyer A. S., 2011b.*Review:* 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, 2106-2130 ; doi:10.3390/md9102106.

Ale, M.T., Maruyana H., Taumachi H., Mikkelsen J. D. and Meyer J. D., 2011c. *Article:* Fucose-Containing Sulfated Polysaccharides from Brown Seaweeds Inhibit Proliferation of Melanoma Cells and Induce Apoptosis by Activation of Caspase-3 *in Vitro.Mar. Drugs.*9, 2605-2621.

Chotigeat, W., Tongsupa, Supamataya K. and Phongdara A., 2004.Effect of fucoidan on disease resistance of black tiger shrimp. *J. Agriculture*. Elsivier 233.23-30.

Claver I. P., Zhang H., Li Q., Kexue Z., and Zhou H., 2010.Optimization of ultrasonic extraction of polysaccharides from chinese malted sorghum using response surface methodology. Pakistan Journal of nutrition 9 (4): 336-342.

Coates J., 2000.Interpretation of infrared spectra, apractical approach.*Encyclopedia of analytical chemistry.* John wiley andsons Ltd. pp. 10815–10837

Cumashi, A., Ushakova N. A., Preobrazhenskaya M. E., D'Incecco A., Piccoli A., Totani L., Tinari N., Morozevich G.E. and Berman A. E., 2007. A comparative study of the anti-inflammatory, anticoagulant, antiangiogenic, and antiadhesive activities of nine different fucoidans from brown seaweeds.*Glycobiology.5*, 541–552.

Ermakova, S, Sokolova R, Kim SM, Um B, Isavakov V. and Zvyaginseva T., 2011.Fucoidans from brown seaweeds *Sargassum hornery, Eclonia cava, Costaria costata*: Structural characteristics and anticancer activity.

Appl Biochem Biotechnol. 164:841–850.

Ji A., Yao Y., Che O., Wang B., Sun L., Li X. and Xu F., 2011.Isolation and characterization of sulfated polysaccharide from the *Sargassum pallidum (Turn) C. Ag.*and its sedative/hypnotic activity. *Journal of Medicinal Plants Research.* Vol. 5(21),pp.5240-5246.

Kadi A., 2004. Rumput laut nilai ekonomis dan budidayanya.Pusat Penelitian Oseonografi Lembaga Ilmu Pengetahuan Indonesia.LIPI. Jakarta. ISBN : 979-3378-19-0.

Kim W. J., Kim S. M., Kim H. Y., Hye-Rim O, Lee K. B., Lee L. K.and Park Y., 2007.Purification and Anticoagulant Activity of a Fucoidan from Korean *Undaria pinnatifida* Sporophyll.Algae. Volume 22(3): 247-252.

Kim W. J., Koo Y. K., Jung M. K., Moon H. R., Kim S. M., Synytsya I., Hye Sook Yun-Choi H. S. Y., Kim Y. S., Park J. K. and Park Y., 2010. Anticoagulating Activities of Low-Molecular Weight Fuco-Oligosaccharides Prepared by Enzymatic Digestion of Fucoidan from theSporophyll of Korean *Undaria pinnatifida. Arch Pharm Res Vol 33, No 1, 125-131*.

Krishnaiah D, Prasad D.M.R., Bono A. and Sarbatly R., 2007.Optimization of ultrasonic extraction parameters of Iota-carrageenan from seaweed *Eucheuma denticulatum*.Caledonian

Journal of Engineering. Volume 03, Number 02, 1-11.

Kwong B. W., Mao Z., and Xiaoyan W., 2011. Kelp fucoidan optimization of ultrasonic extractionof brown algae *Laminaria japonica*. Guangdong College of Pharmacy. 26 (2). 3-6.

Li, B., Wei X. J., Sun J. L. and Xu S.Y., 2006.Structural investigation of a fucoidan containing a fucose-free core from the brown seaweed*Hizikia fusiforme.Carbohydr. Res. 341*, 1135–1146.

MontgomeryD. C., 2005.Response surface methods and designs.John Willy and Sons.Inc.

Nuryanti dan Salimy D. H., 2008.Metode permukaan respon dan aplikasinya pada optimasi eksperimen kimia.Risalah Lokakarya Komputasi dalam Sains dan Teknologi Nuklir: 373-391.

Park K., Cho E., In M., Kim D. C. and Chae H. J., 2012. Physicochemical properties and bioactivity of brown seaweed fucoidan prepared by ultra high pressure-assisted enzyme treatment.*Korean J. Chem. Eng*., 29(2), 221-227.

PT. Marinal Indoprima, 2011.Daftar Harga Ekspor Rumput Laut. Data Internal PT. Marinal Indoprima. Surabaya. tidak dipublikasikan

Qiao D., Hu B., Gan D., Sun Y., Ye.and Zeng X., 2009. Extraction optimized by using response surface methodology, purification and preliminary characterzation of polysaccharide from *Hyriopsis cumingi. Journal Home page.Carbohydrate polymer*.76.422-429.