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Extraction Optimization by Response Surface Methodology and Characterization of Fucoidanfrom Brown Seaweed *Sargassum polycystum*

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Abstract: Fucoidanwere recovered from *Sargassum polycystum* by single-step extraction with ultrasonic wave pretreatment (amplitude 80%, 15 minute).Extraction wereoptimized using central composite design ofrespon surface method. Ultrasonic wave pretreatment conditions were 80%amplitude, for 15 minute. Alga solution in 0.03 MHCl ratio 1:20 (b/v) at70-90^oC for 3-5 hour were evaluated duringthis process to establish a condition to maximize the extraction. The yield (%), total carbohydrate (mg/g), fucose (mg/g) and sulfate contents (mg/g) were also determined for eachexperimental condition.The result showed thatall extraction factors had significant effects ($p<0.05$) onfucoidan yield, fucose, total carbohydrate and sulfate contents. The optimum conditions were 81^oC for 4.04 hours. The validated experimental crude fucoidanyield were7.15±5.58%, 131.15±1.92 mg/g fucose, 396.8±65.58 mg/gtotal carbohydrate and 94.67±1.37 mg/g sulfate contents. Characterization of crude fucoidan by FTIR and HPLC showed that crude fucoidan (*Sargassum polycystum)* is composed by fucose, xylose, galactose, rhamnose, glucose, mannose and sulfate.
Keywords: fucoidan, fucose, sulfate, sargassum polycystum, extraction methode.

Introduction

Fucoidan, a cell-wall matrix polysaccharide in brown seaweed, is composed by L-fucose and sulfate including minor amounts of galactose, xylose, glucose and mannose[21]. Fucoidan exert various biological activities such as antitumor and anticancer[2]. Fucoidan can be extracted from *Sargassum polycystum*. Extraction of fucoidan from brown seaweeds generally involves multiple, extended aqueous extractions, usually with hot acid (hydrochloric acid), and may include addition of$CaCl_2$ to promote alginate precipitation[3,16]. It has long been known that extraction time and temperature may influence both yields and composition of the fucoidan extract[1].Extraction of fucoidan (*Sargassumhornery*)at 60°C with 0.1M HCl for 2 hours in 2 extraction step, and precipitation with 4volumes 96% ethanol[7].Fucoidan extraction*Hizikiafusiforme*3 step extraction at temperature 70°Cfor 2h, precipitation with ethanol and $CaCl_2$[13].Each step lasting several hours and resulted lower fucose contents.

Ultrasonic waveextraction techniques have been applied[12] for extracting fucoidan from the brown seaweed *Laminariajaponica*[11] and *Eucheumadenticulatum*. The extraction process were fast but produced a low yield. Extraction combination using degradation by ultrasonic waves followed by water bath heating can produce more optimal fucoidan yield and fucose content. However, little attention devoted to the combination of extraction method followed bywaterbath heating and characterization of fucoidan (*Sargassum polycystum).* Herein, we report the detail of combination extraction, ultrasonic degradation and single-step optimization extraction by using response surface methodology and characterization of fucoidan *Sargassum polycystum*.

Material and Method

Material and Reagents

Brown seaweed *Sargassum polycystum*was obtained from Madura island, crude fucoidan commercial was obtained from PT. SOHO IndustriPharmasi Jakarta, chloroform, methanol, aquades, hydrochloric acid (HCl) 37%, etanol 99.8%, trifluoroacetic acid (TFA) 99%, trichloroacetic acid (TCA) 99%,H_2SO_4,fenol, cisteinhidrochloride, NaOH, $CaCl_2$, perchlorate acid ($HClO_3$), $BaCl_2$, K_2SO_4, D-glucoseand D-xylose, L-rhamnose, D-galaktose, D-mannose (Sigma-Aldrich) danL-fucose were purchased from Santa Cruz. All chemicals used were analytical grade.

Single-step extraction of fucoidan

General extraction process *Sargassum polycystum*.Algal were washed with fresh water to remove salt, sand and epiphytes, dried at sun rice and milled using an Coffee Mill 100 watts to pass through a 500-µm sieve, pretreated with a MeOH-$CHCl_3$-H_2O (4:2:1), and mixedat room temperature to remove colored matter and phenol compounds prior to extraction.

Sargassum polycystum pretreatment diluted in 0.03 M HCl(1:20 w/v), degradated by ultrasonic extraction (amplitude 80%, 15 minute) and than extracted with *waterbath*at 70-90^{o}C for 3-5 hours. The suspensions were filtrated (supernatant 1) through nylon fiber to separate the residual alga. The residual alga were washed with aquades (1:5 w/v) and filtrated (supernatant 2). The first and second supernatant were combined (Extract A). A liquid fraction from Extract A was precipitated with 1 M $CaCl_2$ and the mixture was maintained overnight at 4°C to release and precipitate alginate. The fraction obtained byionization of $CaCl_2$ was separated by filtration. Tree volume of ethanol absolute were added to the resultant filtrate and the mixture stored at 4°C for 8 hours. Ethanol-precipitatedfucoidan was recovered by centrifugation (8,500 rpm, 15 minute, 4°C), dried at 50ºC overnight, milled and stored for further analysis.

Experimental Design of RSM

Second-orderexperiment were the extraction process optimization using central composite design of RSM with 2 variables: temperature(x1) andtime(x2). Optimization extraction step has 13 randomly ordered treatment with5 replicates center point (9-13 treatments), each condition following the extraction process central composite rotatabled esign(CCRD) ofRSMare presented in Table1below.Based onexperimental data, regression analysis and fit models polynomial equation second-order:

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

WhereYisthe response variable, $\beta_0$is the intercept coefficient; β_i, β_{ii}, $\beta_{ij}$are the regression coefficients of each linear, quadratic, interaction, andx_i, x_j, code from two independent variable amplitude and time(i≠j).

The accuracy of the data analysis performed with polynomial equations model of Design-Expert software version7to determine thecorrelation coefficient(R) and coefficient of determination(R2) of data extraction, total carbohydrate, fucose and sulfatecontents. Correlation coefficient(R) and coefficient determination (R^2) were tested for statistical significance byF-test at the probabilityp=0.05.

Analysis of fucoidan characterization

The fucose content were analyzed by cistein hydrochloride method[4], sulfate content by bariumchloride-gelatin method[5],total carbohydrate content byphenol-sulfuric acid method using L-fucoseas standard[6].

Functional group was analyzed by Infra Red Spectroscopy(FT-IR) spectrometer (shimadzumodel 8400S) using16scansand 400-4000cm^{-1}frequency range. The crude extractfucoidanwas conducted using potassium bromide pellets (KBr), fucoidan were smoothed by potassium bromide powder and then pressed to 1mm pellets. Vibrational transition frequency of each spectrum corrected absorbance at base line and normalized between 0 and 1.

Monosaccharide composition ofcrude fucoidanextract were analyzed using High Performance Liquid Chromatography (HPLC),10-15 mg crude extract fucoidan were hydrolyzed with 2MT rifluoroacetic acid(0.5 ml) at 121^{o}Cfor 2h, in aglass tube closed withN_2gas. The glass tube were cooled with ice-water, centrifuged at5000 rpm for 5 minute, the liquid fraction was neutralized to pH 7 with2MNaOH. The sampleis injected in the HPLC system with two column. System HPLC Knauermanager 5000, pump 1000, 4050 columnoven, refractive index detector S2300, aminex HPX87P column, temperature 85^{o}C,aquabide smobile phase. Aminex HPX 87H column, temperature 65^{o}C, $H_2SO_4$0.005 M mobile phase, flow rate of0.6ml/min, injection volume of 20 µl.

Result and Discussion

Effect temperature and time extraction on the yield fucoidan

Reseach the effects of temperature and time extraction different on the yield of crude fucoidan *Sargassum polycystum*, extractions were performed at 70-90^{o}C temperature and extraction time of 3-5 h. The yield of crude fucoidan increased the extraction temperature 60-80^{o}C and 2-4 h, decreased the extraction temperature of 90^{o}C and 3-5 h (Figure 1b). This is consistent with reports of extracting fucoidan and other polysaccharides of brown algae[1,18]. Statistical analysis showed significant differences between 70°C, 80^{o}C and 90^{o}C ($p<0.05$).

The results showed that the treatment temperature (80^{o}C) and extraction for 4 h had positive effect on the yield of fucoidan, meanwhile extraction at 90°C within 3-5 h decreased the yield of fucoidan. Cell walls of brown algae *Sargassum polycystum* more porous with increasing extraction temperature (70-80^{o}C) and time (3-4 h) brown algae increased degraded, which is out intercellular tissue of more soluble in the solvent HCl 0.03 M, whereas at 90^{o}C and 5 hours in a 0.03 M HCl solution partially degraded fucoidan. Cell wall matrix *Sargassumhenslowianum* in a mild acid solution (0,2 M HCl) tend to be porous and wrinkled due to increased temperature and extraction time, the yield increased at 30-60^{o}C and 1-3 hours, at 90^{o}C and 5 h extraction,fucoidan were partially degraded[1].

Effect of temperature and extraction time on the fucose contents

Treatment at 60-90^{o}C for 2-5 hours obtained 62.60 mg/g-145.28 mg/g fucoidan *Sargassum polycystum* fucosecontents. **Figure 1b'** showed that the results of treatmentat 70-90^{o}C for 3-5 hours had positive effect on the increased fucose contents of crude fucoidan *Sargassum polycystum.*Fucose contents increased at 60-80^{o}C and 3-4 hours, and then declined at 90^{o}C,3-5 hours. Statistical analysis showed that treatment temperature and time extraction and interaction significantly affect fucose contents of fucoidan *Sargassum polycystum* ($p<0.05$). Fucose contents at 70°C, 3 h were 81.13 mg/g, the highest fucose content(145.28 mg/g)achieved at 80°Cand 4 hours extraction, whereas the extraction temperature of 90^{o}C and 5 hfucose content decreased 84.91 mg/g. Low fucose content were obtained because the damage of Molecules integrity in 0.03 M HClat 90^{o}C and 5 hours extraction. Higher temperature, time and acid solvent of fucoidan extraction broke the cell wall matrix contract followed by acid penetration into the intercellular tissue resulting in partial degradation of fucoidan fucose damage[1].

Effect temperature and time extraction on the total carbohydrate contents

To investigate the effects of temperature and time extraction difference on the total carbohydrate content of fucoidan *Sargassum polycystum,* the extraction process were carried out at 70-90^{o}C for 3-5 hours. The results figure 1b'' showed the treatment temperature and time extraction on the total carbohydrate contens.Total carbohydrate content of fucoidan tends to increase with increasing temperature and extraction time, after reaching the optimal point (80°C, 4 h) the total carbohydrate contents decline. Total carbohydrate contents increased at 70-80^{o}C for 3-4 h extraction, and decline at 90^{o}C,3-5 h. Statistical analysis showed that temperature and extraction time significantly affect the total carbohydrate content ($p<0.05$). Total carbohydrate contents were 312.34 mg/g at 70°C and 3 h extraction, the highest total carbohydrate content were 408.74 mg/g obtained at 80°C for4 h extraction, andat 90^{o}C, 5 h extraction the total carbohydrate content was decreased (353.21 mg/g). The state alleged integrity polysaccharide molecules are relatively stable at 70-80^{o}C and 3-4 h extraction in 0.03 M HClsolution, whereas at 90^{o}C and 3-5h, polysaccharides undergo depolymerization into free sugars and solubility was decreased in 0.03 M HClsolution. Higher temperature and extraction time causes depolymerization of polysaccharide into free sugars and decreased solubility.[14]

Effect of temperature and time extraction on the sulfate content

Temperature and time extraction has negatively significant effect on the sulfate contents of crude extract fucoidan *Sargassum polycystum*, sulfate contents tend to decrease with increasing temperature (70-90^{o}C) and extraction time 3-5 h (Figure 1b’’’). Statistical analysis showed that treatment temperature and extraction time significantly affect the sulfate contents ($p<0.05$). The sulfate content at 70°C and 3 hours were 124.46 mg/g, sulfate content at 80°C and 4 h were 98.93 mg/g, sulfate content decreased rapidly at 90^{o}C and 5 hours were 82.55 mg/g. Fucoidanhas a sulfate contents that were relatively unstable in acidic solvent (0.03 M HCl), ultrasonic wave, increasing temperature (70-90^{o}C) and time (3-5 h).Sulfate bond severedsulfate contents were decreased. High-temperature treatment in 0.03 M HCl solvent causes partial degradation of fucoidan *Sargassumhenslowianum* severed bond sulfate, sulfate contents decreased rapidly at a temperature extraction 90^{o}C and extraction time of 5-10 h[1].

Prediction models and statistical analysis

Predictive models of quadratic polynomial equations response yield fucoidan, fucose contents, total carbohydrate contents and linear regression the response sulfate content result analysis regression of experimental data fucoidan *Sargassumpolycystum*. Results of analysis of variance experimental data model polynomial multiple responses fucoidanhas value coefficient determination R^2 between 0.9055 and 0.9840 fit between the experimental results with the predicted value of the program, a second-order polynomial equation multiple responses: yield, fucose, total carbohydrate and sulfate contens (variable code) as follows:

$$y = 7.01 + 0.37x_1 + 0.29x_2 - 1.03x_1^2 - 0.40x_2^2 - 0.022x_1x_2 \qquad R^2=0.984$$

$$y = 132.45 + 2.24x_1 - 4.20x_2 - 29.32x_1^2 - 12.18x_2^2 - 14.15x_1x_2 \qquad R^2=0.905$$

$$y = 132.45 + 2.24x_1 – 4.20x_2 - 29.32x_1^2 - 12.18x_2^2 - 14.15x_1x_2 \qquad R^2=0.939$$

$$y = 97.36 – 11.65x_1 – 8.23x_2 \qquad R^2=0.919$$

Examined lack of fit test multiple responses fucoidan *Sargassum polycystum*has yield as a value p ($p = 0.0567$), fucose contents ($p=0.1646$), total carbohydrate contents ($p=0.0522$), sulfate contents ($p=0.0522$), each response has p value higher than 0.05 indicates that the model is not significant. This result in accordance to the result of Liu et al.[15] lack of fit test has a p-value higher than 0.05 indicates that the inaccuracy model of the pure error were not significant mean equation polynomial models was accurate.

Respon surface and contour plot

The 3Dcurve response surface and 2 Dcontour plots representative of the regression equationsre sponse yield, fucose content sand total carbohydrate contents, whereas the sulfate contents has linear regression equation. Response surface and contour plot showing the relationship between variable experiment with the response and the type of interaction between the two variables tested. Circular shape or contour plot indicating elliptical quality of interaction between the independent variables significant or not. Circular contour plot indicates that the interaction between the corresponding variable are negligible, while elliptical contour plot sindicate that the quality interaction between the corresponding variables are significant on the response[18]. Response surface plot sand contour plots multiple response fucoidan using Design-Expert software is presented in Figure1. Effect of interaction temperature and time extraction is notsignificant on the yield (Figure 1a) and total carbohydrate content (Fig.1a") the shaped contourplot are circular. Interaction of temperature and extraction time are significant on the fucose contents (Fig. 1a') evident from the shaped contourplots are elliptical.

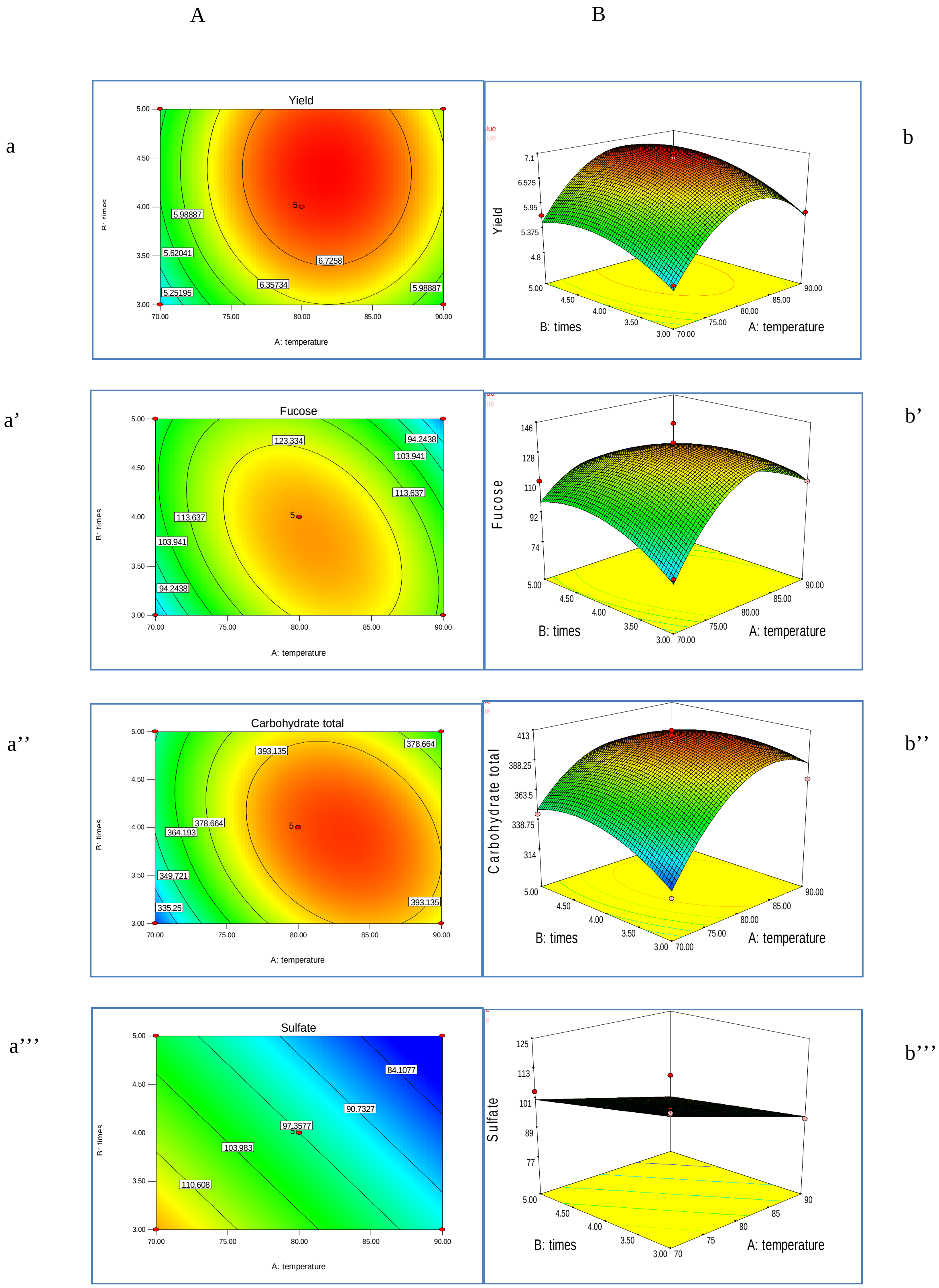

Figure 1. *Contour plot*(a, a’, a’’and a’’’) and response surface plot (b, b’, b’’and b’’’) showing the effect of temperature and time extraction on the yield, fucose, carbohydrate total and sulfate contents of crude fucoidan *Sargassum polycystum*

Optimization Extraction and Validation

Optimization extraction

The optimum extraction point fucoidan. *Sargassum polycystum* initially degradation ultrasonic waves (amplitude 80%, 15 minute) the calculation Design Expert software was the extraction temperature of 81.00°C and extraction time 4.04h. At the optimum conditions, the maximum predicted yield fucoidan were 7.03%, 131.94 mg/g fucose contens, 406.10mg/g total carbohydrate content and 96.50mg/g sulfate contents. The optimal extraction point of fucoidan *Sargassum polycystum* initia ldegraded ultrasonic waves (amplitude 80%, 15 minute) occurred at a temperature(81^{o}C), this were lower than the optimal point extraction fucoidan *Sargassumhenslowianum* results by Aleetal.[1]which obtained at 90^{o}C,and 4 h, but faster than Qiaoetal (4.46h)[18]. This suggests that the combination extraction of pretreatment ultrasonic wave degradation and extraction of water bath heating is more effective than manually extracting water bath heating. This condition were caused by vibration of the ultrasonic waves on the initial degradation break down the cell walls of *Sargassum polycystum* and increase the penetration of the solvent into the cell so that fucoidan is present in the cell can extracted easily. The effect of ultrasonic cavitation produces broken power will mechanically break down the cell wall, increasing penetration of fluids towards the cell membrane and increase the transfer of material so that the components in the cell extracted easily[20].

Validation optimum point

In order to validate the adequacy of the the model equation, five verification experiment validation were carried out the optimum extraction point under these variable extraction condition: degradated ultrasonic wave (amplitude 80%, 15 menute), temperature extraction 81.00^{o}C and time extraction 4.04 h. The Result validate experiment the optimum extraction point fucoidan (Table 2) was obtained: yield 7.15±0.07%, 131.15±1.92 mg/g fucose content, 396.86±5.58mg/g carbohydrate total content and 94.67±1.37 mg/g sulfat content. The difference between experiment validate and predicted Design-Expert software: 1.45%yield, 0.89%fucose content, 2.27%total carbohydrate content and 1.27% sulfate content. Levels error at less than 5% between experimentwere validated and predicted by software design Expert, indicated that independent variable of the optimum extraction point was satisfactory and accurate. The experiment validation and predicted value result has levels error less than 5% showed that optimum variable point value is accurate[22].

Tablel 1. *Central Composite Rotatable Design* (CCRD) matrix and respon fucoidan *Sargassum polycystum*

Extraction variable		Code variable		Crude fucoidan			
Temperature (^{o}C)	Time (hour)	x_1	x_2	Yield (%)	Total carbohydrate Content (mg/g)	Fucose content (mg/g)	Sulfate content (mg/g)
70	3	-1	-1	4.99	314.65	81.13	124.46
70	5	-1	1	5.68	343.96	111.32	81.90
90	3	1	-1	5.76	373.26	111.32	92.82
90	5	1	1	6.36	353.21	84.91	82.55
80	4	0	0	7.02	399.49	126.42	95.23
80	4	0	0	6.98	398.71	130.19	94.59
80	4	0	0	7.10	412.60	145.28	95.07
80	4	0	0	6.88	408.74	133.96	97.64
80	4	0	0	7.06	402.57	126.42	98.93
80	2.59	0	-1.414	5.84	380.21	115.09	106.64
80	5.41	0	1.414	6.46	380.98	88.68	81.90
65.86	4	-1.414	0	4.27	312.34	94.34	110.17
94.14	4	1.414	0	5.36	379.43	62.26	81.74

Table 2. Validation experimental and predicted

Variable Extraction		Yield (%)		Fucose contents (mg/g)		Carbohydrate total contents (mg/g)		Sulfate contents (mg/g)	
Temperature (^oC)	Time (h)	Actual	Predicted	Actual	Predicted	Actual	Predicted	Actual	Predicted
81,00	4,04	7,15	**7,05**	130,19	132,16	392,54	**406,10**	94,15	**95,87**
81,00	4,04	7,04	**7,05**	133,30	132,16	393,32	**406,10**	94,46	**95,87**
81,00	4,04	7,23	**7,05**	129,41	132,16	402,57	**406,10**	96,84	**95,87**
81,00	4,04	7,20	**7,05**	133,16	132,16	403,34	**406,10**	93,10	**95,87**
81,00	4,04	7,12	**7,05**	129,67	132,16	392,54	**406,10**	9478	**95,87**

Fungtional group characterization

Functional group characterization of fucoidan *Sargassum polycystum* analyzed by using fourier tranfer infrared (FTIR) in4000-400 cm^{-1}wavelength. The analysis of functional groups fucoidan *Sargassumpolycystum* compared with fucose standards and commercial fucoidan *Fucusvesiculosus* is presented in Figure 2.

Absorption band at 3409.10 cm^{-1} and 3533.35 cm^{-1}in fucoidan*Sargassumpolycystum*, 3425.34 cm^{-1}in commercial fucoidan standart *Fucusvesiculosus* and 3407.02 cm^{-1}in fucose showed the stretching vibrations of OH groups of carbohydrates[8,17].Peaks at 2935.46 cm^{-1} in $^{-f}$ucoidan *Sargassumpolycystum*, 2989.46 cm^{-1} and 2939.31 cm^{-1}in commercial fucoidan *Fucusvesiculosus*, 2987.53 cm^{-1} and 2920 cm^{-1} of fucose showed CH vibration[8]. Peak 1622,02 cm^{-1}infucoidan *Sargassumpolycystum*, 1645.17 cm^{-1}in commercial fucoidan and 1645.17 cm^{-1}in fucosestandart showed vibration C=C indicated absorbance for uronat acid.

Absorption band at 1610 cm^{-1}in fucoidan *Sarggassumhenslowianum* and 1620 cm^{-1}in fucoidan commercial *Fucusvesiculosus* indicates the absorbance of uronat acid[2]. Peak at 1417.58 cm^{-1} in fucoidan *Sargassum polycystum*, 1421.44 cm^{-1}in commercial fucoidan *Fucusvesiculosus* showed the CH stretching fucose and indications of sulfate groups attached to fucoseat C2 and C4 as well as a variety of vibration shadow of polysaccharide consisting of glucose, mannose, xylose and rhamnose. Fucose has a CH group absorption the wave numbers 1452.30 and 1414.69 cm^{-1}. Peak at 1420.3~1384.4 cm^{-1} shows a variety of CH vibration of the polysaccharide composed of D-glucose, D-mannose,D-xylose and acid galakturonat[24]. Peak in the range 1470-1400 cm^{-1} indicates the scissoring vibration of CH_2 (galactose and mannose)[2]. Peak at 1139.85 cm^{-1} and 1118.64 cm^{-1} in fucoidan *Sargassum polycystum* and absorption band at 1080.06 and 1053.06 cm^{-1}in commercial fucoidan *Fucusvesiculosus* shows the CH stretching vibration of fucose and S=O bound to the axial position of C-4[8,9], fucose has a strong absorbance at wave number 1200-1050 cm^{-1}. Peak at 898.77 cm^{-1}in fucoidan*Sargassum polycystum* and 904.55 cm^{-1}in commercial fucoidan *Fucusvesiculosus* showed CH bend vibration of polysaccharide composed of galactose, rhamnosa, mannose, glucose[24].Peak 850 cm^{-1} and 820 cm^{-1} is a sulfate group COS, sulfate bound at the equatorial position of C-2 and C-3 of L-fucose, and the axial position of C-4[2]. Aleet al.[2], reported that sulfate groups of fucoidan*Sargassumhenslowianum* and *Fucusvesiculosus* contained in the wave number 817 cm^{-1} and 822 cm^{-1},sulfate bound at C-2 and C-3 of L-fucose , absorption band 840-850 cm^{-1} sulfate bound to fucose axial position C-4[10].Commercial fucoidan *Fucusvesiculosus* sulfate groups contained in wave number 848.62 cm^{-1} bound to the fucose axial C-4 position. Sulfate groupsfucoidan *Sargassum polycystum* at wave number 817.76 cm^{-1} bound to the L-fucose equatorial positions C-2 and C-3. Peak 669.25 cm^{-1}fucoidan, *Sargassum polycystum* and commercial fucoidan *Fucusvesiculosus* at wavenumber 690.47, 669.26 showed CH_2-S vibration sulfate bound to fucose and indicated xylose[23].Peak 599.82 cm^{-1}fucoidan *Sargassum polycystum* and commercial fucoidan *Fucusvesiculosus* at wave number 576.68 cm^{-1} showed the vibration CH_3-S,fucose has a strong absorption the wave number 610.43 cm^{-1}.

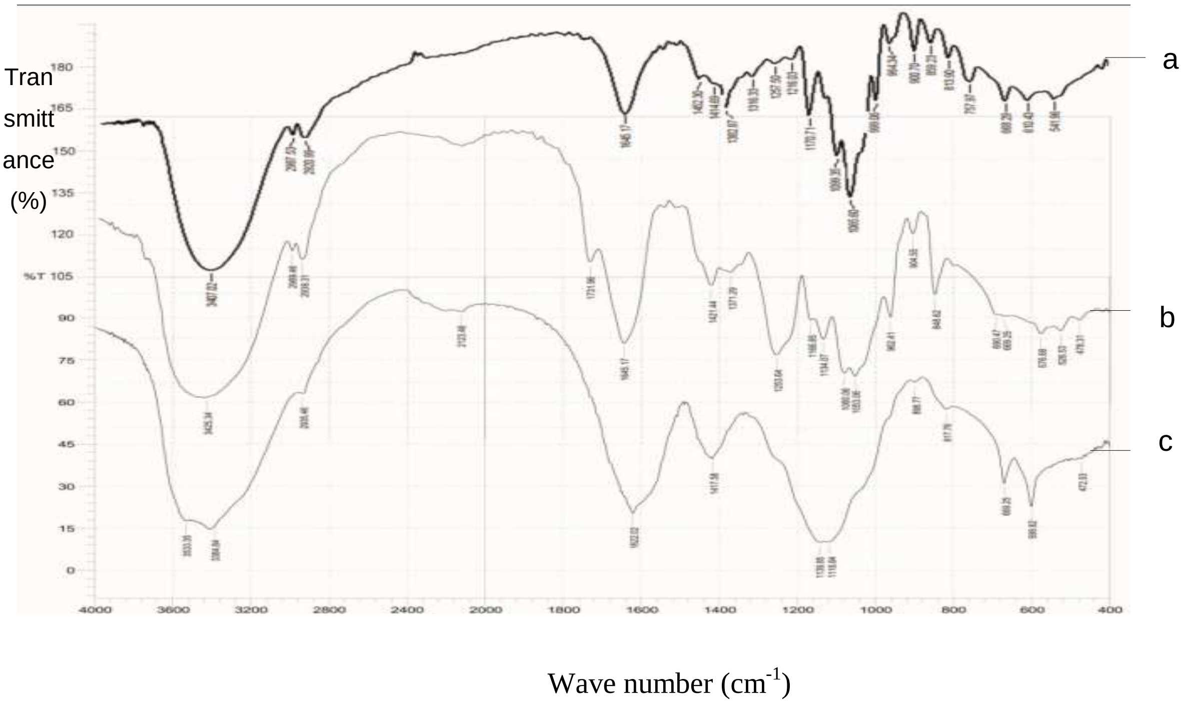

Figure 2. Spectrum FT-IR fucosestandart (a).Spectrum FT-IR crude fucoidan commercial *Fucusvesiculosus*(b). Spectrum FT-IR crude fucoidan*Sargassumpolycystum* (c)

Monosaccharide composition

HPLC analysis of fucoidan*Sargassumpolycystum* showed that the polysaccharide consist mainly offucose, galactose, rhamnose, xylose, mannoseandglucose.

Concentration of each component monosaccharides fucoidan *Sargassum polycystum* as follows: 48.64mg/g fucose, 35.29mg/g xylose, 34.33mg/g galactose, 21.93mg/g rhamnose, 5.72mg/g glucoseand5.72mg/g mannose. Relative percentage monosaccharide composition of fucoidan *Sargassum polycystum* HPLC analys is the highest of fucose 32.08%, 22.64% galactose, xylose23.27%, 14.47% rhamnose, glucose3.77% and3.77% mannose. Monosaccharide composition and retention time fucoidan *Sargassumpolycystum* HPLCanalysis results are presented in Table3.

Tabel 3. Monosaccharide composition of crude fucoidan*Sargassumpolycystum* after hidrolysis with 2M TFA

Peak	Composition monosaccharide	Retention time (minute)	Contents (mg/g)	Relatifepercentace (%)
1	Glucose	8.32	5.72	3.77
2	Xylose	8.90	35.29	23.27
3	Rhamnose	9.33	21.93	14.47
4	Fucose	10.27	48.64	32.08
5	Galactose	14.10	34.33	22.64
6	Mannose	17.35	5.72	3.77

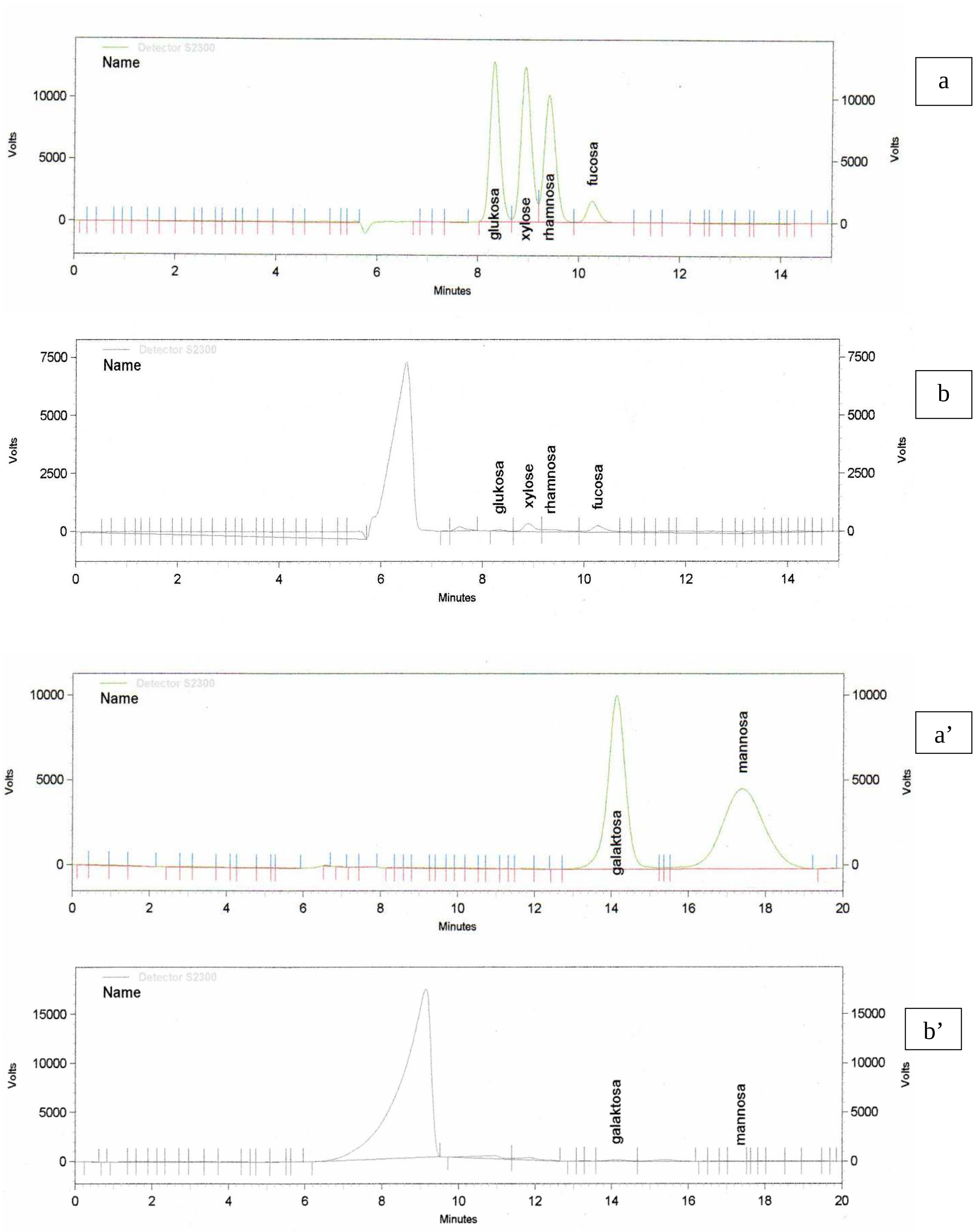

Figure 3. Chromatogram of glucose, xylose, rhamnose, fucose(a) galactose, mannose (a'), (b) from hydrolyzate fucoidan*Sargassum polycystum* by AMINEX HPX 87H Column, (b') by AMINEX HPX 87P Column

Conclusion

The optimal extraction point fucoidan*Sargassumpolycystum* occurred at81.00^{o}C4.04h. Experimental validated results showed the optimal point extraction of crude fucoidan were 7.23%, 131.15mg/g fucose, 403.34mg/g total carbohydrate and 94.67mg/g sulfate content.FT-IR andHPLC analysis showed that crude fucoidan *Sargassumpolycystum* were composed by fucose, galactose, xylose, glucose, mannoseandsulfate.

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