Extraction Of Sequential Fucoidan And Alginate Biorefinery For Pre-Extraction Acid Treatment Of Intrinsic Alginate Viscosity Of Brown Algae

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Abstract

The main process of alginate extraction from brown algae is pre-extraction by acid treatment. Pre-extraction conditions of acid treatment (temperature, time and pH) have an effect on the chemical and physical properties of alginate from brown algae. The aims of this study was to determine the effect of pre-extraction of acid treatment (temperature, time and pH) on the intrinsic viscosity of alginate and determine the pre-extraction conditions which was gave the maximum intrinsic viscosity response. The 2^k factorial design was used to evaluate the effect of pre-extraction of acid treatment (temperature, time and pH) on intrinsic viscosity and determine the accuracy of the first-order polynomial model. The results showed that the temperature, time and pH of the pre-extraction treatment gave a real quadratic effect on intrinsic viscosity. Intrinsic viscosity increased with higher temperature, time and pH degree, then decreased after reaching maximum. The curvature test was significantly different (α=0.05) by first order polynomial of quadratic model. The maximum value of the intrinsic viscosity response of alginate was 502.05 ml/g and occurred in the pre-extraction conditions acid treatment temperature of 35^{o}C, time of 60 minutes, and pH 3. Thus, it concluded that the best pre-extraction acid treatment temperature was 35^{o}C for 60 minutes and under pH 3.

Keywords**:** pre-extraction, alginate, intrinsic viscosity, *Sargassum cristaefolium*

Introduction

Alginates are polysaccharides that found in the matrix of brown algal cell walls ranging from 8-40%, composed of linear polymers β- (1-4)-D-mannuronate (M) and ɑL-guluronate (G) (Boisseson et al., 2004 ; Draget and Taylor, 2011). Alginate is widely used in the food and non-food industries as an additive to stabilize the emulsion, thickener and gel forming system (Torres et al., 2007; Hernandez-Carmona, 2013). Alginate needs for the domestic industry are currently estimated at more than 2000 tons per year all of which are met from imported products (Laksmono et al., 2013). Indonesia has a large potential of alginophyte resources, but not optimally utilized considering that the development of domestic alginate extraction methods can't produce alginate with high rheological quality.

The first step in the alginate extraction protocol is acid treatment. Pre-extraction treatment of brown algae with acid solvents affects the physical characteristics of brown algae in alkaline solvents and alginate quality (Hernandez-Carmona et al. 1999; Lorbeer et al. 2015; Silva et al. 2015). Therefore, it is important to understand the effect of pre-extraction of acid treatment on the viscosity of intrinsic alginate based on the biorefinery concept of fucoidan and alginate extraction from brown algae. The purpose of this study was to observe the effect of pH, temperature and time of fucoidan extraction as a pre-

extraction treatment of acid solvents on the intrinsic viscosity of alginates from brown algae *Sargassum cristaefolium*.

Material and Method

Material

*Sargassum cristaefolium wa*s obtained from the Poteran Island of Sumenep, Madura. All chemicals; KOH, formaldehyde, aquades, hydrochloric acid (HCl) 37%, ethanol 99.8%, and Na_2CO3 had a degree of technical purity (CV. Makmur Sejati & CV. Krida Tama Persada)

Experimental Design

The alginate extraction design used a 2k factorial design with 3 variables, namely temperature (x1), time (x2), and pH (x3). Each variable consisted of 2 levels that was coded with -1 and +1 and was expanded by 3-replicate center points coded 0 (Gazpersz, 1992), the experimental design was presented in **Table 1**.

Table 1. Factorial design 2^3 with expansion of center point

No	Actual Variable			Code Variable x 2			Intrinsic Viscosity (ml/g)
	Temperature (°C)	of Time (minutes)	pH	x1		x3	
1	45	30	5	+1	0	-1	152.07
2	45	90	5	+1	+1	+1	198.92
3	45	30	1	+1	-1	-1	276.65
4	25	30	5	-1	-1	+1	187.87
5	25	30	1	-1	-1	-1	265.91
6	45	90	1	+1	+1	-1	195.41
7	25	90	1	-1	+1	-1	105.89
8	25	90	5	-1	+1	+1	223.06
9	35	60	3	0	0	0	502.05
10	35	60	3	0	0	0	403.35
11	35	60	3	0	0	0	400.31

Based on experimental data, a regression analysis and the suitability of the first order polynomial equation model were performed:

$$Y=\beta0+\beta1x1+\beta2x2+\beta1x3 \quad \text{.....} \quad (1)$$

Where Y was the response variable, β_0 was the intercept coefficient; βi, βii, βij were the linear regression coefficients, and x123 was the code of three independent variables of temperature, time and pH.

Sample Preparation

Brown algae washed with fresh water until clean and soaked in 0.1% KOH for 1 hour, then was washed to remove alkaline residues (Subaryono and Apriani, 2010). Furthermore, brown algae dried by sun drying, milled and filtered with 60 mesh filter. Then, brown algae soaked in 0.1% formaldehyde solution overnight and washed thoroughly and dried by cabinet dryer on 45°C for 6 hours (Wedlock and Fasihuddin, 1990; Hernandez-Carmona et al. 1999).

Pre-extraction

Brown algae dissolved in 0.03 M HCl on pH 1-5, temperature 25-45^{o}C, time 30-90 minutes, solvent ratio 1:20 (w / v) then stirred constantly with a 500 rpm homogenizer, and washed with distilled water to neutral the pH then the water drained by pressed (HernandezCarmona et al. 1999).

Alginate Extraction

Brown algae acid pre-extraction results was added Na_2CO_3 (pH 10) solution with 1:20 (w / v) ratio on 70^{o}C for 2 hours. The filtrate was filtered and centrifuged at 5000 rpm for 10 minute, then the supernatant was taken. The alginate filtrate was added with a 96% ethanol with 1: 2 (v / v) ratio, then left for 2 hours and filtered. Alginate was washed twice with 70% ethanol and 96%, filtered and pressed. The alginate was dried in an oven at 45^{o}C for 24 hours and grinded 60 mesh.

Intrinsic Viscosity

Measurement of viscosity of alginate samples was carried out with Ubbelohde capillary viscometer (Canon, USA) with a capillary diameter of 0.56 mm at 25^{o}C. Alginate solution was prepared by dissolving 30 mg of alginate in 10 ml distilled water for 5 hours at room temperature (25^{o}C), then a series of alginate concentrations of 0.05-0.3 g / dL was made. Solution flow time t, measured relative to the solvent flow time, t0. Intrinsic viscosity was determined by extrapolation from ηsp/c to zero concentration (Chee et al. 2011).

Relative viscosity, $\eta = \frac{t}{t_0}$ ..... (2)

Specific viscosity, $\eta sp = \eta - 1$ ..... (3)

Viscosity reduction, $\frac{\eta_{sp}}{c} = \frac{\eta_{-1}}{c}$ ..... (4)

Intrinsic Viscosity, $[\eta] = \lim_{c \to 0} \frac{\eta_{sp}}{c}$ ..... (5)

Data analysis

Data analysis and the accuracy of the polynomial equation model were carried out with the expert design program version 7 for analysis of the intrinsic viscosity range.

Results and Discussion

Intrinsic viscosity

Research on the effect of temperature, time and pH of pre-extraction of different acid treatments on intrinsic viscosity of *Sargassum cristaefolium* found that intrinsic viscosity was higher at pH 3 and time 60 minutes then decreased at pH 5 and time 30-90 minutes, highest intrinsic viscosity is 502, 05 ml / g at 35^{o}C, 60 minutes and pH 3, while the lowest intrinsic viscosity was 105.89 ml / g at 25^{o}C, pH 1 and 90 minutes **(Figure 1)**. The intrinsic viscosity of the results of this study was relatively similar to that of the literature (Rahelivao et al. 2013; Fenorodosa et al. 2010; Torres et al. 2009), and higher than the results of Mahmood and Siddique (2010), Fertah et al. (2014) and Sellimi et al. (2015).

Treatment temperature 25-45^{o}C, time 30-90 minutes and pH 1-5 significantly affected the intrinsic alginate viscosity of *Sargassum cristaefolium* (P = 0.05). The pre-extraction treatment had a very positive effect on intrinsic viscosity of alginate at 35^{o}C, 60 minutes and pH 3, intrinsic viscosity decreased rapidly at pH 1 and 5 within 30-90 minutes. This is related to the degradation of the alginate polymer chain due to the β

elimination reaction in the 4-α-glycosidic bond and the hydrolytic breakdown of the proton catalyst (Smidsrod et al. 1969; Haug et a1, 1967). The degradation of the alginate polymer chain is increasingly severe at pH 1 and the pre-extraction time is 30-90 minutes (Sugiono et al. 2018; Lorbeer et al. 2015).

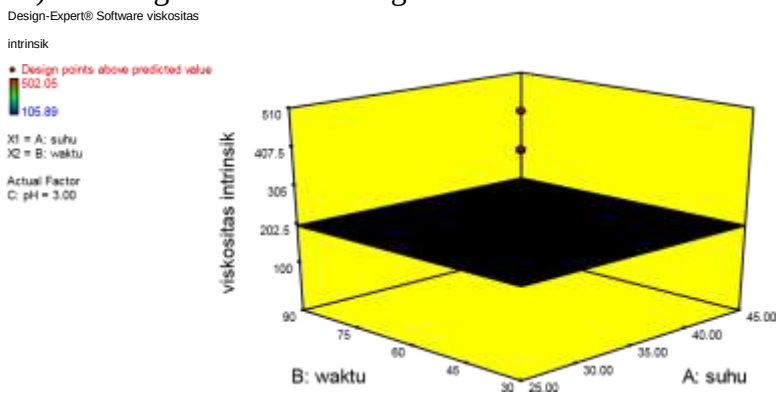

Figure 1. Effect of temperature and time of pre-extraction of acid treatment on intrinsic viscosity of alginate of *Sargassum cristaefolium* (A: Temperature; B: Periode of Time)

Model accuracy

The prediction of the polynomial model of the first-order experimental calculation results of intrinsic viscosity response as follows:

y = 244,942-10.08x1-39.80x2-20.48x3 + 22.61x1.x2-40.05x1.x3 + 80.82x2.x3-16.78x1.x2.x3

Variance analysis results **Table 2.** found that the model was significantly different at the level of confidence α = 0.05, this showed that the treatment temperature, time and pH of the extraction significantly influence the intrinsic viscosity response. The influence of single factors and the interaction of temperature, time and pH of pre-extraction of acid treatment negatively affect intrinsic viscosity.

Table 2. Analysis of various first order polynomial regressions

A source of diversity	Number of squares	df	Middle Squared	F-value	p-value
Model	0.000	0			
Curvature	1.200E+005	1	1.200E+005	37.54	0.0002*
Residual value	28770.94	9	3196.77		
Lack of Fit	22070.29	7	3152.90	0.94	0.6046ns
Error	6700.65	2	3350.33		
Sum of total squares	1.488E+005	10			

Note: * = Significantly different, ns = not significantly different

Curvature test was significant at the level of confidence α = 0.05. This showed that the first-order experimental polynomial model was quadratic (Gazpersz, 1992; Montgomery 2005). It was explained that if the curvature test was significant it meant that the polynomial model was quadratic (Sugiono et al. 2014). The midpoint of

planning was appropriate, the optimal response is around 35oC, 60 minutes and pH 3 **(Figure 2).**

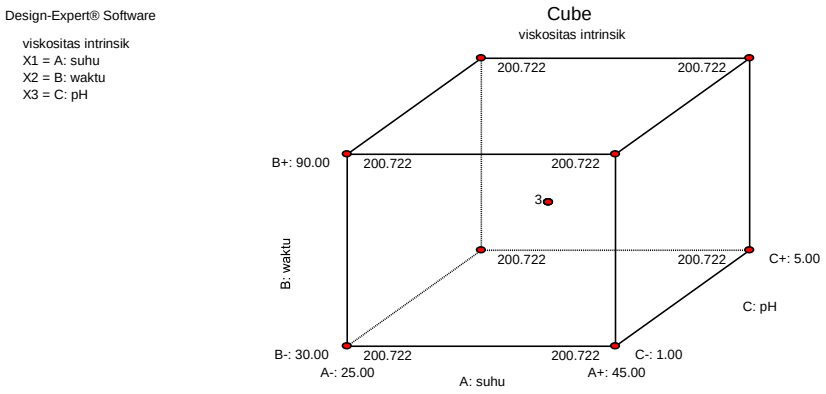

Figure 2. Effect of temperature, time and pH on intrinsic viscosity (A: Temperature; B Periode of time; C: pH)

Conclusion

Based on the results of the study it can be concluded that (1) the temperature, time and pH treatment have a significant effect on the viscosity of intrinsic alginate Sargassum cristaefolium and (2) the best result of the maximum intrinsic viscosity value (502.05 ml/g) by the quadratic test of first order polynomial model was 35oC for 60 minutes and pH 3.

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