BIOREFINERY FOR SEQUENTIAL EXTRACTION OF FUCOIDAN AND ALGINATE FROM BROWN ALGA Sargassum cristaefolium

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ABSTRACT

The critical step in the sequential extraction of fucoidan and alginate from brown algae is acid treatment, since it noticeably affects physicochemical properties of the both components. This study aimed to investigate the effects of acid treatment on the multiple responses of alginate and fucoidan yield from brown alga Sargassum cristaefolium. Box Behnken Design (BBD) from Response Surface Methodology (RSM) was established to understand the effects of temperature, time and pH in acid treatment on the fucoidan yield and multiple-response alginate as follows: yield, intrinsic viscosity, and molecular weight. The experimental results revealed that temperature, time and pH significantly affected fucoidan yield, alginate yield, intrinsic viscosity, and molecular weight of alginate. The optimum acid treatment was found at temperature 33.75 °C, time 58.22 min, and pH 3.07, resulting in fucoidan yield 1.22±0.068%, alginate yield 29.85±0.24%, intrinsic viscosity 409.72±8.23 ml/g and molecular weight 194.08±3.77 kDa with the desirability value 0.805.

Keywords: Fucoidan; Alginate; Biorefinery; Acid Treatment; Sargassum cristaefolium

1. Introduction

Brown algae is inarguably one of important sources for polysaccharides, e.g. fucoidan and alginate, with different physicochemical properties (Rioux et al., 2007; Ale et al. 2011a; Torres et al., 2007; Draget and Taylor, 2011). Alginate derived from brown algae is thermostable component, which is widely applied in a variety of industries as thickener, emulsifier, stabilizer, and gelling agent (Poncelet et al. 1999; Gomez et al. 2009; Rahelivao et al. 2013; Sellimi et al. 2015), while it is also massively used in food supplementation, pharmaceutical industry (producing slow-release characteristics), and antitumor (Sousa et al. 2007; Moebus et al. 2012; Jensen et al. 2012). Fucoidan isolated from brown seaweed is also reported capable of exerting beneficial health effects mainly on antitumor, anticancer, anti-inflammation, and immunomodulator (Asker et al., 2007; Ye et al. 2008; Kim et al. 2010; Ale et al. 2011b; Costa et al., 2011).

Although seaweed is industrially important, the processing has been currently hindered by some serious constraints, mainly related to low efficiencies and huge amount of waste. To deal with this, the use of integrated biorefinery for producing various products could gradually eliminate the mentioned constraints, while also rising economic benefits (Lorbeer et al. 2015). Such biorefinery processing is greatly possible for brown algae due to presence of fucoidan and alginate with existing or future potential applications (Jung et al. 2013; Ruiz et al. 2013). The parallel processing technology for producing fucoidan and alginate from brown algae could be a great opportunity in biorefinery industry (Sugiono and Ferdiansyah, 2019).

In general, acid treatment was applied to extract fucoidan and alginate from brown algae. It enables to induce destruction of cell walls, hydrogen bond cleavage, and solubilization of
extracted fucoidan (Kim et al. 2010; Ale et al. 2012; Ermakova et al. 2011; Sugiono et al. 2014), while the acid also simultaneously plays role in converting alginate-salts to alginate-acids, avoiding them from production of insoluble contaminants (Myklestad, 1968; Arvizu et al. 2007). During extraction of alginate, acid treatment serves to remove contaminants (fucoidan, laminaran and polyphenol) and produce alginate-acids which then increase their extractability using sodium carbonate (Hernandez-Carmona et al. 1999; Torres et al. 2007; Gomez et al. 2009; Sellimi et al. 2015; Rahelivao et al. 2013; Fertah et al. 2014; Sugiono et al. 2019a). Based on this mechanism, acid treatment becomes a basic principle for sequential biorefinery in extraction of fucoidan and pre-extraction of alginate.

Previous studies have reported the application of acid treatment for sequential extraction of fucoidan and alginate with regard to characterization of the components (Rioux et al. 2007), but their works were not exclusively directed to biorefinery processing. Therefore, the use of acid treatment with optimum levels allows us to perform biorefinery processing for extracting fucoidan and alginate from brown seaweed, resulting in high yield and quality. In low acid concentration, the yield was also poor; on the other hand, the excessive level of acid would degrade alginate structure, causing reduction of its viscosity. This present work aimed to determine optimum level of pH, temperature, and time in the acid treatment for isolating fucoidan and alginate from brown alga *Sargassum cristaefolium* with regard to biorefinery industry.

2. Materials and methods

2.1. Materials and reagents

Brown algae *Sargassum cristaefolium* was obtained from Poteiran Island in Sumenep, Madura, and collected in December 2018. Chemicals (distilled water, HCl 37%, NaOH, ethanol 99.8%, Na₂CO₃) for extraction and analyses were analytical grade.

2.2. Sequential extraction of fucoidan and alginate

2.2.1. Pre-treatment of brown algae

Brown algae was washed using fresh water, dried, ground, and sieved at 60 mesh (Sugiono et al. 2014). The powder was then soaked in a solution containing ethanol: CHC{l₃}: distilled water (4:2:1), stirred overnight to remove phenol and protein. Last, the mixture was washed and dried at 45 °C for 6 h (Ale et al. 2012).

2.2.2. Fucoidan extraction

The pre-treated algae (7.5 g) was added with HCl (1:20, b/v; pH 1-5) and incubated in a shaking waterbath at 25 – 45 °C for 30 – 90 min. Subsequently, vacuum filtration was used to separate residue (A) from filtrate. The filtrate was mixed with ethanol 96% (1:2, v/v) and left overnight at room temperature until producing precipitate. Fucoidan was collected following centrifugation at 7000 rpm for 10 min, and dried using vacuum dryer at 45 °C for 18 h (Ale et al. 2012).

2.2.3. Alginate extraction

Residue A (collected from previous process) was added with Na₂CO₃ 2.5% (1:20, b/v) and incubated in a shaking waterbath at 70 °C for 2 h, then followed by filtration to collect filtrate. The filtrate was centrifuged at 5000 rpm for 10 min, mixed with ethanol 96% (1:2, v/v) and filtered after incubation for 2 h. The alginate was washed twice using ethanol 70% and 96%, respectively, filtered and dried using vacuum dryer at 45 °C for 24 h. Ultimately, the dried alginate was ground and sieved at 60 mesh (Gomez et al. 2009).

2.3. Experimental design

Box-Behnken Design in Response Surface Methodology (RSM) was used, consisting of 3 variables, i.e. temperature (X₁: 25, 35, 45°C), time (X₂: 30, 60, 90 min), and pH (X₃: 1, 3, 5). The coded (+1 and 0) and actual of independent variables used in this experiment was presented in Table 1. A totally amounting of 15 experimental runs with three replicates in center
point (Table 2) (Montgomery, 2005). The center points were fixed according to preliminary study.

Regression analysis and model adjustment at the second order was carried out as follows:

\[ Y = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum_{i=1}^{3} \sum_{j>i}^{3} \beta_{ij} x_i x_j \]  

(1)

where \( Y \) = response, \( \beta_0 \) = intercept coefficient, \( \beta_i, \beta_{ii}, \beta_{ij} \) = regression coefficient for linear, quadratic, and interaction, and \( x_i, x_j \) = variables of pH and temperature, and time \((i \neq j)\).

The data analysis was performed in Design-Expert version 7 software in order to find correlation coefficient (R) and determination coefficient (R²), while the significance was set at \( P = 0.05 \). Accuracy between validation and estimated data by Design Expert was compared using paired sample t-test in Minitab 16 software.

**Table 1.** Coded and actual of independent variables

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Symbols</th>
<th>Variables</th>
<th>Coded</th>
<th>Actual</th>
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<tr>
<td>Temperature (°C)</td>
<td>( x_1 )</td>
<td>-1</td>
<td>25</td>
<td></td>
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<td></td>
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<td>0</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+1</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Time (min)</td>
<td>( x_2 )</td>
<td>-1</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>pH</td>
<td>( x_3 )</td>
<td>-1</td>
<td>1</td>
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</tr>
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<td></td>
<td></td>
<td>0</td>
<td>3</td>
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<tr>
<td></td>
<td></td>
<td>+1</td>
<td>5</td>
<td></td>
</tr>
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**Table 2.** Box-Behnken Design from RSM and responses

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<thead>
<tr>
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<th>Responses</th>
</tr>
</thead>
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<td>Time (min)</td>
</tr>
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<tr>
<td>15</td>
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</tr>
<tr>
<td>Pred.</td>
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<tr>
<td>Valid</td>
<td>33.75</td>
<td>58.22</td>
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</table>
2.4. Characterization

2.4.1. Yield
Yield was determined according to ratio of alginate or fucoidan weight over initial weight of brown algae, then multiplied by 100% (Torres et al. 2007).

2.4.2. Intrinsic viscosity
For alginate viscosity, viscometer capillary Ubbelohde (Canon, USA) with capillary diameter of 0.56 mm was employed at 25 °C. Alginate solution was made by dissolving 30 mg of alginate in 10 ml of aquabides, stirred for 5 h at room temperature (25 °C) and diluted at serial concentration of 0.05-0.3 g/dL (Chee et al. 2011). Relative viscosity $\eta$ was determined according to ratio of flow time $t$ over flow time for solvent $t_0$. Meanwhile, intrinsic viscosity $[\eta]$ was calculated as follows:

Relative viscosity, $\eta = \frac{t}{t_0}$ (2)
Specific viscosity, $\eta_{sp} = \eta - 1$ (3)
Reduction viscosity, $\eta_{rp} = \frac{\eta - 1}{c}$ (4)
Intrinsic viscosity, $[\eta] = \lim_{c \to 0} \frac{\eta_{sp}}{c}$ (5)

2.4.3. Molecular weight
Molecular weight of alginate was determined according to relationship between averaged intrinsic viscosity and molecular weight. Calculation of molecular weight referred to Mark-Houwink, where $k = 0.023$ dL/g and $a = 0.984$ (Clementi et al. 1998). In this case, $[\eta]$ represented intrinsic viscosity (dL/g), while $M_w$ represented molecular weight (kDa).

$[\eta] = kM_w^a$ (6)

3. Results and discussions

3.1. Yield of fucoidan
The results showed that all studied variables showed linear relationship to the yield of fucoidan (Figure 1). The yield seemed to raise as increase in temperature, time, and acidity. The highest fucoidan extract (1.5%) was obtained at temperature 45 °C, time 90 min, and pH 1. This is in accordance with previous result reported by Ale et al. (2012), finding that a high temperature causes swelling of the algae cell wall due to thermal expansion, resulting in enhancement of fucoidan extractability (Sugiono et al. 2014). Meanwhile, the use of low pH and longer acid treatment enabled to soften cell wall of the algae, which increased solubility of fucoidan in HCl (Silva et al. 2015). Rodriguez et al. (2011) reported that the increase in temperature and time could improve extractability of fucoidan, then it gradually decreased after reaching the optimum level. Lorbeer et al. (2015) stated that fucoidan yield was higher when extracted under higher temperature and longer extraction time, while the low pH could destroy fucoidan structure.

3.2. Yield of alginate
In this present work, we found that all the variables demonstrated quadratic effects on the alginate yield (Figure 2), ranging from 26.21 – 31.12%. This was similarly reported by Silva et al. (2015) and Sugiono et al. (2019a). The yield showed an increase with the increased temperature, longer time, and reduced pH during pre-extraction with acid treatment. This is understandable since an increase in pH level and time could enhance conversion of Ca/H ions, thereby improving the solubility of alginate in Na$_2$CO$_3$. In addition, higher temperature and longer exposure to acid treatment noticeably contributed to formation of porous and softened structure in surface of the cell walls, which in turn also enabled to increase extractability of alginate (Sugiono et al. 2018b). Fertah et al. (2014) asserted that alginate extract was relatively increased with the increasing extraction temperature, after this condition it was continuously to decrease due to a degradation of alginate chain molecules. Nevertheless, pre-extraction of algae using acid treatment at pH 5 showed a contrary result. The exchange of Ca/H ions was logarithmically in proportional with acid concentration and pre-extraction time (Mykleasted et al. 1968; Lorbeer et al. 2015).
Figure 1. Response surface plots of fucoidan yields from brown algae *Sargassum cristaefolium* as a function of temperature and time (A), pH and time (B), temperature and pH (C).

Figure 2. Response surface plots for alginate yield from brown alga *Sargassum cristaefolium* as a function of temperature and time (A), pH and temperature (B), pH and time (C).
Figure 3. Response surface plots for alginate intrinsic viscosity from brown alga *Sargassum cristaefolium* as a function of temperature and time (A), pH and temperature (B), pH and time (C).

Figure 4. Response surface plots alginate molecular weight from brown alga *Sargassum cristaefolium* as a function of temperature and time (A), pH and temperature (B), pH and time (C).
3.3. Intrinsic viscosity of alginate
The results demonstrated that difference in temperature, time, and pH showed quadratic effect on intrinsic viscosity of alginate (Figure 3). An increase in temperature, time, and pH resulted in a growing viscosity, while it tended to be lower after reaching optimum condition. In this present work, intrinsic viscosity reached 103.15 ml/g to 446.39 ml/g, which was relatively similar to that reported by Torres et al. (2007), Fenoradosoa et al. (2010) and Rahelivao et al. (2013). The incrementation of alginate intrinsic viscosity was found at pH 3 and time 60 min; however, it showed a decrease at pH 1 and pH 5 in 30 – 90 min. The rising viscosity is associated with the increasing conversion of Ca/H ion exchange occurring at pH 3 within 30 – 90 min, thus improving the extractability of long-chain alginate.

Additionally, pre-extraction carried out at pH 5 seemed to be ineffective in reducing phenol compounds, in which their existence differently contributed to the increasing cleavage of main polymer chains of alginate during extraction in alkaline condition (Wedlock and Fasihuddin, 1990). Jayasankar (1996) reported that viscosity of alginate was higher after treated with acid compared to that without acid treatment. Meanwhile, extraction at pH 1 could induce degradation of alginate polymer chains (Haug et al. 1963; Smidsrod et al. 1969). Furthermore, the increase in temperature and time during acid treatment would induce cell wall to soften and swell, which remarkably enhanced the extractability of long-chain molecules of alginate (Sugiono and Ferdiansyah, 2018).

3.4. Molecular weight of alginate
Present work successfully found that concentration of alginate with high molecular weight tended to increase with a rising temperature, time and acidity (Figure 4). This is augmented by previous studies reported by Torres et al. (2007) and Lorbeer et al. (2015). It is noteworthy that pH levels in pre-extraction phase strongly caused positive effects on the incrementation of molecular weight, occurring up to pH 3 and time 60 min, although it was then declined at pH 1 and pH 5 within 30 – 90 min. At condition of pH 3, temperature 35 °C and time 60 min, the conversion of Ca/H ions was higher, thus escalating the solubility of alginate in Na$_2$CO$_3$. Myklestad (1968) found that Ca/H ion exchange occurred at a higher level with the increase in acidity and time during acid treatment. The higher temperature and longer period of acid treatment was responsible for degradation of cell wall, ultimately contributing to enhanced level of extracted alginate. Sugiono et al. (2018a) mentioned that the extraction of high molecular weight alginate could achieve more desirable results when carried out at high temperature, longer time, and low pH during pre-extraction acid treatment, after that, it tended to attenuate due to destruction of alginate polymer chains. At low pH, the polymer chain of alginate was destroyed because of β-elimination and hydrolytic reaction which was catalyzed by proton (Hernandez-Carmona et al. 1999; Silva et al. 2015). However, at pH 5, molecular weight of alginate was reduced due to oxidative depolymerization triggered by phenolic compounds through auto-oxidation process to release hydrogen peroxide; this free radical was capable of cleaving main chain of alginate molecules (Smidsrod et al. 1963). Wedlock and Fasihuddin (1990) also reported that acid treatment at pH close to 7 seemed to be less effective in phenolic compound removal; as commonly known, the component was not desired since it promoted degradation of alginate polymers in alkaline extraction stage. Furthermore, previous study found that molecular weight of alginate was higher at acid treatment of pH 3.5 compared to that extracted at pH 5 (Lorbeer et al. 2015).

3.5. Model accuracy
Box Behnken Design was used to evaluate the effects of temperature, time, and pH on yield (fucoidan and alginate), intrinsic viscosity, and molecular weight of alginate. The second order polynomial for biorefinery of sequential extraction was presented in Table 3.
The model accuracy on each response was evaluated using model significance, lack of fit, and correlation coefficient which are presented in Table 3. The acceptable model was achieved according to following criteria, i.e. significance of \( P<0.05 \), \( R^2 \geq 0.8 \) and lack of fit>0.1 (Montgomery, 2005). In this case, the second order polynomial for fucoidan yield and multiple response of alginate fitted entire criteria, suggesting that it is acceptable for predicting optimum response.

### 3.6. Optimization and verification

The optimum condition for extraction was achieved at temperature 33.75 °C, time 58.22 min, pH 3.07. In such condition, the response was predicted to reach fucoidan yield 0.71%, alginate yield 29.63%, intrinsic viscosity 448.8 ml/g, and molecular weight 212.6 kDa, with desirability value of 0.805 (Figure 5). Desirability ranges from 0 to 1, in which desirability close to 1.0 indicates that prediction of optimum condition generated by Design Expert possesses high validity (Sugiono et al. 2019b).

The predicted optimum condition was verified using 3 replicates, while the experimental value of response was described as follows: fucoidan yield 1.22±0.068%, alginate yield 29.85±0.24%, intrinsic viscosity 409.72±8.23 ml/g, and molecular weight 194.08±3.77 kDa. Based on paired sample t-test, the data obtained from prediction and validation did not differ significantly (\( P>0.05 \)), suggesting that the experimental data showed a desirable suitability with optimum point as predicted by the model.

### Table 3. Polynomial models, significance codes and fitting models

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Fucoidan yield (%)</th>
<th>Alginate yield (%)</th>
<th>Intrinsic viscosity (ml/g)</th>
<th>Molecular weight (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>β_0 +0.74</td>
<td>+29.85</td>
<td>+446.38</td>
<td>+211.46</td>
</tr>
<tr>
<td>Linear</td>
<td>β_1 -0.051^ns</td>
<td>+0.87^*</td>
<td>-25.96^ns</td>
<td>-12.33^ns</td>
</tr>
<tr>
<td></td>
<td>β_2 +0.31^ns</td>
<td>+0.57^**</td>
<td>-16.03^ns</td>
<td>-7.61^ns</td>
</tr>
<tr>
<td></td>
<td>β_3 -0.49^*</td>
<td>-1.57^*</td>
<td>+17.64^ns</td>
<td>+8.34^ns</td>
</tr>
<tr>
<td>Quadratic</td>
<td>β_11 -</td>
<td>+0.86^ns</td>
<td>-8.54^ns</td>
<td>+3.53^ns</td>
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<tr>
<td></td>
<td>β_22 -</td>
<td>-0.17^ns</td>
<td>+42.02^ns</td>
<td>+19.98^ns</td>
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<tr>
<td></td>
<td>β_33 -</td>
<td>-0.21^ns</td>
<td>+54.85^ns</td>
<td>+26.04^ns</td>
</tr>
<tr>
<td>Cross product</td>
<td>β_12 -</td>
<td>-1.57^ns</td>
<td>-99.53^**</td>
<td>-47.73^*</td>
</tr>
<tr>
<td></td>
<td>β_13 -</td>
<td>-0.91^*</td>
<td>-125.27^*</td>
<td>-59.93^*</td>
</tr>
<tr>
<td></td>
<td>β_23 -</td>
<td>-0.44^***</td>
<td>-122.96^**</td>
<td>-58.82^*</td>
</tr>
<tr>
<td>Fitting model</td>
<td>P value 0.0157^*</td>
<td>0.0062^**</td>
<td>0.0417^*</td>
<td>0.0423^*</td>
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<tr>
<td></td>
<td>Lack of Fit 0.0577^ns</td>
<td>0.1722^ns</td>
<td>0.7914^ns</td>
<td>0.7961^ns</td>
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<tr>
<td></td>
<td>R^2 0.9558</td>
<td>0.9575</td>
<td>0.9038</td>
<td>0.9032</td>
</tr>
</tbody>
</table>

Equation of the type \( Y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_1x_1x_2 + \beta_1x_1x_3 + \beta_2x_2x_3 + \beta_3x_3x_1 + \beta_1x_1x_2x_3 \)

Significance codes: *** = \( P < 0.001 \)

** = 0.001<\( P < 0.01 \)

* = 0.01<\( P < 0.05 \)

ns = \( P > 0.05 \)
Figure 5. Response surface (A) and contour plot (B) of desirability for optimal points in biorefinery of fucoidan and alginate sequential extraction.

4. Conclusions

Biorefinery process for the sequential extraction of brown algae polysaccharides has been developed, this process can produce two products of fucoidan and alginate. The experimental results showed that all studied variables (temperature, time, pH) demonstrated linear effects on fucoidan yield, but displayed quadratic effects on alginate yield, intrinsic viscosity, and molecular weight. From the optimization, the best condition for acid treatment would be as follows: temperature 33.75 °C, time 58.22 min, pH 3.07. Such condition reached fucoidan yield 1.22±0.068%, alginate yield 29.85±0.24%, intrinsic viscosity 409.72±8.23 ml/g, and molecular weight 194.08±3.77 kDa.

5. References


Structure and properties of three alginates from madagascar seacoast algae. *Food Hydrocolloids*, 32(1), 143–46.


Sugiono, S., Ferdiansyah, D. (2018). Fucoidan and alginate sequential biorefinery extraction: Effect of Pre-extraction Acid-treatment Against Intrinsic viscosity of alginate from brown alga *Sargassum cristaefolium*. *Food Science and Technology Journal*, 1(2), 44-51


Sugiono, S., Masruri, M., Estiasih, T., Widjanarko S.B. (2019b). Optimization of extrusion-assisted extraction parameters and characterization of alginate from brown algae (*Sargassum cristaefolium*). *Journal of Food Science and Technology*, 56 (8), 3687-3696

Sugiono, S., Masruri, M., Estiasih, T., Widjanarko S.B. (2019b). Structural and rheological characteristics of alginate from *sargassum cristaefolium* extracted by twin
screw extruder. *Journal of Aquatic Food Product Technology*, 28 (9), 944-959


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