FOODSCITECH
Food Science and Technology Journal

Microwave-Assisted Extraction in A Sequential Biorefinery of Alginate and Fucoidan From Brown Alga *Sargassum Cristaefolium*

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ABSTRACT

The brown alga has bioactive alginate and fucoidan, a potential for raw materials the biorefinery industry with core processing of fucoidan and alginate extraction. The research on sequential biorefinery of fucoidan and alginate was conducted in conventional and hydrothermal methods using a smart pressure-cooker, but the yield of fucoidan is low. The improvement was carried out by extracting fucoidan and alginate using microwave-assisted extraction (MAE). This study aimed to obtain the MAE processing conditions that produced the maximum yield of fucoidan and alginate. The factorial design of 2^k was used to determine the effect of MAE processing conditions, i.e. temperature, time, and alga/water ratio on fucoidan and alginate yields. The parameters observed were fucoidan and alginate yield. Data analysis and prediction of first-order model and model accuracy was a regression modelling using a design expert program. The result showed that the elevate of temperature MAE processing to 80 ^oC,for 20 minutes, and alga/water ratio of 2:20 (w/v) increased fucoidan (4.5%) and alginate (40.6%) yield, but it decreased the yield at 100 ^oC, for 30 minute, and alga/water ratio of 3:30 (w/v). The equation of the first-order model was quadratic, by wich the model test in curvature was found significant at α=0.05. The MAE sequential biorefinery extraction improved fucoidan and alginate yield and had a potential efficiency for commercialization. The maximum response of fucoidan and alginate yield was obtained from a MAE sequential biorefinery at 80 ^oC,for 20 minutes,and alga/water ratio 2:20 (w/v); further research is needed to optimizing the process.

Keywords*: Alginate; Biorefinery; Fucoidan; Microwave-Assisted Extraction; Sargassum Cristaefolium*

INTRODUCTION

Brown algae has bioactive fucoidan and alginate with different characteristics (Rioux et al., 2007). Alginates are widely used in food and non-food industries as thickeners and gelling agents. In pharmaceutical field, alginate and alginate oligosaccharides (AOs) are used as agent for slow-released drugs, antioxidant, antitumors, anticancer, and anti-inflammatory (Moebus et al., 2012). Fucoidan functions as an anticancer, immunomodulatory, and anti-inflammatory compound (Ale et al., 2012). Brown algae has great potential eedstocks for anintegrated fucoidan and alginate biorefinery extraction process (Jung et al., 2013), but this potential has not been utilized optimally.

The domestically developed fucoidan and alginate extraction methods are still conventional, needed a high temperature, takes a long time and the yield was still low. Nurhidayati et al. (2020) extracted fucoidan from *Sargassum cinereum* at 100 ^oC for 5 h and obtained a yield of 2.78%. Sinurat and Kusumawati (2017) reported the extraction of fucoidan from *Sargassum binderi* at 85 ^oC for 4 h obtained a yield of 2.57%. Skriptsova, (2016) extracted fucoidan with a solvent of 0.1 N HCl which obtained a yield of 2.5%. Trica et al. (2019) reported that alginate extraction from *Cystoseira barbata* in 3% Na_2CO_3 solution at

FOODSCITECH, 5 No. 2 December 2022
ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
DOI: http://dx.doi.org/10.25139/fst.vi.4976

60 ^oC for 2 h obtained a yield of 19%. Ardalan et al. (2018) also extracted alginate from *Sargassum angustifolium* conventionally in a 2% Na_2CO_3 solution at 90 ^oC for 2 h and the yield of 22.4%. While the fucoidan extracted by microwave-assisted extraction (MAE) at a pressure of 120 psi, a time of 31 minutes and an algae/solvent ratio of 1/25 g/ml obtained a fucoidan yield of 6.93%, higher than the conventional method (Rodriguez-Jasso et al. (2011). However this fucoidan and alginate extraction method was conducted with a separated purpose so that the efficiency of using raw materials is low and produces a lot of waste. This condition not support the growth of domestic fucoidan and alginate industries. Therefore, the development of an integrated fucoidan and alginate extraction method according to the industrial biorefinery concept is the right solution for this problem.

Previous research had carried out the integrated extraction of fucoidan and alginate according to the conventional and hydrothermal biorefinery concept by using a smart pressure cooker. Two products have been produced, i.e., alginate and fucoidan (Sugiono & Ferdiansyah, 2019). However, this method is ineffective because the yield of fucoidan is relatively small and takes a long time. Therefore, it is necessary to develop an integrated and more effective method using microwave-assisted extraction (MAE). Dobrincic et al. (2020) report that extraction by MAE is more selective, quick, high yield, and less energy and solvent consumption so that it is considered more environmentally friendly. However, the effect of MAE processing condition such as temperature, time, and alga/water ratio on the alginate and fucoidan yielded from brown alga *Sargassum cristaefolium* has still not been reported. The research aimed to obtain MAE process conditions to produce maximum yield on a sequential integrated extraction for fucoidan and alginate.

METHODS

Materials

Brown algae *Sargassum cristaefolium* used in this research was obtained from Poteran Island, Sumenep Regency, Indonesia. Chemicals such as aquades, methanol, chloroform, $CaCl_2$, Na_2CO_3, NaOCl, and 96% ethanol, had pro analysis grades purchased from local distributors in Indonesia.

Research Design

A factorial design of 2^k was used in this research, where k is the number of variables studied. Three studied variables were temperature (x_1), time (x_2), and alga/water ratio (x_3). These research variables consisted of two levels, coded -1 and +1. The center point is coded 0 with three times of replications (Gaspersz, 1992). The experimental design of this research is shown in Table 1.

The response data of yield of fucoidan and alginate in this research was conducted through regression analysis and model accuracy using the equation of:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_1 X_3 \quad (1)$$

In which Y = the response variable, β_0 = the intercept coefficient, β_1, β_2, and β_3 = linear regression coefficients, and X_1, X_2, and X_3 = the codes of the three variables of temperature, time, and alga/water ratio.

FOODSCITECH, Vol. 5 No. 2 December 2022
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Table 1. The factorial Design 2^3 and responses of fucoidan and alginate MAE-sequential biorefinery

No	Actual variable			Code variable			Yield	
	Temperature (°C)	Time (minute)	Alga/water ratio (w/v)	X_1	X_2	X_3	Fucoidan (%)	Alginate (%)
1	100	10	1	+1	-1	-1	3.30	18.60
2	100	30	3	+1	+1	+1	3.20	15.80
3	100	10	3	+1	-1	+1	3.10	22.40
4	60	10	3	-1	-1	+1	1.39	25.30
5	60	10	1	-1	-1	-1	1.47	28.20
6	100	30	1	+1	+1	-1	3.60	14.90
7	60	30	1	-1	+1	-1	2.13	33.40
8	60	30	3	-1	+1	+1	2.40	26.10
9	80	20	2	0	0	0	3.90	40.60
10	80	20	2	0	0	0	4.50	39.50
11	80	20	2	0	0	0	4.05	36.80

Sample Preparation

Brown algae was washed with freshwater until it was clean then sun-dried until their water content reached 13%. The dried brown algae was ground by using a coffee grinder and sieved using a 60 mesh screen.

Sequential Extraction Of Fucoidan And Alginate
Pretreatment Of Brown Algae

Brown alga powder was soaked in a solution containing ethanol: $CHCl_3$: distilled water in a ratio of 4:2:1, then the mixture was stirred overnight to remove phenol and protein. Last, the mixture was washed and dried at 45°C for 24 h followed a method by Ale et al. (2012).

Fucoidan Extraction by Microwave-Assisted Extraction

Ten grams of brown algae was mixed with water at alga/water ratio of (1-3):20 (w/v). The fucoidan was extracted using Microwave-Assisted Extraction (Microwave Reaction System SOLV Anton Paar) at temperature range of 60-100 ºC for 10-30 minutes. The products were added with water to reach alga/water ratio of 1:20 (w/v) and then stirred for 30 minutes, cooled and filtered using filter press to obtain residue (A) and liquid fraction. The liquid fraction was added with 1 M $CaCl_2$ solution and the mixture was left overnight at 4 °C to release and precipitate alginate. The fraction obtained separated by filtration. Around three-folds volume of 96% ethanol was added to the filtrate and the mixture was stored at 4 °C for 8 h. Ethanol-precipitated fucoidan was collected by centrifugation (5.000 rpm, 10 minutes, 4 °C), vacuum dried at 50 ºC overnight, milled, and stored for further analysis.

Alginate Extraction

Residue A from the previous process was added with 2.5% Na_2CO_3 solution (1:20, b/v) and incubated in a shaking water bath at 70 °C for 2 h, then followed by filtration to collect the filtrate. The filtrate was centrifuged at 5.000 rpm for 10 minutes and added with 10% NaOCl solution (1:10 v/v) and then stirred for 45 minutes, mixed with 96% ethanol (1:2, v/v), and filtered after rested for 2 h. The alginate was washed twice using 70% ethanol and 96%, respectively, filtered, and dried using a vacuum dryer at 45 °C for 24 h. Finally, the dried alginate was ground and sieved with a 60 mesh screen.

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Data Analysis

The data were analyzed for regression and accuracy polynomial model using the Design-Expert version 7.

RESULT AND DISCUSSIONS

Fucoidan Yield

The results showed that the temperature treatment, time, and alga/water ratio in the fucoidan extraction process with microwave-assisted extraction (MAE) resulted in fucoidan yields ranging 1.39-4.5% (Table 1). The fucoidan yields were increased with increasing temperature (60-100 ^{o}C), time (10-30 minutes), and alga/water ratio (1-3:20 w/v). The highest fucoidan yield occurred at a processing set temperature of 80 ^{o}C for 20 minutes and an alga/water ratio of 2:20 (w/v), and the lowest was at 60 ^{o}C, for 10 minutes, and an alga/water ratio of 3:20 (w/v). The yield of fucoidan obtained from MAE in this study was higher than fucoidan yield from *Ecklonia radiata* was 3.05% and fucoidan yield from *Sargassum cristaefolium* was 1.8%, as previously reported by Lorbeer et al. (2015) and Sugiono and Ferdiansyah (2020), respectively. Meanwhile, fucoidan yield from *Sargassum cristaefolium* extracted by MAE in this research was lower than fucoidan yield from *Fucus vesiculosus* was 12.53% reported by Rodriguez-Jasso et al. (2011).

Several studies have reported that extraction with MAE can produce biopolymers with high molar mass, shorter time, and higher yields (Chen et al., 2005; Leonelli & Mason, 2010). Microwaves emit electromagnetic waves that have magnetic properties and have two polar ions, positive and negative poles (Quitain et al., 2013). Water molecules and materials rotate quickly, causing strong friction and generating heat (Silva et al., 2015). Microwaves rotate because of the repulsion from the same pole; materials with positive and negative ions rotate at a speed of 2.450 times per second.

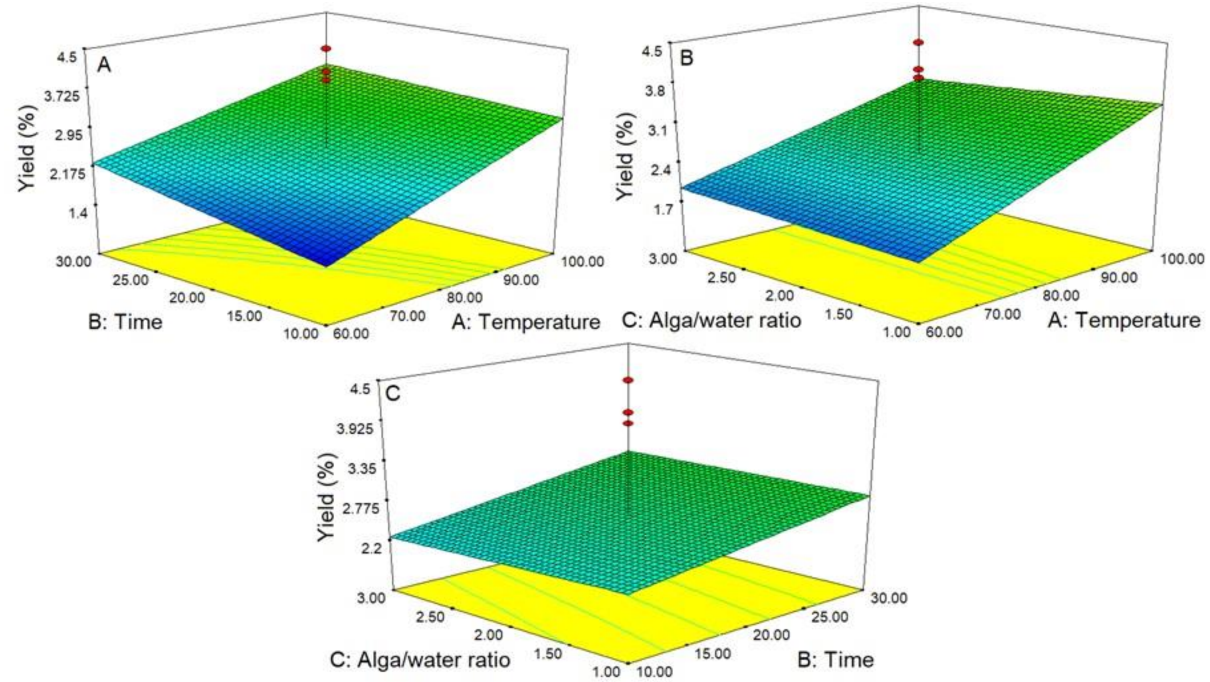

Figure 1. Response surface of fucoidan yields from *Sargassum cristaefolium* as effects of MAE temperature and time (A), temperature and alga/water ratio (B), alga/water ratio and time (C).

The observations of fucoidan extraction with MAE-sequential biorefiner showed that the processing conditions of 60-100 ^oC, with processing time course of 10-30 minutes, and alga/water ratios of 1-3:20 (w/v) had significant effects on the increasing fucoidan yields from *Sargassum cristaefolium*. The yield of fucoidan tended to increase with increasing temperature, time, and alga/water ratio until it reached the maximum yields and then decreased (Figure 1). The fucoidan yield peacked at 80 ^oC, for 20 minutes applied on, alga/water ratio of 2:20 (w/v) but the yield decreased at 100 ^oC for 30 minutes and alga/water ratio of 3:20 (w/v). The increase in temperature during the extraction process with MAE caused an increase in the evaporation process of the liquid in the cell and an increase in moisture pressure. This condition increased the number of porous cell walls of algae. The increase of porosity and porousness of algae cell wall matrix combined with the increase in temperature, time, and pressure makes mass transfer faster so that the extractability of the biomaterial is greater (Sugiono et al., 2014; Wang et al., 2010). Dobrinčić et al. (2020) explained that added time of MAE process rapid internal heating was increased cell wall porous and the intracellular compounds was release into the solvent. Microwave radiation also created cuticle layer destruction which was observed as a very rough surface of alga with many cavities after aplication of high power and pressure MAE.

Alginate Yield

The effect of different MAE-sequential biorefinery processing conditions on fucoidan and alginate yield indicated that the alginate yield tended to increase with increasing temperature (60-100 ^oC), time (10-30 minutes), and alga/water ratio (1-3:20 w/v). The alginate yield of *Sargassum cristaefolium* were 14.9-40.6% (Figure 2). The highest alginate yield was for the MAE processing condition at 80 ^oC, for 20 minutes, and alga/water ratio of 2:20 (w/v) whereas, the lowest was at 100 ^oC, time 30 minutes, alga/water ratio 1:20 (w/vl). The yield of alginate *Sargassum cristaefolium* from the sequential extraction of fucoidan and alginate resulted from this study was relatively the same as that found by Fertah et al. (2014), alginate yield from *Laminaria digitata* was 40.1%. Meanwhile, alginate yield from *Sargassum cristaefolium* extracted by MAE in this research was higher than alginate yield from *Ecklonia radiata* was 38.3% and alginate yiled from *Saccorhiza polyschides* was 23.8% as these data were reported by Lorbeer et al. (2015) and Silva et al. (2015).

The results showed that the different MAE processing conditions in the sequential integrated extraction of fucoidan and alginate significantly affected the alginate yield with a quadratic curve. Alginate yield increased rapidly at 80 ^oC, for 20 minutes, on alga/water ratio of 2:20 (w/v) but decreased significantly at 100 ^oC, for 30 minutes, on alga/water ratio of 1:20 (w/v). This was because the increasing temperature of the MAE to 80 ^oC caused the brown algae cell walls to expand and became more porous so that the alginate dissolved easily in sodium carbonate solution. Meanwhile, for MAE at 100 ^oC for 30 minutes and alga/water ratio of 1:20 (w/v), alginate yield decreased because it was suspected that alginate was extracted and co-dissolved with fucoidan as impurity (Quitain et al., 2013). The increase in temperature caused an increase in pressure to drive mass transfer, the extractability of fucoidan increased, and alginate was also extracted and co-dissolved as impurities according to findings by Rodriguez-Jasso et al. (2011). This condition indicated by the increasing filtrate resulting from fucoidan separation, and it was cloudy and viscous.

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ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
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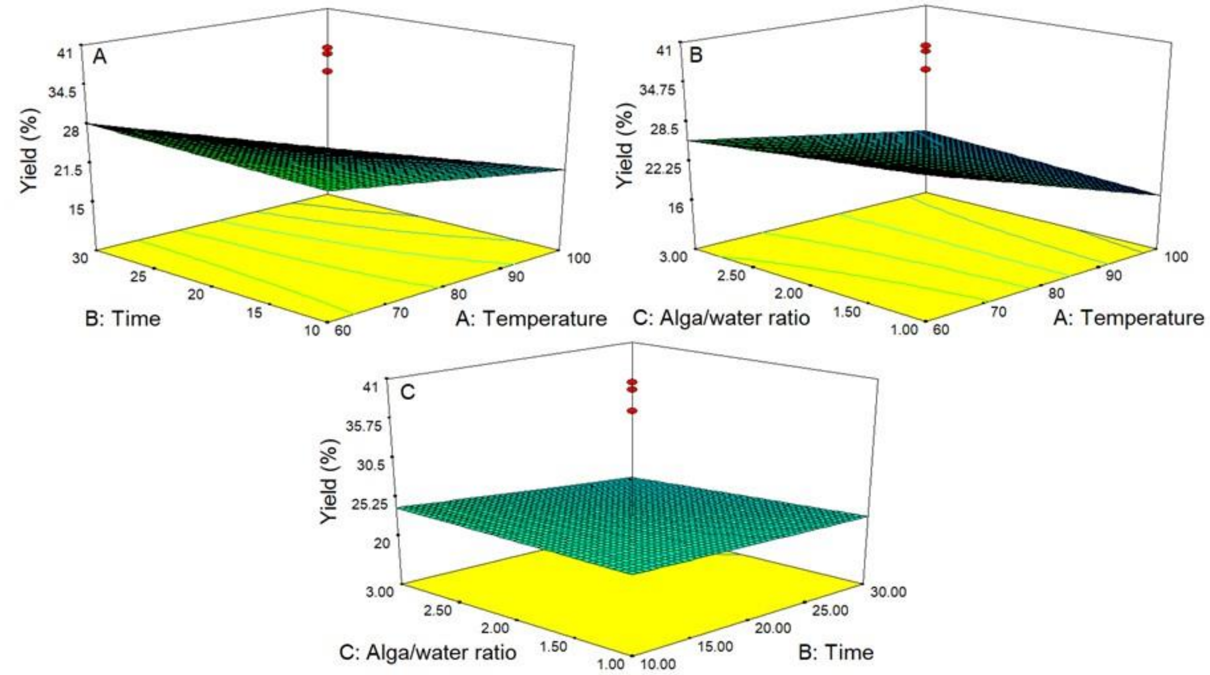

Figure 2. Response surface of alginate yields from *Sargassum cristaefolium* as effects of MAE temperature and time (A), temperature and alga/water ratio (B), alga/water ratio and time (C).

Model Accuracy

Two level factorial design was used to evaluated the effect of MAE parameters, i.e., temperature, time, and alga/water ratio on fucoidan; and alginate or fucoidan yield. The first-order model of the fucoidan and alginate yield response was obtained bellow:

$$Y_{1\ (fucoidan)} = 2.57+0.7x_1+0.26x_2+0.05x_3-0.14x_1x_2-0.12x_1x_3+0.018x_2x_3 \qquad (R^2=0.9576)$$
$$Y_{2\ (alginate)} = 22.71-4.79x_1-0.91x_2-0.31x_3-1.66x_1x_2+1.49x_1x_3-0.54x_2x_3 \qquad (R^2=0.9679)$$

The accuracy polynomial model of fucoidan and alginate yield was evaluated based on parameters model significance, correlation coefficient, and lack of fit. The analysis of variance showed that the model was significant at the confidence level (α) of 0.05. This indicateds that the condition of the sequential extraction process of fucoidan and alginate integrated with MAE significantly affected the yield of fucoidan and alginate.

The model test results showed that the linear model and its interactions were insignificant, whereas the curvature test significantly affected the yields at α=0.05 (Table 2). This showeds that the first-order model of sequential extraction of fucoidan and alginate was found quadratic in agreement with models found by Gazpersz (1992), Montgomery (2005). Rodriguez-Jasso et al. (2011) and Kadam et al. (2015) explained that if curvature hads a significant effect at α=0.05 so the first-order model was quadratic. The accuracy and acceptable model based on parameters, i.e., significance of $P<0.05$, $R^2 \geq 0.8$ and lack of fit>0.1 (Montgomery, 2017). The accuracy of polynomial model base on these parameters for fucoidan and alginate yield was fitted entire criteria are presented in Table 2. The center point is correct, the maximum response of fucoidan and alginate yield sequential extraction by MAE at 80 ^{o}C, 20 minutes, and alga/water ratio 2:20 (w/v) (Figure 3).

ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
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Table 2. Analysis of variance first-order regression model and model accuracy

Variety Source	**Fucoidan**	**Alginate**
Model	11.29*	15.07*
Linear		
A-Temperature	*56.08*	71.18
B-Time	*7.53*	2.59
C-Alga/water ratio	*0.28*	0.30
Interaction	*2.07*	8.58
AB	*1.71*	6.87
AC	*0.035*	0.90
BC	76.88*	223.77**
Curvature		
Model accuracy		
P-value	0.0363*	0.0242*
Lack of Fit	0.7230^{ns}	0.28^{ns}
R^2	0.9576	0.9679

Note: ** = High significant 0.001<P<0.01
* = significant 0.01<P<0.05
ns = not significant P>0.05

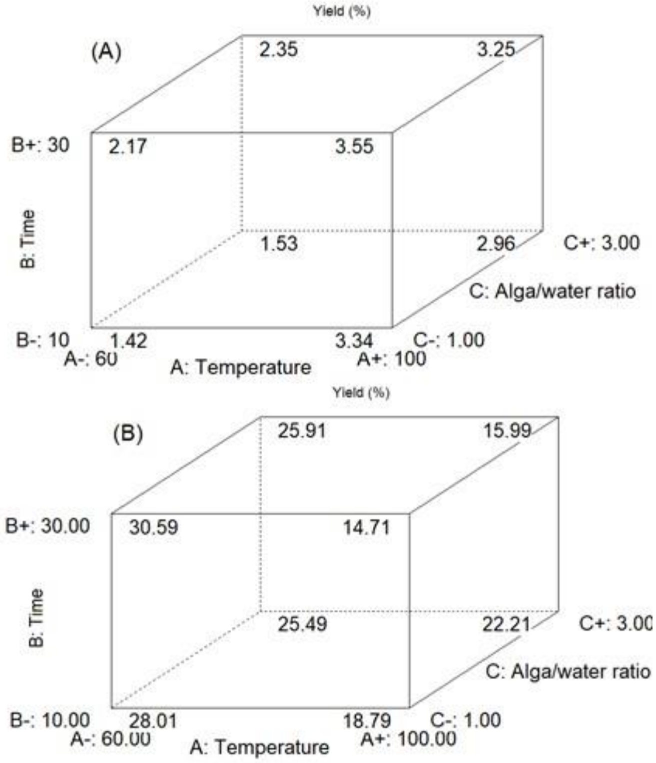

Figure 3. Response surface of fucoidan (A) and alginate (B) yield from *Sargassum cristaefolium* as effects of MAE temperature, time, and alga/water ratio mapped with cube model relationship of those three variables.

ISSN 2622-1985 (print) | ISSN 2622-4127 (online)
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CONCLUSION

MAE processing conditions of temperature, time, and alga/water ratio significantly affected the fucoidan and alginate yields. The highest fucoidan (4.5%) and alginate (40.6%) yield was obtained from a processing condition at 80 ^{o}C, for 20 minutes, and alga/water ratio of 2:20 (w/v). Analyzing regression model obtained that linear model was found insignificant whereas, curvature analysis was found significant as first-order quadratic model. Biorefinery sequential of integrated extraction of fucoidan and alginate using MAE improved fucoidan and alginate yield and has potential efficiency for commercialization. Further research is needed to find the optimum point.

ACKNOWLEDGMENTS

The authors would like to thank the Kemdikbudristek and LPDP for providing financial support for this research through the Research Grant Funds for the Lecturer Independent Grant 2021, contract number 182/E4.1/AK.04.RA/2021.

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