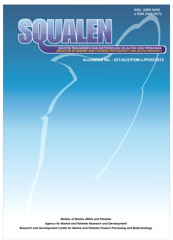

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Optimization of Processing Conditions of Alkali Treated Cottonii (ATC) from Sap-free *Eucheuma cottonii*

Fateha[1,2], Singgih Wibowo[2], Joko Santoso[1], Agusman[2] and Uju[1]

[1]Department of Aquatic Products Technology, Faculty of Fisheries and Marine Sciences
Bogor Agricultural University, Dramaga IPB Campus, Jalan Agatis, Bogor 16680 West Java, Indonesia
[2]Research and Development Center for Marine and Fisheries Product Processing and Biotechnology,
Jalan KS. Tubun, Petamburan VI, Slipi, Central Jakarta 10260, Indonesia

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Abstract

Alkali treated cottonii (ATC) is a derivative product of *Eucheuma cottonii* that is treated with alkali. This study used raw material of sap-free seaweed for ATC processing. Sap-free seaweed is a seaweed which thallus has been extracted with liquid known as sap. The use of sap-free seaweed as an ATC product is constrained by its low quality, so an effort to improve its quality is needed. Therefore the obejcetive of this Research was to get the optimal conditions of ATC processing. Experimental data were designed and analyzed using Response Surface Methodology-Central Composite Design (RSM-CCD) using Design Expert 10.0.7® program. The optimization of ATC processing involved three components that were considered influential, namely KOH concentration, temperature, and processing time. Recommendation for optimal conditions issued by RSM-CCD on ATC processing from sap-free seaweed were 6 % KOH concentration at 75 °C for 120 minutes (93.1% desirability). The results of the response analysis showed a yield of 39.47% and a gel strength of 595.32 g/cm^2. As a research control, the recommendation of RSM-CCD used in ATC processing from nonsap-free seaweed was obtained yield of 36.81% and gel strength of 574.44 g/cm^2. ATC from sap-free seaweed has higher yield and gel strength than that from nonsap-free seaweed. This might be due to the sap-free seaweed was obtained using the proper sap extraction process and ATC processing under optimal conditions, so that the quality of seaweed was better maintained.

Keywords: **alkali treated cottonii (ATC), *Eucheuma cottonii*, gel strength, respons surface methodology**

1. Introduction

Seaweed is one of the biological resources of the Indonesian water that can be found along the coast. FAO (2018) reports that the growth of Indonesian seaweed production has continued to increase from 2010 to 2016 with a number ranging from 4 million tons to 11 million tons. One of Indonesia's main seaweed potentials is *Eucheuma cottonii*. Indonesia has supplied *E. cottonii* globally more than 30 million tons in 2016. This makes Indonesia the largest producer of *E. cottonii* in the world.

Eucheuma cottonii with the new scientific name *Kappaphycus alvarezii* is red algae (Rhodophyceae) known as a producer of carrageenan. Carrageenan forms that are widely used in industry include refined carrageenan (RC), semi refined carrageenan (SRC), and alkali treatment cottonii (ATC). ATC has the simplest technology compared to RC and SRC and is easier to be developed. ATC is a semi-finished product of carrageenan processing.

On the other hand, in several regions of Indonesia, *E. cottonii* processing activities for food has been found by removing sap from thallus seaweed. The sap, the liquid extracted from seaweed thallus is usually discarded because it is considered useless (Purnomo et al., 2017). The sap-free seaweed is usually handled by bleaching and drying which is then used as local food such as candy seaweed, seaweed drink, and other related foods. There are indications that seaweed sap has potential use for fertilizer (plant growth hormone), food supplements (biosalt), and other active substances (Basmal, Sedayu, & Utomo, 2009; Layek et al., 2015; Sedayu, Basmal, & Utomo, 2013).

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*Corresponding author.
E-mail: fateh_1504@yahoo.com

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Utilization of sap-free seaweed and non-sap-free seaweed can be developed from the same production series. The development of sap-free seaweed as fertilizer or supplements is closely related to the quality of seaweed produced. Low quality of sap-free seaweed is the obstacle in developing the products (Purnomo et al., 2017; Sedayu et al., 2013). Efforts are needed to improve the quality of sap-free seaweed to improve the quality of seaweed, especially the yield and the gel strength.

Alkali treatment in ATC processing can increase gel strength, yield, and viscosity (Suryaningrum, Murdinah, & Erlina, 2003). Alkali treatment is a process of reorientation of molecules to form a stable structure on the transfer of sulphate, so that it can improve the mechanical properties of the carrageenan gel (Campo, Kawano, Silva, & Ivone, 2009; Moses, Anandhakumar, & Shanmugam, 2015). The use of KOH as an alkali solvent in ATC processing is more efficient than NaOH (Romenda, Pramesti, & Susanto, 2013). This is because kappa carrageenan is sensitive to K^+ ions in gel formation (Hakim, Wibowo, Arfini, & Peranginangin, 2011). ATC quality is also influenced by several factors including KOH concentration, temperature, and cooking time (Ninghidayati, Nurlaili, Gunardi, & Roesyadi, 2017; Nurmiah, Syarief, Sukarno, Peranginangin, & Nurtama, 2013).

To the best of our knowledge, the research on the optimization of ATC processing conditions using raw materials of sap-free *E. cottonii* has never been published. Thus, the results of this optimization are expected to provide useful information to improve the quality of ATC products which in turn will be beneficial for developing the utilization of sap and sap free seaweed in the same production series. This study is designed using Response Surface Methodology (RSM) to obtain the optimum ATC processing conditions from KOH concentration, temperature, and time to produce optimal yield and gel strength.

2. Materials and Methods

2.1. Materials

The raw material used was the 47-day-old *E. cottonii* obtained from seaweed cultured in Kalianda waters, South Lampung, Indonesia. The materials used include pro-analysis potassium hydroxide (KOH) and potassium chloride (KCl), as well as chemicals for analysis. The tools used were included analytical scales (Mettler Toledo, Toledo, Ohio, United Stated), waterbath (Memmert, Germany), Texture Analyzer (Stable Micro System, Goldamig, Surrey, UK), and oven (Memmert, Germany).

2.2. Preparation of Raw Materials

The preparation of *E. cottonii* seaweed as a raw material for processing ATC products was carried out at the seaweed harvesting location on the edge of the coast of South Lampung. The raw material was divided into two parts, a sap-free and nonsap-free seaweed. The preparation method for sap-free seaweed, which was washed with sea water repeatedly until it was clean, drained and put in a transparent clear plastic bag that has been designed with a little hole on corner of the plastic with a cover. This hole serves as a discharge of seaweed extracted that has been incubated for 24 hours as described in Purnomo et al. (2017). Furthermore, seaweed was removed from the plastic and dried in the sun on a *para-para*; made form a bamboo pad that is given a polyethlen webbed or woven bamboo with a cavity. In drying by using para-bases are placed using bamboo poles so that they do not directly touch the surface of the ground. Preparation of nonsap-free seaweed was done in the same way as the first but only through the washing, draining, and drying process without being extracted again. Dried seaweed was packaged for further quality characterization and will be processed into ATC products. ATC processing was conducted according to Basmal, Syarifudin, and Ma'ruf (2003). ATC processing methods include washing seaweed, heating (modification of processing conditions according to the results of RSM-CCD optimization), washing, cutting, and drying of the sun.

2.3. Characteristic of ATC Products

Product characterization was carried out on the initial raw materials and ATC products produced. Characterization parameters such as yield, moisture content (AOAC, 2005), Clean Anhydrous Weed (CAW) SNI 8170: 2015 (BSN, 2015); and gel strength (FMC Corp. 1977) were analyzed. The ATC formulation used a method that was developed by Basmal et al. (2003) with modification.

2.4. Experiment Design (Montgomery, 2001)

Response Surface Methodology was used to analyze the processing conditions of KOH concentration, temperature, and time which had an influence on ATC processing specifically to yield and gel strength parameters. The RSM design used in this study was Central Composite Design (CCD) using Design Expert 10.0.7® software.

Determination of independent variables was obtained based on the previous studies of researchers regarding general ATC processing under conditions using KOH concentrations in the range of 6 to 10 %, temperatures of 60 to 75 °C and time of 60 to 120 minutes (Basmal et al., 2003; Junaidi, Hutajulu, Sudibyo, Lestari, & Aviana. 2018; Nurmiah et al., 2013; Suryaningrum et al., 2003) presented in Table 1.

2.5. Statistical Analysis

Analysis of the results data were conducted using analysis of variance (ANOVA). The experimental design data was analyzed with DX 10.0.7® software. The initial estimation of the data was done by looking at the *lack of fit* section to determine the equation model suggested by the program. Furthermore, ANOVA was carried out with the selected model. The model that produced in significance to ANOVA and not significant on *lack of fit* was chosen to analyze variables. The model is significant if p-value is <0.05 (probability of error is less than 5%). Besides the model, the p-value "Prob> F" is also analyzed in *lack of fit*. The accuracy of the polynomial model equation is expressed by the coefficient of determination R^2 and the adjusted-R^2 value of the model. Both are evaluated to check the adequacy of the model. After obtaining the appropriate model, it will be displayed in a three-dimensional graph. The next optimization step was determined by criteria that include variables and each response that affects. At this stage, the objectives the goal limits, and the importance of the interests were determined. The final step was the DX 10.0.7® program will display several optimal solutions with different desires. The optimal solution that has desirable values close to 1 tends to be chosen as the best solution. The statistical analysis of the T-test was used to compare seaweed samples sap-free seaweed and nonsap-free seaweed.

3. Results and Discussion

3.1. Characteristics of Dried Raw Material Eucheuma cottonii

Characteristics of sap-free and nonsap-free *E. cottonii* include yield, moisture content, and CAW. Moisture content is one of the quality indicators considered in dried seaweed products because it is related to product damage. CAW reflects the quality of cleanliness and dryness of seaweed as a whole. A high CAW value will also be accompanied by a high yield value. The results of the quality parameter analysis are presented in Table 2.

The yield of dried sap-free *E. cottonii* is lower than the nonsap-free treatment. This phenomenon occurred due to sap-free treatment allowing the seaweed tissue to become lysed easily along with mineral substances. The sap-free treatment also influenced the appearance of the final product which was looked cleaner. The appearance of nonsap-free seaweed looks more dull with salt and sand. Different values of moisture content may be caused by the drying process which was not carried out simultaneously. The high value of CAW obtained from sap-free seaweed treatment about 52.29% which was probably caused by the treatment of sap extracting. Sap extracting would also release salt and other impurities. The value of moisture content and CAW of sap-free seaweed still meets the requirements according to SNI 2690: 2015 (BSN, 2015).

3.2. Optimization of ATC Processing Conditions with RSM

The results of the quality analysis of each response variable were included in the DX10.0.7® software design of the RSM-CCD and displayed in the response column as input data (Table 3). The ATC formulation used a method that was developed by Basmal et al. (2003) with modification.

3.2.1. Response Analysis of Yield and Gel Strength

Analysis of variance (ANOVA) is carried out to evaluate the quality of the appropriate model. The prediction model for the recommended yield of RSM was a linear model while the gel strength was a quadratic model. Based on ANOVA analysis, the polynomial model was statistically significant value (p <0.0001) indicating that linear and quadratic models

Table 1. Independent variables and the level of each variable

Independent variable	**Symbol**	**Coded Level**		
		Low (-1)	**Middle (0)**	**High (1)**
KOH concentration (%)	X_1	6	8	10
Temperature (^{o} C)	X_2	60	67.75	75
Time (minutes)	X_3	60	90	120

Table 2. Parameters of quality analysis of seaweed *E. cottonii*

Paramater	*E. cottonii*		SNI 2690:2015
	Sap-Free	Nonsap-free	
Yield (%)	6.88 ± 0.66	9.23 ± 0.22	-
Moisture content (%)	25.93 ± 0.46	21.58 ± 1.90	Max. 30.0
Clean Unhydrous Weed (%)	52.29 ± 1.79	34.36 ± 0.95	Min. 50.0

were able to explain the diversity of yield and gel strength well with the coefficient of determination 0.9245 and 0.9813, respectively. This showed that the concentration of KOH, temperature, and time had an effect on the yield and gel strength of ATC. The lack of fit values of the linear and quadratic models showed not significant with values of 0.1936 and 0.5646, respectively. The recapitulation of the analysis results for the two measured responses is presented in Table 4.

The yield is the percentage of the ratio between the final material divided by the initial material. Yield of dried seaweed is obtained from how much its original material (wet seaweed) is dried. Product yield is one of the important factors to see the importance of processing and processing efficiency. The approved ATC in this study was the ATC final weight obtained after the initial seaweed weight was divided and verified in percent. ATC yield response test results was obtained with a range between 37.20% and 50.20% with an average value of 44.31% ± 0.92. According to Ilias, Ismail, and Othman (2017) stated that the yield value is influenced by the extraction method and the type of seaweed used. Santoso, Sukri, and Uju (2007) reported that seaweed harvested after 45 days can be used as raw material to produce the best ATC. The yield of ATC produced in this study had a higher value than the standard requirements set by Indonesia of at least 28% (Dakay, 2008). The mathematical model for the ATC rendering response of yield is $Y = 85.60746 - 0.28096X_1 - 0.55586X_2 - 0.017028X_3$.

Figure 1 shows the interactions between the process variables KOH concentration, temperature, and time depicted through the response surface of the yield. A high yield was obtained when the KOH concentration, temperature, and cooking time were moving lower. The low concentration of KOH and temperature causes the cellulose tissue in seaweed to become soft for a long time, so it is not easily hydrolyzed (Griffin, 1970). This may also be caused by the low contact time between heat and seaweed, causing the carrageenan content did not come off and resulted in the increase of ATC yield. The yield became lower when the KOH concentration, time, and temperature increase. Rizal, Mappiratu, and Razak (2016) also reported a decrease in the yield value accompanied by an increase in KOH concentration (6-10%). KOH is a demineralization agent that also functions as a hydrolysis agent (Griffin, 1970). Foust (1959) stated that the higher temperature will widen the distance between molecules in a solid. The looseness of the molecule makes the tissue in the seaweed thallus easily degraded. The combination of components influences the yield response value which is marked with different colors. The lowest yield of recovery is 37.20% indicated by blue mark, while the highest yield of 50.20% is indicated by red mark.

Gel strength is an important physical property in gel formation in ATC as a product between carrageenan processing. The gel strength was affected by, among others, the KOH concentration and extraction time (Tunggal & Hendrawati, 2015). The mathematical model for gel strength response is $Y = -1315.35439 + 84.99125X_1 + 3.07267X_2 + 3.07267X_3) + (0.45217X_1X_2) - (0.12833X_1X_3) - (0.030022X_2X_3) - (7.06416X_1^2) - (0.13879X_2^2) + (6.09806E\ {-003}X_3^2)$.

Figure 2 shows the surface shape of the interactions between the variable components and gel strength response. The red color indicates the highest gel strength value and the blue color is the lowest gel strength value. The gel strength movement increases with the increasing of KOH concentration, temperature, and time. This is reinforced by Distantina, Wiratni, Fahrurrozi, and Rochmadi (2011) which reported that the increase of gel strength was influenced by an increase of extraction time. The highest value of gel strength occured at 8% KOH concentration, but decreased at 10% KOH concentration. This is likely due to the transformation of the sulfate group by the K^+ ion which is accelerated with an increase in temperature to form volatile potassium sulfate. The gel strength increased with the elimination of sulfate which forms 3,6-anhydrogalactose completely (Musthapa, Chandar, Abidin, Saghravani, & Harun 2011).

Table 3. Formulation of ATC optimization and results of variable analysis and response

Run	Variable			Response	
	Concentration of KOH	Temperature	Time	Yield	Gel strength
	(%)	(^oC)	(minute)	(%)	(g/cm^2)
	X_1	X_2	X_3	Y_1	Y_2
1	6	75	60	40.63	550.50
2	6	60	120	50.20	480.40
3	8	67.5	90	44.30	534.68
4	10	60	120	46.63	402.24
5	8	54.9	90	50.10	362.26
6	10	60	60	49.84	352.27
7	10	75	60	40.05	530.27
8	11.4	67.5	90	42.50	422.07
9	8	80.1	90	37.20	650.25
10	10	75	120	40.20	572.13
11	8	67.5	90	44.71	505.48
12	8	67.5	39.5	43.90	480.70
13	8	67.5	140.6	41.50	607.02
14	8	67.5	90	45.73	544.49
15	8	67.5	90	44.55	505.71
16	8	67.5	90	45.75	518.34
17	6	60	60	49.80	380.72
18	8	67.5	90	43.66	510.55
19	6	75	120	40.35	604.25
20	4.6	67.5	90	44.53	474.76

Tabel 4. Analysis model for response of yield and gel strength

Response	Model	Significant ($p<0.05$)	Lack of fit ($p>0.05$)	R^2	Adj-R^2	PredR^2
Yield	Linear	<0.0001	0.1936	0.9245	0.9104	0.8742
Gel strength	Quadratic	<0.0001	0.5646	0.9813	0.9644	0.9169

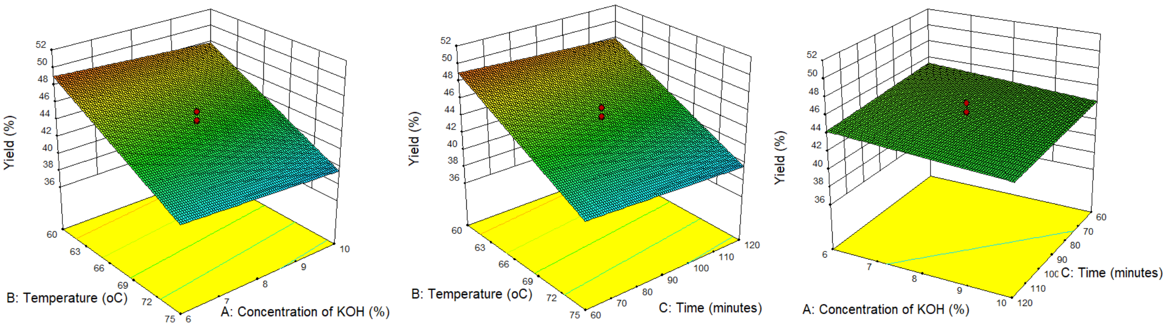

Figure 1. Three-dimensional curve of the interaction of process variables on the yield response

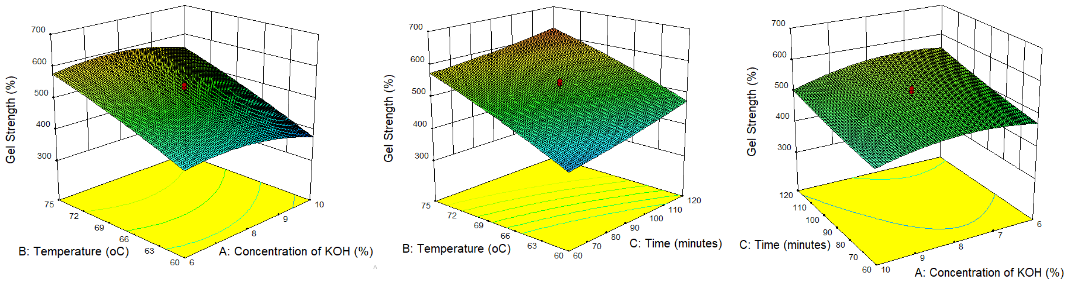

Figure 2. Three-dimensional curve of the interaction of process variables on the gel strength response

Table 5. Components and responses optimized at the formula optimization stage

Component/Response	**Goal**	**Low Value**	**High Value**	***Importance***
KOH concentration	Minimize	6	10	+++
Temperature	In range	60	75	+++
Time	In range	60	120	+++
Yield	In range	37	50.20	+++++
Gel strength	Maximum	352.27	630.30	+++++

3.2.2. Formula Optimization and Verifying Model Prediction Value

The optimization of the formula was done after the mathematical model was obtained from each response by setting the criteria depending on the importance of the response variable. Optimization of components aims to reduce the lowest possible operational costs and maximize the desired variable or response components. Determination of optimization of KOH concentration with Minimized Goal is related to the application of this component in ATC processing in the field. The lower the KOH concentration used is expected to be more efficient and safe for the environment without reducing the function and purpose of optimizing the quality of the resulting ATC product. The components of ATC temperature and processing time are related to the quality of ATC and the optimization is determined on the Goal In range. The yield response is related to the final result of the ATC product, so Goal In range optimization is determined. The gel strength is closely related to the functional properties that are needed by the industry, so Goal Maximize optimization is determined. Determination of the results of the processing optimization criteria which include variable components, responses, targets, and their importance are presented in Table 5.

The DX 10.0.7®program designed by RSM-CCD will recommend an optimal formula solution and the highest desirability was 0.931. The variable components chosen by the program were a 6% KOH concentration, temperature 75 °C and a processing time of 120 minutes. The final result of this formula was predicted to produce a yield of 40.19 % and a gel strength of 618.00 g/cm^2.

Verification is the proof stage for the prediction of the optimum solution response value from the DX 10.0.7® program designed by the RSM-CCD. The response value of the verification results obtained is compared with the predicted response value by the software. The results of ATC processing conditions verification were yield of 39.47% results and gel strength of 595.32 g/cm^2. Verification values and predictive values of the yield response and gel strength are presented in Table 6.

Analysis of the verification results from the recommended formula solution of the software was within the range set by the software. The formula solution predicted by the software can be said to be in accordance with the actual response test results.

As a comparison, ATC processing of nonsap-free seaweed had been processed using the recommended RSM-CCD formulation obtained. Nonsap-free seaweed used for this trial was a dried seaweed with normal drying treatment without sap-free treatment. ATC processing from nonsap-free seaweed produced yield values of 36.81±0.46 and gel strength of 574.44±6.73. Further analysis by T-test showed that the quality value of ATC produced was not significantly different ($p<0.05$). This might be due to the ATC processing conditions were optimal

Table 6. Value of predictive and verification respons of ATC processing formula

Response	Prediction	Verification	95%CI Low	95%CI High	95% PI Low	95% PI High
Yield (%)	40.19	39.47	38.96	41.22	37.52	42.86
Gel strength (g/cm^2)	618.00	595.32	589.42	635.49	527.87	663.13

for sap-free seaweed but not optimal for non-sap-free seaweed. However, it is possible that the yield and ATC gel strength value of ATC from nonsap-free seaweed can be higher if the process is conducted under optimal conditions. Based on ATC quality analysis, it can be said that the quality of ATC sap-free seaweed was still able to compete the carrageenan market.

4. Conclusion

Optimizing ATC using RSM-CCD produces interactions between the variables. Components such as KOH concentration, temperature, and time have an influence on the yield and gel strength of ATC products. The best processing conditions for sap-free seaweed based on RSM-CCD were 6% KOH concentration, temperature 75 °C and 120 minutes.

5. Suggestion

Research on the use of sap-free seaweed as an ATC product opens its own opportunities for *E. cottonii* seaweed, but this research is still limited to the laboratory scale. Suggestion for this research is the need for further research on a larger scale for ATC processing of seaweed free from the results of optimization of the RSM-CCD.

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