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Production of refined carrageenan from *Kappaphycus alvarezii* on pilot plant scale: optimization of water extraction using Response Surface Methodology

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Abstract

A refined carrageenan is a form of carrageenan, extracted from red algae and purified. Important factors affecting the commercial production of carrageenan after alkaline extraction are the ratio of seaweed to water, temperature, and extraction time. In this study, extraction of refined carrageenan from *Kappaphycus alvarezii* was conducted on pilot plan scale. Extraction conditions were varied, affecting the final characteristics of the carrageenan product. The optimum conditions investigated for the extraction process included the ratio of seaweed to water, temperature, and extraction time determined using Response Surface Methodology (RSM). Box-Behnken was used to investigate the interaction effects of three independent variables, namely seaweed to water ratio, extraction temperature and extraction time. The results showed that based on the RSM approach, ratio of seaweed to water, temperature and extraction time had a significant influence on the carrageenan. Optimum extraction conditions obtained were seaweed to water ratio of 1:25.22, extraction temperature of 85.80^{o}C and extraction time of 4 h. Under these optimal conditions, the yield obtained was 31.74 % and gel strength was 1833.37 g.cm^{-2}.

Introduction

Carrageenan, a hydrocolloid, is an important material which has found applications in human food and the pet-food industry. It is used as a stabilizer, thickener, gelling agent and emulsifier (Van de Velde *et al.*, 2002; Campo *et al.*, 2009; Pereira *et al.*, 2009, Robledo and Freile-Pelegrín 2011). Demand for gelling additives for food and non-food applications is steadily increasing and alternative sources for carrageenan production is highly in demand (McHugh, 2003).

Carrageenan is extracted from the red algae (Rhodophyta) which have undergone a process of purification. The extraction process is done in two stages, the first extraction with alkaline is utilized commercially to change the precursor residues that lead to increased gel strength in the final product; this removes some of the sulphate groups from the molecules and increases the formation of 3,6-anhydro-D-galactose (Van de Velde *et al.*, 2002; McHugh, 2003; Freile-Pelegrín and Robledo, 2008). This is then followed by extraction with water after neutralization. Extraction with water dissolves or withdraws the thallus polysaccharides from seaweed in the form of a slurry, facilitates the process of filtration and improves the yield of the carrageenan (Montolulu *et al.*, 2008; Distantina *et al.*, 2009).

Important factors related to water extraction affecting the commercial production process are the seaweed to water ratio, temperature and extraction times. The use of disproportionate amounts of water in the extraction process is problematic in the process of polysaccharide withdrawal and quality of the resulting product (Rees, 1969; Basmal *et al.*, 2005). Similarly, the temperature and time process of all fractions of carrageenan extracted from seaweed using water affects the speed and the power of dissolution.

Various reports describe the processing and functional properties of carrageenan extraction, including Tuvikene *et al.*, (2006). The results

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demonstrate that alkaline treatments gave higher extraction yields compared to aqueous treatment. Hayashi *et al.*, (2007), studied the effects of different protocols of mariculture on the yield and quality of carrageenan produced by *K.alvarezii* cultivated in Brazil. Refilda *et al.*, (2009), studied some parameters influencingextraction process of carrageenan from red algae *(Eucheuma cottonii)*; Montolulu *et al.*, (2008), found that extraction with water at a temperature of 50-70°C makes it possible to produce a high yield of carrageenan with high molecular weight. However, these studies are limited to laboratory scale. Additionally, the quantity and quality of the resulting carrageenan are different, limiting their direct application.

There are many different methods used to extract carrageenans from seaweed and processing conditions are usually considered trade secrets by the manufacturers as these affect the final carrageenan properties. Therefore research and development of carrageenan are subsequently directed more towards trials at the pilot plant scale. Production of carrageenan on a pilot plant scale is necessary before being applied on the industrial scale. The geometric difference between process conditions of laboratory scale and industrial scale production allows for the differences in resulting carrageenan. Hence, the important factors affecting the carrageenan production process are seaweed to water ratio, temperature and time of extraction. These are the process conditions that need to be controlled, in order to obtain optimum conditions for carrageenan production at the pilot plant scale.

Optimization of the extraction condition of carrageenan is done using Response Surface Methodology (RSM) with the Box-Behnken design. RSM is a collection of statistical and mathematical methods that are useful for modeling and analyzing engineering problems (Montgomery, 2001; Bas and Boyaci 2007; Raissi and Farsani 2009). The main idea of this method is to determine the effect of independent variables on the response, to get a model of the relationship between independent variables and the response and to get process conditions that produce the best response (Giovanni 1983).

Data is quite limited on the properties of pilot plant scale-extracted carrageenan using water after alkaline extraction. Hence the present study aims to use RSM with Box-Behnken design as a tool for optimizing the extraction conditions on pilot plant scale to obtain maximum yield and gel strength. This research can be used as a reference for the development industry in the form of Small Medium Business.

Materials and methods

Sample preparation

Dried *K. alvarezii* seaweed was harvested in Bali, Indonesia. In the laboratory, all of the seaweeds were washed with tap water to remove salt, sand and attached epiphytes. The process was repeated twice. The 'clean seaweed' sample was processed further.

Design of carrageenan extraction on pilot plant scale

Extraction tank (1000 L) used on pilot plant scale was made of a stainless steel vessel and cylindrical double jacket with a water heater between the cylinder. The vessel was designed as 1000 L in volume (diameter = 2000 mm, height = 1000 mm), an agitator located in the center of the vessel was driven by an electromotor. A temperature sensor was placed inside the vessel to control the temperature. The extraction tank was heated by a burner (diesel oil) placed outside of the extraction tank.

Carrageenan extraction

The 'clean seaweed' samples (20 kg) were extracted in 6% hot potassium hydroxide solution for 2 h at 85ºC. After the alkaline extraction, the algae were neutralized by washing with running tap water until they had a pH of about 8-9 (Hoffman *et al*., 1995). The material was then transferred to an extraction tank set with extraction conditions at different seaweed to water ratio, temperature and time (Table 1). The resultant paste was filtered in a rotary vacuum filter, using diatomaceous earth (celite) as a filter aid. During filtration, the filtrate was pumped into the precipitation tank and simultaneously a solution of 1% KCl was added to precipitate the carrageenan. The carrageenan was separated on a filter press and pressed in a hydraulic press to remove as much water as possible. Finally, the carrageenan fibers were recovered and dried at 50^{o}C for 18 h, weighed and milled into a fine powder to pass an 80 mesh. A flow chart of the carrageenan extraction process is shown in Figure 1.

Determination of carrageenan yield

The carrageenan yield (%) was determined according to the formula:

Yield (%)= Wc/Wm.100

where Wc is the extracted carrageenan weight (g) and Wm is the dry seaweed weight (g) used for extraction.

Determination of gel strength of carrageenan

Gel strength of carrageenan was determined using a Texture Analyzer (TAXT Plus, Stable Micro Systems Ltd., Surrey, England). Carrageenan solution was prepared by dissolving 3 g of carrageenan powder and 0.6 g KCl to 197 ml of distilled water with continuous magnetic stirring at 80°C for 15 min. For the gel strength analysis, this solution was placed in plastic tubes (40 mm diameter, 50 mm height, flat bottom) which were kept under refrigeration at 10°C for 16 hours before analysis. All analyses were carried out in duplicates.

Experimental design and statistical analysis

Before optimization of the process using RSM, the first set of tests were performed to select relevant independent variables including seaweed to water ratio (A), temperature (B), and time (C) as shown in Table 1. The range of seaweed to water ratio 1:20-1:35 (w:v), temperature (85-95°C), and time (2-4 h) were obtained based on the results of preliminary experiments. The model proposed for the response Y is given below:

$$Y=x_0+x_1A+x_2B+x_3C+x_4A2+x_5B2+x_6C2+x_7AB+x_8AC+x_9BC$$

Where x_0 was the offset term, x_1, x_2 and x_3 were related to the linear effect terms, x_4, x_5 and x_6 were connected to the quadratic effects and x_7, x_8 and x_9 were associated with the interaction effects.

The production of carrageenan includes several important processes, such as extraction with water (hot-water extraction) after the alkaline extraction. The ratio of seaweed to water (w:v, A), temperature (ºC, B) and time (h, C) were independent variables studied to optimize yield and gel strength of K. alvarezii extracts. The extraction yield (%) and gel strength (g.cm^{-2}) are the most important properties for carrageenan production. These two responses were selected together as the dependent variables for the combination of independent variables. Experimental runs were randomized to minimize the effects of unexpected variability in the observed responses. A Box-Behnken design consisting seventeen experimental runs was employed including five replicates at the center point (Montgomery, 2001). Seventeen extraction condition combinations were generated using Design Expert software version 7.0® (DX 7.0®) as shown in Table 1.

The carrageenan processing included important processes, extraction with water (hot-water extraction) after the alkaline extraction. The ratio of seaweed to water (w:v, A), temperature (°C, B) and time (h, C) were independent variables studied to optimize yield and gel strength of K. alvarezii extracts. The extraction yield (%) and gel strength (g.cm-2) are the most important characteristic for carrageenan production. These two responses were selected together as the dependent variables for the combination of the independent variables. Experimental runs were randomized to minimize the effects of unexpected variability in the observed responses. A Box-Behnken design consisting seventeen experimental runs was employed including five replicates at the center point (Montgomery 2001). Seventeen extraction condition combinations were generated by the software program of Design-Expert version 7.0® (DX 7.0®) as shown in Table 1.

Verification of model

The adequacy of the polynomial model was expressed by the multiple coefficients of determination, R2. The significance of each coefficient was determined by using the F values and P values. Optimization of extraction conditions including the ratio of seaweed to water, temperature, and extraction duration for maximizing yield and gel strength were calculated by using the predictive equation from RSM. The optimum condition was verified by conducting experiments under these conditions. The response was monitored and the results were compared against model predictions.

Results and discussion

The experimental results are also shown in Table 1. The result indicated that yield of carrageenan was in the range of 25.38-30.88% and the gel strength values were 1136.1-2062.6 gr.cm^{-2}. The yield value obtained were within ranges reported by Paula and Pereira (2003) for *K. alvarezii* brown strain from Sao Paulo, Brazil (25–35%); Al-Alawi *et al.* (2006) for Hypnea bryoide in Oman (30.05-33.16%); Tuvikene *et al*. (2006) for *Furcellaria lumbricalis* and *Coccotylus truncatus* (27-31%); Webber *et al.* (2012) for *K. alvarezii* (31.17%). Meanwhile, the gel strength obtained in the present study was considerably higher than that reported by Tuvikene *et al.* (2006) (350 g.cm^{-2}); (Hayashi *et al*. 2007) (168.86 g.cm^{-2}) and Basmal *et al.* (2009) (1279 g.cm^{-2}).

The ANOVA of the quadratic regression model indicated the model to be significant. There was only a 0.05% chance that a "Model F-Value" this large could occur due to noise. Model P value (Prob>F) was very low (<0.0005). The lack of fit is an indication of the failure of a model representing the experimental data at which points were not included in the regression.

Table 1. Results of experiment

	A	B	C	Response	
Runs	Seaweed to water ratio (w:v)	Temperature (ºC)	Time (h)	Yield (%)	Gel strength (g.cm^{-2})
1	1:20.0	85	3	27.96	1291.45
2	1:35.0	85	3	26.81	1136.10
3	1:20.0	95	3	25.68	1261.95
4	1:35.0	95	3	25.49	1220.85
5	1:20.0	90	2	25.69	1326.40
6	1:35.0	90	2	26.49	1497.25
7	1:20.0	90	4	25.99	2062.60
8	1:35.0	90	4	26.77	1281.95
9	1:27.5	85	2	27.29	1502.40
10	1:27.5	95	2	28.22	1259.30
11	1:27.5	85	4	30.36	1785.75
12	1:27.5	95	4	25.38	1790.35
13	1:27.5	90	3	29.49	1490.60
14	1:27.5	90	3	30.86	1434.85
15	1:27.5	90	3	30.88	1405.75
16	1:27.5	90	3	30.51	1357.40
17	1:27.5	90	3	28.42	1553.60

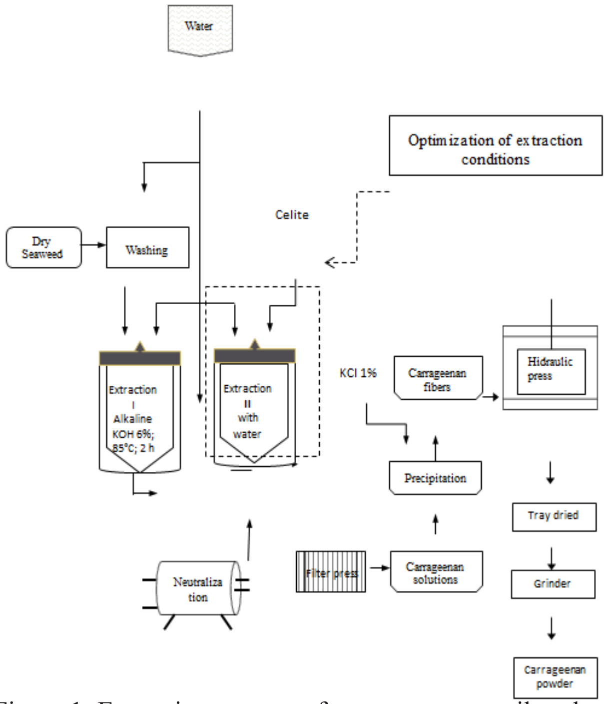

Figure 1. Extraction process of carrageenan on pilot plant scale

Variations in the models cannot be accounted for random error (Montgomery 2001). If there is a significant lack of fit indicated by a low probability value, the response predictor is discarded. The lacks of fits in the model were not significant (P>0.05), meaning that these models were sufficiently accurate for predicting the relevant responses (Koocheki *et al.*, 2009).

The coefficient of determination R^2 is the proportion of variation in the response attributed to the model rather than to random error and is suggested for a well fitted model (Sita Kumari *et al.*, 2008; Koocheki *et al.*, 2009). The value of determination coefficient R^2 = 0.8362 (yield) and 0.8370 (gel strength) implied that the sample variations of 83.62% for the yield and 83.70% for the gel strength of carrageenan were attributable to the independent variables, namely the ratio of seaweed to water, extraction temperature and extraction time. Thus, analysis of variance showed that the predicted second order models were statistically suitable.

Effect of extraction conditions on yield of carrageenan

The parity plot analysis showed a satisfactory correlation between the experimental and predicted values of carrageenan yield. Points clustered around the diagonal line indicated a good fit of the model, since the deviation between the experimental and predicted values was minimal (Sita Kumari *et al.*, 2008). Results also show the combinations of components that affect yield values. The blue color indicates the lowest yield, whereas the red color indicates the highest yield. A line consisting of the points on the contour plot is a combination of three components at different levels that generates a response value of the same yield. Response surface for the effect of extraction temperature and time on the yield of carrageenan is presented in Figure 2.

The analysis of the results showed that the extraction temperature was the only highly significant factor affecting the yield of carrageenan. Carrageenan yield showed strong positive correlation with extraction temperatures. It was found that with increasing extraction temperature, the yield increased. It has been reported that increase in polysaccharide yield is due to the strong effect of extraction time–temperature on the mass transfer rate of the water-soluble polysaccharides in the cell wall (Wu *et al.*, 2007). Further increase of extraction temperature leads to decrease in the yield of carrageenan. The higher extraction temperatures may have resulted in degradation of the polysaccharide (Webber *et al.*, 2012). The low yield of carrageenan products has been reported to be a result of heating at higher temperatures. This gave the solution a high concentration and made it difficult to separate between filtrate and residue (Refilda *et al.*, 2009).

Effect of extraction conditions on gel strength of carrageenan

The parity plot analysis also showed a satisfactory correlation between the experimental and predicted values of carrageenan yield wherein the points clustered around the diagonal line

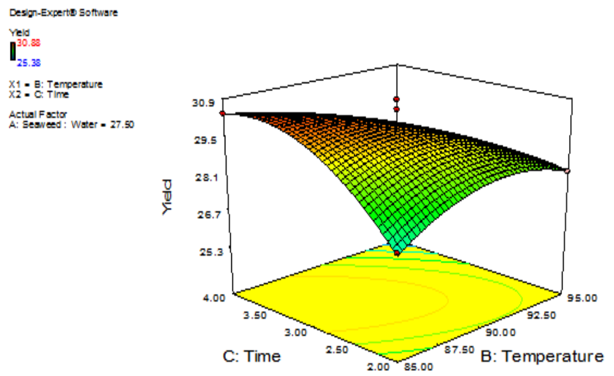

Figure 2. 3D graphic surface optimization of carrageenan yield versus extraction temperature and extraction time

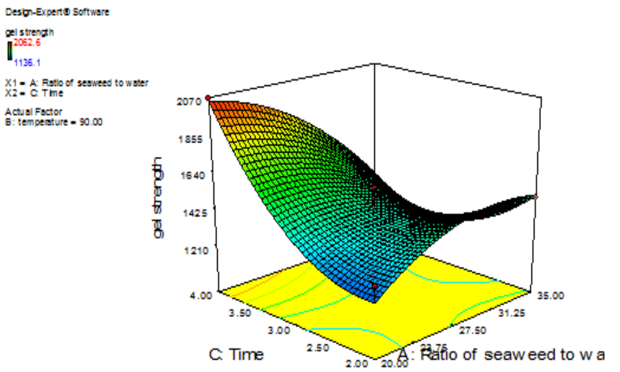

Figure 3. 3D graphic surface optimization of carrageenan gel strength versus ratio of seaweed to water and extraction time

indicated the good fit the model, since the deviation between the experimental and predicted values was minimal (Kumari *et al*., 2008). Results show the combination of components that affect gel strength values. The blue color indicates lowest gel strength, whereas the red color indicates highest gel strength. The line consisting of the points on the contour plot is a combination of three components at different proportions that generates same gel strength. Response surface for the effect of the ratio of seaweed to water and time on gel strength of carrageenan is presented in Figure 3.

The effect of the ratio of seaweed to water and extraction time indicated increased gel strength up to certain values. However, a further increase in the ratio of seaweed to water and extraction time showed a decrease in gel strength. This was suspected due to higher extraction temperature causing carrageenan degradation, thereby producing carrageenan fragments and lowering gelling ability. Similar results have been reported for Gracilaria cliftonii and other samples (Cho *et al*., 2005; Kumar and Fotedar, 2009). The greater the volume of water, the better the swelling of seaweed, thus allowing the agar to be extracted easily. Dried marine algae can swell

Table 2. Comparison of predicted and actual response value for optimum process conditions with Box-Behnken design

Response	Formula			
	Predicted	Verified	95%PI Low	95%PI High
Yield(%)	30.00	31.74	27.71	32.29
Gel strength (g.cm^{-2})	1887.92	1833.37	1626.09	2149.76

to about 20 times of their dry matter volume when exposed to water (Jiménez-Escrig and Sánchez-Muniz 2000).

Optimization of extraction conditions under pilot plant scale

The optimization of multiple responses was developed using desirability functions with the responses to be maximized (Wangtueai and Noomhorm, 2008). The final result of the optimization phase in the form of improved extraction conditions is determined based on predetermined targets. The DX 7.0® program builds solutions for a different desired value. The higher the desirability value (approaching 1), the closer the the optimal conditions for carrageenan extraction.

For the optimization process, the recommended extraction conditions are seaweed to water ratio of 1:25:22, the temperature of 85.80°C and extraction time of 4 h. Under the optimal conditions obtained, the yield of carrageenan was 30.00% and gel strength was 1887.92 g.cm^{-2}.

Verification of model

In the verification stage, the actual response values obtained were compared against predicted values. Table 2 shows the suitability of the model equation for predicting optimum response values tested using the selected optimal conditions. Results of the verified solutions were not exactly the same, but all the values were within the range of 95% low prediction interval and 95% high prediction interval. Thus the empirical models developed were reasonably accurate. The 95% prediction interval is the range in which we can expect any individual value to fall into 95% of the time (Noordin *et al*., 2004).

Conclusion

Extraction with water after alkaline extraction is a treatment that affects the carrageenan quality.

Different extraction conditions affect the yield and gel strength of carrageenan. Response Surface Methodology (RSM) can effectively be used to determine the optimum extraction conditions for carrageenan production from K. alvarezii on a pilot plant scale. Extraction with water after alkaline extraction was optimized to maximize yield and gel strength content. Second-order polynomial models were obtained for predicting extraction yield and gel strength. Using the contour plots, the optimum set of operating variables are graphically shown in order to obtain the desired properties of carrageenan suitable for further development and applied for the construction industry in the form of Small and Medium Businesses. Optimum extraction conditions for maximizing extraction yield and gel strength were: the ratio of seaweed to water (1:25.22), temperature (85.80°C) and extraction time (4 h). Under these conditions, the carrageenan product obtained had a yield of 31.74 % and gel strength of 1833.37 g.cm^{-2}.

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