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Optimization and Characterization of Sea Grape Carrageenan (*Caulerpa sp.*) Using Ultrasonic-Assisted Extraction and Microwave-Assisted Extraction Methods

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Abstract

This study aimed to optimize carrageenan extraction from sea grape (*Caulerpa sp.*) and to characterize physicochemical properties in comparison with commercial carrageenan using Ultrasonic-Assisted Extraction (UAE) and Microwave-Assisted Extraction (MAE) methods. Extraction optimization was conducted using Response Surface Methodology (RSM), a Box-Benhken Design was employed to evaluate the effects of sample amount, solvent concentration, and time on yield, viscosity, and sulfate content. The extracted carrageenan was then analyzed for yield, moisture content, ash content, sulfate content, viscosity, water holding capacity (WHC), and solubility. The results showed that the optimum extraction conditions for the MAE method were obtained at a sample size of 40 g, a solvent concentration of 10%, and an extraction time of 40 minutes, resulting in a predicted carrageenan yield of 15.23%, 3.66 cP viscosity, and 27.61% sulfate content. Experimental validation under the predicted optimum conditions yielded an extraction yield of 14.53%, viscosity of 3.68 cP, and sulfate content of 24.71%. Meanwhile, the UAE method achieved optimum conditions at a sample size of 20 g, a solvent concentration of 9.11%, and an extraction time of 40 minutes, with a predicted yield of 11.01%, 3.54 cP viscosity, and 18.55% sulfate content. Experimental validation under the predicted optimum conditions yielded an extraction yield of 13.12%, viscosity of 3.60 cP, and sulfate content of 17.30 %. Under these conditions, the extracted carrageenan showed a water content of 9.34–9.94%, ash content of 53.01–54.72%, sulfate content of 17.30–24.21%, viscosity of 3.60–3.68 cP, water holding capacity (WHC) of 102.93–110.61 g/g, and solubility ranging from 71.80% to 95.23% depending on temperature. Compared to commercial carrageenan, the extracted product exhibits a much lower viscosity but a comparable sulfate content that is still within the standard range.

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Introduction

Indonesia is the largest archipelagic country in the world with highly abundant marine biological resources, one of which is seaweed distributed across almost all coastal regions of Indonesia (Baransano et al., 2011). One type of seaweed that is widely utilized is red seaweed (*Rhodophyceae*), which serves as a major source of hydrocolloids, particularly carrageenan (Baransano et al., 2011). Carrageenan plays an important role in various industries, especially as a thickening agent, emulsifier, stabilizer, and gelling agent in the food industry, as well as a raw material in the production of cosmetics, paints, textiles, and pharmaceutical products in non-food industries (Kurniawan et al., 2022). The increasing industrial demand for carrageenan has driven global market growth, which reached a value of US$864.3 million in 2019 and is projected to reach US$1.12 billion by 2024, with an annual growth rate of 5.45% (Andhiarto & Pusparini, 2025). The demand for carrageenan in Indonesia has also increased annually, with average growth rates of 3.82% for exports, 23.38% for imports, and 4.46% for consumption (Devi et al., 2020). This condition positions Indonesia as one of the main suppliers of global seaweed production, contributing approximately 38–39% of total global output. National production is estimated to exceed 12 million tons in 2024, increasing from 10.3 million tons in 2010 and 11.7 million tons in 2016 (Basyuni et al., 2024).

Carrageenan has been proven to have

extensive applications with continuously increasing production; however, its primary source still relies on red algae (Rhodophyceae), particularly from the genera *Kappaphycus* and *Eucheuma*. These seaweeds are known to contain relatively high carrageenan content, approximately 30–60% of dry weight (Hasan et al., 2019). The increasing market demand and dependence on a single type of raw material pose risks to raw material availability and production sustainability, particularly in cases of cultivation disturbances or price fluctuations. Therefore, efforts to diversify carrageenan sources are necessary by exploring other seaweed species with potential carrageenan content.

One alternative source worth investigating is sea grapes (*Caulerpa* sp.), a green macroalga known to contain sulfated polysaccharides with diverse biological activities. Studies have reported that *Caulerpa racemosa* contains sulfated galactans and ulvan-type polysaccharides with structural and functional properties that may overlap with those of commercially relevant hydrocolloids, including carrageenan (Premarathna et al., 2024). Although the predominant polysaccharides in *Caulerpa* sp. are xylan and ulvan rather than carrageenan (Nurjanah et al., 2018), the presence of sulfate groups and galactan-type residues in certain fractions suggests that some components may exhibit physicochemical characteristics comparable to those of sulfated galactans from red algae. In this regard, sulfated galactans have been structurally confirmed in several species of the genus *Caulerpa*, including a sulfated (1→4) galactan isolated from *C. racemosa* (Shilpa et al., 2025) and sulfated pyruvated galactans from *C. cupressoides* (Costa et al., 2020), demonstrating that galactan-type sulfated polysaccharides are indeed present within this genus, though their structural identity differs from that of red algal carrageenan. However, direct structural confirmation of carrageenan identity in *Caulerpa* sp. has not been established, and the extent to which its sulfated polysaccharide fractions share functional properties with carrageenan remains to be evaluated.

The commonly used methods for carrageenan extraction are conventional techniques, such as hot alkaline extraction and precipitation. These methods have several limitations, including high water consumption during the cleaning process, high energy requirements, relatively long processing times, the generation of large volumes of liquid waste, and the need for substantial amounts of solvents and raw materials, making them less efficient in terms of energy and cost (Kumalaningrum et al., 2025). To overcome these limitations, various innovative extraction methods that are more environmentally friendly and efficient have been developed, including Ultrasound-Assisted Extraction (UAE) and Microwave-Assisted Extraction (MAE).

The Ultrasound-Assisted Extraction (UAE) method utilizes ultrasonic waves that propagate through the solvent and generate cavitation effects, thereby increasing the contact surface between the solvent and the cell walls of the raw material, facilitating cell disruption and enhancing the diffusion of target compounds into the solvent (Firdaus et al., 2021). Numerous studies have demonstrated the advantages of UAE over conventional methods, including reduced solvent usage, shorter extraction time, faster chemical reactions, higher energy efficiency, and improved characteristics of the resulting extracts (Wang et al., 2020). Meanwhile, the use of microwave radiation in this method enables rapid and efficient heating of the solvent (Puspitaningtyas et al., 2021). Microwave-assisted extraction offers the advantage of more uniform heating, as heat is generated internally within the material rather than transferred from the external environment. The microwave radiation induces a temperature increase in both the material and the solvent, leading to cell wall disruption and the release of intracellular compounds into the solvent, thereby increasing extraction yield (Yulianti et al., 2014).

To date, no study has specifically evaluated the potential of *Caulerpa* sp. as a raw material for the production of carrageenan-like sulfated polysaccharides using Ultrasound-Assisted Extraction (UAE) nor Microwave-Assisted Extraction (MAE) combined with Response Surface Methodology (RSM) optimization. While prior research has demonstrated the presence and bioactivity of sulfated polysaccharides in *Caulerpa* sp (Premarathna et al., 2024), systematic extraction optimization and quality benchmarking against carrageenan standards, particularly under the SNI 8391-1:2017 framework, have not been reported. This research therefore aims to evaluate the physicochemical properties of sulfated polysaccharide extract from *Caulerpa* sp. obtained via RSM optimization, and to assess whether these properties are comparable to those specified for carrageenan under applicable quality standards, with the understanding that structural confirmation would be required to establish polysaccharide identity.

Materials and Methods

Materials

The materials used in this study include Sea Grapes (*Caulerpa sp*), Aquades, KOH, 5% HCl, 0.25M $BaCl_2$, 96% ethanol, filter paper (Whatman 42), and commercial carrageenan. Meanwhile, the tools needed are blender, baking pan, baking paper, oven, analytical balance, funnel, porcelain cup, furnace, desiccator, Erlenmeyer, ultrasonic bath, dehydrator, measuring cup, beaker, brookfield viscometer, hot plate, water analyzer, sentrifuge, funnel, thermometer, condenser, hose, glass adapter/connection, stand and clamp, spatula, and aluminum foil.

Methods

Sample Preparation

Sea grapes (*Caulerpa sp.)* obtained from Kalianda, South Lampung, were first cleaned by washing them with clean water to remove dirt. The washing process was carried out in two stages, the entire plant was washed in the first stage, and then each stem was washed in the second stage. Afterward, the samples were ground using a blender at maximum speed for 4 minutes. The resulting mixture was then placed on a baking sheet lined with baking paper and dried using a dehydrator at 65°C for 8 hours. The dried samples were then ground using a grinder.

Carrageenan Extraction Using MAE and UAE methods

Carrageenan extraction from *Caulerpa sp* begins with the sample powder being soaked in 400 ml of KOH solution. For the MAE method, the mixture is then put into a microwave with medium power (375 W) (Arto, 2021). On the other hand, UAE method will require an ultrasonic cleaner with the highest frequency where the sample that has been soaked in KOH in an Erlenmeyer will be put into the tool and extracted using a temperature of 70°C. Variations in the number of samples, KOH solvent concentration, and time are carried out according to the Box-Behnken Design (BBD). After the extraction is complete, the next stage is heating on a hot plate using a temperature of 80-90°C while stirring for 30 minutes. Filtration was then performed to obtain the filtrate, which was then added with 5% HCl and ethanol 96% as a precipitation step to obtain a precipitate. The filtrate was then filtered to obtain a precipitate. The precipitate was dried in a dehydrator at 50°C for 17 hours (Kumalaningrum et al., 2025).

Yield

The yield analysis in this study was conducted by comparing the weight of the carrageenan obtained with the weight of the dried seaweed used. The results were expressed as a percentage (Firdaus et al., 2021). The extraction yield was calculated according to the following equation:

$$\text{Yield (\%)} = \frac{Dry\ carrageenan\ weight\ (g)}{Dry\ seaweed\ weight\ (g)} \times 100\% \quad (1)$$

Moisture Content

Moisture content of carrageenan was determined using a moisture analyzer. A 2 gram of sample was weighed and placed in a heat chamber of 105°C for 3-4 minutes (Karaman et al., 2014). Moisture content was calculated using the following equation:

$$\text{Moisture Content (\%)} = \frac{W1-W2}{W1} \times 100\% \quad (2)$$

while W_1 = initial sample weight before drying (g); W_2 = sample weight after drying (g)

Ash Content

Ash content testing was carried out gravimetrically by burning 2 g of sample in a furnace at 550–600 °C for 6 hours, then cooling in a desiccator and weighing to constant weight. The water content in percent was calculated by dividing the weight of the ash by the weight of the sample used(Liu, 2019). Ash content was calculated using the following equation:

$$\text{Ash Content (\%)} = \frac{\mathrm{B-A}}{C} \times 100\% \quad (3)$$

while A = weight of the empty porcelain crucible (g); B = weight of the porcelain crucible and ash residue (g); C = weight of the sample (g).

Sulfate Content

The sulfate content used was determined according to the SNI 8391-1:2017 method. A 0.5 g carrageenan sample was added to 10 mL of 0.1 N HCl for 15 minutes at boiling temperature. Next, 10 mL of 0.25 M BaCl2 solution was added in a water bath for 5 minutes. The solution was cooled for 5 hours, the precipitate formed was filtered through ashless filter paper and washed with boiling water until chloride-free. The precipitate was charred before being fired in a furnace at 550°C for 6 hours. The weight of the white ash represents the weight of the barium sulfate (BaSO4) (Badan Standarisasi Nasional, 2017). The weight of the white $BaSO_4$ ash was used to calculate sulfate content according to the following equation:

$$\text{Sulfate Content (\%)} = \frac{\mathrm{P}\times 0.4116}{Sample\ weight\ (g)} \times 100\% \quad (4)$$

while P = weight of $BaSO_4$ precipitate (g); 0.4116 = conversion factor derived from the ratio of the molecular weight of SO_4^{2-} to that of $BaSO_4$.

Viscosity

Viscosity was determined using a Brookfield viscometer. A 1.5% carrageenan solution was heated on a hot plate while stirring until the temperature reached 80°C. The viscometer was then turned on and measured when the temperature reached 75°C. The reading was taken after spindle No. 1 had rotated for one minute at 60 rpm. The results were expressed in mP.S(Fathoni & Arisandi, 2020).

Water Holding Capacity

To determine WHC, 0.5 g of the hydrocolloid sample was placed into a centrifuge tube and mixed with 3 mL of water. The mixture was shaken for 1 minute and then centrifuged at 3200 rpm at 24°C for 30 minutes. The retained water volume was subsequently measured. Water holding capacity (WHC) represents the ability of hydrocolloids to retain water, expressed as the volume of water held per gram of sample (%) (Lastra-Ripoll et al., 2022). WHC was calculated according to the following equation:

$$\text{WHC} = \frac{A-B}{B} \times 100\% \quad (5)$$

while A = weight of the sample after water absorption (g); B = initial sample weight (g).

Solubility

Solubility, expressed as the percentage of dissolved dry sample relative to the initial sample weight, was measured. A 1% (w/v) hydrocolloid dispersion was prepared in 30 mL of distilled water and maintained at temperatures of 25, 45, and 65°C for 30 minutes with continuous stirring. A 10 mL aliquot of the mixture was then centrifuged, and the resulting supernatant was dried in a muffle furnace at 125°C until a constant weight was achieved (Lastra-Ripoll et al., 2022). Solubility was calculated using the following equation:

$$\text{Solubility (\%)} = \frac{M1\times 3}{M0} \times 100\% \quad (6)$$

Table 1. Combination of Sea Grape (*Caulerpa sp*) Extraction Treatments Box-Behnken Design

No	Sample Amount (g)	Solvent Concentration (%)	Extraction Time (minute)
1	20	6	30
2	40	6	30
3	20	10	30
4	40	10	30
5	20	8	20
6	40	8	20
7	20	8	40
8	40	8	40
9	30	6	20
10	30	10	20
11	30	6	40
12	30	10	40
13	30	8	30
14	30	8	30
15	30	8	30

while M_0 = initial sample weight (g); M_1 = weight of dissolved solids after drying (g); 3 = volume conversion factor based on the ratio of total solution volume to the volume of aliquot analysed (30 mL : 10 mL).

Experimental Design and Optimization

The experimental design and optimization of both MAE and UAE processes were conducted using Response Surface Methodology (RSM) with a Box–Behnken Design (BBD). Three independent variables were evaluated, namely raw material quantity (20–40 g), solvent concentration (6–10%), and extraction time (20–40 min), while extraction temperature (70°C for UAE), microwave power (medium level for MAE), and solvent volume (400 mL) were maintained constant. The responses analyzed included carrageenan yield, viscosity, and sulfate content, which were used as criteria for determining the optimal extraction conditions. Statistical modeling, regression analysis, and optimization were performed using Minitab, generating predictive polynomial equations and three-dimensional response surface plots to describe the interaction effects among variables and identify the optimum process parameters. The adequacy of the fitted quadratic models was evaluated using analysis of variance (ANOVA), including model significance and lack-of-fit tests. Model performance was further assessed using the coefficient of determination (R^2), adjusted R^2, and predicted R^2. The combination of extraction model is shown in Table 1.

Results and Discussion

The Statistical significance of the developed models was assessed by analysis of variance (ANOVA), and the results are presented in Table 2 and Table 3.

Analysis of variance (ANOVA) demonstrated that all quadratic models for both MAE and UAE were statistically significant ($p < 0.05$), while the lack-of-fit was not significant ($p > 0.05$), indicating that the developed models adequately fitted the experimental data (Table 2 & Table 3). The coefficient of determination (R^2) ranged from 94.65% to 97.38%, indicating that the models explained a high proportion of the variability in the experimental responses (Iqbal et al., 2025). The adjusted coefficient of determination (adjusted R^2), which accounts for the number of predictors in the model, ranged from 85.02% to 92.66%. Furthermore, the differences between R^2 and adjusted R^2 ranged from 0.0472 to 0.0963, all of which were below the acceptable limit of 0.2 (Permana et al., 2023), indicating good agreement between the two statistics and suggesting that the developed models were not overfitted.

Table 2. Analysis of variance (ANOVA) for the fitted quadratic models of extraction yield, viscosity and sulfate content MAE

Response	F-value	p-value model	p-value lack of fit	R^2	Adjusted R^2	Predicted R^2
Yield	14.57	0.004	0.061	96.33%	89.71%	43.31%
Viscosity	14.27	0.005	0.518	96.25%	89.51%	59.87%
Sulfate content	9.83	0.011	0.335	94.65%	85.02%	31.93%

Table 3. Analysis of variance (ANOVA) for the fitted quadratic models of extraction yield, viscosity and sulfate content UAE

Response	F-value	p-value model	p-value lack of fit	R^2	Adjusted R^2	Predicted R^2
Yield	17.61	0.003	0.204	96.94%	91.44%	56.00%
Viscosity	20.63	0.002	0.923	97.38%	92.66%	87.56%
Sulfate content	13.10	0.006	0.343	95.93%	88.61%	48.58%

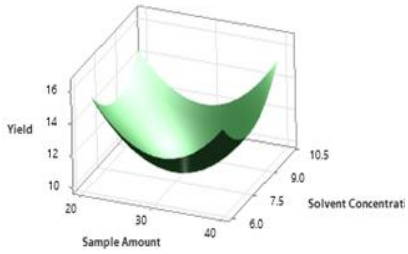
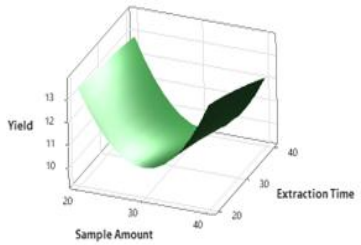
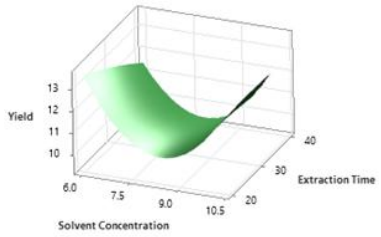
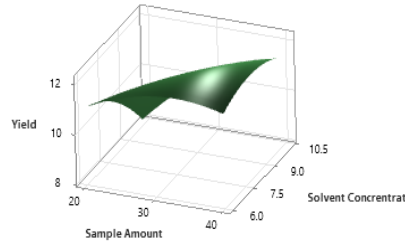
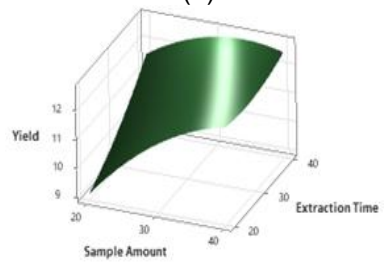
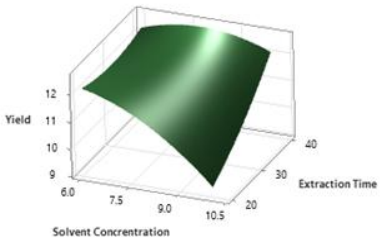
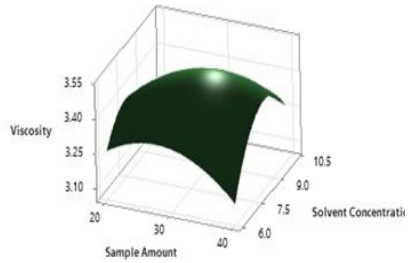
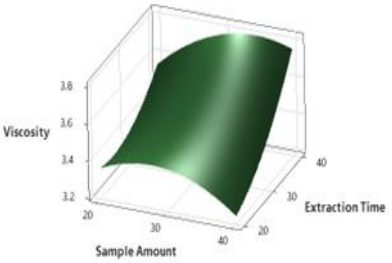
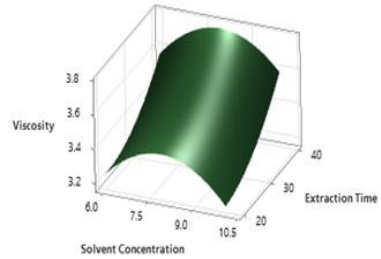
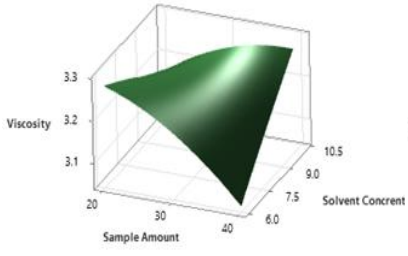
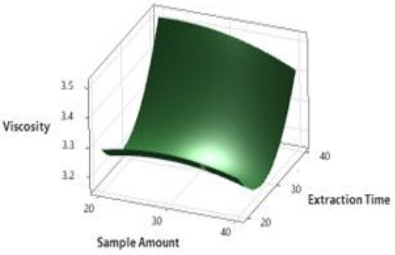
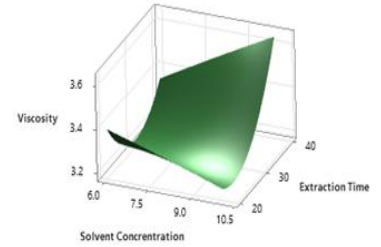
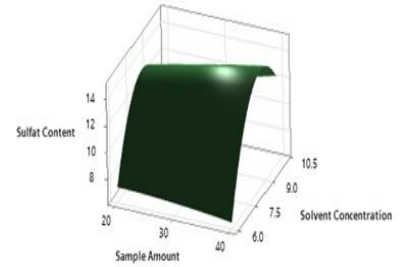
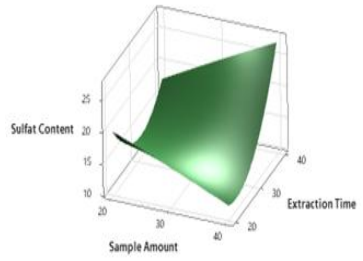
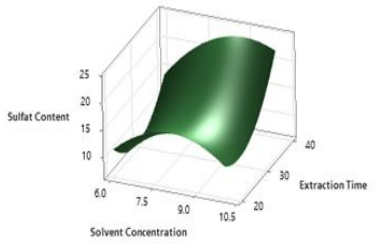
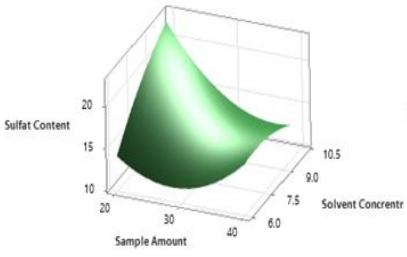
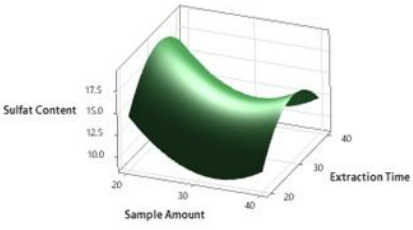
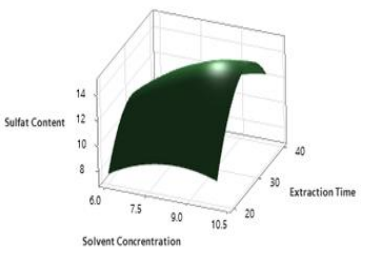
	Sample Amount and Solvent Concentration	Sample Amount and Extraction Time	Solvent Concentration and Extraction Time
Yield (MAE)			
	(a)	(b)	(c)
Yield (UAE)			
	(d)	(e)	(f)
Viscosity (MAE)			
	(g)	(h)	(i)
Viscosity (UAE)			
	(j)	(k)	(l)
Sulfate Content (MAE)			
	(m)	(n)	(o)
Sulfate Content (UAE)			
	(p)	(q)	(r)

Figure 1. Three-dimensional response surface plots of the extraction variables

Extraction yield is an indicator of the effectiveness of an extraction, the higher the yield the higher the output. During the extraction process, ion exchange reactions occur in the sample, influenced by the solvent and the extraction time (Kasim et al., 2023). UAE and MAE enhance extraction yield through distinct mechanisms. In UAE, the collapse of acoustic cavitation bubbles generates shear forces and micro-jets that physically disrupt cell walls, increasing mass transfer from the sample to the solvent, so that a higher sample amount positively contributes to yield. In contrast, MAE operates through dielectric heating, where rapid molecular vibration raises intracellular temperature, generating internal pressure that disrupts the cell wall and releases intracellular compounds, making it more sensitive to extraction time, as prolonged exposure risks thermal degradation of the target compound (Tran et al., 2024).

Based on the results in the surface plot in Figure 1a, the yield increases at certain sample quantities and concentrations, indicating the presence of an optimum point. An increase in solvent concentration causes the yield to rise, as the alkali converts the 6-sulfated galactose group into 3,6-anhydrogalactose through a sulfate elimination reaction. This process increases the polysaccharides that form carrageenan and facilitates the release of carrageenan from the cell wall (Yunus et al., 2026). Meanwhile, an excessive amount of material can reduce the yield. This is because the solvent has a harder time diffusing into the cell wall molecules, resulting in a lower yield. Additionally, an unbalanced ratio of material to solvent prevents the maximum extraction of carrageenan (Hasah et al., 2023).

Based on the surface plot in Figure 1b, the yield is influenced by extraction time and sample volume in a nonlinear manner. The graph shows variations in yield across different combinations of the two variables, with areas of low yield that subsequently increase under specific conditions. Extraction time affects yield, as longer contact with the solvent results in more carrageenan being released from the cell walls, leading to increased yield (Desiana & Hendrawati, 2015). An increase in sample size can initially lead to a decrease in yield (Hasan et al., 2019), however, under certain conditions, the yield percentage may rise again. This pattern may be attributed to the ratio between sample mass and solvent volume reaching a more favorable equilibrium, allowing for more efficient extraction per unit of sample weight, rather than simply reflecting an increase in the total amount of extracted material.

Based on Figure 1c, the yield exhibits a nonlinear relationship with solvent concentration and extraction time, characterized by a pattern of initial decrease followed by a subsequent increase under certain conditions. At certain solvent concentrations, the yield tends to decrease. However, as the solvent concentration increases, the yield rises again because during the extraction process, the alkaline solvent accelerates the formation of 3,6-anhydrogalactose (Andhiarto, 2021). Extraction time also affects yield; a longer extraction time results in a longer contact time between the solvent and the seaweed, leading to a greater amount of extracted carrageenan (Murdiningsih et al., 2018). A combination of higher solvent concentration and longer extraction time yields a higher yield.

Based on Figure 1d, it can be seen that the more sample quantities used in extraction using the UAE method, the more the yield. Meanwhile, the concentration of the solvent in this extraction tends to decrease the yield. This is in contrast to research by Fauziah et al. (2024), who found that yield increased with increasing KOH concentration in seaweed extraction. Optimal conditions for obtaining maximum yield can be achieved by using a high sample volume combined with a solvent concentration that is not too concentrated.

Based on Figure 1e, it can be seen that the yield increases with the increase in sample quantities and extraction time in the UAE method. The more samples used, the higher the yield. This occurs because as the sample increases, the material available for extraction also increases. The combination of the sample number and the extraction time can increase the yield. This increased extraction time allows for optimal contact between the solvent and the sample, which can accelerate the reaction and increase the rate of compound diffusion through the cell wall (Ashfarina et al., 2020).

Based on Figure 1f, it can be seen that the extraction time and solvent concentration in the UAE method are inversely proportional, where the extraction time tends to have a positive effect, where increasing the time will increase the yield, while too high a solvent concentration tends to reduce the yield. The optimum extraction condition for this extraction is to use a long time with a solvent concentration that is not too high.

Effect of Sample Amount, Solvent Concentration and Extraction Time on Viscosity

The surface plot in Figure 1g shows the effect of the interaction between solvent concentration and sample amount on viscosity. Viscosity tends to increase as solvent concentration and sample amount increase until an optimum region is reached, then decreases when both factors are too high. When the sample amount increases while the solvent volume remains constant, the solid-to-solvent ratio becomes higher, making the solvent less effective for optimal polysaccharide extraction. This is related to the extracted sulfate groups; the lower the sulfate content, the lower the viscosity. According to (Bono et al., 2014), the viscosity of carrageenan can be influenced by the sulfate content, which is directly proportional to its concentration. Furthermore, high alkali concentrations increase the repulsive forces between negative charges on the polymer chains; this occurs due to increased ionization of the negatively charged groups. This causes the molecular chains in the sulfated polysaccharide to move farther apart, reducing intermolecular interactions and ultimately resulting in a solution with lower viscosity (more dilute) (Desiana & Hendrawati, 2015).

Based on figure 1h, viscosity increases with increasing extraction time. However, at a certain sample volume, viscosity decreases. A combination of increased extraction time and sample volume under certain conditions can increase viscosity. Extraction time has a

greater effect than sample volume on increasing viscosity. A longer extraction time can increase the extracted sulfate content, resulting in higher viscosity (Hasan et al., 2019).

Based on the surface plot in Figure 1i, the extraction time increases viscosity, and at certain concentrations, viscosity decreases. Increasing the extraction time results in a greater amount of sulfate being extracted (Hasan, 2019). Viscosity is directly proportional to sulfate content, therefore, the higher the sulfate content, the higher the solution viscosity. Increasing concentration under certain conditions can increase viscosity. However, at excessively high concentrations, viscosity decreases because the hydrophilic properties of the polymer weaken (Ega et al., 2016).

Based on Figure 1j, it can be seen that in extraction using the UAE method, the combination of sample amount and solvent concentration provides a cross-interaction effect where the amount of sample used is highly dependent on the solvent concentration in influencing viscosity. The greater the number of samples used tends to decrease the viscosity of the extraction results, in carrageenan extraction this occurs due to a decrease in the number of sulfate groups produced and the high formation of 3,6-anhydrogalactose which can cause a decrease in viscosity (Arzani et al., 2020). The concentration of the solvent used can affect viscosity where an increase in solvent concentration increases the viscosity value, however, an excessive increase in concentration is likely to decrease viscosity because the dissolved salts will reduce the charge along the polymer chain which causes a reduction in the repulsive force between sulfate groups (Andhiarto, 2021). Optimum results can be obtained by using a sample amount and solvent concentration that is not too high.

Figure 1k shows that in extraction using the UAE method, the sample amount and time affect the viscosity value, where viscosity increases with time, while adding too much sample can decrease viscosity. In this extraction process, time plays a greater role in viscosity than the sample amount.

A positive synergy, where increasing both can increase viscosity. The combination of maximum time and maximum solvent concentration can produce the highest viscosity. However, in this study, extraction time is a more influential factor than solvent concentration. Extraction time that is too long can affect the viscosity of carrageenan. In (Ashfarina et al., 2020), it was found that the viscosity of carrageenan extracted for more than 60 minutes decreased due to the desulfatization process which caused the sulfate in seaweed to decrease.

The surface plot in Figure 1m shows the interaction between solvent concentration (KOH) and the amount of material on the response of carrageenan sulfate levels produced through the extraction process. Based on the visualization displayed, the resulting surface shape shows a tendency for an increase in sulfate levels along with an increase in both variables, indicated by the increasing surface area of the graph. However, under certain conditions, the surface of the graph appears to begin to slope, indicating that the system has approached optimum conditions and is experiencing limitations in increasing the response. The combination of KOH and the amount of material can increase sulfate levels. The higher the KOH concentration, the more significant the increase in the amount of material appears to be, with the resulting sulfate levels tending to increase until reaching a certain point. This indicates that the effectiveness of the amount of material depends on the solvent's ability to extract a component. The role of KOH in the alkali treatment process is able to open the cell wall structure and optimize the dissolution of sulfated polysaccharides in the material during extraction (Mendes et al., 2024).

Based on the visual surface plot results, it illustrates the relationship between extraction time and sample size on sulfate levels, where both variables have a significant influence on the resulting response. Increasing extraction time and sample size shows a tendency to increase sulfate levels, where it appears that extraction time has a more dominant influence than sample size. Increasing extraction time is initially able to increase sulfate levels because longer time can optimize the solvent to penetrate the cell wall matrix and dissolve sulfated polysaccharides optimally. This is in line with research. (Heriyanto et al., 2018a), which states that longer extraction times optimize the diffusion of active compounds, thereby increasing yield and sulfate content. However, the surface pattern of the graph does not show a perfectly linear relationship, but rather a curved one. This indicates an optimum extraction time limit, where additional time no longer significantly increases sulfate levels and can even potentially cause a decrease due to degradation of the carrageenan structure, especially in the sulfate groups bound to the polysaccharide chain (Daet et al., 2025).

The surface plot in Figure 1o shows that sulfate levels are significantly affected by the combination of solvent concentration and extraction time. Research has shown that increasing solvent concentration increases sulfate levels because more sulfate compounds can be dissolved and extracted from the material. However, at too high a concentration, sulfate levels actually decrease, which is thought to be due to reduced solvent effectiveness (Mendes et al., 2024). A similar trend is observed with extraction time, indicating that increasing initial extraction time also increases extraction yields. However, too long an extraction time can cause degradation of the target compound, resulting in decreased levels (Heriyanto et al., 2018).

According to the surface plot as shown in Figure 1p, sulfate content is significantly influenced by sample amount as indicated by the strong curvature of the surface. Sulfate content decreases as sample amount increases to a certain level, followed by a slight increase at higher levels, indicating the presence of an optimum point. The same result was shown by Dikianur et al. where the more samples used, the lower the sulfate content is (Alvianto & Arzani, 2025). In contrast, solvent concentration presents less effect, only a slight increase in sulfate content observed at higher concentrations. The curve is weak, which indicate that it is not significant compared to sample amount. It is linear with Distantina et al. which stated that increasing KOH concentration around 0.1–0.5 N lowers sulfate content (Distantina et al., 2011). The surface also reveals a mild interaction between variables, where higher sulfate content is

Table 4. Optimum extraction conditions and predicted responses for UAE and MAE processes obtained using RSM

Method	Sample Amount	Solvent Concentration	Extraction Time	Sulfate Content Fit	Viscosity Fit	Yield Fit	Composite Desirability
MAE	40	10	40	27.6089	3.65903	15.2322	0.892787
UAE	20	9.111	40	18.5461	3.54162	11.0084	0.704909

obtained at lower sample amounts combined with moderate to high solvent concentrations. Overall, the optimal range is low sample amounts and moderate–high solvent concentrations.

The surface plot shows that sulfate content is significantly influenced by sample amount, as indicated by the curvature of factors. Sulfate content decreases as the sample amount increases to intermediate levels, followed by an increase at higher sample amounts. Sulfate content becomes higher when extraction conditions retaining more highly sulfated carrageenan fractions, which also could be influenced by the mass of sample used (Alvianto & Arzani, 2025) Meanwhile, extraction time denotes a slight decrease with increasing extraction time, followed by a modest increase at longer durations, indicating that extraction time is not significant. Sulfate content tend to decreased with a longer time (Andhiarto & Pusparini, 2025). The graph shows that lower sulfate content were obtained at intermediate levels of both variables, while higher sulfate contents were achieved at lower sample amounts with shorter extraction times or higher sample amounts with longer extraction times.

Based on Figure 1r, sulfate content increases with increasing extraction time, presenting that longer extraction gives a higher sulfate result. The curvature suggests that extraction time has a significant positive effect on sulfate content and plays a major role in determining the response. As explained by Andhiarto et al., Longer time often increases yield up to an optimum (Andhiarto & Pusparini, 2025). The solvent concentration shows a less impact on sulfate content. Although sulfate content tend to increase slightly with increasing solvent concentration up to a certain point, the effect is relatively moderate compared to extraction time, indicating that this factor is less significant. It is linear with Distantina et al. which stated that increasing KOH concentration around 0.1–0.5 N lowers sulfate content (Distantina et al., 2011). The combined effect of these two variables shows that the highest sulfate content is achieved at longer extraction times and moderate to high solvent concentrations, indicating the existence of an optimal region under these conditions.

Optimum extraction conditions and predicted responses for UAE and MAE

Based on the research results of 15 variations of Box Behnken Design with analysis of yield, viscosity and sulfate content, the optimum extraction point using the MAE method was obtained at a sample size of 40 grams, a concentration of 10% and an extraction time of 40 minutes. Meanwhile, extraction using the UAE method obtained optimum conditions at a sample size of 20 grams, a concentration of 9.11% and an extraction time of 40 minutes (Table 4).

The composite desirability values were 0.893 for MAE and 0.705 for UAE. Since desirability values range from 0 to 1, with values closer to 1 indicating better optimization performance, these results suggest that the selected conditions satisfactorily fulfilled the optimization criteria (Iqbal et al., 2025). To verify the predictive capability of the developed models, experimental validation was performed under the predicted optimum conditions. The validation results for the MAE method produced an extraction yield of 14.53%, a viscosity of 3.68 cP, and a sulfate content of 24.71%, while the UAE method yielded an extraction yield of 13.12%, a viscosity of 3.60 cP, and a sulfate content of 17.30%. Overall, the experimental results were generally comparable to the predicted responses, confirming that the developed RSM models were suitable for predicting the optimum extraction conditions.

Physicochemical Properties of the Extracted Polysaccharides and Commercial Carrageenan

The moisture content obtained by UAE (9.34%) and MAE (9.94%) was notably higher than that of commercial carrageenan (4.49%); however, all values remained within the maximum limit of 12% stipulated by SNI 8391-1:2017. The elevated moisture content observed in both extraction methods may be attributed to the nature of ultrasonic and microwave energy, which can increase thermal exposure during processing and subsequently affect water retention in the final product. Mahyati & Azis (2019) reported that UAE-derived carrageenan exhibited moisture content ranging from 6.27–14.18%, with higher temperature or prolonged extraction time tending to increase moisture levels; values exceeding 12% were noted to surpass FAO/FCC limits. Similarly, Andhiarto & Pusparini (2025) observed that MAE produced higher moisture content under short extraction durations, suggesting that extraction kinetics influence the degree of water retention in the polysaccharide matrix. Although the moisture values in this study did not exceed the SNI threshold, the relatively higher moisture in UAE and MAE extracts compared to commercial carrageenan warrants attention in the context of product shelf life and microbial stability.

The ash content of UAE (53.01%) and MAE (54.72%) extracts was comparable to that of commercial carrageenan (55.70%); however, all three values substantially exceeded the SNI 8391-1:2017 permissible range of 15–40%, indicating that the extracts did not meet the standard quality requirements for ash content. This is a critical finding that reflects a significant limitation of the extracts produced in this study and warrants further investigation into the contributing factors. Several factors may account for this excessive ash content. First, *Caulerpa* sp. is a green macroalga that naturally accumulates inorganic minerals from its marine environment; higher seawater salinity or elevated mineral deposition in the thallus can substantially

Table 5. Comparison of extract characteristics with commercial carrageenan and SNI 8391-1:2017

Characteristics	UAE	MAE	Commercial carrageenan	SNI 8391-1:2017
Water Content (%)	9.34 ± 0.35	9.94 ± 0.09	4.49 ± 0.05	Maks. 12
Ash Content (%)	53.01 ± 0.25	54.72 ± 0.07	55.70 ± 0.06	15-40
Sulfate Level (%)	17.30 ± 0.27	24.21 ± n0.27	17.67 ± 0.16	15-40
Viscosity (cps)	3.60 ± 0.2	3.68 ± 0.1	185.67 ± 0.6	Min. 5
WHC (g/g)	110.61 ± 11.51	102.93 ± 5.21	588.33 ± 7.51	-
Solubility in 25°C (%)	80.43 ± 6.61	71.80 ± 15.65	78.23 ± 3.87	-
Solubility in 45°C (%)	77.23 ± 3.09	95.23 ± 34.19	2.93 ± 0.15	-
Solubility in 65°C (%)	78.43 ± 9.36	78.67 ± 7.72	0.80 ± 0.10	-

increase the ash content of the resulting extract (Maulidia et al., 2024). Second, inadequate pre-extraction washing of the seaweed may have allowed excess K^+ ions, residual salts, and particulate matter to remain in the biomass, thereby contributing to inflated ash values (Heriyanto et al., 2018). Third, the alkaline KOH treatment used in both UAE and MAE may introduce additional potassium salts into the extract matrix, further elevating ash content if post-extraction purification is insufficient. P. et al. (2023) reported similarly high ash values (55–60%) in some semi-refined carrageenan (SRC) products, suggesting that incomplete refining is a recurring challenge across extraction methods.

The sulfate content of UAE-derived extract was slightly lower than that of commercial carrageenan, yet still satisfied the Indonesian National Standard minimum threshold of 15%. Conversely, the MAE extract yielded a higher sulfate content compared to both the UAE extract and commercial carrageenan. These differences can be attributed to variations in extraction method and the species-specific characteristics of the raw material used. Al-Nahdi et al. (2019) demonstrated that different seaweed species employed in carrageenan extraction yield distinct sulfation levels and carrageenan characteristics, underscoring the importance of species selection in determining the sulfate profile of the final product. The degree of sulfation is particularly significant as it directly influences the functional properties of carrageenan, including its gelling ability, viscosity, and interactions with proteins.

The viscosity values of *Caulerpa* sp. extracts obtained via UAE and MAE were substantially lower than both commercial carrageenan and the minimum requirements prescribed by SNI 8391-1:2017 and FAO (2007), which specify an acceptable viscosity range of 5–800 cP. This represents a critical deficiency, as viscosity is a fundamental rheological parameter that determines the functional applicability of carrageenan as a thickener, stabilizer, or gelling agent in food systems. Furthermore, the sonication energy applied during UAE may have induced chain scission of polysaccharide chains, resulting in lower molecular weight fragments and consequently reduced viscosity of the extract. Tecson et al. (2021) demonstrated that ultrasonic treatment significantly reduced the molecular weight of κ-carrageenan by up to 89.32%, confirming the depolymerizing effect of ultrasonication on carrageenan-type polysaccharides.

The Water Holding Capacity (WHC) of carrageenan extracts from *Caulerpa* sp. revealed that UAE produced extracts with higher water-binding capacity compared to MAE; however, both values remained significantly lower than that of commercial carrageenan. According to Mendes et al. (2024), WHC in carrageenan is influenced by a combination of factors including extraction method, degree of sulfation, molecular weight, and product purity. The lower WHC observed in both UAE and MAE extracts in this study is consistent with the relatively low sulfate content and viscosity values, suggesting that the extracted polysaccharides have not yet attained the structural integrity required for optimal water retention. This further corroborates the possibility that the polysaccharide fractions extracted from *Caulerpa* sp. differ structurally from commercial carrageenan.

The solubility of *Caulerpa* sp. carrageenan was assessed at three temperature levels: 25°C, 45°C, and 65°C. At 25°C, UAE-derived extract exhibited slightly higher solubility than MAE, with both values remaining within a comparable range to commercial carrageenan, indicating acceptable cold-water solubility for both methods. Interestingly, at 45°C and 65°C, the solubility of commercial carrageenan was significantly lower than that of the sea grape extracts. This inverse relationship is explained by the gelation behavior of commercial carrageenan: κ-carrageenan undergoes a conformational transition from random coil chains to double helix structures at elevated temperatures, which subsequently aggregate to form a three-dimensional network that traps water molecules and transitions the material from a solution to a semi-solid gel state (S. Liu et al., 2016). In contrast, the UAE and MAE extracts from *Caulerpa* sp. remained in solution at higher temperatures, while appearing favorable in terms of solubility, may reflect the absence of structured sulfated galactan networks necessary for gel formation, consistent with the low viscosity and WHC results discussed above.

Conclusion

Based on optimization using Response Surface Methodology (RSM) with yield, sulfate content, and viscosity as response variables, the physicochemical properties of the sulfated polysaccharide extract from *Caulerpa* sp. were compared against carrageenan quality standards. Sulfate content and water content were found to fall within ranges generally associated with carrageenan standards, whereas viscosity and ash content did not meet the required thresholds. Compared to commercial carrageenan, WHC values differed considerably, while solubility was relatively acceptable. However, since no structural confirmation was performed, these findings cannot be interpreted as evidence that the extract is carrageenan; rather, they

indicate that certain measured parameters are within comparable ranges. Therefore, further structural analysis such as FTIR, NMR, or monosaccharide composition is needed to confirm the type and identity of polysaccharides present in Caulerpa sp. and to determine whether they exhibit functional properties comparable to those of carrageenan.

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