Jurnal Pena Sains
Jurnal Pendidikan Sains dan Sains Murni
Journal homepage: https://journal.trunojoyo.ac.id/penasains

OPTIMIZATION OF NaOH PRETREATMENT ON AGAROSE QUALITY OF *Gelidum* sp. SEAWEED FROM THE COSTAL AREA OF YOGYAKARTA, INDONESIA

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ABSTRACT

Seaweed of *Gelidium* sp. is a type of red seaweed that can produce agarose. In this study, seaweed *Gelidium* sp. was taken from costal area in Yogyakarta, Indonesia. Agarose can be produced from *Gelidium* sp. using a method including alkali pretreatment and agarose extraction using PEG 6000 at 85°C. Agarose extraction pretreatment of *Gelidium* sp. was carried out in an alkaline environment using NaOH with concentrations of 4%, 6%, 8%, and 10%. The purpose of this research is to obtain the best quality agarose from pretreatment with various NaOH concentrations. The quality of agarose is determined from the results of gel strength, sulfate content, and galactose content. Good quality of agarose has the higher value of gel strength and galactose content, but the lower sulfate content in agarose extracted from *Gelidium* sp. Based on the results obtained, the highest agarose yield was obtained from pretreatment with a NaOH concentration of 4% of 31.09%. Meanwhile, at 6% NaOH concentration, the highest gel strength was obtained at 151.70 grams/cm2, the lowest sulfate content of 0.4%, and the highest galactose content of 35.76%. Based on statistical analysis using ANOVA with a significance level of 95%, it shows that the variation of NaOH concentration in the extraction pretreatment has a significant effect on gel strength, sulfate content, and galactose content ($p < 0.05$) but is not significant on the agarose yield. In conclusion, the optimization of NaOH pretreatment produced the best agarose quality at a NaOH concentration of 6% because it resulted in the highest gel strength and galactose content and the lowest sulfate content in agarose.

Article Info	
Article history:	***Keywords:***
Received: February 26, 2025	Agarose;
Accepted: April 3, 2025	*Geledium* sp;
Published: April 30, 2025	Gel strength;
	NaOH pretreatment;
	Red seaweed

10.21107/jps.v12i1.29381 E-ISSN: 2527-7634

Introduction

Indonesia is an archipelagic country that has a variety of seaweed. Indonesian seaweed is one of the export commodities, such as *Gracilaria* sp., *Eucheuma* sp., *Sargassum* sp., and *Gelidium* sp. In 2020, the export value of seaweed-based hydrocolloid products reached 88 million USD, and for dried seaweed materials reached 183 million USD (Basyuni et al., 2024). Improving the quality of processed seaweed products continues to be carried out to increase added value in the seaweed industry (Sutinah et al., 2020). *Gelidium* sp. is an important red seaweed in Indonesia because it produces high quality agar, therefore it can be used as a mixing material with other low-quality agar, such as from *Gracilaria* sp. (Subaryono et al., 2021).

Agar is a polysaccharide consisting of agarose and agaropectin. The application of agar is widely used in both food and non-food industries, especially in the field of biotechnology (Cebrián-Lloret et al., 2024; Li et al., 2020). In addition, agar can be used as an emulsifier, stabilizer, gelling agent, or thickener due to its ability to form gels. Therefore, gel strength becomes crucial in determining the quality of agarose (Sinurat et al., 2024). The good quality of agarose from seaweed can be shown by its gel strength and high galactose content, but low sulfate content (Pandya et al., 2022). The quality of agarose obtained from seaweed is highly dependent on the seaweed species, extraction method, and environmental conditions in seaweed growth (Bertasa et al., 2020).

One of the methods of agarose extraction that has been used is precipitation with quaternary ammonium. The limitation of this method is the efficiency of agarose extraction and it requires a continuous washing procedure to separate agarose from agaropectin (Sánchez-Flores et al., 2025). The development of agarose extraction methods from red seaweed continues to develop, such as extraction using polyethylene glycol which utilizes the solubility properties of agarose, therefore the extraction process becomes more efficient in separating agarose and agaropectin (Jiang et al., 2023). Pretreatment in the extraction process is very necessary to increase the yield, gel strength, and reduce the sulfate content of seaweed (Mohibbullah et al., 2023). The pretreatment method using alkali NaOH on red seaweed *Gracilaria gracilis* can increase the gel strength from 105.30 grams/cm^2 to 377.39 grams/cm^2 (Belattmania et al., 2021).

One of the areas in Indonesia that produces *Gelidium* sp. is the coastal area of Yogyakarta. *Gelidium* sp. from Yogyakarta produced a high agar yield of 10%, while the Ujung Genteng area produced 4% agar. However, the gel strength of the agar from *Gelidium* sp. Yogyakarta was lower than Ujung Genteng (Sinurat et al., 2024). Therefore, it is necessary to optimize the agarose production process from *Gelidium* sp. in the coastal area of Yogyakarta. This study aimed to analyze the quality of agarose, specifically agar yield, gel strength, sulfate content, and galactose content from red seaweed *Gelidium* sp. found in the waters of Yogyakarta, using alkaline pretreatment with varying concentrations of NaOH. The effect of NaOH concentration variations was analyzed using the statistical Analysis of Variance (ANOVA) method.

Research Methods

Materials

The materials used were *Gelidium* sp. seaweed from Yogyakarta, Indonesia, NaOH, CaO, HCl, K_2SO_4, NaCl, and Polyethylene Glycol (PEG) 6000, all sourced from Merck.

Optimization of NaOH Treatment

Gelidium sp. seaweed was obtained from the waters of Yogyakarta. The seaweed was separated and washed to remove sand, stones, and other impurities, then sun-dried until completely dry. A total of 100 grams of dried seaweed, 1% CaO was added and left to stand for 1 hour. Then the seaweed is rinsed with distilled water until the pH is neutral for further pretreatment with NaOH. Seaweed is mixed with NaOH concentrations of 4%, 6%, 8%, and 10% (1:10) at 60°C for 1 hour. The seaweed is washed back with water until the pH is neutral (Belattmania et al., 2021).

Agarose Extraction from Dried Gelidium sp.

The extraction process was carried out by mixing the seaweed and water (1:20 ratio) at a temperature of 85°C for 2 hours. The mixture was filtered using a 150-mesh sieve. The filtrate was then added with 20% (w/v) PEG 6000 and 1% (w/v) NaCl, and heated at 85°C for 30 minutes. The mixture was filtered again using a 150-mesh sieve.

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The filtrate was then placed into molding pans and left to stand for 24 hours at room temperature, then dried under the heat of the sun for 3 days. After drying, the mixture was ground to obtain agar powder (Junianto et al., 2021).

Agarose Yield

The yield of agarose extraction was calculated by weighing the agarose produced from the extraction and dividing it by the weight of the dry seaweed before extraction (Vuai, 2022). The calculation was as follows:

$$\text{Agarose Yield (\%)} = \frac{\text{Agarose weight}}{\text{Dry Seaweed weight}} \times 100\% \quad [1]$$

Agarose Quality Analysis

Gel Strength

A total of 1.8 grams of the sample, 0.18 grams of KCl was added. Then, 80 mL of distilled water was added to the beaker, and the mixture was heated to 80°C while continuously stirring. The hot solution was then poured into a dish to solidify and stored at 20°C for 1 hour. The strength of the gel was measured using the Texture Analyzer (Pereira et al., 2021).

$$\text{Gel strength} = \frac{\text{Sample length x} \frac{\text{Calibration weight}}{\text{Calibration length}}}{\text{Gel compression diameter}} x100\% \quad [2]$$

Sulphate Content

A total of 1 g of agar was placed into an Erlenmeyer flask and 50 mL of 0.2N HCl was added. The Erlenmeyer flask was placed on a vertical water bath and heated to a boil, then refluxed for 1 hour. A total of 25 mL of 10% H2O2 was added to the solution, and refluxing was continued for another 5 hours. The solution was then added with 10 mL of 10% $BaCl_2$, drop by drop, over a period of 2 hours. The formed precipitate was then filtered using Whatman No. 1 filter paper and washed with boiling distilled water until free of chloride. The filter paper was dried in an oven and then ashed at 800°C in a furnace until white ash was obtained. The ash was then cooled in a desiccator and weighed until a constant weight was achieved (Gomes-Dias et al., 2022).

$$\text{Sulphate (\%)} = \frac{\text{Ash weight (g)} \times 0.0416}{\text{Sample weight (g)}} \times 100\% \quad [3]$$

Galactose Content

A total of 1 g of sample was put into the erlenmeyer and 25 mL of 25% HCl was added. The solution was hydrolyzed for 2.5 hours by attaching a vertical condenser, then cooled and neutralized with 30% NaOH. The solution was transferred into a 100 mL volumetric flask and diluted to the mark with distilled water. The solution was then filtered using filter paper. A total of 10 mL of solution was pipetted and put in a 250 mL erlenmeyer, 15 mL of aquadest was added and 25 mL of the luff solution was boiled for 10 minutes. The solution was added 10 mL of 20% KI and 25 mL of 25% H_2SO_4. Solution was titrated with 0.1 N Na-thiosulfate (which has been standardized). Starch indicator was added until the solution was blue and titration was continued until the solution was milky white (Latimer Jr., 2023).

$$\text{Galactose(\%)} = \frac{\text{Dilution factor x mg Galactose x0.9}}{\text{Sample weight (g)}} \text{x100\%} \quad [3]$$

Statistical Analysis

The effect of NaOH concentration on agarose quality and content was tested using a single-factor Complete Random Design with a linear model. The collected data were calculated so that the average and standard deviation were obtained with a significance level of 95%. ANOVA (P = 0.05) was used to detect significant differences between parameters by comparing F count and F table (Patthamasopsakul et al., 2024).

Result and Discussion

Agarose Yield

Agarose yield is a percentage of the weight of the material that can be used compared to the total weight of the material (Vuai, 2022). Therefore, the agar yield is calculated based on the ratio of the weight of the agar resulting in the dry weight of *Gelidium* sp. The effect of NaOH concentration on the yield results can be seen in Figure 1. The highest yield was obtained in the 4% NaOH pretreatment of 31.09%, whereas the pretreatment with 6%, 8%, and 10% NaOH produced lower yields, namely 27.4%, 26.87%, and 26.79% respectively. Based on the agarose yield results in Figure 1. It shows that an increase in NaOH concentration can lead to a decrease in the agarose yield. Pretreatment with NaOH is

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needed to break the cell wall in red seaweed, therefore more agarose is extracted, but an increase in NaOH concentration will also degrade the polysaccharides from agarose so that the yield decreases (Tatary & Sarabandi, 2025). In another study, acid pretreatment of the extraction of *Gelidium sesquipedale* agar from the south of Europe's Atlantic coast resulted in agarose yield of 5-32% (Gomez Barrio et al., 2023).

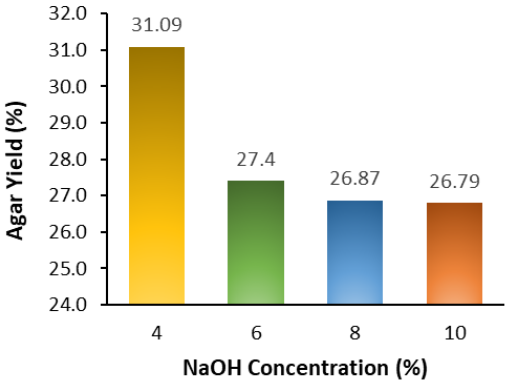

Figure 1. Percentage of yield to NaOH concentration

Based on the statistical results of ANOVA, NaOH Pretreatment on the agarose yield from *Gelidium* sp. resulted in a calculated F value of 1.145 and an α value = 0.388 (Table 1). At the 95% confidence level, it showed an α > value of 0.05, thus statistically the variation in NaOH concentration did not have a significant effect on the results of agarose yield.

Table 1. ANOVA Table of Agarose Quality

No	Parameter	F	α
1	Agarose Yield	1.145	0.388
2	Gel Strength	1898.483	0.000
3	Sulphate	9.761	0.00475
4	Galactose	338.703	0.000

Gel Strength

The strength of the gel is one of the important factors in the quality of agarose and the chemical structure of the polysaccharide affects the gel strength (Shauli & Salomon, 2025). Pretreatment with NaOH may affect the gel strength value of agarose. The gel strength data of the variation in NaOH concentration can be seen in Figure 2. Agarose resulted in the highest gel strength value at a NaOH concentration of 6% at 151.70 grams/cm^2, while NaOH concentrations of 4%, 8%, and 10% the gel strength was lower, producing gel strengths of 61.92 grams/cm^2, 53.46 grams/cm^2, and 25.39 grams/cm^2 respectively. The difference in the strength of the gel is due to the content of galactose and sulfate contained in the large sample, thus the gel strength in the sample is smaller.

The characteristics of gel formation are caused by 3 hydrogen atoms at 3,6 anhydrogalactose which forces the molecules to form helical bonds and the interaction of these helical bonds will form a gel while the presence of sulfate groups forming will cause the polymer to be rigid, therefore helical twists are difficult to form and the quality of the gel decreases (Fittolani et al., 2020). In other studies, treatment without NaOH and with NaOH resulted in an increase in gel strength, from 132.78 ± 2.99 g/cm^2 to 201.33 ± 5.44 g/cm^2 on agarose of red seaweed *Gracilaria tenuistipitata* (Mohibbullah et al., 2023).

However, higher concentrations of NaOH may cause a decrease in gel strength due to the presence of several sulfate groups that have been stabilized to alkali (Xiao et al., 2021). Therefore, it is necessary to optimize the concentration of NaOH in the pretreatment of agar extraction from red seaweed.

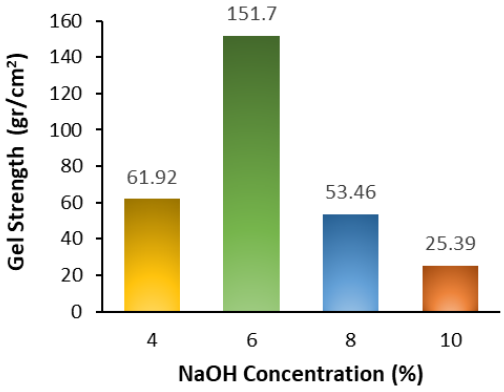

Figure 2. The gel strength of agarose on NaOH concentration

Table 1 shows the statistical value of the variation in NaOH concentration on the gel strength of agarose giving a calculated F value of 1898.483 and the value of α = 0.000. The results indicate that the α < 0.05 at a 95% confidence level, which means that statistically, the treatment of NaOH concentration had a significant effect on the gel strength of the agarose.

Sulfate Content

The presence of sulfate groups can be useful for the biological activity of sulfate

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polysaccharides in seaweed (Torres et al., 2021). However, the sulfate group will decrease the gel strength of the agar after the extraction process from seaweed (Vuai, 2022). Sulfate content from agarose extraction in seaweed *Gelidium* sp. can be seen in Figure 3, the 6% NaOH concentration showed the lowest sulfate content at 0.4%, which is consistent with the gel strength results, as the 6% NaOH concentration also yielded the highest gel strength. Alkaline treatment can reduce the sulfate content in the agar and form a gel of 3,6 anhydrogalactose (Xiao et al., 2021). Meanwhile, at NaOH concentrations of 4%, 8% and 10%, the sulfate content was higher, namely 0.87%, 0.95% and 1.16% respectively. The high sulfate content at a NaOH concentration of 4% is probably due to the alkaline treatment is not enough to convert the sulfate ester to 3,6 anhydrogalactose (Fittolani et al., 2020).

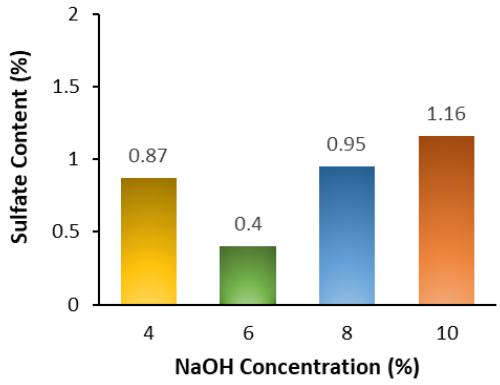

Figure 3. Sulfate content of agarose on NaOH concentration

The results of data processing ANOVA show the statistical value of the variation of NaOH concentration on the sulfate content of agarose giving a calculated F value of 9.76 and α value = 0.00475. These results showed that the $\alpha < 0.05$ at a 95% confidence level, which means that statistically the treatment of NaOH concentration had a significant influence on the sulfate content of agarose.

Galactose Content

The galactose of agarose is essential to give the physical properties of agarose such as being able to indicate the level of its gel strength (Bertasa et al., 2020). The galactose content results from the pretreatment of NaOH concentration gave the highest result at a concentration of 6% of 35.76%, as shown in Figure 4. Meanwhile, at NaOH concentrations of 4%, 8%, and 10%, the galactose content results were 30.89%, 32.86%, and 28.87%, respectively. In Figure 2, the 6% NaOH concentration also resulted in the highest gel strength, namely 151.70 grams/cm^2, thus, the galactose content obtained shows a match with the gel strength results. Meanwhile, at a NaOH concentration of 10%, the galactose content was 28.87%, and the gel strength results also resulted the lowest gel strength, namely 25.39 grams/cm^2.

Alkali treatment is important through breaking the long chain of galactose polysaccharides on the seaweed cell wall, but if the alkali is added in excess, it will also degrade the galactose polymer in agarose, so that the galactose content and gel strength become lower (Zhang et al., 2020). The statistical results in Table 1 of ANOVA show a calculated F value of 338,703 and a value of $\alpha = 0.000$. Pretreatment of NaOH concentration showed a significant effect on galactose content, as the $\alpha < 0.05$ at a 95% confidence level.

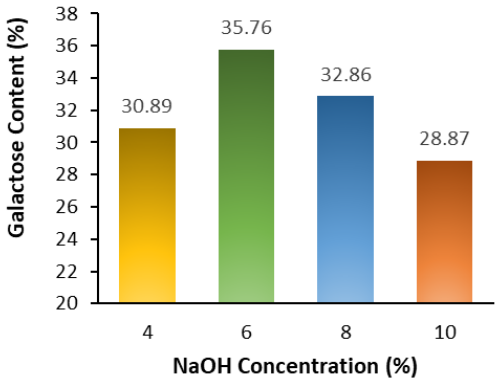

Figure 4. Galactose content of agarose on NaOH concentration

Conclusion

In this study, NaOH pretreatment before the agarose extrusion process of *Gelidium* sp. from the waters of Yogyakarta, the highest yield of 31.09% was obtained at a NaOH concentration of 4%. However, at a NaOH concentration of 6%, the best agarose quality was obtained, namely the highest gel strength and galactose content and the lowest sulfate content, namely 151.70 grams/cm^2, 35.76%, and 0.4% respectively. These results show that NaOH pretreatment has an effect on the agarose quality of *Gelidium* sp.

10.21107/jps.v12i1.29381 E-ISSN: 2527-7634

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