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Agar from Red Algae *(Gracilaria tenuistipitata)* as a Valuable Biopolymer: Extraction and Characterization

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ABSTRACT

Agar, a natural biopolymer extracted from red algae, holds immense potential for revolutionizing healthcare, including biomedical engineering. This study explores the extraction feasibility of agar from red algae (*Gracilaria tenuistipitata)* abundantly available in the coastal area of Cox's Bazar, Bangladesh. Five extraction methods were investigated, including a control group and treatments with water and NaOH solutions at concentrations of 2%, 4%, and 6%. The extraction of agar from algae was characterized through Fourier-transform infrared spectroscopy (FTIR), gel strength testing, melting and gelling temperature assessments, pH value measurement, and sulfate content analysis. Statistical analysis, including ANOVA and Tukey's HSD test, was utilized to assess the impact of the pre-treatment process on the yield and characteristics of agar. The test revealed significant variations among the different extraction methods ($p < 0.05$), highlighting the crucial role that pre-treatment plays in influencing agar yield and its physicochemical properties. While the control group achieved the highest agar yield (16.67 ± 1.44%), the 2% NaOH pre-treatment showed superior physicochemical attributes. Specifically, this treatment resulted in agar with optimal gel strength (3.25 ± 0.67 N/cm²), melting temperature (84.20 ± 0.80°C), gelling temperature (36.13 ± 1.21°C), pH (7.49 ± 0.26), and sulfate content (3.67 ± 0.58 mg/L), all of which are comparable to those of commercial agar. This preliminary study suggests that the red algae (*Gracilaria tenuistipitata)* found in Bangladesh is a promising source of agar for wider applications, including biomedical engineering. The agar extracted from abundant local sources in this country could unlock its potential for advancing healthcare solutions and sustainable national economic growth.

1. INTRODUCTION

The marine algal diversity of Bangladesh contains at least 200 documented seaweed types that grow along its 710 km coastline, especially in the southeastern areas, including Cox's Bazar and Saint Martin's Island (Hossain et al., 2021). Three types of algae exist in Bangladesh: green (Chlorophyta), brown (Phaeophyta), and red (Rhodophyta). The red algae of Gracilaria establish their dominant position in the scientific field due to their ability to produce high-value hydrocolloids. The algal species found in the country present a significant opportunity to extract sustainable biopolymers from renewable sources (A & G, 2024; El-Beltagi et al., 2022; Hossain et al., 2021; Mohibbullah et al., 2023). Biopolymers have wider applications across the packaging, agricultural, pharmaceutical, and biomedical engineering fields (Hoque et al., 2023; Rahmati et al., 2019; Rashid et al., 2023; Tatrishvili, 2025; Vijayan et al., 2016).

Agar, a natural polysaccharide predominantly extracted from red algae (Gracilaria), comprises two primary components: agarose (a linear polymer of repeating β-D-galactose and 3,6-anhydro-α-L-galactopyranose units) and agaropectin (a sulfated, branched polymer). While agarose provides structural neutrality, agaropectin enables gelation through sulfate-mediated interactions (Madadi et

al., 2021; Padmesh & Singh, 2021; Rhein-Knudsen et al., 2017). The application of agar in different products depends on its qualities and physicochemical conditions (sulfate content, gel strength, pH, melting temperature, and gelling temperature) (Zhang et al., 2020, Mohibbullah et al., 2023). The food industry, microbiological media, cosmetics and biotechnology use agar because it creates thermo-reversible gels, has clarity, and operates at higher melting and gelling temperatures (Graham et al., 2019; Martínez-Sanz et al., 2019). In healthcare, agar-based materials have garnered interest for their advantageous properties in wound dressing, tissue engineering scaffold development, diagnostic devices, and drug delivery platforms (Armisén, 1991; Dhivya et al., 2015; Khandwal et al., 2025; Lam et al., 2015). Furthermore, Agar's characteristics, which resemble those of extracellular matrices and its ability to encapsulate cells, make it particularly suitable for biomedical applications. Developers utilize agar hydrogel membranes derived from Gracilaria species to create promising controlled drug delivery systems and regenerative medicine solutions, owing to their biocompatibility, biodegradability, and mechanical strength (Kazimierczak et al., 2019; Park et al., 2020; Sudhakar et al., 2024; Vieira et al., 2025).

Research on agar is progressing globally; however, studies focusing on local algal biopolymers from Bangladesh are still limited. The red algae species *Gracilaria tenuistipitata*, abundant in Cox's Bazar's coastal waters, presents a promising sustainable agar source due to its rapid growth, environmental resilience, and high polysaccharide content (Hossain et al., 2021). However, optimized extraction protocols are crucial for maximizing yield and enhancing agar quality. A critical step is alkali pretreatment, which chemically converts L-galactose-6-sulfate into 3,6-anhydro-L-galactose. This structural modification directly improves agar's gelling capacity by reducing sulfate group interference with polymer network formation (Xiao et al., 2021). Although alkali pretreatment is crucial for obtaining high-quality agar, it can also dramatically reduce the extraction yield (Mohibbullah et al.). To unlock the full industrial potential of *G. tenuistipitata*, a systematic investigation is needed to refine alkali treatment parameters—including concentration, temperature, duration, and seaweed-to-alkali ratio. Such optimization will establish a scalable, efficient extraction methodology tailored to this underutilized local resource, ensuring consistent quality for high-value applications such as edible coatings for fruits/vegetables, tissue engineering scaffolds, and controlled drug delivery systems.

This study aims to examine the extraction feasibility of agar from red algae (*Gracilaria tenuistipitata*), which is abundantly available in the coastal area of Cox's Bazar, Bangladesh. This research involves assessing the pretreatment effects of different NaOH concentrations (2%, 4%, and 6%) in comparison to a control group and a water-treated group, with the goal of identifying optimal extraction conditions that not only maximize agar yield but also improve its gelling properties and overall quality. Ultimately, this study aims to promote the sustainable utilization of local marine resources in Bangladesh, thereby driving progress in both food and biomedical applications, while also contributing to economic growth.

2. MATERIALS AND METHODS

2.1 Materials and Instrumentation

This study used a range of high-grade chemicals and advanced equipment. The red sea algae (*Gracilaria tenuistipitata*) were collected from Nuniarchara Sea Beach, Cox's Bazar. Analytical-grade Sodium Hydroxide and Acetic Acid, sourced from Sigma-Aldrich, were employed for the pretreatment and analysis of the algae samples. The gel strength of the extracted agar was evaluated using a Zwick Roell Z010 Universal Testing Machine (UTM), which applied controlled force through a perforated plate onto cylindrical gel samples. For the drying procedure, a Witeg FD-8 bench-top freeze dryer was utilized. Sample preparation involved a Biobase MS7-H550-Pro magnetic stirrer for precise mixing and heating, while sulfate concentrations were analyzed with a DR 6000 UV-Vis Spectrophotometer (Hach). Additionally, functional group identification in the agar samples was performed using a PerkinElmer Spectrum Two FTIR Spectrometer with a ZnSe system.

2.2 Extraction Procedure of Agar

The overall methodology of the work is illustrated in the following flowchart (Fig. 1).

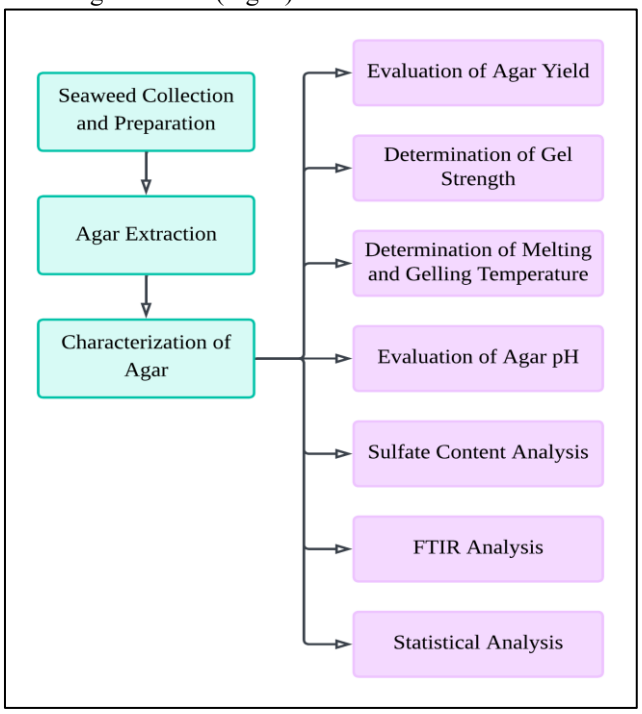

Figure 1: Flow diagram for the extraction and characterization of agar

2.2.1 Collection and Preparation of Algae

Algae samples of the *Gracilaria tenuistipitata* species were collected randomly from the shore and subtidal regions of Cox's Bazar Nuniarchara Sea Beach (21°28′27″ N and 91°57′52″ E). These samples, cultivated by local farmers during the summer season, were placed in poly bags and

transported in a large sack to the Biomaterials Lab at the Department of Biomedical Engineering at MIST (Military Institute of Science and Technology). Upon arrival, the samples were thoroughly washed with tap water to remove any sand and debris. After cleaning, samples were stored in a cold and dark environment in the laboratory for further observation and experimentation.

2.2.2 Extraction of Agar

The extraction process was divided into five distinct pretreatment groups: control, water treatment, and alkali treatments with 2%, 4%, and 6% sodium hydroxide (NaOH). Each group included three replicates, resulting in a total of fifteen extractions. For all extractions, 4 grams of dried algae were accurately weighed and used per sample.

In the control group, the algae samples were boiled directly in distilled water without any pretreatment. For the water treatment group, the seaweed samples were soaked in 200 mL of distilled water at room temperature for two hours, maintaining a seaweed-to-water ratio of 1:50. For the alkali treatments, analytical-grade NaOH was dissolved in distilled water to prepare 2%, 4%, and 6% (w/v) solutions, with corresponding pH values of 12.8, 13.2, and 13.5, respectively. The algae samples were submerged in 200 mL of each alkali solution at room temperature for two hours. Afterward, the samples were neutralized by soaking them in 200 mL of a 0.5% acetic acid solution for 10 minutes followed by thorough rinsing with distilled water to eliminate residual chemicals.

Following pretreatment, all samples, including the controls, were transferred to 500 mL containers filled with distilled water and boiled at 100°C for three hours. This process disintegrated the algae and released the agar content. The hot extracts were filtered through a cotton towel to separate solid residues. The filtrates were poured into plastic containers and allowed to cool at room temperature, where gelation occurred naturally. The gels were subsequently frozen at -20°C for 24 hours. After freezing, the gels were thawed, and excess water was carefully removed by decanting. The gels were then placed on Whatman filter paper for additional drying before being transferred to petri plates and freeze-dried using a Witeg FD-8 bench-top freeze dryer. The yield of each sample was calculated based on the final dried weight. Representative figures of the dried agar samples are shown in Figure 2.

Figure 2: (a) A single dried agar sample, and (b) A collection showcasing all the dried agar samples

2.3 Characterization of Agar

2.3.1 Yield Evaluation

The samples were extracted and poured into 500 mL polypropylene containers after being filtered through two layers of cotton towels. After the filtrate had been subjected to another night of freezing, it was thawed for four hours at room temperature. It was freeze-dried at -80°C using the Bench-top freeze drier FD-8 made by the German manufacturer Witeg. This process continued until no moisture was detected in the filtrate. Agar yield was used to calculate the quantity of agar recovered. It was calculated using the formula below and expressed as a percentage:

$$\%\text{Yield} = \frac{\text{Amount of Dried Extracted Agar}}{\text{Amount of Dried Algae Used}} \times 100\%$$

2.3.2 Gel Strength Measurement

Agar gel was homogenized by heating in a 50 mL beaker. Approximately 20 cc of agar sample was homogenized and poured into cylindrical molds (diameter: 5 cm) to prepare test samples. The gels were allowed to set at room temperature for at least 2 hours. The final gel samples had a thickness of approximately 2.5 cm.

Agar gel strength was tested using a Universal Testing Machine (UTM) at a penetration rate of 1 mm min^{-1}. A perforated disc plate with a diameter of 3.8 cm was placed on top of each gel. A plunger was used to push the disc into the gel, causing the gel to extrude through the perforations. The peak force (in Newton) required for penetration was recorded. Gel strength was calculated by dividing the peak force by the area of the disc and expressed in Newton per square centimeter (N/cm^2).

2.3.3 Melting and Gelling Temperature Measurement

The agar gels were placed in a beaker and heated on a hot plate to increase their temperature gradually. The melting point of the agar was determined using glass beads. The temperature at which the glass beads, placed on the surface of the agar, began to sink into the jelly was recorded as the melting point. The gelling point, on the other hand, was identified as the temperature at which the glass beads ceased to move within the molten agar as it solidified.

2.3.4 pH Measurement

The pH measurement of agar is crucial in scientific and industrial applications, as it influences microbial growth, gel strength, nutrient availability, and the quality control of food and pharmaceutical products (A & G, 2024; El-Beltagi et al., 2022; Hossain et al., 2021; Mohibbullah et al., 2023). Accurate pH determination enables the formulation of customized culture media and the creation of optimal environments for biotechnological and biomedical applications, thus driving advancements in research, technology, and product development. In this study, the pH of agar samples was measured using a calibrated pH and temperature meter (Adma AD8000). All pH measurements were taken while the agar was in its molten state to ensure consistency and accuracy.

2.3.5 Sulfate Content Measurement

The sulfate content in agar, an important indicator of its purity, was measured using a DR 6000 UV-Vis Spectrophotometer (Hach). The presence of sulfate is critical as it reflects the degree of polysaccharide extraction from algae cell walls and can influence the gelation, stability, and biological activity of agar (Sasuga et al., 2017; Xiao et al., 2021). To determine the sulfate content, agar samples were first dissolved in distilled water. Each liquid sample was prepared by taking 10 mL of the dissolved agar solution, and approximately 1 mL of each sample was transferred into UV cells for analysis. A specific reagent, SULFAVER-4, was added to the solutions to chemically react with the sulfate ions and form a colored complex. The solutions were vigorously shaken for approximately three minutes to ensure thorough mixing, followed by a 10-minute period for complete color development. After the reaction, the absorbance of each sample was measured at a wavelength of 420 nm using the UV-Vis spectrophotometer. The sulfate concentration in the solutions was directly quantified and expressed in milligrams per liter (mg/L).

2.3.6 FTIR Analysis

The FTIR analysis aimed to identify and characterize the functional groups present in the agar samples. This analysis was conducted using a Perkin-Elmer Spectrum Two machine. Initially, the agar samples were dried and ground into a fine powder, which was then mixed with potassium bromide to form circular pellets. These pellets were exposed to infrared radiation, inducing molecular vibrations that resulted in the absorption or emission of infrared radiation. The resulting spectral patterns were quantified, enabling the identification of specific functional groups and chemical bonds within the samples. FTIR analysis was performed on control, water-treated, and alkali-treated samples at concentrations of 2%, 4%, and 6%. For each sample, a graph was generated, and the peaks and troughs were compared to those of the standard agar sample to assess their similarities. The functional groups present in the samples were estimated based on the wavenumbers identified in the spectral graphs.

2.3.7 Statistical Analysis

The physicochemical properties of the samples were statistically evaluated using one-way ANOVA, with the support of the Statistical Package for the Social Sciences (SPSS) software. Statistical significance will be determined based on p values predetermined to be less than or equal to 0.05.

3. RESULTS AND DISCUSSIONS

3.1 FTIR Analysis

Agar consists primarily of agarose and agaropectin, two components with distinct properties and roles. These constituents contribute to the versatility and wide-ranging utility of agar. (Hossain et al., 2021; Mohibbullah et al., 2023). The chemical structure of agar is shown in Figure 3. The Fourier-transform infrared (FTIR) spectroscopy analysis of agar samples provides a comprehensive understanding of their chemical structure and composition, revealing distinctive functional groups within the polysaccharide matrix. This study thoroughly analyzed the FTIR band spectra of several agar samples within the range of 4000 cm^{-1} to 400 cm^{-1}. The purpose was to get insights into their molecular structure and possible uses.

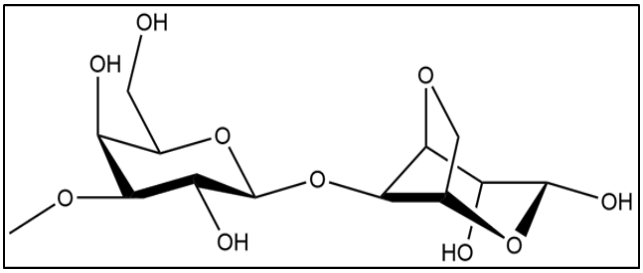

Figure 3: Chemical Structure of Agar

According to the studies, the identification of characteristic peaks at specific wavenumbers in the FTIR spectra suggests the presence of functional groups in the agar samples. The presence of sulfate groups at the C-4 position in D-galactose units, C-6 of L-galactose units, and 3,6-anhydro-D-galactose C-O linkages is indicated by bands at around 820 cm^{-1}, 845 cm^{-1}, and 931 cm^{-1}, respectively (Gómez-Ordóñez & Rupérez, 2011; Souza et al., 2012). Furthermore, the peaks observed at 1026.3 cm^{-1} and 1067.5 cm^{-1} are attributed to the stretching of the C–O bond in primary alcohols (Sobuj et al., 2021). Additionally, the bands within the range of 990 cm^{-1} to 1150 cm^{-1} indicate the presence of glucose (Rhein-Knudsen et al., 2017; Sukhikh et al., 2022; Thombare et al., 2023). Table 1 concisely presents the functional groups detected in agar by FTIR peaks, providing an overview of their chemical composition and importance. Furthermore, Figures 4 to 10 exhibit graphical illustrations of the FTIR study, demonstrating a comparative view of the spectra acquired from various agar samples belonging to distinct treatment groups.

Table 1: Functional groups identified by FTIR peaks in agar

Wavenumbers (cm^{-1})	Functional Group
3391	O-H stretching; vibration of water and hydroxyl groups
2932	C-H stretching; vibration of methyl and methylene groups
1629	C=O stretching; vibration of carboxylic acids and esters
1370	C-H bend
1250	C-O-C stretching; vibration of ether linkages
1072	C-O stretch
931	C-O stretching; vibration of 3,6-anhydrogalactose units, which are unique to agar
845	C-O-C bending; vibration of pyranose rings
820	Sulfate group

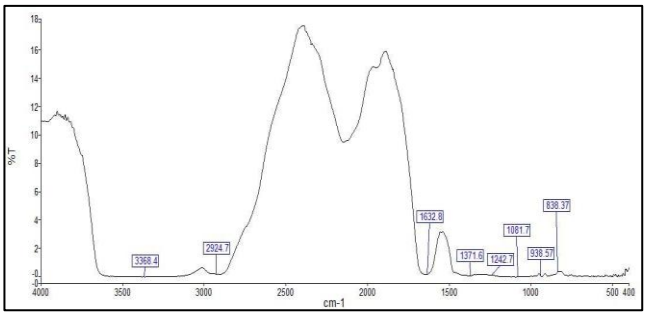

Figure 4: Fourier-Transform Infrared (FTIR) analysis of commercial Agar-Agar

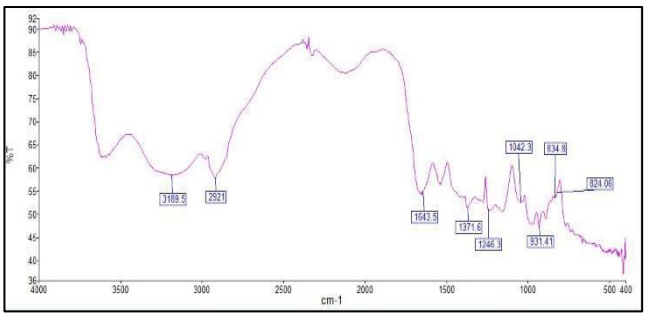

Figure 5: Fourier-Transform Infrared (FTIR) analysis of control sample

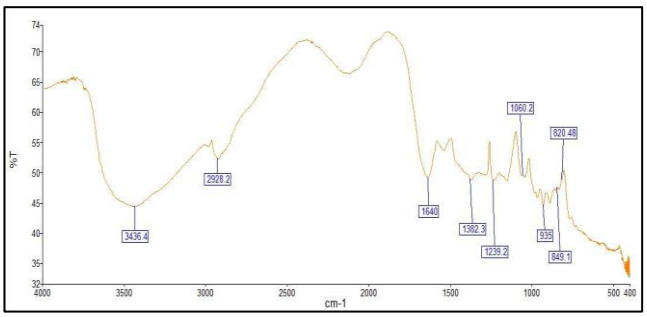

Figure 6: Fourier-Transform Infrared (FTIR) analysis of water-treated sample

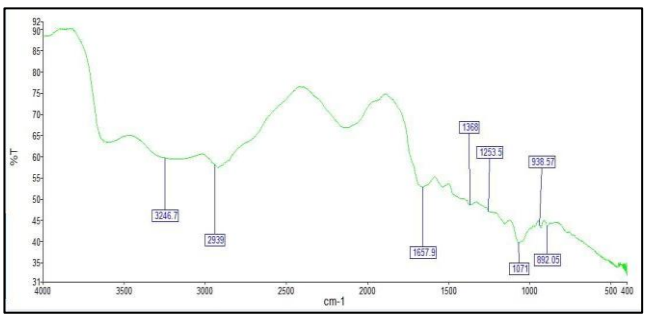

Figure 7: Fourier-Transform Infrared (FTIR) analysis of 2% NaOH-treated sample

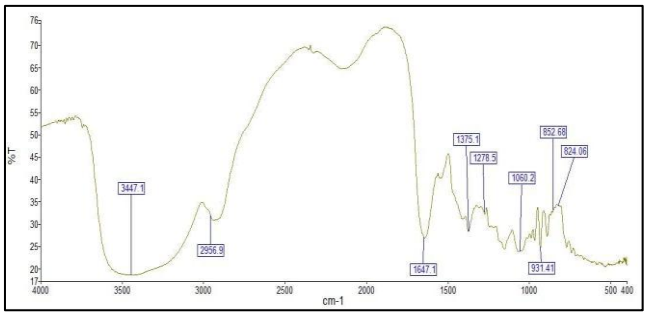

Figure 8: Fourier-Transform Infrared (FTIR) analysis of 4% NaOH-treated sample

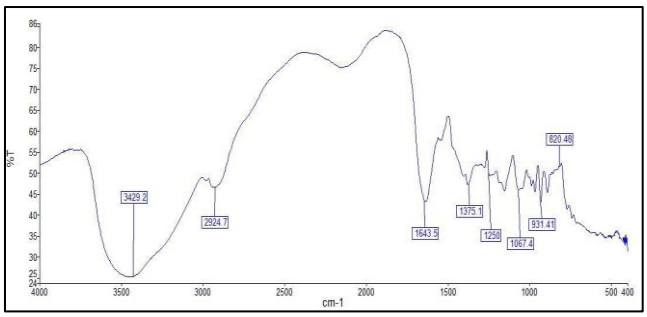

Figure 9: Fourier-Transform Infrared (FTIR) analysis of 6% NaOH-treated sample

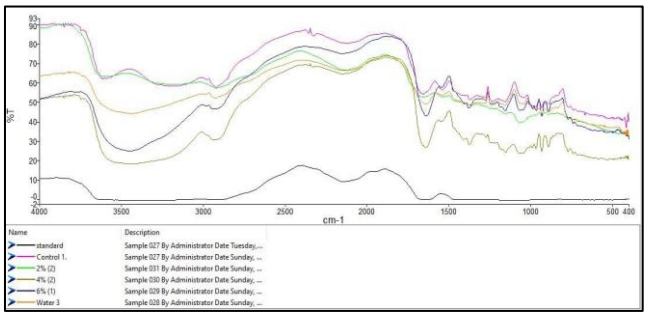

Figure 10: Fourier-Transform Infrared (FTIR) analysis of all samples

Based on the FTIR spectra, the control sample shows stronger and additional absorption bands, including a distinct peak at 845 cm^{-1}, which may indicate the presence of residual impurities. In contrast, the spectrum of commercial food-grade agar-agar appears broader with fewer sharp peaks, likely due to the inclusion of additives or preservatives. Differences in peak sharpness and intensity among samples suggest variations in chemical composition and purity. The water-treated sample exhibits elevated baseline absorbance but less defined peaks, indicating inefficient impurity removal. NaOH-treated samples exhibit more pronounced spectral differences: the 2% NaOH treatment yields fewer sharp peaks, suggesting effective yet gentle purification, while the 4% NaOH treatment provides more distinct peaks, indicating improved clarity and purity. However, the 6% NaOH treatment, despite higher absorbance, may lead to structural degradation due to the harshness of the treatment. FTIR analysis thus provides insight into the

chemical structure and quality of extracted agar (Fujiwara et al., 2023). The presence of similar functional groups in both the extracted and commercial agar samples supports the potential for industrial and biomedical applications (Qari & Haider, 2021).

3.2 Determination of Yield Percentage

The extraction yield percentage is a vital metric that indicates the efficiency of agar extraction and the influence of various pre-treatment conditions on the yield obtained from red algae (*Gracilaria tenuistipitata*). Table 2 presents a summary of the yield percentages for each group.

Table 2: Yield percentage of agar extracted from red sea algae (*Gracilaria tenuistipitata*)

Treatment Groups	Agar Yield (% DW)			Mean ± Standard Deviation
	Extraction 1	Extraction 2	Extraction 3	
Control	15.00	17.50	17.50	16.67 ± 1.44
Water	12.50	15.00	15.00	14.17 ± 1.44
2% NaOH	12.50	12.50	10.00	11.67 ± 1.44
4% NaOH	10.00	12.50	7.50	10.00 ± 2.50
6% NaOH	7.50	10.00	7.50	8.33 ± 1.44

The results demonstrate a significant decrease in yield for the treated agar samples compared to the control group, which exhibited a yield of 16.67 ± 1.44%. This decline can be attributed to structural modifications induced by the pre-treatment processes, as well as the presence of impurities in the control samples that may have artificially inflated their yield measurement. The control group retained its native polysaccharide structure, along with potential contaminants. In contrast, the water treatment yielded 14.17 ± 1.44%, likely resulting in the partial dissolution of some agar components, although it also removed some impurities. The alkali treatments, utilizing concentrations of 2% to 6% NaOH, showed progressively lower yields, ranging from 11.67 ± 1.44% to 8.33 ± 1.44%. This reduction can be attributed to several key factors. Firstly, the alkaline hydrolysis of glycosidic bonds in agarose results in the breakdown of polymer chains. Additionally, the excessive removal of sulfate groups, which are crucial for the gel-forming properties of agar, contributes to the decline in yield. Furthermore, the more thorough elimination of non-agar impurities, which were present in the control group and contributed to its higher yield, also plays a significant role in this reduction. The lowest yield recorded in the 6% NaOH treatment (8.33 ± 1.44%) is particularly noteworthy, as illustrated in Table 2. This reduction is a consequence of the harsh alkaline conditions, which caused significant degradation of polysaccharides, loss of smaller fragments during filtration, and the complete removal of impurities. These findings are consistent with previous studies indicating that higher alkali concentrations can substantially reduce agar yield while enhancing purity and gel strength (Sasuga et al., 2017; Xiao et al., 2021; Mohibbullah et al.,2023). The one-way analysis of variance (ANOVA) confirmed that the pretreatment significantly affected agar yield ($F(4, 10) = 11.29$, $p = 0.0001$). Post-hoc analysis indicated significant differences between the control and treatment groups. The trends are illustrated in the bar chart in Figure 11, which shows the control group with the highest yield, followed closely by the water treatment group. Conversely, the yields for the 2%, 4%, and 6% NaOH treatment groups progressively declined, reaching a minimum in the 6% NaOH group.

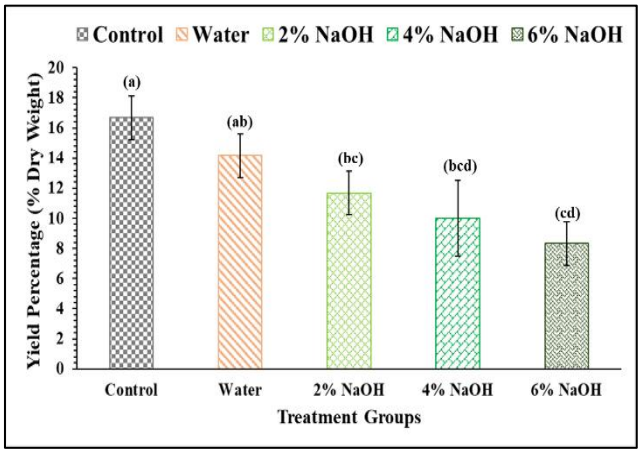

Figure 11: A bar chart illustrating descriptive statistics (mean ± standard deviation) of agar yield percentage and Tukey HSD post-hoc comparisons for each pretreatment group

3.3 Determination of Gel Strength

The gel strength of agar extracted from *Gracilaria tenuistipitata* was evaluated to determine the influence of different pre-treatment conditions on its physical properties. The results, summarized in Table 3, revealed significant variations in gel strength across the treatment groups. The control group exhibited the highest gel strength at 4.67 ± 0.64 N/cm², indicating the retention of a robust gelling network structure. In contrast, the water-treated samples showed the lowest gel strength at 1.44 ± 0.66 N/cm², suggesting that water pretreatment disrupts the agar's gelling network. Among the NaOH-treated groups, agar extracted with 2% NaOH demonstrated optimal gel strength (3.25 ± 0.67 N/cm²), comparable to commercial agar standards (3.38 ± 0.26 N/cm²). This finding highlights the suitability of 2% NaOH treatment for producing agar with industrially relevant gelling properties. However, higher NaOH concentrations led to a progressive decline in gel strength, with values of 3.16 ± 0.38 N/cm² for 4% NaOH and 2.34 ± 0.42 N/cm² for 6% NaOH. The reduction in gel strength at higher NaOH concentrations can be attributed to excessive sulfate group removal and polymer degradation, as supported by previous studies (Sasuga et al., 2017; Xiao et al., 2021; Mohibbullah et al.,2023).

Table 3: Gel strength of agar extracted under different pre-treatment conditions

Treatment Groups	Gel Strength (N/cm²)			Mean ± Standard Deviation
	Extraction 1	Extraction 2	Extraction 3	
Control	5.40	4.45	4.17	4.67 ± 0.64
Water	1.12	0.99	2.20	1.44 ± 0.66
2% NaOH	2.48	3.71	3.55	3.25 ± 0.67
4% NaOH	3.59	2.86	3.03	3.16 ± 0.38
6% NaOH	2.78	1.94	2.30	2.34 ± 0.42

The one-way ANOVA confirmed that pretreatment significantly influenced agar gel strength ($F(5, 12) = 12.53$, $p = 0.0002$). Tukey HSD post-hoc comparisons further highlighted the significant differences among groups. The 2% NaOH group ranked third in gel strength, statistically similar to the commercial agar and 4% NaOH groups, but significantly higher than the water treatment group. The 4% NaOH-treated group showed the fourth-highest gel strength, significantly exceeding the water-treated group but not differing substantially from the 6% NaOH group. The 6% NaOH group exhibited the fifth-highest gel strength, reflecting the detrimental effects of excessive alkali treatment on gelling properties. Figure 12 provides a graphical representation of the gel strength data, emphasizing the significant variations across groups. The results demonstrate that while alkali pretreatment reduces agar yield (as shown in Table 1), moderate NaOH concentrations, particularly 2%, optimize gelling properties, making the extracted agar suitable for applications requiring specific textural characteristics. These applications include food preservation (as coatings for fruits and vegetables) and pharmaceutical uses, such as stabilizing drug solutions to enhance shelf life.

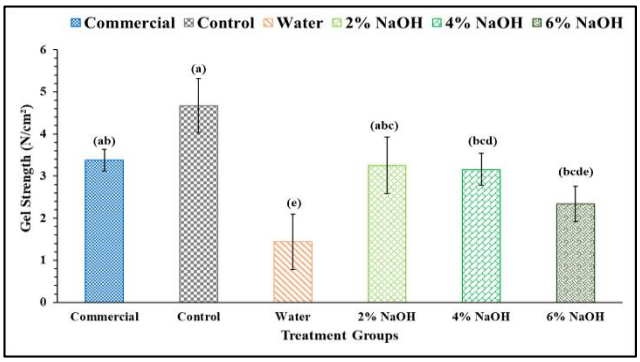

Figure 12: A bar chart illustrating descriptive statistics (mean ± standard deviation) of agar gel strength and Tukey HSD post-hoc comparisons for each pre-treatment group

3.4 Determination of Melting Temperature

The melting temperature of the extracted agar was determined to assess the influence of various pretreatment conditions. The melting temperature was measured for each of the 15 extractions, as well as for three commercial agar samples, and the results are summarized in Table 4.

Table 4: Melting temperature of agar extracted under different pre-treatment conditions

Treatment Groups	Melting Temperature (°C)			Mean ± Standard Deviation
	Extraction 1	Extraction 2	Extraction 3	
Control	92.50	90.60	90.80	91.30 ± 1.04
Water	81.20	80.50	79.20	80.30 ± 1.01
2% NaOH	83.40	84.20	85.00	84.20 ± 0.80
4% NaOH	79.20	80.10	82.30	80.53 ± 1.59
6% NaOH	80.10	81.00	79.40	80.17 ± 0.80

The melting temperature reflects the structural integrity and purity of agar, both of which are influenced by pretreatment conditions. The findings revealed significant differences in melting temperatures among the groups. The control group exhibited the highest melting temperature at 91.30 ± 1.04°C, indicating the presence of impurities that increased the melting point. In contrast, the water-treated group showed the lowest melting temperature at 80.30 ± 1.01°C, suggesting that water treatment may have disrupted the agar's structure and reduced its thermal stability. Similarly, the 4% NaOH- and 6% NaOH-treated groups had melting temperatures of 80.53 ± 1.59°C and 80.17 ± 0.80°C, respectively, reflecting a slight decrease in thermal stability due to the excessive removal of structural components, consistent with the observed trends in gel strength. The 2% NaOH-treated group demonstrated a melting temperature of 84.20 ± 0.80°C, which was comparable to that of commercial agar 85.03 ± 0.21°C. This indicates that mild alkali treatment effectively removes impurities while maintaining the structural integrity of agar, making it well-suited for applications demanding high thermal stability, such as in the food industry, microbiological media, cosmetics, and biotechnology. The one-way ANOVA confirmed that pretreatment significantly influenced the melting temperature of agar ($F (5, 12) = 56.37$, $p = 6.45E-08$). Tukey HSD post-hoc comparisons highlighted significant differences among the groups. The control group had a significantly higher melting temperature compared to all other groups, including the commercial, water-treated, and NaOH-treated groups. The commercial agar group exhibited a higher melting temperature than the water, 4% NaOH, and 6% NaOH groups, but was statistically similar to the 2% NaOH group. The melting temperatures of the water, 4% NaOH, and 6% NaOH groups were statistically identical, indicating a similar level of structural disruption across these treatments. Figure 13 illustrates the descriptive statistics (mean ± standard deviation) for the melting temperature of agar under each pretreatment condition, along with Tukey HSD post-hoc comparisons.

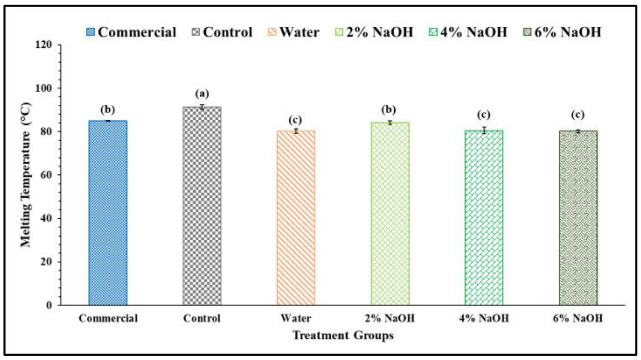

Figure 13: A bar chart illustrating descriptive statistics (mean ± standard deviation) of melting temperature and Tukey HSD post-hoc comparisons for each pretreatment group

3.5 Determination of Gelling Temperature

The gelling temperature of agar, a critical parameter influencing its application in various industries, was measured for samples extracted under different pretreatment conditions and compared to commercial agar. The gelling temperature was determined by monitoring the point at which the agar solution transitioned into a gel. A detailed summary of the gelling temperatures for all groups is provided in Table 5.

Table 5: Gelling temperature of agar extracted under different pretreatment conditions

Treatment Groups	Gelling Temperature (°C)			Mean ± Standard Deviation
	Extraction 1	Extraction 2	Extraction 3	
Control	39.50	36.00	39.10	38.20 ± 1.91
Water	35.00	35.80	33.40	34.73 ± 1.22
2% NaOH	37.40	36.00	35.00	36.13 ± 1.21
4% NaOH	37.50	37.80	36.00	37.10 ± 0.96
6% NaOH	35.80	33.40	34.60	34.60 ± 1.20

The results revealed that pretreatment conditions significantly influenced the gelling temperature of the extracted agar ($F(5, 12) = 3.38$, $p = 0.0388$). The control group exhibited the highest gelling temperature at 38.20 ± 1.91°C, indicating that impurities may have contributed to the higher value. The water-treated group showed a slightly lower gelling temperature of 34.73 ± 1.22°C, reflecting the impact of water pretreatment on the agar's gel-forming properties. Among the NaOH-treated groups, the 2% NaOH treatment yielded a gelling temperature of 36.13 ± 1.21°C, which closely aligns with the gelling temperature of commercial agar (36.17 ± 1.07 °C). The 4% NaOH treatment slightly increased the gelling temperature to 37.10 ± 0.96°C, whereas the 6% NaOH treatment resulted in the lowest gelling temperature at 34.60 ± 1.20°C, indicating that excessive alkali treatment may negatively impact gelling properties. Figure 14 graphically presents the descriptive data, including the mean and standard deviation, for the gelling temperature of agar in each group, alongside the Tukey HSD post-hoc comparisons. The analysis revealed that the 6% NaOH treatment group exhibited a significantly lower gelling temperature compared to the control group, indicating that harsh alkali conditions disrupt the structural integrity necessary for gelling. However, no significant differences were observed between the control group and the water, 2% NaOH, 4% NaOH, or commercial agar groups. Similarly, the gelling temperatures of the water-treated group and the NaOH-treated groups (2%, 4%, and 6%) were statistically comparable to that of commercial agar, except for the 6% NaOH group.

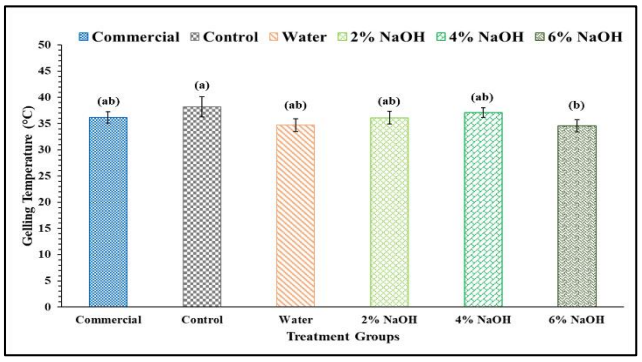

Figure 14: A bar chart illustrating descriptive statistics (mean ± standard deviation) of gelling temperature and Tukey HSD post-hoc comparisons for each pre-treatment group

3.6 Determination of pH

The pH of agar is a crucial parameter that determines its quality, stability, and suitability for various applications. In this study, the pH of extracted agar samples was measured using a digital pH meter calibrated with standard buffer solutions to ensure accuracy. The pH values of the samples, subjected to different pretreatment conditions, are summarized in Table 6.

Table 6: pH of agar extracted under different pre-treatment conditions

Treatment Groups	pH			Mean ± Standard Deviation
	Extraction 1	Extraction 2	Extraction 3	
Control	6.80	6.75	6.82	6.79 ± 0.04
Water	7.90	8.00	8.10	8.00 ± 0.10
2% NaOH	7.20	7.58	7.70	7.49 ± 0.26
4% NaOH	7.20	7.03	7.23	7.15 ± 0.11
6% NaOH	7.44	7.74	7.58	7.59 ± 0.15

The results revealed that the pretreatment conditions significantly influenced the pH of the extracted agar ($F(5, 12) = 26.03$, $p = 4.74E\text{-}06$). Agar derived in the control

group exhibited a slightly acidic pH of 6.79 ± 0.04, indicating the natural acidity of the algae. In contrast, the water-treated group had the highest pH value at 8.00 ± 0.10, reflecting a shift toward alkalinity due to the removal of specific acidic components during water pretreatment. The NaOH-treated groups exhibited intermediate pH values, with the 2% NaOH treatment yielding a pH of 7.49 ± 0.26, the 4% NaOH treatment producing a pH of 7.15 ± 0.11, and the 6% NaOH treatment resulting in a pH of 7.59 ± 0.15. These values indicate that the concentration of alkali used during pretreatment directly affects the pH of the resulting agar. The pH of the commercial agar sample (7.51 ± 0.06) closely aligned with that of the 2% NaOH treatment group, suggesting that mild alkali treatment is likely employed during commercial agar production. This consistency highlights the suitability of 2% NaOH pretreatment for producing agar with a pH similar to commercial standards, balancing both quality and functionality. Figure 15 presents a bar chart illustrating the descriptive statistics for the pH values, including the mean and standard deviation for each group, alongside Tukey HSD post-hoc comparisons. The statistical analysis revealed significant differences among groups. The water-treated group had a significantly higher pH than all other groups. In contrast, the 6% NaOH group exhibited the second-highest pH, considerably higher than the control and 4% NaOH groups, but not statistically different from the 2% NaOH and commercial groups. The commercial group's pH was significantly higher than the control group, but not substantially different from the 2% NaOH and 4% NaOH groups. No significant differences were observed between the 2% NaOH and 4% NaOH groups, although the 2% NaOH group had a significantly higher pH than the control group.

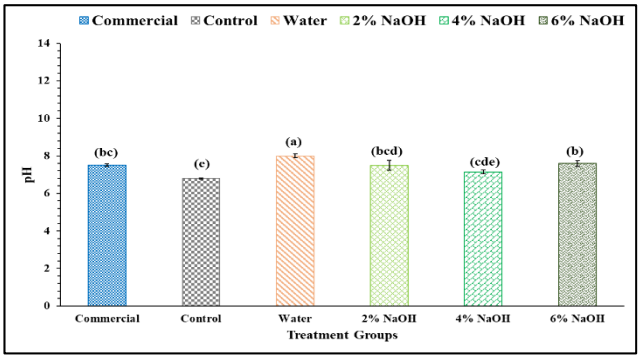

Figure 15: A bar chart illustrating descriptive statistics (mean ± standard deviation) of pH and Tukey HSD post-hoc comparisons for each pre-treatment group

3.7 Determination of Sulfate Concentration

The sulfate content in agar is a critical parameter that reflects its purity and significantly impacts its gelling capacity, stability, and biological activity (Sasuga et al., 2017; Xiao et al., 2021). This study evaluated the sulfate concentration in agar extracted from *Gracilaria tenuistipitata* under different pretreatment conditions, with results summarized in Table 7. The findings revealed that pretreatment had a significant influence on the sulfate concentration of the extracted agar ($F(5, 12) = 517.18$, $p = 1.54E-11$). The control group exhibited the highest sulfate content at 24.00 ± 1.00 mg/L, similar to the water-treated group, which had a sulfate concentration of 23.00 ± 1.00 mg/L. The marginal reduction in the water-treated group may be attributed to the mechanical agitation during the process, which likely removed some surface contaminants and salts. In contrast, the NaOH-treated groups demonstrated a substantial reduction in sulfate content, confirming the effectiveness of alkali treatment in improving agar purity.

Table 7: Sulfate concentration of agar extracted under different pre-treatment conditions

Treatment Groups	Sulfate Concentration (mg/L)			Mean ± Standard Deviation
	Extraction 1	Extraction 2	Extraction 3	
Control	25.00	23.00	24.00	24.00 ± 1.00
Water	23.00	22.00	24.00	23.00 ± 1.00
2% NaOH	3.00	4.00	4.00	3.67 ± 0.58
4% NaOH	3.00	4.00	2.00	3.00 ± 1.00
6% NaOH	2.00	3.00	2.00	2.33 ± 0.58

Precisely, agar treated with 2% NaOH showed a significant reduction in sulfate concentration, measuring 3.67 ± 0.58 mg/L. Further reductions were observed with higher concentrations of NaOH, as the 4% NaOH-treated group had a sulfate content of 3.00 ± 1.00 mg/L, and the 6% NaOH-treated group exhibited the lowest sulfate concentration at 2.33 ± 0.58 mg/L. Notably, there was no statistically significant difference in sulfate concentrations among the NaOH-treated groups, suggesting that even mild alkali treatment is highly effective in sulfate removal. Figure 16 illustrates a bar chart of the sulfate concentrations, including mean values, standard deviations, and Tukey HSD post-hoc comparisons. The chart highlights the stark contrast between the control and water treatment groups, which had the highest sulfate levels, and the NaOH-treated groups, which exhibited significantly lower levels. Therefore, the results emphasize the role of NaOH pretreatment in enhancing the quality of extracted agar by reducing sulfate content, thereby improving its gelling characteristics and overall performance.

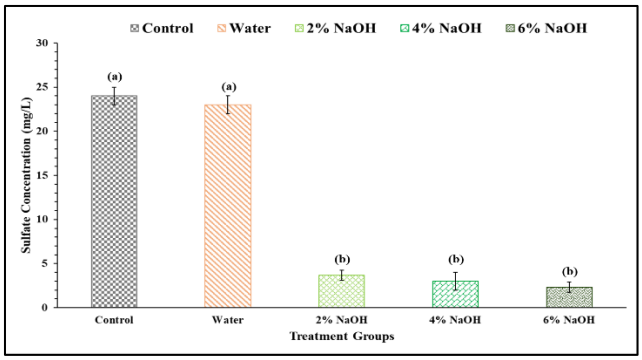

Figure 16: A bar chart illustrating descriptive statistics (mean ± standard deviation) of sulfate concentration and Tukey HSD post-hoc comparisons for each pre-treatment

group

9. CONCLUSIONS

Bangladesh, being endowed with extensive marine biodiversity, offers a sustainable avenue for extracting valuable biopolymer agar from red algae (*Gracilaria tenuistipitata)* abundantly available on Cox's Bazar beach. The preliminary results of this work recognize the high potential of red algae as a promising source of agar. The comparative evaluation of different extraction processes indicates that pre-treatment conditions greatly influence the yield, and physicochemical characteristics and overall quality of the extracted agar. Among all methods studied, the 2% NaOH pre-treatment yielded the most favorable results—producing agar with high gel strength, desirable melting and gelling temperatures, and physicochemical properties (such as pH and sulfate content) comparable to those of commercial-grade agar. Furthermore, the FTIR spectroscopy confirmed the structural integrity and the presence of characteristic functional groups (e.g., sulfate esters, hydroxyl, and glycosidic linkages), which are essential for the agar's performance in various biomedical applications. Overall, these findings suggest that the extracted agar possesses the necessary characteristics for potential use in biomedical fields such as edible coatings for fruits and vegetables, tissue engineering scaffolds, and drug delivery systems.

Through the optimization of the extraction process and the utilization of local marine resources, this work not only creates a greater scope for biomedical advancements but also promotes environmental sustainability and economic benefits. The agar derived from this abundant local source in Bangladesh has the potential to revolutionize healthcare solutions while contributing to sustainable national economic growth.

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DATA AVAILABILITY STATEMENT

Datasets generated during the current study are available from the corresponding author upon reasonable request.

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This research was self-funded.

ETHICS APPROVAL

This study is an engineering experimental investigation. The MIJST Research Ethics Committee has confirmed that formal ethical approval was not required.

ETHICS, CONSENT TO PARTICIPATE, AND CONSENT TO PUBLISH

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Author 1: Fawzia Afrin Rimpy- Methodology, Investigation, Writing manuscript

Author 2: Md Enamul Hoque- Conceptualization, Project Administration, Supervision, Manuscript revision

Author 3: Quazi Farsheed Mahmud- Investigation, Data curation, Writing manuscrip

Author 4: Itmam Nowroj- Methodology, Analysis, Writing Manuscript

Author 5: Sazedur Rahman- Validation, Manuscript revision

Author 6: Tarek El-Bialy- Resources, Manuscript revision

Author 7: M. Azam Ali- Visualization, Manuscript revision

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Portions of this manuscript were assisted by an artificial intelligence language model (ChatGPT, OpenAI). The tool was used solely for language editing, text refinement, and clarity improvement. All content, data interpretation, analysis, conclusions, and final decisions were generated, verified, and approved by the authors. The authors take full responsibility for the accuracy and integrity of the manuscript.

CONFLICT OF INTEREST DECLARATION

The authors declare that they have no conflicts of interest.

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