

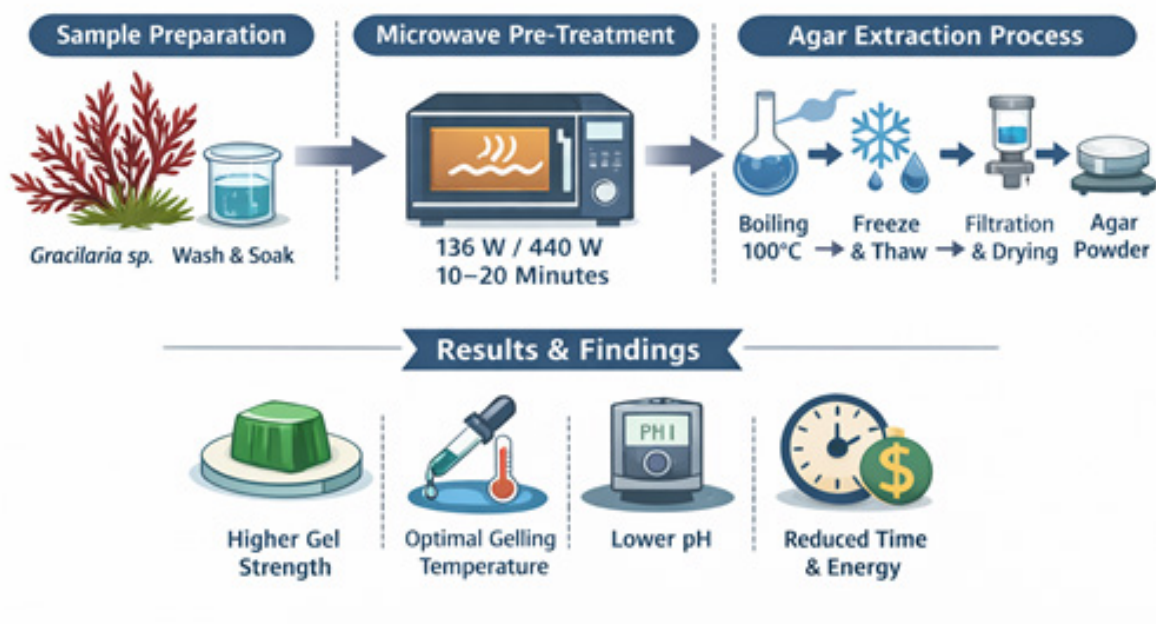
RESEARCH ARTICLE

Effect of Microwave-Assisted Extraction Pre-treatment on the Yield and Quality of Agar from Red Seaweed (*Gracilaria* sp.)

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Graphical Abstract

Microwave-Assisted Extraction of Agar from Red Seaweed (*Gracilaria* sp.)



Highlights

- Microwave-assisted extraction (MAE) significantly reduces processing time compared to conventional boiling methods
- A critical trade-off exists where high microwave power (440 W) increases yield but significantly degrades gel strength
- Optimized conditions (136 W, 10 min) produce agar with superior gel strength outperforming control samples
- FTIR analysis confirms that moderate MAE preserves essential functional groups better than high-power treatments

RESEARCH ARTICLE

Effect of Microwave-Assisted Extraction Pre-treatment on the Yield and Quality of Agar from Red Seaweed (*Gracilaria* sp.)

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Abstract

Conventional agar extraction from *Gracilaria* sp. requires prolonged boiling at high temperatures (100 °C for 2 h) and often yields low-quality agar with unstable gel strength and inconsistent yield. Microwave-assisted extraction (MAE) offers an innovative and sustainable alternative to improve agar recovery from red seaweed while reducing processing time and energy consumption. This study employed a 2 × 3 factorial design using microwave power levels (136 and 440 W) and extraction time (10, 15, and 20 min) to evaluate their effects on the yield and physicochemical properties of agar. Following MAE pre-treatment, the seaweed was boiled (100 °C, 30 min), followed by frozen, thawed, and dried to produce agar, while control samples were proceeded without MAE treatment. Physicochemical characteristics analyses included agar yield, colour, gel strength, gelling and melting temperatures, pH, and syneresis, with functional groups characterised by FTIR spectroscopy. Results indicated that control agar and MAE-treated agar (136 W, 10 min) exhibited significantly higher ($p < 0.05$) gel strength than other treatments. Chroma values were unaffected by MAE, but hue angle decreased significantly ($p < 0.05$) at 136 W (20 min) and 440 W (15 - 20 min). Gelling temperature was significantly higher ($p < 0.05$) in MAE (136 W) samples, while melting temperature increased in MAE (440 W) treatments. Control agar showed significantly higher pH ($p < 0.05$) than MAE-treated agar, and syneresis was greatest at 440 W for 20 min. The optimised MAE condition (136 W, 10 min) produced agar with superior gel strength and substantially reduced processing time. These findings demonstrate the potential of MAE as a time and energy efficient pretreatment for agar extraction from *Gracilaria* sp.

Keywords: Agar; Gel strength; *Gracilaria* sp.; Microwave-assisted extraction; Physicochemical properties

INTRODUCTION

Seaweeds are photosynthetic microalgae that play important ecological roles in marine ecosystems such as oxygen production, carbon sequestration and as the basis of food webs (Yadav, 2022). Seaweeds are commonly classified into three major groups based on their pigmentation and taxonomic characteristics: red, brown, and green seaweeds. Rhodophyta (Red seaweeds) namely *Kappaphycus*, *Eucheuma* and *Gracilaria* are economically important as they are a source of carrageenan and agar (Lim et al., 2017). Several *Gracilaria* species are distributed throughout tropical and subtropical regions. In Malaysia, *G. changii*, *G. blodgettii* and *G. coronopifolia* have been

reported from Sarawak, while *G. manilaensis* is in Johor and *G. changii* has been reported from Selangor (Andriani et al., 2016; Othman et al., 2018). *Gracilaria* sp. is also one of the main sources of agar that is widely cultivated worldwide and utilized for commercial agar production (Mendes et al., 2022).

Agar is a complex polysaccharide extracted from red seaweed, consisting of agarose and agarpectin. The agar content in *Gracilaria* can range from 11.64% to 20.67%, depending on the species, extraction technique and cultivation method (Belattmania et al., 2021; Mendes et al., 2022). Due to its excellent gelling, thickening and stabilizing, and film forming properties, agar is widely

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used in the food industry and has numerous applications in the biomedical and biotechnology fields (Surendran & Sherje, 2023).

Conventional Agar production from *Gracilaria* sp., particularly *Gracilaria gracilis*, is important due to its high yield, abundance, and ease of cultivation (Gioele et al., 2017; Mouedden et al., 2024). Conventionally, agar is extracted from red seaweed using hot water extraction combined with alkali pre-treatment, involving steps such as alkali pre-treatment, extraction at 95–121 °C, filtration, purification by freeze-thaw cycles, and drying (Chung et al., 2011; Gómez Barrio et al., 2022). Alkali pre-treatment softens the thalli and removes sulfate groups, converting galactose-6-sulfate to 3,6-anhydrogalactose to improve gel strength (Vuai, 2022) and helps agar dissolve more easily during hot extraction (Xiao et al., 2019). However, this conventional method often results in low agar yields (4–12%) and consumes high energy, impacting the environment (Gómez Barrio et al., 2022; Xiao et al., 2019).

Microwave-assisted extraction (MAE) has emerged as an efficient extraction technique for recovering valuable from plant and marine biomass. Microwave energy provides rapid and relatively uniform heating, which can enhance mass transfer and facilitate the release of target compounds from the biomass matrix (Belwal et al., 2018). In red seaweeds like *Gracilaria* sp., offering faster processing by breaking cell walls with microwave energy (Thaiphani et al., 2020). MAE improves yield and quality while protecting heat-sensitive compounds from thermal degradation (Belwal et al., 2018). Optimizing microwave power and extraction time is critical to maximize recovery (Zamanhuri et al., 2021). It is also recognized as a greener method, requiring less solvent and energy (Staicu et al., 2024).

Recent research has demonstrated the potential of microwave technology for agar extraction. Deepika et al. (2024) reported that MAE could double agar yield and increase 3,6-anhydro-L-galactose while reducing sulfate content compared to conventional extraction. However, excessive microwave time or power may degrade polysaccharides (Yan et al., 2010). Despite these benefits, there is insufficient information of how microwave power and treatment time used as a pre-treatment influence both the yield and functional quality of agar extracted from *Gracilaria* sp.

MATERIALS AND METHODS

Materials

Sun-dried red seaweed samples (*Gracilaria* sp.) were received from a *Gracilaria* farm located in Laman Alam Jaya, Johor Bahru, Malaysia, in 2025 and transported to the laboratory for further processing and analysis. Prior to extraction, the samples were vacuum-treated to remove trapped air within their internal structure, then stored in a sealed container at 4 °C until further processing. Sodium hydroxide (NaOH; 5%, w/v) was used for the alkaline

pre-treatment. All chemicals and reagents used were of analytical grade.

Alkaline pre-treatment

Agar extraction from dried *Gracilaria* sp. was conducted according to the method described by Ahmad et al. (2011) with slight modifications. Briefly, 30 g of sample was soaked in 1 L of 5% (w/v) sodium hydroxide solution at 70 °C for 1 h. After washing to remove excess alkali and spin-drying (20 spins), the seaweed was blended with distilled water at a 1:30 (w/v) ratio using a smart cooking processor (Samu Giken, P10+, Japan) at 7000 rpm for 1 min. The resulting homogenized suspension was then transferred into a 1 L container for microwave-assisted extraction (MAE) pre-treatment.

Pre-treatment of microwave-assisted extraction (MAE)

Microwave-assisted extraction (MAE) pre-treatment was performed on *Gracilaria* sp. following Deepika et al. (2024) with slight modifications. The alkali-pretreated, blended samples were placed in 1 L containers and treated using a microwave oven (Toshiba MW2-MM21pF (Bk), Japan) operating at a maximum power of 800 W. Control samples were extracted conventionally without MAE. The MAE parameters are listed in Table 1, and each treatment was conducted in triplicate.

Table 1: Microwave power and treatment times used for microwave-assisted extraction (MAE) pre-treatment of *Gracilaria* sp

Sample	Power (W)	Time (min)
Control	-	-
1	136	10
2	136	15
3	136	20
4	440	10
5	440	15
6	440	20

Agar extraction

Agar extraction from *Gracilaria* sp. followed Ahmad et al. (2011) with slight modifications, including adjusting the hot-water extraction time to 30 min for both MAE-pretreated samples and controls. Extraction was carried out at 100 °C for 30 min using a smart cooking processor (Samu Giken, P10+, Japan). The hot agar solution was filtered through muslin cloth, poured into 650 mL capacity containers, covered, and frozen at −18 °C for 24 h. After thawing at room temperature (27 °C), the separated liquid was removed, and the remaining agar was dried in an oven (Mettler, INB500, Germany) at 60 °C for 24 h. The dried

agar was then cut into pieces (2×5 cm) and stored in airtight containers at room temperature until further analysis.

Agar yield analysis

Agar yield was determined following Hii et al. (2016) by comparing the dry weight of extracted agar to the initial dry weight of the red seaweed. The analysis was performed in triplicate for each sample. The result was expressed as a percentage (% w/w) using the following formula:

$$\text{Yield (\% w/w)} = \frac{\text{Dry weight of extracted agar (g)}}{\text{Initial dry weight of seaweed (g)}} \times 100\%$$

Gel strength analysis

Gel strength was measured following Lee et al. (2014). Dried agar was dissolved in distilled water to prepare a 1.5% (w/v) solution, heated at 95 °C for 10 min, then poured into 25 mL moulds and cooled at 4 °C for 24 h to set. Gel samples (20–25 mm thick) were tested using a texture analyser (Shimadzu, AGS-J 500 N, Japan) with a 10 mm diameter cylindrical probe moving at 1 mm/s to a depth of 5 mm. The analysis was performed in triplicate for each sample. Maximum force (g) recorded was divided by the probe's cross-sectional area to express gel strength in g/cm².

Colour analysis

The Colour of dried agar was measured following Menaka & Wijesekara (2025) using a portable colour meter (Konica Minolta, CR-400, Japan). The instrument was calibrated according to the manufacturer's instructions before measurement. L* (lightness), a* (green–red), and b* (blue–yellow) values were recorded from five random points on each evenly levelled sample. The analysis was performed in triplicate for each sample. Chroma (C*) and hue angle (h°) were then computed based on the L*, a*, and b* values using the following equations:

$$C^* = \sqrt{a^{*2} + b^{*2}}$$

$$h^\circ = \tan^{-1} \left(\frac{b^*}{a^*} \right)$$

Gelling and melting point analysis

The gelling and melting temperatures of agar gels were determined to characterize their thermal properties. For gelling point determination, 1.5% (w/v) agar solutions (10 mL) were prepared following Menaka & Wijesekara (2025), cooled at approximately 1 °C/min in glass test tubes (1.5 cm diameter), and the temperature at which the gel stopped flowing when tilted was recorded as the gelling point.

For melting point analysis, following the method described by Freile-Pelegrín and Murano (2005), a 1.5% (w/v) agar solution (10 mL) was poured into test tubes and allowed to gel at room temperature for 24 h. A glass bead was then placed on the gel surface, the test tube was placed

upright in a water bath starting at 50 °C, and the water bath temperature was gradually increased at a rate of 1 °C/min up to 100 °C. The melting point was recorded as the temperature at which the glass bead sank, indicating the gel had completely melted. Both gelling and melting point analyses were performed in triplicate for each sample.

pH analysis

The pH of agar gels was measured following (Menaka & Wijesekara, 2025). A known amount of agar gel was dissolved in 10 mL of distilled water to obtain a final agar concentration of 0.15% (w/v). The mixture was stirred thoroughly until a homogeneous suspension was obtained. The pH was then recorded using a calibrated pH meter (Mettler Toledo, FiveEasy F20, US) equipped with a glass electrode. The pH meter was calibrated using standard buffer solutions before measurements. The analysis was performed in triplicate for each sample.

Gel syneresis analysis

Gel syneresis was determined following (Menaka & Wijesekara, 2025). Hot agar solution (1.5% w/v, 20 mL) was poured into 50 mL centrifuge tubes, and the initial weight (W₁) was recorded. After cooling at 27 °C to form gels, samples were stored at 4 °C for 12 h for stabilization, then equilibrated to 27 °C for 2 h. Samples were centrifuged at 2150 g for 10 min (Kubota 4200, Japan), and the separated water was removed; the final weight (W₂) was recorded. The analysis was performed in triplicate for each sample. The weight was recorded in gram (g). Syneresis (%) was calculated as the percentage water loss based on the difference between W₁ and W₂:

$$\text{Gel syneresis (\% w/w)} = \frac{W_1 - W_2}{W_1} \times 100\%$$

Functional group analysis using Fourier Transform Infrared Spectroscopy (FTIR)

Chemical composition and functional groups of agar extracted from *Gracilaria* sp. were analyzed using Fourier Transform Infrared Spectroscopy (FTIR) following Hussein (2015). Samples were ground, mixed with potassium bromide (KBr), and pressed into transparent pellets for measurement with a spectrometer (Perkin Elmer, Spectrum 400 FT-IR/NIR, USA) over the range 400–4000 cm⁻¹.

Statistical analysis

All treatments were conducted using three independent replicates (n = 3), and the results are presented as mean ± standard deviation (SD). Statistical analysis was performed using two-way analysis of variance (ANOVA), followed by Tukey's post hoc test, to compare the characteristics of agar extracted using the conventional method with those of agar subjected to microwave-assisted extraction (MAE) pretreatment from *Gracilaria* sp. Differences were considered statistically significant at $p < 0.05$,

corresponding to a 95% confidence level. All statistical analyses were performed using Minitab Statistical Software (version 2022).

RESULTS AND DISCUSSION

Agar yield

Based on Table 2, the control sample recorded the lowest mean agar yield ($6.64 \pm 0.68\%$), which was significantly lower ($p < 0.05$) than the yields of all MAE-pretreated samples, except the sample pretreated at 136 W for 10 min. In contrast, the other MAE-pretreated samples showed significantly ($p < 0.05$) higher agar yields, ranging from $8.66 \pm 0.40\%$ (136 W, 15 min) up to 11.09% (440 W, 15 min). The increase in yield correlated with higher microwave power and longer exposure time, highlighting the effectiveness of MAE in breaking cell walls and enhancing polysaccharide release. Moderate microwave power (440 W) proved more effective than lower power (136 W) in improving extraction efficiency. This may be attributed to the volumetric heating generated by microwave energy, which increases internal pressure within the cells and disrupts cellular structures, thereby facilitating the release of water-soluble polysaccharides such as agar (Mussatto, 2015). Furthermore, higher microwave power also produces stronger dielectric heating, promoting solvent penetration and breaking hydrogen bonds and cell walls, as observed in studies on ion transfer and temperature rise under MAE (Dulanlebit & Hernani, 2023).

The lower yield observed in the from the control sample reflects the limitations of conventional extraction, which relies on slow, uneven heat transfer through conduction. This may result in inadequate disruption of the rigid cell-wall structures, thereby limiting solvent penetration and the diffusion of agar into the extraction medium (Xiao et al., 2021). Overall, these findings show that MAE pre-treatment, particularly at 440 W for 15 min, offers clear advantages in improving agar yield from *Gracilaria* sp., while reducing extraction time and energy consumption compared to conventional methods.

Gel strength

Gel strength is an important functional property of agar, reflecting its ability to form firm and elastic gels, which is highly valued in industrial applications. As shown in Table 2, gel strength was influenced by both microwave power and exposure time, with prolonged treatment resulting in a marked reduction in gel strength. Lower-power MAE (136 W, 10–15 min) maintains gel quality comparable to the control, indicating an optimal balance between cell wall disruption and polysaccharide preservation. The slight increase in gel strength at 136 W for 10 min (476.8 vs. 437.2 g/cm² control) suggests that mild microwave heating efficiently releases intact, high-quality agarose while minimizing chain fragmentation.

In contrast, prolonged or high-power MAE treatment significantly compromises gel strength by inducing polysaccharide chain degradation and loss of functional groups (Zhao et al., 2019). Excessive microwave energy generates internal temperatures and pressures high enough to break glycosidic bonds within agarose chains, producing low-molecular-weight oligosaccharides and monosaccharides (Shen et al., 2023). Furthermore, high-power microwave exposure promotes oxidative degradation of sulfate and hydroxyl groups essential for hydrogen bonding and gel network stability, thereby further reducing gel-forming capacity.

Colour of dried agar

From Table 3, the chroma (C*) values representing the color saturation or intensity of dried agar extracted from *Gracilaria* sp. showed no significant change ($p > 0.05$) after microwave-assisted extraction (MAE) pre-treatment.

This stability in chroma is likely because natural pigments such as carotenoids and phycobiliproteins are mainly influenced by the raw material itself and are less affected by MAE, which uses short treatment times and controlled temperatures. The MAE process focuses on increasing agar yield through volumetric heating and efficient mass transfer, without causing notable pigment degradation.

Table 2: Effect of microwave-assisted extraction (MAE) parameters on agar yield and gel strength of *Gracilaria* sp

Sample	Power (W)	Time (min)	Agar Yield (%)	Gel Strength (g cm ⁻²)
Control	-	-	6.64 ± 0.68^c	437.20 ± 22.40^a
1	136	10	7.90 ± 0.37^{bc}	476.80 ± 21.10^a
2	136	15	8.66 ± 0.40^b	435.27 ± 11.31^{ab}
3	136	20	10.36 ± 0.36^a	334.10 ± 21.00^c
4	440	10	10.36 ± 0.36^a	390.13 ± 9.54^b
5	440	15	11.09 ± 0.68^a	291.19 ± 11.76^c
6	440	20	10.65 ± 0.16^a	290.65 ± 15.73^c

Values indicate mean $\pm$ standard deviation (n=3). ^{a-c} Means with different superscript letters indicate significant differences ($p < 0.05$) between samples within the same column

Table 3: Effect of microwave-assisted extraction (MAE) on chroma and hue angle of dried agar from *Gracilaria* sp

Sample	Power (W)	Time (min)	Chroma (C*)	Hue Angle (°)
Control	-	-	27.36 ± 1.15 ^a	94.62 ± 0.17 ^a
1	136	10	33.06 ± 0.88 ^a	90.01 ± 0.45 ^b
2	136	15	28.17 ± 5.34 ^a	92.87 ± 1.11 ^a
3	136	20	36.94 ± 3.95 ^a	87.98 ± 1.06 ^{bc}
4	440	10	28.17 ± 5.34 ^a	92.87 ± 1.11 ^a
5	440	15	34.87 ± 3.58 ^a	87.24 ± 0.33 ^c
6	440	20	34.35 ± 2.52 ^a	87.63 ± 0.88 ^c

Values indicate mean ± standard deviation (n=3). ^{a-c} Means with different superscript letters indicate significant differences ($p < 0.05$) between samples within the same column

In contrast, the hue angle (h°) values, which indicate the type of reflected color, decreased significantly ($p < 0.05$) in most MAE-treated samples compared to the control, especially at 136 W (10 and 20 min) and 440 W (10, 15 and 20 min), indicating a shift from yellowish tones (control: 94.62°) toward increasingly reddish or brownish hues (440 W, 20 min: 87.63°). This color shift is primarily driven by the Maillard reaction, a non-enzymatic browning process between reducing sugars (abundant in *Gracilaria* sp. as D-galactose and L-galactose units) and amino compounds. Microwave-generated heat promotes condensation reactions between carbonyl groups of reducing sugars and amino groups of proteins, producing brown-colored melanoidin polymers (Rupérez & Saura-Calixto, 2001). This process occurs progressively with increasing MAE intensity, explaining the consistent decrease in hue angle. Secondary mechanisms include oxidation of natural pigments promoted by elevated temperatures and polysaccharide chain degradation generating reactive oligosaccharides that readily participate in Maillard-type reactions. Despite these substantial hue angle changes, the chroma (color saturation) remains stable, suggesting that while the color tone shifts dramatically, the overall intensity of color expression is maintained.

Gelling and melting point

The gelling temperature of agar refers to the temperature at which hot agar solution begins to form a gel during cooling and is mainly influenced by its molecular structure and the strength of interactions between polysaccharide chains (Mohibbullah et al., 2023). As shown in Table 4, agar treated with MAE at 136 W for 10, 15, and 20 min had significantly higher gelling temperatures ($p < 0.05$) than the control and samples treated at 440 W. The progressive increase in gelling point from 10 to 20 min at 136 W (34.1°C to 35.0°C) suggests that extended treatment at this lower power further optimizes cell wall disruption while maintaining polysaccharide integrity, leading to increasingly efficient extraction of high-quality, high-molecular-weight agar (Guo et al., 2019). In contrast, MAE at 440 W produced dramatically lower gelling temperatures due to thermal degradation of agarose chains and disruption of critical

hydrogen bonding (Zhao et al., 2020), with excessive heat breaking polymer chains into shorter fragments with fewer hydrogen bonding sites, resulting in weaker gel structures (Zhang et al., 2021).

Meanwhile, the melting temperature of agar represents the temperature at which the gel structure transitions from a gel to a liquid state and is generally reported to be within the range of $85\text{--}95^\circ\text{C}$. As shown in Table 4, there is an inverse relationship between MAE power/duration and melting point. Control agar exhibited a melting temperature of $83.5 \pm 0.3^\circ\text{C}$, while MAE treatments at 136 W and 440 W resulted in progressively higher melting points, with the highest temperatures recorded at 440 W ($86.1\text{--}87.1^\circ\text{C}$). This trend suggests that higher microwave power and longer treatment times enhance thermal stability. The mechanism differs from that of gelling point, where higher power degrades polysaccharide chains and lowers gel strength, but simultaneously extracts thermally stable components, particularly 3,6-anhydro-L-galactose (3,6-AG), which increases melting temperature (Nour et al., 2021; Suzuki et al., 2001). These opposing trends emphasize critical parameter optimization: applications requiring high gel strength demand low-power MAE (136 W, 10-15 min), while applications requiring high thermal stability benefit from high-power MAE (440 W).

pH of agar

Measuring the pH of agar gel is important because pH can directly affect its functional properties, such as viscosity and gel strength, through its effects on hydrogen bonding and double-helix formation (De Alwis & Wijesekara, 2022; Sinurat et al., 2023). Maintaining a neutral to mildly alkaline pH (approximately pH 7–9) helps preserve functional properties without adverse effects associated with excessive alkalinity (Martínez-Sanz et al., 2020). As shown in Table 5, the control sample had a significantly higher pH ($p < 0.05$) than all MAE-treated gels. Gels treated at 136 W and 440 W for 20 min exhibited lower pH values than those subjected to shorter MAE treatments at the respective power levels. This trend suggests that increasing MAE intensity, either through higher microwave power or

Table 4: Effect of microwave-assisted extraction (MAE) parameters on gelling and melting temperatures of agar from *Gracilaria* sp

Sample	Power (W)	Time (min)	Gelling Point (°C)	Melting Point (°C)
Control	-	-	33.57 ± 0.21 ^c	83.50 ± 0.30 ^e
1	136	10	34.10 ± 0.17 ^b	84.17 ± 0.15 ^d
2	136	15	34.27 ± 0.15 ^b	84.73 ± 0.21 ^d
3	136	20	35.00 ± 0.20 ^a	85.43 ± 0.21 ^c
4	440	10	32.00 ± 0.10 ^e	86.10 ± 0.20 ^b
5	440	15	32.87 ± 0.06 ^d	86.80 ± 0.27 ^a
6	440	20	33.07 ± 0.12 ^d	87.10 ± 0.10 ^a

Values indicate mean ± standard deviation (n=3). ^{a-c} Means with different superscript letters indicate significant differences ($p < 0.05$) between samples within the same column

longer treatment duration, may contribute to a reduction in gel pH, potentially due to accelerated polysaccharide hydrolysis and increased release of hydrogen ions (Mamiru & Gonfa, 2023). These effects are supported by previous studies indicating that MAE can alter the chemical environment, promote degradation of polysaccharide side chains, and induce oxidation of small molecules, potentially contributing to a decrease in gel pH (Martiny et al., 2022).

Gel syneresis of agar

Syneresis in agar gel refers to the release of water from the gel matrix due to network contraction, negatively affecting texture, sensory properties, and stability, particularly in food applications (Priyashantha et al., 2019). Table 5 shows that the control sample exhibited low syneresis ($1.578 \pm 0.399\%$), indicating stable water retention in conventionally extracted agar. MAE treatment at 136 W for 10 and 15 min produced similarly low syneresis values ($1.478 \pm 0.292\%$ and $1.847 \pm 0.218\%$, respectively), suggesting good gel stability. Lower-power, shorter-duration MAE treatments maintain tighter gel networks of agarose and agarpectin, effectively reducing syneresis and preserving gel stability (Martínez-Sanz et al., 2020). In contrast, high-power MAE (440 W) causes significant degradation, breaking polysaccharide chains and disrupting hydrogen bonding. This leads to a less structured, more porous gel with reduced water retention capacity. As a result, syneresis progressively increased with extended 440 W treatment (from 2.173% at 10 min to 2.877% at 20 min), indicating cumulative degradation. These findings demonstrate that low MAE parameters (136 W, 10 min) better preserve gel quality and water-holding capacity compared to high-intensity treatments.

Functional group of agar

Fourier Transform Infrared Spectroscopy (FTIR) was used to analyze the chemical composition and functional groups of agar from *Gracilaria* sp. Key absorption bands were identified across all samples (Figure 1). Broad band at $3200\text{--}3400\text{ cm}^{-1}$ (O–H stretching) indicated hydroxyl groups and hydrogen bonding, with intensity decreasing

at higher power and longer MAE durations, particularly 440 W for 20 min, suggesting polysaccharide degradation or water loss. Bands near 2920 cm^{-1} (C–H stretching) and around 1650 cm^{-1} (C=O stretching) were consistently observed across all treatments, showing no significant variation. In contrast, the sulfate ester band at 1250 cm^{-1} was strong in the control and 136 W (10 and 15 min) samples but weakened at longer times and higher power, indicating desulfation likely caused by thermal or hydrolytic cleavage of sulfate ester bonds.

Significant changes were also observed in the $930\text{--}1070\text{ cm}^{-1}$ region, corresponding to C–O–C stretching vibrations of 3,6-anhydro-L-galactose (3,6-AG), a key structural unit responsible for agar's gel-forming capability. Samples treated under low MAE conditions (136 W for 10 and 15 min) exhibited strong, well-defined bands in this region, comparable to the control, indicating effective extraction and preservation of this functional group. Conversely, samples treated at 136 W for 20 min and all 440 W treatments showed progressive reductions in band intensity, with the weakest signal observed at 440 W for 20 min, suggesting breakdown of 3,6-AG structures and loss of gel-forming potential. Overall, FTIR confirms that low MAE conditions (136 W, 10 and 15 min) maintain agar structure and gel properties, while excessive power or duration promotes desulfation and structural degradation.

Correlation between parameters

Pearson correlation analysis (Table 6) explored relationships among physicochemical properties and gel quality of agar extracted from *Gracilaria* sp. Agar yield showed a strong negative correlation with gel strength ($r = -0.804$, $p < 0.01$), indicating that higher yield does not necessarily produce stronger gels. This trade-off arises because intensive MAE (high power, long duration) efficiently releases agar but simultaneously degrades agarose chains, increasing the proportion of oligosaccharides and agarpectin, which are less capable of forming stable gels. Gel strength also correlated positively with pH ($r = 0.809$, $p < 0.01$) and hue angle ($r = 0.656$, $p < 0.01$), suggesting that milder treatment conditions preserve molecular interactions and

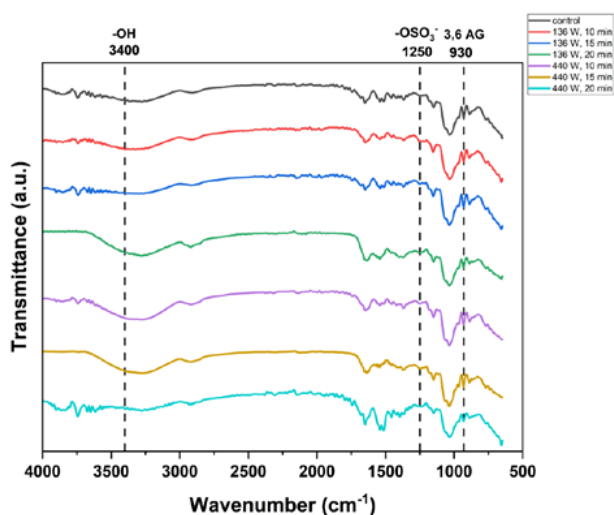
Table 5: Effect of microwave-assisted extraction (MAE) parameters on pH and gel syneresis of agar from *Gracilaria* sp

Sample	Power (W)	Time (min)	pH	Gel Syneresis (%)
Control	-	-	8.79 ± 0.02 ^a	1.58 ± 0.40 ^{de}
1	136	10	8.51 ± 0.09 ^b	1.48 ± 0.29 ^c
2	136	15	8.45 ± 0.08 ^b	1.85 ± 0.22 ^{cde}
3	136	20	8.19 ± 0.15 ^c	2.37 ± 0.29 ^{abc}
4	440	10	8.30 ± 0.04 ^{bc}	2.17 ± 0.06 ^{bcd}
5	440	15	8.26 ± 0.12 ^{bc}	2.59 ± 0.08 ^{ab}
6	440	20	8.05 ± 0.08 ^c	2.88 ± 0.04 ^a

Values indicate mean ± standard deviation (n=3)

Table 6: Pearson correlation coefficients between physicochemical parameters and gel quality attributes of agar from *Gracilaria* sp.

Parameters	Gel Strength (g cm ⁻²)	Agar Yield (%)	Hue Angle (°)	Melting point (°C)	Gelling point (°C)	pH
Agar Yield (%)	-0.804					
Hue Angle (°)	0.656	-0.378				
Melting point (°C)	-0.879	0.857	-0.474			
Gelling point (°C)	0.261	-0.524	-0.149	-0.608		
pH	0.809	-0.625	0.483	-0.733	0.175	
Gel Syneresis	-0.878	0.752	-0.561	0.895	-0.329	0.767

**Figure 1:** FTIR spectra showing the functional group characteristics of agar extracted using microwave-assisted extraction

structural features, enhancing gel stability. In contrast, gel strength showed strong negative correlations with melting temperature ($r = -0.879$, $p < 0.01$) and syneresis ($r = -0.878$, $p < 0.01$), while melting temperature correlated positively with agar yield ($r = 0.857$, $p < 0.01$) and syneresis ($r = 0.895$, $p < 0.01$).

These trends indicate that high-power MAE produces thermally stable but structurally compromised agar fractions, enriched in 3,6-anhydro-L-galactose, which melt at higher temperatures but are prone to water loss (Sousa et al., 2012, 2013). pH negatively correlated with yield ($r = -0.625$, $p < 0.01$) and syneresis ($r = -0.767$, $p < 0.01$), reflecting that higher power and prolonged treatment times lowers pH, promotes denser gel packing, (Sousa et al., 2012, 2013) and increases water expulsion (Ghebremedhin et al., 2021). Overall, these correlations highlight the importance of optimizing MAE parameters to balance extraction yield, gel strength, thermal stability, and functional quality.

CONCLUSIONS

This study successfully evaluated the effects of microwave-assisted extraction (MAE) pre-treatment on the physicochemical properties and functional characteristics of agar extracted from *Gracilaria* sp. The results remonstrated that particularly 136 W for 10–15 min, provided a favourable balance between extraction efficiency and agar quality. Under these conditions, agar exhibited comparatively higher gel strength and desirable gelling and melting properties while maintaining stable colour characteristics. Conversely, higher power (440 W) and prolonged treatment times caused polysaccharide degradation, reducing gel strength, thermal stability, and

increasing syneresis. FTIR analysis confirmed that essential functional groups such as sulfate and 3,6-anhydrogalactose remained largely intact under moderate MAE conditions, whereas higher power (440 W) and prolonged treatment times weakened these signals. Correlation analysis demonstrated that maximizing yield alone does not ensure superior gel properties, emphasizing the critical need to balance extraction efficiency with agar quality preservation. These findings provide valuable guidance for optimizing MAE parameters in agar production from *Gracilaria* sp., enabling the agar processing industry to achieve superior product quality and functional performance based on specific application requirements.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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AUTHOR CONTRIBUTION STATEMENT

M.I.M.: Conceptualization, Formal analysis, Writing – original draft. **N.S.Z.:** Investigation, Methodology, Writing – original draft. **N.S.S.-S.:** Validation, Writing – review and editing. **N.S.M.R.:** Supervision, Validation, Funding acquisition, Project administration, Writing – review and editing. **S.N.L.M., S.A.-H., N.M.T., and S.J.L.:** Writing – review and editing.

DATA AVAILABILITY STATEMENT

All data supporting this study are available from the corresponding author upon reasonable request.

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