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Original Article

Comparative Study of Antioxidant and Physicochemical Properties of *Gracilaria fisheri* Agar Obtained using Ultrasound-assisted Extraction without and with Enzymatic Treatment

Noorain Nasuha Omar[1], Nor Hayati Ibrahim[1,2*], Nizaha Juhaida Mohamad[1], Nurmahani Mohd Maidin[1], Herlina Marta[3], Shamini Nair Achudan[4]

[1] Faculty of Food Science and Agrotechnology, Universiti Malaysia Terengganu, 21030, Kuala Nerus, Terengganu, Malaysia

[2] Functional Food RIG, Food Security in a Changing Climate SIG, Food Security Research Cluster, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

[3] Department of Food Technology, Faculty of Agro-Industrial Technology, Universitas Padjadjaran, Sumedang 45563, Jawa Barat, Indonesia

[4] Farm's Best Food Industry Sdn. Bhd., Lot 37 & 38, Masjid Tanah Industrial Area, 78300 Masjid Tanah, Melaka, Malaysia

[*] **Correspondence email:** yati@umt.edu.my

Abstract

Ultrasound-assisted extraction (UAE) is increasingly utilised in agar extraction as an alternative to conventional methods. However, the UAE alone may not be sufficient to maximise the functional quality of agar. In this study, we compared the antioxidant and physicochemical properties of *Gracilaria fisheri* agar (AGF) extracted using UAE, without and with enzymatic treatment (0.006% Viscozyme® L), referred to as UAEE. The extraction was done using a solid-to-water ratio of 1:98, an ultrasonic power of 506 W, and a temperature of 60 °C. The AGF from UAEE exhibited significantly ($p < 0.05$) lower IC_{50} value (0.13 mg/mL) than that from UAE (0.49 mg/mL), indicating a reduced amount of sample necessary to neutralise half of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals. Additionally, UAEE provided AGF with a significantly ($p < 0.05$) higher ascorbic acid equivalent antioxidant capacities (AAEC) of 11125.78 mg AA/100 g, compared to 2829.82 mg AA/100g for UAE. The enhanced antioxidant activity of AGF from UAEE may be attributed to its higher contents of total phenolic compounds, total sugars, and sulfate. Despite its higher sulfate content, it exhibited significantly ($p < 0.05$) higher melting properties, signifying its superior gelling properties over AGF from the UAE. However, it showed significantly ($p < 0.05$) lower ferric reducing antioxidant power, likely due to its lower galacturonic acid content relative to AGF from UAE. Overall, combining the UAE with enzymatic treatment resulted in a significantly

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higher AGF functional quality, offering a promising approach for the efficient and sustainable AGF extraction.

1. Introduction

Red seaweed (Rhodophyta) is important in food applications due to sulfated galactans, which are commercially recognised as agar and carrageenan. Agar is a gelatin-like substance that is a great vegetarian alternative to animal-derived gelatin. Yarnpakdee et al. [1] conducted a study demonstrating the extensive use of agar as an economical and accessible thickening and gelling agent in various food formulations. This is due to its capability to form gels when combined with other liquid foods, such as pudding and jelly, as they cool, resulting in a firm and smooth texture. Agar can also stabilise canned foods, jams, desserts, ice creams, and sauces [2]. The ability of agar to form gels is influenced by a range of factors, including the type, arrangement, and extent of substitution, as well as the molecular weight [3]. It is worth noting that agar obtained from *Gracilaria* species possessed a lower quality of gel due to the high sulfate content present in native agar (4.3%) compared to those that underwent alkaline pre-treatment (1.6-2.6%) [4].

Nonetheless, Imjongjairak et al. [4] discovered the importance of sulfate groups and phenolic compounds in determining the antioxidant activity of agar extracts. The existing conventional extraction method, however, has a noticeable impact on the content of these compounds. This is due to various external factors, including the adverse effects of extraction temperature. It is important to highlight that many studies focused primarily on achieving the extracted agar's maximum yield and gel strength or elastic modulus. For this reason, high extraction temperatures were commonly utilised, with relatively little attention given to preserving antioxidant-related compounds. Furthermore, Imjongjairak et al. [4] reported that increasing the temperature in hot water extraction of *Gracilaria fisheri* agar up to 70 °C led to a higher sulfate content. However, it also led to an undesirable reduction in phenolic compounds in the agar extract, consequently lowering its antioxidant activity. Conversely, employing higher extraction temperatures between 70–100 °C or boiling water led to substantial disruption of sulfate bonds and polysaccharide chains during the extraction process, resulting in a reduction in sulfate content found in the extracts, as observed by Marinho-Soriano and Bourret [5]. Therefore, as previously suggested by Wang et al. [6], using low extraction temperatures (<70 °C) can yield agar extracts with a higher sulfate content. This characteristic is associated with desirable bioactivity effects, including enhanced antioxidant activity.

The extraction parameters applied in conventional methods could yield agar with satisfactory gel strength, but harmed its antioxidant capabilities. Alternative approaches such as ultrasound-assisted extraction (UAE) and enzymatic-assisted extraction (EAE) have been explored to address this issue, but each has limitations. For instance, Khalil et al. [2] reported that excessive sonication during UAE may degrade extract quality, while EAE is less cost-effective due to the high consumption of buffer solutions. To overcome these drawbacks, an ultrasound-assisted extraction combined with enzymatic treatment (UAEE) could maintain the antioxidant properties while preserving necessary gel strength. According to Hamed et al. [7], agars are embedded within the polysaccharide-polymer matrix of the cell wall, containing cellulose microfibrils. In this context, ultrasound treatment could effectively break down the cell walls, enhancing mass transfer and penetration during extraction [8]. Simultaneously, enzymatic treatment can aid in extracting the desired compounds, improving the extraction yield of bioactive agar extract with fewer chemical residues compared to conventional methods [9].

In our recent work, UAEE applied to *G. fisheri* resulted in a notable agar yield of 32.02%, with acceptable gel strength (83.48 g/cm²) and antioxidant activity, as indicated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity (58.05%) [10]. Despite these promising results, further investigation is required to differentiate the antioxidant and physicochemical properties of *G. fisheri* agar obtained using UAE alone versus UAE combined with enzymatic treatment. Such a comparison is significant as it establishes whether enzymatic treatment provides tangible benefits over UAE alone, thereby ensuring scientific clarity and practical relevance in refining agar extraction strategies for improved yield, quality, and bioactivity. This knowledge is also valuable for guiding food, pharmaceutical, and nutraceutical industries in selecting efficient and cost-effective approaches for agar production. Therefore, the objective of this study was to compare the physicochemical and antioxidant characteristics of *G. fisheri* agar extracted using UAE and UAEE, thereby providing a clearer understanding of the added value of enzymatic treatment in UAE-based agar extraction.

2. Methodology

2.1. Materials

Red seaweed (*G. fisheri*) was obtained from a local supplier in Kelantan, Malaysia. A cellulolytic enzyme mixture (Viscozyme® L) was procured from Sigma Chemical Company in St. Louis, Missouri, United States. Only chemicals of analytical grade were employed in all instances.

2.2. Seaweed Powder Preparation

Seaweed was washed using tap water to remove contaminants and sand. Subsequently, it was soaked overnight in water and sun-dried for 3 days. Once fully dry, the seaweed was ground into a fine powder for approximately 10 min and sieved through a 250 µm sieve to achieve a uniform particle size. The resulting sample was stored at 4 °C in a sealed plastic bag before agar extraction.

2.3. Extraction of Agar

The UAE was carried out at a solid-to-liquid ratio of 1:98, with an ultrasonic power of 506 W and a temperature of 60 °C. 9 g of *G. fisheri* seaweed powder was agitated for 6 h in an ultrasonic water bath (JeioTech UC-10 Ultrasonic Cleaner, Korea). Specifically, for the UAEE, agar extraction was performed using Viscozyme® L under conditions adapted from Omar et al. [10]. Before extraction, the seaweed powder was homogenised in an enzymatic extraction solution prepared by diluting 50 µL of Viscozyme® L in distilled water to achieve a final enzyme concentration of 0.006%. After the first hour of agitation in the ultrasonic water bath, the pH of the extraction solution was adjusted to the optimal enzyme performance (Viscozyme® L: pH 4.5) using 0.1 N HCl. After another 5 h of agitation, the enzyme in the UAEE solution was primarily inactivated at 65 °C for 5 min. Next, the residue was separated from both the UAEE and UAE solutions by squeezing it between two muslin cloth layers, then subjected to centrifugation at 10,000 rpm for 10 min at 3 °C (Hettich Zentrifugen D-78532 Tuttlingen, Germany). Following centrifugation, the supernatant was mixed with an equal volume of 95% denatured ethanol and stored overnight in a chiller. The next day, the precipitate was recovered by centrifugation and oven-dried on plastic at 40 °C overnight. The dried agar was then collected and stored in a chiller for further analysis. Figure 1 summarises the extraction process. The agar yield was calculated using the following equation:

$$\text{Agar Yield}(\%) = \frac{\text{Weight of dried agar (g)}}{\text{Weight of dried seaweed (g)}} \times 100 \tag{1}$$

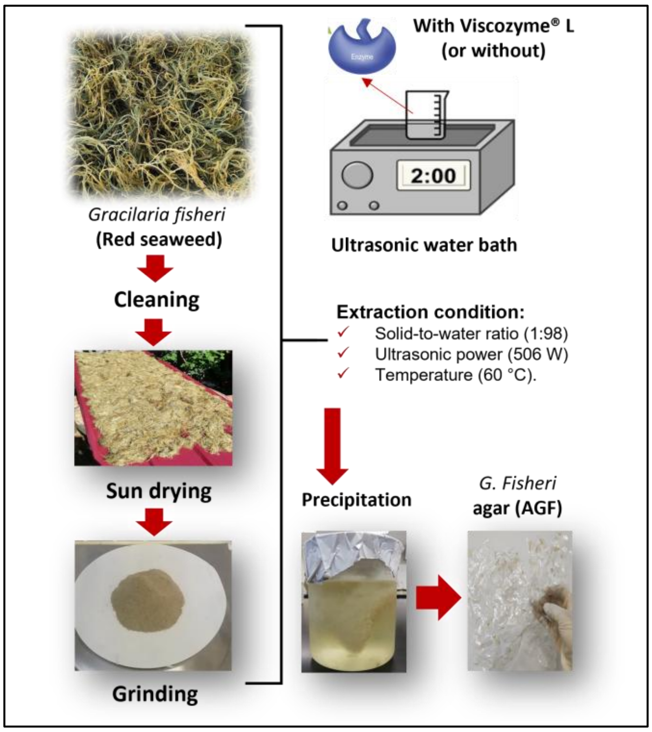

Figure 1. Summary of agar extraction procedure using ultrasound-assisted extraction without or with enzymatic treatment.

2.4. Determination of Antioxidant Properties

2.4.1. Total phenolic content

The phenolic content in *G. fisheri* agar (AGF) was evaluated utilising the Folin-Ciocalteu method. The reaction mixture was prepared by combining 0.5 mL of AGF solution with 2.5 mL of a 10% (v/v) Folin-Ciocalteu reagent, vortexed for 15 s, and then incubated for 2 min at room temperature. Subsequently, 2 mL of a 7.5% (w/v) sodium carbonate (Na_2CO_3) solution was added to the reaction mixture, vortexed again, and incubated for 1 h at room temperature. The absorbance of the resultant solution was measured at 760 nm via a Shimadzu UV-1900i spectrophotometer (Kyoto, Japan). Distilled water (0.5 mL) served as a blank rather than an AGF solution. A calibration curve constructed from gallic acid solutions at concentrations of 2, 5, 20, 40, 60, and 80 ppm ($R^2 = 0.999$) guided the calculations. All measurements were conducted in four replicates, and the total phenolic content (TPC) was reported as gallic acid equivalents (GAE) in milligrams per gram of AGF extracts. A slightly modified version of the method described by Dewanto et al. [11] was employed, and TPC was calculated using the following formula, where c = X/1000 (concentration of gallic acid in mg/mL), v = volume of AGF solution (mL), and m = mass of AGF extract (g).

$$\text{TPC (mg GAE/g)} = \frac{c \times v}{m} \tag{2}$$

2.4.2. Total phenolic content

Total flavonoid content (TFC) in AGF was assessed using the aluminium chloride colourimetric method. 280 μL of AGF solution was combined with 1200 μL of distilled water and 90 μL of a 5% (w/v) sodium nitrate ($NaNO_3$) solution. After vortexing for 1 min, the reaction mixture was left to incubate for 5 min.

Thereafter, 90 µL of a 10% (w/v) aluminium chloride ($AlCl_3$) solution was added to the reaction mixture, which was subsequently vortexed once again and incubated for another 5 min. Finally, 1200 µL of a 0.5 M sodium hydroxide (NaOH) solution was added, and the mixture was incubated for 30 min. Throughout this analysis, all incubation processes occurred at room temperature. Subsequently, the absorbance of the resulting solution was measured at 510 nm using a spectrophotometer (Shimadzu UV-1900i, Kyoto, Japan), with 280 µL of distilled water serving as a blank instead of an AGF solution. The TFC was expressed as quercetin equivalent (QUE) in milligrams per gram of AGF extracts, and the method was slightly modified from de la Rosa et al. [12]. The flavonoid content was calculated using the following formula: c = X/1000 (concentration of quercetin in mg/mL), v = volume of AGF solution (mL), and m = mass of AGF extract (g). The measurements were conducted in four replicates, and a standard curve obtained with quercetin at concentrations of 50, 150, 200, and 250 ppm (R^2 = 0.985) was used for calculations.

$$\text{TFC (mg QUE/g)} = \frac{c \times v}{m} \tag{3}$$

2.4.3. DPPH antioxidant activity and ascorbic acid equivalent antioxidant capacity

The AGF extract's antioxidant activity was assessed using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. A DPPH reagent was prepared at a concentration of 0.1 mM/L in 95% methanol. In the assay, 1 mL of AGF solution at various concentrations (ranging from 50 to 1000 ppm) was thoroughly homogenised with 2 mL of freshly prepared DPPH reagent. Subsequently, the mixture was incubated in the dark for 30 min at room temperature. Following incubation, the absorbance was measured at 517 nm using a UV/Vis spectrophotometer (Shimadzu UV-1900i, Kyoto, Japan). The absorbance of the AGF solution combined with the DPPH reagent (A_{sample}) was calculated by subtracting the absorbance value of the AGF solution without DPPH reagent (A_{blank}). A control sample comprising a mixture without the AGF solution was also prepared. The method used was slightly modified from Brand-Williams et al. [13] and Duan et al. [14], and the percentage inhibition was calculated using the following equation:

$$\text{DPPH inhibition (\%)} = \frac{1-\left(A_{sample} - A_{blank}\right)}{A_{control}} \times 100 \tag{4}$$

The results were also reported as IC50 values. These values were acquired from the linear segment of the graph illustrating DPPH inhibition (%) relative to the concentration of the AGF solution (measured in parts per million, ppm), utilising the following trendline equation: [$y = ax + b$]. The IC_{50} values were calculated by modifying this equation and applying the expression x when the y value equalled 50.0%.

$$\text{AEAC (mg AA/100g)} = \frac{IC_{50}\left(AA\right)}{IC_{50}\left(Sample\right)} \times 100 \tag{5}$$

Subsequently, the antioxidant activity of DPPH was compared to the ascorbic acid equivalent antioxidant capacity (AEAC). This was calculated based on the amount of ascorbic acid equivalent to 100 g of the sample, with AA representing ascorbic acid and IC50 representing the inhibition concentration at 50%.

2.4.4. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was conducted on AGF using freshly prepared FRAP reagent, which comprised a mixture of 0.3 M sodium acetate buffer (pH 3.6), 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), and 20 mM ferric chloride in a ratio of 10:1:1 (v/v). The FRAP reagent was warmed to 37°C for 30 min, following which 1800 µL of the pre-warmed FRAP reagent was added to 240 µL of AGF solution. The mixture was then incubated for 30 min at room temperature. The absorbance was measured at 595 nm using a Shimadzu UV-1900i spectrophotometer from Kyoto, Japan, with 240 µL of distilled water as a blank instead of the AGF solution. The antioxidant capacity was calculated using a standard curve generated using Trolox at concentrations of 2, 4, 8, and 50 µM ($R^2 = 0.9915$). The method used was slightly modified from Benzie and Strain [15]. The calculations were performed using the following formula, where c = X (concentration of Trolox in µM), v = volume of AGF solution (L), and m = mass of AGF extract (g).

$$\text{FRAP } (\mu\text{mol TE/g}) = \frac{c \times v}{m} \tag{6}$$

2.5. Determination of Physicochemical Properties

2.5.1. Total Sulfate Content

The total sulfate content in AGF was determined turbidimetrically using the $BaCl_2$-gelatine method. A capped vial containing 5 mg of AGF was added to 2.5 mL of HCl (1 M) to hydrolyse the sample for 3 h at 100 °C. Upon reaching room temperature, the sample was filtered using Whatman No. 1 filter paper, then mixed with 3.8 mL of a 3% (w/v) trichloroacetic acid (TCA), and 1 mL of $BaCl_2$-gelatine reagent (40 mM $BaCl_2$ and 0.3%, w/v gelatine). The mixture was vigorously homogenised to ensure homogeneity and incubated for 15 min at room temperature. The absorbance of the AGF hydrolysate (A_1) and the control solution (A_2) was measured against the blank solution at 360 nm using a spectrophotometer (Shimadzu UV-1900i, Kyoto, Japan). Distilled water was used as a blank in place of AGF hydrolysate, and gelatine solution was utilised as a control instead of $BaCl_2$-gelatine reagent. The final absorbance of AGF was calculated by subtracting A_2 from A_1. A standard curve was constructed using K_2SO_4 (at 200 to 700 ppm, equivalent to 0.11 – 0.37 mg sulfate/mL). The results of the four replications were expressed in percentages. The method used was modified from Dodgson and Price [16], Lee et al. [17] and Peasura et al. [18].

2.5.2. Total Sugar Content

A phenol-sulfuric acid method was employed to measure the total sugar content of AGF. Approximately 0.2 mL of a 5.0% (w/v) phenol solution and 1.0 mL of concentrated sulfuric acid were homogenised with 0.2 mL of both the AGF solution and standard glucose solutions at concentrations of 20, 40, 60, 80, 100, and 140 ppm. After vortexing the mixtures for 1 min, they were incubated for 20 min at room temperature. The absorbance of the mixtures was measured at 490 nm using a spectrophotometer (Shimadzu UV-1900i, Kyoto, Japan), with distilled water serving as a blank. Glucose standards were applied to construct a calibration curve, enabling estimation of the total sugar content in AGF. Measurements were taken in four replicates, and results were expressed in percentages. The method was modified from DuBois et al. [19].

2.5.3. Galacturonic acid content

Both the AGF solution and galacturonic acid (GalA) standard were prepared at different concentrations (250, 750, 1000, 1250 and 1500 ppm). A fixed volume of AGF solution (750 µL) and GalA standard (300 µL) were mixed with a sodium tetraborate sulfuric acid solution (0.0125 M). After heating at 90 °C for 15 min, the mixture was cooled in an ice bath to stop the reaction. Subsequently, 45 µL of 0.15% (w/v) m-hydroxy biphenyl was incorporated, and the reaction mixture was incubated at ambient temperature for 40 min. The absorbance of GalA in AGF was evaluated at 520 nm using a Shimadzu

UV-1900i spectrophotometer from Kyoto, Japan. All analyses were completed in quadruplicate, and outcomes were reported as percentages. The method was slightly modified from Khan et al. [20].

2.5.4. Fourier transform infrared spectra

Firstly, AGF samples were prepared as KBr pellets (consisting of 2 mg AGF powder and 200 mg KBr) under a hydraulic pressure of 5t cm^{-2} for 5 min. Before acquiring the biopolymer mixtures, the sample holder (KBr crystal) was thoroughly cleaned with acetone, and the background reading or value of the samples was recorded. FTIR spectroscopy (using Perkin Elmer, USA, Spectrum Software V 6.0.1) was employed to acquire the IR spectra of AGF. Each spectrum represented the average of 16 scans, encompassing a spectral range of 4000 – 400 cm^{-1} at a resolution of 2 cm^{-1}, focusing particularly on functional groups, including sulfate, phenolic, and monosaccharide constituents. All measurements were performed in four replicates, and the results were reported in specified ranges of absorbance wavelength associated with these functional groups. The method used was adapted from Khongthong et al. [21].

2.5.5. Gel strength

The agar gel was prepared by mixing agar with boiling distilled water at a concentration of 1.5% (w/v) and stirring on a hot plate stirrer (IKA Labortechnik stirrer, Selangor, Malaysia) until fully dissolved. Subsequently, the agar solution was poured into a plastic container (3 cm in diameter and 3 cm in height) to create a gel that was 15 mm thick. The container was covered and chilled at 4 °C for 12 h. Before analysing, the agar gel was allowed to reach 25–30 °C for 2 h. Gel strength was determined using a Texture Analyser (Stable Microsystem, TA.XT Plus, Godalming, Surrey, United Kingdom) equipped with a P/0.5R cylindrical Delrin plunger and a 5000 g load cell. The analyser operated at a penetration speed of 1 mm/s, with the plunger penetrating the gel to a distance of 5 mm. All measurements were conducted in four replicates, and results were expressed in g/cm^2. The method was modified from Kumar and Fotedar [22] and Lee et al. [23].

2.5.6. Melting behaviour

The thermal characteristics of AGF were assessed through differential scanning calorimetry (DSC) using DSC-Q 2000 from TA, USA. In this analysis, 2 mg of solid agar was precisely weighed and placed in a 100 µL aluminium pan, with an empty pan as the reference. Thermal scanning was carried out under a nitrogen atmosphere at a 50 mL/min flow rate, using the cool-heat method within a temperature range of -10 °C to 220 °C and a heating rate of 5 °C/min. The acquired experimental data were analysed using the TA-Universal software. The measurements were performed in four replicates, and the outcomes were recorded in terms of onset and offset melting temperatures (°C) along with the enthalpy change (ΔH) in J/g. The method was modified from Chen et al. [24].

2.6. Statistical Analysis

The significant differences between AGF extracted using UAEE and UAE regarding their antioxidant and physicochemical properties were established through an independent (two-sample) t-test. The data obtained from four independent replications were analysed using Minitab (Release 14) (Minitab Inc., USA). The significance level is denoted as $p < 0.05$.

3. Results and Discussion

3.1. Yield of Agar

Agar is known to be present within the complex structure of seaweed cell walls, and agar colloids can be effectively diffused out by initially decomposing the epidermis of seaweed [25]. Irrespective of the type of seaweed, the agar yield achieved also differs based on the extraction method employed. In the present study, UAE resulted in a pronouncedly lower AGF yield of 8.52% (± 2.27%), compared to the much higher yield of 32.02% (± 5.05%) achieved through UAEE in our previous work [10]. Although both values fall within the reported yield range for *Gracilaria* species (4.66% to 36.7%) [26,27], the substantial gap between the two highlights the limitations of UAE as a standalone method. While effective in enhancing mass transfer through cavitation, UAE lacks the biochemical capacity to sufficiently disrupt the seaweed cell wall's complex polysaccharide–cellulose matrix. This limitation restricts the release of agar, resulting in a lower extraction efficiency.

In contrast, including enzymatic treatment in UAEE significantly enhances cell wall degradation. Viscozyme® L, a multi-enzymatic complex containing arabinase, cellulase, β-glucanase, hemicellulase, and xylanase, facilitates the hydrolysis of structural carbohydrates, allowing ultrasonic waves to act more efficiently. This synergistic interaction between enzymatic hydrolysis and ultrasonic disruption leads to more complete release and solubilization of agar. Supporting the importance of enzymatic assistance, previous studies have reported improved agar yields when other enzymes were employed. For instance, using catalase in extracting agar from *G. lemaneiformis* yielded 16.08% agar [25], while amylase-assisted extraction from the same species yielded as high as 49.15% [28]. These findings reinforce the crucial role of enzyme selection and its compatibility with extraction conditions in maximising agar recovery. Compared to the UAE alone, UAEE offers a more effective and targeted approach, particularly when using enzyme mixtures like Viscozyme® L specifically tailored for lignocellulosic material degradation.

3.2. Antioxidant Properties of Agar

3.2.1. Total phenolic content

Table 1 reveals that the TPC (2.23 mg GAE/g) of AGF extracted through UAEE using Viscozyme® L was significantly ($p < 0.05$) higher compared to the TPC (1.86 mg GAE/g) of AGF extracted through UAE alone. This finding aligns with the results obtained by Habeebullah et al. [29], who reported lower TPC values in agar attained from non-enzymatic extraction due to phenolic compounds bound to the seaweed cell wall, limiting their extractability. However, our study revealed that combining sonication and enzymatic treatment effectively degraded the cell wall, releasing agar extract and phenolic compounds from *G. fisheri*. This was probably achieved by modifying the pH in UAEE using hydrochloric acid to optimise Viscozyme® L functionality, which operates optimally at pH 4.5. This modification potentially impacted the enzyme's catalytic activity by altering protein configuration and substrate affinity, as Gligor et al. [30] explained, increasing bound phenolic compounds within the UAEE agar. However, the TPC found in AGF extracted through UAEE (2.23 mg GAE/g) was relatively lower compared to the TPC present in agar extracted via EAE using the same enzyme from *P. tenera* (9.40 mg GAE/g) and *G. gracilis* (28.20 mg GAE/g), as reported by Senevirathne et al. [31] and Sapatinha et al. [32], respectively. The difference in TPC values might be influenced by the type of seaweed and the extraction method applied, which is consistent with the observation made by Sapatinha et al. [32] in their research. Furthermore, the phenolic compound within this UAEE agar is anticipated not to withstand the ultrasonic waves (at an ultrasonic power of 506 W) exerted in UAEE. This agrees with Khalil et al. [2], who stated that excessive sonication could potentially reduce the quality of the extracts.

Table 1. The antioxidant properties of *G. fisheri* agar (AGF) extracted using ultrasound-assisted extraction without and with enzymatic treatment.

Antioxidant properties	AGF obtained from		p-value
	UAE	UAEE	
Total phenolic content (mg GAE/g)	1.86 ± 0.07	2.23 ± 0.07	0.001*
Total flavonoid content (mg QUE/g)	8.51 ± 0.29	7.93 ± 0.45	0.083
DPPH antioxidant activity (IC_{50} values) (mg/mL)	0.49 ± 0.01	0.13 ± 0.03	0.000*
Ascorbic acid equivalent antioxidant capacity (AEAC) (mg AA/100g)	2829.82 ± 86.10	11125.78 ± 2589.84	0.008*
Ferric reducing antioxidant power (FRAP) assay (µmol TE/g)	3.57 ± 0.20	1.96 ± 0.10	0.000*

Note: UAE, ultrasound-assisted extraction without enzymatic treatment; UAEE, ultrasound-assisted extraction with enzymatic treatment using Viscozyme® L; The results are expressed as mean ± standard deviation of four replicates (n=4). *The values are significantly different ($p < 0.05$).

On the other hand, the TPC (1.86 mg GAE/g) of AGF extracted through UAE was found to be higher than the TPC previously seen in the polysaccharide of *Gracilaria sp*., extracted using a similar technique but with a different ethanol and water mixture [33]. It has been previously found that about 1.51 mg GAE/g of phenolic compounds were discovered in the extract obtained via a mix of 80% ethanol and 20% water (E80:20). In comparison, roughly 1.79 mg GAE/g of phenolic compounds were present in the extract when a mixture of 50% ethanol and 50% water (E50:50) was utilised. Thus, the current results obtained using 100% water as an extraction medium contradict the general trend reported in earlier investigations. López et al. [34] concurred that, besides the extraction method, the polarity of the solvent also plays a crucial role in influencing the yield of polyphenols. These factors may also affect the type of phenolic groups extracted, either as glycosides or bound to the cellular structure [35]. As previously observed by Sasadara and Wirawan [36], contrary to the extraction yield, water's presence reduces the extracts' phenolic content. Their results indicated a significant inverse correlation between solvent polarity and TPC. Besides the factors described above, the variety of observed TPC could also be influenced by environmental conditions and the geographical locations of seaweed cultivation [37].

According to Maadane et al. [38], phenolic compounds are secondary metabolites usually found in plants and are associated with various biological activities, including protection against oxidative stress damage. Seaweeds commonly contain these compounds as they are regularly subjected to challenging environmental conditions, prompting the synthesis of various metabolites to protect against abiotic and biotic factors [30]. Several previous studies have discussed the antioxidant capacity related to phenolic compounds. In this present study, the bioactive potential of this compound was observed in DPPH antioxidant activity, as further discussed in the following section.

3.2.2. Total flavonoid content

Flavonoids represent a category of plant secondary metabolites known for their polyphenolic structure [39]. This study observed that there was no significant ($p > 0.05$) difference in the total flavonoid content (TFC) between the AGF extracts, as shown in Table 1, even though the value of TFC in AGF extracted through UAE (8.51 mg QUE/g) was comparatively higher than that obtained through UAEE using

Viscozyme® L (7.93 mg QUE/g). The lower TFC in the UAEE extract might stem from the possibility of flavonoid compounds binding to Viscozyme® L, causing enzyme aggregation and subsequent denaturation. Consequently, this hindered the optimal performance of Viscozyme® L in efficiently breaking down the complex structure of seaweed cell walls, thus preventing the release of flavonoids into the extraction medium. This similar finding is previously noted by Nagappan et al. [40], wherein they identified a similar phenomenon, attributing the inhibition of α-amylase and α-glucosidase by the crude extract from *Sargassum* species to the elevated polyphenol content in the extract.

Despite this, the TFC attained in AGF extracted via UAEE still surpassed the TFC found in the polysaccharide of *P. tenera* (1 mg QUE/g) that was also extracted using Viscozyme® L, as reported by Senevirathne et al. [31]. The present values, however, are lower than TFC levels in *Porphyra sp.* (10.74 mg QUE/g), *S. latissimi* (16.49 mg QUE/g), *A. esculenta* (19.44 mg QUE/g), and *G. gracilis* (22.94 mg QUE/g), which were extracted using a similar enzyme, as demonstrated by Sapatinha et al. [32]. These differences in TFC were readily expected due to differences in seaweed species [41]. Besides, the TFC found in AGF extracted through UAE was lower than in *F. vesiculosus* (286.30 mg QUE/g) and *P. canaliculata* (122.60 mg QUE/g), as stated by Garcia-Vaquero et al. [42]. This discovery aligns with Cox et al. [43], who proposed that brown seaweed generally contains higher TFC than red seaweed. Interestingly, flavonoid compounds are acknowledged for their antioxidant attributes, which offer protection against various reactive oxygen species and the capacity to inhibit lipid oxidation [44].

3.2.3. DPPH antioxidant activity and ascorbic acid equivalent antioxidant capacity (AEAC)

DPPH was utilised as a free radical to evaluate the antioxidant characteristics of different compounds by assessing their ability to either scavenge or donate hydrogen to these radicals [45]. Demonstrated through the 50% inhibition capacity (IC_{50}) value, the DPPH antioxidant activity of AGF extracted via UAEE was determined as 0.13 mg/mL. This result was significantly ($p < 0.05$) lower than the AGF extract obtained through UAE, which was recorded as 0.49 mg/mL. The result indicates that the UAEE extract exhibited stronger antioxidant capability, requiring only 0.13 mg/mL of its antioxidant compound to neutralise 50% of DPPH radicals in a sample solution, as opposed to the UAE extract, which required 0.49 mg/mL of antioxidant concentration to achieve the same radical-scavenging efficacy.

The observed results can be predominantly ascribed to utilising a dual approach that combines sonication and enzymatic treatment in UAEE. This method effectively promoted the release of AGF extract, including compounds associated with antioxidants, such as phenolic compounds. This is supported by higher TPC results for AGF obtained from UAEE compared to UAE. Athukorala et al. [46] emphasised in their study that the antioxidant properties of phenolic compounds in polysaccharide extracts can be attributed to their redox characteristics, functioning as both reducing agents and hydrogen donors while neutralising free radicals. This observation is consistent with Devi et al. [47], who observed a strong correlation between antioxidant activity and phenolic content, demonstrating their potential as effective DPPH radical scavengers. Moreover, similar trends were reported by Wang et al. [6] in the polysaccharide extract derived from *Rhodomela condevoides* (red algae) and by de Sousa Oliveira Vanderlei et al. [26] in the polysaccharide extract obtained from *G. birdiae* and *G. cornea*.

The trend of results obtained in this study showed that a higher phenolic compound led to a decreased IC_{50} value but a significantly ($p < 0.05$) higher level of AEAC in the UAEE extract (11125.78 mg AA/100 g). In contrast, the UAE extract with a lower phenolic compound exhibited an increased IC_{50} value and a lower AEAC level of 2829.82 mg AA/100 g (Table 1). This finding aligns with a previous study conducted by Chew et al. [48], who revealed that when the level of phenolic compounds was elevated, the value of IC_{50} was reduced, resulting in a heightened level of AEAC. Furthermore, the superior DPPH antioxidant activity detected in the UAEE extract can be ascribed to its excellent performance in extracting high-quality AGF with desirable sulfate groups, as depicted in Table 1. The study by Imjongjairak et al. [4] validates the correlation between sulfate groups and antioxidant activity, emphasising that polysaccharide extracts with higher sulfate group concentrations exhibit enhanced scavenging activity compared to those with lower sulfate content. This concept aligns with findings by Wang et al. [49], who discussed the ability of sulfate groups in crude sulfated polysaccharides to activate hydrogen atoms in the anomeric carbon, thereby influencing potent hydrogen atom-donating capabilities. The superior antioxidant capacity of AGF from UAEE is strongly anticipated due to the pronounced synergy between the enzymatic treatment and sonication applied in this method. This is supported by Kadam et al. [50], who demonstrated that extracts obtained using enzyme-assisted extraction methods exhibited greater antioxidant capacity than those obtained through traditional extraction techniques.

3.2.4. Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) assay measures the capacity of antioxidants in sample extracts to convert ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) within an acidic environment, indicated by an intense blue colour [51]. During this process, antioxidants transfer electrons, thereby reducing the oxidised intermediates of lipid peroxidation and forming ferrous complexes, thus serving as primary and secondary antioxidants. Based on the results of this study, the average absorbance for AGF extracted through UAE was 0.39, whereas for UAEE, it was 0.27 (data not shown). The higher sample absorbance in the UAE extract reflects the superior reducing capacity of the specific antioxidant compound found in this extract, corresponding to approximately 3.57 µmol TE/g of FRAP value. This value is significantly ($p < 0.05$) higher than the UAEE extract, which reported only 1.96 µmol TE/g.

Notably, the increased reduction capacity observed in the UAE extract is likely attributed to the presence of flavonoid compounds, as the increase in FRAP value corresponded with the elevated flavonoid content in this extract. This finding contrasts with the UAEE extract, which has a low level of flavonoid compounds, as indicated in Table 1. This observation aligns with Steenkamp et al. [52], who noted in their study that flavonoids can scavenge various reactive oxygen species, demonstrating antioxidant properties by mechanisms such as free radical scavenging, chelation of metal ions like iron and copper that could trigger radical reactions, and inhibition of enzymes involved in free radical generation. However, the absorbance values reported for both UAE and UAEE extracts were significantly lower compared to the absorbance of *G. gracilis* extract obtained through EAE using Viscozyme® L (EAV: ~1.000), as reported by Sapatinha et al. [32]. This is supported by Francavilla et al. [53], who demonstrated that FRAP compounds from *G. gracilis* extracted in an aqueous medium were 9 µmol TE/g.

3.3. Physicochemical Properties of Agar

3.3.1. Total Sulfate Content

The current study discovered that the agar extraction method is crucial in determining the amount of sulfate groups in the extract. This distinction was evident as the total sulfate content of AGF extracted through UAEE (1.60%) was significantly ($p < 0.05$) higher compared to AGF extracted via UAE (1.30%), as shown in Table 2. This occurrence is potentially attributed to the synergistic effect of sonication and enzymatic treatment employed in the UAEE process, which could enhance its efficiency in extracting AGF along with the sulfate group, instead of solely using sonication treatment (UAE). Additionally, the variation in sulfate content observed between UAEE and UAE extracts may be due to the high temperature used in the additional procedure (i.e. deactivation of enzymes) implemented in

UAEE. The temperature for this process, set at 65 °C with continuous manual stirring for 5 min, likely had dual effects. It could not only deactivate the enzyme but also promote the further rupture of hydrogen bonds between hydrophilic polymer chains, thereby facilitating the release of agar extract and sulfate groups into the extraction medium.

The present finding is consistent with an observation in the previous study conducted by Imjongjairak et al. [4], who noted a positive correlation between the yield and sulfate content with the temperature employed. Specifically, they reported an enhancement in sulfate content as the extraction temperature elevated from 25 to 55 °C, particularly in samples derived from the same seaweed species. Although the sulfate content obtained in this study was considerably lower than their reported results, it still fell within the acceptable range for the global food market, which stipulates a sulfate content of less than 4%, as indicated by Sousa et al. [54]. Comparatively, the sulfate content found in UAEE extracts exceeded the sulfate content reported for *G. lemaneiformis* agar extracted using other methods, such as EAE and alkaline extraction, which yielded 1.39% and 0.84% sulfate content, respectively [55]. Furthermore, a study by Wang et al. [56] established a positive relationship between sulfate content and scavenging effect, which can be attributed to the strong hydrogen-donating capacity of sulfate groups. This correlation is supported by our findings, as the increased sulfate content in the UAEE extract corresponded to higher DPPH antioxidant activity, as depicted in Table 1.

Table 2. Physicochemical properties of *G. fisheri* agar (AGF) extracted using ultrasound-assisted extraction without and with enzymatic treatment.

Physicochemical properties	AGF obtained from		p-value
	UAE	UAEE	
Total sulfate content (%)	1.30 ± 0.04	1.60 ± 0.01	0.001*
Total sugar content (%)	66.18 ± 0.33	66.71 ± 0.19	0.049*
Galacturonic acid content (%)	22.11 ± 1.22	17.61 ± 1.78	0.009*
Melting behaviour			
T_{onset} (°C)	161.26 ± 8.31	179.56 ± 6.40	0.017*
T_{offset} (°C)	163.99 ± 6.32	180.14 ± 5.91	0.014*
ΔH (J/g)	190.00 ± 20.02	240.30 ± 16.33	0.011*

Note: UAE, ultrasound-assisted extraction without enzymatic treatment; UAEE, ultrasound-assisted extraction with enzymatic treatment using Viscozyme® L; The results are expressed as mean ± standard deviation of four replicates (n=4). *The values are significantly different ($p < 0.05$).

3.3.2. Total sugar content

Agar is composed of repeating units of the sugar galactose, interconnected to form a complex polysaccharide structure. Table 2 reveals that AGF extracted using UAEE exhibited a total sugar content of 66.71% glucose, significantly higher ($p < 0.05$) than AGF obtained through UAE, which contained 66.18% glucose. The increased glucose content in the UAEE-extracted AGF might be attributed to using the enzyme, namely Viscozyme® L, during the extraction process. This enzyme contains various carbohydrases such as xylanase, arabinase, β-glucanase, hemicellulose, and cellulase [57]. These carbohydrases likely facilitated the breakdown of bonds between sugar units, converting them into individual sugar molecules, thereby increasing the glucose content in UAEE extract. This finding, in parallel with the observations made by Meinita et al. [58], indicates that enzymatic saccharification (a process in which complex carbohydrates, such as polysaccharides, are broken down into simpler sugars using enzymes) is more effective in producing glucose than galactose from agar waste during *G. latifolium* agar extraction. Furthermore, Trigueros et al. [59] discovered an enhanced release of glucose, galactose, and arabinose with increased cellulase concentration. Nevertheless, they found that the minimal sugar compounds released occurred when water was used as a solvent without enzyme addition due to the absence of hydrolytic effects. Apart from that, the dominance of glucose in both AGF extracts can be linked to the conversion of galactose to glucose aided by ultrasound, aligning with the findings of Essa et al. [60]. Consequently, the elevated glucose content in both AGF extracts exceeds the glucose content of *G. chouae polysaccharide* (52.63%) extracted via hot water extraction, as reported by Khan et al. [20].

Additionally, the higher glucose content found in the UAEE extract may have contributed to the increased DPPH antioxidant activity compared to the UAE extract (Table 1). This relationship is supported by Essa et al. [60], who emphasised that the type and composition of sugars significantly influence the antioxidant potential in algal extracts. Their study suggests that both glucose and galactose possibly contribute to the antioxidant capabilities of *Jania rubens* extracts. Besides, it is known that sugar compounds are one of the gel-supporting constituents that could influence the gel properties of agar. This is demonstrated in the UAEE extract, where a higher glucose content is consistent with its higher gel strength [10]. Khalil et al. [2] reported a similar outcome and stated that agar is recognised as 'sugar active' due to its capacity to enhance gel strength when combined with sugars.

3.3.3. Galacturonic acid content

In this study, the total GalA content in AGF obtained via UAE was significantly ($p < 0.05$) higher (22.11%) than that in AGF extracted through UAEE, which amounted to 17.61% (Table 2). It is likely that the UAE extract primarily contained galactose, with the observed GalA content possibly resulting from the ongoing oxidation process of this sugar monomer. This stands in contrast to the UAEE extract, which, as discussed earlier, showed a higher composition of glucose content. Moreover, the study revealed that ultrasound effectively disrupted the complex cell wall structures of *G. fisheri*, enhancing the release of AGF containing GalA, a sugar acid. Imjongjairak et al. [4] observed that various factors, including the type and arrangement of sugars, influence the antioxidant activity. Moreover, Jridi et al. [61] noted in their study that the presence of GalA in polysaccharides is crucial for the chelation of ferrous ions. It is believed that the antioxidant activity in the UAE extract was attributed to the ability of GalA to surround and capture the ferrous ions generated during the reduction of ferric ions. This is evidenced by the increased GalA content, aligning with the elevated FRAP value observed in the UAE extract (Table 1). Conversely, the temperature applied in both the UAEE and UAE methods (set at 60 °C) seems to be conducive for the conversion of galactose into GalA through oxidation. This observation aligns with Essa et al. [60], suggesting that higher temperatures may facilitate the conversion of glucose and galactose into glucuronic acid. This understanding extends to the GalA compound within the AGF. Consequently, both agars exhibit higher GalA content compared to that reported by Khan et al. [20] and Chang et al. [62] for *G. chouae* polysaccharides (9.62 and 6.3%, respectively), as well as by Tian et al. [63] for *Sargassum carpophyllum* polysaccharide (6.2%). However, the previous study provided limited insights into the GalA compound, which makes it challenging to substantiate the obtained outcomes with concrete data or explanation.

3.3.4. Fourier transform infrared (FTIR) – spectra of functional groups

Fourier transform infrared (FTIR) spectra in the 4000–400 cm^{-1} region of AGF extracted through UAE with and without enzymatic treatment of four replications are shown in Table 3. Based on spectra, the dominant band was observed at 3417.26–3447.99 cm^{-1} for the UAEE extract and at 3411.35–3441.51 cm-1 for the UAE extract, corresponding to the vibration of O-H groups. Moreover, the bands that are equivalent to the ester sulfate group (-S=O), the skeleton of galactan, and the vibration of the C-O-C bridge of 3,6-anhydro-L-galactose in the UAEE extract were visible at absorbance ranges of 1363.53–1384.96 cm^{-1}, 1088.82–1097.98 cm^{-1} and 927.84–939.74 cm^{-1}, respectively. In the UAE extract, these bands were found at absorbance ranges of 1380.92–1385.76 cm^{-1}, 1075.23–1087.93 cm^{-1} and 930.42–932.16 cm^{-1}, respectively. Furthermore, the phenolic group in the UAEE extract showed absorbances in the 1419.60–1419.70 cm^{-1} range, while the band related to this group might have been too weak to be detected in the UAEE extract. In a previous study by Chen et al. [25], the most important bands associated with the vibration of the sulfate at C-4 of D-galactose units, C-6 of L-galactose units, and C-2 of 3,6-anhydro-L-galactose units were found at 845 cm^{-1}, 820 cm^{-1}, and 805 cm^{-1}, respectively. In the UAEE extract, these bands were observed at the absorbance range 831.54–843.78 cm^{-1}, 825.81–826.40 cm^{-1} and 800.90–811.36 cm^{-1}, respectively. The UAE extract also exhibited vibration in the sulfate group, except for the sulfate at C-6 of L-galactose units. The undetectability of phenolic compounds and the vibration of the sulfate group in the UAE extract could explain the lower values of phenolic content (Table 1) and sulfate content (Table 2) determined turbidimetrically in this AGF extract. Thus, the identified functional groups supported the effectiveness of ultrasound-assisted extraction and enzymatic treatment in extracting high-quality AGF and these valuable compounds.

3.3.5. Gel strength

The AGF obtained via UAE demonstrated lower gel strength (66.20 ± 4.04 g/cm^2) along with reduced sulfate content (1.30%) (Table 2). In contrast to our previous report, the AGF extracted through UAEE exhibited appreciably higher gel strength (85.22 ± 8.75 g/cm2) [10], in line with its elevated sulfate content (1.60%). Similarly, Chen et al. [25] discovered that agar with higher sulfate content displayed superior gel properties, attributed to its increased water retention capacity and viscosity. Nevertheless, the current results contradict Rocha et al. [64], who observed an inverse relationship between gel strength and sulfate content in agar extracts. They stated that agars derived from *Gracilaria* species tend to have higher sulfate levels, consequently reducing gelling capacity. This was also evidenced by the identification of relatively weak gel strength (< 50 g/cm^2) in native agar sourced from *G. lemaneiformis* due to its higher sulfate content, as noted by Xiao et al. [55]. This is because the interference caused by sulfate groups during the gelation process causes significant disruptions in helix formation. These disruptions result in decreased stability and strength of the gel structure, as established by GomesDias et al. [65]. Based on our findings, it appears that the choice of extraction method likely contributes to the variation in the gel properties of AGF. The combined application of ultrasound and the enzyme Viscozyme® L in UAEE synergistically impacts the composition or structure of the AGF extract, leading to enhanced gel strength. In contrast to using UAE alone, which showed reduced gel strength, this observation aligns with the findings of Gómez Barrio et al. [66], who noted a tenfold reduction in the gel strength of agar extracted with ultrasound.

3.3.6. Melting behaviour

The differential scanning calorimetry was employed to examine the melting characteristics of AGF extracted through UAE with and without enzymatic treatment. The analysis revealed that the UAE extract exhibited an onset temperature of 161.26 °C and an offset temperature of 163.99 °C during the melting process, with an enthalpy change of 190.00 J/g. This value was significantly lower ($p < 0.05$) than the UAEE extract, which exhibited a melting onset temperature of 179.56 °C, an offset temperature of 180.14 °C, and an associated enthalpy change of 240.30 J/g. Despite this, both AGF extracts met the commercial standards for melting temperature (>85 ºC). Notably, the melting properties of AGF in this study surpassed those reported by Archana et al. [67] for *G. corticata* agar, where the onset temperature, peak temperature, and enthalpy change were 40.31°C, 77.88 °C, and 144.3 J/g, respectively.

Furthermore, Sousa et al. [54] highlighted a positive relationship between melting temperature and molecular weight, indicating stable interactions within polymer gelling sequences with increased temperature. The film-like structure and thinness of the AGF sample in this study likely contributed to the higher observed melting temperature. A survey by Ganesan et al. [68] also reported high endothermic peaks for edible film from Acanthophora spicifera, ranging between 114.78 and 129.94 °C. It is essential to emphasise the correlation between agar's melting temperature, gel strength, and its 3,6-anhydro-L-galactose content, as evidenced in a study by Chen et al. [25]. This study observed a similar trend where the higher gel strength of the UAEE-extracted AGF corresponded with a higher melting temperature (Table 2). This relationship was anticipated for the 3,6-anhydro-L-galactose content detected in the extract through FTIR analysis (Table 3), suggesting that additional energy was required to disrupt agar's more stable gel network [1].

Table 3. FTIR bands found in *G. fisheri* agar (AGF) extracted using ultrasound-assisted extraction without and with enzymatic treatment.

Specific bands from the previous studies	AGF obtained from		Features
	UAE	UAEE	
3430 cm^{-1} (a)	3411.35 - 3441.51 cm^{-1}	3417.26 - 3447.99 cm^{-1}	Vibration of O-H groups
1650 cm^{-1} (a)	1636.86 - 1654.62 cm^{-1}	1647.50 - 1654.25 cm^{-1}	Amide I vibration
1433.4cm^{-1} (b)	1458.15 - 1459.16 cm^{-1}	1437.69 - 1458.43 cm^{-1}	Uronic acid
1424.2 cm^{-1} (b)	-	1419.60 - 1419.70 cm^{-1}	Phenolic group
1380 - 1355 cm^{-1} (c)	1380.92 - 1385.76 cm^{-1}	1363.53 - 1384.96 cm^{-1}	Ester sulfate group (-S=O)
1080 cm^{-1} (a)	1075.23 - 1087.93 cm^{-1}	1088.82 - 1097.98 cm^{-1}	Skeleton of galactan
1158, 1072 and 1035 cm^{-1} (d)	1035.22 - 1035.26 cm^{-1}	1073.18 - 1077.63 cm^{-1}	Existence of galactose and glucose
963.6 - 927.6 cm^{-1} (b)	969.14 cm^{-1}	950.97 - 968.01 cm^{-1}	Glycosidic linkage
940 - 930 cm^{-1} (c)	930.42 - 932.16 cm^{-1}	927.84 - 939.74 cm^{-1}	Vibration of the c-o-c bridge of 3,6-anhydro-L-galactose
900 - 890 cm^{-1} (c)	887.45 - 896.02 cm^{-1}	886.63 - 890.62 cm^{-1}	Agar-specific band
845 cm^{-1} (e)	840.95 - 855.09 cm^{-1}	831.54 - 843.78 cm^{-1}	Vibration of the sulfate at C-4 of D-galactose units
820 cm^{-1} (e)	-	825.81 - 826.40 cm^{-1}	Vibration of the sulfate at C-6 of L-galactose units
805 cm^{-1} (e)	811.08 cm^{-1}	800.90 - 811.36 cm^{-1}	Vibration of the sulfate at C-2 of 3,6-anhydro-L-galactose units

Note: UAE, ultrasound-assisted extraction without enzymatic treatment; UAEE, ultrasound-assisted extraction with enzymatic treatment using Viscozyme® L.

References: [a]Xiao et al., [55], [b]Essa et al., [60], [c]Souza et al., [69],[d]Gong et al., [70], [e]Chen et al. [24].

4. Conclusion

This study demonstrated that combining ultrasound-assisted extraction with enzymatic treatment (UAEE) using Viscozyme® L offers a sustainable and efficient method for extracting agar from *G.*

fisheri. This approach overcame the UAE's limitations, specifically the low yield and gel strength, and significantly improved TPC, DPPH antioxidant activity, AEAC, total sulfate and sugar content, and melting properties. Although the UAE resulted in higher FRAP and galacturonic acid content, the overall performance of UAEE was superior in producing higher-quality agar with improved functional and bioactive properties. The comparative evaluation between UAE and UAEE is particularly valuable, as it demonstrates the added benefits of enzymatic treatment, thereby informing both scientific understanding and practical decision-making in the development of the extraction process. Furthermore, UAEE aligns with the principles of green technology by minimising chemical inputs and energy demand, supporting the responsible valorisation of *G. fisheri*. Beyond advancing sustainable marine resource management, these findings hold potential for application in food, pharmaceutical, and nutraceutical sectors, strengthening small-scale, seaweed-based industries in tropical coastal regions and contributing to local socio-economic resilience.

Declaration of Conflict of Interest

The authors declared no conflict of interest with any other party in the publication of the current work.

ORCID

Nor Hayati Ibrahim https://orcid.org/0000-0002-6091-9081
Nizaha Juhaida Mohamad https://orcid.org/0000-0003-4984-2324
Nurmahani Mohd Maidin https://orcid.org/0000-0002-1344-8109

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