

Original Article

Enhancing the growth, hydrocolloid, and mineral content of *Gracilaria verrucosa* by selecting seedstock beyond local sources and various cultivation methods

Lestari L. Widowati*, Sri Rejeki, Restiana W. Ariyati, Rosa Amalia

Faculty of Fisheries and Marine Science, Universitas Diponegoro. Jl. Prof. Jacob Rais, Tembalang, Semarang, 50275. Indonesia.

Abstract: The quality of seedlings is essential for optimal growth rate and seaweed quality. The availability of local seedlings often poses constraints on quality and sustainability. This research aims to determine the optimal cultivation method for *Gracilaria verrucosa* using seeds from multiple locations. Seedlings of *G. verrucosa* were sourced from Semarang (S), Brebes (B), and Demak (D). Observations were conducted by cultivating the three seedlings using the off-bottom (O), broadcast (B), and longline (L) methods. A completely randomised design with a combination of seedling origin and cultivation method (3x3), namely SO, SB, SL, BO, BB, BL, and DO, DB, DL, was conducted in Brebes Regency, Central Java. The highest growth rate of *G. verrucosa* was observed in SL (3.21 ± 0.07 % day⁻¹), while the lowest was observed in BB and DB. The broadcast cultivation method was shown to increase agar content across all seed sources, but this increase did not always correlate with gel strength. SB seedlings showed the best combination with high agar content (34.33 ± 0.58 %) and strong gel strength (294.7 ± 6.05 g.cm⁻²), while SO produced the highest gel strength (324.7 ± 5.21 g.cm⁻²) despite having lower agar content (26.65 ± 0.80 %). Integrating selective seed sources and optimised cultivation methods is crucial for enhancing growth, ensuring hydrocolloid quality, and achieving sustainable production of *G. verrucosa*.

Article history:

Received 20 August 2025
Accepted 20 November 2025
Available online 25 April 2026

Keywords:

Agar
Gel strength
Indonesia
Mineral
Seaweed

Introduction

Indonesia's rise as a leading seaweed producer, ranging from 10 to 11.6 million tonnes, significantly impacted the global market, providing a substantial portion of the world's supply from 2016 to 2019 (FAO, 2018). As a result, many coastal communities experienced improved livelihoods and increased job opportunities. *Gracilaria* species are pivotal to global agar production, a gelatinous substance widely used in the food, pharmaceutical, and biotechnology industries. Comprising nearly 175 species, *Gracilaria* is renowned for producing unique galactans known as agar, which are integral to various industrial applications. The agar yield and quality from *Gracilaria* can vary depending on the species and environmental factors. For instance, a study on *Gracilaria gracilis* reported agar yields ranging from 19.19% to 23.31%, influenced by the site of production site. Another study highlighted that

optimising environmental conditions could enhance the yield of *G. tenuistipitata* (Mustafa et al., 2024). Recent studies have further elucidated the factors influencing agar yield and quality in various *Gracilaria* species. For instance, research on *G. gracilis* indicates that environmental factors, including nutrient availability, light intensity, salinity, and water temperature, significantly affect agar yield. Higher agar yields, ranging from 20 to 30%, were associated with elevated salinity levels and optimal light conditions (Banik et al., 2023). Another study, focusing on *G. verrucosa* cultivated in acid-sulfate soil-affected brackish water ponds in South Sulawesi, Indonesia, found that soil iron content and pH, as well as water salinity and nitrate levels, play crucial roles in determining agar content. Specifically, increased soil iron content was associated with reduced agar content, whereas higher soil pH, salinity, and nitrate concentrations were correlated with enhanced agar

*Correspondence: Lestari L. Widowati
E-mail: rrwidowati@yahoo.com

yields (Mustafa et al., 2024). These findings underscore the importance of optimising environmental conditions to improve agar production from *Gracilaria* species.

Recent literature continues to highlight the nutritional richness of seaweeds and the factors influencing their composition. The nutritional composition of seaweeds is significantly influenced by their geographical origin, environmental conditions, and species-specific characteristics. A comprehensive review by Jha et al. (2021) emphasises that seaweed is abundant in proteins, vitamins, minerals, and dietary fibre. The study also notes that bioactive compounds such as polyphenols, polysaccharides, and sterols contribute to the health benefits associated with seaweed consumption. The nutritional profile of seaweeds can vary significantly depending on the species, environmental conditions, and harvesting period (Smith, 2020).

Seaweeds are well-known for their nutritional balance and high concentrations of essential minerals, including Mg, Cu, Fe, and Zn. They also provide macronutrients that function as coenzymes, along with minerals, antioxidants, and vitamins (Filippini et al., 2021). A further highlight was that seaweeds sourced from Italian markets showed differences in mineral content based on whether they were wild-harvested or farm-cultivated. These findings suggest that both the origin and cultivation method significantly impact the nutritional and biochemical properties of seaweeds, underscoring the need for more comprehensive studies in diverse environments. The chemical composition of seaweeds remains inadequately investigated, leaving significant gaps in our understanding of their nutritional and biochemical properties. The limited scope of these studies means that the chemical profiles of seaweeds from other regions, such as tropical or polar environments, remain largely unexamined. As a result, there is a need for more comprehensive research to fully understand the potential of seaweeds as a nutritional resource across various geographic locations and environmental conditions (Wong and Cheung, 2000).

Several techniques are employed in seaweed



Figure 1. The location of seaweed cultivation (Brebes) with seeds comes from Semarang, Demak, and Brebes itself.

farming, including broadcast, longline, and off-bottom approaches (Hayashi et al., 2017). Each region's specific coastal topography shapes the implementation of these methods. The off-bottom technique involves securing *Gracilaria* sp. seeds, bound with raffia rope, to a length of nylon rope or net suspended above the bottom of the water, and using wooden stakes for support. In line with Banik et al. (2023), the long-line method offers several advantages, including lower costs and potentially faster growth of *Gracilaria* sp. compared to traditional stocking methods used by farmers. This method not only minimises but can also prevent pests and diseases affecting *Gracilaria* sp., resulting in quicker growth and reduced material costs. Therefore, this research aims to determine the optimal cultivation method for *G. verucosa* using seeds from multiple locations, and to compare their quality using parameters such as agar yield, gel strength, and mineral composition.

Materials and Methods

Study area: The study was conducted at a brackish-water pond in Randusanga Village, Brebes district, Central Java, Indonesia. The experiment was conducted over one seaweed cultivation cycle (60 days) from May to June in 2-ha ponds. The *G. verucosa* used in this experiment was collected from Demak, Brebes, and Semarang, Central Java (Fig. 1).

Research design: This experiment evaluated three cultivation methods: long-line, off-bottom, and

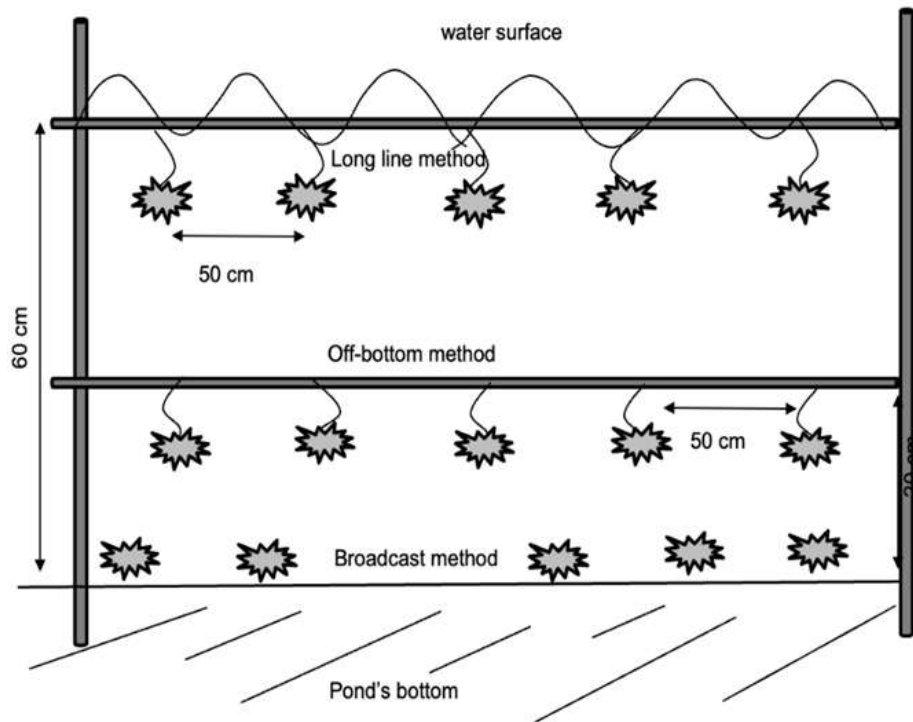


Figure 2. Design of long line, off-bottom and broadcast method to be used in *Gracilaria verrucosa* cultivation.

broadcast (Fig. 2). Bamboo poles 2 m long were embedded in the substrate as main stakes. The horizontal bamboo poles were tied to the main post to support hanging seaweed. For the long-line method, the horizontal bamboo was installed 60 cm from the bottom of the pond, whereas for the off-bottom system, it was installed 20 cm from the bottom. *Gracilaria verrucosa*, each weighing 100 g, were tied to a rope and then hung on the horizontal bamboo with a distance of 50 cm between each tie. The broadcast method involved placing *G. verrucosa* directly on the substrate.

The treatment was a 2-factor design, with the source from different locations (Factor A) and the cultivation method (Factor B). Factor A consists of three treatments: seaweed from Semarang (S), Brebes (B), and Demak (D). Factor B concerned the cultivation method: long line (L), off-bottom (O), and broadcast (B). Thus, the combination of the factors creates the factorial pattern with 3x3 as follows: SL, SO, SB, BL, BO, BB, DL, DO, and DB.

Data collection

Growth: Every cluster of seaweeds was wiped with paper towels to remove excess water, weighed, and

returned to the pond at the beginning and end of cultivation time. Daily growth rate was calculated applying the formula (Ricker, 1975) of $SGR (\%/day) = ((\ln W_t - \ln W_0) / T) \times 100$, where W_0 is the initial wet weight, W_t indicates the final wet weight, and T denotes the days of cultivation.

Agar content and gel strength: Dried seaweeds (500 g) were subjected to pre-treatment with 5 litres of 6% sodium hydroxide (w/w) at 85°C for three and a half hours. Then, it was rinsed several times with normal water and neutralised with 200 mL of 0.5% acetic acid (w/w) for one hour at ambient temperature. After extracting with 200 mL of distilled water at 85°C for 2 hours, the mixture was filtered using a 100% cotton filter cloth. After passing the seaweed combination through a fresh filter cloth, it was left to gel for a full day at room temperature, and the desiccated samples were separated and ready for additional examination.

The air-dried weight was used to calculate the agar yields (Yarnpakdee et al., 2015). The Brookfield CT3 4500 was used to measure gel strength using the TPA Bloom technique. Dried agar (1.5) flour was solubilised in 100 ml of purified water by magnetic stirring for 20 to 30 minutes at 90°C. To eliminate

waves, the solvent was stabilised in an 80-90°C water bath for fifteen minutes (Rejeki, 2018). Twenty-two millilitres of the embedding solution were placed in specially prepared 30-millilitre cups; the cups were refrigerated for 20-30 minutes, then wrapped in aluminium foil and kept overnight at 28°C. The full cups were put on a weighing scale, and a stainless steel cylinder rod (0.786 cm²) was placed on top of the samples. The rod was then squeezed using a rheometer until the gel cracked, at which time the force at the breakage was measured. The samples were placed on top. The gel strength was calculated as the weight at which the gel fractured divided by the surface area of the stainless steel cylinder (Yarnpakdee et al., 2015). The gel's break force in g.cm⁻² was calculated by averaging the results from three cups.

Minerals: The 100-gram sample of *G. verucosa* from each treatment was rinsed with purified water and subsequently dehydrated in an oven at 70°C for 48 hours. The dried *G. verucosa* was ground to a fine powder with a particle size of less than 75 µm. *Gracilaria verrucosa* was employed to ascertain the presence of both primary and secondary elements using the Rigaku NEXCG X-ray fluorescence (XRF) spectrometer. For each sample, a minimum of 3 pellets were produced by compressing the powder at 10 tons for 2 minutes. The resultant pellets were cylindrical, measuring 1.0 mm in thickness and 20.0 mm in diameter. Subsequently, the pellet was secured to a Mylar sheet within a plastic frame and positioned above the X-ray beam for analysis (Chaqmaqchee et al., 2017).

Water quality: Analyses of nitrate, nitrite, ammonia, and orthophosphate were conducted using spectrophotometry according to Indonesian National Standards. SNI 6989-79:2011 measured nitrate by reducing cadmium–zinc to nitrite, diazotising with sulfanilamide, and coupling with NED at 543 nm against potassium nitrate standards. SNI 06-6989.9:2004 detected nitrite at 543 nm using the sulfanilamide–NED azo dye technique with sodium nitrite as reference. The phenate technique (SNI 06-6989.30:2005) quantified ammonia by measuring indophenol blue at 640 nm against ammonium

chloride standards in the presence of phenol, hypochlorite, and sodium nitroprusside. SNI 06-6989.31:2005 identified orthophosphate by generating a phosphomolybdate–ascorbic acid combination and reading at 880 nm with KH₂PO₄ as reference.

Statistical analysis: The analysis was performed using SPSS version 20.0.0. The normality of the data was assessed using Shapiro-Wilk tests, and the homogeneity of the data was examined using Levene's tests at the 95% significance level. A two-way ANOVA was conducted to determine the effects of seed source and cultivation method on SGR, agar content, gel strength, and mineralization. The Duncan Multiple Range Test (DMRT) was employed to compare group means post hoc, with differences assessed as significant at the acceptable level of $P < 0.05$. Meanwhile, the water quality in the pond in *G. verucosa* is described by descriptive analysis.

Results

The assessment of normality and homogeneity indicated that the data were normally distributed and homogeneous. The results showed that both location and cultivation method had significant effects ($P < 0.05$) on SGR, agar content, and gel strength. However, no significant interaction effect (location × method) was observed ($P > 0.05$), indicating that the influence of cultivation method on growth and agar quality was consistent across all seed origins.

Growth: The specific growth rate (SGR) (% day⁻¹) revealed significant variations among sites ($P < 0.05$) (Fig. 3). The highest SGR was observed at SL (3.27±0.18), and the second-highest values were recorded at SO (2.68±0.12) and SB (2.84 ± 0.15), which are considerably higher than those in Semarang. Accordingly, seedlings from Semarang had better growth than those from Bebes and Demak in all cultivation methods. At the intermediate growth stage, seaweeds harvested from DO, BO, DL, and BL had SGR values ranging from 2.14 to 2.41(% day⁻¹). The seeds from Brebes and Demak, cultured using the broadcast method (DB and BB), showed the lowest growth rate (Fig. 3).

Agar content: The agar concentration of *G. verrucosa*

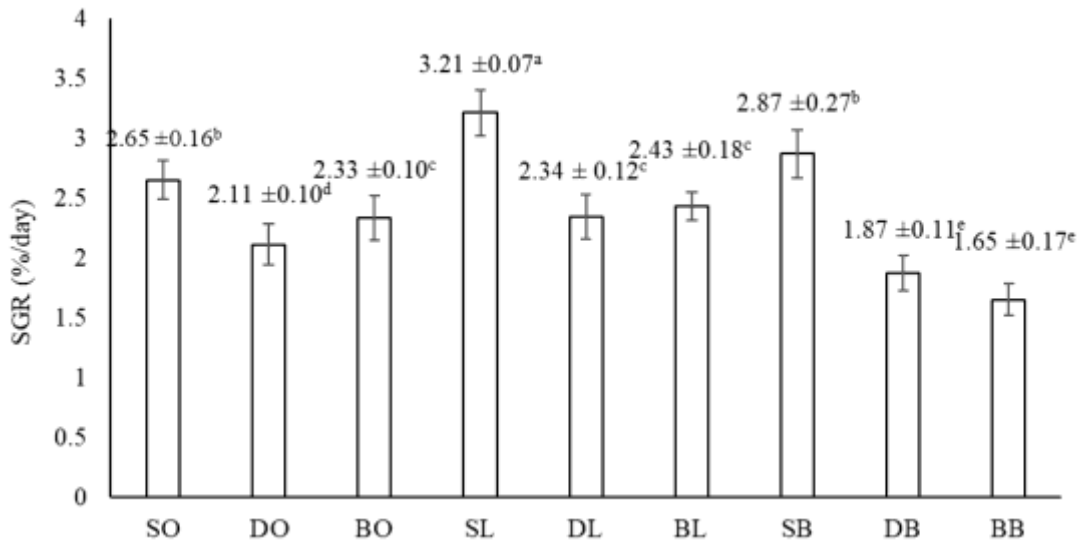


Figure 3. The specific growth rate (%.day⁻¹) of *Gracilaria verrucosa* from some places of origin, which were cultivated in long lines, off-bottom, and broadcast. Different uppercase letters denote a statistical difference ($P < 0.05$), analyzed by Duncan's Multiple Range test. Error margins represent a standard deviation (SD) ($n = 25$).

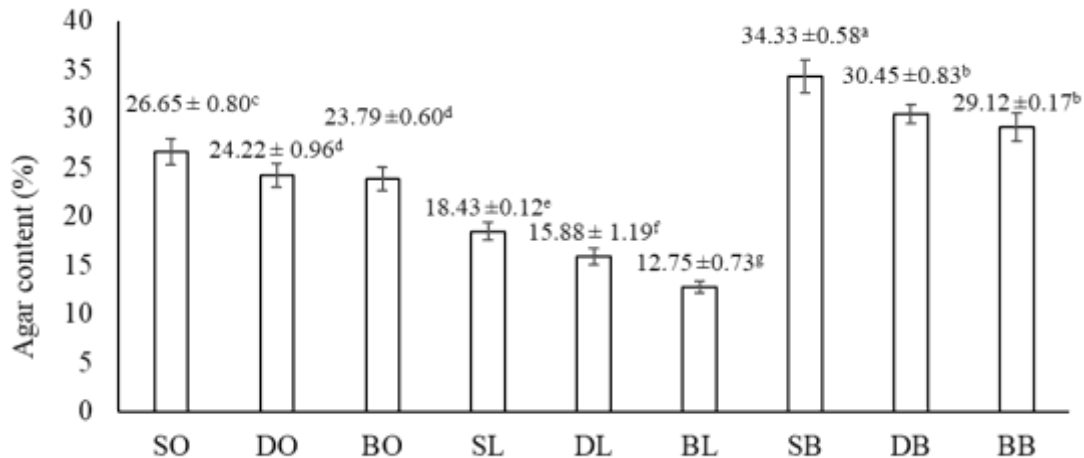


Figure 4. The agar content (%) of *Gracilaria verrucosa* from some places of origin, which were cultivated in long lines, off-bottom, and broadcast. Different uppercase letters denote a statistical difference ($P < 0.05$), analyzed by Duncan's Multiple Range test. Error margins represent a standard deviation (SD) ($n = 25$).

varied considerably across locations and culture methods. The broadcast cultivation method produced much higher agar content when used with seeds from all locations. The results recorded for SB, DB, and BB were $34.33 \pm 0.58\%$, $30.45 \pm 0.83\%$, and $29.12 \pm 0.17\%$, respectively. In more detail, SB had the highest agar content among the treatments. Meanwhile, for DB and BB, as the second-highest, there was no significant difference. Lower agar contents were obtained in SL ($18.43 \pm 0.12\%$), DL ($15.88 \pm 1.19\%$), and BL ($12.75 \pm 0.73\%$), with BL exhibiting the lowest yield (Fig. 4). The agar concentration in seedlings from

Semarang, Demak, and Brebes varies according to the culture method employed. Nonetheless, when assessing the same growing technique, seedlings from Semarang exhibit much higher agar content than those from Demak and Brebes. For instance, in the off-bottom method, SO revealed significantly higher agar content ($26.65 \pm 0.80\%$) than DO ($24.22 \pm 0.96\%$) and BO ($23.78 \pm 0.60\%$).

Gel strength: The highest gel strength (g.cm^{-2}) was observed for SO (324.7), which was significantly higher than the second-highest, SB (294.7), BB (263.1), DO (227.4), and BO (245.6). The long line

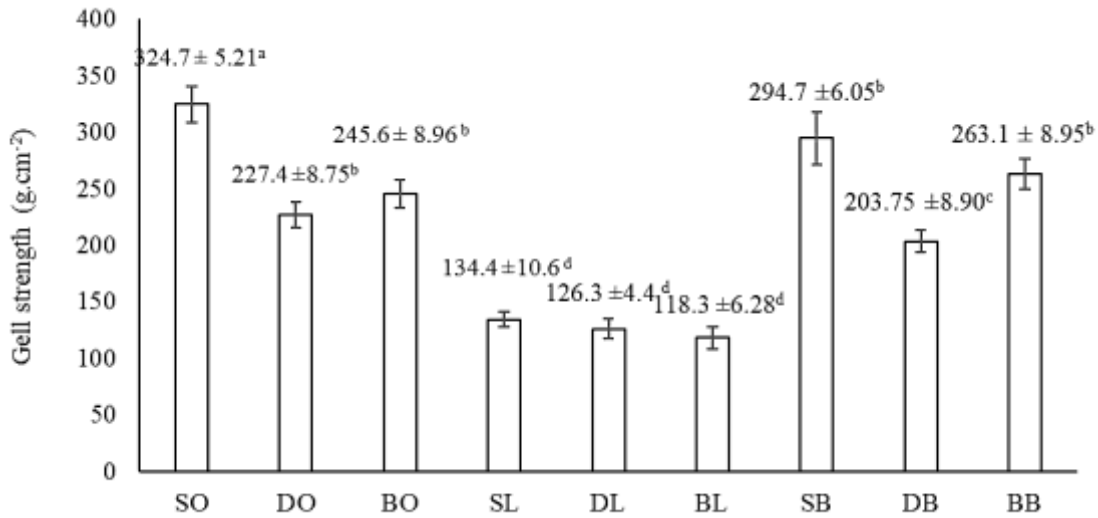


Figure 5. The gel strength (g. cm⁻²) of *Gracilaria verrucosa* from some places of origin, which were cultivated in long lines, off-bottom, and broadcast. Different uppercase letters denote a statistical difference ($P < 0.05$), analyzed by Duncan's Multiple Range test. Error margins represent a standard deviation (SD) (n = 25).

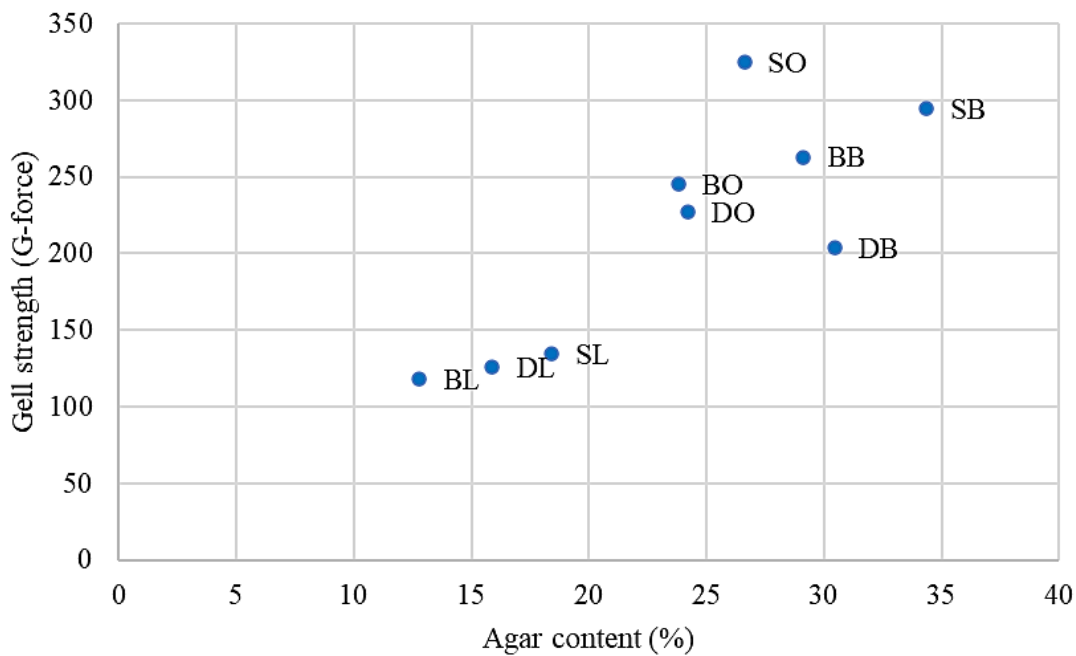


Figure 6. The correlation between agar concentration and gel strength in *Gracilaria verrucosa* from various seed origins and cultivation techniques.

method combined with seeds from Semarang, Brebes, and Demak showed the lowest gel strength (Fig. 5). There was no difference in gel strength for the Brebes seed, which was cultivated using the broadcast and off-bottom method. However, the seed from Semarang showed significant differences across all cultivation methods.

Interaction of gel strength and agar content: The agar content and gel strength of *G. verrucosa* exhibited significant variation across several seed

source combinations and growth techniques (Fig. 6). In general, there was a positive, although not perfectly linear, relationship between agar content and gel strength. Interestingly, SO (Semarang-Off-bottom) produced the highest gel strength (~325 g-force) with a moderate agar content (~27%), indicating that the physical quality of the gel is not only determined by the agar content, but also by its molecular composition, such as the ratio of agarose to agaropectin.

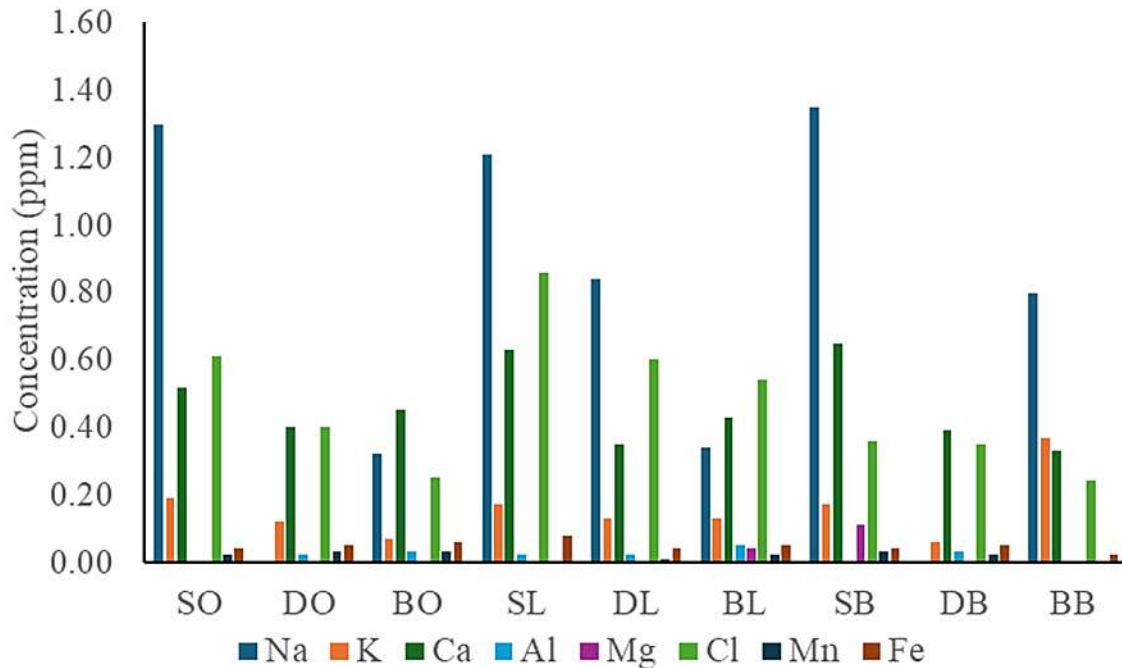


Figure 7. Mineral profile of *Gracilaria verrucosa* from Semarang, Demak, and Brebes cultivated with long line, off-bottom, and broadcast

The SB (Semarang–Broadcast) combination exhibited the highest agar content (~35%) and a gel strength approaching 300 g-force, followed by BB (Brebes–Broadcast) and DB (Demak–Broadcast), both at approximately 30% and with gel strengths above 200 g-force. This verifies that the broadcast method tends to produce superior quality. Conversely, BL (Brebes-Longline) produced the lowest agar content (~13%) with a gel strength of approximately 120 g-force, whilst DL and SL had similarly low values (17-19% and <150 g-force, respectively). This indicates that although longline supports biomass growth, the resulting agar quality is lower.

Minerals: The differences in macro- and micro-mineral content were noted across all combinations of the two parameters, underscoring the significance of site of origin conditions and culture system design. The mineral composition is primarily characterized by Na (0.32- 1.35%), followed by Ca (0.33-0.63%) and Cl (0.24-0.61%), and then by K (0.06-0.37%). The presence of Ca, Cl, and K is verified in every treatment. The greatest Na concentrations were observed in SB (1.35%) and SO (1.30%), suggesting that seaweed originated from Semarang promoted increased salt absorption. Conversely, no measurable Na was identified in the DO and DB systems (Demak

with off-bottom and broadcast). SL showed a markedly higher Cl content (0.86%) than all other treatments, suggesting that the long-line method in Semarang maximizes Cl uptake. In the same frame, Ca (0.63%) was followed. Regarding micro minerals, Cl and Fe were identified in all treatments, with concentrations of 0.86% and 0.08%, respectively, in seaweed from Semarang using the long-line method (SL). Conversely, seaweed from Brebes cultivated using the broadcast technique exhibited the lowest concentrations of Cl and Fe. Meanwhile, the micro-minerals Al, Mg, and Mn were not detected in any seaweed samples from Semarang, Brebes, and Demak (Fig. 7).

Water quality: The physicochemical characteristics of the culture environment during *Gracilaria* cultivation remained within the optimal ranges required for growth (Table 1). Water temperature ranged from 28.5 to 30.5°C, with an average of 29.5±2.11°C, well within the reported tolerance range of 25-31°C for *Gracilaria* spp. (Noor et al., 2021). The pH was relatively stable, ranging from 7.50 to 8.20 (7.8±0.93), falling within the optimal range of 7.6-8.0 (Weizhou et al., 2012). Salinity fluctuated slightly between 28.2-30.3‰ (29.3±4.22‰), which also conformed to the recommended range of 20-35‰ for

Table 1. Physicochemical characteristics of the culture environment during *Gracilaria verucosa* cultivation.

Parameter	Min	Max	Average±SD	Optimal	Reference
Temperature (°C)	28.5	30.5	29.5±2.11	25.00 - 31.00	Noor et al. (2021)
pH	7.50	8.20	7.8±0.93	7.6 - 8.0	Weizhou et al. (2012)
Salinity (‰)	28.2	30.3	29.3±4.22	20.00 - 35.00	
Nitrite (ppm)	0.01	0.05	0.03±0.004	0.005 - 0.05	
Nitrate (ppm)	0.92	3.51	2.47±0.29	1.20 - 2.70	Badraeni et al. (2020)
Amonia (ppm)	0.03	0.07	0.04±0.005	0.02 - 0.028	Sawant et al. (2024)
Phosphate (ppm)	0.45	2.56	2.35±0.25	1.30 - 2.50	

seaweed cultivation.

For nutrient parameters, nitrite concentrations were low (0.01-0.05 ppm; mean 0.03±0.004 ppm), remaining within the acceptable threshold (0.005-0.05 ppm) (Badraeni et al., 2020). Nitrate levels ranged from 0.92 to 3.51 ppm (2.47±0.29 ppm), within the optimal range of 1.20-2.70 ppm reported by Sawant et al. (2024). Ammonia concentrations were maintained at 0.03–0.07 ppm (0.04±0.005 ppm), within the safe range of 0.02-0.028 ppm for *Gracilaria*. Orthophosphate concentrations ranged from 0.45 to 2.56 ppm (2.35±0.25 ppm), within the favorable range of 1.30-2.50 ppm.

Discussions

Growth: Our results showed that *Gracilaria* seedlings from Semarang consistently had higher specific growth rates (SGR) than those from Demak and Brebes, regardless of cultivation method. This suggests that the source of seedstock significantly influences growth performance, potentially more so than the method of cultivation employed (Ho et al., 2019). The enhanced growth of Semarang seedlings is likely associated with the environmental characteristics of their native habitat. The waters of Semarang are typically rich in nutrients, including nitrogen and phosphorus, due to coastal dynamics and river inputs. This nutrient enrichment creates conditions conducive to seedling growth, with improved physiological capabilities for nutrient uptake and assimilation (Noor et al., 2021). Consequently, these seedlings exhibit strong growth even under various farming methods. This observation is consistent with earlier research on *Gracilaria* sp., which indicated that nutrient-rich environments can yield seedstock exhibiting enhanced physiological

plasticity and competitive growth performance (Chen et al., 2017). The lower growth performance of seedlings from Brebes and Demak compared to those from Semarang across various cultivation methods can be explained by differences in the environmental conditions of origin and seedling quality. Seedlings from Brebes and Demak are likely to grow in environments with greater fluctuations in water quality, including changes in salinity, turbidity, and relatively limited nutrient availability. These conditions can suppress nutrient absorption efficiency and reduce photosynthetic activity, leading to a lower growth rate. Conversely, seedlings from Semarang develop in waters with a more stable nutrient supply and more supportive hydrodynamic conditions, resulting in seedlings with better physiological capacity and high plasticity when applied to various cultivation methods (Kim, 2016).

The higher growth in the longline method for seedlings from Semarang and Demak can be attributed to more favourable hydrodynamic conditions. The longline system facilitates the free-hanging of *Gracilaria* thallus in the water column, promoting optimal water circulation, increasing dissolved oxygen, and ensuring even light distribution (Banik, 2023). Seedlings from Semarang and Demak, having adapted to waters with higher currents and dynamic nutrient levels, can leverage these conditions to enhance photosynthetic efficiency and nutrient absorption. In contrast, seedlings from Brebes exhibited higher growth with the off-bottom method. This may result from the characteristics of the water in Brebes, which is shallower, more prone to tidal fluctuations, and more turbid. Seedlings that grow under these conditions exhibit enhanced adaptation to low light intensity near the substrate (Ho et al., 2019;

Badraeni et al., 2020). Consequently, when recultivated using the off-bottom method, they exhibit a more optimal growth response than those suspended freely in the water column. Contrary to the broadcast method, which is frequently employed for its ease of use, it yields suboptimal growth across all seed origins. The presence of thallus at the water's bottom restricts light penetration, heightens the likelihood of sediment buildup, and diminishes water circulation around the thallus. The identified factors lessen the efficiency of photosynthesis and nutrient absorption, resulting in reduced growth rates. Sediment accumulation may diminish *Gracilaria* productivity by obstructing light penetration and reducing the thallus' metabolic capacity (Chen et al., 2017; Noor et al., 2021).

Agar content: The research findings indicate that the broadcast cultivation method produces greater agar content than other methods across all *Gracilaria* seed sources. This condition is attributable to the characteristics of the broadcast method, wherein the seaweed thallus is directly positioned on the seabed. This position subjects the thallus to more challenging environmental conditions, including reduced light intensity due to turbidity, possible sediment coverage, and greater fluctuations in temperature and dissolved oxygen compared to longline or off-bottom methods (Rejeki et al., 2018; Banik et al., 2023). Exposure to more stressful environmental conditions led *Gracilaria* to enhance polysaccharide synthesis as a physiological adaptation mechanism (Kim, 2016; Ho et al., 2019). Structural polysaccharides, such as agar, are essential for preserving cell integrity, safeguarding tissues against osmotic damage, and supporting physiological functions in suboptimal conditions. Environmental stress, specifically light limitation and nutrient fluctuations, can enhance polysaccharide accumulation in red seaweed (Smith et al., 2010).

Research on *G. lemaneiformis* and *G. tenuistipitata* indicates that various strains or seed sources exhibit considerable genetic diversity, affecting polysaccharide production, including agar (Lee, 2016). Research has identified variability in sulfation patterns and galactan structures among *Gracilaria*

species and their populations, influencing functional attributes and agar content. This suggests that seeds from Semarang may possess a better genetic ability to synthesise galactan sulphate than those from Demak or Brebes, even when grown under the same conditions (Mustafa et al., 2024). The environmental conditions in Semarang, characterised by consistent nutrient availability (nitrate, ammonia, and phosphate) and favourable water dynamics, might affect the first physiological state of seedlings. Seedlings that grow in nutrient-rich environments generally exhibit enhanced metabolic capacity, including the ability to allocate energy to the synthesis of structural polysaccharides (Jha et al., 2021).

Gel strength: This study demonstrates that gel strength in *Gracilaria* seedlings is strongly influenced by cultivation site and method, with Semarang off-bottom culture (SO) yielding the highest values. The superior performance of SO is likely due to favorable hydrodynamic conditions and nutrient availability, which enhance photosynthesis and polysaccharide biosynthesis (Bixler and Porse, 2011). Previous studies similarly reported higher agar quality due to stable, nutrient-rich environments (Debbarma et al., 2016). In contrast, long line culture (SL, DL, and BL) consistently yielded lower gel strength, indicating that this method may subject thalli to suboptimal conditions, including mechanical stress and diminished nutrient flux. Stress conditions have been shown to modify cell wall composition and reduce the extent of polysaccharide polymerisation, thereby diminishing gel strength. Bottom culture (SB and BB) exhibited relatively high gel strength values, ranking just below SO. Nevertheless, bottom culture in Demak (DB) produced lower values, suggesting that specific environmental factors, such as turbidity, sediment load, or eutrophication, may inhibit optimal polysaccharide synthesis in that area.

The identified spatial variation underscores the significance of site-specific environmental factors. Coastal waters in Semarang exhibit elevated nutrient input from terrestrial runoff, potentially leading to increased carbohydrate accumulation. Demak waters exhibit high turbidity and suspended solids, which

may restrict light penetration and photosynthetic efficiency, consequently diminishing agar gel strength. Brebes exhibits intermediate values, indicating transitional conditions in which environmental input partially influences agar quality (Sawant and Jaiswar et al., 2024).

Interaction of gel strength and agar content: Agar content and gel strength of *G. verrucosa* differed markedly among seed sources and cultivation techniques, with a generally positive, though not strictly linear, relationship between the two traits. The non-linearity is expected because gel strength depends not only on total agar yield but on polymer fine structure, especially the agarose: agaropectin ratio, degree of sulfation, and 3,6-anhydro-L-galactose (3,6-AG) formation (Siregar, 2024). Fractions rich in agarose and low in sulfate produce stronger and clearer gels, while fractions with more agaropectin or higher sulfate substitution tend to weaken the gel structure, even if the total agar content is high (Rejeki, 2018).

The SO (Semarang-off-bottom) treatment produced the highest gel strength (~325 g-force) at moderate agar (~27%), implying a more favorable polymer architecture (higher agarose fraction and/or lower sulfate). Off-bottom culture typically moderates irradiance and shear, reduces thallus abrasion and fouling, and stabilizes diel temperature–salinity swings—conditions linked to increased 3,6-AG formation and stronger gels in *Gracilaria* (Hurtado et al., 2019). By contrast, Broadcast places thalli on (or near) the pond bottom, where lower turbulence and slightly elevated nutrient levels can favor polysaccharide accumulation (higher agar yield). Yet gel strength remained below in SO, consistent with reports that bottom-settled thalli may accumulate more sulfated agaropectin or experience microanoxia and sediment contact, which biases biosynthesis toward weaker-gel fractions (Wong et al., 2000; Kim et al., 2016).

The longline treatments (BL, DL, and SL) showed lower agar yield (~13-19%) and weaker gels (<150 g-force). Suspended thalli experience higher light, flushing, and hydrodynamic stress, which can increase

sulfate substitution and reduce agarose content, thereby depressing gel strength even when biomass growth is high (Yarnpakdee et al., 2015). The observed decoupling between growth performance and hydrocolloid quality suggests that environmental conditions promoting high biomass accumulation may alter carbohydrate metabolism in ways that do not favor the synthesis or structural refinement of agar polymers, particularly the agarose fraction that governs gel strength.

Seed origin significantly influenced both agar yield and functional quality. Across cultivation methods, seed derived from Semarang consistently outperformed those from Demak and Brebes, either in agar content, gel strength, or both. This trend suggests the presence of strain-level (genetic) variation and/or superior physiological pre-acclimation, which may regulate key biochemical pathways, particularly those controlling sulfation patterns and the biosynthesis of 3,6-anhydro-L-galactose (3,6-AG), a structural determinant of gel strength. (Kim et al., 2016). Such genetic or adaptive advantages may explain the superior performance of Semarang seed regardless of cultivation techniques.

Minerals: Biological and environmental factors significantly influence seaweed's capacity to absorb minerals. As internal components, seaweed's characteristic cell wall polysaccharides and proteins serve as highly effective binding sites for nutrient retention (Smith et al., 2010; Jha et al., 2021). Based on research data, seaweed from Semarang has higher macro-mineral content across all cultivation methods. This is possible because the internal factors in seedlings from Semarang have a typical cell wall that can bind Na, K, and Ca. Given external factors, all treatments were cultivated in the same waters, so the surrounding waters had the same quality. The bioavailability of the mineral in the surrounding water is considered an external factor (Peñalver et al., 2020). The Na content in SO, SL, and SB was 1.3, 1.2, and 1.35% higher than the macro mineral content in *Gracilaria edulis* ($0.42 \pm 0.01\%$); *Sargassum* sp. ($0.39 \pm 0.009\%$), and *Ulva lactuca* ($0.35 \pm 0.009\%$) (Debbarma et al., 2016).

Mineral concentrations in various seaweeds vary by location, growth stage, and seasonal fluctuations. In micro minerals, Al in this study was observed at 0.02-0.03%, which is lower than in the previous research in brown seaweed (0.128%) and green seaweed (0.554%) (Jha et al., 2021). In the current study, the upper value concentration of Fe showed in SL (0.08%), which is at the same level as the previous study 0.08-0.09% (Jha et al., 2021). The present research location is situated in proximity to residential areas and some chicken farms, though not particularly close. This kind of activity might support the mineral content of seaweed (Apadym, 2010). Alongside anthropogenic activity, natural forces such as flooding and soil erosion can also contribute to increased mineral concentration, leading to greater bioaccumulation in seaweed.

Conclusion

Strategic integration of seed origin and cultivation method is critical, as both jointly determine the growth performance, biochemical composition, and hydrocolloid quality of *Gracilaria verrucosa*. Semarang seed consistently outperformed local seed across sites, reflecting genetic or adaptive advantages and confirming that high-quality production does not depend on local seedstock. Off-bottom culture with Semarang seed produced the strongest gels, broadcasting maximized agar content, and longline favored biomass but required quality screening. Mineralogical analysis further revealed higher Na, K, and Ca accumulation in Semarang seed, enhancing nutritional and industrial value. Collectively, these findings demonstrate that selective seed sourcing combined with optimized cultivation methods is essential for meeting production goals and ensuring quality in seaweed farming.

Acknowledgements

Thank you to the Faculty of Fisheries and Marine Sciences, with sources of funds other than the State Budget of Diponegoro University, for the Academic Year 2021, with contract number 92/UN7.5.10.2/PP/2021. Thanks, are also given to Dr. Ir. Titik

Susiliwati, who has assisted in the research design.

References

- Apadym G., Ayliker V., Cengiz E., Saydam M., Kup N., Tirasoglu E. (2010). Analysis of metal contents of seaweed (*Ulva lactuca*) from Istanbul, Turkey by EDXRF. *Turkish Journal of Fisheries and Aquatic Sciences*, 10: 215-220.
- Banik U., Mohiuddin M., Wahab M., Rahman M., Nahiduzzaman M., Sarkar S., Wong L., Asaduzzaman M. (2023). Comparative performances of different farming systems and associated influence of ecological factors on *Gracilaria* sp. seaweed at the south-east coast of the Bay of Bengal, Bangladesh. *Aquaculture*, 574: 739675.
- Badraeni, Azis H., Tresnati J., Tuwo A. (2020). Seaweed *Gracilaria changii* as a bioremediator agent for ammonia, nitrite and nitrate in controlled tanks of Whiteleg Shrimp *Litopenaeus vannamei*. IOP Conference Series: Earth and Environmental Science, 564.
- Bixler H.J., Porse H. (2011). A decade of change in the seaweed hydrocolloids industry. *Journal of Applied Phycology*, 23: 321-335.
- Chen B., Zou D., Zhu M., Yang Y. (2017). Effects of CO₂ levels and light intensities on growth and amino acid contents in red seaweed *Gracilaria lemaneiformis*. *Aquaculture Research*, 48: 2683-2690.
- Chaqmaqchee F., Baker A., Salih N.F. (2017). Comparison of various plastics wastes using X-ray fluorescence. *American Journal of Materials Synthesis and Processing*, 2(2): 24-27.
- Debbarma J., Rao B.M., Murthy L.N., Mathew S., Venkateshwarlu G., Ravishankar C.N. (2016). Nutritional profiling of the edible seaweeds *Gracilaria edulis*, *Ulva lactuca* and *Sargassum*, sp. *Indian Journal of Fisheries*, 63(3): 81-87.
- FAO. (2018). *The Global Status of Seaweed Production, Trade and Utilization* Vol. 124. Roma.
- Filippini M., Baldisserotto A.M., Simonetta., Giorgio F.R., Silva G., Domenico V., Giorgia B., Raissa., Stefano M., Silvia V. (2021). Heavy metals and potential risks in edible seaweed on the market in Italy. *Chemosphere*, 263: 127983.
- Hayashi L., Leonardi P.I., Critchley A.T. (2017). The cultivation of *Kappaphycus* and *Euclima* in tropical and subtropical waters. *Advances in Botanical Research*, 78: 121-156.

- Hurtado A.Q., Neish I.C., Critchley A.T. (2019). Phyconomy: The seaweed industry in the Asia-Pacific region. *Journal of Applied Phycology*, 31(2): 797-818.
- Ho C.L., Lim Y-y., Lee W-K. (2019). PH affects growth, physiology, and agar properties of agarophyte *Gracilaria changii* (Rhodophyta) under low light intensity from Morib, Malaysia. *Regional Studies in Marine Science*, 30: 100738.
- Indonesian National Standard (SNI). 2011. Water quality: Determination of nitrate with spectrophotometric method using cadmium reduction. SNI 6989.79:2011. National Standardization Agency of Indonesia (BSN), Jakarta.
- Indonesian National Standard (SNI). (2004). Water quality—Determination of nitrite by spectrophotometric method. SNI 06-6989.9:2004. National Standardization Agency of Indonesia (BSN), Jakarta.
- Indonesian National Standard (SNI). (2005). Water quality—Determination of ammonia with spectrophotometric phenate method. SNI 06-6989.30:2005. National Standardization Agency of Indonesia (BSN), Jakarta.
- Indonesian National Standard (SNI). (2005). Water quality—Determination of orthophosphate by spectrophotometric ascorbic acid method. SNI 06-6989.31:2005. National Standardization Agency of Indonesia (BSN), Jakarta.
- Jha A.K., Panda S.K., Kishore P., Mathew S., Ravishankar C.N. (2021). Trace-minerals and lipid quality indices in seaweeds growing at Okhta, India: A health risk assessment. *Regional studies in Marine Science*, 47: 101966.
- Kim J.K., Yarish C., Pereira R. (2016). Tolerances to hypo-osmotic and temperature stress in native and invasive species of *Gracilaria* (Rhodophyta). *Phycologia*, 55(3): 257-264.
- Lee W., Lim Y., Leow A., Namasivayam P., Abdullah J., Ho C. (2016). Factors affecting yield and gelling properties of agar. *Journal of Applied Phycology*, 29: 1527-1540.
- Mustafa A., Athirah A., Ratnawati E., Paena M., Kamariah K., Asaad A., Tarunamulia T., Asaf R., Syaichudin M., Taukhid I. (2024). Identifying the environmental factors that determine the agar content of seaweed *Gracilaria verrucosa* in acid sulfate soil-affected brackishwater ponds. *Polish Journal of Environmental Studies*, 33(4): 4673-4686.
- Noor M., Hidayat N., Mohamad M., Abdullah N., Rusly S., Saad S., Mukai Y., Susanti D. (2021). Growth of *Gracilaria manilaensis* Yamamoto et Trono (Rhodophyta) under different light intensities, salinities and pH. *Borneo Journal of Marine Science and Aquaculture (BJoMSA)*, 5(2): 53-56.
- Peñalver R., Lorenzo J.M., Ros G., Amarowicz R., Pateiro M. (2020). Seaweeds as a Functional Ingredient for a Healthy Diet. *Marine Drugs*, 18(6): 301.
- Rejeki S., Ariyati R., Widowati L.L., Bosma R.H. (2018). The effect of three cultivation methods and two seedling types on growth, agar content and gel strength of *Gracilaria verrucosa*. *Egyptian Journal of Aquatic Research*, 44(1): 65-70.
- Ricker W.E. (1975). Computation and interpretation of biological statistics of fish populations. *Fisheries Research Board of Canada, Bulletin*, 191: 1-382.
- Sawant O., Jaiswar S. (2024). Assessing the nitrate and phosphate uptake kinetics potential and growth performance of *Gracilaria corticata* var. *cylindrica* in shrimp farm water (SFW). *Discover Oceans*, 1: 7.
- Siregar R., Permadi A., Wijaya V. (2024). Physicochemical characteristics of agar from *Gracilaria verrucosa* at different locations. *Postharvest and Marine Biotechnology Journal of Fisheries and Marine Affairs*, 19(2): 93.
- Smith J.L., Summers G., Wong R. (2010). Nutrient and heavy metal content of edible seaweeds in New Zealand. *New Zealand Journal of Crop and Horticultural Science*, 38: 19-28.
- Weizhou C. (2012). Effects of different temperature and salinity on growth and biochemical constituents of *Gracilaria chouae*. *South China Fisheries Science*, 8(2): 51-57.
- Wong K.H., Cheung P.C.K. (2000). Nutritional evaluation of some subtropical red and green seaweeds Part I – proximate composition, amino acid profiles and some physico-chemical properties. *Food Chemistry*, 71: 475-482.
- Yarnpakdee S., Benjakul S., Kingwascharapong P. (2015). Physico-chemical and gel properties of agar from *Gracilaria tenuistipitata* from the lake of Songkhla, Thailand. *Food Hydrocolloids*, 51: 217-226.