## SHORT COMMUNICATION

# Effect of light colour on Gracilaria edulis growth and agar

Ayesha U. Kuragodage . Palawinnege R.T. Cumaranatunga . Kariyawasam H.M.A. Deepananda 💿

Received: 31 May 2022 / Accepted: 26 August 2022 / Published online: 02 September 2022 © The Author(s) 2022

**Abstract** The seaweed industry is a rapidly growing global industry. The present study aimed at empirically ascertaining the effect of different colours of visible light spectrum on morphology and growth rate of red algae (*Gracilaria edulis*), and agar yield and quality of the *G. edulis* to elucidate the most suitable depth range for culturing *G. edulis* commercially. Changes in colour and biomass of fresh *G. edulis* were recorded fortnightly, and the characteristics of agar extracted from *G. edulis* exposed to different colours of light (natural, red, green, and violet) were evaluated. *G. edulis* exposed to green light showed the highest growth rate, while the highest agar yield could be obtained from *G. edulis* exposed to red light. Comparatively higher gel strength, viscosity, and water holding capacity of extracted agar were reported in *G. edulis* exposed to red light. The growth rate & agar quality of *G. edulis* cultured under natural light were comparatively lower. The study confirms that *G. edulis* should be cultured under green light to obtain a significant growth rate and, *G. edulis* should be cultured under red light to obtain agar of better quality and quantity. Penetration depths of red and green light in the water column are up to 5m and 30m, respectively. Present findings unfold that culture facilities for *G. edulis* should be established at 1-5m water depths for obtaining a comparatively higher growth rate and high-quality agar. The study warrants empirical studies *in-situ* to make a robust conclusion.

Keywords G. edulis . Growth rate . Agar yield . Gel strength . Viscosity . Water holding capacity

# Introduction

The seaweed industry that produces an extensive range of products is a rapidly growing global industry. Total seaweed production tonnage has increased from 2.2 million tonnes to 35.8 million tonnes between 1950 and 2019 (Cai et al. 2021). In 2019, 34.7 million tonnes of world seaweed cultivation production for various food and non-food uses have generated a USD 14.7 billion first-sale value of which sales for human consumption make up the greatest share (FAO 2020; Cai et al. 2021). Hydrocolloids are the major products extracted from seaweeds and minor products of seaweeds include fertilizers, cosmetics, and biofuel. USD 2.65 billion of foreign exchange have been earned by 98 countries in 2019 by exporting seaweeds (USD 909 million) and seaweed-based hydrocolloids (USD 1.74 billion) (McHugh 2003; Cai et al. 2021). Wild seaweed production remains at 1.1 million tonnes, and total aquaculture production of seaweeds by 49 countries/territories has increased to 35.8 million tonnes, the majority of which (97%) are from Asian countries including China, Indonesia, the Republic of Korea, Philippine, Democratic People's Republic of Korea, Japan and Malaysia. Production of seaweeds in Asia, Africa and Oceania is dominated by cultivation (Cai et al. 2021).

Ayesha U. Kuragodage . Palawinnege R.T. Cumaranatunga . Kariyawasam H.M.A. Deepananda (🖂)

Department of Fisheries and Aquaculture, Faculty of Fisheries and Marine Sciences & Technology, University of Ruhuna, Matara 81000, Sri Lanka e-mail: ashoka@fish.ruh.ac.lk

Agar, a kind of strong gelling hydrocolloid is a linear galactan polysaccharide extracted from red algae (Rhodophyceae), specifically from *Gracilaria*, *Gelidium* and *Pterocladia/Gelidiella* (McHugh 2003; Glicksman 1987). To date, *Gelidium* species are not commercially cultivated and, 3.7 million tonnes of world production of agar-containing seaweeds (agarophytes) in 2019 was almost entirely supplied by *Gracilaria*, which were mostly provided by cultivation in China (95.63%) with a small amount (53 955 tonnes) of wild collection from Chile (Cai et al. 2021). In 2019, 3.6 million tonnes of farmed *Gracilaria* (10.5 per cent of all seaweeds) has been produced by 11 countries (Cai et al. 2021).

*Gracilaria* has been the main source of food-grade agar used for industrial food production (Armisen 1995). Agar consists of above 94% of highly soluble fibre which is not metabolized within the human body (Galatas 2006). Thus, they do not add calories to foods (Armisen 1991; 1995). Global production of agar was 9600 tonnes in 2009 (Bixler and Porse 2011), and agar has a high market demand and retail price, compared to other seaweed hydrocolloids such as alginates and carrageenans (Rhein-Knudsen et al. 2015). Agar has become the most usable and beneficial hydrocolloid and about 80% of agar produced globally is used for food applications including water gels, dairy products, fermented products, canned meat, and fish products, soups and sauces, and fining agent for clarifying wine, juice and vinegar, and bakery products, while remaining 10–20% is used in pharmaceutical and biotechnology industries (Imeson 2010; Madhavan and Abirami 2015). The commercial value of agar in the phycocolloid market depends largely on the agar yield and quality which can be affected by many factors including light intensity, water depth, season, salinity, temperature, epiphytes & epibionts, nutrient status, physiological state, extraction method and storage (Lee et al. 2017).

Seaweed culture techniques have been explored in recent years. When culturing seaweed in open water, the most suitable water depth is important to be determined for high return from the industry. The visible light spectrum consists of different colours having specific wavelengths and energy. When visible light travels through a water column, waves having different wavelengths are absorbed in different depths. Long wavelengths of the light spectrum red, orange, and yellow can penetrate to approximately 5, 10, and 30 meters, respectively, while the short wavelengths of the light spectrum blue and green can penetrate beyond 30 meters (Łuczyński and Birk 2018). Scientists have studied the growth and pigment composition of different red algae species under different light qualities (Godínez-Ortega et al. 2008; Marinho-Soriano et al. 2012; Wu 2016), and at different depths (Indriatmoko et al. 2015; Oliveira and Freire 2012). Nevertheless, experimental studies on growth rate and yield and quality of agar extracted from *G. edulis* exposed to different colours of light are scarce. The present study ascertains the effect of different colours of light (wavelengths) on morphological characters and growth rate of *G. edulis* thallus, and the yield and quality of agar extracted from *G. edulis* cultured under different colours of light to elucidate the most suitable wavelength and water depth for culturing *G. edulis* commercially.

## Materials and methods

*Gracilaria edulis* samples collected from the seaweed farming centre of Halyes Aquagri (Private) Ltd, Kenniya, Sri Lanka were brought to the marine research facility of the University of Ruhuna, Sri Lanka. Plastic transparent culture bottles (5L capacity) were used to culture seaweeds, and 200L fibreglass (opaque) tanks were used to dip the culture bottles to maintain a constant temperature within replicates. Filtered seawater was used as a culture medium which was continuously aerated and renewed daily. Culture bottles and tanks were filled with filtered seawater. Each fibreglass tank consisted of three culture bottles. Four fibreglass tanks were indiscriminately arranged in the open area of the marine research facility, and three tanks were covered by cellophane sheets (14.2gsm) with three different colours (red, green & violet) separately to provide light at different ranges of wavelengths and the rest was covered by transparent polythene (Fig. 1). Fibreglass tanks covered with red, violet, and green cellophane received light with wavelengths of  $\approx$ 635-700nm,  $\approx$ 490-560nm, and  $\approx$ 380-450nm, respectively in the visible spectrum and transparent polythene filtered light similar to the natural light of the visible light spectrum.

Wavelength ranges of the emitted light through different coloured cellophane sheets to the experimental tanks were confirmed using a HACH DR 3900 spectrophotometer under the wavelength range 320nm–700nm. Based on the wavelength emitted to the experimental tank, the depth of seawater at which each of the above light wavelengths would be available was determined in accordance with Łuczyński and Birk



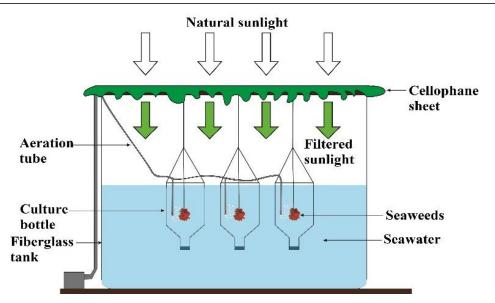


Fig. 1 Schematic diagram of the experimental setup

Table 1 Wavelength range emitted through cellophane sheets and their respective depth ranges in calm seawater

Cellophane sheet colour	Emitted wavelength	Respective colours of the spectrum	VisThe visiblelour emitted to the experimental setup	Approximate penetration depth ranges (m) (Łuczyński and Birk 2018; Ikelite 2021)	
	range (nm)	1	1 1		
Red	600-700	Red	Red	≈5	
Violet	320-464	Violet & blue	Violet	≈20	
Violet	620-700 Red	VIOL	~20		
Green	493-550	Green	Green	≈30	
Natural light (control)	320-700	White	White	≈0-1	

(2018) and Ikelite (2021). Wavelength ranges of the light, filtered through different coloured cellophane sheets (red, green & violet), used during the experiment and the possible depth range in seawater for each light wave are summarized in Table 1.

Healthy and strong seedlings of acclimatized *G. edulis* plants (initial weight, 25-30g) were selected for the six weeks experiment. The temperature of the culture medium was measured twice a day (morning and afternoon) using a thermometer, while salinity and pH were measured daily with an environmental multiprobe. Biomass (fresh weight) of *G. edulis* was recorded once in two weeks with an analytical balance (Mettler PI3600 DeliaRange). Colour changes of the thallus, if any, were observed and photographed at two-week intervals through the experimentation. The growth rate (GR) of the *G. edulis* was determined by following Schmidt et al. (2010).

GR [% per day] = [(Wt/Wi) - 1] \* 100/t

Where, Wi = initial wet mass, Wt = wet mass after t-days (14 days) and t = internal time in days.

After 6 weeks, seaweeds were harvested, and cleaned samples were cut into small pieces about 1-1.5cm in length and kept overnight in acetone to remove pigments and again washed with fresh acetone until the supernatant became colourless. The method described by Praiboon et al. (2006) was used to extract the agar. Samples of 5g dried *G. edulis* received different treatments were incubated in 333mL of 5% NaOH at 80°C for 2 hours in a reciprocal shaking water bath (JSSB-30T- Korea). Then, the seaweed sample was washed in running tap water for 30 mins to eliminate excess NaOH. The alkali-treated seaweed sample was soaked in a 2% H<sub>2</sub>SO<sub>4</sub> solution for 1 hour to neutralize the alkali-treated seaweeds. After that, the sample was washed in running water overnight to completely remove the acid. The sample was boiled with 150ml of distilled water for 2 hours. The extracted agar solution was filtered through a piece of muslin cloth while hot. Extracted agar was kept at room temperature to form a gel. The solidified agar was frozen at -20°C for 48 hours. Then the sample was thawed in tap water and kept in the oven (Type UL 30 Memmert West German) at 60°C for 24 hours to dry. Dry agar was ground and packed in airtight containers.

The percentage yield of agar (W/W %) was determined by calculating the weight of agar against the weight of dried seaweeds used for agar extraction. Moisture, ash, and crude protein content of extracted agar from *G. edulis* cultured under four light treatments were determined by employing the method in AOAC (1995), and the crude fat contents of each were determined separately following the method described by Folch et al. (1957). The gel strength of extracted agar was determined by adopting a modified method described by Falshaw et al. (1998). In this context, 30 mL of agar solution (1.5% w/v) was filled into a petry dish (diameter and height of container were 3.2 cm, 1.5 cm, respectively) and kept at room temperature overnight. The gelled agar was kept on a balance. A stainless rod (surface area 1.5 cm<sup>2</sup>) that contained the small container at the upper part was taken. The rod was put on the solid gel and pressed the gel by adding weights into the container until the gel collapsed and a reading of the balance was taken at that point. Gel strength is defined using the ratio of weight difference of before and after gel collapse to the surface area of the rod using the following equation.

## Gel strength $(g/cm^2) = (final weight (g) - Initial weight (g)) / surface area of the rod (cm<sup>2</sup>)$

The viscosity of extracted agar was determined at 80°C using the Ostwald viscometer following Distantina et al. (2011) with minor modifications.

Absolute viscosity of agar  $(kg/m^3s) = (\eta 1 \times \rho 2 \times t2) / (\rho 1 \times t1)$ 

Where,  $\rho 1 = \text{Density}$  of water (kg/m<sup>3</sup>);  $\rho 2 = \text{Density}$  of agar (kg/m<sup>3</sup>);  $\eta 1 = \text{Viscosity}$  of water (kg/m<sup>3</sup>s) ( $\eta 1=0.355$ kg/m<sup>3</sup>s = 0.862cP for water at 80°C); t1 = Time taken for the water to move between upper and lower marks of the upper reservoir (s); t2 = Time taken for the agar solution to move between upper and lower marks of the upper reservoir (s).

The water holding capacity of extracted agar solution was determined following the modified method described by Cho et al. (2004) in which the following equation was used.

Water holding capacity (%) =  $((Wx - Wy)/Wz) \times 100$ 

Where, Wx =Weight of the centrifuge tube with sample before centrifuging (g), Wy =Weight of the centrifuge tube with sample after centrifuging (g), and Wz = Weight of the sample (g).

Agar solutions of 1.5% strength were used in assessing the viscosity, gel strength, gelling temperature, melting temperature, and pH. Gel pH 1.5% (w/v) of extracted agar was determined using an environmental multiprobe. Gelling temperature and melting temperature of extracted agar powder were determined using a thermometer following the modified method described by Chung et al. (2011). Physical and chemical parameters of agar extracted from *G. edulis* cultured under different light conditions were compared. Data obtained from three replicates of each experimental system was expressed as mean $\pm$ standard deviation (SD). Statistical analyses were performed using the SPSS software (version 21), and Tukey's honest significant difference (HSD) test was used to compare mean values.

### Results

Salinity, pH, and temperature in the experimental groups were not significantly different from each other (P >0.05). The salinity and pH of the culture medium throughout the experiment were  $35.14\pm0.40$  ppt and  $7.74\pm015$ , respectively, while the temperature of the culture media was  $30.07\pm1.96$  °C throughout the experimentation. *G. edulis* treated with red and green light resulted in a greenish-coloured thallus and among them, red light treated algae showed a comparatively brighter green colour, while natural and violet light treated algae showed a reddish-brown (bleached appearance) and reddish-green thallus, respectively at the end of the experimentation. The colour changes in fresh *G. edulis* with natural light, red light, green light, and violet light through the experimentation are shown in Fig. 2.

There was a significant difference (P < 0.05) in the relative growth rate of *G. edulis* cultured under natural light when compared with that of the *G. edulis* cultured under red, green, and violet colour lights. *G. edulis* treated with green light, and violet light showed the highest and lowest growth rate, respectively. The growth rate of *G. edulis* cultured under red, violet, and natural light conditions showed a decreasing trend,



Fig. 2 Changes in the colour of fresh G. edulis treated with different wavelengths of the visible light spectrum

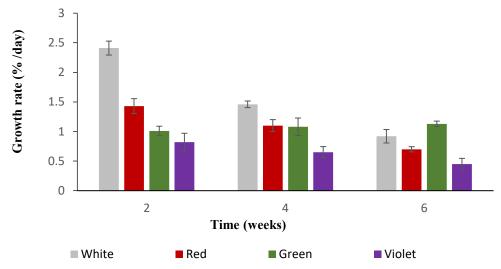


Fig. 3 Relative growth rate in G. edulis cultured under different coloured light conditions

while green light treated plants exhibited a significant increment in growth (P < 0.05) through the culture period. Relative growth rates of *G. edulis* cultured under different coloured light treatments are depicted in Fig. 3.

Percentage yields of agar extracted from G. edulis cultured under different coloured light treatments were

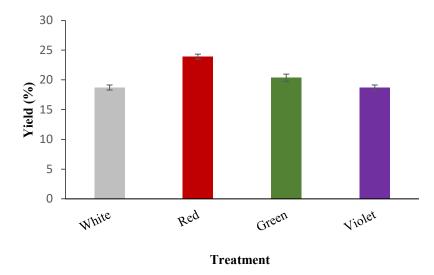


Fig. 4 Percentage yield of the agar extracted from G. edulis cultured under different colours of lights

significantly different (n=12, P <0.05), and the yield (%) varied between 18.67 $\pm$ 0.48% and 23.91 $\pm$ 0.42%. The highest percentage yield (23.91 $\pm$ 0.42%) was recorded in red light treatment, while the lowest percentage yields (18.72 $\pm$ 0.42% and 18.67 $\pm$ 0.48%) were recorded in natural light (Control) and violet light treatments. The percentage yield of the experimentally extracted agar from *G. edulis* is depicted in Fig. 4.

There was no significant difference (n=12, P >0.05) in the percentage of ash, moisture, crude protein, and crude lipid composition of agar extracted from *G. edulis* cultured under natural (control), red, green, and violet coloured light. Crude protein composition and moisture content of experimentally extracted agar from *G. edulis* cultured under normal light and red, green, and violet coloured light treatments were quite similar. The proximate nutrient composition of agar extracted from different light conditions is summarized in Table 2.

The solubility of extracted agar among treatments was indistinguishable. The colour of the extracted agar from red and violet light treated *G. edulis* was yellow. The agar of *G. edulis* treated with normal light was light yellow, while the agar extracted from green light treated *G. edulis* exhibited greenish-yellow colour. The water holding capacity of the extracted agar, which ranged between  $57.68\pm3.26\%$  and  $63.01\pm0.83\%$ , was quite similar among *G. edulis* cultured under all light treatments except the red light. The viscosity of agar extracted from *G. edulis* cultured under different colours of light treatments ranged between  $10.83\pm0.04$  cP and  $11.88\pm0.01$  cP and exhibited a significant difference among the four treatments (P <0.05). The gel strength of agar, experimentally extracted from *G. edulis* cultured under different colours of light treatments was significantly different (P <0.05), and the highest gel strength was observed from red-light treatments were not significantly different (P >0.05). The appearance, solubility, water holding capacity, viscosity, gel strength, gelling temperature, melting temperature, and pH of experimentally extracted agar are given in Table 3.

#### Discussion

The present study unfolds the effect of different wavelengths of the visible spectrum on the growth rate, morphological characters of *G. edulis*, and the yield and quality of agar produced by *G. edulis*. The study is significant as previous studies have only focused on the growth and pigment composition of different species of red algae under different light qualities (Godínez-Ortega et al. 2008) and at different depths (Jayasankar and Varghese 2002; Oliveira and Freire 2012).

The impact of different light treatments on *G. edulis* over to natural lights is shown by differences in morphological characteristics of the thallus through the experimentation. The colour of the thallus exposed to natural light has shown reddish-brown (bleached appearance) over the thalli grown under red-violet



Table 2 Proximate composition of agar extracted from G. edulis cultured under different colours of lights

Light treatment	Proximate composition					
	Ash (%)	Moisture (%)	Crude protein (%)	Crude lipid (%)		
Natural	3.20±0.48ª	16.52±0.27 <sup>ab</sup>	2.08±0.13ª	7.51±0.33ª		
Red	$3.72{\pm}0.04^{b}$	$16.30\pm0.32^{\rm a}$	2.15±0.06 <sup>a</sup>	$6.92\pm0.56^{\rm a}$		
Green	3.53±0.02 <sup>ab</sup>	17.16±0.26 <sup>b</sup>	2.09±0.07 <sup>a</sup>	7.04±0.33ª		
Violet	$3.71 \pm 0.01^{b}$	16.67±0.35 <sup>ab</sup>	$2.02{\pm}0.10^{a}$	$6.87{\pm}0.57^{a}$		

Values in the same column with different superscripts were significantly different (P < 0.05)

Table 3 Characteristics of agar extracted from G. edulis cultured under different colours of light

	Treatment				
Physical characteristic	Natural	Red	Green	Violet	
Appearance	Light yellow	Yellow	Greenish-yellow	Yellow	
Solubility	Boiling water	Boiling water	Boiling water	Boiling water	
Water holding capacity (%)	58.87±2.81ª	$63.01 {\pm} 0.83^{b}$	57.68±3.26ª	57.96±3.31ª	
Viscosity at 80°C (cP)	10.83±0.04ª	11.88±0.01°	10.97±0.01 <sup>b</sup>	$10.84{\pm}0.02^{a}$	
Gel strength at 20°C (g/cm <sup>2</sup> )	89.12±1.33ª	$97.24{\pm}0.98^{b}$	86.12±2.02ª	87.22±1.53ª	
Gelling temperature (°C)	33.83±0.29ª	33.33±0.58 ª	32.67±0.58 ª	34.33±0.29 ª	
Melting temperature (°C)	88.50±0.5 <sup>ab</sup>	87.67±0.58 <sup>a</sup>	$88.83{\pm}0.76^{b}$	$88.67{\pm}0.58^{ab}$	
рН	7.95±0.17 ª	7.87±0.07 ª	7.80±0.09 <sup>a</sup>	7.91±0.03 ª	

Values in the same row with different superscripts were significantly different (P < 0.05)

and green lights. Different colours of light are reported to result in differences in metabolic activities, changes in pigment content and their activities, and energy absorbance (Wu 2016). Thus, colours of light are responsible for the changes in the colour of the thallus of *G. edulis* in the study. These observations indicate that the culture of *G. edulis* close to the surface of the water column (0-1m) at which thallus is exposed to the highest intensity of natural light can cause bleaching in the thallus, indicating that surface water up to 1 m is not suitable for commercial farming of *G. edulis*.

The veracity of this argument is further confirmed by the significant differences in the relative growth rate of G. edulis cultured in experimental aquaria. The growth rate of G. edulis exposed to green light has increased significantly, which is in agreement with the findings of Godínez-Ortega et al. (2008) and Wu (2016). Moreover, Leukart and Lüning (1994) have reported that green light results in a several-fold effect on the growth of the thallus rather than red and blue light and, Saffo (1987) has found that red algae exhibit a higher absorption of green light than green algae. G. edulis exposed to green light has shown the highest growth rate over red, violet, and normal lights owing to the presence of high Chlorophyll-a concentration and higher efficiency of light absorption. Most green algae grow in the upper zone of water, but brown algae grow often in deeper water and many red algae are subtidal algae (Wu 2016; Macusi et al. 2011). In the subtidal zone, where green light predominates, the specific photosynthesis pigments of the red algae allow efficient absorption (Wu 2016). And growth rates and photosynthesis of several red algal species depend on the quality of the light and pigment composition during the cultivation period (Leukart and Lüning 1994). However, the light requirements are very low in green light for all red algae, the action spectrum of growth is synchronized with the photosynthetic action spectrum with maximum efficiencies in green wavelength (Leukart and Lüning 1994). Contrary to the present study, Ghedifa (2021) has reported that Gracilaria gracilis presented the highest growth rate under red light over those grown under ultraviolet, blue and red light. This could happen due to absorption characteristics of algae that depend on several other factors including the thallus morphology, thickness, and structure of the photosynthetic system (Larkum et al. 1967)

Agar yields are highly determined by environmental conditions and growth stage of algae. Agar yielded from seaweeds is reported to vary between 6-71% and 20-30% of the yield can be obtained from most agarophytes (Mclachlan and Bird 1986). *Gracilaria* sp. normally provides 10-25% of agar yield (Hoyle 1978) and, Jayasinghe et al. (2016) have reported a yield of  $20.5\pm0.39\%$  of agar from alkaline-treated *G. edulis*. The agar yield obtained from *G. edulis* exposed to a red light in the present work is higher than the

yield reported by Jayasinghe et al. (2016), but the agar yield obtained from *G. edulis* cultured under green light and agar yielded by Jayasinghe et al. (2016) are approximately similar. Although the agar yielded from the *G. edulis* exposed to natural light and violet light in the present study are comparatively lower, *G. edulis* exposed to red light results in the highest agar yield than those cultured under natural, green, and violet lights. These findings confirm that the colour of light to which *G. edulis* is exposed affects significantly on agar production. Red light range in the visible light spectrum is important to drive photosynthesis (Kang et al. 2013). Therefore, the high agar yield from *G. edulis* exposed to red light could be due to the high photosynthesis rate which helps produce the polysaccharide component of agar. Certain wavelengths of ultraviolet radiations of solar light cause harmful effects on the DNA of the cells. This could be the reason for the low agar yield under natural light. *G. edulis* treated with red light performs low growth rate than thallus exposed to green light. The growth rate is a result of cell division, and red light treated *G. edulis* must be saving a comparatively high amount of energy for agar production. This may be the possible reason for the significant difference in the yield of *G. edulis* treated with red and green light.

High ash content in extracted agar indicates the presence of inorganic salts generated during the treatment process. In addition, a high amount of inorganic minerals present in the raw seaweeds may also be responsible for the higher ash content. Differences in moisture content vary with the extent of drying and exposure to humidity during storage. Permeability to moisture through materials used for packaging may affect the moisture content. However, the moisture content of extracted agar from *G. edulis* and *G. verrucosa* (17.1±0.61%) by Jayasinghe et al. (2016) is in the range of moisture content of agar extracted from *G. edulis* in the present work. The crude protein contents of agar extracted in the present study are much lower than the crude protein content of extracted agar (6±0.35%) from *Gracilaria sp.* (Jayasinghe et al. 2016). The low-fat composition of agar shows efficient removal of the fat and water during the extraction process (Songchotikunpan et al. 2008) and therefore, higher fat content in extracted agar in the present study over the fat content recorded from *G. edulis* and *G. verrucosa* by Jayasinghe et al. (2016) could be due to the insufficient removal of water and fat during the extraction process.

The amount of hydrophilic substances contained in agar may change the water holding capacity of agar in the present study. Extraction conditions, agar concentration, and surrounding temperature are greatly affecting on the viscosity of the agar solution and are proportional to its molecular weight (Murano 1995; Praiboon et al. 2006). Hence, the agar extracted from *G. edulis* in the present work may consist of low molecular weight due to lower viscosity in extracted agar. Furthermore, alkali treatment may cause a reduction in the viscosity of extracted agar.

The gel strength of extracted agar prepared from different light-treated *G. edulis* reflects one of the essential qualities of agar. However, the gel strength in the present study are much lower than those of alkalitreated *G. fishery* (228.27 $\pm$ 48.18g/cm<sup>2</sup>), *G. edulis* (239.95 $\pm$ 28.35g/cm<sup>2</sup>), and *Gracilaria* sp. (334.50 $\pm$ 14.1g/cm<sup>2</sup>) recorded by Praiboon et al. (2006). The gel strength of agar is affected mainly by species, different environmental parameters, extraction methods or treatment, and measuring method of gel strength (Lee et al. 2016). Some studies (Freile-Pelegrin and Murano 2005; Meena et al. 2008) have reported that alkalit treatments fail to improve the gel strength of agar in some cases. Daugherty and Bird (1988) have reported that agar extracted from seaweeds cultivated at  $23\pm3^{\circ}$ C and  $31\pm1^{\circ}$ C exhibit lower gel strengths than seaweeds cultured at normal temperature ( $29\pm1^{\circ}$ C). It can be assumed that alkaline treatment and water temperature of culture units in the present work may reduce the gel strengths of agar.

The mean Gelling and melting temperatures of extracted agar in the present experiment are not in accordance with the findings of Jayasinghe et al. (2016). Changes in agar extraction methods such as soaking time, soaking temperature, extraction temperature, and alkaline/non-alkaline treatments may affect the melting and gelling temperatures. The alkaline treatments used in the agar extraction procedure during the present study have decreased the melting temperature of the extracted agar.

## Conclusion

The present experimental setup helps identify the most effective colour of the visible light spectrum to enhance the growth rate and harness a higher yield of high-quality agar from G. *edulis*. The study unfolds that surface (up to 1m) and deeper waters are not suitable for culturing G. *edulis* commercially, and a higher growth rate and high-quality agar can be obtained by culturing G. *edulis* under a mixture of red and



green light. Also, the culture depth of *G. edulis* should not be too close to the surface and deeper than 5m. Findings of the present experimental study help elucidate that the 1-5m depth range is suitable for culturing *G. edulis* commercially for agar production and, the study warrants *in-situ* experiments in open water to affirm the present findings.

Acknowledgement The Faculty of Fisheries and Marine Sciences & Technology, University of Ruhuna, Sri Lanka is acknowledged for facilitating the experimental work.

Conflicts of interest The authors declare no conflict of interest.

#### References

AOAC (1995) Official methods of analysis of the association of official analytical chemists, Washington, DC, USA

Armisen R (1991) Agar and agarose biotechnological applications. Hydrobiologia 221:157–166. https://doi.org/10.1007/BF00028372

Armisen R (1995) World-wide use and importance of *Gracilaria*. J Appl Phycol 7:231–243. https://doi.org/10.1007/BF00003998

- Bixler HJ, Porse H (2011) A decade of change in the seaweed hydrocolloids industry. J Appl Phycol 23:321–335. https://doi. org/10.1007/s10811-010-9529-3
- Cai J, Lovatelli A, Aguilar-Manjarrez J, Cornish L, Dabbadie L, Desrochers A, Diffey S, Garrido-Gamarro E, Geehan J, Hurtado A, Lucente D, Mair G, Miao W, Potin P, Przybyla C, Reantaso M, Roubach R, Tauati M, Yuan X (2021) Seaweeds and microalgae: an overview for unlocking their potential in global aquaculture development. FAO Fisheries and Aquaculture Circular No. 1229. Rome. https://doi.org/10.4060/cb5670en
- Cai J, Lovatelli S, Stankus A, Zhou X (2021) Seaweed revolution: where is the next milestone. FAO Aquaculture Newsletter 63:13–16 Cho SM, Kwak KS, Park DC, Gu YS, Ji CI, Jang DH, Lee YB, Kim SB (2004) Processing optimization and functional properties of
- gelatin from shark (*Isurus oxyrinchus*) cartilage. Food Hydrocoll 18(4):573–579. https://doi.org/10.1016/j.foodhyd.2003.10.001
  Chung K, Soo S, Teo SS, Phang S (2011) Analysis of native and alkali-treated agar from species of *Gracilaria* (Gracilariaceae, Rhodo-phyta) collected from Morib, Malaysia. Malaysian J Sci 30(2):83-91. https://doi.org/10.22452/mjs.vol30no2.1
- Daugherty BK, Bird KT (1988) Salinity and temperature effects on agar production from Gracilaria verrucosa strain G-16. Aquaculture 75:105–113. https://doi.org/10.1016/0044-8486(88)90025-7
- Distantina S, Wiratni, Fahrurrozi M, Rochmadi (2011) Carrageenan properties extracted from *Eucheuma cottonii* Indonesia. Int J Chem Mol Eng 5:487-491. doi.org/10.5281/zenodo.1333328
- Falshaw R, Furneaux RH, Stevenson DE (1998) Agars from nine species of red seaweed in the genus *Curdie* (glacilariaceae, rhodo-phyta). Carbohydr Res 308:107-115. https://doi.org/10.1016/S0008-6215(98)00049-4
- FAO (2020) The State of world fisheries and aquaculture 2020, Sustainability in action. Rome. https://doi.org/10.4060/ca9229en
- Folch J, Lees M, Stanley GHS (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226(1):497-509. https://doi.org/10.1016/S0021-9258(18)64849-5
- Freile-Pelegrin Y, Murano E (2005) Agars from three species of *Gracilaria* (Rhodophyta) from Yucatán Peninsula. Bioresour Technol 96(3):295-302. https://doi.org/10.1016/j.biortech.2004.04.010
- Galatas F (2006) Agar-gulaman-kanten: The king of fibers. In: Abstracts of the 6th Asia-Pacific conference on algal biotechnology, Manila, Philippines, 12-15 October 2006
- Ghedifa AB, Vega J, Korbee N, Fethi M, Figueroa FL, Sadok S (2021) Effects of light quality on the photosynthetic activity and biochemical composition of *Gracilaria gracilis* (Rhodophyta). J Appl Phycol 33(5):1-13. https://doi.org/10.1007/s10811-021-02496-y
- Glicksman M (1987) Utilization of seaweed hydrocolloids in the food industry. Hydrobiologia 151:31–47. https://doi.org/10.1007/ BF00046103
- Godínez-Ortega JL, Snoeijs P, Robledo D, Freile-Pelegrin Y, Pedersen M (2008) Growth and pigment composition in the red alga *Halymenia floresii* cultured under different light qualities. J Appl Phycol 20:253-260. https://doi.org/10.1007/s10811-007-9241-0
- Hoyle MD (1978) Agar studies in two Gracilaria species (G. bursapastoris (Gmelin) Silva and G. coronopifolia J. Ag.) from Hawaii. II. seasonal aspects. Bot Mar 21:347–352. https://doi.org/10.1515/botm.1978.21.6.347
- Ikelite (2021) Why you need strobes underwater. https://www.ikelite.com/blogs/buying-guides/why-you-need-strobes-underwater. Accessed 27 Nov 2021

Imeson A (ed) (2010) Food stabilisers, thickeners and gelling agents. Blackwell

- Indriatmoko, Heriyanto, Limantara L, Brotosudarmo THP (2015) Composition of photosynthetic pigments in a red alga *Kappaphycus* alvarezi cultivated in different depths. Procedia chem 14:193-201. https://doi.org/10.1016/j.proche.2015.03.028
- Jayasankar R, Varghese S (2002) Cultivation of marine red algae *Gracilaria edulis* (Gigartinales, Rhodophyta) from spores. Indian J Mar Sci 31(1):75-77. http://eprints.cmfri.org.in/id/eprint/7016
- Jayasinghe PS, Pahalawattaarachchi V, Ranaweera KKD (2016) Effect of extraction methods on the yield and physiochemical properties of polysaccharides extracted from seaweed available in Sri Lanka. Poult fish wildl sci 4:150. doi:10.4172/2375-446X.1000150
- Kang JH, Krishnkumar S, Atulba SLS, Jeong BR, Hwang SJ (2013) Light intensity and photoperiod influence the growth and development of hydroponically grown leaf lettuce in a closed-type plant factory system. Hortic Environ Biote 54:501-509. https://doi. org/10.1007/s13580-013-0109-8
- Larkum AWD, Drew EA, Crossett RN (1967) The vertical distribution of attached marine algae in Malta. J Ecol 55(2):361–371. https://doi.org/10.2307/2257881
- Lee WK, Lim P, Phang S, Namasivayam P, Ho C (2016) Agar properties of *Gracilaria* species (Gracilariaceae, Rhodophyta) collected from different natural habitats in Malaysia. Reg Stud Mar Sci 7:123–128. https://doi.org/10.1016/j.rsma.2016.06.001

Lee W, Lim Y, Leow AT, Namasivayam P, Abdullah JO, Ho C (2017) Factors affecting yield and gelling properties of agar. J Appl Phycol 29:1527-1540. https://doi.org/10.1007/s10811-016-1009-y

Leukart P, Lüning K (1994) Minimum spectral light requirements and maximum light levels for long-term germling growth of several

red algae from different water depths and a green alga. Eur J Phycol 29(2):103-112. https://doi.org/10.1080/09670269400650551 Łuczyński T, Birk A (2018) Underwater image haze removal and colour correction with an underwater-ready dark channel prior.

arXiv:1807.04169. https://arxiv.org/abs/1807.04169. Accessed 20 Nov 2021 Macusi ED, Katikiro RE, Deepananda KA, Jimenez LA, Conte AR, Fadli N (2011) Human induced degradation of coastal resources in Asia Pacific and implications on management and food security. J Nat Studies 9:13-28

Madhavan S, Abirami (2015) A review on hydrocolloids-agar and alginate. J Pharm Sci Res 7(9):704-707

Marinho-Soriano E (2012) Effect of depth on growth and pigment contents of the macroalgae Gracilaria bursapastoris. Braz J Pharmacogn 22(4):730-735. https://doi.org/10.1590/S0102-695X2012005000059

McHugh DJ (2003) A Guide to the seaweed industry, FAO Fisheries Technical Paper 441. Rome, 105 p.

- Mclachlan J, Bird CJ (1986) Gracilaria (Gigartinales, Rhodophyta) and productivity. Aquat Bot 26:27–49. https://doi.org/10.1016/0304-3770(86)90004-5
- Meena R, Prasad K, Ganesan M, Siddhanta AK (2008) Superior quality agar from *Gracilaria* species (Gracilariales, Rhodophyta) collected from the Gulf of Mannar, India. J Appl Phycol 20:397–402. https://doi.org/10.1007/s10811-007-9272-6
- Murano E (1995) Chemical structure and quality of agars from *Gracilaria*. J Appl Phycol 7:245. https://doi.org/10.1007/BF00003999 Oliveira VP, Freire F (2012) Influence of the depth on the growth of the seaweed *Gracilaria birdiae* (Rhodophyta) in a shrimp pond. Braz J Aquat Sci Technol 16(1):33-39. doi:10.14210/bjast.v16n1.p33-39
- Praiboon J, Chirapart A, Akakabe Y, Bhumibhamon O, Kajiwara T (2006) Physical and chemical characterization of agar polysaccharides extracted from the Thai and Japanese species of *Gracilaria*. Sci Asia 32:11–17. doi: 10.2306/scienceasia1513-1874.2006.32(s1).011
- Rhein-Knudsen N, Ale MT, Meyer AS (2015) Seaweed hydrocolloid production: an update on enzyme assisted extraction and modification technologies. Mar Drugs 13(6):3340-3359. https://doi.org/10.3390/md13063340
- Saffo MB (1987) New light on seaweeds. Bio Sci 37(9):654-664. doi:10.2307/1310712
- Schmidt EC, Nunes BG, Maraschin M, Bouzon ZL (2010) Effect of ultraviolet-B radiation on growth, photosynthetic pigments, and cell biology of *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) macroalgae brown strain. Photosynthetica 48:161–172. https:// doi.org/10.1007/s11099-010-0022-7
- Cho SM, Kwak KS, Park DC, Gu YS, Ji CI, Jang DH, Kim SB (2004) Processing optimization and functional properties of gelatin from shark (Isurus oxyrinchus) cartilage. Food Hydrocolloids, 18(4):573–579
- Chung KM, Soo SS, Teo SS and Phang SM (2011) Analysis of native and alkali-treated agar from species of Gracilaria (Gracilariaceae, Rhodophyta) collected from Morib, Malaysia. Malaysian J Sci 30(2):83-91
- Godínez-Ortega JL, Snoeijs P, Robledo D, Freile-Pelegrín Y, Pedersén M (2008) Growth and pigment composition in the red alga Halymenia floresii cultured under different light qualities. J Appl Phycol 20:253-260
- Jayasankar R, Varghese S (2000) Growth and pigment constituents of the red alga *Gracilaria edulis* (Gmelin) Silva, grown at different depths. Indian J Fish 47(4):365-369
- Kumar K, Fotedar R (2009) Agar extraction process for Gracilaria cliftonii. Carbohydrate Polymers, 78(4):813-819
- Oliveira VP, FREIRE FA and Soriano EM (2012) Influence of depth on the growth of the seaweed *Gracilaria birdiae* (Rhodophyta) in a shrimp pond. Braz J Aquat Sci Techno 16(1):33-39
- Songchotikunpan P, Tattiyakul J, Supaphol P (2008) Extraction and electrospinning of gelatin from fish skin. Int J Biol Macromol 42:247–255
- Sousa-Pinto I, Lewis R, Polne-Fuller M (1996) The effect of phosphate concentration on growth and agar content of *Gelidium robustum* (Gelidiaceae, Rhodophyta) in culture. Hydrobiologia 326/327:437-443

#### **Publisher's Note**

IAU remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.