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Extraction of agar from *Eucheuma cottonii* and *Gelidium amansii* seaweeds with sonication pretreatment using autoclaving method*

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Abstract The effect of sonication pretreatment condition on *Eucheuma cottonii* and *Gelidium amansii* seaweed towards agar extraction wae studied. Four parameters were changed during sonication to investigate the effects on agar yield and quality. These parameters include the time interval, concentration ratio, frequency, and intensity. The highest amount of agar extracted from *Eucheuma cottonii* species could be obtained from the time interval of 30 min, seaweed weight to solvent volume ratio of 1:20, the frequency of 35 Hz, and the sonication power intensity of 30%. For *Gelidium amansii* species, the best agar yield also could be obtained from the time interval of 30 min, 1:20 of seaweed weight to water volume ratio, the frequency of 35 Hz, and power intensity of 30%. From the experiment, sonication pretreatment significantly influenced the yield and properties of extracted agar. The sonication with autoclaved seaweed produced agar containing less sulfate content, which is an excellent chemical property for gel electrophoresis applications. The gel strength of sonication with autoclaving for both seaweeds, *Eucheuma* and *Gelidium* species was the highest among those by sonication with direct heating, which proved that sonication pretreatment with autoclaving could enhance the physical properties of the agar.

Keyword: *Gelidium amansii*; *Eucheuma cottonii*; autoclaving; sonication pretreatment; water extraction

1 INTRODUCTION

Agars are derived from marine macroalgae, particularly red seaweeds. They contain strong gelling polysaccharides and linear polymers can be characterized by its chemically repeating units of 3-6, anhydro L-galactose and alternating D-galactose bond (Francavilla et al., 2013). The structure of agar comprises two separate polymers that can be fractionated. One of them is agarose, which has better gelling properties, and the other is agaropectin, which is the non-gelling sulfated fraction (Nishinari and Fang, 2017). Red seaweed can also peculiarly produced sulfated galactans, which consist of sulfate esters in very low levels and some methoxy groups (Recalde et al., 2016). However, the pattern of sulfate groups and the type of glycosidic linkage in the polysaccharides differ among the species.

Agars are widely used for various applications, which include food industry, dentistry, bacteriological and biotechnological uses (Hernández-Carmona et al., 2013; Abdul Khalil et al., 2018). In the food industry, agars are used as a gelling and thickening

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agent, where the gelling properties of agars are used as a stabilizer in acidic dairy products. In the dentistry and biotechnology application, derivatives of agars can be used as dental prosthetics, molding of materials, and plant culture tissues (Chew et al., 2018a; Ouyang et al., 2018). There are three categories of algae: red algae, brown algae (Phaeophyceae) and green algae (Chlorophyceae) (Abdul Khalil et al., 2018). Brown macroalgae consist of practically 1 800 species, with an olive-green to dark brown color derived from an abundance of fucoxanthin. This type of macroalgae consumes a yellow-brown pigment that masks the green color of chlorophyll. Green algae contain approximately 1 500 species all over the world, which are mainly used as a starch in the food industry due to the capability of the polysaccharides structures containing cellulose and pectin. Meanwhile, for red algae, they consist of 6 000 species, which commonly grow in deep waters. Red algae were specifically called as red due to the phycocyanin and phycoerythrin pigments present in this marine macroalgae type (Fuse and Goto, 1971). Additionally, the composition of red algae imitates their species, which contains cellulose, glucan, and galactan. Two types of structural polysaccharides that are useful in gel formation can be found in these algae, which are carrageenan and agar. The red alga *Gelidium amansii*, is mainly used for agar extraction, due to its high quality of agars to be extracted and the compositions contained in these type of species (Hernández-Carmona et al., 2013). While for *Eucheuma cottonii* (Kappaphycus Alvarezii), they are commonly used for carrageenan extraction (Distantina et al., 2011). These types of macroalgae obtained a high yield of carrageenan on previous studies (Loureiro et al., 2010; Chan et al., 2013; Recalde et al., 2016). To date, however, none of the researchers has studied the structures of these macroalgae. Therefore, one of the objectives of this study was to investigate and study the presence of agar structures and their properties in *Eucheuma cottonii* species compared than *Gelidium amansii* species.

There are various of methods that have been developed and currently being used by the industries to produce agar, such as an eco-friendly agar extraction method called agar photobleaching extraction process (Li et al., 2008). In their study, the photo-bleached agar gelling temperature means that the temperature is within the acceptable range of the international market. They also discover that the photolysis process used by them was useful for sulfate group removal with alkaline hydrolysis while increasing the gel strength of agar that produced during the photolysis (Li et al., 2008). Recently, the optimization and quality of agar polysaccharide isolated from the marine red macroalgae *Pyropia yezoensis* by using alkaline pretreatment have been studied (Sasuga et al., 2017). It was claimed that alkaline pretreatment of agar extraction process helps the conversion of L-galactose sulfate to 3,6-anhydrogalactose, by increasing their average molecular weight. Alkali-pretreated agar also contains less sulfate content and higher gel strength values than that of native agar. Therefore, their study proved that alkaline pretreatment for *Pyropia yezoensis* species could produce industrial grade agar comparable to that by *Gelidium* species (Sasuga et al., 2017).

Nowadays, most of the currently used methods are green and safe to the environment, apart from being efficient and suitable for an industry used (Li et al., 2008). However, some of the methods used require long processing time, complicated operation, and using harsh chemicals. This creates the need for some innovation on the processing of agar to improve the quality and yield of agar extracted, whereby the utilization of an advanced pretreatment technology that could improve the extraction of agar would benefit the overall economics of agar processing (Chew et al., 2018b). The traditional process of agar extraction involves steps like alkali-treated seaweeds in an extraction solution, filtration of the extract, and jellification, freezing, thawing, and drying etc. Therefore, the extraction methods can thus vary in terms of the type and parameter used to get various properties of the agar-extracted product (Souza et al., 2012).

In this study, agar was extracted from the red macroalga *Eucheuma cottonii* species, and then was compared to existing agar sources of *Gelidium amansii* in terms of the yield, and physical and chemical properties of the resulting agar. On the other hand, we focused on the effect of autoclaving in ultrasonic pretreatment to extract agar from both red macroalgae species. Agar was subsequently extracted using the eco-friendly and simple method. The effects of different sonication pretreatment parameters used on agar extraction were evaluated where the parameters include (time interval, frequency, intensity, and concentration). Our study was done to verify the feasibility of agar extraction by applying sonication pretreatment with autoclaving method proposed. The

Table 1 Condition of sonication pretreatment process

Type of sample	Sample	Parameter	
		Type	Value
Gelidium (sonication + autoclaving)	GPA30	Intensity (%)	30
	GPA60		60
	GPA90		90
	GCA1	Seaweed to water ratio (g/mL)	1:60
	GCA3		3:60
	GCA5		5:60
	GFA35	Frequency (Hz)	35
	GFA130		130
	GTA15	Time (min)	15
	GTA30		30
	GTA45		45
Eucheuma (sonication + autoclaving)	EPA30	Intensity (%)	30
	EPA60		60
	EPA90		90
	ECA1	Seaweed to water ratio (g/mL)	1:60
	ECA3		3:60
	ECA5		5:60
	EFA35	Frequency (Hz)	35
	EFA130		130
	ETA15	Time (min)	15
	ETA30		30
	ETA45		45
Gelidium (sonication + direct heating)	GC1	Seaweed to water ratio (g/mL)	1:60
	GC3		3:60
	C5		5:60
	GF35	Frequency (Hz)	35
	GF130		130
	GT15	Time (min)	15
	GT30		30
	GT45		45
Eucheuma (sonication + direct heating)	EC1	Seaweed to water ratio (g/mL)	1:60
	EC3		3:60
	EC5		5:60
	EF35	Frequency (Hz)	35
	EF130		130
	ET15	Time (min)	15
	ET30		30
	ET45		45

comparison of agar yield and quality for both seaweeds are further discussed.

2 MATERIAL AND METHOD

2.1 Material

Two types of red macroalgaes, *Gelidium amansii*, and *Eucheuma cottonii*, were used. The seaweeds were collected in South Korea (33°6′–43°0′N / 124°11′–131°50′E). The samples were washed thoroughly with clean water and dried using the air-drying oven (Memmert, Germany) for 3 to 4 days at 50°C. The samples were peeled into smaller pieces and stored in dry place for further experiments. HCl, K_2SO_4, H_2SO_4 and other chemicals were obtained from R&M Chemicals (Malaysia).

2.2 Method

2.2.1 Synthesis of agar

Agar extraction was performed as per Mollet et al. (1998). The seaweeds were extracted by using a water extraction method, with a water ratio of 1:20 to 1:30 [w/v] (Mollet et al., 1998). In brief, the pH of the solution was adjusted to 6.0–6.5 using 0.0025% H_2SO_4 solution and measured using a pH meter (Sartorius, model PB-10). Then, pretreatment was operated by using an ultrasonic processor (Hielscher, UP400S) fitted with a standard sonotrodes H_3, in which the process was divided into two conditions, (i) sonication pretreatment with autoclaving; and (ii) sonication pretreatment with direct heating. The parameters used for sonication are shown in Table 1. All experiments were performed at room temperature.

The hot water extraction after the sonication was divided into two conditions: using direct heating (Fisher Scientific, hotplate stirrer) and autoclaving. For direct heating, the temperature used was 99°C for 1.5 h, while for autoclaving, the temperature used was 120°C for 1 h. After the extraction, the seaweed solution was filtered to remove insoluble particles from the aqueous solution by using filter cloth and cellulose nitrate membrane filtration (3 μm). The filtrate was then allowed to cool down to ambient temperature, and being frozen overnight at -20°C. Thereafter, the solution was thawed at room temperature and was subjected to centrifugation (5 500 r/min, 15 min) to separate the water content and desired agar mixture. This separation step was important to obtain the pure agar content. Then, the mixture was oven dried (VO200-Memmert, Germany) at 50–60°C for 2 or 3 days until a constant weight was obtained. The dried agar sample was weighed and recorded.

2.2.2 Characterization of agar

The structural characterization of agar was carried out in Fourier Transmission Infrared Spectroscopy FTIR (Perkin Elmer, Germany). The characterization was conducted in order to observe the purity and chemical bonding of the agar structures. The frequency range was between 4 000–400 cm^{-1}. The resolution used is 4 cm^{-1}, which is 8 times of scanning rate. The force gauge chosen for agar characterization was between 80–100 N.

2.2.3 Measurement of gelling and melting temperatures

Gelling and melting temperatures of the agar samples were measured as described by Wang et al. (2017), with some modifications (Wang et al., 2017). For gelling temperature, 5 mL of hot agar solution (2.0% w/v) was prepared in a 50-mL test tube. The agar solution was cooled at a rate of 0.5–1.0°C/min. At the same time, a thermometer was placed in the hot agar solution to record the temperature. The test tube containing the agar solution was slanted by 45° and returned back to the vertical position, for each 0.5°C drop in temperature. The gelling temperature was obtained when the surface of the agar solution does not go back to its horizontal position and was completely congealed.

While for the melting temperature, 5 mL of agar gel (2.0% w/v) was prepared in a 10-mL test tube. The sample was placed at room temperature and kept overnight, covered with aluminum foil. An iron ball of 4 mm diameter was placed on the surface of the agar gel. The sample was submerged into a beaker containing water and placed on a hotplate with adjustable heat. The range of temperature adjusted to reach 100°C (with a gradual increase of 1°C/min). The melting temperature was recorded when the iron ball touched the bottom of the test tube.

2.2.4 Measurement of agar gel strength

Gel strength of agar was measured by using a TA-XT plus texture analyzer (Stable Micro Systems Ltd., Surrey, UK). The agar gel was prepared by using 2.0% (w/v) of agar gel with gel depth approximately 15–20 mm in a test tube. The agar gel was placed at room temperature and left overnight. The 75 mm diameter cylindrical plunger p/75 probe was used for analysis, with a 5 000-g load cell and the penetration speed of 1 mm/s for a distance of 5 mm. The gel strength was measured and expressed in g/cm^2.

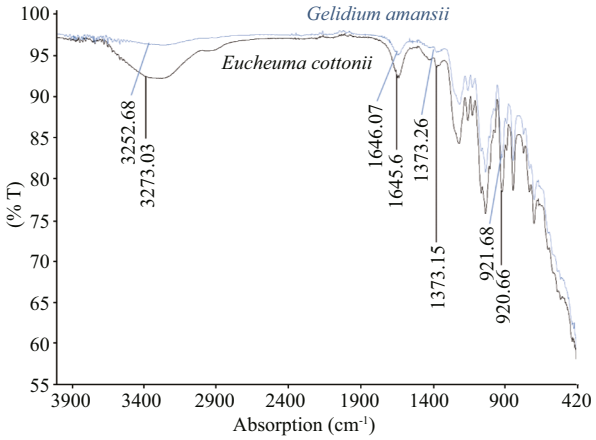

Fig.1 FTIR spectra of extracted agar from *Gelidium amansii* and *Eucheuma cottonii* species

2.2.5 Quantification of sulfate content

Sulfate content was measured as per Yarnpakdee et al. (2015), in which $BaCl_2$-gelatin reagent was used immediately after HCl hydrolysis. Firstly, agar powder (40 mg) was hydrolyzed at 105–110°C with 6 mL of 0.5 mol/L HCl solution. Then, the solution was mixed and filtered using Whatman No. 1 filter paper (0.22 μm). Next, 0.2 mL of solution was transferred into a 10-mL test tube containing 3.8 mL of trichloroacetic acid, TCA (3%w/v) and 1-mL $BaCl_2$-gelatin reagent. The solution was mixed and left at room temperature for 20 min. Sample blank was prepared, except the distilled water was used instead of the agar sample. The absorbance at 360 nm was measured using a UV-Vis spectrophotometer (Shimadzu, model UV-1800). Finally, the sulfate content was measured and expressed by a standard curve prepared using solutions of K_2SO_4 in concentration from 0.1 to 1.0 mg/mL (0.053 3–0.533 mg/SO_4^{2-}/mL) as a reference.

3 RESULT

3.1 Characterization of Agar

Agar was successfully characterized by using the FTIR spectra as shown in Fig.1. Two types of agars that extracted from *Gelidium amansii* and *Eucheuma cottonii* have gone through sonication pretreatment in autoclaving were successfully studied. For *Gelidium* seaweed, strong absorption peaks can be seen at 3 252.68 cm^{-1}, 1 646.07 cm^{-1}, 1 373.26 cm^{-1}, and 921.68 cm^{-1}. While for *Eucheuma*, high absorption peaks were shown at 3 273.03 cm^{-1}, 1 645.6 cm^{-1}, 1 373.15 cm^{-1}, and 920.66 cm^{-1}. These peaks represent specific chemical bonding and vibration resulted from

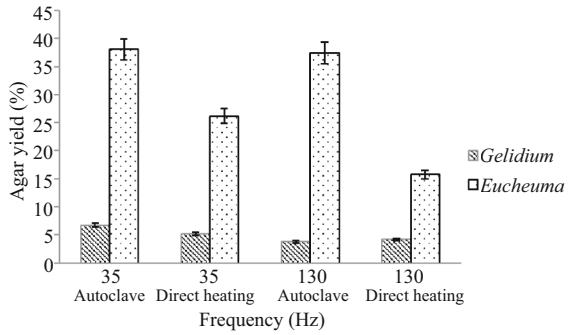

Fig.2 Agar yield obtained from two types of seaweed in two different frequencies

The yield is expressed as a percentage of dry weight.

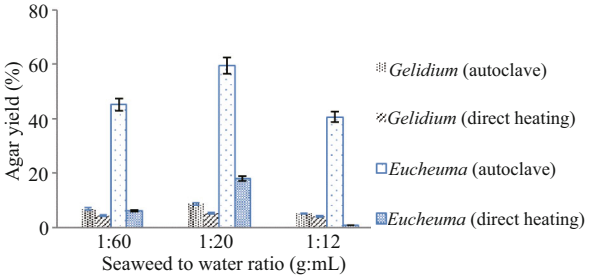

Fig.3 Agar yield obtained from the different ratio of seaweed weight and water volume used

The yield is expressed as a percentage of dry weight.

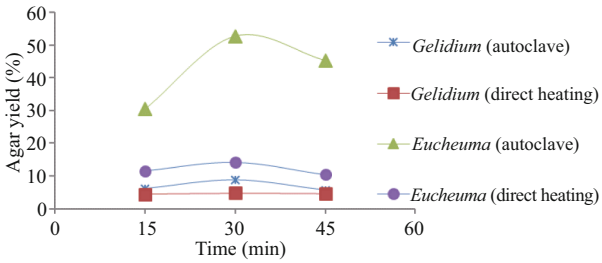

Fig.4 Agar yield obtained from different time interval for two types of seaweeds that sonication-pretreated with autoclaving and with direct heating

The yield is expressed as a percentage of dry weight.

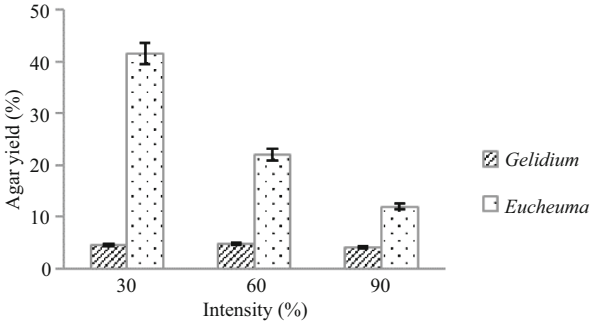

Fig.5 Agar yield obtained from two types of seaweed used with different values of intensity

The yield is expressed as a percentage of dry weight.

the agar structures. Both seaweeds showed higher absorption bands at range 920–921 cm^{-1}, while less vibration occurred at the range of 1 373.15 cm^{-1} and 1 373.26 cm^{-1}.

3.2 Effect of frequency on agar yield

The agar yield obtained is shown in Fig.2. The overall agar yield was calculated based on the percentage of dry weight. In overall, Fig.2 shows that the value of the highest agar yield occurred at frequency 35 Hz for both *Gelidium* and *Eucheuma* species by sonication pretreatment with autoclaving, which was 6.78% and 38.06%, respectively. The lowest agar yield for *Gelidium* and *Eucheuma* species was obtained when the frequency was 130 Hz, which as 3.76% and 15.75%, respectively.

3.3 Effect of seaweed weight to water volume ratio on agar yield

Figure 3 shows the effect of seaweed weight to water volume ratio on agar yield produced. The highest agar yield for *Eucheuma cottonii* seaweed was 59.47%, which obtained from 1:20 ratio by sonication pretreatment with autoclaved agar, where the lowest agar yield from the same species was 0.88%, which was obtained from 1:12 ratio by sonication pretreatment with direct heating agar. For *Gelidium amansii* seaweed, 1:20 ratio at sonication pretreatment with autoclaving showed the highest agar yield compared than 1:12 ratio at sonication pretreatment with direct heating, which was 8.77% and 4.16%, respectively.

3.4 Effect of time on agar yield

Figure 4 shows the effect of time interval on agar yield. For *Eucheuma cottonii* species, the highest value of agar yield was at 30 min time interval by sonication pretreatment with autoclaving while the lowest at 45 min time interval of sonication pretreatment with direct heating, which was 52.70% and 10.37%, respectively. Whereas for *Gelidium amansii* species, the highest value of agar yield was at 30 min time interval by sonication pretreatment with autoclaving and the lowest at 15 min of sonication pretreatment with direct heating, which was 8.70% and 4.45%, respectively.

3.5 Effect of power intensity on agar yield

Figure 5 showed the power intensity on agar yield produced. Based on the results, the highest value was

Table 2 The optimization results obtained from two types of seaweeds, where the yield is expressed as a percentage of dry weight

Species	Pretreatment method	Agar yield (%)	Physical parameter			Chemical parameter
			Gel strength (g/cm^2)	Gelling temperature (°C)	Melting temperature (°C)	Sulfate content (mg/mL)
Gelidium amansii	Autoclaving	49.10	840	26.9	65.1	0.310 8
	Direct heating	13.26	390	25.7	59.8	0.477 5
Eucheuma cottonii	Autoclaving	56.49	190	25.2	68.0	0.301 3
	Direct heating	16.02	17	25.5	51.4	0.417 4

41.58% of power intensity, 30% for sonicated *Eucheuma cottonii* species while the lowest value was 4.22% of power intensity, 90% for sonicated *Gelidium amansii* species. For *Eucheuma* species, the trend of the agar yield was decreasing as the intensity increased, which were 41.58%, 22.02% and 12.02% for the power of 30, 60, and 90, respectively. As for *Gelidium* species, the agar yield for the power of 30, 60, and 90 were slightly different, which were 4.73%, 4.53%, and 4.22%, respectively.

3.6 Optimization condition

The agar yields depend on physical and chemical properties, such as gelling strength, gelling and melting temperature, and sulfate content. The optimization conditions for agar yield are shown in Table 2. For *Gelidium amansii* species, the yield for sonication pretreatment with autoclaved agar showed a higher value than that of sonication pretreatment with direct heating, being 49.10% and 13.26%, respectively. For *Eucheuma* species, the yield for sonication pretreatment with autoclaving was 56.49%, which is higher than that of sonication with direct heating for about 16.02%.

In Table 2, the gel strength values of each condition are in different ranges. Consequently, agars from *Eucheuma* and *Gelidium* species by sonication pretreatment with autoclaving showed the highest values than those with direct heating, which was 190 g/cm^2 and 840 g/cm^2, respectively. For sonication pretreatment with direct heating, the values for *Eucheuma* and *Gelidium* were 17 g/cm^2 and 390 g/cm^2, respectively. Meanwhile, for gelling and melting temperature of both seaweeds tested, the values ranged from 26.9°C to 25.2°C and from 51.4°C to 68.0°C, respectively. Where for the sulfate content, according to the results, all the samples attained with almost similar sulfate content (0.477 5 to 0.301 3 mg/mL). The sulfate contents of sonication pretreatment with autoclaving for *Eucheuma* and *Gelidium* species were quite low, for 0.301 3 and 0.310 8 mg/mL, while that of sonication with direct heating was 0.477 5 and 0.417 4 mg/mL, respectively.

4 DISCUSSION

4.1 Characterization of agar

As shown in Fig.1, agar extracted from *Gelidium amansii* species showed a high peak absorption at 1 373.26 cm^{-1}, which is due to the sulfate ester (S=O group) vibration. For the vibration peak at 3 252.68 cm^{-1}, it was caused by the interaction of the oxygen atom of OH groups of D-galactopyranose. Thereafter, the other chemical groups for the characteristics of a given agar type, namely 3,-6-anhydro-L-galactopyranose at 921.68 cm^{-1} and carbonyl group (C=O stretching) were observed at 1 646.07 cm^{-1}. On the other hand, the FTIR spectra of *Eucheuma cottonii* agar showed a very strong beam absorption in the region 3 273.03 cm^{-1} (for OH stretching of the hydroxyl group) and 1 645.6 cm^{-1} region (C=O stretch peak). The agar extracted from *Eucheuma cottonii*, showed a strong absorption band in the region 1 373.15 cm^{-1}, which was caused by the sulfate ester (S=O groups) vibration. Gómez-Ordóñez and Rupérez (2011) stated that the peaks produced are correlated to the agar quality, and the presence of α-L-galactose, -6-sulfate units may lessen the gel strength of the polygalactans. Whereas, both of the agar products displayed high peaks at 920.66 cm^{-1}, reflecting the vibration of the C-O-C bridge of 3,6-anhydro-L-galactopyranose. This C-O-C group band is the utmost representative of the agar structure because infrared spectra of other polysaccharides contained in red macroalgae like cellulose and xylans would not be observed at this wave range. This band is also associated with the degree of sulphatation, due to the 1,4-linked residue that may be replaced by 3,6-anhydro-L-galactose residue or be switched by other sulfate groups (Stanley, 1995).

4.2 Effect of frequency on agar yield

The data (Fig.2) show that the agar yield of sonication pretreatment with autoclaving was higher than those of direct heating. Previously, Fidelis et al. (2014) studied the effect of sonication frequency and enzymatic proteolysis, as well as an alkaline pretreatment on chemical composition, structure, and antioxidant activity of sulfated polysaccharides extracted from *Glacilaria birdiae*. They found that sonication frequency at a lower value as well as alkali treatment done on seaweed was the best condition to extract anticoagulant and antioxidant sulfated polysaccharides from *Glacilaria birdiae*. The high yield at 35 Hz is related to changes in agar structures, thus allowing the release of most polysaccharides when extracted at this frequency. In other words, the intracellular hydrogen bonding in polysaccharides can be devastated due to trivial frequency waves, thus altering the structures.

4.3 Effect of seaweed to water ratio on agar yield

As seen in Fig.3, the highest agar yield was at ratio 1:20 for both seaweeds *Gelidium* and *Eucheuma*, where ratios 1:60 and 1:12 were low in agar yield, which is because, at the ratio of 1:12, the smaller weight ratio caused a low agar yield due to lack of volume of water available to extract the agar from the raw seaweed. These results indicated that small amount of solvent used for polysaccharide extraction will cause the diffusion driving force to become small and difficult to spread, hence slowing down the agar extraction process (Kumar and Fotedar, 2009). While the increase of water volume can result in less agar yield. Therefore, both of the seaweed species that affected by the sonication pretreatment with autoclaving could increase the agar yield. On the other hand, the effect of seaweed-water ratio during agar extraction process on agar properties was studied instead of the seaweed-water ratio used during pretreatment process (Arvizu-Higuera et al., 2008; Orduña-Rojas et al., 2008). Based on their results, the greater the volume of water, the easier the agar to be extracted. Dried seaweed can surge to about 20 times of their dry matter volume when exposed to water. Among the three values of seaweed to water ratio, 1:20 of both seaweeds showed the highest agar yield, indicating the suitable ratio for agar extraction condition.

4.4 Effect of time on agar yield

Seen from Fig.4, the effect of time interval on agar yield produced from *Gelidium* and *Eucheuma* seaweeds is shown. The purpose of the time setting for pretreatment is to hydrate the seaweeds and promote the accessibility of soluble polysaccharides. The main constituents of seaweeds are polysaccharides as a hydrophilic polymer. Hydrogen bonding was used for water and polysaccharides of seaweeds reaction, in which the water-holding capacity of seaweeds varies in each species used. Time parameters have a gradual effect on *Gelidium amansii* agar yield, but for *Eucheuma cottonii* species, had mostly affected the agar yield. This can be seen in the results at a time interval of 30 min, agar yield increased drastically from sonication pretreatment with direct heating to sonication pretreatment with autoclaving, which was also the optimum time interval for a higher agar yield (Ahmad et al., 2011). Our best result for time interval was 30 min but 45 min, which is the same, the best time interval. It is because, at a higher time interval, biopolymer degradation may occur in the yield of agar. A longer time would affect the sulfate-binding process by alkaline materials. In addition, the longer pretreatment time (45 min) could result in diffusion between agar and water and lowered the yields, as agar structures were altered when sulfate was one of the important parts of agar molecular chains (Orduña-Rojas et al., 2008).

4.5 Effect of power intensity on agar yield

The effect of power intensity on agar yield produced is shown in Fig.5. The agar yield of seaweeds *Gelidium* and *Eucheuma* showed that the power intensity affected the yield produced, especially for *Eucheuma* species. It can be seen that as the intensity increased, the yield of *Eucheuma* species decreasing drastically. On the other hand, the agar yield of *Gelidium* species decreased on a small scale when the intensity increased gradually. It can be explained that the effect of sonication on agar extraction yield was attributed to the collapsing bubble and cavitation that tempted the stress on the cell wall of macroalgae structure. This is because, during sonication pretreatment, the prompts of cell interference caused ultrasonic waves. Arvizu-Higuera et al. (2008) mentioned that high-intensity ultrasonic waves would cause a small trembling collapse of cavitation bubbles between agar cells (Arvizu-Higuera et al., 2008). This can be explained why the agar yields of the two

seaweeds decreased with the power intensity increase. To the best of our knowledge, power intensity may affect agar yield because the mechanical and chemical energy catastrophic processes throughout the cell wall could release intracellular compounds into solution, and produced the emission of shockwaves. This explanation gains support from Bleakley and Hayes (2017); they stated that the cavitational effect in sonication might enhance the extraction of chemical compounds from macroalgae, which was done by facilitating solvent access through cell disruption and proper mass transfer during the sonication process in a high power intensity.

4.6 Optimization conditions

The agar yields and the physical and chemical properties for the optimization conditions are shown in Table 2. The proficiency of agar produced for food industry application depends on its capability in forming a thermoreversible gel. Therefore, gelling and melting temperatures are significantly important in agar quality control (Stanley, 1995). From the present study, the gelling temperature (25.2–26.9°C) is the standard range used in biotechnology applications. Previously, Oyieke (1993) obtained a similar gelling temperature (27.1°C) as reported using other species, *Glacilaria gracilis*. Intracellular hydrogen bonding in polysaccharides can be devastated due to the trivial frequency waves that could cause fluctuation of the structures, which explained the gelling temperature of sonication pretreatment with autoclaving was higher than that of direct heating. In previous studies, it was reported that the gelling temperature increased as the increase of methoxyl content (Duckworth and Yaphe, 1971; Andriamanantoanina et al., 2007). However, some other studies on different seaweeds used applied higher gelling temperatures for *G. tenuistipitata* (42.3°C) and *G. arcuata* (63.5°C) (Montaño et al., 1999). These authors explained that the high gelling temperature of *G. arcuata* is uncommon and was likely due to the colloids' high molecular weight. Meanwhile, the melting temperature of the two seaweeds we tested, the values ranged from 51.4°C to 68.0°C. The maximum melting temperature obtained (68.2°C) in the present study is similar to that of *G. blodgetti* (Bird and Hinson, 1992).

For *Eucheuma* species, the agar yield for sonication pretreatment with autoclaving was higher than that with direct heating. For *Gelidium* species, the agar yield for sonication pretreatment with autoclaving was higher than that with direct heating. This shows that sonication-autoclaving pretreatment yielded a better quality of agar than non-autoclaving sonicated agar. The gel produced was also very clear and transparent. The gel strength values of each condition were in different ranges (Table 2). As a result, agars of sonication pretreatment with autoclaving of the two seaweeds, *Eucheuma* and *Gelidium* species showed the greater values than those by direct heating. The higher extraction of agarose composition indicates the greater ability to separate agarose from agaropectin in the pretreatment process. Improvement in terms of high gelling extraction can be done in the future to enhance the gelling strength. While for the sulfate content, according to the results, all the samples attained with a very small range (0.301 3–0.477 5 mg/mL). The sulfate contents of agar by autoclaving for both *Eucheuma* and *Gelidium* species were quite low than those by direct heating. The values obtained are within the range of standard values for both tests, which means that the chemical composition of the agars was suitable for the industry, especially the food industry (Recalde et al., 2016). Higher sulfate content of agars can affect the gel strength as well as the agar yield as reported in some studies (Navarro et al., 2007; Arvizu-Higuera et al., 2008). In these studies, the increase of sulfate content could be related to the changes in the molecular structures of the agar. Therefore, it was successfully proved that sonication pretreatment with autoclaving is able to reduce sulfate content in the agar gels produced.

5 CONCLUSION

In conclusion, we successfully studied the properties of *Eucheuma cottonii* seaweed with *Gelidium amansii* and compared them in terms of their yield percentage of agar, and their physical and chemical properties. The results show that *Eucheuma* had a higher yield of agar than *Gelidium* species after sonication pretreatment with autoclaving. *Eucheuma cottonii* can produce a high yield of agar, featuring low sulfate content, good gelling, and higher melting temperatures (65–68°C). The optimal conditions of sonication pretreatment with autoclaving are: time interval of 30 min, power intensity of 30%, seaweed to water ratio of 1:20 w/v, and the frequency of 35 Hz. Improvement in using a different range of parameters during the pretreatment process will be realized to enhance the physical properties of agar production in near future.

6 DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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