Relationship of the viscosity of carrageenan extracted from *Kappaphycus alvarezii* with seawater physical and chemical properties at different planting distances and depth

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Abstract. The carrageenophyte seaweed *Kappaphycus alvarezii* is important both as an export commodity and for the livelihoods of coastal communities. Seawater physical and chemical properties can influence the viscosity of carrageenan produced by *K. alvarezii*. This study analysed the relationship between this viscosity and seawater physical and chemical properties. The study site was Laikang Bay in Jeneponto District, South Sulawesi, Indonesia. A nested factorial design was used. The two factor levels were seedling planting distance (10, 20 and 30 cm), and depth (2 and 5 m). Treatments were grouped by planting time (2 periods) with three replicate stations. A floating raft culture method was used. Seawater parameters measured were light intensity (in-situ), sediment, nitrate, orthophosphate, ammonia, calcium, and magnesium concentrations (ex situ). Carrageenan extraction and viscosity measurement were performed at the Hasanuddin University Industrial Engineering Laboratory in Makassar. There was a medium strong (64%) correlation between physical and chemical seawater parameters and viscosity. Viscosity range was 41-80 cP (mean 62.78±12.99 cP). The lowest viscosity was obtained with 30 cm planting distance at 5 m, and the highest with 10 cm planting distance at 2 m. We recommend planting distance and depth for seaweed farming in areas with coral substrate.
Key Words: *Kappaphycus alvarezii*, carrageenan, light intensity, water quality, viscosity.

Introduction. The seaweed *Kappaphycus alvarezii* is one of the priority aquaculture commodities developed in Indonesia, both for export and to improve the welfare of people living in coastal communities across the archipelago (Parenrengi & Sulaeman 2007). The carrageenan extracted from *K. alvarezii* is widely used for industrial purposes, in food, cosmetics, pharmaceuticals and organic fertilizers (Parenrengi & Sulaeman 2007; Bindu & Levine 2011; Bono et al 2011; Tavassoli-Kafrani et al 2015; Neish & Suryanarayan 2017; Hurtado et al 2019).

The development of seaweed cultivation is progressing rapidly because seaweed cultivation can be carried out with the use of simple technology, low production costs, and there is high market demand (Neish et al 2017). According to the United Nations Comtrade Database (http://comtrade.un.org/data/), in 2014 the volume of seaweed commodities imported into Indonesia was 239 601 kg, indicating that domestic demand for certain types of seaweed was not met by domestic production. *K. alvarezii* is a very important producer of carrageenan for industry (Bindu & Levine 2011). Carrageenan is widely used in the food industry because of its physical and functional properties, e.g. as a thickener, gelling agent, and stabilizer (Bono et al 2011).

In addition to seaweed cultivation methods, environmental factors such as physical and chemical parameters and the seabed substrate can strongly influence the quality of farmed seaweed (Armita 2011). Viscosity of the carrageenan produced is one of the parameters measured and controlled to determine the quality of the seaweed produced, being thick and sticky. It is as the measure of the resistance of the carrageenan fluid to flow, and its gradual deformation by shear or tensile stress. Viscosity value needs to be in accordance with the quality standards of the Food and Agriculture Organisation of the United Nations (FAO), i.e. greater than 5 cP (FAO 2014). The purpose of this study was to analyse the relationship between physical and chemical parameters of the ambient seawater and the viscosity of carrageenan extracted from farmed *K. alvarezii*.

Material and Method

Description of the study sites. The research was conducted from October 2013 to March 2014. The research site was Laikang Bay, Garassikang Village, West Bangkala District, Jeneponto Regency, South Sulawesi Province, Indonesia (Figure 1). The shallow protected waters of Laikang Bay are suitable for seaweed farming, with relatively stable conditions and an environment which has changed little in recent years.

Figure 1. Map of the study area in Laikang Bay, South Sulawesi, Indonesia showing the experimental layout with arrows indicating Stations I, II and III.

Equipment and materials used. The seaweed planted during this study was a brown variety of the carrageenophyte seaweed K. alvarezii. Standard laboratory equipment and chemicals were used for the extraction and viscosity analysis of the carrageenan, as well as for the ex-situ measurement of seawater physical and chemical parameters. The frames of the floating rafts used for seaweed culture were made from bamboo (Figure 2C), while the planting lines were made from polyethylene No. 6 and No. 1.5 rope. The frames were kept in place through attachment to wooden stakes which were driven into the seabed.

Research procedures. The initial stages included preliminary field observations to determine the research stations in the fringing coral reef ecosystems of Laikang Bay. The floating raft units (2m x 2m) were deployed at 3 stations (Figure 1). At each station, rafts were deployed in two depths (2 m and 5 m). Seaweed seedlings (50 g initial weight,) were spaced at three planting distances (10, 20, and 30 cm) with two controls (one for each depth) at each station. Various aspects of the procedure are shown in Figure 2. The treatment codes and the geographical coordinates of each raft are shown in Table 1.

Figure 2. Scenes from the *Kappaphycus alvarezii* seaweed cultivation during the study period. A. Example of a 50 g seedling; B. Unit with 10 cm spacing; C. unit with 30 cm spacing; D. Weighing seedlings; E. attaching seedlings to the ropes; F. Seedling after 42 days cultivation.

Table 1

Geographical coordinates of the research stations in Laikang Bay, Indonesia

Station and treatment codes (station/seedling spacing /depth)[a]	*Latitude (South)*	*Longitude (East)*
I / B1 / A1 and A2	05°35'550''	119°31'198''
I / B2 / A1 and A2	05°35'539''	119°31'212''
I / B3 / A1 and A2	05°35'526''	119°31'218''
1 / K 1 and K2	05°35'513''	119°31'221''
II / B1 / A1 and A2	05°35'616''	119°31'006''
II / B2 / A1 and A2	05°35'612''	119°31'028''
II / B3 / A1 and A2	05°35'617''	119°31'013''
II / K 1 and K2	05°35'621''	119°31'009''
III / B1 / A1 and A2	05°35'298''	119°31'306''
III / B2 / A1 and A2	05°35'291''	119°31'306''
III / B3 / A1 and A2	05°35'277''	119°31'292''
III / K 1 and K2	05°35'272''	119°31'292''

[a] Station numbers: I, II, III; Seedling spacing: B1 = 10 cm, B2 = 20 cm, B3 = 30 cm; Depth: A1 = 2 m, A2 = 5 m; Controls: K1 and K2 at each station.

Variables measured

Viscosity. Viscosity is a measure of the rate at which molecules flow in fluid or semifluid material due to internal friction; the higher the viscosity, the higher the resistance to deformation. The viscosity test was carried out to determine the level of viscosity of the carrageenan when in solution at a set concentration. Carrageenan was extracted using standard procedures. Viscosity was analysed at the end of the 42 day planting period for each treatment. The procedures were carried out at the Industrial Engineering Laboratory of Hasanuddin University, Makassar. Viscosity (cP) was measured with a viscometer following the procedures of Raharjo (2009). A carrageenan solution was made with a concentration of 1.5% through heating in a boiling water bath while stirring regularly until the temperature reached 75°C.

Water quality parameters. Water quality measurement procedures and analysis were carried out in accordance with the American Public Health Association (APHA) Standard Methods (APHA 2005). Physical and chemical parameters measured included light intensity, measured at the research site (in situ), while sedimentation rate as well as nitrate, orthophosphate, ammonium, calcium (Ca) and magnesium (Mg) concentrations were measured ex situ at the Chemical Oceanography Laboratory of the Marine Science Department, Faculty of Marine Sciences, Hasanuddin University, Makassar. The parameters measured along with units and equipment or methods used are shown in Table 2.

Table 2

Water quality parameters measured, with units and methods

No.	*Parameter*	*Unit*	*Equipment/method*
1	Light intensity	lux	Lux meter
2	Sedimentation rate	$mg \cdot cm^{-2} \cdot day^{-1}$	Sediment trap
3	Nitrate	ppm	Spectrophotometer
4	Orthophosphate	ppm	Spectrophotometer
5	Ammonia	ppm	Spectrophotometer
6	Calcium (Ca)	ppm	Titration
7	Magnesium (Mg)	ppm	Titration

Water samples were collected at a predetermined depth using sampling bottles. These were stored in a cool box with ice to preserve the condition of the water samples during transport to the laboratory. Nitrate, orthophosphate and ammonium were measured through a spectrophotometric process, while Ca and Mg were measured through titration. Sedimentation rates were measured by placing 3 sediment traps with a diameter of 5 cm and a length of 30 cm at each treatment site. The sediment traps were placed between coral colonies under the seaweed cultivation floating rafts at the beginning of the study and then removed at the end of the study. The trapped sediment particles were measured by filtering suspended particles which had been deposited in the sediment trap using filter paper; the process was assisted by the use of a vacuum pump. The filtered sediment was then oven dried at 105°C to obtain the dry weight of the suspended particles deposited in the sediment trap. Concentrations of nitrate, orthophosphate, ammonium, Ca and Mg were measured at the surface and close to the seabed.

Data analysis. To analyse the relationship between the physical and chemical parameters of the waters and the viscosity of the carrageenan obtained from *K. alvarezii* seaweed, the data were analysed using multiple linear regression analysis using the backward method implemented in SPSS version 16. The significance of between treatment differences was evaluated using analysis of variance (ANOVA) at the 95% confidence level ($\alpha = 0.05$) followed by Tukey honestly significant difference (HSD) post-hoc tests if $p < 0.05$.

Results. The range, mean and standard deviation of the recorded values of the physical and chemical parameters (light intensity, sedimentation rate, concentrations of nitrate, orthophosphate, calcium (Ca) and magnesium (Mg)) measured in the sea surface layer and at just above the sea bed at all experimental units (three stations, three seedling spacing and two depth treatments) over two planting periods (N = 42) are shown in Table 3. The multiple regression analysis of the correlation between the viscosity of carrageenan extracted from the seaweed *K. alvarezii* and these parameters could explain 64% of the observed variation in viscosity (R^2 = 0.640), with partial correlation and regression coefficients as shown in Table 4.

Table 3

Range, mean value and standard deviation of physical and chemical parameters measured at the experimental units in Laikang Bay, Jeneponto Regency, Indonesia

Physical and chemical parameters	*Unit*	*Minimum*	*Maximum*	*Mean*	*Standard deviation (SD)*	*N*
Surface						
Light intensity	lux	3242.00	4119.00	3710.97	317.30	42
Sedimentation rate	mg cm^{-2} day^{-1}	13.82	66.16	32.69	11.07	42
Nitrate	ppm	0.75	2.39	1.50	0.46	42
Orthophosphate	ppm	0.24	0.54	0.40	0.08	42
Ammonia	ppm	0.23	0.64	0.39	0.11	42
Calcium (Ca)	ppm	888.87	999.01	914.49	25.35	42
Magnesium (Mg)	ppm	1047.06	1176.60	1097.88	31.36	42
Sea bed						
Light intensity	lux	105.00	956.00	516.83	400.24	42
Sedimentation rate	mg cm^{-2} day^{-1}	4.34	66.16	26.45	14.56	42
Nitrate	ppm	0.00	0.00	0.00	0.00	42
Orthophosphate	ppm	0.01	0.07	0.04	0.02	42
Ammonia	ppm	0.01	0.10	0.02	0.01	42
Calcium (Ca)	ppm	421.75	669.00	512.41	54.14	42
Magnesium (Mg)	ppm	1005.96	1228.23	1103.43	78.59	42

Table 4

Multiple regression partial correlation coefficients for the correlation between carrageenan viscosity and several physical and chemical seawater parameters

Physical and chemical parameters and constants	*Partial correlation*	*Regression coefficient*
Slope (constant)		379.5274
Light intensity	0.2311*	0.0048
Sedimentation	0.4499*	0.2406
Nitrate	-0.2145*	18.1676
Orthophosphate	-0.5010*	-64.425
Ammonia	-0.3097*	0.0000
Calcium (Ca)	-0.6381*	43.3615
Magnesium (Mg)	-0.5591*	0.0000

* Statistically significant correlation ($p < 0.05$).

The viscosity of carrageenan obtained from the seaweed samples harvested throughout the study ranged from 41 to 80 cP with an average of 62.78±12.99 cP. The mean viscosity of carrageenan extracted from the seaweed after 42 days of cultivation based on the combination of spacing and depth treatments is presented in Table 5.

Table 5
Mean viscosity of carrageenan from *Kappaphycus alvarezii* with different seedling spacing and depth treatments (two 42 day planting cycles)

Planting distance (cm)	*Viscosity (cP) at different water depths*	
	2 m	*5 m*
10	77.50	62.67
20	72.67	53.83
30	65.50	44.50

The lowest viscosity was obtained from the treatment combination of 30 cm spacing with 5 m depth, while the highest was from the combination of 10 cm spacing and 2 m depth. The carrageenan viscosity values obtained can be considered relatively high, and were higher than those reported from Southeast Maluku which ranged from 32.24 to 54.39 cP (Kumayanjati & Dwimayasanti 2018). Other studies have also found high gel strength in carrageenan from *K. alvarezii* cultivated in Takalar (Fahrur et al 2019).

The analysis of variance (ANOVA) for the viscosity of carrageenan from the cultivated *K. alvarezii* showed that seedling spacing and the depth of the cultivation area combined had a significant ($p < 0.05$) effect on viscosity. The partial analysis of variance for the first and second planting periods showed that the viscosity was not affected by the interaction of spacing and depth, but the main effect of spacing and depth on viscosity was significant. The consistency in yields across two planting periods indicated that the differences in environmental factors between planting periods 1 and 2 were not great enough to cause significant differences in carrageenan viscosity.

The results show a pattern of decreasing gel strength with increasing seedling spacing. The post hoc Tukey HSD test showed that the mean viscosity of carrageenan from *K. alvarezii* at seedling spacings of 10 cm and 20 cm were significantly different from that obtained at a seedling spacing of 30 cm (Table 6).

Table 6
Significance of differences in mean viscosity between seaweed planting distance treatments (both depths combined)

Planting distance (cm)	*Mean viscosity±SD (cP)*	*Number of replicates (n)*
10	70.08 ± 10.54^{a}	12
20	63.25 ± 10.72^{a}	12
30	55.00 ± 13.67^{b}	12

Notes: SD = standard deviation; different superscripts indicate significantly different mean values based on the Tukey HSD post hoc test at the 95% confidence level ($\alpha = 0.05$).

Discussion. The correlation regression showed that physical and chemical parameters (light intensity, sedimentation, nitrate, orthophosphate and calcium) could explain 64.0% of the observed variation in the viscosity of carrageenan extracted from Kappaphycus alvarezii. The partial correlation between viscosity and physico-chemical parameters was moderate ($p < 0.05$). This indicates that the viscosity of carrageenan extracted from *K. alvarezii* is significantly influenced by physical and chemical parameters as well as other parameters that were not analysed.

The viscosity of a hydrocolloid is influenced by several factors, namely carrageenan concentration, temperature, carrageenan type, molecular weight and the presence of other molecules (Towle 1973; FAO 2014). An earlier study (Suryaningrum et al 1991) reported that an increase in gel consistency resulted in a lower carrageenan viscosity. A high water content in seaweed was also found to have a negative impact on the viscosity. Although Muñoz et al (2004) found no significant difference in carrageenan viscosity between different in seaweed strains, they did report that seaweed with higher growth rates tended to produce carrageenan with higher viscosity values.

Seaweed requires sunlight as an energy source for the formation of organic matter necessary for normal growth and development. Light plays an important role in the process of photosynthesis, with an initially positive relationship, which the levels off and above a certain threshold photosynthesis will decrease with further increases in light intensity (Neish 2008). Most algae have a light intensity tolerance range, and high light intensity can result in bleaching or even mortality, while growth is slow under low light intensity. As an example, Isnansetyo & Kurniastuty (1995) report that increasing light intensity from 500 to 12,000 lux increases algal growth with a decline in growth when light intensity exceeds 12,000 lux. High sedimentation caused by natural processes or anthropogenic activities has the potential to reduce light penetration (Dahuri et al 2001). This can inhibit the primary productivity of the coral ecosystem as well as that of cultivated seaweeds such as *K. alvarezii*.

Nitrate is the main form of nitrogen in natural waters and is the main nutrient for algae growth while phosphate is more widely used in the activities of forming plant cells (Effendie 2003). Phosphate is a constituent of the pyrophosphate bond of adenosine triphosphate (ATP) which is rich in energy and is a fuel for all activities in all living cells and is an important constituent of cells. Total phosphorus describes the total amount of phosphorus (particulate and dissolved, organic and inorganic) in the form of orthophosphates. Orthophosphate is a form of phosphorus that can be used directly by aquatic plants, while polyphosphate must undergo hydrolysis to form orthophosphate first, before it can be used as a source of phosphorus.

Sediment is the main storage place for phosphorus in cycles that occur in the oceans, generally in the form of particulates that bind to iron oxides and hydroxide compounds (Effendie 2003; Paytan & McLaughlin 2007). High levels of orthophosphate are generally due to the high diffusion of phosphate from the sediment (Effendie 2003). Phosphorus compounds bound in sediments can undergo decomposition with the help of bacteria or through an abiotic process to produce dissolved phosphate compounds that can diffuse back into the water column (Paytan & McLaughlin 2007).

Phosphate can be a limiting factor in seaweed biomass and gel production (Buschmann et al 2004). The presence of phosphorus and nitrogen in the waters has an important role as nutrients for coral reef ecosystems. Although a sufficiency of phosphorus is important for seaweed farming, excessive presence of phosphorus, accompanied by nitrogen, can stimulate an explosion in algal growth, including undesirable epiphytes (Dahuri et al 2001; Neish 2008). Calcium is needed for seaweed for growth, reproduction, and for the formation of food reserves in the form of organic compounds.

Conclusions. The physical and chemical parameters measured at the experimental site under different planting spacings and depths showed a moderate correlation (64.0%) with the viscosity of the carrageenan extracted from *Kappaphycus alvarezii* seaweed at the study site in the waters of Laikang Bay, Jeneponto Regency, Indonesia. Carrageenan viscosity, a key quality criterion, was significantly higher when planting in relatively shallow waters at close seedling spacings of 10-20 cm. The results can be used to inform the development of recommendations for seaweed cultivation in waters with coral substrates, in particular for the adjustment of seedling spacing and depth for best performance.

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