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Exploring the Growth Dynamics, Carrageenan Yield, and Bioactive Properties of *Eucheuma spinosum* Cultivated with Different Methods

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ABSTRACT

Eucheuma spinosum, a type of macroalga, is cultivated in Indonesia for applications in food, medicine, and cosmetics. This alga produces iota carrageenan, which is softer than kappa carrageenan. The objective of this study was to analyze the growth, carrageenan yield, and bioactive compounds of *E. spinosum* grown using different cultivation methods. Conducted in Gerupuk Bay, Central Lombok, West Nusa Tenggara, Indonesia, from June to September 2024, this research employed an experimental method with a completely randomized design. The treatments compared two cultivation methods: floating rafts and bottom-off. Observed parameters included growth, carrageenan yield, thallus slices, proximate composition, chlorophyll-a, phycoerythrin, and carotenoids. The results showed that the absolute weight of *E. spinosum* was 74.7g on floating rafts and 150.775g on bottom-off. The specific growth rates were 2.01 and 3.99%/day, respectively. Carrageenan yields were 24% for the bottom-off method and 11% for floating rafts. Carotenoid contents were 6.69 mol/L on floating rafts and 4.23 mol/L on bottom-off. Phycoerythrin levels measured 2.69µg/ L on floating rafts and 0.91µg/ L on bottom-off. Chlorophyll-a content was 5.42mg/ L on floating rafts and 1.38mg/ L on bottom-off. Proximate analysis revealed the following for floating rafts: water content 28.2098%, ash 32.0270%, crude fat 0.0199%, crude fiber 4.5991%, and protein 7.9170%. For the bottom-off method: water content was 31.6676%, ash was 30.7466%, crude fat was 1.7061%, crude fiber was 6.0548%, and protein was 4.3919%. The study concluded that *E. spinosum* cultivated using the bottom-off and floating raft methods exhibited differences in growth, carrageenan yield, pigment content, and proximate composition.

INTRODUCTION

In Indonesia, seaweed cultivation mainly focuses on *Kappaphucus alvarezii*, *Gracilaria*, and *Eucheuma spinosum* **(Purnomo *et al.*,, 2018)**. The choice of species

cultivated is driven by consumer demand, which spans the food, medicine, and cosmetics industries, including a notable demand for *E. spinosum* **(Purbosari *et al.*, 2022)**. According to **FAO (2018)**, China, Indonesia, and the Philippines are the top three countries known for large-scale seaweed production and the greatest diversity of species. China at 17.53 million tons in 2017, Indonesia at 9.12 million tons in 2021, and the Philippines at 1,499,961.25 tons in 2019 **(Aprilia *et al.*, 2023)**. The three countries that produce seaweed on a large scale and with the greatest species diversity are China, Indonesia, and the Philippines **(Aprilia *et al.*, 2023)**. 42% of seaweed production is contributed by the algal genera *Eucheuma* spp. and *Kappaphycus* spp., also referred to as eucheumatoid and carrageenanophytes **(Efendi *et al.*, 2015; FAO, 2018)**. Species that produce carrageenan include *Eucheuma* spp. and *K. alvarezii*, and the genus *Gracilaria* that produces agar **(Komariyah *et al.*, 2019; Arrosyad & Alamsjah, 2020)**. Seaweed is utilized for human consumption. According to **Chapman *et al.* (2015)** and **Gomez Pinchetti (2016)**, seaweed is believed to be a nutritious health food and is favored by consumers who choose organic food.

According to **Kasim *et al.* (2020)**, *E. spinosum* is a macroalgae that is cultivated and widely distributed in shallow coastal waters of Indonesia. The genus *Eucheuma* is part of the Family Solieriaceae within the Order Gigartinales. The thallus of *E. spinosum*, resembling cartilage, typically faces downward, with cylindrical branches and thorn-like structures. *E. spinosum* produces iota carrageenan, which is softer than kappa carrageenan and is used in the pharmaceutical industry as a medicinal ingredient. According to **Bartlova *et al.* (2021)**, iota carrageenan from *E. spinosum* has finer properties, high purity, and high clarity. Iota carrageenan is used in high-economic-value products such as toothpaste. Purified iota carrageenan has a higher selling value than kappa carrageenan. Iota carrageenan which has been purified and pressed has twice the price of processed kappa carrageenan. **Khalid *et al.* (2018)**, **Goswami *et al.* (2019)** and **Hans *et al.* (2021)** stated that iota carrageenan from *E. spinosum* is a valuable source of bioactive compounds due to its various biological activities. Iota carrageenan is a potential source of biologically active compounds for drug development. Bioactive compounds in the cell wall of *E. spinosum* have a variety of pharmacological activities such as anti-inflammatory, antiviral, antitumor, anticoagulant, antioxidant, and antimicrobial. *E. spinosum* seaweed contains bioactive compounds such as flavonoids, saponins, alkaloids, terpenoids and tannins. This seaweed also has antioxidant and antibacterial activity, including flavonoids, alkaloids, steroids, saponins, phenolics, and tannins **(Sari *et al.*, 2022)**.

Cultivation of *E. spinosum* can be done using the bottom peg and floating raft method. Both methods have their own advantages and disadvantages. Basic stakes have the advantage of being able to protect seedlings from waves, which can be done by seaweed planting in a row. While the disadvantages of this method are that seaweed

control can only be done at low tide, must have a depth of about 0.5 meters at the lowest tide and 3 meters at the highest tide. The floating raft method has the advantage that it can be applied to locations where the currents and waves are low or usually in the bay area, but can also be applied to the location of the waters that are quite high waves so that this method is quite flexible. Meanwhile, the disadvantages of this method are that the cost is quite expensive for the scale of cultivators, and the service life of the raft construction can only last up to 1 year **(Sunarpi *et al.,* 2020)**. The selection of the cultivation method is based on the physical characteristics of the sea waters where the cultivation takes place. The floating raft method is appropriate for bay-shaped sea waters with a depth of less than 5m and calm water movement. The bottom peg method is suitable for tidal areas with coral and white sand bottoms. **Veeragurunathan *et al.* (2015)**, **Mantri *et al.* (2020)** and **Prasad *et al.* (2022)** explained that various seaweed cultivation methods including the raft method, net method, bottom stakes, and longlines have been practiced in all countries that cultivate seaweed. Each cultivation method has advantages, limitations, and disadvantages. The off-bottom method, floating raft method, and longline method are only suitable in shallow waters.

Gerupuk Central Lombok is a producer of *Eucheuma spinosum* in Indonesia. *E. spinosum* is cultivated by farmers using the floating raft method and the bottom peg method. The use of both methods is to optimize the utilization of marine space. The production of *E. spinosum* from the two methods is different, hence it is necessary to analyze the growth and bioactive compound. The aim of this research was to analyze the growth, carrageenan yield, and bioactive compounds of *E. spinosum* grown using various cultivation techniques.

MATERIALS AND METHODS

Time and place

This research was conducted in the waters of Gerupuk, Central Lombok, West Nusa Tenggara, Indonesia from June to September 2024. The research location is shown in Fig. (1).

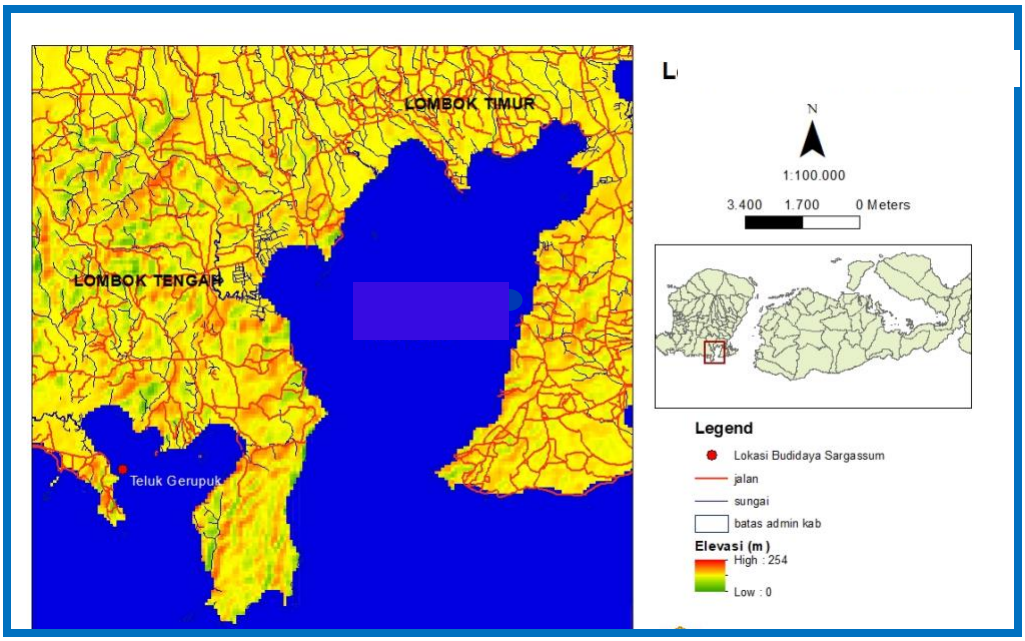


Fig. 1. Research location

Material

The materials used were *E. spinosum*, bamboo, polyethylene rope, raffia, alcohol, filter cloth, float bottles, and sacks. The tools used were a microscope, razor blade, prepared glass, knife, scissors, oxygen meter, refractometer, pH pen, lux meter, secchi disk, blender, pot stove, wooden stirrer, digital scale, and stainless tray.

Experimental design

This research used an experimental method with a complete randomized design. The treatment of this study was the difference in cultivation methods, namely floating rafts and bottom off. The number of sample clumps of *E. spinosum* on one unit of floating raft is 40 clumps. The number of sample clumps of *E. spinosum* on the basic is 40 clumps.

Absolute growth

Measurement of absolute weight growth was done to determine the total difference between the increase in seaweed biomass that has been planted. Measurement was done using the final weight data that has been taken and then reduced by the initial weight. The following formula was used to calculate the absolute growth **(Nasmia *et al.*, 2022)**:

$$W = Wt - Wo$$

Where:
W: Seaweed weight gain (g)

Wt: Final weight of seaweed (g)
Wo: Initial weight of seaweed (g)

Specific growth

Specific growth rate is the value of growth in daily time. The specific growth rate was calculated using the following formula **(Nasmia *et al.*, 2022)**:

$$\textbf{LPS = Ln (Wt=Wo)/t x 100\%}$$

Where:
LPS: Specific weight loss rate(%/day)
Wo: Initial weight of seaweed (g)
Wt: Final weight of seaweed (g)
t: Maintenance Time (days)

Analysis of carrageenan

Carrageenan extraction from *E. spinosum* involves several steps. Initially, the sample was sun-dried to reduce its water content and then re-soaked for 24 hours. The soaked samples were cut into small pieces for easier blending. The blended material was then cooked for 15 minutes at medium heat and mixed with 96% alcohol. The mixture was filtered through a cloth and sun-dried. The percentage of carrageenan was calculated using the formula outlined by **Dhewang *et al.* (2023)**.

$$\textbf{Yield (\%) = (Carrageenan weight)/(Dry seaweed weight) x 100\%}$$

Proximate analysis

Observations of proximate analysis was carried out in several stages, according to **AOAC (1990)** and **Martini (1997)**, namely:

a. Determination of moisture content

The clean porcelain cup was dried in a drying oven at 105ºC for 1 hour. Then the porcelain cup was cooled in a desiccator for 1 hour (equivalent to room temperature), and then weighed in a closed state (A g). Samples as much as 1.5-2.0g were put into a porcelain cup (B g), then dried in an oven at 105ºC for 8-12 hours. After that, the cup containing the sample was cooled in a desiccator for 1 hour, and then weighed (C g).

Calculation:

1. Water content $= \frac{B-C}{B-A} X100\%$

2. Dry matter content 100% - % Moisture content

b. Determination of ash content

Samples in porcelain cups previosly dried in an oven (105ºC weighed) (C g) were put in a furnace at 600ºC for 2-4 hours (until they became white). The porcelain cup was cooled in a desiccator for 15-30 minutes and was then weighed (D g).

Calculation:

1. Ash content $= \frac{D-A}{B-A} X100\%$

2. Organic matter content 100% - % Ash content

c. Determination of crude protein content

The sample material weighed approximately 0.25, then the sample was put into the Kjeldhal flask and 1.5g of a mixture of $CuSO_4$ and $K_2 SO_4$ (1:7) were added as well as 2 boiling stones. Furthermore, 7.5ml of concentrated $H_2 SO_4$ was added carefully in Kjeldahl flask and contents were deconstructed in a fume hood until it was clear and smokeless for approximately 45 minutes. The results of the deconstruction were diluted with 100 ml cold distilled water, then 40% NaOH (50ml) was added carefully as well as2 boiling stones. Then the Kjeldahl flask was installed on the distillatory device previously installed with a 250ml Erlenmeyer containing $H_3 BO_3$ 3% as much as 25ml. Subsequently, the distillation process took place and was stopped when the Erlenmeyer container reached 100ml. The distillate was immediately titrated with $H_2 SO_4$ 0.1 N standard solution, and the titration was stopped when the color of the solution turned pink/original color.

Calculation:

1. Crude protein $= \frac{mil\ titrasi\ x\ 0.1\ x\ 0.014\ x\ 6.25}{berat\ sempel} X100\%$

2. Organic matter without N 100% - % Crude protein

d. Determination of crude fat content

The fat-free filter paper was placed in a drying oven at 105º C for 1 hour. Then, it was cooled in a desiccator for 1 hour and weighed (A g). 1.5-2g of sample wrapped in filter paper (B g) was placed in a drying oven for 8 hours at 105ºC, then cooled in a desiccator for 30-60 minutes and weighed (C g). filter paper containing the sample was placed in a Soxhlet extraction device. The collection flask, upright cooler, and Soxhlet extraction apparatus were assembled in such a way and placed in a water bath. The Soxhlet extraction apparatus was filled with petroleum benzene or other fat solvents until it all went down and entered the collection flask. This was repeated until the extraction apparatus was fully charged. The extraction process

continued and was stopped when the Soxhlet flask was clear. The sample was removed from the extraction tool, and the remaining petroleum benzene was evaporated then put in a drying oven at 105ºC for 4 hours and cooled in a desiccator for 1 hour, then the sample was weighed (D g).

Calculation:

1. Crude fat $= \frac{C-D}{B-A} X100\%$

2. Carbohydrate 100% - % crude fat

e. Determination of crude fiber content

The fat-free sample (A g) was put into a 500ml beaker glass and 100ml of $H_2 SO_4$ 0.255 N was added, then boiled on a hotplate for 30 minutes. A bulb flask (cooler) containing water was placed above the beaker glass. After boiling, it was filtered with a linen funnel and rinsed with hot water several times on the sample residue. Then, into the beaker glass 100ml NaOH 0.313 N was added, then boiled again for 30 minutes. After boiling, the sample was filtered with a gooch crucible previously filled with glass fiber as a filter. A vacuum pump was used to facilitate the filtering process. Next, the gooch crucible was rinsed with hot water several times and finally rinsed with enough absolute ethanol until the filtrate was colorless. Gooch crussible containing the next sample was let in the oven at 105ºC for 12 hours. Then, it was cooled in a desiccator for about 1 hour and weighed (B g). The sample in the gooch crussible was incinerated in a furnace at 600ºC for 2 hours. Subsequently, the sample was placed in a desiccator for 1 hour and weighed (C g).

Calculation:

1. Crude fibre $= \frac{B-C}{A} X100\%$

2. Extract material without N 100% - % crude fibre

Thallus slices

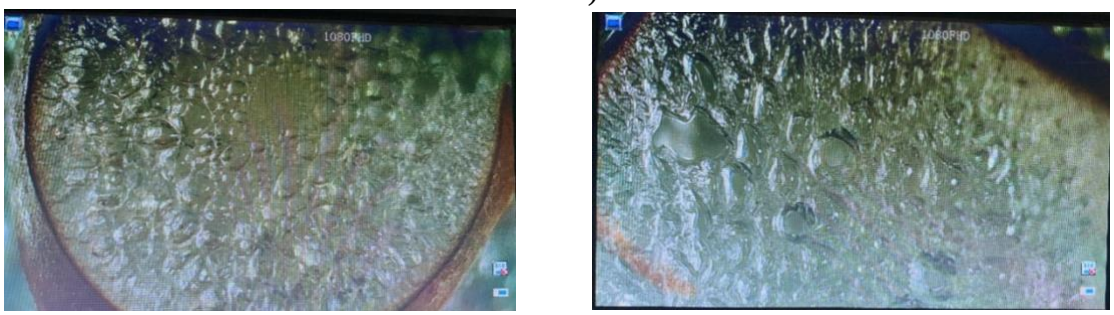
Observation of thallus tissue was done by taking samples and making thallus slices of *K. alvarezii* seaweed as thin as possible. Then thalus slices were put on a cover glass to be observed under a microscope. The observed thallus were old and young.

RESULTS AND DISCUSSION

The cross-sectional condition of *E. spinosum* seaweed tissue cultivated with different methods can be seen in Fig. (2).

1. Thallus slices

a) b)



c) d)

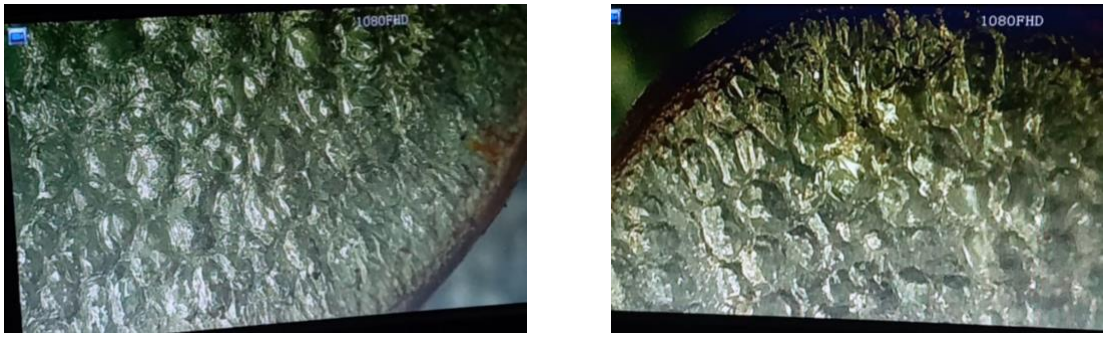


Fig. 2. Slices of thallus a) Slices of young thallus in the floating raft method; b) Slices of old thallus in the floating raft method; c) Slices of young thallus in the bottom peg method; d) Slices of old thallus in the bottom peg method

The results of observations of slices of *E. spinosum* grass tissue showed that the visible seaweed cells showed different forms, namely some are round, and some are oval. Young thallus tissue have a round formation and is slightly smaller than the old thallus tissue. The thallus network is getting bigger toward the center. In the thallus tissue *E. spinosum* has a different structure as seen in the picture above, the difference in tissue structure is thought to be due to several factors, namely environmental conditions and water quality. According to **Maulani *et al.* (2017)**, one of the factors affecting the success of *K. alvarezii* seaweed cultivation is the condition of sea waters that fluctuate and tend to be extreme, such as salinity below 20ppt and above the optimal range of 30ppt, water temperature above 35ºC, pH that is too acidic and alkaline, the level of water brightness above 5m, resulting in infecting seaweed with disease (ice-ice). While the young ones look small, it marks that the thallus will experience growth as seen in the picture of young thallus tissue. This is in line with the statement of **Darmawati (2012)** The results of histological studies of *K. alvarezii algae* tissue show that these algae cells are oval on the outside / behind the thallus wall and enlarged and arranged irregularly in the middle of the thallus. The parts of the thallus tissue consist of cortical and medular. Modular is a large cell located in the center and cortical is a small cell located at the edge near the cell

wall. **Yatin *et al.* (2023)** stated that the results of histological observations of fresh or healthy *E. spinosum* algae tissue are characterized by cells outside or behind the cell wall that are small, oval, and rather dense. However, in the central part of the thallus, cell size increases, and cells closer to the cell wall become less dense. The newly formed young cells are smaller in size.

Observations of thallus tissue carried out on the basic peg method can be seen in Table (1). It can be seen that the slice of thallus tissue in seaweed is said to be good, it is characterized by a tight network of cells without any space. In young thallus tissue, the visible cells have a small round cell shape, regular and not tenuous in the epidermis. Likewise, in the old thallus, the visible cells show the shape and size of cells that are not much different from the young thallus slices where the cells on the outside have a smaller size, slightly oval and slightly compacted, then in the middle of the thallus the size of the cells will get bigger. Axis thalli consist of layers of epidermis, cortex, and medulla. Thick cells with a uniform shape formed the epidermal layer. Deeper parts are followed by a layer of the cortex that is smaller than the medulla **(Widyartini *et al.*, 2021)**.

2. Absolute weight

Based on results, an absolute weight of 74.7g floating rafts and 150.775g basic stakes were obtained. It was noticed that, the basic peg method has the highest value compared to the floating raft method. Absolute growth in seaweed can be caused by several factors, such as research location, planting method, and water quality. In line, **Raihanun *et al.* (2022)** stated that the absolute growth of seaweed is influenced by several factors such as different cultivation locations, currents, nutrients, and light. High absolute weight growth has a location that is close to optimal for cultivation activities. **Roleda and Hurd (2019)** and **Windartoto *et al.* (2021)** added that an essential nutrient requirement of seaweed is nitrogen. **Simatupang *et al.* (2021)** stated that water quality that has a value more or less than the optimal value in light intensity, salinity and temperature can result in the development of ice-ice disease, which is often associated with epiphytic growth on seaweed, resulting in low production or crop failure.

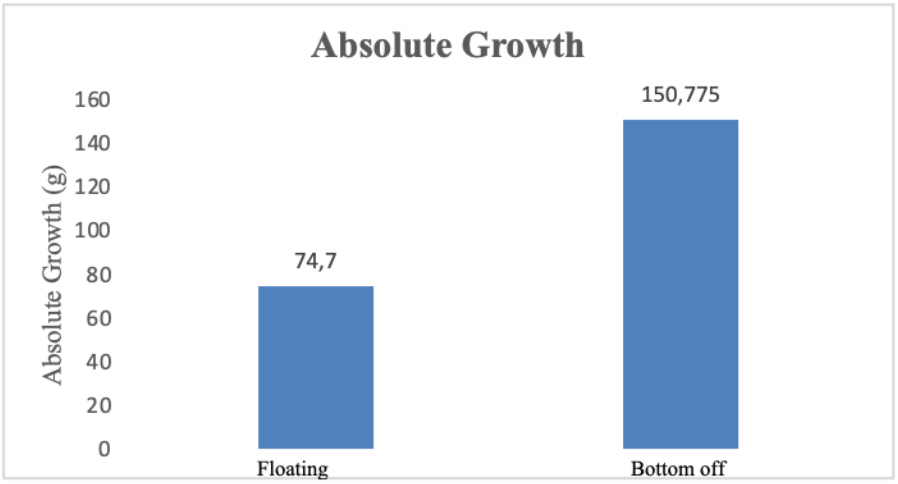


Fig. 2. Graph of absolute weight in *E. spinosum* seaweed cultured under different methods

Sapitri *et al.* (2016) postulated that environmental factors that affect the growth of seaweed include sunlight, nutrients in marine waters, water temperature, current speed (waves), pH waters, pests, or diseases including the presence of large fish. According to **Afandi *et al.* (2020)**, differences in the location of maintenance also affect the growth of seaweed. Selection of the right location greatly affects seaweed cultivation activities, since it is closely related to water quality conditions and the growing season. **Kasim and Mustafa (2017)** explained that environmental factors play an important role in the growth of *K. alvarezii.* According to the opinion of **Arjuni *et al.* (2018)**, the low growth of seaweed in the floating raft method is due to several conditions that affect both physical, chemical, and other ecological conditions impacting the growth of seaweed.

3. Specific growth rate

The results of the maintenance of *E. spinosum* seaweed maintained for 30 days with the floating raft method and the basic peg method showed a specific growth rate of around 2.01 and 3.99%/day, respectively. Based on the specific growth rate graph, the growth of seaweed on the basic peg method is relatively fast because the thallus on the seaweed is less and not too lush, making the seaweed get more nutrients so that the growth of seaweed increase and develop faster than in the floating raft method. In addition to the factor of lush thallus, planting seaweed at a close distance can also affect the growth of seaweed in taking nutrients. A specific growth rate at the base peg above 3% can be said to be optimal for its growth. According to **Chairun *et al.* (2024)**, specific growth rate of more than 3.3% is considered good for seaweed growth.

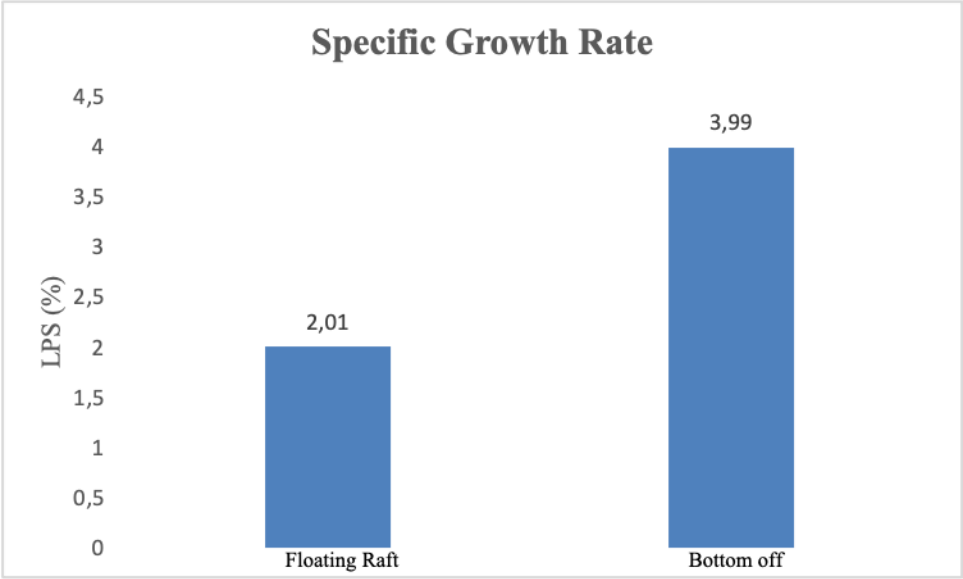


Fig. 3. Graph of specific growth rate of *E. spinosum* seaweed cultured with different methods

Munoz *et al.* (2004) and **Kasim and Mustafa (2017)** recorded that the daily growth rate of *K. alvarezii* in Yucatan Peninsula, Mexico was 2.0-7.1%/day. According to **Cokrowati *et al.* (2018)**, the optimal growth rate of *Kappaphycus alvarezii* exceeds 3%. This is supported by **Erpin and Ruslaini (2013)**, who elucidated that seaweed aquaculture becomes profitable when the daily growth rate surpasses 3%. **Supiandi *et al.* (2020)** highlighted that specific growth rates are influenced by competition for nutrients in the water, particularly when planting distances are too tight. Overcrowding can also lead to the accumulation of dirt or mud within the seaweed clumps due to restricted water movement.

Lobban and Paul (2000), in their study, identified several factors affecting seaweed growth, including phenotype, genotype, age, reproductive conditions, nutrient availability, and environmental conditions. **Arjuni *et al.* (2018)** further explained that variations in specific growth rates result from differences in ecological characteristics across cultivation sites. These differences in nutrient availability and water quality directly impact the growth of *K. alvarezii.*

4. Carrageenan content

E. spinosum is reddish brown with a smaller thallus diameter than *E. cottonii. E. spinosum* has a cylindrical thallus, and pointed thallus branches, and is covered by soft spines which are the new thallus. According to **Lestari *et al.* (2023)**, carrageenan is a hydrocolloid compound consisting mostly of galactose and 3,6-anhydro-Dgalactose and contains esters of sodium sulfate, ammonium, calcium, magnesium, and potassium which can be extracted from seaweed from the Rodhophyceae class, one of which is *E.*

spinosum. There are three types of carrageenan: iota carrageenan from *E. spinosum*, kappa carrageenan from *E. cottonii*, and lambda carrageenan.

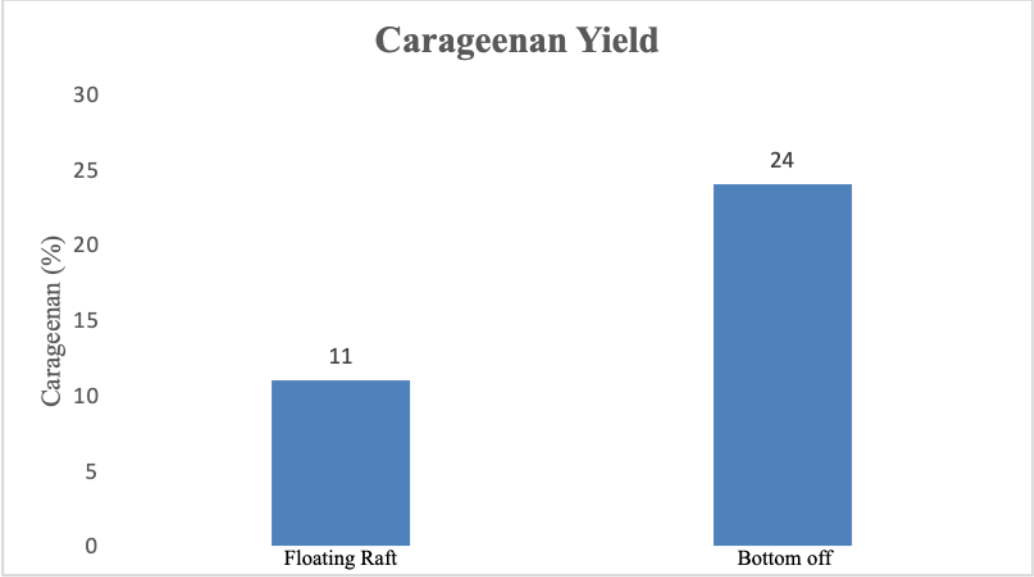


Fig. 4. Carageenan yield graph of *E. spinosum* seaweed cultivated with different methods

Fig. (4) shows the carageenan yield of *E. spinosum* from different cultivation methods. According to **Setijawati (2014)**, carrageenan from *E. spinosum* and *E. cottonii* has differences in gel strength and gel viscosity. Kappa-carrageenan has strong gel properties, is rigid, easy to crack, and has high viscosity. Iota-carrageenan is soft and elastic and has a low viscosity.

Carrageenan is one of the parameters that determine the quality of algae. The quality of carrageenan will be better if the seaweed grows well, and in good water quality. There are several parameters that can be used to assess the quality of carrageenan, including yield, ash content, water content, viscosity and elongation or gel strength. To produce these parameters, a good seawater characteristics is required **(Fathoni & Arisandi, 2020)**. The higher the carrageenan content the better because it can increase the economic value of the algae **(Tuiyo & Zulkifli, 2023)**. The results of the carrageenan test on *E. spinosum* seaweed reared for 30 days with the floating raft method and basic pegs can be seen in Fig. (4) Cultivation methods between floating rafts and basic pegs carrageenan obtained has a high enough difference, which is where the basic peg method has the highest value of 24% and the lowest floating raft of 11%. Carrageenan levels in each species of *Kappaphicus* sp. range from 54-73%, depending on species and location, whereas seaweed carrageenan content in Indonesia ranges from 61.5-67.5% **(Parenrengi *et al.*, 2012; Asni & Najamuddin, 2021)**. *E. spinosum* on floating rafts is at the surface of the water so that the thallus expends more energy to adapt to the movement of sea surface water. *E. spinosum* on the bottom pegs remains at the water's base, minimizing energy expenditure for thallus adaptation to water

movement. This allows more energy to be directed toward carrageenan formation. According to **Alimudin (2013)**, the yield of carrageenan from each seaweed is very diverse, it is strongly influenced by several factors including species, location of cultivation, and climate where cultivation. In line with **Dean *et al.* (2023)**, this is due to differences in environmental conditions in each cultivation site that can affect the growth and quality of seaweed. Some environmental factors that can affect the content of carrageenan extracts and nutrients in seaweed include water temperature, light intensity, salinity, and water quality.

Table 1. Chlorophyll-a, phycoerythrin, and carotenoid contents of *E. spinosum* seaweed cultivated by different methods

No.	Sample Code	Chlorophyll-a Level (mg/L)	Phycoerythrin Level (µg/L)	Carotenoid Content (µmol/L)
1	*E. spinosum* Floating Raft	5,41	2,69	6,69
2	*E. spinosum* Basic Stake	1,38	0,91	4,23

5. Chlorophyll-a content

Chlorophyll is a pigment owned by seaweed, one of which is seaweed species *E. spinosum*. Based on the results of the analysis of chlorophyll-a levels obtained from *E. spinosum* seaweed in the floating raft method (5.42mg/ l) and in the method of basic pegs (1.38mg/ l), the floating raft method can be seen to have a higher chlorophyll-a content than the basic peg method. High and low value of chlorophyll-a content is influenced by the intensity of light, it is in the floating raft method that seaweed receives a lot of sunlight compared to the basic peg method so that seaweed *E. spinosum* can stimulate to produce more chlorophyll-a. According to **Astriana *et al.* (2019)**, different water depths mean that different waters receive different amounts of sunlight. This is because the waters become deeper and the intensity of sunlight entering the waters decreases thus it also causes a decrease in the rate of photosynthesis in plants; chlorophyll has health benefits. Research by **Ebrahimi *et al.* (2023)**, showed that chlorophyll pigments have biological effects that improve health, including antioxidant, anti-inflammatory, and anticancer effects. According to **Limantara and Rahayu (2008)** as cited in **Merdekawati and Susanto (2009)**, chlorophyll is widely used in food, beverages, medicine, and some home industries. Chlorophyll helps repair tissues, cleanses the blood, helps the liver produce red blood cells, and acts as a cleansing agent in the body.

6. Carotenoid content

Carotenoids are red to orange-colored pigments that are usually bound to chlorophyll in chloroplasts. These pigments are abundant in nature and are found in

higher plants, algae, fungi, and bacteria in both photosynthetic and non-photosynthetic tissues **(Maleta *et al.*, 2018)**. **Adrian *et al.* (2021)** clarified that carotenoid pigments are a group of yellow, orange, and orange-red pigments. Pigments are natural colorants found in plants and animals. Carotenoid pigment extracts can be separated using chromatographic methods. Common chromatographic methods for determining pigment types are column chromatography (CT) and thin-layer chromatography (TLC). Algae *E. spinosum* contains carotenoids up to 6.69mol/ L in floating rafts and 4.23mol/ L in bottom stakes. Carotenoids play an important role in supporting human health and survival. These pigments are associated with enhanced immune response and protection against cancer and are potent antioxidants. One of the most important physiological functions of carotenoids is acting as precursors of vitamin A. The function of carotenoids as provitamin A means that the pigments can be used to prevent and treat eye diseases such as cataracts, xerophthalmia, night blindness, and macular degeneration. In line with **Johra *et al.* (2020)**, β-carotene is a precursor of vitamin A that essentially functions in many biological processes including vision. The human macula lutea and eye lens are rich in lutein, zeaxanthin, and meso-zeaxanthin, collectively known as macular xanthophylls, which help maintain eye health and prevent ophthalmic diseases. Carotenoids provide chlorophyll energy at depth and help protect plants from excess ultraviolet radiation. Carotenoids are potential antioxidants that can protect cells and organisms from oxidative damage caused by free radicals produced by the body during metabolism **(Minsas *et al.*, 2023)**.

7. Phycoerythrin content

Phycoerythrin is an oligomeric protein that has 3 sub-units, namely, αβ, and γ with the form (αβ) 6γ hexamers and chromophore (phycobilin) with a tetrapyrrole structure **(Pan *et al.*, 2013; Charrier *et al.*, 2018)**. Phycoerythrin is a pigment from the red alga *E. spinosum* that has antioxidant activity and has the potential to be developed in the nutraceutical field. One of the main pigments of red algae is phycobilin, which is composed of phycoerythrin, phycocyanin, and allophycocyanin **(Pagulendren *et al.*, 2012)**. The results showed the value of phycoerythrin levels of 2.69µg/ L on floating rafts and 0.91µg/ L on basic stakes. Ficoerythrin has a high stability compared to other pigments, with a pH range between 3.5 to 9.5 according to the results of the study **(Kawsar *et al.*, 2011)**. Phycoerythrin, like phycobilin, is a protein that acts as a complementary pigment in red and blue-green algae and helps chlorophyll absorb light in the process of photosynthesis in algal cells. Light absorbed by phycoerythrin is efficiently transferred to phycoerythrin, then to allophycocyanin, then to allophycocyanin B, and finally to chlorophyll **(Abfa *et al.*, 2013)**. The chemical properties of phycoerythrin allow this compound to stop reactions through oxidation and reduction to produce reactive oxygen species (ROS), so it can reduce the negative effects of free radicals, strengthen the defense system against oxidants, and protect against damage due to oxidation

processes **(Lailani *et al.*, 2020)**. Phycoerythrin has the potential as an antioxidant. In this way, this pigment can slow down and even inhibit the oxidation of substances, protecting cells from the effects of free radicals. Apart from being an antioxidant, it also has the potential as a natural pigment and fluorescent marker that is stable at high temperatures **(Pumas *et al.*, 2012)**.

8. Proximate analysis

Table 2. Results of proximate analysis of *E. spinosum* seaweed cultivated with different methods

Code	Water (%)	Ash (%)	Crude fat (%)	Crude fiber (%)	Crude protein (%)
Floating Raft	28,2098	32,0270	0,0199	4,5991	7,9170
Base Stake	31,6676	30,7466	1,7061	6,0548	4,3919

The results of the proximate analysis test can be seen in Table (2). These results have different contents in each parameter. The water content in the floating raft is 28.2098% and the base peg is 31.6676%. According to **Ananda (2019)**, dry red seaweed with a drying process for 40 hours has a moisture content of 40%. Water content is very influential on the quality of a material. The lower the water content in seaweed, the better the quality of the seaweed. The high water content is caused by the drying process that is less stable or not optimal. Therefore, the water content obtained is higher **(Lifendro *et al.*, 2021)**. In congruent with **Manteu *et al*, (2018)**, the high and low water content is influenced by the drying process.

The test results of ash content in this study were obtained on floating rafts at 32.0270% and on the base peg at 30.7466%. These results are different from the seaweed *K. alvarezii* studied by **Dean *et al.* (2023)**, where the ash content obtained was 31.572%. High ash content in seaweed is associated with the absorption of mineral nutrients, as well as being form of adaptation to environmental conditions of marine waters containing various minerals with high concentrations **(Dharmananda, 2002)**.

Based on the results of research on the red seaweed *E. spinosum*, it has a crude fat content in the floating raft method of 0.0199% and the basic peg method of 1.7061%. According to **Kumar *et al.* (2011)**, the fat content of seaweed is generally less than 4% and generally lower than land plants. The low-fat content is due to the form of storage of food reserves in plants in the form of carbohydrates, especially polysaccharides, hence vegetable fats generally have a low percentage.

Fiber is the part of a plant component that cannot be absorbed by the body. Fiber is generally divided into two, namely dietary fiber and crude fiber. The results of crude fiber analysis on *E. spinosum* seaweed recorded 4.5991% on floating rafts and 6.0548% for the basic peg method. **Nurjanah *et al.* (2018)** noted that seaweed fiber content reaches 30-40% dry weight, and seaweed is known as a source of fiber that can be used

as a functional food to prevent obesity and degenerative diseases. **Ortiz *et al.* (2006)** stated that seaweed fiber content is influenced by season, geographic location, species type, harvest age, and environmental conditions.

The protein content in this study in the floating raft was 7.9170%, and the base peg was 4.3919%. **Fardiaz *et al.* (2011)** analyzed the proximate composition of the red seaweed (*Rhodophyceae*) *Eucheuma spinosum* in Nusa Penida Waters, reporting a protein content ranging from 4.85% to 5.95%, which is relatively low. Similarly, a study by **Dean *et al.* (2023)** on *Kappaphycus alvarezii* seaweed carrageenan found a protein content of 3.848% based on proximate analysis.

9. Water quality

The value of water quality parameters is still in the optimal range for the growth of *E. spinosum* in both cultivation methods, namely floating rafts and basic stakes, which in conducting seaweed cultivation activities have a range of water quality values supporting its growth. The temperature values obtained in the cultivation activities are still in the optimal range. This is in accordance with the statement of **Zou *et al.* (2018)** that the optimal temperature for aquaculture growth is 30.3 - 31.7°C where the water temperature has warmer water conditions. According to **Piazzi *et al.* (2002)**, the optimal temperature range to support seaweed growth is between 25-31°C. Temperature is very important for aquaculture growth, extreme temperature changes can result in the death of seaweed.

Table 3. Water quality results of *E. spinosum* seaweed cultivation media

Parameter	Floating Raft	Base Stake	Eligibility
Temperature (°C)	33	28	26 - 32 **(SNI, 2010)**
pH	-	-	7 - 8.5 **(SNI, 2010)**
Dissolved Oxygen (mg/l)	5,6	6,5	5-10 mg/l **(Cokrowati *et al.*,, 2020)**
Salinity (ppt)	29	29	28 - 34 **(SNI, 2010)**
Phosphate (mg/l)	<10	<10	0.14-0.72 mg/l **(Hoang *et al.*,, 2016)**
Nitrate (mg/l)	<10	<10	>0.04 mg/l **(SNI, 2010)**
Light intensity (lux)	750	580	500 - 1000 **(Sitorus *et al.*,, 2020)**

The optimal salinity range for seaweed growth according to **Atmanisa *et al.* (2020)** ranged from 28 to 31ppt. **Dawes (1987)** reported that macroalgae can still live on salinity between 5 - 35ppt. Dissolved oxygen in the waters if it reaches 5mg/ l then seaweed metabolism can run optimally. According to **Cokrowati *et al.* (2020)**, the optimal DO concentration for sargassum is 5-10mg/ L. Moreover, according to **Madina (2022)**, the DO quality standard for seaweed is more than <4mg/ l. Furthermore, according to **Widyartini *et al.* (2017)**, the optimal phosphate content in water is 0.1-0.2mg/ L.

Phosphate concentration for the fertile water should range from 0.051 to 0.1mg/L. Additionally, phosphate concentration also controls the growth **(Nursidi *et al.*, 2017)**. The optimal nitrate content for *Sargassum* growth ranges from 0.200-0.420ppm **(Widyartini *et al.*, 2015)**, hence it can be said that the waters have a good level of fertility and can be used for seaweed cultivation activities. According to **Kautsari and Ahdiansyah (2015)** and **Sarjito *et al.* (2022)**, the feasible nitrate concentration for seaweed cultivation ranges from 0.01 to 0.07mg/L, while the concentration of <0.01 is not suitable for seaweed cultivation. The light intensity obtained ranged from 7500 lux floating rafts and 5800 lux base stakes. According to **Sitorus *et al.* (2020)**, good light intensity for cultivation is around 5000 lux. Additionally, **Apriliyanti *et al.* (2024)** stated that when light intensity is reduced, seaweeds tend to experience slower growth because their photosynthesis process is disrupted. Photosynthesis in seaweed requires light as one of the main factors to produce the energy needed for growth.

CONCLUSION

The conclusion of this research is that *E. spinosum* cultivated in the basic peg and floating raft method experiences differences in growth, carrageenan yield, color pigment content, and proximate.

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