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Growth, Carottenoid, Fiber and Mineral Content of the Sea Grapes (*Caulerpa lentillifera*) Cultivated With Different Methods

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ABSTRACT

One of the main cultivation commodities currently being developed by the government is seaweed. *C. lentillifera* has a profitable selling value on the market, while the highest demand comes from Japan. Therefore, in order to develop *C. lentillifera* cultivation optimally, a comprehensive study was needed. Seaweed growth is influenced by several factors including the physics and chemistry of the cultivation environment. In addition, choosing the right cultivation method is based on the characteristics of the cultivation location. This research aimed to determine the best cultivation method for the sake of growth, with a positive effect on the fiber and ash content of *C. lentillifera* sea grapes. The method used in this research was an experimental research method with a completely randomized design (CRD) with 3 treatments, bottom method, off-bottom method and surface method, as well as 3 replications. The parameters observed were growth, carotenoid content, fiber and ash. Analysis of variance was carried out using the SPSS version 16.0 program. The highest absolute growth of *C. lentillifera* seaweed (98.33± 5.69) was obtained using the off-bottom method. Meanwhile, the lowest absolute growth (11.00± 3.61) was obtained using the bottom method. The fiber and mineral content of *C. lentillifera* is quite high at all depths. In general, the carotenoid content is strongly influenced by the intensity of light reaching the seaweed thallus. The measured water quality parameters are sufficient to support the physiological process of seaweed growing and developing with high quality. Different cultivation methods have significant differences in the absolute growth and quality of cultivated *C. lentillifera* seaweed. The best method in this research is using the off-bottom method.

INTRODUCTION

One of the main cultivation commodities currently being developed by the government is seaweed. Marine plants are classified as macroalgae which live attached to rocks and coral reefs at the bottom of the water. This macroalgae cannot be distinguished between the roots, stems and leaves, so this part of the plant is called the thallus.

Therefore, it is classified as a lower plant **(Ordóñez *et al*., 2022)**. Seaweed is not only human food but is also used as animal feed **(García-Vaquero & Hayes, 2016)**. Seaweed has many other commercial applications, such as acting as a bioindicator, aiding in the bioremediation of agricultural wastewater, and serving as a soil fertilizer **(Hong *et al.*, 2007; Sharma *et al*., 2014; Cole *et al*., 2016)**.

One seaweed that has the potential to be developed is *C. lentillifera* because it has a fairly high nutritional content and does not contain substances harmful to the body, hence this plant is very safe for daily consumption **(Hasbullah *et al*., 2016)**. This type of seaweed contains 0.16- 18% crude fiber, 0.08- 0.80% crude fat, 94- 96% wet content and 0.43- 0.55% crude protein **(Nofiani *et al*., 2018)**. Seaweed growth is influenced by several factors including the physics and chemistry of the cultivation environment. In addition, choosing the right cultivation method is based on the characteristics of the cultivation location. The planting methods used in this research were the base surface method, off-bottom method and bottom method. Differences in seaweed planting methods affect the growth and quality of seaweed. At the surface of the pond, the water absorption of sunlight is quite strong, but as the depth increases, the absorption of light becomes weaker **(Saffo, 1987)**.

One of the factors that determines the success of a seaweed cultivation business is the use of cultivation methods. Initially, people obtained *C. lentillifera* directly from the sea, but now *C. lentillifera* has begun to be cultivated in ponds, although it has not yet been developed like milkfish, shrimp or other fisheries commodities. *C. lentillifera* has a profitable selling value on the market, while the highest demand comes from Japan. Therefore, in order to develop *C. lentillifera* cultivation optimally, a comprehensive study was needed. However, there are several obstacles hindering the efforts to increase the production of *C. lentillifera* seaweed, such as the growth rate and quality of the *C. lentillifera* seaweed content. Seaweed growth is influenced by several factors including the physics and chemistry of the cultivation environment. In addition, choosing the right cultivation method is based on the characteristics of the cultivation location. The planting methods used in this research were the surface method, off-bottom method and bottom method.

Nutrients can be absorbed through water and soil, therefore the closer to the bottom of the pond, the greater the absorption of nutrients. Differences in photosynthesis and nutrient absorption processes in seaweed can affect the growth rate and the quality of the *C. lentillifera* seaweed content.

Based on previous research conducted by **Saffo (1987)** at the surface of pond water, the absorption of sunlight is quite strong, but as the depth increases, the absorption of light becomes weaker. The deeper the water , the lesser the intensity of sunlight , thus affecting the photosynthesis process. **Ega *et al*. (2016)** concluded that the intensity of sunlight received affects the growth; both low and high sunlight levels can impact the growth. Apart from that, nutrients are also needed at every depth **(Serdiati & Widiastuti,**

2010). To improve the quality of the *C. lentillifera* seaweed cultivation, research should be carried out using different methods in ponds based on the distance from the bottom of the pond to determine its effect on the growth, carotenoid content, fiber and ash content of *C. lentillifera* seaweed.

MATERIALS AND METHODS

This research was conducted over a period of 6 weeks, from December 2023 to January 2024, at the Brackish Water Aquaculture Fisheries Center in Takalar Regency. The seaweed seeds used were *C. lentillifera*. The seeds were cleaned to remove dirt and any attached organisms. Each basket contained 200g of *C. lentillifera*, totalling 1.8kg. The selected seaweed seeds were young, fresh, clean, and free from other types of seaweeds.

The container used for this research was a plastic basket with dimensions 45 x 32 x 16cm (Fig. 1).

Fig. 1. Container for seaweed test

The weight of the *C. lentillifera* seaweed at the start of the research was 200 per container with the treatment of placement of growth at different cultivation depths, namely bottom method, off-bottom method and surface method. The depth of the water in the cultivation pond was 100cm (Fig. 2).

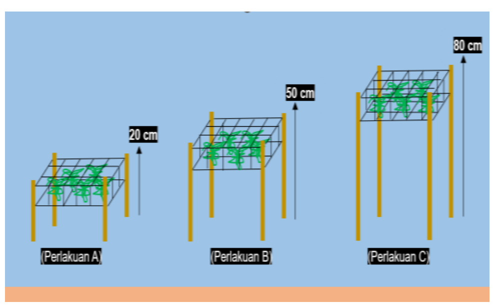

Fig. 2. Layout of the research container

The seaweed was maintained for 6 weeks (±42 days), and data collection (weighing) on the weight of the seaweed was carried out once. Thus, there was 1 time collection of seaweed weight data during the research.

The experimental design used was a completely randomized design (CRD) with 3 treatments and 3 repetitions, hence there were 9 experimental units. The treatments were:

Treatment A : Bottom method (20cm above the bottom)
Treatment B : Off-bottom method (50cm above the bottom)
Treatment C : Surface method (20cm below the water surface)

Parameters observed

1. **Growth**

Total growth of *C. lentillifera* was calculated using the formula from **Supriadi *et al.* (2016)**:

$W = Wt - Wo$

where:

- W = Absolute growth (g)
- Wt = Final measurement weight (g)
- Wo= Initial weight (g)

2. **Carotenoid content**

Carotenoid content was analyzed at the end of the study using the method proposed by **Shahidi and Brown (1998)**, with the following formula:

$C(ppm) = (E \times B)(A460 \times Vextract)$

Where:

- C = Carotenoid pigment concentration (from seaweed extract)
- A460 = Absorbance at 460nm
- $V_{extract}$ = Extract volume (mL)
- E = Extension coefficient of 1% standard in acetone (2200)
- B = Weight of extracted sample (g wet weight)

3. **Fiber content**

Fiber content of *C. lentillifera* was analyzed at the end of the study using the method from **Syamsuddin and Azis (2019)**, calculated with the following formula:

$\text{Crude fiber content} = c(a - b) \times 100\%$

Where:

- a = Weight of cup with dry residue
- b = Weight of empty cup
- c = Weight of sample

4. **Ash content**

Ash content was analyzed at the end of the study using the method from **Syamsuddin and Azis (2019)**, calculated with the following formula:

$\text{Ash content} = W(W1 - W2) \times 100\%$

Where:

- W1 = Weight of cup + sample after ashing
- W2 = Weight of empty cup

- W = Sample weight before ashing

Measurement of water quality parameters was carried out every day starting at the beginning of cultivation until the end of the maintenance period. The physical parameters measured were salinity, temperature and pH. Regarding the measurement of chemical parameters, they were measured at the beginning, middle and end of the maintenance. The chemical attributes were measured in several ways: phosphate, nitrate, ammonium and carbon dioxide (CO2).

The absolute growth data were analyzed and carried out using the analysis of variance (ANOVA), and further W-Tukey tests using SPSS version 16.0 software. Data related to water quality parameters were analyzed descriptively according to the needs of the *C. lentillifera* cultivation.

RESULTS

The results of the analysis of variance (ANOVA) showed that each method tested produced a significant absolute growth of *C. lentillifera*. The results of the Tukey test showed that the off-bottom method produced the highest absolute growth of the *C. lentillifera* seaweed, followed by the surface method, while the bottom method resulted in the lowest absolute growth (Table 1).

Table 1. Absolute growth of *C. lentillifera* at different depths

Treatment (Method)	Mean Absolute Growth (g±SD)
Bottom Method	11,00 ± 3,61^{b}
Off-Bottom Method	98,33 ± 5,69^{b}
Surface Method	69,00 ± 8,19^{b}

Description: numbers with different letter codes in the absolute growth column showed a significant difference (P <0.05).

The following data on the analysis of carotenoid, fiber and ash content of *C. lentillifera* sea grapes which were analyzed at the end of the study (Table 2).

Table 2. Carotenoid, fiber and ash content at different cultivation method

Treatment (Method)	Carotenoid content (%)	Fiber %	Ash %
Bottom method	0,85	7.97	25.12
Off-bottom method	0,83	6.56	35.95
Surface method	0,82	6.66	34.40

The following data are the results of water quality measurements in the *C. lentillifera* maintenance media during cultivation, which can be seen in Table (3).

Table 3. Water quality parameters during the study

Parameter	Range
Salinity	25-35ppt
Temperature	29- 32ºC
pH	8.0- 8.7
Nitrate	0.237- 15.672ppm
Phosphate	0.051- 1.00ppm
Ammonium	0.01- 0.03ppm

DISCUSSION

The absolute low growth in the bottom method is caused by the presence of moss plants attached to the seaweed *C. lentillifera* which is thought to be the cause of slow growth due to competition for nutrients between seaweed and moss plants. This is in accordance with the findings of **Anggadiredja *et al.* (2006)** who stated that plants around the cultivated plants are competitors that can disrupt the growth of seaweed.

In addition, the low absolute growth is thought to be influenced by different light intensity factors in different cultivation methods. Growth in the off-bottom method and surface method is higher than on the base method, because the ability of light to penetrate on the off-bottom method and surface method is more optimal than on the bottom method. **Susilowati *et al*. (2012)** stated that depth is one of the determining factors in the growth rate of seaweed, because the increasing depth of planting causes the intensity of light reaching the water column to decrease. This is because the photosynthesis process decreases with depth; as you go deeper, the intensity of sunlight diminishes, which affects photosynthesis. According to **Ega *et al.* (2016)**, the intensity of sunlight received affects growth since both low and high sunlight intensity affects the growth of seaweed itself. Apart from that, nutrients are also needed at every depth **(Serdiati & Widiastuti, 2010)**.

Table 2. Carotenoid, fiber and ash content at different cultivation method

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Green seaweed (including mfera) generally contains carotenoids, which function as antioxidants **(Tamat *et al.*, 2007)**, preventing the process of free radical oxidation, which is beneficial for human health. In this study, the highest carotenoid content of *C. lentillifera* was found in the bottom method, lower when compared to the off-bottom method and the surface method, which had relatively the same carotenoid content (Table 2). The differences in the results of carotenoid content in *C. lentillifera* obtained in this study were due to the seaweed's absorption of sunlight not being the same in each cultivation method. **Olaizola and Duerr (1990)** elucidated that, the low levels of carotenoid pigments occur because the ideal light intensity reaches the seaweed thallus. In green algae, high carotenoid content occurs when the algae is exposed to an excessive light intensity (quantity) or ultraviolet light, which can cause damage (due to photoinhibition, photooxidation, photodamage) to chlorophyll **(Glenn & Doty, 1981)**, because carotenoids function to protect chlorophyll damage by this phenomenon **(Kabinawa, 2006)**.

C. lentillifera grown using the bottom method has a relatively higher fiber content when compared to *C. lentillifera* grown using the off-bottom method and the surface method (Table 2). The fiber content of *C. lentillifera* in this study was higher compared to the carotenoid content of *C. lentillifera* due to the high polysaccharides in seaweed cells. According to **Ruperez and Saura-Calixto (2001)**, the high fiber content of seaweed cannot be separated from its carbohydrate components. Variations in content are caused by environmental conditions **(McDermid *et al.*, 2005)**. These include nutrient concentrations **(Wong & Cheung, 2000)**. Other influencing factors are the algae's ability to absorb nutrients, growth phase in addition to the photosynthetic activity **(Wong & Cheung, 2000; Matanjun *et al*., 2009)**.

The ash content of seaweed, as defined by **Winarno (1990)**, is the inorganic residue left after burning and consists of minerals, including various metal salts. These minerals are essential for human nutrition **(Winarno, 1990)** and include iron, zinc, magnesium, calcium, potassium, and sodium **(Krishnaiah *et al.*, 2008)**, as well as silica, arsenic, copper, cobalt, cadmium, molybdenum, lead, and chromium **(Hampel, 2013)**. For *C. lentillifera*, the macro minerals present are potassium (K), calcium (Ca), magnesium (Mg), and sodium (Na), while the micro minerals include iron (Fe), zinc (Zn), and manganese (Mn) **(Tapotubun, 2018)**.

The highest ash content was found in the off-bottom method treatment, followed by the bottom method treatment, then in the surface method (Table 2). In this study, the high ash content was thought to be because the sea grapes used had quite high mineral content **(Idham & Isamu, 2018)**.

The results of salinity measurements during the research ranged from 28- 30ppt, which is still in the ideal range for growing the *C. lentillifera* seaweed. According to **Guo *et al.* (2015)**, *C. lentillifera* seaweed can survive at a salinity of 20- 50ppt, but its growth can only occur at a salinity of 20- 45ppt. Salinity plays a crucial role in the success of seaweed cultivation. A significant increase or decrease in salinity, beyond the optimal range, can reduce the quality of the seaweed and lead to the death of many plant cells **(Serdiati & Widiastuti, 2010)**.

The temperatures measured during the study ranged from 29- 31°C, which still allows *C. lentillifera* to grow and develop well. According to **Indriani and Suminarsih (2003)**, seaweed can grow and develop well at water temperatures between 26- 30°C.

However, **Guo *et al*. (2015)** stated that temperatures of 25– 30°C can cause branch formation in the seaweed *C. lentillifera* and can increase the specific growth rate.

The results of pH measurements during the research ranged between 6- 8.5, and the ideal pH for seaweed growth is 6- 9. According to **Widyorini (2010)**, a pH of less than 6.5 will suppress the growth rate, the acidity level can even be deadly, and there will be no reproduction rate, while the ideal pH for cultivation is between 6.6 and 9.

Based on nitrate data collected in this study, which ranged between 0.1064-0.1973ppm, nitrate is one of the most common forms of nitrogen found in natural waters and is the main nutrient for plant growth. For the growth of marine algae, the range of nitrate data collected in this study is not ideal since a range of 0.9 to 3.5ppm is required **(Atmadja *et al*., 1996)**. This is in line with the opinion of **Nuraini (2006)**, who assessed that the ideal nitrate content conditions in waters for algae growth are 0.9– 3.5ppm. In addition, research by **Pong-Masak and Sarira (2015)** found that the generally ideal nitrate range for seaweed growth is 0.95– 3.5ppm.

C. lentillifera really needs phosphate to grow, develop and produce. To encourage algae growth, the ideal phosphate range is 0.1– 3.5ppm **(Kapraung, 1987)**. However, after measuring the water quality during the research, phosphate was found to be 0.0097-0.0089ppm. Nitrogen compounds influence the amount of phosphate that *C. lentillifera* requires for an optimal growth. In situations where the nitrogen is in the form of ammonium salts, the highest phosphate concentration will be lower.

Ammonium is the main product of aquatic organisms that produce nitrogen in waters. Based on the ammonium data collected from this research, it ranges from 0.0021-0.0028ppm, where the ammonia range is not optimal, and this range is included in the lower category. According to **Andarias (1992)**, the ammonium levels for the survival of marine algae are between 0.01 and 0.03ppm. **Setiaji *et al*. (2012)**, found that an ammonia content of approximately 0.5ppm is beneficial for the growth of *C. lentillifera*.

CONCLUSION

Differences in cultivation methods affect the absolute growth of *C. lentillifera* seaweed significantly. The off-bottom method yielded the highest growth of *C. lentillifera*. The bottom method resulted in the highest carotenoid content at 0.85ppm, while the off-bottom method produced the highest fiber content at 7.97%. The bottom method also recorded the highest ash content at 35.95%. Water quality measurements, including temperature, salinity, and pH, remained within the ideal conditions for the growth of *C. lentillifera*.

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